

Review

Open Access

Novel insights into the differential functions of Notch ligands in vascular formation

Tsutomu Kume

Address: Feinberg Cardiovascular Research Institute, Feinberg School of Medicine, Northwestern University, 303E Chicago Ave, Chicago, IL 60611, USA

Email: Tsutomu Kume - t-kume@northwestern.edu

Published: 16 November 2009

Received: 19 August 2009

Journal of Angiogenesis Research 2009, 1:8 doi:10.1186/2040-2384-1-8

Accepted: 16 November 2009

This article is available from: <http://www.jangiogenesis.com/content/1/1/8>

© 2009 Kume; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

The Notch signaling pathway is a critical component of vascular formation and morphogenesis in both development and disease. Compelling evidence indicates that Notch signaling is required for the induction of arterial-cell fate during development and for the selection of endothelial tip and stalk cells during sprouting angiogenesis. In mammals, two of the four Notch receptors (Notch1 and Notch4) and three of the five Notch ligands (Jagged1, Dll1, and Dll4) are predominantly expressed in vascular endothelial cells and are important for many aspects of vascular biology. During arterial cell-fate selection and angiogenesis, the roles of Notch1 and Notch4 are thought to be similar, and the function of Dll4 is well-characterized. However, the molecular mechanisms that determine the functional similarities and differences of Notch ligands in vascular endothelial cells remain largely unknown; consequently, additional research is needed to elucidate the ligand-specific functions and mechanisms associated with Notch activation in the vascular endothelium. Results from recent studies indicate that Dll1 and Dll4 have distinct roles in the specification and maintenance of arterial cell identity, while Dll4 and Jagged1 have opposing functions in tip- and stalk-cell selection during sprouting angiogenesis. This review will focus on the newly discovered, distinct functions of several Notch ligands in the regulation of blood vessel formation and will provide perspectives for future research in the field.

Introduction

Notch signaling is evolutionarily conserved and critical for cell-fate determination, differentiation, and many other biological processes [1]. The mammalian Notch signaling pathway is composed of four Notch receptors (Notch1-4) and five ligands (Jagged1 and 2 and Delta-like [Dll] 1, 3, and 4). All of the ligands are transmembrane-type proteins and, consequently, Notch signaling is often mediated by cell-cell interactions. Transmission generally occurs between neighboring cells that express high levels of either the receptor or the ligand, although receptor-ligand coexpression occurs in some cells, such as vascular endothelial cells. Over the last decade, numerous studies

have demonstrated that Notch signaling is critically involved in vascular development and disease [2-6]. For example, Notch signaling is required for arterial cell-fate determination during embryonic development, and the Notch pathway controls both developmental and pathological angiogenesis by modulating the selection of endothelial tip and stalk cells in newly sprouting blood vessels. Regulation of the Notch pathway in blood vessels has been well characterized; however, the specific roles of each Notch ligand during vascular formation and morphogenesis are unknown. Recent studies provide insight into the distinct functions of Notch ligands in blood vessels, and this review summarizes the current understand-

ing of how several ligands differentially activate Notch signaling in the vasculature.

Basic mechanisms of the Notch signaling pathway

Notch signaling is initiated by interactions between a Notch ligand expressed on the surface of one cell (the signaling cell) and a Notch receptor expressed on the surface of a neighboring cell (the receiving cell). Upon ligand binding, Notch is sequentially cleaved, and the Notch intracellular domain (NICD) is released into the cytoplasm. The NICD enters the nucleus, where it interacts with the transcription factor CSL (named after mammalian CBF1, *Drosophila* Su(H), and *Caenorhabditis elegans* LAG1) to form a transcriptional activation complex that induces expression of the bHLH transcription factors (Hes and Hey families) (Figure 1). This signaling mechanism is considered the "canonical" Notch pathway; non-canonical Notch signaling has also been reported [7].

The extracellular domains of mammalian Notch ligands have several distinct features that participate in receptor binding (Figure 2). Their N-terminal regions contain a conserved module, and a second conserved module, the DSL (Delta/Serrate/LAG-2) domain, is located adjacent to the N-terminal region. Both Notch ligands and receptors contain multiple EGF-like repeats, and the ligands

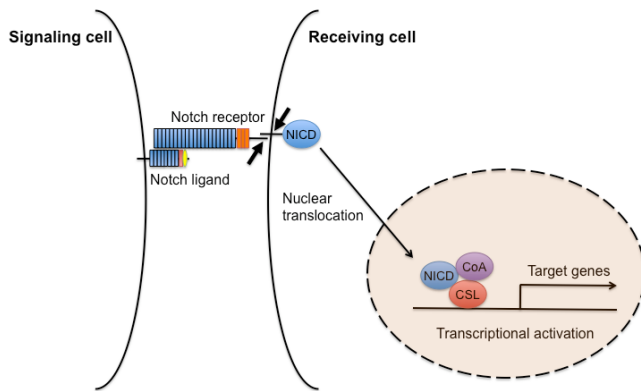


Figure 1
A diagram of the canonical Notch signaling pathway.
 This schematic shows a simplified overview of the main components of Notch signaling. Upon Notch ligand binding, a two-step proteolysis cleavage process (small arrows) within the juxtamembrane region and transmembrane domain of the Notch receptor is catalyzed by a member of the disintegrin and metalloproteases (ADAMS) family and the γ -secretase containing complex, respectively, then the Notch intracellular domain (NICD) is released from the membrane and translocates to the nucleus, where it forms a transcriptional activation complex with CSL and coactivators (CoA), thereby inducing the transcription of target genes.

