VEGF and EG-VEGF


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Vascular endothelial growth factor (VEGF) plays an essential role in angiogenesis. This includes developmental angiogenesis as the loss of a single allele is embryonic lethal due to lack of development of endothelial cells. During early development, endothelial cells depend on VEGF for survival and if it is withdrawn, massive apoptosis results. This is not true in adult organisms; withdrawal of VEGF does not result in widespread apoptosis of endothelial cells, but angiogenesis still appears to be dependent upon VEGF. This includes cyclical angiogenesis in the female reproductive system, skeletal growth, and pathologic angiogenesis, such as tumor angiogenesis.

There are three receptors for VEGF, VEGFR1, R2, and R3. VEGFR3 mediates lymphangiogenesis. VEGFR2 is the major mediator of mitogenesis of endothelial cells. It binds VEGF-A and proteolytic fragments of VEGF-C and –D. VEGFR1 is not as well understood. It binds VEGF-A and –B, and placental growth factor (PlGF). Depending on the setting, VEGFR1 may act as a decoy or as a signaling receptor. It doesn’t mediate mitogenesis, but seems to promote differentiated functions of endothelial cells such as release of growth factors. It also has a role in hematopoiesis and recruitment of monocytes/macrophages. Neuropilin 1 and 2 are also receptors for VEGF; they are isoform specific receptors/coreceptors.

The VEGF gene has eight exons and alternative splicing of mRNA results in several isoforms. The different isoforms have different properties: VEGF189 and VEGF206 bind very tightly to heparin, so they are sequestered in extracellular matrix. VEGF165 is the predominant isoform. It binds heparin less tightly and therefore has some solubility but also associates with matrix. VEGF121 lacks a heparin-binding domain and so it is very soluble.

Other active moieties of VEGF are generated by proteolytic degradation. Plasmin cleaves VEGF165 resulting in a N-terminal 110 amino acid peptide which lacks a heparin-binding domain. VEGF110 can associate with VEGF165 to form VEGF110-VEGF165 heterodimers, and then with more digestion, VEGF110 homodimers can result. Matrix metalloproteinase-3 (MMP3) cleaves VEGF165 resulting in an N-terminal 113 amino acid peptide. Inflammation causes increased levels of these enzymes and therefore increased levels of the VEGF fragments.

In tumor xenograft models, tumors are obtained when VEGF null cells are implanted, because VEGF derived from the host is sufficient. Tumor growth still occurs when up to 70% of VEGF activity is eliminated. It appears that blockade of all VEGF isoforms and fragments is necessary to achieve sufficient reduction of VEGF activity to prevent tumor growth.

In ocular fluids obtained from patients with diabetic retinopathy, total VEGF activity is significantly higher than activity attributable to heparin binding VEGF. This means that VEGF165, which is included within the heparin binding fraction, is partially responsible for VEGF activities and the remaining, mediated by VEGF121 and/or by proteolytic products of heparin-binding VEGF, is likely to be responsible for a significant amount of ocular pathology.
The first ocular disease that has been clinically targeted with anti-VEGF treatment is neovascular age-related macular degeneration (NVAMD). Clinical trials are underway testing Ranibizumab (Lucentis), an affinity-matured Fab variant of bevacizumab (Avastin). The affinity of Lucentis is increased by ~100-fold above that of Avastin Fab. Like Avastin, Lucentis inhibits all isoforms and active fragments of VEGF. Its molecular weight is 48 kD. It has been demonstrated to suppress CNV at Bruch’s membrane rupture sites in monkeys.

Endothelial gland-derived VEGF (EG-VEGF) is an angiogenic protein that is structurally unrelated to VEGF. It is a selective mitogen for adrenal endothelial cells and has no effect on human umbilical vein endothelial cells. It is expressed in steroidogenic tissues such as adrenal gland, ovary, testis, and placenta. Like VEGF it can induce fenestrae in endothelial cells. It is a mediator of tissue-specific angiogenesis; it induces angiogenesis in the ovary, but if it is expressed in the cornea, there is no effect. Transgenic mice in which the rhodopsin promoter drives expression of EG-VEGF in the retina develop sprouting of neovascularization from choroidal vessels, which are fenestrated, but not from retinal vessels.

Prokineticin 2 (BV8) is highly homologous to EG-VEGF. It is selectively expressed in testis, bone marrow, and circulating leukocytes. It can induce hematopoiesis and it is a potent chemoattractant for monocytes.