Int. J. Dev. Biol. 55: 365-375
doi: 10.1387/ijdb.103214ec

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

The role of angiogenic growth factors in organogenesis

ENRICO CRIVELLATO*

Department of Medical and Morphological Research, Anatomy Section, University of Udine, Italy

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 morphogenic and patterning instructions during organ formation (Coultas *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

***Address correspondence to:** Enrico Crivellato. Department of Medical and Morphological Research, Anatomy Section, P.le Kolbe, 3, I-34100 Udine, Italy. Fax: +39 0432-49-4201. e-mail: enrico.crivellato@uniud.it

Final, author-corrected PDF published online: 26 July 2011.

ISSN: Online 1696-3547, Print 0214-6282

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)-β, 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 (PlGF), 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, $VEGF_{123}$ being most diffusible acts over a long range and $VEGF_{188}$ over a short range (Ruhrberg *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 haemotopoietic 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). PlGF binds VEGFR-1 and neuropilin-1. This angiogenic molecule does not seem to play a fundamental role in vascular formation during embryo life as PlGF knockout mice do not express significant phenotype (Nagy *et al.*, 2003). It appears that PlGF is mostly active in post-natal vascular formation. VEGF-B is also a ligand for both VEGFR-1 and neuropilin-1 and, like PlGF, 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 (Flt-1) and VEGFR-2 (Flk-1) present an overall similar structure. VEGFR-1 binds VEGF, VEGF-B and PlGF 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 orthologes Ang-3 in mouse and Ang-4 in human. All Angs are ligand for the receptor tyrosine kinase Tie-2 (also known as TEK) 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-β and TGF-β 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 Krieglstein, 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-βRi receptor (Lebrin *et al.*, 2005). TGF-β 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 (Betsholtz *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 embryogenic 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, neuropilins 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). Neuropilin-1 is the receptor for the soluble ligand semaphorin-3A. In addition to neural-patterning defects, neuropilin-1-deficient embryos also exhibit aberrant cardiovascular formation and defective vessel branching (Kawasaki *et al.*, 1999). Neuropilin-2 is a receptor for semaphorin-3F but also binds $VEGF_{165}$. 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). Ephrin2B 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 intersomitic vascular guidance among somites because disruption of ephrin/ephrin receptor signalling functions determines aberrant growth of intersomitic branches into the somites (Helbling *et al.*, 2000). Molecules of the ephrin/ephrin receptor family have been recently implicated in the regulation of lymphatic vessel proliferation (Makinen *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 signalling 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 $\alpha 3\beta 1$ 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 precardic 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 endothelial 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 (Garrat *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 (Gouysse *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 endoderm (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 homozigous 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 (Esni *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 zebrafish. 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 glorerulogenesis 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

patterning (Gerber *et al.*, 1999a). In addition, the use of neutralizing antibodies against VEGFR-2 injected intraamniotically impairs fetal lung maturation (Compernolle *et al.*, 2002). Remarkably, injection of anti-flt1 antibodies is ineffective. VEGF-A is secreted by peripheral respiratory epithelial cells at the tip of developing respiratory tubes, creating a VEGF gradient that guides the vascular network to follow and surround the growing bronchi (de Mello *et al.*, 1997; Ng *et al.*, 2001). *In vitro* experiments with embryonic lung cultures treated with an antisense oligodeoxynucleotide to the VEGFR-2 receptor have shown that loss of VEGF function leads to reduced epithelial branching, decreased epithelial and mesenchymal proliferation index as well as downregulation of BMP-4 expression (Del Moral *et al.*, 2006). Remarkably, isolated E 19 rat alveolar type II pneumocytes have been shown to express VEGFR-2 (Raoul *et al.*, 2004). In addition, VEGF-A stimulation increases surfactant protein B transcripts in this experimental system. Recent work by Ahlbrecht *et al.* (2008) has demonstrated in the mouse that VEGFR-2 expression is restricted to the early vascular primitive network from E 12.5 through E 15.5 while VEGFR-2 is detectable in the epithelial system from E 16.5 on and persists there postnatally. At postnatal stages, VEGFR-2 expression is increasingly restricted to individual cells in the alveolar septa. Isolation and *in vitro* cultivation of alveolar epithelial cells confirm VEGFR-2 expression and show VEGF secretion into the supernatant.

The close developmental linkage between vascular development and lung morphogenesis is further demonstrated by hyperexpression experiments of murine VEGF in the respiratory epithelium of transgenic mice. This genetic manipulation causes altered lung vascularization, which is associated with marked disruption of the lung acinar structure (Zeng *et al.*, 1998). The pulmonary architecture in these animals exhibits defect of branching morphogenesis. In mouse embryonic lung cultures, exogenous $VEGF_{164}$, one of the three isoforms generated by alternative splicing of the *Vegf-A* gene, stimulates branching morphogenesis and increases the index of proliferation in both epithelium and mesenchyme (Del Moral *et al.*, 2006). In conclusion, these data indicate that lung morphogenesis is critically dependent on lung neovascularization and VEGF plays a key role in lung patterning (Zeng *et al.*, 1998).