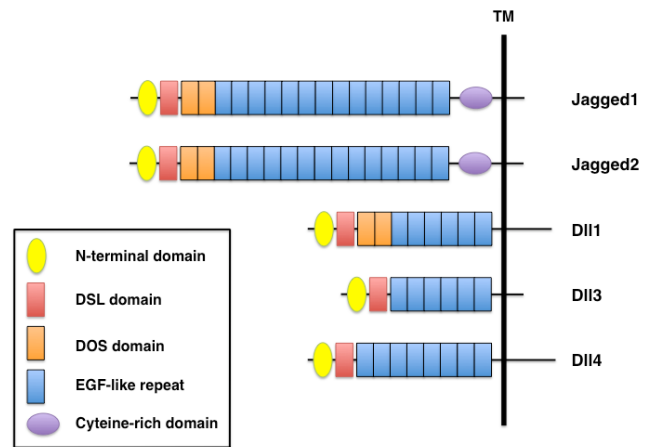


Figure 2
Domain organization of mammalian Notch ligands.
 Five mammalian ligands are classified into two categories, Delta-like (Dll1, Dll3, Dll4) and Serrate-like (Jagged1, Jagged2), based on structural homology to the two *Drosophila* ligands, Delta and Serrate. All Notch ligands have an N-terminal domain, a DSL (Delta/Serrate/LAG-2) domain and EGF-like repeats. Jagged1 and Jagged2 contain a cysteine-rich domain, whereas Jagged1, Jagged2, and Dll1 have two DOS (Delta and OSM-11-like proteins) domains located immediately following the DSL domain.

Jagged1, Jagged2, and Dll1 have tandem EGF repeats that form the DOS (Delta and OSM-11-like proteins) domain [8]. Jagged1 and Jagged2 also contain a cysteine-rich domain located between the EGF-like repeats and the transmembrane domain. Both the DSL and DOS domains are critical for receptor binding [9], and the structural diversity of Notch ligands is determined by the presence or absence of the cysteine-rich DOS domains.

Activation of Notch signaling through cell-cell interactions (*trans*-interactions) has been well characterized; however, Notch ligands also regulate the Notch pathway by binding to Notch receptors within the same cell (*cis*-interactions) [10,11]. In general, *trans*-interactions between Notch ligands and receptors activate Notch signaling, whereas *cis*-interactions are believed to inhibit Notch signaling [9]. The precise mechanisms that mediate Notch activation by the *cis*-interactions remain unclear, and further studies need to be performed [12].

Notch receptor and ligand expression in blood vessels

Notch1 is broadly expressed in many tissues, including the heart and vascular endothelial cells, while Notch4 expression is restricted to vascular endothelial cells [13-15], and Notch3 is predominantly expressed in vascular smooth muscle cells [16]. Transcriptional regulation of

Notch4 in vascular endothelial cells is controlled by fibroblast growth factor 2 (FGF2), the signal-dependent transcription-factor activator protein 1 (AP-1), and the glucocorticoid receptor [14,15,17]. Four of the five known mammalian Notch ligands (Jagged1, Jagged2, Dll1, and Dll4) are expressed in vascular endothelial cells [13,18-20]; Jagged1 is also expressed in smooth muscle cells surrounding the arteries and plays an important role in smooth muscle cell maturation [21]. The molecular mechanisms that control the expression of Notch ligands in vascular endothelial cells and smooth muscle cells have been frequent topics of recent research (Table 1). For example, Dll4 expression during arterial specification and for tip-cell selection during vessel sprouting [22-28] is mediated by vascular endothelial growth factor (VEGF). Importantly, and as described below, the expression patterns of the Notch ligands vary both spatially and temporally and, consequently, the localization of each ligand is likely to be critical for Notch activation in blood vessels (Figures 3, 4, 5, 6, and 7).

Notch1, Notch4, and the ligands Dll1 and Dll4 during arterial specification and maintenance

Results from recent studies in zebrafish suggest that activation of Notch signaling by the Sonic hedgehog (Shh) and VEGF pathways is essential for arterial specification during development [29,30]. Two Notch receptors, Notch1 and Notch4, are predominantly expressed in arterial endothelial cells of early mouse embryos. *Notch1* mutant mice die with cardiovascular defects during early development [13], and endothelial-specific ablation of *Notch1* in mice leads to embryonic lethality and vascular abnormalities that are associated with angiogenesis [31]. An endothelial-specific *Notch1*+/- mutation has also been associated with impaired postnatal neovascularization in a marine hind-limb ischemia model [32]. These results demonstrate the cell-type specific role of Notch1 in the vascular endothelium during development and postnatal life (Table 2). *Notch4* mutant mice display no conspicuous phenotype, but the vascular defects observed in compound *Notch1*; *Notch4* mutant embryos are more severe

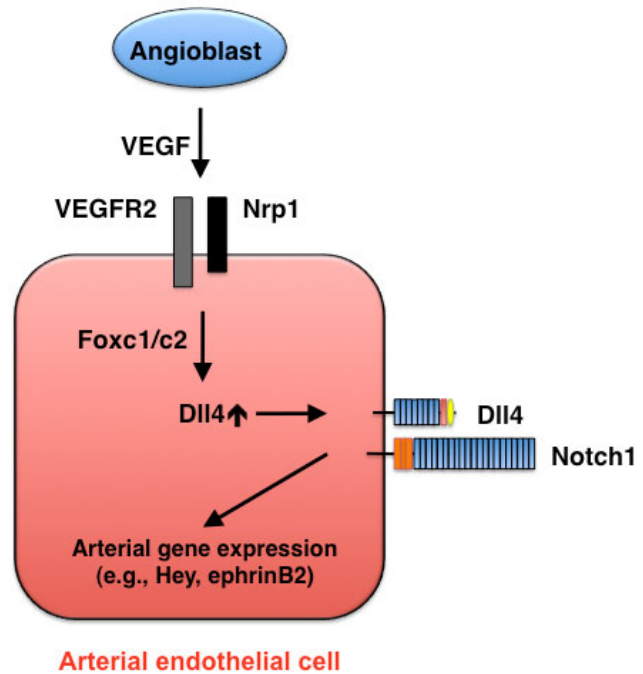


Figure 3
Arterial cell specification mediated by Dll4-Notch signaling. During early development, VEGF (in concert with Foxc1/c2 transcription factors) induces Dll4 expression in endothelial cells, and Dll4-Notch signaling promotes arterial gene expression.

than those in *Notch1* mutants [13], which suggests that the functions of Notch1 and Notch4 overlap during vascular development. Endothelial expression of a constitutively active Notch4 mutant from the *Flk1* (*VEGFR2*) locus results in embryonic vascular abnormalities such as disorganized vascular networks and dilated blood vessels [33], and in adult mice, constitutively active Notch4 expression has been associated with arteriovenous malformations (AVMs) (i.e., abnormal connections between arteries and veins) that are accompanied by ectopic expression of the arterial marker ephrinB2 in veins [34]. Furthermore, the

