VEGF as a Therapeutic Target in Cancer

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Abstract
Tumors require nutrients and oxygen in order to grow, and new blood vessels, formed by the process of angiogenesis, provide these substrates. The key mediator of angiogenesis is vascular endothelial growth factor (VEGF), which is induced by many characteristics of tumors, most importantly hypoxia. Therefore, VEGF is an appealing target for anticancer therapeutics. In addition, VEGF is easy to access as it circulates in the blood and acts directly on endothelial cells. VEGF-mediated angiogenesis is rare in adult humans (except wound healing and female reproductive cycling), and so targeting the molecule should not affect other physiological processes. Tumor blood vessels, formed under the influence of VEGF, are disorganized, tortuous and leaky with high interstitial pressure, reducing access for chemotherapies. Inhibiting VEGF would reduce the vessel abnormality and increase the permeability of the tumor to chemotherapies. Several approaches to targeting VEGF have been investigated. The most common strategies have been receptor-targeted molecules and VEGF-targeting molecules. The disadvantage of receptor-targeted approaches is that the VEGF receptors also bind different members of the VEGF super-family and affect systems other than angiogenesis. The best-studied and most advanced approach to VEGF inhibition is the humanized monoclonal antibody bevacizumab (Avastin®), which is the only anti-angiogenic agent approved for treatment of cancer.

Introduction
The development of an adequate vasculature to deliver nutrients and oxygen to tumor cells is essential for tumor growth. The process of angiogenesis, the development of new blood vessels, occurs by stimulation of host vasculature to sprout new capillaries [1, 2]. Recognition of the crucial role of angiogenesis in a variety of diseases has led to intensive research of its regulatory factors, with a range of molecules that affect angiogenesis being identi-

Table 1. Regulators of angiogenesis

<table>
<thead>
<tr>
<th>Promoters</th>
<th>Inhibitors</th>
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<td>VEGF</td>
<td>Thrombospondin</td>
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<td>Acidic fibroblast growth factor</td>
<td>Angiostatin</td>
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<td>Basic fibroblast growth factor</td>
<td>Endostatin</td>
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<td>Transforming growth factor-α, β</td>
<td>Vasostatin</td>
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<td>Epidermal growth factor</td>
<td>Prolactin</td>
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<td>Tumor necrosis factor-α</td>
<td>Growth hormone</td>
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<td>Angiogenin</td>
<td>Canstatin</td>
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<td>Interleukin-8</td>
<td>Tumstatin</td>
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<td>Angiopoietin-1, 2</td>
<td>Interferon-α</td>
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fied (table 1). Most of these molecules, such as basic fibroblast growth factor and matrix metalloproteinases, have a relatively broad range of action, with effects on other systems in addition to their potential role in angiogenesis.

Vascular endothelial growth factor (VEGF) has been identified as the central mediator of angiogenesis [3, 4; reviewed by Ferrara et al.; 5, 6]. The role of VEGF as a key mediator of angiogenesis is discussed earlier in this issue [7].

VEGF expression is elevated in many cancers including colorectal cancer (CRC), breast, lung and other tumors [8–10]. The level of VEGF expression also correlates with microvessel density and metastatic spread in some tumor types, including colorectal, breast and cervical cancer and melanoma. Given the central role of VEGF in tumor angiogenesis and the correlation with tumor growth, VEGF has emerged as the most promising therapeutic target for angiogenesis inhibition.

**Targeting VEGF as a Therapeutic Strategy**

The most powerful rationale for targeting VEGF is its central role in tumor angiogenesis and its expression by many tumors. Several other characteristics also make VEGF an attractive target. Because VEGF circulates in the blood, and acts directly on endothelial cells, it is not necessary to penetrate tumor tissue to inhibit tumor angiogenesis through VEGF. While VEGF is a potent mitogen for endothelial cells, it has little effect on other cell types, and so should not affect other physiological processes. Angiogenesis has limited importance in normal physiology except wound healing and female reproduction and therefore inhibition of VEGF would not be expected to cause the range of side effects that can occur with other cancer treatments, particularly chemotherapy.

In addition, VEGF acts on endothelial cells, which are relatively stable, quiescent in adults and have a lifespan of many years. This stability means that the cells are less likely to mutate to a treatment-resistant phenotype than genetically unstable tumor cells, making them a more attractive target for long-term therapy.

