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Notch Signaling in Vascular Endothelial and Mural Cell Communications

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The Notch signaling pathway is a highly versatile and evolutionarily conserved mechanism with an important role in cell fate determination. Notch signaling plays a vital role in vascular development, regulating several fundamental processes such as angiogenesis, arterial/venous differentiation, and mural cell investment. Aberrant Notch signaling can result in severe vascular phenotypes as observed in cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and Alagille syndrome. It is known that vascular endothelial cells and mural cells interact to regulate vessel formation, cell maturation, and stability of the vascular network. Defective endothelial–mural cell interactions are a common phenotype in diseases characterized by impaired vascular integrity. Further refinement of the role of Notch signaling in the vascular junctions will be critical to attempts to modulate Notch in the context of human vascular disease. In this review, we aim to consolidate and summarize our current understanding of Notch signaling in the vascular endothelial and mural cells during development and in the adult vasculature.

OVERVIEW OF NOTCH SIGNALING

The Notch pathway is a highly conserved signaling mechanism that has been studied for over a century, beginning with the identification of Notch as the gene responsible for wing margin development within *Drosophila melanogaster* (Dexter 1914; Morgan 1916; Mohr 1919). The subsequent cloning of the gene for the Notch receptor paved the way for the identification of components of the Notch signaling pathway (Kidd et al. 1983; Wharton et al. 1985). The Notch signaling pathway is one of a relatively small number of conserved mechanisms that mediate several fundamental physiological processes including cell proliferation, differentiation, and apoptosis during development (Bray 2006). In recent years, studies have identified critical roles for Notch signaling in the processes of vasculogenesis and angiogenesis during development and in maintenance of vascular homeostasis in the adult (Akil et al. 2021). Vascular endothelial cells [VSMCs] and mural cells (vascular smooth muscle cells and pericytes) work in unison to promote the development of durable blood vessels that can support blood flow (Sweeney and Foldes 2018). Notch signaling plays an important role in regulating cell fate determination, cell proliferation, and maturation during these processes (Baeten and Lilly 2017).

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Notch receptors are transmembrane proteins that interact with membrane-bound ligands. In mammals, the canonical signaling pathway involves the interaction of one of the four Notch receptors (Notch 1, Notch 2, Notch 3, and Notch 4) with five known Delta/Serrate/Lag (DSL)-2 ligands, including Jagged 1, Jagged 2, Delta-like 1 (Dll1), Delta-like 3 (Dll3), or Delta-like 4 (Dll4) (Alabi et al. 2018). Initially formed as single-chain precursors, the Notch receptors undergo cleavage by a furin-like convertase in the Golgi apparatus to form extracellular and transmembrane regions that are held together at the cell membrane by noncovalent bonds (Tian et al. 2017). Activation of the canonical Notch signaling pathway is initiated with the extracellular interaction of a Notch receptor with a Notch ligand, resulting in two sequential proteolytic cleavage events by disintegrin and metalloproteinase domain-containing protein 10 (ADAM 10) and γ-secretase, giving rise to the release of the Notch intracellular domain (NICD) from the cell membrane (Fig. 1). The NICD subsequently translocates to the nucleus where it exerts its function in cooperation with recombination signal-binding protein for immunoglobulin κJ (RBPJK) and coactivators including Mastermind. The NICD-RBPJK-activated transcriptional complex drives the up-regulation of Notch target genes including, but not limited to, hairy and enhancer and split (HES) 1, 5, and 7 and HES-related repressor protein (HERP) 1 to 3 (Hofmann and Iruela-Arispe 2007). The response to Notch receptor activation is exquisitely sensitive to dosage. Initially subtle differences in Notch expression and signaling can distinguish single cells from a group of seemingly equivalent neighboring cells and therefore drive those cells to an alternate differentiation fate (Lai 2004; Schweisguth 2004; Fouillade et al. 2012).

Importantly, regulation of Notch signaling can occur at several steps; for example, post-translational modifications can occur within the Golgi apparatus. Fringe glycosyltransferases modify the sugar moieties within the epidermal growth factor (EGF)-like repeats through the extension of *O*-linked glycans on the Notch receptors and associated ligand extracellular domains (Moloney et al. 2000). In vertebrates, three fringe orthologs have been extensively characterized including lunatic fringe, manic fringe, and radical fringe. Knockout mouse models of lunatic fringe bear resemblance and display key pathological features seen in mouse models deficient in components of the Notch signaling pathway (Shen et al. 1997; Zhang and Gridley 1998; Barrantes et al. 1999). Modifications of Notch by fringe leads to differential modulation of the interaction of Notch with the DSL ligands. Lunatic fringe inhibits Jagged 1–mediated signaling and conversely increases the strength of Dll1-mediated signaling through Notch 1. In contrast, lunatic fringe potentiates both Jagged 1 and Dll1 signaling through Notch 2 (Hicks et al. 2000). These modifications have the ability to modify the strength of response of Notch receptors to their ligands and may help to better understand the context-dependent functions of Notch signaling (Hicks et al. 2000; del Álamo et al. 2011; Shen et al. 2021).

Clinical and experimental evidence has shown that Notch signaling plays a key role in both development and maintenance of the vasculature, whereas disrupted Notch signaling can have dramatic effects on vascular development and stability (Table 1). Abnormal Notch signaling components have been implicated in many developmental human pathologies including Alagille syndrome (AGS), tetralogy of Fallot, syndactyly, spondylocostal dysostoisis, and aortic valve disease (Gridley 2003; Garg et al. 2005). The disorders, however, are not limited to development, as mutations in Notch 3 lead to cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a progressive neurovascular disorder that is characterized by ischemic stroke of adult onset (Joutel et al. 1996).

