Notch signalling in smooth muscle cells during development and disease

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Received 10 November 2011; revised 30 December 2011; accepted 16 January 2012; online publish-ahead-of-print 19 January 2012

Abstract

The Notch signalling pathway is a highly conserved cell–cell signalling mechanism that plays a central role in the development and maturation of most vertebrate organs. In vertebrates, Notch receptors, several ligands, and components of the downstream signalling machinery are expressed in the vessel. Over the past decade, numerous studies have highlighted the critical role of the Notch pathway in the vasculature. The goal of this review is to summarize our current understanding of the contribution of Notch signalling in smooth muscle cells to vascular development and physiology. We further discuss the growing clinical importance of this pathway in human pathological conditions involving the vasculature, namely cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, Alagille syndrome, and pulmonary arterial hypertension.

Keywords

Notch • Smooth muscle cell • Development • Artery • CADASIL

This article is part of the Spotlight Issue on: Smooth Muscle Cells and Vascular Diseases

1. Introduction

The Notch pathway is an evolutionary conserved intercellular signalling mechanism that operates in most vertebrate organs during development.1 Over the past decade, it has become clear that the Notch pathway plays a key role in the development and homeostasis of the vasculature, coordinating multiple aspects of endothelial and smooth muscle cell (SMC) behaviour. Involvement of the Notch pathway in endothelial cells has been the subject of several excellent reviews.2,3 This article will review recent progress in deciphering the contribution of the Notch pathway in SMC to vascular development, physiology, and pathology in mice and humans.

The vascular system comprises an extensive network of arteries, veins, and capillaries of different types and calibres with specialized functions. Full elaboration of this system involves precisely coordinated multistep processes. During the early stages of development, endothelial cell precursors differentiate and aggregate into a primary vascular network, which is subsequently expanded and reshaped into a highly branched hierarchical vascular tree.4,5 Recruitment of mural cells and their differentiation into SMC or pericytes are important for the stabilization and maturation of vessels into fully functional arteries, veins, or capillaries, respectively.4,5 Furthermore, acquisition of artery or vein identity, at the level of endothelial and SMC, plays an important role in the development and maturation of the blood vessel network.6,7 In the mature vessel, fully differentiated SMC are contractile cells that play a critical role in the regulation of vessel tone, blood pressure, and blood flow distribution.

Within normal adult organisms, SMC show extensive differences in morphology, function, and gene expression, although they transcribe a common set of contractile SMC genes. This may depend on their position in the vascular bed (artery vs. vein, conduit vs. resistance vessel), their embryological origin, or the organ context. Molecular determinants controlling the expression of SMC genes have recently been reviewed, showing the importance of CArG–SRF–myocardin-dependent mechanisms and epigenetic control in the regulation of vascular SMC lineage.8,9 Importantly, SMC in the adults still exhibit some plasticity and can undergo extensive phenotypic changes following vascular injury or in certain disease states.9

Mammals have four Notch receptors (Notch1, Notch2, Notch3, and Notch4) and five Delta/Serrate/Lag-2 (DSL) ligands: Delta-like1 (Dll1), Delta-like3 (Dll3), Delta-like4 (Dll4), Jagged1 (Jag1), and Jagged2 (Jag2) (Figure 1). Both Notch receptors and DSL ligands are transmembrane proteins with a large extracellular domain that consist primarily of epidermal growth factor-like repeats (EGFR). Notch receptor is synthesized as a precursor protein, which is cleaved in two by furin during transport to the cell surface, at a site called S1. This yields a large ectodomain (NotchECD) and a membrane-tethered intracellular domain (NotchICD) which are held together non-covalently by a juxtamembrane heterodimerization domain. Ligand binding to Notch triggers additional proteolytic

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cleavages including an ADAM metalloprotease-mediated cleavage within the extracellular juxtamembranous region followed by a Presenilin/γ-secretase-dependent cleavage releasing the Notch intracellular domain (NICD) from the membrane. Once cleaved, the NICD translocates to the nucleus where it forms an active transcriptional complex with transcription factor RBP-Jκ and recruitment of Mastermind (MAM) and coactivators such as histone acetylases (HAc). Notch signalling occurs through heterotypic interactions between endothelial cells (EC) as signal-sending cells and SMC as receiving cells, as well as through homotypic SMC–SMC interactions. SMC may switch from a receiving to sending cell state by up-regulation of Jag1 expression upon Notch activation.

