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REVIEW

The role of microenvironment in tumor angiogenesis

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Abstract Tumor microenvironment is essential for tumor cell proliferation, angiogenesis, invasion and metastasis through its provision of survival signals, secretion of growth and pro-angiogenic factors, and direct adhesion molecule interactions. This review examines its importance in the induction of an angiogenic response in tumors and in multiple myeloma. The encouraging results of pre-clinical and clinical trials in which tumors have been treated by targeting the tumor microenvironment are also discussed.

Keywords Angiogenesis · Anti-angiogenesis · Metastasis · Microenvironment · Multiple myeloma · Tumor growth

Tumor growth and metastasis are angiogenesis dependent

The current wisdom is that both solid and hematological tumors are endowed with angiogenic capability and their growth, invasion and metastasis are angiogenesis dependent [1]. Judah Folkman, a highly rated pioneer and researcher in this field wrote: "Once tumor take has occurred, early increase in tumor cell population must be preceded by an increase in new capillaries that converge upon the tumor" [2]. Solid and hematological tumors are endowed with angiogenic capability and their growth, invasion and metastasis are angiogenesis dependent.

Angiogenesis is important for supplying oxygen, nutrients, growth factors, hormones, and proteolytic enzymes which control the coagulation and fibrinolytic systems, as well as dissemination of tumor cells to distal sites. Angiogenesis is controlled by the balance between molecules that have positive and negative regulatory activity. This concept led to the notion of the "angiogenic switch", which depends on an increased production of one or more positive regulators of angiogenesis [3]. Endothelial cell turnover in the healthy adult organism is low, the quiescence being maintained by the dominant influence of endogenous angiogenesis inhibitors over angiogenic stimuli. In pathological conditions, angiogenesis may be triggered not only by the overexpression of pro-angiogenic factors, but also by the down-regulation of inhibitory factors.

About 30 angiogenic factors have been identified, including vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), transforming growth factor alpha and beta (TGF-α and -β), platelet derived growth factor (PDGF), tumor necrosis factor alpha (TNF-α), angiogenin, interleukins (ILs), chemokines and angiopoietins (Angs). On the other hand, several anti-angiogenic factors have been described, such as angiostatin, endostatin and thrombospondin.

Neoplastic cell populations can only form a clinically observable tumor if the host produces a vascular network sufficient to sustain their growth. Furthermore, new blood vessels provide them with a gateway through which to enter the circulation and metastasize distant sites.

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The importance of microenvironment in tumor growth

Studies on neoplastic transformation have focused on events that occur within transformed cells. They have addressed the microenvironment of tumor cells and documented its importance in supporting tumor progression. The pathogenesis of most cancers, in fact, includes complex and mutual interactions that affect the number and phenotype of the tumor cells and various normal stromal cells. The intricate tumor–microenvironmental interactions are increasingly recognized as critical features of several neoplasias.

Tumor cells are surrounded by an infiltrate of inflammatory cells: lymphocytes, neutrophils, macrophages and mast cells, which communicate via a complex network of intercellular signaling pathways, mediated by surface adhesion molecules, cytokines and their receptors. This infiltrate, particularly macrophages, may contribute to tumor angiogenesis, and there are many reports of associations between macrophage infiltration, vascularity and prognosis. Tumor-associated macrophages accumulate in poorly vascularized hypoxic or necrotic areas [4], and respond to experimental hypoxia by increasing the release of VEGF and FGF-2 and a broad range of other factors, such as TNF-α, urokinase and matrix netalloproteases (MMPs) [5]. Moreover, activated macrophages synthesize and release inducible nitric oxide synthase (NOS), which increases blood flow and promotes angiogenesis [6].

The importance of microenvironment in tumor metastasis

It has long been accepted that most malignant tumors show an organ-specific pattern of metastasis. For example, colon carcinomas metastasize usually to liver and lung but rarely to bone, skin or brain and almost never to kidneys, intestine or muscle. In contrast, other tumor entities, such as breast carcinomas, frequently form metastases in most of these organs. This specific formation of secondary tumors at distant sites appears to require a number of steps which must be successfully completed by metastazing tumor cells [7].

