Cerebral arteriovenous malformations. Part 1: cellular and molecular biology

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Object. The scientific understanding of the nature of arteriovenous malformations (AVMs) in the brain is evolving. It is clear from current work that AVMs can undergo a variety of phenomena, including growth, remodeling, and/or regression—and the responsible processes are both molecular and physiological. A review of these complex processes is critical to directing future therapeutic approaches. The authors performed a comprehensive review of the literature to evaluate current information regarding the genetics, pathophysiology, and behavior of AVMs.

Methods. A comprehensive literature review was conducted using PubMed to reveal the molecular biology of AVMs as it relates to their complex growth and behavior patterns.

Results. Growth factors involved in AVMs include vascular endothelial growth factor, fibroblast growth factor, transforming growth factor β, angiopoietins, fibronectin, laminin, integrin, and matrix metalloproteinases.

Conclusions. Understanding the complicated molecular milieu of developing AVMs is essential for defining their natural history. Growth factors, extracellular matrix proteins, and other molecular markers will be the key to unlocking novel targeted drug treatments for these brain malformations. (DOI: 10.3171/2009.2.FOCUS09316)

Key Words • arteriovenous malformation • molecular biology • vascular biology

Brain AVMs, first described as “erectile tumors” > 200 years ago, are defined as the direct communication of arteries to abnormally tortuous and dilated veins without interposing capillaries. The natural history of these malformations remains uncertain, as they are usually treated soon after diagnosis. Traditionally, they have been thought of as congenital lesions resulting from abnormal vascular development, and their association with well-defined genetic disorders, such as ataxia telangiectasia, Wyburn-Mason syndrome, Osler-Weber-Rendu-Sheehan disease, and Sturge-Weber syndrome, appears to support this conclusion. However, many authors have suggested that they are not static congenital lesions, but are dynamic with the ability to grow, regress, and even reappear as de novo brain AVMs after complete resection or radiosurgery. This behavior has historically led AVMs to be classified as “proliferative capillaropathies,” “erectile tumors,” “metamorphotic dysplastic vessels,” or lesions with “autonomic growth.”

Twelve cases of recurrent brain AVMs have been reported in the literature between 1966 and 1998. By defining recurrence as the reappearance of an AVM after a posttreatment angiogram has documented complete obliteration via surgery (with or without adjunctive endovascular therapy) or radiosurgery, another 22 cases can be counted (1998–2007). One case involved a brain AVM that recurred at a different location after radiosurgery. Although these data show the existence of recurring AVMs, the actual rate of recurrence is unknown because of the scarcity of long-term follow-up data in the literature. The growth or regression of a sporadic AVM in the
brain has been described in several case reports, challenging the concept that these malformations are static entities. One series of 106 patients with AVMs has demonstrated that > 50% of the lesions increased in size, whereas 9% decreased over a mean period of 8 years without intervention. Remodeling within these lesions can lead to changes in their angiographic appearance over time.

To appreciate why these changes occur, it is necessary to review the different forces acting on the growth and behavior of individual AVMs. These forces begin during vasculogenesis and angiogenesis in the fetal brain, when a number of cellular behaviors (apoptosis, migration, differentiation, and proliferation) affect the potential development of vascular lesions. The embryology relevant to vascular development will be reviewed in this paper, and the molecular factors, including growth factors and extracellular proteins, will be explored. Finally, we consider the relevant physiology and vascular architecture in reference to the behavior of brain AVMs.

Vasculogenesis and Angiogenesis

The vascular and nervous systems are the 2 earliest developing organs in vertebrates. There are 2 neuronal cerebral cortical migrating populations: radial and tangential. Cerebral cortical vascular development has 2 components—a ventriculopetal (most abundant) and a ventriculofugal (least abundant). Both develop along and parallel to the radial neurogenesis. Thus, the early cerebral vascular system and cortical glial/neuronal development of the ventricular, subventricular, subcortical plate, and cortical plate zones progress through morphogenesis predominantly in a parallel anatomical fashion. The cellular titers of the VEGF family within the ventricular zone are high during the early period of corticogenesis, which is a key factor in the migration of the centripetally directed vessels.