The intricate and dynamic expression of Notch receptors and ligands in vascular cells further highlights the importance of Notch signaling. Notch 1 and Notch 4 are strongly expressed by the endothelium and Notch 3 expression is largely localized to VSMCs and pericytes within the brain (Joutel et al. 2000; Domenga et al. 2004). Interestingly, activated Notch 1 has been identified in the arterial vasculature during pulmonary development. Notch 2 is

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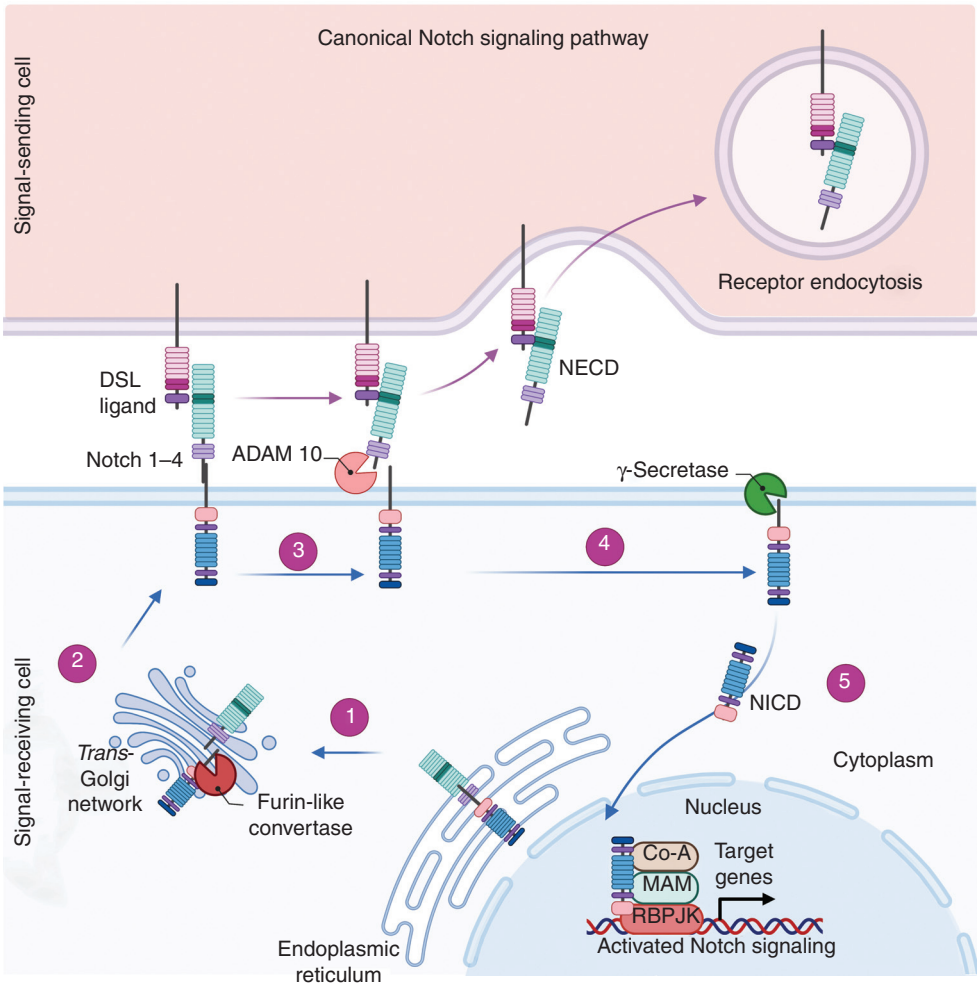

Figure 1. Schematic of the canonical Notch signaling pathway. (1) Notch receptors (1–4) are initially synthesized as single-chain precursors within the endoplasmic reticulum and subsequently transported to the Golgi apparatus. (2) The Notch precursor then undergoes cleavage by furin forming an extracellular and transmembrane domain and is modified by the glycotransferases fringe. These modified proteins are transported and inserted into the cell membrane. The Notch receptor interacts with Delta/Serrate/Lag (DSL)-2 ligands Jagged 1, Jagged 2, Delta-like 1 (Dll1), Delta-like 3 (Dll3), or Delta-like 4 (Dll4). (3) Upon binding, the Notch receptor is cleaved by ADAM 10 releasing the Notch extracellular domain (NECD) that is then degraded by receptor endocytosis. (4) There is then a subsequent proteolytic cleavage by γ-secretase releasing the Notch intracellular domain (NICD) into the cytoplasm. (5) The NICD is then translocated to the nucleus and forms a coactivator complex with factors including recombination signal-binding protein for immunoglobulin κJ (RBPJK) and Mastermind-inducing transcription of *Hes* and *Hey* genes. (Created in BioRender.com.)

found at high levels within mesenchymal tissue and smooth muscle cells that surround the pulmonary arteries and aorta (High et al. 2007). Vascular endothelial cells also express the ligands Dll1, Dll4, and Jagged 1. The Notch ligand Jagged 2, Notch 1–4, Dll4, Jagged 1, and Jagged 2 are largely expressed in arteries but not veins (Villa et al. 2001). We will review the role of the Notch signaling in endothelial cells and mural cells individually during development and in the adult vasculature, then assess their cumulative interactions.

