<Barresi12e, Further Development_c20_10>

Arterial, Venous, and Lymphatic Vessels

Arteries and veins differ substantially from one another even though they are made from the same endothelial precursor cells. Arteries have an extensive coating of smooth muscle and a rich and elastic extracellular matrix. Veins have less extensive musculature and are characterized by valves that direct the flow of blood. A key to our understanding of the mechanism by which veins and arteries form was the discovery that the primary capillary plexus in mice actually contains two types of endothelial cells. The precursors of the arteries contain ephrin B2 in their cell membranes, and the precursors of the veins contain one of the receptors for this molecule, Eph B4 tyrosine kinase, in their cell membranes (Wang et al. 1998). If ephrin B2 is knocked out in mice, vasculogenesis occurs but angiogenesis does not. It is thought that during angiogenesis Eph B4 interacts with its ligand, ephrin B2, in two ways. First, at the borders of the venous and arterial capillaries, it ensures that arterial capillaries connect only to venous ones. Second, in nonborder areas, it ensures that the fusion of capillaries to make larger vessels occurs only between the same type of vessel (Figure 1). As we saw before, the same proteins involved in neural patterning are involved in endothelial patterning.

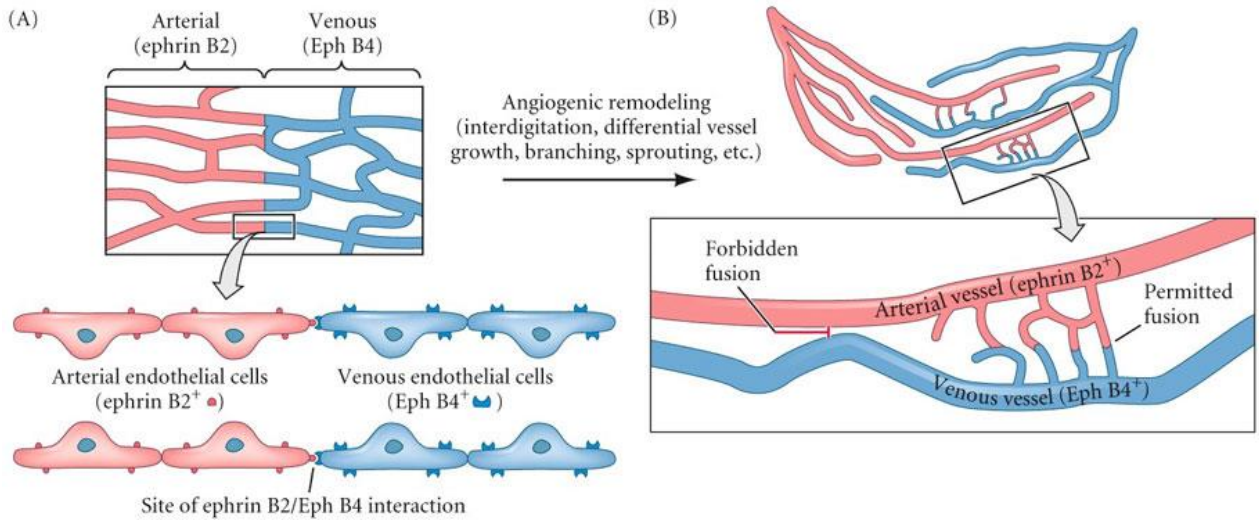

Figure 1 Roles of ephrin and Eph receptors during angiogenesis. (A) Primary capillary plexusproduced by vasculogenesis. The arterial and venous endothelial cells have sorted themselvesout by the presence of ephrin B2 or Eph B4 in their respective cell membranes. (B) A maturingvascular network wherein the ephrin-Eph interaction mediates the joining of small branches (futurecapillaries) and may prevent fusion laterally. (After Yancopoulos et al. 1998.)

