VEGF Gradients


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VEGF is central to normal angiogenesis and pathological angiogenesis. What goes wrong in pathological angiogenesis? One hypothesis is that VEGF isoforms contribute to the multifunctional nature of VEGF. The heparin-binding domain of VEGF has many types of activities. Evidence for this is derived from mice that express only VEGF_{120}, which have no heparin-binding VEGF. They have normal organ development until late in gestation and then develop problems related to vascular insufficiency due to abnormal density of blood vessels. There is a deficiency in branching of blood vessels. Microvessels are about 60% larger in diameter than normal. The number of dividing vascular cells is the same as in wild type mice, but they are organized differently. Larger vessels have less complex branching than normal. There are stunted blood vessel sprouts that are bulbous and lack filopodia. There is proliferation at ends of vessels, which is normally not a characteristic of tip cells at the ends of vessels. This increase in proliferation may give rise to larger vessels. We have hypothesized that heparin-binding VEGFs are needed to set up VEGF gradients, which promote pathfinding for vessels and branching morphogenesis.

In contrast to mice with only VEGF_{120}, mice that express only VEGF_{188} have ectopic branching, but mice that express only VEGF_{164} are normal. Mice that express both VEGF_{120} and VEGF_{188}, but not VEGF_{164} are also normal. Both soluble and matrix-binding VEGFs are needed, which are provided by VEGF_{164} alone or the combination of VEGF_{120} and VEGF_{188}.

There is more to the heparin-binding region story than just interaction with extracellular matrix. Heparin-binding domain also mediates neural cell migration via neuropilins. VEGF_{120/120} mice have another phenotype, abnormal assembly of the facial motor nucleus. There is a delay in assembly and change in morphology. A similar phenotype is present in neuropilin1 knockout mice. Implantation of a soaked bead also results in an abnormal facial motor nucleus. This suggests that VEGF_{164} is involved in motor neuron migration, which in fact is isoform-specific.

In contrast, neuroprotection is not isoform-specific. In a model of ischemia-induced retinal cell death, retinal neurons can be rescued by either VEGF_{120} or VEGF_{164}.

What about the heparin-binding domain in the context of disease? In xenograft tumor models, tumor growth is best when VEGF_{164} or a combination of VEGF_{120} and VEGF_{188} are available. In some way the heparin-binding domain provides growth advantage.

Mice depleted of leukocytes show less ischemia-induced retinal neovascularization than wild type mice. In ischemic retina, VEGF_{164} is preferentially upregulated and mice lacking VEGF_{164} do not develop ischemia-induced neovascularization that breaks through the internal limiting membrane. Mice lacking VEGF_{164} also develop less leukostasis.
Alanine scanning mutation analysis was performed in the heparin-binding domain of VEGF. There are three amino acids required for heparin binding and the same three amino acids are also required for leukostasis. VEGF proteins mutated in these three amino acids bind to VEGFR2, but have diminished interaction with VEGFR1. Leukostasis is mediated through VEGFR1. So it appears that the heparin-binding domain helps to mediate inflammation and amplify the pathologic effects of VEGF.

Summary of functions of the heparin-binding domain of VEGF:

1) Critical for setting up gradients needed for branching morphogenesis of vessels during development.
2) Acts through neuropilin 1 for proper motor neuron nucleus assembly.
3) Amplifies inflammation in disease.