Assembly and Patterning of Vertebrate Blood Vessels

Victoria L. Bautch* and Carrie A. Ambler

The mechanisms that regulate blood vessel assembly at appropriate sites in the organism are poorly understood, yet understanding this regulation is critical to the ability to design therapeutics around vessel production in vivo. Classic embryologic studies have yielded descriptive analyses of vascular pattern formation, and they show that angioblasts and endothelial cells respond to environmental cues to assemble at precise embryologic sites. The present study incorporated a genetic model, the mouse, into these studies to obtain mechanistic information regarding vessel assembly and patterning. The data show that both embryo-derived and stem-cell-derived mouse angioblasts respond to host cues in the avian embryo and pattern properly, and also highlight a critical role for the vascular endothelial cell growth factor signaling pathway in these patterning events. (Trends Cardiovasc Med 2004;14:138–143)

The last several years have seen great progress in our understanding of how endothelial precursor cells called angioblasts differentiate and assemble into vessels at proper places in the embryo. In particular, solidifying the importance of circulating endothelial precursor cells in adult neoangiogenesis (for reviews, see Masuda and Asahara 2003, Rafii and Lyden 2003) has invigorated investigations into regulation of the embryonic angioblast. However, important questions regarding both differentiation and patterning of early endothelial cells remain. For example, the signals that control vascular pattern formation, the developmental stage of the cells that respond to these patterning signals, and how the signals are transduced by the responding cells are largely unknown. Understanding how spatiotemporal aspects of vessel formation are regulated at the molecular and cellular level is critical to the ability to reconstitute vessels in patients. Others and we have developed models to dissect vascular patterning, and these models are currently in use to investigate the assembly and patterning of embryonic angioblasts (Pardanaud et al. 1989, Witting et al. 1995, Cleaver and Krieg 1998, Ambler et al. 2001, Weinstein 2002, Rupp et al. 2003).

Victoria L. Bautch is at the Department of Biology, the Carolina Cardiovascular Biology Center, and the Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA. At the time of this study, Carrie A. Ambler was at the Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA. She is now at Cancer Research UK, Lincoln's Inn Fields, London, United Kingdom.

*Address correspondence to: Victoria L. Bautch, PhD, Dept. of Biology, CB#3280, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA. Tel.: (+1) 919-966-6797; fax: (+1) 919-962-8472; e-mail: bautch@med.unc.edu.

1050-1738/04/$-see front matter

• Overview

Early elegant descriptions of vascular development by Florence Sabin (Sabin 1917 and 1920) and the Clarks (Clark and Clark 1939) set the stage for a model of blood vessel formation that is still useful today (for reviews, see Drake et al. 1998, Poole and Coffin 1989, Risau 1997). Blood vessels form developmentally by two processes—vasculogenesis and angiogenesis. Vasculogenesis is the coalescence and differentiation of mesodermal precursor cells to form vessels, and angiogenesis involves the migration and division of already differentiated endothelial cells by sprouting to form new vasculature. The primary vascular plexus is then remodeled by interactions between endothelial cells and other mesodermal cells—primarily smooth muscle cells and pericytes. Historically, vasculogenesis and angiogenesis were thought to be discrete in space and time. However, recent evidence for circulating endothelial precursor cells that contribute to adult vascularization in disease suggests that both vasculogenesis and angiogenesis may be operative throughout the life of the organism (Asahara et al. 1997). Moreover, the work of several laboratories, including ours, suggests that many vessels form embryologically by some combination of vasculogenesis and angiogenesis (Ambler et al. 2001, Brand-Saberi et al. 1995, Childs et al. 2002, Feinberg and Noden 1991). Thus, these distinctions may represent the ends of a continuum rather than discrete processes, and it is predicted that vascular patterning signals will be shared between embryos and adults. Although primary plexus formation and remodeling are both subject to vascular patterning cues, this review focuses on how the primary vascular plexus is patterned in the embryo.

