Published in final edited form as:
Stroke. 2013 March ; 44(3): 795–798. doi:10.1161/STROKEAHA.112.671974.

Endoglin Deficiency in Bone Marrow Is Sufficient to Cause Cerebrovascular Dysplasia in the Adult Mouse after VEGF Stimulation

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Abstract

Background and Purpose—Bone marrow-derived cells (BMDCs) home to vascular endothelial growth factor (VEGF)-induced brain angiogenic foci, and VEGF induces cerebrovascular dysplasia in adult endoglin heterozygous (*Eng*$^{+/-}$) mice. We hypothesized that *Eng*$^{+/-}$ BMDCs cause cerebrovascular dysplasia in the adult mouse after VEGF stimulation.

Methods—BM transplantation was performed using adult wild-type (WT) and *Eng*$^{+/-}$ mice as donors/recipients. An adeno-associated viral vector expressing VEGF (AAV-VEGF) was injected into the basal ganglia 4 weeks after transplantation. Vascular density, dysplasia index (vessels >15 μm/100 vessels), and BMDCs in the angiogenic foci were analyzed.

Results—The dysplasia index of WT/*Eng*$^{+/-}$ BM mice was higher than WT/WT BM mice (p<0.001) and was similar to *Eng*$^{+/-}$/*Eng*$^{+/-}$ BM mice (p=0.2). Dysplasia in *Eng*$^{+/-}$ mice was partially rescued by WT BM (p<0.001). WT/WT BM and WT/*Eng*$^{+/-}$ BM mice had similar numbers of BMDCs in the angiogenic foci (p=0.4), most of which were CD68^{+}. *Eng*$^{+/-}$ monocytes/macrophages expressed less matrix metalloproteinase-9 and Notch1.

Conclusions—ENG-deficient BMDCs are sufficient for VEGF to induce vascular dysplasia in the adult mouse brain. Our data support a previously unrecognized role of BM in the development of cerebrovascular malformations.

Keywords

arteriovenous malformation; adult mouse; brain angiogenesis

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Disclosures None

Introduction

Mutations in endoglin cause Hereditary Hemorrhagic Telangiectasia 1 (HHT1). Telangiectases and arteriovenous malformations (AVM) have been viewed as a disorder of the extant endothelium.[1] VEGF induced cerebrovascular dysplasia in adult *Eng*$^{+/-}$ mice;[2] the majority of BMDCs in the angiogenic foci were monocytes/macrophages (Mø),[3] which contribute to vascular repair and angiogenesis.[4] We hypothesized that *Eng*-deficiency in BMDCs causes cerebrovascular abnormalities in mice after VEGF stimulation.

Methods

After institutional approval, the design and groups listed in Supplemental Figures S1 and S2, and methods described in the online-only Data Supplement were used.

Results

AAV-VEGF induced brain angiogenesis in all groups and caused abnormal cerebrovascular morphology in mice with *Eng*$^{+/-}$ BM (Figure 1). Vascular densities (mean±SD) were: 820±153 (WT/WT BM), 720±150 (*Eng*$^{+/-}$/WT BM), 653±120 (WT/*Eng*$^{+/-}$ BM), and 674±76 vessels/mm^2 (*Eng*$^{+/-}$/*Eng*$^{+/-}$ BM). Mice carrying *Eng*$^{+/-}$ somatic or BM cells showed a trend towards lower vascular density compared to WT/WT BM mice (p=0.06, Figure1B). WT/*Eng*$^{+/-}$ BM mice had more than five-fold greater dysplasia index than WT/WT BM mice (1.7±0.3 vs. 0.3±0.3, p<0.001, Figure 1C), comparable with the dysplasia index of *Eng*$^{+//}$*Eng*$^{+/-}$ BM mice (1.9±0.4, p=0.2). Transplantation of WT BM to *Eng*$^{+/-}$ mice partially rescued dysplasia (p<0.001, Figure 1C).

Using EGFP expressing donors, WT/WT BM and WT/*Eng*$^{+/-}$ BM mice had similar BMDC counts in the angiogenic foci (400±125 vs. 339±112/mm^2, p=0.4) (Supplemental Figures S3A and S3B). The majority BMDCs was CD68$^+$ (WT/WT BM: 67%±8 vs WT/*Eng*$^{+/-}$ BM: 64%±10, p = 0.6) (Figures 2A and 2C, Supplemental Figure S3C). About 7% of BMDCs in both groups were CD31$^+$ endothelial cells (Figure 2B and 2D).