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, vasoconstrictor 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 epiphyseal growth plate, ii) remodelling of hypertrophic cartilage, and iii) ossification of newly formed bone matrix by osteoblasts (trabeculae) (Gerber *et al.*, 1999b). A crosstalk between endothelial cells and bone-forming cells, such as osteoblasts and osteoprogenotor 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 epiphyseal 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 osteoblasts (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

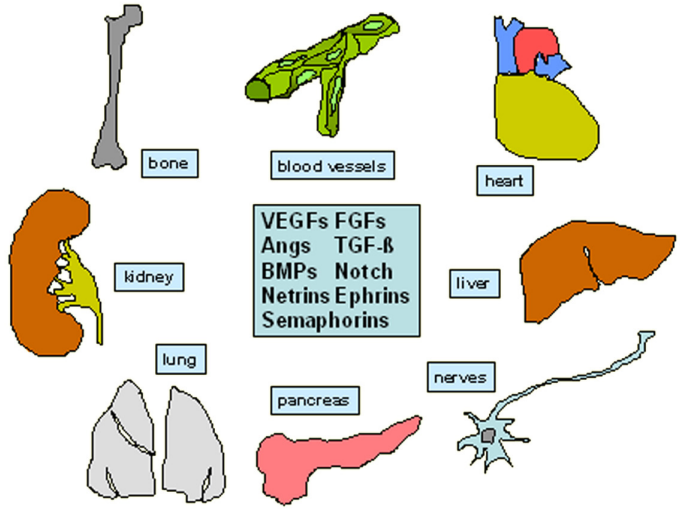

Fig. 1. Angiogenic growth factors are a class of molecules which promote fundamental cues for cell fate specification, embryo patterning, organ differentiation and tissue remodelling during embryo development. *See text for details.*

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. Axonal 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 embryo 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 support development of both projection neurons and interneurons, stimulating neuroepithelial cell contacts and activating Notch and Hes1 to promote neural stem cell self-renewal. Thus, endothelial cells appear as critical components of the neural stem cell niche and emerge as crucial elements for both stem cell maintenance and neurogenic potential.

Endothelial cell signalling has been implicated in the early stages of autonomic neuron development. During early mouse development, the first major arteries formed are the dorsal aorta and the carotid system. Smooth muscle cells surrounding these vessels release diffusible factors that direct growth of sympathetic nerves along these arteries (Glebova and Ginty, 2005; Larrivée *et al.*, 2009). Among these factors, endothelin-3 may be a possible candidate for sympathetic neuron axonal extension along the wall of the external carotid artery (Makita *et al.*, 2008). Artemin and neurotrophin 3 are other molecules secreted by embryonic blood vessels with a role in axon extension along vessels of sympathetic chain ganglia neurons (Honma *et al.*, 2002; Kuruvilla *et al.*, 2004). In avian embryos, the endothelium and enveloping mesenchymal cells of the dorsal aorta express BMP proteins, namely BMP-4 and BMP-7, in the immediate vicinity of the site where the sympathoadrenergic precursor cells aggregate and start to differentiate into adrenergic ganglionic cells. BMP-4 and BMP-7 are able to induce the expression of the adrenergic marker enzyme tyrosine hydroxylase in neural crest cultures (Schneider *et al.*, 1999). BMP-2 induces autonomic cell differentiation in rat neural crest stem cell cultures, which is recognizable by expression of *Mash1*, a transcription factor required for autonomic neurogenesis. Overall, these studies document that endothelial cells provide instructive signals for differentiation of adjacent neural crest cells.

Concluding remarks

In this review article, we have presented the most recent data concerning the role of angiogenic factors as promotors of cell fate specification, embryo patterning, organ differentiation and tissue remodelling during organogenesis (Fig. 1). The majority of these effects are linked to endothelial cells. Endothelial cells release in a paracrine fashion and express to the cell surface many signalling molecules that can affect the destiny of developing tissue cells intimately associated to them. The emerging scenario is that of a general developmental model whereby cross-talk between endothelial cells and tissue cells would be responsible for a series of sequential inductive and differentiating events. Angiogenic factors, in particular the members of the VEGF family, represent molecular effectors which provide pleiotropic effects, being able to influence not only vascular development but also a series of tissue-linked differentiating properties.

Acknowledgements

This work has been supported by local funds from Ministero dell'Istruzione, dell'Università e della Ricerca, Rome, to the Department of Medical and Morphological Research, Anatomy Section, University of Udine.

References

ABRAHAMSON D.R., ROBERT B., HYINK D.P., ST JOHN P.L. and DANIEL T.O. (1998). Origins and formation of microvasculature in the developing kidney. *Kidney Int.* 54: S7-11.

AHLBRECHT K., SCHMITZ J., SEAY U., SCHWARZ C., MITTNACHT-KRAUS R., GAUMANN A., HABERBERGER R.V., HEROLD S., BREIER G., GRIMMINGER

F., SEEGER W. and VOSWINCKEL R. (2008). Spatiotemporal expression of flk-1 in pulmonary epithelial cells during lung development. *Am J Respir Cell Mol Biol* 39: 163-170.

AKHURST R.J., LEHNERT S.A., FAISSNER A. and DUFFIE E. (1990). TGF beta in murine morphogenetic processes: the early embryo and cardiogenesis. *Development* 108: 645-656.

ALTIERI D.C. (2008). New wirings in the surviving networks. *Oncogene* 27: 6276-6284.

ALVA J.A. and IRUELA-ARISPE M.L. (2004). Notch signalling in vascular morphogenesis. *Curr Opin Hematol* 4: 278-283.

BAGRI A. AND TESSIER-LAVIGNE M. (2002). Neuropilins as Semaphorin receptors: *in vivo* functions in neural cell migration and axon guidance. *Adv Exp Med Biol* 515: 13-31.

BARON J., KLEIN K.O., YANOVSKI J.A., NOVOSAD J.A., BACHER J.D., BOLANDER M.E. and CUTLER G.B. JR. (1994). Induction of growth plate cartilage ossification by basic fibroblast growth factor. *Endocrinology* 135: 2790-2793.

BATES D., TAYLOR G.I., MINICHIELLO J., FARLIE P., CICHOWITZ A., WATSON N., KLAGSBRUN M., MAMLUK R. AND'NEWGREEN D.F. (2002). Neurovascular congruence results from a shared patterning mechanism that utilizes Semaphorin3A and Neuropilin-1. *Dev Biol* 255: 77-98.

