Perspectives

Vascular Endothelial Growth Factor—A Positive and Negative Regulator of Tumor Growth

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Abstract

Over the past decade, the well-documented role of vascular endothelial growth factor (VEGF) in tumor angiogenesis has led it to become one of the leading therapeutic targets for the treatment of cancer. Emerging evidence from genetically modified animal models, however, suggests that elevated levels of VEGF, or a proangiogenic phenotype, may impede, rather than promote, early tumor development and progression. For example, hypermorph VEGF transgenic mice display delayed progression of a retroviral-induced murine leukemia, and knockdown of VEGF expression within the myeloid compartment accelerates tumor progression. Several mechanisms have been proposed to explain this paradox, whereby VEGF induces changes within the hematopoietic compartment and tumor microenvironment through recruitment of tumor inhibitory monocytic cells and the negative regulation of tumor angiogenesis. Thus, it is apparent that the levels of VEGF expression in both tumor and nontumor tissues, as well as the context and timing of its modulation relative to cancer induction, play an important role in determining the effects of VEGF expression on tumorigenicity. In light of these recent findings, the various mechanisms underlying the negative role of VEGF during early tumor development, progression, and metastasis will be discussed. Cancer Res; 70(3); 863–7. ©2010 AACR.

Introduction

A growing body of experimental and clinical evidence has emerged in the last decade to support the concept that mediators of angiogenesis, particularly vascular endothelial growth factor (VEGF), contribute to the development and progression of various malignancies and the formation of metastases. Numerous studies have reported high levels of VEGF expression in a wide variety of tumors that have been associated with poor prognosis (1, 2). This evidence has led to the principle that inhibition of VEGF can block tumor angiogenesis, remove the main nutrient supply, and thereby incapacitate tumor growth. However, evidence from recent clinical trials targeting VEGF challenges this well-accepted principle because monotherapy with anti-VEGF agents has only modestly improved overall survival of cancer patients and tumor resistance is often observed (3–5). Accordingly, recent experimental evidence from Stockmann et al. and Greenberg et al. suggests that, although anti-VEGF therapy has been modestly successful at inhibiting tumor growth, VEGF can act as a negative regulator of angiogenesis and tumor progression (6, 7). This work questions the idea that maximally sustained levels of VEGF and potentially other proangiogenic factors are required for tumor growth (6, 7). From these and other findings, it is apparent that manipulation of the host toward a proangiogenic phenotype, either systemically or within the hematopoietic compartment, manifests into a protective role against tumor growth in vivo. Although evidence exists in the literature to support the idea that VEGF is a positive regulator of tumor growth, what we would like to elaborate on here is the concept that VEGF also acts as a negative regulator of tumor growth.

VEGF Acts as a Negative Regulator of Tumor Progression

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. Intriguingly, Stockmann and colleagues (7) observed that deleting VEGF in myeloid cells accelerates tumorigenesis in a mouse model of breast and lung cancer, depicting the protective role of VEGF. This study suggests that VEGF, derived from innate immune cells, plays an intricate role in orchestrating angiogenesis and tumorigenesis. The study by Greenberg et al. (6) also suggests that VEGF, on binding to its receptor, VEGF-R2, acts as a negative regulator of angiogenesis by impeding the function of vascular smooth muscle cells (VSMC) and pericytes through induction of a receptor complex with the platelet-derived...
growth factor receptor (PDGF-Rα), leading to the loss of vessel stabilization. Consistent with these works, our group reported a tumor inhibitory role for VEGF by showing that a 2-fold overexpression of systemic levels of VEGF in mice heterozygous for a VEGF “hypermorphic” allele decelerates tumorigenesis in a retroviral-induced, spontaneous murine leukemia model (8). 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 (8). Similarly, in the same study, the capacity of erythropoietin to confer deceleration of leukemia progression was also shown (8). This correlation was not unexpected because Epo, like VEGF, is known to be a proangiogenic hormone and exhibits pleiotropic properties (9–11). Recently, Nagy et al. (12) have similarly used VEGF transgenic mice to show an inhibitory role for VEGF in a murine breast cancer model. Taken together, these studies have provided evidence to support several mechanisms whereby VEGF inhibits the growth and progression of various cancer types through recruitment of tumor inhibitory monocytic cells and the negative regulation of tumor angiogenesis.

