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Direct evidence that ventral forebrain cells migrate to the cortex and contribute to the generation of cortical myelinating oligodendrocytes

Eiko Nakahira [a], Tetsushi Kagawa [a,b,c,*], Takeshi Shimizu [c], Martyn D. Goulding [d], Kazuhiro Ikenaka [a]

[a] *Laboratory of Neurobiology and Bioinformatics, National Institute for Physiological Sciences, 5-1 Higashiyama, Myodaiji, Okazaki 444-8787, Japan*
[b] *Laboratory of Active Transport, National Institute for Physiological Sciences, 5-1 Higashiyama, Myodaiji, Okazaki 444-8787, Japan*
[c] *Division of Cell Fate Modulation, Institute of Molecular Embryology and Genetics, Kumamoto University, 2-2-1 Honjo, Kumamoto, Kumamoto 860-0811, Japan*
[d] *Molecular Neurobiology Goulding Lab, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92186-5800, USA*

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Abstract

Cortical neuroepithelial cells generate neurons, astrocytes, and oligodendrocytes (OLs) in vitro. However, whether cortical OLs are derived from the cortical neuroepithelium or migrate from the ventral forebrain is under severe debate yet. This is due to the fact that OL progenitor cells (OPCs), as marked by the expression of PDGFRα or NG2, are generated at around embryonic day (E) 11 or 12 in the mouse ganglionic eminences, but the myelinating OLs appear during the second week postnatally in the cortex. There has been no labeling method for long-term glial cell-lineage tracing. Thus, we developed a new strategy: plasmid DNA encoding Cre recombinase was introduced into the Cre/loxP reporter forebrain in ventral- or dorsal-specific manner by in utero DNA electroporation. The reporter *gfp* gene is expressed permanently owing to the chromosomal DNA recombination. The GFP-labeled myelinating OLs were detected in the adult cortex when electroporation was targeted to the ventral neuroepithelium, demonstrating at least some of the myelinating OLs are derived from the ventral forebrain. However, when electroporation was targeted to the dorsal, we could not find GFP-labeled myelinating OLs. This suggests that the progenitors of cortical OPCs are absent or located at restricted regions in the dorsal forebrain (cortex) at E12.

Keywords: Oligodendrocyte; Forebrain; Migration; In utero electroporation; Olig2; PDGFRα

Introduction

Oligodendrocytes (OLs) are myelin-forming cells and widely observed in the adult central nervous system (CNS). It had long been believed that OL progenitor cells (OPCs) were generated from all parts of ventricular zone (VZ) because OL differentiation was observed in the primary culture prepared from various regions of embryonic CNS. Recently developed neurosphere and clonal analyses also indicated that OLs could be generated from the multipotent neural stem cells in various regions of the embryonic brain (Davis and Temple, 1994; Gritti et al., 1996; Johe et al., 1996; Reynolds and Weiss, 1996; Tropepe et al., 1999; reviewed in Gage, 2000; Temple, 2001). However, the in vivo expression of the OPC markers, Olig2, myelin proteolipid protein (PLP)/DM-20, and platelet-derived growth factor receptor α subunit (PDGFRα), is initially detected at a restricted region in the ventral neuroepithelium and, as development proceeds, distributes to the dorsal cortex (Timsit et al., 1995; Pringle et al., 1998; Takebayashi et al., 2000; Spassky et al., 2000; Tekki-Kessaris et al., 2001; Ivanova et al., 2003), suggesting that some populations of cortical OLs are derived from the ventral forebrain. Nevertheless, it has remained unclear whether the developmental change of marker-positive cell distribution corresponds to OPC migration.

To address this question, several attempts have been made to identify the origin of cortical OLs in vivo. Olivier et al. (2001) applied chick-quail grafting experiments and demonstrated that

* Corresponding author. Division of Cell Fate Modulation, Institute of Molecular Embryology and Genetics, Kumamoto University, 2-2-1 Honjo, Kumamoto, Kumamoto 860-0811, Japan. Fax: +81 96 373 6614.
E-mail address: kagawa@kaiju.medic.kumamoto-u.ac.jp (T. Kagawa).

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all OLs in the avian cortex were derived from precursors in the anterior entopeduncular area of the basal forebrain. However, since the structure of chick cortex is much simpler than that of the mammalian cortex, there might be a difference between chick and mammalian cortices. Marshall and Goldman (2002) analyzed Dlx2/tauLacZ knock-in mouse and confirmed that subpallium-derived Dlx2-expressing cells give rise to OLs in the white matter and cerebral cortex. These experiments supported the idea of ventral origin of OLs. In contrast, Gorski et al. (2002) demonstrated using Emx1/Cre knock-in mouse that most cortical OLs originate from a pallium (cortex)-derived Emx1-expressing cells. The latter two experiments depended on the promoter activities of *dlx2* and *emx1* genes for labeling cells in a specific region of the brain: Dlx2 to label cells with ventral origin and Emx1 to label cells with dorsal origin. In these cases, it is always possible (although these have not been proven) that the dorsally derived cells later express Dlx2/tauLacZ or ventrally derived cells express Emx1 after migrating into the cortex. In another example, it has recently been shown in the late embryonic spinal cord that dorsal cells begin to express Olig2, which has been believed to be a ventral marker, in a shh-independent manner (Cai et al., 2005; Fogarty et al., 2005; Vallstedt et al., 2005). Thus, we must be careful in interpreting the results obtained by the genetic strategies. In this sense, it has not been directly demonstrated yet whether the cells with ventral origin contribute to the generation of mammalian cortical OLs.

