The Vascular Endothelial Growth Factor (VEGF) Signaling Pathway: A Therapeutic Target in Patients with Hematologic Malignancies

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ABSTRACT

Angiogenesis is an important component in the progression and metastasis of solid tumors. We now appreciate that angiogenesis is also critically involved in the pathogenesis of hematologic malignancies. Current data suggest important prognostic and therapeutic implications of angiogenesis in a variety of malignancies of the hematopoietic system, including acute and chronic leukemias, myeloproliferative diseases, multiple myeloma, non-Hodgkin's lymphomas, and Hodgkin's disease. Vascular endothelial growth factor (VEGF) is a major angiogenic factor that regulates multiple endothelial cell functions, including mitogenesis. Cellular and circulating levels of VEGF are elevated in hematologic malignancies and are adversely associated with prognosis. Angiogenesis is a very complex, tightly regulated, multistep process, the targeting of which may well prove useful in the creation of novel therapeutic agents. Current approaches being investigated include the inhibition of angiogenesis stimulants (e.g., VEGF), or their receptors, blockade of endothelial cell activation, inhibition of matrix metalloproteinases, and inhibition of tumor vasculature. Preclinical, phase I, and phase II studies of both monoclonal antibodies to VEGF and blockers of the VEGF receptor tyrosine kinase pathway indicate that these agents are safe and offer potential clinical utility in patients with hematologic malignancies. The Oncologist 2001;6(suppl 5):32-39

INTRODUCTION

Leukemias, myelodysplastic syndromes (MDS), lymphomas, and multiple myeloma (MM) are complex diseases with a wide range of clinical, morphologic, biologic, cytogenetic, molecular, and immunophenotypic features [1-5]. With this multitude of disease-associated variables, it is not surprising that response to treatment differs considerably among patients [6]. Although significant progress has been made in the management of these disorders, the majority of patients with leukemia or lymphoma who fail front-line therapies or who relapse after an initial response, die from progressive disease. No effective therapy has been developed for the majority of patients with advanced myeloproliferative disorders (MPD). As the relative ineffectiveness and toxicities of traditional cytotoxic therapies become more appreciated, the search for therapeutic advances is increasingly focused on affecting the critical steps involved in the development and mutation of malignant clones.

A major component in the growth and metastasis of solid tumors is angiogenesis—the formation of new blood vessels. Recently, however, angiogenesis has been found to play a role in hematologic malignancies [7-16]. This article will briefly review current data on the role of vascular endothelial growth factor (VEGF) and its receptors in hematologic malignancies that provide the rationale for ongoing studies of novel therapeutic interventions directed at VEGF and its receptors.

THE BIOLOGY OF VEGF AND ITS RECEPTORS

VEGF regulates several endothelial cell functions, including mitogenesis, permeability, vascular tone, and the production of vasoactive molecules [17]. The VEGF gene is located on the short arm of chromosome 6 [18]. This gene has eight exons and seven introns [19]. Transcription of the VEGF gene is physiologically regulated by hypoxia-mediated control of gene transcription, and production of differing
isoforms of VEGF is controlled by alternative mRNA splicing and proteolytic processing. The predominant isoforms are VEGF189, VEGF165, VEGF121, and VEGF205.

The activity of VEGF is mediated through three receptor tyrosine kinases: VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), and VEGFR-3 (Flt-4) (Fig. 1) [20]. VEGFR-1 is expressed on endothelial cells and monocytes and mediates cell motility [21]. The proliferative and mitogenic activities of VEGF, as well as vascular permeability, are mediated primarily through VEGFR-2 [22]. Lastly, VEGFR-3, also known as Flt-4, which is homologous with the neuropilin-1 receptor, is believed to mediate lymphoangiogenesis [23-25].