Table 1. Vascular expression of key Notch signaling components and their role in vascular smooth muscle cell (VSMC) function and associated pathologies

Notch signaling component	Vascular expression	Function in VSMCs	Associated vascular disease
Notch 1	Endothelial cells (Takeshita et al. 2007), pericytes (Kofler et al. 2015), VSMCs (low basal expression) (Li et al. 2009)	Promotes proliferation + cell survival (Sweeney et al. 2004)	Adams–Oliver syndrome (Stittrich et al. 2014) Aortic valve disease (Garg et al. 2005)
Notch 2	VSMCs (large caliber vessels) (High et al. 2007; Varadkar et al. 2008)	Promotes differentiation (Baeten and Lilly 2015) + Jagged 1–mediated proliferation inhibition (Boucher et al. 2013)	Alagille syndrome (McDaniell et al. 2006) Hajdu–Cheney (Simpson et al. 2011)
Notch 3	Pericytes (Joutel et al. 2000), VSMCs (Domenga et al. 2004)	Promotes differentiation + mural cell investment (Domenga et al. 2004), promotes proliferation + cell survival (Liu et al. 2010)	Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) (Joutel et al. 1996) Infantile myofibromatosis (Wu et al. 2021)
Notch 4	Endothelial cells (Wu et al. 2005)	-	-
Jagged 1	VSMCs (High et al. 2007), endothelial cells (Lindner et al. 2001), pericytes (Kofler et al. 2015)	Promotes differentiation (Manderfield et al. 2012)	Alagille syndrome (Kamath et al. 2004) Tetralogy of Fallot (Eldadah et al. 2001)
Dll1	Endothelial cells (Sörensen et al. 2009)	-	-
Dll4	Endothelial cells (Liu et al. 2003)	-	Adams–Oliver syndrome (Meester Josephina et al. 2015)

Supportive references are included in the table.

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THE ROLE OF NOTCH SIGNALING IN ENDOTHELIAL CELLS DURING DEVELOPMENT

Blood vessel formation is a dynamic and complex process that has a critical role in homeostasis to ensure adequate and appropriate supply of oxygen, nutrients, and trophic factors as well as the removal of waste products in disease processes (Sweeney and Foldes 2018). Endothelial cell proliferation and sprouting are tightly controlled, organized processes involved in the development of new blood vessels, which takes place by two distinct processes: vasculogenesis or angiogenesis. During development of an embryo, the initial growth of blood vessels in an avascular environment (e.g., de novo) is referred to as vasculogenesis (Potente et al. 2011).

During vessel development, Notch signaling is important for vascular remodeling and in the process of arterial specification through its interactions with Notch ligands and association with other pathways such as vascular endothelial growth factor (VEGF) signaling (Krebs et al. 2000; Shawber et al. 2003). The embryonic vasculature is formed from vascular

endothelial cells derived from mesodermal precursor cells (Goldie et al. 2008; Dyer and Patterson 2010). Early endothelial progenitor cells known as angioblasts arise at embryonic day 7.5 and coalesce to form a vascular plexus (Vokes and Krieg 2002). Mesodermal cells expressing sonic hedgehog produce VEGF that interacts with VEGF receptor 2 (VEGFR2) and neuropilin 1 in arterial precursor cells leading to the expression of Dll4 and Notch (Morrow et al. 2009). Once an endothelial precursor cell reaches a critical threshold level of Dll4, it induces strong Notch signaling in an adjacent cell and establishes a cell fate decision selecting an arterial over venous endothelial cell fate (Gridley 2010). Conversely, venous endothelial progenitor cells lack neuropilin 1, and low amounts of VEGF trigger expression of chicken ovalbumin upstream promoter transcription factor II (COUP-TFII). COUP-TFII represses neuropilin 1 and Notch signaling promoting a venous cell fate and EphB4 expression (Fig. 2). During development of the yolk sac, the vasculature is formed by de novo vasculogenesis. Vascular endothelial cells are produced from hemangioblasts that give rise to blood islands containing hematopoietic cells and endothelial progenitor cells (Caolo et al. 2012).

The vascular endothelium primarily expresses Notch 1 and Notch 4 receptors. The initial Notch 1 knockout model clarified its critical role in development as the knockout mice died during embryogenesis on day 11.5 (Swiatek et al. 1994). Vascular endothelial cell–specific Notch 1 knockout mice were then developed and displayed early embryonic lethality and robust vascular defects within the embryonic and yolk sac vasculature, similar to the global Notch 1 knockout phenotype suggesting that Notch 1 is critical for embryonic vascular development (Swiatek et al. 1994; Limbourg et al. 2005). Conversely, Notch 4 knockout mice do not display any obvious vascular defects. Interestingly, a double-knockout of Notch 1 and Notch 4 produces a more severe vascular phenotype than the Notch 1 knockout alone, suggesting possible compensatory mechanisms (Krebs et al. 2000). Vascular network modification and expansion occurs through the process of angiogenesis, whereby endothelial cells proliferate and sprout from the existing vasculature generating a network that remodels into arteries and veins (Potente et al. 2011). The earliest genes identified to differentiate arterial and venous endothelium were EphrinB2 and EphB4 that belong to the Eph-ephrin subclass of receptor tyrosine kinases (Pasquale 2008). During development, factors such as Notch, Hedgehog, and Coup-TFII have been identified to play important roles in arterial specification (Lawson et al. 2001; You et al. 2005). Notch signaling promotes an increase in EphrinB2 expression in arterial endothelial cells while repressing the expression of EphB4 in venous endothelial cells (Chen et al. 2021). An overexpression of Notch 4 in adult mice results in arteriovenous malformation and expression of EphrinB2 in veins that was reversible upon repression of Notch 4 expression. Dll4 knockout mice exhibit severe vascular defects and are associated with a reduction in EphrinB2 expression and an increase in EphB4 expression that indicate a failure in arterial differentiation (Duarte et al. 2004). These data show that Notch signaling is required for the proper development of arterial and venous blood vessels, and is involved in the specification of arterial endothelial cells and in the suppression of venous cell fate (Lawson et al. 2001; Zhang et al. 2014).