Spatiotemporal cell labeling by DiI is used as a direct approach for fate mapping analyses. However, the proliferating glial cells, including OPCs, are difficult to trace in vivo because DiI would be quickly diluted after several rounds of proliferation. Retroviral infection cannot define the precise position of the initially infected cells. The slice culture system is also inadequate for this purpose because the appearances of OPC markers begin at E11 or E12, and the mature (myelinating) OLs appear at the second week postnatally. There has been no slice culture system that reproduces developmental course of the forebrain from E12 to P10. Here, we established a new strategy for tracing the cell fate of ventral or dorsal forebrain cells. Mouse embryos of a Cre reporter strain in utero were electroporated selectively to the dorsal or ventral forebrain with a plasmid encoding Cre recombinase. This study provides direct evidence that a population of myelinating OLs in the cortex originates from the VZ of the ventral forebrain.

Materials and methods

Animals

To maintain the ROSA-GAP43-EGFP reporter mouse colony, the following PCR primers were used for genotyping: forward primer, 5′-CTTACTTGTA-CAGCTCGTCC-3′; reverse primer, 5′-GCAGAAGAACGGCATCAAGG-3′. ICR mice were purchased from Japan SCL (Shizuoka, Japan). The day of insemination was designated embryonic day zero (E0).

Plasmids

pCX-EGFP, which contains the EGFP cDNA under the control of the CMV enhancer and chick β-actin promoter, was a gift from Dr. M. Okabe (Osaka University, Osaka, Japan). The EGFP cDNA in pCX-EGFP was replaced by an 1100-bp *Mlu*I fragment encoding the NLS-Cre recombinase that was prepared from pCre-Pac (a gift of Dr. T. Yagi, Osaka University, Osaka, Japan) to construct pCX-Cre.

In utero DNA transfer by electroporation

Plasmid DNA was purified using the CONCERT plasmid maxi prep kit (Invitrogen, Carlsbad, CA) and dissolved in 1 mM Tris–HCl and 0.1 mM EDTA (pH 8.0) at a concentration of 3–4 μg/μl. The DNA solution also contained 0.05% Fast Green for monitoring the injection. Pregnant mice at E12 were deeply anesthetized with sodium pentobarbital. The uterine horns were exposed, and approximately 1–1.5 μl of DNA solution was injected into the lateral ventricle (LV) of the embryonic forebrain through the uterus with a glass micropipette (type G-1, Narishige, Tokyo, Japan). After injection, the embryo in the uterus was placed between the electrodes. The forceps-type electrodes were custom-made with a thin tungsten needle as an anode (CUY661-3X7 with CUY661N in a recent catalogue of NEPA GENE, Chiba, Japan). An electroporator (CUY21, NEPA GENE) was used to deliver six 50-ms pulses of 33–35 V, with 75-ms intervals. The uterus was placed back into the abdominal cavity for continued embryonic development.

Electroporation with FITC-conjugated oligonucleotides

E12 ICR embryos were electroporated with 10 mg/ml of FITC-conjugated oligonucleotide (5′-ATGCATGCATGCAT-3′). Soon after electroporation, the brains were dissected. Several holes were made in the brain to flush out any remaining oligonucleotides in the lateral ventricles with PBS 3 times. Brains were fixed in 4% paraformaldehyde (PFA) and 0.1 M phosphate buffer (pH 7.4) overnight at 4°C. Brains were sliced with a Vibratome (DTK-3000, DOSAKA EM, Kyoto, Japan) (300-μm thickness) and mounted onto glass slides with 80% glycerol.

Immunostaining of vibratome sections

Embryos were perfused and postfixed with 4% PFA overnight at 4°C. Brains were dissected and embedded in 3% agar in PBS and sliced with a vibratome, producing 300-μm sections. Free-floating sections were incubated with rabbit anti-GFP polyclonal antibody (Molecular Probes, Eugene, OR) overnight and subsequently, with Alexa Fluor 488-labeled goat anti-rabbit antibody (Molecular Probes).

Primary culture and immunocytochemistry

The cerebral cortices were isolated from E17 ROSA-GAP43-EGFP embryos whose ventral forebrains were electroporated at E12. The cells were dissociated in 0.25% trypsin with 0.01% DNase I for 15 min at 37°C with shaking. The cells were seeded onto polyethyleneimine-coated 8-well glass slides (8 mm in diameter) at a density of $3 \times 10^5/cm^2$ and cultured in 10% FCS DMEM for 4 days. To induce dorsal cells to generate OLs in vitro, the cerebral cortices were isolated from E14 ROSA-GAP43-EGFP mice that were dorsally electroporated at E12. Olfactory bulbs were carefully removed to avoid a contamination of olfactory-derived oligodendrocytes (Spassky et al., 2001). Each tissue was triturated in calcium- and magnesium-free HBSS and cultured on ornithine/fibronectin-coated dishes for 4 days in N2-supplemented DMEM/F-12 containing 10 ng/ml basic fibroblast growth factor (bFGF PeproTech 100-18B). The cells were replated on ornithine/fibronectin-coated chamber slides and treated with 30 ng/ml triiodothyronine (T3) and 40 ng/ml thyroxine (T4) in the absence of bFGF to induce OL differentiation for another 3 days. Rabbit polyclonal anti-GFP antibody (Molecular Probes) was used with the following cell-specific markers: O4 antibody (provided by Dr. S. Pfeiffer, University of Connecticut Medical School, Farmington, CT) for OLs, mouse monoclonal anti-GFAP antibody (Sigma, St. Louis, MO) for astrocytes, and mouse monoclonal anti-MAP2 antibody (Sigma) for detecting neurons. Alexa Fluor® secondary antibodies (Molecular Probes) were used for visualizing the stained cells.

