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Molecular, Cellular, and Genetic Determinants of Sporadic Brain Arteriovenous Malformations

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Brain arteriovenous malformations (AVMs) are a significant cause of hemorrhagic stroke in children and adults.¹ They can occur as part of hereditary syndromes, such as capillary malformation–arteriovenous malformation syndrome and hereditary hemorrhagic telangiectasia, in which they result from mutations in genes with known or plausible roles in angiogenesis and vascular remodeling, such as *RASA1* and *ACVRL1*.^{2–6} Less is known about the cause of sporadically occurring AVMs, which account for the vast majority of lesions in the general population. The formation of these sporadic lesions is theorized to result from several possible diverse elements that could contribute to a common AVM phenotype, including gain or loss of function genetic mutations,^{7,8} inflammation,^{9–11} impaired blood-brain barrier integrity,^{12,13} and/or a response to injury phenomenon.¹⁴ Current research by the authors is underway to investigate sporadic AVM pathogenesis on these related fronts.

GENETICS

The process by which AVMs develop, progress, and eventually hemorrhage is poorly understood. However, aberrations in blood vessel formation and segregation during embryonic development or adult life are thought to be primordial factors.¹⁵ In vascular development, the establishment of a vascular identity (be it arterial or venous) initiates from molecular signals that result in functional, and subsequently structural, changes.^{16,17} There are several hierarchical signaling pathways that promote or inhibit divergent endothelial cell fates, including hedgehog,¹⁸ vascular endothelial growth factor,^{19,20} notch,^{21,22} TGF-beta,^{23,24} and the ephrin ligand-receptor pathway,²⁵ among others.²⁶ These pathways are crucial regulators of vascular assembly, differentiation, and boundary formation and have

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complex interactions, during both development and adult life. Syndromes with Mendelian inheritance patterns, such as hereditary hemorrhagic telangiectasia, are associated with known mutations in the TGF-beta super-family signaling pathways that result in AVMs. This provides the first clue that a causative genetic basis for the sporadic lesions is possible. However, patients with sporadic AVMs have failed to demonstrate germline mutations or structural variation, and causative somatic mutations in candidate genes (*ACVRL1*, *ENG*, *SMAD4*) have not been identified.²⁷ Several studies have characterized various single-nucleotide polymorphisms in these genes, although their role in affecting gene function, gene expression, or conferring disease susceptibility is not well elucidated.^{28–31} Further study with available tools, namely, next-generation sequencing technology, holds the potential to aid in the discovery of genetic alterations not previously identified and to overcome many of the limitations of conventional techniques, such as hypothesis-driven genotyping and DNA microarray.^{32,33}

In other diseases of the central nervous system in which both sporadic and hereditary forms occur, advanced genomic techniques have been used to identify mutations in genes acting in similar pathways (Table 1). Analysis of next-generation sequencing data has been able to identify focal genetic alterations in sporadic hemangioblastoma,³⁴ port wine stain,³⁵ and meningioma,^{36,37} which are shared with known germline mutations in their syndromic forms. This confirms the long-standing hypothesis that these sporadic phenotypes are genetically related to their hereditary counterparts.

Could sporadic AVMs be linked to the mutations that are known to cause hereditary hemorrhagic telangiectasia or other vascular malformation syndromes? There are 2 main advantages of next-generation sequencing that are being used to answer this question. First, a comprehensive and unbiased analysis of the genome is able to detect alterations in gene pathways outside of those typically implicated in angiogenesis and vasculogenesis, yet result in a similar AVM phenocopy. Second, next-generation sequencing has the sensitivity to detect rare mutations in heterogeneous AVM samples. Surgical AVM samples are a diverse mix of vascular cells (endothelial cells, pericytes, and smooth muscle cells), circulating cells (lymphocytes), glial cells, and fibroblasts with the exact pathologic cell population being unknown (Table 2).^{38–41} Therefore, if the potential causative mutation occurs in only a single cell type (ie, endothelium), the purity of the sample analyzed is low, and the chance of finding that mutation in any single reading of the DNA sequence is proportionally low. Next-generation sequencing overcomes this limitation by reading DNA sequences many times over, with “deep sequencing” of several-hundred-fold coverage being used to effectively identify low frequency events in heterogeneous samples.^{34,42} With the spectacular 6-log drop in the cost of sequencing over the last 12 years, next-generation sequencing platforms are a powerful and cost-effective means for discovering the seemingly cryptic genetic basis of sporadic AVMs.

