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REVIEW

Endothelial signaling and the molecular basis of arteriovenous malformation

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Abstract Arteriovenous malformations occur when abnormalities of vascular patterning result in the flow of blood from arteries to veins without an intervening capillary bed. Recent work has revealed the importance of the Notch and TGF-β signaling pathways in vascular patterning. Specifically, Notch signaling has an increasingly apparent role in arterial specification and suppression of branching, whereas TGF-β is implicated in vascular smooth muscle development and remodeling under angiogenic stimuli. These physiologic roles, consequently, have implicated both pathways in the pathogenesis of arteriovenous malformation. In this review, we summarize the studies of endothelial signaling that contribute to arteriovenous malformation and the roles of genes implicated in their pathogenesis. We further discuss how endothelial signaling may contribute to vascular smooth muscle development and how knowledge of signaling pathways may provide us targets for medical therapy in these vascular lesions.

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Introduction

Arteriovenous malformations (AVMs) are vascular developmental anomalies wherein the blood flow through an artery or arteriole is directed into a vein or venule without an intervening capillary bed. The existence of a shunt from the high-pressure arterial system into the normally low-pressure venous system exposes these vascular lesions to the risk of hemorrhagic rupture. AVMs can be found anywhere in the body, including the brain and lungs, where a spontaneous bleed can result in grave consequences.

Patients who present with AVMs most commonly do so between 30 and 40 years of age [1–4]. While genetically normal individuals may present with this pathology, AVMs are particularly common in patients with the disease hereditary hemorrhagic telangiectasia (HHT). Up to 35 % of individuals with HHT harbor at least one AVM, although multiple AVMs occur in these patients [5–7]. The sporadic and hereditary nature of AVM suggests that both inherited signaling abnormalities and environmental exposures are involved in their pathogenesis.

Arteriovenous malformations can spontaneously disappear or recur after treatment with surgery, radiosurgery or endovascular therapy. This plastic nature indicates that such lesions are actively remodeling [1]. Therefore, they must be dependent on continuous abnormal cell signaling for their maintenance and elaboration. In particular, the signaling defects of AVMs likely culminate in failures of appropriate arterial and venous fate specification as well as microvascular branching and remodeling.

Table 1 Notch pathway mutants that exhibit AVMs

References	Gene	Mod.	Sp.	Description (time of embryonic lethality, if applicable)
Krebs et al. [13]	*Notch1*	KO	Mm	(E9.5) Narrow, collapsed vessels with poor remodeling of primitive vasculature
	Notch4	KO	Mm	Moderate changes suggestive of a functional overlap with Notch1
	Notch1 and *Notch4*	KO	Mm	(E9.5) Changes similar to, but more pronounced than Notch1 KO
Lawson et al. [20]	*SuH, mib*	DN	Dr	Abnormal arterial markers, ectopic venous markers, and AV shunts
Krebs et al. [16]	*Notch1*	CA	Mm	Defective remodeling, fusion of dorsal aorta and common cardinal vein, similar to RBPJ-KO and ephrinB2/EphB4-KO mice
Uyttendaele et al. [22]	*Notch4*	EC–CA	Mm	(E10) Diminished branching, particularly in the brain, with associated tissue necrosis, dilated vasculature without appropriate microvasculature
Carlson et al. [17]	*Notch1* and *Notch4*	EC–CA	Mm	Adult activation induced lethal AVMs that can be suppressed with target suppression
Murphy et al. [23]	*Notch4*	CA	Mm	Activation at birth induced AVMs, decreased capillary branching, and hemorrhage
Murphy et al. [18]	*Notch4*	CA	Mm	Intravital repression of active Notch4 caused AVM regression, return of capillary flow and the re-establishment of appropriate vessel identity
Gale et al. [26]	*Dll4*	Het	Mm	(E9.5) Fusion of the arterial and venous systems decreased body size, diffuse tissue necrosis
Duarte et al. [25]	*Dll4*	KO	Mm	(E9.5) Poor arteriogenesis, great vessel atresia, absence of arterial markers, ubiquitous venous markers
Krebs et al. [27]	*Dll4*	KO	Mm	(E9.5) Fistulization between dorsal aortae and posterior cardinal veins
Benedito et al. [31]	*Dll4*	KO	Mm	Diffusely reduced arterial caliber. Abnormal EC morphology with dysfunctional basement membrane. Increased EC migration and proliferation contributing to AVM formation
Trindade et al. [32]	*Dll4*	OE	Mm	(E9.0) Excessive arterialization with VSMC and basal laminae, ectopic arterial markers

SuH Suppressor of Hairless, *mib* mindbomb, *EC* endothelial cell, *KO* knockout, *DN* dominant negative, *CA* constitutive activity, *Het* heterozygous, *OE* overexpressed, *Mm Mus musculus*, *Dr Danio rerio*

In this review, we outline the abnormalities in endothelial cell (EC) signaling associated with AVM that have been used to create animal models or observed in primary human samples, with a focus on the Notch and TGF-β pathways.

Notch signaling

Notch signaling is an evolutionarily conserved pathway that has been implicated in cell fate specification in many tissues, wherein signals are transmitted by direct cell-to-cell interaction. Notch family cell-surface receptors bind their cell-surface ligands—including Delta-Like Ligands (DLL) and JAGGED (JAG)—on neighboring cells. Binding induces conformational change in NOTCH receptors. This leads to the cleavage of the Notch intracellular domain (NICD) from the full-length receptor. NICD is then translocated to the nucleus, where it binds RBP-J (also known as CSL, CBF1 and *Suppressor of hairless* (SUH)) and potentiates transcription of effector molecules, including HES and HEY transcription factors [8–10]. Notch pathway alterations have an established role in the human diseases CADASIL (mutant *NOTCH3*) and Alagille syndrome (mutant *JAGGED1*) in which vascular smooth muscle and biliary epithelial cell development, among other processes, are compromised [11, 12]. In the endothelium, Notch signaling has a particularly important role in the control of arterial fate specification and vascular patterning [10]. Both of these functions are involved in the pathogenesis of AVMs.

Interestingly, models that induce both constitutive activity of Notch signaling as well as complete or partial interruption of the pathway have proven sufficient to induce AVMs in animal models. The former result in AVMs with increased arterial diameter, whereas the latter produce AVMs with decreased growth. Both types illustrate functions of Notch in the vasculature, but models with constitutively active Notch signaling more closely mimic AVMs in humans [13–18]. We will discuss NOTCH1 and NOTCH4 receptors and the ligand DLL4 in the context of AVM (Table 1).

Notch1 and Notch4 receptors

Notch1 homozygous deletion leads to embryonic lethality in mice at embryonic day 9.5 (E9.5) with defects in vascular patterning that include diffuse vascular underdevelopment, hypersprouting, and poor remodeling of the primitive vascular plexus [19]. These mice exhibit direct anastomoses between arterial and venous circulations within the embryo, a feature that is characteristic of AVM [13, 16].