In zebrafish, the separation of arterial cells and venous cells occurs very early in development. The angioblasts develop in the posterior part of the lateral plate mesoderm, and they migrate to the midline of the embryo, where they coalesce to form the aorta (artery) and the cardinal vein beneath it (Figure 2). Zhong and colleagues (2001) followed individual angioblasts and found that, contrary to expectations, all the progeny of a single angioblast formed either veins or arteries, never both. In other words, each angioblast was already specified as to whether it would form aorta or cardinal vein. This specification appears to be controlled by the Notch signaling pathway[i] (Lawson et al.

2001, 2002). Repression of Notch signaling resulted in the loss of ephrin B2-expressing arteries and their replacement by veins. Conversely, activation of Notch signaling suppressed venous development, causing more arterial cells to form. Activation of the Notch proteins in the membranes of the presumptive angioblasts causes the activation of the transcription factor Gridlock. Gridlock in turn activates ephrin B2 and other arterial markers, while those angioblasts with low amounts of Gridlock became Eph B4-expressing vein cells.

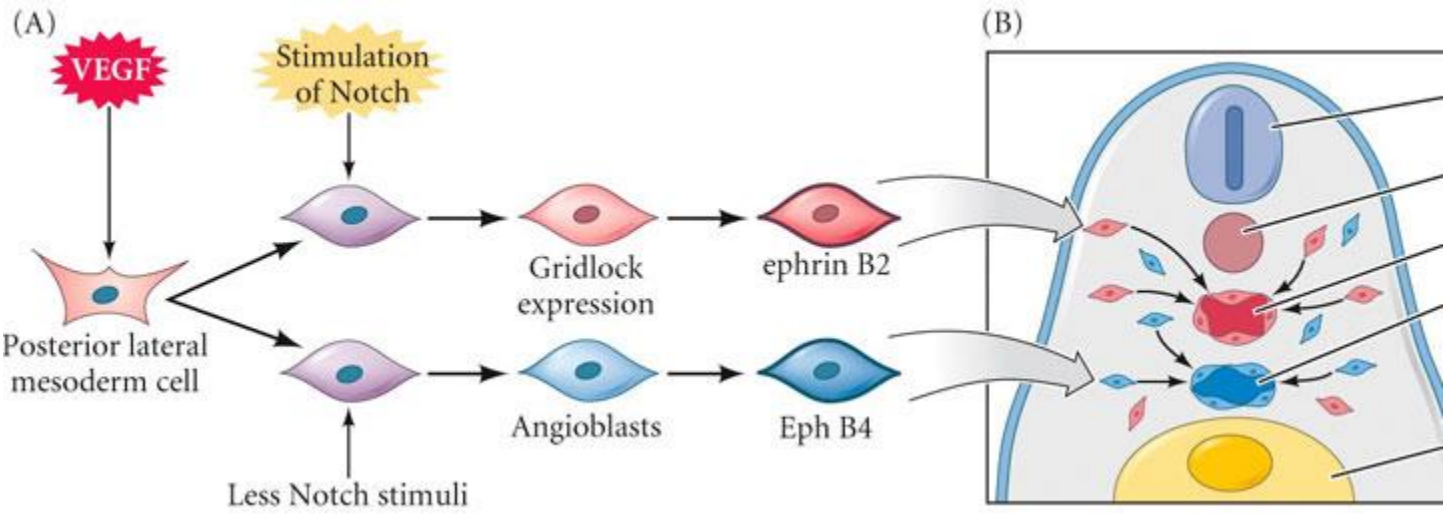

Figure 2 Blood vessel specification in the zebrafish embryo. (A) Angioblasts experiencing activation of Notch upregulate the Gridlock transcription factor. These cells express ephrin B2 and become aorta cells. Those angioblasts experiencing significantly less Notch activation do not express Gridlock, and they become Eph B4-expressing cells of the cardinal vein. (B) Once committed to forming veins or arteries, the cells migrate toward the midline of the embryo and contribute to forming the aorta or cardinal vein.