• Molecular Control of Vascular Development

Vascular development and pathologic blood vessel formation proceed via input to endothelial cells or their precursors, called angioblasts. The best-characterized signaling pathways involve receptor tyrosine kinases, although other classes of signaling inputs are also important (for reviews, see Folkman and D'Amore 1996, Ilan and Madri 1999, Yancopoulos et al. 1998). In avian models, fibroblast growth factor is involved in the induction of angioblasts, whereas vascular endothelial growth factor (VEGF) supports the assembly and patterning of vessels (Poole et al. 2001 #126; Drake et al. 2000 #29]. The

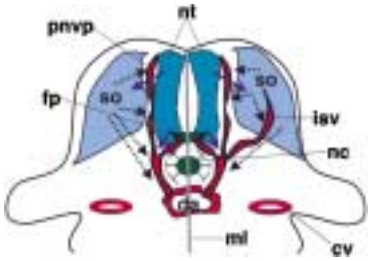

Figure 1. Model of vascular patterning at the axial midline. Schematic of a transverse section through the vertebrate (avian and mouse) trunk at limb level. The neural tube produces a positive signal (dark blue arrows), whereas the notochord produces a restrictive signal to midline crossing (black stop lines), and both act on vessels near the midline. The dotted arrows show hypothetical migration patterns of somitic angioblasts in response to these cues to form midline vessels. cv, cardinal vein; da, dorsal aorta; fp, floor plate; isv, intersomitic vessel; ml, midline; nt, neural tube; nc, notochord; pnvp, perineural vascular plexus; so, somite.

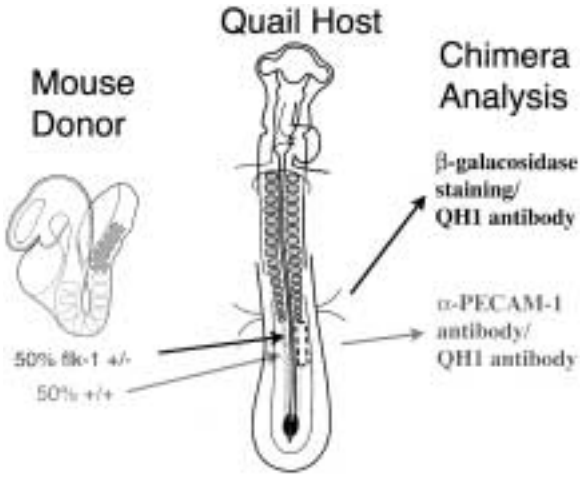

Figure 2. Mouse-avian graft experimental design. Mouse donor embryos (embryonic day 8.5) were from matings with mice carrying lacZ in one copy of the flk-1 locus, and yolk sacs were genotyped after the surgery. Presomitic mesoderm was excised and placed into the presomitic mesoderm cavity of an HH stage 10-12 quail embryo. After incubation for 48 or 72 h, the chimeric embryos were processed for β galactosidase or platelet-endothelial cell-adhesion molecule 1 (PECAM-1) reactivity in whole mount, depending on the genotype, to visualize graft-derived mouse endothelial cells. After sectioning, the sections were reacted with QH1 antibody to visualize host endothelial cells. For more details, see Ambler et al. (2001).

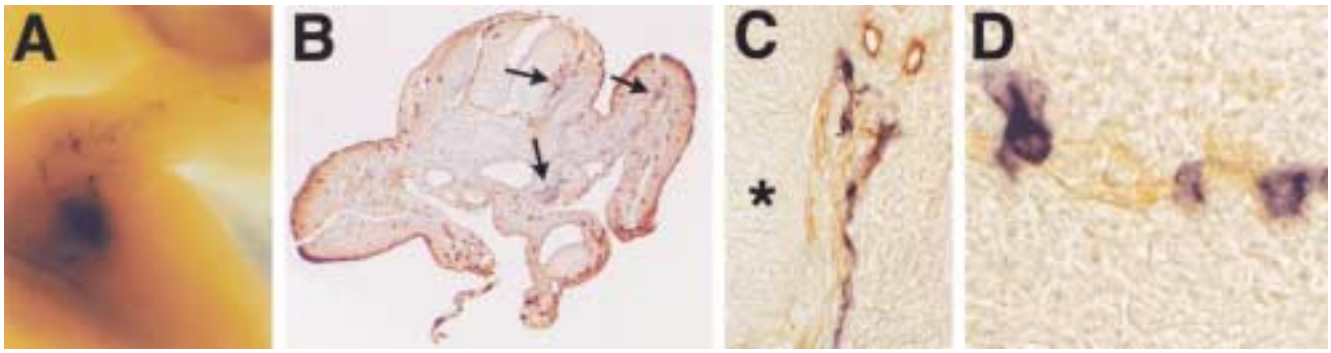

Figure 3.

