Pathogenesis of non-hereditary brain arteriovenous malformation and therapeutic implications

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Abstract
Brain arteriovenous malformations (bAVM) represent a high risk of intracranial hemorrhages, which are substantial causes of morbidity and mortality of bAVMs. Although a variety of genetic factors leading to hereditary bAVMs are investigated extensively, their pathogenesis is still not well elucidated, especially in sporadic bAVMs. In this presentation, the author reviewed the updated data of not only genetic aspects of sporadic bAVMs but the architecture of microvasculature, the roles of the angiogenic factors and the signal pathways.[1]

Introduction

Two hypothesized mechanisms for bAVM formation have been postulated:[2] 1) abnormal sprouting angiogenesis leading to an anomalous direct arterial-venous connection. 2) the progressive dilation of existing capillary beds resulting in high-flow shunt from arterial to venous circulations. Both mechanisms have been described and appear specific to the rodent model, but pathogenesis of bAVM is still not elucidated. BAVMs can occur as part of hereditary syndromes, such as hereditary hemorrhagic telangiectasia (HHT) and capillary malformation (CM)-AVM, where they result from germline mutations in genes that have known or plausible roles in angiogenesis and vascular remodeling, such as ENG, ALK1, SMAD4 and RASA1, among others. Similarly, familial inheritance of sporadic bAVMs has been ascribed to mutations in ALK1.[3] Less is known about the cause of sporadic AVMs, which account for the majority of disease burden in the general population.

Studies of AVM tissue suggest a dynamic and biologically active angiogenic and inflammatory lesion rather than a static congenital vascular malformation. Molecular biology and gene expression studies have been performed to investigate the nature of AVMs.

Vasculogenesis and Angiogenesis

During embryonic development, formation of blood vessel networks relies on two processes: **vasculogenesis** (de novo vessel formation during embryogenesis) and **angiogenesis** (the expansion of a pre-existing vascular network through sprouting or splitting of vessels). Subsequent growth of the vertebrate vasculature occurs entirely by angiogenesis, the first phase of which involves vascular endothelial cell (EC) proliferation and migration, the second phase of angiogenesis is vascular stabilization, during which ECs form capillary tubes, strengthen their intercellular junctions and recruit smooth muscles cells (SMCs) to their walls.[4] (Figure 1)

Brain AVMs form at the interface between arterial and venous endothelium where capillary endothelium normally lies. The angiogenic process, most severely disrupted by the vascular malformations, is that of vascular stabilization, the process whereby vascular ECs form capillary tubes, strengthen their intercellular junctions, and recruit smooth muscle cells SMCs to the vessel wall. AVM nidus express markers specific to arteries and veins as well as capillaries. AVM nidus are thought to constitute of aberrant vessels which are not finally differentiated and inadequately matured,[5] and histopathologically lacks a true capillary bed.

Microvasculature

Cerebral blood vessels are anatomically comprised of ECs, vascular SMCs, and pericytes. To date, most bAVM research has focused on the endothelium. On histologic evaluation, significant endothelial heterogeneity has been described.[2] Disruption of the blood-brain barrier (BBB) is well documented,[6] and

microhemorrhages are frequently observed in unruptured bAVMs and may predict future rupture[7]. (Figure 2, 3)

EC s

Brain ECs form monolayer cell lining of the vascular lumen, serving as the vital interface between blood and brain parenchyma known as the BBB. At a molecular level, ECs express higher levels of pro-angiogenic factors[8], and as a result frequently assume a pro-angiogenic phenotype in bAVMs. The experimental models reproduce some features observed in human bAVM (e.g. dilated vessels, an arteriovenous shunt, a high-flow lesion, and a formation of nidus). Adenovirus-mediated EC-selective ALK1 deletion and overexpression of VEGF induces lesions resembling human bAVM as well,[9] suggesting involvement of changes in EC function and angiogenesis in the pathogenesis.

