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Short Note

Surfactant protein A promoter directs the expression of Cre recombinase in brain microvascular endothelial cells of transgenic mice

Fanwei Meng [a,c,1], Ling Shi [a,1], Xuan Cheng [a], Ning Hou [a], Yongliang Wang [a], Yan Teng [a], Anming Meng [c], Xiao Yang [a,b,*]

[a] *Genetic Laboratory of Development and Diseases, Institute of Biotechnology, Beijing 100071, PR China*
[b] *Model Organism Division, E-institutes of Shanghai Universities, Shanghai Jiaotong University, PR China*
[c] *Laboratory of Developmental Biology, Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing 100084, P.R. China*

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Abstract

Brain microvascular endothelial cells (ECs) have unique characteristics distinguished from peripheral ECs and play important roles in blood–brain barrier (BBB). To investigate the physiological control of the brain ECs, we generated a transgenic mouse line in which the expression of Cre recombinase was driven by the promoter of the mouse surfactant protein A (*SP-A*) gene. The Cre activity was detected in blood vessels of brain, alveolar type II cells of lung and epithelium of gland stomach. In brain ECs, the Cre activity started at embryonic day 11.5, indicating that the subpopulation of ECs in brain could be molecularly defined at early embryonic stages. The use of *SP-A-Cre* mice should facilitate analysis of gene function in the brain ECs.

Keywords: Surfactant protein A; Blood–brain barrier; Cre recombinase; Brain microvascular endothelial cells; Transgenic mouse

1. Introduction

There is increasing evidence that endothelial cells (ECs) exhibit remarkable heterogeneity, which makes the endothelium uniquely adapted to communicate with specific cells in different tissues (Cleaver and Melton, 2003). Although molecular differences have been characterized between EC populations of arteries and veins, large and small vessels and normal and tumor vessels, very few organ-specific antigens expressed in endothelium of specific tissues have been confirmed (Cleaver and Melton, 2003).

Blood–brain barrier (BBB) is a complicated system formed by the endothelial cells that line cerebral microvessels. It functions as a "physical barrier" and a "metabolic barrier", permitting the entry of required nutrients and excluding potentially harmful compounds (Abbott et al., 2006). Compared with peripheral endothelial cells, brain microvascular endothelial cells have unique characteristics: tight junctions, very few pinocytotic vesicles, and special enzymes that are capable of metabolizing drug and nutrients. However, the genetic control of the physiological functions of the brain microvascular endothelial cells is still largely unknown.

The Cre–*loxP* system provides a powerful means to enable cell- or tissue-deletion of a targeted gene in specific tissues of interest. *Tie-1* and *Tie-2* promoters have been used to direct the Cre recombinase to express when the first endothelial cells arise and throughout development (Gustafsson et al., 2001; Kisanuki et al., 2001). The transgenic mice in which the Cre recombinase expression is restricted to brain endothelial cells will be new genetic tools for the investigation of the molecular mechanisms governing the physiological functions of the brain endothelium that is involved in regulation of the BBB.

Surfactant protein A (*SP-A*) is widely detected in alveolar of lung, blood vessel walls, epithelia of colon and stomach (Luo et al., 2004). The expression of *SP-A* is strong in blood vessels of brain, and barely detectable in blood vessels of other tissues

* Corresponding author. Institute of Biotechnology, 20 Dongdajie, Beijing 100071, P.R. China. Tel./fax: +86 10 63895937.
E-mail address: yangx@nic.bmi.ac.cn (X. Yang).
[1] F. Meng and L. Shi contributed equally to this work.

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(Luo et al., 2004). Here, we report the generation of a transgenic mouse in which the Cre recombinase driven by the *SP-A* promoter is expressed in brain microvascular endothelial cells.

2. Results and discussion

A transgenic mouse (*SP-A-Cre*) was generated in which the Cre recombinase expression is under the control of the 1.4 kb promoter of the mouse *SP-A* (Bruno et al., 1999). To check the tissue distribution and the excision activity of Cre recombinase, the *SP-A-Cre* transgenic line was bred with a mouse strain that carries *Smad4* conditional alleles ($Smad4^{co/co}$) (Yang et al., 2002). The Cre-mediated excision of exon 8 of *Smad4* (Fig. 1A) in different tissues of the $SP\text{-}A\text{-}Cre;Smad4^{Co/+}$ offspring at postnatal day 4 (P4) was evaluated by PCR. PCR analyses revealed that the Cre mediated recombination was detected in lung, stomach, intestine and brain (Fig. 1B). The Cre transcripts were also detected in these tissues of 8-month-old transgenic mice by RT-PCR (Fig. 1C).