NOTCH SIGNALING DURING ANGIOGENESIS AND ENDOTHELIAL CELL FATE DETERMINATION

Angiogenesis is an essential process that is involved in development, tissue growth, and wound healing processes and broadly refers to the formation of new blood vessels from the existing vasculature (Takeshita et al. 2007; Akil et al. 2021). Notch signaling has been identified as an important mediator of angiogenesis through interaction with Notch ligands. Dll4 has been identified as an important ligand for stimulating angiogenesis (Benedito et al. 2009; Naito et al. 2020). Conversely, Jagged signaling has been found to compete with Dll4 to negatively regulate angiogenesis (Benedito et al. 2009). The Notch signaling pathway has been

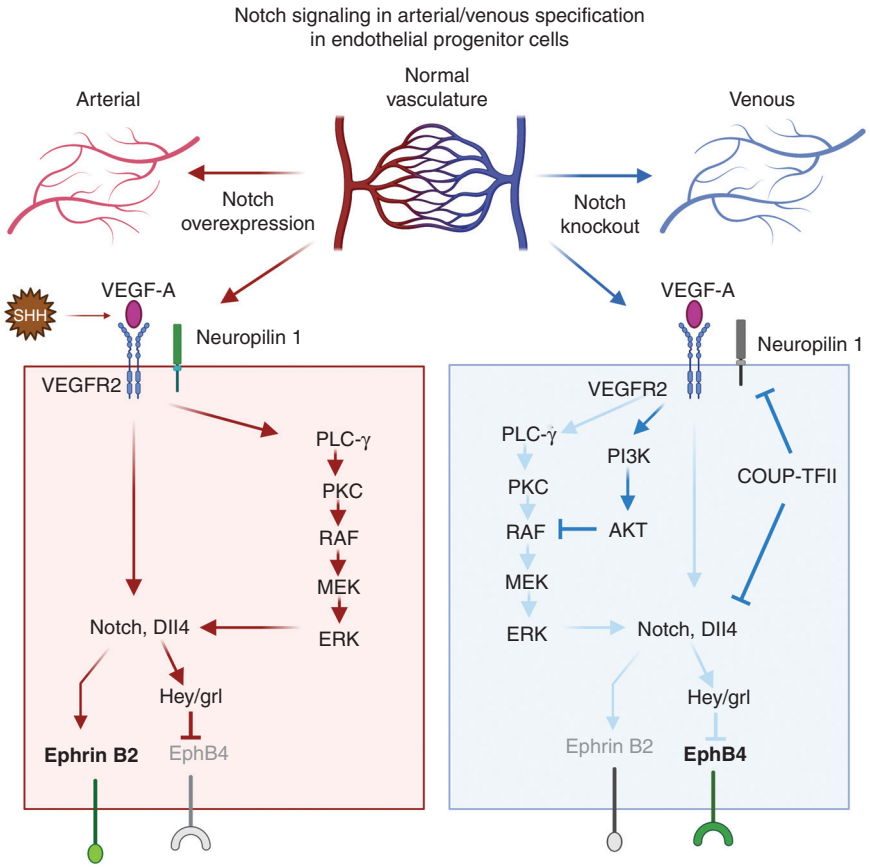

Figure 2. The role of Notch signaling in arterial specification during development. The *top* panel is a schematic showing how changes in Notch expression impact arterial/venous specification in the arterial/venous network. The two major signaling pathways operate downstream of vascular endothelial growth factor (VEGF)-A, induce arterial differentiation, the Notch pathway, and the PLC/MAPK pathway. In the arterial specification pathway (red arrows), sonic hedgehog–expressing mesodermal cells produce VEGF-A and stimulate endothelial precursor cells. Mesodermal cells expressing sonic hedgehog produce VEGF that interacts with VEGFR2 and neuropilin 1 in arterial precursor cells leading to the expression of Dll4 and Notch. Once an endothelial precursor cell reaches a critical threshold level of Dll4, it causes strong Notch signaling in an adjacent cell and establishes a cell fate decision selecting an arterial over venous endothelial cell fate. There are two distinct mechanisms that drive vein differentiation (blue arrows). The venous endothelial progenitor cells lack neuropilin 1 and low amounts of VEGF trigger expression of chicken ovalbumin upstream promoter transcription factor II (COUP-TFII). COUP-TFII represses VEGFR2, neuropilin 1, and Notch signaling and promotes a venous cell fate and promotes EphB4 expression; additionally, the activation of the PI3K/AKT pathway blocks arterial specification by preventing ERK activation. (Created in BioRender.com.)

proposed as a therapeutic target for pathological angiogenesis. Inhibition of the Notch ligand Dll4 in the retinal ischemia model promoted the formation of normal vasculature that was partially able to reverse the ischemic environment (Lobov and Mikhailova 2018). Additionally, targeting Dll4 has become a focus in cancer research as Dll4 inhibition leads to excessive, nonproductive angiogenesis and consequently results in impaired tumor growth and may reduce metastasis (Kuhnert et al. 2011; Mendonça et al. 2019; Yang et al. 2020).