Table 2 TGF-β pathway mutants that exhibit AVMs

References	Gene	Mod.	Sp.	Description (time of embryonic lethality, if applicable)
Johnson et al. [60]	*ACVRL1*	N/A	Hs	Responsibility of *ACVRL1* in HHT2
Oh et al. [54]	*acvrl1*	KO	Mm	(Mid-gestation) fusion of capillary plexi, vascular dilation
Urness et al. [74]	*Acvrl1*	KO	Mm	(E9.5–11.5) shunting from aortic arches to cardinal veins, poor VSMC recruitment, reduced arterial markers, hemogenic venous endothelium
Srinivasan et al. [65]	*Acvrl1*	Het	Mm	Survive to adulthood, age-dependent incidence of AVMs, unpredictable organ involvement
Seki et al. [67]	*Acvrl1*	Het + LacZ	Mm	Demonstrates arterial specificity of ALK1, notably in developing/remodeling vessels
Park et al. [36]	*Acvrl1*	EC–KO	Mm	(E18.5) diminished arterial wall development, irregular VSMC, shunts
Hao et al. [78]	*Acvrl1* + *Eng*	Het + VEGF	Mm	Stimulation with VEGF and vasodilator produces neurovascular dysplasia, EC proliferation, greater sensitivity of Endoglin mutants
Park et al. [75]	*Acvrl1*	KO	Mm	Adult deactivation sufficient for organ AVMs. Skin lesions required injury also
Walker et al. [112]	*Acvrl1*	KO + VEGF	Mm	Local deactivation of ALK1 and VEGF stimulation produces AVMs and abnormal arterial and venous markers
Corti et al. [68]	*acvrl1*	KO	Dr	AVMs secondary to stabilization of normally transient shunts, enlarged arterial caliber
Milton et al. [76]	*Acvrl1*	SM–KO	Mm	Age-dependent brain and spinal cord AVMs leading to paralysis in 10–15 weeks of life
Larrivée et al. [83]	*Acvrl1*	Fc-ALK1	Mm	Soluble ALK1 receptor, blockade of BMP9, dense dilated plexi with poor remodeling
McAllister et al. [59]	*ENG*	N/A	Hs	Responsibility of *ENG* in human HHT1
Li et al. [91]	*Eng*	KO	Mm	(E11.5) poor VSMC development, atretic major vessels, poor remodeling
Bourdeau et al. [64]	*Eng*	Het	Mm	Develop clinical HHT over months; telangiectases, hemorrhage, and poor weight gain
		KO	Mm	(E10) diffuse hemorrhage, poor remodeling, vessel rupture
Jonker and Arthur [90]	*Eng*	LacZ	Mm	Expression begins E6.5, widespread in endothelium
Satomi et al. [94]	*Eng*	Het	Mm	Cerebral AVMs develop in endoglin heterozygotes, not homozygous-wild type
Torsney et al. [95]	*Eng*	Het	Mm	6 % of animals with large AVM, 25 % lethal, 44 % with hemorrhage, infertility, symptomatic at 36 weeks, many uterine AVMs
Sorensen et al. [107]	*Eng*	KO	Mm	Hemogenic venous endothelium, endoglin null does not change in ephrinB2
Mahmoud et al. [92]	*Eng*	KO	Mm	Poor remodeling of retinal plexi, endothelial proliferation, decreased capillary investment of gel matrix implant
Choi et al. [97]	*Eng* + *Acvrl1*	KO	Mm	Local knockout of endoglin with VEGF is more dysplastic than the same for ALK1
Cole et al. [61]	*HHT3*	N/A	Hs	Identification of locus mutated in HHT3
Bayrak-Toydemir et al. [58]	*HHT4*	N/A	Hs	Identification of locus mutated in HHT4
Gallione et al. [62, 63]	*SMAD4*	N/A	Hs	Identification of *SMAD4* as gene responsible for JP–HHT

EC endothelial cell, *KO* knockout, *LoF* loss of function, *CA* constitutive activity, *Het* heterozygous, *OE* overexpressed, *Hs Homo sapiens*, *Mm Mus musculus*, *Dr Danio rerio*

Mice with *Notch4* homozygous deletion, however, develop without abnormality, survive through adulthood and are fertile. *Notch1/Notch4* double-null mutants also exhibit lethality at E9.5, but display a more severe phenotype than mice with only *Notch1* gene deletion. The amplification of defects in combined-null mutants suggests overlap in the developmental responsibilities of *Notch1* and *Notch4* [13] (Table 2).

Complete interruption of Notch signaling in zebrafish with dominant negative Notch downstream mediators induced the downregulation of arterial markers and upregulation of venous markers, as well as shunting of blood from the arterial to venous system [20]. Interestingly, the alteration of EC identity was observed prior to the onset of blood flow, which suggested that EC fate specification is a primary process mediated by Notch signaling [20].

The discovery that Notch determines EC fate led to further study with the aid of the constitutively active *Notch4*-based oncogene *int3* [21]. In light of the fact that *Notch1* and *Notch4* have overlapping effects in ECs, *int3* has been

employed to assay the effects of activated Notch signaling. Mice that expressed *int3* in the endothelium to stimulate Notch signaling exhibited embryonic lethality at E10 with diffuse AVMs [22]. Early postnatal [23] and adult expression [17] of *int3* induced AVMs, followed by lethality, within weeks. As opposed to Notch knockout models, wherein venous markers were induced in natively arterial cells, constitutive Notch activation produced vascular enlargement, increased expression of arterial marker EPHRINB2 in natively venous endothelial cells and increased recruitment of vascular smooth muscle cells (VSMCs). This was associated with increased endogenous Notch activity (i.e., Notch activity via *int3*-independent signaling) through *Hey1*, endogenous *Notch4* and *Dll4* [17].

The neurovasculature in this animal model was particularly notable. The presence of constitutively active *Notch4* signaling induced AVMs in mouse brains accompanied by neurologic deficits and hemorrhage. Brains were specifically noted to harbor dilated vessels with poor tissue capillary infiltration and associated tissue necrosis, reminiscent of human cerebral AVM [4, 22–24]. In one study, hemorrhage was observed in all transgenic mice and was always secondary to the presence of an AVM [23].

Arteriovenous malformations have also been observed secondary to the overexpression of *Notch1*-ICD. These AVMs demonstrated dilatation unlike hypoplastic AVMs in *Notch1* knockout mice. Systemic AVMs were observed in these animals as early as E9.5 and closely mirrored AVMs observed in *EphrinB2* and *EphB4* knockout mice [16].

Postdevelopmental alterations of Notch signaling cause remodeling of the vasculature and may also result in AVMs. *Int3* activation in the neonatal and adult mouse induced AVMs with subsequent hemorrhage, neurologic sequelae, and ultimately death. Furthermore, regression of AVMs was achieved upon the suppression of *int3*- [17, 23]. AVM regression was observed with in vivo microscopy that revealed the restoration of capillaries, the regression of shunt enlargement and re-establishment of arterial and venous endothelial markers. Interestingly, marker restoration and shunt regression occurred without a change in endothelial cell number. The observed change in the arterial/venous marker expression, without a change in their number, suggests that Notch signaling is sufficient for cell fate specification [18].

