The role of angiogenic growth factors in organogenesis

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ABSTRACT  Angiogenic growth factors are a class of molecules which exert a fundamental role in the process of blood vessel formation. Besides vasculogenic and angiogenic properties, these compounds mediate a complex series of patterning activities during organogenesis. Angiogenic factors cooperate in the growth and development of embryo tissues in a cross-talk between endothelial cells and tissue cells. It is well established that many tissue-derived factors are involved in blood vessel formation, but there is now emerging evidence that angiogenic factors and endothelial cells themselves represent a crucial source of instructive signals to non-vascular tissue cells during organ development. Thus, angiogenic factors and endothelial cell signalling are currently believed to provide fundamental cues for cell fate specification, embryo patterning, organ differentiation and postnatal tissue remodelling. This review article will summarize some of the recent advances in our understanding of the role of angiogenic factors and endothelial cells as effectors in organ formation.

KEY WORDS: angiogenic factor, endothelial cell, vasculogenesis, angiogenesis, signalling pathway, organ formation

Introduction

The vascular system of vertebrates consists of an organized, branched network of arteries, veins and capillaries that penetrates virtually all the tissues of the body. From the developmental point of view, the circulatory system is the first functioning physiological system to emerge during vertebrate embryogenesis in combination with the haematological system (Risau, 1995; Ribatti, 2006). Parallel formation of the cardio-vascular and haematopoietic systems initiates as the size of the embryo increases such that simple diffusion of oxygen and essential molecules is insufficient to guarantee normal cell growth. Thus, formation of the vascular system is crucial to ensure proper growth and differentiation of all tissues of the developing organism because it functions in delivering oxygen and nutrients while removing waste. Apart from granting blood supply, there is increasing evidence that blood vessels are endowed with the ability to provide instructive regulatory signals to surrounding non-vascular target cells during embryo development. This interesting perspective reflects the increased recognition of the significance of the vascular endothelium as a source of developmental cell signalling in early embryo life. Indeed, it is becoming eminently clear that vasculogenesis and angiogenesis play primary determinative roles in patterning developing structures during embryogenesis, primarily through the paracrine actions of endothelial cells. Thus blood vessels are currently recognized as crucial factors in embryo development, organ differentiation as well as postnatal tissue remodelling. A reciprocal influence between organ cells and penetrating tissue vasculature may be postulated. On the one hand, organ instructions to endothelial cells provide cues for vascular development and acquisition of local specialities; on the other hand, vascular signals back to organ cells provide morphogenetic and patterning instructions during organ formation (Coults et al. 2005; Cleaver and Melton, 2003). This review will highlight recent advances on the role of angiogenic growth factors during organogenesis and cell differentiation. It will focus, in particular, on the cross-talk between endothelial cells and distinct populations of developing tissue cells.

Angiogenic growth factors involved in early vascular development

Early in embryo life, vascularization is established by a process called vasculogenesis, which implies the de novo formation of blood vessels from proliferation and differentiation of mesoderm-derived angioblasts into endothelial cells (Risau, 1995). Vascular development begins very early after the initiation of gastrulation with the formation of blood islets in the yolk sac and angioblast precursors in the head mesenchyme and posterior lateral plate mesoderm. Angioblasts give rise initially to the major blood vessels of the trunk, the dorsal aorta and axial vein, as well as the endocardium.

Abbreviations used in this paper: FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor.

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of the heart. Thus, vasculogenesis provides the body with a primary vascular network before the heart even begins to beat. The term angiogenesis "summarizes a set of morphogenic events that expand and fine-tune the initial, more primitive, embryonic vascular network of arterioles, venules and highly branched capillaries to provide efficient blood supply and organ specific vascular functions" (Gerhardt, 2008). Angiogenesis intervenes mostly during later stages of embryogenesis and increases the pre-existing vascular bed through sprouting, bridging and intussusceptive growth. The nascent vasculature formed by vasculogenesis and angiogenesis is stabilized via the recruitment of mural cells, namely pericytes in capillaries and smooth muscle cells in large vessels, and the generation of the extracellular matrix. Then, primitive vessels undergo branching, pruning and specialization to acquire features suitable for the function of each respective organ (Carmeliet, 2003, 2005).

