Arteriovenous malformations in hereditary haemorrhagic telangiectasia: looking beyond ALK1-NOTCH interactions

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Abstract

Hereditary haemorrhagic telangiectasia (HHT) is characterized by the development of arteriovenous malformations—enlarged shunts allowing arterial flow to bypass capillaries and enter directly into veins. HHT is caused by mutations in ALK1 or Endoglin; however, the majority of arteriovenous malformations are idiopathic and arise spontaneously. Idiopathic arteriovenous malformations differ from those due to loss of ALK1 in terms of both location and disease progression. Furthermore, while arteriovenous malformations in HHT and Alk1 knockout models have decreased NOTCH signalling, some idiopathic arteriovenous malformations have increased NOTCH signalling. The pathogenesis of these lesions also differs, with loss of ALK1 causing expansion of the shunt through proliferation, and NOTCH gain of function inducing initial shunt enlargement by cellular hypertrophy. Hence, we propose that idiopathic arteriovenous malformations are distinct from those of HHT. In this review, we explore the role of ALK1–NOTCH interactions in the development of arteriovenous malformations and examine a possible role of two signalling pathways downstream of ALK1, TMEM100 and IDs, in the development of arteriovenous malformations in HHT. A nuanced understanding of the precise molecular mechanisms underlying idiopathic and HHT-associated arteriovenous malformations will allow for development of targeted treatments for these lesions.

Keywords

NOTCH • Alk1 • Arteriovenous malformation • TMEM100 • Inhibitors of differentiation

1. Development of arteriovenous malformations in hereditary haemorrhagic telangiectasia is caused by loss of ALK1 or Endoglin

Arteriovenous malformations (AVMs)—direct connections between arteries and veins, bypassing the capillary bed—are characteristic features of hereditary haemorrhagic telangiectasia (HHT), also known as Osler–Weber–Rendu Syndrome. HHT has an estimated prevalence ranging from 3 to 29.6 cases per 100 000 people and causes a two-fold increase in mortality in patients younger than 60 years old.1–4 AVMs are thought to arise from enlargement of a capillary vessel, creating a shunt between an artery and a vein.5,6 The AVM and venous vessels downstream from it are directly exposed to high-pressure arterial flow, which can lead to vessel wall rupture, though if and when an individual AVM will rupture remains unpredictable.7 Secondary to ruptured AVMs, patients with HHT experience a range of symptoms from nose-bleeds (epistaxis) and telangiectasias of the skin and mucosa, to gastrointestinal bleeding, chronic anaemia, stroke, or brain abscesses, as well as AVMs in the lung and liver.8

HHT is caused by autosomal dominant (heterozygous) mutations in Endoglin (ENG) or activin receptor-like kinase (ALK)-1 also known as ACVRL1, both members of the BMP signalling pathway.9–11 Mutations in SMAD4, a downstream effector of this and other pathways, cause a combined syndrome of HHT and Juvenile Polyposis.12 Though mutations in this signalling pathway are known to cause AVMs, the pathogenesis of these lesions is not fully understood. The exact locations where AVMs arise are unpredictable, and the severity of HHT is highly variable, even within the same family.13,14 Additionally, AVMs grow and regress dynamically throughout the patients’ lives.15,16 The prevalence of pulmonary AVMs is greater with Endoglin mutations, while gastrointestinal bleeding is more common with ALK1 mutations.13 Yet, despite this genetic correlation, most AVMs are non-familial and
idiopathic, occurring in people without HHT-associated mutations. The variable presentation of HHT-related AVMs, along with the higher incidence of idiopathic AVMs, suggests that interactions with downstream factors must also be involved in the pathogenesis of these lesions.

