Role of VEGF/VEGFR in the pathogenesis of leukemias and as treatment targets (Review)

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Abstract. Angiogenesis plays an important role in solid tumor growth, progression and metastasis. Evidence suggests that the progression of hematolymphoid malignancies also depends on the induction of new blood vessel formation under the influence of acute leukemia, myelodysplastic syndromes, myeloproliferative neoplasms, multiple myeloma and lymphomas. The vascular endothelial growth factor (VEGF) is the most important proangiogenic agent that activates receptors on vascular endothelial cells and promotes blood vessel regeneration. It has been demonstrated that VEGF/VEGF receptor (VEGFR) expression is upregulated in several types of hematolymphoid tumor cells accompanied with angiogenesis. The levels of VEGF/VEGFR are correlated with the treatment, relapse and prognosis of hematolymphoid tumors. In order for VEGF family and their receptors as antiangiogenic targets to treat solid tumors, several antiangiogenic agents targeting VEGF-related pathways have been used for the treatment of hematolymphoid malignancies in clinical trials. The results demonstrate a promising therapeutic intervention in multiple types of hematolymphoid tumors. This review aims to summarize recent advances in understanding the role of VEGF and angiogenesis in leukemias, mainly focusing on their upstream transcriptors, downstream targets and the correlation of VEGF/VEGFR with the treatment, relapse or prognosis of leukemia. The progress of VEGF and its receptors as attractive targets for therapies are also discussed in clinical application.

Contents

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1. Introduction

Angiogenesis is a tightly regulated process dealing with the development of new blood vessels from a pre-existing vascular network. In solid tumor growth, angiogenesis is critical for tumor development, progression and metastasis (1,2). Several types of leukemias similar to solid tumors were also reported to have high in bone marrow microvessel density (MVD) (3). This suggests that angiogenesis plays an important role in the progression of hematolymphoid malignancies. Often, angiogenesis is maintained by a balance of endogenous antiangiogenic and proangiogenic factors. However, the exact mechanism triggering vascular endothelial growth factor (VEGF) expression in hematolymphoid tumors is unknown; different mechanisms similar to those observed in solid tumors are anticipated. In recent years, the expression level of VEGF/VEGF receptor (VEGFR) in patients with different hematolymphoid tumors has been detected and related to reduced survival and lower remission rates (4). Studies have demonstrated that VEGF/VEGFR-related pathways are the most relevant regulators of neoangiogenesis, vasculogenesis and recruitment of endothelial progenitor cells. Furthermore, VEGF/VEGFR interactions may stimulate proliferation, migration and survival of leukemia/lymphoma cells by autocrine and paracrine loops (3). According to a number of studies, acute leukemia cells secrete significant amounts of VEGF in the serum and malignant hematopoietic cells were found to express VEGF and its receptors (5). The clinical outcome of this approach has also been confirmed, and recently coordinated efforts in research have resulted in a number of novel antiangiogenic agents (6). This review focuses on the current knowledge of angiogenesis and antiangiogenic therapies in hematolymphoid malignancies. It mainly demonstrates the role of VEGF/VEGFR in hematological malignancies including its important role in growth, proliferation, survival, as well as the correlation of VEGF/VEGFR with the treatment, relapse or

prognosis of leukemia. The progress of VEGF and its receptors as attractive targets for therapies are discussed together with their clinical application in leukemic diseases.

2. Brief review of VEGF/VEGFR

VEGF, a glycoprotein, was first purified from the *in vitro* culture medium of bovine pituitary folliculo-stellate cells by Ferrara and Henzel (7). Named after its mitogenic activity for vascular endothelial cells, VEGF was considered a highly specific co-mitogen for the vascular endothelial cells that promoted vascular permeability (8). The five subtype members in the VEGF family are VEGF-A, PlGF, VEGF-B, VEGF-C and VEGF-D. VEGF-A is the prototype of the ligand and is commonly known as VEGF. All these ligands promote endothelium regeneration and increase vascular permeability via binding to three transmembrane receptor tyrosine kinases which are VEGFR-1, -2 and -3. VEGFR-1 ligands include VEGF-A, -B, and placental growth factor (PlGF). VEGFR-2 (also known as KDR in human and Flk-1 in mice) has ligands of VEGF-A, -C, -D and is predominantly expressed in vascular endothelial cells. The activation of VEGFR-2 is necessary and sufficient in order to mediate VEGF-dependent angiogenesis and the induction of vascular permeability (8,9). The binding of VEGFR-3 to the VEGF homologues VEGF-C and VEGF-D is largely restricted to lymphatic endothelial cells and plays an important role in the regulation of lymphangiogenesis (9,10).

Receptor tyrosine kinases are expressed in adult endothelial cells except the brain. VEGFR-1 is also expressed in hematopoietic, monocytes and smooth muscle cells (11,12). VEGFR-2 is expressed mostly in vascular endothelial cells, and also in neuronal, megakaryocytes and hematopoietic stem cells (13). Although the exact contribution of VEGFR-1 signaling to angiogenesis is unclear, it has been shown that VEGFR-1 directly cooperates with VEGFR-2 via heterodimerization similar to the binding of two additional VEGF homologues, VEGF-B and PlGF (12). It has been discovered by Brekken *et al* (14) that VEGF-2 plays a significant role in VEGF-induced angiogenesis and the binding between VEGF and the VEGFR-2 may activate the mitogen-activated protein kinase (MAPK) system via protein kinase C (PKC) or the Ras protein to induce the proliferation of vascular endothelial cells.

