

REVIEW

(Lymph)angiogenic influences on hematopoietic cells in acute myeloid leukemia

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The purpose of this review is to provide an overview of the effect of (lymph)angiogenic cytokines on hematopoietic cells involved in acute myeloid leukemia (AML). Like angiogenesis, lymphangiogenesis occurs in pathophysiological conditions but not in healthy adults. AML is closely associated with the vasculature system, and the interplay between lymphangiogenic cytokines maintains leukemic blast survival in the bone marrow (BM). Once AML is induced, proangiogenic cytokines function as angiogenic or lymphangiogenic factors and affect hematopoietic cells, including BM-derived immune cells. Simultaneously, the representative cytokines, VEGFs and their receptors are expressed on AML blasts in vascular and osteoblast niches in both the BM and the peripheral circulation. After exposure to (lymph)angiogenic cytokines in leukemogenesis and infiltration, immune cell phenotypes and functions are affected. These dynamic behaviors in the BM reflect the clinical features of AML. In this review, we note the importance of lymphangiogenic factors and their receptors in hematopoietic cells in AML. Understanding the functional characterization of (lymph)angiogenic factors in the BM niche in AML will also be helpful in interrupting the engraftment of leukemic stem cells and for enhancing immune cell function by modulating the tumor microenvironment.

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INTRODUCTION

Acute myeloid leukemia (AML) is aggressively malignant and is closely associated with increased microvessel density in the bone marrow (BM).^{1–3} Most hematologic malignancies, including AML, are associated with angiogenesis in peripheral organs as well as the BM. The human body contains two major vasculatures: the blood vessels and the lymphatic vessels. In embryonic mouse development, blood vessels are formed by the differentiation of endothelial precursors, called angioblasts. This process, called vasculogenesis, is the formation of new blood vessels from stem/progenitor cells. Angiogenesis, the process of the formation of new blood vessels from preexisting blood vessels, commonly occurs postnatally in tumors and in inflammation. Folkman *et al.*⁴ first addressed the concept of angiogenesis, leading to many studies, from basic science to clinical applications, to treat both hematologic malignancies and solid tumors. Similarly, Sabin FR, who described the embryonic origin of lymphatic vessels in 1902, addressed the venous origin of lymphatic vasculature in mammals as well as blood vessel formation.⁵ Lymphatic vessel formation is also divided into two processes, called lymphangiogenesis and lymphovasculogenesis.⁶ Lymphangiogenesis in the embryo and

in postnatal stages initiates from the cardinal vein and preexisting lymphatic vessels, respectively, in response to VEGF-C stimulation.^{7–9} The VEGF-C or D/VEGFR-3 axis is a dominant driver of lymphangiogenesis under normal and pathologic conditions.¹⁰ Abundant expression of angiogenic and lymphangiogenic cytokines in tumors HAS bifunctional roles in terms of receptor binding.^{9,11–14} Lymphangiogenic cytokines contribute to the deterioration of immune cells as well as promote the protection of tumor cells and metastasis.^{14–16} Like solid tumors, AML blasts highly express receptors and proangiogenic factors such as VEGF-A, VEGF-C and VEGF-D,^{17–19} implying a direct or indirect function to protect the blasts from anti-cancer drugs. Several cytokines, including VEGF-C and angiopoietin (ANG)-2, are linked to clinical AML outcome.^{20–22} Figure 1 illustrates the interaction of specific cytokines with their receptors, which are expressed in AML and solid tumors. VEGFR-3-expressing natural killer cells in AML, with abundant expression of VEGF-C, are functionally suppressed with low levels of IFN- γ , suggesting the effects of a lymphangiogenic promoted microenvironment in hematopoietic cells.¹⁵ The BM, a pivotal organ in AML, is considered to be a secondary organ for mature and homed

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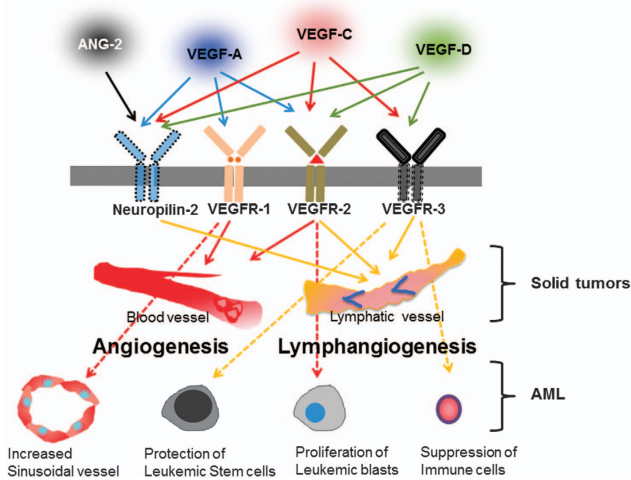