While heterozygous deletion of \( \text{Alk1} \) in mice can model the development of AVMs in HHT, complete loss of this gene causes lethal failures in embryonic vascular development. \( \text{Alk1} \) heterozygous mice are viable, but develop AVMs and secondary bleeding reminiscent of HHT, including the formation of cutaneous and mucosal telangiectasias, haemorrhages in internal organs, and bleeding from the gastrointestinal tract. In \( \text{Alk1} \) homozygous mutants, arteriovenous shunts begin to form between the dorsal aorta and the cardinal vein at E8.5.19 By E9.5, they have well-established shunting between the dorsal aorta and cardinal vein, dilation of vessels and the heart tube, a lack of mature blood vessels in the yolk sac, severe loss of capillaries in the embryo proper, and growth retardation, and die between E10.5 and 11.5.19–21 Localized deletion of \( \text{Alk1} \) specifically in endothelial cells results in vessel dilation and AVM formation in vascular beds lacking \( \text{Alk1} \).24,25 Endothelial-specific inducible deletion of \( \text{Alk1} \) in neonatal pups (P6) is lethal within \( \sim 48 \) h due to haemorrhages in the lung capillaries.23 The capillary plexuses in the retinas of these mice have enlarged vessels and hyperbranching, with increased endothelial cell proliferation and filopodia formation.

The equivalent zebrafish mutant, known as violet beauregard (vbg), develops AVMs as part of a phenotype that is related to, but distinct from, murine \( \text{Alk1} \) knockout mutants. In vbg fish, the patterning of the vascular tree is normal; however, circulation becomes largely restricted to a loop of dilated cranial vessels by \( 2 \) days post-fertilization (dpf).24,25 Increased endothelial cell number and dilation of the caudal division of the internal carotid artery result in increased blood flow through downstream vessels, which subsequently retain normally transient vascular connections, resulting in AVMs.5,24,25 In the absence of blood flow, the AVM does not form, offering a biomechanical explanation for AVM localization in this model.5 vbg fish secondarily develop oedema in the head, yolk sac, and pericardium, and die between 7 and 10 dpf.25 Mouse models with Endoglin mutations have closely similar but less severe phenotypes to \( \text{Alk1} \) mutants. Deletion of Endoglin is lethal by E10.5–11.5.26,27 As with \( \text{Alk1} \) mutants, before E8.5, Endoglin knockout mice are indistinguishable from their littermates and undergo vasculogenesis normally; however, from E9.0 onward, anastomoses are present between the dorsal aorta and cardinal vein, but with less dilation than in \( \text{Alk1} \) knockout embryos.20 By E9.5, distinct vessels fail to form in the yolk sac, although some dilated vascular channels are present, and the yolk sac displays clusters of red blood cells but is otherwise anemic.24,27 Because Endoglin functions as a co-receptor to enhance ALK1 signalling,5,28 impaired, but not completely absent ALK1 signalling is present, which may explain the Endoglin mutants’ less severe phenotype.

2. Activation of ALK1 signalling during vascular development

Since the development of AVMs is an error in vascular remodelling, understanding the onset and role of ALK1 signalling in vascular development is essential. ALK1 is a transmembrane receptor that is activated by the circulating factors BMP9 and BMP10.29–31 TGF \( \beta \)1 is also able to weakly stimulate ALK1 signalling, but this has been demonstrated only to occur in immortalized, but not primary, endothelial cells in vitro, so likely has little biological effect in vivo.32 Upon ligand binding, the cytosolic proteins SMAD 1/5/8 are phosphorylated by a complex composed of ALK1, Endoglin, and a Type II Receptor.11,31,33 Phosphorylated SMADs 1/5/8 then complex with SMAD4 and move into the nucleus, where they bind to BMP response elements to modify gene transcription.31 \( \text{Alk1} \) and Bmp10 are first expressed at E8.5,24,25,26 (Figure 1), while Bmp9 expression begins 2 days later, at E10.5.20 Before the onset of \( \text{Alk1} \) expression, a primitive vascular plexus has already formed in the yolk sac surrounding the embryo and is connected to the cardiac circulation within the embryo proper.33 From E8.5 to E9.5, the embryonic vessels undergo an initial remodelling into a hierarchical structure of arteries, veins, and capillaries, essential for survival and continued development of the embryo.33 \( \text{Alk1} \) and Bmp10, but not Bmp9, are expressed during this critical time for vascular remodelling, from E8.5 to E9.5, when phenotypes begin to arise in \( \text{Alk1} \) and Endoglin knockout embryos (Figure 1). Supporting the role of BMP10 in vascular remodelling, Bmp10 knockout mice also demonstrate failed vascular remodelling in the yolk sac, fusions between the dorsal aorta and cardinal vein, and die at E9.5.29,36 The role of BMP10 must be interpreted cautiously, however, since these mice also have a primary heart defect that may result in altered blood flow and contribute to the phenotype of impaired vascular remodelling.36