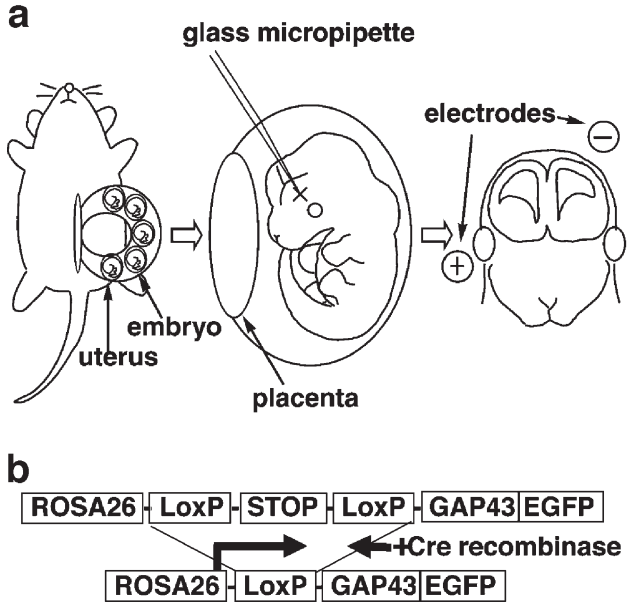

Fig. 1. Schematic representation of in utero electroporation and Cre-assisted DNA recombination in the ROSA-GAP43-EGFP reporter mouse. (a) The DNA solution was injected into the telencephalic vesicles through the uterine wall with a glass micropipette on E12. The DNA-injected embryos, with the surrounding uterus, were held by forceps-type electrodes and received electrical pulses. Manipulated embryos were born and developed in good physical shape. Nearly 90% of the electroporated embryos were alive at E15, and most of them were born. Fifty percent grew normally thereafter and survived for over a year. (b) The ROSA-GAP43-EGFP reporter mouse carries a GAP43-EGFP fusion gene connected to downstream transcriptional stop signals flanked by two loxP sites in the ROSA26 locus. Once Cre recombinase is expressed, the transcriptional STOP signals are removed, and the GAP43-EGFP fusion gene starts to be expressed under the control of the ROSA26 promoter in the transformed cells.

Cryosections and immunohistochemistry

Brains were perfused and postfixed in 4% PFA, cryoprotected in 20% sucrose, and sectioned at 10- or 25-μm thicknesses using a cryostat. For immunohistochemistry, the sections were incubated with the following primary antibodies: rabbit anti-GFP polyclonal (Molecular Probes), mouse anti-glutathione-*S*-transferase-π (GST-π) monoclonal (BD Transduction Laboratories, San Jose, CA), rat anti-myelin proteolipid protein (PLP) monoclonal (provided by Dr. M. Lees, University of Queensland, Brisbane, Australia), and rat anti-PDGFRα monoclonal (BD PharMingen, San Diego, CA) antibodies. Alexa Fluor® secondary antibodies (Molecular Probes) were used for visualizing the stained cells. For double staining of Olig2 and GFP, sections were first incubated with rabbit anti-Olig2 polyclonal antibody (a gift from H. Takebayashi, National Institute for Physiological Sciences), followed by incubation with Alexa Fluor 594-conjugated goat anti-rabbit IgG antibody. The sections were then incubated with anti-GFP antibody, followed by Alexa Fluor 488-conjugated goat anti-rabbit IgG antibody. All sections were counterstained with 0.1 μg/ml DAPI (Wako, Osaka, Japan). Fluorescence microscopy images were obtained using a BX51 microscope (Olympus, Tokyo, Japan) and Axio Cam (Carl Zeiss, Oberkochen, Germany).

Results

Establishment of the region selective electroporation system

To study the migration of OPCs in the embryonic mouse brain, we established a region selective electroporation system. In utero, DNA electroporation is useful for introducing DNA in a spatially and temporally restricted manner (Tabata and Nakajima, 2001; Saito and Nakatsuji, 2001). Since DNA is negatively charged, the injected DNA moves towards the anode in the electronic field (Fig. 1a); thus, DNA can be introduced in a dorsal- or ventral-specific manner by electrode positioning. Indeed, FITC-conjugated oligonucleotides were successfully introduced into the desired regions: 28/32 ventral forebrains (87.5%; Fig. 2a) and 28/35 dorsal forebrains (80.0%; Fig. 2b) received DNA into the desired region. These scores were counted at the beginning of the experiments, and our technique improved over time to target a specific region at a higher efficiency. Another remarkable feature of in utero DNA electroporation is the transient retention of plasmid DNA in actively proliferating cells, which is useful for temporal gene expression. However, this turns out to be a disadvantage for fate-mapping studies, since permanent cell labeling is required for this purpose. Indeed, plasmid DNA was quickly diluted in the mitotic cells, including the OPCs, during their proliferation. For example, the ventral forebrain was electroporated at E12 with pCX-EGFP, which contains EGFP cDNA under the control of the cytomegalovirus enhancer and the chicken β-actin promoter (CAG promoter, Niwa et al., 1991), and analyzed at E15. The GFP signals were mainly detected in the mantle layer of the basal ganglia, which consisted of postmitotic neurons, but not in the mitotic-active VZ (Fig. 3a).

Since this temporal expression of the marker gene had been predicted, we utilized a Cre/loxP reporter mouse strain, the ROSA-GAP43-EGFP mouse (Fig. 1b). *GAP43-EGFP* is a fusion gene encoding a membrane location signal of GAP43 and EGFP, enabling the visualization of cell morphologies (Ono et al., 2001). This mouse does not express the GAP43-EGFP fusion gene under normal conditions. When plasmid DNA encoding *Cre* recombinase derived from the CAG promoter is introduced into the embryo by electroporation, GAP43-EGFP becomes continuously expressed in the transformed cells and their progeny (Fig. 1b). The constant expression of GAP43-EGFP in the VZ cells of the ganglionic eminence (GE) supported this theory (Fig. 3b). After the electroporated brain cells were cultured, GAP43-EGFP expression was observed in the 3 major CNS cell types examined: neurons, astrocytes, and OLs (ventral electroporation in Figs. 3e, f, and g; and dorsal

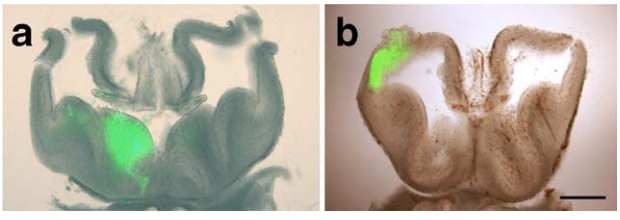

Fig. 2. Introduction of FITC-conjugated oligonucleotides to either the ventral or dorsal forebrain with high efficiencies by electroporation. E12 ICR mouse embryos were electroporated with FITC-conjugated oligonucleotides. Shortly after electroporation, embryo brains were dissected, and the remaining "free" oligonucleotides were washed away with PBS. FITC oligonucleotides were selectively introduced into the ventral forebrains (a) and the dorsal forebrains (b). Scale bar: 500 μm.

