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.1 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 coworkers2 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.3,4 FAK, localized to focal adhesions, regulates cell motility by inducing actin polymerization and the turnover of contacts between cells and the ECM.5,6 However, other studies have implicated FAK in the transduction of soluble signals including VEGF-A7–10 and PDGF-B.6 FAK associated with the cytoplasmic domains of growth factor receptors, is phosphorylated in response to ligand binding7,8 and recruits signaling molecules such as Src, PI3K, and Grb2.9–12

The Ilic studies revealed that embryos deficient for FAK5,13 die at ∼E8.5 to 9.5 days with multiple defects, including disrupted vascular development. The process of vessel assembly in these mutants is thought to be arrested after endothelial cell differentiation because CD31 (PECAM)-positive cells were found clustered in FAK−/− yolk sacs and in embryoid bodies derived from FAK−/− embryonic stem cells. These cells were unable to form a capillary plexus, and the authors conclude that the primary role of FAK during vessel assembly is to modulate endothelial cell migration and tube formation (also referred to as tubulogenesis). This idea is consistent with the previous finding that cells derived from FAK null embryos exhibit decreased motility and turnover of focal adhesions.5 However, FAK may also play a role in earlier stages of vessel assembly, as discussed in the following sections.

Endodermal Induction of Mesodermal Progenitors to an Endothelial Fate

Ilic and coworkers propose that the primary role of FAK is in the modulation of mesodermal migration; however, FAK is also expressed in endodermal cells.13–15 In wild-type mice, the visceral endoderm of the yolk sac elicits soluble signals including VEGF-A16 and Indian Hedgehog (IHH)17 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 mobility 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.2

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.18 Furthermore, RT-PCR analysis and immunostaining revealed significantly reduced

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expression of PECAM in FAK−/− embryos and embryoid bodies, respectively, compared to wild type. Thus, until more specific markers of mature endothelial cells are localized within FAK−/− embryos and yolk sacs, the possibility that endothelial cell differentiation is defective in the absence of FAK remains.

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 αβ3, αβ5, and αβ1, 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.10 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 αβ5, 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−/− versus wild-type embryos, there was a significant increase in apoptosis in endothelial cells in vitro when FAK levels within focal adhesions were reduced. Thus, although reduced FAK activity is associated with increased apoptosis in some systems, this is not proposed to be a primary defect of vascular cells in FAK−/− embryos.

In summary, the data presented by Ilic and coworkers in their study published in this issue, as well as other published reports, provide support for the hypothesis that FAK controls endothelial cell migration and tube formation. However, sufficient data are not provided to rule out other earlier roles of FAK in endodermal migration and induction of mesodermal commitment to an endothelial fate. Therefore, FAK activity may modulate vascular development at multiple stages of vessel assembly.

References


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