ANGIOGENESIS IN THE PROGRESSION OF HEMATOLOGIC MALIGNANCIES

Microvessel density ([MVD]—the average number of microvessels seen in a microscopic field [15]) is increased in a variety of hematologic malignancies [7]. MVD can be assessed by staining paraffin-embedded sections of bone marrow with antibodies against vascular-associated antigens such as factor VIII, CD34, Ulex europaeus, CD31/PECAM, and thrombomodulin [15]. The number of blood vessels in multiple fields is then counted, either directly through a microscope or following digitalization of an image. MVD is significantly greater in patients with advanced MDS, (refractory anemia with excess blasts [RAEB], RAEB in transformation) than in normal individuals, and is even greater in patients with acute myeloid leukemia (AML) (i.e., MVD in AML > in MDS > normals) [16], in whom it has been shown to decrease significantly following successful induction chemotherapy [14]. MVD is also increased significantly in the bone marrow of children with acute lymphocytic leukemia (ALL) [15]. While normal bone marrow has a predominance of straight, nonbranching microvessels, bone marrow from patients with ALL demonstrates a complex, arborizing architecture. Increased MVD is also found in the bone marrow of patients with chronic leukemias (myelogenous [CML] and B-lymphocytic [CLL]), and MPD, [7, 13, 26, 27] as well as in patients with MM, in whom MVD is a prognostic marker that correlates with the plasma cell labeling index [28].

PROGNOSTIC VALUE OF ANGIGENIC FACTORS

The process of angiogenesis is governed by a complex balance of positive and negative regulatory factors. Stimulatory molecules include VEGF, basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), tumor necrosis factor-α, tumor growth factor-β, angiogenin, epidermal growth factor, and the angiopoietins [29]. The two most potent positive regulatory molecules are VEGF and bFGF, which may act synergistically to stimulate the formation of new blood vessels. Negative regulatory factors include platelet factor 4, thrombospondin-1, tissue inhibitors of metalloproteinases, proactin, angiotatin, endostatin, bFGF soluble receptor, interferon alpha, and placental proliferin-related protein. Increased levels of positive regulatory molecules have been correlated with a poor prognosis in patients with solid tumors [30-33]. Studies of human hematopoietic cell lines have demonstrated the presence of VEGF mRNA, bFGF mRNA, or both in all cell lines examined [8].

ELEVATED VEGF LEVELS IN HEMATOLOGIC MALIGNANCIES

Significantly elevated levels of VEGF are observed in a variety of hematologic malignancies [7, 10, 34]. A study of 417 patients showed similarly elevated levels of VEGF in patients with AML (n = 115) and advanced MDS (n = 40) compared with controls [7]. Increased concentrations of VEGF and other angiogenic factors in this population correlated directly with increased MVD in the bone marrow. In stored serum samples, increasing levels of cellular VEGF showed a positive correlation with shorter overall and disease-free survival times (Table 1) [35]. Furthermore, VEGF levels were an independent predictor of outcome in AML patients who had higher white blood cell and blast counts.
Intracellular levels of VEGF have also been correlated with prognosis in patients with CLL [36]. A study of 225 patients with B-cell CLL demonstrated a median intracellular VEGF level that was 7.26 times higher than that detected in normal controls. Patients with lower levels of VEGF protein showed a trend toward shorter survival. In a subgroup of patients with conventional clinical and laboratory features of early-stage disease (Rai stages 0 to II, Binet stages A and B) and good prognosis (β2 microglobulin [β2 M] ≤2.8 mg/dl), lower levels of VEGF were again associated with shorter survival times. However, no correlation among VEGF, disease stage, and β2 M levels was found for the entire group. In contrast, levels of serum VEGF above the median have shown an indirect correlation with the duration of progression-free survival (PFS) in patients with early-stage disease (Binet stage A) [37]. When added to the Rai classification, serum VEGF levels identified two groups with different PFS within stages I and II. Rai stage I-II patients with VEGF levels greater than the median value experienced very short PFS (median 9.5 months), whereas Rai stage I-II patients with low VEGF levels had substantially longer PFS (median not reached at 15 months).