Decreased branching, consistent with well-characterized anti-angiogenic functions of Notch signaling, is also observed in animals with endothelial expression of *int3* [22]. This may contribute to AVM development due to reduced size and growth of the capillary bed, along with vascular enlargement, which potentiate direct communication between arteries and veins.

While it is true that both Notch pathway inhibition and activation can induce vascular shunting, AVMs from patients typically demonstrate NOTCH1 activation as suggested by histological features and greater expression of effector molecule HES1 compared to non-diseased vessels. This latter finding was mirrored by *int3*-driven animal models of Notch activation, which suggests similarity in the underlying signaling [14]. Another series of human primary samples demonstrated NOTCH1 activation in ECs as well as VSMCs with elevated levels of JAG1, DLL4, and HES1 [15]. While findings in diseased tissues do not demonstrate the causality of Notch activity, their histological and molecular similarity to mouse models of Notch activity is convincing enough to suggest a parallel mechanism of AVM.

Delta-like ligand 4 (DLL4)

Delta-like ligand 4 is a ligand for both NOTCH1 and NOTCH4 [13, 22]. It is involved in arterial and venous specification during development in addition to its established role in tip cell formation in the remodeling vasculature [25–27]. VEGF signaling induces DLL4 expression, which is used by tip cells to suppress tip fate of neighboring ECs in order to prevent excessive vessel branching [28–30]. *Dll4* is expressed in arterial endothelia during development, but only in remodeling small arteries and microvessels in the adult mice, where it has a "salt and pepper" expression pattern. These patterns are suggestive of its role in both primary vascular morphogenesis and signal-driven remodeling [29].

Heterozygous or homozygous-null *Dll4* mutant mice exhibit a phenotype similar to, but more extensive than, *Notch1*-null mice. Mice that are homozygous-null or heterozygous for *Dll4* die at approximately E9.5 (depending on genetic background). At this time point, mutants exhibit a decrease in somite number, atretic vasculature and tissue necrosis [26]. Shunting in AVMs was specifically identified in only one of three studies that initially described *Dll4* haploinsufficiency [27], but findings in these three studies were similar in many other respects [25, 26].

Apart from the aforementioned abnormalities, EC identity is also compromised in *Dll4* mutants. Arterial markers such as EPHRINB2 and CONNEXIN40 are not detectable in arterial EC, while the venous marker EPHB4 is expressed in endothelial cells of arteries and veins despite normal expression of lineage-independent EC markers. These changes were observed prior to the onset of blood flow [25–27].

Flow-dependent structural abnormalities have also been noted. Impaired branching and remodeling of the vessels were accompanied by diminished arterial caliber, and atresia or fusion of the aorta and other large vessels [25–27]. On the cellular level, *Dll4* mutants also displayed underdeveloped basement membranes and EC adhesion [31]. In

each of these studies, it appeared that none of these defects were attributable to abnormal vasculogenesis.

As one might expect, mice in which *Dll4* was overexpressed in the endothelium exhibited excessive arterialization [32]. EPHRINB2 and CONNEXIN37 were strongly expressed in both arterial and venous endothelia while EPHB4 expression was diminished in veins. These mice also displayed enlarged vessels, shunting through AVMs, and died by E9. Increased extracellular matrix deposition was also observed with increased ECM protein synthesis and decreased matrix metalloprotease production [32].

In summary, developmental effects of the ligand DLL4 are broad and reciprocate the effects of its receptors NOTCH1 and NOTCH4. These encompass vascular patterning, endothelial cell identity and function. Such findings imbue the reviewed studies with internal consistency. The endothelial roles of *Dll4* further display acute sensitivity to gene dosing, which suggests the importance of its time- and site-specific regulation in the developing vasculature.

Insights from notch findings

What do the phenotypes of *Notch1/4* and *Dll4* mutants teach us about Notch signaling in the vasculature and the development of AVMs? One set of findings concerns EC identity and the achievement of arterial and venous specification. Notably, the expression of arterial markers correlates with the degree of Notch activity: upregulation induces arterial markers in natively venous endothelia, whereas downregulation suppresses their expression in natively arterial endothelia. Several groups noted that altered expressions of arterial and venous endothelial markers were observed *before* the onset of blood flow. The timing of these aberrations suggests that vascular identity is intrinsically determined, at least in part, by Notch signaling [25, 31, 32]. EPHRINB2 and EPHB4 are expressed in arterial and venous endothelia, respectively, as a consequence of Notch activity. They are markers, rather than determinants, of cellular identity. Expression of EPHRINB2, for instance, is also controlled by Notch signaling in cardiac development [20, 33]. Blood flow, which has been shown to influence intracellular signaling and secondary vascular specification [34], may also be involved in vascular remodeling, but seems uninvolved in the initial determination of vascular cell fate.

The timing of embryonic lethality in Notch mutants is likely attributable to its role in vascular remodeling. Until around E8.5, Notch pathway mutants are histologically indistinguishable from wild-type mice, but all prove lethal around E10. By this point vasculogenesis, or the de novo formation of the vasculature, is complete. Arteriogenesis, the process of remodeling, branching and elaboration of these vessels, is initiated at this time and is critical to sustain perfusion of the growing fetus [35]. Notch pathway mutants, with either gain- or loss-of-function, share this time point of embryonic lethality because of defective arteriogenesis.

Notch activation requires an appropriate middle ground with respect to its effects on vascular branching and remodeling. Tissues need appropriate investment with vasculature for sustenance. Excessive Notch activation limits capillary sprouting, thereby limiting adequate blood vessel network formation. This results in tissue necrosis in the area surrounding an AVM. Conversely, Notch hypoactivity produces vessels that are too atretic to provide vascular supply. Interestingly, the degree of endothelial Notch activity correlates with arterial caliber [33]. Notch gain-of-function mutants, either via constitutive receptor activity [17] or DLL4 overexpression [36] demonstrate increased arterial lumen diameter which may predispose to rupture.

While the exact mechanism of Notch-induced lumen enlargement remains unclear, several mechanisms can be considered. Diminished endothelial cell migration may leave parent vessels with greater numbers of endothelial cells, thereby producing enlargement [36]. Alternatively, alterations in the balance of the endothelial cell population between arterial and venous fate may lead to direct arteriovenous connection with subsequent lumen enlargement [37]. In a mouse model, gain-of-function Notch mutations as well as loss of *EphrinB2* or *EphB4* suggested that reciprocal changes in size of arteries and veins occur secondary to distribution shifts in ECs, without a change in population size [37]. Similar changes were seen in a mouse model of constitutive RAF1/ERK activation that leads, among other things, to increased DLL4 expression. In the mutants, a shift in arterial and venous identities resulted in formation of enlarged arteries and diminished veins [38].

Notch may also contribute to the determination of individual cell size. Intravital regression of AVMs observed after interruption of constitutively active Notch signaling is not associated with a change in cell number [18]. In animals with DLL4 overexpression, as in animals with constitutive Notch activity, arteries are found to be enlarged [32]. When hyperactivation of Notch signaling is reversed, however, enlarged arteries show regression to normal size [18]. Thus, cellular density and cell size must also be determined, in part, by Notch activity.

