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Olig2-positive progenitors in the embryonic spinal cord give rise not only to motoneurons and oligodendrocytes, but also to a subset of astrocytes and ependymal cells

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Abstract

Motoneurons and oligodendrocytes in the embryonic spinal cord are produced from a restricted domain of the ventral ventricular zone, termed the pMN domain. The pMN domain is the site of expression of two basic helix–loop–helix transcription factors, Olig1 and Olig2, which are essential for motoneuron and oligodendrocyte development. Previous lineage-tracing experiments using *Olig1-Cre* and *Olig2-GFP* mice suggested that motoneurons and oligodendrocytes, but not astrocytes, are produced from the pMN domain. However, important questions remain, including the fate of neuroepithelial cells in the pMN domain, and specifically whether motoneurons and oligodendrocytes are the only types of cells produced in the pMN domain. We performed lineage-tracing experiments using a tamoxifen-inducible Cre-recombinase inserted into the *Olig2* locus. We demonstrated that motoneurons and oligodendrocyte progenitors are derived from the $Olig2^{+}$ progenitors in the pMN domain, and also found that a subset of astrocytes at the ventral surface of the spinal cord and ependymal cells at the ventricular surface are also produced from the pMN domain. These findings demonstrate that motoneurons and oligodendrocytes are not the only cell types originating from this domain.

Keywords: Olig2; CreER; Recombination; Lineage tracing; Radial glia; Astrocyte; Glia limitans; Motoneuron; Oligodendrocyte; Ependymal cell

Introduction

Transcription factors are known to regulate cell type-specific differentiation during formation of the central nervous system (CNS). For example, in early developing spinal cord, expression of basic helix–loop–helix (bHLH) and homeobox transcription factors demarcates and yields distinct dorsoventral domains, in which cells undergo cell type specification to become various neuronal or glial subtypes (Briscoe et al., 2000; Jessell, 2000; Schuurmans and Guillemot, 2002). Motoneurons and oligodendrocyte progenitors (OLPs) are produced sequentially from a common domain of the ventral ventricular zone, termed the pMN domain in the developing spinal cord (Richardson et al., 2000). Olig1 and Olig2 are bHLH transcriptional factors specifically expressed in the pMN domain (Lu et al., 2000; Takebayashi et al., 2000; Zhou et al., 2000). Gain-of-function (Lu et al., 2000; Mizuguchi et al., 2001; Novitch et al., 2001; Zhou et al., 2000, 2001) and loss-of-function (Lu et al., 2002; Park et al., 2002; Takebayashi et al., 2002; Zhou and Anderson, 2002) studies have established that Olig2 is essential for motoneuron and oligodendrocyte development during early embryogenesis, and Olig1 has been

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shown to play roles in oligodendrocyte development postnatally (Lu et al., 2002; Xin et al., 2005).

Previous lineage-tracing analyses of Olig-expressing progenitors using *Olig1-Cre ROSA26R* and *Olig2-GFP* mice have suggested that Olig1- and Olig2-expressing cells generate only motoneurons and oligodendrocytes and do not produce astrocytes (Lu et al., 2002; Zhou and Anderson, 2002). These results suggest the hypothesis that oligodendrocytes and motoneurons share common precursors, separate from astrocytes, and that there are no tripotential (neuron/astrocyte/oligodendrocyte) progenitors in the developing spinal cord (Lu et al., 2002; Zhou and Anderson, 2002; reviewed by Rowitch et al., 2002). However, in *Olig2-GFP* mice (Zhou and Anderson, 2002), only short-term lineage analysis can be carried out, and it is difficult to track Olig2 lineage cells after cessation of *Olig2* gene expression, since GFP-expression largely mimics Olig2-expression. Since Olig1 expression in early spinal cord is biphasic and weaker than that of Olig2 (Lu et al., 2000; Mizuguchi et al., 2001), findings for *Olig1-Cre Rosa26R* mice (Lu et al., 2002) may apply to only a fraction of Olig2 lineage cells in early development. In addition, Cre recombinase is active in all Olig1$^+$ cells including migrating OLPs in parenchyma, not only those derived from pMN domain, but also those derived from dorsal domains at later stages (Cai et al., 2005; Fogarty et al., 2005; Vallstedt et al., 2005). To overcome these problems and to label progenitors exclusively in the pMN domain within a limited time window, we employed tamoxifen (TM)-inducible Cre recombinase, CreER™, for lineage-tracing experiments. Using mice with *CreER™* knocked-in at the *Olig2* locus and *loxP*-flanked (floxed) reporter mice, we examined whether motoneurons and oligodendrocytes are the only derivatives of Olig2$^+$ cells and whether astrocytes or other types of cells are also generated from Olig2$^+$ cells. We first demonstrated that motoneurons and OLPs are derived from Olig2$^+$ progenitors in the pMN domain. We also found other labeled cell types: astrocytes at the ventral pial surface of the spinal cord and ependymal cells at the ventricular surface. Our findings demonstrated that not only motoneurons and OLPs, but also a subset of astrocytes and ependymal cells, are produced from the pMN domain after down-regulation of Olig2 expression. In addition, we found that there are Olig2$^+$ radial glial cells in the pMN domain at both neurogenic and gliogenic stages.