BETSHOLTZ C., KARLSSON L. and LINDAHL P. (2001). Developmental roles of platelet-derived growth factors. *Bioessays* 23: 494-507.

BJARNEGÅRD M., ENGE M., NORLIN J., GUSTAFSDOTTIR S., FREDRIKSSON S., ABRAMSSON A., TAKEMOTO M., GUSTAFSSON E., FÄSSLER R. and BETSHOLTZ C. (2004) Endothelium-specific ablation of PDGFB leads to pericyte loss and glomerular, cardiac and placental abnormalities. *Development* 131: 1847-1857.

BOULETREAU P.J., WARREN S.M., SPECTOR J.A., PELED Z.M., GERRETS R.P., GREENWALD J.A. and LONGAKER M.T. (2002). Hypoxia and VEGF up-regulate BMP-2 mRNA and protein expression in microvascular endothelial cells: implications for fracture healing. *Plast Reconstr Surg* 109: 2384-2397.

BURRI P.H. (1984). Fetal and postnatal development of the lung. *Ann Rev Physiol* 46: 617-628.

CARLEVARO M.F., ALBINI A., RIBATTI D., GENTILI C., BENELLI R., CERMELLI S., CANCEDDA R. and CANCEDDA F.D. (1997). Transferrin promotes endothelial cell migration and invasion: implication in cartilage neovascularization. *J Cell Biol* 136: 1375-1384.

CARLEVARO M.F., CERVELLI S., CANCEDDA R. and DESCALZI CANCEDDA F. (2000). Vascular endothelia growth factor (VEGF) in chartilage neovascularization and chondrocyte differentiation: auto paracrine-role during endochondral bone formation. *J Cell Sci* 113: 59-69.

CARMELIET P. (2003). Angiogenesis in health and disease. *Nat Med* 9: 653-660.

CARMELIET P. (2005). Angiogenesis in life, disease and medicine. *Nature* 438: 932-936.

CARMELIET P. and TESSIER-LAVIGNE M. (2005). Common mechanisms of nerve and blood vessel wiring. *Nature* 436: 193-200.

CHOI M.E. and BALLERMANN B.J. (1995). Inhibition of capillary morphogenesis and associated apoptosis by dominant negative mutant transforming growth factor-beta receptors. *J Biol Chem* 270: 21144-21150.

CLEAVER O. and MELTON D.A. (2003) Endothelial signalling during development. *Nat Med* 9: 661-668.

COMPERNOLLE V., BRUSSELMANS K., ACKER T., HOET P., TJWA M., BECK H., PLAISANCE S., DOR Y., KESHET E., LUPU F., NEMERY B., DEWERCHIN M., VAN VELDHOVEN P., PLATE K., MOONS L., COLLEN D. and CARMELIET P. (2002). Loss of HIF-2alpha and inhibition of VEGF impair fetal lung maturation, whereas treatment with VEGF prevents fetal respiratory distress in premature mice. *Nat Med* 8: 702-710.

COULTAS L., CHAWENGSAKSOPHAK K. and ROSSANT J. (2005). Endothelial cells and VEGF in vascular development. *Nature* 438: 937-945.

COX C.M. AND POOLE T.J. (2000). Angioblast differentiation is influenced by the local environment: FGF-2 induces angioblasts and pattern vessel formation in the quail embryo. *Dev Dyn* 218: 371-382.

DARLAND D.C. and D'AMORE P.A. (2001). Cell-cell interactions in vascular development. *Curr Top Dev Biol* 52: 107-149.

DAVIS S. and YANCOPOULOS G.F. (1999). The angiopoietins: yin and yang in angiogenesis. *Curr Top Microbiol Immunol* 273:173-185.

DEL MORAL P.M., SALA F.G., TEFFT D., SHI W., KESHET E., BELLUSCI S. and WARBURTON D. (2006). VEGF-A signalling through Flk-1 is a critical facilitator of early embryonic lung epithelial to endothelial crosstalk and branching morphogenesis. *Dev Biol* 290: 177-188.

DELVAEYE M., DE VRIESE A., ZWERTS F., BETZ I., MOONS M., AUTIERO M. and CONWAY E.M. (2009). Role of the two zebrafish *survivin* genes in vasculo-angiogenesis, neurogenesis, cardiogenesis and haematopoiesis. *BMC Dev Biol* 9: 25.

DE MELLO D.E., SAWYER D., GALVIN N. and REID L.M. (1997). Early fetal development of lung vasculature. *Am J Resp Cell Mol Biol* 16: 568-581.

DICKSON B.J. (2002.) Molecular mechanisms of axon guidance. *Science* 298: 1959-1964.

DÜNKER N. and KRIEGLSTEIN K. (2000). Targeted mutations of transforming growth factor-beta genes reveal important roles in mouse development and adult homeostasis. *Eur J Biochem* 267: 6982-6988.

DU PASQUIER D., PHUNG A.C., YMLAHI-OUAZZANI Q., SINZELLE L., BALLAGNY C., BRONCHAIN O., DU PASQUIER L. and MAZABRAUD A. (2006). Survivin increases vascular development during *Xenopus* ontogenesis. *Differentiation* 74: 344-253.

DUMONT D.J., GRADWOHL G., FONG G.H., PURI M.C., GERTSENSTEIN M., AUERBACH A. AND BREITMAN M.L. (1994). Dominant-negative and targeted null mutations in the endothelial receptor tyrosine kinase, tek, reveal a critical role in vasculogenesis of the embryo. *Genes Dev* 15: 1897-1909.