The highly stereotyped architecture of the vascular tree emerges as the result of a combination of genetic and epigenetic factors (Coultas et al., 2005). The specification of angioblasts into arterial or venous lineages is genetically determined and occurs already before the onset of blood circulation. Interestingly, vessel guidance in intersomitic vessels is clearly dependent upon genetic programs as these vessels form before perfusion and independent of oxygen signalling. Oxygen tension and haemodynamic forces are further critical factors in shaping the intricate patterns of local vascular circuits. The primitive vascular labyrinth undergoes progressive expansion, branching and remodelling, leading to differentiation into arterial, venous and capillary channels with precise directional polarity and distinct left/right-side patterning.

Many angiogenic growth factors and signalling pathways are involved in early vascular development as well as organ patterning during embryogenesis. Among these, we will consider the following: vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF)-2, angiopoietins (Angs), transforming growth factor (TGF)-β1, netrins, semaphorins, ephrin, Notch, survivin.

The vascular endothelial growth factor protein family

Probably, the most important tissue factors responsible for angioblast differentiation and tube formation are the members of the VEGF protein family. VEGF intervenes in the very early steps of blood vessel patterning and later modulate endothelial cell maintenance in normal tissues. Remarkably, endothelial cells are a source of VEGF, thus an endothelial cell-mediated autocrine VEGF loop contributes to vascular growth (Yonekura et al., 1999).

The VEGF protein family encompasses the following molecules: VEGF (or VEGF-A), VEGF-B, placental growth factor (PIGF), VEGF-C and VEGF-D and their receptors VEGFR-1, -2 and -3 (Tammela et al., 2005). VEGF has been established as the prime angiogenic molecule during organogenesis, as well as post-natal physiological and pathological angiogenesis. It is the most potent stimulator of endothelial cell proliferation, sprouting, migration and tube formation. It is also a powerful survival factor and permeability factor for endothelial cells. VEGF binds VEGFR-1 and VEGFR-2 as well as the neuropilin-1 and -2 receptors for semaphorins. Early in mouse embryo, it is expressed at sites of active vasculogenesis and angiogenesis, whereby it directs the migration of VEGFR-1 and VEGFR-2 positive cells (Ferrara et al., 2003). A series of at least six VEGF isoforms has been described which differ for tissue diffusion properties and binding to extracellular matrix. Thus, VEGF_{121} is diffusible, VEGF_{189} binds to the matrix, VEGF_{165} has an intermediate profile, VEGF_{120} being most diffusible acts over a long range and VEGF_{186} over a short range (Ruhrborg et al., 2002). Differential sequestration of different VEGF isoforms in the extracellular matrix is crucial for the balance between capillary branching and enlargement of vessel size. VEGF is a key regulator of both vasculogenesis and angiogenesis. Homozygous VEGF knockout mice as well as mice lacking a single VEGF allele die in utero during the early developmental stages due to defects in blood island formation and vascular development (Ferrara et al., 1996). VEGF is strongly induced in hypoxic conditions and stimulates inflammatory cell recruitment as well as angiogenesis-related matrix degradation (Pugh and Ratcliffe, 2003). Although best characterized as a critical mediator of vasculogenesis and angiogenesis, VEGF not only acts on endothelial cells but also binds to a series of other cells. VEGF interacts with haemopoietic stem cells, causing mobilization of blood elements from the bone marrow (Liang et al., 2001). It also promotes monocyte chemoattraction and altered regulation of VEGF results in profound defects in heart development (Stalmans, 2005). VEGF also binds to neurons stimulating neuroprotection. VEGF protects neural cells from hypoxia, facilitates axonal outgrowth, promotes endothelial cell release of neurogenic factors and induces neural stem cell proliferation (Rosenstein and Krum, 2004; Shen et al., 2004; Schanzer et al., 2004). PIGF binds VEGFR-1 and neuropilin-1. This angiogenic molecule does not seem to play a fundamental role in vascular formation during embryonic life as PIGF knockout mice do not express significant phenotype (Nagy et al., 2003). It appears that PIGF is mostly active in post-natal vascular formation. VEGF-B is also a ligand for both VEGFR-1 and neuropilin-1 and, like PIGF, may have subtle angiogenic effects in adult life (Silvestre et al., 2003). VEGF-C acts on the VEGFR-3 and induces selective lymphangiogenesis without accompanying angiogenesis (Karkkainen et al., 2004). Indeed, mice lacking both VEGF-C alleles fail to develop lymphatic vessels.