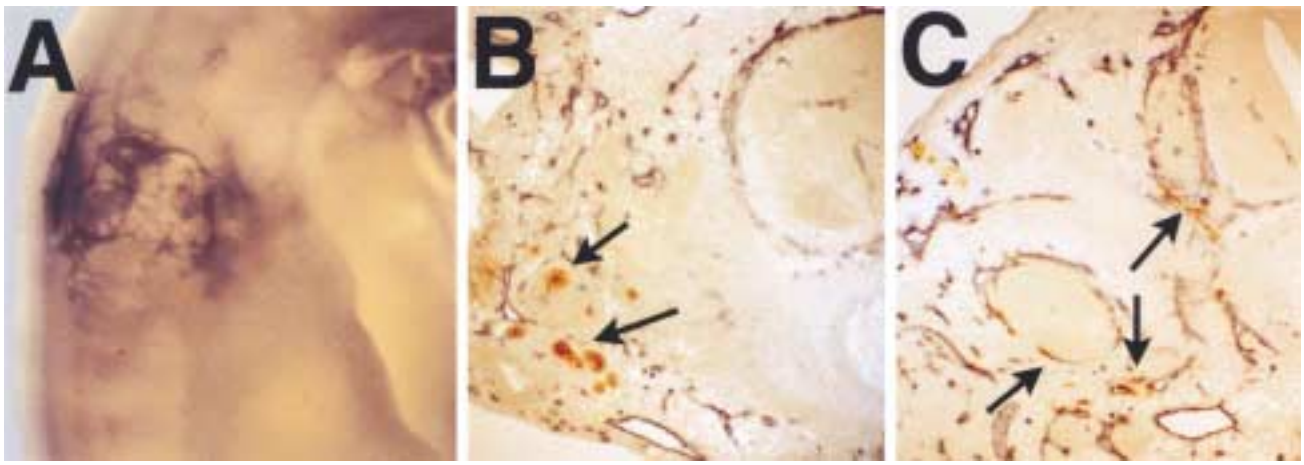

Figure 4.

VEGF signaling pathway consists of multiple related ligands (VEGF-A, VEGF-B, VEGF-C, and placenta growth factor [PlGF]), receptor tyrosine kinases (VEGFR-1, VEGFR-2, and VEGFR-3) and coreceptors (neuropilin-1 and neuropilin-2). VEGF-A interactions with VEGFR-2 and VEGFR-1 are the focus of our current work because they are critical for many early endothelial cell responses such as cell division, filopodia formation, migration, and survival. Moreover, these cellular responses are coordinated in vascular patterning.

Both flk-1 (VEGFR-2) and flt-1 (VEGFR-1) are essential for development, because targeted mutations are embryonically lethal in the mouse. However, the vascular phenotypes are quite distinct (Fong et al. 1995, Shalaby et al. 1995). Analysis of flk-1 null mutant embryos, as well as biochemical and cell culture studies, support the conclusion that flk-1 can mediate the full repertoire of endothelial responses to VEGF (for reviews, see Cross et al. 2003, Ferrara et al. 2003). The role of flt-1 in vascular development is much less clear. Flt-1 null mutant embryos have vascular disorganization and an overabundance of endothelial cells (Fong et al. 1995). Our recent work (Kearney et al. 2002) revealed an endothelial cell proliferation defect in flt-1 mutant embryos and embryonic stem (ES) cell cultures, but the molecular mechanism underlying the cellular response to flt-1 is unclear. Most published work supports one of two models: a splice variant produces a soluble version of the flt-1 extracellular domain (sflt-1) that can bind VEGF, so that this isoform and/or the intact receptor may act as a "sink" to bind VEGF and dampen VEGF signaling through flk-1 (Kendall et al. 1996); or, alternatively, because VEGF signaling through the flt-1 receptor can occur (Waltenberger et al. 1994), flt-1 may inhibit cell division by this mechanism. A recent study (Autiero et al. 2003) implicates flt-1 signaling through another ligand, PlGF, in abrogating myocardial ischemia. However, a "knock-in" mouse lacking most of the cytoplasmic domain of flt-1 is viable, suggesting that flt-1 signaling may not be required for vascular development and patterning (Hiratsuka et al. 1998).

