Regional- and temporal-dependent changes in the differentiation of Olig2 progenitors in the forebrain, and the impact on astrocyte development in the dorsal pallium

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A B S T R A C T
Olig2 is a basic helix–loop–helix transcription factor essential for oligodendrocyte and motoneuron development in the spinal cord. Olig2-positive (Olig2+) cells in the ventricular zone of the ventral telencephalon have been shown to differentiate into GABAergic and cholinergic neurons. However, the fate of Olig2 lineage cells in the postnatal forebrain has not been fully described and Olig2 may regulate the development of both astrocytes and oligodendrocytes. Here, we examined the fate of embryonic Olig2+ progenitors using a tamoxifen-inducible Cre/loxP system. Using long-term lineage tracing, Olig2+ cells in the early fetal stage primarily differentiated into GABAergic neurons in the adult telencephalon, while those in later stages gave rise to macroglial cells, both astrocytes and oligodendrocytes. Olig2+ progenitors in the diencephalon developed into oligodendrocytes, as observed in the spinal cord, and a fraction developed into glutamatergic neurons. Olig2 lineage oligodendrocytes tended to form clusters, probably due to local proliferation at the site of terminal differentiation. In spite of the abundance of Olig2 lineage GABAergic neurons in the normal neocortex, GABAergic neurons seemed to develop at normal density in the Olig2 deficient mouse. Thus, Olig2 is dispensable for GABAergic neuron specification. In contrast, at the late fetal stage in the Olig2 deficient mouse, astrocyte development was retarded in the dorsal neocortex, but not in the basal forebrain. Olig2 functions, therefore, in gliogenesis in the dorsal pallium. Short-term lineage tracing experiments revealed that the majority of late Olig2+ cells were not direct descendants of early Olig2+ progenitors in the fetal forebrain. These observations indicate that embryonic Olig2+ progenitor cells change their differentiative properties during development, and also that Olig2 plays a role in astrocyte development in a region-specific manner.

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Introduction

During the development of the central nervous system (CNS), neuroepithelial cells with neural stem cell characteristics produce a variety of progenitors for both neurons and macroglial cells. A specific progenitor is derived from a specific domain which is delineated by the expression of specific transcription factors (TFs). TFs regulate and determine cell fates as well as proliferation, migration and axonal growth. For example, targeted disruption or improper expression of certain TF changes cell fate (Deneen et al., 2006; Lu et al., 2002; Mizuguchi et al., 2001; Sharma et al., 2000; Shirasaki et al., 2006; Takebayashi et al., 2002). Therefore, some TFs are regarded as cell type-specific markers for the embryonic progenitor cells. However, detailed relationships between embryonic progenitors and their terminal phenotypes remain largely unknown.

Olig2 is a basic helix–loop–helix transcription factor essential for oligodendrocytic and motoneuron differentiation in the spinal cord (Lu et al., 2000, 2002; Takebayashi et al., 2000, 2002; Zhou et al., 2000; Zhou and Anderson, 2002), and oligodendrocytic specification in the ventral forebrain (Tekki-Kessaris et al., 2001; Parras et al., 2007; Petryniak et al., 2007). We have been performing lineage analysis of embryonic cells expressing Olig2 (Olig2+ cells). Olig2+ progenitors in the early embryonic spinal cord differentiate into motoneurons, and those in late stages primarily differentiate into oligodendrocytes, with a lesser number of astrocytes and ependymal cells (Masahira et al., 2006). In the forebrain, Olig2 expression is initially localized in the...
ventricular zone (VZ) of the ventral telencephalon such as the medial and lateral ganglionic eminence (MGE and LGE) and zona limitans intrathalamica (ZLI) (Nery et al., 2002; Takebayashi et al., 2000).

Recently, it was shown that at an early fetal stage of development, Olig2+ cells in the MGE and LGE differentiate into GABAergic neurons and cholinergic neurons (Furusho et al., 2006; Miyoshi et al., 2007). The initially restricted expression of Olig2 in the ventral forebrain also suggests oligodendrocyte differentiation of Olig2+ progenitor cells from the MGE (Tekki-Kessaris et al., 2001). Indeed, loss of Olig2 results in markedly reduced oligodendrocytic development in the forebrain (Lu et al., 2002; Takebayashi et al., 2002). By contrast, recent evidence by others suggests that Olig2 positively regulates gliogenesis, including astrocytogenesis in the early postnatal telencephalon (Marshall et al., 2005; Cai et al., 2007). These findings are contrary to the view that Olig2 inhibits astrocytic differentiation (Fukuda et al., 2004; Setoguchi and Kondo, 2004). Therefore, although Olig2+ cells in the embryonic CNS include oligodendrocyte precursor cells (OPCs) and subsets of neurons (Masahira et al., 2006; Furusho et al., 2006), further analysis is necessary to characterize the differentiation of Olig2+ progenitors in the developing forebrain.

In this study, we investigated the fates of embryonic Olig2+ cells in the forebrain by performing comprehensive lineage tracing experiments using a tamoxifen-inducible Cre/loxP system. Our results demonstrate that Olig2+ cells in the early telencephalon primarily differentiate into GABAergic neurons and later into macroglial cells, while those in the diencephalon develop into excitatory neurons as well as oligodendrocytes. Unexpectedly, Olig2+ cells in the late fetal telencephalon primarily give rise to astrocytes. In addition, Olig2 deficiency results in a decrease in number of astrocytes in the dorsal pallium, but not in the basal forebrain area, at a late fetal stage. These observations indicate that embryonic Olig2+ progenitor cells change their differentiation properties during development, and also that Olig2 plays a role in astrocyte development in a region-specific manner.

Materials and methods

Animals

Mice used in this study and the genotyping protocols were described previously (Novak et al., 2000, Sakai and Miyazaki, 1997, Takebayashi et al., 2002). Olig2^{CreERT2} heterozygous male mice with an ICR background (Japan SLC, Hamamatsu, Japan) and a reporter allele were mated with ICR females. Midday of the plugged day was termed embryonic day (E) 0.5. We used reporter mouse lines: ZEG mice (Novak et al., 2000), which express GFP upon Cre-mediated recombination, and CAG-CAT-Z mice (Sakai and Miyazaki, 1997), which express LacZ upon recombination. Pregnant dams received an intraperitoneal injection of 4-hydroxytamoxifen (4-OHT; Sigma-Aldrich, St. Louis, MO; 2–3 mg/animal) at E9.5–E7.5 to induce Cre-mediated recombination in Olig2+ cells, as described previously (Masahira et al., 2006). Double-heterozygous mice treated with 4-OHT were analyzed at fetal stages, postnatal day 7 (P7) or adult (P90–12 month old). Some pregnant mice received 5-bromo-2′-deoxyuridine (BrdU; 50 mg/kg body weight; Sigma-Aldrich) 2 h prior to fixation of fetal (E18.5) mice. To observe the effect of Olig2 deficiency, Olig2^{CreERT2} heterozygous mice were crossed to the heterozygous female to obtain Olig2+/- mice. As Olig2 deficient mice were stillborn, the fetal brain was analyzed at E18.5.

