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# Intravenous transfusion of endothelial colony-forming cells attenuates vascular degeneration after cerebral aneurysm induction

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ABSTRACT

Cerebral aneurysm (CA) rupture is a major cause of subarachnoid hemorrhage with high morbidity and mortality. Using an animal model, we examined the potential of endothelial colony-forming cells (ECFCs) transfusion on vascular degeneration after CA induction and underlying mechanisms. CA was induced in the right anterior cerebral artery–olfactory artery (ACA/OA) bifurcations in Sprague–Dawley rats with or without ECFCs transfusion. The degeneration of internal elastic lamina (IEL), media thickness and CA size were evaluated. Expression of matrix metalloproteinase-2 and 9 (MMP-2 and 9), tissue inhibitor of metalloproteinase-1 (TIMP-1), macrophage chemoattractant protein-1 (MCP-1), vascular cell adhesion molecule-1 (VCAM-1), nuclear factor κB (NF-κB), endothelial nitric oxide synthase (eNOS), B-cell leukemia/lymphoma-2 (Bcl-2), and inducible nitric oxide synthase (iNOS) were analyzed by quantitative real-time polymerase chain reaction. The macrophages infiltration and apoptosis of smooth muscle cells (SMCs) were examined immunohistologically. Rats in

Abbreviations: CA, cerebral aneurysm; MMPs, matrix metalloproteinases; SMCs, smooth muscle cells; EPCs, endothelial progenitor cells; ECs, endothelial cells; ECFCs, endothelial colony-forming cells; MNCs, mononuclear cells; PBS, phosphate-buffered solution; DiI-acLDL, diI-acetylated low-density lipoprotein; UEA-1-FITC, ulex europaeus agglutinin-1 labeled with fluorescein isothiocyanate; vWF, von willebrand factor; KDR, kinase domain receptor; VE-cadherin, vascular endothelial cadherin; LCCA, left common carotid artery; NS, normal saline; ACA/OA, anterior cerebral artery/olfactory artery; IEL, internal elastic lamina; RT-PCR, real-time reverse transcription polymerase chain reaction; TIMP-1, tissue inhibitors of metalloproteinases-1; eNOS, endothelial nitric oxide synthase; MCP-1, macrophage chemoattractant protein-1; NF-κB, nuclear factor kappa B; Bcl-2, B-cell leukemia/lymphoma-2; ANOVA, one-way analysis of variance; LSD, least significant difference

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CA+ECFCs transfusion group showed a notable reduction in IEL degeneration, media thinning and CA size compared with those in CA+saline group. ECFCs transfusion inhibited the MMP-driven wall destruction by downregulating MMP-2, MMP-9 expression and upregulating TIMP-1. ECFCs transfusion dramatically decreased VCAM-1 and NF-κB expression, increased eNOS expression and caused no change in MCP-1 expression, which was accompanied by reduced macrophages infiltration. Moreover, ECFCs transfusion reversed downregulation of Bcl-2 expression and upregulation of iNOS expression, and decreased SMCs apoptosis. Collectively, these findings suggest that ECFCs transfusion confers protection against degeneration of aneurysmal wall by inhibiting inflammatory cascades and SMCs apoptosis.



## 1. Introduction

Cerebral aneurysm (CA) rupture is a major cause of subarachnoid hemorrhage with high morbidity and mortality (Sencer and Kiris, 2006). Due to the catastrophic consequences of CA rupture, it is necessary to understand mechanism underlying CA initiation, progression and rupture. Vascular remodeling coupled with inflammation is considered to be an essential part in the pathogenesis of intracranial aneurysms (Aoki et al., 2007a; Hashimoto et al., 2006). Current studies show that vascular endothelium and internal elastic lamina (Deanfield et al., 2007) dysfunction or damage make vessel wall vulnerable to hemodynamic stress (Fukuda et al., 2000; Hazama et al., 1986). Sustained hemodynamic stress triggers endothelial activation and inflammatory cells infiltration into the vessel wall (Aoki et al., 2007a; Jamous et al., 2007). The subsequent digestion of extracellular matrix components caused by matrix metalloproteinases (MMPs) and apoptosis of medial smooth muscle cells (SMCs) lead to abnormal vascular remodeling and initiation of CA (Aoki et al., 2007a; Kondo et al., 1998; Tulamo et al., 2010).

