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Review Article

Current Endoglin-Deficient Mouse Models for Brain Arteriovenous Malformation

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Abstract

The *Endoglin* (*ENG*) gene encodes a major glycoprotein that belongs to the TGFβ superfamily. In humans, mutations in the *ENG* gene are associated with an autosomal dominant disease, hereditary hemorrhagic telangiectasia (HHT). HHT patients have a higher incidence of brain arteriovenous malformation (bAVM) than the normal population. Because bAVM has a tendency to rupture and cause intracranial hemorrhage, having a better understanding of bAVM pathogenesis is crucial. In recent years, *Eng*-deficient bAVM mouse models have been established. This review looks at current models and how they are being utilized to study disease mechanisms and test new therapies.

ABBREVIATIONS

Eng: Endoglin; Alk1: Acvrl1 Oractivin Receptor-Like Kinase 1; bAVM: Brain Arteriovenous Malformation; Tgfβ: Transforming Growth Factor B; Tgfβr: Transforming Growth Factor B Receptor; HHT: Hereditary Hemorrhagic Telangiectasia; Huvecs: Human Umbilical Cord Endothelial Cells; VEGF: Vascular Endothelial Growth Factor; AAV: Adeno-Associated Virus; Ad: Adenovirus; ER: Estrogen Receptor; SM22α: Smooth Muscle Action A; BM: Bone Marrow

INTRODUCTION

Endoglin (ENG) is found in the human chromosome 9q34.11 and encodes a homodimeric transmembrane protein, a major glycoprotein of the vascular endothelium. ENG belongs to the TGFβ superfamily and is a type III TGFβ receptor. It requires association with TGFβRI (TGFβ receptor, type I) and/or TGFβRII (TGFβ receptor, type II) to bind with TGFβ1 and TGFβ3 (but not TGFβ2) with high affinity [1-4]. ENG also binds to activin-A, BMP2 and BMP7 in association with corresponding receptors [1], and requires the presence of other types of TGFβ receptors for complete down signaling. Interestingly, *in vitro* studies using COS-7 cells show that ENG overexpression reduces TGFβRII phosphorylation, but enhances TGFβRI phosphorylation, indicating ENG's important modulating role in the TGFβ signaling pathway [4].

Gene expression analyses have shown that Eng expresses in endothelial cells [5,6], and activates monocytes/macrophages [7], mesenchymal cells, fibroblasts [8], and vascular smooth muscle cells [9,10]. Mutations in the *ENG* gene are associated with an autosomal dominant disease, hereditary hemorrhagic telangiectasia (HHT), type 1, also known as Osler-Rendu-Weber Syndrome 1 [11]. In the endothelium, ENG interacts with the activin receptor-like kinase 1(*ALK1* or *ACVRL1*) gene, a type 1 TGFβR. *ALK1* mutations cause HHT, type 2.

Immunoprecipitation assays using cultured human umbilical cord endothelial cells (HUVECs) from normal and HHT patients reveal that ENG also interacts with ALK1in the absence of TGFβ [12], indicating that both genes function in the same signaling pathway. The clinical features in HHT patients are telangiectases in mucocutaneous membrane and arteriovenous malformation (AVM) in multiple organs, e.g., skin, liver, lung, intestine and brain.

Brain AVMs (bAVMs) are abnormal vessels shunting blood directly from arteries to veins [13]. Normally, arteries and veins are connected by capillaries where oxygen is exchanged. Since the structure of AVM vessels is abnormal and cannot sustain the hemodynamic pressure coming directly from the arteries, they tend to rupture, which can cause life-threatening intracranial hemorrhage [13]. A limited understanding of the disease occurrence and mechanism delays the development of effective therapies. Since HHT patients develop bAVM with higher frequency than the normal population, genetic mouse models with *Eng* deletion have been used to establish bAVM animal models. In this review, we discuss current *Eng*-deficient mouse models and their applications.