A hallmark of Notch signaling is regulation of cell fate decisions. A clear example of this can

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be observed in the vasculature during the specification of stalk/tip cells phenotype in the growing blood vessel sprout (Fig. 3). During angiogenesis, expression of Dll4 at the cells at the end of the sprout (tip cell) activates Notch 1 in the stalk cell (Blanco and Gerhardt 2013). Vascular sprout formation therefore relies on a coordinated temporal and spatial localization of Notch signaling. This is shown through Notch signaling inactivation at the onset of angiogenesis resulting in an increase in the number of tip cells at the expense of stalk cells. Tip cells alone are insufficient to form tubes or stable junctions (Mack and Iruela-Arispe 2018). Notch is required for stabilization of the vasculature and differentiation and suppression of endothelial cell proliferation (Ehling et al. 2013; Mack et al. 2017). Notch-mediated inhibition of proliferation requires tumor suppressor phosphatase and tensin homolog (PTEN) (Serra et al. 2015), which blocks stalk cell proliferation downstream of Notch signaling. Notch signaling suppresses excessive sprouting via lateral inhibition, a process by which Notch signaling inhibits its neighboring cells from assuming a similar differentiated adult cell state (Sjöqvist and Andersson 2019).

VEGF signaling promotes the up-regulation of Dll4 in the tip cells that then activates Notch 1 in the neighboring stalk cells. The formation of vascular sprouts requires a highly organized and spatial localization of Notch signaling. Ultimately, during the process of angiogenesis, the activation of Notch signaling reduces endothelial cell proliferation through blunting the cell's responsiveness to VEGF (Siekmann and Lawson 2007; Eelen et al. 2020). Additionally, stalk cells express Jagged 1 that can compete with Dll4 for *cis* binding of Notch receptors within the tip cells. Jagged binds but does not activate the Notch receptor in the tip cell and therefore prevents Notch signaling in the tip cell (Blanco and

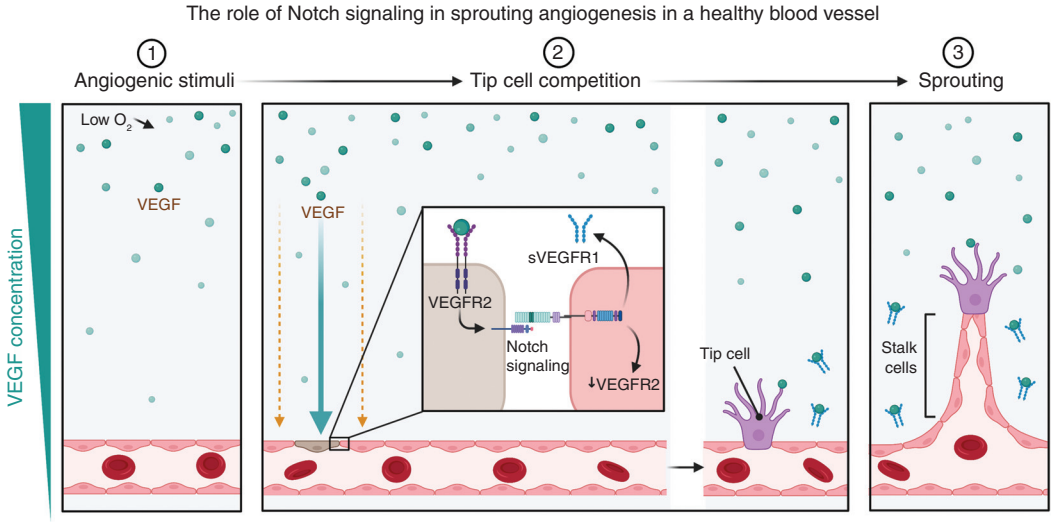

Figure 3. Schematic of Notch signaling in sprouting angiogenesis. (1) Angiogenesis is initiated by a stimulus such as hypoxia, which leads to an increase in vascular endothelial growth factor (VEGF) expression in tissues. (2) The presence of VEGF (green circles) leads to the binding of VEGF receptor 2 (VEGFR2) on the surface of the endothelial cells. A VEGF/Notch-regulated mechanism ensures a limited number of tip cells are formed through a process known as lateral inhibition. After VEGF binds to the VEGFR2 receptor, it promotes the formation of a tip cell (brown cell) and promotes an increase in the Notch ligand Dll4 expression, while simultaneously inhibiting the formation of tip cells by its neighbors through Notch signaling. Activating Notch signaling results in the down-regulation of VEGFR2 and promotes the production of soluble VEGFR (sVEGFR) that can then act to scavenge extracellular VEGF and hence prevent overvascularization. (3) These cells will then become stalk cells that form the body of the sprouting vessel. (Created in BioRender.com.)

Gerhardt 2013). Interestingly, the ability of ligands to activate Notch is dependent on the glycosyltransferase fringe. Without fringe, the nonglycosylated Notch receptor can interact with Jagged 1 resulting in inhibition, but in the presence of fringe, a glycosylated Notch receptor is strongly activated by binding with Dll4 (Benedito et al. 2009). Dysregulation of Notch ligand levels has consequences for the vascular network; for example, mice that are heterozygous for Dll4 have hypervascularized retinas (Lobov and Mikhailova 2018; Suchting et al. 2007).

THE ROLE OF NOTCH SIGNALING IN MATURE VASCULAR ENDOTHELIAL CELLS

Local artery obstruction of blood flow to the periphery can be restored through expansion of arterioles to form collateral vessels in a process known as arteriogenesis (Heil et al. 2006). This process may not be limited to tissue repair but may also have a role in physiological vascular expansion during tissue growth in exercise (Roca and Adams 2007). It has been reported that Dll1, a Notch ligand that is largely localized to the vascular endothelium, has a pivotal role in postnatal angiogenesis and is up-regulated during ischemia-induced arteriogenesis (Limbourg et al. 2007). However, this has been difficult to study due to the embryonic lethality of the Dll1 knockout. Dll1 heterozygous mice display reduced formation of collateral arteries and, in an ischemic hind limb model, fail to restore blood flow (Limbourg et al. 2007).

