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Brothers and Sisters: Molecular insights into arterial-venous heterogeneity

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Abstract

The molecular differences between arteries and veins are genetically predetermined and are evident even before the first embryonic heart beat. Although ephrinB2 and EphB4 are expressed in cells that will ultimately differentiate into arteries and veins respectively, many other genes have been shown to play a significant role in cell fate determination. The expression patterns of ephrinB2 and EphB4 are restricted to arterial-venous boundaries, and Eph/ephrin signaling provides repulsive cues at arterial-venous boundaries that are thought to prevent intermixing of arterial- and venous-fated cells. However, the maintenance of arterial-venous fate is susceptible to some degree of plasticity. Thus, in response to signals from the ambient microenvironment and shear stress, there is flow-mediated intercalation of the arteries and veins that ultimately leads to the formation of a functional, closed-loop circulation. In addition, cells in the blood vessels of each organ undergo epigenetic, morphologic and functional adaptive changes that are specific to the proximate function of their cognate organ(s). These adaptive changes result in an inter-organ and intra-organ vessel heterogeneity that manifest clinically in a disparate response of different organs to identical risk factors and injury in the same animal. In this review, we will focus on the molecular and physiologic factors influencing arterial-venous heterogeneity between and within different organ(s). We will explore arterial-venous differences in selected organs as well as their respective endothelial cell architectural organization that results in their inter- and intra-organ heterogeneity.

Keywords

molecular heterogeneity; arterial-venous specification; vascular development

Introduction

In vertebrates, blood vessels play a significant role in establishing a circulation that is essential for gas exchange, delivery of nutrients, removal of metabolic waste, leukocyte trafficking and providing a means for inter-organ communication [1]. The circulatory vasculature is divided into two distinct and separate networks comprised of arteries and veins. The inner layers of these blood vessels are ubiquitously lined by endothelial cells whose functions have evolved beyond nutrient delivery and oxygen transport. In response to cues from the microenvironment, endothelial cells from these vessels undergo epigenetic, morphologic and functional adaptive changes that are specific for enhancing the proximate function of their cognate organ(s). These tissue-specific adaptive changes in vessel architecture provide a fitness advantage that allows

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the endothelium to conform to the diverse needs of the various ambient microenvironments within and between different tissues/organs. The end result of these adaptive changes is an inter-organ and intra-organ vessel heterogeneity that manifests pathophysiologically in a disparate response of blood vessels from different organs to identical risk factors and injury in the same animal. In this review, we will focus on the molecular and physiologic factors influencing arterial-venous heterogeneity between and within different organ(s).

Molecular differences among blood vessels

In the vertebrate embryo, arteries and veins have evolved as anatomically distinct but closely interconnected blood vessels. The formation of the molecular identity of arteries and veins is a complex process that is genetically predetermined by signaling from several genes. However, arterial-venous differentiation is susceptible to a significant degree of plasticity. Hemodynamic changes and ambient physiologic requirements of the microenvironment mediate differentiation of arterial and venous cells at the arterial-venous boundaries as well as intercalation of arteries and veins to allow blood to flow from arteries to veins in a closed loop circulation. Numerous genes play an important role in the complex signaling cascade that result in arterial-venous differentiation; some of these genes are depicted in Figure 1.

Ephrin/eph-receptor family

Of all the receptor tyrosine kinases that are found in the human genome, the Eph-receptor family, which has 13 members, constitutes the largest family. Their ligands, the ephrins, are divided into two subclasses, the A-subclass (ephrinA1-ephrinA5), whose members are tethered to the cell membrane by a glucosylphosphatidylinositol, and the B-subclass (ephrinB1-ephrinB3) ligands, which have a transmembrane domain that is followed by a short cytoplasmic region. Similarly, the Eph receptors are divided into an A-subclass (EphA1-EphA8) and a B-subclass (EphB1-EphB4, and EphB6). Originally, it was assumed that ephrin subclasses A and B strictly interacted with their respective Eph receptors; however, the binding preference of ephrins is now known to be promiscuous as they are not class-restricted [2-4]. Since Eph receptors and ephrin ligands are both membrane bound, binding and activation of Eph and ephrins require cell-cell interactions. Unlike the ligands of other receptor tyrosine kinases, which act as agonists in both their soluble and membrane-bound forms, only membrane-bound ephrins are active— the soluble ephrins act as dominant negative molecules [5, 6]. A unique feature of ephrins is that they are also capable of "receptor-like" signaling resulting in bi-directional signal transduction with both forward (ephrinB-EphB) signaling and reverse (EphB-ephrinB) signaling, where the ephrin cytoplasmic tail is phosphorylated and recruits signaling effectors [7, 8] (Figure 2A). In addition to being markers of arteries and veins, other functions that have been attributed to Eph and ephrins include cell migration, axonal guidance and tissue-border formation by repulsion of neighboring cells— a function that is important in defining the arterial-venous borders [[9-11]].

Arterial and venous specification in the developing embryo is genetically identified through the distinctive expression of ephrinB2 in cells that will ultimately become arteries and EphB4 (the ephrinB2 receptor) in cells that will ultimately become veins [[12-14]]. Surprisingly, the arterial-venous expression of ephrinB2 and EphB4 precedes the formation of morphologically distinct arteries and veins (that is before the onset of blood flow) [9, 10]. Although the expression patterns of arterial ephrinB2 and venous EphB4 are distinct, interactions between these two markers are essential for proper vascular development. Mouse mutants defective for both the ligand ephrinB2 and the receptor EphB4 die at embryonic day 9.5 (E9.5) as a result of defective remodeling— the primitive blood vessels are formed but the arrangement of the primary vascular plexus into a hierarchically organized vascular system that consist of large vessels and capillaries fails to occur [[12, 15, 16]] (Figure 2B). Consequently, this defect disrupts the differentiation of blood vessels into morphologically distinguishable arteries and veins.

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Although ephrins and Ephs can restrain cell movement at arterial-venous boundaries (across which the migration of endothelial cells are restricted), grafted cells retain some degree of plasticity permitting them to colonize both arteries and veins [11]; however, these cells can change the expression of markers (ephrinB2) to match arterial-venous properties of their host vessels [12].

Although ephrins and Eph are markers of arteries and veins, studies in zebrafish and mice have led to the discovery of other critically important genes that are upstream of ephrins and Ephs. These include sonic hedgehog, notch and its ligands, VEGF, gridlock (*grl)*, neuropilins (*nrp*), Sox7/Sox18 and COUP-TFII (Figure 1). Targeted disruption of one or more of these genes or their ligand leads to perturbation in arterial-venous differentiation. Thus, the intricate genetic processes that establish the complex vascular system during development depend on the combinatorial effects of the intrinsic interaction between ephrins and Ephs and their upstream genes discussed below.