VEGF-D activates VEGFR-2 and VEGFR-3. It is both angiogenic and lymphangiogenic (Saharinen et al., 2004). VEGFR-1 (Flk-1) and VEGFR-2 (Flik-1) present an overall similar structure. VEGFR-1 binds VEGF, VEGF-B and PIGF with high affinity. VEGFR-2 binds VEGF, VEGF-C and VEGF-D. VEGFR-1 is first expressed in angioblasts and endothelial cells particularly in early developmental stages. VEGFR-2 is the primary receptor transmitting VEGF signals in endothelial cells. It is expressed not only on endothelial cells but also by the primitive endoderm, embryonic angioblasts, osteoblasts and retinal progenitor cells (Ferrara et al., 2003). VEGFR-1 gene targeted mice die at E 8.5 due to disorganization of blood vessels and overgrowth of endothelial cells (Ferrara et al., 2003). VEGFR-2 gene targeted mice die at E 8.5-E 9.5 due to lack of development of the blood islands, embryonic vasculature and haematopoietic cells (Shalabi et al., 1995).

The fibroblast growth factor protein family

FGFs are a family of heparin-binding growth factors. More than twenty structurally-related members of the FGF family have been identified (Presta et al., 2005). FGFs exert their angiogenic activity by interacting with various endothelial cell surface receptors, including the high affinity tyrosine kinase receptors (FGFRs), heparin-sulphate proteoglycans, and integrins. In vitro endothelial cells of different origin express FGFR1 and, under some circumstances,
FGFR2. Although FGFs can stimulate endothelial cells and induce angiogenesis, these cytokines are mitogenic for multiple cell types. FGF-2 is a crucial factor for inducing pluripotent cells of the quail blastodisc to undergo vasculogenesis, and experiments in the chick have suggested that FGF signalling is important for initiation of angioblast specification (Flamme et al., 1997; Cox and Poole, 2000). Apart its critical role in early vessel formation, cytokines of the FGF family play a relevant role in different organogenesis settings, in particular they are involved in controlling branching morphogenesis (Metzger et al., 2008).

**Tie-1, Tie-2 and angiopoietins**

The Angs family consists of Ang-1, -2, and the orthologues Ang-3 in mouse and Ang-4 in human. All Angs are ligand for the receptor tyrosine kinase Tie-2 (also known as TIE) and play a critical role in endothelial sprouting, vessel wall remodelling and mural cell recruitment (Thurston, 2003). Tie-1 is an orphan receptor tyrosine kinase. Ang-1 and-4 activate Tie-1, although Tie-1 does not directly bind these ligands (Saharinen et al., 2005). Ang-1 is produced by pericytes and smooth muscle cells, activates endothelial Tie-2, maximizes interactions between endothelial cells and pericytes and is expressed behind the leading edge of angiogenic vessels, a position consistent with vessel maturation. (Sundberg et al., 2002). Mutation of either Ang-1 or Tie-2 does not affect initial formation of blood vessels but embryos die in mid-gestation with major defects in vascular remodelling and stability (Dumont et al., 1994; Sato et al., 1995). Ultrastructural analysis suggests that Tie-2-knock out blood vessels lack mural cells (Patan, 1998). Knockout embryos lacking Tie-2, display failure of endothelial cell adherence and interaction with perivascular cells and extracellular matrix (Davis and Yancopoulos, 1999). In PDGF-B deficient mice, Ang-1 restored the vascular structure and permeability (Uemura et al., 2002). Ang-1 also counteracts VEGF-induced endothelial leakiness (Thurston et al., 1999). During angiogenesis, Ang-2 is expressed by endothelial cells located at the leading edge of proliferating vessels (Maisonpierre et al., 1997) and acts as a destabilizing factor which is restricted to endothelial cells in areas of vascular remodelling and binds Tie-2 without inducing signal transduction (Maisonpierre et al., 1997). During endothelial activation, Ang-2 is secreted from endothelial cells, where it is stored in Weibel-Palade bodies (Fiedler et al., 2004). In general, the Ang family of angiogenic growth factors play a critical role in endothelial sprouting, vessel wall remodelling, and mural cell recruitment. Tie-2 activation induces vascular remodelling, but also maintains vessel stability and endothelial cell survival.

