Biochemical and Biophysical Research Communications 414 (2011) 539–544

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Ebf1 deficiency causes increase of Müller cells in the retina and abnormal topographic projection at the optic chiasm

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ARTICLE INFO

Article history:
Received 15 September 2011
Available online 28 September 2011

Keywords:
Ebf1
Early B-cell factor
Knockout
Retinal development
Retinal ganglion cell
Axon projection
Optic chiasm

ABSTRACT

The Ebf transcription factors play important roles in the developmental processes of many tissues. We have shown previously that four members of the Ebf family are expressed during mouse retinal development and are both necessary and sufficient to specify multiple retinal cell fates. Here we describe the changes in cell differentiation and retinal ganglion cell (RGC) projection in *Ebf1* knockout mice. Analysis of marker expression in *Ebf1* null mutant retinas reveals that loss of *Ebf1* function causes a significant increase of Müller cells. Moreover, there is an obvious decrease of ipsilateral and retinoretinal projections of RGC axons at the optic chiasm, whereas the contralateral projection significantly increases in the mutant mice. These data together suggests that Ebf1 is required for suppressing the Müller cell fate during retinogenesis and important for the correct topographic projection of RGC axons at the optic chiasm.

1. Introduction

The mouse retina is composed of six classes of neuronal cells, including the ganglion, amacrine, horizontal, bipolar, rod and cone cells, and one class of glia cells, the Müller cells. The seven classes of cells can be further divided into more than 50 subgroups with distinct morphologies and functions [1,2]. All retinal cell types arise from the same group of multipotent progenitors during retinogenesis. The process of retinal development is under the tight and delicate controls of both intrinsic and extrinsic factors [3,4]. The rod and cone photoreceptors reside in the outer nuclear layer and are responsible for collecting the light signal from the environment. The horizontal, bipolar and amacrine cells are interneurons located in the inner nuclear layer and are responsible for integrating and relaying the signal from the photoreceptors. The retinal ganglion cells (RGCs) integrate the signals from amacrine and bipolar cells, and then transmit them through their long exons, which bundle into the optic nerve and cross the optic chiasm before finally projecting into the brain.

Visual signals from each retina are transmitted to the thalamus and cerebral cortex on both sides of the brain. One of the crucial steps to establish the binocular and 3-dimensional vision is the proper RGC axon projection at the optic chiasm region. In the wild type mouse, there are about 3–5% RGC axons projecting ipsilaterally at the optic chiasm, while more than 95% RGC axons project contralaterally to the other side of the brain [5,6]. Interestingly, there is a small portion of RGC axons originated from the nasal part of the retina that project through the chiasm into the contralateral optic nerve, which is called the retinoretinal projection [7–10]. The retinoretinal projection normally disappears soon after birth, and its mechanism and developmental significance is still not clear. Whether RGC axons would extend to cross the midline at the optic chiasm, and at which direction they will further project into the brain, are under the dynamic control of interactions between a group of factors, such as EphB1/Ephrin B2 [11,12], NrCAM [13], Slit1/Slit2 [10], and transcription factors Zic2 [14,15], Foxd1 [16], etc.

The Ebf/Olf (Early B-Cell Factors) family of proteins are helix-loop-helix (HLH) transcription factors including four members, Ebf1 through Ebf4, that are important for neural development [7,17–19], adipogenesis [20,21], and lymphocyte development [22,23]. Previously we reported that all four members of the Ebf family are involved in retinal cell specification [24]. In the retinas, *Ebfs* are expressed in horizontal, ganglion, type 2 OFF-cone bipolar, and non-AII glycinergic amacrine cells [24]. However, the loss-of-function study with the knockout mice has not been done yet. Here we report the increase of Müller cell in the retina and abnormal RGC axon projection at the optic chiasm in the *Ebf1* null mouse, and reveal an essential role of *Ebf1* in regulating these developmental events.

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doi:10.1016/j.bbrc.2011.09.108

