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Possible roles of Plexin-A4 in positioning of oligodendrocyte precursor cells in developing cerebral cortex

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ABSTRACT

Molecular mechanisms regulating positions of oligodendrocyte precursor cells (OPCs) remain unclear in developing cerebral cortex. To explore the mechanisms, we investigated how Plexin-A4, receptor of Semaphorin in OPCs, is involved in the positioning. We found that Plexin-A4 knockout mice exhibited (1) an increased number of OPCs in both the upper- and middle-regions of the cortical plate, where both indirect- and direct-ligands of Plexin-A4, Sema3A and Sema6A, respectively, were continuously expressed, and (2) aberrant distributions of OPCs in both the intermediate zone and corpus callosum, where Plexin-A4 was richly expressed in wild-type mice. These results suggest that Plexin-A4 is involved in the precise positioning of OPCs in developing cerebral cortex.

1. Introduction

During the development of the central nervous system (CNS), oligodendrocyte precursor cells (OPCs) are generated at the ganglionic eminence, and then migrate a long distance to their final position, where they form myelin sheaths and wrap target axons. The wrapped axons enable neurons to facilitate rapid conduction. Successful myelination, therefore, is dependent on the precise positioning of OPCs in CNS. Ablation of oligodendrocytes in the cerebellum causes a distorted development of granule and Purkinje cells [5,17], indicating that the precise positioning of OPCs is critical for the regular development of CNS.

A recent study has reported molecular mechanisms in positioning of OPCs in the spinal cord [34], while no report is available on molecular mechanisms in the cerebral cortex, in which the developmental origin and migration path of OPCs have been well documented [9,12,19,24,25,26,38].

We previously reported that: (1) Plexin-A4, a member of class A Plexins, is expressed in developing cerebral cortex and in cells of FBD-102b line, a clonal OPC line originally established in our laboratory [8,20], and; (2) Plexin-A4 is involved in the signaling of both Sema3A and Sema6A [22]. These reports indicate that potential guidance molecules in developing cerebral cortex are class 3 Semaphorins which are secretory proteins and their signals can travel through the receptor complexes consisting of Neuropilins and class A Plexins [7,23]. Class 3 Semaphorins have also been reported to act as repellent or attractant proteins for OPCs [3,28,29].

However, the question remains unanswered what roles Plexin-A4 plays in OPCs in developing cerebral cortex. In order to answer the question, the present study investigated the distribution of OPCs in developing cerebral cortices of Plexin-A4 knockout mice.

2. Experimental procedures

2.1. Animals

Plexin-A4 knockout mice [30,31] were provided by RIKEN BRC (BRC No. 02099) with the support of National BioResource Project of Ministry of Education, Culture, Sports, Science and Technology, Japan. CD-1 (Charles River Japan, Yokohama, Japan) and Plexin-A4 knockout mice were maintained in the experimental animal facility of Tokyo University of Science. Care and handling conformed to the NIH guidelines for animal research, and experimental protocols were approved by the Institutional Animal Care and Use Committee.

2.2. Immunohistochemistry

The protocol for immunohistochemistry was described previously [33]. Briefly, brain sections were overlaid with primary

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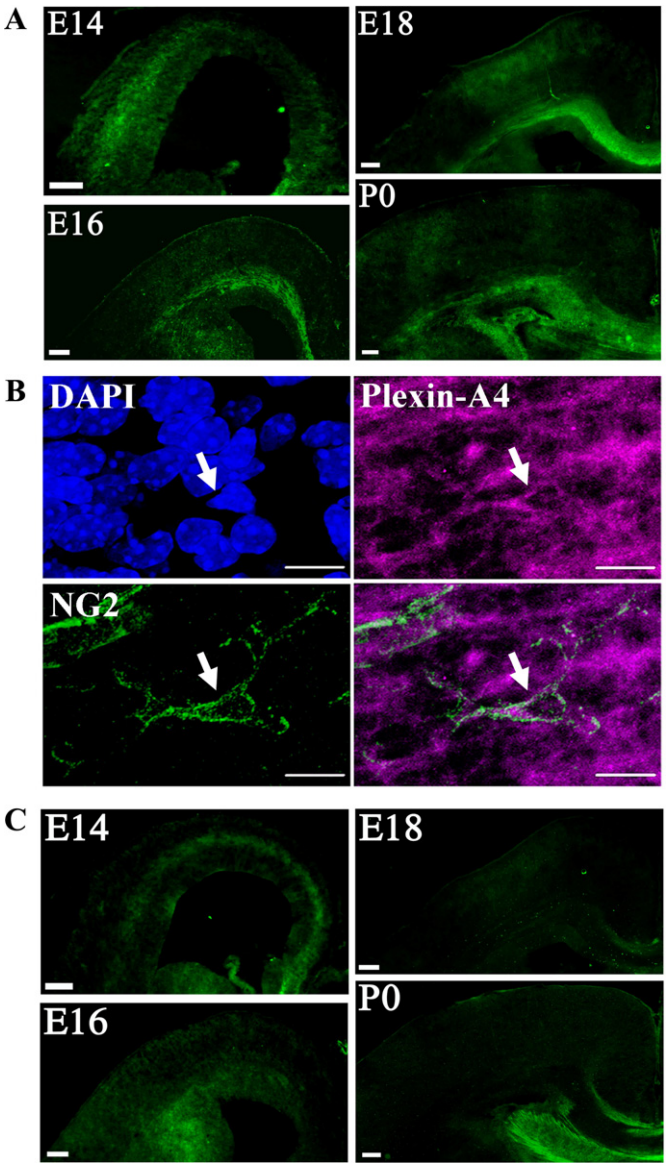