**The transforming growth factor protein family**

TGF-β1 and TGF-β2 receptors are expressed by endothelial cells and mural cells. Three isoforms of TGF-β have been recognized: TGF-β1, TGF-β2 and TGF-β3. TGF-β plays a crucial role in blood vessel formation. In mice, genetic deletion of the TGF-β signalling pathway leads to animal death in utero due to malformed vascular phenotype (Choi and Ballermann, 1995; Dünker and Kriegstein, 2000). Endothelial cells have been shown to express different TGF-β receptors. Apart from the TGF-βRI receptor, called ALK5, which is expressed on most cell types, endothelial cells possess two unique receptors: endoglin, a TGF-βRIII receptor and ALK1, a TGF-βRII receptor (Lebrin et al., 2005). TGF-β1 is a critical factor in blood vessel construction. Indeed, mural cell specification and proliferation are mediated by TGF-β and platelet-derived growth factor (PDGF)-B expressed by endothelial cells (Betscholtz et al., 2001). When a mesenchymal cell come in contact with endothelial cells, activation of latent TGF-β1 contributes to differentiation of precursor cells into pericytes or smooth muscle cells (Darland and D’Amore, 2001). Mice deficient for endoglin, a TGF-β1 co-receptor, display reduced association with smooth muscle cells and pericytes (Li et al., 1999). TGF-β1 inhibits endothelial cell proliferation and migration, and mice deficient for TGF-β1 signalling components show dilated and irregularly shaped microvessels (Lebrin et al., 2005). Vessels with the slowest rate of endothelial cell proliferation have the greatest association with pericytes.

The bone morphogenic protein (BMP) family is the largest within the TGF-β superfamily of growth factors, which also includes activins, inhibins, myostatin, and others (Massague, 1998). BMPs bind to two major types of membrane-bound serine/threonine kinase receptors, the type I and type II receptors (Wan and Cao, 2005). BMPs are potent osteoblast differentiation factors but they are involved in a number of different embryonic functions.

**Netrins**

Netrins are matrix binding proteins implicated in axon attraction and guidance during nervous system development (Dickson, 2002). Netrins have also been implicated in axon repulsion. This effect is mediated by receptors of the Unc5 family. Recent studies have shown that netrins is also involved in blood vessel development. Netrin1 and Unc5b, one of the four mammalian Unc5b receptors, regulate blood vessel repulsion (Lu et al., 2004). A role for Unc5b in mediating endothelial cell repulsion was confirmed by analysis of the developing intersegmental vessels in zebrafish embryos. Unc5b is expressed in endothelial tip cells. Loss of Unc5b in mice results in aberrant extension of tip cell filopodia and excessive branching of many vessels. During embryogenesis, netrin1 seems to prevent intersegmental vessels from entering adjacent somites by activating Unc5b (Lu et al., 2004).

**Semaphorins**

Semaphorins are a family of cell-associated and secreted proteins that signal through multimeric receptor complexes comprised of plexins, neurophilins and other molecules (Bagri and Tessier-Lavigne, 2002). Membrane-associated semaphorins bind to plexins, whereas secreted semaphorins bind to neuropilins. Genetic studies in *Drosophila* and mice indicate that semaphorin signalling acts as a repulsive cue in axon guidance and neuronal cell migration (Raper, 2000). Neurophilin-1 is the receptor for the soluble ligand semaphorin-3A. In addition to neural-patterning defects, neurophilin-1-deficient embryos also exhibit aberrant cardiovascular formation and defective vessel branching (Kawasaki et al., 1999). Neurphilin-2 is a receptor for semaphorin-3F but also binds VEGF. Several studies indicate that class-3-semaphorins function as inhibitors of angiogenesis. Semaphorin-3A antagonizes the effects of VEGF in an in vitro angiogenesis assay as well as in vivo (Miao et al., 1999; Bates et al., 2003) and both semaphorin-3A and -3F were shown to inhibit vascular remodelling during embryonic development through an effect on integrin-mediated cell adhesion (Serini et al., 2002). Furthermore, it was observed that semaphorin-3F
can inhibit FGF-2- as well as VEGF-induced angiogenesis in vivo (Kessel et al., 2004). Neuropilin-2-null mice survive to adulthood but do not form normal lymphatic vessels and capillaries (Yuan et al., 2002). Several semaphorins and their receptors have also been implicated as direct regulators of vessel guidance and branching. Semaphorin-4D, which interacts with plexinB1, induces endothelial cell migration and tubulogenesis in vitro and stimulates blood vessel formation in vivo (Suchting et al., 2006).

**Ephrins**

Ephrins are axonal guidance molecules that provide repulsive cues for growing axons. A role of ephrin in vascular patterning has also been proposed. Indeed, deletion of ephrin signalling results in a general failure in angiogenic remodelling of the primary vascular plexus and subsequent embryonic lethality at midgestation (Gerety et al., 1999). In addition, interaction between ephrinB2 and its receptor ephB4 are supposed to help establishing and maintaining boundary formation between arteries and veins in the embryonic vasculature (Wang et al., 1998). EphrinB2 expression specifically in the arterial and ephrinB4 receptor in the venous endothelium provide one of the earliest known molecular distinctions between arteries and veins. The ephrin/ephrin receptor pathways are also believed to participate in intersomatic vascular guiding among somites because disruption of ephrin/ephrin receptor signaling functions determines aberrant growth of intersomatic branches into the somites (Heibling et al., 2000). Molecules of the ephrin/ephrin receptor family have been recently implicated in the regulation of lymphatic vessel proliferation (Makinum et al., 2005).