Animals were perfused transcardially with phosphate buffered saline (PBS) followed by 4% paraformaldehyde in PBS under deep anesthesia with pentobarbital (100 mg/kg body weight). The brains were removed from the skulls and maintained in the same fixative overnight at 4 °C, and then immersed in PBS containing 20% sucrose. Some specimens were fixed with Bouin’s fixative and processed for paraffin preparation (Ono et al., 2004). All animal procedures were conducted in accordance with guidelines described by the National Institutes of Health for Care and Use of Laboratory Animals, and approved by the Animal Research Committee of the National Institutes of Health for experimental protocols. Fixed brains were cut coronally or sagittally (as seen above). Sections from double-heterozygous mice (Olig2^{CreERT2}/ZEG) were double-labeled by an immunofluorescent method. A mixture of primary antibodies was applied to the sections for one to two overnight. After washing the sections with PBS, they were incubated for 1 h with a mixed solution of species-specific secondary antibodies that were conjugated to Alexa Fluor 488, 594 or 633. Nuclei of cells were counterstained with Hoechst33342 (0.1 µg/ml, 10–30 min). The following primary antibodies were used: rat anti-GFP (1:2000, Nakalai Tesque, Kyoto, Japan), rat anti-platelet-derived growth factor receptor α (PDGFRα;1:500, BD Bioscience, San Diego, CA), rabbit anti-GABA for inhibitory neurons (1:2000, Sigma-Aldrich), rabbit anti-GSTpi for mature oligodendrocytes (1:2000, IBL, Gunma, Japan; Takeyama and Cammer, 1991), rabbit anti-GRAP for astrocytes (1:2000, Dako, Denmark), rabbit anti-GLAST for astrocyte progenitors (1:5000, a gift from Dr. Masahiko Watanabe, Hokkaido University; Shibata et al., 1997), mouse anti-NeuN for mature neurons (1:1000, Chemicon, Temecula, CA), mouse anti-IIIβ-tubulin for immature neurons (1:4000, Tu1; Covance, Berkeley, CA), rabbit anti-nestin for radial glia (1:1000, IBL, Gunma, Japan; Takeyama and Cammer, 1991), rabbit anti-NG2 for OPCs or synantocytes (1:1000, Chemicon, Temecula, CA; Butt et al., 2005a), mouse anti-phosphate activated glutaminase (PAG) for excitatory neurons (a gift from Dr. Takeshi Kaneko, Kyoto University; final conc. 2 µg/ml; Kaneko et al., 1995), mouse anti-CAM kinase II for excitatory neurons (1:500, Chemicon), mouse anti-Brdu (1:5000, BD Bioscience), rat anti-Brdu (1:200, Abcam, Cambridge, UK), rabbit anti-Iba-1 for microglia (1:1000, Wako, Osaka, Japan), mouse anti-ZO1 for adherens junction in the VZ cells (1:20, a gift from Dr. Shoichiro Tsukita, Kyoto University; Yonemura et al., 1995), rabbit anti-Olig2 (1:200, IBL, Takasaki, Japan), rabbit or goat anti-Olig2 (1:1000, Chemicon) and rabbit anti-nestin for radial glia (1:500, IBL).

In situ hybridization

The method for ISH on coronal sections was modified slightly from a previously described method (Ding et al., 2005). The following cDNAs were used as probes: GAD67 (GAD1; GenBank accession number, NM_000877, nt_281–821, a gift from Dr. Yanagawa, Gunma University) and GAD65 (GAD2; NM_000872, nt_602–1414, a gift from Dr. Yanagawa, Gunma University) were used as markers for GABAergic interneurons. Vesicular glutamate transporter 1 (VGLUT1; NM_182993, nt_618–1266) and VGLUT2 (NM_008083, nt_735–1324) were used as markers for excitatory neurons. Sonic hedgehog (Shh; NM_000917, nt_69–1890, a gift from Dr. Andy McMahon, Harvard University, and Dr. Sumihare Noji, Tokushima University) was used for identification of the ZLI. Fibroblast growth factor receptor 3 (FGFR3; NM_000810, nt_205–2703) and GLAST (Ma et al., 2006; Ogawa et al., 2005; a gift from Dr. Koichi Tanaka, Tokyo Med. Dent. University) were used to identify astrocytes or their precursor cells. Colony stimulating factor 1 receptor (c-fms; Ma et al., 2006) was utilized as a microglial marker and PLP (Kagawa et al., 1994) was used for oligodendrocyte. VGLUT1, VGLUT2, and FGFR3 cDNAs were amplified by reverse transcriptase polymerase chain reaction. After ISH, some brains from ZEG mice were further subjected to GFP immunohistochemistry with an ABC method. To obtain maximal sensitivity for GABAergic neurons, probes for GAD65 and GAD67 were sometimes used as a mixed probe solution.

Quantitative analysis

The total number of recombinant cells in the adult brain was estimated by counting GFP+ cells in every fourth section at 50 µm in thickness and multiplying by four. The forebrain forebrain analyzed here encompassed the telencephalon and the diencephalon. The telencephalon included the cerebral cortex, olfactory bulb and basal forebrain rostral to the optic chiasm. The diencephalon included the thalamus, ventral thalamus and hypothalamus between the optic chiasm and caudal hypothalamus. The telencephalon included the cerebral cortex, olfactory bulb and basal forebrain rostral to the optic chiasm. The diencephalon included the thalamus, ventral thalamus and hypothalamus between the optic chiasm and caudal hypothalamus. The telencephalon included the cerebral cortex, olfactory bulb and basal forebrain rostral to the optic chiasm. The diencephalon included the thalamus, ventral thalamus and hypothalamus between the optic chiasm and caudal hypothalamus. The telencephalon included the cerebral cortex, olfactory bulb and basal forebrain rostral to the optic chiasm. The diencephalon included the thalamus, ventral thalamus and hypothalamus between the optic chiasm and caudal hypothalamus.
labeled cells and that of the reporter positive cells in randomly chosen areas of the forebrain.

To analyze the effects of Olig2 deficiency, the density of inhibitory neurons and astrocyte progenitors was examined by counting GAD mRNA+ cells in the cerebral cortex and GLAST or FGFR3 mRNA+ cells in the telencephalon, respectively, in unit areas (290 μm x 220 μm, or 580 μm x 440 μm) of randomly chosen areas. Results were compared between Olig2 deficient and wild/heterozygous mice and analyzed with the Student’s t-test. At least three animals were used in each group and at least five sections were examined in each animal.

