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REVIEW

The role of blood flow in vessel remodeling and its regulatory mechanism during developmental angiogenesis

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Abstract

Vessel remodeling is essential for a functional and mature vascular network. According to the difference in endothelial cell (EC) behavior, we classified vessel remodeling into vessel pruning, vessel regression and vessel fusion. Vessel remodeling has been proven in various organs and species, such as the brain vasculature, subintestinal veins (SIVs), and caudal vein (CV) in zebrafish and yolk sac vessels, retina, and hyaloid vessels in mice. ECs and periendothelial cells (such as pericytes and astrocytes) contribute to vessel remodeling. EC junction remodeling and actin cytoskeleton dynamic rearrangement are indispensable for vessel pruning. More importantly, blood flow has a vital role in vessel remodeling. In recent studies, several mechanosensors, such as integrins, platelet endothelial cell adhesion molecule-1 (PECAM-1)/vascular endothelial cell (VE-cadherin)/vascular endothelial growth factor receptor 2 (VEGFR2) complex, and notch1, have been shown to contribute to mechanotransduction and vessel remodeling. In this review, we highlight the current knowledge of vessel remodeling in mouse and zebrafish models. We further underline the contribution of cellular behavior and periendothelial cells to vessel remodeling. Finally, we discuss the mechanosensory complex in ECs and the molecular mechanisms responsible for vessel remodeling.

Keywords Zebrafish · EC rearrangement · Vessel pruning · Hemodynamics · Wnt signaling

Introduction

The vasculature is the earliest organ formed during embryonic development and provides nutrients for the development of other tissues. The functional vascular network is a closed circulating lumen composed of arteries, veins and capillaries that delivers oxygen, nutrients and hormones to tissues and organs and excretes metabolic wastes. In the early stage of embryonic development, the primary vascular plexus is formed by vasculogenesis and angiogenesis with increased vessel density [1]. This immature plexus is followed by complex vessel remodeling to form a tree-like vascular network through rearranging endothelial cells (ECs) [1]. The process of vasculogenesis, angiogenesis, and vessel remodeling to form a functional vascular network is called "angioadaptation" [2, 3]. At the same time, the vasculature always adjusts its morphology to meet the needs of the body at different developmental stages. In this review, we classified vessel remodeling into vessel pruning, vessel regression and vessel fusion based on the EC's behaviors. Vessel pruning is defined here as the segment-by-segment reshaping of a vascular bed, characterized by loss of ECs and retention of type IV collagen (Col. IV) positive empty sleeves [4, 5]. The result of vessel pruning is that the break of two previously connected vessels and then merge into the existing adjacent vessels. Vessel regression is defined here as the complete involution of a vascular bed dependent on the programmed apoptosis of ECs [4, 5], such as the pupillary membrane (PM) and hyaloid vessels, which usually contributes to nourishing the development of retinal vascular beds.

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Vessel fusion is defined as two adjacent vessels that fuse into one, accompanied by rapid dilation of the vessel diameter [6]. In addition, vascular remodeling not only occurs in the process of angiogenesis but also exists in pathological conditions, such as pulmonary hypertension, diabetes and tumors.

In this review, we aim to summarize the vessel remodeling process in vertebrates, the role of ECs, pericytes and astrocytes in vessel remodeling, the effect of hemodynamics on vessel remodeling, and the mechanisms that regulate vessel remodeling.

The way of vessel remodeling

EC programmed apoptosis-mediates vessel regression

Different from vessel pruning, programmed vessel regression, such as the PM and hyaloid vessels of the developing eye, mainly refers to the loss of blood vessels of the entire network. The hyaloid capillary network is a transient vascular network that completely regresses during ocular development and is mediated by macrophage-dependent programmed EC apoptosis [7–10]. Lobov et al. found that the PM, tunica vasculosa lentis and hyaloid vessels are more abundant at P8 in *PU.1* mutant mice, which lack macrophages, than in WT mice [7]. Endothelial conditional knockout of *Bim* ($Bim^{Flox/Flox}$, VE-cadherin-cre, Bim^{EC}), a proapoptotic Bcl-2 family member, decreases retinal vascular apoptosis and hyaloid vessel regeneration [11]. Periostin, secreted by intraocular macrophages, promotes hyaloid vessel regression by enhancing the adhesion of macrophages to hyaloid vessels [12]. These results strongly support that macrophages contribute to the regression of PM and hyaloid vessels. During vessel regression in PM, the first apoptotic vessel EC (VEC) is macrophage dependent. Then, this VEC enters the vessel lumen to cause angiostenosis and blood flow stasis, which trigger subsequent apoptosis of the remaining VECs in the affected segments [13, 14]. These results demonstrated that not only are macrophages indispensable for programmed vessel regression, but blood flow also contributes to VEC apoptosis.

Vessel fusion contributes to vessel dilation

Vessel fusion is an autonomous activity of ECs in which two small vessels fuse into one vessel with a larger diameter [15] (Fig. 1a, b). Vessel fusion plays a vital role in the dilation of vessel diameter [15, 16]. Vessel fusion occurs at the early stage of embryo development, even in the absence of vessel smooth muscle cells (vSMCs) [15]. Vessel fusion between the dorsal aorta and lateral capillary plexus is regulated by vascular endothelial growth factor (VEGF) during Japanese quail embryo development [17]. In vitro models of three-dimensional vascular microtissue or uniluminal vascular spheroids can fuse to form a larger diameter spheroidal structure depending on VEGF and preserve the morphological architecture of the cultured spheroids [18, 19]. Ryan et al. found that vessel fusion was restricted to the highest blood flow near the vitelline artery and vein, resulting in a rapid increase in the vessel diameter of the cultured mouse embryo [6]. The ECs of capillaries with low blood flow migration to larger vessels with higher blood flow also contribute to enlarging the vessel diameter (Fig. 1a) [6]. Knockout of

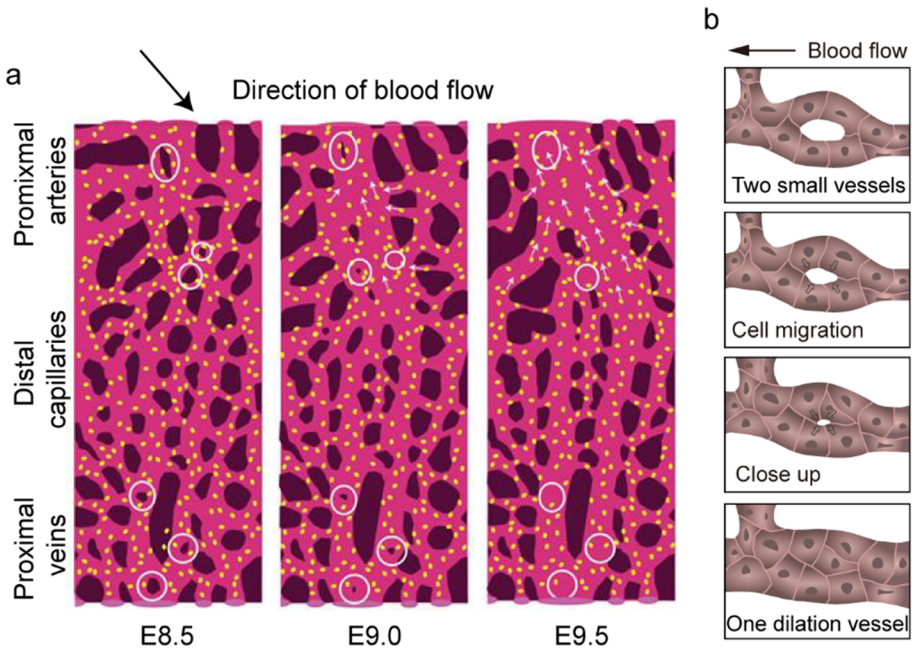

Fig. 1 Vessel fusion contributes to engaging the vessel diameter. **a** Vessel fusion in the cultured mouse embryo. Blood enters the vascular plexus through the vitelline artery to the distal capillaries and is ultimately collected by the vitelline vein at E8.5. Vessel fusion (marked with circles) occurs in the proximal vessels that are exposed to high blood flow, resulting in a rapid change in vessel diameter. Vessel hierarchy is established through vessel fusion by E9.0–9.5, resulting in different flow velocity distributions in arteries, capillaries and veins. **b** Vessel fusion is mediated by the redistribution of ECs. Figure **a** adapted from Ryan et al. [6]