• Vascular Patterning

There is some information on the cellular and molecular processes that control where and when blood vessels form. Genetic analyses in mice and zebrafish suggest that the Notch, ephrin, cadherin, and endothelial differentiation gene (EDG) pathways, as well as unidentified genes such as out-of-bounds, are involved in patterning the primary vascular plexus (Childs et al. 2002, Dorrell et al. 2002; for a review, see Hogan and Kolodziej 2002). Unfortunately, the early lethality of mice carrying VEGF pathway mutations has prevented rigorous genetic evaluation of VEGF signaling in mammalian vascular patterning. However, endothelial cells have morphogenetic properties that are regulated by VEGF, suggesting involvement in vascular patterning. For example, the extension and retraction of filopodia, the formation and dissolution of adhesions and junctions, and the coordination of these events with the overall movements of migration are often cellular responses to patterning cues. Moreover, recent studies link VEGF expression by retinal cells and neurons to local vessel patterning in the retina (Ruhrberg et al. 2002) and limb (Mukuoyama et al. 2002).

Additional evidence that VEGF-A is involved in blood vessel patterning comes from analysis of mice that express only one VEGF isoform (Carmeliet et al. 1999, Ng et al. 2001, Stalmans et al. 2002). VEGF-A is alternatively spliced to give three major isoforms of 120, 164, and 188 amino acids in the mouse. VEGF120 is freely diffusible, VEGF188 is bound to the matrix, and VEGF164 is intermediate in these properties. Analysis of retinal vascularization showed that $VEGF^{120/120}$ mice had fewer vessels that were more dilated and $VEGF^{188/188}$ mice had fewer arterioles that were smaller than normal, whereas $VEGF^{164/164}$ mice were normal (Stalmans et al. 2002). A recent pair of studies (Gerhardt et al. 2003, Ruhrberg et al. 2002) investigated the role of VEGF isoforms on formation of filopodia by endothelial cells, which is often the initiation step for directional migration. In the retina and in the hindbrain, directional filopodia formation required the proper configuration of VEGF-A isoforms, suggesting that local gradients of VEGF-A may be

Figure 3. Mouse graft-derived endothelial cells migrate and pattern properly in quail hosts. (A) Whole-mount view of a chimeric embryo with an flk-$1^{+/-}$ presomitic mesoderm graft. The dark area to the left is the graft, and extensive migration of flk-1^{+} cells is seen in the limb. (B) Transverse section of a chimera with a wild-type presomitic mesoderm graft, stained for platelet-endothelial cell-adhesion molecule 1 (PECAM-1) (purple) and QH1 (brown). Mouse graft-derived endothelial cells (purple) are seen in the perineural vascular plexus (PNVP), the limb, and the kidney rudiment (arrows). (C) Higher magnification of a transverse section of a chimera with a wild-type presomitic mesoderm graft, stained for PECAM-1 (purple) and QH1 (brown). This PNVP has extensive graft-derived contribution (purple), suggesting that graft cells expanded by angiogenesis. The asterisk denotes the neural tube. (D) Vessel in the limb comprised of finely interdigitated graft endothelial cells (PECAM-1, purple) and host endothelial cells (QH1, brown), suggesting that this vessel formed by vasculogenesis. For more details, see Ambler et al. (2001). (Reprinted from Developmental Biology, Ambler CA, Nowicki JL, Burke AC, Bautch VL, Assembly of trunk and limb blood vessels involves extensive migration and vasculogenesis of somite-derived angioblasts, 234:352–364, Copyright 2001, with permission from Elsevier.)

Figure 4. Stem-cell-derived endothelial cells migrate and pattern in the avian host using vascular endothelial growth factor (VEGF) signaling. Partially differentiated embryoid bodies (EBs) were placed in the presomitic mesoderm cavity of quail hosts and incubated for 48 or 72 h. (A) Whole-mount view of a wild-type EB graft in a quail host, stained for platelet-endothelial cell-adhesion molecule 1 (PECAM-1) (purple). Extensive stem-cell-derived vascular cells have migrated and patterned. (B) Transverse section through an flk-$1^{-/-}$ EB graft in a quail host, stained for PECAM-1 (brown) and QH1 (purple). Arrows point to small clumps of presumptive graft-derived angioblasts that have not migrated. (C) Transverse section through a VEGF-$A^{-/-}$ EB graft in a quail host, stained for PECAM-1 (brown) and QH1 (purple). Arrows point to areas of graft-derived vascular contribution in the host, suggesting that host signals can compensate for the lack of EB-derived VEGF-A. For more details, see Ambler et al. (2003). (Reprinted from Developmental Biology, Ambler CA, Schmunk GM, Bautch VL, Stem cell-derived endothelial cells/progenitors migrate and pattern in the embryo using the VEGF signaling pathway, 257:205–219, Copyright 2003, with permission from Elsevier.)

critical to the proper migration that presages patterning in some vascular beds. It should be noted, however, that much early developmental patterning is likely to result from a more diffuse presentation of VEGF. For example, in early development, VEGF signals from endoderm may more uniformly impinge on nascent angioblasts to induce a vascular plexus (for a review, see Rupp et al. 2003). In summary, these findings suggest that both local availability and diffusion of VEGF-A are important for vascular development and patterning.