**Notch**

Notch signalling is required for remodelling the primary vascular plexus into the hierarchy of mature vascular beds and maintaining arterial fate (Alva and Iruela-Arispe, 2004). The Notch-3 receptor is highly expressed in pericytes and disruption of Notch-3 signaling in Notch-3-/- mutant mice results in enlarged vessels due to the lack of pericytes (Wang et al., 2007). Immature pericytes express the nerve/glial antigen-2 (NG2) proteoglycan during early stages of angiogenesis and soluble NG2 promotes endothelial cell motility and angiogenesis via engagement of galectin-3 and α3β1 integrin (Fukushi et al., 2004). Both blocking by antibodies as well as knocking out of the gene encoding NG-2 abrogated vascular growth (Ozerdem and Stallcup, 2004). Recent findings suggest that the Notch signalling pathway regulates how endothelial cells respond to VEGF stimulation. Tip cell formation depends on the balance between VEGF and Notch signalling. Disruption of the Notch pathway leads to excessive tip cell specification. Thus, Notch signalling functions to repress tip formation. In this way, endothelial cells in the sprout become stalk cells. Notch signalling thus acts as a negative feed-back mechanism downstream of VEGF to select for single endothelial tip cells at the head of the sprouting capillary.

**Survivin**

Survivin is a small inhibitor of apoptosis proteins. It prevents cell death, promotes cell cycle progression and is highly expressed in proliferating cells, while being barely detectable in quiescent adult tissues (Altieri, 2008). In mice, conditional gene inactivation studies indicate that surviving is essential for brain development, haematopoiesis, angiogenesis and cardiogenesis (Leung et al., 2007; Jiang et al., 2005; Zwerts et al., 2007). In Xenopus laevis, overexpression of one of the two surviving genes induces endothelial cell proliferation (Du Pasquier et al., 2006). During zebrafish development, survivin contributes to normal vasculogenesis, angiogenesis, cardiogenesis, neurogenesis and haematopoiesis (Ma et al., 2007; Delvaeye et al., 2009).

In mice depleted of endothelial cell survivin, embryonic heart development is abnormal, and the mutant endocardial lineage cells cannot support epithelial-mesenchymal transformation (Zwerts et al., 2007). Studies in the zebrafish model also highlight the importance of survivin in heart development (Delvaeye et al., 2009).

**Angiogenic factors and heart development**

In the embryo, the heart develops from the precardiac lateral folds to form the primitive heart tube (Sugy and Markwald, 1996). The cardiac endothelium, which in all vertebrates is continuous with the endothelium of the major blood vessels, derives from endothelial cell precursors, which appear at mouse embryonic day (E) 7. Endothelial cells of the endocardium are critical for heart development. Indeed, several experimental data indicate that endothelial cells of the endocardium take part to heart organogenesis. In the vertebrate embryonic heart, the cushion tissue is of endocardial origin. Starting from E 15, endocardial cells localized within the atrioventricular and conotruncal regions transform from an endothelial to a migratory mesenchymal cell phenotype and invade the underlying cardiac jelly (Icardo, 1989; Markwald et al., 1996). Endothelial/mesenchymal transformation is under control of different factors originating from endocardial cells. TGF-β is one of these factors which play a critical role during early steps of heart development. Changes in TGF-β expression occur in endocardial cells and their mesenchymal progeny which correlate with the onset of cushion formation (Akhurst et al., 1990; Nakajima et al., 1994). TGF-β-3 signalling is capable to stimulate and maintain endothelial/mesenchymal conversion (Ramsdell and Markwald, 1997). Neuregulin-1, a member of epidermal growth factor (EGF) family, is another molecule produced and secreted by endocardial cells, which provides essential signals during heart development (Garratt, 2006). Neuregulin-1 is a growth and differentiation factor that acts via tyrosine kinase receptors of the ErbB family, namely ErbB2 and ErbB4 (Meyer et al., 1997). Mice carrying homozygous mutations in Neuregulin-1, ErbB2 or ErbB4 die around E 10.5 due to embryonic cardiac failure. Heart observation shows lack of trabeculae formation in the ventricular walls and aberrant, slow contraction of the cardiac chambers (Lemke, 1996). Thus, Neuregulin-1 provides an essential paracrine signal to myocardial cells resulting in the formation of a normally trabeculated heart ventricle (Garratt et al., 2003). Also in zebrafish, knock-down expression of Neuregulin-1 determines a drastic perturbation of the developing heart (Milan et al., 2006).

