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Perspective

The VEGF signaling pathway in cancer: the road ahead

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Abstract

The vascular endothelial growth factor (VEGF) family of soluble protein growth factors consists of key mediators of angiogenesis and lymphangiogenesis in the context of tumor biology. The members of the family, VEGF-A (also known as VEGF), VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PlGF), play important roles in vascular biology in both normal physiology and pathology. The generation of a humanized neutralizing antibody to VEGF-A (bevacizumab, also known as Avastin) and the demonstration of its benefit in numerous human cancers have confirmed the merit of an anti-angiogenesis approach to cancer treatment and have validated the VEGF-A signaling pathway as a therapeutic target. Other members of the VEGF family are now being targeted, and their relevance to human cancer and the development of resistance to anti-VEGF-A treatment are being evaluated in the clinic. Here, we discuss the potential of targeting VEGF family members in the diagnosis and treatment of cancer.

Key words Angiogenesis, vascular endothelial growth factor (VEGF), VEGF receptors, monoclonal antibody, cancer metastasis

The theory that blood vessels might be critical for the growth of tumors has its roots throughout the early and mid-20th century[1,2]. Initially, observations by pathologists and cancer biologists suggested an association between blood vessels and tumors[3,4]. Using experimental models, cancer biologists observed that blood vessels were essential to support tumor growth beyond the size allowed by oxygen diffusion alone[5]. The concept of blood vessels supporting tumor growth gained critical support from the theory and work of Judah Folkman, who proposed the relationship between neo-vessels and tumor growth[6]. While Folkman's work highlighted the importance of angiogenic factors in tumors, it was not until the discovery and characterization of vascular endothelial growth factor-A (VEGF-A; also known as VEGF), and the development of inhibitory monoclonal antibodies that blocked the binding of VEGF-A to key receptors, that an *in vivo* proof-of-principle experiment targeting a known tumor angiogenic factor was performed[2,7].

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The VEGF family of proteins first came to the attention of cancer biologists in the early 1980s when vascular permeability factor (VPF) was discovered in the ascites fluid of cancer patients[8]. It was another 6 years before this molecule was discovered to be identical to VEGF-A, a mitogen in endothelial cells capable of promoting angiogenesis *in vivo*[9,10]. With the isolation of the VEGF-A cDNA, the entire predicted amino acid sequence for this multifunctional cytokine was defined. Structural similarity to the transforming growth factor-β (TGF-β) family and a highly conserved cysteine-rich motif quickly assigned VEGF-A to the burgeoning family of cystine-knot growth factors[11]. Since then, related genes encoding other members of the VEGF family have been isolated, and their protein products have been characterized in developmental, vascular, and cancer biology[12-18]. As is commonly observed with ligands of a growth factor family, the related growth factors share a common series of receptors for signal transduction. The VEGF family signals predominantly through the receptor tyrosine kinases VEGF receptor (VEGFR)-1, VEGFR-2, and VEGFR-3, in combination with the co-receptors neuropilin (NP)-1 and NP-2, and, in some cases, can use other receptors such as integrins[19].

VEGF-A

VEGF-A has become the most widely studied tumor

angiogenic factor. The generation of a humanized monoclonal antibody (mAb) that inhibits the interaction of VEGF-A with its receptors VEGFR-1 and VEGFR-2[20] and the development of small-molecule protein tyrosine kinase (PTK) inhibitors to the cytoplasmic regions of the VEGF receptors[21] have provided avenues to explore the efficacy of targeting the VEGF-A signaling pathway in cancer. Extensive mouse tumor models developed through the 1990s[7] led to the success of the VEGF-A mAb known as Avastin (or bevacizumab) in treating a range of human cancers including colorectal cancer, lung cancer, and renal cell carcinoma. However, the recent withdrawal of Avastin for treating breast cancer in the United States has highlighted the limitations of anti-angiogenic treatments[22], as well as the need for a careful cost-benefit analysis to ensure that scarce healthcare resources are well spent in the treatment of cancer. While Avastin is clearly beneficial in a subset of cancer patients, the development of resistance to Avastin and the pre-existing refractoriness of some tumors suggest the role of other growth factors in sustaining tumor angiogenesis. Alternative ligands (other than VEGF-A) for the angiogenic receptor VEGFR-2 (i.e., VEGF-C and VEGF-D; see below) and other angiogenic factors from distinct growth factor families [e.g., fibroblast growth factor (FGF)] are logical candidates for promoting resistance to Avastin. In addition, other mechanisms, such as vascular mimicry, the involvement of endothelial precursor cells (EPCs) or altered tumor metabolism leading to reduced oxygen requirement, may also contribute to the development of resistance[21].

