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Review

Endothelium as master regulator of organ development and growth ☆

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ABSTRACT

Development of the vasculature is one of the earliest events during embryogenesis, preceding organ formation. Organogenesis requires a complex set of paracrine signals between the vasculature and the developing nonvascular tissues to support differentiation and organ growth. However, the role of endothelium in controlling organ growth and, ultimately, size is little-understood. In this review, we summarize new data regarding the endothelium function in order to provide a more comprehensive understanding of the communication between the endothelium and the organ's tissue.

Contents

1. Introduction

1.1. Cardiovascular system formation in a mouse embryo

Embryonic vascular development comprises a highly organized sequence of events that requires a correct spatial and temporal expression of specific sets of factors that lead to formation of a complex network of capillary plexuses and blood vessels. *Vasculogenesis* refers to the initial events in vascular development in which endothelial cell precursors (angioblasts) migrate, differentiate, and assemble a primitive vascular network. The subsequent process of growth, expansion, and remodeling of primitive vessels into mature vascular tree is referred to as *angiogenesis*.

In the mouse, blood vessels develop early, once hemangioblasts arise from mesodermal cells (Coffin et al., 1991; Hatzopoulos et al., 1998). At E7.5, the extra-embryonic mesodermal cells of the yolk-sac aggregate into clusters forming the initial blood islands with endothelial and hematopoietic precursors defined by shared expression of CD34, CD31, and Flk-1 (VEGF receptor 1) (Shalaby et al., 1997). Shortly thereafter, blood islands segregate, with endothelial precursors lining spaces containing the hematopoietic progenitors. In contrast, the intra-embryonic vessels form in paraxial mesoderm directly from random endothelial precursor cells/angioblasts expressing Flk-1 and SCL/TAL-1 (Drake et al., 1997). The angioblasts proliferate locally and interconnect in a loose meshwork that undergoes both cranio-caudal and dorso-ventral progression into a primary vascular plexus of cells expressing CD31, CD34 and Tie-2 receptor.

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Later, at E8.0, the paired dorsal aortas are visible, and at E9.0, the heart starts to pump regularly and forms together with the major blood vessels the first functioning organ system in the embryo.

2. Endothelium and blood vessel formation

Studies over the last decade have elucidated roles of many signaling pathways in vascular developments. While a detailed analysis of this complex subject is beyond the scope of this review, we will concentrate on genes involved in VEGF signaling pathways, including VEGF-A itself, its receptors, Flt-1 (VEGF-R1) and Flk-1/KDR (VEGF-R2), and other related genes including neuropilins 1 and 2 and VE-cadherin, as well as genes involved in vessel wall maturation and arterial/venous specification.

The endothelial cell-specific mitogen vascular endothelial growth factor (VEGF) and its two receptors, Flt-1 and Flk-1/KDR, are involved in all morphogenic events associated with blood vessel formation in the mouse embryo. However, despite overlapping affinities of Flk-1 and Flt-1 for VEGF, targeted disruption of either receptor leads to a distinctly different phenotype. Flk-1 deficient mice die between E8.5 and E9.5 as a result of an early defect in the development of haematopoietic and endothelial cells that leads to absence of blood islands in the yolk sac and organized vessels in the embryo (Shalaby et al., 1997; Shalaby et al., 1995). On the other hand, Flt-1 deficient mice die from vascular overgrowth, caused by aberrant endothelial cell division and abnormal vascular channel morphology due to defects in endothelial cell–cell or cell–matrix interactions and reduced vascular sprout formation (Fong et al., 1995; Kearney et al., 2002; Kearney et al., 2004). Flt-1 affinity for VEGF-A is higher than that of Flk-1, and the excessive Flk-1 activation in Flt-$1^{-/-}$ embryonic vessels suggests that Flt-1 primary function may be in modulating Flk-1 activity during blood vessel formation by sequestering VEGF-A (Roberts et al., 2004). Furthermore, the loss of a single VEGF-A allele is lethal, indicating that the concentration of VEGF is an important factor for endothelium proliferation and normal vasculature development (Carmeliet et al., 1996; Ferrara et al., 1996). Finally, deletion of VEGF-A_{165} co-receptors, neuropilin-1 and 2, is lethal at E8.5 due to deficient vessel organization in the yolk sac and the embryo proper (Takashima et al., 2002).

Vascular endothelial (VE)-cadherin plays an essential role in mediating cell–cell recognition in adherens-type junctions. Targeted deletion of VE-cadherin in mice is associated with impairment in endothelial cell survival and angiogenesis that leads to lethality at E9.5 (Carmeliet et al., 1999). Another study has demonstrated that extra-embryonic vasculogenesis is dependent on VE-cadherin activity, whereas intra-embryonic vasculogenesis (i.e. formation of the dorsal aortas) is not (Gory-Faure et al., 1999). However, VE-cadherin is not required for de novo blood vessel formation but rather acts to prevent the disassembly of nascent vessels (Crosby et al., 2005). VE-cadherin is also intimately involved in VEGF signal transduction via regulation of VEGF-R2 activity. VE-cadherin deletion inhibits VEGF-stimulated Akt activation induced by the formation of a VEGF-R2/VE-cadherine/β-catenin/phosphatidylinositol 3-kinase (PI3 kinase) complex (Carmeliet et al., 1999) consistent with VEGF-induced regulatory mechanism of vascular permeability and angiogenesis induction. Therefore, the integration of Flk-1 signaling and VE-cadherin function confers on the endothelium the ability to modulate cell–cell adhesion in response to VEGF role (Crosby et al., 2005; Yamaoka-Tojo et al., 2006).