**FIG. 1. A:** Schematic showing 2 neuronal cerebral cortical migrating populations: radial and tangential. Generally the radial glia and radial neuronal migration are perpendicular to the tangent of the cortical surface. The radial neuronal migration projects from the ventricular surface of the cortical mantle to the pia marginal layer. The tangential neuronal migration from the ganglionic eminence of the striatum moves parallel to the cortical surface and is unassociated with any significant counterpoint vascular development. The cerebral cortical vascular development has 2 components: a ventriculopetal (most abundant) and a ventriculofugal (least abundant). Both develop along and parallel to the radial neurogenesis. Thus, the early cerebral vascular system and cortical glial/neuronal development of the ventricular, subventricular, subcortical plate, and cortical plate zones progress through morphogenesis predominantly in a parallel anatomical fashion. The cellular titers of the VEGF family within the ventricular zone are high during the early period of corticogenesis, which is a key factor in the migration of the centripetally directed vessels. The molecular environment is critical to cell fate and vascular development (Figs. 1A and B and 2).

There are 2 phases to vascular development: vasculogenesis and angiogenesis. Vasculogenesis is the formation of primitive endothelial cells (VEGF is the earliest vascular marker). Angiogenesis is defined by morphogenic changes hallmarking by ongoing “pruning” of the premature vascular plexus, the formation of new branches, and stabilization of the definitive vessel. Over the past 2 decades, an accumulating body of information has demonstrated some of the chemical and molecular factors critical to vasculogenesis and angiogenesis (Table 1). The progenitor cells destined to evolve into angioblastic cells can be modulated by FGF. The roles of HIF and sonic hedgehog are suspected of influencing the induction of these vascular progenitor cells that become endothelial and hematopoietic cells. The hemangioblastic progenitor cells clump together and are the earliest cells to express...
VEGF-A ligands and VEGFR-2. These angioblastic cells can form angiocysts, which then migrate and ultimately fuse forming a primitive capillary plexus. This complex of an immature VEGF/VEGFR–positive vascular plexus defines the end of vasculogenesis and a downregulation of VEGF. The foundation for specific regions of new branching or pruning of primitive plexus radicals, caused by apoptosis, emerges. This pruning of the vessels marks the transition to angiogenesis.

The primitive capillary plexus has 3 basic characteristics. The surrounding mesenchymal cells migrate around the primitive capillary plexus and differentiate into SMCs and pericytes. As noted, there is programmed cell death to specific regions of the primitive capillary plexus, causing a remodeling of the vascular architecture. Finally, new branches form, and stabilization of the mature vascular structure occurs. The molecular aspects of vasculogenesis and angiogenesis are complex; however, aberrations of molecular signaling can lead to abnormal vascular development.

Normal embryogenesis requires the timely expression of a balance between anti- and proapoptosis, cell attraction and repulsion, cell migration and cell fixation, and cell proliferation and cell differentiation. For endothelial cells to enter regions of organogenesis, the activation of migration, branching, and proliferation factors as well as substrates is required. However, differentiation into more stable and mature vascular elements cannot occur during the proliferation phase. Thus, downregulation of the complex processes responsible for these events must occur. The subsequent upregulation of factors influencing differentiation and maturation is eventually required to become the key and major players. The magnitude and complexities of growth factors, morphogens, and cytokines; the critical timelines of upregulation and downregulation; and the spectrum of isoforms in any given family of activators or inhibitors is just beginning to be understood in vasculogenesis and angiogenesis.

**Molecular Biology of AVMs: VEGF, TGFβ, and ANGs**

The altered expression of up to 900 genes has been associated with AVMs. Perhaps > 300 genes are upregulated and almost 560 are downregulated in cerebral vascular malformations. These genes encode growth factors, cell adhesion and ECM factors, inflammatory factors, MMPs, and endocrine hormones. Different cell types, including endothelial cells, vascular SMCs, and inflammatory cells, have been examined to understand the pathogenesis of AVMs. Tables 1–3 summarize several molecular factors involved in vascular development and their role in AVMs.

