Pathogenesis of Brain Arteriovenous Malformations

Masaki KOMIYAMA

Abstract

Brain arteriovenous malformations (bAVMs) represent a high risk of intracranial hemorrhages, which are substantial causes of morbidity and mortality of bAVMs, especially in children and young adults. Although a variety of factors leading to hemorrhages of bAVMs are investigated extensively, their pathogenesis is still not well elucidated. The author has reviewed the updated data of genetic aspects of bAVMs, especially focusing on clinical and experimental knowledge from hereditary hemorrhagic telangiectasia, which is the representative genetic disease presenting with bAVMs caused by loss-of-function in one of the two genes: endoglin and activin receptor-like kinase 1. This knowledge may allow us to infer the pathogenesis of sporadic bAVMs and in the development of new medical therapies for them.

Key words: animal model, brain arteriovenous malformation, gene mutation, hereditary hemorrhagic telangiectasia, pathogenesis

Introduction

Brain arteriovenous malformations (bAVMs) consist of abnormal tangles of dilated vascular structure called nidus, which connects arteries and veins directly without intervening capillary beds. They are one of the major causes of intracranial hemorrhage and/or subarachnoid hemorrhage, which lead substantial morbidity and mortality of bAVMs, especially in children and young adults. Primary rationale for treatment of bAVMs is to prevent new or recurrent hemorrhage. Current treatment modalities include surgical removal, endovascular treatment, and stereotactic radiosurgery.1) However, it is a current controversy that risks of these interventions for unruptured bAVMs may exceed that of best medical management.2) There is no medical treatment available to prevent development or rupture of bAVMs. Although pathogenesis of bAVMs is not yet well elucidated, genetic mutations and genetic risk factors are increasingly identified. Appropriate animal models of bAVMs are prerequisite for understanding of the pathogenesis and development of new therapies. The author reviewed pathogenesis of bAVMs using the current updated data, especially the new knowledge from clinical cases and animal experimental models of hereditary hemorrhagic telangiectasia (HHT), which is also known as Osler-Weber-Rendu disease.3–5) Since it is conceivable that bAVMs in HHT have similar genetic backgrounds to sporadic bAVMs, knowledge gained from bAVMs in HHT may allow us to infer the pathogenesis of sporadic bAVMs and to develop new medical therapies from them.

Is AVM Congenital or Acquired?

It is classically believed that bAVMs are “congenital” lesions, which means that AVMs exist at birth or exist as a primordial vascular structure due to developmental failure of the embryos in the 40-mm to 80-mm length interval (approximately 10–14 weeks of gestation).6) In this context, bAVMs are conceived to be “static” lesions. However, there is little evidence to support this concept. If most bAVMs were “congenital,” defined as an existence at birth, phenotypic presentation such as hemorrhage and/or seizure might occur more frequently among younger population. This does not hold true since the average age of the initial diagnosis of bAVMs is about 30–40 years old.7,8) The fact that many routinely performed antenatal ultrasound screenings of the fetus fail to detect bAVMs also suggests that developmental AVM formations are rare except for some specific forms of bAVMs such as vein of Galen aneurysmal malformations and dural sinus malformations with arteriovenous (AV) shunts.9,10) Increasing evidences support postnatal growth of bAVMs.11) De novo formation, growth, regression, recurrence after complete resection, and development...
after infarction were infrequently reported. These facts indicate a subset of bAVMs is “dynamic” lesions even in adulthood. This means that bAVMs can grow, remodel, and regress in addition to rupture. In fact, as described below, bAVMs do develop in adult-onset mice experimental model under certain conditions.

Sporadic and Familial bAVMs

Most of the bAVMs (more than 95%) are sporadic, but some have apparently genetic backgrounds. It is reported that about 3% of bAVMs are caused by HHT. The HHT and capillary malformation (CM)-AVM are well-known familial bAVMs with known causative gene mutations. Although most familial AVMs are related to HHT, a small number of them are related to CM-AVM, which is caused by mutation of RASA1 gene. Excluding bAVMs due to these two diseases, familial bAVMs are extremely rare. In the latest review of familial bAVMs without HHT, clinical characteristics of familial bAVMs are not significantly different from sporadic bAVMs except for the age at diagnosis. The mean age at diagnosis in 53 patients with familial bAVMs among 25 families was 8 years younger than sporadic bAVMs.