In addition to the known canonical interactions of Notch with its ligands, there are also noncanonical roles for Notch in the vasculature. It is known that shear stress plays an important role in the maintenance of the blood–brain barrier via a noncanonical Notch 1 activation driving the assembly of adherens junctions through the regulation of Rac1. The process is initiated when shear stress causes the activation of Notch 1 via the Dll4 ligand, leading to the exposure of the transmembrane domain of Notch 1. The Notch 1 transmembrane domain affects barrier function by promoting the formation of a receptor complex at the membrane that is comprised by VE-cadherin, tyrosine phosphatase LAR, and a Rac guanine–nucleotide exchange factor (GEF) trio that promotes the activation of Rac1 (Polacheck et al. 2017).

Other fundamental mechanisms that are regulated by Notch signaling include apoptosis and cell survival, key events during the process of vascular remodeling (Akil et al. 2021). Notch 2 sensitizes endothelial cells by inhibiting the expression of survivin, a key antiapoptotic regulator. During the process of vascular remodeling, activation of Notch signaling can trigger apoptosis (Quillard et al. 2009).

NOTCH SIGNALING IN MURAL CELL DEVELOPMENT

Vessels formed in the early stages of vasculogenesis are composed primarily of endothelial cell tubes; to support blood flow and withstand pressure changes, they recruit mural cells through the release of PDGFB (Hellström et al. 1999). Mural cells act as support cells in vascular function. The most studied types of mural cells include VSMCs and pericytes that have distinct roles within the vasculature. VSMCs provide support and are important for contractility of large vessels, whereas pericytes are associated with smaller caliber capillaries and enhance endothelial cell stability and vessel maturation during sprouting angiogenesis (Armulik et al. 2011; Kofler et al. 2015). Different mural cell types have distinct expression of Notch receptors; VSMCs have been reported to express primarily Notch 2 and Notch 3 and may also express Notch 1 (Fouillade et al. 2012). Pericytes have been reported to express Notch 1 and Notch 3 as well as low levels of Notch 2 (Kofler et al. 2015; Diéguez-Hurtado et al. 2019). Together, mural cells play numerous roles to maintain vessel homeostasis including regulating vasopermeability and controlling blood flow. Notch receptor expression is heterogenous and has unique roles in different vascular beds. Notch 2 expression is highly expressed in large caliber vessels; within these vessels, Notch 3 is not sufficient to completely compensate for loss of Notch 2 and, additionally, loss of Notch 3 results in an exaggerated phenotype (McCright et al. 2001; Wang et al. 2012).

Haploinsufficiency of Notch signaling is causative of several genetic conditions (Table 1) including AGS, an autosomal-dominant disease caused by heterozygous mutations in either Notch 2 (McDaniell et al. 2006), Jagged 1 (Warthen et al. 2006), or, in rare instances, both (Brennan and Kesavan 2017). It is commonly characterized by developmental defects affecting the heart, skeleton, liver, and eyes (Li et al. 1997). Dysfunctional Notch signaling in VSMCs during AGS is associated with vascular phenotypes affecting the cardiac outflow tract and great vessels, and in certain circumstances mutations in Jagged 1 can result in congenital heart defects known as tetralogy of Fallot that can occur during AGS or in the absence of an AGS diagnosis (Eldadah et al. 2001; McElhinney et al. 2002; Bauer et al. 2010). Adams–Oliver syndrome (AOS) is another congenital condition that is caused by Notch 1 haploinsufficiency leading to scalp defects and limb defects and is often associated with cardiovascular abnormalities (Southgate et al. 2015; Suarez et al. 2021).

It is important to highlight that VSMCs have been shown to display a high degree of plasticity and are therefore not regarded as terminally differentiated. In response to molecular stimuli, VSMCs have been found to adapt their responses, a fact that has been implicated in vascular disease (Wu and Zhang 2009). It has been reported that deficiency in Notch signaling in pericytes leads to arteriovenous malformations in a murine model (Nadeem et al. 2020). Arteriovenous malformations are focal vascular lesions in arteries that shunt into veins with no intervening capillary bed. These vascular lesions have been reported to occur in several organ systems; however, they are of particular seriousness in the brain where they are known to result in stroke and seizures (Solomon and Connolly 2017). Additionally, the loss of Notch signaling in pericytes causes a reduction of PDGFRβ levels and increased pericyte apoptosis (Nadeem et al. 2020). Therefore, tight regulation of Notch signaling is vital to pericyte survival (Arboleda-Velasquez et al. 2014). Further supporting this, Notch3 signaling has been implicated in the expansion of brain pericyte populations in zebrafish, whereby it promotes pericyte proliferation and vascular integrity (Wang et al. 2014). Recent studies have shown that pericytes play an important role in the promoting of endothelial sprouting in postnatal vasculature. The expression of VEGFR1 by pericytes leads to a spatial restriction of VEGF signaling (Eilken et al. 2017).

