Angiogenesis: A Target in Solid Tumors, Also in Leukemia?

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Targeting angiogenesis has become an established therapeutic approach to fighting solid tumor growth in cancer patients. Even though increased angiogenesis has long been recognized in various types of hematologic malignancies, the molecular basis underlying this angiogenic switch in leukemias remains poorly understood. The BM stroma is gaining increasing attention for its role in promoting leukemia growth and resistance against current treatments with tyrosine kinase inhibitors. This article provides a brief overview of the role of angiogenesis in leukemias, discusses recent insights into the role of placenta growth factor (PIGF), a VEGF family member, as a novel disease candidate in chronic myeloid leukemia (CML), and highlights the therapeutic potential of PIGF blockade for imatinib-resistant CML.

Introduction

Small organisms, such as the fruit fly Drosophila melanogaster or the worm Caenorhabditis elegans, lack a proper vascular system because oxygen can diffuse from the environment to all parts of their bodies. In contrast, higher organisms need a system to transport oxygen and nutrients because these substances cannot be distributed by simple diffusion. Therefore, the establishment of a vascular system was essential evolutionarily in organisms in which the limits of oxygen diffusion were outgrown. Functional blood vessels provide the basis of tissue homeostasis and growth with the supply of oxygen and nutrients and the elimination of metabolic degradation products. During development, endothelial progenitor cells form a primitive vascular network of small capillaries in a process termed “vasculogenesis.” In subsequent steps, this primitive network expands by a process termed “angiogenesis,” in which endothelial cells (ECs) sprout and form a more complex and elaborated vascular system. Finally, in a process termed “arteriogenesis,” the coverage of EC channels with pericytes and smooth muscle cells provides vessel maturation and stabilization.

In addition to wound healing, the cycling ovary, and the placenta during pregnancy, most blood vessels are kept in a quiescent state under physiological conditions in adulthood by a balanced microenvironment of pro- and anti-angiogenic factors.¹ However, in pathological conditions, numerous disorders are caused or characterized by excessive or insufficient angiogenesis. The best known of these conditions are diseases with abnormal excessive angiogenesis such as cancer, arthritis, chronic inflammation, infectious or autoimmune diseases, psoriasis, choroidal neovascularization, and others.¹ Conversely, several diseases are characterized by insufficient angiogenesis or vessel regression, such as preeclampsia, Alzheimer disease, stroke, amyotrophic lateral sclerosis, diabetic neuropathy, peripheral artery disease, osteoporosis, and ischemic heart disease. Additional diseases are being categorized as angiogenic disorders.¹ The basic mechanisms of vascular branching and tumor angiogenesis have been extensively reviewed in earlier publications.¹,² The first part of this review focuses mainly on the key target, VEGF and its receptors, because they are currently the targets of the US Food and Drug Administration (FDA)—approved anti-angiogenic drugs.

The process of angiogenesis is initiated in conditions under which cells experience low oxygen tension and mount a hypoxia inducible factor 1α (HIF-1α)—mediated response.³ HIF-1α is a transcription factor that is stabilized in hypoxic conditions and activates several genes, including the prototypic angiogenic VEGF-A (from here on termed VEGF). VEGF is the founding member of the structurally related VEGF family of growth factors comprising VEGF, VEGF-B, VEGF-C, VEGF-D, and the viral homolog VEGF-E, as well as placenta growth factor (PIGF) (Figure 1).⁴ These growth factors can bind and signal through the tyrosine kinase (TK) receptors VEGFR-1, VEGFR-2, and VEGFR-3, each with different affinities and selectivities. VEGF binds to VEGFR-1 and VEGFR-2, PIGF and VEGF-B bind to VEGFR-1, and VEGF-C and VEGF-D bind to VEGFR-3 and—depending on the species and with a lower affinity—to VEGFR-2.³ VEGF is the most prominent of these factors because it has a crucial role in angiogenesis and vessel maintenance. Indeed, VEGF expression levels need to be tightly regulated because VEGF haploinsufficiency leads to embryonic lethality.³ VEGF induces a pro-angiogenic and permeability-enhancing signal via VEGFR-2, which is abundantly expressed on ECs, whereas signaling through VEGFR-1 and the regulation of angiogenesis by this receptor is more complex.