Weinstein and Lawson (2003) speculate that vascular beds are formed in a two-step process. First, new arteries form in response to VEGF. Second, these arteries then induce neighboring angioblasts (possibly through the ephrin-Eph interactions) to form the venous vessels that will provide the return for the arterial blood. This speculation fits well with the detailed observations of chick vascular development done by Popoff (1894) and Isida (1956). These researchers found that the vitelline arteries appeared first within the capillary network and that these capillaries appeared to induce veins on either side of them.

The lymphatic vessels

In addition to the blood vessels, there is a second circulatory system, the **lymphatic vasculature**. The lymphatic vasculature forms a separate system of vessels that is essential for draining fluid and transporting lymphocytes. In most cases, the development of the lymphatic system commences when a subset of endothelial cells from the jugular vein (in the neck) sprout to form the lymphatic sacs. After the formation of these sacs, the peripheral lymphatic vessels are generated by further sprouting (Sabin 1902; van der Putte 1975). The lymphatic vessels lack both the pericytes and the extracellular matrix that surround the blood vessels, making the lymphatic circulation much more permeable to interstitial fluid (Wang and Oliver 2010). In certain organs, however, there appear to be discrete endothelial progenitor cells specified for generating lymph vessels (Klotz et al 2015; Nicenboim et al 2015; Martinez-Corral et al 2015).

Commitment to the lymphatic lineage appears to be mediated through the **Prox1** transcription factor, which downregulates blood vessel-specific genes and upregulates genes involved in forming

lymphatic vessels (Wigle and Oliver 1999; Wigle et al. 2002; Françoise et al. 2008). Indeed, it appears that the ground state is to make venous blood vessel endothelial cells, and that upon Prox1 expression, these vessels acquire the lymphatic fate (Wigle et al. 2002; Srinivasan et al. 2007). Moreover, if Prox1 expression is downregulated, the lymphatic endothelial cells return to a venous-like condition (Johnson et al. 2008). This makes the lymphatic vessel cell one of the few cell types whose maintenance is dependent on continued expression of a particular gene that initiated its differentiation (Figure 3).