EDSBAGGE J., JOHANSSON J.K., ESNI F., LUO Y., RADICE G.L. and SEMB H. (2005). Vascular function and sphingosine-1-phosphate regulate development of the dorsal pancreatic mesenchyme. *Development* 132: 1085-1092.

EREMINA V., SOOD M., HAIGH J., NAGY A., LAJOIE G., FERRARA N., GERBER H.P., KIKKAWA Y., MINER J.H. and QUAGGIN S.E. (2003) Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. *J Clin Invest* 111: 707-716.

EREMINA V. and QUAGGIN S.E. (2004). The role of VEGF-A in glomerular development and function. *Curr Opin Nephrol Hypertens* 13: 9-15.

ESNI F., JOHANSSON B., RADICE G.L. and SEMB H. (2001). Dorsal pancreas agenesis in N-cadherin-deficient mice. *Dev Biol* 238: 202-212.

FERRARA N., GERBER H.P. AND LECOUTER J. (2003). The biology of VEGF and its receptors. *Nat Med* 9: 669-676.

FIEDLER J., LEUCHT F., WALTENBERGER J., DEHIO C. and BRENNER R.E. (2005). VEGF-A and PlGF-1 stimulate chemotactic migration of human mesenchymal progenitor cells. *Biochem Biophys Res Commun* 334: 561-568.

FIEDLER J., BRILL C., BLUM W.F. and BRENNER R.E. (2006) IGF-I and IGF-II stimulate direct cell migration of bone-marrow-derived human mesenchymal progenitor cells. *Biochem Biophys Res Commun* 345: 1177-1183.

FIEDLER U., SCHARPFENECKER M., KOIDL S., HEGEN A., GRUNOW V., SCHMIDT J.M., KRIZ W., THURSTON G. and AUGUSTIN H.G. (2004). The Tie-2 ligand angiopoietin-2 is stored in and rapidly released upon stimulation from endothelial cell Weibel-Palade bodies. *Blood* 103: 4150-4156.

FLAMME I., FROLICH T. AND RISAU W. (1997). Molecular mechanisms of vasculogenesis and embryonic angiogenesis. *J Cell Physiol* 173: 206-210.

FUKUSHI J., MAKAGIANSAR I.T. and STALLCUP W.B. (2004). NG2 proteoglycan promotes endothelial cell motility and angiogenesis via engagement of galectin-3 and alpha 3 beta 1 integrin. *Mol Biol Cell* 15: 3580-3590.

GARRATT A.N. (2006). "To erb-B or not to erb-B…" Neuregulin-1/ErbB signalling in heart development and function. *J Mol Cell Cardiol* 41: 215-218.

GARRATT A.N., OZCELIK C. and BIRCHMEIER C. (2003). ErbB2 pathways in heart and neural diseases. *Trends Cardiovasc Med* 13: 80-86.

GERBER H.P. and FERRARA N. (2000). Angiogenesis and bone growth. *Trends Cardiovasc Med* 10: 223-228.

GERBER H.P., HILLAN K.J., RYAN A.M., KOWALSKI J., KELLER G.A., RANGELL L., WRIGHT B.D., RADTKE F., AGUET M. and FERRARA N. (1999a). VEGF is required for growth and survival in neonatal mice. *Development* 126: 1149-1159.

GERBER H.P., VU T.H., RYAN A.M., KOWALSKI J., WERB Z. and FERRARA N. (1999b). VEGF couples hypertrophic chartilage remodelling, ossification and angiogenesis during endochondral bone formation. *Nat Med* 5: 623-628.

GERETY S.S., WANG H.U., CHEN Z.F. and ABDERSON D.J. (1999). Symmetrical mutant phenotypes of the receptor EphB4 and its specific transmembrane ligand sphrin-B2 in cardiovascular development. *Mol Cell* 4: 403-414.

GERHARDT H., GOLDING M., FRUTTIGER M., RUHRBERG C., LUNDKVIST A.,

ABRAMSSON A., JELTSCH M., MITCHELL C., ALITALO K., SHIMA D. and BETSHOLTZ C. (2003). VEGF guides angiogenic sprouting utilizing endothelial tip cell philopodia. *J Cell Biol* 161: 1163-1177.

GERHARDT H. (2008). VEGF and endothelial guidance in angiogenic sprouting. *Organogenesis* 4: 241-246.

GLEBOVA N.O. and GINTY D.D. (2005). Growth and survival signals controlling sympathetic nervous system development. *Annu Rev Neurosci* 28: 191-222.

GOUYSSE G., COUVELARD A., FRACHON S., BOUVIER R., NEJJARI M., DAUGE M.C., FELDMANN G., HÉNIN D. and SCOAZEC J.Y. (2002). Relationship between vascular development and vascular differentiation during liver organogenesis in humans. *J Hepatol* 37: 730-740.

GRELLIER M., BORDENAVE L. and AMEDEE J. (2009a). Cell-to-cell communication between osteogenic and endothelial lineages: implications for tissue engineering. *Trends Biotechnol* 27: 562-571.

GRELLIER M., GRANJA P.L., FRICAIN J.C., BIDARRA S.J., RENARD M., BAREILLE R., BOURGET C., AMÉDÉE J. and BARBOSA M.A. (2009b). The effect of co-immobilization of human osteoprogenitors and endothelial cells within alginate microspheres on mineralization in a bone defect. *Biomaterials* 30: 3271-3278.

GUALDI R., BOSSARD P., ZHENG M., HAMADA Y., COLEMAN J.R. and ZARET K.S. (1996) Hepatic specification of the gut endoderm *in vitro*: cell signalling and transcriptional control. *Genes Dev* 10: 1670-1682.