Figure 1 Schematic illustration showing the interaction of specific cytokines and their receptors, which are expressed in AML and solid tumors. Postnatally, blood vessels express VEGFR-1 and VEGFR-2 and the lymphatic endothelium expresses VEGFR-2, VEGFR-3 and Neuropilin-2. On the basis of receptor binding, their signaling causes phosphorylation in specific vessels in tumors. Thus, VEGF-A, VEGF-C, VEGF-D and ANG-2 are important cytokines that promote lymphangiogenesis in solid tumors. In addition, these four (lymph)angiogenic cytokines are abundantly expressed in the BM in AML, indicating their relevance in AML. Solid arrows and dotted arrows indicate solid tumors and AML, respectively.

CD4⁺ and CD8⁺ cells, as well as a primary lymph organ for lymphogenesis and myelogenesis.²³ Notably, the BM contains a sinusoidal vessel that permits the circulation of mature blood cells and stem/progenitor cells. Sinusoidal vessels consist of a thin basal lamina and a single layer of endothelial cells without pericytes,²⁴ and differ from blood vessels in other organs without possessing the definitive properties of lymphatic vessels. Obscured sinusoidal endothelial cells are supported by myeloid F4/80⁺ cells without lymphatic capillaries and sustain hypoxic conditions in the BM.^{24,25} In solid tumors, hypoxia-related factors, such as hypoxia inducible factor-1, encourage lymphangiogenic promoting conditions.^{26,27} Recent papers revealed the function of lymphangiogenic cytokines in the direct modulation of the immune response in pathologic conditions.^{28–30} The BM is under the influence of a huge amount of (lymph)angiogenic cytokines of diverse cell types in AML. These (lymph)angiogenic promoting conditions affect all the host cells of AML, including immune cells and stem cells. Although the BM is an organ lacking lymphatic vasculature, it has not been demonstrated whether properties of the sinusoidal endothelium in the BM are restricted to blood vessels, or whether they are similar to those seen in lymphatic vessels in other organs in AML. Thus, we selectively present an overview of the current understanding of the interaction between (lymph)angiogenic cytokines and hematopoietic cells, and provide basic insight into strategies of the advanced lymphatic related factor-targeted therapy in AML.

LYMPHATIC ENDOTHELIAL CELL MARKERS AND LYMPHANGIOGENIC CYTOKINES

In the past, blood vessel data have been rapidly amassed, whereas lymphatic vessels have been slowly targeted since their pivotal role in tumor biology was revealed. The specific receptor for VEGF-C, VEGFR-3, is a representative marker for the lymphatic endothelium. VEGFR-3 knockout mice die because of cardiac defects and the pivotal role in the lymphatic vasculature with its ligand, VEGF-C.³¹ VEGFR-3 is the first lymphatic marker expressed in lymphatic endothelial cells (LECs), but not in blood endothelial cells, in normal adult tissues. In tumors, VEGFR-3 is abundantly expressed in blood endothelial cells, strongly implying that it contributes to the formation of new blood vessels as a lymphangiogenic factor.³² VEGF-C is a representative lymphangiogenic cytokine, and it induces lymphatic vessel enlargement. VEGF-C^{-/-} mice fail to sprout lymphatic vessels, and are rescued by VEGF-D and the VEGF-C/VEGFR-3 axis.^{7,33,34} The VEGF-C/VEGFR-3 axis is a representative pathway in the development of the lymphatic sac and lymphatic vessels in the postnatal stage.³⁵ In the BM, Sca-1⁻VEGFR-3⁺VEGFR-2⁺ sinusoidal vessels are restricted to the vasculature, supporting BM angiogenesis and hematopoietic stem cells (HSCs).³⁶ The VEGFR-3⁺ sinusoidal endothelium expresses VEGF-C through the regulation of cytokines such as fibroblast growth factor (FGF) from blasts,^{14,37} demonstrating the complexity of VEGFR-3 as a marker. Specific LECs initiate Sry-related HMG box (SOX-18) and Prospero homeobox-1 (Prox-1) transcription factors, known as master regulators for lymphatic vessels,^{38–40} in cardinal veins at embryonic day 9.5. Defects in SOX-18 induce lymphatic dysfunction and stimulate Prox-1 expression in lymphatic precursor cells. In 1902, Prox-1 was first proposed by Sabin FR, and was later identified by Wigle JT and Oliver G,³⁹ as a marker for LECs. Prox-1 knockout mice die at embryonic day 14.5 with an unstable respiratory system. Conditional depletion of Prox-1 converts the cell fate from LECs into blood endothelial cells,⁴¹ indicating that Prox-1 is a key molecule in lymphatics. Podoplanin is a mucin-type transmembrane glycoprotein that is expressed in both LECs and podocytes.^{42,43} In 2003, Schacht *et al.*⁴⁴ reported the malformation of lymphatic vessels, but not blood vessels, in Pdpn-knockout mice. These mice died at birth because of respiratory failure. Podoplanin mutant mice display lymphedema and impaired lymphatic vasculature. Podoplanin induces platelet activation through the CLEC-2 receptor, which separates lymphatic vessels from blood vessels.^{45,46} Lymphatic vessel endothelial hyaluronan receptor-1 (Lyve-1) was identified as a lymph-specific receptor by Banerji *et al.*⁴⁷ in 1999. Although Lyve-1 knockout mice have no abnormal lymphatic phenotypes,⁴⁸ Lyve-1 is expressed in the lymphatic endothelium and macrophages, but not in blood vessels.^{49,50} Recent studies identified new markers for LECs, such as EphB2, FOXC2, ANG-2, Integrin α 9, Syk and COUP-TFII, implying pivotal roles for these proteins in the formation of new lymphatic vessels.^{51–57} Another lymphangiogenic cytokine, VEGF-D, is a ligand of VEGFR-3, which induces lymphatic vessel formation and promotes tumor metastasis.^{33,58}