Vascular endothelial growth factors consist of 6 subtypes labeled VEGF-A to VEGF-E and placental growth factors. The main isoform is VEGF-A. Furthermore, each subtype can have several splice variants. Vascular endothelial growth factor–A is expressed in astroglia adjacent to AVMs, whereas VEGF-C and -D expression is higher in malformations with larger niduses. Moreover, VEGF-C and -D may also contribute to AVM growth. All VEGF subtypes and their variants bind vascular endothelial tyrosine kinases (VEGFRs). Specific receptors include Flt-1 (VEGFR-1) and KDR (Flk-1 or VEGFR-2).
TABLE 1: Chemical and molecular factors critical to vasculogenesis and angiogenesis* 

<table>
<thead>
<tr>
<th>Factor</th>
<th>Receptor</th>
<th>Actions</th>
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<tbody>
<tr>
<td>VEGF subtypes: A–E &amp; placental growth factor†</td>
<td>TKR: FLT1, KDR</td>
<td>endothelial cell replication, migration, differentiation, survival</td>
</tr>
<tr>
<td>TGF‡</td>
<td>Type I/II</td>
<td>biphasic: in early stages of development, TGFα is inhibitory to vascular endothelial proliferation; in later stages, it becomes stimulatory; activates differentiation &amp; migration of surrounding mesenchymal cells in intercellular matrix to differentiate into pericytes &amp; SMCs</td>
</tr>
<tr>
<td>TGFα§</td>
<td>EGF</td>
<td>stimulates cell growth pathways</td>
</tr>
<tr>
<td>bFGF¶</td>
<td>TKR: FGFR-1 (highest affinity)</td>
<td>stimulates cascade of secondary messengers including MAPKs, resulting in angiogenesis; acts on fibrocytes, myocytes, endothelial cells, &amp; neuronal cells</td>
</tr>
<tr>
<td>ANG-1***</td>
<td>TIE2 agonist</td>
<td>regulates recruitment of pericytes &amp; smooth muscle precursors; promotes interactions between endothelial cells &amp; support cells to stabilize vessels; like VEGF, heavily involved in angiogenesis</td>
</tr>
<tr>
<td>ANG-2***</td>
<td>TIE2 antagonist</td>
<td>regulates recruitment of pericytes &amp; smooth muscle precursors; deconstructive signal that promotes vascular remodeling/destabilization</td>
</tr>
<tr>
<td>Delta††</td>
<td>Notch</td>
<td>arterial development; mutated delta/notch associated with CADASIL</td>
</tr>
<tr>
<td>endoglin‡‡</td>
<td>response to TGFα + SMADS</td>
<td>increase endothelial proliferation; activates mesenchymal cells to differentiate with intercellular matrix to pericytes &amp; SMCs; these migrate &amp; encircle endothelial conduits secondary to TGFα influence.</td>
</tr>
<tr>
<td>neuropilin 1 &amp; 2§§</td>
<td>cofactor for KDR</td>
<td>neuropilin-1 is involved in arterial development, while neuropilin-2 is involved in venous development</td>
</tr>
<tr>
<td>ephrin B2¶¶</td>
<td>ephrin B4</td>
<td>regulation of angiogenesis by Eph/ephrin &quot;repulsive&quot; interactions</td>
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* CADASIL = cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; EGF = epidermal growth factor; FGFR-1 = fibroblast growth factor receptor 1; FLT1 = also known as VEGFR-1; KDR = also known as FLK1 or VEGFR-2; TIE2 = also known as TEK; TKR = tyrosine kinase receptor.
† References 46, 59, 71.
‡ Reference 78.
§ Reference 1.
¶ References 5, 44, 67.
** References 45, 81, 92.
†† Reference 69.
‡‡ Reference 54.
§§ Reference 81.
¶¶ Reference 49.

On ligand binding, the activated pathway leads to the replication, migration, differentiation, and survival of endothelial cells (Figs. 3–5). Vascular endothelial growth factor is a soluble factor that has a potent mitogenic effect on vascular endothelial cells in large and small vessels both in vivo and in vitro. Notably, the development of the embryonic vascular system is highly dependent on the actions of soluble factors. Vascular endothelial growth factor is expressed at high levels during embryonic development, but its expression is normally suppressed in the adult cerebral vasculature. Interestingly, VEGF is highly expressed in children with recurrent cerebral AVMs. The expression of VEGF is high in the endothelial layer and media of vessels in AVMs. Pathological studies have shown that almost three-quarters of AVMs resected following incomplete embolization express VEGF and Flk-1, whereas the endothelium of only one-quarter of AVMs not preoperatively embolized expresses these factors. This finding may explain why partially obliterated AVMs recur.