Insufficient Notch activation, conversely, results in atretic vasculature with poor remodeling from primitive plexi. This leads to formation of AVMs via the persistence of multiple small-vessel anastomoses between arteries and veins [16] as well as increased venous markers at the expense of arterial markers [20, 25–27, 37]. While there may appear to be an excess of vascular quantity in such tissues, such vessels fail to adequately perfuse tissues to support their growth and survival [39, 40].

Taken together, these results speak to pleiotropic functions of the Notch pathway in vascular development: the establishment of EC identity, vascular branching and microvascular investment, EC migration and population determination. So how do Notch abnormalities produce AVMs? AVMs may occur secondary to the elimination of the capillary bed normally interposed between the arterial and venous circulations as a consequence of Notch's potential for microvascular suppression. They may occur due to arterial enlargement and the subsequent need for vascular outflow, given Notch's effects on arterial growth and cell migration. Lastly, AVMs may occur due to rerouting of blood flow due to abnormalities in cell identity precipitated by Notch and EphrinB2/EphB4 signaling. To assign AVM to any of these specific malfunctions is not possible at the present. Further work is needed to determine whether these are distinct functions and, if so, to identify the mechanisms by which each function is influenced by Notch.

TGF-β signaling and Hereditary Hemorrhagic Telangiectasia

Transforming growth factor-β (TGF-β) signaling is critical to numerous aspects of development and includes a large family of molecules. In mammals, ligands of this family consist of closely related proteins designated TGF-β1-3, as well as a number of related proteins that include Bone Morphogenetic proteins (BMPs). TGF-β and BMP family members elicit their effects by activating serine/threonine kinase receptor heterotetrameric complexes composed of two type I and two type II receptors. There are seven type I receptors, named activin receptor-like kinase (genes *Acvrl1 to Acvrl7*, which encode the receptors ALK1–7) and five type II receptors (ACTRIIA, ACTRIIB, BMPRII, TGF-βRII, and AMHRII) [41]. In addition there are a number of accessory receptors including, ENDOGLIN (ENG; CD105) and TGFBR3, which do not transmit signals directly, but influence TGF-β cascade activity by their interaction with TGF-β receptors. Type I and type II receptors display similar structural properties, comprised of a relatively short extracellular domain, a single membrane-spanning domain and an intracellular domain containing a serine-threonine kinase domain. Upon ligand binding, the type II receptors phosphorylate and activate the type I receptors [42].

Classically, TGF-β signaling involves phosphorylation and nuclear translocation of SMAD transcription factors, but there are also SMAD-independent pathways. Activated type I receptors recruit and phosphorylate receptor-regulated SMADs (R-SMADs); SMADs 2 and 3 are specifically activated by TGF-β type I receptors (ALK4, ALK5, ALK7), whereas SMADs 1, 5, and 8 are activated by BMP type I receptors (ALK1, ALK2, ALK3, ALK6) [43–47]. R-SMAD*s* then form complexes with SMAD4 (Co-SMAD) and translocate to the nucleus, where they regulate the transcription of target genes [48]. SMAD6 and SMAD7, the inhibitory SMADs (I-SMAD*s*) inhibit signaling downstream of TGF-β type I receptors, thereby acting as negative regulators of signaling mediated by TGF-β [49, 50]. SMAD6 is known to be more specific for BMP type I receptor-mediated signaling, while SMAD7 is able to block signaling mediated by multiple TGF-β type I receptors, including type I receptors for BMP and TGF-β/ACTIVIN [51]. In addition, I-SMADs can compete with SMAD4 for association with R-SMADs, resulting in an inactive complex [52].

Transforming growth factor-β family members can also elicit SMAD-independent signaling that leads to activation of MAP kinases (ERK, P38, JNK), PI3K/AKT, TAK1 and RHO GTPases [52, 53]. The effects of these pathways in the vasculature are broad and unclear, complicated in no small part by the numerous molecules that participate in signal transduction with pleiotropic and antagonistic effects [54].

Inadequate TGF-β signaling, and BMP signaling via its known dependence on TGF-β-pathway molecules, has been associated with vascular pathologies that can lead to the formation of AVMs. Hereditary hemorrhagic telangiectasia (HHT, also known as Osler-Weber-Rendu disease) is an autosomal-dominant disorder characterized by the age-dependent development of focal AVMs, capillary telangiectasia and vascular dysplasia [7, 55, 56]. HHT affects one in five thousand people [56] and is diagnosed by the presence of three out of four possible traits: an affected kindred, recurrent nosebleeds, multiple mucocutaneous telangiectasias, or AVMs on major organs [57]. At least four forms of HHT have been described [55, 56, 58]. HHT1 and HHT2 develop in individuals who have defects in genes encoding components of the TGF-β signaling pathway: *ENG* [59], and *ACVRL1*, respectively [60]. The loci of genes abnormal in HHT3 [61] and HHT4 have been identified, but remain uncharacterized [58]. An additional disease, Juvenile Polyposis with HHT (JP–HHT) has been characterized as a result of mutation in *SMAD4* [62, 63].

Animal models of *Acvrl1* and *Eng* mutations have shown that abnormal TGF-β signaling is sufficient to induce certain features of HHT, namely vascular fragility, hemorrhage and the production of age-dependent AVMs [64, 65]. Endothelial cell-specific deletion of *Smad4* has not been found to induce AVM, but it does produce blood vessel fragility, increased endothelial cell proliferation and defective pericyte recruitment [66].

Activin-like receptor kinase 1 (*Acvrl1*; ALK1)

Expression of ALK1 in mice is limited to the arterial endothelium in the settings of vascular development and remodeling but is induced during angiogenesis, following

inflammation or wound healing [67–69]. ALK1 signaling is initiated by TGF-β [70] and more potently by BMP9 and BMP10 [71, 72]. With the latter two, it acts to inhibit VEGF expression and its angiogenic sequelae [71–73]. Genetic mouse models have shown that *Acvrl1* deficiency results in early embryonic lethality at E11.5 as a result of defects in the remodeling of the primary capillary plexus into a functional network of arteries, capillaries, and veins [54, 74]. Prominent shunts exist between the dorsal aorta and posterior cardinal vein. Further vascular defects include poor VSMC recruitment, diminished arterial EPHRINB2 expression and the appearance of hemogenic venous endothelium [74]. Interestingly, AVMs following loss of *Acvrl1* more closely mirror those found in Notch gain-of-function than Notch loss-of-function [17].

When *Acvrl1* deletion is limited to the endothelium in an *Acvrl1-Cre*, *Acvrl1*$^{fl/fl}$ mouse lethality was delayed to E18.5 [36]. This is likely due, at least in part, to the delayed complete endothelial expression of this Cre as well as the absence of its endocardial expression. Notwithstanding, these animals still display AVM characteristics with poorly developed and disordered vascular smooth muscle within a grossly tortuous arterial system [36]. Endothelial-specific deletions of *Acvrl1* in postnatal and adult mice prove lethal at 5 and 9–21 days, respectively, secondary to diffuse AVMs and hemorrhage [75].