INFLAMMATION

AVMs are dynamic lesions, and even though the majority of them appear to remain stable, they are well known to disappear,^{43–46} grow,^{47–49} and even recur after removal.^{50–52} If genetic alterations are the predisposing events in sporadic AVM pathogenesis, inflammation

and/or the consequences thereof may be one propagating force that advances growth, maintains their architecture, or even results in AVM hemorrhage. Genetics and inflammation are closely tied to each other, as multiple single-nucleotide polymorphisms have been associated with increased levels of pro-inflammatory cytokines such as tumor necrosis factor alpha, interleukin-6, interleukin-1alpha, and 1beta, among others.⁹ How this hyper-zealous, local, pro-inflammatory milieu, presumably incited through some form of injury such as stroke or trauma, results in AVM development or propagation is yet to be determined. It is possible that these local cytokines result in the chemotaxis of neutrophils and macrophages, which are frequently identified in the vascular wall of resected AVM tissue.⁵³ Clinically, advanced MRI imaging (ferumoxytol-enhanced MRI) has been able to noninvasively identify macrophage localization to AVM vessel walls.^{10,54} These recruited inflammatory cells then secrete a number of signaling or enzymatically active molecules, leading to further inflammation and/or destabilization of the vascular wall. Local secretion of IL-6 and IL-8 increases local matrix metalloproteinase expression and increase proliferation, migration, and survival of endothelial and smooth muscle cells.^{55–57} Secretion of leukocyte-derived myeloperoxidase and matrix metalloproteinase-9 leads to degradation of a number of vascular basement membrane components, including collagen, fibronectin, laminin, and other adhesion molecules.⁵⁸ Inflammatory cytokines are also known to increase expression of VEGF⁵⁹ and ANG2,⁶⁰ which are key downstream ligands responsible for promoting angiogenic responses in neoplasia and other conditions.^{61,62} These downstream pathways are also possible targets for therapeutic intervention, since inhibition with agents in clinical use and preclinical development have the potential to disrupt the key steps in AVM propagation by normalizing the structure and function of abnormal blood vessels.^{63,64} A human clinical trial of bevacizumab for brain AVM is currently underway and recruiting patients (ClinicalTrials.gov Identifier:NCT02314377).

Alternatively, a secondary local inflammatory state (reactionary phenomenon) as a result of pathological hemodynamic forces may be responsible for the observed local changes in cytokines and leukocytes. Altered hemodynamic shear stress has been shown to be a direct determinant of endothelial function and phenotype in a range of vascular pathophysiologies,^{65,66} and the turbulent high-flow nature of AVMs is unlikely to be an exception. This makes the cause-and-effect nature of inflammation and AVM pathology difficult to discern, and the pathologic hemodynamics within AVM tissue likely alter the gene expression profile sufficiently to complicate interpretation of observational studies (Figure 1).

An emerging area of interest in AVM-related inflammation is the contribution of the adaptive, cell-mediated immune response. The abnormal presence of T cells has been demonstrated in AVM vessels, but their nature or purpose remains an enigma.^{11,53} Using immunohistochemistry and flow cytometry, work is being done to differentiate subtypes of T cells (eg, Th1, Th2, Th17, Treg) in previously untreated brain AVMs. Further examination of local effector secreted cytokines, intracellular cytokine staining, and transcription factors in the AVM microenvironment will be targeted based on cell populations discovered. Since inflammation, hemodynamic stress, genetic variation, and angiogenesis are closely tied with one another, the exact contribution of inflammation to AVM pathogenesis remains an area of intense investigation.⁶⁷

BLOOD-BRAIN BARRIER

Normal cerebrovascular structure and function is dependent upon coordinated signaling of multiple cell types including endothelial cells, mural cells (vascular smooth muscle cells and pericytes), immune cells, glia, and neurons.^{68–70} Pericytes (PCs) are the principal mural cell population at the capillary level, covering roughly 80%–90% of the capillary wall. PCs fulfill a modulatory role in a number of integral cerebrovascular functionalities that are disrupted in AVMs, including regulation of brain angiogenesis, blood vessel diameter, vascular wall stability, and integrity of the blood-brain barrier (BBB).^{68,69,71} Not surprisingly, PCs are reduced in murine AVM models.^{72,73} Taken a step further, when PC-deficient rodents are examined, vessels lacking PCs display focal and/or diffuse dilatations with increased tortuosity and multiple findings consistent with heightened vascular fragility and/or permeability, including microaneurysms, overt hemorrhage, chronic leakage of circulating plasma-derived proteins, and heightened extravasation of blood-borne immune cells,^{68,74–80} findings that are consistently observed in human AVM tissue specimens.^{81–84} In one qualitative study to date, PCs were shown to be reduced in human perinidal tissue specimens.^{72,73} However, pericytes are normally confined to smaller vessels, and the relative contributions of vascular smooth muscle cells and PCs to AVM development and/or propagation is unclear. To date, no studies have quantified mural cells (PCs and vascular smooth muscle cells) in AVM specimens, and the relationship between a hypothesized loss of mural cells with increased AVM permeability (ie, microhemorrhage) is unknown.