Vascular endothelial growth factor receptors, normally expressed only by endothelial cells of the blood vessels and vascular wall SMCs, may play a pivotal role. The increased expression of the Flt-1, Flt-4, and Flk-1 receptor subtypes occurs around AVMs. The Flk-1 epitope is biphasic. In the early stages of development, TGFβ is inhibitory to vascular endothelial proliferation; in later stages, it promotes the differentiation of surrounding mesenchymal cells to differentiate into pericytes and SMCs. With the influence of PDGF, ANG, and TIE receptors, these cells encircle the primitive vascular plexus endothelium as part of angiogenesis.

The roles of the TGF family in vascular development are complex and multidimensional; for example, TGFβ is biphasic. In the early stages of development, TGFβ inhibits vascular endothelial proliferation; in later stages, it promotes the differentiation of surrounding mesenchymal cells to differentiate into pericytes and SMCs. With the influence of PDGF, ANG, and TIE receptors, these cells encircle the primitive vascular plexus endothelium as part of angiogenesis.
TABLE 2: Molecular factors involved in vascular development

<table>
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<tr>
<th>Factor</th>
<th>Function</th>
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<tr>
<td>fibronectin &amp; laminin†</td>
<td>found in BM of vascular beds, providing structural support to vessel wall by anchoring endothelial cells to underlying internal elastic membrane &amp; SMCs; ratio of laminin to fibronectin provides indicator of vessel wall maturity</td>
</tr>
<tr>
<td>integrins‡</td>
<td>anchor themselves to the ECM &amp; transducer signals from the ECM to the cell interior; regulates cell adhesion, migration, invasion, proliferation, survival, &amp; apoptosis</td>
</tr>
<tr>
<td>MMP§</td>
<td>zinc-dependent endopeptidases that degrade variety of ECM proteins</td>
</tr>
</tbody>
</table>

* BM = basement membrane.  † References 23, 28, 52, 54, 63, 65, 77. ‡ References 12–14, 30, 31, 42, 43, 80. § Reference 88.

complex, is mutated in hereditary hemorrhagic telangiectasia. Cerebral AVMs are found in up to 10–25% of patients with this genetic disorder. The ENG mutation is associated with a higher prevalence of cerebral AVMs. An important downstream cofactor, endoglin, enhances ALK1 effects on endothelial proliferation. Mutations of endoglin and/or ALK1 can result in vascular abnormalities such as hereditary hemorrhagic telangiectasia and arteriovenous fistulas. The failure of ALK5/endoglin, which normally enhances endothelial development, may lead to a deficiency in the capillary plexus maturation bridging the arteriovenous interval, which results in arteriovenous fistula development. The ALK5 activation may modulate the endothelial cellular inhibition of proliferation and migration. Transforming growth factor–β plays a complex role in angiogenesis, and the effects of its downstream substrates may be critical in de novo AVM formation (Figs. 6–8).

Angiopoietins regulate the recruitment of pericytes and smooth muscle precursors and are involved in angiogenesis and vascular stability. More specifically, ANG-1 uses an endothelial cell–specific tyrosine kinase (TIE2, or TEK), whereas ANG-2 is a TIE2 antagonist. Angiopoietin 1 promotes interactions between endothelial and support cells to stabilize vessels, whereas ANG-2 is a deconstructive signal that promotes vascular remodeling or destabilization. Although downregulated in the mature quiescent vasculature, ANG-2 expression is upregulated in brain AVMs. Phenotypes observed in vivo because of the overexpression of ANG-2 are similar to brain AVM vessels, with abnormally dilated vessels that lack a mature perivascular cellular support structure. Furthermore, TIE2 has upregulated autophosphorylation in AVMs (Fig. 9). This gene has been found to be mutated in the systemic familial venous malformation syndrome, which has many similarities to AVMs. Angiopoietin 1 protein levels are 30% lower, ANG-2 protein levels are 800% higher, and ANG-2 mRNA levels are 40% higher in brains with AVMs compared with controls.