Acvrl1 heterozygous mice survive into adulthood, but greater than 40 % of these animals developed AVMs within 1 year of age. AVMs and shunt-associated tissue necrosis in these animals can result in high-output cardiac failure [65]. Arguably, this genotype best mirrors the natural history of HHT2 by demonstrating multi-organ AVMs with an age-dependent incidence in the manner of other autosomal dominant diseases.

Interestingly, *Sm22*α-directed knockout of *Acvrl1*, which preferentially deleted *Acvrl1* in VSMCs, did not produce embryonic lethality. It did, however, induce AVMs in the central nervous system that were lethal in a fraction of each litter, between 2 and 15 weeks of age, due to inconsistent *Cre* activation [76]. This model suggests that VSMCs may also be implicated in AVM pathogenesis, but further work is required to better evaluate the contribution of VSMCs to AVM.

When defects in ALK1 signaling are combined with stress, such as VEGF stimulation or wound healing, vasodilation or injury and inflammation, blood vessels take on dysplastic traits that can result in AVMs [75]. Remarkably, the use of an anti-VEGF agent was shown to reduce vascular dysplasia in an adult *Acvrl1* knockout model [77]. It appears that dysplastic traits brought on by vascular remodeling stimuli in *Acvrl1* heterozygotes represent a precursor lesion to the development of large AVMs that are observed in the setting of disordered ALK1 signaling, such as in HHT2 [75, 78, 79].

Recent studies have attempted to elucidate the downstream cellular effects mediated by ALK1 signaling, but show conflicting results. One model states that ALK1 and ALK5 may balance the effects of TGF-β family ligands in response to different ligand concentrations, with ALK1 promoting the initiation of angiogenesis, and ALK5 promoting its resolution [80, 81]. However, it has also been reported that ALK1 and ALK5 have mutually exclusive expression patterns in blood vessels, which suggests that each type I receptor has a physically isolated function during vascular development [67]. Importantly, endothelial *Tgfbr1* (encoding ALK5) and *Tgfbr2* deletions in mice and zebrafish ECs do not phenocopy *Acvrl1* deletions [36]. Because ALK5 is reportedly required for ALK1 signaling via TGF-β, there is limited support for a balance model in which the ALK5 kinase activity is required for appropriate ALK1 activation as it pertains to AVM [36].

Other studies show that ALK1 signaling can promote endothelial quiescence in response to BMP9 stimulation. Reports indicate that BMP9 and BMP10 prevent EC migration in an ALK1-dependent manner [71]. Furthermore, zebrafish studies in which *acvrl1* has been mutated show that loss of receptor function leads to increased numbers of ECs in cranial vessels, suggesting that *acvrl1* may normally act to prevent EC proliferation [69]. Studies in mice, using *LacZ* reporter genes, also show that ALK5 is more restricted to the vessel media, particularly VSMCs, while ALK1 is expressed in the endothelium [36]. Therefore, it appears that ALK1 may act as a suppressor of endothelial cell migration and possibly proliferation, maintaining a quiescent endothelial state. Further recent in vitro studies have supported this interpretation [72, 82, 83]. ALK1 may also regulate the behavior of ECs and participate in the prevention of AVM through its regulation of gene expression. For example, in cultured ECs and zebrafish, BMP9/ALK1 reportedly upregulates expression of the potent vasoconstrictor *Endothelin-1* [68, 84] and downregulates expression of *Cxcr4* and *Apelin* [83, 85], which are involved in migration and proliferation of ECs. These may contribute to the phenotypic changes, such as vessel enlargement, observed in conditions of reduced ALK1 signaling. Therefore, it is possible that gene dysregulation, due to loss of ALK1 signaling in HHT2 patients, is also involved in AVM pathogenesis.

Additionally, AVMs associated with ALK1 signaling deficiency may also arise as a consequence of blood vessel enlargement. For example, zebrafish with *acvrl1* deficiency have increased cranial blood vessel diameter, which may allow the persistence of normally transient arteriovenous connections, resulting in AVMs [69]. It has been suggested that alterations of blood flow in the setting of such persistent vasculature dilation might contribute to AVM [68], but given the strength of blood flow in animals at this stage, as

well as the frequency of low-shear stress pulmonary AVMs in HHT patients, it is unclear if AVM might be assigned to abnormal flow conditions.

ALK1 may also exert its effects on angiogenesis in cooperation with Notch signaling [83]. Inhibition of ALK1 and Notch signaling in retinas resulted in increased endothelial tip cell proliferation and excessive angiogenesis; a finding consistent with the previously-noted importance of BMP9 and BMP10 signaling in retinal development [86]. Combined Notch and ALK1 inhibition produced more profound vascular abnormality than blockade of these pathways individually. These defects were rescued by BMP9 in a SMAD-dependent manner. Furthermore, ALK1 signaling resulted in upregulation of several factors involved in Notch signaling, such as *Hey1*, *Hey2*, and *Jag1*. These findings indicate that Notch activity in the setting of mutant *Acvrl1*, such as in HHT2, may contribute to AVM directly through pathway crosstalk [83].

Endoglin

Endoglin (*ENG*) is the gene that encodes the transmembrane glycoprotein and accessory receptor for TGF-β ligands which is mutated in HHT1 [59]. While HHT1 is similar to HHT2, there is a particular enrichment of cerebral and pulmonary AVMs in HHT1 patients [7, 87]. Developmentally, ENG is expressed throughout the endothelium whereas ALK1 is limited to the arterial circulation [67]. It appears sequentially in the vasculature, beginning with major vessels and progressing into distal vessels. Adult expression of ENG is mostly restricted to the quiescent arterial endothelium, but can be re-expressed under conditions of endothelial stress [88, 89]. Notably, ENG has a relatively higher expression in the primitive neurovasculature [90].

Homozygous global deletion of *Eng* results in embryonic lethality at E10.5 [64, 91]. Animals appear normal until E9.5, but by E10.5 there is a drastic reduction in body size relative to wild-type mice. There are no defects in vascular differentiation, but arterial and venous endothelia display abnormalities in their respective cell identities before the onset of blood flow. These mice also demonstrate defective vascular remodeling, wherein blood vessels fail to remodel from a primitive plexus to a segmental and branched vasculature. Additionally, there is visible hemorrhage within the embryo. As in *Acvrl1* mutants, *Eng* mutants displayed smooth muscle cells were differentiated, but they were underdeveloped and disorganized [64, 91].

Neonatal *Eng* knockout also results in AVM formation, increased endothelial cell proliferation, decreased migration and poor vascular remodeling [92]. AVMs in these animals express venous markers EPHB4 and Apelin receptor (APLNR), but not arterial markers JAG1 or EPHRINB2. Interestingly, these changes occurred without alteration in levels of activated SMAD2 or SMAD1/5/8 [92]. This indicates that SMAD-independent pathways downstream of ENG may also play a role in the defects associated with ENG dysfunction, and HHT1 pathogenesis.