It is well known that circulating endothelial progenitor cells (EPCs), as committed linage specific stem cells, play an important role in maintaining vascular integrity by replacing injured and dysfunctional endothelial cells (ECs) (Deanfield et al., 2007; Gill et al., 2001). Our previous studies have demonstrated that decreased level and impaired function of EPCs in CA patients shifted the balance between vascular injury and repairs which correlated with CA formation (Wei et al., 2011a). Conditions, such as erythropoietin and surgery, could increase circulating EPCs level and slow down the rate of CA formation and progression (Wei et al., 2011b; Xu et al., 2011). Given the previous findings, we believe that EPCs are involved in the formation and progression of CA. Endothelial colony-forming cells (ECFCs), a subtype of EPCs with high proliferation and tube formation potential (Ingram et al., 2004), are accepted as a reliable source of EPCs for the treatment of vascular diseases (Lavergne et al., 2011). The purpose of the present study was to investigate the effects of exogenous ECFCs transfusion on abnormal vascular remodeling after CA induction and the possible underlying mechanisms.

## 2. Results

### 2.1. Characteristics of cultured ECFCs

After 14 days, cultured cells formed typical cobblestone-like colonies (Fig. 2A). The tube formation capacity was confirmed by Matrigel assays (Fig. 2B). The ECFCs were further determined by expression of the stem cell marker, CD34 (Fig. 2C), and the EC markers, vWF, VE-cadherin and KDR expression (Fig. 2D), and exhibiting the ability of DiI-acLDL uptake and FITC-UEA-1 binding (Fig. 2E).

### 2.2. ECFCs transfusion inhibited the degeneration of aneurysmal wall

The results of Verhoeff's Van Gieson staining were that elastic fibers and nuclei showed black, collagen displayed red (Figs. 3A–C). In the CA+saline group, 95% CI for the median of IEL score was 0.94–1.81 ($n=8$) 2 months after aneurysm induction. ECFCs transfusion significantly reduced the IEL score after 2 months (0.34–1.41, 95% CI for the median, $n=8$; $P<0.01$ versus CA+saline; Fig. 3D). The media thickness was significantly thicker in the ECFCs group ($0.71\pm0.12$, $n=8$) compared with the CA+saline group ($0.48\pm0.11$, $n=8$, $P<0.01$; Fig. 3E). The aneurysm size was smaller in the ECFCs group ($35.38\pm9.43$ μm, $n=8$) than that in the CA+saline group ($52.35\pm11.09$ μm, $n=8$, $P<0.01$; Fig. 3F).

### 2.3. ECFCs transfusion reduced the MMPs-driven wall destruction

In RT-PCR analysis, compared with the normal group, mRNAs expression of MMP-2 ($P<0.01$; Fig. 3G) and MMP-9 ($P<0.01$; Fig. 3H) were quantitatively elevated in the process of CA formation in the CA+saline group, while TIMP-1 ($P<0.01$; Fig. 3I) expression was downregulated. Compared with CA+saline group, MMP-2 ($P=0.039$), MMP-9 ($P<0.01$) and TIMP-1 ($P=0.031$) mRNA expression were reversed after ECFCs transfusion.

### 2.4. ECFCs transfusion decreased inflammation in aneurysmal wall

Compared with the normal group, MCP-1, VCAM-1 and NF-κB mRNA expression were upregulated and eNOS mRNA expression was downregulated 2 months after CA induction in the CA+saline group (MCP-1, $P=0.014$; VCAM-1, $P<0.01$; NF-κB, $P<0.01$; eNOS, $P<0.01$; Figs. 4E–H). ECFCs transfusion after CA induction caused a significant decrease in mRNA levels of VCAM-1 ($P<0.01$), NF-κB ($P=0.041$) and eNOS ($P<0.01$), and no change in MCP-1 mRNA level ($P=0.546$) compared with the CA+saline group. The number of macrophages infiltrated into CA walls per 100-μm square was $4.25\pm1.04$ cells in the