Of the Notch receptors, Notch1 and Notch4 are primarily present in endothelial cells and Notch3 in SMC and brain pericytes. Activated Notch1 is also detected in arterial SMC of the pulmonary vasculature during development and high levels of Notch2 are observed in the mesenchymal tissue and SMC surrounding the aorta and pulmonary arteries. Of the ligands, Dll1, Dll4, and Jag2 are present in endothelial cells while Jag1 is expressed by both endothelial and SMC. Of interest, most studies have highlighted the preponderance of Notch components in the arteries.

2. Vessel wall formation/maturation, Notch signalling, and SMC phenotype

Contribution of the Notch pathway in the SMC phenotype has been initially investigated in cultured cells. However, contrasting results were obtained, with independent studies showing that Notch activation can promote the expression of contractile SMC genes while several others demonstrated robust inhibition. Conversely, gene inactivation strategies in mice provided more consistent data.
clearly demonstrating that Notch signalling positively regulates multiple aspects of vascular SMC specification, differentiation, and maturation during development. Below we review this role according to the vascular bed and embryological origin of SMC.

2.1 Notch in aortic arch arteries and aorta

The origin of SMC from the major arteries of the trunk is complex with contributions from several independent cell lineages. SMC of the developing arch artery, ductus arteriosus, and the proximal regions of the major aortic arch branches derive from the cardiac neural crest. The second heart field gives rise to SMC at the root of the aorta and pulmonary artery and the somites to SMC of the descending aorta. Notch signalling is required within neural crest precursors for proper patterning of the outflow tract region and aortic arch artery development. In mice, inhibition of Notch activity in neural crest derivatives, using a dominant-negative version of Mastermind-like protein 1 (DNMAML) whose expression is controlled by a Pax3-cre driver line, causes congenital heart defects including aortic arch patterning defects and pulmonary artery stenosis. These are associated with defective formation of the smooth muscle layer of the aortic arteries, whereas the nascent endothelial tubes form normally. Mutant embryos exhibit strongly reduced expression of a LacZ transgene knocked into the SM22α locus and localized down-regulation of SMα-actin (SMA) staining in the aortic arch arteries, particularly in the sixth one, which contributes to the pulmonary artery and ductus arteriosus. Moreover, ex vivo studies using neural tube explants from mutant embryos show a dramatic reduction in the number of cells differentiating into contractile SMC. These findings thus provide evidence that Notch signalling positively regulates differentiation of cardiac neural crest cells into SMC, in a cell autonomous manner. Moreover, the data suggest that Notch signalling in neural crest precursors is dispensable for their migration and expansion into the cardiac outflow tract. Notch signalling is also required in the second heart field for proper patterning of the outflow tract. Inhibition of Notch signalling in the second heart field, using the same DNMAML and an islet-1-cre driver line, strongly impairs the behaviour of neighbouring cardiac neural crest cells which exhibit faulty migration into the outflow tract resulting in congenital heart defects with aortic arch arteries anomalies. In this process, the data suggest that Notch could stimulate the expression of Fgf8 and BMP4, two critical factors involved in outflow tract development. 

