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Developmental defects

Notch: cell fate determination from vascular development to human vasculopathy

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Advances in understanding vascular development have fostered new drug development to control ANGIOGENESIS (see Glossary). Control of vessel formation directly impacts numerous pathologic processes, from cancer to degenerative disorders. However, a comprehensive knowledge of how cell-fate decisions occur during vascular development is needed to allow rational manipulation of vascular growth. Notch signaling governs cell-fate decisions, and disruption of Notch genes result in cardiovascular defects. This review discusses the phenotypes of Notch mutant mice that relate to human cardiovascular defects, fragile-vessel syndromes, and tumor angiogenesis. Target molecules for therapeutic interventions in these vascular pathologies are discussed.

Introduction

In mammals, development and maintenance of the cardiovascular system entail multiple cell-fate decisions that specify a variety of vascular structures, including veins, arteries, capillaries, and lymphatics. Similarly, organogenesis relies on the development of specialized vascular beds, such as those supplying the cardiac and renal circulation. Although many of the proteins that function in this complex process of vascular specialization have been identified, little is known about how the myriad of cell-fate decisions is regulated. One candidate set of regulators includes the Notch family of signaling receptors, which are known to function as modulators of cell-fate determination. Thus, the findings that Notch genes are robustly expressed in the vasculature and that mice deficient in components of the Notch signaling cascade display cardiovascular defects, suggest a role for Notch in guiding the multiple cell-fate decisions needed to form the cardiovascular system.

Notch signaling

The Notch pathway is an evolutionarily conserved signaling mechanism that functions to modulate a variety of cell-fate decisions. Depending on the cellular context, Notch signaling has been proposed to inhibit as well as induce differentiation, proliferation, and cell survival [1]. In *Drosophila*, a single

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Glossary

Alagille's syndrome: a pleiotropic autosomal dominant developmental disorder with phenotypic abnormalities of the liver, heart, kidney, eye, vertebrae, limbs and facial features.
Angioblast: immature precursor stem cell of endothelial cells.
Angiogenesis: the process of remodeling or growing blood vessels from established vessels to generate large arteries and veins and microcapillaries that involves endothelial cell proliferation, apoptosis, migration, tubulogenesis and accessory cell recruitment.
Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL): a late-onset (average age of 45) autosomal dominant disorder that is characterized by migraines with aura and recurrent strokes leading to psychiatric symptoms, progressive cognitive decline and dementia which arise from a slowly developing arteriopathy associated with the disorganization and destruction of the vascular smooth muscle cells surrounding the small cerebral arteries.
Cerebral cavernous malformation (CCM): vascular lesions characterized by enlarged and thin-walled vascular structures in the central nervous system without intervening brain parenchyma, lined by endothelial cells and lacking supporting vascular smooth muscle cells leading to pediatric strokes because of intercranial hemorrhaging.
Granular osmophilic material deposition (GOM): abnormal granular osmiophilic materials that is positive for acid polysaccharides that is deposited within the basal lamina of the vascular smooth muscle cells in CADASIL patients.
Mesangial cell: specialized smooth muscle cell that stabilize the capillaries within the renal glomeruli.
Tetralogy of Fallot: a combination of four congenital heart defects: pulmonic stenosis, ventricular septal defect, dextroposition of the aorta and hypertrophy of the right ventricle.
Vasculogenesis: the process by which uniform blood vessels develop de novo.

Notch gene product is activated by two ligands, Serrate and Delta. In mammals, these families have expanded to four Notch genes (Notch1–4) and five ligands, two Serrate-like (Jagged1–2) and three Delta-like (Dll1, 3 and 4) [1]. Consistent with regulating cell-fate decisions via direct cell-to-cell interactions, both receptors and ligands are membrane-spanning cell-surface proteins (Fig. 1). Additional control of Notch signaling is provided by Fringe proteins and O-fucosyltransferase 1 (Pofut), which function to regulate productive ligand–receptor interactions [2].