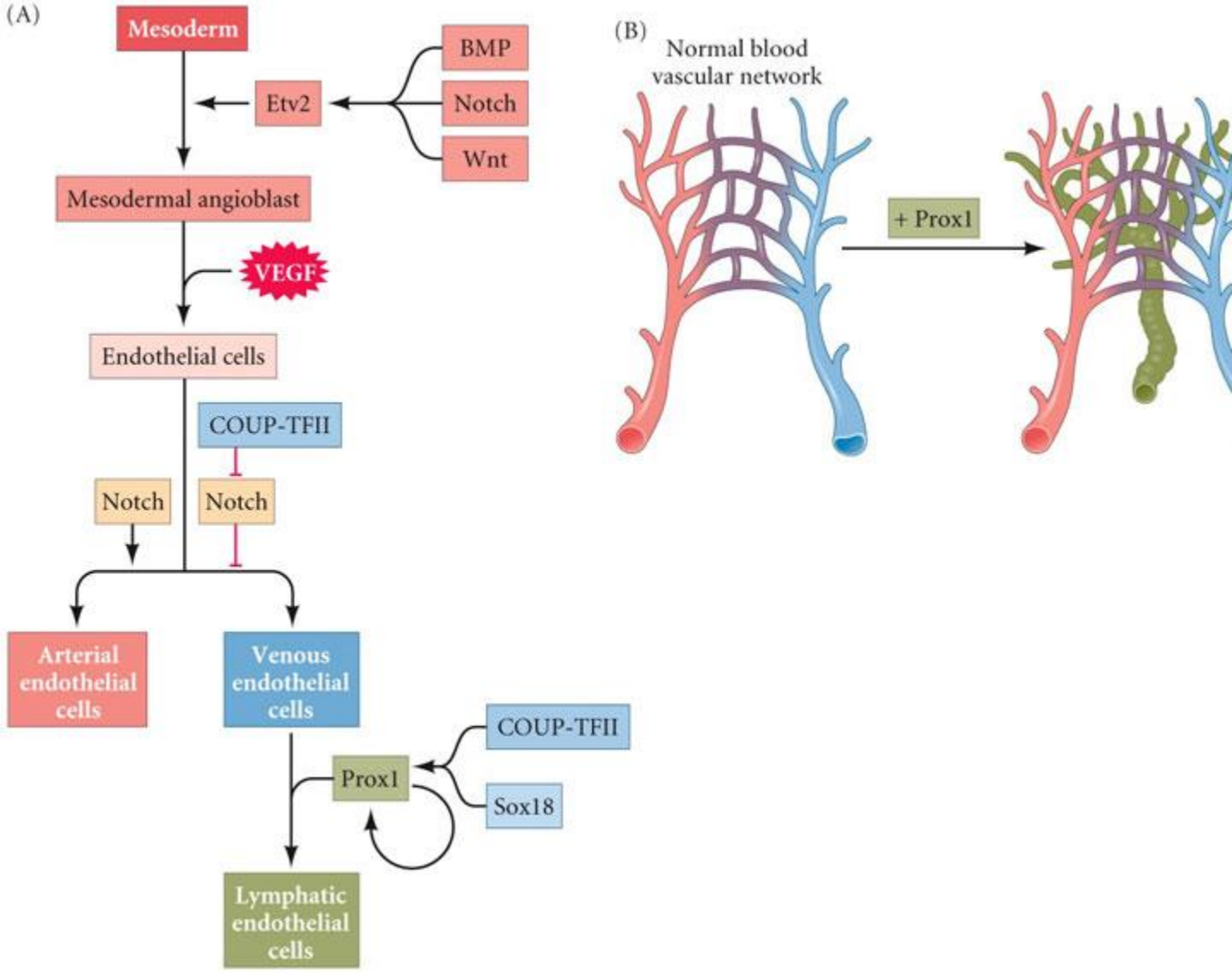

Figure 3 Origin of the lymphatic vessels. (A) The endothelial precursor (angioblast) cells are determined to be arterial or venous depending on their response to Notch signals. In veins, the COUP-TFII transcription factor inhibits Notch signaling. The venous endothelial cells can be further transformed into lymphatic vessels by the expression of Prox1. The Sox18 gene appears to be needed prior to Prox1 expression. (B) Upon Prox1 signaling in the venous endothelial cells, lymphatic vessels emerge. (After Adams and Alitalo 2007; Oliver and Srinivasan 2010.)

One of the genes upregulated by Prox1 is VEGFR-3, which encodes the receptor for the paracrine factor VEGF-C. As important as VEGF-A is for blood vessel development, VEGF-C is equally necessary for proper lymphatic development (Figure 4; Karkkainen et al. 2004; Alitalo et al. 2005). VEGF-C produced in the area of the jugular vein attracts Prox1-positive endothelial cells out from the vein and then promotes their proliferation and development into the lymphatic sacs (see Adams and Alitalo 2007; Hosking and Makinen 2007)