It has been identified that VEGF is the specific growth factor for angiogenesis, while other growth factors including fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) have no specificity since they act on several different types of cells including vascular endothelial cells. Moreover, VEGF may regulate the development of hemopoietic stem cells and remodel both the extracellular matrix and the regeneration of inflammatory cytokines (15,16). Several leukemic cell strains and primary cells synthesizing and secreting VEGF may affect and modulate the malignant biological behavior of leukemic cells by two positive-feedback loops which are paracrine and autocrine (17,18). VEGF secreted by leukemic cells interacts with relevant receptors on the endothelial cell surface and stimulates endothelial cells to produce growth factors that act on leukemic cells resulting in an increase in their proliferative activity and drug resistance. Hence, antiangiogenesis therapy based on the principle of inhibiting the physiological function of VEGF has become a novel target for antiangiogenic therapy (19).

3. Signaling pathway of VEGF/VEGFR in leukemia

Upstream regulators target VEGF/VEGFR expression in leukemic diseases. Much research has focused on the factors regulating VEGF expression and their functions in leukemic diseases from different aspects. VEGF expression is regulated by several intrinsic and extrinsic factors, with hypoxia and hypoglycemia being the major stimuli (20). Often, hypoxia-induced transcription of VEGF mRNA is mediated by the binding of hypoxia-inducible factor-1 (HIF-1) (21); intratumoral hypoxia and HIF-1 mediation have been discovered to be a key angiogenesis triggering event (22,23). This basic helix-loop-helix transcription factor may not only contribute to hypoxia-induced VEGF production, but may also play a critical role in the oncogene-dependent expression of VEGF (24). Additional recent data suggest that HIF-1α may also be involved in BCR/ABL-dependent expression of VEGF in Ba/F3 cells (25,26). HIF-1α also accounts for the molecular mechanism of autocrine regulation of VEGF in chronic lymphocytic leukemia (CLL) B cells. Ghosh *et al* (24) reported that CLL B cells express constitutive levels of HIF-1α under normoxia. The stabilized HIF-1α may form an active complex with the transcriptional coactivator p300 and phosphorylated-STAT3 at the VEGF promoter and recruit RNA polymerase II to upregulate VEGF transcription. Consequently, VEGF is secreted at higher levels in CLL B cells. The authors examined the status of the von Hippel-Lindau gene product (pVHL) that dealt with HIF-1α degradation and discovered it was at a notably lower level in CLL B cells compared with that in normal B cells. This initial evidence explained the aberrant autocrine VEGF secretion in CLL cells. Besides BCR/ABL, microvesicles (MVs) released by malignant cancer cells constituting an important part of the tumor microenvironment may also activate the HIF-1α pathway with VEGF production in B-cell CLL patients. Ghosh *et al* (27) demonstrated that MVs circulating in the plasma of B-cell CLL patients exhibited a phenotypic shift from the predominant platelet derived in early stage to leukemic B-cell derived at advanced stage. Furthermore, the total MV level in patients with CLL was higher compared to that of healthy patients. Apart from being a factor in angiogenesis these results indicate that VEGF is also an essential mediator in other clinical phenomena.

As described, VEGF production is associated with the constitutive activity of Janus kinase 3 (Jak3) and the c-Jun N-terminal kinases (JNKs). Jak3 has been suggested to play a key role in the transformation of CTCL T cells since Jak3 inhibitors trigger apoptosis and inhibit cell growth and spontaneous cytokine production of malignant T cells (28-30). It is proposed that the oncogene Stat3, the primary target of Jak3-mediated transformation, requires coactivators such as HIF-1α to induce VEGF expression. Additionally, c-Jun phosphorylation and its ability to bind to a VEGF promoter element relating to JNK activity and VEGF production indicate that JNK induced VEGF expression is caused by an increase in c-Jun/AP-1 activity. Activation of the JNK/AP-1 signaling pathway has previously been implicated in the induction of VEGF transcription (31). However, the JNKs have been shown to promote VEGF expression through other mechanisms, such as increasing VEGF mRNA stability (32). Therefore, inhibition of VEGF-inducing pathways or neutralization of VEGF

itself may imply novel therapeutic modalities in cutaneous T-cell lymphoma (CTCL) (33). In a previous study, researchers discovered that lysophosphatidic acid (LPA) protected CLL cells from apoptosis through a higher expression of LPA receptors and autocrine production of VEGF. Kumar *et al* (34) reported that an increase in VEGF by LPA was mediated through the activation of JNK and transcription factor NF-κB since blocking JNK or NF-κB activation may inhibit LPA to induce VEGF expression. Furthermore, it was demonstrated that LPA protected cells from apoptosis by blocking the activation of both VEGFR-1 and VEGFR-2 via the VEGF receptor kinase inhibitor. Knocking down the expression of VEGFR-1 and inhibiting the activation of NF-κB and JNK may also block LPA to avoid apoptosis. We hypothesize that LPA contributing to VEGF production in B cell malignancies leads to cell survival (35).