Single-nucleotide Polymorphisms (SNPs) in bAVMs

The SNPs are variations of deoxyribonucleic acid (DNA) sequence that differ between members of the same species. Evidence of SNPs in sporadic bAVMs has been accumulated (Table 1). Some SNPs in the inflammatory cascades and in the regulation of angiogenesis play a role in the development of hemorrhage of bAVMs nonspecifically. Identification of SNPs related to hemorrhagic risk of bAVMs or bAVM susceptibility enables stratification and prognostication of high-risk patients and selection of the better management. Apolipoprotein E (APOE) genotype may influence the bleeding risk of bAVMs. APOE ε2 genotype carriers had five-fold increased risk of new hemorrhage than those with the other genotypes. Similarly, SNPs in inflammatory cytokine interleukin-6 (IL-6) (homozygous IL-6 −174G>C) are also associated with hemorrhagic presentation of bAVMs. Tumor necrosis factor (TNF)-α is a pro-inflammatory cytokine and TNF-α −238G>A polymorphism is associated with increased risk of hemorrhage in the natural course of bAVMs. activin receptor-like kinase 1 (ALK1) intervening sequence (IVS) 3′−35A>G polymorphism is associated with an increased risk (susceptibility) for bAVMs. All these genetic associations to hemorrhage and susceptibility of bAVM require replication in larger samples. Recently, Weinsheimer et al. reported genome-wide association study to investigate the association of common SNPs with risk of sporadic bAVM in Caucasians, and found that no SNPs including ALK1 IVS3 −3A>G were replicated in the large bAVM replication cohort, suggesting that common SNPs do not contribute strongly to bAVM susceptibility.

Angiogenesis and Inflammation in bAVMs

Molecular and histopathological analysis of bAVM specimen revealed the higher level of angiogenic factors and inflammatory cytokines. In fact, angiopoietin-2, matrix metalloproteinase (MMP)-9, vascular endothelial growth factor (VEGF) are highly expressed in sporadic bAVMs, and concerted effects

<table>
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<tr>
<th>SNP</th>
<th>Authors</th>
<th>Year</th>
<th>Risk/referent genotype</th>
<th>OR</th>
<th>95%CI</th>
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<td>2004</td>
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<td>AG/GG</td>
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<td>1.31–12.29</td>
</tr>
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<td>Pawlikowska et al.</td>
<td>2006</td>
<td>ε2/not ε2</td>
<td>4.97</td>
<td>1.43–17.3</td>
</tr>
<tr>
<td>Ds disease susceptibility</td>
<td></td>
<td></td>
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<tr>
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<td>Pawlikowska et al.</td>
<td>2005</td>
<td>AA; AG/GG</td>
<td>2.47</td>
<td>1.38–4.44</td>
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<tr>
<td></td>
<td>Simon et al.</td>
<td>2006</td>
<td></td>
<td>1.73</td>
<td>1.19–2.51</td>
</tr>
</tbody>
</table>

of these angiogenic factors may maintain the angiogenic phenotype in bAVMs.  

Homeobox gene of Hox D3 upregulates the expression of several proangiogenic molecules including integrin α₅β₃ and urokinase plasminogen activator and may contribute bAVM formation.  

Inflammatory cells (neutrophils and macrophages) are also found in bAVMs in the tissue removed during microsurgery.  

Inflammatory biomarker of IL-6 is increased in bAVMs with hemorrhagic presentation.

HHT

HHT is an autosomal dominant vascular disorder characterized by vascular dysplasia in multiple organs leading to hemorrhage, stroke, high-output heart failure, and death.  

It has a prevalence of 1:5,000–8,000.  