Elevated levels of angiogenic growth factors are also associated with an adverse prognosis in patients with non-Hodgkin’s lymphoma (NHL). In a single-institution study of 200 patients, levels of serum VEGF were negatively correlated with prognosis [34]. However, the highest prognostic power was observed when VEGF and serum bFGF levels were examined in combination. The risk of death in patients whose VEGF and bFGF levels were both within the highest quartiles was nearly three times greater than it was for other patients. This risk was independent of other variables used in the International Prognostic Index [38]. We have recently analyzed the clinical significance of serum levels of VEGF, bFGF, HGF, and angiogenin in untreated patients with NHL or Hodgkin’s disease (HD) (M. Albitar and F. Giles, unpublished data). In HD and NHL patients, there were statistically significant increases in VEGF and HGF as compared with normal controls. HGF was significantly increased in patients with NHL and was marginally increased in those with HD. In contrast, angiogenin levels were significantly decreased in patients with either NHL or HD. In patients with NHL, higher levels of VEGF and bFGF correlated with more advanced disease stage and with higher levels of lactate dehydrogenase. The levels of these angiogenic factors did not correlate with survival in NHL. In HD, high levels of VEGF correlated with shorter survival. Levels of VEGF returned to normal in patients with NHL in which pre- and post-therapy samples were available, while no significant change in the levels of other factors was noted. In post-therapy samples from patients with HD, HGF and bFGF returned to normal levels, while VEGF remained high. Although angiogenin levels remained lower than those of normal controls after therapy in patients with either HD or NHL, patients with NHL with higher angiogenin levels after therapy had significantly shorter survival. In addition, VEGF levels after therapy remained predictive of survival in patients with HD. These data suggest that angiogenic factors may have distinct roles in the biology of HD and NHL.

In patients with MM, levels of VEGF, bFGF, and HGF parallel disease activity [10]. In one study, levels of VEGF correlated with other features of aggressive disease, including levels of C-reactive protein and β2 M [10]. Patients with MM demonstrated 58% higher levels of VEGF in their bone marrow than did other patients, suggesting that the VEGF in patients with MM is of bone marrow origin. Myeloma cells have been shown to express and secrete VEGF [10]. As marrow neovascularization parallels disease activity in MM, it is reasonable to postulate that the vascular growth factor is acting in an autocrine fashion. However, MM cells express VEGF receptors only weakly, if at all. Therefore, the mechanism may be paracrine and result from a VEGF-induced time- and dose-dependent increase in stromal cell secretion of interleukin-6, a known MM growth factor [9]. Interactions between proangiogenic molecules and bone marrow stromal elements may also contribute to the growth of malignant cells from patients with AML. For example, incubation of human umbilical vein endothelial cells with VEGF results in an increased secretion of GM-CSF, a known stimulator of myeloid blast cell growth, by the endothelium [39].
VEGF Receptors in Hematologic Malignancies

We have recently evaluated the clinical significance of VEGFR-1 and VEGFR-2 protein levels in both AML and MDS and assessed their relationship to VEGF protein levels (M. Albitar and F. Giles, unpublished data). Western blot analysis and radioimmunoassay (RIA) were used to confirm and quantify specific protein levels in bone marrow samples from previously untreated patients with AML or MDS. VEGFR-1 levels were significantly higher in AML than in MDS, but no significant difference was found in the VEGFR-2 levels. No significant correlation between VEGFR levels and duration of survival was found. VEGF protein levels were significantly higher in MDS than in AML. Cox proportional hazard regression model showed increasing VEGF levels to significantly correlate with shorter survival of patients with MDS, consistent with our previous report of the inverse relationship between VEGF levels and survival of patients with AML [35]. There was also a significant correlation between VEGF and VEGFR-2 levels in both AML and MDS, but not between VEGF and VEGFR-1 levels. These data suggest that VEGF expression, rather than the expression of its receptors, is the determining factor in the biological behavior of AML and MDS, and that VEGFRs are differentially expressed in AML and MDS.

