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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, preceeding 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 controling 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.

Keywords

Endothelium; angiogenesis; development; organ size; growth

1. Introduction. 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 extraembryonic 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 intraembryonic 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

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dorso-ventral progression into a primary vascular plexus of cells expressing CD31, CD34 and Tie-2 receptor. 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 demosntrated that extraembryonic vasculogenesis is dependent on VE-cadherin activity, whereas intraembryonic 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 VEGFR2/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 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).

TGFβ signaling is essential for vascular smooth muscle cell differentiation. Endothelial specific deletion of the TGFβ type II receptor or its other receptor, ALK5, disrupts TGFβ 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 TGFβ 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 vascular SMCs 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 Notch 4 exhibit a more severe phenotype associated with angiogenic remodeling than Notch1 homozygous mutant embryos (Krebs et al., 2000). Moreover, heterozygous embryos DII4$^{+/-}$, 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).

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

Hypoxia controls VEGF expression by HIF-1a 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-1a 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 local hypoxic stimulation of VEGF production which in turn stimulates blood vessel development (Maltepe et al., 1997).

VEGF downstream signaling is thought to involve activation of transcription factors in the Ets family. Among Ets transcription family members, Ets1, Ets2, Tel, Erg1, Net, and Fli1 are involved in the transcriptional regulation of endothelial specific genes such as those encoding Tie1 and -2, VEGFR1 and -2, VE-Cadherin, MMPs, urokinase-type plasminogen activator (u-PA), and protease inhibitors maspin and TIMP 1. In the early embryo, Ets1 expression is detected in immature blood islands suggesting that Ets1 is expressed in hemangioblasts, the common progenitors of endothelial cells and erythroid cells. Later, when these blood islands mature, the expression of Ets1 becomes restricted to peripheral endothelial cells (Pardanaud and Dieterlen-Lievre, 1993). Through the embryo's development, Ets1 is detected in endothelial cells during the formation of new blood vessels, and its expression decreases with the increase of layers of mural cells and is no longer detected in endothelial cells of large blood vessels or adult capillaries (Vandenbunder et al., 1989). Despite Ets endothelial expression, its homozygote deletion has no effect on vascular development (Barton et al., 1998). This could be explained by endothelial expression of Erg and Fli which may relay Ets1 activity in $Ets1^{-/-}$ mice. Tel expression is detected from E7.0 in various tissues of the mouse embryo and in the yolk sac. $Tel^{-/-}$ mice are embryonic lethal and die between E10.5–11.5 with defective yolk sac angiogenesis and intra-embryonic apoptosis of mesenchymal and neural cells (Wang et al., 1997). However, hematopoiesis at the yolk sac stage appears unaffected in $Tel^{-/-}$ embryos. The increase in mesenchymal cell apoptosis suggests that Tel plays a critical role in endothelial survival.

The MADS (MCM1, Agamous, Deficiency, Serum-response factor) box transcription factor Myocyte Enhancer Factor 2 (MEF2) plays multiple roles in differentiation and patterning of endothelial cells or smooth muscle cells during vessel formation. Among four MEF2 genes expressed in vertebrates (MEF2a-d), MEF2a and MEF2c are expressed in early embryonic vasculature. In endothelial cells, MEF2C transcriptional activity may be activated directly by Ets transcription factors via an evolutionary conserved transcriptional enhancer, expressed as early as E7.5 (De Val et al., 2004). The presence of an intronic endothelial cell specific enhancer in MEF2c gene identifies MEF2 as a target of Ets factors and establishes a link between these two regulatory pathways. Homozygote deletion of MEF2C gene results in severe vascular abnormalities and lethality by E9.5 (Lin et al., 1998). The vascular malformations in $MEF2C^{-/-}$ embryo were related to the failure of endothelial cells to organize normally into a vascular plexus and the lack of smooth muscle cell differentiation. Interestingly, endocardial defect in $MEF2C^{-/-}$ embryo featured reductions in Ang 1 and VEGFmRNA, suggesting a possible role of MEF2C in regulating Ang1 and VEGF expression in the myocardium (Bi et al., 1999).

Lung Kruppel Like Factor (LKLF), a zinc finger transcription factor, is expressed as early as E9.5 in vascular endothelial cells throughout the developing mouse embryo. $LKLF^{-/-}$ mice display normal angiogenesis, vasculogenesis, and cardiac development. However, $LKLF^{-/-}$ embryos died between E12.5 and E14.5 from severe intra-embryonic and intra-amniotic hemorrhaging as result of the defect in VSMCs and pericyte recruitment (Kuo et al., 1997). Interestingly, PDGF B, Tie1, Tie2, TGF-β, and heparin-binding epidermal growth factor (HB-EGF) were normally expressed. Therefore, hypothetically, it can be assumed that LKLF defines

a distinct transcriptional pathway in which endothelial cells control the assembly of mural cells and concomitant vessel wall stabilization during embryogenesis.