The growth potential of AVMs and their regression may involve opposing forces supporting or inhibiting angiogenesis and vascular remodeling. A phenomenon noted in tumor angiogenesis is the need for both VEGF and ANG-2; if VEGF is absent, vessels will undergo regression if ANG-2 is present. Furthermore, the 2 opposing actions of the ANGs, ANG-1 and ANG-2, can actually change depending on vessel size and concentration levels. For example, ANG-2 at high concentrations can actually switch from an antagonist to agonist for the TIE2 receptor. In general, ANG-1 and ANG-2 appear to have opposing effects: one aids the development of a structurally sound vascular network, while the other creates a disorganized and defective network.

**Hyperangiogenic Environment**

The hypoxic environment surrounding the brain AVM is thought to stimulate the secretion of all the aforementioned angiogenic factors and their levels of activity. It is known that both hypoxia and ischemia lead to the secretion of VEGF, its receptors, and other growth factors. Astrocytes have been shown to secrete VEGF when exposed to a hypoxic environment. The VEGF gene promoter contains a response element called HIF-1, which can increase VEGF expression 30-fold within minutes of being exposed to hypoxic conditions (Fig. 10). This hypoxic environment may be induced by arteriovenous shunting through the AVM.

Several studies have supported the idea of hypoxic/ischemic angiogenic stimulation in brain AVMs. Vascular endothelial growth factor and HIF are expressed in a much higher percentage of embolized AVMs compared with emergently surgically treated malformations, alluding to hypoxic stimulation of VEGF within the local AVM environment. In cases of partial embolization (or occlusion), local endothelial hypoxia can result within the obliterated portion of the AVM nidus. This hypoxic condition may
Fig. 3. Schematic illustrating the VEGF ligand family and VEGFR family. Vascular endothelial growth factor/VEGFR-1 and -2 predominantly service vasculogenesis (and somewhat lymphogenesis). Vascular endothelial growth factor 3 is essential to lymphogenesis.

Fig. 4. Schematic demonstrating the VEGF-A ligand initiating a homodimerization of VEGFR-2. This activates receptor kinases and autophosphorylation of tyrosines on the cytoplasmic portion of the VEGFR-2, which activates a series of cytoplasmic and membrane-bound substrates (Grb, Ras, Raf, and MAPK). The phosphorylation of MAPK initiates entry into the nucleus and the activation of transcription factors for endothelial proliferation, endothelial survival, endothelial fenestration/permeability, and development of primitive capillary plexuses. Note that PAL-E is present in nonbarrier vessels and absent in barrier vasculature elements such as the brain (blood-brain barrier) and retinal vessels (retinal blood barrier). With the overexpression of VEGF-2, PAL-2 endothelial marker increases with an associated capillary permeability. Neuropilin-1/neuropilin-2 are obligate coreceptors that enhance VEGF-A/VEGFR-2. In contrast, VEGFR-1 has some inhibitory effect on VEGF-2.
mediate neovascularization and underscores the need for complete obliteration of the nidus. Note, however, that VEGF and HIF can also be upregulated independently of embolization, indicating other mechanisms at work.\textsuperscript{57}

It is also important to consider that while the environment surrounding the brain AVM may be facing an ischemic/hypoxic insult, the nidus is actually well perfused with arterial blood at high flow rates. Since the nidus does not generally contain any neuronal tissue and thus has little potential to become ischemic, factors affecting the growth or regression of the nidus may be different from those that affect the surrounding vessels. It is also possible that microhemorrhage or frank hemorrhage act as a stimulus for angiogenesis. In a process described as “hemorrhagic angiogenic proliferation,” certain blood factors, such as hemosiderin, fibrin, and fibrin split products, are known (experimentally) to stimulate vascular growth.\textsuperscript{3,17,132}