Various explanations have been proposed for the site selectivity of blood–bone metastases, including tumor cell surface characteristics [8, 9], response to organ derived chemotactic factors [10], adhesion between tumor cells and the target organ components [11, 12] and response to specific host tissue growth factors [13]. The relative importance of pre-existing tumor subpopulations with specific metastatic properties and the organ environment characteristics in determining metastatic homing have been debated [14–16].

An alternative explanation for the different sites of tumor growth involves interactions between the metastatic cells and the organ environment, possibly in terms of specific binding to endothelial cells and responses to local growth factors. Endothelial cells in the vasculature of different organs express different cell surface receptors and growth factors that influence the phenotype of the corresponding metastases. Greene and Harvey [17] first suggested that the organ distribution patterns of metastatic foci were dependent on the formation of sufficient adhesive bonds between arrested tumor cells and endothelial cells, and they hypothesized that these interactions were similar to lymphocyte/endothelial cells at the sites of inflammation.

The development of organ-derived microvascular endothelial cell cultures has allowed more specific studies on the preferential homing of tumor cells. Auerbach and co-workers [18, 19] found that different tumors showed differences in their adhesive propensity and preference for different endothelial cells, and in a few cases preferential adhesion was observed to the endothelial cells derived from the organ of origin and the target organ.

The “seed and soil” theory

In 1889, the English surgeon Stephen Paget published his “seed and soil” explanation of the non-random pattern of metastasis, and was the first to suggest that interactions between tumor cells and host cells in the microenvironment are critical in regulating tumorigenesis [20]. Certain favored tumor cells (the “seed”), he said, had a special affinity for the growth-enhancing milieu within specific organs (the “soil”), and hence metastasis only occurred when the “seed” and the “soil” were compatible.

The importance of several components of the “soil” in regulating tumor growth has since been emphasised: (1) the extracellular matrix (ECM); (2) stromal cells and their growth factors and inhibitors; (3) microvessels and angiogenic factors; (4) inflammatory cells. There is now substantial evidence that tumor growth and progression depend on the cross talk between malignant cells and their adjacent stromal compartment.

Angiogenesis in multiple myeloma

Multiple myeloma (MM) is a B-cell neoplasm characterized by clonal expansion of malignant plasma cells in the bone marrow, where they proliferate and acquire resistance to apoptosis and eventually lead to osteolysis, renal dysfunction and anemia. It is still incurable with a median survival of approximately 4 years.

Bone marrow angiogenesis plays an important role in the pathogenesis and progression of MM, as in other hematological malignancies [21]. Growth is halted and a dormancy state is induced in the avascular phase (such as monoclonal gammopathy of undetermined significance (MGUS), or non-active MM) [22], whereas with clonal expansion and epigenetic modifications (hypoxia, shear stress) of the microenvironment tumor plasma cell subsets switch to an angiogenic phenotype that generates the "vascular phase" (active MM), and involves changes in the local balance between pro- and anti-angiogenic factors.

The cause of induction of the vascular phase is the subject of current investigation [23]. Several studies show overexpression and secretion of VEGF by the clonal plasma cells. VEGF stimulates proliferation and chemotaxis in both endothelial cells (EC) via VEGF receptor-2 (VEGFR-2) and stromal cells via VEGFR-1. These cells are rapidly phosphorylated by the interaction with VEGF, and signal via extracellular signal-related kinase-2 (ERK-2) [24].

A murine model for MM indicates that the switch is preceded by the expression of mRNA for VEGF and secretion of the protein by plasma cells, and by a shift from CD45 positive to CD45 negative plasma cells that are the VEGF producers [25]. Accordingly, CD45 expression (as the CD45 positive percentage) by a patient's bone marrow plasma cells is inversely correlated with the degree of bone marrow angiogenesis [26]. Other studies demonstrate that the expression levels of VEGF, FGF-2 and their receptors overlap between MGUS, smoldering MM (SMM) and newly diagnosed MM (NMM). However, 63% of MGUS samples inhibit angiogenesis in vitro compared to 43% SMM and 4% NMM. Hence the angiogenic switch from MGUS to NMM is partly refefable to an increasing tumor burden rather than increased expression of VEGF and/or FGF-2 and by a loss of the MGUS anti-angiogenic activity [27].

