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RETROSPECTIVE

Vascular Endothelial Growth Factor: Much More than an Angiogenesis Factor

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Vascular endothelial growth factor (VEGF) is best known as a cytokine essential for embryonic vasculogenesis and for the angiogenesis associated with various pathologies including cancer. However, VEGF also serves other functions that are less widely recognized. An early study (Berse *et al.*, 1992) revealed widespread expression of VEGF transcripts in adult tissues devoid of ongoing neovascularization, thereby predicting additional VEGF functions distinct from angiogenesis. More recent studies have confirmed that VEGF does indeed serve multiple additional functions, including normal maintenance of endothelial and neural cell compartments. These findings have important implications for the use of VEGF antagonists and VEGF receptor antagonists in patients for which inhibition of pathological angiogenesis is the therapeutic goal.

In 1989, reports of the cloning and sequencing of VEGF generated considerable excitement. VEGF had all the hallmarks of an important angiogenesis factor, including significant sequence homology to platelet-derived growth factor (PDGF; Leung *et al.*, 1989; Keck *et al.*, 1989). Although it was recognized that cytokines such as the acidic and basic fibroblast growth factors (FGFs) could stimulate endothelial cell proliferation in vitro and induce angiogenesis in vivo, VEGF was clearly different. First and foremost, VEGF was an "endothelial-specific mitogen," whereas the FGFs were mitogenic for multiple cell types (Ferrara, 2002). This apparent specificity toward endothelial cells was consistent with a specific and important role for VEGF in angiogenesis. Moreover, VEGF transcripts encoded a classical signal sequence that directed active secretion of VEGF from the cell; in contrast, the FGFs lacked this sequence and presumably remained cell-associated unless released through cell injury or death (D'Amore, 1990). Thus, a signal sequence for secretion and specificity for endothelial cells conferred VEGF with key characteristics expected for a diffusible angiogenesis factor. Subsequently, the identification of VEGF receptors on endothelial cells and direct demonstration that VEGF was pivotal for both embryonic and tumor angiogenesis confirmed the initial optimism about the importance of this molecule (Ferrara, 2002).

Several years before the cloning of VEGF, we had identified a protein that we designated tumor-secreted vascular permeability factor (VPF; Senger *et al.*, 1983). Expression of VPF by tumor cell lines was associated with the formation of prominent ascites in animals bearing peritoneal tumors. Moreover, elevated expression of VPF correlated with malignant transformation of cell lines (Senger *et al.*, 1986). After purification to homogeneity, the N-terminal protein sequence and structural characteristics associated with VPF revealed that VEGF and VPF were the same protein (Senger *et al.*, 1990). Thus, VEGF became known as a cytokine that potently induces both angiogenesis and vascular hyperpermeability.

After the cloning of VEGF in 1989, research in the field remained focused primarily on the relationship of VEGF to angiogenesis. Nonetheless, we became interested in the possibility of additional functions. To get a "wide-angle view" of what such functions might include, we surveyed for expression of VEGF transcripts in a variety of normal adult tissues and also in tumors. Surprisingly, we found widespread expression of VEGF transcripts in normal adult tissues (Berse *et al.*, 1992); expression was generally less than in tumors but nonetheless readily detectable. These observations were published in *Molecular Biology of the Cell*, in an article that has been widely cited as researchers have continued to study the many functions of VEGF (Berse *et al.*, 1992).

The highest levels of VEGF expression were found in normal lung, kidney, heart, and adrenal gland. Lower, but significant levels of VEGF transcript were found in liver, spleen, gastric mucosa, and breast. As determined with in situ hybridization, VEGF transcript was found in cardiac myocytes and in epithelial cells of lung alveoli, renal glomeruli, and adrenal cortex. Because angiogenesis is not a feature of normal adult lung, kidney, heart, or adrenal gland, findings that VEGF transcript was expressed in these organs required another explanation. Consequently, we postulated that VEGF could be responsible for inducing and maintaining basal permeability of the normal microcirculation, as required for transport of nutrients from the blood. Also, we proposed that VEGF could be important for maintaining the existing density of endothelial cells in normal adult tissues (Berse *et al.*, 1992).

To date, definitive proof linking VEGF to regulation of basal permeability is lacking, although there is supportive evidence. In particular, antagonism of VEGF or VEGF

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receptors in mice results in a ~ 50% reduction in fenestrations in renal glomerular capillaries and significant loss of fenestrations in other vascular beds, including adrenal cortex (Kamba *et al.,* 2006). Given that a single fenestra (area ~0.003 μm^2) exhibits hydraulic conductance and permeability greater than 1 mm^2 of nonfenestrated endothelium (Levick and Smaje, 1987), it would seem that significant loss of fenestrae would result in sharply reduced basal capillary permeability. Other supportive evidence for VEGF involvement comes from transgenic mice overexpressing VEGF in skin. In these mice, skin blood vessels exhibit hyperpermeability under basal conditions (Thurston *et al.,* 1999). However, interpretation of this observation must be tempered by the caveat that VEGF is expressed at higher than normal levels in this transgenic model.