There is also a body of work focused on vascular patterning in the context of the whole embryo. This work centers on graft experiments that provide both unique spatial information regarding migration of angioblasts, and some evidence that midline signaling centers affect vascular pattern formation. In the midgestation avian and murine embryo, the neural tube, notochord, and dorsal aorta straddle the axial midline in a dorsal-to-ventral configuration (see Figure 1). Signals from the neural tube proper, the notochord, and a specialized part of the ventral neural tube called the floor plate are required to pattern most of the embryonic trunk, including the neural tube and the somites that form alongside the neural tube. The seminal work of Noden established that avian angioblasts were intrinsic to most embryonic tissues and highly migratory (Noden 1989). Subsequent elegant work from several groups using quail-chick chimeras (Pardanaud et al. 1989, Wilting et al. 1995) showed that (1) avian somites are a source of angioblasts that migrate extensively and colonize vascular beds of the trunk and limb, and (2) avian angioblasts are restricted from crossing the axial midline and colonizing the contralateral side of the embryo. Removal of axial structures showed that the notochord was required for the midline barrier (Klessinger and Christ 1996). Although these axial centers produce well-characterized molecular signals (Sonic hedgehog, Wnts, BMPs, and so forth) that are critical to patterning most embryonic structures in the vicinity, the relationship between these signals and vascular patterning information has not been examined. In the frog, VEGF-A provides a long-range midline migration/patterning signal for formation of the dorsal aorta via expression from a structure called the hypochord (Cleaver and Krieg 1998). However, the hypochord is not present in avian and mammalian embryos, and midline vascular patterning signals in these organisms are not identified.

- Vessel Assembly and Patterning Studies in Mouse-Avian Chimeras

Mouse Angioblasts Assemble and Pattern in the Avian Host

To take advantage of the genetic manipulability of the mouse, we examined patterning properties of mouse endothelial cells by analysis of mouse-avian chimeras (Ambler et al. 2001). Briefly, presomitic mesoderm grafts from day 8.5 mouse embryos were placed orthotopically in the avian (quail or chick) host (see Figure 2 for details). Mouse embryos were genetically altered to express lacZ from the flk-1 locus, so that mouse angioblasts/endothelial cells would turn blue on development of the chimeras, or the mouse-specific vascular marker platelet-endothelial cell-adhesion molecule 1 was used. After incubation in ovo for 72 h, chimeric embryos were analyzed for number and location of graft-derived mouse endothelial cells. Representative grafts are shown in Figure 3. Somite-derived endothelial cells migrated extensively and contributed to several vascular beds, including the vessels of the limb, kidney, the cardinal vein, intersomitic vessels, and the blood vessels surrounding the neural tube, called the perineural vascular plexus (Figures 3A–C). These results show that mouse endothelial cells can migrate extensively and respond to avian vascular patterning cues in the avian host.

Because quail angioblasts/endothelial cells can be distinguished from mouse endothelial cells through use of the quail-specific vascular marker QH-1 (Pardanaud et al. 1987), we were in a unique position to investigate the relationship between graft and host endothelial cells. We noted many vessels composed of both mouse and quail endothelial cells (Ambler et al. 2001). In some cases, the mouse component was extensive in a given area, suggesting the possibility that these vessels arose by angiogenesis or expansion of preexisting vessels (Figure 3C). In other vessels, the mouse and quail cells were finely interdigitated, suggesting that these vessels arose from a vasculogenic process (Figure 3D). Thus, it appears that both processes contribute to vessel assembly and patterning in beds colonized by somite-derived endothelial cells, and that both processes are utilized in the same spatial compartment.