DISCUSSION AND CONCLUSION

Discussion

Homozygous and heterozygous *Eng* deletion mouse models: Initial HHT bAVM models mimicked gene mutation

in HHT patients. The Eng gene was heterozygously inactivated ($Eng^{+/-}$) by adding a premature stop codon into one allele of the mouse *Eng* gene [14]. After birth, these mice exhibit many phenotypes resembling those of HHT1 patients, including mucocutaneous telangiectases, external bleeding, and AVM in the liver, lungs, brain and gastro intestines [15]. $Eng^{+/-}$ mice have been widely used to study tissue-specific gene expression, gene function in the endothelium, and pathophysiological abnormalities of AVM in affected organs, as well as to test new therapies [16-22]. However, penetrance of bAVM in $Eng^{+/-}$ mice is only 7%, suggesting that the $Eng^{+/-}$ mouse is not a robust model for bAVM [15]. Inactivation of both *Eng* alleles ($Eng^{-/-}$) causes embryonic lethality at mid-gestation due to vascular and cardiac anomalies [14].

Angiogenic stimulation for bAVM formation: By analyzing surgically resected bAVM lesions in sporadic bAVM patients, an elevated level of an angiogenic factor, vascular endothelial growth factor (VEGF), was found [23,24]. VEGF increases in the plasma of HHT patients [25]. Therefore, a response-to-injury theory has been proposed for the initiation of bAVM [13,26]. Our group tested whether angiogenic stimulation could enhance bAVM formation in $Eng^{+/-}$ mice. Through intra-brain injection of an adeno-associated virus expressing VEGF (AAV-VEGF), vascular dysplasia (abnormal vessels) was found at the injection sites in almost all of the mice [26]. However, the vascular dysplasia in the brain of $Eng^{+/-}$ was at the capillary level [26] and could not fully recapitulate the human bAVM phenotype (Table 1). This suggests that homozygous deletion of *Eng* may be needed for the development of more severe bAVM phenotypes.

Conditional homozygous knockout of the *Eng* gene in adult mice plus angiogenic stimulation cause bAVM formation: In the Cre-loxP system, Cre recombinase induces deletion of the DNA sequence between two loxP sites that have the same orientation. This system can be used to conditionally inactivate targeted genes in certain tissues or cells and at a specific developmental stage, and therefore, embryonic lethality caused by homozygous mutation of critical genes can be avoided.

Allinson et al., generated *Eng*-floxed ($Eng^{2f/2f}$) mouse lines that have the *Eng* gene exons 5-6 flanked by loxP sites [27]. To test whether homozygous *Eng* gene deletion plus angiogenic stimulation can initiate bAVM formation, we co-injected Ad-Cre and AAV-VEGF into the brain of $Eng^{2f/2f}$ mice [27,28]. The mice developed vascular dysplasia beyond the capillary level around the AAV-VEGF injection site (Table 1) [28]. However, the lesion was less severe than those in the brain of similarly treated $Alk1^{2f/2f}$ mice [29], most likely due to the low gene deletion efficiency of *Eng* compared with *Alk1* (1% vs 16%) [28]. Thus, more effective *Eng* gene deletion may be needed.

In addition to expressing Cre with a viral vector, a Cre expression cassette can be introduced into the mouse genome. Cre expression can be regulated in the cassette by inducible systems, e.g., estrogen receptor (ER) [30]. In this expression system, an estrogen receptor ligand-binding domain is fused with Cre recombinase. When tamoxifen is present, Cre ER-fused protein enters the nucleus to implement gene deletion [30], this system allows more flexibility to execute the deletion process.

To delete the *Eng* gene in adult mice, the R26CreER mouse line, in which the R26 promoter drives ubiquitous Cre expression upon tamoxifen treatment [31,32], was bred with $Eng^{2f/2f}$ mice to produce R26CreER; $Eng^{2f/2f}$ [28]. Global deletion of *Eng* in adult R26CreER; $Eng^{2f/2f}$ mice alone through a daily intraperitoneal injection of tamoxifen (2.5mg/25 g of body weight) for 3 consecutive days did not cause bAVM formation [33]. BAVM developed only when brain angiogenesis was induced [33] (Table 1), supporting the notion that angiogenic stimulation is needed to induce bAVM.

Developmental bAVM model: In addition to adult onset bAVM models established in *Eng* gene-mutated mice, we also established developmental bAVM models in SM22αCre; $Eng^{2f/2f}$ mice. BAVMs spontaneously developed in 90% of SM22αCre; $Eng^{2f/2f}$ mice by 5 weeks of age and with a mortality of 50% by 6 weeks [33] (Table 1). In addition to bAVMs, these mice also had spinal and intestinal AVMs [33]. Although SM22α (smooth muscle action α) is predominantly expressed in smooth muscle cells, the transgene SM22αCre also expresses in other cell types, including endothelial cells [34,35]. Compared with adult bAVM mouse models, this model does not require exogenous VEGF stimulation to induce the bAVM phenotype and is therefore a better model to test new therapies for bAVMs.