The observation that specific deletion of Jag1 in neural crest cells results in aortic arch remodelling defects with deficient SMC differentiation and that deletion of Jag1 in the second heart field phenocopies the second heart field DNMAML mutants strongly supports the possibility that Jag1 acts as an important ligand in this context. Similarly, Notch2 appears as the key Notch receptor implicated in the differentiation of neural crest precursors into SMC. The role of Notch signalling in the development of the aorta and contractile differentiation of SMC after the onset of SMC differentiation is not entirely clear yet. On the one hand, SM-specific deletion of Jag1 using an SM22α-Cre driver line produces patent ductus arteriosus, causing early postnatal death. These mice have prominent defects in contractile SMC differentiation in the vascular wall of the ductus arteriosus and adjacent descending aorta, with strongly reduced expression of SMA, SM22α, and SM-myosin heavy chain contractile proteins. Moreover, SM-specific deletion of Notch2 causes narrow aorta and pulmonary arteries due to SMC defects. On the other hand, SMC differentiation of the aortic arch arteries is not affected when Notch is inhibited using the DNMAML and the SM22α-cre driver line described above. This might, however, result from inefficient inhibition of Notch and/or a lower dosage requirement of Notch activity in this context.

2.2 Notch in organ arteries

SMC of organ arteries also have diverse embryonic origins. For example, the neural crest gives rise to mural cells (SMC and pericytes) of most brain vessels, while the mesothelium and proepicardium contribute to mesenteric and coronary vessels, respectively. In the heart, Notch signalling regulates the differentiation of epicardium-derived cells (EPDC) into SMC. Lineage tracing showed that EPDC give rise to fibroblasts and coronary SMC. Complete suppression of Notch signalling in EPDC results in severe morphological changes of the coronary vasculature with enlargement of arteries and veins. In the absence of Notch signalling, EPDC form and surround the developing vessels but fail to differentiate into SMC. Conversely, conditional activation of Notch signalling in EPDC results in premature differentiation of SMC. Experiments using the DNMAML and the SM22α-cre driver line, as described above, indicate that Notch signalling is also required after the onset of SMC differentiation. Inhibition of Notch activity in SMC results in enlarged brain arteries with marked thinning of the tunica media. Unfortunately, analysis of arteries within other organs is not reported in this study. The expression pattern of Jag1 suggests that it may be acting as an important ligand in this process but this remains to be tested. Expression mapping of Notch receptors and RBP-Jκ activity as well as structural and functional studies of Notch3 knockout (Notch3KO) mice provide ample evidence that Notch3 is the essential Notch receptor involved in this process (Figure 2). First, Notch3 is predominantly restricted to SMC of organ vessels and is most prominently expressed in arterial SMC. Secondly, lacZ expression in NAS (Notch activity sensor) mice, which carry a nls-lacZ reporter gene driven by an artificial promoter containing multiple RBP-Jκ-binding motifs, abundantly marks SMC of organ arteries, particularly the brain and tail arteries, from late gestation. Importantly, this β-galactosidase activity is almost abrogated in Notch3KO mice arguing that Notch3 is the major contributor to SM-RBP-Jκ activity in distal arteries. Thirdly, in mice completely lacking Notch3, parenchymal arteries, particularly in the brain, lung, heart, and tail, are enlarged and have a thinner muscular coat, although expression of common contractile SMC genes is roughly preserved. Electron microscopy analysis shows that SMC are present, but have an abnormal and extremely thin shape, and are not cohesive. Further studies provided evidence that Notch3 controls arterial differentiation and postnatal maturation of SMC. Consistent with these rather specific SMC alterations caused by loss of Notch3, contraction and relaxation in response to pharmacological agents seem preserved in mutant arteries while myogenic tone, a fundamental property of distal arteries, is strongly decreased. Accordingly, auto-regulation of cerebral blood flow as well as the regulation of renal haemodynamics are severely impaired in Notch3KO mice. In the brain, Notch signalling in SMC is also required to pattern the vasculature. In higher vertebrates, the cerebral circulation is derived from the carotid and vertebral-basilar arteries that communicate at the base of the brain to form the circle of Willis. Suppression of Notch signalling using the DNMAML expressed in an SMC lineage restricted fashion results in defective patterning of the circle of
Wills, particularly in its anterior part, with loss of arterial symmetry, variable dilations, and constrictions of arterial segments, leading to a lack of functional communication between the left and right cerebral circulation.20 Again, the receptor involved in this process appears to be Notch3.37