Neuropilin-2 binds to VEGF-C and VEGF-D and is required for lymphatic vessel development.^{59,60} Proangiogenic cytokines such as platelet-derived growth factor-BB, FGF, hepatocyte growth factor, insulin-like growth factors (IGF) 1 and 2, and VEGF-A, along with the receptor VEGFR-2, also contribute to lymphangiogenesis as direct lymphangiogenic cytokines in changeable pathophysiological conditions,^{12,13,61–64} demonstrating the dynamic and complicated behavior of angiogenic/lymphangiogenic cytokines in diseases. As mentioned above, these markers have been used for LECs and lymphangiogenic factors.

BM CELLS AND CONTRIBUTION OF BM CELLS INTO LECs

The BM, comprised of flexible tissues, consists of heterogeneous cells including HSCs, mesenchymal stromal cells, osteoblasts, osteoclasts, fibroblasts, red blood cells, lymphocytes (natural killer (NK) cells, T cells, B cells), monocytes and DCs along with the extracellular matrix (ECM, fibronectin, collagen). Among these cells, the stem/progenitor cells can differentiate into LECs and macrophages, which contribute to the proliferation of LECs.^{6,65–67} Although it is arguable that stem cells in the BM have plasticity to other cell lineages such as skeletal myoblasts and neural cells, the contribution of BM-derived HSCs and endothelial progenitor cells to blood cells and vessels is commonly accepted.^{68,69} Since the description of endothelial progenitor cells as a therapeutic source by Asahara *et al*,⁶⁹ they are commonly thought to treat impaired tissues via neovasculature.^{70,71} Lymphangiogenesis is a more accepted concept than lymphvasculogenesis.^{72,73} However, recent evidence shows that lymphvasculogenesis can occur in the postnatal stage by stem/progenitor cells derived from the BM,^{6,65,66,74} implying that BM-derived cells can function as contributors to LECs. In particular, CD11b⁺ macrophages and myelomonocytes are incorporated into new lymphatic vessels in an inflammation-induced cornea model and a tumor model.^{65,75} These papers show the participation of myeloid cells in LEC. CD133⁺CD34⁺VEGFR-3⁺ cells, as endothelial cell precursors, have dual functions as lymphatic endothelial progenitor cells and endothelial progenitor cells.⁶⁶ VEGFR-3⁺ CD14⁺ monocytes in circulation also produce the lymphangiogenic cytokine VEGF-C, which may serve lymphatic endothelial progenitor cells from hematopoietic cells.^{66,76} To note, CD11b⁺ and Flt3⁺ cells can differentiate into cells of several lineages as well as LECs. CD11b⁺ B220⁺ cells differentiate into B lymphocytes, and Flt3⁺ cells can function as progenitor cells for NK cells.⁷⁷ Cells in the hematopoietic system, in particular macrophages and monocytes, have the plasticity to form LECs and cells of other lineages in pathologic situations. As described above, myeloid cells strongly contribute to lymphangiogenesis via direct or indirect mechanisms, as well as to angiogenesis in pathophysiological conditions. However, the relationship between BM cells and LECs in the extramedullary organ, as well as to the BM of hematologic malignancies, remains to be investigated.