Results

Gradual dispersal of Olig2+ cells in the developing forebrain

We previously demonstrated expression of Olig2-mRNA in the E9.5 and E12.5 forebrain (Furusho et al., 2006). To better understand the distribution of Olig2+ cells that underwent recombination, the spatio-temporal expression pattern of Olig2 protein was immunohistochemically determined with high precision in the fetal forebrain (telencephalon and diencephalon). In the E9.5 telencephalon, Olig2 expression was reminiscent in the ventral part (Fig. 1A, arrowhead). Olig2+ cells appeared in the ventral telencephalon by E10.5 (not shown), and, until E12.5, they were mostly localized in the VZ of the MGE, septum and anterior entopeduncular area with a faint labeling in the LGE (Fig. 1B). No or few labeled cells were found in the mantle layer or dorsal pallium. By E14.5, Olig2 immunoreactivity in the LGE gradually intensified and Olig2+ cells were distributed throughout the VZ of the ventral telencephalon (Fig. 1C). In addition, labeled cells were scattered in the mantle layer of the ventral telencephalon. However, a very limited number of Olig2+ cells were detected in the dorsal neocortex at this stage (Figs. 1C, G). By E17.5, Olig2+ cells were widely distributed throughout the telencephalon including the neocortex (Figs. 1D, H). The VZ of the striatum still contained densely-packed Olig2+ cells. It is worth noting that, at this stage, the dorsal VZ and/or subventricular zone (VZ/SVZ) of the hippocampus and that underneath the corpus callosum contained Olig2+ cells (Figs. 1D, H, arrowhead; Supplement Fig. 1). These Olig2+ cells in the VZ/SVZ incorporated BrdU during 2 h pulses, expressed nestin and were directly facing the ventricle with the expression of ZO-1, an adherens junction protein, all of which are characteristics of radial glia or VZ cells (Supplement Figs. 1E–M). Thus, in a later fetal stage, the dorsal VZ of the telencephalon also seemed to generate Olig2+ lineage cells.

In the diencephalon, Olig2+ cells appeared from E9.5. They were detected in the restricted region of the diencephalon, and formed bilateral spots in the neuroepithelial layer of the ventral diencephalon (Fig. 1E, arrowhead) and a few in the dorsal portion. By E12.5, Olig2+ cells were observed in the VZ of the ventromedial telencephalon including medial ganglionic eminence (B, MGE), and in the prethalamus (F, Preth) that is ventral to the zona limitans intrathalamica (ZLI, arrowhead). Olig2+ cells were also distributed in the caudal hypothalamus (K, arrow). Hipp, hippocampus. LGE, lateral ganglionic eminence. NCx, neocortex. Scale bars in panels A, E=100 μm, in panels B–D and F–H, K, 500 μm.

Fig. 1. Immunohistochemical localization of Olig2+ cells in the developing forebrain. Coronal and sagittal sections of fetal forebrain showing distribution of Olig2+ cells. (A, E) [E9.5], Olig2 expression in the telencephalon was reminiscent in the ventral part (arrowhead in panel A) while that in the diencephalon was found in bilateral spots (arrowhead in panel E). (B, F) [E12.5], Olig2+ cells were observed in the VZ of the ventromedial telencephalon including medial ganglionic eminence (B, MGE), and in the prethalamus (F, Preth) that is ventral to the zona limitans intrathalamica (ZLI, arrowhead). (C, G) [E14.5], Olig2+ cells were distributed in the VZ of the striatum (Str) and septum (Spt) in the telencephalon (C) and in the VZ of the hypothalamus (G, Hypth). Note that the mantle layer of the ventral telencephalon and diencephalon contained labeled cells while the dorsal neocortex and the dorsal thalamus (dth) did not. (D, H) [E17.5], Olig2+ cells were widely distributed throughout the forebrain. Note that the VZ/SVZ of the dorsal telencephalon contained Olig2+ cells (arrowheads in panels D, H) which are magnified in Supplement Figs. 1C, D. (I–K), Sagittal sections showing Olig2 and Shh expression in the E12.5 telencephalon, arranged in a medial (I) to lateral order (K). Olig2+ cells were observed ventrally to the ZLI which itself did not express Olig2 (I, asterisks) but did express morphogen Shh (J, arrows). Olig2+ cells were also distributed in the caudal hypothalamus (K, arrow). Hipp, hippocampus. LGE, lateral ganglionic eminence. NCx, neocortex. Scale bars in panels A, E=100 μm, in panels B–D and F–H, K, 500 μm.
cells were observed in the intermediate part, probably adjacent to the ZLI, which secretes the morphogen Shh, while Olig2+ cells were not observed in the ZLI itself (Figs. 1F, I, J). Cells ventral (or rostral) to the ZLI showed stronger immunoreactivity to Olig2 than those dorsal (caudal) to the ZLI, the former corresponding to the prethalamus derived from prosomere 3 (p3; future ventral thalamus; Puelles and Rubenstein, 2003) (Figs. 1F, I). In addition, the most caudal region of the ventral diencephalon contained numerous Olig2+ cells (Fig. 1K, arrow) as observed at E14.5 (Fig. 1G). In the diencephalon at E14.5, Olig2+ cells were found in the prethalamus and hypothalamus, especially in the VZ (Fig. 1G). Far fewer labeled cells were observed in the dorsal thalamus. By E17.5, Olig2+ cells were widely distributed throughout the diencephalon, including the dorsal thalamus mantle layer. The neuroepithelial cells facing the third ventricle showed

Fig. 2. Neuronal and macroglial differentiation of embryonic Olig2+ cells in the adult forebrain. Detailed morphology of recombinant cells in the adult brain of Olig2KICreER:Z/EG double-heterozygous mice stained with GFP immunohistochemistry. Animals were treated with 4-OHT at fetal stages (see Materials and methods). (A) Neuron-like cell. (B) Astrocyte-like cell. (C) Oligodendrocyte-like cell. (D–F) A neuron-like GFP+ cell (D) expressed NeuN (arrow in panel E); Blue color in panel F (as well as in panels I, L, O, P, S) is Hoechst33342 staining of cell nuclei. (G–I) An astrocyte-like bushy cell (G) was immunoreactive for GFAP (arrow in panel H) indicative of protoplasmic astrocyte. (J–L) GFP+ thick processes (arrow) in the optic tract express GFAP (K), indicative of fibrous astrocyte. (M–O) Oligodendrocyte-like cells (M) expressed Olig2 (N) in the adult brain. Double-positive cells are indicated by arrowheads. (P) Oligodendrocyte-like GFP+ cells formed a loosely-arranged cluster in the basal forebrain. (Q–S) Three oligodendrocyte-like cells (arrows in panel Q) in the cluster expressed GSTpi (R). (T–W) Projection pictures arranged from optical sections taken by a CLSM. Oligodendrocyte-like GFP+ cells with round perikarya (U, arrows) expressed GSTpi (T) while those with irregular contours did not. (V, W) GFP+ cells expressed NG2 proteoglycan. Scale bars in panels C, I, and W=20 μm, in panels F, L, O, S, and U=50 μm, and in panel P=500 μm.
intense Olig2 immunoreactivity (Fig. 1H). The results showed an initially restricted localization and then a subsequent dispersal of Olig2+ cells in the forebrain, both in the telencephalon and diencephalon.