### 2.3 Signal-sending and signal-receiving cells

In classical Notch signalling, the extracellular domain of the Notch receptor expressed on the surface of one cell (signal-receiving cell) interacts with the extracellular domain of the ligand expressed on an adjacent cell (signal-sending cell). Several studies support the existence of heterotypic interactions between the endothelial cells as signal-sending cells and SMC as signal-receiving cells (Figure 1). In embryos with endothelial-specific deletion of Jag1, there is a strong deficit in SMC in the aortic arch arteries and dorsal aorta due to a failure of adjacent SMC to differentiate.38 Also, mice with post-natal deletion of Jag1 in the endothelial cells exhibit defects of the retinal vasculature including reduced coverage of retinal arteries by SMC.39 Moreover, three-dimensional (3D) endothelial–mural cell co-cultures show that endothelial cells can promote, in adjacent mural cells, activation of Notch signalling and subsequent expression of SMC contractile genes. This process requires direct cell–cell contact between endothelial and mural cells, and expression of jag1 in endothelial cells and of Notch3 in mural cells.40 Heterotypic interactions between endothelial cells and SMC are likely to occur primarily during the early stage of vascular development. Indeed, as vessel maturation proceeds, SMC become separated from endothelial cells by the lamina elastica, thus creating a physical barrier precluding such interactions. In addition to heterotypic interactions, impaired differentiation of SMC arising from SM-specific deletion of jag1 suggests the existence of homotypic interactions between adjacent SMC.29 Overall, there is converging evidence for a model where endothelial cells initiate the signalling cascade, through heterotypic interactions, thus enabling mural cells to maintain and distribute the signal to the adjacent layers of mural cells through homotypic interactions (Figure 1).

In the common model of Notch signalling, ligand–receptor interaction occurring within the same cell results in Notch inhibition. Such interaction is presumed to reduce the ability of a cell to receive the signal from neighbouring cells by a process called ‘cis-inhibition’ of the receptor by the ligand.41,42 However, a recent study using cultured pulmonary artery SMC suggests that in these particular cells, Notch signalling might involve intracellular interaction between the ligand (Jag1) and the receptor (Notch3).43

Interaction of Notch receptors with ligands distinct from the Delta/jagged proteins has been reported and may provide yet another source of activation. Studies have shown that MAGP1/2 (microfibril-associated glycoproteins-1 and -2), which covalently associate with fibrillins in elastic fibres can bind to the extracellular domain of Notch1 and activate Notch signalling in vitro.44 However, the in vivo relevance of this finding remains to be demonstrated.

### 2.4 Notch target genes in SMC

The best-characterized direct Notch targets are the bHLH genes of the Hes/Hey families, which function as transcriptional repressors. Hes/Hey have been shown to function downstream of various Notch receptors in many distinct organs and contexts.12 Hey1, Hey2, and HeyL genes are up-regulated in the developing SM layer of the aortic arch arteries and strongly down-regulated when Notch is inactivated, suggesting that Hey genes are relevant targets of Notch in SMC during aortic development.26 This is further substantiated by the finding that combined loss of Hey1 and Hey2 results in severe

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**Figure 2** Notch3/RBP-Jk activity is functionally important in arterial SMC of organ arteries. (A–C) LacZ (blue) in Notch Activity Sensor mice is abundantly expressed in arterial SMC. In the absence of Notch3, lacZ expression is almost abrogated while SMA expression (brown) is roughly preserved. (D and E) Electron micrographs show that SMC of the Notch3KO artery are not cohesive (arrows) and have an abnormal shape and a smaller profile. Scale bar represents 29 μm (A and C), 12 μm (B), and 1.7 μm (D and E); a, artery; v, vein.
loss of SMC in the aorta of embryos and that loss of Hey2 causes thinning of the tunica media in the aorta and pulmonary artery.\(^{45,46}\)