Table 1: Signaling pathways/factors that regulate Notch ligand expression in vascular endothelial and smooth muscle cells

Pathway/factor	Ligand	Cell type	Biological effect	References
VEGF	Dll4 (↑)	Endothelial cells Endothelial tip cell formation in sprouting angiogenesis	Arterial specification	[22,24,27] [23,25,26,28]
TNFα	Dll4 (↓) Jagged1 (↑)	Endothelial cells	Tip cell and stalk cell selection	[52]
VEGF + FGF2 (synergistic)	Dll1 (↑)	Endothelial cells	Ischemia-induced postnatal arteriogenesis	[38]
PDGF/angiotensin II	Jagged1 (↓)	Smooth muscle cells	Growth regulation	[45]
Jagged1 (endothelial)	Jagged1 (↑)	Mural cells	Smooth muscle cell maturation	[21]

↑, Upregulation; ↓, Downregulation.

VEGF, vascular endothelial growth factor; TNF, tumor necrosis factor; FGF, Fibroblast growth factor; PDGF, platelet-derived growth factor

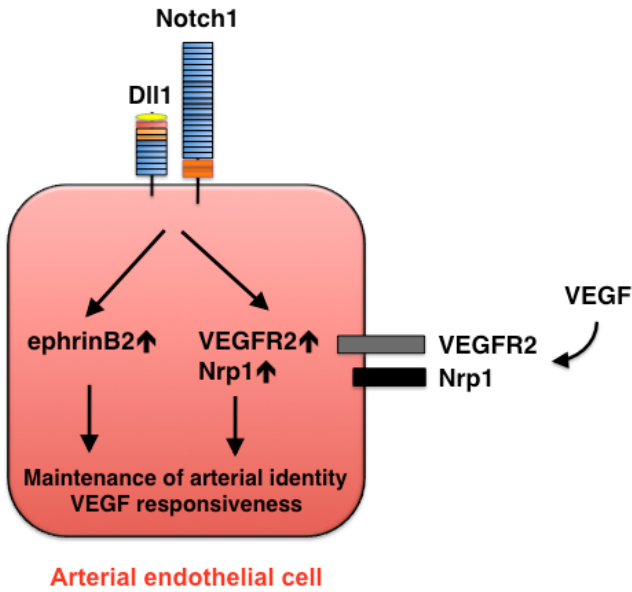


Figure 4
Maintenance of arterial identity mediated by Dll1-Notch signaling. At a later stage of development, Dll1 expression is induced in arterial endothelial cells and is required for maintenance of the arterial phenotype. Dll1 also acts upstream of VEGF by regulating the expression of VEGFR2 and its co-receptor neuropilin 1 (Nrp1).

impaired arterial and venous differentiation associated with constitutively active Notch4 expression can be reversed by suppressing Notch4 activity in the endothelium [34]. Collectively, these findings suggest that both reduced and elevated Notch signaling can lead to vascular abnormalities and, consequently, that the maintenance of proper Notch signaling is critical during vascular development.

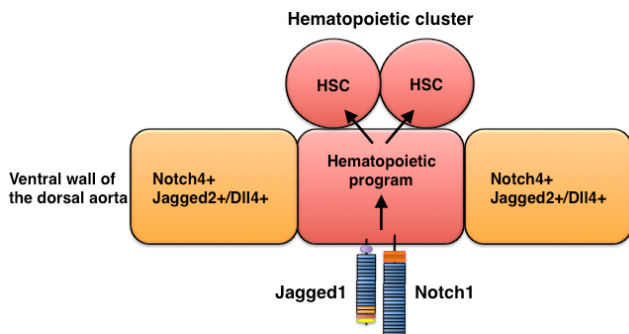


Figure 5
Jagged1-mediated hematopoietic program in the dorsal aorta during development. Hematopoietic stem cells (HSC) descend from Jagged1 + Notch1 + endothelial cells in the aorta.

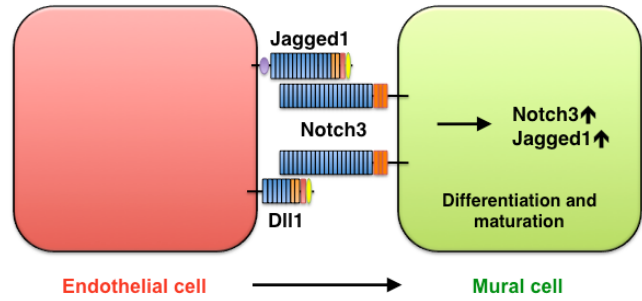


Figure 6
Smooth-muscle maturation mediated by Jagged1/Dll1-Notch3 signaling. Jagged1 and Dll1 in endothelial cells activate Notch3 on mural cells, thereby promoting mural-cell maturation.

Of the four Notch ligands (Jagged1, Jagged2, Dll1, and Dll4) that are expressed in arterial endothelial cells, Dll4 alone is expressed in the dorsal aorta of mice at embryonic day 8.5 (E8.5), and its expression is restricted to vascular endothelial cells [13]; thus, Dll4 is believed to be the ligand for Notch1 and Notch4 during early vascular develop-