GUIDOLIN D., CRIVELLATO E. AND RIBATTI D. (2011). The "self-similarity" logic applied to the development of the vascular system. *Dev Biol* 351: 156-162.

HALLENE K.L., OBY E., LEE B.J., SANTAGUIDA S., BASSANINI S., CIPOLLA M., MARCHI N., HOSSAIN M.,ÄBATTAGLIA G. and JANIGRO D. (2006). Prenatal exposure to thalidomide, altered vasculogenesis, and CNS malformations. *Neuroscience* 142: 267-283.

HELBLING P.M., SAULNIER D.M. and BRANDLI A.W. (2000). The receptor tyrosine kinase EphB4 and ephrin-B ligand restrict angiogenic growth of embryonic veins in *Xenopus laevis*. *Development* 127: 269-278.

HONMA Y., ARAKI T., GIANINO S., BRUCE A., HEUCKEROTH R., JOHNSON E. and MILBRANDT J. (2002). Artemin is a vascular-derived neurotropic factor for developing sympathetic neurons. *Neuron* 35: 267-282.

HORNER A., BISHOP N.J., BORD S., BEETON C., KELSALL A.W., COLEMAN N. and COMPSTON J.E. (1999). Immunolocalisation of vascular endothelial growth factor (VEGF) in human neonatal growth plate cartilage. *J Anat* 194: 519-524.

ICARDO J.M. (1989). Changes in endocardial cell morphology during development of endocardial cushion. *Anat Embryol* 179: 443-448.

JACQUEMIN P., LEMAIGRE F.P. and ROUSSEAU G.G. (2003). The Onecut transcription factor HNF-6 (OC-1) is required for timely specification of the pancreas and acts upstream of Pdx-1 in the specification cascade. *Dev Biol* 258: 105-116.

JACQUEMIN P., YOSHITOMI H., KASHIMA Y., ROUSSEAU G.G., LEMAIGRE F.P. and ZARET K.S. (2006) An endothelial-mesenchymal relay pathway regulates early phases of pancreas development. *Dev Biol* 290: 189-199.

JIANG Y., DE BRUIN A., CALDAS H., FANGUSARO J., HAYES J., CONWAY E.M., ROBINSON M. and ALTURA R.A. (2005). Essential role for survivin in early brain development. *J Neurosci* 25: 6962-6970.

JUNG J., ZHENG M., GOLDFARB M. and ZARET K. (1999). Initiation of mammalian liver development from endoderm by fibroblast growth factors. *Science* 284: 1998-2003.

KARKKAINEN M.J., HAIKO P., SAINIO K., PARTANEN J., TAIPALE J., PETROVA T.V., JELTSCH M., JACKSON D.G., TALIKKA M., RAUVALA H., BETSHOLTZ C. and ALITALO K. (2004). Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins. *Nat Immunol* 5: 74-80.

KAWASAKI T., KITSUKAWA T., BEKKU Y., MATSUDA Y., SANBO M., YAGI T., FUJISAWA H. (1999). A requirement for neuropilin-1 in embryonic vessel formation. *Development* 126: 4895-4902.

KESSLER O., SHRAGA-HELED N., LANGE T., GUTMANN-RAVIV N., SABO E., BARUCH L., MACHLUF M. AND NEUFELD G. (2004). Semaphorin-3F is an inhibitor of tumor angiogenesis. *Cancer Res* 64: 1008-1015.

KITAMOTO Y., TOKUNAGA H. and TOMITA K. (1997). Vascular endothelial growth factor is an essential molecule for mouse kidney development: glomerulogenesis and nephrogenesis. *J Clin Invest* 99: 2351-2357.

KUMAR M., JORDAN N., MELTON D. and GRAPIN-BOTTON A. (2003). Signals from lateral plate mesoderm instruct endoderm toward a pancreatic fate. *Dev Biol* 259: 109-122.

KURUVILLA R., ZWEIFEL L.S., GLEBOVA N.O., LONZE B.E., VALDEZ G., YE H. and GINTY D.D. (2004). A neurotropin signalling cascade coordinates sympathetic neuron development through differential control of TrkA trafficking and retrograde signalling. *Cell* 118: 243-255.

LAMMERT E., CLEAVER O. and MELTON D. (2001). Induction of pancreatic differentiation by signals from blood vessels. *Science* 294: 564-567.

LAMMERT E., CLEAVER O. and MELTON D. (2003). Role of endothelial cells in early pancreas and liver development. *Mech Dev* 120: 59-64.

LARRIVÉE B., FREITAS C., SUCHTING S., BRUNET I. and EICHMANN A. (2009). Guidance of vascular development. Lessons from the nervous system. *Circ Res* 104: 428-441.

LEBRIN F., DECKERS M., BERTOLINO P. and TEN DIJKE P. (2005). TGF-beta receptor function in the endothelium. *Cardiovasc Res* 65: 599-608.

LECOUTER J., MORITZ D.R., LI B., PHILLIPS G.L., LIANG X.H., GERBER H.P., HILLAN K.J. and FERRARA N. (2003). Angiogenesis-independent endothelial protection of liver: role of VEGFR1. *Science* 299: 890-893.

LEMKE G. (1996). Neuregulins in development. *Mol Cell Neurosci* 7: 247-262.

LEUNG C.G., XU Y., MULARSKI B., LIU H., GURBUXANI S. and CRISPINO J.D. (2007). Requirements for survivin in terminal differentiation of erythroid cells and maintenance of hematopoietic stem and progenitor cells. *J Exp Med* 204: 1603-1611.

LI D.Y., SORENSEN L.K., BROOKE B.S., URNESS L.D., DAVIS E.C., TAYLOR D.G., BOAK B.B. and WENDEL D.P. (1999). Defective angiogenesis in mice lacking endoglin. *Science* 284: 1534-1537.