NOTCH SIGNALING IN ADULT MURAL CELLS

Notch 3 is the predominant Notch receptor expressed by mural cells and has been proposed as an important mediator of maturation of VSMCs (Domenga et al. 2004; Liu et al. 2010; Kofler et al. 2015). Notch 3 knockout mice have an increase susceptibility to ischemic stroke upon challenge (Arboleda-Velasquez et al. 2008). The importance of Notch 3 expression within adult mural cells is highlighted by the hereditary disorder CADASIL, a progressive small vessel disease that is caused by mutations within the Notch 3 gene, which is often debilitating and leads to dementia and an early mortality (Schoemaker and Arboleda-Velasquez 2021). CADASIL is characterized by VSMC pathology and ischemic stroke (Joutel et al. 1996, 2000; Arboleda-Velasquez et al. 2011). A major hallmark of CADASIL is the accumulation of granular osmophilic material deposits within the basement membrane of the vasculature (Joutel et al. 2000).

CADASIL is caused by mutations within the Notch 3 gene that commonly lead to an odd number of cystine residues in the EGF-like repeats in the Notch 3 extracellular domain (Notch 3^{ECD}) (Joutel et al. 1997), which compromises the stability of the Notch 3^{ECD} due to an improper formation of disulfide bridges. This has been proposed to result in protein misfolding and accumulation of the Notch 3^{ECD} (Spinner 2000). Key pathological features of CADASIL include the progressive loss of mural cells. Murine models of Notch 3 knockout do not accurately recapitulate the CADASIL phenotype despite reduced VSMC coverage and impaired VSMC maturation (Domenga et al. 2004; Liu et al. 2010; Henshall et al. 2015). Interestingly, a double transgenic murine model of Notch 3 knockout and heterozygous expression of Notch 1 leads to pericyte dysfunction and arteriove-

nous malformations that mimic some of the hallmarks of CADASIL (Kofler et al. 2015). Notch 3 is highly expressed in mural cells when mural cells are cocultured with endothelial cells. Notch 3 has been reported to promote its own expression and increases the expression of Jagged 1 (Liu et al. 2009). Modulation of Notch signaling has been proposed as a therapeutic target in CADASIL using a Notch 3 agonist antisera that was effective at preventing SVD phenotypes in Notch 3 knockout and CADASIL mice (Li et al. 2008; Machuca-Parra et al. 2017).

Notch signaling in mural cells also has a role in regulating cell survival and apoptosis. It has been reported in a variety of cell types that Notch signaling prevents apoptosis (Sweeney et al. 2004; Dror et al. 2007; Arboleda-Velasquez et al. 2014), and it has been proposed that noncanonical interactions of Notch and MAPK/ERK promote cell survival (Wang et al. 2002; Baeten and Lilly 2017).

NOTCH SIGNALING BETWEEN ENDOTHELIAL AND MURAL CELLS

It is widely known that vascular endothelial and mural cells have regions of direct communication through myoendothelial gap junctions and microprojections (Sandow et al. 2009; Nagaraja et al. 2013; Tian et al. 2017). At least three types of endothelial cell–pericyte contacts have been reported: (1) peg and socket contacts whereby endothelial and pericyte processes interdigitate, (2) adherent plaques, and (3) cell–cell contacts that resemble gap junctions (Carlson 1989; Armulik et al. 2005; Zhang et al. 2020). Two-way communication between endothelial cells and pericytes is important to regulate the development and function of the vasculature. During angiogenesis, as endothelial cells form capillaries, they recruit pericytes that stabilize the vessel and prevent capillary sprouting, which helps direct basement membrane formation while also promoting pericyte differentiation (Stratman et al. 2009; Liu et al. 2010; Armulik et al. 2011; Trost et al. 2016). One of the major factors in ongoing communications between endothelial and mural cells is Notch signaling. It is tantalizing to think that cell–cell interactions mediated by Notch signaling take place at this location where the plasma membrane of vascular endothelial cells and mural cells come in close proximity.

Within the context of endothelial–mural cell communication, evidence indicates that endothelial cells act primarily as signal-sending cells and VSMCs behave as signal-receiving cells (High et al. 2008). This is supported by several in vitro and in vivo studies, where coculture experiments of endothelial cells and mural cells highlighted the role of endothelial cells in promoting the activation of Notch signaling in the adjacent mural cells leading to the up-regulation of several contractile genes as well as increased expression of Jagged 1 (Fig. 4; Liu et al. 2009). Additionally, an endothelial cell–specific knockout of Jagged 1 leads to impaired VSMC differentiation in the aortic arch arteries, and postnatal endothelial knockout of Jagged 1 results in a reduction in coverage of retinal arterioles by VSMCs (High et al. 2008; Benedito et al. 2009). Intriguingly, the VSMC-specific knockout of Jagged 1 also results in improper differentiation of VSMCs indicating that Notch/Jagged 1 signaling between VSMCs is also required (Feng et al. 2010). During vascular development, Notch receptors expressed by the VSMCs bind Jagged 1 expressed by the endothelium leading to an up-regulation of the integrin αvβ3 that promotes maturation of the VSMCs and enabling adhesion of VSMCs to the endothelial basement membrane (Scheppke et al. 2012; Tian et al. 2017). In the adult vasculature, it has been shown that knockout of AKT results in a reduction in Jagged 1 expression in the endothelium and a progressive loss of VSMCs due to impaired Notch signaling (Kerr et al. 2016). Conditioned media from endothelial cells or the addition of soluble Jagged 1 are insufficient to stimulate VSMC differentiation in vitro; conversely, coculture of endothelial and VSMCs promotes differentiation (Xia et al. 2012).