VEGF FAMILY IN AML

The human VEGF family consists of VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor. There are three VEGF receptor tyrosine kinases: VEGFR-1, VEGFR-2 and VEGFR-3. VEGF-A has several isoforms, known as VEGF₁₁₀, VEGF₁₂₁, VEGF₁₄₅, VEGF₁₄₆, VEGF₁₄₈, VEGF₁₆₅, VEGF₁₆₇, VEGF₁₈₃, VEGF₁₈₆, VEGF₁₈₉ and VEGF₂₀₆, and their functions are based on the binding to the extracellular matrix.⁷⁸ The most important factors in AML are VEGF-A and VEGF-C, which are expressed on BM cells in AML. VEGF was isolated in the HL-60 myeloid leukemia cell line, and its AML expression was first reported by Fiedler *et al*.^{17,79} Increased microvessel density is associated with angiogenic factors such as VEGF and is seen in the BM of AML patients.^{1–3} Most AML BM cells greatly increase expression of VEGFs and their receptors, depending on factors such as AML subtype (i.e., t(15;17) or t(8;21) AML), polymorphism score and vascular morphology in remission status.^{80–83} In particular, VEGFR-2 is constitutively phosphorylated and is relocated intracellularly to the nucleus from the surface in leukemia, t(8;21) and MLL, but not in other cytogenetic groups.^{83,84} In addition, the proliferation of AML is blocked by anti-VEGFR-2 drugs via the inhibition of VEGF/VEGFR-2 phosphorylation. This suggests that the VEGF signal is dependent on the cytogenetic subgroup, and that therapy using a VEGF inhibitor should be performed based on individual characteristics of AML. VEGF in AML cells can act via autocrine and paracrine signaling. The activated VEGFA/VEGFR-2 axis regulates blast survival and growth by internal loops via an intracellular receptor without secretion. This loop is not dependent on cell density, and exogenous neutralizing antibodies cannot arrest the proliferation of blasts by this autonomous machinery. External autocrine loops are also involved in preventing blast survival and releasing VEGF-A from the blasts (autocrine), activating VEGF receptors on AML blasts which bind to VEGF from other stromal cells (paracrine).^{81,84} Like VEGFR-2, VEGFR-3 also has two types of receptors for activation, an intracellular receptor and a surface receptor. Internalized VEGFR-3, which can be relocated into the cytoplasm by ephrin-B2, occurs in lymphangiogenesis,⁸⁵ implying that VEGF receptors act via internal loops. Autocrine signaling in AML also maintains the survival of blasts.¹⁸ Simultaneously, VEGF-C from endothelial cells can conserve VEGFR-3⁺ blasts after chemotherapy via paracrine signaling.¹⁴ VEGF-C-releasing endothelial cells and blasts can affect all the hematopoietic cells in the BM as well as VEGFR-3⁺ blasts. VEGF-C is abundantly expressed on BM in AML with its receptor VEGFR-3, and these molecules support leukemic cell proliferation against chemotherapy.^{14,18} Both factors, VEGF-A and VEGF-C, contribute to the proliferation of the lymphatic endothelium and tumors in a tumor model,^{12,14} suggesting that they function as (lymph)angiogenic factors. Direct interactions (juxtacrine loops) between blasts and stromal cells are important for blast survival in AML BM.⁸⁶ These proteins have a clinical correlation in AML. Patients with VEGF-A and VEGF-C overexpression showed reduced survival and poor prognosis.^{14,22,87,88} It is necessary to further address whether

proangiogenic VEGF in the BM can affect extramedullary lymphatics and immune cells and act as a (lymph)angiogenic stimulator via endocrine signaling in AML, which is likely for solid tumors.