Although Hes/Hey genes execute multiple key roles in Notch signalling, they do not explain all Notch functions. In organ arteries for instance, expression of Hes and Hey genes is unchanged in the absence of Notch3.\(^{34}\) So far, very few other Notch targets have been identified. First, cell culture experiments and expression analyses in Notch mutant mice suggest that SMA is a direct target of Notch, providing a possible mechanism for the positive role of Notch in the contractile SMC differentiation.\(^{47}\) Interestingly, Tang et al.\(^{48}\) showed that in cultured SMC, forced expression of Hey1 and Hey2 can repress basal SMA expression and Notch-induced SMA up-regulation, through suppression of NICD/RBP-Jk binding to the SMA promoter. The SMA/Hey pair may be one example, among Notch targets, conforming to the conventional transcriptional incoherent feed-forward loop, where the Notch stimulus regulates both a gene and a repressor of this gene.\(^{49}\) Based on these results and others,\(^{50}\) a model was recently discussed whereby Notch signalling could activate SMC differentiation until the expression level of Hey proteins rises above a critical threshold required to antagonize and terminate this signal.\(^{51}\) This model remains to be validated in vivo. It might explain part of the contradictory results observed when manipulating Notch activity in cultured SMC since many parameters can modulate the output from such a regulatory loop, including the level of Notch activation, the rate of synthesis and stability of targets, and the thresholds required for activation and repression. Secondly, platelet-derived growth factor receptor-beta (PDGFR-\(b\)) appears to be another direct target of Notch.\(^{52}\) Indeed, cell culture data have demonstrated Notch/RBP-Jk-dependent regulation of PDGFR-\(b\) expression, and analysis of Notch3KO mice shows attenuation of PDGFR-\(b\) expression in SMC of the mutant tail arteries. The link between Notch and PDGF signalling seems complex and may indicate a negative feedback loop between Notch and PDGF signalling. On the one hand, Notch-induced increase in PDGFR-\(b\) leads to an augmented intracellular response to PDGF stimulation. On the other hand, Notch and PDGF signalling exert opposite effects on SMC migration, at least in cultured cells, and agonist stimulation with PDGFB-BB decreases the expression of both Notch3 and PDGFR-\(b\). Thirdly, the microRNA cluster miR-143/miR-145 has been recently added to the list of candidate Notch direct targets.\(^{53}\) miR-143 and miR-145 are strongly expressed in visceral and vascular SMC during late gestation and in adult mice.\(^{54,55}\) In cultured SMC, the Jag1/Notch pathway positively regulates miR-143/miR-145 expression and the 5 kb-proximal promoter activity of this miRNA cluster, in a pathway that is independent and parallel to the SRF pathway. Jag1-induced up-regulation of SMC contractile genes is attenuated by miR-143/miR-145 specific antagonirs, suggesting that miR-143/miR-145 may be important intermediates in the response to Notch activation during SMC differentiation.\(^{53}\) However, the in vivo relevance of these results, in the context of vessel development, remains to be clarified since mice lacking both miRNAs express contractile SMC genes at levels comparable with wild-type mice.\(^{55}\) Finally, components of the Notch pathway are themselves direct targets. Like Notch1 in other cellular contexts,\(^{56}\) Notch3 has been shown to autoregulate its own expression in cultured mural cells, providing a feedback mechanism that may reinforce signalling.\(^{40}\) Also, Epstein's lab recently demonstrated that Jag1 is a Notch target gene, with a relevant role in the developing aortic arch arteries. This lab identified a Notch responsive enhancer within the second intron of Jag1, which can direct in vivo transgenic expression to SMC precursors of the aortic arch arteries. Importantly, these results provide a mechanism for propagation of the Notch signal between adjacent layers of mural cells by Notch/Jag1 lateral induction in newly differentiated SMC, at least in the context of developing aortic arch arteries\(^{28}\) (Figure 1).