LIANG D., CHANG J.R., CHIN A.J., SMITH A., KELLY C., WEINBERG E.S. and GE R. (2001). The role of vascular endothelial growth factor (VEGF) in vasculogenesis, angiogenesis, and haematopoiesis in zebrafish development. *Mech Dev* 108: 29-43.

LU X., LE NOBLE F., YUAN L., JIANG Q., DE LAFARGE B., SUGIYAMA D., BRÉANT C., CLAES F.,ÄDE SMET F., THOMAS J.L., AUTIERO M., CARMELIET P., TESSIER-LAVIGNE M. and EICHMANN A. (2004). The netrin receptor UNC5B mediates guidance events controlling morphogenesis of the vascular system. *Nature* 432: 179-186.

MA A., LIN R., CHAN P.K., LEUNG J.C., CHAN L.Y., MENG A., VERFAILLIE C.M., LIANG R. and LEUNG A.Y. (2007). The role of survivin in angiogenesis during zebrafish embryonic development. *BMC Dev Biol* 7: 50.

MAES C., CARMELIET P., MOERMANS K., STOCKMANS I., SMETS N., COLLEN D., BOUILLON R. and CARMELIET G. (2002). Impaired angiogenesis and endochondral bone formation in mice lacking the vescular endothelial growth factor isoforms Vegf164 and Vegf188. *Mech Dev* 111: 61-73.

MAISONPIERRE P.C., SURI C., JONES P.F., BARTUNKOVA S., WIEGAND S.J., RADZIEJEWSKI C., COMPTON D., MCCLAIN J., ALDRICH T.H., PAPADOPOULOS N., DALY T.J., DAVIS S., SATO T.N. and YANCOPOULOS G.D. (1997). Angiopoietin-2, a natural antagonist for Tie 2 that disrupts *in vivo* angiogenesis. *Science* 277: 55-60.

MAKINEN T., ADAMS R.H., BAILEY J., LU Q., ZIEMIECKI A., ALITALO K., KLEIN R. and WILKINSON G.A. (2005). PDZ interaction site in ephrinB2 is required for the remodelling of the lymphatic vasculature. *Genes & Dev* 19: 397-410.

MAKITA T., SUCOV H.M., GARIEPY C.E., YANAGISAWA M. and GINTY D.D. (2008). Endothelins are vascular-derived axonal guidance cues for developing sympathetic neurons. *Nature* 452: 759-763.

MAJUMDAR A. and DRUMMOND L.A. (1999) Podocyte differentiation in the absence of endothelial cells as revealed in the zebrafish avascular mutant, cloche. *Dev Genet* 24: 220-229.

MARKWALD R., EISENBERG L., EISENBERG C. and SUGI Y. (1996). Epithelial-mesenchymal transformations in early avian heart development. *Acta Anat* 156: 173-186.

MASSAGUE J. (1998). TGF-beta signal transduction. *Annu Rev Biochem* 67: 753-791.

MATSUMOTO K., YOSHITOMI H., ROSSANT J. and ZARET K.S. (2001). Liver organogenesis promoted by endothelial cells prior to vascular function. *Science* 294: 559-563.

MAYR-WOHLFART U., WALTENBERGER J., HAUSSER H., KESSLER S., GUNTHER K.P., DEHIO C., PUHL W. and BRENNER R.E. (2002) Vascular endothelial growth factor stimulates chemotactic migration of primary human osteoblasts. *Bone* 30: 472-477.

MEYER D., YAMAAI T., GARRATT A., RIETHMACHER-SONNENBERG E., KANE D., THEILL L.E. and BIRCHMEIER C. (1997). Isoform-specific expression and function of neuregulin. *Development* 124: 3575-3586.

METZGER R.J., KLEIN O.D., MARTIN G.R. AND KRASNOW M.A. (2008). The branching programme of mouse lung development. *Nature* 453: 745-751.

MIAO H.Q., SOKER S., FEINER L., ALONSO J.L., RAPER J.A. AND KLAGFBRUN M. (1999). Neuropilin-1 mediates collapsin-1/semaphorin III inhibition of endothelial cell motility. Functional competition of collapsin-1 and vascular endothelial growth factor-165. *J Cell Biol* 146: 233-242.

MILAN D.J., GIOKAS A.C., SERLUCA F.C., PETERSON R.T. and MACRAE C.A. (2006). Notch 1b and neuregulin are required for specification of central cardiac conduction tissue. *Development* 133: 1125-1132.

MUKOUYAMA Y.S., SHIN D., BRITSCH S., TANIGUCHI M. and ANDERSON D.J. (2002). Sensory nerves determine the pattern of arterial differentiation and blood vessel branching in the skin. *Cell* 109: 693-705.

NAGY J.A., DVORAK A.M. AND DVORAK H.F. (2003). VEGF-A(164/165) and PlGF: roles in angiogenesis and arteriogenesis. *Trends Cardiovasc Med* 13: 169-175.

NAKAJIMA Y., KRUG E.L. and MARKWALD R.R. (1994). Myocardial regulation of transforming growth factor-beta expression by out-flow tract endothelium in the early embryonic chick heart. *Dev Biol* 165: 615-626.

NG Y.S., ROHAN R., SUNDAY M.E., DE MELLO D.E. and D'AMORE P.A. (2001). Differential expression of VEGF isoforms in mouse during development and in the adult. *Dev Dyn* 220: 112-121.

OTOMO H., SAKAI A., UCHIDA S., TANAKA S., WATANUKI M., MORIWAKI S., NIIDA S. and NAKAMURA T. (2007). Flt-1 tyrosine kinase-deficient homozygous mice result in decreased trabecular bone volume with reduced osteogenic potential. *Bone* 40: 1494-1501.