Mlc2a reduced blood flow velocity, which blocked vessel fusion and inhibited the increase in vessel diameter mediated by EC migration against blood flow. In conclusion, vessel fusion can not only simplify the vascular network, but also, more importantly, automatically select the fused vessel segment according to the magnitude of blood flow velocity, which contributes to enlarging the vessel diameter and forming a tree-like structure vascular network.

EC rearrangement mediates vessel pruning

The primary vasculature is formed by angiogenesis, endothelial sprouting and proliferation and contains a large number of redundant and nonfunctional vessels. These redundant vessels are remodeled into a mature and functional tree-like network, which is characterized by a large number of excess capillaries undergoing physiological regression [20, 21]. In addition, vessel pruning is also accompanied by a reduction in the number of vascular loops.

The retinal vasculature develops postnatally in mice, which is a good model to study sprouting angiogenesis and vessel remodeling (Fig. 2). The mature retinal vasculature consists of three distinct vascular plexuses. The primary pluxus with few vessels sprouts from the optic nerve head at postnatal day (P) 1 and then outgrows to reach the periphery at P8 [22], which localizes to the inner retina ganglion cell layer [9]. The veins of the primary pluxus insert into the deeper pluxus at the outer edge of the inner nuclear layer around P7 [22, 23]. The vessel density, segment length and number of branch points peaked at P10 (Fig. 2a). After angiogenesis of the retinal vasculature, vessel pruning is necessary to simplify the primary vascular network (Fig. 2a), which is characterized by an increase in IB4-/Col. IV + antibody staining (Fig. 2b) [24]. At P18, the retinal vasculature was more hierarchical and functional (Fig. 2a). Research has shown that 95% of vessel pruning events in the mouse retina were not related to EC apoptosis but through EC rearrangement [20]. Our group found that Tecr, a very-long-chain fatty acid synthesis, contributes to retinal angiogenesis. Endothelial-specific deletion of *Tecr* caused vascular defects with decreased vessel density and branch points at P7, but this phenotype disappeared at P5 and P10 [25]. We speculated that a transient effect on vessel density after abolishing *Tecr* was caused by excessive vessel pruning.

Zebrafish embryos develop in vitro, and their transparent characteristics make them an excellent model for studying vascular development. Researches have shown that the process of vessel pruning is present in zebrafish Cranial Division of the Internal Carotid Artery (CrDI) [26], zebrafish intersegmental vessels (ISVs) [20], zebrafish subintestinal veins (SIVs) [27] and zebrafish caudal veins (CV) (Fig. 3a) [28].The formation of zebrafish ISVs and endothelial lumen involved cell divisions, cell arrangements and dynamic alterations in intercellular junctional complexes by anastomosis (Fig. 3a) [29]. Claudio et al. found that arterial cells disconnected and retracted from the aorta where venous sprouts

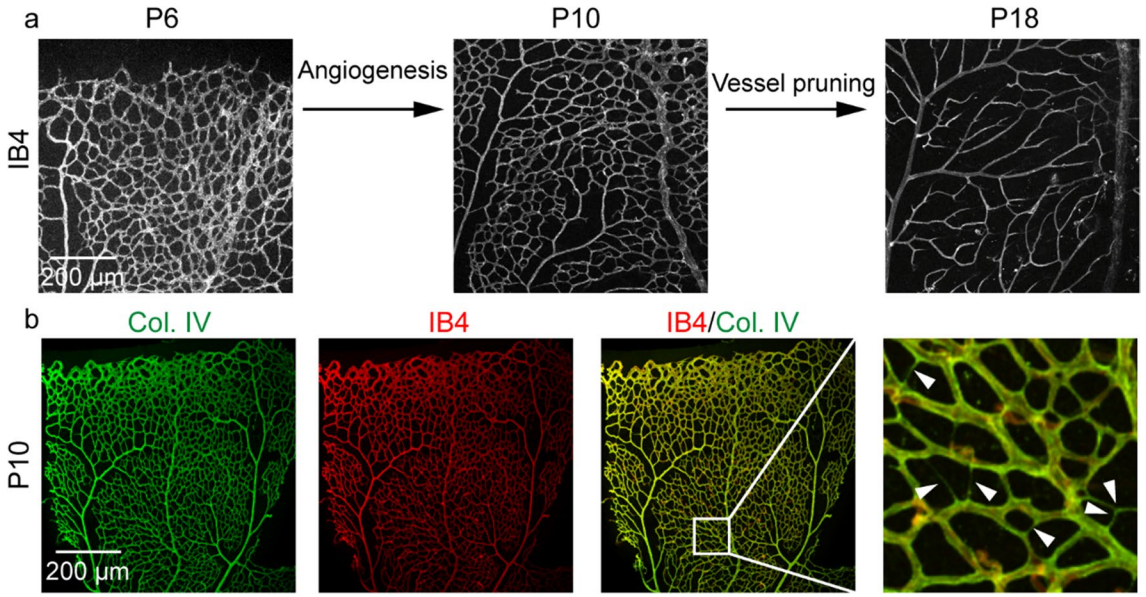

Fig. 2 Vessel pruning in the mouse retinas. **a** Fluorescence immunohistochemistry of the mouse retinas using isolectin B4 (IB4, grey) to show the vasculature. The process of angiogenesis and vessel pruning in the mouse retina from P6 to P18. The vessel density reaches a pink at P10. Vessel pruning contributes to decreasing the vessel density from P10 to P18. Scale bar 200 μm. **b** Fluorescence immunohistochemistry of the mouse retinas using anti-collagen type IV (Col. IV, green) and IB4 (red) to visualize vessel pruning at P10. Arrowheads indicate the segments undergoing vessel pruning, characterized by IB4-/Col. IV + antibody staining. Scale bar 200 μm