Notch proteins exist as heterodimeric receptors, with an extracellular and an intracellular peptide held together by non-covalent interactions (Fig. 1). This processing of Notch occurs via a furin-like protease prior to ligand activation [3]. Upon ligand-binding, the cytoplasmic domain of Notch is released from the cell-surface by a Presenilin (PS)/γ-secretase-dependent proteolytic cleavage [3]. The intracellular Notch protein then translocates to the nucleus, interacts with the CSL (CBF1, Su(H), Lag-2) transcriptional repressor, and converts it to transcriptional activator [3]. The hairy/enhancer of split (HES) and HES-related (Hey, CHF, HRT, HESR) transcriptional repressors are the direct targets of Notch/CSL-dependent signaling [4]. Notch also signals by a CSL-independent pathway that is poorly defined [5]. The turnover, and thus activity, of Notch proteins is regulated by a SEL-10 (Fbw7)/ubiquitin-dependent pathway [6].

In vivo studies

The *in vitro* vascular models of Notch function published to date provide conflicting results, and often have depended on a single endothelial cell line. Cardiovascular development and maintenance entail the interaction of endothelial and mural cells, which has not been reproducibly replicated *in vitro*. Thus, the extensive mouse models available provide a crucial perspective into the function of Notch signaling in regulating the complex interactions of the cardiovascular system. Although disruption of Notch signaling genes can cause diverse effects, often altering neurogenesis, somitogenesis and organogenesis, vascular defects are frequently observed. We will review four general classes of vascular defects: (1) vascular remodeling, (2) vascular stabilization, (3) arterial–venous specification, and (4) cardiac development. A description of the various vascular defects that arise from mutation of Notch or Notch ligand genes

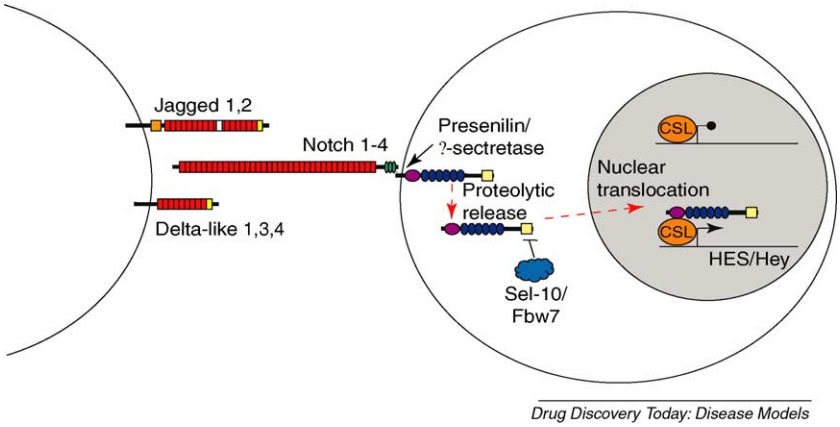

Figure 1. Schematic of Notch signaling pathway. CSL – CBF1, supressor of hairless, Lag-2; HES – hairy enhancer of split; Hey – HES related protein.

are summarized in Table 1, and those phenotypes that arise from mutation of Notch signaling components are summarized in Table 2. We will also discuss the implications of Notch signaling in human diseases and drug development.