Hedgehog

The Hedgehog family is composed of three ligands—sonic hedgehog, Indian hedgehog and desert hedgehog, and all three hedgehog ligands are not only expressed in the embryonic and adult heart (with sonic hedgehog being the most abundant) but also signal through the same receptors called patched-1 and patched-2 [13]. Sonic hedgehog induces arterial differentiation indicating that it is critical for blood vessel development [14, 15]. Activation of sonic hedgehog signaling in the adult heart promotes coronary neovascularization [13]. Zebrafish embryos lacking sonic hedgehog also lack arterial ephrinB2 expression and fail to undergo arterial differentiation—they retain a single big blood vessel that expresses venous markers [16, 17]. Sonic hedgehog's regulation of arterial fate determination is critically dependent on, and linked to VEGF expression in the somite [18]. VEGF signaling is downstream of sonic hedgehog and upstream of the notch pathway (Figure 1) in determining arterial cell fate. Loss of VEGF or sonic hedgehog results in loss of arterial identity, and embryos lacking sonic hedgehog activity fail to express VEGF within their somites [19]. Furthermore, exogenous expression of VEGF and sonic hedgehog causes ectopic expression of arterial markers, and microinjection of VEGF mRNA into embryos lacking sonic hedgehog activity rescues arterial differentiation [19]; thus, sonic hedgehog and VEGF play a coordinated role in a complex signaling cascade that establishes arterial fate.

VEGF-A

In mice, VEGF-A is alternatively spliced into a soluble $VEGF_{120}$ (which lacks exons 6 and 7) [20, 21], $VEGF_{164}$ (which lacks exon 6) and $VEGF_{188}$ (which contains all exons, is insoluble and binds strongly to cell surface and extracellular matrix). Genetic studies have demonstrated that specific VEGF isoforms mediate a complex interplay of signaling events that regulates arterial and venous differentiation. Mice heterozygote for all 3 isoforms and mice that are homozygous for the $VEGF_{164}$ isoform develop normal retinal vasculature indicating that these genetic make-ups confer normal cues for vessel pattern. On the other hand, $VEGF_{120/120}$ mice have reduced viability and histologic analysis shows that these mice have evidence of severely impaired retinal arterial development. Furthermore, $VEGF_{188/188}$ mice have reduced viability and normal retinal venous development but a dysfunctional arterial differentiation [21]. These vascular remodeling defects exhibited by $VEGF_{120/120}$ and $VEGF_{188/188}$ mice are likely due to a combinatorial dysfunction in guidance cues from matrix-associated $VEGF_{188}$ and diffusible $VEGF_{120}$ as a result of a genetically induced change in the normal concentration gradients of VEGF proteins.

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Notch

Notch proteins are comprised of four members in mammals (Notch1-Notch4), and bind to one of five different ligands—Jagged 1-2 or *Delta-like* (*Dll*) 1-3 [18] and are involved in cell fate decisions and patterning during embryonic development [22, 23]. VEGF-A up-regulates Notch-1 and one of its ligands, *Dll-4*, in endothelial cells [[23, 25, 28-31]]. This upregulation of Notch-1 occurs in mice through the activity of transcription factors Foxc1 and Foxc2 [24]. Notch signaling leads to the expression of the bHLH transcription factors *grl* in zebrafish and *hesr1/hesr2* in mice [25, 26]. The signaling mechanism of these genes is highly integrated during vessel development for appropriate vessel identity. For example, deletion of one or both copies of the Notch effector genes *Hey1* or *Hey2* results in a defect in ephrinB2 expression and arterial development while venous specification is generally unaffected [27]. Furthermore, both copies of the *Dll-4* gene are important for arterial fate determination since mice with only one copy suffer from lethality due to abnormalities in the development of the arteries [[31, 36, 37]]. In addition, arteriovenous malformations develop when Notch signaling is either reduced [[25, 33, 37]] or constitutively active [28]. Mice defective in the Notch ligand Jagged-1 die from hemorrhage early during embryogenesis, exhibiting defects in remodeling of the embryonic and yolk sac vasculature [29].

Several studies have shown that Notch signaling plays a central role in regulating sprouting angiogenesis [30]. When Notch is activated, membrane associated γ-secretase (GSI) cleaves Notch intracellular domain which becomes translocated to the nucleus where it stimulates transcription of target genes. Inhibition of Notch signaling either by administration of DAPT (a GSI inhibitor), endothelial cell-specific deletion of Notch 1, inactivation of one allele of Notch ligand (*Dll-4*) [31] or by knockdown of Rbpsuch (a DNA-binding protein that mediates transcription of Notch target genes) [32] enhance sprouting angiogenesis and vascular density. Conversely, angiogenic sprouting is inhibited either by activation of Notch signaling by jagged 1 peptide [31] (a Notch ligand), or by simultaneous inhibition of *Dll-4* and VEGF-A signaling [33]. Taken together, these data indicate that endothelial cells are dependent on VEGF-A from the microenvironment to turn on angiogenic sprouting and on *Dll-4*/Notch signaling to be resistant to VEGF-A signaling and turn off sprouting angiogenesis. It is tempting to speculate that the differential effects of Notch-induced alteration in angiogenic sprouting can lead to variegation in vessel patterning that may be a catalyst for arterial-venous heterogeneity.

Gridlock

Notch activation leads to the expression of the bHLH transcription factors *grl* in zebrafish and *hesr1/hesr2* in mice [[33, 34, 39]]. *Grl* is downstream of the Notch pathway and is involved in cell fate decisions made by Notch pathways [34, 35]. Zebrafish embryo with homozygote deletion of *grl* have characteristic localized vascular defects in the paired lateral dorsal aorta of the anterior trunk at 24 hours post fertilization, resulting in absence of blood flow in the tail while retaining normal functioning heart and normal cranial circulation. These localized vascular defects subsequently lead to development of arterial-venous shunts and development of collateral blood flow in an attempt to restore blood flow to the abnormally developed distal aorta. Histologic analysis shows that both caudal artery and caudal veins are present but collapsed due to absence of circulation; furthermore, these caudal vessels can be recruited (when collateral vessels have matured) suggesting that *grl* causes a localized vascular defect and not a generalized deficiency of caudal vessels [36]. These data indicate that *grl* plays a critical role in arterial fate determination in the developing embryo.