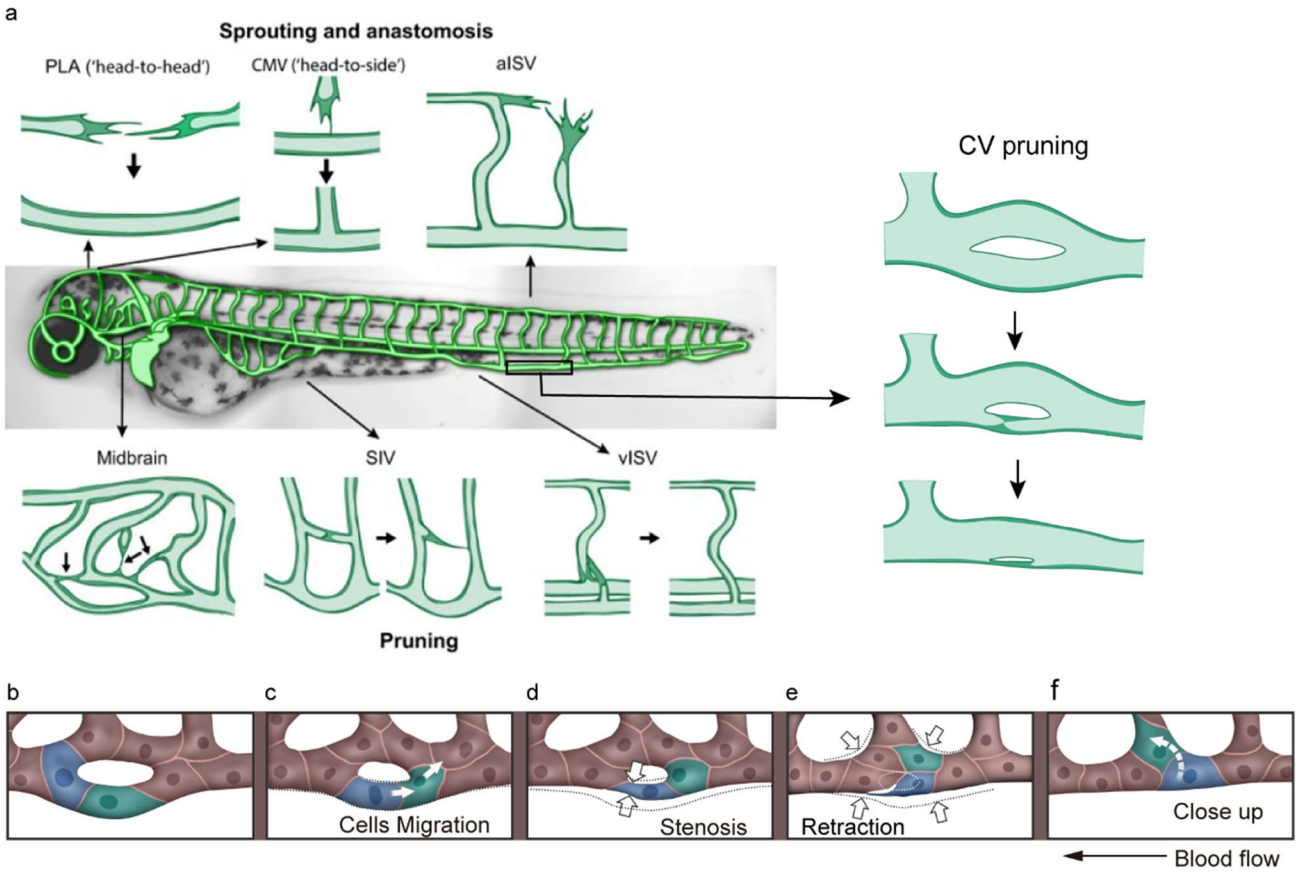

Fig. 3 Anastomosis and vessel pruning events in the zebrafish model. **a** The process of anastomosis and vessel pruning in zebrafish vasculature. The central panel shows an overview of the zebrafish vascular beds. Sprouting and anastomosis have been studied in the palatocerebral artery (PLA), the communicating vessel (CMV) and the segmental arteries (aISV). Vessel pruning has been studied in the midbrain vasculature, the subintestinal vein (SIV), the segmental veins (vISV) and the caudal vein (CV). Figure **a** adapted from Charles et al. [34]. The process of zebrafish CV pruning is mediated by EC rearrangement, which includes the stages of selection pruning segment (**b**), cell migration (**c**), stenosis (**d**), retraction (**e**) and close-up (**f**). **b** The diameters of the lower branch and the upper branch are the same at the beginning of vessel pruning. **c**, **d** The ECs marked with blue and green migrate against the blood flow, resulting in vessel stenosis at the lower branch. **e**, **f** The ECs marked with blue and green migrate into the adjacent vessel, finishing the pruning of the lower branch. The arrow indicates the direction of blood flow. The rearrangement of ECs during vessel pruning is marked with blue and green. The arrows in (**c**, **f**) indicate the direction of EC migration. The arrows in (**d**, **e**) indicate vessel stenosis. Figure **b–f** adapted from Wen et al. [28]

connected to the ISV during ISV pruning by monitoring the dynamics of ECs with mosaic endothelial expression of membrane-bound eGFP in Tg(*kdrl:mCherry-CAAX*) embryos (Fig. 3a) [20]. The disconnection of the ISVs involved cell migration but was unrelated to EC apoptosis. The parallel and vertical branches of SIVs in zebrafish are remodeled from the reticular structure with multiple vascular loops, accompanied by an increase in nuclei number(Fig. 3a) [27]. Therefore, SIV pruning is a synergistic effect involving the dynamic migration of cells and the collapse of the lumens. During 1.0–4.0 days postfertilization (dpf) of the zebrafish embryos, midbrain vasculature formed by angiogenesis with a large number of vascular loops and redundant segments. Vessel pruning plays a critical role in the development of midbrain vasculature to reduce the complexity of the vascular network by migrating ECs from pruned segments to adjacent unpruned segments (Fig. 3a) [30]. Furthermore, vessel pruning of zebrafish midbrain vascular networks was preferentially restricted to segments that were either located between two parallel primary vessels (H-type) or were one of the two nearby segments that formed a small local loop (O-type) [30]. zebrafish CV is remodeled from ventral capillaries of the CV plexus (CVP) as a novel animal model to study vessel pruning [28]. We showed that CV formation is accompanied by a decrease in vascular loops from 36 h postfertilization (hpf) to 72 hpf through vessel pruning (Fig. 3a) [28].

Vessel pruning is a process of EC rearrangement, which resembles anastomosis in reverse in morphology [20, 27, 31]. Franco et al. proposed that vessel pruning includes four distinct steps: initial selection, stenosis, retraction and resolution [20, 32]. Our results showed a similar

process of zebrafish CV pruning driven by EC rearrangement (Fig. 3b–f) [28]. *Tg(kdrl:mCherry; fli1a:nEGFP)* and *Tg(fli1a:nEGFP);Ki(cdh5-mRFP)* transgenic fish were used to explore the relationship between EC migration and vessel stenosis. At the beginning of CV pruning, the diameters of the two branches were almost the same (Fig. 3b). Then, EC nuclei, marked with blue and green at the lower branch, migrated against the blood flow (Fig. 3c), resulting in vessel stenosis (Fig. 3d). After that, the ECs retracted to the neighboring segments from the lower branch (Fig. 3e, f). Finally, the lower branch was pruned (Fig. 3f) [28]. The results demonstrated that the migration of EC nuclei contributes to vessel stenosis. In vessels without perfusion, both vessel stenosis and EC migration to the neighboring branch contribute to vessel pruning [27, 28]. Lowell et al. found that the preservation of vessel segments is determined by cellular decision-making behavior at bifurcations. The pruned cells prefer to choose vessel segments with larger shear stress or more cells [33].

Usually, vessel pruning is irrelevant to EC apoptosis. However, Eva et al. found that CrDI pruning in zebrafish is accompanied by EC death [26]. In contrast to the regression of hyaloid vessels in mice [7], EC death during CrDI pruning is independent of macrophages [26]. During dorsal CrDI pruning, 1–2 of a total of 3–4 ECs undergo apoptosis, and the remaining ECs migrate toward the dorsally located Primordial Midbrain Channel or the ventral CrDI [26]. Zhang et al. reported that EC apoptosis was indeed associated with brain vessel pruning in zebrafish [35]. Microglia, but not macrophages, contribute to the clearance of apoptotic ECs. However, microglia are dispensable for brain vessel pruning. The EC apoptosis-accompanied pruned vessels had two important characteristics: 1) most of these segments were longer than those without apoptosis, and 2) the nuclei of adjacent blood vessels occupied both ends [35]. Although EC apoptosis occurs during vessel pruning, it is not absolutely required for the completion of this process [26, 36]. EC-specific deletion of *Caspase-8* or *Bak/Bax* in mice did not affect vessel pruning, even though it decreased cell death [37, 38]. These results strongly proved that EC apoptosis is not essential to vessel pruning and cannot trigger vessel pruning under physiological conditions.