Stem Cell-Derived Angioblasts/ Endothelial Cells Migrate and Pattern in the Embryo via VEGF Signaling

We next took advantage of the ability of mouse ES cells to differentiate to form angioblasts/endothelial cells in vitro (Wang et al. 1992). By grafting partially differentiated embryoid bodies into avian hosts, we showed that ES cell-derived angioblasts, which were not programmed in the embryo prior to grafting, essentially behaved as somite-derived angioblasts and responded to avian patterning cues (Ambler et al. 2003). A representative example is shown in Figure 4A. This finding allowed us to test the effects of genetically manipulating the VEGF signaling pathway on vessel patterning (Ambler et al. 2003). Deletion of the VEGF receptor flk-1 eliminated vessel patterning, although the presence of some angioblasts in the grafts suggested that the target population was present (Figure 4B). In contrast, deletion of VEGF in the stem cells did not eliminate vessel development and patterning (Figure 4C). These results show that this pathway is critical to proper vessel patterning, and also show that a signal or signals from the avian can rescue the genetic lesion in VEGF-A.

The Neural Tube Patterns Vessels at the Midline via VEGF-A

The rescue of the VEGF mutant endothelial cells/progenitors in the avian host suggested that embryonic structures emit signals that pattern vessels, and that VEGF-A is a candidate to be a positive patterning signal. Based on the fact that graft-derived endothelial cells were found surrounding the neural tube in 100% of chimeric embryos (Ambler et al. 2001), we hypothesized that the neural tube was the source of a positive patterning signal. Results from a series of embryologic and genetic experiments supported this hypothesis (Hogan et al.

2004). Moreover, we recently set up a three-dimensional explant culture system and used it to show that VEGF-A is an important component of the neural-tube-derived vascular patterning signal (Hogan et al. 2004).

• Concluding Remarks

Previous work by others showed that blood vessels obey patterning cues, and VEGF-A was implicated in at least some patterning pathways. Our work has built on these seminal studies, and we investigated specifically how mouse endothelial cells assemble and pattern in the embryo. We have also implicated the VEGF-A signaling pathway in patterning around the midline in higher vertebrates. However, numerous important questions remain. It is highly likely that VEGF-A cooperates with other signals and pathways to effect vascular patterning; the nature of these additional signals is largely unexplored. It is still unclear which developmental stage(s) of precursors/endothelial cells are competent to respond to vascular patterning cues. Finally, a better understanding of local specificity versus universality in vascular patterning cues is needed. Is one set of vascular patterning cues used for all or most patterning, or do vascular bed-specific patterning signals lend specificity to regional patterning events? Answers to these questions will impact the understanding of the basic processes of blood vessel formation and patterning. An understanding of how vascular pattern is controlled also has multiple ramifications with regard to medical therapies and disease treatments. Specifically, the ability to induce appropriate neovascularization in patients to treat blockage of coronary and other vessels depends on understanding what cues normally pattern vessels, and how endothelial cells and their precursors respond to these cues. Likewise, the medical goal of reconstituting blood vessels outside of the body for grafting purposes will be facilitated with a better understanding of vascular patterning events.

• Acknowledgments

The authors apologize to colleagues whose work could not be cited due to space constraints. They thank the Bautch laboratory members and colleagues of the Carolina Cardiovascular Development Group for informative and lively discussion, and Susan Whitfield for printing. This work was supported by grants from the National Institutes of Health (R01 HL43174 and R21 HL 71993) to V.L.B.

References

Ambler CA, Nowicki JL, Burke AC, Bautch VL: 2001. Assembly of trunk and limb blood vessels involves extensive migration and vasculogenesis of somite-derived angioblasts. Dev Biol 234:352–364.

Ambler CA, Schmunk GM, Bautch VL: 2003. Stem cell-derived endothelial cells/progenitors migrate and pattern in the embryo using the VEGF signaling pathway. Dev Biol 257:205–219.

Asahara T, Murohara T, Sullivan A, et al.: 1997. Isolation of putative progenitor endothelial cells for angiogenesis. Science 275: 964–967.

Autiero M, Waltenberger J, Communi D, et al.: 2003. Role of PlGF in the intra- and intermolecular cross talk between the VEGF receptors Flt1 and Flk1. Nat Med 9:936–943.

Brand-Saberi B, Siefert R, Grim M, et al.: 1995. Blood vessel formation in the avian limb bud involves angioblastic and angiotrophic growth. Dev Dyn 202:181–194.