VEGF and the recruitment of tumor inhibitory myeloid cells. Over the recent past, the contribution of various bone marrow–derived cells, primarily from the myeloid lineage, to tumor angiogenesis has been the subject of intense investigation. In tumors, infiltrating monocytes differentiate into tumor-associated macrophages (TAM) that are persuaded by the tumor microenvironment to secrete proangiogenic and prometastatic cytokines stimulating cell migration, invasion, and metastasis (13, 14). Additionally, these recruited monocytes are capable of undergoing trans-differentiation into endothelial-like cells, expressing the angiotropin receptor Tie2, that directly participate in the formation of blood vessels required for tumor growth (15, 16). Mouse tumor models that are refractory to anti-VEGF therapy display a CD11b+/Gr1+ tumor inhibitory monocytic cell population within the tumor microenvironment (17). These cells, which are associated with tumor refractoriness, carry alterations in the gene expression of both proangiogenic and antiangiogenic factors, such as downregulation of the angiogenic inhibitor thrombospondin-1 (17).

Interestingly, unlike the tumor promoting ability of the above-mentioned monocytic cells or TAMs, in a murine leukemia model it was recently identified that induction of a proangiogenic phenotype, achieved through in vivo Epo administration, results in the accumulation of a different monocytic cell population (Sca1+/cKit+/Mac1+) capable of leukemia inhibition in a nitric oxide (NO)–dependent manner (18). These tumor inhibitory monocytic cells differentiate into macrophages and dendritic cells capable of NO production (18). The production of NO has been implicated in the destruction of tumor cells by macrophages (19) as well as induction and accumulation of hypoxia-inducible factor 1α (HIF-1α; refs. 20, 21). These findings suggest that recruitment of different subsets of myeloid-lineage cells into the tumor microenvironment leads to the acceleration or inhibition of tumor growth.

It is clear that tumor-infiltrating monocytes can have separate and contradictory functions, although the mechanisms regulating the recruitment and activation of these cells in the tumor microenvironment remain to be fully understood. Indeed, several proangiogenic cytokines such as granulocyte-colony stimulating factor and monocyte chemoattractant protein-1 have been implicated in the mobilization of TAMs and myeloid cells to the tumor microenvironment (14, 17). However, additional studies are necessary to provide evidence of direct correlation between the activity of these chemoattractants and the recruitment of tumor-infiltrating myeloid cells. Conceivably, the recruitment of monocyteic cells with opposing functions is dependent on the concentration of oxygen within the tumor microenvironment. This notion can be supported by studies reporting that TAMs, which exert proangiogenic and prometastatic phenotypes, are attracted to and accumulate under hypoxic conditions (14). The state of hypoxia can be induced by the modulation of VEGF, such as anti-VEGF therapy. Conversely, normoxic conditions, maintained by a proangiogenic phenotype, may explain the accumulation of tumor inhibitory myeloid cells (8, 18).

As mentioned above, we provided evidence to suggest that the tumor inhibitory role of this myeloid population is attributed, at least in part, to its ability to produce NO (18). In turn, this induces the activation of HIF-1α (20) and prevents the degradation of these tumor inhibitory myeloid cells (21). Recent studies have discovered that HIF-1α expression promotes myeloid cell survival and inflammatory and immune functions (22, 23), potentially enhancing the antitumoral phenotype. Moreover, HIF-1α has been implicated in the regulation of growth factor–induced VEGF expression as well as Epo expression (24). The accumulation of HIF-1α thereby sustains the proangiogenic conditions in the tumor microenvironment by promoting the expression of VEGF or other mediators of angiogenesis, which in turn maintains the normoxic conditions, therefore leading to further recruitment of tumor inhibitory myeloid cells. The interpretation of these findings has led us to propose this intriguing positive feedback loop model, which may explain the inhibitory role of tumor-associated myeloid cells recruited to the tumor microenvironment under normoxic conditions maintained by increased levels of VEGF or a proangiogenic phenotype (Fig. 1).

VEGF and the regulation of tumor angiogenesis. The key regulatory role of VEGF in angiogenesis that leads to suppression of early tumor growth may not arise from alterations within the hematopoietic compartment alone. Angiogenesis is dependent on the formation of vascular sprouts, which requires the proliferation and invasion of endothelial cells and subsequent vessel stabilization that is provided by vascular smooth muscle cells and pericyte coverage of the newly formed vessels (6). Interestingly, Stockmann et al. (7) reported that the cell lineage–specific targeted deletion of VEGF in myeloid cells accelerates tumor progression in multiple subcutaneous isograft models of lung cancer and an autochthonous transgenic model of breast cancer. In the absence of myeloid cell-derived VEGF, an atypical high-density vessel network is formed. Quantitative analysis
showed that these mice display decreased intratumoral blood vessel length, tortuosity, and permeability, as well as increased levels of pericyte coverage. The lack of this malignancy-associated increased vascularization facilitates more rapid tumor development. This study suggests that myeloid-derived VEGF plays a unique role in the management of the tumor "angiogenic switch," facilitating changes in tumor vessel function and normalization, thereby indicating the ability of VEGF to act as a negative regulator of tumor progression.