To identify the exact cell types where Cre recombinase performs its excision function, we bred the *SP-A-Cre* transgenic mouse with a reporter mouse *ROSA26* (Soriano, 1999). *SP-A-Cre;ROSA26* double transgenic mice will express LacZ in the tissues where Cre-mediated recombination occurs. We compared the LacZ staining between *SP-A-Cre;ROSA26* and *ROSA26* littermates at P1. In consistent with the PCR results, LacZ activity was detected in blood vessels of brain, alveolar type II cells of lung and epithelial cells of gland stomach in *SP-A-Cre;ROSA26* transgenic mice, while no Cre activity was found in the counterparts of *ROSA26* littermates (Fig. 1D). Other tissues tested did not express LacZ (data not shown). All these data indicate that Cre recombinase is expressed specifically in blood vessels of brain, alveolar type II cells of lung and epithelial cells of gland stomach in the *SP-A-Cre* transgenic mice at a level allowing recombination at the *ROSA26* and other loci.

To determine if the LacZ positive cells in brain of *SP-A-Cre; ROSA26* transgenic mice are endothelial cells rather than other type cells, analysis by fluorescence microscope was performed using antibody against β-Gal, and CD31 (Gustafsson et al., 2001) which can mark the vascular endothelial cells. We found that all β-Gal-positive cells were also stained by CD31 antibody ($n=108$) (Fig. 2A), suggesting that the Cre recombinase is specifically expressed in brain microvascular endothelial cells.

In order to determine the initial expression time of Cre recombinase, we carried out whole-mount LacZ staining of double transgenic embryos at E10.5, E11.5 and E12.5. The activity of Cre recombinase was first detected in primitive brain vascular endothelial cells at E11.5, but not in blood vessels in any other tissues including heart (Fig. 2B). Previous studies have shown that ECs of the first embryonic vessels are morphologically homogeneous and express same early vascular markers (Drake and Fleming, 2000). Heterotopic transplantation experiments show that peripheral vessels growing into brain tissue adopt tight junctions, characteristic of brain vessels (Stewart and Wiley, 1981). Our results provided new evidence demonstrating that the subpopulation of ECs in brain of the early embryos could be molecularly defined.

In conclusion, we have first generated a transgenic mice line in which an *SP-A* promoter directs the expression of the Cre recombinase only in brain microvascular endothelial cells through embryonic, neonatal and adult phases in the mouse. It would enable the disruption of *loxP* flanked genes in these cells and

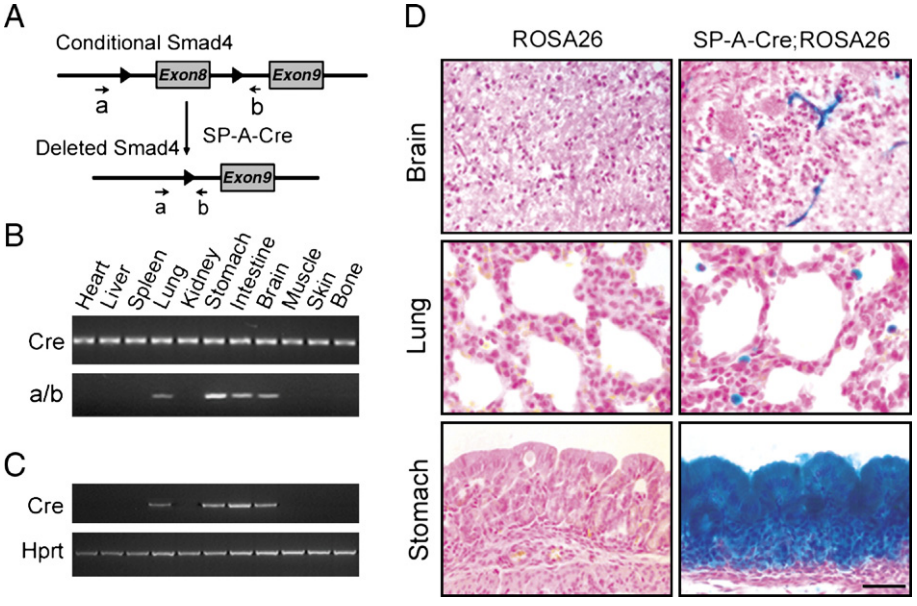

Fig. 1. Tissue distribution of Cre activities in *SP-A-Cre* transgenic mice. (A) Schematic diagram of pre- and post-Cre-mediated recombination of the floxed *Smad4* alleles. A 234 bp fragment could be amplified from the deleted *Smad4* allele by PCR using primers a and b after the Cre-mediated excision of exon 8 of *Smad4*. (B) Tissue distribution of Cre recombinase. A 234 bp fragment was amplified from tissues with Cre activities. A 481 bp fragment was amplified using primers Cre-1 and Cre-2 from all tissues that carried the *SP-A-Cre* transgene in the genome. (C) Distribution of Cre mRNA in the tissues of 8-month-old transgenic mice was revealed by RT-PCR. Loading sequence of tissues was the same with the one in B. (D) Cre activity was visualized by LacZ staining on sections from P1 *SP-A-Cre;ROSA26* double transgenic (right panels) and *ROSA26* mice (left panels). Scale bar: (D) 50 μm.