EC junction/F-actin dynamic rearrangement contribute to vessel remodeling

Vessel pruning is a process of cell rearrangement that involves lumen collapse and cell-to-cell junction remodeling. Lenard et al. characterized vessel pruning as type I and II pruning depending on the perfusion of the pruning branches [27, 39]. Type I pruning is characterized by lumen collapse before cell rearrangement with nonlumenized, multicellular tube with continuous junctional connections. Type II pruning is cell rearrangement resulting in a unicellular tube by cell self-fusion that then collapses, and the bridging cell is incorporated into the major branch [27]. In the mouse retina, lumen disconnections occur in nonperfused vessels with discontinuous vascular endothelial junctions labeled by VE-cadherin or zona occludens proteins (ZO-1) [20]. Usually, the junctions form an isolated ring structure surrounding a patch of apical endothelial membrane with a continuous Col. IV basement membrane, which is similar to morphological anastomosis in reverse [20].

Research has proven that dynamic F-actin rearrangement at EC junctions and assembly of endothelial filopodia are indispensable to angiogenesis sprouting and lumen formation [40, 41]. Actin dynamics interact with both the VE-cadherin/catenin complex and the membrane cytoskeleton to control cell–cell adhesion, cell shape change or cell motion [42]. F-actin or stress fibers aligned parallel to the direction of flow when ECs were exposed to high blood flow in vivo or shear stress in vitro [43]. Once blood flow is reduced, ECs will reorganize the actin skeleton with a decreased number of stress fibers and change their position to the cells' peripheral band [44]. To dissect the role of EC junctions and F-actin in vessel pruning, we generated the knock-in (KI) zebrafish *Ki(cdh5-mRFP)* and the transgenic line *Tg(Fliep:lifeact-EGFP)*, in which VE-cadherin is marked with red fluorescence and EC F-actin is marked with green fluorescence. We showed that the dynamic polymerization and depolymerization of VE-cadherin and F-actin were observed during CV pruning. Usually, discontinuous VE-cadherin occurred before disruption of F-actin at the multicellular to unicellular stage and retraction stage. Moreover, deletion of *klf6a* or *tagln2* in zebrafish resulted in abnormal CV pruning caused by disruption of VE-cadherin and F-actin rearrangement [28]. Therefore, it is clear that vessel pruning is a dynamic process of EC junction and F-actin cytoskeleton rearrangement.

The contribution of periendothelial cells to vessel remodeling

The role of periendothelial cells, such as pericytes and astrocytes, in vascular development and maturation is well studied. Their contribution during vessel remodeling is rather unclear. The interaction between pericytes and ECs is one of the important factors affecting vascular morphology and vessel remodeling [45]. As early as 1998, Benjamin et al. found that disruption of pericyte-EC associations leads to excessive vessel pruning in a hyperoxia-induced mouse model [46]. During the regression of hyaloid vessels, apoptosis is observed in ECs and pericytes. Furthermore, the apoptosis of pericytes is more frequent than that of ECs [10]. *Bim*

alleles ($Bim^{Flox/Flox}$) and pericyte *pdgfrb*-cre (Bim^{PC}) mice were used to reduce pericyte apoptosis, which inhibited hyaloid vessel regression and retinal vessel pruning [11]. Suppression of VEGF production leads to an immature vascular network because of reduced pericyte coverage [47]. Pericytes secrete CXC receptor 3 (CXCR3) ligands, which activate the CXCR3 signaling pathway in ECs and promote vessel pruning [48]. Pericyte maturation is regulated by phosphoinositide 3-kinase (PI3K) signaling. Pericyte conditional knockdown of *PI3K* blocks pericyte proliferation and enhances pericyte maturation, resulting in fewer branch points of retinal vessels compared to the control at P6 [49]. The above results demonstrated that pericytes function not only directly in vessel pruning but also in pericyte-EC interactions.

Retinal astrocytes colocalize with the inner layer of retinal blood vessels as oxygen sensors during neonatal development and vascular remodeling [9]. Astrocyte prolyl hydroxylase domain proteins (PHDs) are oxygen sensors, and their deletion results in elevated hypoxia inducible factor (HIF)-2α protein levels and fewer mature astrocytes. Immature astrocytes cause increased retinal vascular density due to defective vessel pruning [50]. Astrocytes express not only VEGF but also β- and γ-crystallins, which function together in regulating vessel regression of the developing eye [51–53]. The expression of Aquaporin 4 (AQP 4), the major water channel in astrocytes, is elevated in astrocytes in human persistent fetal vasculature (PFV) disease. The loss of AQP4 leads to astrocytes ensheathing the hyaloid artery, thus preventing regression of the hyaloid vessels [54]. Conditional knockout of *Vhl* in retinal astrocytes using floxed alleles in *glial fibrillary acidic protein (GFAP)-Cre* mice resulted in transient accelerated vessel regression, followed by increased vessel branch points of primary hyaloid vessels [9]. In the process of vessel pruning in the zebrafish brain, microglia act as scavengers to clear apoptotic ECs but are dispensable for vessel pruning [35]. These results demonstrate the contribution of astrocytes during vessel remodeling.

The role of hemodynamics in vessel remodeling

The inner layer of the vascular structure is composed of a monolayer of ECs that directly sense and transduce hemodynamic forces into molecular signaling to regulate vascular development. Endothelial mechanic sensors such as integrins, platelet endothelial cell adhesion molecule-1 (PECAM-1)/vascular endothelial cell (VE-cadherin)/vascular endothelial growth factor receptor 2 (VEGFR2) complex, and notch1 contribute to angiogenesis, vessel integrity and vessel remodeling [55–59]. Although the early stages of vascular development, such as vasculogenesis and angiogenesis, have been well studied, the maturation of vascular networks, such as vessel remodeling, needs to be further studied.

The segment with lower blood flow will be pruned

Hemodynamics plays a very important role in vascular development, vessel remodeling, maturation, and vessel quiescence under physiological conditions. The magnitude of flow shear stress (FSS) is coincident with blood flow velocity and inversely correlated with vessel diameter. Correspondingly, arteries, capillaries, and veins present different magnitudes of blood flow to adapt to tissue development. We have previously reported that decreasing blood flow by *tnnt2a* MO inhibits CVP angiogenesis in zebrafish embryos and leads to an oversimplified CVP vasculature, which is regulated by the *ERK5-klf2a-nos2b* axis [60]. When zebrafish embryos were exposed to a simulated microgravity (SM) environment from 24 to 36 hpf, the heartbeat of zebrafish was significantly reduced. This SM environment resulted in an increased intercapillary number and a wider CVP in zebrafish embryos [61, 62]. Several groups have identified that vessel pruning preferentially occurs at segments under low blood flow and the stabilization of segments under high blood flow [20, 28, 30]. The average blood flow velocity in unpruned segments is higher than that of pruned segments during vessel pruning of zebrafish midbrain vasculature [30]. To prove this theory in vivo, time-lapse imaging of CV pruning in *Tg(flk1:EGFP;gata1:dsRed)* transgenic fish was performed to clarify the relationship between the magnitude of blood flow and vessel pruning [28]. The average velocity of red blood cells (RBCs) in the vascular branch was calculated to assess the magnitude of blood flow. The data showed that the diameters of the two branches were almost the same before the initiation of CV pruning in zebrafish (Fig. 4a). However, the blood flow of the lower branch is slower than that of the upper branch at this stage (Fig. 4a). Then, vessel stenosis occurs at the lower branch (Fig. 4b). The difference in blood flow gradually increased between the upper and lower branches until there was no blood perfusion in the lower branch (Fig. 4c). Finally, the lower branch is pruned, while the upper branch remains (Fig. 4d, e). This result revealed that a decrease in blood flow occurred before vessel stenosis in the pruned segment, which may trigger the segment with lower blood flow to prune [28]. Furthermore, we found that *klf6a* could respond to blood flow to regulate CV pruning [28]. Kochhan et al. found that maintaining blood flow through the dorsal CrDI by laser ablation of the adjacent nasal ciliary artery (NCA) prevents dorsal CrDI pruning [26]. SIV plexus pruning, CV pruning, and CrDI pruning are blocked after slowing blood flow by triciane treatment or *tnnt2a* MO injection at the single-cell stage in zebrafish embryos [26–28]. CrDI