### 3. Vessel injury, Notch signalling, and SMC phenotypic switching

In response to vessel injury, SMC undergo a phenotypic transition whereby they proliferate, migrate from the medial layer to the intima, secrete metalloproteases, increase their expression of matrix proteins, and decrease their expression of contractile proteins.\(^{57}\)

The development of intimal hyperplasia, in response to vascular injury, is associated with a temporal and spatial change in Notch1, Notch2, and Notch3 receptors, Jag1 and Jag2 ligands, as well as Hey target genes. Expression of Notch pathway components is downregulated within the first days following injury and then up-regulated 7–14 days after injury at the locus of neointima.\(^{19,58–63}\) There have been several studies supporting a functional role for Notch signalling in SMC phenotypic switching during repair of vascular injury; however, there still remain discrepancies about whether Notch promotes or inhibits neointimal formation. On the one hand, three studies have reported an inhibition of neointima formation after vascular injury in mice with reduced Notch activity, arising from heterozygous deletion of Notch1, homozygous deletion of Hey2, or adenoviral transfection with a soluble Jag1, which presumably inhibits the Notch pathway.\(^{60,61,64}\) Noteworthy, inhibition of neointima formation is comparable between global and SMC-specific Notch1 haploinsufficient mice. Consistently, primary cultures of SMC, derived from aortas of SMC-specific Notch1 haploinsufficient mice, Hey2KO mice, or soluble Jag1-treated coronary artery SMC, all show reduced cell chemotaxis and proliferation, as well as increased apoptosis. Moreover, Notch3 does not appear to be involved in this process since vessel remodelling is not affected by total loss of Notch3. Taken together, these results suggest that Notch1 in SMC, but not Notch3, mediates SMC proliferation, migration, and survival and neointimal formation after vascular injury and that the effects are likely mediated by Hey2. Physiological regulation of PDGFR-\(b\) and miR-143/miR-145 by Notch signalling may be relevant in this context,\(^{57}\) but this remains to be investigated. On the other hand, Wu et al.\(^{65}\) recently showed that endothelial-specific Jag1 heterozygous deletion produces the opposite phenotype with an exaggerated intimal and medial thickening after carotid artery ligation. They also present results of studies in cultured SMC consistent with the in vivo data.\(^{65}\) The reasons for these apparently contradictory results are yet unclear. Precise identification of the cells in which Notch is inactivated, particularly in this latter experimental setting, may help clarify this issue.

### 4. Notch and vascular diseases

#### 4.1 Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)

Small vessel disease (SVD) of the brain is recognized as a major public health burden, being responsible for at least 25% of all strokes and 40% of vascular dementia.\(^{66}\) Dominant mutations in NOTCH3 cause
CADASIL, the most common hereditary SVD. On neuropathological examination, there are diffuse lesions of the hemispheric white matter and multiple small lacunar infarcts in the white and deep grey matter as well as in the brain stem. Magnetic resonance imaging of the brain indicates that white matter lesions are the earliest changes preceding the onset of symptoms by 10–15 years and are always present after the age of 35, whereas lacunar infarcts occur later in life. The underlying vascular lesion is a unique arteriopathy characterized by progressive loss of SMC, thickening of the wall by various types of collagens and laminin and a material of yet unclear composition appearing on electron micrographs as multiple deposits of granular osmiophilic material (GOM), located extracellularly but close to the cell surface of SMC and pericytes. Virtually, no therapies are available to modulate the progression of this disease, which leads to premature death of the patients around the age of 65–70.