Leukemia is an angiogenesis-dependent malignancy (36,37) and angiogenesis is strictly dependent on Akt/NF-κB activation. The inhibitors of the NF-κB pathway decreasing VEGF secretion in leukemic cells and inhibiting endothelial cell activities may cause the interruption of a reciprocal stimulatory loop between leukemic and endothelial cells. Different reports demonstrating the activation of NF-κB in lymphoid and myeloid malignancies underscore the implication in malignant transformation (38). While the overexpression of NF-κB may lead to chemoresistance, the appropriate inhibition of this pathway may lead to successful therapy. Moreover, the transcription of VEGF by the classical NF-κB target gene may be repressed, which is one aspect of the participation in tumorigenesis of adult T-cell leukemia (ATL) (39). NF-κB is also activated by the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway, which is also crucial to several aspects of cell growth, survival and apoptosis. PI3K/Akt activation has been implicated in both the pathogenesis and the progression of a variety of neoplasms which includes leukemias (40). Accumulated evidence over the years has indicated that the PI3K/Akt signal transduction pathway is a major factor for cancer resistance in conventional therapies. Indeed, pharmacologic inhibitors of PI3K/Akt have been found to potentiate the apoptotic action of anti-leukemic drugs (41). Therefore, targeting NF-κB activation as well as its upstream regulator Akt may constitute an additional strategy to improve conventional therapies.

Several extensively characterized transcription factors which include activator protein-1 (AP-1), NF-κB and stimulatory protein-1 (SP-1) modulate VEGF expression. The activation and binding of these transcription factors in tumor cells contribute to VEGF transcription and tumor metastasis (42-44). Among those, AP-1 is a critical factor in the regulation of VEGF gene expression. Pollmann *et al* (45) determined that VEGF gene expression is mostly regulated by AP-1 and c-Jun in human promyelocytic leukemia (HL-60) cells. Poulaki *et al* (46) demonstrated that the insulin-like growth factor-1 receptor (IGF-1R) in thyroid tumor cell membranes may promote VEGF production by enhancing the activity of transcription factor AP-1 (46). Rutin in combination with vitamin E has been demonstrated to synergistically inhibit oxidative damage. Chuang *et al* (47) reported that rutin in combination with vitamin E attenuated VEGF expression in HL-60 cells by decreasing the activity of AP-1.

The BTB domain (named after the *Drosophila* transcription factors Bric-a-brac, Tramtrack and Broad) of promyelocytic leukemia zinc finger (PLZF) as a novel apoptotic and anti-angiogenic protein, may directly inhibit tube formation and migration of endothelial cells on Matrigel *in vitro*. To date, the BTB domain is reported to reduce the expression of VEGF, p-Ak, and p-eNOS in HUVECs. Akt and eNOS play significant roles in angiogenesis stimulated by VEGF which is known to stimulate Akt-dependent phosphorylation of eNOS. These observations reveal that the BTB domain has little or no effect on non-phosphorylated Akt and eNOS. These data indicate that VEGF is essential to the BTB domain function and they form a positive feedback loop to facilitate leukemia-related disease (48) (Fig. 1).

BCR/ABL (an oncogene fusion protein consisting of BCR and ABL, which is associated with the Philadelphia chromosome) functions as a constitutive tyrosine kinase leading to autophosphorylation (49) and activates multiple signaling molecules including p21Ras (50), signal transducer and activator of transcription 5 (STAT5) (51-53) and phosphoinositide 3-kinase (PI3-kinase) (54). Using both single-marker analysis and haplotype analysis, BCL-2 SNP was found to demonstrate consistent association with susceptibility to chronic myeloid leukemia (CML) (55). Recently, it was reported that treatment with Bcr-Abl-targeting siRNAs and imatinib resulted in an enhanced VEGF suppression in K562 cells (56). Little is known about the biochemical mechanisms and signaling pathways contributing to BCR/ABL-induced expression of angiogenic growth factors in CML cells. Böhm *et al* (57) reported that BCR/ABL induces VEGF production in CML cells through a pathway involving PI3-kinase and mammalian target of rapamycin (mTOR). mTOR has recently been implicated in leukemic cell growth, tumor-associated angiogenesis and the expression of VEGF in acute myeloid leukemia (AML). mTOR-targeting drugs exert anti-leukemic effects on AML cells *in vitro* through multiple actions, including direct inhibition of proliferation, induction of apoptosis and suppression of VEGF (57).

Downstream target genes of VEGF in leukemias. In addition to stimulating angiogenesis, other studies have demonstrated that VEGF may directly stimulate proliferation of several types of leukemia cells. For example, VEGF stimulates multiple myeloma (MM) cells to migrate, proliferate and survive on fibronectin via autocrine and paracrine loops, which usually contributes to the binding of VEGF and VEGFR-2 (58). As to the modulation of its downstream target gene, a great deal of progress has been made. VEGF induces the expression of heat shock protein 90 (Hsp90), which binds Bcl-2 and Apaf-1 to increase leukemic cell resistance to serum deprivation-induced apoptosis (59). Moreover, VEGF may play an important role in the growth of hematologic neoplasms via a paracrine mechanism. When endothelial cells are exposed to recombinant human VEGF, they may increase mRNA expression of several hematopoietic growth factors, including G-CSF, GM-CSF, stem cell factor (SCF) and IL-6, which act as growth factors for myeloid and lymphoid cells (60,61). In other words, VEGF may promote tumorigenesis by enhancing the production of hematopoietic growth factors. Both VEGF and PKCβII were reported to have higher levels of expression