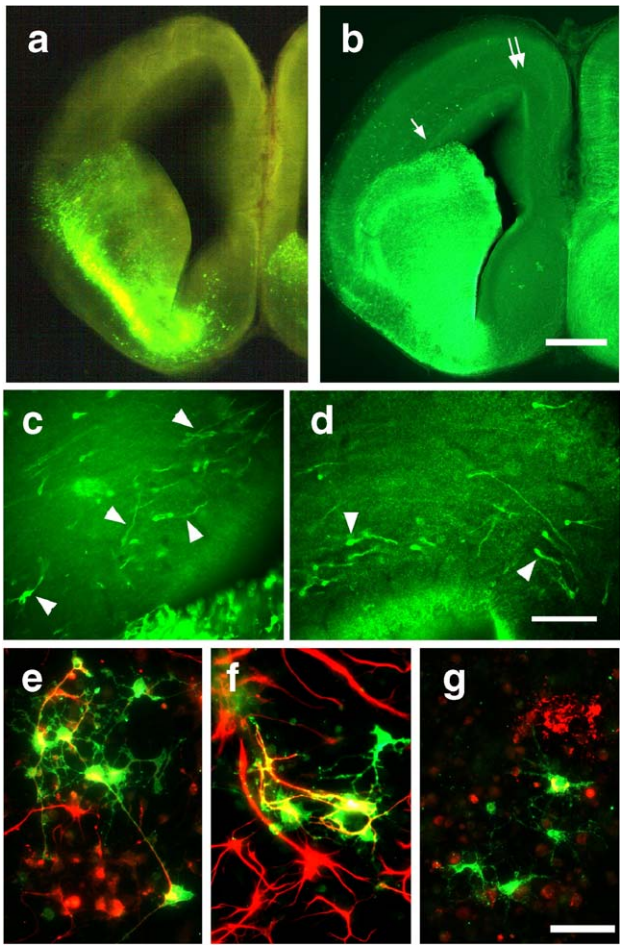

Fig. 3. Permanent GFP labeling by the Cre-EP system. (a) The ventral forebrain of the left side was electroporated with pCX-EGFP at E12 and analyzed at E15. GFP signals were not in the mitotic-active VZ. (b) ROSA-GAP43-EGFP mice were electroporated with pCAG-Cre at E12 and analyzed at E16. The VZ cells of the GE began to express EGFP. Notably, the surface of the ventricular cortex was not labeled by GFP. (c) A magnified image of the area indicated by a single arrow in panel b. Many GFP$^+$ cells, presumably originating from the VZ of the GE, were observed in the cortical SVZ and directed their processes towards the cortex (arrowheads). (d) A magnified image of the area indicated by the double arrows in panel b. GFP$^+$ cells were even observed in the dorsal arch area (arrowheads). ROSA mice were electroporated with pCAG-Cre at E12. (e–g) GFP expression in various cell types in vitro. The mouse cortices were dissociated at E17 and cultured for 4 days. The cells were double immunostained by anti-GFP antibodies (green) and cell-type specific makers (red): anti-MAP2 antibody (e), anti-GFAP antibody (f), and O4 antibody (g). GFP expression was detected in the three major cell types of the CNS. Scale bars: 500 μm (a, b), 100 μm (c, d), and 50 μm (e–g).

electroporation in Supplemental Fig. 1). Thus, the ROSA26 promoter is active in these terminally differentiated progeny. In addition, the ventricular lumen of the cortices, which later developed into the ependyma, was never labeled by GAP43-EGFP in the ventrally electroporated mice (Figs. 3b and c), even at postnatal day 30 (Figs. 5d and g). Thus, the position of the GAP43-EGFP$^+$ ependymal cells most likely represents the site of initial DNA introduction. This idea was supported by avian grafting studies, in which ependymal cells did not actively disperse on the ventricular surface, but instead formed cell clusters (Cameron-Curry and Le Douarin, 1995; Pringle et al., 1998; Olivier et al., 2001).

At E16, many GAP43-EGFP$^+$ cells were observed in the cortical subventricular zone (SVZ) of mice ventrally electroporated at E12. They exhibited bipolar morphologies with long leading processes oriented toward the dorsal direction, indicating their active tangential migration (Fig. 3c). Some cells were even found near the hippocampal region (Fig. 3d). As previous studies demonstrated, the cells migrating from the GE into the SVZ were most likely neural precursors (Anderson et al., 1997; Wichterle et al., 2001). The Cre-EP system is especially favorable for tracing mitotic cells, and we next applied this technique to investigate the migratory pathways of OPCs.