Vascular remodeling

Notch signaling effects remodeling of the primary vascular network of uniformly sized vessels into functionally and morphologically distinct arteries, veins and capillaries. Pathologic remodeling has been implicated in the abnormal vasculature observed in tumors, which might coopt and/or remodel existing vasculature during the invasion of normal tissues [7]. Pathologic remodeling is also implicated in the abnormally assembled vessels of congenital vascular malformations. In mice, disruption of *Jagged1* (GenBank accession number NM_0138602), *Dll4* (GenBank accession number NM_019454), *Notch1* (GenBank accession number NM_008714), *Notch1/Notch4* (GenBank accession number NM_010929), *CSL/rpbsuh* (GenBank accession number NM_009035.1), *Pofut* (GenBank accession number AF375885), or the *PS1* (GenBank accession number AF149111.1) and *PS2* (GenBank accession number AF038935.1) results in embryonic lethality between E9.5 and 10.5, characterized by a disruption of branching morphogenesis or angiogenesis [9–14]. Although Notch4$^{-/-}$ mice are viable and fertile, Notch1$^{-/-}$; Notch4$^{-/-}$ embryos develop more severely disorganized vasculatures than Notch1$^{-/-}$ embryos, suggesting a potential redundancy of Notch1 and Notch4 function in vascular development [10]. Transgenic mice that activate Notch4 signaling within the embryonic endothelium (Tie1$^{+/LacZ}$; Flk1$^{N4/int\text{-}3}$ [GenBank accession numbers X71426; X70842]) also die midgestation with defects in vascular remodeling [15]. A similar phenotype is seen in Fbw7$^{-/-}$ (GenBank accession number AF391192) mice that are defective in turning over Notch proteins, and thus overexpress intracellular Notch1 and Notch4 proteins [16,17]. In these loss- and gain-of-function embryos, the initial vascular plexus forms in the head and yolk sac, but fails to reorganize into large vessels and capillaries. Similarly, endothelial cells are recruited to the intersomitic vessels, yet the formation of branched capillary networks is disrupted. In the Dll4$^{-/-}$ embryos, endothelial cells accumulate at the apical end of the intersomitic vessels [8]. In the brains of PS1$^{-/-}$; Flk1$^{+/LacZ}$ embryos, a reduction in capillary sprouting was associated with increased endothelial cell proliferation as well as apoptosis, suggesting that Notch signaling regulates the survival and differentiation of capillary endothelial cells [18]. Taken together, current evidence suggests that Notch signaling is not required for ANGIOBLAST (see Glossary) development and VASCULOGENESIS (see Glossary) to progress, but is necessary during the later process of vascular remodeling. Moreover, loss or gain of Notch activity results in a similar remodeling defect, suggesting that appropriate levels of Notch signaling are crucial for proper maturation of the embryonic vasculature.

Another intriguing aspect of Notch function in vasculature in human disease is its potential role in tumor angiogenesis. Increased expression of the Notch ligand Dll4 has been identified in proliferating vasculature of experimental tumors [19]. More recently, breast cancer cells exposed to estrogen have been shown to up-regulate Notch1 and Jagged1, with Notch1-dependent activation of hypoxia-inducible factor 1α and enhanced tumor vessel formation [20]. These findings raise the possibility that Notch deregulation is not only linked with oncogenesis, but specifically with the formation of tumor vasculature, requisite for tumor progression beyond a microscopic size. In recent studies Dll4 was found to be selectively expressed in the capillaries of tumor xenografts in mice, but was not detected in larger, presumably stabilized vessels [14]. In addition, Dll4 was found to be downregulated in tumors of mice treated with the VEGF blocking agent VEGF-Trap, consistent with its function as a downstream effector of VEGF [14,21]. Critically, unlike VEGF, Dll4 was not expressed in the vessels in the surrounding stroma, suggesting that it represents a novel, attractive target for therapeutic intervention.

Vascular stabilization

Recent studies demonstrate defective vascular mural cell recruitment, with associated progressive ectasia, in the great vessels of infants with transposition of the great arteries. The cause of these linked structural and microscopic anomalies is unknown [22]. However, an intriguing clue could be provided by studies of Notch mutant embryos. In these animals, the dorsal aortae and anterior cardinal veins develop, but the maturation or stabilization of these major blood vessels is disrupted. In Notch loss-of-function embryos, the caliber of the dorsal aortae and anterior cardinal veins are reduced, and in some cases are entirely absent [8,10,23]. By contrast, the dorsal aortae and anterior cardinal veins are dilated in the Notch gain-of-function embryos [15–17]. Thus, appropriate Notch signaling appears requisite for appropriate stable assembly of the major blood vessels of the embryo. Consistent with this hypothesis, smooth muscle cells are recruited to the aortae of Tie1$^{+/LacZ}$; Flk1$^{N4/int\text{-}3}$ and Hey1$^{-/-}$; Hey2$^{-/-}$ (GenBank accession numbers AF151521; AB093589) embryos, but fail to encircle the artery [15,23]. Similarly, expression of alpha-smooth muscle actin was reduced in Dll4$^{+/-}$ embryonic aortae, indicating deficient recruitment or organization of vascular mural cells [13,14]. Thus, a common result of altered Notch signaling is defective assembly of perivascular cells during aortic maturation. This defect could arise from deficient arterial and venous specification (as discussed below). By contrast, the defects in adult vessels as a result of Notch mutations may arise because of progressive