Smooth Muscle Cells

Vascular SMCs are the predominant cellular constituent of the vessel wall in arteries and veins. SMCs derived from bAVMs formed tubes in culture, which were longer than those formed by normal brain vascular SMCs. The migration and proliferation of SMCs in bAVMs exceeded those of normal brain vascular SMCs. The reductions in vascular SMCs in bAVMs have been described.[10]

Pericytes

Pericytes are embedded within a vascular basement membrane that is shared with the adjacent endothelium. The close spatial proximity during vascular remodeling between sprouting ECs and neighboring pericytes suggests a crosstalk, and potentially a reciprocal signaling relationship between these two cell types during vascular remodeling.[11]

Pericytes are important in promoting vascular stability and maturation throughout the human body.[12] Reduction in pericytes has been described in both human bAVMs and rodent models, and is greatest in ruptured human AVMs.[6] In unruptured AVMs, the magnitude of pericyte loss correlates with the severity of BBB disruption and microhemorrhage.[6]

Angiogenic factors

EC behavior during vascular development is regulated by prominent growth factor families such as VEGF, Fibroblast growth factor (FGF), angiopoietin (ANGPT) and transforming growth factor beta (TGF beta)/ BMP families. One important pathway for EC–vascular SMC and pericytes crosstalk is the PDGFB/ PDGFRβ pathway. In addition to growth factors, vascular development and homeostasis are regulated by many other signaling inputs including ligand-receptor signaling pathways such as Wnt and Notch. (Figure 4)

VEGF

VEGF is a potent EC mitogen that is thought to play a key role in angiogenesis,[13] and abnormal expression of VEGF has been repeatedly observed, making it increasingly more evident that a disruption in angiogenic signaling systems is involved in the formation and progression of bAVMs. Increased VEGF expressions in the endothelium and other cells of the surgical specimens of bAVM implied that VEGF was involved in EC proliferation and angiogenesis in bAVMs.[8] Furthermore, the nidus size is larger in lesions positive for VEGF-A or Flt-1(VEFR-A receptors) staining than in those without positive signals, suggesting the pathogenic role of VEGF-mediated angiogenesis in the enlargement of the lesion.[8]

TGF-β

Transforming growth factor beta (TGF-β) is a multifunctional cytokine which has multiple effects on brain vascular development implicated in vascular malformations – including both bAVMs and cavernous malformations.[14] In humans, it is suggested that single nucleotide polymorphism (SNPs) in ALK1 or ENG may be associated with heightened risk of sporadic AVMs.[15]

Notch

The Notch pathway is a critical mediator for the differentiation of arteries and veins.[16] AVM arises due to impaired arterial or venous differentiation during early angiogenesis consequent to imbalanced Ephrin proteins, particularly EPHB2 and EPHB4.

Notch components are considered to be critical mediators of EC fate decisions and vascular lumen formation and both loss-of-function and gain-of-function Notch mutations result in arteriovenous shunting. Thomas et al reported that an augmented expression of EphrinB2, Hey2 and DLL4 in the nidus structures from in bAVM when compared to normal brain arteries,[5] and they suggested that deregulated arterial specification signaling might have a significant role in the pathogenesis of AVM. Recent works have suggested a role for Notch signaling in the formation of non-syndromic, sporadic bAVMs.[17]

Gene mutations

The identification of gene mutations and genetic risk factors associated with bAVMs has enabled understanding of the genetics for this disease. The genetic hypothesis of the formation of AVMs is a "two-hit" mechanism in which an inherited mutation in one copy of a cerebrovascular malformation gene is followed by a somatic mutation in a second counterpart. The second "hit" could be environmental in the form of a localized physiological or pathological perturbation.

KRAS

A recent study revealed that the majority of sporadic bAVMs also harbored somatic activating KRAS mutations driving the downstream mitogen-activated protein kinase (MAPK)-ERK signaling.[18] Activating KRAS mutations were noted in 62.5% of 72 bAVMs, but in none of 21 paired blood samples.[18] The presence of activating KRAS mutations in more than half of bAVM tissue samples may indicate the pathogenic role of these KRAS mutations.[19] In sporadic bAVMs, KRAS mutations are also detected in 9 of 15 specimens (60%) and 7 of them are G12V or G12D mutations.[20] Priemer DS et al demonstrated the first reported instance of a KRAS p.G12C mutation in a bAVM.[21]

KRAS mutations were detected in ECs from human bAVMs in vitro and it was noted that mutant KRAS expression initiated increased ERK activity that was counteracted by inhibition of MAPK–ERK signaling.[18] Interestingly, KRAS is mostly implicated in tumorigenesis and cancer, where mutations promote unregulated activation of growth-promoting signal transduction pathways resulting in cell transformation and genomic instability.