**Actions of VEGF on Tumor Vasculature**

In physiological angiogenesis, VEGF stimulates the formation of new blood vessels and maintains immature vessels in coordination with other factors to ensure blood vessels have a normal structure and function. This coordination is lost in tumor blood vessels stimulated by VEGF, leading to prolific growth of disordered vessels with blind ends. VEGF also increases the permeability of blood vessels, resulting in poorly perfused tumors, with subsequent hypoxia stimulating further VEGF production. In addition, the leaky blood vessels result in high tumor interstitial pressure. These effects make it difficult for chemotherapy to access to tumor tissue. Inhibition of VEGF results in normalization of permeability and reduced interstitial pressure, improving accessibility for treatments such as chemotherapy.

**Regulators of VEGF Production**

The most significant regulator of VEGF production is hypoxia. As a tumor increases in mass and becomes hypoxic, VEGF is induced and stimulates growth of new vessels. Transcription of VEGF mRNA is up-regulated in hypoxia through transcription factors known as hypoxia-inducible factors (HIFs) that bind to the VEGF promoter [11, 12]. The up-regulation of VEGF in the hypoxic state is highly specific: HIFs do not increase expression of other members of the VEGF gene superfamily [13]. Under normal oxygen tension, VEGF is suppressed by the product of a tumor suppressor gene known as the von Hippel Lindau (VHL) gene, which is involved in the degradation of VEGF protein. Patients with a defective VHL gene suffer from VHL disease, a condition in which there is excessive blood vessel formation resulting in angiomas in the retina and cerebellum as well as other areas. Notably, patients with VHL disease also have a markedly increased susceptibility to renal and brain cancers, suggesting an important role for VEGF in the pathogenesis of these tumors [14].

VEGF production is up-regulated by several major growth factors which are frequently expressed by tumors, including epidermal growth factor, transforming growth factor-α and -β, fibroblast growth factor and platelet-derived growth factor [6]. Hormones such as estrogen and thyroid-stimulating hormone [14] and inflammatory cytokines including interleukin-1 and interleukin-6 also induce VEGF expression in many cell types [13]. Mutations in tumor suppressor genes including p53 [15] and oncogenes such as ras [16] have also been shown to up-regulate VEGF. Induction of VEGF expression appears to be characteristic of many tumor types and it is likely that inhibition of VEGF would inhibit the angiogenic activity of these tumors.
Other Activities of VEGF

The effects of VEGF on endothelial cells and vasculature are well documented. However, VEGF has other roles that influence tumor growth and progression, including inhibiting functional maturation of dendritic cells [17] and enhanced adhesion of natural killer cells to microvessels [18]. The importance of these effects in human tumors is not fully established, but data suggest that induction of VEGF may contribute to the evasion of host immune responses by growing tumors.

Direct effects of VEGF on apoptosis in tumor cells have also been described. In murine and human tumor cell lines, addition of VEGF up-regulated expression of the anti-apoptotic gene $bcl-2$ and addition of anti-VEGF antibodies induced apoptosis [19]. Similarly, in human breast cancer cell lines, induction of VEGF expression by hypoxia provided an autocrine survival signal, while addition of anti-VEGF expression induced apoptosis [20]. These data suggest that VEGF expression may have direct, autocrine effects in promoting tumor cell growth and survival, in addition to promoting angiogenesis.

Prognostic Significance of VEGF Expression

Increased neovascular formation and intratumoral microvessel density in human tumors are associated with poorer prognosis [10]. These findings appear to be correlated with the degree of VEGF expression, as VEGF expression was also found to be a powerful prognostic indicator in a range of solid tumors [10]. The prognostic significance of VEGF expression has subsequently been confirmed in a variety of different solid tumors and hematological malignancies [10, 21–24].

Approaches to Targeting VEGF

VEGF is an ideal therapeutic target; it is crucial for tumor growth and progression with limited applications in normal physiology. Several approaches have been investigated, including agents that target either VEGF or its cell surface receptors. Receptor-targeted molecules include monoclonal antibodies and inhibitors of VEGF-receptor tyrosine kinases. Molecules targeting VEGF include monoclonal antibodies and soluble receptor constructs. A disadvantage of receptor-targeted approaches is that the VEGF receptors (VEGF receptors 1 and 2) may also bind different members of the VEGF superfamily and affect systems other than angiogenesis [25, 26]. The same argument applies to soluble receptor constructs, which may also bind to factors other than VEGF. Therefore, one of the major advantages in targeting VEGF, the fact that effects on normal physiological processes are minimized, may be reduced by approaches that do not target the VEGF molecule with high specificity.