Endothelial *Eng* gene deletion is necessary for bAVM development: As mentioned above, *Eng* not only expresses in endothelial cells [5,6], but also in activated monocytes/macrophages [7], mesenchymal cells, fibroblasts [8], and smooth muscle cells [9,10]. Using cell type-specific expression of Cre recombinase, the *Eng* gene was conditionally deleted in different cell types to determine which one is most crucial for bAVM development [36,37].

Table 1: Comparison of Eng-deficient bAVM mouse models.

Strain	Method of Establishment		bAVMPhenotype
	Intra-brain Injection	Intraperitoneall njection	
Eng+/-	N/A	N/A	7%
Eng+/- plus intra-brain injection	AAV-VEGF	N/A	Capillary level of abnormal vessels in nearly 100% of mice
$Eng^{2f/2f}$ plus intra-brain injection	Ad-Cre and AAV-VEGF	N/A	Vascular dysplasia beyond capillary level in nearly 100% of mice
R26CreER;$Eng^{2f/2f}$	AAV-VEGF	Tamoxifen for 3 consecutive days	100%
SM22αCre;$Eng^{2f/2f}$	N/A	N/A	90%

In Scl Cre ER; *Eng*$^{2f/2f}$ adult mice, which have *Eng* deletion only in endothelial cells, AVM formed in the skin around the ear and back wound [36,37]. However, Myh11 Cre ER-mediated *Eng* deletion in smooth muscle cells in adult mice did not cause AVM formation in the skin, including the wound area [36]. Furthermore, Lys MCre; *Eng*$^{2f/2f}$ adult mice, which have a macrophage-specific promoter driven Cre, did not develop AVM in any organ or in the brain angiogenic region [33], indicating that *Eng* deletion in endothelial cells is essential for AVM formation [33,36].

Applications of *Eng*-deficient bAVM models: *Eng*-deficient bAVM mouse models can be used to study the mechanisms involved in bAVM formation and to test new therapies. Using *Eng*$^{+/-}$ mice, the influence of bone marrow (BM)-derived cells has been studied. Transplantation of BM cells from *Eng*$^{+/-}$ to WT mice induces vascular dysplasia in the brain angiogenic region, similar to that in the brain angiogenic region of *Eng*$^{+/-}$ mice [26]. Transplantation of BM cells from WT to *Eng*$^{+/-}$ mice reduces the severity of vascular dysplasia in the brain angiogenic foci of *Eng*$^{+/-}$ mice [26]. These data suggest that *Eng* gene mutation in BM cells is sufficient to cause bAVM formation, and that transplantation of normal BM cells to bAVM patients could be a therapeutic option.

Eng-deficient bAVM mouse models have also been used to analyze the role of macrophages in bAVM pathogenesis. Although *Eng* deficiency has been shown to impair monocyte migration into injured tissue [38-40], a high number of BM-derived macrophages and activated microglia has been found in the bAVM lesion in mice. Compared with normal macrophages, *Eng*-deficient macrophages in R26CreER; *Eng*$^{2f/2f}$ mice show slower and more persistent infiltration into the brain angiogenic region, as well as delayed clearance that causes unresolved inflammation, which in turn enhances abnormal vascular remodeling and the severity of the bAVM phenotype [41].

Conclusion

Eng-deficient models, established through conventional and conditional knockout techniques, are valuable tools for studying bAVM mechanisms and testing new therapies. Data gathered from studies using these models not only advance our knowledge of the disease but also contribute to the advancement of therapeutic approaches.

However, despite their value and importance in bAVM research, some limitations exist when using mouse models. Mice are too small to monitor progression and hemodynamic changes using conventional non-invasive imaging techniques. Future studies should therefore generate models in larger animals, e.g., rabbit and swine. In addition, although *Eng*-deficient mouse models recapitulate many bAVM phenotypes found in HHT1 patients, these models cannot fully reflect the pathogenesis and disease mechanisms of sporadic bAVM. Next-generation gene sequencing methods are invaluable tools to uncover the genes involved in sporadic bAVM. Future research using animal models that mimic sporadic bAVM would thus offer further opportunities to investigate the disease.

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