The vast majority of CADASIL-associated NOTCH3 mutations are missense mutations. Remarkably, all mutations are located in the extracellular domain, lead to an odd number of cysteine residues within an EGFR, and cause abnormal accumulation of the extracellular domain of NOTCH3 (NOTCH3ECD). NOTCH3ECD accumulates at the plasma membrane of SMC and brain pericytes in close vicinity to GOM deposits and also within GOM deposits. This observation and the finding that Notch3 is primarily expressed in SMC strongly suggest that SMC is the primary target cell.

All types of genetic manipulations (knock-out, knock-in, and transgenesis) have been attempted, in order to reproduce CADASIL in the mouse. However, only transgenic mice over-expressing (approximately four-fold over Notch3 endogenous levels) a mutant Notch3 protein, with an endogenous-like expression pattern, recapitulate most CADASIL features, in a chronological order consistent with the known natural history of the human disease. Specifically, PAC-Notch3R169C transgenic mice exhibit first Notch3ECD accumulation and GOM deposits, between 1 and 6 months of age, and then develop white matter lesions by 20 months of age. Prior to the appearance of brain lesions, mutant mice exhibit moderate and widespread reduction (10–20%) in the resting cerebral blood flow. Importantly, neuropathological changes in this mouse model occur in the absence of noticeable blood–brain barrier breakdown, SMC degeneration, or vessel stenosis. Instead, results of experiments strongly suggest that brain lesions arise from a combination of capillary reduction and arterial dysfunction.

While it has long been thought that CADASIL was caused by reduced Notch signalling, findings from various experiments do not support this assumption. The complete absence of Notch3 is not associated with CADASIL features in the mouse. Several CADASIL-associated NOTCH3 mutant alleles, including those located in the mutational hotspot EGFR2–5, are able to activate RBP-Jκ transcription at wild-type levels, in vitro in cultured cells, or in vivo in brain arteries. A few mutations, detected in about 5% of patients, are located in the EGFR10–11, which are required for DSL ligand binding. While these mutations result in a loss of Notch3 signalling and function, in vitro or in vivo, genotype–phenotype correlations suggest that they are associated with a milder clinical phenotype. Importantly, all mutations are associated with NOTCH3ECD accumulation and GOM deposits, and the data rather suggest a model that invokes novel pathogenic roles for mutant NOTCH3, potentially through sequestration of components essential for SMC function and viability within the aggregates.

### 4.2 Alagille syndrome

Alagille syndrome (ALGS) is an autosomal dominant multisystem disorder, which is one of the most common genetic causes of chronic liver disease in childhood. Patients typically present during the first 3 months of life with an intrahepatic cholestasis, due to a paucity of interlobular bile ducts, which may eventually lead to progressive liver failure. Other manifestations include a large range of congenital heart defects including cardiac outflow tract defects as the most frequent ones and skeletal, renal, and ophthalmological abnormalities.

The literature also documents intracranial and peripheral vessel abnormalities that are significant causes of morbidity and mortality, accounting for up to 34% of mortality in ALGS patients. JAG1 is the major gene involved in ALGS. The majority of JAG1 mutations are nonsense mutations or small deletions/insertions that lead to frameshift and premature termination codons, consistent with JAG1 haploinsufficiency being one major mechanism for ALGS. Heterozygous loss-of-function mutations in NOTCH2 have been identified in a small proportion of patients.

Only mice that are compound heterozygous carriers of a Jag1 null allele and a hypomorphic Notch2 allele or mice with a missense mutation of Jag1 are able to reproduce the major features of ALGS. Upon investigation of the role of Jag1 in SMC in mice, Iruela-Arispe’s lab recently provided important insight into the specific cellular events causing liver defects in ALGS. They made the unexpected observation that conditional deletion of Jag1 in SM22α-expressing cells of the developing portal vein mesenchyme was sufficient to recapitulate the hepatic defects of ALGS. Additional in vivo and in vitro studies using 3D co-cultures demonstrate that Jag1-dependent signalling in the portal vein mesenchyme is essential for the morphogenesis of biliary progenitor cells, expansion of the mesenchyme, and organization of mature bile ducts. These results underscore the critical and instructive role of the vasculature in the organization and differentiation of the liver parenchyma.