The basic helix-loop-helix (bHLH) transcription factor HAND1 (also called eHAND) is expressed in numerous tissues during development including heart, limbs, neural crest derivatives, and extra-embryonic membranes. Hand1 deficient mice survive to the nine somite stage, at which time they succumb as result of defects in the yolk sac vasculature and vascular smooth muscle recruitment. Interestingly, in these embryos, vasculogenesis occurs but vascular refinement is arrested (Morikawa and Cserjesi, 2004).

3. Endothelium initiates step-wise processes in organ formation

Shortly after cardiovascular system development begins, other organs emerge. Every developing organ is connected to the circulatory system and exposed to a range of vascular signals. Endothelial cells are unique from other inducers of differentiation, such as cardiac mesoderm or notochord, in that they remain associated with the growing organ. This would suggest that the formation of a specific organ is the outcome of a paracrine signal between vascular and nonvascular cells during the organ's formation.

Liver development starts at E8.5 when cardiac mesoderm initiates hepatic cell differentiation in ventral endoderm. After liver specification, mesenchymal cells of the septum transversum surround the liver endoderm and form multilayered epithelium. Endothelium-endoderm interaction exists even before liver morphogenesis as angioblasts aggregate between the thickening hepatic epithelium and the septum transversum mesenchyme. At E9.5, endothelial cells surround the liver endoderm at the time when hepatic cells migrate into the septum transversum and intermingle with endothelial cells. In Flk1-deficient mouse embryos, liver specification exists, but liver morphogenesis fails due to deficient angioblast migration and the lack of endothelial cell maturation. This is not due to secondary effects resulting from the impaired embryonic growth or absence of blood flow, since explanted liver buds cultured *in vitro*, in an embryo tissue explant system that supports liver vasculogenesis, do not grow. The lack of endothelial cells in Flk-$1^{-/-}$ explants specifically affects the outgrowth of the hepatic endoderm and has no effect on the growth of the surrounding fibroblast cells or on the expression of the early liver genes in the endoderm (Matsumoto et al., 2001).

Pancreatic development is initiated at E8.5–E9.5 when dorsal and ventral buds develop from the gut endoderm. The buds express homeobox gene Pdx1 (Pancreas-duodenum homeobox gene 1) and form precisely at locations that maintain close contact with the endothelium of large vessels, the aorta dorsally and the vitelline veins ventrally. At E9.5–E10.5, both dorsal and ventral Pdx1 expressing buds evaginate and insulin expression begins at sites where the dorsal pancreatic endoderm contacts portal vein endothelium (Lammert et al., 2001). Several experiments demonstrate the role of endothelium in pancreatic development. Pdx1 and insulin expression are initiated when isolated dorsal endoderm (E8.5) is recombined in culture with dorsal aorta. However, insulin expression is not induced by recombination with notochord or neural tube, whereas the notochord induces Pdx1 (Lammert et al., 2001). Removal of the dorsal aorta in Xenopus laevis embryos results in the failure of insulin expression (Lammert et al., 2003a). Pancreatic VEGF-A deletion driven by Cre recombinase under Pdx1 promoter confirms that VEGF-A is required for the capillary network formation inside of the islets but not for the pancreatic islet development (Lammert et al., 2003b). Data supports a two-step model: the first step is VEGF-A independent, involving signaling from vessel endothelium to the pancreatic epithelium, and the second step requires VEGF-A as a paracrine signal from the islet to the endothelium (Lammert et al., 2003b).

The developing kidney represents another example of endothelial cell-driven morphogenesis. In mammals, the kidney develops in three stages: pronephros, mesonephros, and metanephros.

The definitive kidney develops from the metanephros starting at E10.5 and becomes functional after birth. In the early kidney development, in addition to the classically recognized inductive interaction between the metanephric mesenchyme and the ureteric bud, recent data identified an interaction between angioblast and mesenchyme. The mesenchyme produces Gdnf (glial cell line derived neurotrophic factor) that stimulates branching and growth of the ureteric bud via Pax2 regulation. Pax2 and Gdnf expression are induced by a yet-unidentified signal from a population of Flk1-expressing cells located in the periphery of the induced mesenchyme and adjacent to the ureteric bud (Gao et al., 2005). It is possible that VEGF-A produced by the mesenchyme mediates angioblast–mesenchyme interaction and plays a role in morphogenesis and nephrogenesis in the early kidney (Gao et al., 2005; Tufro, 2000).