OTHER (LYMPH)ANGIOGENIC CYTOKINES AND AML

AML is a heterogeneous blood disorder, which has complex gene and cytokine expression. Like solid tumors, AML presents with overexpression of angiogenic cytokines, which can convert their function into (lymph)angiogenic processing. IGF I promotes AML blast survival via the phosphoinositide 3-kinase/Akt pathway,⁸⁹ and IGF binding protein 2 is expressed on AML blast and HSCs. This molecule supports blast migration and promotes survival in an autocrine manner, along with the differentiation of osteoclasts,^{90,91} suggesting an association with the BM. Hepatocyte growth factor is an angiogenic cytokine and a pivotal element in AML blast and cell lines. Its receptor c-MET is also activated in AML. IGF, hepatocyte growth factor and FGF, which are not VEGF family members, function as (lymph)angiogenic factors in various solid tumors, predicting the lymphatic potential in AML. FGF influences myeloid progenitor cells as well as HSCs in survival. In particular, the MLL-ELL oncoprotein, also known as MLL fusion protein, strongly induces FGF in AML by autocrine stimulation.^{37,92} As mentioned above, almost all lymphangiogenic and angiogenic cytokines have an important role in conserving leukemic cells, and these cytokines are autonomously released from blasts in AML. These cytokines have bifunctional roles to form vasculature in solid tumors. Although the BM has no lymphatic capillaries in AML, many lymphangiogenic cytokines influence hematopoietic cells in the BM microenvironment.

RECIPROCAL INTERACTION BETWEEN (LYMPH)ANGIOGENIC FACTORS AND IMMUNE CELLS

Traditionally, the lymphatic system has been considered a transportation system for tissue fluid homeostasis and immune cells, including DCs and macrophages.⁹³ It is known that lymphangiogenesis and immune cell suppression are involved in cancer. However, little is known about how lymphangiogenic factors affect immunity in pathological conditions. Increasing evidence has shown that the lymphatic endothelium and cytokines from LECs can regulate immune cell function. Podgrabinska S *et al.* report that the lymphatic endothelium directly suppresses DC function under inflammatory conditions, indicating that LECs are active participants in immune cell function.²⁸ Lund *et al.*³⁰ addressed the protection from antitumor immunity in melanoma afforded by VEGF-C. VEGF-C induces the immune tolerance of tumor antigen-specific CD8⁺ T cells. The escape of tumors from the immune response occurs by lymphatic and interstitial flow.⁹⁴ In a maternal-fetal interface, VEGF-C induces NK cell tolerance with a CD56^{high}CD16^{dim} phenotype in the uterus.⁹⁵ Recent papers address the direct communication between the

lymphatic endothelium or lymphangiogenic factors and immune cell function. VEGF-C can recruit VEGFR-3⁺ tumor-associated macrophages, which simultaneously encourage immune suppression and blast protection from chemotherapy.^{14,96,97} Krebs *et al.*⁹⁸ provide evidence that VEGF-C/VEGFR-3 signaling is also crucial for active, innate and adaptive immune responses as well as neovasculature in lung injury. Under high VEGF-C levels, high VEGFR-3⁺ NK cells in AML display lower levels of cytotoxicity in response to low levels of IFN- γ than VEGFR-3⁻ NK cells. In mouse experiments, KLRG1+/CX3CR1-GFP+ NK cells, which reside in VEGFR-3+ sinusoids, have lower killing potential than NK cells in the parenchyma,^{15,99} suggesting a relationship between VEGFR-3 expression and NK cell function. IFN- γ is a main cytokine that attacks tumor cells by immune cells such as T and NK cells. IFN- γ is known as an anti-angiogenic or lymphangiogenic factor in the tumor microenvironment and *in vitro* experiments.^{50,100,101} It can be inferred that opposing regulation exists between IFN- γ releasing immune cells and neo lymphangiogenesis. Other immune cells and mast cells (MCs) are influenced by the lymphatic endothelium and are located near vascular and lymphatic vessels.²⁹ The function of MCs has been restricted to the allergic response. However, their importance in innate and adaptive immunity was magnified in pathogen infection and in immunotherapy in tumors.^{102,103} AML, which is a diagnosis of systemic mastocytosis, is not thought to be of mast cell lineage. Although there is no detection of MCs in *de novo* AML, MCs appear at the relapse stage, which highly stimulate the lymphangiogenic conditions in t(8;21)(q22;q22) patients or AML1/ETO-positive AMLs after HSCT.^{104,105} Whether high levels of lymphangiogenic factors lead to the dysfunction of MCs with a VEGFR-3⁺ phenotype, unbalanced immunity, and the induction of relapse status remains to be investigated. Furthermore, the feasibility of VEGFR-3⁺ MCs as predictors of relapse in AML needs to be examined. On the other hand, Syk⁺ leukocytes have been shown to induce lymphangiogenesis, but not endothelial progenitor cells.¹⁰⁶ This shows the capacity of mature hematopoietic cells to control lymphangiogenesis. B cells and CD11b+/GR1+ macrophages produce VEGF-A, C and D in the periphery under inflammatory conditions.^{67,107} Murakami *et al.*¹⁰⁸, using Vegfr1 tk^{-/-} mice, reported that VEGF-A recruited BM-derived macrophages via the activation of VEGFR-1 on macrophages in subcutaneous tissues. Cursiefen *et al.*¹⁰⁹ also showed that VEGF-A stimulates lymphangiogenesis and macrophage recruitment, using a VEGF trap in an inflammatory corneal model. These papers demonstrated that VEGF-A induces the mobilization of monocytes, which then leads to the production of VEGF-C and D in differentiated monocytes. Although VEGF-A stimulation initiates angiogenesis, the function of VEGF-A as a lymphangiogenic cytokine has gradually been revealed in pathological conditions. A new challenge for lymphatics is to understand how they act as active immunosuppressants and function as regulators in tumor microenvironments.^{110,111} Once AML is induced, VEGFR-3 and other LEC markers such as Prox-1, Lyve-1 and podoplanin are