Neuropilins

Neuropilins, which are cell surface receptors for soluble semaphorins, are part of a large gene family that controls axon guidance in the nervous system [37, 38]. The expression of nrp-1 (an isoform-specific receptor for human $VEGF_{165}$ 39 or mouse $VEGF_{164}$ 40) and nrp-2 becomes

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restricted to arteries and veins respectively as soon as arteries and veins become morphologically distinguishable [[18, 45]]. This restrictive expression pattern of nrp-1 and -2 is critical to the identity of arteries and veins and is temporally related to the reported expression of ephrinB2 and EphB4 respectively [[12, 15, 16]]. Targeted deletion of the *nrp-1* gene in mouse results in embryonic lethality at different time points depending on the genetic background — E10.5 on a C57BL/6 background and E13.5 on a CD1 background [41–43]. On a CD1 background, *nrp-1*-null mouse embryos have an abnormal vascular network formation in the yolk sac, defects in aortic arches, abnormality of the outflow tract [42] and defective sprouting of hindbrain vessels [44]. On a C57BL/6 background, *nrp-1*-null mouse embryos show severe vascular defects that are due to *nrp-1* deletion but which only become apparent after the onset of blood flow [43]. Histologic analysis shows formation of the dorsal aorta at the normal time point (E8.5) that subsequently regresses at E9.5 due to abnormal remodeling, partly due to the lack of recruitment of smooth muscle cells to larger vessels. Furthermore, *nrp-1*-null mice on a C57BL/6 background undergo normal yolk sac vasculogenesis but fail to undergo vascular remodeling that normally begins after the onset of blood flow, resulting in vascular defects consisting of poorly branched and enlarged yolk sac vessel with large avascular spaces.

Sox

Recently, *Sox7* and *Sox18* have been shown to play a significant role in arterial fate determination [45]. Members of the Sox-F family play crucial roles during the formation of definitive endoderm [46], hematopoietic stem cell regulation, cell fate determination [47] and cardiovascular development [47]. Simultaneous knockdown of *Sox7* and *Sox18* induces a loss of posterior circulation and development of arterial-venous shunt; however, loss of either gene independently does not result in any observed vascular defect [45, 48]. In addition, embryos with *Sox7/Sox18* double knockdown have a dramatic decrease in arterial markers like Notch3, ephrinB2a and *Dll*-4 and increased expression of venous markers like dab2 and flt4 in arterial tissues [45]. These observations suggest that although Sox7 and Sox18 are individually dispensable during vascular development, they play a synergistic role in arterial specification and remodeling and that they are upstream of Notch and *Dll-4*.

COUP-TFII

The discovery of the arterial specification pathways raises the question of whether venous development is the default state. This possibility was refuted with the identification of COUP-TFII, a member of the orphan nuclear receptor superfamily that plays a critical role in active vein specification [49]. Studies of the role of COUP-TFII using COUP-TFII/lac*Z* "knock-in" mouse models have shown that COUP-TFII expression occurs in the venous endothelium at embryonic E8.5. High levels of COUP-TFII are detected in the anterior cardinal vein, umbilical vein and vitelline vein, but not arterial endothelial cells including dorsal aorta, internal carotid artery and umbilical artery [49]. Genetic ablation of COUP-TFII in endothelial cells enables veins to acquire arterial characteristics, including the expression of arterial markers (nrp-1) and Notch signaling molecules. Ectopic expression of COUP-TFII in endothelial cells results in inhibition of nrp-1 expression and effectively the downstream Notch pathway [49] (Figure 1). These data indicate that COUP-TFII plays a critical role in active vein specification and in repressing Notch signaling to maintain vein identity.

The dichotomous specification of molecular markers of arteries and veins prior to the first heart beat does not explain how the attractive and repulsive forces within the boundaries of arteries and veins allow intercalation of these genetically distinct vessels to form a closed-loop circulation. Fluorescence data have shown that individual angioblasts give rise to either arterial or venous (but not mixed) clones [25], which is consistent with the genetic predisposition theorem. However, data from the avian model showing that endothelial cells can still become incorporated into the venous system after acquiring the expression of arterial-specific genes

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(*ephrinB2* and *nrp-1*) suggest that vascular identity, although genetically predetermined, is reversible during the embryonic period [12, 50]. Modern techniques using time-lapse video microscopic analysis have shown small caliber arteries can disconnect from the growing artery only to be subsequently reconnected to the veins [51] — thus providing a mechanism for the formation of a closed loop circulation. Taken together, these data suggest that, prior to the onset of the first heartbeat, endothelial cells expressing arterial- and venous-specific markers are genetically predetermined. However, with onset of blood flow, vessels demonstrate a significant amount of plasticity with hemodynamic changes within the microenvironment allowing vessels originally part of the arterial tree to contribute to the venous tree. Due to the fact that the complex interplay between genetic, hemodynamic and environmental factors in determining the molecular heterogeneity between arteries and veins is not well understood, the above review of genetic influences governing vessel identity represents only what is currently known, and almost certainly represents a gross simplification of this complex process.

Physiological heterogeneity among vessel types

The physiologic differences defining arteries and veins are, in part, predicated on differences in the type and structural assembly of their underlying endothelial cells, types of gap and tight junctions as well as presence or absence of basement membrane and fenestrations. In general, arteries carry oxygenated blood (with the exception of pulmonary arteries), have tighter endothelial junctions, have thicker tunica media and pulsate. In contrast, veins carry deoxygenated blood (with the exception of pulmonary veins), have looser endothelial junctions, have thinner tunica media, do not pulsate and have greater capacity to mediate inflammatory responses [52]. In addition to the primary physiological differences demarcating arteries from veins, each organ influences the architectural organization of its specific vessels such that it is best suited for the cognate organ-specific function. We will now examine the physiologic heterogeneity in selected organs and discuss organ-specific plasticity when appropriate.

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Renal vessels

The kidney is a highly vascular organ that has three identified ontogenic stages during development [53]. Blood enters the kidneys through the renal artery that subsequently branches into afferent arterioles before blood exits the kidney via efferent arterioles. In the mature functioning kidney, renal vessels have evolved as size- and charge-specific filters in order to provide organ-specific function [54]. These specialized vessels are not only permeable to water and small solutes but are also relatively permeable to certain macromolecules like renin (~40 kDa) through specialized filtration barriers. The filtration barrier in the glomerular vessels consist of glomerular endothelial cells covered with a surface layer of membrane-associated proteoglycans, glycosaminoglycans, glycoproteins, glycolipids and associated plasma proteins (known collectively as the glycocalyx [55]), the glomerular basement membrane, glomerular epithelial cells and podocytes [56] (Figure 3A). The glomerular endothelial cells, which form the initial filtration barrier to blood flow through the kidney, possess continuous fenestrations [57, 58] with specialized slit-pore diaphragms [59]. The pores have uneven diameters ranging between 60 and 240 nm that allow bulk fluid and anionically charged molecules to be filtered and facilitate movement of cationically charged molecules [60, 61].