The importance of microenvironment in regulating MM angiogenesis

Since MM mainly progresses in the bone marrow, signals from this microenvironment play a critical role in maintaining plasma cell growth, migration and survival. The pathogenesis of most cancers includes complex and mutual interactions that affect the number and phenotype of the tumor cells and host stromal cells [28]. Reciprocal positive and negative interactions between plasma cells and bone marrow stromal cells (BMSC), namely hematopoietic stem cells, fibroblasts, osteoblasts/osteoclasts, chondroclasts, EC, EC progenitor cells, T lymphocytes, macrophages and mast cells, are mediated by an array of cytokines, receptors, and adhesion molecules. The MM microenvironment is formed by clonal plasma cells, ECM proteins and BMSC, which are intimately involved in all biological stages of intramedullary growth [29]. Interactions between these components determine the proliferation, migration and survival of plasma cells, as well as their acquisition of drug resistance and the development of diseases [30–32]. Receptors expressed by plasma cells, such as $\alpha_V\beta_3$ integrin, are crucial for their relationships with each other (homotypic interrelationships) and with ECM proteins [33]. Very late activating antigen-4 (VLA-4), leukocyte function-associated antigen (LFA-1), mucin-1 antigen (MUC-1) expressed by plasma cells, and vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) expressed by BMSC mediate their heterotypic and homotypic interactions. All these interactions result in enhanced expression and release of cytokines and growth factors needed for the plasma cell survival [34].

Plasma cells in the bone marrow microenvironment secrete cytokines, such as TNF-α [35], TGF-β, VEGF, FGF-2 [36], hepatocyte growth factor/scatter factor (HGF/SF) [37], Ang-1 and MMPs [36, 38]. Moreover, binding of plasma cells to BMSC triggers transcription and secretion by the latter of cytokines, such as IL-6 [39], insulin-like growth factor (IGF-1) [40] and VEGF [41] and CXCL12/stromal cell derived factor-1α (SDF-1α) [42], that mediate cell growth (IL-6, IGF-1, VEGF), survival (IL-6, IGF-1), drug resistance (IL-6, IGF-1, VEGF), migration (IGF-1, VEGF, MMPs, SDF-1α) and angiogenesis (VEGF) in the bone marrow. MM endothelial cells (MMEC) differ from umbilical vein EC (HUVEC). They produce growth and invasive factors for plasma cells, including VEGF, FGF-2, MMP-2, and MMP-9 [43].

Bone marrow MMEC express more mRNA and secrete larger amounts of the CXC-chemokines CXCL8/IL-8, CXCL11/interferon-inducible T-cell alpha chemoattractant (I-TAC), CXCL12/SDF-1α and CCL2/monocyte chemotactic protein-1 (MCP-1) than HUVEC, and paired plama cells express cognate receptors to a variable extents, which suggests that paracrine loops between MMEC and plasma cells involving CXC-chemokines and their receptors are operative in MM patients and mediate plasma cell proliferation and chemotaxis [42].

This tumor–host interplay highlights a reciprocal relationship that sustains and promotes the progression of MM by inducing pathological developments, such as angiogenesis and osteolysis [29].

How the pro-angiogenic activity of the cytokines in bone marrow microenvironment may be counteracted

Since the cytokine network between plasma cells and BMSC in the bone marrow milieu promotes plasma cell

growth, survival and migration, and plasma cells in the bone marrow are resistant to conventional agent treatment, targeting this network constitute a rationale to the treatment of MM.