In an accompanying study published simultaneously with that of Stockmann et al., Greenberg and colleagues (6) presented intriguing evidence also suggesting that VEGF acts as a negative regulator of angiogenesis by disrupting the function of vascular smooth muscle cells and pericytes. Vessel stabilization and maturation is dependent on the migration of vascular smooth muscle cells and pericytes to vessel walls, which is mediated by binding of PDGF to its cognate receptor, PDGF-Rβ (6). The capacity of VEGF to act as a negative regulator of angiogenesis can be attributed to the formation of a previously undescribed VEGF-R2/PDGF-Rβ receptor complex resulting in the suppression of PDGF-Rβ signaling. On binding to its receptor, VEGF-R2, present on the surface of vascular smooth muscle cells and pericytes, VEGF blocks PDGF-mediated phosphorylation of PDGF-Rβ and in turn ablates vessel stabilization (6). The deletion of tumor cell-derived VEGF blocks the induction of this receptor complex and thereby increases pericyte coverage and tumor vessel maturation (6). Thus, although VEGF is known to promote sprouting angiogenesis by endothelial cell activation, it can also modulate vessel integrity and/or vascular tone by interfering with accessory cell function.

Conclusions

VEGF may act as a negative regulator by initiating changes in the microenvironment or tumor niche. Such changes include alterations in the host innate inflammatory response...
Greenberg JI, Shields DJ, Barillas SG, et al. A role for VEGF as a
by Greenberg et al. has confirmed the ability of VEGF to
in the formation of malignancy-associated high-density vas-
ulated through genetic modification before cancer induction.
ent because the studies described above suggest that VEGF
of tumor growth is dependent on its concentration in the
vels of VEGF in both tumor and nontumor tissues play im-
ent and differential roles in determining the effects of
VEGF on tumorigenicity. However, additional analyses of
consequences of VEGF deletion or activation after tumor
initiation, using conditional alleles, are necessary to provide
critical insight into the effect of this angiogenic factor on tu-
progression and metastasis.
Overall, the evidence presented above suggests that VEGF
plays an important role in governing the mechanisms that
control tumor growth and confer a survival advantage to
the host. Consequently, it may be an oversimplification that
tumor growth is largely dependent on maximally sustained
levels of VEGF and potentially other proangiogenic factors,
particularly during the early stages of disease. Moreover,
the ability of VEGF to act as either an enhancer or inhibitor
of tumor growth is dependent on its concentration in the
host microenvironment, as well as the context and timing
of its modulation relative to cancer induction. This is appar-
ent because the studies described above suggest that VEGF
acts as an inhibitor of tumor growth when its levels are mod-
ulated through genetic modification before cancer induction.
The study by Stockmann et al. (7) has established a critical
role for VEGF derived from tumor-infiltrating immune cells
in the formation of malignancy-associated high-density vas-
culature, signaling to the tumor endothelium, and the sensi-
tization and efficacy of chemotherapeutic agents. The study
by Greenberg et al. has confirmed the ability of VEGF to
modulate tumor vessel maturation by interfering with peri-
cyte function. Recently, preclinical studies have shown that
mice treated with VEGF-targeted therapies, before tumor in-
duction, display increased tumor invasion and metastasis
and decreased overall survival (25, 27). Moreover, clinical
evidence also challenges this well-accepted theory because
monotherapy with VEGF-targeted agents has resulted in only
modest objective responses with short-term survival benefits
(4, 5), whereas the combination of antiangiogenic agents with
chemotherapy improves overall survival rates (28, 29). As
such, the combination of anti-VEGF therapy with antimeta-
static therapy would likely suppress the role of VEGF in tu-
mor growth to provide a more effective treatment option and
ameliorate clinical outcomes.
These above findings also implicate that the hematopoiet-
ic compartment is a pivotal player in the control of tumor
growth through the regulation of VEGF. A better understand-
ing of the proangiogenic phenotype of the host, particularly
with respect to the myeloid compartment and accessory cells
of the vasculature, as presented herein, may likely lead to im-
proved cancer therapies and/or preventative interventions in
the future. Therefore, further experimental and clinical stud-
ies are required to clarify the controversy surrounding the
dichotomous roles of VEGF in tumor angiogenesis.

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