Embryonic Olig2+ cells persist in the adult brain as neurons and macroglial cells

We next performed long-term lineage tracing of embryonic Olig2+ cells (Figs. 2–4; Supplement Figs. 2 and 3). Olig2+ cells in the fetal forebrain were labeled with GFP or LacZ by Cre-mediated recombination after the 4-OHT injection, and these brains were then analyzed at adult stages. Some of the recombinant cells failed to express cell type-specific markers (see below). Therefore, we categorized the recombinant Olig2 lineage cells based on a standard morphologic classification (Hosokawa and Mannen, 1963; Leibnitz et al., 1982; LeVine and Goldman, 1988), in the adult Olig2CreERZ/EG forebrain. The morphological categorizations of neurons, astrocytes, or oligodendrocytes were as follows. Neuron-like cells had a relatively large, round or oval cell body with smooth contours, and extended multiple longer processes. Their thicker processes tapered towards their tips and sometimes bore varicose protrusions which are apparently dendrites and spines (Fig. 2A). Astrocyte-like cells show a bushy profile in the gray matter (Figs. 2B, G) and extend thick processes in the white matter (Fig. 2J). Oligodendrocyte-like cells have a smaller cell body (usually less than 10 μm) with round or irregular contour. They extend

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**Fig. 3.** Distribution of Olig2 lineage recombinant cells in the adult forebrain following treatment with tamoxifen at fetal stages. Recombinant GFP+ cells were plotted on montage pictures of adult forebrain coronal sections. Each dot indicates a recombinant cell. Time of 4-OHT (TM) treatment is indicated at the left side of the pictures. Note that neuronal cells (yellow spots) are uniformly distributed in the forebrain, while glial cells, especially oligodendrocytes (red spots), tended to form clusters in E12.5TM and E14.5TM animals (C–F). An arrow in panel D indicates one such cluster in the fimbria. Scale bar = 1 mm.

**Fig. 4.** Proportion of neuronal and macroglial cells derived from Olig2+ cells. Percentages of neuronal and glial cell types among recombinant cells after 4-OHT treatment at fetal stages are shown in each graph (means±S.E.M). N, neuron-like cells (black). As, astrocyte-like cells (red). OL, oligodendrocyte-like cells (green). In the telencephalon, 4-OHT treatment at E12.5 resulted in appearance of more neuron-like cells (B) while that in later fetal stages resulted in more astrocyte-like cells (D, F). In the diencephalon, 4-OHT treatment at E12.5 resulted in appearance of more neuron-like cells (B) while that in later fetal stages resulted in more astrocyte-like cells (D, F). In the diencephalon, macroglial cells, especially oligodendrocyte-like cells, appeared most abundantly from Olig2+ cells (C, E, G), except at very early stages (A). Neurogenesis in Olig2+ cells lasted even after E16.5 in the telencephalon (F) while that had ceased completely by E16.5 in the diencephalon (G), (H, I) Sequential changes of proportions of neuronal or macroglial cell generation from Olig2+ cells in the telencephalon (H) and the diencephalon (I).
multiple processes that were much thinner and shorter than processes of neuron-like cells (Fig. 2C) and they never had protrusions. A few recombinant cells were occasionally detected in the ependymal layer (not shown), and they were excluded from enumeration because their appearance was sporadic and they were rare compared with other kinds of cells. In this way, nearly all of the recombinant GFP+ cells observed at the adult stage could be categorized into three cell types. We did not observe recombinant cells with a typical microglial morphology.

To confirm the validity of the morphological classification, we next examined the expression of cell type-specific markers. Neuron-like cells were frequently immunoreactive to NeuN (Figs. 2D–F; 78.6 ±9.04% of the neuron-like cell population; mean ±S.E.M.) and expressed enzymes for neurotransmitter synthesis (see below). Astrocyte-like bushy cells were immunoreactive to GFAP in the gray matter, corresponding to protoplasmic astrocytes (Figs. 2G–I). In addition, GFAP+, thick processes in the white matter (mostly in the optic tract) were apparently from fibrous astrocytes (Figs. 2J–L). Approximately 40% of astrocyte-like cells expressed GFAP, which is consistent with a previous report that showed that only some astrocytes express GFAP (Tanaka et al., 2007). Oligodendrocyte-like cells expressed Olig2 (Figs. 2M–O; 53.4 ±7.51% of the oligodendrocyte-like cell population), GSTpi (Figs. 2Q–U; 28.1 ±11.9%), or NG2 (Figs. 2V, W; 25.7 ±9.51%). Therefore, oligodendrocyte-like cells categorized by a profile included OPCs or NG2+ cells/synantocytes/polydendrocytes (Butt et al., 2005a; Nishiyama et al., 2002; Nishiyama et al., 2005; Zhu et al., 2007). GSTpi+ cells usually had a round perikaryon and expressed enzymes for neurotransmitter synthesis (Butt et al., 2005a; Nishiyama et al., 2002; Nishiyama et al., 2005; Zhu et al., 2007). GSTpi+ cells usually had a round perikaryon and expressed enzymes for neurotransmitter synthesis (Butt et al., 2005a; Nishiyama et al., 2002; Nishiyama et al., 2005; Zhu et al., 2007). GSTpi+ cells usually had a round perikaryon and expressed enzymes for neurotransmitter synthesis (Butt et al., 2005a; Nishiyama et al., 2002; Nishiyama et al., 2005; Zhu et al., 2007). GSTpi+ cells usually had a round perikaryon and expressed enzymes for neurotransmitter synthesis (Butt et al., 2005a; Nishiyama et al., 2002; Nishiyama et al., 2005; Zhu et al., 2007). GSTpi+ cells usually had a round perikaryon and expressed enzymes for neurotransmitter synthesis (Butt et al., 2005a; Nishiyama et al., 2002; Nishiyama et al., 2005; Zhu et al., 2007). GSTpi+ cells usually had a round perikaryon and expressed enzymes for neurotransmitter synthesis (Butt et al., 2005a; Nishiyama et al., 2002; Nishiyama et al., 2005; Zhu et al., 2007). GSTpi+ cells usually had a round perikaryon and expressed enzymes for neurotransmitter synthesis (Butt et al., 2005a; Nishiyama et al., 2002; Nishiyama et al., 2005; Zhu et al., 2007). GSTpi+ cells usually had a round perikaryon and expressed enzymes for neurotransmitter synthesis (Butt et al., 2005a; Nishiyama et al., 2002; Nishiyama et al., 2005; Zhu et al., 2007). GSTpi+ cells usually had a round perikaryon and expressed enzymes for neurotransmitter synthesis (Butt et al., 2005a; Nishiyama et al., 2002; Nishiyama et al., 2005; Zhu et al., 2007). GSTpi+ cells usually had a round perikaryon and expressed enzymes for neurotransmitter synthesis (Butt et al., 2005a; Nishiyama et al., 2002; Nishiyama et al., 2005; Zhu et al., 2007). GSTpi+ cells usually had a round perikaryon and expressed enzymes for neurotransmitter synthesis (Butt et al., 2005a; Nishiyama et al., 2002; Nishiyama et al., 2005; Zhu et al., 2007). GSTpi+ cells usually had a round perikaryon and expressed enzymes for neurotransmitter synthesis (Butt et al., 2005a; Nishiyama et al., 2002; Nishiyama et al., 2005; Zhu et al., 2007). GSTpi+ cells usually had a round perikaryon and expressed enzymes for neurotransmitter synthesis (Butt et al., 2005a; Nishiyama et al., 2002; Nishiyama et al., 2005; Zhu et al., 2007). GSTpi+ cells usually had a round perikaryon and expressed enzymes for neurotransmitter synthesis (Butt et al., 2005a; Nishiyama et al., 2002; Nishiyama et al., 2005; Zhu et al., 2007). GSTpi+ cells usually had a round perikaryon and expressed enzymes for neurotransmitter synthesis (Butt et al., 2005a; Nishiyama et al., 2002; Nishiyama et al., 2005; Zhu et al., 2007). GSTpi+ cells usually had a round perikaryon and expressed enzymes for neurotransmitter synthesis (Butt et al., 2005a; Nishiyama et al., 2002; Nishiyama et al., 2005; Zhu et al., 2007). Olig2 was not expressed in astrocyte-like cells of the adult brain, except in the olfactory bulb. While less than 5% of astrocyte-like GFP+ cells expressed Olig2 in the telencephalon (3.58 ±4.50% in the telencephalon, 3.62 ±2.48% in the diencephalon), approximately one fourth of the astrocyte-like cells expressed nuclear Olig2 expression in the olfactory bulb of 5 week old animals (27.4 ±8.49%). After Olig2+ cells were distributed widely and had started active proliferation by this stage, Neuron-like cells accounted for less than 5% of the recombinant cell population, and most of them were observed in the olfactory bulb. Approximately half of the recombinant cells revealed a neuron-like profile in the adult olfactory bulb following E15.5 or E17.5 4-OHT treatment (51.5 ±6.65%, n=5 brains; Supplement Figs. 3B–D).