**Structural Proteins and the ECM**

Integrins, immunoglobulins, cadherins, and selectins, along with the ECM milieu, may have significant effects on angiogenesis, vasculogenesis, and brain AVM growth. Arteriovenous malformations in the brain exhibit a mature vessel wall phenotype, as evidenced by intense laminin expression localized in and around the internal elastic lamina and the absence of significant fibronectin expression.\textsuperscript{58,106} Fibronectin and laminin are found in basement membranes of normal vascular beds, providing structural support to the vessel wall by anchoring endothelial cells to the underlying internal elastic membrane and smooth muscle layers. The relative ratio of laminin to fibronectin expression provides a partial indicator of vessel wall maturity. Immature vessels are associated with a fibronectin-rich matrix deficient in laminin. Mature vessels express laminin more consistently and contain little to no fibronectin in their matrix.\textsuperscript{58,105} The presence of laminin in AVMs probably contributes to the resilience of these vessels in the face of hemorrhage compared with CMs (immature vessels), which are prone to repetitive microhemorrhages.\textsuperscript{58,61}

Integrins, a superfamily of receptors, are anchored to the ECM and transduce signals from the extracellular environment to the cell’s interior.\textsuperscript{13,49,50,106} At least 16 distinct $\alpha$ and 8 distinct $\beta$ integrin subunits have been identified as having the ability to regulate a variety of cellular responses, including adhesion, migration, invasion, proliferation, cell survival, and apoptosis (Fig. 11).\textsuperscript{11,12,34,35} Integrins are required for the optimal activation of growth factor receptors including VEGF and bFGF—2 factors important in the growth of brain AVMs.\textsuperscript{2,12,15,20,28,51,52} The ligand-receptor interaction in these pathways becomes optimal only under appropriate conditions for cell attachment, a process strictly regulated by integrins.\textsuperscript{11,20,34,35}

Both integrin $\alpha_\beta_i$ and $\alpha_\beta_\beta$ are strongly associated with AVMs.\textsuperscript{74,109} Integrin $\alpha_\beta_i$ is an important regulator of angiogenesis from the early/immature phases to the late maturation phases, whereas $\alpha_\beta_\beta$ is mainly responsible for coordinating the early stages of angiogenesis.\textsuperscript{11,12,28,109} The expression of $\alpha_\beta_i$ has been shown to be greater in brain
Fig. 6. Schematic showing the ligand TGFβ activates a Type II receptor (cytoplasmic domain kinase) that phosphorylates and forms a heterodimer with a Type I transmembrane receptor (there are 2 types of Type I receptors: ALK1 and ALK5). Type III receptors are inhibitory.

Fig. 7. Schematic tracing the process through which the TGF-1/receptor complex can activate 2 subtypes of Type I receptors: ALK1 and ALK5. Activation of different cytoplasmic SMADs then activates specific nuclear transcription. Endothelial-specific subtype ALK1 TGFβ receptor Type I activates SMAD1/SMAD5. Subtype ALK5 TGFβ receptor Type I activates SMAD2/SMAD3.
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Fig. 8. Schematic revealing the path by which both SMAD1/SMAD5 and SMAD2/SMAD3 activate SMAD4 that enters the nucleus and initiates specific transcription factors. Endothelial-specific subtype ALK1 TGFβ receptor Type I increases endothelial migration and proliferation. The ALK5 TGFβ receptor Type I inhibits proliferation and migration of endothelial cells. Endoglin blocks the ALK5 pathway and enhances the ALK1 pathway.

Fig. 9. Schematic showing processes associated with TIE2. The TIE2 receptor protein kinase is activated by ligand ANG-1 and inhibited by ANG-2. Activation of this endothelial protein tyrosine kinase receptor possibly activates the transcription of PDGF, EGF, and VEGF. This activates mesenchymal cells to migrate toward the primitive endothelial vessel and also elaborates ANG isoforms. Transforming growth factor-β is also secreted by mesenchymal cells and in early embryogenesis inhibits endothelial proliferation. Moreover, in an autocrine manner mesenchymal participates in its differentiation into SMCs and pericytes. In TIE2-null animals (−/−) in the presence of VEGF-A/VEGFR-2, there is the formation of large blood vessels with fewer branches, clumping of the endothelial cells, and loss of supporting cells approximating to the endothelial cells. The same is observed with ANG-1 ligand−null animals. Angiopoietin-2 also binds with TIE2 with high affinity but does not activate the tyrosine kinase receptor; however, it inactivates the receptor and blocks its availability to ANG-1 ligand. If ANG is transgenically overexpressed, it will yield a phenotype equivalent to an ANG-1−null animal. This complex relationship of ANG-1 and -2 with TIE receptors can be related to angiogenic defects and brain AVMs.
AVMs compared with that in CMs specifically in the endothelium, subendothelium, and media.\textsuperscript{109} The \(\alpha_\text{v}\beta_3\) subtype is expressed in both brain AVMs and CMs.\textsuperscript{132,133} The \(\alpha_\text{v}\beta_3\) antagonists, potential pharmacological agents in the treatment of AVMs, have a minimal effect on preexisting blood vessels\textsuperscript{13,14,16} and are limited in cellular distribution. They are completely absent in epithelial cells, which are usually nonspecifically targeted with other drugs.\textsuperscript{9,66}