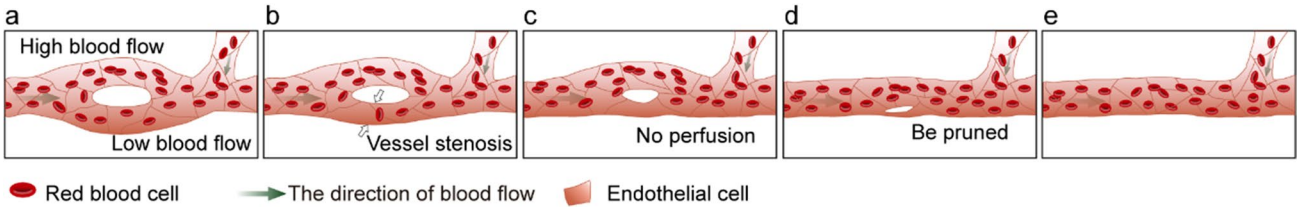

Fig. 4 The role of blood flow during vessel pruning, in which the segment with lower blood flow is pruned. **a** The diameter of the upper branch and the lower branch is the same before vessel pruning, while the blood flow velocity of the lower branch is lower than that of the upper branch. **b** Vessel stenosis occurs at the lower branch with low blood flow. **c** No blood perfusion in the lower branch. **d**, **e** The lower branch is pruned, while the upper branch remains

pruning is blocked during heartbeat blocking with tricaine treatment but recovers vessel pruning after drug withdrawal [26]. Lucitti et al. found that vessel remodeling is defective in *Mlc2a*$^{-/-}$ mouse embryos, in which both plasma and erythroblast flow in the circulation are disrupted [63]. In conclusion, hemodynamics is the key factor in regulating vessel remodeling.

Shear stress induces ECs to align in their direction and to polarize [64, 65]. Cell polarization in the direction of blood flow plays a vital role in cell migration [65]. Franco et al. found that ECs polarized toward adjacent vessels during vessel pruning [20]. However, there are misaligned or nonpolar ECs at the lower wall shear stress segments relative to adjacent segments with higher wall shear stress [20]. Our results demonstrated that ECs did not exhibit polarity in pruned segment during zebrafish CV pruning [28]. EC polarity is tightly associated with the magnitude of blood flow. However, CV exhibits low blood flow from 48 to 60 hpf compared to arteries, when arterial ECs already become polar [64]. This may be one of the reasons to explain the nonpolarity ECs during CV pruning.

Mechanical sensors of ECs in vessel remodeling

Blood flow coordinates the behavior and function of ECs to form a mature vascular network, but how ECs read and interpret the signals generated by hemodynamics is not clear. The branching structure of the arterial tree is based on the adaptive response of the vessel diameter induced by wall shear stress [66]. ECs are exposed to different magnitudes of FSS, which in turn sense and respond to changes in blood flow to regulate the morphology and function of blood vessels. Our understanding of how ECs recognize the distributions of the different magnitudes of blood flow to regulate gene expression and adjust vascular morphology is limited. Recently, scientists have gained some insights into EC mechanosensors and signal transduction, including integrins, the PECAM-1/VE-cadherin/VEGFR2 complex, and notch1 (Fig. 5).

In the event of mechanotransduction, the extracellular matrix (ECM) plays a pivotal role in the interaction of the matrix and cells (Fig. 5). The matricellular protein thrombospondin-1 (Thbs1) binds to integrin αVβ1 to regulate the focal adhesion-actin complex by promoting the nuclear shuttling of YAP. Deletion of *Thbs1* in mice disrupted mechanotransduction by inhibiting Thbs1/integrin/YAP signaling, resulting in abnormal vessel remodeling [58]. Integrins mediate FSS-induced AKT phosphorylation by PI3K and ERK1/2 activation [56, 67, 68]. FSS stimulates the activation of integrins and binding to ECM proteins, which induces the transient inactivation of Rho and the activation of downstream JNKs. The transient inactivation of Rho is adequate for cytoskeletal alignment in the direction of flow [57]. Integrins also interact with cadherins to regulate actin cytoskeleton alignment, intracellular forces, endothelial integrity, focal adhesion remodeling and cell contractility, which are critical for cell migration [57, 69–71]. These results indicate that integrins may participate in FSS-involved vessel remodeling by regulating the actin cytoskeleton and junction rearrangement.

The mechanosensory complexes PECAM-1, VE-cadherin and VEGFR2 transmit mechanical force into cell signaling to regulate vessel remodeling, cardiac development and atherogenesis (Fig. 5) [55]. PECAM-1 directly senses mechanical force and then activates Src, whereas VE-cadherin functions as an adaptor with its binding partner β-catenin to bind with VEGFR2 and activate downstream PI3K and integrins [55, 72, 73]. Another study from the same group showed that VEGFR3 is also a component of this complex and is involved in regulating the hemodynamic response of ECs [74]. The shear stress "set point" at which ECs have a preferred level of FSS is related to the level of VEGFR3 in the cells. Increasing the level of VEGFR3 in ECs decreases the "set point", but this is the opposite of lymphatic ECs [75, 76]. PECAM-1$^{-/-}$ mice exhibited thinner intima-media and adventitia induced by partial carotid ligation, which implied that PECAM-1-dependent signaling is necessary for flow-induced vessel remodeling [77]. The downstream target of low shear stress (LSS), a small