Placenta Growth Factor (PlGF)

PlGF is a ligand for VEGFR-1, which has been reported to play a dual role in cancer as a stimulator of angiogenesis and as an autocrine or paracrine factor promoting proliferation of tumor cells expressing VEGFR-1[23,24]. It has also been proposed that PlGF may modulate the effect of VEGF-A by displacing VEGF-A from VEGFR-1 expressed on endothelial cells. A range of animal tumor models have provided supportive or contradictory data on the role of PlGF in driving tumor angiogenesis and tumor growth[25,26]. These findings suggest a context-dependent activity for PlGF in cancer, either due to the presence of other VEGF family members or the diverse expression of VEGF receptors on various cell types including endothelial cells, tumor cells, or associated immune cells[27,28].

VEGF-B

While VEGF-B, a ligand for VEGFR-1, has been shown to be expressed in human cancer[29], its functions in arteriogenesis[30] and in fatty acid uptake by cells[31] have highlighted its role in other pathologies, predominantly cardiovascular disease. Recently, VEGF-B was shown to regulate endothelial cell uptake and transport of fatty acids in muscle, leading to the hypothesis that VEGF-B antagonists could be used as novel pharmacologic agents in the treatment of type 2 diabetes[31]. Although inhibitory antibodies against VEGF-B have been developed[32], so far there is little data on the role of this growth factor in tumor biology; it was reported, however, that overexpression of VEGF-B in a mouse model of pancreatic cancer suppressed tumor growth, highlighting the differences in biological functions between the VEGF family members[33].

VEGF-C

VEGF-C and VEGF-D form a subfamily within the VEGF family, consisting of highly related polypeptides that require post-translational cleavage of the N- and C-terminal propeptides to generate mature forms with enhanced binding affinities for VEGFR-2 and VEGFR-3[16,34-36]. Notably, VEGFR-3 is expressed on lymphatic endothelium, where it can promote lymphangiogenesis[37], and it is also expressed on angiogenic blood vessels[38]. VEGF-C is expressed in a range of human cancers, including solid tumors such as nasopharyngeal, liver and gastric cancers, with a preponderance in Asian populations[39]. Mouse tumor studies have displayed the ability of VEGF-C to promote tumor angiogenesis and lymphangiogenesis *in vivo* and to drive tumor growth and metastatic spread[40-42]. Given that the mature form of VEGF-C is a high affinity ligand for VEGFR-2 and that VEGF-C is expressed in many human cancers, VEGF-C is likely to be an alternative ligand to VEGF-A for VEGFR-2 –binding, which could in turn promote tumor angiogenesis. Therefore, VEGF-C, in combination with anti-angiogenic drugs such as Avastin, may be a viable target for anti-cancer therapy.

VEGF-D

The growth factor VEGF-D is closely related in structure to VEGF-C, and it includes a central VEGF homology domain (VHD) related to other VEGF family members, with N- and C-terminal propeptides[16] that can be proteolytically cleaved by enzymes such as proprotein convertases and plasmin[36,43,44]. VEGF-D is expressed in a range of human cancers[39,45,46] and has been associated with poor patient outcome in some tumor types[47,48]. Importantly, animal models of cancer have demonstrated that VEGF-D can promote tumor angiogenesis and lymphangiogenesis, solid tumor growth, dilation of collecting lymphatic vessels, and lymphatic and distant

organ metastasis[49-53]. The proteolytic processing of VEGF-D is required for promoting tumor growth and spread[54]. Opportunities for targeting VEGF-D signaling in cancer could involve mAbs specific to the VHD of VEGF-D that are capable of inhibiting binding to VEGFR-2 and VEGFR-3[49,55,56]. Alternatively, PTK inhibitors that block VEGFR-2 and VEGFR-3 signaling would interfere with VEGF-D-mediated signal transduction[21]. Further, mAbs to VEGFR-2 and VEGFR-3[57,58] that would prevent the binding of VEGF-D, or a soluble form of VEGFR-3 that could sequester both VEGF-C and VEGF-D[59], could be employed. Targeting the VEGF-D signaling pathway would likely have the merit of inhibiting both tumor angiogenesis and lymphangiogenesis[60], which could, in turn, restrict both solid tumor growth and metastatic spread.