We have also recently examined the role of VEGFR-2 in CLL (M. Albitar and F. Giles, unpublished data). Western blot analysis was used to determine that VEGFR-2 is present in peripheral blood CLL cells. Cellular levels of VEGFR-2 were then quantified using a solid-phase RIA. Peripheral blood cells from patients with CLL were compared with peripheral blood mononuclear cells from controls. Patients with elevated VEGFR-2 levels had greater elevations in lymphocyte counts, severe anemia, elevated β₂M, and advanced-stage disease. Elevated VEGFR-2 levels were also associated with significantly shorter survival. These data indicate that cellular VEGF-2 levels may serve as a prognostic factor in CLL. Further studies are indicated to investigate the biologic implications of these findings, particularly the interactive effects of VEGF and VEGFR-2 on CLL proliferation.

Non-VEGF Angiogenic Factors in Hematologic Malignancies

A number of other angiogenic factors also play a role in the pathophysiology of hematologic malignancy. Levels of bFGF and/or HGF are significantly increased in patients with AML, MDS, CML, chronic myelomonocytic leukemia (CMML), CLL, and MM [7, 40]. Among hematopoietic malignancies, the highest levels of bFGF were found in patients with CLL while the highest levels of HGF were found in patients with CMML [7]. Notably, while levels of bFGF and HGF were found to be increased in patients with ALL, levels of VEGF were not. The therapeutic implications of this observation are not currently clear.

It is particularly important to note that, although very high levels of VEGF and bFGF have been documented in patients with CLL, bone marrow MVD is not overtly increased in most of these patients. This illustrates the fact that, aside from any direct stimulation of new vessel formation, factors involved in angiogenic modulation have independent significant relationships with the hematologic malignancies. In patients with MM, HGF is overproduced, and the MM cells possess the HGF receptor, c-Met [40]. This suggests that an autocrine loop may be contributing to disease progression. These studies indicate that angiogenic factors play an important role in the progression of hematologic malignancies. However, the relationship is complex and incompletely understood [16].

Antiangiogenic Interventions

Angiogenesis is a multistep process. It begins with an imbalance between proangiogenic and antiangiogenic factors. Under the influence of angiogenic factors such as VEGF and bFGF, endothelial cells change their structure and begin to proliferate [41]. Leading cells secrete matrix metalloproteinases that degrade the extracellular matrix (ECM) along a front. During this process, the ECM is remodeled. Interactions between components of the ECM and endothelial cells are mediated by integrins, heterodimeric transmembrane protein members of a diverse family of more than 15 alpha and eight beta subunits [42]. Endothelial cells express multiple integrins that mediate the adhesion of ECM vitronectin, collagen, laminin, von Willebrand factor, and fibrinogen. In addition, integrin binding regulates cell survival, proliferation, and migration.

An analysis of this process demonstrates several steps that might be useful targets for antiangiogenic intervention. These include the inhibition of angiogenic growth factors (e.g., VEGF, bFGF) or their receptors, inhibition of matrix metalloproteinases, blockade of endothelial cell activation, and inhibition of the tumor vasculature [43, 44]. Inhibition of growth factor signaling could be accomplished by decreasing the production of VEGF (antisense strategy or ribozymes), blocking the binding of VEGF to its receptor (monoclonal anti-VEGF), and blocking signal transduction. Angiogenesis could also be inhibited by treatment with direct inhibitors of endothelial cell production, blocking integrin and endothelial survival signaling, and inhibiting the activity of the matrix metalloproteinases. This article focuses on inhibition of VEGF through antibodies or VEGF receptor inhibitors. The reader is referred to several excellent review articles on other antiangiogenesis interventions in development [43, 44].
STUDIES OF ANTI-VEGF (BEVACIZUMAB)

Bevacizumab is an anti-VEGF monoclonal antibody (mAb). The mAb is a humanized murine antibody with antigen binding complementary determining regions from murine VEGF A.4.6.1 [43, 45, 46]. It recognizes all VEGF isoforms without binding to FGF, endothelium-derived growth factors, HGF, platelet-derived growth factor (PDGF), or nerve growth factor. It has exhibited indirect and potent antitumor activity in experimental models.