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The individual renal vessels that form the conduit for this passage of blood display considerable functional heterogeneity amongst themselves depending on which region of the kidney they serve. For instance, the glomerular capillaries serve primarily to filter fluids and solutes out of the blood that enters the kidneys before integrating with the efferent arterioles. The efferent arterioles subsequently terminate in vasa recta that enter the medulla of the kidney as descending vasa arterioles (descending vasa recta) before exiting the medulla as ascending veins (ascending vasa recta). These parallel vascular tubes are structurally distinct (Figure 3B).

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For instance, the descending vasa recta are lined by the continuous and non-fenestrated endothelium while the ascending vasa recta are lined by lined by fenestrated endothelium. Furthermore, while blood is flowing through the descending vasa recta into the medulla, osmotically active solutes, like sodium chloride and urea, are first absorbed into the interstitium of the descending vasa recta and subsequently released through the interstitium into the ascending vasa recta. Thus, osmotically active solutes are shuttled between the ascending and descending capillary within the microcirculation of the medulla as a mechanism for effective prevention of a wash out of medullary hypertonicity and preservation of medullary countercurrent exchange which is essential for gradient-mediated filtration in kidneys [62, 63]. This functional heterogeneity between the descending vasa recta and ascending vasa recta also facilitates shunting of oxygen and other nutrients from the descending arteriole to the ascending vasa recta [64] within the medulla. Physiologic and immunologic measurements have shown that aquaporin-1 water channels and the facilitated urea carrier are significantly concentrated in the descending vasa recta [63, 65, 66] where they facilitate efflux of water from the descending vasa recta to the papillary interstitium [67-69]. Taken together, these studies show that there is a complex vascular relationship as well as heterogenous transport properties within the renal vasculature.

Some diseases are due to abnormalities in renal vessels. For example, congenital Finnish nephritic syndrome, an inherited autosomal recessive trait, results from a defect in Nephrin, a 136 kDa protein that assembles to form an isoporous filter in the glomerulus that is essential for maintenance of the filtration barrier [70]. Mice and humans with defective Nephrin develop severe proteinuria and diffuse foot-process effacement [71, 72].

Cardiac vessels

In vertebrates, the lumen of the ascending aorta is continuous with that of the coronary arteries that supplies the myocardium with blood during diastole. As the coronary arteries course through the epicardium, they branch into several smaller arteries that penetrate the myocardium (myocardial vessels) and ultimately branch into arterioles and the capillary network that surround cardiomyocytes. The endocardium which forms the inner lining of cardiac chambers, on the other hand, is perfused by the entire blood volume inside the heart cavity and has been shown to act as a sensor of circulating blood volume and as a modulator of cardiac performance, rhythmicity, and growth via autocrine and paracrine signaling [73, 74].

Although the coronary artery ostium is continuous with the ascending aorta, coronary arteries do not develop as outgrowths of the ascending aorta [75]. Rather, they arise separately from the proepicardium, a primordium that arises from the septum transversum mesenchyme [76, 77]. The precursor cells in the proepicardium subsequently give rise to the epicardium, coronary endothelium as well as coronary smooth muscle cells [78, 79]. Prior to embryonic day 9.5 in mouse, the heart is divided into two layers consisting of an outer myocardial layer and inner endocardial layer. At about embryonic day 10.5, a third layer (epicardium) migrates to the embryonic heart and invades its outer surface to form a uniform layer of epithelium [78, 80, 81]. In response to fibroblast growth factors, bone morphogenic factors and transforming growth factor signaling from the myocardium, some of the epicardial derived cells (EPDCs) undergo epithelial-to-mesenchymal transformation (EMT). Subsequently, thymosin-β4 (Tβ-4) — a G-protein monomer-binding protein [82] — is secreted from the myocardium and stimulates EPDCs in a paracrine fashion to migrate to the myocardium where they respond to angiogenic (VEGF/bFGF) or arterogenic (PDGF/TGF-β) factors that provide the stimulus for onward differentiation into endothelial or smooth muscle cells that form the coronary vessels [82-87]. The essential role of Tβ-4 in the formation of coronary vessels is evidenced by the fact that cardiac-specific Tβ-4 knockdown by conditional RNA interference resulted in EPDCs that undergo EMT but fail to migrate into the myocardium, which not only results in impaired

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coronary vasculogenesis and defective collateral growth but also in the development of ventricular non-compaction [83] — a thin spongiform appearing myocardium. Friends of GATA (FOG-2) — a protein that physically associate with the N-terminal zinc finger of GATA-4 — is also important in the formation of the coronary vessels. FOG-2-deficient mouse embryos have normally developed epicardium but die at midgestation with a complete absence of coronary vessels but with no phenotypic defects in systemic vessels [88, 89]. Further analysis shows that the epicardial cells of *FOG-2*-deficient mice fail to undergo epithelial-to-mesenchymal transformation that is a critical step in the formation of coronary vessels [89].

The endocardium arises from cardiogenic mesoderm that is derived from the rostral portion of the primitive streak [90]. There is a significant amount of heterogeneity between endocardial and coronary vascular endothelial cells. The endocardium is typically characterized by the presence of deeper and more tortuous intercellular clefts, trabeculae and furrous microvilli. These features of the endocardium allow the myocytes adjacent to the endocardium to be exposed to a significant amount of blood and nutrients because of markedly increased surface area. In contrast, myocardial capillaries are characterized by a continuous endothelium, and the capillary endothelium is in intimate contact with cardiomyocytes. They possess shallower intercellular clefts and fewer tight junctions. It has been estimated that this intramyocardial capillary network is so vast that myocardial endothelial cells outnumber cardiomyocytes by a ratio of 3:1 [84]. This architectural design is well suited for delivery of adequate blood supply and nutrients to the fastidious cardiomyocytes.

The cardiac venous system is also unique in its systemic connections. The greater coronary veins generally parallel the epicardial arteries and drain into the coronary sinus, which in turn communicates with the inferiolateral portion of the right atrium, where its orifice is guarded by the Thebesian valve. As with the coronary arteries, the coronary venous system derives from mesenchymal transformation of the proepicardial organ, although maturation of coronary veins is delayed in comparison with the coronary arteries [91]. The signals that differentiate coronary arterial and venous differentiation have not been clearly defined, but activation of Notch-dependent pathways appears to be crucial for this distinction [92]. Abnormalities of arteriovenous communication within the coronary circulation, especially when fistulas result in communication of the right coronary artery directly with the right ventricle or atrium, are not uncommon and can be clinically hazardous [93].