With regard to the diencephalon, treatment with 4-OHT at E9.5 revealed a moderate number of GFP+ cells (519 ±232 cells/diencephalon). They were mostly observed in the caudal diencephalon such as the ventral thalamus and hypothalamus (Figs. 3A, B), and were a mixed population of neuronal and macroglial cells (Figs. 4A, I). 4-OHT treatment at E12.5 resulted in more than 1000 recombinant cells in the diencephalon (1274 ±305). Oligodendrocyte-like cells were the most abundant recombinant cell type induced by the E12.5 or later 4-OHT treatment, while the next most abundant were astrocyte-like cells (Figs. 4C, E, I). They were distributed in the ventral thalamus and hypothalamus, while a very few cells were found in the dorsal thalamus. The distribution of neuron-like cells was similar to that of macroglial cells, though the proportion of neuron-like cells decreased. In animals treated with 4-OHT at E14.5 or later, the recombinant cell number in the diencephalon further increased, and both types of macroglial cells were observed throughout the diencephalon. No or few recombinant cells in the diencephalon showed neuron-like morphology when 4-OHT was administered at E16.5 or later (Figs. 4G, I).

The relationship between the embryonic age at which recombina-
tion was initiated and the final morphological phenotype of Olig2+ cells is summarized in Figs. 4H, I. This suggests that forebrain Olig2+ cells change their differentiative properties during early development.

**Differentiative properties of embryonic Olig2+ cells are spatio-temporally different in the forebrain**

We next examined the spatio-temporal distribution of Olig2 lineage cells and their differentiation in the adult forebrain (Fig. 3). Each dot in Fig. 3 indicates a recombinant cell, classified by morphology. The total number of recombinant cells in the adult forebrain gradually increased as the time of 4-OHT treatment was delayed to later developmental times (Supplement Fig. 2). When 4-OHT was injected at E9.5, no or few recombinant cells appeared in the telencephalic region (4.25 ±1.65 cells/telencephalon, n=4 brains). Recombinant GFP+ cells sporadically appeared in the cerebral cortex and basal forebrain (Fig. 3A). As expected from the Olig2+ cell distribution (Figs. 1B, F), 4-OHT treatment at E12.5 induced the appearance of more recombinant cells in the telencephalon (Figs. 3C, D; 4363 ±278 cells/telencephalon, n=3 brains). Neuron-like cells were the most abundant type of recombinant cells, as approximately 70% of recombinant cells showed a neuron-like profile (Fig. 4B). They were widely distributed throughout the telencephalon, including the olfactory bulb, neocortex, striatum, amygdala, hippocampus and other telencephalic areas (Figs. 3C, D). Nearly all of the recombinant cells in the olfactory bulb were neuron-like cells (94.1 ±1.25% of recombinant cells; n=4 brains; Supplement Fig. 3A). Macrogold-like cells also appeared in the telencephalon of the same brain. Interestingly, most of the macrogold cells, especially oligodendro-

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That is, neurogenesis from Olig2+ cells gradually decreased while gliogenesis increased. Olig2+ cells during late fetal stages generated glial cells, including abundant astrocytes in the telencephalon, while those in the diencephalon tended to give rise to oligodendrocytes. Therefore, the differentiative properties of Olig2+ cells seem to depend upon the developmental stage and the specific anatomical region.

Cessation of Olig2 expression in the Olig2 lineage cells

Long-term lineage tracing experiments demonstrated that the differentiative properties of early Olig2+ progenitor cells are apparently different from those of late Olig2+ progenitors. If Olig2+ progenitor cells in later fetal stage are direct descendants of those in the early fetal stage, recombinant cell population induced by E12.5 4-OHT treatment (Figs. 3C, D) should include more glial cells (Figs. 3G, H), as reporter expression was continuous after recombination. We next performed short-term lineage analysis to examine continuous Olig2 expression in the Olig2 lineage cells.

When Olig2+ cells were marked with LacZ in Olig2KICreER:CAT-Z mice by 4-OHT injection at E9.5 and the labeled cells were analyzed at E14.5, LacZ+ cells were mainly distributed in the parenchyma of the diencephalon. However, less than 1% of X-gal+/+ cells were immunopositive for Olig2 (Figs. 5A, B; 0.60±0.25 %, n=4 brains). When Olig2+ cells at later stages such as E13.5 or E14.5 were labeled with GFP in Olig2KICreER:CAT-Z/Eg mice by the 4-OHT treatment and analyzed 3 days later, recombinant GFP+ cells were widely observed in the basal forebrain, and fewer in the dorsal pallium. Approximately one third of GF+ recombinant cells continued nuclear Olig2 expression 3 days after the recombination (Fig. 5C; 36.8±4.70%; n=3 brains). While a very limited number of GF+Olig2+ cells were seen in the intermediate zone of the cortex and hippocampus (Fig. 5D), none of the GFP+ cells was Olig2+ in the V2/SVZ of the dorsal pallium (Fig. 5E). These results indicate that Olig2 expression is not always continuous and that more than half of the Olig2 lineage cells cease Olig2 expression within a few days after the initiation of recombination. Therefore, although Olig2+ cells gradually spread from the ventral to dorsal regions of the forebrain (Fig. 1), not all of the Olig2+ cells in late fetal stages were descendants of Olig2+ cells at early stages.

In order to understand cell types of GFP+/Olig2+ and GFP+/Olig2− cells in the short-term lineage analysis, we examined expression of cell type markers in the over 2000 GFP+ cells in the late fetal stage. When fetuses were treated with 4-OHT at E13.5 and analyzed at E16.5, one third of GFP+ cells expressed Olig2. Most of the GFP+/Olig2− cells in the forebrain expressed either PDGFRα (42.9 % of GFP+/Olig2− cell population) or GLAST (58.8 %), markers for oligodendrocyte and astrocyte precursors, respectively (Figs. 5G, H), whereas class III β tubulin, a marker for immature neurons, was expressed by a small number of GFP+/Olig2− cells (9.42 %; not shown). The above value suggests the presence of cell co-expression of both progenitor markers, which is supported by the observation that approximately 15 % of GLAST+ cells co-expressed PDGFRα and vice versa in the late fetal stage (not shown). More than half of the GFP+/Olig2− cells failed to express any cell type-specific markers, while approximately one-fifth of GFP+/Olig2− cells were immunopositive for class III β tubulin (Fig. 5F) and a lesser number expressed GLAST (not shown). Therefore, Olig2 lineage cells that ceased Olig2 expression included neuronal cells while those retaining Olig2 expression were mostly a glial precursor population, both astroglial and oligodendroglial.