The following is a brief update on the status of anti-angiogenic therapy in solid tumors and how angiogenesis influences hematopoietic tumors. The role of PIGF in solid tumors and leukemia is also discussed, and its therapeutic potential as a novel anti-angiogenic target highlighted.

Anti-angiogenic therapy in solid tumors

In solid tumors, it is well established that the angiogenic switch from an initial avascular tumor nodule to a rapidly growing, highly vascularized tumor is a critical step in the process of carcinogenesis.⁷ The concept that tumor growth and metastasis are angiogenesis dependent and that blockade of angiogenesis could be a target in cancer therapy was postulated in 1971 by Judah Folkman. Anti-angiogenic therapy has now become an additional pillar in the treatment options for several cancers.

Due to its prominent role in angiogenesis, VEGF has been in the spotlight of research efforts that resulted in the approval of 5 FDA
Another concern, raised by at least some recent preclinical findings in particular experimental conditions, is that VEGF signaling inhibition might inhibit the primary tumor growth, but at the same time also evoke an adaptation in tumor cells to a more metastatic phenotype. However, these findings are highly debated, and not reproduced by other preclinical results or large clinical studies. Further research will be needed to personalize anti-angiogenic medicine by more optimally matching the pharmacological profile of an anti-angiogenic therapy with correctly selected patients. Predictive biomarkers would therefore be of enormous value, but are sorely lacking to date.

Currently, a new vascular-targeting therapeutic strategy is gaining increasingly more attention. It is well known that tumor blood vessels are highly abnormal in structure and function, characterized by a tortuous, chaotic, and irregular branching network (Figure 2A-B). In the tumor vasculature, ECs are highly activated, lose their polarity and alignment, and detach from the basement membrane, all resulting in a leaky, fenestrated network that facilitates bleeding and increases the interstitial fluid pressure. Apart from the ECs, the entire vessel wall, including the basement membrane and the covering pericytes, becomes abnormal in most tumors. Tumor ECs are typically covered with fewer and more abnormal pericytes, and their associated basement membrane is only loosely associated and inhomogeneous in structure. It is suspected that this abnormal vasculature impedes the distribution of chemotherapy and oxygen. Traditional anti-angiogenic therapy aims to maximally inhibit angiogenesis and to prune existing tumor vessels; however, this strategy can also increase the risk of aggravating hypoxia and enhancing tumor cell invasiveness.

Recent genetic and pharmacological studies have revealed that targeting abnormal tumor vessel function by the induction of vessel normalization can offer alternative options for anti-angiogenic therapy (Figure 2C). Vessel normalization can be achieved by several different approaches, including blockade of VEGF, genic modulation of the oxygen sensors prolyl hydroxylase domain containing protein 2 (PHD2), targeting of mechanisms that affect the pericyte coverage and vessel maturation, and targeting myeloid cells via blockade or genetic loss of PIGF. Vessel normalization could provide a means to increase the responsiveness to chemotherapy, immunotherapy, or radiation, and may contribute to restricting tumor dissemination.

Angiogenesis and anti-angiogenic therapy in leukemia

With the BM and the lymphatic organs being the main location for hematologic malignancies, and leukemic cells circulating in the peripheral blood, angiogenesis was initially considered to not or only minimally contribute to the pathogenesis of “liquid tumors.” However, by now it has become apparent that the BM vasculature plays an important role in hematopoiesis in health and disease. For example, endothelial and mural cells (pericytes and smooth muscle cells) provide a specialized “vascular niche” for hematopoietic stem cells (HSCs). Different from the “osteoblastic niche,” which provides a microenvironment for long-term quiescent HSCs, the vascular niche supports proliferation and differentiation of short-term hematopoietic progenitors. It is postulated that HSCs first leave the osteoblastic niche and are then mobilized to the vascular niche before being released into the bloodstream.