Eng heterozygous mice developed normally, but older animals displayed clinical signs of HHT, such as nosebleeds and telangiectasia [64, 93]. These animals develop AVMs of the brain as early as 25 weeks of age [94, 95]. Such vascular morphology is correlated with a poorly organized and underdeveloped smooth muscle layer, a 70 % reduction in smooth muscle coverage and an increase in mast cell-driven inflammation [95].

Like ALK1, ENG is upregulated in the setting of vascular injury or repair [89]. Expression of ENG is also upregulated in states of hypoxia in mouse brain endothelium where its expression is driven by ERK, P38 and JNK signaling [96]. In endothelial cells that are not remodeling, however, ENG is weakly expressed or altogether undetectable [88]. VEGF is capable of inducing capillary dysplasia in *Eng* heterozygotes in a similar way, but more intensely, than in *Acvrl1* heterozygotes [79]. Dysplasia was increased by 33 % in *Eng* heterozygotes [79], but when normalized to efficacy of transduction, a later study reported an 800 % increase in dysplasia [97]. This greater sensitivity may explain the increase in frequency of manifestations of HHT1 over HHT2, but its biological origin remains unclear [7, 79].

In sporadic (HHT-unrelated) AVMs, ENG protein is observed at levels comparable to those in non-diseased endothelium [98]. ENG levels in AVMs of HHT1 patients, given the heterozygosity of these individuals at the *ENG* locus, and decreased by half from levels observed in tissues of non-diseased patients. [99–101]. Importantly, however, these levels are consistent throughout the endothelium, which suggests that there is not a complete absence of ENG within AVMs of HHT1 [101], but there may exist foci of "second hits" in genes with parallel function that potentiate AVM formation at an individual site [98].

Other TGF-β family members

Smad 4

SMAD4 mutation leads to the syndrome of juvenile polyposis: a portion of patients with this syndrome also present with the signs of HHT, producing a compound disease state (JP–HHT) [62, 63]. The full extent of clinical variability and frequency of features is not well understood, as clinical information on *SMAD4* mutation carriers is relatively limited (*SMAD4* mutations represent 2–3 % of cases of HHT). Although it had been suspected that variability

in the presentation of JP–HHT correlated with mutation in exons 8, 9 or 11 of *SMAD4*, it now appears that causative mutations may occur in any exon of the gene [102]. Published studies show that a significant proportion of JP–HHT patients develop AVMs of the lung and liver, capillary telangiectases of the brain, and oral telangiectases [62].

The pathogenesis of JP–HHT might be due to different effects of lowered levels of SMAD4 within endothelial cells, leading to decreased TGF-β/BMP signaling, which may produce vascular dysplasia. Deletion of *Smad4* in mouse cerebrovascular ECs demonstrated impaired interaction with pericytes and increased EC proliferation. Interestingly, *Smad4* appears to stabilize cerebrovascular EC-pericyte interactions by regulating the transcription of *N-cadherin* through its association with Notch transcriptional machinery at the *N-cadherin* promoter [66]. Despite its modeling of a human mutation and evidence for cooperation between TGF-β and Notch signaling, this animal notably lacked AVM and resemblance *Alk1/Eng* mutants.

Matrix GLA protein

The knockout of the BMP antagonist *Matrix Gla protein* (*Mgp*) has also been shown to induce AVMs in lungs and kidneys. MGP is a small, gamma-carboxylated protein that binds and inhibits BMP2 and BMP4. *Mgp* knockout produced elevated BMP activity, elevated *Alk1/2/5* expression, SMAD1/5/8 activation in lung epithelial cells and stimulated their VEGF production, with similar effects in the renal mesangium [103]. In endothelial cells, MGP natively increases VEGF expression through TGF-β, associates with ALK1 and provides context-dependent stimulation and inhibition of ALK1 expression and signaling [104–106].

At this time, the mechanism by which *Mgp* mutations induce AVM remains unclear. Simply put, MGP appears to have context-dependent effects on TGF-β signaling. These context-dependent effects directly alter native ALK1/5 signaling, which appear to serve as an entry point for AVM pathogenesis. Further investigation into the function of MGP in AVM and its potential to modulate TGF-β signaling will be important and useful, as MGP represents another link in a pathogenic signaling pathway that already includes receptors (i.e., ALK1, ENG) and a transcription factor (SMAD4).

Insights from TGF-β-driven models

These models leave us with several questions about the vascular functions of ALK1, ENG and TGF-β signaling in addition to some insight. Although it appears that ALK1 and ENG share some transduction pathways, the observed differences between mutants for these receptors are not clearly understood, both in animal models and in patients with HHT. In humans, heterozygosity for *ENG* results in more pulmonary and cerebral AVMs, while heterozygosity for *ACVRL1* results in more hepatic AVMs [7, 94]. Furthermore, mouse models demonstrate that *Eng* mutants do not display the degree of dilatation observed in *Acvrl1* mutants, show vascular shunts later than *Acvrl1* mutants, and do not display the downregulation of arterial EPHRINB2 [107]. It is unclear whether these manifestations carry across species or if there is an organ-specific variation in the expression of these proteins that manifests in differential AVM appearance. There is also little insight into how these differences are contingent on signaling, and this area requires further investigation. To some extent, differences in signaling may be attributed to the known interaction of ALK1 signaling with the Notch pathway [83, 86], that is apparently absent for ENG signaling, given that Notch is known to have effects on dilatation and the expression of *EphrinB2* (discussed above).

It is important to note that Notch gain-of-function, rather than loss-of-function studies, more closely mirror TGF-β loss-of-function mutants [17]. This may indicate that AVMs formed following disruption of ALK1/ENG and Notch signaling occur through different mechanisms. Several studies have described interplay between the Notch and SMAD-dependent cascades, but many questions remain as to the specific roles of such crosstalk in AVM. For instance, Notch intracellular domain has been shown to interact with the SMAD transcriptional complex [108]. Studies by different groups have also shown reduced expression of Notch downstream targets (*Hey1, Hey2, Hes1*) following inhibition of SMAD signaling [83, 109, 110]. Furthermore, Notch has been shown to partially compensate for loss of ALK1 signaling in restoring endothelial gene expression of arterial markers such as EPHRINB2, and in preventing excessive sprouting [83, 109]. Lastly, a mouse model demonstrated reduced Notch signaling in *Acvrl1* knockout embryos [111]. Further studies will be needed to understand the consequences of disrupted ALK1 signaling on Notch activation, the effects of inadequate Notch signaling on ALK1 signaling, and the counterpart alterations with changes in ENG signaling.

One may also speculate that the differences in ALK1- and ENG-driven AVMs can be accounted for by differences in sensitivity to VEGF as a dysplasia-inducing agent. The features of vascular dysplasia—increased cellularity, dilatation and tortuosity—appear to be a precursor lesion of AVM, as mutants manifest these features secondary to vascular insult or comparable stimuli. VEGF signaling has many downstream effectors, some of which are implicated in TGF-β-induced SMAD-independent signaling. Perhaps there exists crosstalk between these VEGF-dependent pathways and the SMAD-independent downstream signals of

ALK1 and ENG. This is likely given that abnormalities in neonatal *Eng* knockout mice appeared to have no change in SMAD activation patterns [92]. More work is required in this area to clarify the contextually-relevant downstream effectors of these receptors, as well as to identify the physiologic ligand(s) whose signaling is disturbed in TGF-β pathway-driven AVMs.