Coronary vessels are capable of a certain degree of plasticity. For example, data from coronary artery bypass grafting surgery and peripheral vascular bypass surgery indicates that placement of veins into the higher pressure and flow of the arterial circulation results in adaptation of the vein to the arterial environment; this “arterialization” consists of thickening, fibrosis and intimal proliferation of intimal cells [84, 94]. Thus, saphenous veins that are grafted into arteries adapt arterial properties over time as the grafted veins develop thicker walls and reduced permeability, reminiscent of the coronary arteries into which they are grafted [95] and demonstrate loss of EphB4 expression without increased expression of ephrinB2 [96]. Furthermore, human saphenous veins perfused *ex vivo* under arterial flow conditions produce increased matrix metalloproteinase-2 [97], reduced thrombomodulin [98] and increased endothelial nitric oxide synthase [99], properties that are reminiscent of arteries. In summary, the origin of the coronary vessels is unique and distinct from that of the systemic arteries and endocardium. This may, in part, explain why coronary vessels are more prone to atherosclerotic disease — the leading cause of mortality in the western world — compared to systemic vessels that are exposed to similar risk factors.

Hepatic vessels

It is believed that the liver diverticulum forms from a thickening in the ventral foregut endoderm after induction mediated by the cardiac mesoderm at around E8 in the mouse [100-101]. The liver

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has a dual blood supply — hepatic arteries, which deliver well-oxygenated blood to the liver and portal veins, which deliver poorly oxygenated but nutrient-rich blood from the intestine to the liver (Figure 4A). The hepatic artery branches into hepatic arterioles before draining into the capillary bed in the liver parenchyma referred to as the hepatic sinusoid. Blood from the sinusoid empties into the central vein (Figure 4B), hepatic venules, hepatic veins and then into inferior vena cava where the blood is recycled to the heart. In contrast to the usual arrangement of veins in the body, blood in the portal vein empties into the portal venules which drain into the hepatic sinusoid. As described above, blood in the sinusoid drains into the central vein, hepatic venules, hepatic veins and then into the inferior vena cava where blood is recirculated via the heart to the systemic circulation [102].

There is a significant amount of heterogeneity across the liver vasculature. Within the portal venule, the endothelium is spindle shaped, non-fenestrated and has short microvilli, and as the portal venules transitions into hepatic sinusoid, the endothelial cells becomes smooth, large and are enriched with actin fibers [103]. This suggests that the site of transition of the portal venule to sinusoid acts as an inlet sphincter that provides an essential regulatory site for hepatic sinusoidal blood flow. The endothelial cells within the hepatic sinusoids are characterized by the presence of large number of sieve-like pores that are about 100 nm in diameter. In addition, the endothelium is discontinuous and has large membrane-bound, non-diaphragmatic fenestrae referred to as sinusoidal endothelial fenestrae [103, 104]. These sinusoidal fenestrae are dynamic structures that form complex invaginations in the endothelium and regulate not only the permeability of the hepatic sinusoids but also sinusoidal blood flow by actomyosin-dependent contraction and dilation of the sinusoidal fenestrae—an adaptation that provides a mechanism for regulating blood flow within the sinusoid [102]. In addition, large-diameter fenestrae are more numerous in the portal region of the sinusoid where they rapidly transport plasma into the space of Disse, thus unloading the effects of arterial jet streams [103]. Taken together, these data suggests that there is a significant amount of intra-organ heterogeneity within the liver vasculature. Perturbation in liver vasculature is a pathophysiologic mechanism in certain disease processes of the liver. For example, in fibrosis of the liver, the sinusoids undergo progressive loss of fenestrae and the formation of continuous basement membrane—a process involving capillarization and venularization of sinusoids that depends in part on changes in the activity of angiopoietins 1 and 2 [105-107].

Pulmonary vessels

The lung has a dual circulation consisting of a low-pressure, high-volume pulmonary vascular network that is involved in gas exchange and a high-pressure, low-volume bronchial vascular system that not only delivers oxygen to the bronchial tree but also play an active role in thermoregulation, humidification of ambient air and facilitation of immune responses in the airway. Although knowledge of their embryonic origin is incomplete, it is becoming increasingly appreciated that the distal microvascular pulmonary vascular networks arise from blood islands where pluripotent lung mesenchymal cells are induced to differentiate into endothelial cells. In contrast, proximal pulmonary arteries and veins arise from proliferation and migration of endothelial cells from existing endothelial-lined vessels. Pulmonary macrovascular (arteries and veins) and microvascular (capillaries) segments arise independently and are fused together at the pseudoglandular phase of development [108, 109]. In order to accomplish the diverse functions described above, endothelial cells lining bronchial vessels undergo adaptive changes that result in vessels that are more permeable and more responsive to inflammation than their pulmonary vascular counterparts [110, 111]. Although there is less leukocyte margination in the bronchial vessels, they exhibit increased permeability in response to inhaled antigens and constitutively express E-selectin, suggesting that bronchial vessels are chronically activated [112, 113].

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During embryogenesis, the pulmonary vessels, which normally receive less than 10% of the cardiac output, undergo a sudden transition from a low flow, high pressure/resistance state to a high flow, low pressure/resistance states that allows it to be perfused by highly oxygenated blood from the lung. A remarkable heterogeneity exists throughout the pulmonary vasculature, in part, due to the unique tissue and blood microenvironment that exist in different lung compartments. For instance, endothelial cells in the pulmonary arteries are exposed to mixed venous blood that is low in oxygen whereas capillary endothelial cells are exposed to arterial blood gases with high oxygen content. In the intact circulation, pulmonary artery endothelial cells are aligned in the direction of blood flow, and in vitro pulmonary endothelial cells similarly align in the direction of flow. Vessel bifurcations introduce non-laminar flow condition as well as complex transmural pressures that represent highly distinctive environmental condition that can alter the endothelial cell function. Endothelial cells at bifurcations are not aligned in the direction of flow where flow is turbulent. Furthermore, lung microvascular endothelium possesses superior barrier properties in vivo when compared with either pulmonary arterioles or venules leading to an excess of 100-fold greater restriction to water permeability [114]. Thus, pulmonary arterial and microvascular endothelial cells are clearly phenotypically distinct.

Although the bronchial and pulmonary vessels are very distinct, pathologic diseases can affect them equally. For instance, hereditary hemorrhagic telangiectasia, which affects both pulmonary and bronchial vessels and is associated with arteriovenous malformation, is caused by mutations in endoglin — an ancillary receptor for transforming growth factor-β_1 and -β_3 [115]. Abnormalities in bronchial vessels are implicated in diseases such as asthma [116, 117] where vascular engorgement and neoangiogenesis in airways contribute to airflow obstruction. Furthermore, abnormality of the pulmonary endothelium leads to other diseases like sepsis-induced lung injury where there is increased permeability of the pulmonary endothelium [118].