Similarly to HSCs, a small fraction of leukemic stem cells possess stem-cell properties with long-term self-renewal, especially in

Notwithstanding these achievements, clinical findings have been less overwhelming than many preclinical results, and anti-angiogenic therapy is currently facing several challenges, in particular the intrinsic refractoriness and acquired evasive escape against VEGFR blockers. VEGF-targeted anti-angiogenic therapy prolongs the survival of patients with certain types of tumors by months, but it fails to induce a survival benefit in others. Surprisingly, bevacizumab elicits better clinical results when combined with chemotherapy, in contrast to receptor TK inhibitors. An increasing number of trials show a transient stabilization of the disease with even tumor regression and a prolonged PFS, but no prolongation of the more important and clinically relevant overall survival (OS). Why prolongation of PFS does not always translate to prolongation of OS in patients undergoing anti-angiogenic therapy remains largely speculative, and several recent overviews have discussed various types of resistance mechanisms.
chronic myeloid leukemia (CML). Both the osteoblastic and vascular niche are of importance for the support and maintenance of leukemic stem cells. BM ECs in this vascular niche are capable of secreting several soluble cytokines such as G-CSF, GM-CSF, VEGF, and IL-6, which promote leukemia cell proliferation and survival.

The BM vasculature is different from the vasculature in other organs. Large-nutrient-supply arteries enter the bone, where they ramify into smaller radial arterioles that first nourish the cortical bone (Figure 3). This cortical bone capillary network then drains into venous sinuses in the BM that extensively anastomose with each other and merge into larger central sinuses, from which an emissary vein leaves the bone. The microvascular capillary network consists of thin-walled, fenestrated sinusoidal ECs (SECs), allowing access, transport, and egression of nutrients and cells across the microvascular wall. These SECs have a unique VE-cadherin–VEGFR2–VEGFR3–Sca1– signature. Due to the unique expression of adhesion molecules, SECs allow hematopoietic stem and progenitor cells to home and traffic through the BM. This sinusoidal network consists only of a thin basal lamina with a single layer of ECs, but typical pericytes are missing, instead, they can be covered by SDF-1+ reticular cells and clusters of myeloid F4/80+ cells. The abundant densely packed surrounding hematopoietic cells and adipocytes further support this fragile network of SECs. Despite its extensively branched vascular network, the adult BM

Figure 2. Scheme illustrating a normal blood vessel (A), the abnormalities of a tumor blood vessel (B), and a partially normalized tumor blood vessel (C). BM indicates basement membrane; phalanx ECs, quiescent ECs; IFP, interstitial fluid pressure; PC, pericytes; M1-TAM, M1-polarized tumor-associated macrophages. (Reprinted with permission from Carmeliet and Jain.)

Figure 3. Diagram and photograph of the BM microvasculature. (A) Scheme illustrating the BM vasculature with an arterial supply ramifying into a capillary network and drained by a venous sinusoidal network. (Source: http://www.accesmedicine.com.) (B) Vascular casts showing the BM microvasculature with slender arterioles supplying an anastomosing venous network. (Source: http://visualsunlimited.photoshelter.com.)
An increase in the BM vascular density, indicative of the induction of angiogenesis, has been observed in various hematologic diseases such as leukemia, multiple myeloma, and myelodysplastic syndromes. Angiogenesis is stimulated upon infiltration of the BM with leukemia cells in various types of human hematological malignancies, including B-cell chronic lymphocytic leukemia, acute lymphoid leukemia (ALL), acute myeloid leukemia (AML), CML, myelodysplastic syndrome, multiple myeloma (MM), and others. In accordance, an increase in microvascular density has been used as an independent prognostic parameter for OS in CML. However, currently, the molecular basis of the angiogenic switch in leukemia in general and CML in particular is still poorly characterized, and other than VEGF and angiopoietin-2, only a few other molecules have so far been implicated in this process. Some of those molecules include soluble (s)VEGFR-1, sVEGFR-2, basic fibroblast growth factor, IL-6, IL-8, TNF-α, angiogenin, tissue factor, and HIF-1α.

Even though elevated VEGF levels are a marker of poor prognosis in hematological malignancies, and angiogenesis is induced in the leukemic BM, much less attention has been paid to evaluate (pre)clinically the possible benefits of VEGF-targeted treatments of leukemia. VEGF, secreted by leukemia cells, can activate VEGF receptors on both the leukemia cells and ECs. VEGF is also able to induce the proliferation of these cells and can chemoprotect leukemia cells against cytotoxic agents such as etoposide and doxorubicin. In CML, the disease-causing Bcr-Abl1 fusion kinase up-regulates the expression levels of VEGF, whereas the Bcr-Abl1 TK inhibitor imatinib down-regulates VEGF.