CLINICAL STUDIES

The safety of bevacizumab was evaluated in a phase I dose-escalation study involving 25 patients who had a variety of tumors, including sarcomas (n = 8), renal cancer (n = 7), breast cancer (n = 5), and lung cancer (n = 2) [43, 47]. Bevacizumab was administered as an infusion of 0.1 to 10 mg/kg over 90 minutes on days 0, 28, 35, and 42. Dose-limiting toxicities were not observed at weekly doses of ≤10 mg/kg, although some patients experienced asthenia, mild headache, fatigue, nausea, and low-grade fever on the day of administration. In addition, bleeding at tumor sites developed in 3 (12%) of the 25 patients. No objective responses were seen; two patients demonstrated a minor response. In addition, 12 (52%) of 23 patients had stabilization of disease during the 70-day study period. A number of studies with bevacizumab are under way including a National Cancer Institute-sponsored phase II study in patients with blast-phase CML in which bevacizumab is given prior to and with idarubicin and cytosine arabinoside.

VEGF-RECEPTOR ANTAGONISTS

A number of receptor tyrosine kinases (RTKs) are directly or indirectly involved in signal transduction during angiogenesis. These include receptors for VEGF, FGF, and PDGF. VEGFR-1 and VEGFR-2 are the RTKs for VEGF. A number of small molecules that inhibit RTKs are in development, including SU5416, SU6668, ZD6474, ZKI22584, and CGP41251. SU5416 is an indolin-2-one that binds in the ATP binding site of the VEGF RTK for VEGFR-1 and VEGFR-2 [43, 48]. In addition, it binds to the PDGF receptor, which is also involved in the transduction of angiogenesis signals. Finally, it binds to c-kit, a related kinase receptor for stem cell factor, and a hematopoietic growth factor that promotes the survival of early hematopoietic progenitor cells and acts synergistically with other hematopoietic growth factors across multiple lineages [43, 49]. The inhibition of c-kit may be an important therapeutic target in patients with AML, as more than 10% of the blasts express c-kit in 30%-80% of AML cases [43, 50, 51].

SU5416

SU5416 produces a dose-dependent inhibition of tumor growth in a variety of xenograft models, including malignant melanoma, glioma, fibrosarcoma, and carcinomas of the lung, breast, prostate, and skin [43, 52]. In a human colon cancer xenograft model, SU5416 inhibited tumor metastases, microvessel formation, and proliferation [43, 53]. In a model of AML, SU5416 inhibited the stem cell factor-induced proliferation of MO7e cells (Fig. 2). Incubation of MO7e cells with SU5416 induced apoptosis through activation of caspase-3 and increased poly(ADP-ribose) polymerase cleavage [43, 54]. Importantly, SU5416 has not produced bone marrow suppression in preclinical models.

A phase I study of SU5416 was conducted to evaluate the toxicity and pharmacokinetics of escalating doses of the angiogenesis inhibitor in 69 patients with a variety of solid tumors [55]. Patients were treated at 13 dosage levels ranging from 4.4 to 190 mg/m²/day intravenously twice weekly for 4 weeks. At the maximum dosage, dose-limiting toxicities were projectile vomiting, headache, and nausea, all of which were reversible over 24 to 48 hours. Mild to moderate toxicities included headache, pain at the infusion site, phlebitis, change in voice, and elevated amino transferase levels. Pharmacokinetic analyses demonstrated a volume of distribution of 22 ±8 l/m², a clearance of 52 ±16 l/hour after eight infusions, and an elimination half-life of 50 ±16 minutes. SU5416 also has a large distributive volume indicating extensive tissue penetration and dose-independent clearance. In combination with 5-fluorouracil, leucovorin, and irinotecan, SU5416 is now under evaluation in a phase III clinical trial for patients with metastatic colorectal cancer.