Tangential migrations of PDGFRα$^+$ and Olig2$^+$ OPCs from the ventral forebrain

We chose E12 for electroporation because at this age, *pdgfrα*$^+$ and *Olig2*$^+$ OPC localization was restricted to the ventral region, but not yet observed in the cortex. Ages later than E12 were inadequate for the region-selective cell labeling because both *pdgfrα*$^+$ and *Olig2*$^+$ OPCs, which might derive from the ventral VZ, started to appear in the cortical SVZ over the next few days (Tekki-Kessaris et al., 2001; Ivanova et al., 2003). First, we investigated whether the dorsal appearance of these marker-expressing cells reflected the tangential migration of OPCs. The pCX-Cre plasmid was injected into the LV of the ROSA-GAP43-EGFP mice and ventrally electrotransferred at E12. Brain sections were prepared at E16 and double immunostained with antibodies to GFP and PDGFRα. Many GAP43-EGFP$^+$ cells were detected in the cortical SVZ, and a few were in the intermediate zone (IZ; Fig. 4a). Many PDGFRα$^+$ cells were detected in the IZ (Figs. 4a and b), and some of them were also positive for GAP43-EGFP (arrow and arrowheads in Figs. 4a and b). Typical double-positive cells exhibited bipolar morphologies and extended their processes tangentially, indicating that these cortical PDGFRα$^+$ cells originated from the basal forebrain. Many of GAP43-EGFP$^+$ bipolar and unipolar cells in the cortical SVZ were positive for Olig2 (arrowheads in Fig. 4c), which is an earlier marker than *pdgfrα* for OPCs (Lu et al., 2000; Takebayashi et al., 2000; Zhou et al., 2000). We previously reported that Olig2$^+$/PDGFRα$^+$ cells were mostly found in the IZ and Olig2$^+$/PDGFRα$^-$ cells were mainly observed in the SVZ at E16 (Ivanova et al., 2003). Current data indicate that both Olig2$^+$/PDGFRα$^+$ and Olig2$^+$/PDGFRα$^-$ cells originate and migrate from the basal forebrain. Since we are permanently labeling the cells and we did not cut the uterus, we can observe the fate of the labeled cells even after birth. Thus, we analyzed whether these cells differentiated into myelinating OLs in the adult cortices.

Myelinating OLs originated from the ventral forebrain

To study the fate of ventral-derived cells, ROSA-GAP43-EGFP mouse brains were ventrally electroporated with pCX-Cre at E12, and the distribution of the GFP-labeled cells was

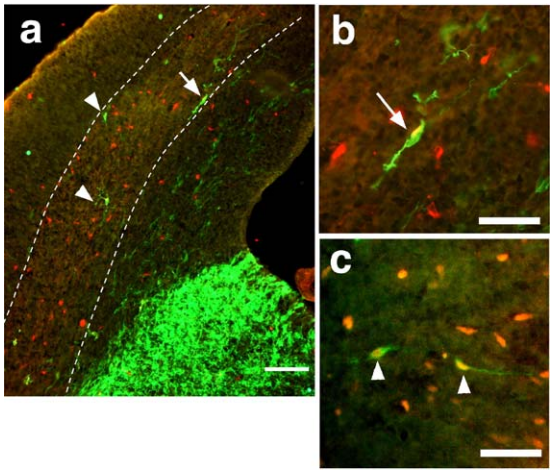

Fig. 4. Subpallial-originated PDGFRα$^+$ and Olig2$^+$ OPCs in the developing cortex. The ROSA-GAP43-EGFP mouse was electroporated at E12 and analyzed at E16. (a) The cryosection was immunostained by anti-GFP antibody in green and anti-PDGFRα antibody in red. The GE ventricular surface was specifically labeled by GFP, indicating the ventral-specific DNA introduction. White dotted lines represent the borders of the CP, IZ, and SVZ. PDGFRα-expressing cells were found mainly in the IZ, and some of them co-expressed GFP. Arrowheads and an arrow indicate GFP/PDGFRα double-positive cells found in the IZ (a, b). (b) A higher magnification of the cell indicated by the arrow in panel a. (c) A cryosection was immunostained by anti-Olig2 (red) and anti-GFP (green) antibodies. Many GFP$^+$ bipolar and unipolar cells with Olig2$^+$ nuclei were observed in the cortical SVZ. Arrowheads indicate GFP/Olig2 double-positive cells in the SVZ. Scale bars: 100 μm (a) and 50 μm (b, c).

analyzed at P9 and P30. To confirm the ventral-specific introduction of the DNA, the following morphologic criteria were examined in each mouse: (1) ependymal cells of the striatum and/or basal ganglia, but never the cortex, were labeled with GAP43-EGFP (Figs. 5d and g). (2) The olfactory bulb contains many GAP43-EGFP-labeled cells in the granular layer, as most olfactory granule neurons are supposed to derive from the SVZ of basal forebrain. (3) Absence of GFP$^+$ radially projecting axons in the cortex (Fig. 5d). We defined such mouse brains as ventrally labeled. The GAP43-EGFP-labeled cell bodies were distributed mainly in the striatum and showed divergent morphologies, including neurons and glial cells. In most cases, less than 5% of the total GFP$^+$ cells were found on the cortical side. Myelin proteolipid protein (PLP) was used as a marker for detecting the mature OLs at P9 because PLP is expressed not only in the extended processes, but also in the OL cell bodies during the early postnatal days. Cells double-positive for anti-GFP and anti-PLP antibodies were clearly detected in the external cortex capsule (Figs. 5a–c), indicating that these OLs originated and migrated from the basal forebrain. At P30, the myelinating OLs that were labeled by GAP43-EGFP became easily identified by their typical morphology, with several processes and myelin structures along the axons (Figs. 5e and h). In addition, the GST-π antibody (Tansey and Cammer, 1991) was auxiliary for the precise classification of mature OLs (Figs. 5f and i). We analyzed 6 ventrally labeled brains, and all contained GFP-expressing OLs in the cortical plate (Fig. 5f) and corpus callosum (Fig. 5i). On average, 26.9 ± 9.9 GFP-expressing cells were found in each cortex coronal section (10 μm thick). The number of cells double-positive for GFP and GST-π was 9.3 ± 4.6 per section, which was 34.8% ± 13.6% of the total GFP$^+$ cells found in the cortex. Because transfection efficiencies by electroporation varied significantly, the standard deviation was relatively high. Similar results were obtained with anti-PLP antibody staining (Fig. 6). These results demonstrated that a population of ventral cells migrated into the cerebral cortex and gave rise to myelinating OLs by P30.