As discussed above, many of the cardiac outflow tract defects seen in patients with ALGS, including aortic arch patterning defects, pulmonary artery stenosis, and ventricular septal defects, are recapitulated in the mouse by Notch inhibition in the neural crest derivatives and can thus be attributed to cell autonomous defects of Notch signalling in these cells. The mechanistic origin of intracranial and peripheral vessel abnormalities remains to be elucidated.

### 4.3 Pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is characterized by structural remodelling of small pulmonary arteries (sPA) via the proliferation of SMC and endothelial cells, causing vessel wall thickening and luminal occlusion. This leads to elevation of pulmonary vascular resistance, right ventricular failure, and death. PAH can be associated with mutations in the gene encoding bone morphogenic protein receptor II (BMPR2) and various other risk factors. A recent study suggests that the Notch3/Hes5 pathway is crucial for the development of rodent PAH and possibly human PAH. Li et al. have shown that Notch3 and Hes5 are abnormally elevated in sPA SMCs in patients with non-familial PAH, and in both hypoxia- and monocrotaline-induced PAH in mice and rat, respectively. Importantly, the amount of Notch3 and Hes5 correlates with disease severity. In vitro studies argue that the Notch3/Hes5 pathway is a crucial mediator of sPASMC proliferation.
A mechanistic link between Notch3 signalling and PAH is supported by the observation that Notch3KO mice are protected from hypoxia-induced PAH and that PAH can be treated by a γ-secretase inhibitor that inhibits the Notch pathway.

5. Concluding remarks and future directions

Research on the contribution of Notch signalling in SMC has accelerated in the past few years. Studies using genetic inactivation strategies in the mouse have demonstrated that Notch signalling is functionally important at various stages of vessel wall maturation, all along the arterial tree. Notch signalling is reiteratively used in mural cell precursors and SMC to positively regulate differentiation, arterial specification, and maturation. Pericytes express Notch3, particularly in the brain capillaries. Recent work has uncovered a critical role for brain pericytes in the maturation and maintenance of the blood–brain barrier. Brain pericytes mediate capillary constriction but the physiological relevance of pericycle contractility is still controversial. Moreover, some pericytes are probably mesenchymal stem or progenitor cells. Whether Notch signalling is functionally important in pericytes is unknown to date.

The molecular machinery that operates upstream and downstream of Notch in SMC is highly context-dependent and yet incompletely understood. A few specific target genes have been identified, indicating the necessity for further studies. Furthermore, recent in vitro studies suggest that Notch Crosstalks with other pathways, such as the TGF-β signalling pathway to regulate mesenchymal stem cell differentiation to SMC or the PDGF signalling pathway to modulate SMC migration. Much remains to be learned in this area, and validating results of studies in cultured cells through in vivo studies is of critical importance.

The connection between Notch and the vasculature was first recognized when dominant mutations in NOTCH3 were found to be responsible for CADASIL, a prototypic SVD of the brain. Deciphering the contribution of the Notch pathway in the adult vessel in various pathological situations is undoubtedly an important future challenge. Given the emerging importance of the Notch3 pathway, we anticipate that mutations in components of this pathway may be involved in other vascular diseases.

Acknowledgements

We thank Stéphanie Debette for careful reading of the manuscript. We apologize to those in the field whose important work was not cited here due to space limitation.

Conflict of interest: none declared.

Funding

This work was supported by grants from Agence Nationale de la Recherche Française to A.J. (ANR Genopath 2008- R08174HS, ANR Genopath 2009- R09065HS; ANR blanc 2010-R11011HH). C.F. is a recipient of a fellowship from the French Ministry of Education and Research.

References


86. Peters N, Opheerk C, Zacherle S, Capell A, Gempel P, Dichgans M. CADASIL-associated Notch3 mutations have differential effects on ligand