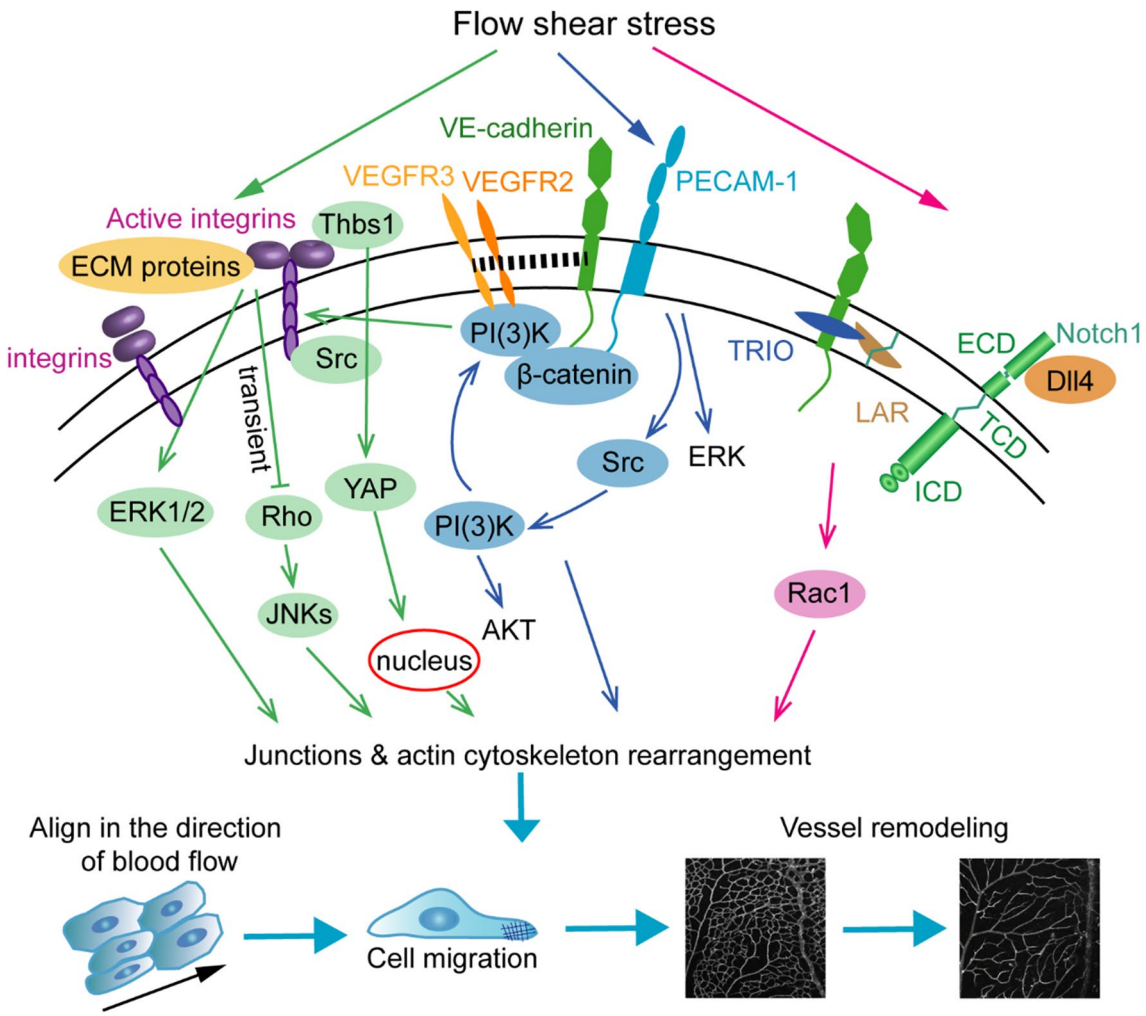

Fig. 5 Mechanosensors regulate vessel remodeling, including integrin signaling, VE-cadherin, VEGFR2/3, PECAM-1 complex and Notch1 signaling. Integrins interact with Thbs 1 to promote YAP nuclear translocation and regulate vessel remodeling by promoting cell migration, junctions and actin cytoskeleton rearrangement. Flow shear stress (FSS)-induced ERK1/2 activation and Akt phosphorylation depend on integrin binding to extracellular matrix (ECM) proteins. The combination of integrins and ECM proteins induces a transient inhibition of Rho and the activation of downstream JNKS, which is necessary for cytoskeletal alignment in the direction of flow. The mechanosensory complex, PECAM-1-VE-Cadherin-VEGFRs, activates the PI3K-Akt pathway to promote cell migration. PECAM-1 directly senses mechanical force and then activates Src, and VE-cadherin binds with β-catenin and VEGFR2/3 to activate downstream P13K and integrin. The NOTCH1 mechanosensory complex senses FSS and regulates junctions and actin dynamics, which includes the processes of (i) FSS-induced endocytosis of DLL4; (ii) cleavage of NOTCH1 to expose the transcellular domain (TCD); and (iii) binding to the LAR with VE-cadherin and TRIO to activate the downstream target RAC1

fraction of VE-cadherin phosphorylation on Y658, causes the dissociation of P120ctn and binds to polarity protein LGN to mediate FSS sensing [78]. VE-cadherin phosphorylation by treatment with LSS and DAPT (*N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-*S*-phenylglycine *t*-butyl ester, an inhibitor of the Notch pathway, inhibits vessel fusion [79]. However, Src inhibition can prevent VE-cadherin phosphorylation in ECs to rescue hyperfusion [79]. VEGF stimulation promotes the fusion of blood vessels in mouse allantoic-derived vascular spheroids or in avian embryos [15, 18]. Overexpression of VEGF by implanting VEGF-soaked heparin chromatography beads causes an increase in vascularity and an enlarged dorsal aorta in quail embryos because of increased vessel fusion events between the dorsal aorta and lateral capillary plexus [17]. Inhibition of circulating VEGF by the fusion protein Flk1/KDR receptor leads to increased capillary density but no difference in cell apoptosis during the programed regression of the PM [13]. The transmembrane semaphorin6A (Sema6A)-null mice shows a reduction of hyaloid network complexity and branch points due to increased cell death by downregulating VEGFR2 [80]. The deficiency of metabolic enzyme CDP-diacylglycerol synthetase-2 (CDS2) induces the secretion of VEGFA, which in turn promotes vessel remodeling by

regulating PIP3 and FOXO1 nuclear accumulation [81]. In conclusion, the mechanosensory complexes PECAM-1, VE-Cadherin and VEGFRs not only perform mechanotransduction but are also important for vessel remodeling.

The transmembrane receptor Notch1, as an important signal pathway in the process of angiogenesis and vessel remodeling, has been proven to be a mechanosensor [39, 59, 82, 83]. The transmembrane domain of Notch1, together with VE-cadherin, the transmembrane protein tyrosine phosphatase LAR and the RAC1 guanidine-exchange factor TRIO, forms a receptor complex that senses mechanical force and contributes to vascular barrier function by promoting the assembly of adherens junctions (Fig. 5) [59]. During ISV differentiation in zebrafish, venous blood flow or weak pulsatility induces upstream migration of ECs to replace arterial ECs and transformation of the vISV. However, arterial blood flow or strong pulsatility of the two adjacent ISVs prevents venous EC migration by activating Notch signaling in ECs [84]. Therefore, blood flow magnitude-induced Notch signaling activation is necessary for the differentiation of aISVs and vISVs. Notch signaling is only responsible for postnatal vein and perivenous capillary plexus remodeling but not for artery remodeling in a mouse retina model [85]. Lobov et al. demonstrated that the Delta-like ligand 4 (Dll4)/Notch pathway is involved in vessel remodeling and regression in oxygen-induced retinopathy (OIR) or the maturation of the neoretina [86]. Dll4/Notch inhibits the perfusion of microvessels by reducing the expression of the vasodilator adrenomedullin and promoting the expression of the vasoconstrictor angiotensinogen to regulate vessel remodeling [86]. Loss of *Notch-regulated ankyrin repeat protein (Nrarp)* leads to excessive vessel pruning events regulated by Notch-dependent cell cycle arrest and Cyclin D1-induced Lef1/ctnnb1/Wnt signaling in retinal vessel segments [87]. Overall, Notch1 functions as a mechanosensor and plays an indispensable role in angiogenesis and vessel remodeling.

Wnt signaling regulates vessel remodeling

The Wnt family consists of 19 highly conserved glycoproteins, which are classified into canonical (β-catenin-dependent) and non-canonical (β-catenin-independent) signaling. Wnt signaling plays a pivotal role in vascular angiogenesis, vessel remodeling and vascular regeneration during vascular development and disease [88–90]. In this section, we will illustrate the role of Wnt signaling in vessel remodeling (Fig. 6).