For HHT, three gene mutations are known: endoglin (ENG) for HHT1 (Online Mendelian Inheritance in Man (OMIM) #187300), activin A receptor type II-like kinase 1 (ACVRL1) or ALK1 for HHT2 (OMIM #600376) and SMAD family member 4 (SMAD4). Gene mutation of SMAD4 is responsible for a combined syndrome of HHT and juvenile polyposis (OMIM #175050). HHT3 (OMIM #601101) and HHT4 (OMIM #610655) are also described, but their genes are not yet identified.  

Recently, it is reported that mutations in bone morphogenetic protein 9 can cause similar HHT phenotype, thus called HHT5 (OMIM #615506). Clinical variations in HHT are significant with intra- and interfamilial variations in severity of complications, age of onset, and location of the lesions. It is conceivable that sporadic bAVMs may have similar genetic backgrounds to HHT. In this context, HHT is a good clinical and experimental model for the investigation of pathogenesis of bAVMs. Actually, there are many experimental studies using HHT transgenic animals.

I. Clinical diagnosis of HHT

HHT is caused by gene mutations in transforming growth factor-β superfamily receptors. ENG is the causative gene for HHT type 1, and ALK1 is for HHT type 2. About 85–90% of HHTs are either HHT type 1 or HHT type 2. Small number of HHT is caused by SMAD4 mutation, which is HHT-related polyposis syndrome. Clinically, HHT is diagnosed by the so-called Curaçao criteria. The following four items of diagnostic criteria show the characteristics of HHT: (1) recurrent, spontaneous nosebleeds; (2) mucocutaneous telangiectasia at tongue, lips, face, fingertips, etc.; (3) visceral AVFs (including AV fistulas) at lungs, brain, liver, and gastrointestinal tract (telangiectasia); and (4) family history of HHT within the first-degree relatives. When patient has more than three items, clinical diagnosis of HHT is definite. Two items are regarded as probable. Only one or no item is regarded as unlikely. Clinical-genetic correlation, in other words, validation of these clinical criteria is very high when adopted to the patients above the age of 16 years.

II. HHT-related bAVMs: angiographic subtypes

The bAVMs in HHT are morphologically classified into three groups previously: micro-AVMs less than 1 cm in size, regular AVMs usually smaller than 3 cm, and arteriovenous fistulas (AVFs) without nidus. However, recently different classification is proposed: capillary (vascular) malformations, AVMs (with nidus), and AVFs. Capillary malformations have no AV shunts on angiography, but show small stains on angiography and “fluffy” enhancement on gadolinium-enhanced magnetic resonance (MR) images. According to this classification, capillary malformations are the most commonly observed lesions (61%). AVM with nidus less than 1 cm in size (micro-AVM by the previous classification) is classified as AVM if the lesion has a nidus and AV shunts. Hemorrhagic risk of capillary malformations might be very low in contrast to that of AVMs and AVFs. Further accumulation of data on hemorrhagic risk of capillary malformations is necessary to provide appropriate therapeutic indication. In general, bAVMs in HHT have characteristic features of superficial location, small size, and multiple lesions. Especially, multiplicity is a specific feature of HHT-related bAVMs. However, it is impossible to distinguish each HHT-related AVMs from sporadic, non-HHT AVMs on the basis of their angioarchitecture.

III. Genetic backgrounds of HHT

The ENG codes for accessory protein receptors of the TGF-β receptor complex and ALK1 encodes for transmembrane kinase which participates the TGF-β signaling. They are primarily expressed in endothelial cells. ALK1 regulates endothelial proliferation and migration, and ENG promotes ALK1’s function in general. Loss-of-function mutations of these genes leading to “haploinsufficiency” are believed to cause HHT. Haploinsufficiency means a reduction of protein to half of the normal levels. When pathogenic proteins are expressed, they could also act in dominant-negative fashion, which means...
dominant mutation acts in opposition to normal gene function. Since only normal ENG is expressed on the cell surface at the level of 50% in HHT type 1, dominant-negative is less likely actually.53)