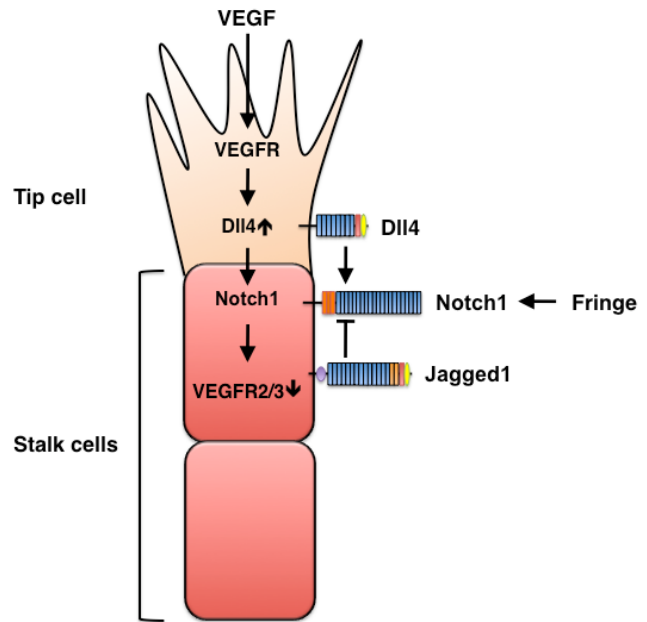


Figure 7
Opposing effects of Dll4 and Jagged1 on sprouting angiogenesis. VEGF signaling induces Dll4 expression in tip cells, and Dll4, in turn, activates Notch signaling in stalk cells, which reduces stalk-cell sensitivity to VEGF stimulation and, consequently suppresses the tip-cell phenotype. Conversely, Jagged1 antagonizes Dll4-mediated Notch activation in stalk cells to increase tip cell numbers and enhances vessel sprouting. The antagonistic effects of the two ligands are controlled by Fringe-dependent modulation of Notch signaling.

ment (Figure 3). *Dll4* mutant mice display early embryonic lethality with impaired arterial specification and AVMs that appear in a genotype-dependent manner (i.e., the severity increases with the number of mutant alleles) [35-37]. These observations further emphasize the importance of maintaining proper Notch activity levels during vascular development. *Foxc1* and *Foxc2* transcription factors directly activate the *Dll4* promoter in endothelial cells, and their induction of *Dll4* expression is enhanced by VEGF, which suggests that *Foxc1* and *Foxc2* act upstream of Notch signaling during arterial-cell specification [22,27].

Dll1 expression is detected in arterial endothelial cells at a later stage (E13.5) of mouse development [19] and continues to be restricted to arterial endothelial cells in adults [38]. *Dll1* is not critically involved in arterial-cell specification; however, analyses in hypomorphic and endothelial-specific *Dll1* mutant mice indicate that *Dll1* is required for the maintenance of arterial identity [19]. Expression of the arterial marker ephrinB2 is reduced, and the venous marker COUP-TFII is upregulated, in endothelial-specific *Dll1* mutant mice, despite *Dll4* expression in the mutant endothelial cells [19]; thus, *Dll4* appears to be essential for initiating the arterial program, whereas *Dll1* is required to maintain arterial identity during embryonic development. In addition, Sorensen et al. have shown that *Dll1*-mediated Notch1 activation upregulates VEGF receptor 2 (VEGFR2) and its coreceptor, neuropilin-1, which suggests that *Dll1* enhances the responsiveness of arterial endothelial cells to VEGF signaling. Thus, *Dll4*-mediated Notch signaling occurs downstream of VEGF during arterial specification, whereas *Dll1*-mediated Notch signaling acts upstream of VEGF to maintain arterial identity (Figures 3 and 4). *Dll1* is also important for ischemia-induced postnatal arteriogenesis and the induction of ephrinB2 [38].

Jagged1 does not play a critical role in arterial development [39-41] but is required for the definitive hematopoietic program in the dorsal aorta. After arterial and venous endothelial cells differentiate, the ventral region of the dorsal aorta, located in the aorta-gonad-mesonephros (AGM) region of the mid-gestation mouse embryo (around E10-11), generates the first adult hematopoietic stem cells (HSCs). Notch4 is broadly expressed throughout the aortic endothelium of the AGM, whereas Notch1 expression is restricted to the ventral region of the dorsal aorta [40,42]. Importantly, three Notch ligands (*Jagged1*, *Jagged2*, and *Dll4*) have distinctive expression patterns in the dorsal aorta of the AGM: *Jagged1* and Notch1 expression overlap in the dorsal aorta, *Jagged2* expression occurs in endothelial cells adjacent to Notch1-positive endothelial cells, and *Dll4* is expressed in both Notch1-positive and Notch1-negative endothelial cells [40,42]. Analyses

in *Jagged1* mutant mice indicate that *Jagged1* is required for Notch1 activation during the induction of intra-embryonic definitive hematopoiesis in the AGM [40]. *Jagged2* and *Dll4* expression in the AGM of *Jagged1* mutant embryos is normal, and hematopoiesis is normal in *Jagged2* mutant mice [40]; thus, the function of *Jagged1* is distinct from *Dll4* and *Jagged2* activity during the hematopoietic program of the newly formed aorta (Figure 5).

Notch3, Jagged1, and Dll1 during smooth-muscle differentiation and maturation

Notch3 is predominantly expressed in the vascular smooth muscle of arteries and is not expressed in veins. Mutations in human *NOTCH3* are associated with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), a disorder that causes stroke and dementia and is accompanied by the degeneration of vascular smooth muscle cells [43]; adult *Notch3* mutant mice display a defect in the maturation of arterial smooth muscle cells [44]. As noted above, *Jagged1* mutant mice exhibit normal arterial development [39,40], yet endothelial-specific *Jagged1* mutants have impaired vascular smooth muscle differentiation [39]. This observation indicates that *Jagged1* expression in the arterial endothelium activates Notch in neighboring cells, and that this function is critical for smooth muscle cell differentiation. *Jagged1* expression by endothelial cells induces mural cells (pericytes in the microvasculature or smooth muscle cells in larger vessels) to express Notch3 and *Jagged1*, which subsequently promotes and maintains the differentiation phenotype of mural cells [21], whereas platelet-derived growth factor (PDGF) and angiotensin II downregulate Notch3 and *Jagged1* expression in vascular smooth muscle cells [45]. Furthermore, a recent study found that expression of the arterial smooth muscle marker smoothelin is impaired in *Dll1* mutant mice [19], and this decline has also been observed in *Notch3*-mutant arteries [44]. Taken together, these findings suggest that *Jagged1* and *Dll1* are the primary ligands that regulate Notch3 activity during smooth-muscle differentiation and maturation (Figure 6).