Vascular remodelling is a flow-dependent process, and \( \text{Alk1} \) expression and SMAD 1/5 phosphorylation can be induced by flow.26,27 In zebrafish, the onset of blood flow induces an up-regulation of \( \text{Alk1} \) expression in arteries, with the greatest expression in arteries closest to the heart where the forces from blood flow are strongest.37 The mouse heart begins to beat at E8.0, but initially only blood plasma is circulating (Figure 1).35 Beginning at E8.5, erythroblasts are released into circulation, which increases the blood viscosity and shear stress (a mechanical force from blood flow) against the vessel wall.35 This increase in shear stress is necessary for vascular remodelling to occur.35 Since the presence of both flow and ALK1 is required for vascular remodelling, this raises the possibility that flow may be mediating ALK1 signalling during early vascular development. In support of this, endothelial cells exposed to flow in vitro show strong increases in SMAD1/5 phosphorylation, compared with static controls, though which BMP receptor is responsible for this effect has yet to be elucidated.37 The important role of flow in vascular development points to the possibility that impaired flow-induced signalling may be playing a role in the development and/or localization of AVMs.

3. NOTCH signalling is up-regulated in idiopathic but not in HHT-related AVMs

Arteriovenous shunts occur in mutants for components of the NOTCH signalling pathway; hence, a role for NOTCH signalling in AVM pathogenesis was proposed. The NOTCH pathway is an intercellular signalling pathway: both the receptor and the ligand are membrane bound on adjacent cells. Upon ligand–receptor interaction, NOTCH is cleaved and the Notch Intercellular Domain (NICD) is released into the cytoplasm. NICD translocates into the nucleus where it associates with the DNA-binding protein RBPJ to initiate transcription of its downstream targets.38 Ablation of Notch in mouse39 or zebrafish40 results in fusions of the dorsal aorta and cardinal vein. Ablation of Rbpj...
also results in defects in arterial specification and AVM development.\textsuperscript{39} Postnatal deletion of endothelial Rbpj in mice causes AVMs in the brain, intestine, and heart by day 14.\textsuperscript{41} Partial loss of the NOTCH ligand Dll4 also results in embryonic lethality with severe vascular defects reminiscent of those seen in mouse embryos lacking NOTCH receptors.\textsuperscript{42}

Although ablation of NOTCH signalling results in AVMs in mice, investigation of this pathway in human patients with idiopathic AVMs found that increased, rather than decreased, NOTCH signalling is present in a number of idiopathic AVMs.\textsuperscript{43–45} This was associated with elevated NOTCH1,\textsuperscript{45} NOTCH4,\textsuperscript{44} and NICD protein levels, as well as increased expression of both NOTCH ligands (JAG1, DLL4)\textsuperscript{45} and downstream mediators of NOTCH signalling (HES1). In the adult mouse, expression of a constitutively active Notch4 (Tie2-tTA; TRE-int3) results in AVMs in the liver, uterus, skin, and brain.\textsuperscript{46–49} Similar to Notch4, endothelial cell-specific, constitutively active Notch1 results in vascular defects and AVM formation.\textsuperscript{50} AVMs formed in these gain-of-function mice are dynamic and can reduce in size if the expression of the constitutively active Notch4 is turned off.\textsuperscript{46,51}