Inhibition of NF-kB activity by specific IkB kinase inhibitor down-regulates IL-6 secretion in BMSC and related plasma cell growth [44]. Furthermore, inhibition of either p38MAPK or TFG-β by specific inhibitors down-regulates IL-6 secretion in BMSC [45, 46]. Targeting inhibition of Ras/Raf/MEK/ERK signaling by using the farnesyltransferase inhibitors (FTI) SCH66336 and R115777 abrogates plasma cell growth [47]. The proteasome inhibitor bortezomib (formerly PS-341) can overcome the protective effect of IL-6 against dex-induced apoptosis in MM by inducing caspase-8, -9, -3 activation, which results in caspase-dependent gp130 cleavage [48, 49]. Moreover, bortezomib inhibits DNA repair and may restore the sensitivity of MM cells to DNA damaging chemotherapeutic agents, suggesting that its combination with conventional chemotherapy may augment clinical effectiveness and overcome resistance in patients with relapsed or refractory MM [50].

VEGF and VEGFR inhibitors may offer therapeutic promise. Thalidomide directly inhibits the growth and survival of MM cells and/or BMSC, modulates adhesive interactions between them, and alters the secretion and bioactivity of cytokines they release into the bone marrow milieu [51]. Treatment with two classes of thalidomide analogs, namely selected cytokine inhibitory drugs (SelCiDs) and immunomodulatory drugs (IMiDs), may alter MM adhesion to BMSC and fibronectin, and abrogate the up-regulation of IL-6 and VEGF induced by tumor cell binding. Ongoing studies are evaluating the efficacy of a humanized monoclonal antibody against VEGF, bevacizumab (Avastatin) in patients with relapsed or refractory MM (with or without thalidomide) [52].

PTK 787/ZK222584 is an oral tyrosine-kinase inhibitor that also binds VEGFR-1. Hence it acts directly on MM plasma cells and inhibits the autocrine VEGF/VEGFR-1-induced plasma cell growth and migration and the paracrine (IL-6-mediated) growth. PTK/ZK (1.25 mg/day) is currently being evaluated in clinical phase I trials [53]. The indazolypyrimidine GW654652 inhibits all three VEGF receptors with similar potency and the VEGF-triggered migration activity and proliferation of MM cell lines, including those sensitive and resistant to conventional therapy [54]. GW654652 also acts in the bone marrow milieu, since it inhibits both IL-6 and VEGF secretion, as well as proliferation of plasma cells induced by their binding to BMSC; it is anti-angiogenic too since blocks HUVEC proliferation [54].

The therapeutic action of bortezomib-induced inhibition of the proteasome in MM is probably a result of direct cytotoxicity and effects on the bone marrow milieu [49]. The anti-angiogenic effect of bortezomib is another potential mechanism of its anti-MM activity [55, 56]. Moreover, bortezomib down-regulates caveolin-1 expression and inhibits caveolin-1 tyrosine phosphorylation, which are required for VEGF-mediated MM cell migration on fibronectin, and blocks VEGF-induced tyrosine phosphorylation of caveolin-1 in HUVEC, thereby inhibiting ERK-dependent EC proliferation [52].

High local VEGF concentration in the MM bone marrow milieu suppresses the anti-proliferative effect of several chemotherapeutic agents, hence promoting multidrug resistance [57]. Combination of these agents along with drugs that block VEGF signaling may enhance anti-MM efficacy by normalizing and sensitizing the tumor vasculature and improving oxygenation and delivery of such agents to tumor cells and EC.

Inhibition of wild-type and constitutively activated FGFR-3 autophosphorylation in human MM cell lines by the FGFR-specific tyrosine kinase inhibitors SU5402, SU10991, PD173074 or CHIR258 is associated with decreased viability and tumor cell growth arrest, both in vitro and in vivo in a murine model [58–60].

IGF-1 receptor (IGF-1R) inhibition whether by neutralizing anti-IGF-1R specific monoclonal antibodies, antagonistic peptides, or selective IGF-1R kinase inhibitors prevents MM cell proliferation by blocking the Ras/Raf/MAPK and Pl3K/AKT-1 pathways; induces phosphorylation of proapoptotic FKHR; down-regulates intracellular anti-apoptotic proteins, and also increases telomerase activity [40, 61–65].

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