BRAF

Hong et al reported the first evidence of activating BRAF mutations in bAVMs and spinal AVMs (sAVM). The total prevalence of KRAS/BRAF mutations was 87.1% (27 of 31 patients) in their cohort.[22] The prevalence of KRAS/BRAF mutations was 81.0% (17 of 21 patients) in bAVM and 100% (10 of 10 patients) in sAVM. KRAS p.G12D and p.G12V were mutation hotspots both in sAVMs and bAVMs, with a prevalence of 30.0% and 30.0% in sAVMs, and 52.4% and 19.0% in bAVMs, respectively, whereas BRAF p.V600E was rare and found in only one bAVM and one sAVM patients.[22] They found that mutation variant frequencies correlated negatively with nidus volumes and largest diameters, but not with age.[22]

Signal pathway

Increased KRAS activity can potentially affect multiple downstream signal pathways. Most of vascular malformations are associated with mutations commonly found in cancer, mainly in the phosphoinositide 3-kinase (PI3K)-AKT-mTOR in low-flow vascular malformations including venous and lymphatic malformations and RAS-MAPK-ERK pathway in high -flow lesions including bAVMs.[18] BAVMs without detectable KRAS mutations also had high levels of phosphorylated ERK1/2, suggesting that the RAS-MAPK-ERK pathway activation is a hallmark of all bAVMs.[18] The PI3K signaling pathway is a critical regulator of the angiogenic process by controlling proliferation, migration and survival of ECs. Germline autosomal dominant RASA1 mutations have been identified in 50% of CM-AVM1 patients including those with Parkes-Weber syndrome. RASA1 encodes p120-RasGAP protein that inhibits activity of RAS protein. Loss of function mutations of RASA1 therefore may lead to activation of RAS and increased downstream signaling via MEK-ERK1/2 and PI3K-AKT-mTOR pathways that can be targeted potentially.[23]

Somatic mutations not only in the RAS-MAPK pathway have been noted in bAVMs, but in the PI3K pathway as well as in other vascular malformations. The RAS-MAPK signaling pathway is the most

promising therapeutic target for bAVMs. The PI3K-AKT-mTOR pathway have a possibility of therapeutic target for bAVMs, although previously the somatic mutations in the PI3K pathway in high-flow AVMs is not emerged. Further study will demonstrate the increasing importance of genetic diagnosis for both germ-line and somatic mutations for future molecular target therapies. (Figure 5)

Future therapeutic target

Considerable efforts have been placed on using existing therapies to target molecular pathways disrupted in bAVMs – including TGF-β, Notch and VEGF. In HHT, treatment with thalidomide was shown to restore endothelial PDGF-B expression leading to recruitment of mural cells and vessel stabilization.[24] Thalidomide or lenalidomide treatment reduced the number of dysplastic vessels and hemorrhage, and increased mural cell (vascular SMCs and pericytes) coverage in bAVM lesion.[10] (Figure 6)

VEGF neutralization prevented and normalized AVM in an animal model for HHT2, an autosomal–dominant disorder characterized by telangiectasia and AVMs in multiple organs.[25] Others have begun to explore a direct approach using **bevacizumab** – a humanized VEGF monoclonal antibody. Bevacizumab has an established safety profile and has been trialed as anti-angiogenic therapy in a number of neoplastic conditions – including glioblastoma.