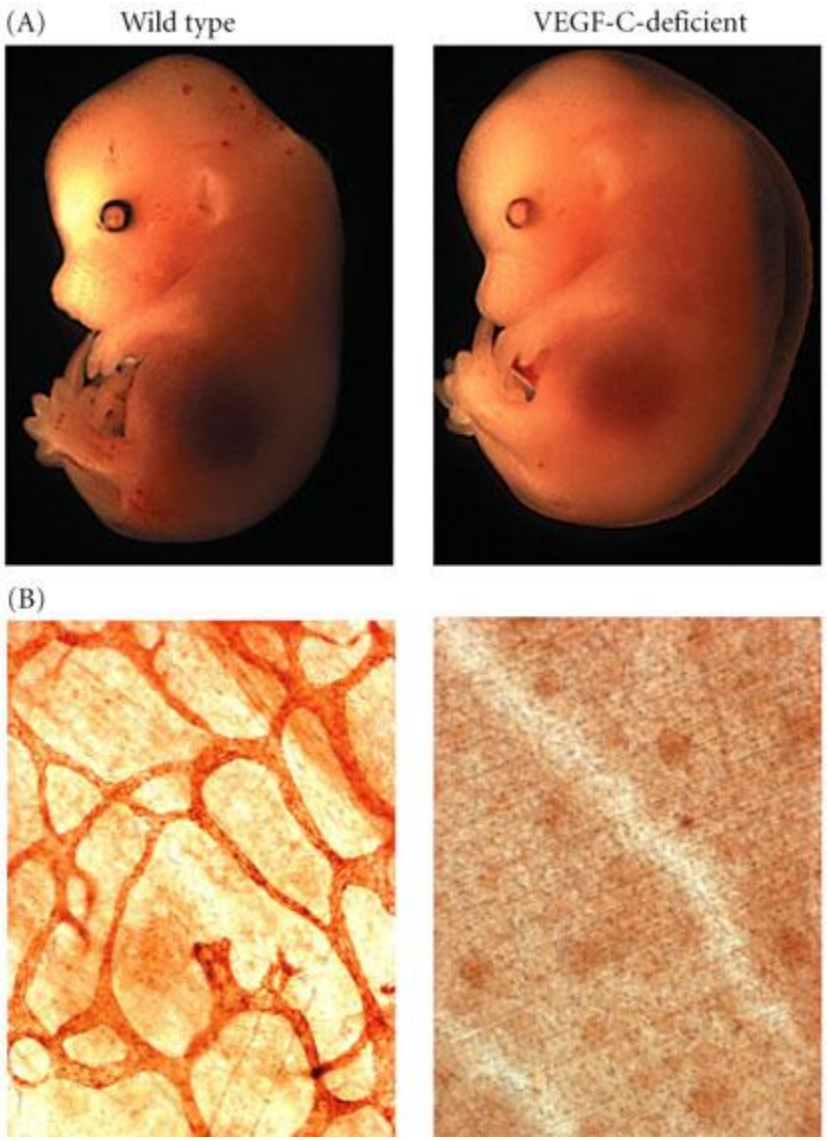

Figure 4 VEGF-C is critical for the formation of lymphatic vessels. (A) Compared with the wild-type control, a 15.5-day mouse embryo heterozygous for a VEGF-C deficiency suffers from severe edema (bloating with excess fluid). (B) 16.5-day mouse embryos stained for lymphatic vasculature. The lack of lymphatics in the skin of the VEGF-C mutant (right) is obvious when compared with that of the wild-type embryo (left). (From Karkkainen et al. 2004, courtesy of K. Alitalo.)

Organ-specific capillary formation

As mentioned for the cases of the brain and the placenta, several organs induce vasculogenesis and angiogenesis in their own mesenchyme. One of the main inducers of VEGF proteins is hypoxia (low oxygen). The HIF-1a transcription factor that activates the VEGF-A gene (among others) is functional only at lower oxygen levels (Cramer et al. 2004). The competence of the mesenchyme cells to respond to this signal is governed by their extracellular matrices. Some extracellular matrices can stress the cell membranes, activating certain GTPases. These GTPases can activate transcription factors (such as GATA2) that activate the genes encoding VEGF receptors, while inactivating the transcription factors that inhibit VEGF receptors[ii] (Mammoto et al. 2009).

The kidney vasculature is mainly derived from the sprouting of endothelial cells from the dorsal aorta during the initial steps of nephrogenesis. The developing nephrons secrete VEGF, thus allowing the blood vessels to enter the developing kidney and form the capillary loops of the glomerular apparatus (Kitamoto et al. 2002). Another striking example of organ-specific angiogenesis is produced by the developing peripheral nerves. Anatomists have known for decades that blood vessels follow peripheral nerves (see Greenberg and Jin 2005). Their proximity allows the nerves to obtain oxygen and allows hormones in the blood to regulate vasoconstriction and vasodilation.