Carmeliet P, Ng Y-S, Nuyens D, et al.: 1999. Impaired myocardial angiogenesis and ischemic cardiomyopathy in mice lacking the vascular endothelial growth factor isoforms $VEGF_{164}$ and $VEGF_{188}$. Nat Med 5:495–502.

Childs S, Chen J-N, Garrity DM, Fishman MC: 2002. Patterning of angiogenesis in the zebrafish embryo. Development 129:973–982.

Clark ER, Clark EL: 1939. Microscopic observations on the growth of blood capillaries in the living mammal. Am J Anat 64: 251–299.

Cleaver O, Krieg P: 1998. VEGF mediates angioblast migration during development of the dorsal aorta in Xenopus. Development 125:3905–3914.

Cross MJ, Dixelius J, Matsumoto T, Claesson-Welsh L: 2003. VEGF-receptor signal transduction. Trends Biochem Sci 28:488–494.

Dorrell MI, Aguilar E, Friedlander M: 2002. Retinal vascular development is mediated by endothelial filopodia, a preexisting astrocytic template and specific R-cadherin adhesion. Invest Ophthalmal Vis Sci 43: 3500–3510.

Drake CJ, Hungerford JE, Little CD: 1998. Morphogenesis of the first blood vessels. NY Acad Sci 857:155–180.

Drake CJ, La Rue A, Ferrara N, Little CD: 2000. VEGF regulates cell behavior during vasculogenesis. Dev Biol 224:178–188.

Feinberg RN, Noden DM: 1991. Experimental analysis of blood vessel development in the avian wing bud. Anat Rec 231:136–144.

Ferrara N, Gerber HP, LeCouter J: 2003. The biology of VEGF and its receptors. Nat Med 9:669–676.

Folkman J, D'Amore PA: 1996. Blood vessel formation: what is the molecular basis? Cell 87:1153–1155.

Fong GH, Rossant J, Gertsenstein M, Breitman ML: 1995. Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. Nature 376: 66–70.

Gerhardt H, Golding M, Fruttiger M, et al.: 2003. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. J Cell Biol 161:1163–1177.

Hiratsuka S, Minowa O, Kuno J, et al.: 1998. Flt-1 lacking the tyrosine kinase domain is sufficient for normal development and angiogenesis in mice. Proc Natl Acad Sci USA 95:9349–9354.

Hogan BLM, Kolodziej PA: 2002. Molecular mechanisms of tubulogenesis. Nat Rev Genet 3:513–523.

Hogan KA, Ambler CA, Chapman DL, Bautch VL: (2004). The neural tube patterns vessels developmentally using the VEGF signaling pathway. Development 131:1503–1513.

Ilan N, Madri JA: 1999. New paradigms of signaling in the vasculature: ephrins and metalloproteases. Curr Opin Biotech 10: 536–540.

Kearney JB, Ambler CA, Monaco KA, et al.: 2002. Vascular endothelial growth factor receptor Flt-1 negatively regulates developmental blood vessel formation by modulating endothelial cell division. Blood 99: 2397–2407.

Kendall RL, Wang G, Thomas KA: 1996. Identification of a natural soluble form of the vascular endothelial growth factor receptor, FLT-1, and its heterodimerization with KDR. Biochem Biophys Res Commun 226: 324–328.

Klessinger S, Christ B: 1996. Axial structures control laterality in the distribution pattern of endothelial cells. Anat Embryol 193: 319–330.

Masuda H, Asahara T: 2003. Post-natal endothelial progenitor cells for neovascularization in tissue regeneration. Cardiovasc Res 58:390–398.

Mukuoyama Y, Shin D, Britsch S, et al.: 2002. Sensory nerves determine the pattern of arterial differentiation and blood vessel branching in the skin. Cell 109:693–705.

Ng Y-S, Rohan R, Sunday ME, et al.: 2001.

Differential expression of VEGF isoforms in mouse during development and in the adult. Dev Dyn 220:112–121.

Noden DM: 1989. Embryonic origins and assembly of blood vessels. Am Rev Respir Dis 140:1097–1103.

Pardanaud L, Altman C, Kitos P, et al.: 1987. Vasculogenesis in the early quail blastodisc as studied with a monoclonal antibody recognizing endothelial cells. Development 100:339–349.

Pardanaud L, Yassine F, Dieterlen-Lievre F: 1989. Relationship between vasculogenesis, angiogenesis, and haemopoiesis during avian ontogeny. Development 105: 473–485.