Malformations due to mutations affecting RAS/BRAF/MEK/ERK pathway (e.g. capillary malformation, CM-AVM, bAVM, sAVM) could perhaps be targeted by BRAF inhibitor (e.g. vemurafenib) and/or MEK inhibitors (e.g. trametinib, cobimetinib) that are available.[22] AVM overlying the left scapular region in a child was treated by genotype-guided. Exome sequencing from a specimen of the AVM and saliva revealed in-frame deletion of MAP2K1, therefore a MELK inhibitor, trametinib treatment was done, and significant reduction in overall volume after 6 months.[26] Initial experiments with MAPK-ERK pathway promoted vascular barrier properties and quiescence in patient-derived KRAS-mutant ECs in vitro.[18]

Vascular anomalies with mutations affecting the PI3K-AKT-mTOR pathway (e.g. venous malformation, venous malformation cutaneo-mucosal, multifocal venous malformation, and lymphatic malformation) are known to respond to mTOR inhibitors (sirolimus, everolimus, and temsirolimus). In vivo animal models showed that **sirolimus** inhibits angiogenesis via downregulating the PI3K/AKT signaling pathway and the expression of vascular endothelial growth factor.[27] All 6 head and neck AVM patients (4 male and 2 female patients) responded favorably to the combination of sirolimus therapy followed by endovascular embolization, and 4 patients exhibited a near-complete response.[28] Maynard et al. reported a 10-year-old girl with thalamic AVM with encephalo-cranio-cutaneous lipomatosis, who sirolimus was administered.[29]

Only recently have contributions to other cell-types – such as pericytes, vascular SMCs and inflammatory cells – have begun to be appreciated in bAVMs.[6] How molecular cross-talk between these cell types is disrupted remains poorly understood in bAVMs, and systematic characterization of other cell types, including astrocytes and resident microglia, has yet to be performed.[2] A more comprehensive understanding of the dysfunction of the neurovascular unit in its entirety will likely yield additional targets for therapeutic development.

Conclusions

Pathogenesis of non-hereditary bAVMs is not clearly understood, however, the identification of gene mutations and genetic risk factors associated with bAVMs has enabled understanding of the genetics for this disease and provided new insights. Knowledge from several research aspects such as gene mutation, signal pathway, and molecular cross-talk of microvasculature may deepen our understanding of the pathogenesis and provide novel therapeutic approaches to bAVMs in the near future.

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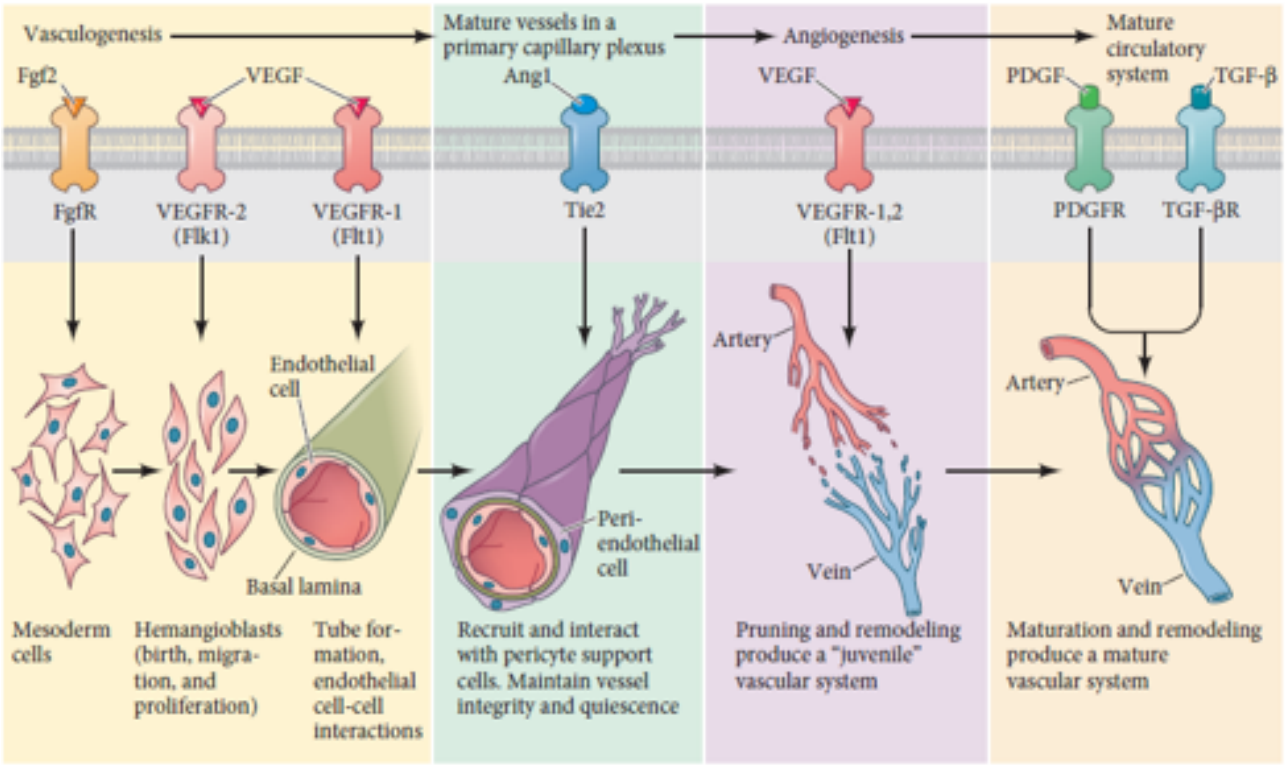