The mechanism allowing the nerves and blood vessels to become adjacent is a reciprocal induction: the nerves secrete an angiogenesis factor, and the blood vessels secrete a nerve growth factor. Mukouyama and colleagues (2002, 2005) have demonstrated that arteries become associated with peripheral nerves, although veins do not (Figure 5). Moreover, peripheral nerves induce arteries to form near them. If the peripheral nerves in the skin fail to form (because of mutations that specifically target peripheral neurons), the arteries likewise fail to form properly. If other mutations cause the peripheral neurons to form haphazardly, the arteries will follow them. This property is due to the secretion of VEGF by the neurons and their associated Schwann cells, which is necessary for arterial formation. Thus, the peripheral nerves appear to provide a template for organ-specific angiogenesis through their ability to secrete VEGF. This interaction is not a one-way street; in some instances, the blood vessels are formed in an area first, before the neurons enter. In those cases, the vascular smooth muscle cells can secrete a compound (most likely GDNF) that allows the neuron to grow alongside it. In this way, neurons can reach their destinations by following the blood vessels (Honma et al. 2002; Li et al. 2013).

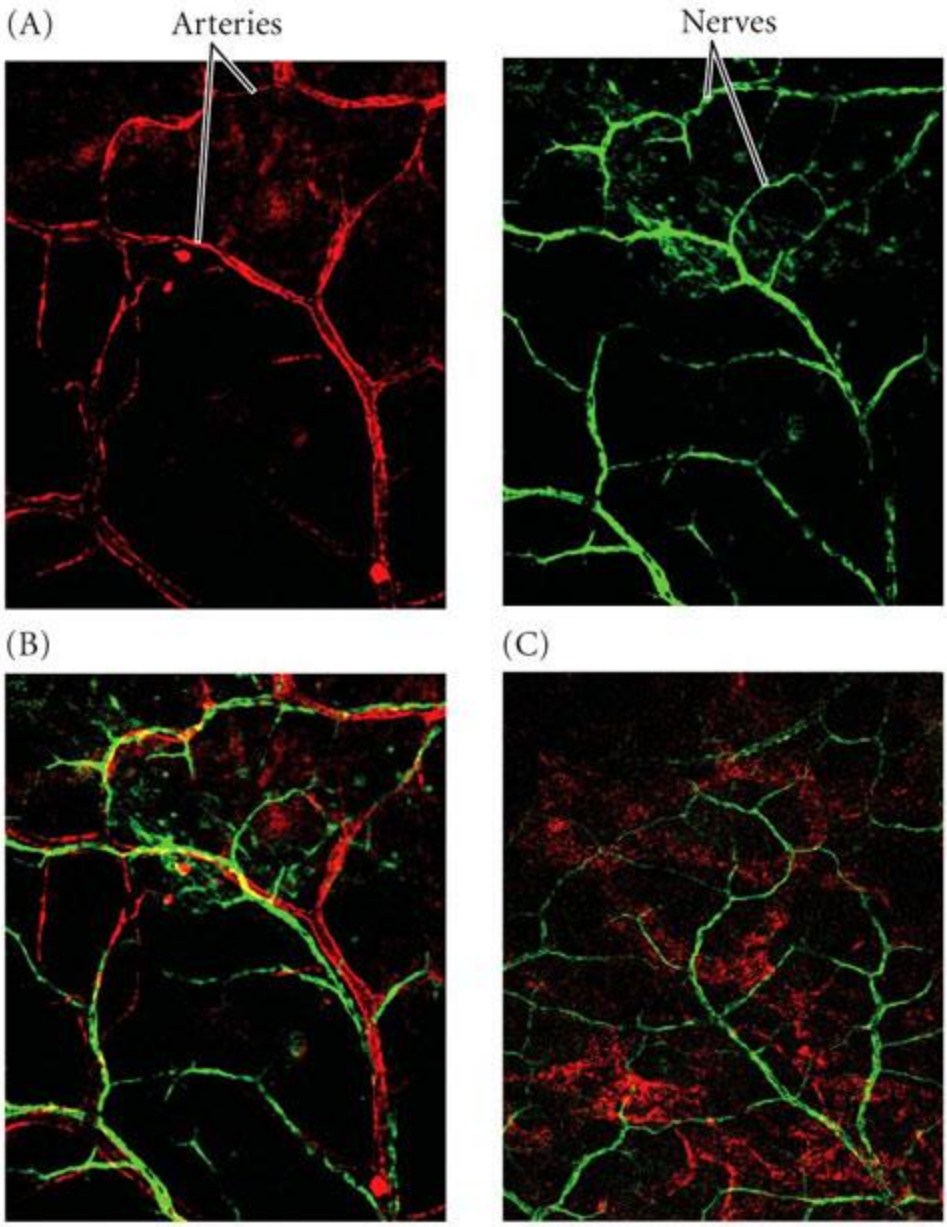

Figure 5 Arteries are specifically aligned with peripheral nerves in mouse limb skin. (A) Antibody staining of arteries (red; left) and nerves (green; right). (B) Placing the photographs together reveals that the arteries and nerves coincide. (C) Doing the same operation with stained nerves and veins reveals that the veins and nerves do not follow one another. (From Mukouyama et al. 2002, courtesy of Y. Mukouyama.)

