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MafB is required for development of the hindbrain choroid plexus

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ABSTRACT

The choroid plexus (ChP) is a non-neural epithelial tissue that produces cerebrospinal fluid (CSF). The ChP differentiates from the roof plate, a dorsal midline structure of the neural tube. However, molecular mechanisms underlying ChP development are poorly understood compared to neural development. MafB is a bZip transcription factor that is known to be expressed in the roof plate. Here we investigated the role of MafB in embryonic development of the hindbrain ChP (hChP) using *Mafb*-deficient mice. Immunohistochemical analyses revealed that MafB is expressed in the roof plate and early hChP epithelial cells but its expression disappears at a later embryonic stage. We also found that the *Mafb*-deficient hChP exhibits delayed differentiation and results in hypoplasia compared to the wild-type hChP. Furthermore, the *Mafb*-deficient hChP exhibits increased apoptotic cell death and decreased proliferating cells at E12.5, an early stage of hChP development. Collectively, our findings reveal that MafB play an important role in promoting hChP development during embryogenesis.

1. Introduction

The choroid plexus (ChP) is an epithelial tissue that produces the cerebrospinal fluid (CSF). The ChP forms a papillary structure accompanied by highly vascularized mesenchyme within each ventricle of the brain, namely, the lateral ventricle (LV), third ventricle (3V) and fourth ventricle (4V). The CSF flows through the ventricular system in the central nervous system (CNS) which it cushions against shock from the outer environment [1]. The CSF also contains several signaling molecules, including insulin-like growth factors (IGFs) and sonic hedgehog (Shh), that stimulate proliferation and differentiation of embryonic neural cells [2,3].

The ChP originates from the roof plate, a non-neural structure transiently emerging at the dorsal midline of the neural tube. The roof plate is an organizing center that controls specification of dorsal interneurons via secretory molecules including bone morphogenetic protein (BMP) and WNT families [4,5]. During mouse embryogenesis, the hindbrain ChP (hChP) in the 4V emerges first at E12.5, followed by the generation of the telencephalic ChP in the LV, and finally the diencephalic ChP in the 3V [6]. A few studies have revealed that some transcription factors such as Lmx1a [7] and Otx2 [8] play an essential role in ChP development. However, the underlying mechanisms are poorly understood.

MafB is a bZip transcription factor that belongs to the large Maf family [9] and is expressed in several regions of the brain [10,11]. Its well-known function is to guide hindbrain segmentation at the early embryonic stage, which is evidenced by studies using *Kreisler* mice that carry a mutation in *Mafb* gene [12,13]. MafB is expressed in the roof plate as well [14]. Previous studies have shown that BMP signaling induces Lmx1a expression in the roof plate, which in turn induces MafB expression [5,7]. These findings suggest that the BMPs–Lmx1a–MafB axis is involved in roof plate development. However, to our knowledge, the role of MafB in the roof plate and its progeny, the ChP, has never been studied.

Here we report that MafB is required for development of the hChP, including differentiation, proliferation and survival. Our findings provide a new molecular mechanism underlying hChP development.

2. Materials and methods

2.1. Mice

Mafb-deficient ($Mafb^{-/-}$) mice in which the GFP gene is inserted into the endogenous *Mafb* locus were described previously [16]. The day when a vaginal plug was found after mating was defined as the embryonic day 0.5 (E0.5). All experiments were performed according to the Guide for the Care and Use of Laboratory Animals

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at the University of Tsukuba.

2.2. Hematoxylin and Eosin (H&E) staining and immunohistochemistry

For H&E staining, embryos were fixed with 10% formalin and embedded in paraffin. Parasagittal sections were prepared at 7 μm thickness. For immunohistochemistry, embryos were fixed with 4% paraformaldehyde (PFA) in PBS overnight at 4 °C, cryoprotected in 30% sucrose/PBS and embedded in Tissue-Tek OCT compound (Sakura Finetek). Frozen sections were prepared at 20 μm thickness using a cryostat. Sections were permeabilized with 0.3% Triton X-100/PBS and blocked with 10% normal goat serum/PBS. Primary antibodies were as follows: Lmx1a (1:2000, AB10533, Millipore), aquaporin 1 (AQP1) (1:5000, AB2219, Millipore), cleaved caspase-3 (1:500, #9661, Cell Signaling Technology) and Ki-67 (1:500, NCL-Ki67p, Leica Biosystems). Sections were incubated with primary antibodies at 4 °C overnight. After washing with PBS, Alexa 568 conjugated secondary antibodies were added. Hoechst 33342 was used for nuclear staining. Fluorescent images were acquired using a Biorevo BZ-9000 fluorescence microscope (Keyence) or a LSM 510 confocal laser scan microscope (Carl Zeiss). For quantification of cleaved caspase-3- or Ki-67-positive cells, three sections were prepared for each sample.