Conditional knockout of *Evi*, the Wnt secretion factor, causes decreased microvessel density in the mouse retina and tumor angiogenesis [91]. Costaining of IB4 and Col. IV revealed increased empty basement membrane sleeves in *Evi*-ECKO mouse retinas, indicating that the decreased microvessel density was caused by increased vessel pruning. Further studies have shown that downregulation of the apoptosis-related gene *Tek* and upregulation of the proliferation-related gene *Cdkn1a and* apoptosis-related genes *Bax* and *Stat2* account for increased vessel pruning [91]. Wnt signaling enhancer R-spondins 3 (*Rspo3*) mutant mice die at approximately E10, causing the primary capillary plexus of the placenta to fail to undergo proliferation and remodeling [92]. Scholz et al. found that the phenotype of *Rspo3*-ECKO was consistent with that in *Evi*-ECKO mouse retinas, showing decreased vascular density, excessive vessel pruning, and increased EC apoptosis [91, 93]. *Rnf213*, *Usp8*, and *Trim30α* expression is increased in *Rspo3*-ECKO ECs, which inactive non-canonical WNT/calcium signaling at the level of NFAT, thus causing excessive vessel pruning. During the development of the mouse retina, the loss of the non-canonical Wnt ligands *Wls*, *Wnt5a* or *Wnt11* leads to an increase in the sensitivity of ECs to shear stress, resulting in endothelial polarization and EC migration against blood flow under LSS, thus aggravating vessel pruning [94]. APCDD1 is a negative regulatory protein of Wnt/β-catenin signaling. *Apcdd1* knockout mice show a transient increase in vascular density during P10–P12 due to decreased vessel pruning in the retinal vasculature. However, there was no difference between $Apcdd1^{-/-}$ and WT mice in retinal vascular density at P14 [95]. Treatment with the canonical Wnt inhibitor DKK-1 or sFRP-1 prevents microvessel pruning and increases vascular density in a rat mesentery model [96]. Deletion of Frizzzed (*Fz)4,* the coreceptor low-density lipoprotein receptor-related protein (*Lrp)5 or Ndp* results in decreased vascular density during postnatal retinal vascular development in mice [97, 98]. However, deficiency of *fzd4* in adult zebrafish increases the vascular density of the ventral retina, not the dorsal retina [99]. Endothelial-specific deletion of β-catenin leads to embryonic death, causing abnormal vascular morphogenesis and an inability to remodel to a tree-like structure, such as enlarged or irregular lumen, abnormal branching of umbilical vessels, lacunae-like bifurcations, and blind ending [100]. β-Catenin gain-of-function (GOF) embryos show the absence of a perineural vascular plexus due to a lack of correct remodeling of small vessels. Dll4/Notch signaling and downstream effectors are significantly increased in β-catenin GOF mutants, which impair EC migration and vessel pruning in the yolk sac and head [101]. *Nrarp* acts as a downstream target of Dll4/Notch signaling in ECs to regulate Notch- and Lef1-dependent Wnt signaling, thus contributing to vessel stability during angiogenesis. Therefore, the balance of Notch signaling is the key factor for vessel pruning in this event. *Nrarp* is specifically expressed at newly formed branch points, and its deletion leads to excessive vessel pruning with reduced vessel density and branch points. Loss of *Lef1* and endothelial-specific deletion of *Ctnnb1* phenocopy the deficient retinas

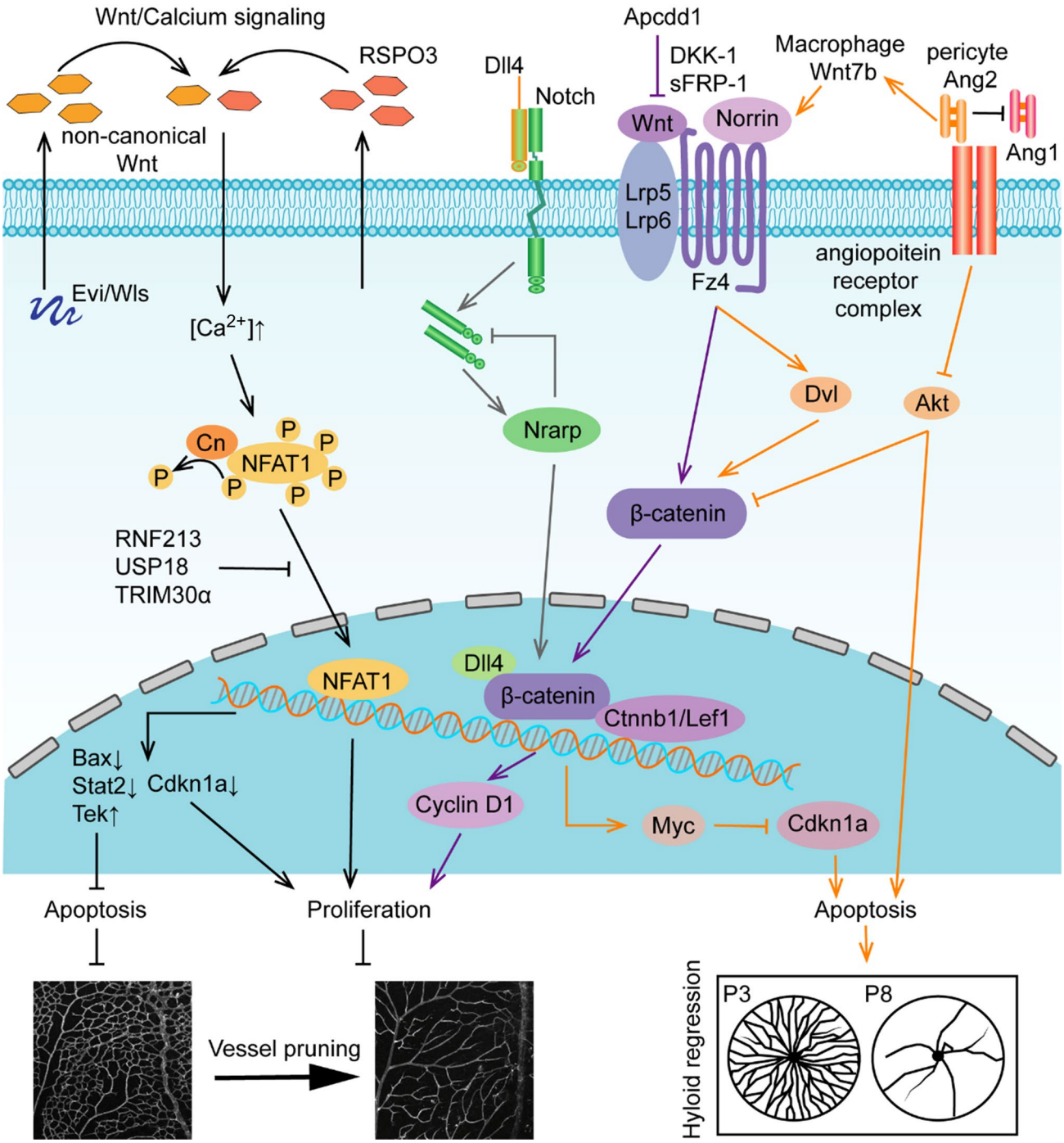

Fig. 6 Role of Wnt signaling in vessel remodeling. Non-canonical Wnt ligands activate Wnt/Ca^{2+} signaling and regulate vessel remodeling at the transcriptional level of apoptosis- and proliferation-related genes. *Evi/Wls*/R-spondin3 (Rspo3) activate non-canonical WNT/calcium signaling at the level of NFAT1 by downregulating *Rnf213*, *Usp18*, and *Trim30α*, which balance the level of cell survival genes to regulate vessel pruning in the retina. Canonical Wnt signaling receptors, coreceptors and ligands cooperate with Dll4/Notch signaling, and pericytes secrete Ang II to balance the progress of vessel remodeling. Canonical Norrin/Fz4/Lrp5/6 accelerate β-catenin nuclear translocation and control the transcription of Cyclin D1 or Myc-Cdkn1a to regulate cell survival. The negative regulatory factor Apcdd1 controls vessel density transiently in retina during P10-12. Dll4/Notch signaling stimulates expression of *Nrarp* and contributes to canonical Wnt signaling by interacting with *Lef1/Ctnnb1*. Ang2 produced by pericytes has a dual identity in the regulation of cell death. On the one hand, Ang2 suppresses Akt to permit cell death. On the other hand, Ang2 promotes the secretion of Wnt7b by macrophages to activate the Wnt/β-catenin pathway, which inhibits cell death by promoting cell cycle entry to regulate hyaloid regression