A limited number of preclinical studies have shown that VEGFR blockade can slow down the progression of hematologic malignancies in a tumor type–specific and context-dependent manner. For example, in a murine xenograft T-leukemia/lymphoma model, bevacizumab combined with doxorubicin delayed tumor growth more than doxorubicin monotherapy alone. Delivery of an anti-VEGF-2 antibody or VEGF-antisense approaches also slowed down leukemia growth in models of promyelomonocytic leukemia and subcutaneous implanted erythroleukemia. In CML, the disease-causing Bcr-Abl1 fusion kinase up-regulates the expression levels of VEGF, whereas the Bcr-Abl1 TK inhibitor imatinib down-regulates VEGF.

Initial human studies have shown that bevacizumab is ineffective as monotherapy in patients with refractory pretreated AML, whereas combination therapy with cytostatic agents provided only a slight survival advantage in ALL. In further studies, therapeutic approaches targeting the VEGF pathway in AML were tested in phase 1 and 2 trials, with promising results for several agents leading to current phase 3 trials. Currently, the therapeutic potential of only a limited number of other agents with anti-angiogenic activity is being explored. For example, thalidomide and lenalidomide, agents with a complex mechanism of action that indirectly inhibit angiogenesis by lowering VEGF secretion from BM ECs, are being evaluated for the treatment of MM. Both agents were FDA approved together with dexamethasone in different settings for the treatment of MM after showing an increased response rate in this disease. In addition, the proteosome inhibitor bortezomib, which induces EC apoptosis and inhibits VEGF production by HIF-1α suppression, improved OS and increased the median time to progression compared with dexamethasone alone and was FDA approved for MM treatment. The VEGF-family member PIGF as a disease-specific cytokine and its role in angiogenesis in solid tumors and leukemia are discussed below.

**A disease-specific cytokine: role and therapeutic potential of PIGF**

PIGF is a member of the VEGF family of growth factors that was originally identified in the placenta, where it controls trophoblast growth and differentiation. PIGF-knockout mice are born without any abnormalities, indicating that PIGF is redundant for vascular development and physiological vessel maintenance in adult health. However, in pathological conditions, PIGF contributes to the pathogenesis of various malignant, inflammatory, and ischemic disorders. The expression of PIGF, unlike that of VEGF, is low/undetectable in most organs in healthy conditions, but it becomes highly up-regulated in pathological disease conditions. Clinical studies show that the expressions of PIGF and of its receptor VEGFR1 (also known as Flt1) are increased in various solid tumors, and in certain tumors can be correlated with disease progression and predict poor prognosis, metastasis, and recurrent disease in humans.

PIGF binds and signals through Flt1 in various cell types (including ECs, smooth muscle cells, fibroblasts, myeloid progenitor cells, macrophages, and tumor cells) to promote tumor angiogenesis, tumor growth, and the formation of the premetastatic niche. Therefore, in tumor biology, PIGF is a multitasking cytokine involved in many different biological processes (Figure 4). Deletion of the TK activity of Flt1 (Flt1TK−/− mice) or treatment with Flt1 and PIGF-specific inhibitors, such as a mAb against PIGF (oPIGF), impairs inflammation and pathological angiogenesis and suppresses tumor growth and metastasis without affecting healthy vessels. Myeloid cells can confer resistance to current anti-angiogenic therapies (such as anti-VEGF therapy) by secreting additional pro-angiogenic factors. Interestingly, oPIGF treatment inhibits the recruitment of macrophages and BM progenitor cells, and therefore may help to reduce the source of angiogenic factors that contribute to the anti-angiogenic escape of tumors. Tumor studies in
PIGF-knockout mice showed that PIGF deficiency delays carcinogen-induced HCC and skin papilloma formation. Moreover, PIGF silencing retards HCC in a transgenic onco-mouse model, consistent with reports that PIGF levels are correlated with poor outcome in HCC patients. These genetic and pharmacological studies identify PIGF and Flt1 as therapeutic targets for anticancer therapy. Furthermore, unlike currently used VEGFR inhibitors, αPIGF is not expected to cause side effects resulting from effects on healthy blood vessels.