Dll4 and Jagged1 in tip- and stalk-cell specification during sprouting angiogenesis

The formation of new blood vessels, a process known as angiogenesis, involves the sprouting of endothelial cells. In response to VEGF stimulation, filopodia extend from a migratory endothelial cell at the vessel's tip (i.e., the tip cell), and proliferative endothelial cells (i.e., stalk cells) form the trunk of the new vessel. Recent studies in mice and zebrafish clearly demonstrate that Notch signaling interacts with VEGF signaling during tip-cell and stalk-cell specification [5]. VEGF induces *Dll4* expression in tip cells, then *Dll4* activates the Notch pathway in adjacent

endothelial cells to reduce expression of VEGFR2 and VEGFR3, thereby suppressing the tip-cell phenotype, and tip-cell phenotype suppression cell-autonomously promotes the stalk-cell phenotype. Together, these mechanisms balance tip-cell and stalk-cell selection and, consequently, limit the number of sprouting vessels (Figure 7). Genetic or pharmacological disruption of Dll4-Notch signaling leads to excessive tip-cell formation and vessel sprouting in cultured cells, in zebrafish and mouse embryos, and during tumor angiogenesis [23,25,46-51].

By using endothelial-specific *Jagged1* mutant mice and mice that overexpress *Jagged1* in vascular endothelial cells, Benedito et al. demonstrated that *Jagged1* enhances angiogenesis and antagonizes the effects of Dll4-mediated Notch signaling during sprouting angiogenesis [52]. *Jagged1* is strongly expressed in stalk cells, whereas Dll4 is predominantly detected in tip cells [52], and the antagonistic interaction between Dll4 and *Jagged1* in endothelial cells is mediated by the glycosyltransferase Fringe, which regulates the posttranslational modifications of Notch receptors in a ligand-dependent manner. Fringe enhances Notch activation in response to Delta-like ligands and reduces Notch activity in response to *Jagged* ligands [12]; consequently, Fringe increases Dll4-induced endothelial Notch signaling and reduces Notch signaling in response to *Jagged1* [52]. *Jagged1* also appears to promote vascular sprouting by regulating VEGFR3 expression in tip cells [52]. Taken together, these results illustrate the opposing effects of Dll4 and *Jagged1* on sprouting angiogenesis.

Notch ligands in pathological angiogenesis

Dll4 is expressed in tumor vasculature [26,36,53,54], and as observed in studies of developmental angiogenesis, the blockade of Dll4-mediated Notch signaling (via systemic administration of Dll4-neutralizing antibodies [47,48] and systemic or local administration of modified Dll4 proteins [47,55]) increased tumor-vessel sprouting, which indicates that Dll4-Notch signaling is critical for tip- and stalk-cell selection during tumor angiogenesis. Remarkably, the inhibition of Dll4-Notch signaling increased neovascularization but impaired tumor growth, because the non-productive angiogenesis reduced tumor perfusion. Conversely, Dll4 activation of endothelial Notch signaling reduces tumor angiogenesis, but increases vessel diameter and perfusion, which enhances tumor growth [47,56]. For these reasons, Dll4 is now recognized as a potential therapeutic target for tumor angiogenesis [57].

As described above, *Jagged1* antagonizes Dll4 during sprouting angiogenesis [52], and overexpression of *Jagged1* in tumor cells has been shown to enhance neovascularization and tumor growth [58]; however, the role of *Jagged1* in pathological angiogenesis (including tumor angiogenesis) is not yet fully understood. Current find-

ings suggest that angiogenic sprouting in the tumor is tightly controlled by positive and negative regulation of *Jagged1* and Dll4 in both endothelial and non-endothelial cells. Recent studies have shown that a soluble form of Notch1 (Notch decoy) acts as an antagonist of ligand-dependent Notch signaling by (potentially) interfering with Dll1, Dll4, and *Jagged1* [59,60]. Importantly, the Notch decoy reduces tumor growth without increasing vessel growth, which suggests that the effects of the Notch decoy differ from those induced by Dll4 blockade. It is therefore likely that the proangiogenic function of *Jagged1* in tumor cells and endothelial cells could also influence tumor angiogenesis.

Notch signaling in peripheral ischemia

Notch signaling is also required for angiogenesis in peripheral ischemia models [32,38] (Table 2). Blood flow recovery and postnatal neovascularization in response to hind-limb ischemia are impaired in both global and endothelial-specific *Notch1*^{+/-} mice, but not in *Notch4*^{-/-} mice [32]. Dll1 is strongly induced in arterial endothelial cells during ischemia-induced arteriogenesis, and *Dll1*^{+/-} mice display reduced collateral-artery growth and impaired blood-flow recovery after hind-limb ischemia [38]. Notch activation and ephrinB2 induction are not observed in the collateral arteries of *Dll1*^{+/-} mice [38].

Concluding remarks and future perspectives

Studies performed in the past few years clearly demonstrate that the different Notch ligands have distinct functions in vascular development and disease. This understanding has prompted numerous investigations into the mechanisms by which Notch signaling is essential for multiple aspects of vascular biology. However, given that the effects of Notch pathway activation on endothelial cells are context-dependent [4], many questions remain to be answered. First, the upstream signaling pathways that control the expression of Notch ligands in blood vessels remain largely unknown; VEGF induces Dll4 expression in endothelial cells (Table 1), but *Jagged1* is absent in tip cells where Dll4 is highly expressed, which suggests that the two ligands are regulated differently. Second, the selective activation of Notch in vascular endothelium remains unclear; for example, Notch signaling is not activated in arteries of *Dll1* mutant mice, despite the presence of *Jagged1* and Dll4 [19]. Third, the role of non-canonical Notch ligands, such as microfibril-associated glycoprotein (MAGP)-2 [9], is poorly understood. MAGP-2 binds to *Jagged1*, *Jagged2*, Dll1, and Notch1 [61,62], and is known to modulate Notch signaling in sprouting angiogenesis [63,64], but the mechanistic basis for the function of MAGP-2 in ligand-dependent Notch activation has yet to be elucidated. Finally, given that Dll4 and *Jagged1* have opposing effects on angiogenesis, experiments that specifically inhibit each ligand with selective

Table 2: Mammalian Notch receptors and ligands involved in vascular development and disease

Component	Phenotype/Role	References
Notch receptors		
Notch1	Proper vascular development; Postnatal neovascularization	[13,31,32]
Notch3	Maturation of vascular smooth muscle cells	[44]
Notch4	Null mice show normal vascular development; <i>Notch1</i> ; <i>Notch4</i> mutant mice have severe vascular defects; Gain-of-function experiments show vascular abnormalities in development and postnatal life	[13,33,34]
Notch ligands		
Jagged1	Dispensable for arterial specification; Formation of hematopoietic stem cells from the aorta; Smooth muscle differentiation and maturation; Proangiogenic regulation	[21,39-41,52]
Dll1	Maintenance of arterial identity; Arterial smooth muscle differentiation; Postnatal arteriogenesis	[19,38]
Dll4	Arterial specification; Tip cell and stalk cell selection during sprouting angiogenesis; Regulation of tumor angiogenesis	[23,25,35-37,46-52,55,56]

neutralizing antibodies [65] may be important not only for understanding how Notch is activated in the vasculature, but also for the development of therapeutic strategies designed to control angiogenesis by targeting Notch signaling.