BM-derived Mø from WT and *Eng*$^{+/-}$ mice were cultured and treated with 4 doses of VEGF (0, 10, 50, and 100ng/ml) for 18 hours. Compared to WT, *Eng* expression was 50% lower in *Eng*$^{+/-}$ Mø (Supplemental Figure S4A). The presence of both *Vegfr1/Flt1* and *Vegfr2/Flk1/Kdr* indicate that Mø can be stimulated by VEGF (Supplemental Figure S4B and C). *Mmp9* was up-regulated in WT but not *Eng*$^{+/-}$ cells at 50ng/ml of VEGF (p=0.003, Figure 3A). *Notch1* expression in *Eng*$^{+/-}$ Mø decreased at 100ng/ml VEGF treatment compared to WT (p<0.001, Figure 3B).

Discussion

This is the first demonstration that *Eng*-haploinsufficiency in BMDCs was sufficient to cause cerebrovascular dysplasia in the adult mouse after angiogenic stimulation. The abnormal angiogenic response was associated with altered expression of angiogenesis-related genes in Mø. These findings are consistent with prior work in *Eng*$^{+/-}$ myocardial infarction mice showing that transfusion of normal, but not HHT1, human mononuclear cells (MNCs) rescued the defect.[4]

TGF-β, VEGF, and Notch pathways act either synergistically with or antagonistically against each other during angiogenesis in a context-dependent manner.[5] Notch signaling in Mø plays a critical role in angiogenesis and repair. Abrogation of monocytic Notch1 adversely affected repair after myocardial injury.[6] Conditional deletion of Mø Notch1 caused abnormal anastomosis between angiogenic sprouts.[7] Further study is needed to

examine whether reduced Notch1 signaling in *Eng*$^{+/-}$ Mø contributes to a dysplastic phenotype.

VEGF dose-dependent effect on Mø depends on culture conditions. Chemotactic response of human Mø to VEGF peaked at 12ng/ml and decreased after 40ng/ml with 2-hour incubation.[8] We found that 50ng/ml VEGF up-regulated *Mmp9* in murine Mø, whereas neither 10 nor 100ng/ml had any effect. Possible explanations are: (1) human cells response to VEGF differently from mouse cells; and (2) various VEGF doses differentially trigger various signaling pathways to regulate diverse monocytic functions. *Notch1* is induced by VEGF in arterial ECs.[5] However, its expression in mouse Mø was not affected by VEGF (10–100ng/ml) in our study, possibly because only a subpopulation of Mø expresses Notch1 during angiogenesis.[7]

Growth factors and cytokines produced by BMDCs can affect local angiogenesis via systemic signaling. We showed that the mobilization of *Mmp9*$^{-/-}$ BMDCs into the circulation in response to VEGF was reduced, which resulted in less BMDC homing and brain angiogenesis.[9] VEGF may affect macrophage polarization by effects on Notch signaling.[10] Further studies should address the indirect/systemic effects of endoglin-deficiency on the BMDC function and the effect VEGF on Mø polarization.

Eng-deficiency in EC precursors may also play a role and deserves further study. In tumors, very few EC precursors are capable of triggering the angiogenic switch.[11] Further, only a small number of homozygously *Eng*-deleted ECs (~ 1%) was sufficient to induce macroscopic cerebrovascular dysplasia after VEGF stimulation.[12]

In summary, one or more subpopulations of *Eng*$^{+/-}$ BMDCs are sufficient to induce an abnormal vascular response to brain angiogenic stimulation. Highly relevant to HHT1, it may be possible to envision development of a rescue strategy using BM transplantation therapy. The role of BMDCs in sporadic brain AVM needs further study, as Mø are associated with the lesion[13] and EC precursors incorporate into the abnormal vascular structures.[14] Consideration should also be given to the role of endoglin in other cerebrovascular diseases such as stroke.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Jeffrey Nelson for statistical consultation, Voltaire Gungab for manuscript preparation, and UCSF BAVM project members (http://avm.ucsf.edu) for support.

Sources of Funding Supported by grants from National Institutes of Health (R01NS027713 to W.L.Y., R21NS070153 to H.S., and P01NS044155 to W.L.Y., H.S.), American Heart Association (SDG0535018N to H.S.), Leslie Munzer Foundation (H.S.); Aneurysm and AVM Foundation (H.S.); and Michael Ryan Zodda Foundation (W.L.Y., J.P.S.).