Table 1. Summary of cardiovascular defects in mice and humans with alteration in Notch and Notch ligands

Gene	Vascular remodeling	Vascular stabilization	Arterial/venous specification	Heart development
Notch1 (LOF[a])	Yolk sac primary plexus Placental labrynthine Intersomitic vessels	Collapsed dorsal aortae	Arterial markers: reduced EphrinB2 and neuropilin1 expression No CD44 expression	ND[b]
Notch2 (LOF)	Hyaloid artery Subcutaneous vessels	Glomerular capillary tuft defect	NPD[c]	Thin myocardial wall Reduced myocardial trabeculation
Notch3 (Hypo[f])	NPD	Adult onset artiopathy, endothelial and smooth muscle cell death	NPD	NPD
Notch4 (LOF)	NPD	NPD	NPD	NPD
Notch1 (LOF) Notch4 (LOF)	Yolk sac primary plexus Placental labrynthine Intersomitic vessels	Severe constriction or loss of anterior cardinal vein and dorsal aortae	ND	ND
Notch4 (GOF[d])	Yolk sac: vitelline vessels enlarged Cranial vasculature	Enlarged dorsal aortae and cardinal veins	ND	Enlarged heart
Notch2 (Het[e]) Jagged1 (Het)	NPD	Glomerular capillary tuft defect	NPD	Narrowing of pulmonary artery Right ventricular hypoplasia Atrial and ventricular septal defects Aorta dextroposition
Jagged1 (LOF)	Yolk sac: loss of large vitelline vessels Cranial vasculature	ND	ND	ND
Delta-like4 (Het)	Reduced size of placental blood vessels	Constriction of dorsal aortae and sometime posterior and anterior cardinal veins	Yolk sac: reduced caliber vitelline arteries and arterial branching Reduced size of umbilical artery	Pericardial swelling
Delta-like4 (LOF)	Yolk sac primary plexus Intersomitic vessels Cranial vasculature	Dorsal aorta absent or reduced to rudimentary capillary plexus Anterior cardinal vein and *sinus venosus* reduced Reduced α smooth muscle actin expression Fusion of dorsal aorta and anterior cardinal vein	Arterial markers: no EphrinB2, Connexin37 and Connexin40	Reduced atrial and ventricular chambers Reduced ventricular trabeculation

[a] Loss-of-function.
[b] No data because of early embryonic death or not described.
[c] No phenotype detected/reported.
[d] Gain-of-function.
[e] Heterozygous.
[f] Hypomorph.

Table 2. Summary of cardiovascular defects in components on the Notch signaling cascade

Gene	Vascular remodeling	Vascular stabilization	Arterial/venous specification	Heart development
CSL/Rbpsuh (LOF[a])	Yolk sac primary plexus Placental labrynthine	Reduction of dorsal aortae Reduced α smooth muscle actin expression	Arterial markers: no EphrinB2 and CD44 in the aorta Arteriovenous malformation as seen by a loss of intermediate capillary beds	Heart looping defect
Presenelin1 (LOF)	NPD[b]	NPD	NPD	NPD
Presenilin1 (LOF) Flk1 (Het[c])	Ventral cranial vasculature Capillary stenosis Subcutaneous vessels Intersomitic vessels	NPD	NPD	NPD
Presenilin2 (LOF)	NPD	Lung hemorrhaging	NPD	NPD
Presenilin1 (LOF) Presenilin2 (LOF)	Yolk sac primary plexus Chorioallantoic fusion	ND[d]	ND	Enlarged pericardial sac
Sel-10/Fbw7 (LOF)	Yolk sac primary plexus Cranial vasculature	Aortae dilation Constriction or loss of cardinal veins		Ventricular and atrial differentiation Reduced Nkx2.5
Hey2 (LOF)	NPD	NPD	NPD	Ventricular septal defect and enlargement Tetralogy of Fallot Tricuspid atresia
Hey1 (LOF) Hey2 (LOF)	Yolk sac primary plexus Placental labrynthine Intersomitic vessels Cranial vasculature	Reduction or loss of dorsal aortae and cardinal veins	Arterial markers: no EphrinB2, CD44 and neuropilin1 in the aorta	Thin myocardium Reduced ventricular trabeculation
Foxf1 (Het)	NPD	Lung hemorrhaging Defects in peripheral lung microvasculature	ND	NPD
Ccm1 (LOF)	NPD	Enlargement intersomitic arteries Aortic defects	Constriction of branchial arch arteries Arterial markers: no Dll4, Notch4 and EphrinB2	NPD
Pofut (LOF)	Yolk sac primary plexus Intersomitic vessels Cranial vasculature	NPD	ND	Heart looping defect