Fig. 1. Expression of Plexin-A4 and Neuropilin-1 in developing cerebral cortices. (A) Expression of Plexin-A4 in developing cerebral cortices. (B) Plexin-A4 expression (magenta) in NG2-positive OPC (green) localized in IMZ. (C) Expression of Neuropilin-1 in developing cerebral cortices. Scale bar = 200 μm (A and C), 10 μm (B).

antibodies. The primary antibodies were rabbit polyclonal anti-NG2 (Chemicon, Temecula, CA), anti-Semaphorin (anti-Sema3A; Abcam, Cambridge, UK), anti-Neuropilin-1 (a kindly gift from Dr. H. Fujisawa), rat monoclonal anti-platelet/endothelial cell adhesion molecule-1 (anti-PECAM; BD, Franklin Lakes, NJ), anti-Semaphorin 6A (anti-Sema6A; R&D Systems, Minneapolis, MN) and Armenian hamster monoclonal anti-Plexin-A4 [31] antibodies. After washes, sections were incubated with species specific secondary antibodies (Cy3-conjugated antibodies; Jackson Immunoresearch, Alexa488 conjugated antibodies; Molecular Probes, Eugene, OR). Sections were incubated with 4′,6′-diamidino-2-phenylindole hydrochloride (DAPI; Sigma) to visualize nuclei. Fluorescence specimens were viewed with a fluorescence microscope IX71 (Olympus, Tokyo, Japan) or a confocal laser scanning microscope LSM510 (Carl Zeiss, Oberkoken, Germany).

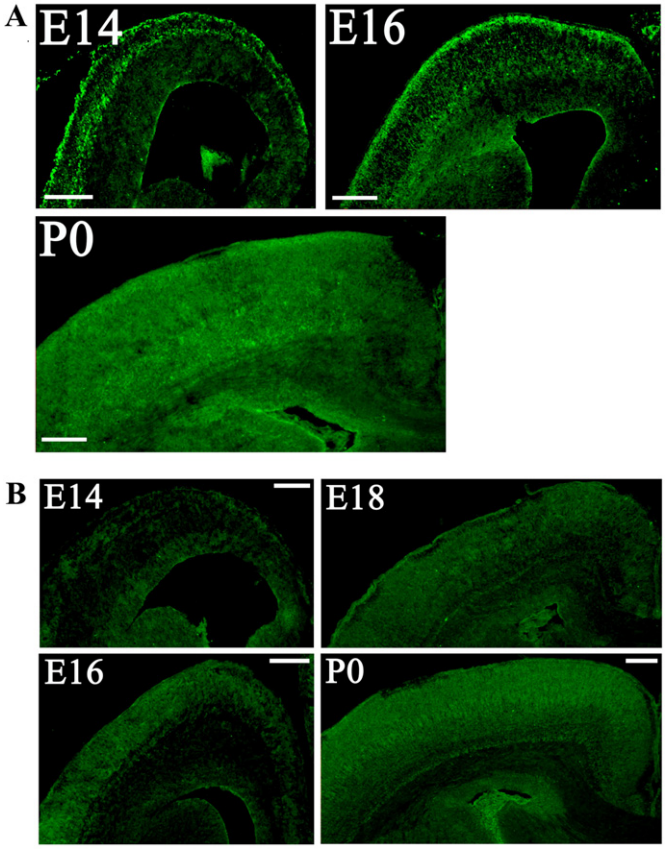

Fig. 2. Localization of Sema3A and Sema6A proteins in developing cerebral cortices. Immunohistochemical analysis of Sema3A (A) and Sema6A (B). Scale bar = 200 μm.

3. Results

3.1. Distribution of OPCs in developing cerebral cortices

The distribution of OPCs in developing cerebral cortices was examined by double-immunostaining with anti-NG2 and -PECAM antibodies (Fig. S1). Our observation confirmed that OPCs arising from the ganglionic eminence (GE) enter into the cerebral cortex through the intermediate zone (IMZ) during embryonic day 14 (E14) to E16, and disperse from IMZ into the cortical plate (CP) during E16–E18, and then the final positioning of OPCs appeared to be completed by postnatal day 0 (P0).

3.2. Localization of Plexin-A4 and Neuropilin-1 in developing cerebral cortices

In IMZ and GM, strong signals of Plexin-A4 protein were detected at E14 and continuously detected till P0 (Fig. 1A). Observation with a confocal laser scanning microscope confirmed the expression of Plexin-A4 protein in OPC located in IMZ at E16 (Fig. 1B). Neuropilin-1, co-receptor of Plexin-A4 was clearly expressed in IMZ at E14 and E16, and the expression was dramatically decreased in IMZ thereafter (Fig. 1C).

3.3. Localization of Semaphorins in developing cerebral cortices

Sema3A and Sema6A, ligands of Plexin-A4 were immunohistochemically localized (Fig. 2). At E14 and E16, Sema3A was detected strongly in the most upper and deepest regions of CP, and weakly in IMZ (Fig. 2A). At P0, Sema3A was detected strongly over the all areas of CP, and weakly in GM (Fig. 2A). Sema6A was clearly detected