OZERDEM U. and STALLCUP W.B. (2004). Pathological angiogenesis is reduced by targeting pericytes via the NG2 proteoglycan. *Angiogenesis* 7: 269-276.

PALMER T.D., WILLHOITE A.R. and GAGE F.H. (2000). Vascular niche for adult hippocampal neurogenesis. *J Comp Neurol* 425: 479-494.

PATAN S. (1998). TIE1 and TIE2 receptor tyrosine kinases inversely regulate embryonic angiogenesis by the mechanism of intussusceptive microvascular growth. *Microvasc Res* 56: 1-2.

PEREZ-POMARES J.M., CARMONA R., GONZALEZ-IRIARTE M., MACIAS D., GUADIX J.A. and MUNOZ-CHAPULI R. (2004). Contribution of mesothelium-derived cells to liver sinusoids in avian embryos. *Dev Dyn* 229: 465-474.

PRESTA M., DELL'ERA P., MITOLA S., MORONI E., RONCA R. AND RUSNATI M. (2005). Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. *Cytokine Growth Factor Rev* 16: 159-178.

PUGH C.W. and RATCLIFFE P.J. (2003.) Regulation of angiogenesis by hypoxia: role of the HIF system. *Nat Med* 9: 677-684.

RAMSDELL A.F. and MARKWALD R.R. (1997). Induction of endocardial cushion tissuein the avian heart is regulated, in part, by TGF-β-3-mediated autocrine signalling. *Dev Biol* 188: 64-74.

RAOUL W., CHAILLEY-HEU B., BARLIER-MUR A.M., DELACOURT C., MAITRE B. and BOURBON J.R. (2004). Effects of vascular endothelial growth factor on isolated fetal alveolar type II cells. *Am J Physiol Lung Cell Mol Physiol* 286: L1293-L1301.

RAPER J.A. (2000). Semaphorins and their receptors in vertebrates and invertebrates. *Curr Opin Neurobiol* 10: 88-94.

RIBATTI D. (2006). Genetic and epigenetic mechanisms in the early development of the vascular system. *J Anat* 208: 139-152.

RISAU W. (1995.). Differentiation of the endothelium. *FASEB J* 9: 926-933.

ROSENSTEIN J.M. and KRUM J.M. (2004). New roles for VEGF in nervous tissue – beyond blood vessels. *Exp Neurol* 187: 246-253.

ROSSI J.M., DUNN N.R., HOGAN B.L. and ZARET K.S. (2001). Distinct mesodermal signals, including BMPs from the septum transversum mesenchyme, are required in combination for hepatogenesis from the endoderm. *Genes Dev* 15: 1998-2009.

RUHRBERG C., GERHARDT H., GOLDING M., WATSON R., IOANNIDOU S., FUJISAWA H., BETSHOLTZ C. and SHIMA D.T. (2002). Spatially restricted patterning cues provided by heparin-binding VEGF-A control blood vessel branching morphogenesis. *Genes Dev* 16: 2684-2698.

SAHARINEN P., TAMMELA T., KARKKAINEN M.J. AND ALITALO K. (2004). Lymphatic vasculature: development, molecular regulation and role in tumor metastasis and inflammation. *Trends Immunol* 25: 387-395.

SAHARINEN P., EKLUND L., MIETTINEN J., WIRKKALA R., ANISIMOV A., WINDERLICH M., NOTTEBAUM A., VESTWEBER D., DEUTSCH U., KOH G.Y., OLSEN B.R. and ALITALO K. (2008). Angiopoietins assemble distinct Tie2 signalling complexes in endothelial cell-cell and cell-matrix contacts. *Nat Cell Biol* 10: 527-537.

SATO T.N., TOZAWA Y., DEUTSCH U.,'WOLBURG-BUCHHOLZ K., FUJIWARA Y., GENDRON-MAGUIRE M., GRIDLEY T., WOLBURG H., RISAU W. AND QIN Y. (1995). Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation. *Nature* 376: 70-74.

SCHANZER A., WACHS F.P., WILHELM D., ACKER T., COOPER-KUHN C., BECK H., WINKLER J., AIGNER L., PLATE K.H. and KUHN H.G. (2004). Direct stimulation of adult neural stem cells *in vitro* and neurogenesis *in vivo* by vascular endothelial growth factor. *Brain Pathol* 14: 237-248.

SCHNEIDER C., WICHT H., ENDERICH E., WEGNER M. and ROHRER H. (1999). Bone morphogenic proteins are required *in vivo* fort he generation of sympathetic neurons. *Neuron* 24: 861-870.

SERINI G., VALDEMBRI D., ZANIVAN S., MORTERRA G., BURKHARDT C., CACCAVARI F., ZAMMATARO L., PRIMO L., TAMAGNONE L., LOGAN M., TESSIER-LAVIGNE M., TANIGUCHI M., PÜSCHEL A.W. and BUSSOLINO F. (2003). Class 3 semaphorins control vascular morphogenesis by inhibiting integrin function. *Nature* 424: 391-397.

SHALABY F., ROSSANT J., YAMAGUCHI T.P., GERTSENSTEIN M., WU X.F., BREITMAN M.L. and SCHUH A.C. (1995). Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature* 376: 62-66.

SHEN Q., GODERIE S.K., JIN L., KARANTH N., SUN Y., ABRAMOVA N., VINCENT P., PUMIGLIA K. and TEMPLE S. (2004). Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science* 304: 1338-1340.