Figure 1

Vasculogenesis and angiogenesis. Vasculogenesis involves the formation of blood islands and the construction of capillary networks from them. Angiogenesis involves the formation of new blood vessels by remodeling and building on older ones. Angiogenesis finished the circulatory connections begun by vasculogenesis. The major paracrine factors involved in each step are shown at the top of the diagram, and their receptors (on the vessel-forming cells) are shown beneath them. (Gilbert Scott F, Barresi Mechael J, Developmental Biology, 11th Ed, Sinauer Associates, 2016)

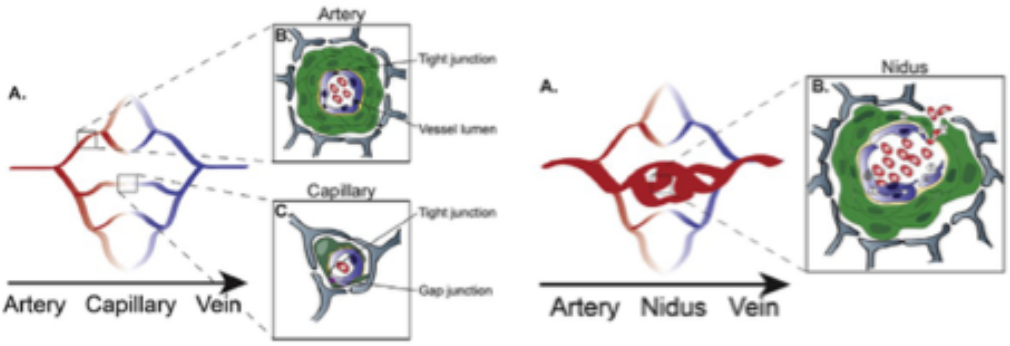

Figure 2
Lt: cells composition of the cerebrovasculature along the arterial-venous axis[2]
Endothelial cells (blue) are embedded within a protein rich basement membrane (yellow).
In arteries, concentric rings of vascular smooth muscle (green) and then astrocyte end-feet (gray) surround the engothelium.
Rt: Alterations in cerebrovascular structure and cytoarchitecture in brain arteriovenous malformations. Heterogeneity of the endothelium (blue) is observed: (1) endothelial degeneration; and (2) endothelial

hyperplasia. (3) vascular smooth muscle cells (green) are reduced. (4) alterations in vascular basement membrane proteins (yellow) (5) loos or alteration of structural components of the vascular wall and disruption of endothelial tight junctions.