αPIGF treatment is not, however, effective in all tumors. Indeed, whereas αPIGF therapy is beneficial for cancers such as HCC or skin epithelial tumors (papillomas), PIGF blockade or deficiency does not inhibit tumor growth in the transgenic Rip1Tag2 model of pancreatic β-cell carcinogenesis despite expression of PIGF in this tumor. The resistance of this tumor model might be due in part to the inability of PIGF loss or inhibition to block the infiltration of neutrophils, key mediators of the angiogenic switch in this model. Therefore, further testing of αPIGF therapies and identifying resistance-predictive markers will be required to determine those tumor types for which αPIGF therapy is beneficial.

A new role for PIGF in CML

CML is characterized by an unregulated growth of predominantly myeloid cells in the BM and the subsequent appearance of these cells in the peripheral blood. It is caused by chromosomal translocation t(9;22)(q34;q11) (“Philadelphia chromosome”) that gives rise to the Bcr-Abl1 fusion kinase. This leukemogenic TK promotes the survival and proliferation of CML cells. Because of its key role in the pathogenesis of CML, most therapies have been focused on targeting Bcr-Abl1, and the Bcr-Abl1 TK inhibitor imatinib is currently the main therapy for the treatment of CML patients. However, whereas imatinib can induce molecular remission in most patients, it fails to completely eradicate the leukemic stem cell pool, and in some cases, patients fail imatinib therapy due to poor tolerance, loss of response, or acquired resistance due to mutations in the Bcr-Abl1 TK domain. Whereas current anti-CML therapies are largely “leukemia cell-centered,” emerging evidence highlights the importance of the BM stroma for the growth, survival, and TK inhibitor resistance of leukemia cells.

In vitro studies showing that PIGF promotes the survival of hematopoietic precursors and stimulates the growth of ALL and AML cells provided initial evidence for a role for PIGF in leukemia. VEGFR-1 (Flt1) is also abundantly expressed by human CML cells. This suggestive link between leukemia and PIGF triggered interest in investigating the in vivo role of PIGF and the therapeutic potential of pharmacological PIGF inhibition in CML. Of a large set of angiogenic candidates, PIGF levels in the peripheral blood and BM plasma were up-regulated the most in a CML mouse model compared with each monotherapy alone. This PIGF-dependent stromal cell–leukemia cell cross-talk favored increased vascular supply and an altered interstitial matrix milieu. In turn, the increased CML expansion further stimulates additional BMSCs to produce more PIGF, thereby fueling a feed-forward amplification loop in a self-sustaining cycle.

Mechanistically, PIGF stimulated matrix deposition in the BM and BM angiogenesis and induced the accumulation of BMSCs. Furthermore, PIGF had direct effects on leukemia cells and stimulated their proliferation, migration, and glycolytic metabolism (Figure 5). This PIGF-dependent stromal cell–leukemia cell cross-talk favored an expansion of both cellular populations. Therefore, by inducing BMSCs to up-regulate PIGF production, leukemia cells create for themselves a fertile pro-tumorigenic “soil” characterized by an increased vascular supply and an altered interstitial matrix milieu. Overall, these preclinical studies suggest novel therapeutic opportunities for CML, specifically for imatinib-resistant patients. Although the translational applicability of PIGF blockade for CML still requires further testing, the current findings stimulate further interest in the therapeutic potential of the blockage of BM angiogenesis and targeting of the leukemia-supportive BM stroma in the future.

Figure 5. Scheme illustrating the bidirectional communication between CML leukemia cells and BMSCs. This interaction produces PIGF, resulting in the increased metabolism and growth of CML cells.

**Conclusion**

Despite formidable progress in our current understanding of (tumor) angiogenesis with the successful translation of anti-angiogenic
therapy “from the bench to the bedside” in clinical practice, there are still many outstanding challenges. Parallelsisms between angiogenesis in solid tumors and in hematological diseases have been recognized in recent years, but we are still lacking fundamental insight into how vessels support hematological diseases and how this can be successfully targeted in clinical settings. Nonetheless, further study of the molecular basis of angiogenesis in hematological malignancies and elucidating how some of these molecules can regulate the BM stroma promises to generate new fundamental insights required for the design of improved anti-leukemia therapy.

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