It has been hypothesized that loss of arterial–venous markers is present in AVMs.\textsuperscript{46} This mechanism explains why both gain-of-function and loss-of-function in Notch result in fusion of arteries and veins, since both would result in abnormal arterial and venous specification. Both mice and zebrafish with impaired Notch pathway signalling show loss of arterial marker expression in their vasculature and AVM formation in some cases.\textsuperscript{39,40,42,52,53} In contrast, vessels in Notch gain-of-function and Dll4 overexpression models are characterized by ectopic venous expression of Ephrinb2, increased smooth muscle cell coverage, and AVM formation.\textsuperscript{46,51,54,55}

Abnormal connections between arteries and veins are a possible source of AVMs; however, the exact mechanism by which loss of arteriovenous identity leads to vessel fusion is unclear. During development, between E8.5 and E9.5, endothelial cells migrate from the dorsal aorta to the cardinal vein.\textsuperscript{56} In the constitutively active Notch mutant mouse, the total number of endothelial cells in the dorsal aorta and cardinal vein is the same between control and mutant mice, but the distribution of cells between the two vessels is altered such that the dorsal aorta has more endothelial cells than normal.\textsuperscript{57} Impaired identification and migration of venous endothelial cells in the constitutively active Notch mutant would explain the increased number of endothelial cells

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**Figure 1** Alk1 knockout phenotype appears during vascular remodelling, concurrent with onset of blood flow and expression of Alk1 and Bmp10. The initial capillary plexus of the mouse embryo yolk sac forms between embryonic day (E) 7.5 and E8.5. The heart begins to beat at E8.0, but initially only plasma is flowing through the capillaries, while the erythroblasts are confined to the blood islands of the yolk sac. At E8.5, erythroblasts are released into circulation, marking the beginning of true blood flow. At the same time, vascular remodelling is initiated, and Bmp10 and Alk1 begin to be expressed. Vascular remodelling is completed by E10.5, at which point Bmp9 begins to be expressed. The phenotype of Alk1 knockout mice arises from E8.5 to E9.5 as a failure of vascular remodelling.
in the dorsal aorta of these mice. A similar process has not been reported for AVMs that form later in development or postnatally. Rather, when AVF formation was induced postnatally in the inducible constitutively active Notch mutant mouse, no significant cell migration was reported as the vessel expanded.

Besides NOTCH signalling components, AVMs have also been reported in transcription factors upstream of NOTCH. Mice with deletions of Foxc (Forkhead) transcription factors, Foxc1+/−; Foxc2−/−, have AVMs and their endothelial cells fail to express Dll4. These mice show reduced expression of other arterial markers such as Notch4, Dll4 and EphrinB2. Though FOXC1 and FOXC2 are required for Dll4 expression, deletion of the Forkhead Binding Element (FBE) on the Dll4 promoter does not attenuate Dll4 expression by FOXC.58 We previously showed that NICD1 and NICD4 can still activate Dll4 promoters that lack the FBE but contain RBPJ binding sites.59 FOXC2 and NICD can act synergistically on the Hey2 promoter60 and thus it is possible that a similar mechanism is present with the Dll4 promoter where, in the absence of the FBE, FOXC2 interacts with NICD to bind the RBPJ binding sites and induce Dll4 expression.

ALK1 and NOTCH signalling pathways interact and can partially compensate for each other to promote normal vascular development. In a neonatal mouse retina model, either injection of the ligand trap ALK1-Fc, which inhibits ALK1 signalling by sequestering BMP9/10, or inhibition of NOTCH signalling results in a hyperfused and hyper-sprouting vascular plexus.61 NOTCH inhibition by DAPT in the retinal vasculature causes hypopersprouting, which can be counteracted by injection of the ALK1 ligand BMP9.62 Moreover, activated SMADs downstream of ALK1 and Endoglin co-immunoprecipitate with NICD, demonstrating that there is a physical interaction between these signalling pathways.62 Not only do the signalling components of these pathways bind to one another, but SMAD1/5/8 binding sites are also present in the regulatory region of many NOTCH target genes, including HEY1, HEY2, and HES1.63 In zebrafish embryos, inhibition of Notch signalling using a gamma-secretase inhibitor (LY411575) does not prevent expression of the Notch target gene, hey2; however, concurrent inhibition both of Notch and Smad signalling does reduce hey2 expression significantly and abolishes efnb2a expression in the dorsal aorta.24 In these models, changes in gene expression were context-dependent; the specific changes that were observed in the dorsal aorta were not replicated in the cranial arteries and vice versa.24 This raises the possibility that location-specific modifiers of NOTCH/BMP signalling are present in the endothelial cells of various vascular beds.