GABAergic and glutamatergic neuron differentiation from Olig2+ cells

Olig2+ progenitor cells in the early GE are known to differentiate into GABAergic (Miyoshi et al., 2007) and cholinergic neurons (Furusho et al., 2006). However, it is not clear whether Olig2+ cells differentiate into other neuronal subsets such as excitatory neurons in the telencephalon and in the diencephalon. We next examined neuronal subtypes in the forebrain.

In the adult Olig2KICreER:Z/Eg cerebral cortex after E12.5 4-OHT treatment, approximately 60% of the neuron-like GFP+ cells were immunoreactive for GABA (60.8±5.91 %, n=5 brains; Figs. 6A–C), and more than three-quarters of neuron-like cells expressed GAD mRNA (Fig. 6D). Thus, the majority of neuronal Olig2 lineage cells were GABAergic neurons in the dorsal cortex including the hippocampus (Figs. 6E–G). In the diencephalon, recombinant GFP+ cells induced E12.5 or earlier were distributed in the thalamic reticular nucleus or other prethalamus areas, showing overlapping distribution with GAD+ cells (Figs. 6E, H, I, J). In addition, approximately 35% of total recombinant cells expressed GAD67 mRNA in the basal forebrain of Olig2KICreER:CAT-Z postnatal mice (37±12.7 %, n=7 brains; Supplement Fig. 4). Thus, GABAergic neurons may be the most abundant neuron subtype derived from the early embryonic Olig2+ cells in GE and in the area surrounding the ZLI.

VGluT1 and VGluT2 are expressed in glutamatergic excitatory neurons in the telencephalic region and diencephalic and lower brainstem regions, respectively (Kaneko and Fujiyama, 2002). Pyramidal neurons in the cerebral cortex and the hippocampus, as well as dentate granule cells, expressed VGluT1 mRNA intensely. However, a very limited number of neurons co-expressed VGluT1 and reporter molecules in the cortex. Approximately 2% of X-gal+ recombinant cells expressed VGluT1 mRNA in the dorsal or ventral pallium of the Olig2KICreER:CAT-Z double-heterozygous P7 following E12.5 4-OHT treatment (Figs. 6K–N, arrows). In addition, no or few recombinant neurons showed a pyramidal neuron profile or only a few GFP+ cells expressed other markers for excitatory neurons such as PAG or CAMKII (not shown) (Kaneko et al., 1995; Terashima et al., 1995). In contrast, a considerable number of reporter cells expressed VGluT2 mRNA in the caudal hypothalamus at perinatal and postnatal stages: 31.2±6.85 % of the total recombinant cells observed in the E17.5 hypothalamus expressed VGluT2 mRNA following E9.5 and E10.5 4-OHT treatment (n=3 brains; Figs. 7A–C). In addition, the adult caudal hypothalamus contained a small number of X-gal+ Olig2 lineage cells expressing VGluT2 mRNA (Fig. 7D). Therefore, Olig2 lineage neuronal cells differentiate into excitatory neurons in the ventral diencephalon, which persist in the adult hypothalamus. Since Olig2 is expressed in the ventral diencephalon at fetal stage, Olig2 lineage glutamatergic neurons seem to be derived from the ventral diencephalon (caudal hypothalamus; arrow in Fig. 1K).

Effects of Olig2 deficiency on the development of GABAergic neurons and astrocytes

The results above demonstrated that GABAergic neurons differentiated from early Olig2+ progenitors and astrocytes differentiated from late Olig2+ progenitors in the forebrain. These results raise another question: Is Olig2 essential for GABAergic or astrocytic specification in these areas? We next analyzed Olig2 lineage differentiation in Olig2 deficient mice. As Olig2 deficient mice are stillborn, Olig2+/− fetuses were examined at E18.5. In the Olig2+/− brain at E18.5, the overall pattern of GAD mRNA expression seemed to be identical to that of the wild type/heterozygous brain (Figs. 8A, B). The dorsal cerebral cortex at E18.5 contained a similar number of GAD67 mRNA expressing cells per unit area (Figs. 8C, D; KO, 207±38.2, and wild/heterozygous, 215±23.9, per unit area 580 μm×440 μm, n=3 brains each). Therefore, Olig2+ cells give rise to GABAergic neurons normally in the absence of Olig2.

We also examined astrocyte development in late (E18.5) fetal stages. FGF3, GFAP and GLAST were used as markers for astrocytes or astrocytic progenitors. First, we examined expression of GFAP and GLAST with an immunohistochemical method. Expression patterns
were essentially similar between wild type and Olig2 deficient mice, although individual variation was observed (Supplement Fig. 5). In order to enumerate the cells more easily, we next examined expression of mRNA for FGFR3 and GLAST with an ISH method. In the E18.5 basal forebrain area (Figs. 8E, F, H, J), the numbers of FGFR3+ or GLAST+ cells were similar per unit area. For FGFR3, 144±8.48 were seen in the wild/heterozygous mice and 144±10.4 in the Olig2 deficient mice (580 μm×440 μm unit area, n=4 brains each). For GLAST, 59.2±8.23 were found in wild/heterozygous mice and 56.5±4.81 in Olig2 deficient mice (290 μm×220 μm unit area, n=5 brains each). Thus, astrocyte development seemed to be normal in the basal forebrain area without Olig2 function. However, in the dorsal pallium, especially in the medial part (Figs. 8E, F), it is surprising that GLAST+ cell density in the cortical plate and intermediate zone was lower in the Olig2 deficient mice, compared with the corresponding areas of the wild/heterozygous mice: 30.7±10.3 in the wild/heterozygous and 15.2±4.74 in the Olig2 deficient mice (290 μm×220 μm unit area, p<0.05, n=4 brains each) (Figs. 8E–G, I). The distribution of FGFR3+ was also reduced in number in the Olig2 deficient dorsal pallium compared