The best-studied and most advanced approach to VEGF inhibition is the humanized monoclonal antibody bevacizumab (Avastin®), which is the only anti-angiogenic agent approved for treatment of cancer. In a large randomized controlled trial, the addition of bevacizumab to standard chemotherapy for patients with previously untreated metastatic CRC resulted in a 30% increase in median survival (Hurwitz et al. 2004 [27], described in more detail later in this issue). Bevacizumab was developed from a murine antibody to human VEGF by recombinant DNA technology [28] and was selected for clinical development based on preclinical evidence showing high antiangiogenic and antitumor activity.

Preclinical Evidence for Bevacizumab Activity

The murine parent antibody of bevacizumab, muMaAb A.4.6.1, was first evaluated in mouse models where it completely suppressed neovascularization of rhabdo-
Fig. 2. A4.6.1 plus topotecan in Wilms tumor. Reproduced with permission. Copyright Elsevier 2001 [35].

Fig. 3. Synergistic effect of Avastin® and capecitabine in a preclinical CRC model [36].
myosarcoma [29] and reduced vascular permeability, vessel diameter and tortuosity within tumors [30]. At doses of $\geq 2.5$ mg/kg, muMAb A.4.6.1 was able to suppress tumor growth [31]. Subsequently, the humanized antibody bevacizumab was found to have antitumor effects and to inhibit VEGF-induced growth of endothelial cells in vitro in a similar fashion to the murine parent [28].

In animal xenograft models, bevacizumab was shown to have profound effects on tumor vasculature. Tumor vascular density was markedly lowered and interstitial pressure decreased by 75% in colon xenografts [9], while vascular permeability was decreased in breast tumor xenografts in athymic rats [32]. Bevacizumab has demonstrated synergy in combination with chemotherapy or radiotherapy in vitro, where bevacizumab overcomes VEGF-induced protection of endothelial cells against docetaxel treatment [33], and in vivo, where bevacizumab enhances tumor suppression in animals when added to cisplatin (Platinol®) [34], topotecan (Hycamtin®) [35], capecitabine (Xeloda®) [36] or radiation [37] (fig. 1–3).

The safety and pharmacokinetics of bevacizumab were evaluated in young adult cynomolgus monkeys. Following twice-weekly administration of bevacizumab at doses up to 50 mg/kg, the only side effects seen were physeal dysplasia and reduction in ovarian and uterine weight. Both of these effects were reversible on cessation of treatment and no other treatment-related effects were observed [38]. The pharmacokinetics of bevacizumab were predictable, with a terminal half-life of 1–2 weeks, clearance of 5 ml/day/kg and 100% bioavailability [39]. The encouraging data from these preclinical studies formed the foundation for the extensive clinical trials program, which led to the approval of bevacizumab in combination with chemotherapy for treatment of metastatic CRC and the ongoing evaluation of the antibody in a wide variety of other solid tumors and hematological malignancies.

Conclusions

Angiogenesis, mediated by VEGF, is crucial for tumor growth and normal development, but has limited applications in adults. Anti-angiogenic therapy has therefore been the subject of intensive research, with VEGF representing the best therapeutic target.

Targeting VEGF in cancer therapy has a number of advantages. Because VEGF is a circulating molecule, therapy does not need to penetrate the tumor, and inhibition of circulating VEGF reduces vascular permeability and thus tumoral interstitial pressure, permitting easier penetration of the tumor by conventional chemotherapeutic targets.

The clinical development of anti-angiogenic therapy is now at an advanced stage in a variety of tumors, but the only agent to have demonstrated a significant anticancer benefit is the humanized monoclonal antibody bevacizumab, which targets VEGF directly. Preclinical data demonstrated that bevacizumab has high anti-tumor activity with a favorable and predictable safety profile, and a randomized trial has shown a significant survival advantage for the addition of bevacizumab to chemotherapy in CRC. Trials in other settings are ongoing.

References


Roberts TP, Pham C, Van Bruggen N, Brasch RC: Macromolecular contrast-enhanced MR imaging can measure microvascular permeability reduction in tumours after treatment with antibodies to vascular endothelial growth factor. 82nd Scientific Assembly and Annual Meeting of the Radiological Society of America, Chicago, USA, December 1–6, 1996: abstract 1417.