One of the most interesting exceptions to the rules of blood vessel formation involves the coronary arteries—those arteries that feed the heart and whose malfunctions cause heart attacks that cause more than 7 million deaths in the United States each year. The coronary arteries arise from the venous endothelial cells of the inflow tract (the sinus venosus). These cells dedifferentiate and migrate across the heart. Those vessels that go into the myocardium then redifferentiate as arteries, while the endothelial cells on the surface redifferentiate as veins. The mechanisms responsible for this change of fate are not yet known (Red-Horse et al. 2010.)

Literature Cited

Klotz L, Norman S, Vieira JM, Masters M, Rohling M, Dubé KN, Bollini S, Matsuzaki F, Carr CA, Riley PR. 2015. Cardiac lymphatics are heterogeneous in origin and respond to injury. *Nature* 522: 62- 67.

Martinez-Corral I, Ulvmar MH, Stanczuk L, Tatin F, Kizhatil K, John SW, Alitalo K, Ortega S, Makinen T. 2015. Nonvenous origin of dermal lymphatic vasculature. *Circ Res.* 116: 1649- 1654.

Nicenboim J, Malkinson G, Lupo T, Asaf L, Sela Y, Mayseless O, Gibbs-Bar L, Senderovich N, Hashimshony T, Shin M, Jerafi-Vider A, Avraham-Davidi I, Krupalnik V, Hofi R, Almog G, Astin JW, Golani O, Ben-Dor S, Crosier PS, Herzog W, Lawson ND, Hanna JH, Yanai I, Yaniv K. 2015. Lymphatic vessels arise from specialized angioblasts within a venous niche. *Nature* 522: 56-61.

---

[i] The coordinated use of Notch and Eph signaling pathways is also seen to regulate the production of neuroblasts and somites.

[ii] This mechanotransduction of VEGF receptors helps explain one of the great conundrums of developmental anatomy: why the aortic arches are asymmetrical. The sixth aortic arch develops only on the left side, whereas the right aortic arch degenerates. The Nodal-induced Pitx2 signal (see Chapter 12) causes rotation of the outflow tract, producing an asymmetric blood flow into the left arch. The left side gets the blood, and the shear force from the blood flow activates the VEGFR-2 gene on that side only. Without VEGFR-2, the endothelial cells on the right side degenerate. Thus, the sixth aortic arch forms only on the left side (Yashiro et al. 2007). A related event in zebrafish is regulated by flow-induced microRNAs (Nicoli et al. 2010).