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**Fig. 5.** Cessation of Olig2 expression in the Olig2 lineage cells. (A, B) Coronal section through the diencephalon of E14.5 Olig2<sup>KICreER:CAT-Z</sup> double-heterozygous mouse treated with 4-OHT at E9.5. The section was stained with X-gal histochemistry followed by Olig2 immunohistochemistry. The area marked by an arrow is magnified in panel B. (B) A higher magnification picture of the prethalamus in panel A. The recombinant Olig2 lineage cells were labeled with X-gal histochemistry (blue; arrowheads), and Olig2+ cells were marked by a black reaction product, developed with a DAB/0.1% nickel chloride/H<sub>2</sub>O<sub>2</sub> solution. Note that none of the Olig2 lineage X-gal+ cells co-expressed Olig2 protein. Sections were counterstained with nuclear fast red. (C–E) The E17.5 basal forebrain (C) and hippocampus (D, E) of the Olig2<sup>KICreER:Z/EG</sup> mouse were treated with 4-OHT at E14.5. The sections were double-labeled with GFP (green) and Olig2 (red) immunohistochemistry. Blue color is nuclear staining with Hoechst33342. (C) A GFP+ cell (arrowhead) continued to express Olig2 while the remaining four cells (arrows) were not positive for Olig2. (D, E) GFP+ Olig2 lineage cells appeared in the hippocampus 3 days after recombination. A GFP+ cell in the intermediate zone (IZ) retained Olig2 expression (arrowhead in panel D) whereas GFP+ cells in the VZ/SVZ were not labeled with Olig2 (arrow in panel E). GFP+ recombinant cells are magnified in insets. CP, cortical plate. DG, dentate gyrus. (F–H) Expression of cell type-specific markers in Olig2 lineage GFP+ cells with or without Olig2 expression. Arrowheads indicate GFP+ cells. Animals were treated with 4-OHT at E13.5 and analyzed after 3 days. While Olig2 lineage GFP+ cells that were negative for Olig2 immunoreactivity expressed class III β tubulin (Tuj1, Fc), those with Olig2 immunoreactivity expressed GLAST (Gc) or PDGF receptor α (Hc). Arrows indicate Olig2+ cells expressing GLAST (Gc) or PDGF receptor α (Hc). Scale bars in panel A=500 μm, in panels B, C=50 μm, in panel D=100 μm, in insets, Fd,Gd,Hd=20 μm.
with wild type animals (41.6±8.24 in the wild/heterozygous and 13.3±4.10 in the Olig2 deficient mice in 290 μm×220 μm unit area, p<0.01, n=4 brains each; not shown). These results suggest that Olig2 plays some roles in the differentiation of astrocytes in the dorsal pallium, but not in the basal forebrain. Therefore, as suggested by others (Marshall et al., 2005; Cai et al., 2007), Olig2 functions in gliogenesis in the neocortex, both oligodendrocytic and astrocytic.

**Fig. 6.** GABAergic and glutamatergic neurons in Olig2 lineage cells. (A–C) GABA+/GFP+ cells in the adult neocortex after the 4-OHT treatment at E12.5. A projection picture of optical sections taken with a CLSM. (D) A GAD mRNA+/GFP+ cell (arrow) in the adult neocortex with 4-OHT treatment at E12.5. In situ hybridization (blue) followed by GFP immunoenzymatic staining (brown). (E–H) GAD67 mRNA+/LacZ+ cells in the P7 forebrain following E12.5 4-OHT treatment. X-gal histochemistry (light blue) followed by GAD67 in situ hybridization (blue). (I) A low magnification picture of E17.5 forebrain stained with GAD ISH followed by GFP IHC. The prethalamic region is magnified in panel J. A higher magnification picture of GAD67+/GFP+ cells in the prethalamus. (K–M) VGluT1 mRNA+/LacZ+ cells in the P7 cortical regions following E12.5 4-OHT treatment. A few double-positive cells are observed in the piriform cortex (I, J) and the dorsal part of the neocortex (K, L). Scale bars in panels C, H, J, N=20 μm, in panel D=50 μm, in panels E, I=1 mm, in panel K=500 μm, in panel L=10 μm, in panel M=200 μm.

**Fig. 7.** Glutamatergic differentiation of Olig2 lineage cells in the caudal hypothalamus. (A–C) VGluT2 mRNA+/GFP+ cells in the E17.5 ventromedial hypothalamus following E9.5 and E10.5 4-OHT treatment. VGluT2 in situ hybridization followed by GFP immunostaining. (D) VGluT2 mRNA+/LacZ+ cells in the caudal hypothalamus in the adult brain (D) following E12.5 4-OHT treatment. X-gal histochemistry followed by VGluT2 in situ hybridization. An arrow in panel D indicates localization of a double-labeled cell, which is magnified in inset (arrow in inset). f, fimbria; mt, mammillothalamic tract; PM, premammillary nuclei; VMH, ventromedial hypothalamic nucleus. Scale bars in panel A=200 μm, in panel C and inset D=20 μm, in panel D=500 μm.
Discussion

Embryonic Olig2+ cells constitute a heterogeneous precursor pool

We examined the differentiative properties of embryonic Olig2+ cells in the forebrain with a tamoxifen-inducible Cre/loxP system. In the telencephalon, Olig2+ cells at E12.5 (or earlier) differentiated into neuronal cells, while those in later stages tended to be macroglial cells. By contrast, Olig2+ cells in the late fetal diencephalon (this study) or in the late spinal cord (Masahira et al., 2006) tended to give rise to oligodendrocytes. These results clearly demonstrated stage- and region-specific differentiation of embryonic Olig2+ cells. One of the most important findings in this study is that Olig2+ cells in the late fetal telencephalon generate astrocytes and that this is their most common differentiative fate. Our short-term lineage tracing showed that Olig2+ cells in the late fetal stage are a mixed population of glial progenitor cells (see Figs. 5F–H). In addition, Olig2+ cells in the VZ have characters of radial glial cells in both the dorsal and ventral telencephalon (Petryniak et al., 2007; Supplement Fig. 1). It is well-known that cortical radial glia give rise to astrocyte (Schmechel and Rakic, 1979). We also demonstrated that Olig2 deficiency resulted in a decreased number of astrocyte in the neocortex in the late fetal stage (see Fig. 8). Our results are consistent with recent reports by Marshall et al. (2005) and by Cai et al. (2007), demonstrating that Olig2 regulates gliogenesis, both oligodendrocytic and astrocytic, in the developing telencephalon.

Fig. 8. Normal development of GABAergic neurons and altered development of cortical astrocyte in the absence of Olig2. (A–D) Expression of GAD mRNA in the E18.5 forebrain of Olig2+/+; (A, C) and Olig2−/− (B, D) mice. Sections were counterstained with nuclear fast red. Cortical areas are magnified in panels C and D. Overall patterns of GAD expression (A, B) and the densities of GAD expressing cells in the cortex (C, D) were similar in wild type and Olig2 deficient mice. (E–J) Expression of GLAST mRNA in the E18.5 forebrain of Olig2+/+; (E, G, H) and Olig2−/− (F, I, J) mice. The dorsal pallium (G, I) and basal forebrain (H, J) of panels E and F are magnified in panels G, I and H, J, respectively. Note that GLAST+ cell density in the dorsal pallium (indicated by arrowheads in panel F) was slightly lower in the Olig2 deficient mouse (F, I) than in the wild type (E, G), while that in the basal forebrain was similar in the Olig2 knockout (E, H) and the wild type (F, J) mice. Density of GLAST+ cells were examined in the dorsal pallium indicated by arrowheads, and compared between WT (E, G) and KO (F, I). Asterisks in panels B and F indicate slight enlargement of the third ventricle at the hypothalamic sulcus in the Olig2 deficient mouse. Scale bars in panels A, B, E, F=1 mm, in panel D=100 μm, in panel H=50 μm.
Differentiation of Olig2 lineage cells into both inhibitory and excitatory neurons