Future

What have the past 20 years taught us about targeting VEGF-A signaling? Clearly, this period has provided biochemists, biologists, and clinicians the time to design and evaluate a variety of agents that modify or inhibit these signaling pathways. The agents that were developed have included a broad range of molecules targeting different components of the pathways, including VEGF-A itself, VEGF receptors, VEGF co-receptors, PTKs and signaling intermediates, and transcription factors. During this period, other VEGF family members were also identified, enhancing the diversity of signaling induced by the VEGF family of ligands.

Avastin, a humanized mAb to VEGF-A, has been widely used in a range of prevalent human cancers over the past 8 years, typically in combination with cytotoxic chemotherapy. Although this agent has provided significant benefit to cancer patients, there is a need for other drugs that could be combined with Avastin to deliver improved clinical outcomes. Use of agents targeting other VEGF family members, in combination with Avastin, may be a potential approach. Further, small-molecule PTK inhibitors of VEGF receptors (that are not highly specific) have been employed although dose-limiting toxicity in combination with cytotoxic chemotherapy has restricted their widespread use. Further insights into the structure and function of the PTK domains of VEGF receptors may allow development of more specific small-molecule PTK inhibitors.

Further studies delving into the complex network of signaling cascades that drive angiogenesis, lymphangiogenesis, and resistance to anti-angiogenic drugs such as Avastin are clearly required. Genome-wide functional approaches such as those using small interfering RNA (siRNA) technology, supported by bioinformatics, could help strategize effective targeting of growth factors, receptors, and PTKs.

Agents specifically targeting VEGF family members and their receptors are currently in various stages of development; mAbs to VEGF-B, VEGF-C, VEGF-D, PlGF, VEGFR-1, VEGFR-2, and VEGFR-3 are being evaluated by the biotechnology and pharmaceutic industries for their efficacy as anti-cancer agents. For these mAbs, a key challenge will be identifying specific cancer indications in which clinical benefit can be achieved. Given that many cancers are resistant to Avastin or develop resistance over the course of treatment, these mAbs may be tested in patients whose cancers are Avastin-resistant and/or whose cancers express the appropriate target growth factor. Hence, assays that quantitate the levels of these growth factors or other relevant biomarkers will be required to identify the appropriate patients, although assessing these biomarkers may not be straightforward. VEGF family members are secreted proteins, which may easily allow testing in blood-drawn samples; however, some family members (e.g., VEGF-C and VEGF-D) are proteolytically processed to generate forms with a different bioactivity. Hence, it will be important to fully appreciate which forms are most bioactive and clinically relevant.

It is important to note that inhibiting signaling by VEGF family members could potentially have pro-tumor effects. Recent studies have demonstrated that anti-angiogenic treatments could cause increased tumor metastasis through mechanisms such as the induction of hypoxia (caused by VEGF-A inhibition), resulting in increased tumor cell motility[61,62]. Nonetheless, these issues have been addressed in various tumor models[63] and by comparing the effect of antibodies and small-molecule inhibitors to show that anti–VEGF-A treatment does not promote metastasis[64].

Clearly, other signaling pathways (i.e., not directly involving VEGF family members) such as those driven by members of the platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), and FGF growth factor families, exert control on tumor angiogenesis and may be important in promoting tumor growth. Other alternative molecular targets, such as c-MET, may also be able to affect multiple tumor properties such as metastasis, angiogenesis, tumor growth, and cellular motility upon hypoxia[65-67]. Although not the subject of this review, such pathways may provide valid therapeutic targets for inhibiting angiogenesis and/or lymphangiogenesis in cancer, thereby restricting tumor growth and spread. Furthermore, recent studies from our own laboratory have indicated the role of known inflammatory pathways in enhancing the spread of cancer[51], opening avenues for the use of existing anti-inflammatory drugs in cancer. This possibility is further exemplified by the recent studies published by Rothwell *et al.*[68,69] demonstrating the anti-cancer effect of non-steroidal anti- inflam matory drugs (NSAIDs) such as aspirin. While expensive

drugs such as Avastin allow the treatment of specific cancers in the Western world, the capacity to employ relatively inexpensive "off-patent" small molecules (such as aspirin) with a defined chemistry and known biological effects could provide a cost-effective and population-based preventative approach to cancer in Asian countries.

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