The greater pulmonary venous circulation is unique in that it carries highly oxygenated blood to the left atrium for systemic delivery into the arterial circulation. The pulmonary veins derive as endothelial invaginations in the cranial portion of the sinus venosus during development [119] Abnormalities in development of the pulmonary veins can lead to sinus venosus defects with abnormal draining of the right pulmonary veins, resulting in intraatrial communication. Clinical consequences of abnormal pulmonary venous development are not limited to anatomic defects. After evolving from the sinus venosus, the pulmonary veins become invested with a myocardial sleeve derived from Pitx2-positive cells from the secondary heart field [120]. The pulmonary myocardium depends on the expression of the transcription factor NKX2.5, and abnormalities in the development of this pulmonary venous sheath when NKX2.5 activity is reduced may provide an important substrate for the development of atrial fibrillation [121].

Summary

There are molecular and physiologic distinction between arteries and veins, and the molecular distinction between arteries and veins are predetermined but remain plastic during development. The complexity and heterogeneity of endothelial cells that form the inner layer of these vessels is becoming increasingly apparent as we begin to understand that arterial-venous identity is influenced not just by intrinsic genetic programs but also by local hemodynamic cues. Although we have made significant progress towards understanding the specification of arterial-venous identity and the remodeling process that leads to their heterogeneity, many more studies remain to be done in order to fully understand this complex signaling process. A few candidate genes and pathways have been identified and discussed in this review; however, these pathways are probably not sufficient to fully control the complex arterial-venous fate decision, and a more complex regulatory pathway will likely be discovered in the future. An added complexity is the fact that many of the function of the identified gene

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products like ephrins, Ephs VEGF and Notch are not solely confined to cell fate decision as these same genes are known to mediate multiple other developmental processes in vertebrates. Future studies should be directed at identifying other signaling pathway that are critical to arterial-venous determination and to fully understand how hemodynamic factors that contributes significantly to the remodeling of arteries and veins are fully integrated with genetic factors and mutually regulated.

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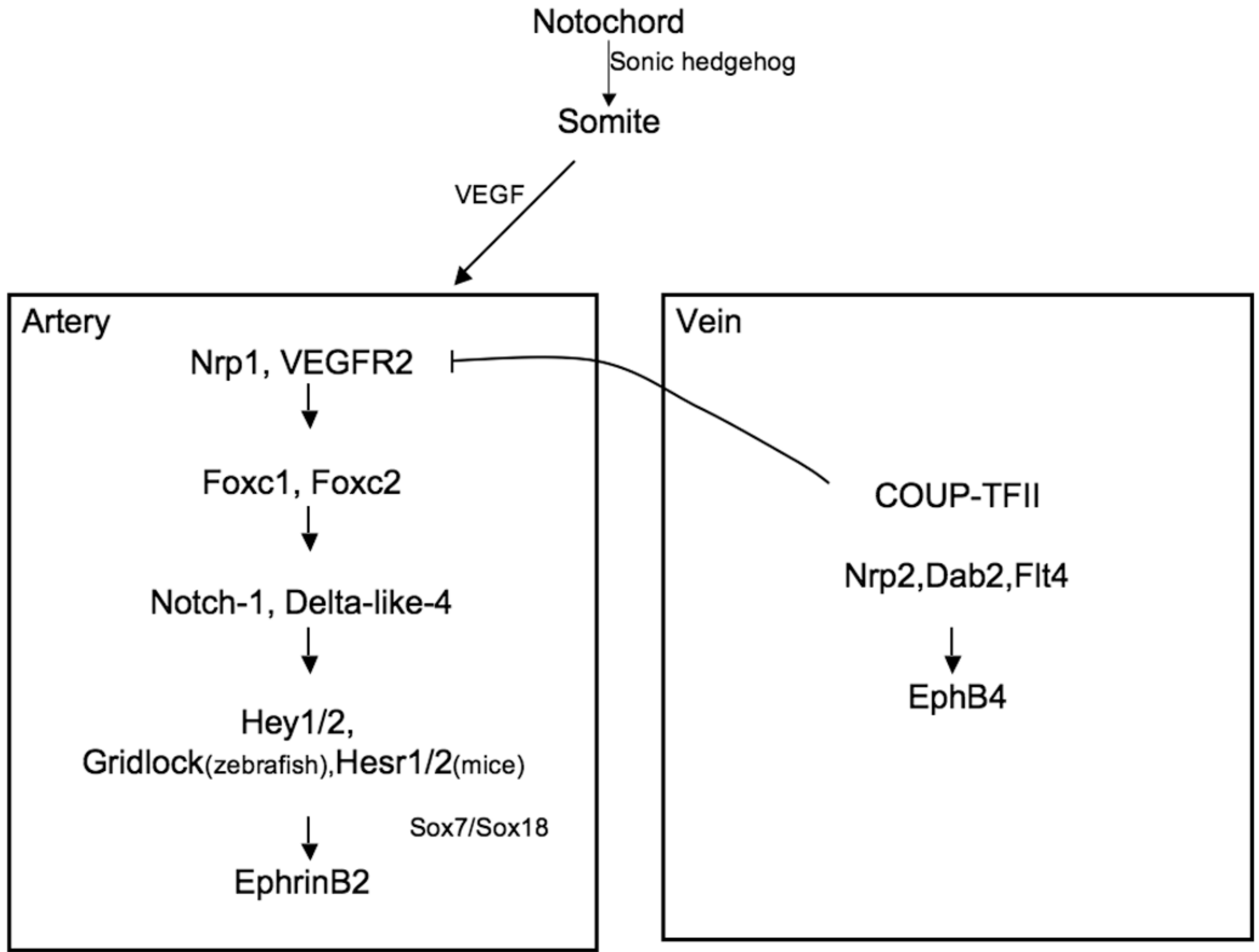

Figure 1. Arterial-venous specification in vertebrate embryo

In response to sonic hedgehog secreted by the notochord, VEGF is secreted by adjacent somite leading to arterialization of the dorsal aorta. VEGF up-regulates Notch pathway including Notch-1 and its ligand, Delta-like-4 in nearby endothelial cells through activity of the transcription factors Foxc1 and Foxc2. Subsequently, there is expression of gridlock (in zebrafish) and hesr1/hesr2 (in mice) which, in addition to the synergistic action of Sox7 and Sox18 leads to arterial specification presumably via EphrinB2 activation. In the cardinal vein, COUP-TFII is required for strong expression of EphB4 and venous differentiation.

