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What, if anything, is an angiogenic factor?

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Multicellular organisms require a functional vascular system for delivery of oxygen, nutrients, and, in general, for tissue and organ homeostasis. What factors are regulating the maintenance and new formation of blood vessels? We proposed that there are at least two mechanisms: one that is independent of physiological tissue requirements, probably, initially genetically encoded and cell autonomous. Vasculogenesis, the formation of blood vessels from *in situ* differentiating endothelial cells from mesodermal precursors called angioblasts is a process determined by these mechanisms. A vascular system (including the primitive hemopoietic system) is laid down before the onset of circulation, i.e. before being functionally necessary. Endothelial cells have differentiated and, due to their specific activated genes and cell surface receptors, are able to respond to environmental or paracrine stimuli by proliferation, migration, maturation or death (for review see [1]). The latter responses play a major role during angiogenesis, the other mechanism by which new blood vessels form, by sprouting from preexisting vessels (for review see [2]). It is logical to assume that, during angiogenesis, factors from the tissue to be vascularized are produced to either directly or indirectly signal the endothelial cell to form a new vessel. What is the nature of these angiogenic factors? Again, it is obvious that a tissue that lacks oxygen, nutrients or, alternatively, a tissue that expands and proliferates needs to signal its demand to the endothelium. Thus, for example, hypoxia, glucose deprivation, hormones that regulate tissue homeostasis, and normal and abnormal increases in cell mass are conditions under which angiogenic factors are likely to be present. So, what are the requirements to call a factor an angiogenic factor?

These are the most important requirements:

1) It should be present when angiogenesis occurs. It should not be present under normal adult conditions when angiogenesis is absent unless there is an inhibitor present at that time.
2) It should be able to diffuse to the endothelial target and, for the ultimate, directly acting factor, should bind to and activate specific receptors on and genes in endothelial cells. Increased levels or overexpression should result in the formation of more blood vessels.
3) Neutralizing the activity of this factor (by its natural or synthetic inhibitors), its redundant like factors, or their receptors, or inactivation of their genes, should inhibit or prevent angiogenesis.

This is asking a lot, and in fact, for none of the so-called angiogenic factors have all these requirements been fulfilled. So, what are the candidates?

Of course, since we work on it, we believe that vascular endothelial growth factor (VEGF) is the best candidate because it actually fulfills almost all of the requirements. Fortunately, this has been confirmed by many different groups and there is no need to go into many details (for review see [3–6]). It is regulated by hypoxia, is upregulated under angiogenic conditions, is rapidly secreted by the producer cells, binds to and activates endothelial spe-

cific tyrosine kinase receptors, and inhibition of its activity or its specific receptor prevents angiogenesis. It was previously argued that the multitude of angiogenic factors then characterized could be easily explained by the fact that angiogenesis was of such crucial importance making a high degree of redundancy necessary. The more surprising it was that inhibition of a single factor or only one of its receptors would inhibit tumor angiogenesis of many different tumors. What are the problems? There is high constitutive expression of VEGF in certain organs which does not correlate with angiogenesis. For example, adult kidney glomerular podocytes, pancreatic beta cells and choroid plexus epithelial cells express a high level of VEGF mRNA and protein, and their neighbouring endothelial cells express the cognate receptors, but new blood vessels do not form [7–10]. One hypothesis was that due to its activity as a vascular permeability factor, VEGF would induce increased vesicular transport or fenestrations (sites of constitutive vascular permeability). Recently, some direct evidence has been provided for this mechanism [11, 12]. However, it is still not clear by what intracellular signaling pathways an endothelial cell responds to VEGF by angiogenesis or vascular permeability.

The problems with the other presumptive angiogenic factors are manifold. This is not to say that they are unlikely to play a role in angiogenesis but just that much less work has been done on these factors, and they do not (yet) fulfill many of the criteria listed above. For example, epidermal growth factor or its cousins transforming growth factor alpha or pleiotrophin have been proposed to play a role in angiogenesis [13]. However, either there is no vascular phenotype in the knockout mice, or receptors have not been demonstrated on endothelium, or neutralization of their activities during angiogenesis has not been achieved. Interleukin 8 [14], tumor necrosis factor (via the B61 ligand of eph receptors; [15]), transforming growth factor beta [16, 17] are multifactorial growth factors and are probably more indirectly involved in angiogenic processes by helping other more directly acting factors to either inhibit or stimulate angiogenesis. The mechanisms by which thymidin phosphorylase (PDECGF) [18, 19] and soluble adhesion receptors [20] regulate angiogenesis are unknown.

One of the most intensely investigated factors that was widely believed to play a role in angiogenesis was fibroblast growth factor (FGF), particularly FGF1 and FGF2. As pointed out many times, it is not yet clear how these factors are released from cells, how their activity is controlled in normal adult organs in which angiogenesis does not occur, and whether any FGF receptors are expressed on endothelial cells *in vivo* (unlike in cultured endothelial cells which express easily detectable levels of FGF receptors). Studies using neutralizing antibodies have been controversial and knockout mice have not yet been published (for discussion see: [21, 22]). *In vivo*, in the cornea model, angiogenesis is reproducibly observed but overexpression in a transgenic limb bud model leads to dysmorphogenesis and pattern duplications [23] but, in contrast to VEGF, not to an increase in vascularization [24].

Have the true angiogenic factors yet to be discovered? As mentioned above, the VEGF/VEGF-receptor system is likely to be of major importance for many different processes of angiogenesis. Other most interesting candidates are the ligand(s) for the tie/tie-2/tek receptors. The knockout phenotype of the tie-2 receptor is most intriguing regarding angiogenesis because it is lethal at a time when angiogenesis becomes more important than vasculogenesis for the vascularization of the embryo [25]. Of course, there may be more yet to be discovered angiogenic factors. These days, analysis of signal transduction pathways in angiogenic endothelial cells, or the analysis of EST sequences from the sequencing of the human genome, or gene trap approaches may lead to their discovery rather than the previously used tedious biochemical purification procedures.

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