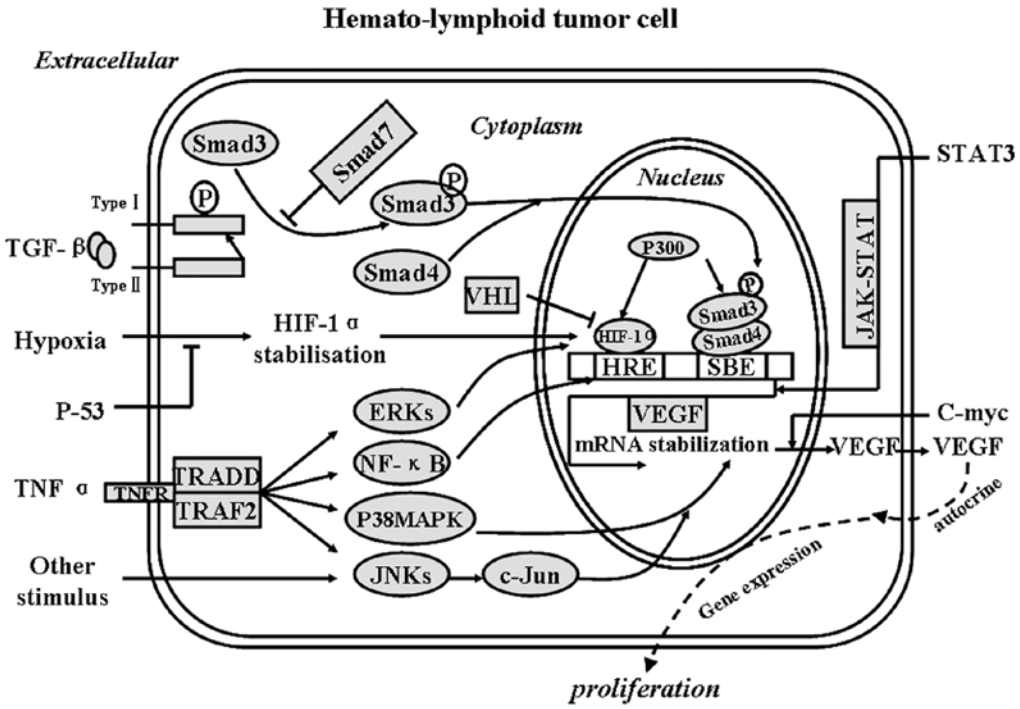

Figure 1. Upstream regulators target VEGF/VEGFR expression in leukemias. Smads, signal transducers and transcription factors in the TGF-β signaling pathway; TRADD, protein which binds adaptor protein TRAF2 and then suppresses TRAF2-mediated apoptosis; TRAF2, TNF receptor-associated factor 2, which mediates the signal transduction from members of the TNF receptor superfamily; VHL, von Hippel-Lindau tumor-suppressor also known as pVHL; ERKs, extracellular signal-regulated kinases; P38MAPK, P38 mitogen-activated protein kinases; JNKs, c-Jun N-terminal kinases; p300, a transcriptional coactivator. TGF-β, hypoxia and TNF-α induce the expression of VEGF and VEGFR in hematolymphoid tumor cells through the Smad family, HIF-1α, ERKs, NF-κB, p38-MAPK and JNKs, respectively, which, ultimately, affects VEGF/VEGFR expression.

related to disease stage and tumor burden (18,62,63). The regulation of PKCβ expression in CLL cells by VEGF was important since the level of PKCβ expression in malignant cells in a Tcl1 mouse model determined the development and progression of the disease. Controlling PKCβ expression is likely to be an important step in CLL pathogenesis. VEGF, due to its role in stimulating PKCβ expression, is therefore central to the pathogenesis of the disease (64). Migration of B-cell chronic lymphocytic leukemia (B-CLL) cells involves several molecules, including matrix metalloproteinase-9 (MMP-9) and VEGF. Downregulation of MMP-9 by VEGF significantly inhibited the migration of B-CLL cells through human umbilical vein endothelial cells. STAT1 was found to be responsible for MMP-9 downregulation since STAT1 gene silencing restored MMP-9 production and B-CLL cell migration in the presence of VEGF. The VEGF/VEGFR-2 axis is upstream of STAT1 tyrosine phosphorylation thus the inhibition of B-CLL cell migration is ultimately due to the effect of VEGF (65).