Competing interests

The author declares that he has no competing interests.

Authors' contributions

The author drafted and wrote the manuscript.

Author's information

The author is an Associate Professor at Northwestern University School of Medicine, USA. He completed his post-doctoral training in the lab of Brigid Hogan at the Howard Hughes Medical Institute at Vanderbilt University Medical Center, USA. He graduated with a Ph.D. in Molecular and Cellular Biology from the University of Tokyo, Japan.

Acknowledgements

The author thanks W. Kevin Meisner, PhD, ELS, for editorial support. This work was supported by a NIH grant (RO1 HL074121) to TK.

References

- Bray SJ: **Notch signalling: a simple pathway becomes complex.** *Nat Rev Mol Cell Biol* 2006, **7**:678-689.
- Alva JA, Iruela-Arispe ML: **Notch signaling in vascular morphogenesis.** *Curr Opin Hematol* 2004, **11**:278-283.
- Gridley T: **Notch signaling in vascular development and physiology.** *Development* 2007, **134**:2709-2718.
- Hofmann JJ, Iruela-Arispe ML: **Notch signaling in blood vessels: who is talking to whom about what?** *Circ Res* 2007, **100**:1556-1568.
- Phng LK, Gerhardt H: **Angiogenesis: a team effort coordinated by notch.** *Dev Cell* 2009, **16**:196-208.
- Roca C, Adams RH: **Regulation of vascular morphogenesis by Notch signaling.** *Genes Dev* 2007, **21**:2511-2524.
- Talora C, Campese AF, Bellavia D, Felli MP, Vacca A, Gulino A, Screpanti I: **Notch signaling and diseases: an evolutionary journey from a simple beginning to complex outcomes.** *Biochim Biophys Acta* 2008, **1782**:489-497.
- Komatsu H, Chao MY, Larkins-Ford J, Corkins ME, Somers GA, Tucey T, Dionne HM, White JQ, Wani K, Boxem M, Hart AC: **OSM-11 facilitates LIN-12 Notch signaling during *Caenorhabditis elegans* vulval development.** *PLoS Biol* 2008, **6**:e196.
- Kopan R, Ilagan MX: **The canonical Notch signaling pathway: unfolding the activation mechanism.** *Cell* 2009, **137**:216-233.
- Fiuza UM, Arias AM: **Cell and molecular biology of Notch.** *J Endocrinol* 2007, **194**:459-474.
- Zolkiewska A: **ADAM proteases: ligand processing and modulation of the Notch pathway.** *Cell Mol Life Sci* 2008, **65**:2056-2068.
- D'Souza B, Miyamoto A, Weinmaster G: **The many facets of Notch ligands.** *Oncogene* 2008, **27**:5148-5167.
- Krebs LT, Xue Y, Norton CR, Shutter JR, Maguire M, Sundberg JP, Gallahan D, Closson V, Kitajewski J, Callahan R, Smith GH, Stark KL, Gridley T: **Notch signaling is essential for vascular morphogenesis in mice.** *Genes Dev* 2000, **14**:1343-1352.
- Wu J, Bresnick EH: **Glucocorticoid and growth factor synergism requirement for Notch4 chromatin domain activation.** *Mol Cell Biol* 2007, **27**:2411-2422.
- Wu J, Iwata F, Grass JA, Osborne CS, Elnitski L, Fraser P, Ohneda O, Yamamoto M, Bresnick EH: **Molecular determinants of NOTCH4 transcription in vascular endothelium.** *Mol Cell Biol* 2005, **25**:1458-1474.
- Joutel A, Andreux F, Gaulis S, Domenga V, Cecillon M, Bataill N, Piga N, Chapon F, Godfrain C, Tournier-Lasserre E: **The ectodomain of the Notch3 receptor accumulates within the cerebrovasculature of CADASIL patients.** *J Clin Invest* 2000, **105**:597-605.
- Wu J, Bresnick EH: **Bare rudiments of notch signaling: how receptor levels are regulated.** *Trends Biochem Sci* 2007, **32**:477-485.
- Beckers J, Clark A, Wunsch K, Hrabe De Angelis M, Gossler A: **Expression of the mouse Delta1 gene during organogenesis and fetal development.** *Mech Dev* 1999, **84**:165-168.
- Sorensen I, Adams RH, Gossler A: **DLL1-mediated Notch activation regulates endothelial identity in mouse fetal arteries.** *Blood* 2009, **113**:5680-5688.
- Villa N, Walker L, Lindsell CE, Gasson J, Iruela-Arispe ML, Weinmaster G: **Vascular expression of Notch pathway receptors and ligands is restricted to arterial vessels.** *Mech Dev* 2001, **108**:161-164.
- Liu H, Kennard S, Lilly B: **NOTCH3 expression is induced in mural cells through an autoregulatory loop that requires endothelial-expressed JAGGED1.** *Circ Res* 2009, **104**:466-475.
- Hayashi H, Kume T: **Foxc transcription factors directly regulate Dll4 and Hey2 expression by interacting with the VEGF-Notch signaling pathways in endothelial cells.** *PLoS ONE* 2008, **3**:e2401.
- Hellstrom M, Phng LK, Hofmann JJ, Wallgard E, Coultas L, Lindblom P, Alva J, Nilsson AK, Karlsson L, Gaiano N, Yoon K, Rossant J, Iruela-Arispe ML, Kalén M, Gerhardt H, Betsholtz C: **Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis.** *Nature* 2007, **445**:776-780.
- Liu ZJ, Shirakawa T, Li Y, Soma A, Oka M, Dotto GP, Fairman RM, Velazquez OC, Herlyn M: **Regulation of Notch1 and Dll4 by vascular endothelial growth factor in arterial endothelial cells: implications for modulating arteriogenesis and angiogenesis.** *Mol Cell Biol* 2003, **23**:14-25.
- Lobov IB, Renard RA, Papadopoulos N, Gale NW, Thurston G, Yancopoulos GD, Wiegand SJ: **Delta-like ligand 4 (Dll4) is induced**