Poole TJ, Coffin D: 1989. Vasculogenesis and angiogenesis: two distinct morphogenetic mechanisms establish embryonic vascular pattern. J Exp Zool 251:224–231.

Poole TJ, Finkelstein EB, Cox CM: 2001. The role of FGF and VEGF in angioblast induction and migration during vascular development. Dev Dyn 220:1–17.

Rafii S, Lyden D: 2003. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. Nat Med 9:702–712.

Risau W: 1997. Mechanisms of angiogenesis. Nature 386:671–674.

Ruhrberg C, Gerhardt H, Golding M, et al.: 2002. Spatially restricted patterning cues provided by heparin-binding VEGF-A control blood vessel branching morphogenesis. Genes Dev 16:2684–2698.

Rupp PA, Czirok A, Little CD: 2003. Novel approaches for the study of vascular assembly and morphogenesis in avian embryos. Trends Cardiovasc Med 13:283–288.

Sabin FR: 1917. Origin and development of the primitive vessels of the chick and of the pig. Contrib Embryol Carnegie Inst 6: 61–124.

Sabin FR: 1920. Studies on the origin of the blood vessels and of red blood corpuscles as seen in the living blastoderm of chick during the second day of incubation. Contrib Embryol Carnegie Inst 9: 215–262.

Shalaby F, Rossant J, Yamaguchi TP, et al.: 1995. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature 376:62–66.

Stalmans I, Ng Y-S, Rohan R, et al.: 2002. Arteriolar and venular patterning in retinas of mice selectively expressing VEGF isoforms. J Clin Invest 109:327–336.

Waltenberger J, Claesson-Welsh L, Siegbahn A, et al.: 1994. Different signal transduction properties of KDR and Flt1, two receptors for vascular endothelial growth factor. J Biol Chem 269:26,988–26,995.

Wang R, Clark R, Bautch VL: 1992. Embryonic stem cell-derived cystic embryoid bodies form vascular channels: an in vitro model of blood vessel development. Development 114:303–316.

Weinstein BM: 2002. Plumbing the mysteries of vascular development using the zebrafish. Semin Cell Dev Biol 13: 515–522.

Wilting J, Brand-Saberi B, Huang R, et al.: 1995. Angiogenic potential of the avian somite. Dev Dyn 202:165–171.

Yancopoulos GD, Klagsbrun M, Folkman J: 1998. Vasculogenesis, angiogenesis, and growth factors: ephrins enter the fray at the border. Cell 93:661–664.

PII S1050-1738(04)00014-3 TCM

Shear-Induced Reorganization of Endothelial Cell Cytoskeleton and Adhesion Complexes

Shannon McCue, Sabrena Noria, and B. Lowell Langille*

Endothelial cells undergo profound morphologic changes in response to alterations in shear stresses that are imposed on them by blood flow, and these responses have important implications for physiologic and pathophysiologic function of blood vessels. Shear-induced changes in cell morphology represent a unique mode of cell motility: elongation of the cells in the direction of shear stress is achieved by a reorientation and assembly of F-actin stress fibers at the basal cell surface that ultimately protrudes the upstream and downstream limits of the plasma membrane. Shape change is also accommodated by dramatic reorganization of cell–substrate and cell–cell junctional complexes. Both of these structures are sites of mechanotransduction, which raises intriguing questions concerning how shear-induced regulation of endothelial cell physiology is integrated with cell morphologic responses. (Trends Cardiovasc Med 2004;14:143–143) © 2004, Elsevier Inc.

Shannon McCue, Sabrena Noria, and B. Lowell Langille are at the Toronto General Research Institute, University Health Network and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada.

*Address correspondence to: B. Lowell Langille, PhD, Toronto General Research Institute, 200 Elizabeth Street, NU 113d Toronto, ON M5G 2C4, Canada. Tel.: (+1) 416-340-4717; fax: (+1) 416-340-4287; e-mail: langille@uhnres.utoronto.ca.

1050-1738/04/$-see front matter

Shear stresses produced by blood flow greatly influences vascular development, structure, and physiologic function, primarily through responses that it induces in vascular endothelium. Shear-induced adjustments in vasomotor tone participate in regulation of tissue perfusion and flow regulates growth and remodeling of developing and mature arteries (Langille 1995, Resnick et al. 2003). Shear stress also affects vascular pathologies. The net effect of physiologic levels of shear that are imposed on most regions of large