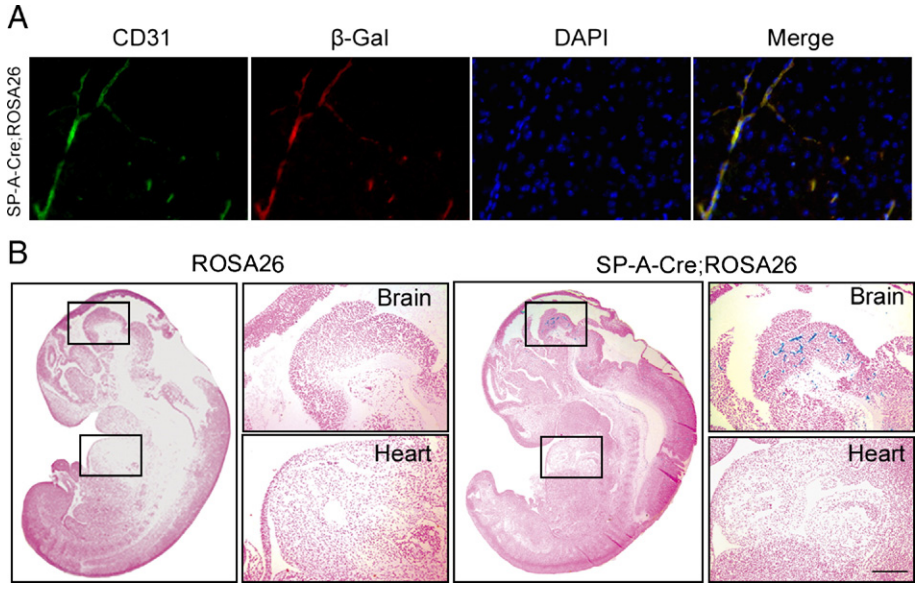

Fig. 2. Cre recombinase specifically expressed in brain microvascular endothelial cells of E11.5 *SP-A-Cre* transgenic embryos. (A) Colocalization of β-Gal (red) and endothelium specific marker CD31 (green) was visualized on frozen brain sections of the *SP-A-Cre;ROSA26* double transgenic mice. The nuclei were stained blue with DAPI. (B) LacZ activity was detected only in brain vessels of E11.5 *SP-A-Cre;ROSA26* double transgenic embryos, but not vessels in other tissues. Scale bar: (A) 50 μm; (B) 650 μm in larger panels and 200 μm in smaller panels.

facilitate the studies on genetic mechanisms controlling brain microvascular endothelium that is involved in formation and function of the BBB. Although the Cre activity was also found in alveolar type II cells of lung and epithelial cells of gland stomach, there is no consequence if the floxed gene is not expressed in this tissue. Therefore, the established *SP-A-Cre* transgenic line will be a valuable tool for studying the genetic control of BBB function, especially for discovering the important signaling pathways that regulate the physiological function of the brain endothelial cells. Previous studies have shown that the extracellular matrix (ECM) is critical during vascular morphogenesis and neovessel stabilization (Davis and Senger, 2005). The use of *SP-A-Cre* mice should greatly facilitate analysis of ECM function in brain blood vessel development and in the blood–brain barrier.

3. Experimental procedures

3.1. Generation of the SP-A-Cre transgenic mice

The 1.4 kb promoter of *SP-A* was obtained from mouse genomic DNA by PCR using the primer 5′-GTGGACAAGTG GACGGCATCC-3′ and primer 5′-GCAACATTTAAGCTTTC CAGGGTC-3′. It was inserted into a vector containing 1.2 kb *Cre* recombinase and 2.1 kb hGH polyadenylation signal (Yang et al., 2005). The linearized fragments were microinjected into the pronuclei of fertilized Kunming mouse oocytes to generate the transgenic mice. The transgenic founder mice were identified by PCR and Southern blot as described (Yang et al., 2005).

3.2. Tissue distribution of the Cre recombinase activity

Multiple tissue genomic DNAs were isolated from P4 *SP-A-Cre;Smad4*$^{co/+}$ mice. Primer a (5′-CCTTAGTTGAAGCTTA TAACTTCG-3′) and primer b (5′-GACCCAAACGTCACCTT CAC-3′) were used to amplify a fragment of 234 bp from the *Smad4* allele after the Cre mediated recombination (Yang et al., 2005). RT-PCR was performed using total RNA isolated from tissues of 8-month-old mice according to the manufacturer's instructions (Takara).

3.3. LacZ staining

LacZ staining was performed using procedures described and counterstained with Nuclear Fast Red (Yang et al., 2005).

3.4. Immunofluorescence

The whole brains were fixed in cold 4% paraformaldehyde and embedded for frozen sections. Sections were incubated with rat monoclonal anti-mouse CD31 (1:100, BD Pharmingen, San Diego, CA) or rabbit polyclonal anti-β-Galactosidase (β-Gal) (1:2000, Abcam, Cambridge, UK), followed by incubation with FITC-conjugated anti-rat IgG or TRITC-conjugated anti-rabbit IgG (1:100, Zhongshan, Beijing). The slides were stained with 4′,6′-diamidino-2-phenylindole (DAPI) to visualize the nucleus. Slides were analyzed using a Nikon fluorescence microscope.

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