IV. Genotype and phenotype correlations of bAVMs in HHT

Prevalence of HHT type 1- and HHT type 2-related bAVMs is a 1,000- and 100-fold increase, respectively, in comparison to sporadic bAVMs29) in general population (10/100,000). Gene mutations and their phenotypes in HHT have been investigated to disclose genotype-phenotype correlations. It is known that bAVMs and pulmonary AVFs are more prevalent in HHT type 1 while hepatic AVMs are more prevalent in HHT type 2.54) There were no clear correlations between genotypes and phenotypes among 109 HHT patients with bAVMs (69% ENG mutation, 17% ALK1 mutation, and 2% SMAD4 mutation) in terms of age at diagnosis, multiplicity of AVMs, and prevalence of brain hemorrhage, and age at brain hemorrhage among gene groups.55) Lack of genotype-phenotype correlations in HHT could be attributable to the currently accepted pathogenesis of HHT, that is, “haploinsufficiency,” which is not related to the specific modes or sites of gene mutation.

Animal Models of bAVMs

I. Classic animal models of bAVMs

Historically, animal AVM models are extradural AV fistulas and are categorized into two types:56) hemodynamic and angiographic models. In hemodynamic models, AV shunts are created surgically from the contralateral extracranial carotid artery through the circle of Willis to the ipsilateral jugular vein, commonly by anastomosing common carotid artery to ipsilateral jugular vein (creating carotid-jugular fistula) with ligation of the jugular vein, commonly by anastomosing common carotid-jugular fistula through the circle of Willis to the ipsilateral jugular vein, commonly by anastomosing common carotid artery to ipsilateral jugular vein (creating carotid-jugular fistula) with ligation of the jugular vein distally.57) In angiographic models, commonly located extracranial “rete mirabile” in artiodactyl (even-toed ungulates) is used as AVM-like structures by surgically created AV shunts.58) Animal models for interventional neuroradiologic techniques have been used to test various devices and embolic materials. These two types of animal models have no intracranial parenchymal nidus, main difference from bAVMs.59)

II. Animal models of HHT-related bAVMs

Transgenic animal models are used for more modern researches on the pathogenesis of bAVMs. Among them, HHT-related transgenic mice are frequently used for this purpose. Two types of animal models are used: developmental (embryological)42,60) and adult-onset models.17,18) Knowledge from the genetic pathways in the HHT models can shed light on the pathogenesis of sporadic bAVMs. Expressivity of both ENG and ALK1 mutations is highly variable among HHT family members who share the same mutant alleles, which indicates that other modifying factors might play an important role in disease progression. Such factors are examined by animal models of HHT type 160) and HHT type 242,61,62) (Table 2).

Homozygous mutation of ENG+/− is lethal at E10–10.5 (embryo at day 10–10.5),60) which is roughly equivalent to human E24–28. In reality in humans, miscarriage occurred at 6–8 weeks of gestation in consanguineous marriage of two HHT type 1 affected first cousins when ENG is essential for cardiovascular development.63) However, the primitive vasculature of the embryo is normal until E9.0 (equivalent to human E20). This indicates that ENG plays an important role in angiogenic process. On the other hand, heterozygous mutation of ENG+/− presents similar symptoms of HHT including nosebleed and telangiectasia in some mice (not all mice) with increasing age, although penetrance is not so high.64) This implies that HHT type 1 is caused by a loss of function of ENG, i.e., haploinsufficiency. If fact, ENG level in ENG+/− mouse was about 50% and 3 of 10 mice developed vascular abnormalities including AVM-like structure.65) Severity and heterogeneity of symptomatology might be associated with the other epigenetic factors such as environment, blood pressure, oxygenation, shear stress, and hormonal levels.60) Homozygous mutation of ALK1−/− in mice is also lethal at E10.5–11.5, exhibiting severe vascular abnormalities. ALK1 in endothelial cells played a crucial role in determining vascular endothelial properties during angiogenesis.51,62) Mice lacking ALK1 developed large AV shunts at the early stage of vascular development (E9.5).62)