VEGF-C has been recognized as a tumor lymphangiogenic factor based on the effects of activated VEGF-R3 on lymphatic endothelial cells. VEGF-C enhances c-Jun binding to the cyclic adenosine 3',5'-monophosphate-response element of the cyclooxygenase-2 (COX-2) promoter and induces COX-2 expression, which may catalyze one of the rate-limiting steps in prostanoid biosynthesis (66) and enhance the survival and proliferation of malignant cells, while negatively influencing anti-tumor immunity. In addition, the VEGF-R3/JNK/AP-1 pathway also participates in the induction of COX-2 by VEGF-C in leukemic cells. VEGF-R2 activation may induce the upregulation of COX-2 via p38 MAPK and JNK signaling pathways in human vascular endothelial cells (67). In line with this study, VEGF-A may also induce the upregulation of COX-2 in leukemic cells. By acting in an autocrine/paracrine manner, VEGF-C may contribute to tumor angiogenesis through the induction of COX-2/prostanoids in subsets of leukemia. A previous study demonstrated that there is a significantly higher induction of VEGF-C by COX-2 in another context (68). Hence, the cross talk between VEGF-C and COX-2 may initiate a positive feedback loop, resulting in enhanced expression of COX-2 and increased synthesis of prostanoids, which lead to a further increase in VEGF-C activity.

The expression of the antiapoptotic myeloid cell leukemia-1 (MCL-1) gene, leading to the enhanced survival of tumor cells, is a novel prognostic factor in B-CLL (69,70). VEGF and interleukin-6 (IL-6) are able to upregulate MCL-1 via autocrine signaling loops. VEGF may be a positive autocrine regulator of MCL-1 in B-CLL. In addition, specific downregulation of MCL-1 gene expression was discovered to promote apoptosis and death of primary B-CLL cells, suggesting the possibility of the inhibition of VEGF, and its pathway may prove useful in the treatment of B-CLL patients (71) (Fig. 2).

4. *In vivo* studies of VEGF/VEGFR in patients with leukemia

VEGF/VEGFR in acute lymphocytic leukemia (ALL). To the best of our knoweldge, Perez-Atayde *et al* (72) first demonstrated that leukemia progression was correlated to increased bone marrow vascularization. It was demonstrated that ALL patients had an increased blood vessel content compared

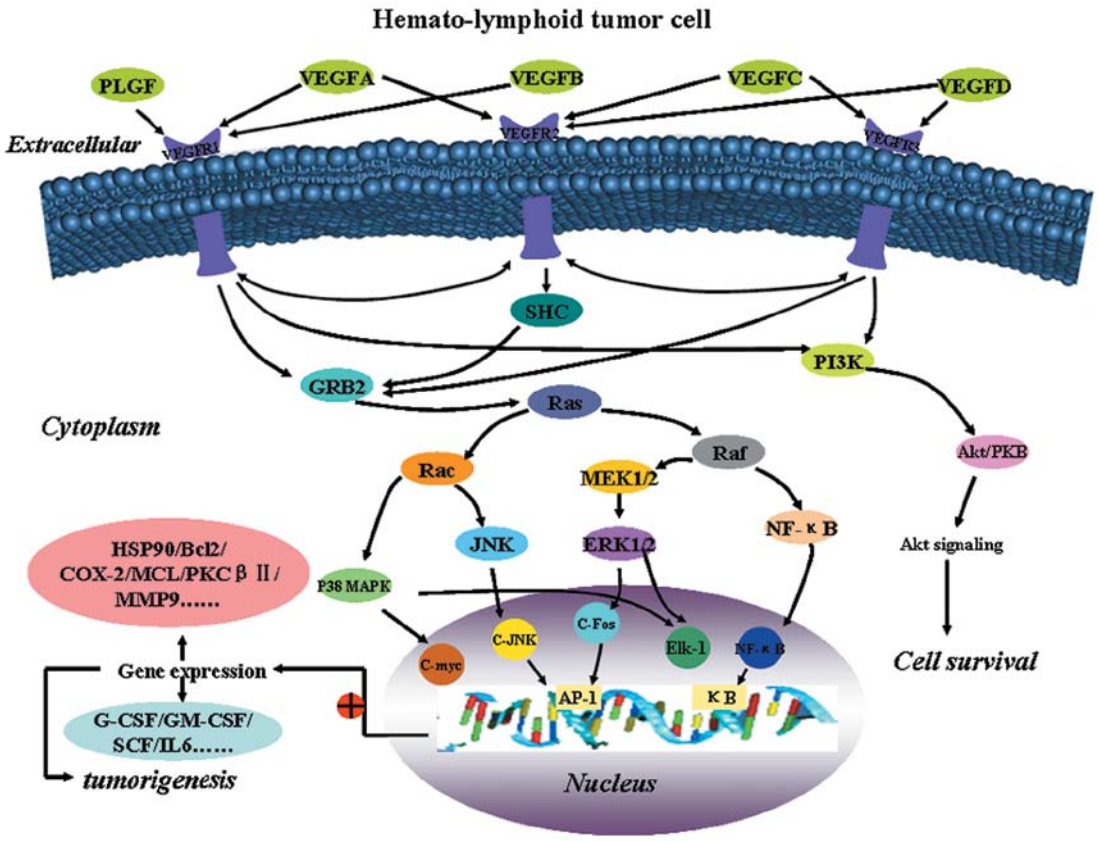

Figure 2. Downstream regulators target VEGF/VEGFR expression in leukemias. After interaction with factors in both the cytoplasm and nucleus, VEGF/VEGFR may effect tumorigenesis by upregulating HSP90, Bcl2, COX-2, MCL, PKCβII, MMP9, and downregulating G-CSF, GM-CSF and IL-6.