[a] Loss-of-function.
[b] No phenotype detected/reported.
[c] Heterozygous.
[d] No data because of early embryonic death or not described.

vascular weakness, independent of endothelial cell specification, as a result of defective cell–cell associations.

In the adult, insight into Notch function in stabilizing arteries and arterioles can be drawn from CEREBRAL AUTOSOMAL DOMINANT ARTERIOPATHY WITH SUBCORTICAL INFARCTS AND LEUKOENCEPHALOPATHY (CADASIL; see Glossary). CADASIL is a late-onset arteriopathy linked to missense mutations in *Notch3* (GenBank accession number XM_009303). In CADASIL, the vascular smooth muscle cells surrounding the cerebral arteries and arterioles regress, decreasing vessel wall thickness [24]. The resulting weakness of the vessel wall can account for the cerebral hemorrhages found in 31–69% of CADASIL patients. Arterial lesions are not restricted to the brain; they are also found in extra-cerebral arteries of the skin and retina [24,25]. The CADASIL phenotype is consistent with the expression of Notch3 in vascular smooth muscle cells [25–27], and suggests that Notch3 could function to maintain cell–cell interactions between the arterial endothelial cells and vascular smooth muscle. In fact, mice that express a mutant *Notch3* (GenBank accession number NM_019029) CADASIL R90C transgene in smooth muscle cells developed hallmark CADASIL arteriopathy, GRANULAR OSMOPHILIC MATERIAL DEPOSITION (see Glossary) and Notch3 extracellular domain accumulation [28]. However, smooth muscle cell anchorage and adhesion appeared to be disrupted prior to these hallmarks, suggesting that Notch3 function can be crucial to adhesion-dependent cell survival. Interestingly, Notch3 is not required for mouse embryonic development or fertility [29].

Mouse genetics has also implicated Notch signaling in the stabilization of specialized vessels of organs. Notch2$^{del1/del1}$ (GenBank accession number D32210), or Notch2$^{+/del1}$; Jagged1$^{+/-}$ mice die postnatally from renal vascular defects [30,31]. In these mice, glomerular development arrests at the capillary loop stage, as seen by the absence of capillary tufts or presence of capillary aneurysms. In these mutant glomeruli, the specialized vascular smooth muscle cells, termed MESANGIAL CELLS (see Glossary), are missing. Thus, this deficiency of mesangial cells could result in impaired stabilization of the vascular beds. Notch signaling can also function in maintaining the homeostasis of the capillaries of the lung, as neonatal FoxF1$^{+/-}$ (GenBank accession number NM_010426) and PS2$^{-/-}$ mice develop pulmonary hemorrhaging [9,32]. In FoxF1$^{+/-}$ mice, Notch2 expression is reduced, as is the downstream target gene HES1 (GenBank accession number D16464), suggesting that the observed vascular lung phenotype might be due to of a loss in Notch2 signaling [32]. Because PS2 is a protease that activates Notch, the hemorrhaging and apoptotic cell death of epithelial and vascular endothelial cells within the lung may also be due to a disruption in Notch signaling [9].