No myelinating OLs were derived from the E12 dorsal forebrain

Next, we examined whether OLs was generated from the cortical VZ. To address this issue, forebrains of ROSA-GAP43-EGFP mice dorsally electroporated at E12 were analyzed at P30. Dorsally labeled brains were classified according to the following morphological criteria: (1) ependymal cells of the cortex side, but never the ventral side, were labeled with GFP (Fig. 7b); (2) presence of radially oriented clusters of GFP-labeled astrocytes in the cortex (Fig. 7a); (3) presence of GFP-labeled axons projecting in a radial direction to the cortex (Fig. 7a).

Sixteen dorsally labeled brains were examined, and none of them contained GFP$^+$ myelinating OLs in the cortical region. On average, 29.2 ± 13.8 GFP-expressing cells were observed in each cortex coronal section (10 μm thick). In some cases, there were a small number of cells double-positive for GFP and GST-π (0.9 ± 1.0 cells per section); however, they did not extend the myelin structure along the axons and were not judged to be myelinating OLs (Figs. 7e and f). Figs. 7g–i provide further evidence that cells double-positive for anti-GFP and anti-PLP were not detected at P9 in the dorsally electroporated cortex.

Discussion

Ventral origin of the mouse cortical OLs

The origin of OLs in the mouse cerebral cortex has been difficult to determine because OPCs are highly motile and mitotic. The established region selective electroporation system combined with Cre recombinase/reporter mouse system is useful for spatiotemporal cell labeling and tracing the migration of proliferating cells including OPCs. In many in situ hybridization studies, cells expressing PDGFRα and/or Olig2 have been first detected in the ventral forebrain and later in the cortex, suggesting that these cells are migrating into the cortex (Timsit et al., 1995; Pringle et al., 1998; Takebayashi et al., 2000; Spassky et al., 2000; Tekki-Kessaris et al., 2001; Ivanova et al., 2003). We first showed that indeed, these OPCs or OPC-related cells in the intermediate zone or subventricular zone of the cortex originated in the ventral forebrain (Fig. 4), thus strongly supported their active migration. We also demonstrated that these ventrally derived cells become myelinating OLs in the adult cortex (Figs. 5 and 6). This study provides direct and in vivo evidence demonstrating that some populations of ventral forebrain cells migrate to the cortex and contribute to the generation of cortical myelinating OLs.

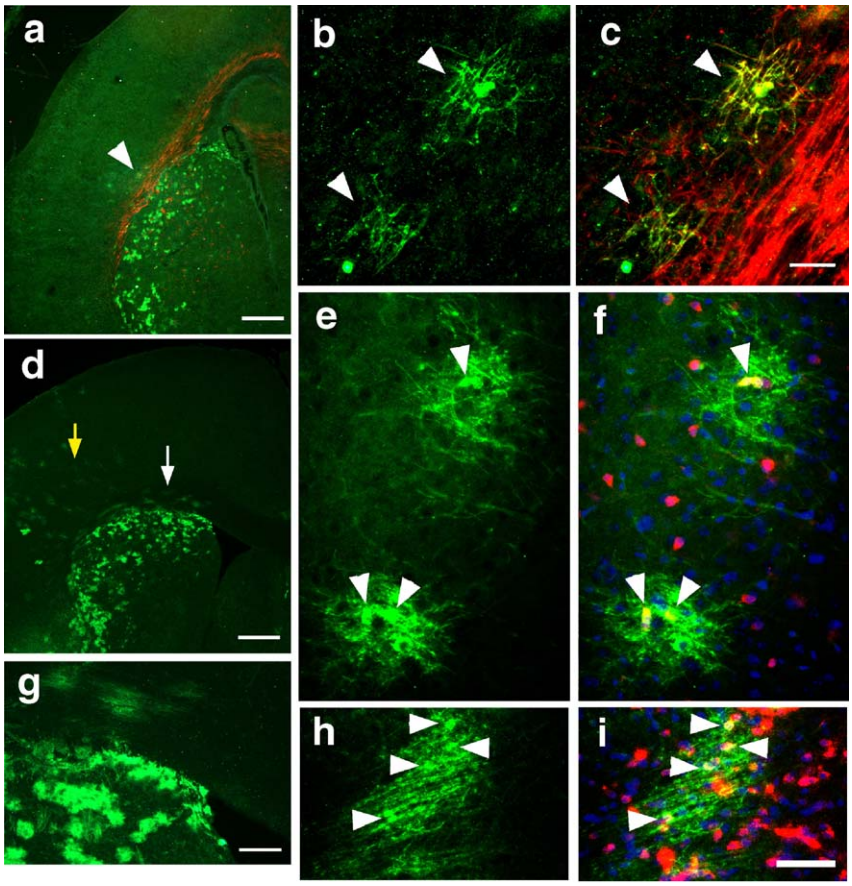

Fig. 5. Myelinating OLs in the cortex originated from the ventral forebrain. (a–c) The ROSA-GAP43-EGFP brain was ventrally electroporated with pCAG-Cre at E12, and the distribution of the GFP labeled OLs at P9 was analyzed. Panels b and c are a higher magnification of the cells indicated by arrowheads in panel a. GFP (green) and PLP (red) double-positive cells were observed in the external capsule area. (d–i) The distribution of the GAP43-EGFP-expressing cells was analyzed at P30. The cryosection was immunostained with anti-GFP (green) and anti-GST-π (red) antibodies. Panel g is a higher magnification of the cells around the LV in panel d. Ependymal cells on the striatum surface are labeled with GFP, indicating the initial site of DNA introduction. (e, f) GFP^+ cells in the cortical plate indicated by the yellow arrow in panel d exhibited characteristic morphologies of myelinating OLs and were double stained by the anti-GST-π antibody (red). Arrowheads indicate GFP^+/GST-π^+ cell bodies. (h, i) GFP^+ cells in the corpus callosum indicated by the white arrow in panel d exhibited characteristic morphologies of myelinating OLs and were double stained by the anti-GST-π antibody (red). Nuclei were stained with DAPI (blue in f and i). Scale bars: 500 μm (a, d), 50 μm (b, c, e, f, h, i), and 100 μm (g).