4. Endothelium supports organ formation

Members of the TGF-β superfamily (including TGF-βs, activins, inhibins, and bone morphogenetic proteins BMPs) play key roles in determining embryonic stem cell fate (Kitisin et al., 2007). BMP-2 secreted from the mouse dorsal aorta induces the basic-helix-loop-helix protein MASH1 and differentiation of neural crest-derived neurons. In contrast, TGF-β1 exclusively drives smooth muscle differentiation of neural crest stem cells. BMP-2 and TGF-β1 act by instructing multipotent cells to commit to one lineage rather than selectively by supporting survival of lineage-committed progenitors (Shah et al., 1996). Another endothelial secreted factor, the brain-derived neurotrophic factor (BDNF), supports the neuronal recruitment and survival contributing to establishment and maintenance of the brain architecture (Leventhal et al., 1999). Furthermore, artemin, a member of the glial cell line-derived neurotrophic factor (GDNF) family expressed by smooth muscle cells, acts as a guidance factor for sympathetic fibers to follow blood vessel paths (Honma et al., 2002). Similar reciprocal signal exists between endothelium and myocardium during heart development. Endocardial-to-mesenchymal transformation that underlies the formation of endocardial cushions is regulated negatively by VEGF (Dor et al., 2001) and mediated by TGF-β (Brown et al., 1999).

Although endothelial cells express many factors required for vascular remodeling, a proper blood flow circulation is essential as well. In fact, when heart development is impaired by specific gene deletions, vessel growth in the yolk sac is affected. Knockout mice deficient in atrial myosin chain 2 (Mlc2a), a contractile myofibril component, demonstrate severely diminished atrial contraction and die between E10.5–11.5 as the result of secondary abnormalities in cardiac morphogenesis and angiogenesis (Huang et al., 2003). However, it has been shown that the embryonic heart begins to beat prior to the requirement for convective bulk transportation to deliver oxygen and nutrients to peripheral tissues for growth (Burggren et al., 2000). That raises the hypothesis that early blood flow is necessary not only as a fluid carrier, but as a mechanical force as well. In an attempt to define the role of hemodynamic forces in vessel remodeling, the blood flow in $Mlc2a^{-/-}$ embryos was visualized and quantified by time-lapse confocal imaging. $Mlc2a^{-/-}$ embryos showed abnormal plasma and erythroblast circulation suggesting that the entry of erythroblasts was a key event (Lucitti et al., 2007). Moreover, the increase in viscosity caused by the entry of blood cells into circulation rescued vessel remodeling defects and eNOS expression in low-hematocrit embryos (Lucitti et al., 2007). Shear stress is thought to be sensed by PECAM (CD31), VE-cadherin, and Flk-1, which in turn could activate eNOS, PI3kinases, and integrins. However, it would be difficult to interpret the involvement of mechano-transduction factors by knockout analysis. One example would be $eNOS^{-/-}$ mice that, despite deficiencies in cardiac maturation and function, vascular dysfunction, and mortality, can survive and reproduce (Feng et al., 2002; Zhu et al., 2007).

Blood flow might be associated with a “paintbrush” in its role in shaping a functional vascular architecture when vessel developmental pattern is genetically predetermined. A recent study

showed that haemodynamics, generated by a Pitx2-induced morphological change in the outflow tract control asymmetric development of the aortic arch (Yashiro et al., 2007; Snider and Conway, 2007). At E10.5, the heart shows symmetrical right-left development of branchial arch arteries (1–4 and 6) and equivalent amounts of blood flow through all arteries. By E11.5, these arch arteries undergo asymmetrical remodeling, the fourth and sixth arteries regress on the right side, while on the left side become aortic arch and pulmonary trunk. At this stage, the blood mainly flows through the left arch arteries. The transcription factor Pitx2, asymmetrically expressed in anterior hearts, induces an uneven distribution of blood flow, left vs. right, supporting left-sided formation of aortic arch. The change in hemodynamic induced by Pitx2 was allied with asymmetrical increased expression of PDGF receptor and VEGF receptor 2. As expected, the ablation of unilateral Pitx2 expression impairs asymmetric remodeling resulting in randomized laterality of the aortic arch {Yashiro, 2007 #504}.

Embryonic vessels display a large homogeneous endothelial cell population, both morphologically and by the expression of early vascular markers. Yet, molecular distinctions between endothelial cells with arterial or venous fates exist at very early stages of vascular development (Lawson et al., 2001). However, during organ development, endothelium-bed heterogeneity is more evident as the result of signal-derived tissue microenvironment and endothelial cell plasticity. As an example, heterotopic transplantation experiments showed that abdominal vessels vascularizing grafted neural tissue formed structural and functional features of the blood-brain barrier, and brain blood vessels growing into grafts of peripheral tissue acquire peripheral vascular morphology with fewer tight junctions (Stewart and Wiley, 1981). In isogeneic transplantation models, heart tissue placed in the ear resulted in new vessels into the graft expressing von Willebrand factor, which is normally expressed in heart capillaries but not in ear vessels (Aird et al., 1997).

Another interesting aspect is related to vessel-organ interaction in defining the organ field boundaries and the organ size. Organ progenitors are thought to arise within “organ fields,” embryonic territories typically larger than the regions destined to contribute to the organ. Still, how the field boundaries are established and what determines the alterations of organ field potential is not well understood. Recently, by determining a fate map of the anterior lateral plate mesoderm (ALPM) in zebrafish, the heart forming regions (HFRs) were shown to overlap with the expression of the transcription factor hand2 (Schoenebeck et al., 2007). During heart development, hand-2 expression was associated with cardiac differentiation and mapped the cardiac field while the mesoderm beyond the rostral limit of hand2 expression gave rise to vessel and blood lineage. Therefore, in this regulatory relationship, embryos deficient in vessel and blood specification generates ectopic cardiomyocytes and, consequently, induction of vessel and blood specification represses cardiac specification and delimits the heart field (Schoenebeck et al., 2007).

5. Vascular endothelium controls adult organ size

Recent evidences support the hypothesis that the control of organ size may be angiogenesis-dependent. We will discuss several such examples:

Adipose tissue

Adipose tissue microcirculation is unique within the vascular system because of its capacity to grow or regress throughout the adult life (Crandall et al., 1997). Moreover, the growth of adipose tissue is angiogenesis-dependent. Obese mice treated with angiogenesis inhibitors demonstrated a loss in adipose tissue and weight reduction (Rupnick et al., 2002). The decline of adipose tissue is associated with the decrease of endothelial cell proliferation and increased apoptosis, and the extent of reduction is dose-dependent and varies by mouse strain and angiogenesis inhibitor. Mice with greater percent of body fat (i.e. *ob/ob* mice) lose more fat

proportionally with their fat content. Angiogenesis-dependent adipose tissue growth/regression might be regulated via angiopoietin/Tie 2 kinases pathway. Adipocytes express angiopoietin-1, while adipose endothelial cells express angiopoietin-2 and Tie2 receptor. If Ang 1 activates Tie2 receptor and promotes vessel maturation, Ang 2 is a competitive antagonist supporting vessel remodeling. Angiopoietin-1 expression and Tie2 activation in different weight-modifying conditions are inversely correlated with the rates of change in body weight (independent of the direction: gain or loss) or etiology (angiogenesis inhibitor TNP-470, leptin, or diet restriction) (Dallabrida et al., 2003). On the other hand, expression levels of angiopoietin-2 and Tie2 receptor are stable. Interestingly, expression profiles of Ang1, Ang2, and activation of Tie2 receptor in heart, lung, and kidney were unaffected by treatment with angiogenesis inhibitor TNP-470. Moreover, *ob/ob* mice injected with ang1/pcDNA has reduced rates of weight gain and fat pad weights, regardless of the route of plasmid administration (subcutaneous, intramuscular, and intravenous). These data suggest that tissue plasticity is enabled by maintaining a relatively immature vasculature able to respond to angiogenic factors.

Prostate gland

Growth of the rat prostate gland after castration is regulated by vascular endothelial cells and subsequent testosterone stimulation (Folkman, 1998). Castration induces a regression of the ventral prostate lobe and decreases the total organ weight as result of the reduced endothelial cells proliferation rate and the loss of vascular mass. Testosterone treatment in castrated rats causes a rapid induction of endothelial cell proliferation and vessel growth that normalizes the vascular mass within 2 days (Franck-Lissbrant et al., 1998). In contrast, the growth of glandular epithelium and the prostate itself is induced several days later. Thus, it seems that the growth of vasculature precedes the growth of the glandular epithelium suggesting that vascular growth is essential for organ growth. The testosterone-dependent factors responsible for vasculature growth stimulation are unknown, but factors derived from epithelial cells, mast cells accumulated during first day of treatment, and tissue macrophages could all be involved (Franck-Lissbrant et al., 1998). VEGF-A is expressed only by glandular epithelial cells and its expression is reduced by castration and increased by testosterone. This suggests that the angiogenic effect of testosterone could be mediated by VEGF-A. Several other factors that stimulate angiogenesis like FGF, TGFβ1, and hepatocyte growth factor are all up-regulated by castration and down-regulated by testosterone (Nishi et al., 1996). Finally, in a recent study in mice, testosterone-stimulated prostate growth was inhibited, but not fully prevented, in the presence of a decoy for VEGF-A, a soluble chimeric VEGFR2 (Lissbrant et al., 2004).

Liver

Certain tissues, such as liver, spleen, and kidney, have the ability to regenerate after injury. Unlike kidney or spleen, which may regenerate 40–100% during an 8 week period, the liver regenerates 100% of its lost mass within 8 days (Michalopoulos and DeFrances, 1997). Angiogenic factors such as VEGF and FGF2 are thought to be important for liver sinusoids regeneration (Assy et al., 1999; Baruch et al., 1995; Kraizer et al., 2001). An angiogenic paracrine signal may control hepatic growth since hepatocytes express VEGF but not VEGF receptors, and the peak of VEGF production corresponds to that of VEGF receptors on endothelial cells (Mochida et al., 1996). Furthermore, endothelial cells secrete HGF, a potent hepatocyte mitogen also proangiogenic (Kinoshita et al., 1991). In the rat, systemic administration of FGF2 after two-third partial hepatectomy accelerates hepatic regeneration during 8 post-operative days, which ultimately stops once the liver reaches its original size (Greene et al., 2003). The FGF-induced control of liver size is regulated by the increase of endothelial cell proliferation at day 2–4 post-injury and the increase of endothelial cell apoptosis at days 6–8. Initiation of endothelial cell apoptosis corresponds to the cessation of the regenerative process. In contrast, administration of the angiogenesis inhibitor TNP-40

reduces hepatic regeneration by decreasing endothelial cell proliferation. This model of hepatic regeneration also suggests that the mass of an adult organ may be controlled by the mass of its endothelium.

Heart

Cardiac hypertrophy in response to thyroid hormone induction is associated with a substantial growth of coronary microvessels. In the rat, myocardial capillary growth during thyroxine treatment has a rapid onset and precedes the heart enlargement (Tomanek and Busch, 1998). The mechanism of effect is not clear, but thyroxine treatment may induce up-regulation of FGF2 expression which in turn may be responsible for angiogenesis stimulation (Tomanek et al., 1998).

The link between angiogenesis and myocardial hypertrophy was addressed more directly in another study in which an angiogenic peptide (PR39) conditionally expressed in myocardium induced myocardial hypertrophy in the absence of any external stimuli (Tirziu et al., 2007). PR39 expression for 3 weeks resulted in a significant increase of endothelial cell mass without changes in the heart size or the heart weight/body weight ratio. However, at 6 weeks post induction, there was an approximately 40% increase in heart weight/body weight ratio associated with a normalization of vascular density and upregulation of hypertrophy markers. Moreover, because PR39 itself is not pro hypertrophic, it seems that the enhanced angiogenesis per se leads to myocyte hypertrophy. The observation that a nitric oxide synthase inhibitor, L-NAME, partially suppressed angiogenesis-driven cardiac hypertrophy and restored cardiac function suggests that nitric oxide may be an endothelium-derived hypertrophy modulator (Tirziu et al., 2007). However, this does not necessarily exclude the possibility that other growth factors secreted by endothelial cells also contribute to angiogenesis-induced cardiac growth. These studies, together with previous reports, suggest the existence of reciprocal signals between the myocardium and the vasculature that promote the growth of each other in a paracrine fashion.

Concluding remarks

In summary, it is becoming evidentially clear that the role of endothelium is more than just serving as a permeable barrier between the blood and organs. Rather, it may function as the key regulator of an organ's development and control an organ's size. This other role of the endothelium is just beginning to be appreciated, and much more needs to be done to generate a coherent picture of cross-talk between endothelium and organ's specific tissue cells. A further elucidation of this reciprocal cross-talk may be of particular interest in developing novel therapeutic strategies in which a correctly chosen pro-angiogenic factor(s) triggers a predetermined sequence of events meant to restore the structure and function of a damaged organ.

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