Vascular Endothelial Growth Factor

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Abstract—Vascular endothelial growth factor (VEGF, VEGF-A) is a major regulator of physiological and pathological angiogenesis. Several VEGF inhibitors have been approved by the FDA for the treatment of advanced cancer and neovascular age-related macular degeneration. This brief review provides a historic account of the challenges associated with the discovery of VEGF and the early steps in elucidating the role of this molecule in the regulation of angiogenesis. (Arterioscler Thromb Vasc Biol. 2009;29:789-791.)

It has been known for a long time that blood vessels are essential to deliver nutrients to tissues. Indeed, the cardiovascular system is the first organ to reach a functional state in an embryo. It is also well established that uncontrolled growth of blood vessels plays a pathogenic role in several disorders including cancer and intraocular neovascular diseases.1

The observation that tumor growth can be accompanied by increased vascularity was reported more than a century ago (reviewed in1). In 1939, Ide et al postulated the existence of a tumor-derived “blood vessel growth stimulating factor.”2 In 1945, Algire et al, on the basis of the observation that growth of tumor xenografts is preceded by local increases in vascular density, concluded that “the rapid growth of tumor transplants is dependent on the development of a rich vascular supply.”3 In 1968 Greenblatt and Shubick provided early evidence that tumor angiogenesis is mediated by diffusible molecules.4 In 1971 Folkman proposed that antiangiogenesis might be a strategy to treat human cancer.5 This key hypothesis gave a major impulsive to angiogenesis research and the search for regulators of blood vessel growth began. However, the identification and isolation of such factors proved difficult. Today the majority of genes from several species have been sequenced, and powerful genomic and proteomic tools are available to aid discovery. However, growth factor discovery in the 1980s, when relatively few genes were known, posed markedly different challenges. After identification of a biological activity from a tissue extract or the supernatants of cultured cells, the protein responsible for the activity had to be purified to near homogeneity through a lengthy series of chromatographic steps. This task could require several years. The major goal was obtaining sufficient amounts of highly purified protein to determine a partial amino acid sequence, which could be compared to available databases to establish whether or not it matched any known protein. Importantly, the amino acid sequence could also enable the design of oligonucleotide probes suitable for cDNA cloning, thus potentially creating critical tools to advance the knowledge of the factor of interest. Indeed, one could find in the literature of that period numerous reports of identification of bioactive factors, which had undergone some biochemical purification, but pending definitive isolation and structural characterization their identity remained unknown.

Several putative angiogenic factors were identified and characterized in that period, including EGF, TGF-α, aFGF, bFGF, angiogenin, etc (reviewed in1). However, although these factors promoted angiogenesis in various bioassays, initial attempts to directly link them to tumor angiogenesis, using neutralizing antibodies or expression analyses, yielded largely negative results.1 Therefore, many investigators felt that in all likelihood key angiogenic molecules had yet to be discovered.

Discovery of VEGF

Independent lines of research contributed to the discovery of VEGF. In 1983 Senger and colleagues reported the identification in the supernatant of a guinea pig tumor cell line of vascular permeability factor (VPF), a protein which induced vascular leakage.6 These authors proposed that VPF might be a mediator of the high permeability of tumor blood vessels.6 However, these efforts did not go as far as fully purifying the VPF protein. The lack of amino acid sequence data precluded cDNA cloning and establishing the identity of VPF. Accordingly, very limited progress in elucidating the role of VPF took place during the following several years.

In 1989, we reported the identification and isolation of a novel heparin-binding endothelial cell mitogen from medium conditioned by bovine pituitary follicular cells.7 This effort began when I was a postdoctoral fellow at the Reproductive Endocrinology Center at the University of California, San Francisco in the early 1980s. I became interested in the regulation of growth of the vascular networks in hypothalamus and pituitary. I was studying a poorly known population of nonhormone secreting cells in the pituitary, the follicular...
or folliculo-stellate cells. Intriguingly, cytoplasmic projections of follicular cells establish intimate contacts with the perivascular spaces, leading some early investigators to suggest that these cells play a role in regulating growth and maintenance of the pituitary vasculature. I was able to isolate and passage homogeneous cultures of follicular cells from bovine pituitary, and then I discovered that their conditioned medium was strongly mitogenic when added to cultured endothelial cells. At that time, bFGF was thought to be the major endothelial cell mitogen in the pituitary. However, in 1986 it was reported that the bFGF gene does not encode a conventional secretory signal peptide (reviewed in1). Accordingly, numerous observations confirmed that bFGF is a poorly secreted protein. Because the follicular cell-derived mitogenic activity I identified was detectable at high level in the medium, I speculated that the molecule responsible for such activity was a truly secreted protein and thus different from bFGF. This hypothesis encouraged me to pursue the isolation of this factor. Also, a soluble endothelial mitogen was particularly appealing, considering the aforementioned early studies implicating diffusible molecules in angiogenesis.4 In 1988 I joined Genentech, where I found not only state of the art technology but also some outstanding colleagues and collaborators. Even though my primary goal was working on art technology but also some outstanding colleagues and collaborators. Even though my primary goal was working on

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Based on the amino acid sequence of bovine vascular endothelial growth factor (VEGF), we designed probes to screen cDNA libraries and identified bovine clones encoding a protein predicted to have 190 amino acids.8 Importantly, a typical signal sequence preceded the NH2-terminus determined by protein sequencing, confirming our initial hypothesis that VEGF was a secreted protein. The mature protein was a homodimer consisting of two subunits of 164 amino acids each. We then screened human cDNA libraries and isolated clones encoding mature monomers of 121, 165, and 189 amino acids, respectively (VEGF121, VEGF165, VEGF189). Alternative mRNA splicing was the most likely explanation for such molecular heterogeneity. Interestingly, recombinant VEGF165 was characterized as a soluble endothelial cell mitogen, similar to native VEGF.8

After our cloning paper was accepted for publication,8 we were informed by the Editor that a group at Monsanto Company led by Daniel Connolly had submitted at about the same time a manuscript reporting the cloning of VPF.9 To our surprise, these investigators described a human clone which encoded a protein identical to VEGF189.9 We learned that Connolly and colleagues had followed up on the earlier work by Senger et al and succeeded at purifying and sequencing VPF. So a single molecule, or at least the products of a single gene, had both mitogenic and permeability-enhancing activities. This was unexpected, because other endothelial cell mitogens such as bFGF do not induce vascular permeability.