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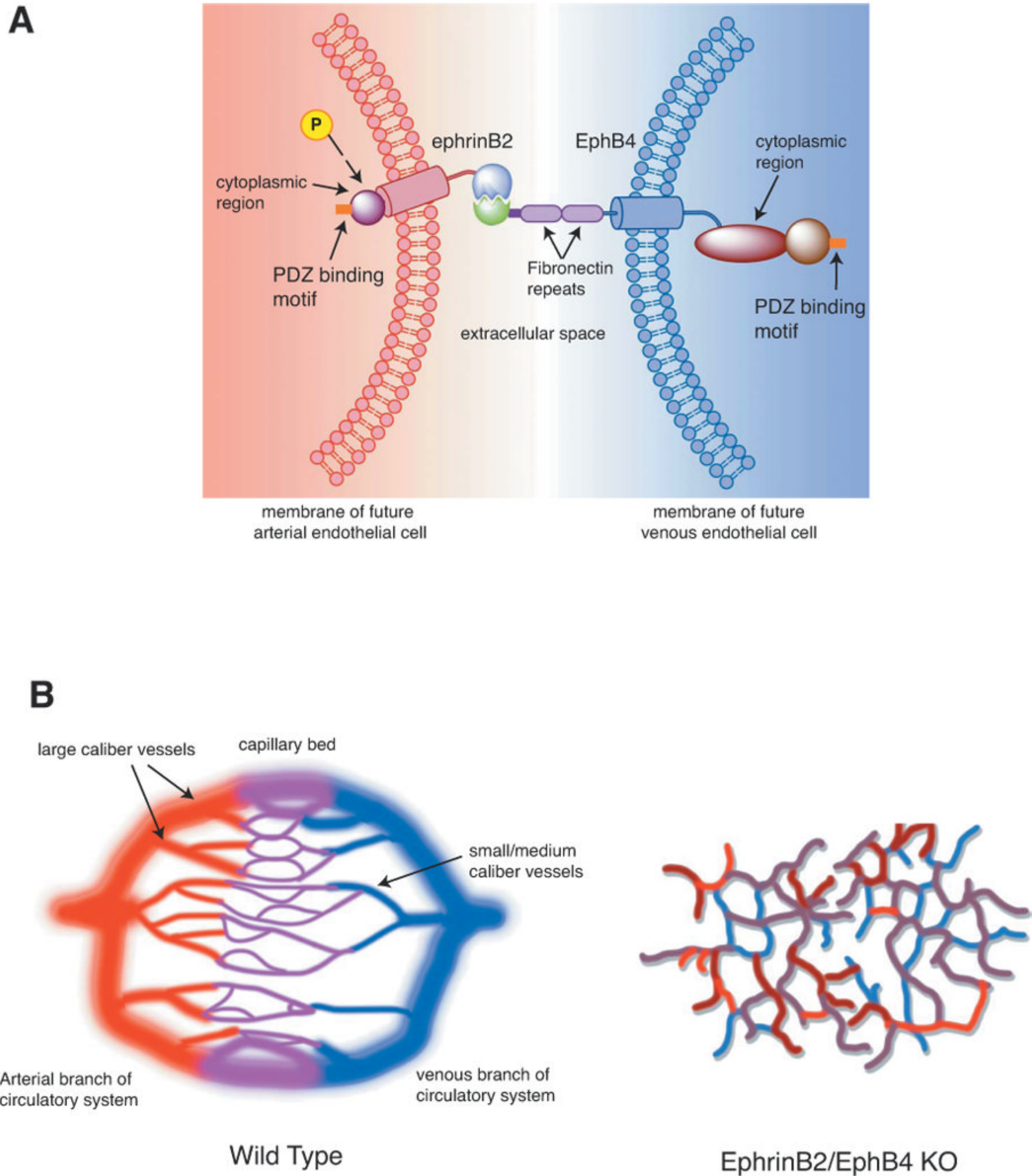

Figure 2. Ephrin B2/EphB4 signaling contributes to the fate of blood vessel identity

A. Schematic diagram showing an ephrinB2-expressing cell interacting with an EphB4-expressing cell. Interactions between the ligand and receptor cause activation and downstream signaling events in both cells. EphrinB2 signaling in endothelial cells contributes to an arterial fate for the cell, while EphB4 signaling results in a venous fate. B. Development of a mature vascular bed requires interaction between ephrinB2-expressing and EphB4-expressing cells. This interaction ensures the formation of a hierarchically-organized system consisting of both arteries and veins of various size as well as a well-defined capillary bed. Mice deficient in both ephrinB2 and EphB4 die *in utero* as a result of defective remodeling of the vascular bed. These mice contain primitive blood vessels that fail to develop into a mature vasculature. These mice

have no distinguishable arteries or veins. Likewise, all vessels are of the same caliber and no capilliaries are present.

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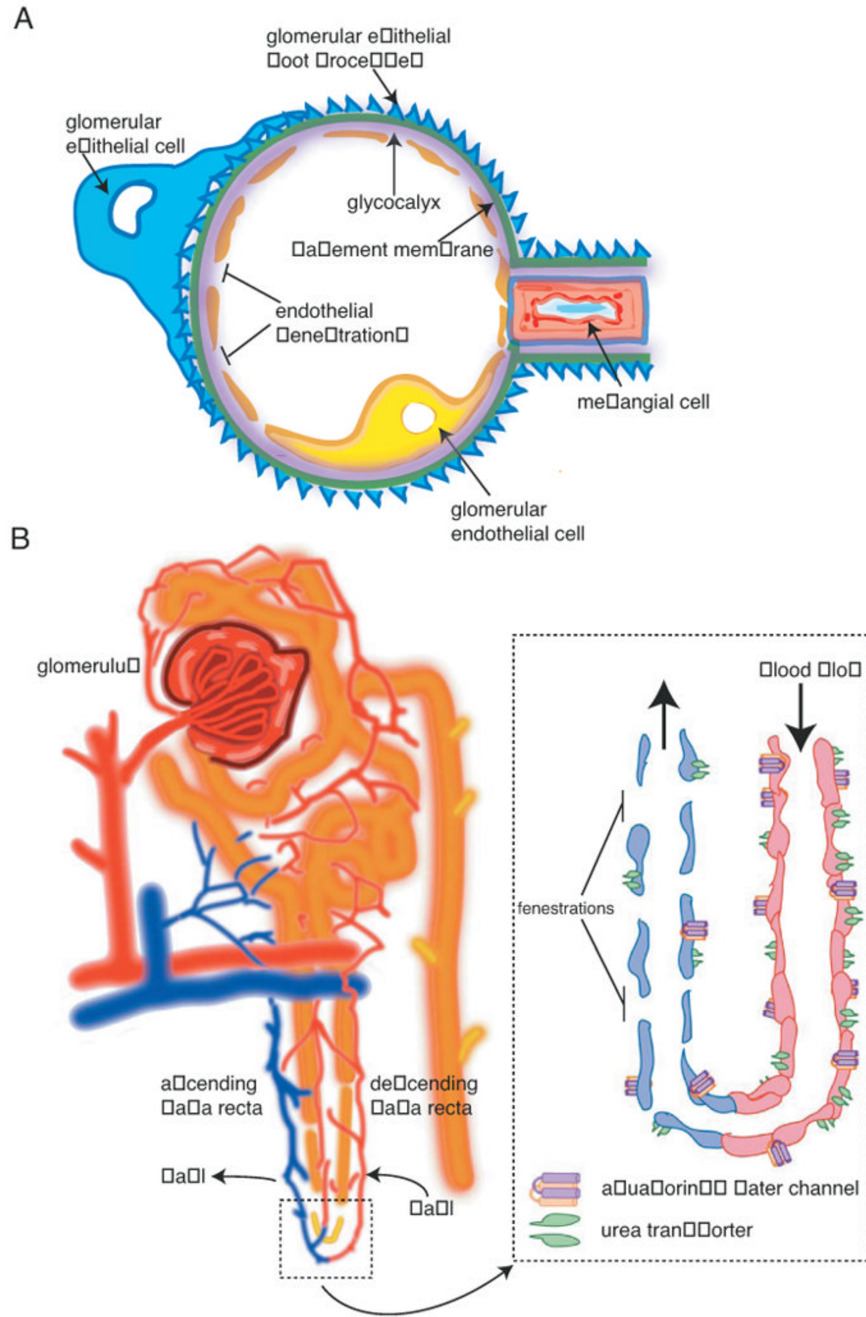