of *Nrarp* mice. The expression of the Notch target *Hey2* is increased, and the expression of *Cyclin D1* is decreased in *Nrarp*$^{-/-}$ mice. Dll4/Notch signaling induces the expression of *Nrarp*, which negatively regulates Notch signaling and interferes with p21CIP-dependent cell cycle arrest. *Nrarp* could significantly induce Lef1/Ctnnb complex-regulated cell cycle arrest by transcriptional activation of *Cyclin D1* [87]. In conclusion, *Nrarp*-mediated cell proliferation by balancing Notch and Wnt signaling may be a main mechanism accounting for vascular stabilization.

In addition, Wnt signaling also regulates hyaloid vessel regression (Fig. 6). *Lrp*5 or *Lrp6* mutant mice show a defect in hyaloid regression and delayed retinal vascular growth caused by a halted cell cycle and decreased cell apoptosis [102]. Knockout of either *Ndp* or *Fz4* leads to delayed hyaloid regression. Norrin activates canonical Wnt signaling by interacting with the Fz4 receptor and Lrp coreceptor to regulate hyaloid regression [97, 103]. *Myc*, Wnt/β-catenin pathway target gene and the gene regulates cell cycle and cell death, deletion of it increases the expression of *Cdkn1a* and resulting in the persistence of hyaloid vessels. *Myc/Cdkn1a* is required for cell cycle entry and proper levels of cell apoptosis to promote hyaloid regression [4]. Lobov et al. found that the expression of *Wnt7b* is increased in hyaloid macrophages from P1 to P5 [7]. Deficiency of *Lrp*5, *Lef1* and *Wnt7b* results in persistent hyaloid vessels, causing reduced EC apoptosis [7]. Macrophage *Wnt7b* activates the WNT pathway in adjacent ECs through cell–cell contact and then regulates EC cell cycle entry, apoptosis and programmed capillary regression [7]. *Wnt7b* in macrophages is stimulated by the suppression of PI3K-Akt survival signaling in ECs through angiopoietin (Ang)2, which triggers ECs to enter the cell cycle and die in the G1 phase of the cell cycle as a result of reduced VEC apoptosis [104].

Conclusions and perspectives

To ensure functional vascular network formation, vessel remodeling is indispensable for the process of vascular development. In this review, we described the differences and characteristics of vessel pruning, vessel regression and vessel fusion. Our previous studies showed that hemodynamic foci are required for EC junctions and actin cytoskeleton rearrangement, EC migration, cell proliferation and cell apoptosis, which are necessary for angiogenesis and vessel remodeling [28, 60, 62, 105]. Studies have found that segments with low blood flow tend to be pruned, while segments with high blood flow are maintained [20, 28]. In addition, the segment that is longer than others also likes to be pruned, which is accompanied by EC apoptosis [35]. The mechanosensory complexes PECAM-1/VE-Cadherin/VEGFRs and Notch1 play an important role in vessel remodeling. PECAM-1 directly senses FSS and transduces the focus to cell signaling. VE-cadherin is an adapter for the mechanic sensor [55]. Furthermore, VE-cadherin and actin cytoskeleton rearrangement contribute to cell migration during vessel pruning. In addition, blood flow connects with Notch signaling, the Wnt pathway and VEGFR to regulate vessel remodeling.

The study of biomechanics and vessel remodeling mechanisms aims to explore scientific issues and principles and to serve clinical medicine. Vessel remodeling is not only indispensable during physiology vasculature development, but essential in pathogenic process of various cardiovascular disorders, including atherosclerosis, hypertension, stroke, tumors metastasis [106]. Vessel remodeling is characterized by EC morphological structure and phenotype changes, such as endothelial-to-mesenchymal transition (EndoMT), under pathological conditions. ECs generally function in migration, hypertrophy, proliferation and apoptosis [16, 23]. Pathological vessel remodeling is also influenced by hemodynamic forces. We aim to elucidate how mechanical factors produce biological effects and regulate cardiovascular development under physiological conditions. In addition, we determined the mechanically sensitive genes to explore their effect on vascular development and the molecular mechanism. Overall, insight into the mechanism of vessel remodeling under physiology may discovery new disease-related genes and cell signaling, which may be the entry point for vessel remodeling-associated disease treatment.

The department of "Mechano-Developmental Biology" was established at Chongqing University in 2010. A series of hemodynamic-vascular developmental biology studies have been carried out, and a number of research papers have been published. Our lab used transgenic zebrafish (*Tg(flk1:EGFP)*, *Tg(kdrl:mCherry)*, *Tg(gata1a:dsRed)*, *Tg(fli1a:nEGFP)*, *Tg(UAS:EGFP)*, *Tg(fli1a:B4GALT1-mCherry)*, *Ki(cdh5-mRFP)*) to explore the effect of blood flow on angiogenesis and vessel remodeling during development and arterial stenosis under physiological and pathological conditions. The combination of confocal microscopy and an in vivo microcirculation real-time tracing system makes zebrafish a powerful tool for studying blood flow-associated vascular development. We found that the reduction in blood flow velocity affects the angiogenesis and pruning process of CVP in zebrafish embryos [28, 60].

However, some issues remain to be studied: (1) The molecular mechanism of the interaction between ECs and perienthelial cells in regulating vessel remodeling; (2) How ECs sense different intensities of FSS to conduct vascular remodeling; and (3) The signaling pathway should be studied to better understand vessel remodeling under both physiological conditions and pathological conditions. In most cases, the vascular system is simplified and simulated, or only a small part of the blood vessels are separated from the whole vascular

network to be studied, which lacks the integrity of the vascular network. In fact, the vascular network is a tree-like structure with complex vascular branches. Previous studies have shown that the differentiation of arteries/veins is regulated by Notch signaling [107]. Arterial, venous or capillary ECs show different polarities in the same period during development, which is regulated by apelin receptor signaling [64]. These results demonstrated that arteries, veins and capillaries are regulated by different signaling pathways. Further studies are needed to explore the molecular mechanisms of blood vessel formation mediated by blood flow. Only by better reconstruction of a full-scale model of circulation can we better analyze its molecular mechanisms. Fortunately, using zebrafish, we can monitor blood flow distribution in the whole blood vessel network in real time, which allows us to investigate in vivo the pathways that modulate flow sensing and response in different types of vessels. Our ultimate aim is to draw a hemodynamics-sensitive gene map covering the vascular network. In addition, this model can be used to explore the initiation and early development of diseases and their molecular mechanisms, including hypertension, atherosclerosis and other pathologic conditions. In general, we hope that our research will contribute to the treatment of numerous pathological conditions in the clinic.

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Availability of data and materials All data generated during this study are included in this published article.

Declarations

Conflict of interest The authors have no relevant financial interests to disclose.

Ethics approval and consent to participate This is a review article, which did not need ethical approval.

Consent for publication Human experiments are not involved in this article.

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