highly expressed in NK cells as well as in BM- and PB-derived mononuclear cells. These markers are generally restricted to lymphangiogenic factors in the postnatal stage; hence, abundant expression suggests the relevance of increased lymphangiogenic factors to immune cells in the progression of AML.¹⁵

(LYMPH)ANGIOGENIC CYTOKINES IN THE BM NICHE

Hematopoietic processing occurs in the BM. BM structure is divided into two main microenvironments. One is the osteoblast niche, which is localized in the inner surface of the bone, lined with osteoblasts from mesenchymal precursors. It serves as a reservoir for long-time HSC with hypoxic conditions. The other is the vascular niche, consisting of sinusoidal vessels that exit the BM and circulate blood cells. In both regions, (lymph)angiogenic cytokines are produced by hematopoietic cells. Cells of osteolineage and reticular perivascular cells are regarded as the major cells in the HSC niches that maintain HSCs. VEGF-A and VEGF-C are necessary factors for HSC retention. Ishikawa *et al.*¹¹² reported that leukemic stem cells homed in the endosteal region after chemotherapy. Because resistant leukemic blasts require the protective niche to relapse, VEGF-A from mesenchymal stromal cells, sinusoidal endothelium and osteoblasts in the endosteal niche may be involved in AML leukemic stem cell niche-associated relapses. However, the interaction between the niche composition and hematopoietic cells should be examined to provide more detailed evidence in a syngeneic mouse model, which can recapitulate the physiologic hematopoiesis in the niche. CD146⁺ sub-endothelial cells, which reside in sinusoidal vessels, produce ANG-1. ANG-1 is a key molecule for HSC quiescence in the BM niche, with its receptor Tie-2.^{113,114} The BM vascular niche was phenotypically and functionally addressed by Hooper *et al.*³⁶ They found that the regeneration of sinusoidal endothelial cells by VEGFR-2 is critical to HSC engraftment, regardless of the osteoblastic niche conditions. In addition, the importance of sinusoidal endothelial cells in reconstructing transplanted BM cells in the BM after irradiation was addressed. Further experimentation with the sinusoidal vessel is needed to investigate whether it is a bifacial regulator in AML, functioning both as a routine exit from the BM and by regulating other immune or leukemic stem cells as enhancers for (lymph)angiogenic factors. By reintroducing the Fanconi anemia-related gene (Fang) by injection of normal mesenchymal stromal cell into *Fancg*^{-/-} mice, Li *et al.*¹¹⁵ showed that mesenchymal stromal cells are a crucial factor in maintaining HSC. Kwon *et al.*¹¹⁶ also first reported a correlation between vascular deterioration and HSC frequency in a genetically manipulated mouse model. Tumor (lymph)angiogenesis is activated and closely involved in disease-free survival in solid tumors. In particular, VEGF-C is a representative predictor for reduced survival via lymph node metastasis in melanoma, breast cancer and lung cancer.¹¹⁷⁻¹¹⁹ Like solid tumors, AML is exposed to (lymph)angiogenic cytokines. VEGF-C and VEGF-A in the BM are representative cytokines for the prediction of poor