SILVESTRE J.S., TAMARAT R., EBRAHIMIAN T.G., LE-ROUX A., CLERGUE M., EMMANUEL F., DURIEZ M., SCHWARTZ B., BRANELLEC D. and LÉVY B.I. (2003). Vascular endothelial growth factor-B promotes *in vivo* angiogenesis. *Circ Res* 93: 114-123

STALMANS I. (2005). Role of the vascular endothelia growth factor isoforms in retinal angiogenesis and DiGeorge syndrome. *Verh K Acad Geneeskd Belg* 67: 229-276.

SUCHTING S., BICKNELL R. AND EICHMANN A. (2006). Neuronal clues to vascular guidance. *Exp Cell Res* 312: 668-675.

SUGY Y. and MARKWALD R.R. (1996). Formation and early morphogenesis of endocardial endothelial precursor cells and the role of endoderm. *Dev Biol* 175: 66-83.

SUNDBERG C., KOWANETZ M., BROWN L.F., DETMAR M. and DVORAK H.F. (2002). Stable expression of angiopoietin-1 and other markers by cultured pericytes: phenotypic similarities to a subpopulation of cells in maturing vessels during later stages of angiogenesis *in vivo*. *Lab Invest* 82: 387-401.

TAMMELA T., ENHOLM B., ALITALO K. AND PAAVONEN K. (2005). The biology of vascular endothelial growth factors. *Cardiovas Res* 65: 550-563.

THURSTON G. (2003). Role of angiopoietins and Tie receptor tyrosine kinases in angiogenesis and lynphangiogenesis. *Cell Tissue Res* 314: 61-68.

THURSTON G., SURI C., SMITH K., MC CLAIN J., SATO T.N., YANCOPOULOS G.D. and MC DONALD D.M. (1999). Leakage-resistant blood vessels in mice transgenically overexpressing angiopoietin-1. *Science* 286: 2511-2514.

TUFRO A., NORWOOD V.F., CAREY R.M. and GOMEZ R.A. (1999). Vascular endothelial growth factor induces nephrogenasis and vasculogenesis. *J Am Soc Nephrol* 10: 2125-2134.

VEILLETTE C.J. and VON SCHROEDER H.P. (2004). Endothelin-1 down-regulates the expression of vascular endothelial growth factor-A associated with osteoprogenitor proliferation and differentiation. *Bone* 34: 288-296.

UEMURA A., OGAWA M., HIRASHIMA M., FUJIWARA T., KOYAMA S., TAKAGI H., HONDA Y., WIEGAND S.J., YANCOPOULOS G.D. and NISHIKAWA S. (2002). Recombinant angiopoietin-1 restores higher-order architecture of growing blood vessels in mice in the absence of mural cells. *J Clin Invest* 110: 1615-1817.

VU T.H., SHIPLEY J.M., BERGERS G., BERGER J.E., HELMS J.A., HANAHAN D., SHAPIRO S.D., SENIOR R.M. and WERB Z. (1998). MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. *Cell* 93: 411-422.

WAN M. and CAO X. (2005). BMP signalling in skeletal development. *Biochem Biophys Res Commun* 328: 651-657.

WANG H.U., CHEN Z.F. and ANDERSON D.J. (1998). Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. *Cell* 93: 741-753.

WANG T., BARON M. and TRUMP D. (2007). An overview of Notch 3 function in vascular smooth muscle cells. *Prog Biophys Mol Biol* 96: 499-509.

WELLS J.M. and MELTON D.A. (1999). Vertebrate endoderm development. *Annu Rev Cell Dev Biol* 15: 393-410.

YONEKURA H., SAKURAI S., LIU X., MIGITA H., WANG H., YAMAGISHI S., NOMURA M., ABEDIN M.J., UNOKI H., YAMAMOTO Y. and YAMAMOTO H. (1999). Placenta growth factor and vascular endothelial growth factor B and C expression in microvascular endothelial cells and pericytes. Implication in autocrine and paracrine regulation of angiogenesis. *J Biol Chem* 274: 35172-35178.

YOSHITOMI H. and ZARET K.S. (2004). Endothelial cell interactions initiate dorsal pancreas development by selectively inducing the transcription factor Ptf1a. *Development* 131: 807-817.

YUAN L., MOYON D., PANDANAUD L., BREANT C., KAKKAINEN M.J., ALITALO K. AND EICHMANN A. (2002). Abnormal lymphatic vessel development in neuropilin 2 mutant mice. *Development* 129: 4797-4806.

ZELZER E., MCLEAN W., NG Y.S., FUKAI N., REGINATO A.M., LOVEJOY S., D'AMORE P.A. and OLSEN B.R. (2002). Skeletal defects in Vegf120/120 mice reveal multiple roles for Vegf in skeletogenesis. *Development* 129: 1893-1904.

ZELZER E., MAMLUK R., FERRARA N., JOHNSON R.S., SCHIPANI E. and OLSEN BR (2003) VEGFA is necessary for chondrocyte survival during bone development. *Development* 131: 2161-2171.

ZENG X., WERT S.E., FEDERICI R., PETERS K.G. and WHITSETT J.A. (1998). VEGF enhances pulmonary vasculogenesis and disrupts lung morphogenesis *in vivo. Dev Dyn* 211: 215-227.

ZHAO R. and DUNCAN S.A. (2005). Embryonic development of the liver. *Hepatology* 41: 956-967.

ZWERTS F., LUPU F., DE VRIESE A., POLLEFEYT S., MOONS L., ALTURA R.A., JIANG Y., MAXWELL P.H., HILL P., OH H., RIEKER C., COLLEN D., CONWAY. SJ. and CONWAY E.M. (2007). Lack of endothelial cell survivin causes embryonic defects in angiogenesis, cardiogenesis, and neural tube closure. *Blood* 109: 4742-4752.