Early Studies on the Role of VEGF in Regulating Endothelial Cell Growth

The discovery of the existence of multiple VEGF/VPF isoforms raised several questions regarding their relative significance. Connolly and colleagues proposed that the 189-aa protein encoded by the clone they identified had characteristics matching those of the native protein.9 Instead, we focused on VEGF165 as potentially the most important isoform and found that, similar to native VEGF, it was a soluble heparin-binding endothelial cell mitogen.8 Subsequent studies confirmed that native VEGF from numerous sources corresponds to VEGF165. VEGF121 was even more diffusible than VEGF165 because of the lack of heparin binding. In contrast, VEGF189 was poorly released because it was largely sequestered in the cell surface and in the extracellular matrix by virtue of a highly basic 24-aa insertion.10 However, it could be made into a soluble form by plasmin-mediated cleavage at the COOH terminus.10 These findings indicated that the VEGF proteins may become available to endothelial cells by at least two different mechanisms: alternative splicing and proteolytic cleavage generating nonheparin binding fragments.10

A key question was whether VEGF plays a role as an angiogenic factor in vivo. The earliest evidence that VEGF expression is correlated to blood vessel growth came from a study published in 1990, showing that VEGF mRNA was expressed at low levels in the avascular granulosa cells in the ovary, whereas it was upregulated in the highly vascularized corpus luteum.11 Furthermore, the high affinity binding sites for VEGF were selectively expressed in endothelial cells in vivo.12 In 1992 two groups reported that the VEGF mRNA is strongly expressed by the highly vascularized glioblastoma multiforme in situ.13,14 The fact that VEGF was a secreted protein, its binding sites were selectively expressed in endothelial cells in vitro and in vivo, the expression of its mRNA was correlated to blood vessel growth, made VEGF an attractive candidate as a potential regulator of angiogenesis (reviewed in11).

Major steps toward a better understanding of VEGF function were the discoveries of two tyrosine kinase VEGF receptors. The first VEGF receptor to be identified was Flt-1, known also as VEGFR-1.15 Subsequently, a highly homologous tyrosine kinase, known as KDR, Flk-1, or VEGFR-2, was also reported to bind VEGF with high affinity.16 Also, several VEGF-related genes were discovered, including placenta growth factor, VEGF-B, VEGF-C, and VEGF-D. Interestingly, VEGF-C and VEGF-D were shown to bind a tyrosine kinase receptor related to VEGFR-1 and VEGFR-2, known as Fms-4 or VEGFR-3, and were implicated in the regulation of lymphatic vessel growth (reviewed in17).

VEGF as an Angiogenic Factor In Vivo and a Potential Therapeutic Target

To advance our understanding of the role of VEGF in vivo, we developed neutralizing anti-VEGF monoclonal antibodies. In 1993 we reported that administration of such anti-
VEGF antibodies substantially reduced growth of several human tumor cell lines implanted in immunodeficient mice, providing the earliest direct evidence that tumor growth is angiogenesis-dependent. These findings were then extended to other tumor models, with a variety of VEGF inhibitors. In 1994 it was reported that VEGF is highly expressed in the ocular fluids of patients with ischemic retinal disorders such as proliferative diabetic retinopathy. These findings suggested that blocking VEGF might be a strategy to treat cancer and possibly other diseases in humans. To test this hypothesis, we developed a humanized anti-VEGF monoclonal antibody, presently known as “bevacizumab.” Subsequently, we developed “ranibizumab,” an affinity-matured Fab variant of bevacizumab.

Inactivation of the vegf gene in mice provided additional evidence for the crucial role of this molecule in angiogenesis. In 1996 our group and Carmeliet’s group reported that vegf is required for normal embryonic vasculogenesis and angiogenesis. Surprisingly, inactivation of even a single vegf allele in mice resulted in developmental abnormalities and early embryonic lethality.

Conclusions
Work over the last two decades has demonstrated that VEGF is a key regulator of angiogenesis. VEGF blockers inhibited physiological and pathological angiogenesis in a variety of models. Importantly, clinical studies have also established that inhibiting VEGF confers benefits to human patients. The first VEGF inhibitor to be FDA approved was bevacizumab (February 2004), after a randomized phase III study showing that adding bevacizumab to cytotoxic chemotherapy resulted in increased median survival and progression-free survival in patients with previously untreated metastatic colorectal carcinoma. Subsequently, two small molecule VEGF receptor tyrosine kinase inhibitors (sunitinib and sorafenib) were approved by the FDA for cancer therapy. Also, blocking VEGF-mediated angiogenesis and vascular permeability with ranibizumab resulted in a substantial benefit, including increased visual acuity, in patients with neovascular age-related macular degeneration. However, the promise that promoting angiogenesis with VEGF (or other angiogenic factors) might have beneficial effects in ischemic disorders such as myocardial or limb ischemia still remains unfulfilled, despite considerable preclinical and clinical efforts.

Acknowledgments
The author regrets that, because of the restriction in the number of references allowed, it was not possible to cite all important papers.

Disclosures
The author is an employee and shareholder of Genetech, Inc.

References
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