outcome and for the definition of AML subgroups.^{22,120} Functional evidence in blast proliferation and survival, as well as the expression of VEGF-C and VEGF-A in AML blasts, have been shown by many reports.^{14,121,122} Both cytokines stimulate the migration of endothelial cells. When AML is induced, expanded BM endothelial cells release leukemic growth factors, including granulocyte-macrophage colony stimulating factor and interleukin-6.^{17,19} Osteopontin (OPN), which is known as early T-lymphocyte activation-1 and was first identified in osteoblasts in 1986, is released by osteoblasts and hematopoietic cells in the BM. Overexpressed OPN, as a lymphangiogenic factor, promotes metastasis via the integrin $\alpha 9$ pathway. OPN is also a prognostic indicator for survival in AML.^{123,124} Although little is known about the potential reciprocal interaction between hematopoietic cells, including leukemic stem cells, immune cells and the BM niche, there is no controversy regarding the importance of the BM niche in AML. Figure 2 presents the BM niche activity in AML. Previous knowledge in AML can lead to advanced therapeutic protocols through understanding the biological activity in the leukemic niche.

PHARMACOLOGIC PERSPECTIVES OF AML TREATMENT

Many anti-angiogenic drugs have been used to treat AML. Because of their broad spectrum of use, from basic science

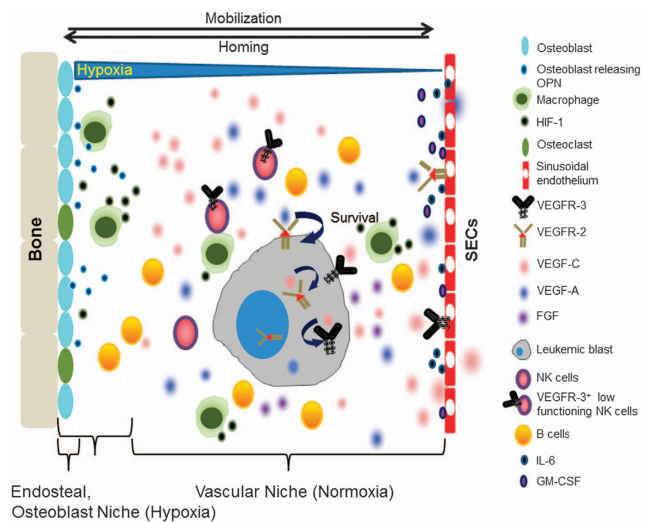


Figure 2 Schematic illustration showing the dynamic BM niche in AML. Abundant expression of lymphangiogenic cytokines, including VEGF-C, VEGF-A, OPN and their receptors, VEGFR-3 and VEGFR-2, are found in BM cells. Under high levels of (lymph)angiogenic cytokines, hematopoietic cells including leukemic stem cells and immune cells are affected, resulting in the dysfunction of immune cells and protection of leukemic stem cells. Abbreviations: OPN, Osteopontin; HIF-1, Hypoxia inducible factor-1; SECs, Sinusoidal endothelial cells; VEGFR-3, Vascular endothelial growth factor receptor-3; VEGFR-2, Vascular endothelial growth factor receptor-2; VEGF-C, Vascular endothelial growth factor-C; VEGF-A, Vascular endothelial growth factor-A; FGF, Fibroblast growth factor; NK cells, Natural killer cells; GM-CSF, Granulocyte macrophage-colony stimulating factor.

to clinical applications, anti-angiogenic drugs including bevacizumab (Avastin) and SU11248 (sunitinib) have been implemented in AML as well as in solid tumors.^{125–127} Bevacizumab is a monoclonal antibody against VEGF-A, and a representative inhibitor of angiogenesis. Although combination therapy has the limitations of dose-limiting toxicity, when chemoreagents and PTK787/ZK222584/Vatalanib (PTK) are simultaneously applied most of the target molecules, including Bevacizumab and PTK, show higher susceptibility rates in combination therapy with imatinib than in monotherapy for complete remission of AML. PTK787/ZK222584 is a tyrosine kinase inhibitor that binds to the ATP-binding sites of VEGF receptors.^{128,129} The small molecules SU5416 and SU11248 inhibit VEGF receptors and are used in refractory AML.^{127,130} Recently, monoclonal antibodies and small molecules targeting VEGF-C/VEGFR-3 have progressed in Phase I clinical trials for solid tumors.^{131–133} On the basis of animal experiments, molecules targeting VEGF signaling in AML have implied that inhibiting lymphangiogenic signaling can block the recurrence of leukemic blasts. In particular, inhibitors targeting VEGFR-3, including monoclonal antibody Hf4-3C5, have been developed and are in phase I clinical trials, numbered NCT01288989.¹³¹ Other small molecules targeting VEGFR, such as Pazopanib, Axitinib and Regorafenib, were reported to restrict both VEGFR-3 and VEGFR-2 or Tie2 inhibition and have been used in solid tumors but not yet in AML.^{134–136} The next-generation strategy, dual functional reagents which can function as anti-angiogenic factors and immunomodulators, such as pomalidomide (originally CC-4047 or 3-amino-thalidomide), needs to be elicited for the advanced treatment of AML.^{137,138} A therapeutic value for IGF-I was confirmed using the NVP-AEW541 inhibitor, which resulted in decreased CD31.¹³⁹ The inhibition of the hepatocyte growth factor receptor MET by crizotinib failed to maintain a blast-free condition unless applied with dual inhibition of FGFR1.¹⁴⁰ The anti-angiopoietin peptibody AMG-386, which inhibits ANG1 and ANG2 with the Tie-2 receptor, is under clinical phase II trials (NCT01290263) for use in a variety of solid tumors, but not for leukemia.¹⁴¹ All cytokines mentioned above have the potential to suppress lymphangiogenic factors as well as angiogenic factors toward AML blasts and tumor environment in the BM. Most therapeutic agents, known as anti-angiogenic drugs, may be applied with other reagents such as HDAC and mTOR inhibitors in AML treatment. Figure 3 summarizes the potential targets and inhibitors in AML.