2.3. In situ hybridization

Embryos were fixed with 4% PFA/PBS overnight at 4 °C and frozen sections were prepared at 20 μm thickness. Sections were incubated with 10 μg/ml proteinase K/PBS at 37 °C for 10 min and post-fixed with 4% PFA/PBS at room temperature for 20 min. After washing, sections were incubated with hybridization buffer (50% formamide, 5 × SSC, 5 × Denhardt's solution, 50 μg/ml yeast tRNA) containing DIG-labeled cRNA probes at 60 °C overnight. After washing, sections were incubated with anti-DIG AP antibody (Roche). Signals were detected with BM purple (Roche). cRNA probe for transthyletin (TTR) were prepared according to the previous report [17]. Three sections were prepared for each sample and TTR-positive area in the hChP was measured with ImageJ software (NIH).

2.4. Statistical analysis

All data are expressed as mean ± SEM. The difference between the two groups was analyzed with Student's t-test. All statistical analyses were performed with R software (http://www.r-project.

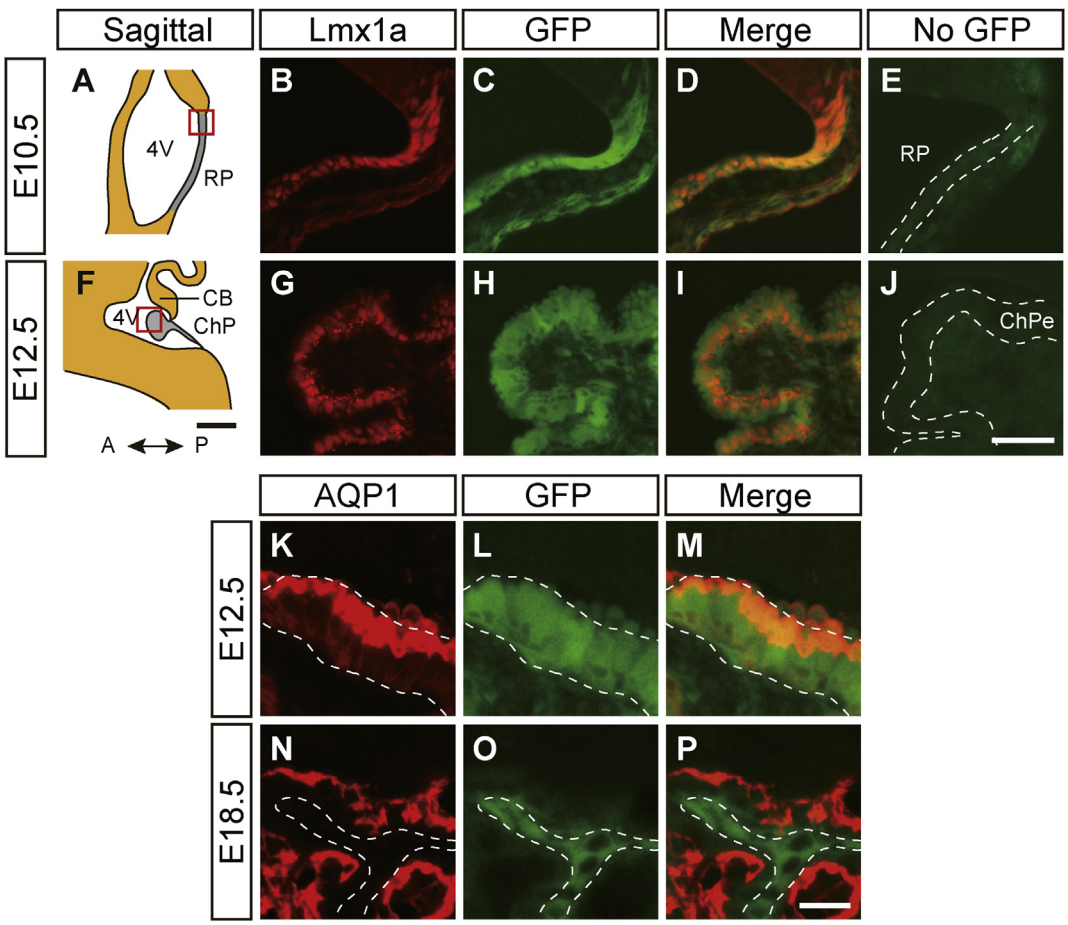

Fig. 1. MafB is expressed in the hindbrain roof plate and early hChP epithelial cells. Schematic images showing parasagittal sections of the mouse hindbrain at E12.5 (A) or E18.5 (F). Gray areas indicate the roof plate (RP) or the ChP. Red squares indicate interest areas. A, anterior; CB, cerebellum; P, posterior. Scale bar: 500 μm. (B–E, G–J) Immunohistochemistry for Lmx1a (red), a well-known marker for the roof plate, using *Mafb*$^{GFP/+}$ mouse embryos. Wild-type mouse embryos (E, J) are used as negative control for GFP fluorescence. Dotted lines mark the roof plate or the ChP epithelium (ChPe). Scale bar: 50 μm. Immunohistochemistry for aquaporin 1 (AQP1, red), a marker for ChP epithelial cells, using *Mafb*$^{GFP/+}$ embryos at E12.5 (K–M) and E18.5 (N–P). At E18.5, GFP fluorescence (dotted lines) is localized in the ChP mesenchyme, not epithelium. Scale bar: 20 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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