Recently identified angiomotin (Amot), a receptor for the angiogenesis inhibitor angiostatin, controls vascular reorganization and endothelial cell chemotactic response to VEGF by a yet-unidentified mechanism. Amot deletion results in severe vascular defects in the intersomitic region and dilated capillaries in the brain causing death at E11–E11.5 (Aase et al., 2007). The migratory defect in Amot$^{-/-}$ endothelial cells was linked to a failure in forming front-rear polarity during migration whereas the response in regard to differentiation and proliferation is unaltered (Aase et al., 2007).

Maturation of primitive endothelial tubes into mature blood vessels requires the recruitment of surrounding mesenchymal cells and their differentiation into vascular smooth muscle cells and pericytes. This process is largely mediated by the angiopoietins and their receptor Tie-2, and PDGF.

Among at least four different angiopoietins identified, Ang-1 activates Tie-2 kinase and promotes vessel maturation and stabilization, whereas Ang-2 binds Tie-2 without activating it, supporting endothelial sprouting. Homozygote deletion of Tie-2 or Ang-1 is lethal before E10.5 as a result of defective vascular network formation and abnormalities in the heart development (Dumont et al., 1994; Suri et al., 1996). On the other hand, transgenic overexpression of Ang-2 is as lethal as a Tie-2 deletion (Maisonpierre et al., 1997). This suggests that vessel maturation/remodeling via Tie-2 receptor activity is tightly regulated by a delicate balance between positive and negative control.

Mural cells associated with newly formed vessels can, in turn, control endothelium proliferation, morphology, and microvessel architecture. Lack of pericytes in PDGF-B and PDGFR-β knockout mice results in endothelial hyperplasia, increased capillary diameter, abnormal endothelial shape and ultrastructure, and increased permeability. Thus, although pericytes deficiency has an early effect on endothelial cell number, the subsequent increased VEGF-A expression may be responsible for the increased vascular permeability which contributes to the edematous phenotype observed at later time during the embryo's gestation (Hellstrom et al., 2001).

TGF-β signaling is essential for vascular smooth muscle cell differentiation. Endothelial specific deletion of the TGF-β type II receptor or its other receptor, ALK5, disrupts TGF-β signaling resulting in endothelium failure to promote smooth muscle cell recruitment and differentiation (Carvalho et al., 2004). Homozygote deletion of either receptor in endothelial cells leads to defects in yolk-sac vasculogenesis and causes embryonic lethality at E10.5, (Carvalho et al., 2007) whereas a specific deletion of TGF-β type II receptor in VSMC results in vascular defects at a later stage allowing the embryo to survive to E12.5 (Carvalho et al., 2007).

Notch signaling is extensively involved in regulation of artery/vein specification, vessel sprouting and branching, and VSMC differentiation (for review see Gridley, 2007). The role of Notch pathway in regulating the early embryonic vascular development is tangled with that of VEGF-A. In mice, Notch4 receptor and Delta like 4 (Dll4) ligand are specifically expressed by arterial and not by venous endothelial cells. Notch4 receptor is dispensable for vascular development (Krebs et al., 2000), while expression of an activated form of Notch4 within the endothelium causes abnormal vessel structure and patterning (Uyttendaele et al., 2001). However, mice lacking Notch1 and Notch4 exhibit a more severe phenotype associated with angiogenic remodeling than Notch1 homozygous mutant embryos (Krebs et al., 2000). Moreover, heterozygous embryos Dll4$^{+/-}$, similar to VEGF-A$^{+/-}$, demonstrate embryonic lethal haploinsufficiency due to major defects in arterial and vascular development (Duarte et al., 2004; Gale et al., 2004).

2.1. Several transcription factors control endothelium specific gene expression during mouse embryo development

Hypoxia controls VEGF expression by HIF-1α stabilization in active complex with ARNT (aryl hydrocarbon receptor nuclear translocator). ARNT$^{-/-}$ embryonic stem cells fail to induce VEGF expression in response to hypoxia, and null embryos exhibit defective yolk-sac angiogenesis and abnormal development of the vitello-embryonic circulation resulting in lethal fetal wasting at and beyond E9.5 (Maltepe et al., 1997). The defect in blood vessel formation is similar to that reported for VEGF$^{-/-}$. Furthermore, homozygote deletion of von Hippel-Lindau (VHL) factor, involved in HIF-1α ubiquitination, is lethal at E10.5 to E12.5 and associated with defects in placental vasculogenesis (Gnarra et al., 1997). Supposedly, during organogenesis, an increase in tissue mass leads to a