CONCLUSION

In this review, we noted the (lymph)angiogenic influences on hematopoietic cells involved in AML. On the basis of conditions in the tumor microenvironment, (lymph)angiogenic cytokines alter their expression and interaction in cells in the BM niche, protecting leukemic cells. (Lymph)

angiogenesis is a common process in tumors. Similar to solid tumors, AML is also closely associated with proangiogenic and lymphangiogenic cytokines in the BM. Sinusoidal vessels in the BM have unusual properties and are different from other vasculatures, including blood vessels and lymphatic vessels. Once leukemia occurs, the BM has a dynamic behavior with leukemia cells and immune cells in the BM niche. Moreover, lymphatic-specific marker proteins such as Prox-1, podoplanin and VEGFR-3, as well as (lymph) angiogenic cytokines including VEGF-C and VEGF-A, are highly increased in BM- and in PB-MNCs. The VEGF and non-VEGF cytokines are important, and are altered to affect all cell types in AML BM. In particular, immune cells such as macrophages, as well as stem/progenitor cells, can contribute to lymphatic-promoting conditions. The pivotal roles of these increased lymphangiogenic factors in the BM niche in AML need to be determined. Because the concept of (lymph) angiogenesis is not foreign to hematologic malignancies and solid tumors, further studies may be needed to elicit the *vivo* machinery linking immune cells, leukemic stem cells and lymphangiogenic function *in situ*. Although it showed the ambilaterality of angiogenic and lymphangiogenic cross talk, the aberrant expression of lymphangiogenic factors in AML is also a feature of pathophysiologic conditions. Lymphangiogenic related study therefore has value as a therapeutic solution to treat AML in the future. New therapeutic strategies focusing on the modulation of lymphangiogenic factors may lead to advanced treatment options by eliciting environmental changes in AML.

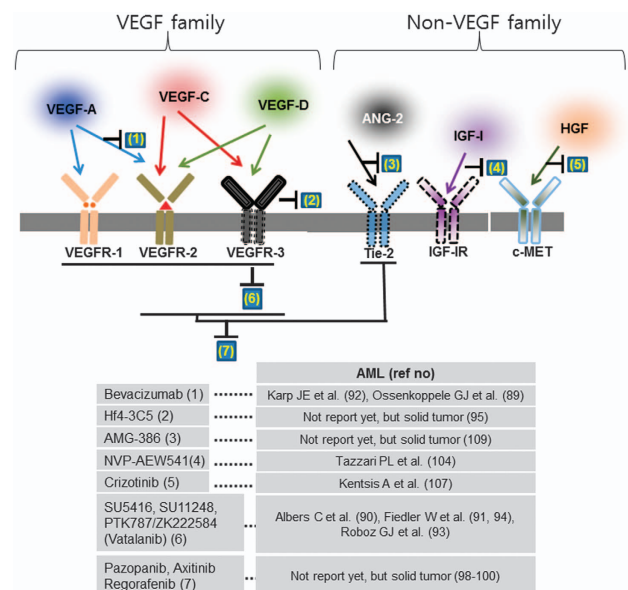


Figure 3 Potential targets and inhibitors for (lymph)angiogenic factors in AML. As target molecules involved in lymphangiogenesis, several factors have been applied in clinical studies. AML focused agents typically target dual or multiple protein kinases, and then chemotherapy combined trials with inhibitors to effectively inhibit AML blasts.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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