Most Olig2 lineage neuronal cells are GABAergic neurons in the telencephalon. GABAergic differentiation of Olig2+ cells was expected because of their restricted localization in the GE VZ. Homotopic and homochronous transplantation of GE cells elicited the temporal and spatial features of inhibitory neuron production (Wichterle et al., 2001; Butt et al., 2005b). Detailed analysis of cortical GABAergic neuron subtypes from Olig2+ cells was reported recently (Miyoshi et al., 2007). While cortical GABAergic neurons were generated from ventral Olig2+ progenitor cells by E14.5, oligodendrocyte intermediates that are also GABAergic were generated by Olig2+ cells at late fetal stage such as E17.5. Therefore, it is probable that some fraction of Olig2+ cells in the late fetal stage are still neurogenic, contributing to generation of the olfactory bulb interneurons. Recently, Ventura and Goldman (2007) elucidated that radial glia in the dorsal telencephalon gives rise to olfactory interneurons in early postnatal stage. Some GABAergic neurons in the diencephalon are also Olig2 lineage cells. Many Olig2 lineage recombinant cells are observed in the thalamic reticular nucleus and ventral thalamus (or prethalamus) where GABAergic neurons are the predominant neuronal subtype (see Figs. 6H and 8A). Since Olig2 is continuously expressed around the ZLI and the prethalamic region from the early to the late fetal stage (see Figs. 1F, G, H), it is highly probable that prethalamic Olig2+ progenitor cells contribute to GABAergic neurons in this area. It is noteworthy that in the human fetal brain, a migratory stream from the GE to diencephalon is observed, and this contributes to GABAergic neurons in the diencephalon (Letinic and Rakic 2001). However, such a cell strand is not observed in the rodent or non-human primate brain (Letinic and Rakic 2001), and therefore, it is less likely that cells in the telencephalon differentiate to neurons in the rodent diencephalon.

It is possible that glutamatergic differentiation from Olig2+ cells may be an exceptional or a transient phenotype in the cortex, since the number of neurons co-expressing VGluT1 and LacZ is limited and also since the adult brain does not contain recombinant neurons with a pyramidal profile, typical of excitatory neurons. In contrast, a considerable number of VGluT2 expressing Olig2 lineage cells appear in the developing caudal hypothalamus, which persisted in the adult brain (see Fig. 7). This is the first direct evidence for glutamatergic neuronal differentiation from Olig2 lineage cells. These cells may be derived from the embryonic hypothalamus (Figs. 1G, K). Thus, our results clearly demonstrated that the final phenotypes of Olig2 lineage neurons are context-dependent. Both the nature of Olig2+ cells and inductive signals from the environment may differ among different regions and stages in the CNS.

Although GABAergic neurons are the major neuronal subtype from Olig2+ cells, GABAergic neurons can develop at normal density in the Olig2 knockout cortex (Figs. 8C, D) (Miyoshi et al., 2007). The function of Olig2 in subtype specification or the development of GABAergic neurons remains unclear.

Migration of Olig2+ precursor cells

Olig2+ cells in the E12.5 telencephalon are mostly localized in the VZ of the MGE, septum and AEP. In the adult telencephalon, Olig2 lineage cells marked with GFP or with LacZ upon recombination at E12.5 are distributed throughout the neocortex. This may reflect dorsal tangential migration of GABAergic neurons (Anderson et al., 1997; Tamanakhi et al., 1997; Tanaka et al., 2003) and, at least in part, of OPCs (Kessaris et al., 2006; Nakahira et al., 2006). The ventral origins and the subsequent dorsal dispersal of GABAergic neurons is conserved in the vertebrate forebrain (Cobos et al., 2001; Kataoka et al., 2000; Mueller et al., 2006; Tuorto et al., 2003). Thus, the GE is an important structure for increasing the number and heterogeneity of neurons, and probably oligodendrocytes as well, in the dorsal pallium. In addition, the olfactory bulb anlagen at E12.5 did not express Olig2 (not shown) whereas recombinant neurons induced at E12.5 resided in the granule and periglomerular layers of the adult olfactory bulb (Supplement Fig. 3), suggesting rostral migration of olfactory neurons from the GE. A considerable number of recombinant neurons were observed in the adult olfactory bulb after E17.5 4-OHT treatment. Many of these may also be immigrants from the GE, as olfactory interneurons are generated in the SVZ, caudal to the olfactory bulb (Hinds, 1968), throughout life (Lois and Alvarez-Buylla, 1994; Ventura and Goldman, 2007).

OPCs have been shown to originate from the MGE or AEP in the fetal forebrain, and then migrate towards the neocortex (Kessaris et al., 2006; Nakahira et al., 2006; Olivier et al., 2001). In addition, recent evidence suggests that OPCs derived from the basal forebrain at early stages are replaced with those derived from the dorsal pallium at later stages during cortical maturation (Kessaris et al., 2006). In our study, dorsal migration of OPCs is suggested by the occasional appearance of Olig2 lineage oligodendrocytes in the adult fimbria and GFP+/Olig2+ cells in the fetal cortex following E12.5 4-OHT treatment. The adult neocortex contains more Olig2 lineage oligodendrocytes when recombination was induced at E16.5 or later. These results might indicate that early-generated OPCs migrate dorsally but are replaced with OPCs generated locally in the neocortex in later stages of corticogenesis during which dorsal cortical VZ generate Olig2+ cells, as demonstrated by Kessaris et al. (2006).

Lineage relationship between early and late Olig2+ progenitors

Olig2+ progenitor cells are initially localized in the ventral VZ such as the MGE and a surrounding area of the ZLI. Olig2, together with other TFs, regulates oligodendrocytic specification in the basal forebrain (Parras et al., 2007; Petryniak et al., 2007). They subsequently undergo gradual dispersal throughout the forebrain. Short-term lineage analysis demonstrated that the majority of Olig2 lineage cells ceased Olig2 expression within a few days of recombination, and the great majority of Olig2+ cells were negative for GFP but positive for PDGFRα or GLAST in the late fetal stage (see Fig. 5), which suggests that some Olig2+ glial progenitors at a late fetal stage are not direct cohorts of early ventral Olig2+ cells. Although certain fractions of ventrally-derived Olig2 lineage cells may reside in the VZ/SVZ of the dorsal pallium, our observations suggest that such cells also cease Olig2 expression in the VZ/SVZ (Figs. 5D, E). Therefore, Olig2+ cells in the VZ/SVZ of the dorsal pallium may acquire Olig2 expression locally in the pallial VZ/SVZ. In addition, Olig2+ cells seemed to be generated locally in the VZ of the dorsal telencephalon (Fig. 1 and Supplement Fig. 1). Indeed, Emx1 lineage cells in the dorsal pallium generate cortical oligodendrocytes (Gorski et al., 2002). Region- and stage-dependent differentiative properties of the Olig2+ progenitor cells may be involved in local generation of Olig2+ progenitors.

In summary, we elucidated the differentiative properties of Olig2+ cells in the fetal forebrain with a stage-specific recombination method. Olig2+ cells in the early stages give rise to inhibitory or excitatory neurons depending on the areas of birth. In late fetal stages, Olig2+ cells differentiate into macroglial cells, astrocytes or oligodendrocytes, again depending on the area of their development. Therefore, Olig2+ cells form dynamic, heterogeneous progenitor pools during the development of the forebrain.

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