Published in final edited form as:
Circ Res. 2011 July 22; 109(3): 246–247. doi:10.1161/CIRCRESAHA.111.249953.

The VEGF165b "ICE-o-form" Puts a Chill on the VEGF Story

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Keywords

Angiogenesis; Antiangiogenesis agents; vascular endothelial growth factor A

Originally described by Harold Dvorak and colleagues at the then Beth Israel Hospital and Harvard Medical School nearly 30 years ago as a vascular permeability factor, vascular endothelial growth factor (VEGF) has been one of the most extensively studied of the cytokine growth factors.[1] This led to investigations where manipulations of VEGF protein, with or without and/or one of its receptors, have provided many advances in our understanding of blood vessel and organ development, while post-natal studies have led to numerous basic discoveries into angiogenesis. However, that is not all. The ability to block VEGF ligand-mediated angiogenesis has resulted in marked improvements in our ability to treat diseases that range from many forms of cancer to previously untreatable diseases within the eye.[2] Unsuccessful efforts have been undertaken to try and use VEGF gene or protein delivery to promote the growth of collateral blood vessels, i.e. therapeutic angiogenesis, to treat patients with ischemic heart and peripheral artery disease.[3,4]. Finally we are beginning to see how VEGF may have a role in normal physiology and pathology in organs like the kidney, in ways that still need to be fully elucidated.[5,6].

In some respects, the VEGF receptor-ligand family is relatively simply.[7,8] There are relatively few ligands with VEGF-A encoded on chromosome 9, VEGF-B on chromosome 11, VEGF-C on chromosome 4, VEGF-D on the X chromosome, and placental growth factor on chromosome 14. Most of these different VEGF genes (isoforms) have 2 or more, transcriptional, splice variants that result in proteins that vary in their relative heparin affinities, receptor binding, and thus potencies. There are 3 major VEGF receptors VEGFR1 (flt-1), VEGFR2 (flk-1 or kdr), and VEGFR3 (flt-4), although transcriptional splice variants and proteolytically cleaved products of the full-length receptors appear to as antiangiogenic agents because these soluble receptors either bind ligand but do not signal or they interact with full-length receptors at the cell membrane to block ligand medicated receptor signaling. This spices up the mix but still the system is far less complex than, for example, the fibroblast growth factor system which has many more ligands and receptors. In situations where have we failed to be successful in developing approaches to modulate/activate VEGF for clinical gain, have we underestimated the complexity of the VEGF system?

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VEGFA has been classically described as having 4 main splice variants (121, 165, 189 and 206), although other splice variants were described as present.[7] Bates and co-workers are largely responsible for describing a form of the VEGF165, termed VEGF165b, which is a splice variant where exon 8 has a 6 amino acid difference from the typical VEGF165.[9] The anti-angiogenic properties of VEGF165b in the human cancers are established where this splice variant does not activate the VEGF receptor but actually inhibits VEGF receptor 2 activation.[9,10] In this issue of *Circulation Research*, Manetti et. al.[11] present what may well be one of the most convincing evidence to date that VEGF165b plays a role in inhibiting angiogenesis in areas other than cancer. Patients with systemic sclerosis (SSc) have, for example, impaired angiogenesis mediated wound healing and the authors analyzed skin biopsy and serum blood samples from 35 individuals with SSc and 23 age- and sex-matched controls. VEGF was up-regulated despite the fact that there was impaired angiogenesis but the VEGF over-expression was of VEGF165b, not the VEGF165. They also showed that the antibodies used to recognize VEGF165 will also detect VEGF165b and thus investigators cannot distinguish between the splice-variants unless they appropriately design their studies. Even at the mRNA level, unless primer probe sets are designed to span the terminal 8th exon of the VEGFA gene, one would not be able to distinguish the pro-angiogenic from the anti-angiogenic form of a VEGF165. They went on and showed that microvascular endothelial cells (MVECs) isolated from the skin of individuals with SSc expressed and released higher levels of VEGF165b than MVECs from healthy individuals. Moreover, MVECs from SSC individuals expressed higher levels of VEGFR-2 but they also showed impaired phosphorylation/activation of that critical receptor and reduced capillary morphogenesis. They further showed that recombinant VEGF165b, and conditioned media from SSC MVECs, inhibited VEGF165 mediated VEGFR-2 phosporylation and capillary morphogenesis in healthy MVECs and these anti-angiogenic effects were abrogated by treatment with an anti-VEGF165b blocking antibodies. They demonstrated that the VEGF165b was functioning as an anti-angiogenic agent, in humans, in an area other than cancer.

Any study that advances our understanding of human disease should give a warm feeling. However, a chill goes through when one considers that Manetti et al.[11] was examining a situation of impaired, VEGF receptor activity and, angiogenesis in the face of excess VEGF ligand. Indeed, one can easily find several recent human studies in patients with peripheral arterial disease[12,13] or ischemic heart disease[14] that also had demonstrated, or inferred that there was, impaired angiogenesis despite higher levels of VEGFA ligand. Conclusions were drawn about the potential mechanism for the VEGF resistance and the impaired angiogenesis. While these conclusions were supported by the presented data and may indeed be correct, those and many other studies did not distinguish between VEGF165 and VEGF165b and thus could not exclude that the up-regulation was of an angiogenesis inhibitor such as VEGF165b. A similar logic applies for any study that looked for changes in VEGF following an intervention or perhaps even gene therapies targeting transcription of the VEGF gene? One is left to conclude that human studies designed to examine expression changes in the VEGF receptor-ligand axis must include studies looking for VEGF165b, and perhaps VEGF121b and VEGF189b as well.[10]

Viewed in another way, the paper by Manetti et al. raises questions about human studies for which there is little non-human, especially mouse, data. A report from my laboratory, for example, just a few years ago in mice with diet-induced diabetes and experimental peripheral arterial disease demonstrated impaired angiogenesis and reduced VEGF receptor signaling, despite higher levels of “VEGF” ligand.[15] We tested (though not exhaustively) for VEGF165b mRNA expression in muscle and could not detect it (data not published). Indeed, published data on mouse VEGF165b is essentially absent. Therefore, one possibility is it that the VEGF165b does not exist in mice; though it has been shown to be present in the

rat.[16] It is chilling and disappointing to consider the possibility that because something does not occur in mice and/or cannot be manipulated within the mouse genome that this should limit its potential significance. The report by Manetti et al.[9] should firmly establish a role for VEGF165b outside of tumor angiogenesis and in conditions in humans where we seek to understand, promote, or inhibit angiogenesis.

Acknowledgments

Funding Sources: Supported by RO1 HL101200 and RO1HL101200-S1 (NHLBI) and 1UO1 DK076136 (NIDDK) to BHA and a Harold Amos Faculty Development Award from the Robert Wood Johnson Foundation to AOD

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