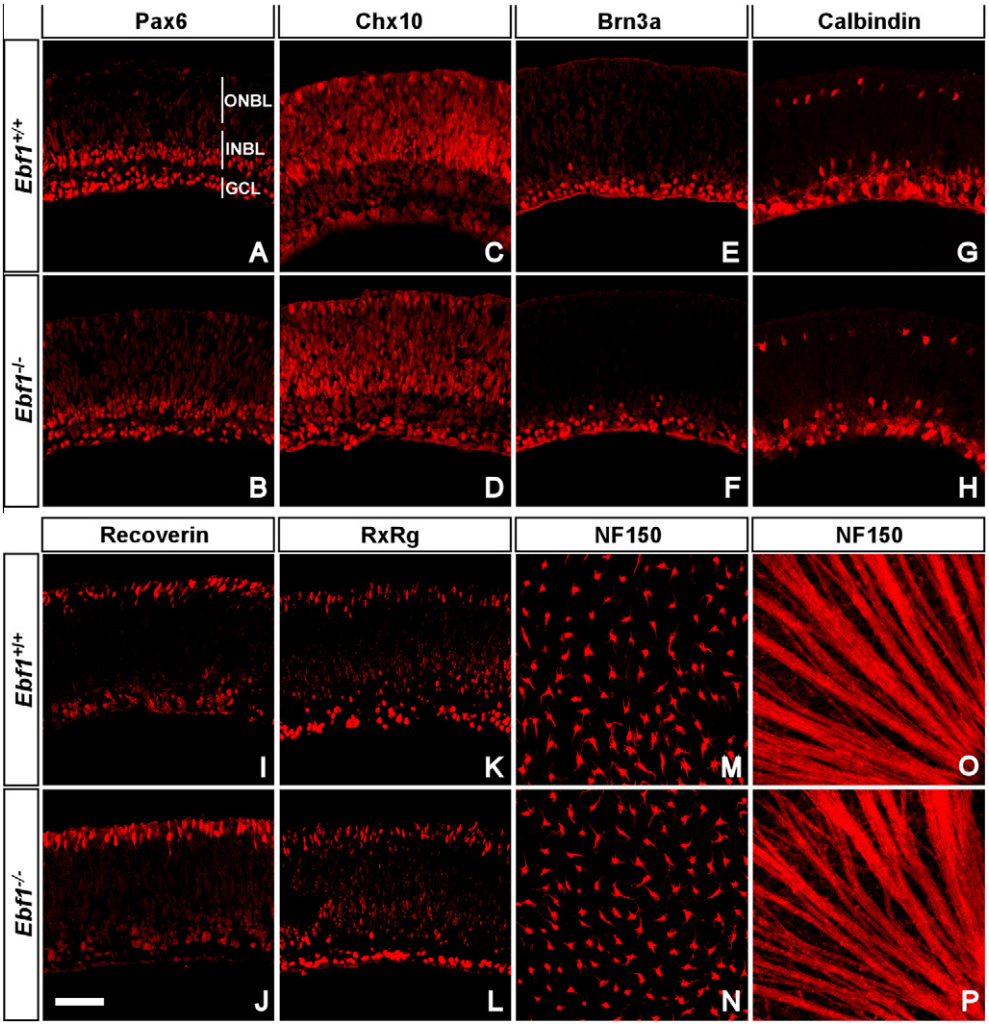

Fig. 1. Expression of cell markers in P0 *Ebf1*$^{+/+}$ and *Ebf1*$^{-/-}$ retinas. (A–L) P0 retinal sections from wild type and mutant retinas were immunolabeled with the indicated antibodies. (A and B) Anti-Pax6 stains RGCs, amacrine and horizontal cells. (C and D) Anti-Chx10 stains progenitor and bipolar cells. (E and F) Anti-Brn3a stains RGCs. (G and H) Anti-calbindin stains horizontal and amacrine cells. (I and J) Anti-recoverin stains photoreceptors. (K and L) Anti-Rxrg stains cone photoreceptors and RGCs. (M–P) NF150 whole-mount staining shows horizontal cells and RGC axon fibers at different layers. There are no significant changes in these markers between wild type and mutant retinas. GCL, ganglion cell layer; INBL, inner neuroblastic layer; ONBL, outer neuroblastic layer. Scale bar: J (for A–P), 50 μm.

2. Materials and methods

2.1. Animals

All experiments with mice were performed in compliance with the IACUC protocols approved by the University of Medicine and Dentistry of New Jersey. The animals were housed and bred at the university facility. The C57BL/6J mice were obtained from the Jackson Laboratory. The *Ebf1* knockout mice were reported previously [22] and maintained by breeding with C57BL/6J mice.

2.2. In vitro retinal explant culture and immunostaining

In vitro retinal explant culture, immunohistochemisty, and whole-mount immunostaining were performed as described previously [24–26]. The following primary antibodies were used: mouse anti-Brn3a (Millipore); goat anti-Bhlhb5 (Santa Cruz Biotechnology); rabbit anti-calbindin D-28 k (Swant); sheep anti-Chx10 (Exalpha); rabbit anti-GABA (Sigma); mouse anti-glutamine synthetase (Millipore); goat anti-GLYT1 (Millipore); rabbit anti-NF150 (Millipore); rabbit anti-Pax6 (Millipore); rabbit anti-recoverin (Millipore); and rabbit anti-RxRg/RxRγ (Santa Cruz Biotechnology). Images were captured with either the Nikon Eclipse 80i Microscope or Leica TCS-SP2 Confocal microscope.

2.3. DiI labeling and tracing

DiIC$_{18}$(3) (1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate) crystal was purchased from Invitrogen (Lot 454239). P0 mice were decapitated and fixed in fresh 4% PFA overnight. After the removal of lens and retina, a DiIC$_{18}$(3) crystal was placed onto the spot of the optic nerve. The heads were kept at 37 °C for 2 weeks in PBS with 0.05% sodium azide in a sealed cell culture plate. They were then dissected and the optic chiasm was exposed for imaging. All images were taken with the same parameters from the Nikon Eclipse 80i microscope.

2.4. Quantification

Retinas from the same litter were used for quantification analysis. Confocal images from matched regions of wild type and mutant retinas were captured with the same scanning thickness. At least 6 regions from each retina and 3 retinas from each genotype were captured. Marker-positive cells were scored from the images.