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Blood Vessel Formation: What Is Its Molecular Basis?

Minireview

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Medical students learning the anatomy of the human cardiovascular system recognize that the blood vessels are named mainly on the basis of luminal diameter, branching, position, and organ supplied. Students and physicians rely upon the general constancy of vascular determinants from one individual to another and take for granted that anatomy books will not go out of date. It is only when they learn that these vessels with their proper diameters and branches are formed in the embryo, mostly before the heart starts beating, that students begin to appreciate the true complexity of the genetic program that governs the development of the vascular system. This appreciation deepens when errors of the basic developmental plan are revealed as 'vascular malformations.'

The genetic and molecular mechanisms that control the development of the vascular system have remained a mystery, until recently. Driven in part by the study of tumor angiogenesis in the 1970s, increased understanding of the growth of capillary blood vessels led to long-term in vitro culture of capillary endothelial cells and to discovery of proteins that are mitogenic for these cells, including basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), among others. The role of these proteins in vascular development is currently the subject of active investigation.

Generation of angioblasts from mesoderm appears to require the action of members of the fibroblast growth factor family (References can be found in Flamme and Risau, 1992). The mesoderm-derived angioblasts then differentiate into endothelial cells which form de novo vessels including the dorsal aorta. VEGF seems to be essential to this process of vasculogenesis, the earliest formation of blood vessels in the embryo. Two receptors for VEGF are expressed by angioblasts and by the endothelial cells, which arise from them. Disruption of the gene for the receptor *VEGFR-2* (*flk-1*), interferes with the differentiation of endothelial cells, leading to death of embryos at day 8.5-9.5 (Shalaby et al., 1995). Disruption of the gene for the other receptor *VEGFR-1* (*flt-1*) permits differentiation of endothelial cells, but interferes with a later stage of vasculogenesis, resulting in thin-walled vessels of larger than normal diameter and the embryo's death at day 9 (Fong et al., 1995). Mice made deficient for VEGF by targeted disruption of the gene die at 8.5-9 days gestation with delayed differentiation of endothelial cells and impairment of both vasculogenesis and angiogenesis (sprouting of new capillary vessels from pre-existing vasculature) (Carmeliet et al., 1996a; Ferrara et al., 1996).

The cloning of TIE1 and TIE2 (also called Tek) tyrosine kinases added another chapter to this story (Dumont et al., 1992). The phenotype of mice deficient for these receptors indicates that they play a role in angiogenesis and remodeling that is subsequent to the action of VEGF, but absence of information about the ligand for either of these receptors has hindered our understanding of their contribution to vascular development.

Three papers in this issue reveal new information about the potential role of the TIE2 receptor and its ligand and raise interesting questions about the details of vessel assembly. The paper by Davis et al. (1996, this issue) reports the isolation and cloning of angiopoietin-1 (a 70 kD glycoprotein), the first known ligand of the TIE2 receptor which is expressed on vascular endothelial cells. Interestingly, unlike VEGF, angiopoietin-1 is neither a mitogen for endothelium, nor does it induce tube formation in vitro. Rather, its pattern of expression in the vicinity of forming vessels suggests that it plays a role in regulating the assembly of non-endothelial vessel wall components. This supposition is supported by observations in the paper by Suri et al. (1996, this issue) in which mice deficient for angiopoietin-1 exhibit abnormal vascular architecture where the principal defect is a failure to recruit smooth muscle and pericyte precursors. In the heart, this defect manifests as poorly developed endocardium, characterized by incomplete association of the endothelial layer with the underlying myocardial wall.

In the paper by Vikkula et al. (1996, this issue), venous malformations in two disparate families were mapped to the *Tie2* receptor where a missense mutation results in an arginine-to-tryptophan substitution. By overexpressing the full-length and wild-type mutant receptors in insect cells, the authors show that the mutant receptor has a 6 to 10-fold increase in autophosphorylation activity. Patients carrying this mutation develop vein-like structures that are deficient in the non-endothelial cells of the vascular wall, mainly a lack of smooth muscle cells (Figure 1). Thus, these malformations are comprised of vein-like lumens lined by a monolayer of endothelial cells, but with thin walls in which smooth muscle layers are markedly reduced, as compared to normal vessels with similar sized lumens. As the luminal diameter of normal veins increases, there is a proportional increase in the number of smooth muscle cell layers (Saenz et al., 1991). In contrast, venous malformations exhibit wide variations in luminal size (including diameters larger than any normal vessels), but without a complementary increase in smooth muscle layers with increasing vessel diameter.

These reports are exciting because a vascular abnormality in both mice and humans is defined by a receptor-ligand system on the vascular endothelial cell. It is intriguing that an apparent defect in vascular remodeling can result either from an activating mutation of the receptor (Vikkula et al., 1996), from the absence of the ligand (Suri et al., 1996), or from a deficiency of the TIE2 receptor itself (previously reported by Sato et al., 1995). However, while each situation reveals a general abnormality in vascular remodeling, there may be subtle but important differences.

The human activating mutation of the TIE2 receptor and the murine ligand (angiopoietin-1) deficiency both

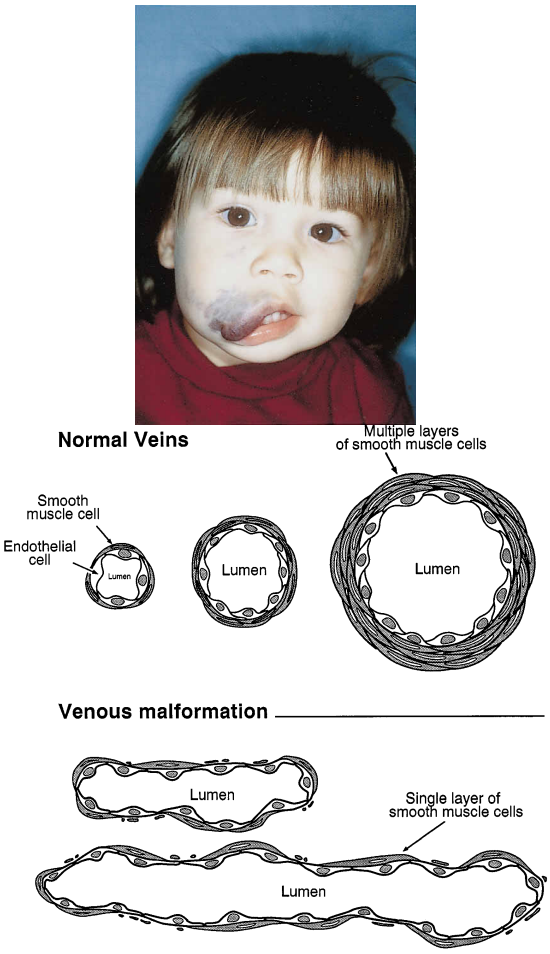

Figure 1. Venous Malformations

Top: Child with a venous malformation.

Bottom: Diagrams to show the histological difference between normal veins and a venous malformation. With increasing luminal diameter, normal veins are lined by increasing numbers of endothelial cells in a monolayer, and increasing numbers of smooth muscle cells in multiple layers. In contrast, as the luminal diameter of venous malformations increases, the number of smooth muscle layers does not increase.

result in defects in recruitment of vascular wall cells. Angiopoietin-1 null mice have "a simplified and less complex vasculature containing fewer branches and more homogeneously-sized vessels" and reveal little evidence of endothelial proliferation. In fact, the authors show no change in the total number of endothelial cells between angiopoietin-null and normal embryos, based on Northern analysis of PE-CAM an endothelial marker. In contrast, in the human venous malformation, the abnormally large lumens suggest that there must have been endothelial proliferation. These differences could be real and reflect differences in the nature of the gene defects, e.g., null versus activating, or they could be due to the fact that one lesion (venous malformation) forms in an adult organism whereas the other (angiopoietin-1 deficiency) is occurring in a developing mouse embryo. We suspect that the increased endothelial proliferation in the venous malformations is secondary to the absence of smooth muscle cells (see below).

Even the assembly of a vascular structure seemingly as simple as a vein, appears to involve several different molecular pathways. When the papers in this issue of *Cell* on TIE2/angiopoietin are considered together with previous reports of vascular phenotypes resulting from targeted disruption of other genes involved in vascular development, a working model of vessel assembly can be constructed. Fifty percent of TGF-β null mice die from a severe defect in yolk-sac vasculogenesis, thought to result from improper interactions between epithelial and mesenchymal cells (Dickson et al., 1995). Platelet-derived growth factor receptor-β (PDGFR-β) and PDGF-B ligand-null mice die perinatally from hemorrhage and have no pericytes (mesangial cells) in the kidney vasculature (Levéen et al., 1994; Soriano, 1994). Furthermore, these animals are reported to lack pericytes throughout the entire microvascular bed (C. Betzholz, submitted). In addition, a recent study reports that mice deficient for tissue factor, die in utero at day 8.5 with vascular abnormalities that appear to be due to a defect in recruitment of smooth muscle/pericytes (Carmeliet et al., 1996b). (Because of our relative lack of knowledge about the actions of tissue factor outside of its function in coagulation, it is difficult to speculate what precise role tissue factor might play in vessel assembly.)

The paper by Suri et al. concludes that the absence of angiopoietin-1 results in vessels that "do not properly recruit supporting cells" to the vascular wall. The Vikkula paper also speculates that the activating mutation of the TIE2 receptor is somehow linked to a deficient recruitment of mesenchymal cells to the vascular wall. They propose that TIE2 receptor expression by endothelial cells regulates ligand expression by smooth muscle cells so that the activating mutation in TIE2 would lead to decreased ligand expression in mesenchymal cells and increased proliferation of the endothelium. Furthermore, Vikkula et al. suggest that, by some as yet unknown mechanism, the TIE2 "receptor ligand loop" is coupled to chemotaxis and proliferation of mesenchymal cells as well as their differentiation into smooth muscle cells. Together these result in "uncoupling between proliferation and differentiation of endothelial cells and smooth muscle cells" and to a "disproportionate number of endothelial and smooth muscle cells in venous malformations."

Incorporating two additional pieces of information provided by the Suri and Davis papers, we suggest a modified interpretation. First, because angiopoietin-1 has no mitogenic effect on endothelial cells, the putative endothelial proliferation that would be necessary for endothelial cells to line a large lumen in the venous malformation, may be a downstream event. Secondly, angiopoietin-1 null embryos have reduced TIE2 receptor mRNA, suggesting that the receptor levels are a function of the ligand and perhaps not the reverse as put forth by Vikkula et al. (See Figure 2 diagram).

In our proposed model, angiopoietin-1 produced by mesenchymal cells activates the TIE2 receptor on endothelial cells, which in turn leads to the production and or release of a recruiting signal for mesenchymal cells. In the case of the pericyte, most data indicate that this recruiting signal is PDGF-BB. But at the level of the smooth muscle, this recruitment also may involve PDGF-AA or HB-EGF (heparin-binding epidermal growth factor), another smooth muscle chemotactic factor and

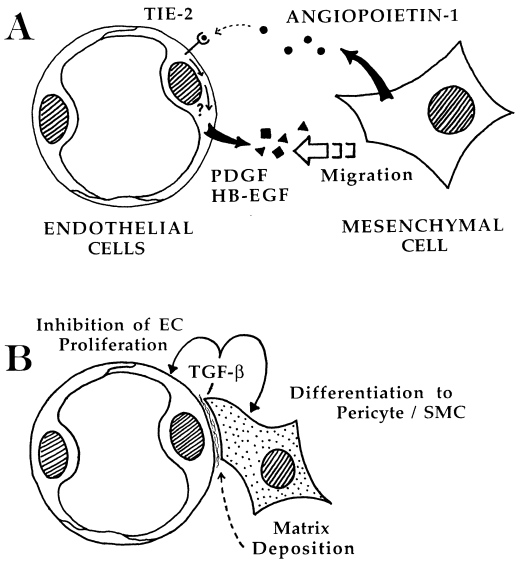

Figure 2. A Proposed Model for the Recruitment of Mesenchmal Cells to Developing Vessels

Angiopoietin-1 released by mesenchymal cells binds TIE2 receptor and activates (or releases) a signal from the endothelial cell that recruits local mesenchymal cells to the forming vessel. Upon contact with the endothelium, TGFβ is activated from its latent state, causing mesenchymal cells to differentiate into pericytes and smooth muscle cells, inhibition of endothelial proliferation (Antonelli-Orlidge et al., 1989), and accumulation of extracellular matrix.

mitogen. Once mesenchymal cells arrive and contact endothelium, TGF-β may be activated (as has been shown to follow endothelial cell-smooth muscle cell interactions [Antonelli-Orlidge et al., 1989]). The TGF-β may then induce differentiation of the mesenchymal cells into pericytes and smooth muscle cells (Rohovsky et al., 1996), inhibit endothelial cell proliferation and stimulate matrix deposition (References found in Hirschi and D'Amore, 1996). Thus, a lack of local TGF-β production could permit increased proliferation of endothelial cells, which line the large lumens of venous malformations. Because TGF-β induces matrix production and alters integrin expression, an absence of endothelial-smooth muscle cell contacts, and the resulting lack of TGF-β, may also explain the poor vascular integrity and reduced remodeling noted in the TIE2 receptor/angiopoietin-null mice.

One key question is: ''Is there a difference between angiopoietin-1 activation of the TIE2 receptor and the activation that is proposed to lead to venous malformations?'' It seems likely that angiopoietin-1 activation of the TIE-2 receptor is functionally different from the activation that occurs as a result of the arginine-to-tryptophan mutation; in the angiopoietin-1 knockout, receptor activity is absent while in venous malformations the receptor may be hyperactivated, yet both lead to apparent defects in mesenchymal cell recruitment and vessel remodeling.

To investigate this question, one could compare the phosphorylation pattern of the TIE2 receptor and its substrates following angiopoietin-1 binding to the phosphorylation pattern of the mutant TIE2. Furthermore, what is the functional result of angiopoietin-1 binding to the TIE2 receptor? Do endothelial cells that express activating mutant TIE2 receptors, or lack TIE2 receptors, have altered production of PDGF-BB or HB-EGF and/or reduced ability to recruit smooth muscle cells or their precursors? Do endothelial cells in venous malformations express normal levels of PDGF or HB-EGF?

What do these new data teach us about the development of blood vessels? The TIE2/angiopoietin system appears to govern maturation and stabilization of blood vessels. 'Stabilization' of a blood vessel is a stage when remodeling has ceased, new branches are not developing, and luminal size is constant, save for physiological vasodilation and vasoconstriction. Thus, the identification and initial characterization of the TIE2 receptor angiopoietin-1 system, begins to elucidate mechanisms for a process which was formerly understood only at a descriptive level. These three papers signify exciting progress in vascular biology because they begin to reveal the molecular basis for vascular remodeling.

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