The finding that TGF-β receptor mutants are susceptible to dysplasia with VEGF stimulation is notable as these experiments likely represent an expedited recapitulation of the mouse models of heterozygosity for *Acvrl1* and *Eng* and, perhaps, HHT patients. Dysplasia seems to serve as an early manifestation of the full AVM disease state. On a background of defective TGF-β signaling (namely receptor heterozygosity), endothelial stress that stimulates VEGF signaling produces vascular dysplasia [112]. Homozygous mutations of *Acvrl1* and *Eng* result in AVMs in the setting of endothelial stimulation: such stimulation may take the form of VEGF or comparable signaling, as occurs during development, injury or inflammation. If both ALK1 and ENG are physiologically upregulated in times of such stress, and the aforementioned dysplasia occurs because of stress in individuals carrying mutations for these genes, then we can infer that the role of TGF-β signaling, in the simplest terms, is to stabilize vessels as they endure angiogenic remodeling.

These findings can be extrapolated to speculate on the origin of AVMs in HHT patients. The fact that AVMs are rare in infants has caused some to contend that they cannot be developmental anomalies [112]. Data from these models would argue against this notion. One may consider the growth of the body as a robust angiogenic stimulus. Such a stimulus, in the setting of TGF-β pathway mutations, may be sufficient to produce an AVM. While such an AVM might develop postnatally, it would still have formed secondary to vascular development. This explanation for AVM reconciles the notions that AVMs form either as consequences of development [23] or as a response-to-injury [113]; the latter being a common explanation for the age-dependent incidence of AVM.

Given these facts, what is particular about HHT biology that results in frequent AVMs? This propensity may be explained by the accumulation of a "second hit" due to an environmental insult [75]; an idea which may be extended to the development of a genetic mutation at the locus of the HHT mutation or partner signaling molecule. Notably, animals heterozygous for *Acvrl1* or *Eng* only demonstrated stress-induced vascular dysplasia or age-dependent incidence of AVMs. Homozygous-null mutants, however, displayed AVMs soon after endothelial stress. If this is true, then it may also be the case that humans genotypically normal for *ALK1* and *ENG* accumulate "first hits" at these loci, but these hits are silent. Only when *two* hits are accumulated at a particular locus, or loci with parallel function, might an AVM manifest. Examples of such parallel loci are numerous, and might include *Mgp*, *Smad4, Smad1/5/8* or any of the TGF-β type II receptors. For example, mutations in the human *BMP9* gene have been found to produce a disorder in the spectrum of HHT [114].

Soluble factors apart from VEGF may also contribute to vascular dysplasia. The soluble ENG receptor (sENG), a protein observed at high levels in women with preeclampsia [115], was also observed at high levels in human-derived AVMs [116]. When expressed in mouse brain vasculature, sENG also produced vascular dysplasia with VEGF coadministration [116]. Similar results have been achieved with the synthetic protein soluble ALK1 (sALK1) [83]. Interruption of signaling upstream of membrane-bound receptors suggests that the phenotypes observed are consequences of ligand-binding of ENG and ALK1, rather than an effect at the cell surface such as co-receptor interaction. Additionally, TNF-α has been shown to cause downregulation of ENG levels, reducing surface expression by 50 % [117]. This functional ENG knockdown may directly lead to vascular dysplasia in the absence of this protein's native function [92], but also in the presence of the associated inflammatory milieu. Alternatively, local induction of VEGF signaling [118–120] in the setting of injury may produce AVMs after TNF-α-associated decrease of endoglin.

RASA1

Capillary malformation-arteriovenous malformation (CM–AVM) is a newly-recognized familial disease wherein patients have cutaneous capillary malformations and large organ AVMs [121]. AVMs in CM–AVM can reside in any tissue and produce similar end-organ effects, such as epilepsy, vein of Galen malformation, various tumors and high-output heart failure [121–123].

This phenotype is defined by heterozygous loss-of-function mutations in the *RASA1* gene, which encodes p120-RasGTPase activating protein (RasGAP) [121]. CM–AVM is caused by polyallelic mutations in *RASA1*, with greater than forty recognized mutations. One-third of individuals with *RASA1* mutations display AVMs [123]. The native function of this protein is to negatively regulate cellular growth and proliferation downstream of receptor tyrosine kinases by accelerating hydrolysis of guanosine triphosphate associated with RAS [124, 125]. Deactivating mutations of GAPs can augment signaling of the Ras oncogene, which is evidenced by mutations of such proteins in basal cell carcinoma and neurofibromatosis [121]. *RASA1* mutations also link the pathogenesis of AVMs to ERK signaling, as RAS is upstream of ERK and diminished RAS deactivation engenders increased ERK activity.

Mice mosaic for *RASA1* deletion show abnormal angiogenesis in conjunction with limited ability to remodel their primitive vasculature [126]. Alterations observed in these vessels could be due to interactions of p120-RasGAP with p190-RhoGAP, which has effects on cytoskeletal remodeling and cell motility [127].

Inflammatory markers

Inflammatory signaling is developing importance in the AVM literature. Animal models and study of primary human samples reveal involvement of inflammatory cells and cytokines, especially TNF-α and IL-1β [76, 95, 128–130]. This correlates with increased tissue markers of inflammation, such as matrix metalloproteases and reactive oxygen species [116, 131]. Inflammation and manipulation of AVMs by treatment also contribute to proliferation, abnormal cellular morphology and alterations in cell adhesion molecules [118–120, 132–136].

Defects in endothelial signaling and smooth muscle development

The manner by which defective endothelial TGF-β signaling leads to disordered development of smooth muscle cells, in spite of their adequate differentiation, remains to be established. It has been previously determined that endothelial cells signal to smooth muscle cells via paracrine signaling in a SMAD2-dependent pathway, but it has not been explained how ALK1 and ENG are involved in the establishment of these smooth muscle architecture [137].

One possible explanation for the smooth muscle effects of *Acvrl1* and *Eng* knockout in endothelium is that these knockouts disrupt endothelial-mesenchymal transition (Endo–MT); a process that has been implicated in both cardiovascular development and disease [138]. In Endo–MT endothelial cells are converted to mesenchymal cells including vascular smooth muscle cells and fibroblasts. The trigger seems to be sustained activation of endothelial TGF-β signaling, which is natively suppressed by fibroblast growth factor (FGF) signals. In the setting of inflammatory cascades such as those involved in TNF-α, IL-1β, and IFNγ, FGF signaling is suppressed and TGF-β is allowed to induce Endo–MT [139]. Notch has also been shown to independently induce Endo–MT [140]. Furthermore, crosstalk between Notch and TGF-β has been implicated in Endo–MT and the development of the vascular smooth muscle cell phenotype [141–144].