Endothelial-mural cell signaling in AVM biology is not a stand-alone entity, and there is significant crosstalk between both cell types, with many angiogenic factors central to inflammatory pathways. For example, the effects of Ang2 on the vasculature can be either pro- or anti-angiogenic, depending on the presence of VEGF.^{85,86} In the presence of VEGF, Ang2 induces PCs to dissociate from existing vessels, making them leaky and allowing the extravasation of other pro-angiogenic factors. These events lead to sprouting and the formation of new blood vessels. In the absence of VEGF, Ang2 induces loss of PC coverage and stimulates vessel regression, culminating in an anti-angiogenic effect. Thus, because of this duality, the balance between Ang1, Ang2, and VEGF is extremely important in mediating angiogenesis and may play a central role in AVM development and progression. In addition to angiopoietin and VEGF cell signaling pathways, the recruitment of mural cells to nascent vascular tubes during angiogenesis is also dependent on platelet-derived growth factor B (PDGF-B) and platelet-derived growth factor receptor β signaling (PDGFR β),^{74–77,79,87–89} further highlighting the complexity of investigations needed to dissect these pathways. A reduction in AVM mural cell population may not only represent a gateway to pathologic angiogenesis, but could also be associated with lesion destabilization and hemorrhage (Figure 2).

CONCLUSION

This multifaceted approach to investigate sporadic brain AVM pathophysiology considers the interconnected, contributory roles of diverse biological processes based on research from the past two decades. Ongoing investigation utilizing human surgical specimens and refinement of animal models will continue to make progress in advancing our understanding

of how AVMs develop and eventually rupture.^{64,90–92} A disease model that incorporates how genetics, inflammation, and impaired BBB integrity conspire to generate AVMs will be essential to the development of screening protocols, the ability to differentiate high versus low hemorrhage risk lesions, and the identification of novel pharmacologic agents.

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FIGURE 1.

Dense cellular extravascular inflammatory infiltrate in brain arteriovenous malformations. Representative confocal microscopy images of (A) control temporal lobe and (B) resected temporal lobe arteriovenous malformation at low magnification [10×] showing endothelium (CD31 platelet endothelial adhesion molecule, green), vascular smooth muscle cells (alpha smooth muscle actin, red), and Hoechst-positive nuclei (blue).

Normal

Arteriovenous Malformation

FIGURE 2.

Cellular constituents of the vascular wall in the normal cerebral vasculature and arteriovenous malformations. **A**, cerebral arteries are comprised of a continuous endothelial cell lining (green) connected via tight and adherens junctions and then further surrounded by a thick vascular basement membrane (yellow) and concentric rings of vascular smooth muscle cells (blue). **B**, in brain arteriovenous malformations, there is hyperproliferation of endothelial cells (green). Focal areas of vascular smooth muscle cell (blue) proliferation and degeneration are also noted. Thinning of the basement membrane (pale yellow) and vascular smooth muscle cell degeneration contribute to the destabilization of the vascular wall, giving rise to micro- and macroscopic hemorrhage.

Table 1

Relationship in gene alterations between sporadic lesions and their associated syndromes

Sporadic Lesion	Syndrome	Common Gene	Discovery Method
Port Wine Stain	Sturge-Weber	GNAQ ³³	Whole genome sequencing
Meningioma	Neurofibromatosis	NF2 ^{34,35}	Whole genome sequencing, whole exome sequencing
Hemangioblastoma	Von Hippel-Lindau	VHL ³²	Whole exome sequencing

Table 2

Cell populations implicated in arteriovenous malformation pathogenesis

Cell Population	Potential Mechanism	Signaling Pathway	Gene of interest (HGNC)	Reference
Endothelium	Vascular remodeling, angiogenesis	Notch, VEGF, TGF-beta, Ras/MAPK	RASA1 (9871), ENG (3349), ACVRL1 (175), SMAD4 (6770), NOTCH3/4 (7883/7884)	Anand et al. 2010, McAllister et al. 1994, Koizumi et al. 2002, Murphy et al. 2014
Fibroblast	Vascular remodeling	TGF-beta	ENG (3349)	Matsubara et al. 2000
Vascular smooth muscle	Vascular remodeling, blood brain barrier	MAPK	ENG (3349), EIF2AK3 (3255)	Uranishi et al. 2001, Torsney et al. 2003, Takagi et al. 2006
Pericyte	Vascular remodeling, blood brain barrier	Notch, TGF-beta, VEGF	NOTCH3/4 (7883/7884), ACVRL1 (175)	Chen et al. 2013
T-cell, B-cell, Macrophage, Microglia, Monocyte, Neutrophil	Inflammation/angiogenesis	Innate & humoral immunity, Ras/MAPK	IL6 (6018)	Chen et al. 2008, Pawlikowska et al. 2004

VEGF = vascular endothelial growth factor, TGF-beta = Transforming growth factor beta 1, MAPK = mitogen-activated protein kinases