A breakdown of communication has been identified in several pathologies such as CADASIL, vascular injury, and diabetic retinopathy. Diabetic retinopathy is associated with an increased deposition of basement membrane that

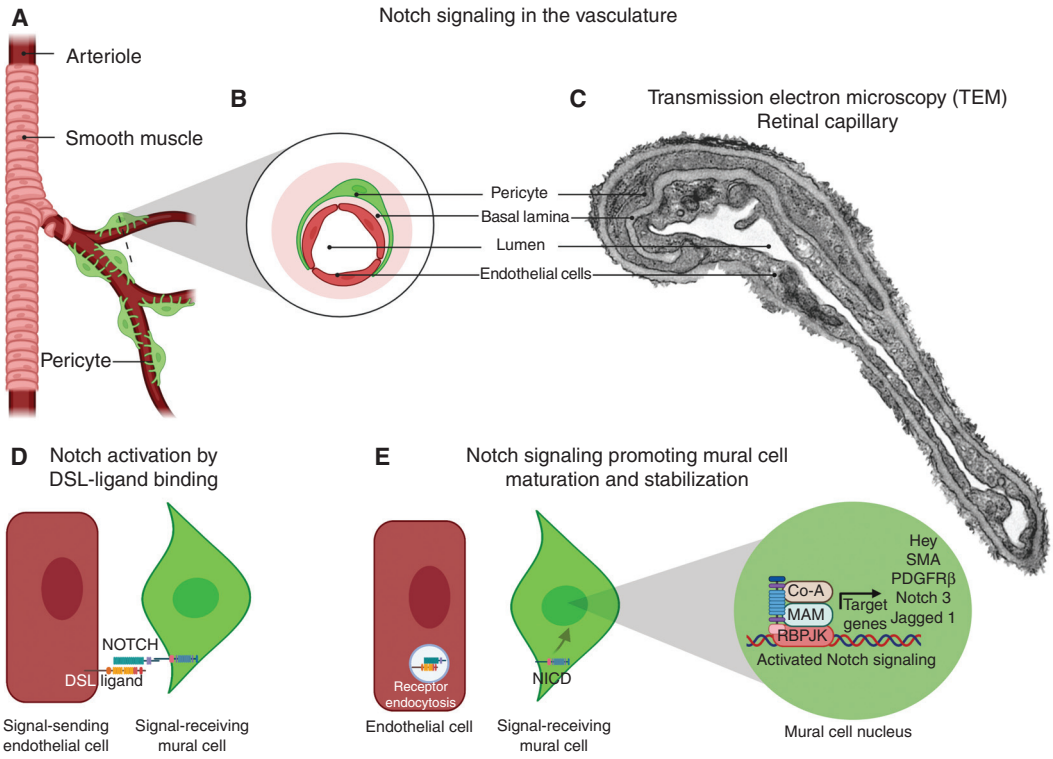

Figure 4. The role of Notch in vascular endothelial and mural cell communication. (*A*) Schematic of mural cell localization within branching arterioles. (*B*) Cross-sectional magnification of a capillary highlight pericytes (green), endothelial cells (red), and basal lamina (pink). (*C*) Transmission electron microscopy (TEM) images of vessels within the retinal ganglion cell layer demonstrate a peg and socket connection between the endothelial cell and pericyte. (*D*) Notch expressed in the mural cell (green) is activated upon ligand binding from the endothelial cell (red). (*E*) After a series of proteolytic events (see Fig. 1), the Notch intracellular domain (NICD) translocates to the nucleus and promotes transcription of a number of genes including PDGFRβ, smooth muscle actin (SMA), Notch 3, and Jagged 1, which promote mural cell maturation and stability. (DSL) Delta/Serrate/Lag, (RBPJK) recombination signal-binding protein for immunoglobulin κJ. (Created in BioRender.com.)

is believed to interfere with direct communication between endothelial and mural cells. In addition, glucose-mediated damage to the pericytes appears to lead to pericyte death and loss of pericyte coverage. Notch 1 expression has been shown to maintain the endothelium in a quiescent state (Noseda et al. 2004), whereas aberrant Notch signaling has been demonstrated to provoke vascular dysfunction (Arboleda-Velasquez et al. 2014; Kofler et al. 2015). In this context, targeting Notch 1 signaling using neutralizing antibodies may hold therapeutic potential and warrants future investigation (Miloudi et al. 2019).

The requirement for coordination of Notch signaling in the vasculature is further highlighted during vascular injury. Many signaling pathways act to repair the damage; however, if not sufficiently regulated, this process can lead to pathology such as neointimal hyperplasia, an exaggerated wound-healing process that is initiated through damage to the endothelial cells and is characterized by proliferation and migration of VSMCs to the lumen of the vessel (Wu and Zhang, 2009; Baeten and Lilly 2017). Additionally, elements of the Notch signaling pathway including Notch 1, Notch 3 and Jagged 1, Jagged 2, Hey 1, and Hey 2 are dysregulated at different

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phases of injury with an initial down-regulation and subsequent overexpression (Lindner et al. 2001; Gridley 2010). In vivo injury models have highlighted an important role for Notch 1 in driving migration and proliferation during neointimal hyperplasia (Li et al. 2009; Wang et al. 2018).

FUTURE DIRECTIONS

Within this article, we have summarized key components and concepts in Notch signaling during endothelial and mural cell communications in development and in the postnatal vasculature. Despite a vast and ever-growing literature regarding Notch signaling and its role in health and disease, several fundamental questions remain. The roles for Notch in the developing vasculature are largely characterized; however, pathologies such as CADASIL develop later in life and present an interesting question regarding the changing requirement for Notch signaling during aging. It is evident that Notch 3 signaling plays an important role in the differentiation of VSMCs during development, and likely during vascular remodeling processes. It remains unclear how these endothelial–VSMC interactions are affected in an adult mature VSMC.

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