to their normal counterparts. There were elevated levels of proangiogenic growth factors including basic fibroblast growth factor (bFGF) and VEGF in urine and peripheral blood samples from ALL patients. The factors were correlated with bone marrow angiogenesis (72,73). The existence of an 'angiogenesis switch' first proposed for solid tumors is suggested to apply to hematolymphoid malignancies (3). The 'angiogenesis switch' in leukemias is indicated to increase bone marrow microvessel density (MVD), the expression of HIF-1, multiple proangiogenic factors (VEGF, bFGF and angiopoietin-2) and soluble VEGFR, but a decrease in the expression of endogenous angiogenesis inhibitors (74,75). In a recent study, MVD was found to be higher in T-ALL compared to B-ALL patients (76). In the B-ALL group, cases with t(12;21) were characterized by a low MVD, while patients with hyperdiploid leukemia displayed a high MVD (3). The correlation between MVD and white blood cell count (WBC) may be a determination of high-risk for B-ALL patients. In addition, patients with a high marrow reticulin fiber density and high MVD exhibit an unfavorable outcome, indicating the possible cellular origin of certain proangiogenic factors (76). The following clinical studies support the capacity of leukemia cells to produce proangiogenic growth factors including VEGF and bFGF *in vitro* (77).

To further detect whether tumor progression is in concert with the induction of tumor angiogenesis in leukemia, angiogenesis was characterized by immunohistochemical staining of factor VIII (FVIII) in bone marrow biopsies and was quantified by MVD assessment in patients with newly diagnosed ALL or after the completion of remission induction chemotherapy. The results suggested that the leukemia was angiogenesis-dependent. This increased the possibility for antiangiogenic drugs to treat leukemia (78). The high plasma VEGF concentration was linked to leukemic cell invasion in adult T-cell leukemia (ATL) and the major source was from ATL cells themselves (79). Noteworthy, all cell lines were observed to express mRNA and protein of VEGFR-1. In clinical specimens, similar results were also detected in all (100%) of 11 and 8 (73%) of 11 ATL patients, respectively. VEGF was effectively bound only to VEGFR-1-expressing cells. Further study demonstrated that the key role of VEGF in ATL is to assist in cell invasion, not proliferation. ATL cells upregulate their own chemotaxis to facilitate the invasion into various organs through the combination of VEGF and VEGFR-1 (80). Besides that, the angiogenic effect of VEGF seems to be dependent on nitric oxide (NO). The associations among functional polymorphisms in VEGF (-2578C>A, -1154G>A and -634G>C) and NOS3 (-786T>C, intron 4 b>a and Glu298Asp) were examined with the prognosis of childhood ALL. The results indicate that polymorphisms of VEGF and NOS3 genes are highly associated with the risk of relapse, therefore it may be a prognostic sign in childhood ALL (81).

VEGF/VEGFR in acute myeloid leukemia. In recent studies, the expression of VEGF/VEGFR in AML patients has been detected. The increased levels of plasma VEGF have been correlated with reduced survival and lower remission rates (4), and the level of plasma/serum VEGF was found to be related to the

number of circulating blasts (82). In addition to the modulation of bone marrow angiogenesis by VEGF from leukemia cells, it was confirmed that the expression of endothelial-specific tyrosine kinase receptors, such as VEGFR-1, -2 and -3, are also observed in leukemic cells (83,84). De Bont *et al* (85) reported that new vessel formation was the result of angiogenesis and vasculogenesis. The degree of neovascularization in the bone marrow was correlated with VEGF expression in the leukemic cells. The bone vessel count (MVC) was higher in 23 cases of AML patients compared to that in the normal controls, and the cell proliferation, bone marrow angiogenesis and expression of VEGF were correlated to each other in patients with AML (85). To further assess cellular VEGF levels and their prognostic significance in newly diagnosed AML, a radioimmunoassay (RIA) was performed to quantify VEGF levels in stored samples from 99 patients diagnosed with AML. Although the patients with an increased VEGF level had a shorter survival, there was no evidence to demonstrate the significant relationship between VEGF level and WBC or blast count in AML. In contrast, the results suggest that the cellular VEGF level is an independent predictor of outcome in AML (86). Interestingly, the co-expression of CD147 and VEGF may indicate a poor prognosis in AML and may be a highly sensitive marker for predicting the clinical outcome of patients (87).

As to the induction of angiogenesis by VEGF in AML, Fielder *et al* (88) investigated the expression of VEGF and its receptors in fresh leukemic blasts and addressed the possible loops for the stimulation of AML blasts. The autocrine VEGF worked through VEGFR-2, by activating eNOS to produce NO through PI3-K/Akt kinase, and maintained clonogenic cell growth in the OCI/AML-2 cell line (89). Also, Fielder *et al* (90) reported that VEGFR-3 represented a cloned member of class III receptor tyrosine kinase with VEGFR-1 and VEGFR-2. The ligand of VEGFR-3 has been identified as VEGF-C that shares sequence homology with VEGF and PlGF. VEGF-C expression was discovered in leukemic samples of 4 out of 7 VEGFR-3-positive and 4 out of 6 VEGFR-3-negative patients (90). MVD was increased in the bone marrow of patients with AML, supporting the hypothesis that angiogenesis plays an important role in AML (61). High VEGF-C mRNA expression in AML blasts is related to drug resistance *in vitro* and *in vivo*. The prognostic significance and associated gene expression profiles of VEGF-C with long-term outcome remain to be defined. However, several effects of VEGF-C on the treatment and gene expression profiles were investigated by using microarray analysis in 525 adult and 100 pediatric AML patients. The results showed that increased VEGF-C predicted adverse long-term prognosis, which provided additional information to well-known factors (91).