4. AVMs that arise due to HHT differ from idiopathic AVMs

Although there is strong evidence of an excess of NOTCH signalling in some idiopathic AVMs, the role of NOTCH in HHT-associated AVMs is less clear. While loss of alk1 up-regulates dll4 expression in zebrafish,24 an increase in NOTCH activation has yet to be demonstrated in HHT patients. In fact, NOTCH1 receptor is down-regulated in endothelial cells isolated from mice with endothelial-specific deletion of alk1 (Acrvl1-iKOe), and NOTCH ligand Jag1 expression is markedly reduced in the retinal arteries in these mice.25 In line with these observations, in a fibrin gel assay JAG1-induced NOTCH signalling was able to partially rescue the hypopersprouting phenotype caused by loss of ALK1 signalling with ALK1-Fc.51 Hence, NOTCH signalling appears to be differentially regulated in HHT-associated AVMs caused by mutations in ALK1, compared with idiopathic AVMs.

Arteriovenous shunts enlarge by different mechanisms in HHT and NOTCH-associated AVMs. In HHT patients, AVMs grow through endothelial cell proliferation.15 This increased proliferation allows for enlargement of the capillary shunt into an AVM. Studies of both zebrafish and mouse models of alk1-deficient AVMs similarly show increased endothelial cell number, consistent with the role of ALK1 in cellular quiescence.5,23,25 Although the zebrafish studies did not conclusively demonstrate whether this increase in endothelial cell number occurred through changes in proliferation, migration, or apoptosis, work in Alk1-deficient mouse models confirms the role of increased endothelial cell proliferation during AVF formation.23

In contrast to HHT-related AVMs, NOTCH gain-of-function models of AVMs do not show any increase in endothelial cell proliferation but rather display endothelial cell hypertrophy.69 One group has noted that two- to three-fold increases in shunt diameter during the first 48 h of AVF formation were accompanied by increases in the area of individual endothelial cells, rather than an increase in cell number in mice with endothelial-specific, constitutively active Notch4.49 A similar increase in cell area is observed when epithelial cells are transplanted with NICD.64 In endothelial cells, overexpression of NICD leads to changes in cell shape and decreases in cortical F-actin as well as functional β-catenin and VE-cadherin.65 This decreased expression of adhesions junction proteins could explain the enlarged cell surface area phenotype and increased vessel diameter observed in NOTCH gain-of-function mutants. In rats with Notch1 over-activation, however, proliferating endothelial cells were found in brain AVMs at 8 or 29 days after AVF formation.65 This implies that the initial AVM pathogenesis in NOTCH gain-of-function occurs through cell hypertrophy, whereas later remodelling of the vessel may indeed involve proliferation of endothelial cells.

Idiopathic and HHT-associated AVMs also differ in location and progression. The majority of brain AVMs have an idiopathic origin,66 while the vast majority of pulmonary AVMs are due to HHT.77 As previously mentioned, AVMs from HHT patients form and regress throughout the person’s life.15 Regression of idiopathic AVMs is rare.16 Furthermore, the risk of rupture in intracranial AVMs is much higher for idiopathic AVMs than for HHT patients.68 Given the numerous differences in mechanism and presentation of AVMs between NOTCH gain-of-function and HHT models, we suggest that these should be regarded as two independent modes by which AVMs develop.