A recent model developed for another form of cerebrovascular disease, cerebral cavernous malformation (CCM), demonstrates the importance of Endo–MT in its pathogenesis as well as the reliance of this process on TGF-β/BMP and Notch signaling. A knockout of proteins known to cause CCM induced dysplastic changes to vascular structure, expression of mesenchymal markers in the endothelium, increased sensitivity to BMP6 and suppression of Notch signaling [145]. Significant alterations in the implicated pathways suggest that there might be parallels in the pathogeneses of CCM and AVM. Certain features of AVM, such as increased neointima and perivascular fibrosis may well be direct consequences of Endo–MT [139]. Endo–MT is an evolving subject of investigation. Studies to determine the extent to which this process contributes to AVM would certainly be of interest.

Intravital signaling as a therapeutic target

As the adult knockout models described above suggest, there appears to be a sustained requirement for appropriate Notch and TGF-β signaling in the vasculature. This implies that perturbations in these signals potentiate the formation of AVMs. In animal models, such perturbations are achieved by genetic manipulation. In humans, they occur by mutation or dysregulation of cell signals. Because the vasculature remodels throughout life, it is placed at risk for such mutations in adulthood and not only during development. Remodeling of the adult vasculature, for instance, takes part in the formation of AVMs or AV fistulae at surgical sites and within large wounds.

Promising observations pertaining to treatment of disordered signaling have been made in both Notch and TGF-β animal models. Interruption of hyperactive Notch signaling in AVMs, by targeting either the Notch receptors or ligands, might be effective to restore vascular identity, improve tissue blood supply, and possibly induce lesion regression [18]. Also, anti-VEGF agents cause regression in AVMs of disordered TGF-β signaling both in animal models and [77, 112], at least initially, in studies of patients with HHT [146]. If intravital regression is possible by disrupting pathologic cell signals, then targeted medical therapy might prove sufficient to treat AVMs. Such therapy may one day complement, or replace, current open surgical, endovascular and radiation therapies.

Summary

Abnormalities in endothelial signaling contribute to the development of arteriovenous malformations. The Notch and TGF-β pathways appear to be particularly central to the development of these lesions. Animal models have enlightened our understandings of the physiological roles of these pathways and how they might contribute to AVM.

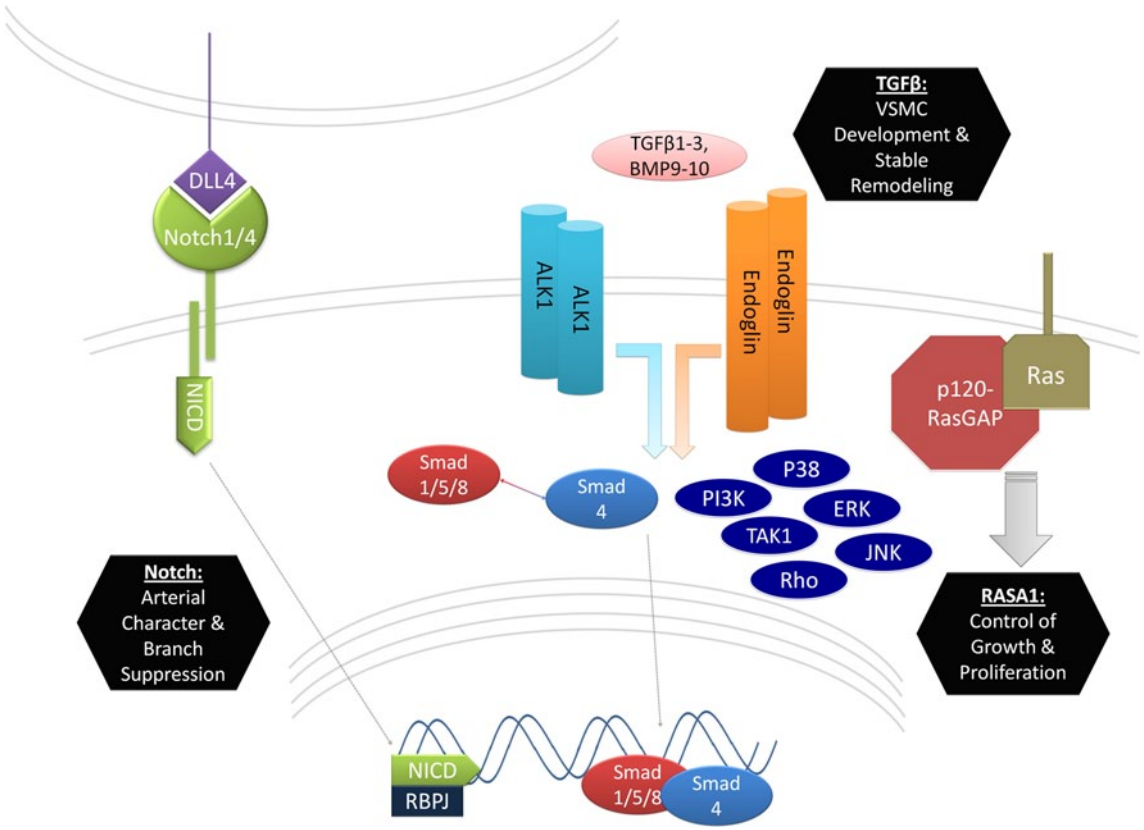

Fig. 1 Summary of major endothelial signaling pathways implicated in AVM. AVM signaling in the endothelium is characterized by abnormalities in Notch and TGF-β signaling with growing contributions from other findings that include abnormalities in Ras signaling. Notch signaling defects in AVMs include those from Notch ligand DLL4 and its receptors NOTCH1 and NOTCH4. Notch receptor intracellular domains (*NICD*) are cleaved upon ligand binding and translocated to the nucleus where they become transcriptionally active with binding partner RBP-J. Functions of this receptor-ligand combination that are pertinent to AVM formation include the determination of arterial character and the suppression of small vessel branching. TGF-β mutants for ALK1, ENG, and SMAD4 correlate with the incidence in AVMs in humans. Mutants for the ALK1 and ENG also produce AVMs in animal models. These TGF-β receptors produce transcriptional activity of SMAD1/5/8 in association with SMAD4 and the activation of SMAD-independent pathways (PI3K, TAK1, RHO, P38, ERK and JNK). Mutants for these genes have abnormal development of VSMCs and marked abnormalities in vascular remodeling. Mutations in p120RasGAP have also been shown to induce AVMs, presumably by failing to limit endothelial cell growth and proliferation

Abnormal activation or inactivation of receptors NOTCH1 and NOTCH4, as well as their ligand DLL4, implicate these molecules in the regulation of endothelial cell identity and branching morphogenesis (Fig. 1, left). TGF-β pathway elements are similarly implicated, but by a different mechanism. These genes appear to be critical to the regulation of vascular smooth muscle development and remodeling, especially in response to angiogenic stressors (Fig. 1, center). New data is also emerging on endothelial functions of other signaling cascades such as those involving p120RasGAP (Fig. 1, right) and inflammatory cytokines. It is possible that AVM represents abnormal signaling in these pathways separately, but also a disruption in the interdependence of these pathways. Considerable work remains to characterize endothelial functions of these pathways and to perhaps realize this investigation in the form of medical therapy.

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