Excessively fragile vasculature has also been associated with altered Notch signaling in other human syndromes. For example, patients with mutations of CEREBRAL CAVERNOUS MALFORMATION-1 (CCM1; see Glossary; GenBank accession number NM_004912.3) have been found to display defective Notch signaling in arterioles. Affected individuals form thin-walled ectatic vascular lesions in the brain, which are prone to rupture, leading to hemorrhagic stroke [33]. Similarly, it has recently been recognized that patients with Alagille's syndrome, caused by mutation of Jagged1 and previously linked with hepatobiliary, cardiac, oculofacial, and bony anomalies, sustain significant mortality from anomalous vasculature. In a recent retrospective review, up to one-third of patient deaths were associated with defective, usually aneurysmal, arteries [34].

Arterial–venous specification

In humans, arteriovenous malformation (AVM) are a clinically important subtype of congenital vascular malformation, associated with physiologically significant arteriovenous shunting and congestive heart failure. The molecular cause of these improper arterial–venous connections is unknown. However, recent studies in animal models provide evidence that Notch participates not only in the formation of distinct arterial and venous circulations, but in the development of the bridging capillary network. In Zebrafish, disruption of Notch signaling results in the loss of the arterial-specific ephrinB2, and an increase in the venous-specific EphB4 within the developing dorsal aorta [35]. Conversely, Notch signal activation inhibits venous development, indicating that Notch participates in arterial–venous specification. Early in mouse development, Notch receptors and ligands are expressed in both venous and arterial endothelial cells, but by midgestation their expression is mainly restricted to arterial endothelium [19,27,36]. A role for Notch in arterial–venous specification has recently been demonstrated in mice. Half of Dl4$^{+/-}$ mice die *in utero* with a reduction in the caliber of the vitelline arteries, umbilical artery, and dorsal aorta, and a loss of arterial branching in the yolk sac [8]. In embryos with severely constricted dorsal aortae, the anterior and posterior cardinal veins were also constricted, suggesting that the venous defect could be secondary to the arterial defect [8,14]. In fact, the arterial markers, ephrinB2 (GenBank accession number U30244), Connexin37 (GenBank accession number NM_008120.2) and Connexin40 (GenBank accession number NM_008121) were not expressed in the dorsal aortae of Dll4$^{-/-}$ embryos. Instead, the dorsal aortae expressed the venous marker, EphB4 (GenBank accession number NM_010144). Consistent with this observation, ephrinB2 and CD44 (GenBank accession number XM_283773) expression was reduced or lost in the dorsal aortae of Hey1$^{-/-}$; Hey2$^{-/-}$, Rbpsuh$^{-/-}$ and Notch1$^{-/-}$ embryos [13,23]. In some of the Dll4$^{-/-}$ embryos, the dorsal aorta was found to be fused with the anterior cardinal vein [8]. AVMs also occur in Dll4$^{+/-}$ mice in which there is an absence of intermediate capillary beds between arteries and veins [13]. Consistent with this phenotype, Dll4 expression is highest where capillaries merge with venules [14]. Taken together, these findings suggest that Notch signaling functions to promote development of the capillary network bridging the arterial and venous circulations, as well as arterial vessel identity and endothelial cell specification [8].

Cardiac development

Congenital defects in the heart and great vessels are a common birth defect in humans, affecting nearly 1% of live-born infants. As above, recent studies demonstrate that microscopic defects could accompany morphologic anomalies, suggesting that a basic defect in the genetic programs directing assembly of the heart and great vessels may be responsible. In humans, a role for Notch signaling in such anomalies is illustrated by Alagille syndrome. This syndrome, associated with mutations in Jagged1 (GenBank accession number U73936) (AGS; see Glossary), is a dominant, pleiotropic developmental disorder with abnormalities of the liver, heart, kidney, eye, vertebrae, limbs and facial features [37]. Most patients present with cardiac malformations that include defects in the pulmonic valve, pulmonary artery and its branches, ventricular and atrial septation, aortic stenosis or coarctation, and TETRALOGY OF FALLOT (see Glossary). Most of these heart defects are recapitulated in mice deficient for components of the Notch signaling pathway [30,31,38–40]. In fact, overlapping and distinct expression patterns have been described for Notch family members during heart development.