5. Exploring alternate molecular players in the development of AVMs in HHT

5.1 TMEM100 and calcium signalling

Of all the ALK1 downstream targets, ablation of Transmembrane Protein 100 (Tmem100) most closely resembles the phenotype of alk1 mutants, including the development of AVMs. Furthermore, Tmem100 expression is down-regulated in alk1 mutants.69 Knockout models of both alk1 and Tmem100 result in heart defects, a failure of vascular remodelling, and abnormal dilation and narrowing of the dorsal aorta, as well as detachment of the endodermal and mesodermal layers in the yolk sac.69 Though the phenotype of Tmem100 knockouts is very similar to alk1 knockouts, they are not identical. When
Tmem100 is ablated postnatally, AVMs form in the lung and intestines, as with the inducible knockout (iKO)-Alk1 mutants. Yet, differently from the iKO-Alk1 mutants, which present AVMs that develop in skin following tissue injury, the iKO-Tmem100 mutants are immune to injury-induced AVM development. Thus, while Tmem100 signalling may contribute to the Alk1 phenotype, it is not the sole contributor.

To date, very little is known about the functional role of Tmem100 other than that it is up-regulated when endothelial cells are exposed to the ALK1 ligand BMP9. Tmem100 has been shown to modulate the interaction between Transient Receptor Potential Channel Vanilloid 1 (TRPV1) and Ankyrin (TRPA1) and loosen their association to promote calcium influx into the cell. This association between Tmem100 and TRPs has currently only been investigated in neurons; however, both TRPV1 and TRPA1 are expressed on endothelial cells. Thus, it would be useful to explore a possible interaction of these proteins in endothelial cells. In support of their potential role in endothelial cells, TRPV1 has been shown to regulate vascular tone. A related family member, TRPV4, enhances arteriogenesis in a femoral artery ligation model. As such, TRPs are understood to play a role in vascular remodelling. This raises the possibility that defective calcium signalling could be involved in the development of AVMs when ALK1 is ablated and is Tmem100 down-regulated. In fact, defective calcium signalling upstream of NFATc1 was observed in cells cultured from whole hearts of Tmem100-null embryos.

Calcium concentration gradients regulate NOTCH activation in endothelial cells. NOTCH is a heterodimeric protein in which the association of the two subunits of the receptors is calcium dependent. In the presence of low extracellular calcium, the subunits of NOTCH dissociate, leading to an increased level of NICD within cells. Hence, cell surface calcium depletion activates NOTCH signalling independently of ligand. If ALK1 and/or Tmem100 affect calcium levels, then it is reasonable to postulate that this would affect NOTCH signalling as well. Indeed, in Tmem100 knockout embryos, a decrease in NOTCH activation is present. Given that a decrease in NOTCH signalling is known to lead to increased endothelial cell proliferation, this could, in part, explain the cell proliferation and vessel enlargement in HHT-related AVMs. It would therefore be informative to investigate calcium signalling in Alk1 and Tmem100 mutants, and determine whether altered calcium gradients would have an impact on NOTCH activation, excessive cellular proliferation, and development of AVMs.

5.2 ID1/3 and cell fate

The inhibitors of DNA-binding/differentiation proteins (IDs) are downstream targets of ALK1 signalling and are regarded to inhibit differentiation and promote cell cycle progression. As such, they could represent possible candidates to explain the aberrant proliferation observed during AVM pathogenesis in HHT. In support of this, mice with a combined loss of both id1 and id3 have normal vasculogenesis at E8.5, but display cranial haemorrhage secondary to the formation of an anastomosing network of dilated capillaries in the brain. Though no group has yet conclusively demonstrated that haemorrhages in these mice arise from ruptured AVMs, the vascular malformations exhibited in this model suggest that ID1/3 may well play a role in AVM formation. Besides regulating cell proliferation, IDs downstream of SMAD 1/5 signalling promote stalk cell phenotype during vascular sprouting. Loss of SMAD/ID signalling results in excessive tip cell formation and impaired stalk cell proliferation, which may contribute to the vessel fusion and dilatation observed in id1/3 knockout mice.