Matrix metalloproteinases, specifically MMP-9, may play a key role in brain AVM growth and spontaneous hemorrhage by affecting vascular stability and angiogenesis as they degrade pericellular substrates.\textsuperscript{120} In murine models of abdominal aortic aneurysms, targeted deletions of the \textit{MMP9} gene specifically suppress experimental aneurysm formation.\textsuperscript{100} Furthermore, MMP-9 is increased relative to tissue inhibitors of metalloproteinases (TIMP-1, -3, and -4) in brain AVMs.\textsuperscript{45} Follow-up experiments have shown that tetracyclines (doxycycline and minocycline) have a dose-response relationship for inhibiting stimulated cerebral MMP activity.\textsuperscript{70} Tetracyclines may have a future role in supporting the vascular stability of these lesions.

\textbf{Cell Types}

Endothelial cells play a fundamental role in vasculogenesis, angiogenesis, and vascular remodeling. Postnatally, endothelial cells are quiescent but possess proliferative potential. Studies using Ki 67 (or MIB-1) and proliferating cell nuclear antigen have demonstrated that brain AVMs have a significantly higher rate of endothelial cell turnover compared with that in control vessels, suggesting the presence of active angiogenesis and/or vascular remodeling in these lesions.\textsuperscript{42,44,121} Hashimoto et al.\textsuperscript{42} have concluded that the rate of endothelial cell turnover in brain AVMs is somewhere between that of normal quiescent cerebrovascular endothelial cells and endothelial cells in progressing tumors.

Vascular SMCs also play an essential role in vessel maturation during vasculogenesis and angiogenesis. The pathogenesis of AVMs partly involves the abnormal assembly or differentiation of vascular SMCs, which have the unique potential to differentiate into various stages of maturity with each stage characterized by the unique expression of phenotypic markers.\textsuperscript{127} Specifically, the varied expression of 3 types of proteins—\(\alpha\)-SMA, myosin heavy chains (including the isoforms that result from alternative splicing of mRNA, SM1, and SM2, and 2 other isoforms, SMemb, and NMHC-A), and a recently identified cytoskeletal protein called smoothelin—leads to a range of vascular smooth muscle morphology. The \(\alpha\)-SMA is
expressed in all stages of vascular SMC differentiation, whereas myosin heavy chains are expressed in only the later stages; within the family of myosin heavy chains, SM2 is expressed later than SM1. Smoothelin is found predominantly in the mature SMCs and provides the contractile properties. In brain AVMs embedded in paraffin tissue, smoothelin expression is significantly decreased in the large vessels. In cells cultured from brain AVMs, smoothelin was absent. The absence of smoothelin in AVMs is thought to reflect the disappearance of contractile properties resulting from the hemodynamic stress that these lesions encounter. The venous components of cerebral AVMs, on the other hand, show prominent expression of α-SMA and myosin, reflecting their new “arterialization.” The level of these proteins (especially smoothelin) may influence AVM rupture rates.