The activity of SU5416 was also studied in a multinational trial of 15 patients with c-kit⁺ AML [43, 56]. Treatment consisted of the intravenous administration of the novel angiogenesis inhibitor at a dose of 145 mg/m².
twice weekly for a 4-week cycle. Patients achieving a partial remission (>50% decrease in peripheral blood or bone marrow blast counts) were eligible for two further 4-week cycles. Forty-four patients were considered evaluable for response. Treatment was generally well tolerated. Major toxicities included one fatal episode of thrombocytopenic gastrointestinal bleeding, one case of grade IV pancreatitis, one case of grade IV hepatotoxicity, and bone pain in three patients. Five patients demonstrated a partial response, three patients failed to respond, and six patients had progressive disease while receiving therapy. Of the responders, one patient received SU5416 for more than 3 months and demonstrated a progressive decrease in blast counts. These data support the continued clinical development of this angiogenesis inhibitor for the management of hematologic malignancies. A multicenter Phase II trial is under way in the U.S. to evaluate SU5416 in patients with advanced or refractory hematologic malignancies. This study is anticipated to accrue 150 patients with MPD, leukemias, MDS, and MM.

**THE FUTURE DEVELOPMENT OF ANGIOGENESIS INHIBITORS**

Angiogenesis inhibitors are predominantly cytostatic agents that target normal dividing endothelial cells [43, 57]. Normal endothelium is more genetically stable than neoplastic cells and is less likely to develop drug-resistant mutations. In addition, normal endothelium is relatively quiescent; therefore, angiogenesis inhibitors are expected to target tumors with a degree of specificity unobtainable with traditional antitumor interventions. Lastly, angiogenesis inhibitors such as SU5416 are less likely to produce myelosuppression and mucositis. Therefore, developmental considerations that apply to traditional cytotoxic agents may not be applicable to SU5416. Because cytostatic therapy produces stable disease, it may be more realistic to substitue time to progressive disease for traditional endpoints such as partial and complete responses.

Approaches to therapy may also have to be modified. Single-agent therapy with a cytostatic agent may be preferable in patients with disseminated but less rapidly growing tumors (e.g., early MDS, smoldering MM). Furthermore, the multiple signaling pathways activated by VEGF (e.g., MAPK, FAK, PI3K/Akt, PLC) are each being separately pursued as therapeutic targets, suggesting that multiple agents that target these pathways may need to be investigated, both as pure noncytotoxic approaches, and in combination with cytotoxic therapy (Fig. 1). This suggestion is based upon the assumptions that these combinations may affect different cellular targets, resistance patterns should not overlap, effects are likely to be additive, and the absence of myelosuppression may facilitate the administration of some cytotoxic agents [43, 57].

**CONCLUSION**

The field of clinical hematology/oncology is in desperate need of additional therapeutic options to treat patients with a variety of malignancies, including those of hematopoietic origin. Evidence supports a clinically significant role for angiogenesis manipulation in patients with hematologic malignancies. One major angiogenic factor is VEGF, which appears to play a role in the progression of acute and chronic leukemias, MM, MPD, and NHL. Various antiangiogenic approaches are under investigation in the treatment of hematologic malignancies and solid tumors. These include inhibition of growth factor signaling by VEGF, direct inhibition of endothelial cell proliferation, integrin blockade, and inhibition of matrix metalloproteinases. Two of the most promising approaches to VEGF inhibition involve the use of mAbs to VEGF and small-molecule inhibitors of RTK, such as SU5416. Data from phase I and II studies indicate that these modalities may be safe and potentially effective and support the need for phase III trials in patients with hematologic malignancies.

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