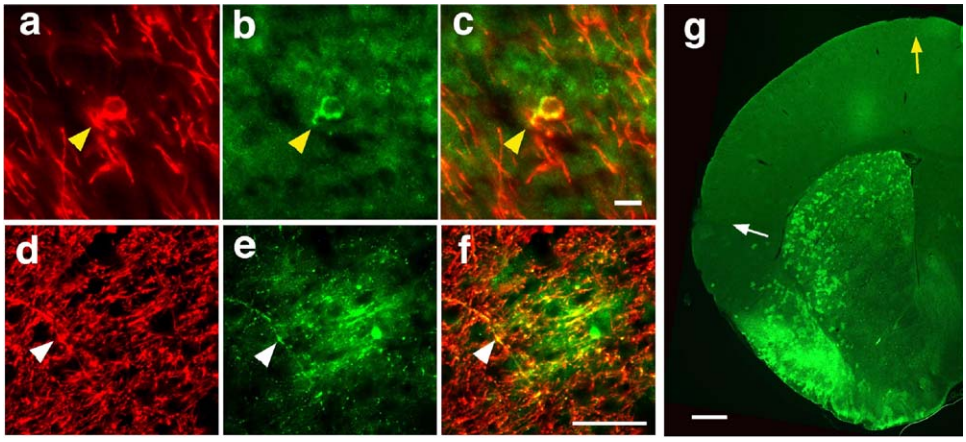

Fig. 6. Myelinating OLs in the cortex originated from the ventral forebrain. The ROSA-GAP43-EGFP brain was ventrally electroporated with pCAG-Cre at E12, and the distribution of GFP-labeled OLs at P30 was analyzed. Cryosections were immunostained with anti-GFP (green) (b, e, g) and anti-PLP (red) (a, d) antibodies. Panel c is a merged image of panels a and b. The position of panels a, b, c in the dorsal cortical plate is indicated by the yellow arrow in panel g. Panel f is a merged image of panels d and e. The position of panels d, e, f in the lateral cortical plate is indicated by the white arrow in panel g. Scale bars: 50 μm (a–c), 50 μm (d–f), and 500 μm (g).