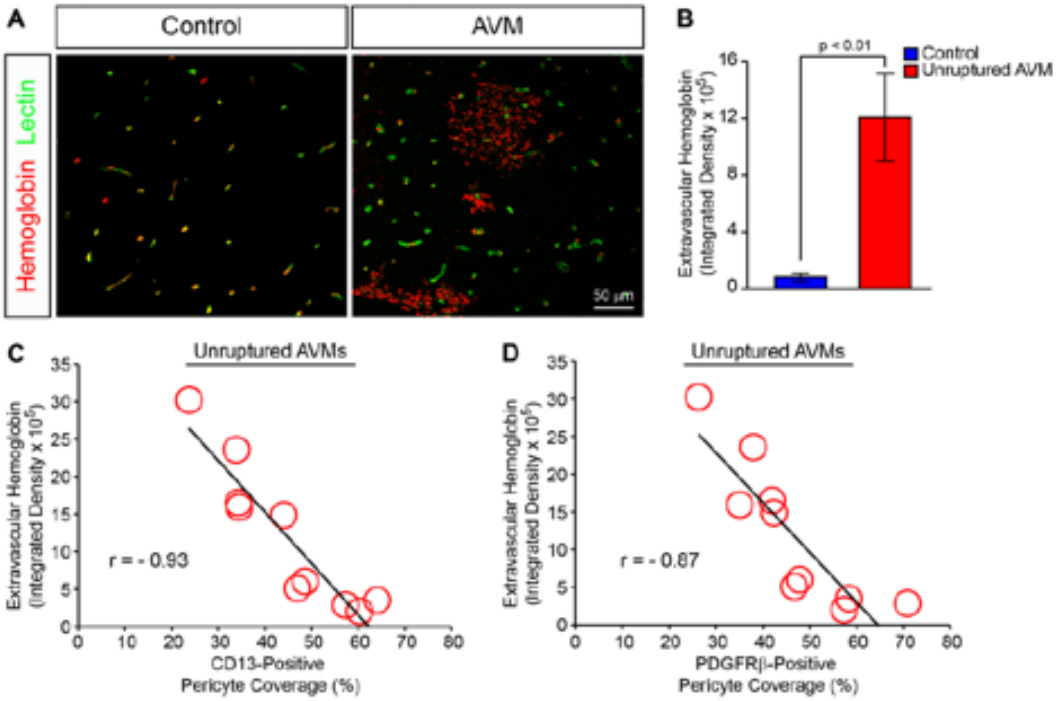

Figure 3
Reductions in vascular pericytes are associated with acute cerebral microhemorrhage in unruptured bAVMs.[6]

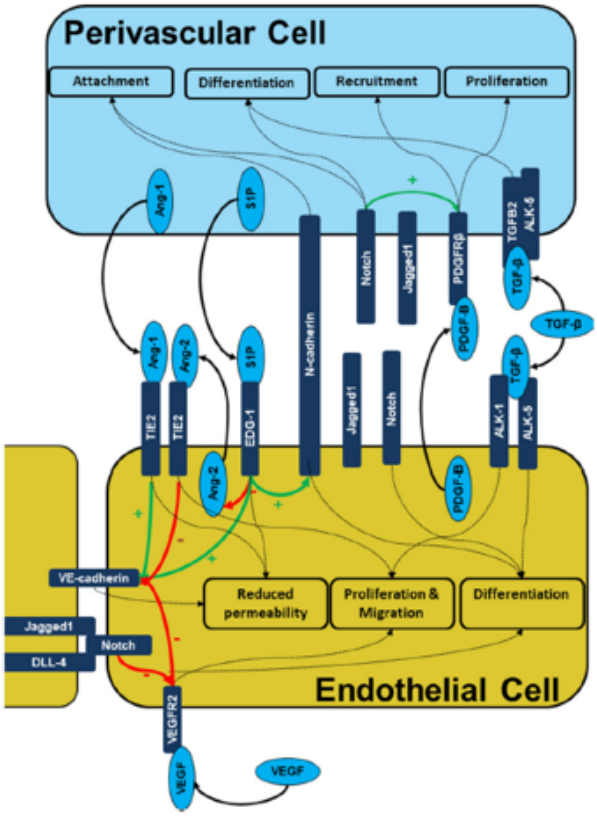

Figure 4
Signaling during endothelial-pericyte cross-talk.[30]
The figure illustrates the intercellular signaling responsible for cell recruitment, differentiation, and maturation as well as vessel stability is built on multiple receptor complexes.