Recently, the role of MAPK pathways in the vascular SMCs of brain AVMs has been elucidated. The MAPKs are a family of serine/threonine kinases that are highly conserved and ubiquitously expressed in response to a variety of stimuli. Specifically, ERKs, members of this MAPK family, may help to explain brain AVM dynamics. Previous studies have shown that ERKs are activated in response to growth factors, oxidative stress, increases in intracellular calcium levels, and/or glutamate receptor stimulation. After stimulation, ERK induces cellular proliferation and transformation. Takagi and colleagues have suggested that the activation of the ERK pathway depends on the chronic increase in blood flow within the vasculature. It has been well established that chronic increases in blood flow can upregulate shear-induced genes, which induce vascular remodeling. In brain AVMs, intranidal vessels are exposed to abnormally high blood flow that creates high shearing forces, which can similarly activate shear-induced genes. Takagi and colleagues have shown that phosphorylated ERK is upregulated in the SMCs of the media layer in brain AVMs. The activation of this pathway signals SMC proliferation, which contributes to the vascular remodeling and growth/regression of these lesions.

The role of inflammatory cells in brain AVMs was considered as early as 1949, when Sorgo hypothesized that the combination of “circulatory factors and perivascular leukocytes” might stimulate the growth of vessels in these lesions. An inflammatory cell infiltrate is present in both ruptured and unruptured cerebral AVMs. After hemorrhage, the inflammatory response in the brain includes persistent infiltration of macrophage/microglia for up to 4 weeks. These inflammatory cells have been found in and around AVM vessel walls as well as in surrounding brain parenchyma. It is conceivable that the presence of inflammatory cells in or around AVM vessels is a gauge for their growth or remodeling.

Interleukin-6, an inflammatory cytokine with a possible role in remodeling the AVM wall, is released by endothelial cells within the AVM nidus. It may contribute
to vascular wall instability by stimulating the release of MMP.\textsuperscript{22,29} An identified polymorphism of this interleukin, IL6-174G allele, reportedly increases the risk of intracranial hemorrhage in patients with brain AVMs.\textsuperscript{97}

\textit{Apoptosis in Brain AVMs}

Apoptosis balances cell proliferation. Possible stimuli activating the proangiogenic pathways in brain AVMs include the following: 1) high local concentrations of oxidants such as oxygen free radicals, NO, and oxidized low-density lipoproteins; 2) inflammatory cytokines such as interleukin-1β, tumor necrosis factor–α, and interferon-γ; 3) direct interaction with inflammatory cells, particularly T lymphocytes and macrophages; and 4) hemodynamic stress and changes in perinidal cerebral blood flow.

In situ DNA fragmentation labeling shows evidence of apoptotic activity within and around brain AVMs.\textsuperscript{122} Two major proangiogenic pathways for AVMs are currently known: the death receptor pathway involving caspase-8, and the mitochondrial pathway involving oxidative stress, the release of cytochrome-C, and the activation of caspase-9. Perinidal neuronal loss around AVMs is associated with Fas-associated death domain proteins and caspase-8.\textsuperscript{123} The expression of caspase-3, a factor in apoptotic pathways, has been observed in the endothelium, media, and perivascular tissue.

Cells cultured from surgical specimens of brain AVMs demonstrate a proliferation rate 1.8–6.4 times greater than that in human umbilical veins, human arterial endothelial cells, and human microvascular cells. Furthermore, this high proliferation rate was sensitive to some well-known inhibitory factors such as dexamethasone.\textsuperscript{131} Brain AVMs are also associated with the ets-1 protooncogene, and ets-1 has been shown to regulate genes involved in the proliferation and apoptotic processes. The low response to cytokines, the higher propensity to proliferate, and the ets-1 expression all suggest that brain AVMs proliferate because of a dysregulated apoptotic process. Although its role is unclear, dysregulated proliferation/apoptosis within and around brain AVMs may have a part in remodeling these lesions over time.

\textbf{Conclusions}

Because > 900 genes are thought to be involved in the pathogenesis of vascular malformations, elucidating the molecular characteristics of these lesions and their growth patterns has proven to be challenging.\textsuperscript{23} Most laboratory work on brain AVMs has been limited to immunohistochemical studies, and thus conclusions have been confined to a biologically inactive system. Dynamic in vivo models, such as lines of endothelial cell cultures and fibrocyte cultures from resected AVMs, are a potential research area. The identification of molecular targets, such as integrins, ANGs, VEGF, HIF, and TGFβ, is critical for targeted drug discovery. The future of endovascular treatment may involve direct infusions of antiangiogenesis agents or embolizations utilizing material coated with antiangiogenic factors.

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