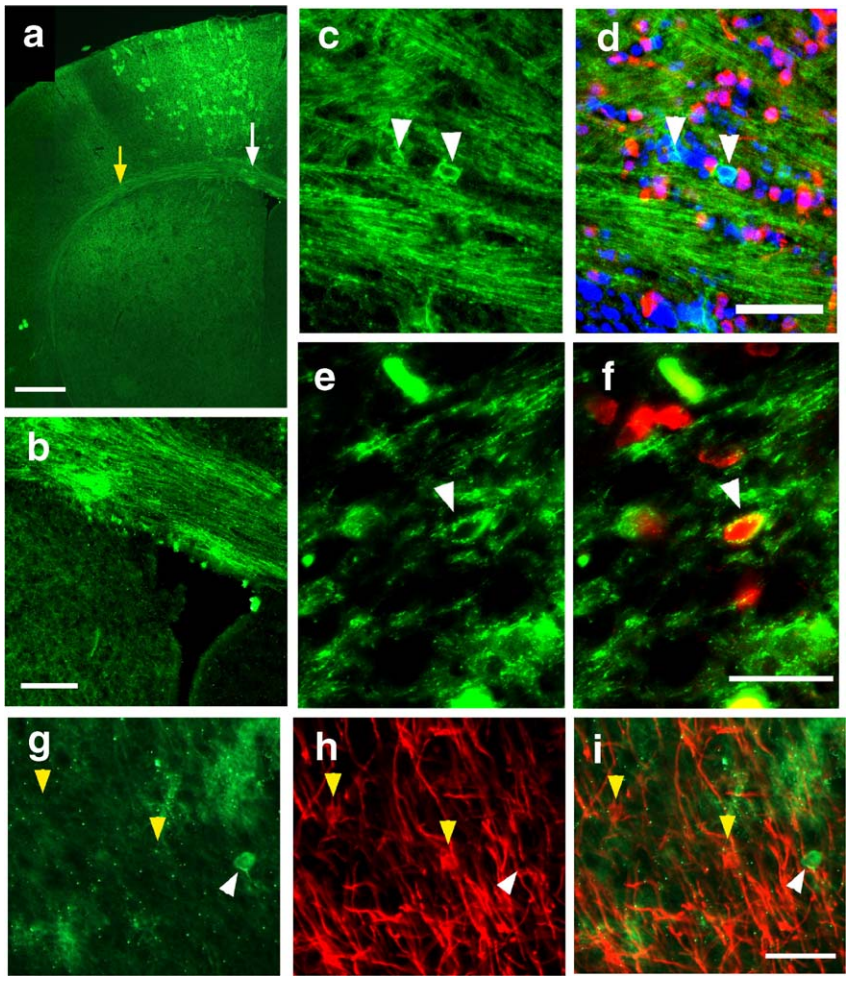

Fig. 7. No myelinating OLs are derived from the E12 cortical VZ. (a) Dorsally electroporated brains at E12 were immunostained with anti-GFP antibodies (green) at P30. Panel b is a higher magnification of the area around the LV of panel a. Ependymal cells of the cortical side were specifically labeled by GFP, indicating the initial site of DNA introduction. Panels c, d are higher magnifications of the corpus callosum area indicated by the white arrow in panel a. GFP^+-positive cell bodies indicated by arrowheads did not express GST-π (red). Nuclei were stained with DAPI (blue in d). Panels e, f are higher magnifications of the cortical plate area indicated by the yellow arrow in panel a. A GFP^+ cell body, indicated by an arrowhead, expressed GST-π, but its morphology was distinct from that of OLs. (g, h, i) At P9, PLP antigen was still accumulated within OL cell bodies as well as newly formed myelin and was useful for identifying OLs. None of the PLP^+ OLs were double-labeled by GFP (yellow arrows in g, h, i), and none of the GFP^+ cells were positive for PLP antibody (white arrows in g, h, i) in the P9 cortices that were dorsally electroporated at E12. Scale bars: Scale bars: 500 μm (a), 100 μm (b), 50 μm (c–f), and 50 μm (h–i).

To ensure the region-specific electroporation, we employed a thin tungsten needle as the electrode and introduced Cre plasmid DNA into the lateral and medial ganglionic eminences where PDGFRα and Olig2 mRNAs were first detected, even though we knew that the miniature electrodes might cause a low transformation efficiency. This could be the reason why there were only a small percentage of the labeled adult cortical OLs when ventral forebrain was electroporated. Since we were able to detect a relatively small number of OLs, our result cannot exclude the possibility that there are OLs derived from regions other than the ventral forebrain (caudal for example). Interestingly, recent studies demonstrated that a population of cortical GABAergic interneurons, which may share the common progenitor cells with OLs (Yung et al., 2002), derived from caudal ganglionic eminence (Ang et al., 2003; Xu et al., 2004). Along with this possibility, although in chick it has been demonstrated that most (if not all) of the cortical OLs originated from a narrow region of anterior entopeduncular area (Olivier et al., 2001), mammalian may have acquired a new source of OLs to supply OLs to large mammalian cortices.

Dorsal origin of the mouse cortical OLs

We examined a total of thousands of GFP-positive cells in 16 cortices that were dorsally electroporated at E12; however we were not able to find GFP-labeled myelinating OLs. This suggests the neuroepithelial cells in the dorsal forebrain (cortex) at E12 do not contribute to the generation of myelinating OLs in vivo. Inconsistent with our result, Gorski et al. (2002) crossbred the Emx1/Cre knock-in mouse and a Cre-loxP reporter mouse and found that a large number of OLs originated from Emx1-expressing cells, which are abundantly present in the embryonic cortical VZ. Several other reports also have indicated a dorsal origin of OLs. Wichterle et al. (2001) mapped the migratory pathway and fate of cells born in the lateral and the medial ganglionic eminence in E13 mice using ultrasound-guided

transplantation in utero. They concluded that neither lateral nor medial ganglionic eminence-derived cells generate cortical glial cells, at least after E13, raising the idea that cortical OLs are from the cortical VZ. McCarthy et al. (2001) injected a modified retroviral lineage library into the LV of the E9.5 mouse guided by ultrasound backscatter microscopy and analyzed each clone at P21. Interestingly, their data also suggested regionally restricted glial generation and distribution. Thus, these in vivo experiments suggested the dorsal origin of OLs.

How can we reconcile these conflicting results? It is possible that OPCs derived from embryonic cortex is sensitive to electroporation that may cause OL lineage cell death or dorsally derived OLs are not able to express GAP43-GFP reporter gene. However, the electroporated ventral VZ cells can differentiate into OLs in vivo, suggesting that the ventral-derived OLs are, more or less, resistant to electroporation. Furthermore, the electroporated cortical VZ cells retained the ability to generate O4-positive OLs in culture (Supplemental Fig. 1 and Supplemental Table 1). These observations do not support the possibility that the OPCs or their progeny are particularly sensitive to electroporation. We also have to discuss whether ROSA promoter is active in the dorsal-derived OLs. Firstly, the expression of GAP43-GFP was detected in both dorsal- and ventral-derived OL lineage cells in vitro (Supplemental Fig. 1 and Figs. 3e–g). In addition, the reporter expression was at least detectable in ventral-derived OLs in vivo. R26R-reporter mouse, which is another cre-reporter mouse that carries a similar loxP construct in the ROSA26 locus, did work to label OLs, though it failed to express reporter activity in GFAP-positive astrocytes for unknown reason (Malatesta et al., 2003). These data support that ROSA promoter is active in the OL lineage cells including dorsally derived OLs.

A remaining possibility is that we missed labeling the OPCs or their precursor cells that were somewhere in the embryonic cortex. The regular (or most abundant) radial glial cells present in the cortical VZ at E12 have been successfully labeled and gave rise to GFP-expressing neurons and/or astrocytes, but not OLs, indicating that these cortical radial glial cells are unlikely the source of cortical OLs. Thus, it is logically proposed that the cells generating OLs must have some specific character in the distribution profile: for example, if the cells are located much inside the cortex, they do not receive the DNA by electroporation. It is also possible that they are present in a restricted region of the E12 cortex where we cannot electroporate. One example is corticostriatal sulcus. When the ventricular surface near the sulcus was electroporated, distribution of Cre-EP-labeled ependymal cells often extended over both the dorsal and ventral parts. We excluded such brains from our experiments because they could not be classified as to dorsally or ventrally electroporated ones. The third possibility is that all Emx1-positive cells are not derived from the cortex, but some Emx1-negative cells (including OL precursors in this scenario) migrate into the cortex after E12 and become Emx1-positive. It is very difficult to prove this because we do not know the origin of these cells.

In either case, our result (although a negative one) strongly suggests that the cortical cells generating OLs have characters (including its spatial distribution) distinct from those of radial glial cells that later give rise to neurons and/or astrocytes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ydbio.2005.12.010.

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