VEGF/VEGFR in chronic myeloid leukemia or chronic lymphocytic leukemia. Aguayo *et al* (36) evaluated the blood vessels in 145 bone marrow biopsies and the levels of VEGF, bFGF, TNF-α, TGF-α and HGF in 417 plasma samples. Except for CLL, vascularity was significantly higher in all leukemias compared with the control bone marrows. The highest number of blood vessels and the largest vascular area were discovered in CML. At the same time the highest levels of VEGF in plasma were detected in CML, while the highest levels of bFGF were in CLL. The level of HGF was highest in chronic myelomonocytic leukemia (CMML). Molica *et al* (92) evaluated the levels of VEGF in serum in B-CLL, and the results indicated that increased serum levels of VEGF may be considered in predicting the risk of disease progression. Ferrajoli *et al* (93) discovered VEGFR-2 was a high-affinity VEGF receptor that plays a role in *de novo* blood vessel formation and hematopoietic cell development. Cellular VEGFR-2 levels may serve as a prognostic factor in CLL. All evidence supports that VEGF is a critical microenvironmental factor, and VEGF inhibition may be a promising new therapeutic approach in CLL. For example, vatalanib and pazopanib seem to be effective and safe candidates. More evaluation will be required to confirm these results.

5. Antiangiogenic therapy in leukemias

Antiangiogenic therapy in animal models with leukemias. According to the relationship between VEGF/VEGFR and leukemias, antiangiogenic therapy was used to treat leukemias in animal models. Dias *et al* (94) reported, using an *in vivo* model of human leukemia, that blocking angiogenesis induced by the interaction of leukemia-derived VEGF with murine VEGFR-2 delays leukemic growth. Although it was not sufficient for its eradication from inoculated mice, these results demonstrated that targeting VEGF-induced angiogenesis may be at least partially effective in delaying the progression of leukemia. Interestingly, long-term remission was achieved only when mice were treated with neutralizing monoclonal antibody (mAb) against murine and human VEGFR-2, blocking the paracrine and autocrine VEGF-VEGFR-2 signaling pathways. On the other hand, mAbs against murine or human VEGFR-1 had no effect towards improving the survival of the leukemic mice, suggesting that the VEGF/VEGFR-2 pathway is more important for the proliferation and engraftment of acute leukemias *in vivo*. However, it is also possible that several leukemias may depend on VEGFR-1 signaling (95). In order to further examine the role of VEGF in AML progression *in vivo*, a number of researchers established two mouse models. In a murine chloroma model, the delivery of VEGF using microencapsulation technology resulted in enhanced tumor growth and vascularization, whereas treatment with a VEGF antagonist soluble NRP-1 (sNRP-1) inhibited tumor angiogenesis and growth. In a systemic leukemia model, the survival of mice injected with adenovirus (Ad) encoding for Fc-sNRP-1 was significantly prolonged as compared with mice injected with Ad-LacZ. Further analysis showed a reduction in circulating leukemic cells and infiltration of liver and spleen as well as bone marrow neovascularization and cellularity (96). Liu *et al* (97) demonstrated that adenoviral gene therapy with antiangiogenic fragments of thrombospondin-1 inhibited leukemic xenograft growth in mice, which addressed the possibility that reduced production of angiogenesis inhibitors by leukemic cells may trigger the onset of the neovascularization process, by shifting the local (bone marrow) angiogenesis balance.

In contrast to the positive roles in leukemia disease, emerging evidence from genetically modified animal models suggests that elevated levels of VEGF, or a proangiogenic phenotype, may impede, rather than promote, early tumor development and progression (98). Researchers reported a tumor inhibitory role for VEGF by demonstrating that a

Table I. Summary of clinical trials and approved antiangiogenic therapies in patients with leukemia.

	Anti-VEGF strategies		RTK inhibitors			Immunomodulators	
Patients	Target	Bevacizumab	Target	Vatalanib	Cediranib	Thalidomide	Lenalidomide
AML	VEGF-A	+	VEGFR1-3	+	+	+	+
CML	VEGF-A	+	VEGFR1-3	+	/	/	/
CLL	VEGF-A	+	/	+	+	+	+

+, There was response to treatment; /, there was no response to treatment. VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; CLL, chronic lymphocytic leukemia.

2-fold overexpression of systemic levels of VEGF in mice heterozygous for a VEGF 'hypermorphic' allele decelerated tumorigenesis in a retroviral-induced, spontaneous murine leukemia model (99). Alterations in the innate immune function, specifically enhanced natural killer cell activity, and increased hematopoietic progenitor cell survival were identified as acquired phenotypes that strongly correlated with and were likely responsible for this leukemic inhibition (99). Accordingly, Ebos *et al* (100) recently showed that blockade of the VEGF pathway, before tumor induction, led to a more aggressive metastasis and shortened survival. Therefore, further experimental and clinical studies are required to clarify the controversy surrounding the dichotomous roles of VEGF in tumor angiogenesis.