- by VEGF as a negative regulator of angiogenic sprouting. *Proc Natl Acad Sci* 2007, **104**:3219-3224.
26. Patel NS, Li JL, Generali D, Poulsom R, Cranston DW, Harris AL: **Up-regulation of delta-like 4 ligand in human tumor vasculature and the role of basal expression in endothelial cell function.** *Cancer Res* 2005, **65**:8690-8697.
 27. Seo S, Fujita H, Nakano A, Kang M, Duarte A, Kume T: **The fork-head transcription factors, Foxc1 and Foxc2, are required for arterial specification and lymphatic sprouting during vascular development.** *Dev Biol* 2006, **294**:458-470.
 28. Williams CK, Li JL, Murga M, Harris AL, Tosato G: **Up-regulation of the Notch ligand Delta-like 4 inhibits VEGF-induced endothelial cell function.** *Blood* 2006, **107**:931-939.
 29. Lawson ND, Scheer N, Pham VN, Kim CH, Chitnis AB, Campos-Ortega JA, Weinstein BM: **Notch signaling is required for arterial-venous differentiation during embryonic vascular development.** *Development* 2001, **128**:3675-3683.
 30. Lawson ND, Vogel AM, Weinstein BM: **Sonic hedgehog and vascular endothelial growth factor act upstream of the Notch pathway during arterial endothelial differentiation.** *Dev Cell* 2002, **3**:127-136.
 31. Limbourg FP, Takeshita K, Radtke F, Bronson RT, Chin MT, Liao JK: **Essential role of endothelial Notch1 in angiogenesis.** *Circulation* 2005, **111**:1826-1832.
 32. Takeshita K, Satoh M, Li M, Silver M, Limbourg FP, Mukai Y, Rikitake Y, Radtke F, Gridley T, Losordo DW, Liao JK: **Critical role of endothelial Notch1 signaling in postnatal angiogenesis.** *Circ Res* 2007, **100**:70-78.
 33. Uytendaele H, Ho J, Rossant J, Kitajewski J: **Vascular patterning defects associated with expression of activated Notch4 in embryonic endothelium.** *Proc Natl Acad Sci* 2001, **98**:5643-5648.
 34. Carlson TR, Yan Y, Wu X, Lam MT, Tang GL, Beverly LJ, Messina LM, Capobianco AJ, Werb Z, Wang R: **Endothelial expression of constitutively active Notch4 elicits reversible arteriovenous malformations in adult mice.** *Proc Natl Acad Sci* 2005, **102**:9884-9889.
 35. Duarte A, Hirashima M, Benedito R, Trindade A, Diniz P, Bekman E, Costa L, Henrique D, Rossant J: **Dosage-sensitive requirement for mouse Dll4 in artery development.** *Genes Dev* 2004, **18**:2474-2478.
 36. Gale NW, Dominguez MG, Noguera I, Pan L, Hughes V, Valenzuela DM, Murphy AJ, Adams NC, Lin HC, Holash J, Thurston G, Yancopoulos GD: **Haploinsufficiency of delta-like 4 ligand results in embryonic lethality due to major defects in arterial and vascular development.** *Proc Natl Acad Sci* 2004, **101**:15949-15954.
 37. Krebs LT, Shutter JR, Tanigaki K, Honjo T, Stark KL, Gridley T: **Haploinsufficient lethality and formation of arteriovenous malformations in Notch pathway mutants.** *Genes Dev* 2004, **18**:2469-2473.
 38. Limbourg A, Ploom M, Elligsen D, Sorensen I, Ziegelhoeffer T, Gossler A, Drexler H, Limbourg FP: **Notch ligand Delta-like 1 is essential for postnatal arteriogenesis.** *Circ Res* 2007, **100**:363-371.
 39. High FA, Lu MM, Pear WS, Loomes KM, Kaestner KH, Epstein JA: **Endothelial expression of the Notch ligand Jagged1 is required for vascular smooth muscle development.** *Proc Natl Acad Sci* 2008, **105**:1955-1959.
 40. Robert-Moreno A, Guiu J, Ruiz-Herguido C, Lopez ME, Ingles-Estevé J, Riera L, Tipping A, Enver T, Dzierzak E, Gridley T, Espinosa L, Bigas A: **Impaired embryonic haematopoiesis yet normal arterial development in the absence of the Notch ligand Jagged1.** *EMBO J* 2008, **27**:1886-1895.
 41. Xue Y, Gao X, Lindsell CE, Norton CR, Chang B, Hicks C, Gendron-Maguire M, Rand EB, Weinmaster G, Gridley T: **Embryonic lethality and vascular defects in mice lacking the Notch ligand Jagged1.** *Hum Mol Genet* 1999, **8**:723-730.
 42. Robert-Moreno A, Espinosa L, de la Pompa JL, Bigas A: **RBPj κ -dependent Notch function regulates Gata2 and is essential for the formation of intra-embryonic hematopoietic cells.** *Development* 2005, **132**:1117-1126.
 43. Joutel A, Corpechot C, Ducros A, Vahedi K, Chabriat H, Mouton P, Alamowitch S, Domenga V, Cecillon M, Marechal E, Maciazek J, Vayssière C, Cruaud C, Cabanis EA, Ruchoux MM, Weissenbach J, Bach JF, Bousser MG, Tournier-Lasserre E: **Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia.** *Nature* 1996, **383**:707-710.
 44. Domenga V, Fardoux P, Lacombe P, Monet M, Maciazek J, Krebs LT, Klonekowski B, Berrou E, Mericskay M, Li Z, Tournier-Lasserre E, Gridley T, Joutel A: **Notch3 is required for arterial identity and maturation of vascular smooth muscle cells.** *Genes Dev* 2004, **18**:2730-2735.
 45. Campos AH, Wang W, Pollman MJ, Gibbons GH: **Determinants of Notch-3 receptor expression and signaling in vascular smooth muscle cells: implications in cell-cycle regulation.** *Circ Res* 2002, **91**:999-1006.