**Angiogenic factors and liver development**

In mammals, the liver anlage develops from the ventral foregut endoderm, a multipotent tissue that also gives rise to the lung, pancreas and thyroid (Gualdi et al., 1996; Wells and Melton, 1999; Zhao and Duncan, 2005). A close structural relationship between
liver organogenesis and the developing hepatic vessels has been proposed in the last decade. Hepatic vessels grow through a combination of angiogenesis and vasculogenesis (Gouysses et al., 2002; Perez-Pomares et al., 2004). In the mouse, generation of the liver bud is concomitant to development of hepatic vasculature, Experimental data indicate that blood vessels, in particular endothelial cells, have an integral part in dictating growth of the hepatic primordium. As early as E 8.5-E 9, prior to liver bud emergence, CD31+ progenitors of endothelial cells or early endothelial cells in the septum transversum closely surround newly specified foregut hepatic endothendrm (Matsumoto et al., 2001). Close interactions occurs between hepatic endoderm and these vascular progenitors, which promote hepatic morphogenesis and delimit the mesenchymal domain into which the liver bud grows. VEGF-R2 homozygous mutant embryos, which lack endothelial cells but still maintain hepatic specification of the foregut endoderm, express defective liver bud outgrowth (Matsumoto et al., 2001). In the absence of endothelial cells, therefore, liver epithelial cells fail to migrate into the surrounding mesenchyme. The signals provided by endothelial cells to promote liver bud morphogenesis are currently unknown but some evidence points to BMP-4 and FGF-8 as potential candidates (Jung et al., 1999; Rossi et al., 2001). In fact, the phenotypes of mice lacking these proteins are similar to the VEGF-R2 mutant phenotype. Liver epithelial cells proliferate within the foregut endoderm in these animals but fail to delaminate into the mesenchyme of the septum transversum.

The assumption that endothelial cells are closely related to early liver development is further supported by the finding that sinusoid endothelial cells supply the hepatocyte mitogen and survival factor hepatocyte growth factor (HGF) (LeCouter et al., 2003).

Angiogenic factors and pancreas development

Pancreas organogenesis is a further confirmation of the crucial role of blood vessel and angiogenic growth factors during embryogenesis (Lammert et al., 2001; Jacquemin et al., 2006). In Xenopus mutant embryos, absence of the dorsal aorta leads to strong reduction or lack of markers indicative of development of the dorsal pancreatic anlage, such as insulin, NeuroD and Pax6 (Lammert et al., 2001). In the mouse, dorsal and ventral pancreatic buds require some different mesodermal inducers and transcriptional effectors (Kumar et al., 2003). Endothelial cell interactions have been shown to be more critical for dorsal than ventral pancreatic development (Yoshitomi and Zaret, 2004). Endothelial cells of the aorta, indeed, are in direct association with Pdx1-positive dorsal endoderm. In VEGF-R2 homozygous null mutant embryos, which lack endothelial cell and a vasculature, Pdx1-positive cells normally appear in the dorsal endoderm (Yoshitomi and Zaret, 2004). However, at the time when a dorsal pancreatic bud normally begins to develop, fewer Pdx1-positive cells are detectable in these animals compared to wild type, and no bud is evident.

A relay pathway has recently been proposed whereby endothelial cells directly support mesenchyme cells which, in turn, stimulate initial differentiation of dorsal pancreatic endoderm (Jacquemin et al., 2006). Indeed, survival of mesenchyme cells which are normally close to the aorta, rostral to the hindgut, is selectively supported by the presence of the aorta (Esnı et al., 2001). Tissue explant assays indicate that aortic endothelial cells support dorsal mesenchyme survival in the absence of circulating factors. Indeed, N-cadherin-deficient mice (Cdh2−/−) exhibit apoptosis of the dorsal mesenchyme and a failure to develop a dorsal pancreatic bud. These embryos also have an abnormal aortic endothelium and deficient blood circulation (Edsbagge et al., 2005).

These data suggest that “patterning and function of blood vessels concomitant with organogenesis may be of general importance for guiding [pancreas] differentiation and morphogenesis” (Edsbagge et al., 2005).