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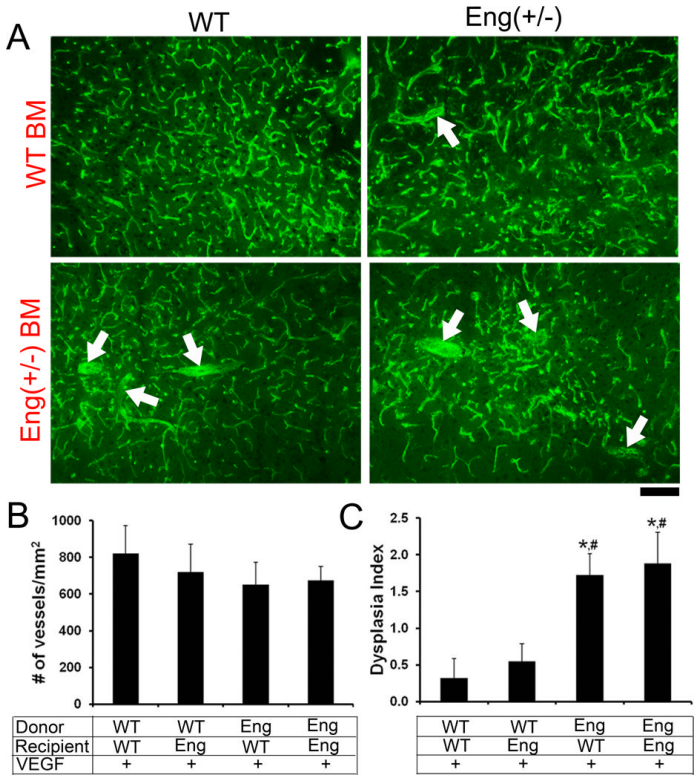

Figure 1. VEGF induced cerebrovascular dysplasia in mice with *Eng*$^{+/-}$ BM

(A) Representative images of VEGF-induced brain angiogenic foci. Arrows indicate dysplastic vessels. Scale bar: 50μm. Quantifications of (B) vascular density and (C) dysplasia index. *: $p<0.001$ compared to WT/WT BM group. #: $p<0.001$ compared to *Eng*$^{+/-}$/WT BM group. Data: mean±SD. n=6 per group.

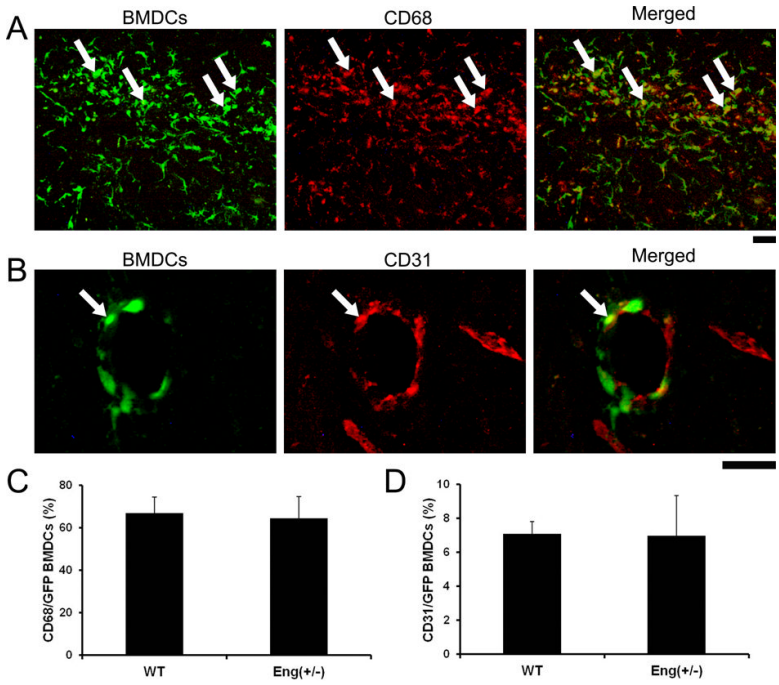

Figure 2. *Eng*-deficiency did not alter BMDC homing ability

(A) & (C) Most of recruited GFP$^+$ BMDCs were CD68$^+$ Mø (arrows). (B) & (D) Few GFP$^+$ BMDCs were CD31$^+$ ECs (arrows). Scale bars: 50μm in (A), 20μm in (B). Data: mean±SD. n=6 per group.

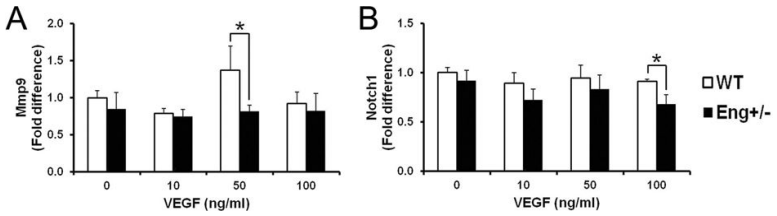

Figure 3. *Mmp9* and *Notch1* expression were reduced in *Eng*$^{+/-}$ monocytes/macrophages after VEGF stimulation

Quantification of (A) *Mmp9* and (B) *Notch1* expression. Expression levels are relative to that of WT-untreated cells. Data: mean±SD from three independent experiments (n=3 per group). *: $p<0.05$.