Heterozygous mice (ENG+−) without stimuli developed less often abnormal microvessel formation than heterozygous mice (ENG+−) stimulated by VEGF.65,66) ENG+−/− mice developed severer cerebrovascular dysplasia than ALK1+−/− mice stimulated by VEGF.67) Inflammatory cells are often found in and around AVMs. Dysmorphic vessels developed in ENG+− and ALK1+−/− mice at the capillary levels, but no AV shunts developed. Although haploinsufficiency of ENG or ALK1 is popularly accepted to cause HHT, heterozygous mutation of ENG or ALK1 is not enough to cause bAVM formation.68) More recently, conditional (tissue and/or time-specific) knockout mice of ENG or ALK1 gene are used as animal models of bAVMs. They have more similarities to human bAVMs in that they have AV shunts and develop spontaneous hemorrhage.17,18)
experiments using conditional mice with ENG^{2f/2f} global cell types and with ENG^{2f/2f} smooth muscle cell/endothelial cell types, it is concluded that homozygous ENG deletion in endothelial cells as well as focal VEGF stimulation might be required for baVM development. Similarly, deletion of ALK1 in endothelium alone with focal VEGF stimulation induced baVM in adult conditional ALK1^{2f/2f} mice. Thus, it seems that homozygous deletion of either ENG or ALK1 in endothelial cells are required for baVM formation.

### Factors Contributing to Pathogenesis of bAVMs

It is known that higher levels of angiogenic factors and inflammatory cytokines are observed in bAVMs than in the normal brain tissues. Also, inflammatory cells are infiltrated to bAVMs. Minor trauma, ischemia, venous hypertension, exogeneous growth factor delivery, high endogenous angiogenic factors, inflammation, and infection are known angiogenic factors contributing to manifest bAVMs. In HHT, development of AVMs may require a copy of inherited mutated gene in particular cells, first. And then, a second hit by the focal somatic mutation in another copy of gene may result in AVM formation in that lesion, as occurred in cerebral cavernous malformation and venous malformation ("second-hit" model). Alternatively, the second hit could be "environmental" in the form of a localized physiological or pathological perturbation. Shedding of ENG from endothelial cells during inflammation reduced endothelial ENG signaling due to increased soluble ENG level and altered blood flow which precipitates a flow-dependent adaptive response involving retention of normally transient AV connections are the examples.

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### Table 2 Mouse models of HHT-related brain AVMs

<table>
<thead>
<tr>
<th>Mutated gene</th>
<th>Authors</th>
<th>Year</th>
<th>Gene deletion</th>
<th>Global/local specific cell</th>
<th>Stimuli</th>
<th>Developmental/adult-onset</th>
<th>Conditional</th>
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<td></td>
<td></td>
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<tr>
<td>ENG</td>
<td>Bourdeau et al.</td>
<td>1999</td>
<td>hetero/homozygous</td>
<td>global</td>
<td>developmental</td>
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<tr>
<td>ENG</td>
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<td>2001</td>
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<td>developmental</td>
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<td>developmental</td>
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<td>VEGF</td>
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<td>homozygous</td>
<td>global/smooth muscle cell/endothelial cell/macrophage</td>
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<td>ALK1</td>
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ALK1: activin receptor-like kinase 1, ENG: endoglin, HHT: hereditary hemorrhagic telangiectasia, VEGF: vascular endothelial growth factor.
From insights into these current bAVM models, it is suggested that both angiogenic stimulation (environmental factors) and regional conditional homozygous gene deletion (genetic predisposition) may promote the ideal bAVM development in the adult mouse brain.\(^{17,70}\)

**Conclusion**

Although pathogenesis of bAVMs is not clearly understood, many researches are underway, especially using HHT animal models. Knowledge from such research works may help deeper understanding of the pathogenesis and provide novel therapeutic approaches to bAVMs in the near future.

**Conflicts of Interest Disclosure**

The author has no conflicts of interest with regard to this manuscript and has registered online Self-reported COI Disclosure Statement Forms through the website for The Japan Neurosurgical Society members.

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