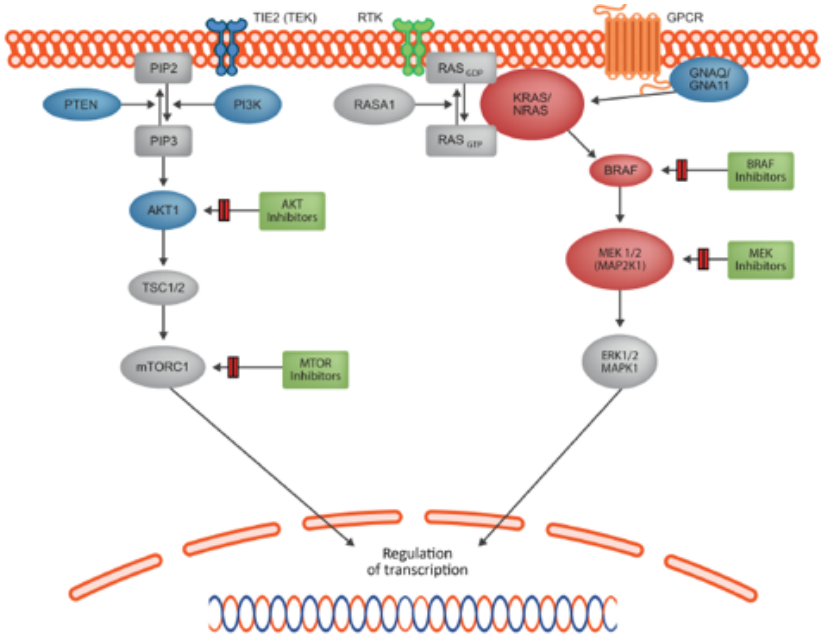

Figure 5
Schematic summary of the known and newly identified signaling proteins affected by mosaic mutations that lead to a vascular malformation phenotype.[20]

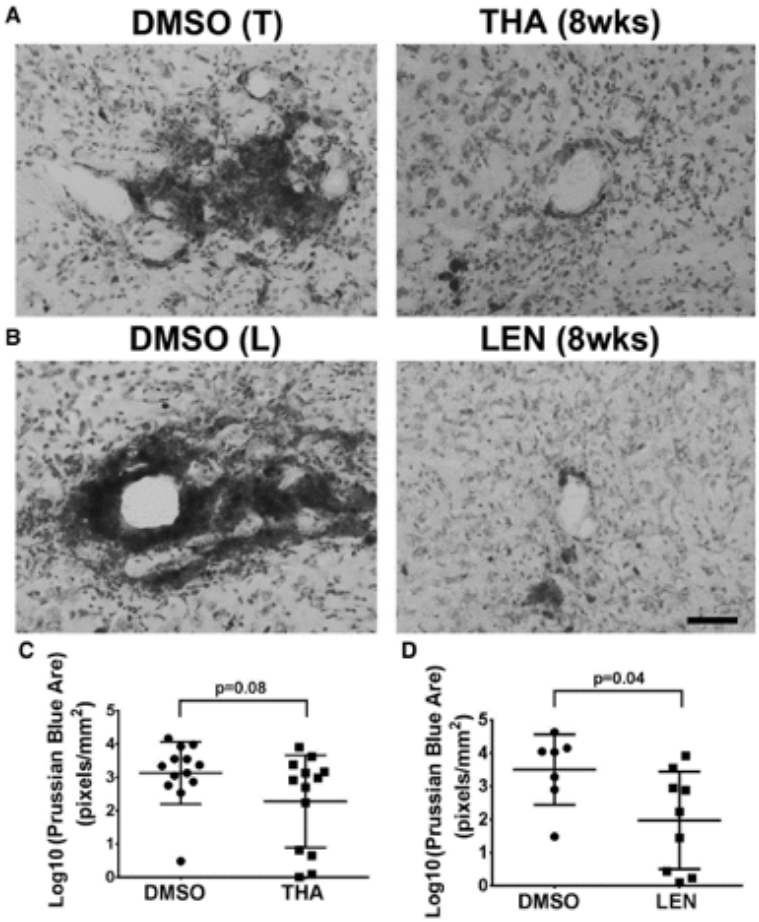

Figure 6
Thalidomide (THA) and lenalidomide (LEN) reduces hemorrhage in the establishment bAVM.[10]