Antiangiogenic therapy in patients with leukemias. The first antiangiogenic agent to be approved was bevacizumab, a humanized anti-VEGF monoclonal antibody. Administration of bevacizumab, in combination with cytotoxic chemotherapy, confers benefits to patients with several types of solid cancers (101-103). Additionally, two small-molecule inhibitors targeting VEGFR and other kinases, sorafenib and sunitinib, have been approved for treating renal cells- and hepatocellular carcinoma (104,105). These are currently under investigation for patients with relapsed and refractory acute leukemia in combination with standard chemotherapy (106). The major classes of antiangiogenic therapy include direct anti-VEGF acting molecules (anti-VEGF antibodies, VEGF-antisense nucleotides), immunomodulatory drugs (IMIDs) with anti-angiogenic properties, receptor tyrosine kinase inhibitors that target VEGFR signaling as well as receptors of other (proangiogenic) factors, the anti-endothelial approach of metronomic therapy, and other new compounds targeting signaling downstream to proangiogenic growth factors, such as mTOR inhibitors, histone deacetylase (HDAC) inhibitors and proteasome inhibitors. Moreover, angiogenesis appears to be targeted even by conventional chemotherapy in different leukemias (61) (Table I). For example, bevacizumab is a humanized murine anti-human VEGF monoclonal IgG1 antibody that blocks the binding of human VEGF to its receptors VEGFR-1 and -2 (107). It was administered after chemotherapy to 48 adults with refractory or relapsed AML. The overall response was 23 of 48 (48%), with complete response (CR) in 16 (33%). MVD decreased in the bone marrow after bevacizumab administration. Currently, bevacizumab is being evaluated as a treatment option for newly diagnosed AML patients in combination with cytarabine and idarubicin in a phase II study. Thalidomide is administered as an antineoplastic agent after the demonstration of its antiangiogenic activity (108). The newer IMIDs lenalidomide, a synthetic compound derived by modifying the chemical structure of thalidomide, was observed to have 2-3 times more potent antiangiogenic activity than thalidomide in various *in vivo* assays (109) and the antiangiogenic activity has been demonstrated to be independent of their immunomodulatory effects (110). In a phase II study by Thomas *et al* (111), thalidomide was analyzed in 16 patients with relapsed or refractory AML; one patient (6%) achieved CR lasting for 36 months. There was no correlation between the reduction in angiogenesis marker levels and responses. In a phase I/II trial by Steins *et al* (112) in 20 AML patients, a partial response was observed in four patients. In parallel, MVD significantly decreased in five patients during treatment with thalidomide. In a study by Barr *et al* (113), thalidomide was examined in combination with fludarabine, carboplatin, and topotecan in 42 patients with poor AML prognosis and 10 of 42 (24%) patients achieved a CR. Small tyrosine kinase inhibitors that target VEGFR are a further important class of antiangiogenic drugs. For example, vatalanib is an oral angiogenesis inhibitor that offers a novel approach to inhibiting tumor growth (114) by interfering with the ATP binding sites of VEGFR. Ongoing studies are now focusing on evaluating the efficacy of vatalanib in combination with imatinib in a phase I/II trial for patients with AML, PMF and blast phase of CML, or in combination with cytosine arabinoside and daunorubicin in patients with AML (115). Cediranib (AZD2171, Recentin) is a potent inhibitor of both VEGFR-1 and -2 (116). In a phase I study with cediranib in 35 AML patients, six patients experienced an objective response. There was a correlation between cediranib exposure and plasma VEGF levels (117). A combination therapy of thalidomide and 5-azacytidine, a hypomethylating drug, was assessed in 40 patients with AML (118). Hematological improvement was observed in 15 of 36 patients (42%), stable disease was observed in 5 of 36 patients (14%), 10 of 36 patients (28%) had disease progression and 6 had CR.

6. Conclusion

This investigation demonstrates that angiogenesis has important biological and prognostic implications in hematolymphoid malignancies. The autocrine regulators of angiogenesis are essential to the development of diseases. VEGF/VEGFR as key

regulators of neoangiogenesis and vasculogenesis have been widely studied. Although the mechanism of the high expression of VEGF in leukemia cells has not yet been identified, we are certain that VEGF/VEGFR interactions may stimulate proliferation, migration and survival of leukemia/lymphoma cells by autocrine and paracrine loops. In clinical studies, the elevated level of VEGF may contribute to the adverse outcome by promoting leukemic cell growth, survival, migration and reduce the sensitivity of cells to therapeutic agent-induced apoptosis. Therefore, the interference of VEGF/VEGFR-related pathway being an ideal candidate in treating leukemia may induce antiangiogenesis and inhibit growth of leukemia cells. All clinical trials causing VEGFR-2 gene polymorphism relates to cytogenetic response (treatment failure following imatinib therapy for CML) and the VEGF genotype relates to the progression of advanced disease. Apart from anti-VEGF molecules, other antiangiogenic therapies include IMIDs, receptor tyrosine kinase inhibitors, anti-endothelial approach to metronomic therapy, mTOR inhibitors, HDAC inhibitors and proteasome inhibitors. A better understanding of the role of VEGF in leukemia and additional trials combining antiangiogenic therapies will provide a greater insight to the mechanisms required for treatment.

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