In mice, the heart develops from a linear tube that at E8.0 loops left to right to establish the positions of the atrial and ventricular chambers. Between E12.5 and 13.5, inter-atrial and -ventricular septation is established, and early development of the cardiac valves is initiated. In Notch mutant mice, there is a failure in atrial and ventricular chamber formation and maturation. Half of Notch2$^{del1/del1}$ embryos develop pericardial effusion and thinning of the myocardial wall [30]. Notch2$^{+/del1}$; Jagged1$^{+/-}$ and Hey2$^{-/-}$ embryos have right ventricular hypoplasia, narrowing of the pulmonary artery, atrial and ventricular septation defects and dextropositioning of the aorta [31,38–40]. In Dll4$^{-/-}$ mice, the arterial and ventricular chambers and ventricular trabeculation is reduced [8]. In the Notch gain-of-function Sel-10/Fbw7$^{-/-}$ mouse, an increase in Hey1 and Hey2 expression correlated with a decrease in the expression of the essential heart specific transcription factor, Nkx2.5 (GenBank accession number NM_008700) [17]. Thus, both over or under-expression of Hey genes are associated with the development of heart defects, suggesting that appropriate levels of Notch signaling are necessary to regulate heart maturation. As Jagged1 and Notch2 are expressed in the endocardium and myocardium, Notch signaling can regulate cell–cell communication between these two cell types during heart development [31].

Conclusions

Mouse models of Notch function have uncovered multiple roles for Notch in the cardiovascular development. These models are informative when considering prenatal and pediatric cardiovascular conditions. However, because of embryonic lethality of Notch mutant mice, current models cannot adequately provide insight into the function of Notch in adult vascular pathologies, such as tumor angiogenesis or atherosclerosis. More sophisticated modeling, such as conditional or hypomorphic gene disruptions, might permit investigation of the effects of altered Notch function in adult vessels.

As we begin to identify the components of the Notch pathway that function in vascular development, potential targets for intervention in human diseases have become apparent. When considering therapeutic approaches, it is informative to focus on Notch signaling components with vascular functions (Fig. 2). Notch is a cell surface receptor, but does not harbor an enzymatic domain. Thus, initial design of inhibitors has focused on using the Notch extracellular domain as a protein-based antagonist or ''decoy''; that binds and sequesters Notch ligands [41,42]. A challenge with Notch decoys, and also neutralizing antibodies, lies in the fact that high sequence conservation among the four Notch proteins precludes easy design of receptor-specific inhibitors. Small molecule inhibitors can be developed to target enzymes that functions in the Notch pathway, such as TACE, fucosyltransferase, or Fringe glycosyltransferase. Antagonists of the pre senilins have been developed, and appear to be powerful inhibitors of ligand-dependent Notch signaling [43]. Thus, development of therapeutic molecules appears feasible, with the major challenge being the identification of inhibitors that are specific for individual targets in the Notch pathway. Given the protean functions of the Notch gene family, such therapeutic targeting of inappropriate Notch signalling – whether deficient in fragile-vessel syndromes, or excessive in growing tumors – can offer significant benefit in a variety of human diseases.

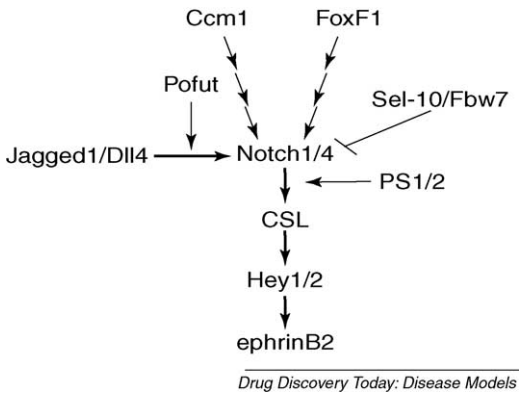

Figure 2. Genetic interactions of the Notch signaling components in cardiovascular development. Mouse genetics suggest that Ccm1 and FoxF1 lie upstream of Notch. Ccm1 – cerebral cavernous malformation 1; FoxF1 – fork heard transcription factor F1; Fbw7 – F box protein w7; Dll4 – Delta like 4; PS1/2 – Presenilin 1 and 2; CSL – CBF1, supressor of hairless, Lag-2; Hey – HES related protein.

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