IDs do not bind to DNA themselves, but instead heterodimerize with basic helix-loop-helix transcription factors (bHLH) to inhibit their binding to the DNA. IDs bind to both ubiquitously expressed and cell-type-specific bHLH proteins. The most well-characterized binding partners of IDs are the E-proteins (E2A, E2-2, and HEB). The E2a knockout mice have a neonatal lethality of 50% of the litter, with surviving pups developing T-cell lymphomas. The cause of the large neonatal lethality has never been reported. Ablation of HEB or E2-2 also results in neo-natal lethality, but, again, the cause of lethality for these mice is unknown. Post-mortem examination of these pups to determine whether AVMs and haemorrhaging are present will begin to clarify the role of IDs and bHLH proteins in AVM development.

Although the E-proteins are the most common binding partners of IDs, IDs can bind other cell-specific bHLH proteins that may be important for the development of AVMs. ID1/2/3 proteins have been shown to directly interact with HES1 and with HEY1, which are known downstream targets of ALK1 and NOTCH signalling, and are involved in arterial cell fate. In particular, HES1 binds to a site on its own promoter to prevent transcription, resulting in HES1 autoinhibition. IDs have been described to inhibit HES1 interaction and prevent the negative autoregulatory effect on its own promoter, without affecting the regulation of other HES1 target genes. Therefore, when IDs are down-regulated, HES1 autoinhibition is permitted, and HES1 expression is decreased. Endothelial-specific Hes1/5 double mutant embryos show defective vascular remodelling in the brain and partial loss of arterial identity in endothelial cells. Thus, it would be useful to explore whether the vascular defects observed in id1/3 mutants and in HHT patients are related to HES1 misregulation.

6. Concluding statements

In this review, we focused on the distinction between HHT-related and idiopathic AVMs. Whereas, in HHT-related AVMs, proliferation of endothelial cells appears to be the major cause of capillary enlargement into a shunt, it is not clear that significant proliferation is present in NOTCH-associated idiopathic AVMs (Figure 2). These phenotypic differences are reflected at the molecular level. HHT-related AVMs are caused by genetic mutations in members belonging to the ALK1 signalling pathway. ALK1 signalling has been described to interact with the NOTCH pathway and to share common targets; however, in animal models of HHT-related AVMs, NOTCH is down-regulated, while in some patients affected by idiopathic AVMs NOTCH is up-regulated. A thorough characterization of NOTCH signalling and ALK1 downstream targets in AVMs sampled from human HHT patients would permit a more detailed comparison to idiopathic AVMs. Given the wide range of mutations likely to be present in idiopathic AVMs, identification of those in which defective NOTCH is present could allow development of more individualized treatments. Two targets of ALK1 signalling have received particular attention during the last years: Tmem100 and ID1/3, respectively (Figure 2). Loss of Tmem100 downstream of ALK1 might be involved in down-regulation of NOTCH signalling in HHT-related AVMs. Similarly, decreased levels of ID1/3 may be responsible for deregulation of bHLH proteins/NOTCH target genes with specific roles in maintaining endothelial cell identity. Further study of ALK1-specific downstream targets and validation of these targets in AVMs from HHT patients will offer additional insight into differences in the pathogenesis of these lesions.
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Figure 2 HHT-related AVMs occur through endothelial cell proliferation, while idiopathic AVMs may occur through endothelial cell hypertrophy. AVMs arise through the enlargement of a capillary into a direct shunt between the artery and vein. In HHT, we propose that loss of ALK1 may lead to decreased NOTCH signalling through impaired TMEM100 and ID1/3 expression and functioning. A decrease in NOTCH signalling permits cellular proliferation and hence enlargement of the capillary into the AVM. In some idiopathic AVMs, there is elevated NOTCH signalling, which leads to cellular hypertrophy and enlargement of the capillary into an AVM.