Figure 3. Heterogeneity within the renal vessels

A. Each glomerulus is surrounded by multiple layers of cells that serve as a specialized filtration barrier. Glomerular endothelial cells form the initial barrier to blood flowing through the kidney and are characterized by numerous fenestrations. B. The vessels of the vasa recta illustrate the complexity and precise specialization of the renal vascular system. The descending vasa recta are lined by a continuous endothelium that is punctuated by a high density of aquarporin-1 water channels and urea transporters (see insert). The ascending vasa recta are lined by a fenestrated endothelium (see insert) and have fewer conducting channels.

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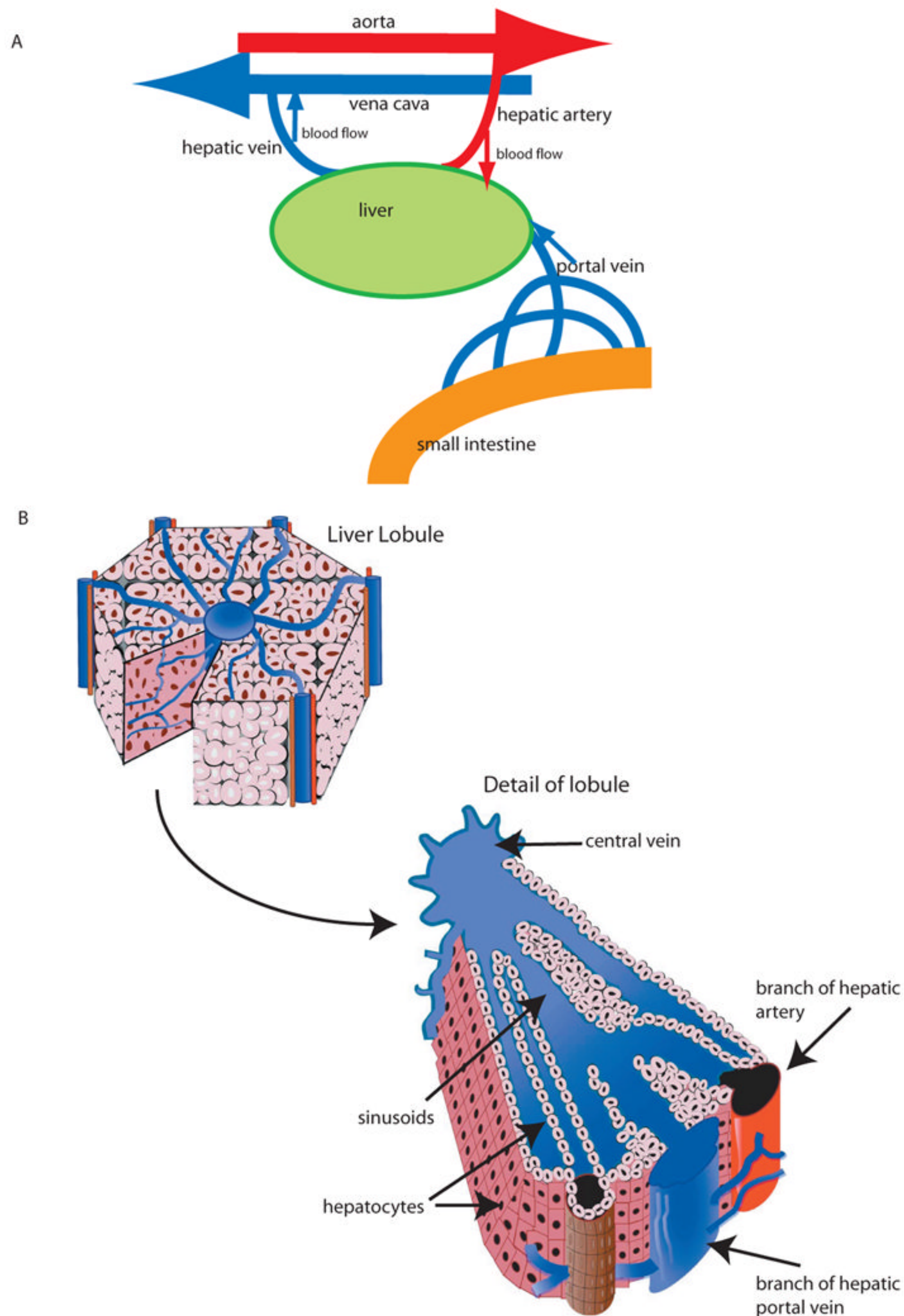

Figure 4. The liver has a dual blood supply

A. Schematic illustration of the arrangement of blood flow in the liver. Blood enters the liver via 2 major vessels: the hepatic artery (carrying oxygenated blood from the aorta) and the portal vein (carrying nutrient-rich blood from the intestine). Blood leaves the liver via the hepatic vein that subsequently drains into the inferior vena cava. B. The vascular arrangement within a liver lobule. Blood enters the lobule through a branch of the hepatic artery and portal vein and flows through the sinusoid to the central vein, which in turn empties into the hepatic vein that leaves the liver and drains into the vena cava.