Angiogenic factors and renal development

Renal blood vessels occur by simultaneous vasculogenic and angiogenic mechanisms (Abrahamson et al., 1998). VEGF is one of the critical factors regulating blood vessel formation in kidney anlagen. This cytokine is responsible for orchestrating renal vasculogenesis, glomerulogenesis as well as tubulogenesis (Tufro et al., 1999). Indeed, the coordinated development of a parenchymal and vascular renal architecture is dependent upon VEGF availability in kidney organ culture (Kitamoto et al., 1997). In the renal anlage, glomerular assembly does not occur in the absence of vessels. These data derive from genetic manipulation in zebrashift. The zebrafish mutant cloche, which fails to develop endothelial cells, does not form a normal pronephric glomerulus (Majumdar and Drummond, 1999). In addition, failure of normal glomerulogenesis occurs in neonatal mice treated with a soluble VEGF receptor chimeric protein, which results in an almost complete VEGF inhibition (Gerber et al., 1999a). Homozygous deletion of VEGF specifically in podocytes prevents recruitment and maturation of endothelial cells in the glomerulus (Eremina et al., 2003). Mice with specific ablation of PDFG-B in endothelial cell exhibit impaired glomerulogenesis due to mesangial cell defects (Bjarnegård et al., 2004). Collectively, these results suggest that endothelial cell recruited by VEGF from podocytes are essential in promoting podocyte and mesangial cell maturation leading to a normal glomerular morphogenesis and filtration barrier function (Eremina and Quaggin, 2004).

Angiogenic factors and lung development

There is a close structural and functional relationship between lung bud development and development of lung vascularization. Early stages of lung vascular development involve both angiogenesis and vasculogenesis (de Mello et al., 1997). Experimental procedures preventing blood vessel formation cause profound disruption of lung morphogenesis. Inhibition of lung endothelial cell development, indeed, is accompanied by inhibition of airway epithelial cell differentiation and maturation. Developing lung vessels comes into increasing proximity to the epithelial cells in the periphery of the lung tubules (Burri, 1984). VEGF-A is a central growth factor regulating physiological and pathological lung formation. Recent studies, indeed, have suggested that the VEGF-A/VEGFR-2 mediates a linkage between the processes of branching morphogenesis, development of the vascular system, and morphogenesis of the pulmonary epithelial system. Experiments of targeted deletion of VEGF-A, or VEGFR-2 have demonstrated the involvement of this ligand-receptor system in lung morphogenesis. The lungs from neonatal mice treated with a soluble VEGF receptor chimeric protein, which causes a high degree of VEGF-A neutralization, appear immature and with less complex alveolar structure.
Angiogenic factors and skeletal growth