 46. Leslie JD, Ariza-McNaughton L, Bermange AL, McAdow R, Johnson SL, Lewis J: **Endothelial signalling by the Notch ligand Delta-like 4 restricts angiogenesis.** *Development* 2007, **134**:839-844.
 47. Noguera-Troise I, Daly C, Papadopoulos NJ, Coetzee S, Boland P, Gale NW, Lin HC, Yancopoulos GD, Thurston G: **Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis.** *Nature* 2006, **444**:1032-1037.
 48. Ridgway J, Zhang G, Wu Y, Stawicki S, Liang WC, Chanthery Y, Kowalski J, Watts RJ, Callahan C, Kasman I, Singh M, Chien M, Tan C, Hongo JA, de Sauvage F, Plowman G, Yan M: **Inhibition of Dll4 signalling inhibits tumour growth by deregulating angiogenesis.** *Nature* 2006, **444**:1083-1087.
 49. Sainson RC, Aoto J, Nakatsu MN, Holderfield M, Conn E, Koller E, Hughes CC: **Cell-autonomous notch signaling regulates endothelial cell branching and proliferation during vascular tubulogenesis.** *FASEB J* 2005, **19**:1027-1029.
 50. Siekmann AF, Lawson ND: **Notch signalling limits angiogenic cell behaviour in developing zebrafish arteries.** *Nature* 2007, **445**:781-784.
 51. Suchting S, Freitas C, le Noble F, Benedito R, Breant C, Duarte A, Eichmann A: **The Notch ligand Delta-like 4 negatively regulates endothelial tip cell formation and vessel branching.** *Proc Natl Acad Sci* 2007, **104**:3225-3230.
 52. Benedito R, Roca C, Sorensen I, Adams S, Gossler A, Fruttiger M, Adams RH: **The notch ligands Dll4 and Jagged1 have opposing effects on angiogenesis.** *Cell* 2009, **137**:1124-1135.
 53. Hainaud P, Contreres JO, Villemain A, Liu LX, Plouet J, Tobelem G, Dupuy E: **The role of the vascular endothelial growth factor-Delta-like 4 ligand/Notch4-ephrin B2 cascade in tumor vessel remodeling and endothelial cell functions.** *Cancer Res* 2006, **66**:8501-8510.
 54. Mailhos C, Modlich U, Lewis J, Harris A, Bicknell R, Ish-Horowicz D: **Delta4, an endothelial specific notch ligand expressed at sites of physiological and tumor angiogenesis.** *Differentiation* 2001, **69**:135-144.
 55. Scehnet JS, Jiang W, Kumar SR, Krasnoperov V, Trindade A, Benedito R, Djokovic D, Borges C, Ley EJ, Duarte A, Gill PS: **Inhibition of Dll4-mediated signaling induces proliferation of immature vessels and results in poor tissue perfusion.** *Blood* 2007, **109**:4753-4760.
 56. Li JL, Sainson RC, Shi W, Leek R, Harrington LS, Preusser M, Biswas S, Turley H, Heikamp E, Haifellner JA, Harris AL: **Delta-like 4 Notch ligand regulates tumor angiogenesis, improves tumor vascular function, and promotes tumor growth in vivo.** *Cancer Res* 2007, **67**:11244-11253.
 57. Thurston G, Noguera-Troise I, Yancopoulos GD: **The Delta paradox: DLL4 blockade leads to more tumour vessels but less tumour growth.** *Nat Rev Cancer* 2007, **7**:327-331.
 58. Zeng Q, Li S, Chepeha DB, Giordano TJ, Li J, Zhang H, Polverini PJ, Nor J, Kitajewski J, Wang CY: **Crosstalk between tumor and endothelial cells promotes tumor angiogenesis by MAPK activation of Notch signaling.** *Cancer Cell* 2005, **8**:13-23.
 59. Dufraigne J, Funahashi Y, Kitajewski J: **Notch signaling regulates tumor angiogenesis by diverse mechanisms.** *Oncogene* 2008, **27**:5132-5137.
 60. Funahashi Y, Hernandez SL, Das I, Ahn A, Huang J, Vorontchikhina M, Sharma A, Kanamaru E, Borisenko V, Desilva DM, Suzuki A, Wang X, Shawber CJ, Kandel JJ, Yamashiro DJ, Kitajewski J: **A notch1 ecto-domain construct inhibits endothelial notch signaling, tumor growth, and angiogenesis.** *Cancer Res* 2008, **68**:4727-4735.
 61. Miyamoto A, Lau R, Hein PW, Shipley JM, Weinmaster G: **Microfibrillar proteins MAGP-1 and MAGP-2 induce Notch1 extracellular domain dissociation and receptor activation.** *J Biol Chem* 2006, **281**:10089-10097.
 62. Nehring LC, Miyamoto A, Hein PW, Weinmaster G, Shipley JM: **The extracellular matrix protein MAGP-2 interacts with Jagged1**

- and induces its shedding from the cell surface. *J Biol Chem* 2005, **280**:20349-20355.
63. Albig AR, Becenti DJ, Roy TG, Schiemann WP: **Microfibril-associated glycoprotein-2 (MAGP-2) promotes angiogenic cell sprouting by blocking notch signaling in endothelial cells.** *Microvasc Res* 2008, **76**:7-14.
64. Albig AR, Roy TG, Becenti DJ, Schiemann WP: **Transcriptome analysis of endothelial cell gene expression induced by growth on matrigel matrices: identification and characterization of MAGP-2 and lumican as novel regulators of angiogenesis.** *Angiogenesis* 2007, **10**:197-216.
65. Hoey T, Yen WC, Axelrod F, Basi J, Donigian L, Dylla S, Fitch-Bruhns M, Lazetic S, Park IK, Sato A, Satyal S, Wang X, Clarke MF, Lewicki J, Gurney A: **DLL4 blockade inhibits tumor growth and reduces tumor-initiating cell frequency.** *Cell Stem Cell* 2009, **5**:168-177.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp

