The FAKs About Blood Vessel Assembly

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The formation of blood vessels during embryonic development is a complex process that requires the coordination of multiple soluble signals, as well as coordinated communication of cells with one another and with their surrounding extracellular matrix (ECM). The process of vessel assembly can be dissected into discernible steps that appear to require unique controls (depicted in the Figure, panel A): induction of mesodermal progenitors to an endothelial fate (differentiation of angioblasts); migration of angioblasts and alignment into a primitive vascular plexus; endothelial tube formation; vascular fusion and plexus remodeling; endothelial cell recruitment of mesenchymal progenitors; and endothelial-induced mural cell differentiation. 

Studies in genetically malleable model systems have enabled the identification of some important soluble effectors, as well as cell junctional and matrix components needed for various stages of vessel formation. However, little is known about how these players are collectively orchestrated to achieve successful assembly of vessel structures. Studies by Ilic and coworkers in this issue suggest that focal adhesion kinase (FAK) may function to receive and transduce signals from both growth factors and surrounding ECM to control cellular movement, replication, and perhaps fate during vessel morphogenesis.

FAK, a cytoplasmic tyrosine kinase, was initially described to mediate integrin signaling as the binding of ECM to integrins enhances FAK phosphorylation. FAK, localized to focal adhesions, regulates cell motility by inducing actin polymerization and the turnover of contacts between cells and the ECM. However, other studies have implicated FAK in the transduction of soluble signals including VEGF-A and Indian Hedgehog (IHH) that are required for vascular induction in the adjacent mesoderm of the yolk sac, the first site of blood vessel formation during embryogenesis. Interestingly, in FAK null embryoid bodies, cells expressing α-fetoprotein (AFP; a marker for visceral endoderm) exhibit decreased motility and increased focal adhesions. Therefore, it is possible that at the earliest stages of vascular development, FAK is needed for appropriate migration and localization of visceral endodermal cells (Figure, panel B). Thus, the clustering of PECAM-positive cells that is observed in the absence of FAK may reflect inappropriate localization of the signal-producing endodermal cells that mediate vascular induction in adjacent mesoderm. Such a problem with endodermal migration, and perhaps differentiated function, could contribute to the observed defects in endothelial tube formation in FAK−/− mutants and is not ruled out in the studies by Ilic et al.

Another potential role for FAK during this stage of vessel assembly is in mesodermal transduction of endodermally derived signals found in previous studies to be necessary for angioblast differentiation, bFGF and VEGF-A. Ilic and coworkers suggest that mesodermally localized FAK does not play a role in the differentiation of endothelial cells because they detect expression of PECAM-positive cells in FAK−/− yolk sacs and embryoid bodies and were able to isolate PECAM-expressing cells from FAK−/− embryos and embryoid bodies and demonstrate that they take up Dil-Ac-LDL dye. However, we would argue that Dil-Ac-LDL uptake is not necessarily endothelial-specific, and PECAM does not specifically mark the endothelial cell lineage, but is also expressed by hematopoietic cells and their progenitors in embryonic (B. Nadin, M.A. Goodell, K.K. Hirschi, unpublished data, 2003) and adult tissues. Furthermore, RT-PCR analysis and immunostaining revealed significantly reduced
Angioblast Migration, Vascular Plexus Formation, and Tubulogenesis

Upon vascular lineage commitment, primordial endothelial cells bind ECM proteins such as fibronectin, vitronectin, collagen, and laminin via cell adhesion molecules including integrins. Integrin receptor signaling, in conjunction with growth factor signaling, promote the proliferation, maturation, and migration of endothelial cells to form tube-like structures that fuse to form a primitive vascular plexus. Ilic and coworkers² suggest that the primary role of FAK in vascular development is the coordination and transduction of these diverse signals. There is mounting evidence, in multiple systems, for crosstalk between integrin and growth factor receptors, and data to suggest that FAK is at the crossroads of these signaling pathways. Of particular relevance to vascular development are potential interactions among VEGF-A and bFGF receptors, integrin receptors α1β1, α3β1, and α5β1, and FAK, because all have been independently demonstrated to play a role in blood vessel assembly in embryonic and/or adult tissues.

There is particularly strong evidence that VEGF receptors physically and functionally interact with integrin receptors. The binding of VEGF-A to Flk-1 induces tyrosine phosphorylation of FAK,⁷,⁸ leading to activation of PI3K and subsequent endothelial cell migration.⁹ Alternatively, VEGF-A signaling through Flt-1 promotes tubulogenesis in a FAK-dependent pathway.⁹ Furthermore, VEGF-A activation of the Src pathway results in the coupling of FAK to integrin α5β1, suggesting that FAK may mediate integrin regulation of VEGF-A induction of endothelial cell adhesion and migration.¹⁹

FAK may also play a role in cell survival and proliferation by regulating progression through G1 in the cell cycle.²⁰ Interestingly, although Ilic and coworkers did not detect differences in apoptotic levels in FAK⁻/+ mutants (B), blood vessel assembly is arrested during tubulogenesis, suggesting that the primary role of FAK in vascular development is the control of endothelial cell migration and tube formation or perhaps earlier during mesodermal induction. However, FAK has also been shown to mediate PDGF-B signaling in mural cell proliferation and migration, which is necessary for the formation of stable, quiescent vessel structures. Therefore, FAK may have other later roles in vessel assembly, which are not revealed in the FAK⁻/+ mutants due to early lethality. Col1 indicates type I collagen; Cx, connexin; Fn, fibronectin; IHH, Indian Hedgehog; Lm, laminin; Ptc, Patched; Smo, Smoothened; and Vn, vitronectin.


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