Literature data suggest that endothelial cells drive mesenchymal stem cells towards an osteoblastic phenotype. Thus, endothelial cells could be considered as "osteoinductive" mediators in coculture models (Grellier et al., 2009a). Therefore, blood vessel invasion of cartilage, which is normally avascular, is the crucial first step in the cascade of events leading to endochondral ossification. Thus, vasculature-driven signals turn out to be essential for correct bone morphogenesis during pre- and post-natal development (Gerber and Ferrara, 2000). Recent studies indicate that endothelial cells could participate at different levels of osteogenesis: as osteoinductive cells by releasing growth factors, by controlling the main three transcription factors required for bone cell differentiation (Dlx 5, cbfa1/Runx2, osterix) and by facilitating a mineralized tissue within a prevascular network (Grellier et al., 2009b). Growth factors produced by endothelial cells include VEGF and BMP-2, vonostrocror endothelin-1 (ET1) and insulin-like growth factor (IGF), which affect the migration and proliferation of osteoblasts and the differentiation of osteoprogenitors cells (Bouletreau et al., 2002; Veillette and von Schroeder, 2004; Fiedler et al., 2006). In 1999, Napoleone Ferrara and co-workers were able to link in a unified conceptual framework the pleiotropic effects of VEGF on bone morphogenesis. This cytokine is a key regulator for i) blood vessel invasion of the epiphysial growth plate, ii) remodelling of hypertrophic cartilage, and iii) ossification of newly formed bone matrix by osteoblasts (trabeculae) (Gerber et al., 1999b). A cross-talk between endothelial cells and bone-forming cells, such as osteoblasts and osteoprogenitor cells, can be postulated in so far as osteoblasts and osteoprogenitors produce high levels of VEGF which promotes angiogenesis and endothelial cell survival. VEGF also influences osteogenic cells because it can promote the recruitment of progenitor cells and their differentiation into osteoblasts possibly by VEGFR-1 activation (Mayr-Wohlfart et al., 2002; Fiedler et al., 2005). Mice deficient for VEGFR-1 exhibit decreased bone volume, mineralizing surface, and mineral apposition rate, and this is accompanied by decreased mineralization of bone marrow stromal cells (Otomo et al., 2007). During endochondral bone formation, in fact, the chondrocytes at the top of the growth plate initially proliferate, then become mature and hypertrophic, and eventually undergo apoptosis, being replaced by mineralization of the extracellular matrix. Hypertrophic chondrocytes are known to secrete angiogenic molecules such as transferrin, FGF-2, MMP-9, VEGF, which recruit blood vessels from the adjacent subchondral bone (Baron et al., 1994; Vu et al., 1998; Horner et al., 1999; Gerber and Ferrara, 2000; Carlevaro et al., 1997, 2000). In mice and primates, blockade of VEGF by intravenously injected soluble VEGF-R proteins almost completely abolishes blood vessel invasion of the epiphysial growth plate. Impaired blood vessel development provokes in turn impaired trabecular bone formation due to unsuccessful induction of apoptotic signals to hypertrophic chondrocytes, and failed recruitment of chondroclasts and osteo-blasts (Gerber et al., 1999b; Maes et al., 2002; Zelzer et al., 2002, 2003). Remarkably, an expansion of the hypertrophic chondrocyte region similar to that reported in VEGF-defective animals can be observed in homozygous mice with a null mutation in the MMP-9...
gene (Vu et al., 1998). The matrix-degrading protease MMP-9 is a potent releaser of angiogenic factors from extracellular matrix. These results provide direct evidence that vascular invasion of cartilage is necessary for proper bone formation.

Angiogenic factors and neural development

There is increasing evidence indicating a close structural and developmental relationship between blood vessels and nerves. This association seems of particular relevance in patterning the peripheral nervous system but contribution in promoting development of central nervous system as well is also to be considered. The molecular mechanisms regulating common wiring of nerves and blood vessels have attracted considerable interest over the past few years. Some common morphogenic signals and mechanisms have been recognized that direct formation of either structures. Indeed, axon-guidance molecules, such as semaphorins, ephrins, Slits and netrins, function also as angiogenic and vessel-guiding factors. Angiogenic growth factors, such as VEGF, express neurogenic potential too. This assumption may explain why in peripheral tissues, blood vessels are often aligned with nerves and display similar branching pattern (Mukouyama et al., 2002). On the one hand, endothelial cells produce signals, such as artemin and neurotrophin 3, that guide axons to track alongside developing vessels (Carmeliet and Tessier-Lavigne, 2005). On the other hand, nerves may also produce signals such as VEGF-A to attract blood vessels and stimulate endothelial cell survival. Thus, mutual guidance in the context of an elegant “self-similarity logic” appears at work in patterning both vascular and neural networks (Guidolin et al., 2011). Both growing vessels and expanding neurons express specialized regions at the tip of their progression front which share some common structural and functional features. In neurons, growth cones project numerous filopodia that actively extend and retract in response to extracellular clues (Gerhardt et al., 2003; Carmeliet and Tessier-Lavigne, 2005). In sprouting vessels, the endothelial tip cells similarly project filopodia that explore the surrounding environment to guide the growth of the nascent vessel. Axon and vascular guidance cues can be divided into attractive or repulsive signals. These molecular signals will determine which direction the extending structure will follow.

Cross-talk between blood vessels and nervous structures has been recognized in both central and peripheral nervous system. In the subgranular zone of hippocampus, an area that maintains an active proliferation throughout adult life, neurogenesis occurs within an “angiogenic niche” (Palmer et al., 2000). Dividing neural cells indeed are intermingled with dividing endothelial cells within dense clusters. This suggests that neurogenesis is intimately associated with a process of active vascular recruitment, development and remodelling. Remarkably, exposure of E 15 embryonic rats to the angiogenesis inhibitor thalidomide results in vascular malformations and abnormal neuronal development in cortical and hippocampal regions (Hallene et al., 2006). In addition, morphological alterations are associated with neuronal hyperexcitability. In co-culture systems, endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells from the mouse cerebral cortex (Shen et al., 2004). Both embryonic (E 10-11) and adult neural stem cells are responsive to soluble factors released by endothelial cells.ENDOTHELIAL CELLS AND ORGANOGENESIS

References


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