As for the involvement of VEGF in maintaining the existing density of endothelial cells in normal adult tissues, direct evidence is compelling and extensive. Antagonism of VEGF or VEGF receptors in mice for 2–3 wk caused significant capillary regression in a variety of adult tissues, including pancreatic islets, thyroid, adrenal cortex, pituitary, choroid plexus, small intestinal villi, epididymal adipose tissue, and trachea (Baffert *et al.,* 2006; Kamba *et al.,* 2006). Vascular densities decreased by as much as 68% (Kamba *et al.,* 2006). Also, in a mouse genetic model, time-specific and selective suppression of VEGF expression by adult kidney podocytes resulted in intracapillary renal thrombosis, renal capillary regression, and proteinuria (Eremina *et al.,* 2008). Furthermore, deletion of *vegf* specifically in the endothelial cell lineage of mice resulted in widespread degeneration of the endothelium and sudden death of 55% of the mutant mice by 25 wk of age (Lee *et al.,* 2007). Such autocrine-VEGF signaling in endothelial cells appeared to be entirely intracellular because only cell-permeable VEGF receptor antagonists, as opposed to nonpermeable antagonists, were inhibitory. Thus, capillary regression caused by nonpermeable VEGF antagonists, as summarized above, is most probably attributable to neutralization of paracrine VEGF that is secreted by nonendothelial compartments. Capillary regression caused by cell-permeable VEGF receptor antagonists likely involves inhibition of signaling by paracrine and autocrine VEGF.

Stimulation of nitric oxide production is yet another critically important VEGF maintenance function, of which we did not speculate, but which became evident subsequently (Ku *et al.,* 1993; Morbidelli *et al.,* 1996; Papapetropoulos *et al.,* 1997; van der Zee *et al.,* 1997). The endothelium of arteries and arterioles uses nitric oxide to signal the surrounding smooth muscle cells to relax, thus resulting in vasodilation and increased blood flow. Also, nitric oxide inhibits platelet activation and inhibits thromboembolism, particularly in venules (Broeders *et al.,* 1998), suggesting a role for VEGF in suppressing undesirable thrombosis.

Finally, VEGF also serves functions that extend far beyond the endothelium. Although originally thought to be endothelial-cell specific, VEGF is now recognized as an important cytokine for other cell types including cells of the immune response and neural cells (Ogunshola *et al.,* 2002; Storkebaum *et al.,* 2004; Maharaj and D'Amore, 2007; Saint-Geniez *et al.,* 2008). In particular, mice with reduced VEGF levels develop adult-onset motor neuron degeneration (Storkebaum *et al.,* 2004), and systemic neutralization of VEGF results in neural cell death in the inner and outer nuclear cell layers of the retina and a decline in retinal function (Saint-Geniez *et al.,* 2008).

Thus, current understanding of VEGF biology has progressed well beyond early predictions that VEGF serves important functions apart from its role angiogenesis. Now that VEGF antibodies have entered the clinic and several other VEGF antagonists and VEGF receptor antagonists are in clinical trials, it is more important than ever to understand the normal maintenance functions served by VEGF and to understand the physiological consequences of long-term interference with VEGF signaling. Indeed, several adverse side effects associated with administration of VEGF antibody have been identified in patients, including systemic hypertension, retinal arterial vasoconstriction, thromboembolism, hemorrhage, proteinuria, and intestinal perforations (Kabbinavar *et al.,* 2003; Eremina *et al.,* 2008; Papadopoulou *et al.,* 2009). Systemic hypertension, retinal arterial vasoconstriction, and thromboembolism likely relate to VEGF function in nitric oxide production as summarized above; hypertension and thromboembolism also may be due to widespread capillary regression, as documented thoroughly in mice (Kamba *et al.,* 2006). Logically, hemorrhage and intestinal perforations may also result from capillary regression, and proteinuria may result from hypertension, possibly in combination with loss of paracrine VEGF support of glomerular endothelium (Eremina *et al.,* 2008). Given the widespread expression of VEGF in adult tissues and the importance of VEGF for neural cells as well as endothelium, additional and yet unrecognized side effects are also likely, particularly with longer term antagonism of VEGF. Thus far, animal studies have focused on relatively short-term antagonism of VEGF; future studies involving longer-term administration of VEGF antagonists and analyses of additional biological parameters will be required to elucidate more completely the side effects to be expected with long-term VEGF antagonism in patients.

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