Materials and methods

Mice

Olig2^{KICreER} mice, *CAG-CAT-Z*, and *Z/EG* mice have been previously described (Novak et al., 2000; Sakai and Miyazaki, 1997; Takebayashi et al., 2002). Heterozygous *Olig2$^{KICreER/WT}$* mice are normal and histologically indistinguishable from wild-type mice. *Olig2$^{KICreER/WT}$ CAG-CAT-Z* or *Olig2$^{KICreER/WT}$ Z/EG* double-heterozygote males were crossed with wild-type C57BL/6J (Japan SLC Inc., Shizuoka, Japan) females to obtain double-heterozygote embryos. Midday of the day on which a vaginal plugs was observed was considered 0.5 days postcoitum (dpc). All animal procedures were performed in accordance with the Policies on the Use of Animals and Humans in Neuroscience Research, revised and approved by the Society for Neuroscience in 1995, guidelines described by the National Institutes of Health Guide for Care and Use of Laboratory Animals, and the local Animal Care and Use Committee.

Genotyping

DNA samples were prepared from tails for normal genotyping, or the caudal part of embryos including the spinal cord for detection of recombined *CAG-CAT-Z* allele. Tissues were incubated at 50°C overnight with gentle agitation in a dissolving solution (50 mM KCl, 10 mM Tris–HCl [pH 8.3], 0.1 mg/ml gelatin, 0.45% NP-40, and 0.45% Tween 20 containing 200 μg/ml proteinase K). This crude genomic DNA solution was directly used as template for polymerase chain reaction (PCR) genotyping. *Olig2$^{KICreER/WT}$* mice were genotyped by PCR as described (Takebayashi et al., 2002). For PCR detection of the *CAG-CAT-Z* allele, the *lacZ* primers ZA4 (5′-CGT TGC ACC ACA GAT GAA ACG C-3′) and ZS4 (5′-CTC AAA CTG GCA GAT GCA CGG T-3′) were used. This pair of primers amplifies a 206 bp fragment from the *CAG-CAT-Z* allele. For detection of Cre-recombined *CAG-CAT-Z* allele, CA1 primer (5′-CTG CTA ACC ATG TTC ATG CC-3′) for the *CAG* promoter and L3 primer (5′-GGC CTC TTC GCT ATT ACG-3′) for the *lacZ* gene were used to amplify 580 bp fragments from the recombined *CAG-CAT-Z* allele (Sakai and Miyazaki, 1997). For detection of the *Z/EG* allele, EGFP1 primer (5′-GAC GTA AAC GGC CAC AAG TT-3′) and EGFP2 primer (5′-GAA CTC CAG CAG GAC CAT GT-3′) for the *EGFP* gene were used to amplify 609 bp fragments. PCR was performed at 95°C for 9 min, followed by 95°C for 20 s, 56°C (for detection of *CAG-CAT-Z* allele), 55°C (for detection of recombined *CAG-CAT-Z* allele), or 60°C (for detection of *Z/EG* allele) for 30 s, and 72°C for 30 s; PCR was run for 37 cycles (for *CAG-CAT-Z*) or 27 cycles (for *Z/EG*) on a thermal cycler (GeneAmp PCR Systems 9700, Perkin-Elmer, Wellesley, MA) using AmpliTaq Gold (Applied Biosystems, Foster City, CA).

Tamoxifen treatment

The *CreER™* gene was constructed in accordance with a previous report (Danielian et al., 1998). We fused *Cre* recombinase and mutated mouse estrogen receptor α isoform (*ER*) gene, which has a FLAG tag at the C terminus (Takebayashi et al., 2002). For lineage analysis of Olig2$^+$ cells, it is important that these cells be labeled during a narrow, defined time window. Since the half-life of 4-hydroxytamoxifen (4-OHT) is relatively short (6 h, compared with 12 h for tamoxifen (TM)) (Robinson et al., 1991), we used 4-OHT (H-6278, Sigma, St. Louis, MO) as a ligand to activate CreER™, dissolving it in a DMSO–ethanol–sesame oil (4:6:90) mixture at a concentration of 10 mg/ml. Pregnant mice were injected intraperitoneally at E9.5, E10.5, E12.5, E14.5, or E15.5 with 1.5 mg of 4-OHT. The injections were performed between 8 a.m. and noon. Embryos were analyzed at E10.5, E11.5, E12.5, E14.5, and E18.5. A single 3.0 mg injection of 4-OHT led to death of embryos.

Histology

All analyses in this study were performed on the spinal cord at thoracic levels. The images were collected with an Olympus microscope (Olympus BX51; Olympus, Japan) and digital camera system (Olympus DP70; Olympus), or with confocal microscopy (Zeiss LSM-510, Nussloch, Germany).

For in situ hybridization (ISH) on cryosections, we used a modified version of methods previously described (Ding et al., 2006). After perfusion-fixation, tissues were fixed with 4% PFA, cryoprotected by 10% and 20% sucrose in phosphate-buffered saline (PBS), embedded in OCT compound (Sakura Finetechnical Co. Ltd., Tokyo, Japan), frozen, and sectioned at 18 μm thickness. After ISH staining, the sections were counterstained using nuclear fast red. The following plasmids were used for generation of probes: mouse *Olig1*, *Olig2* (Takebayashi et al., 2000), *CreER™* (Takebayashi et al., 2002), mouse *Neurogenin2* (*Ngn2*) (obtained as an EST clone from Open Biosystems, Huntsville, AL; Genbank accession number BC055743), and rat *Islet2* (*Isl2*) (provided by Dr. Yasuto Tanabe, Mitsubishi Kagaku Institute of Life Sciences, Tokyo, Japan). Digoxigenin-labeled single-stranded riboprobes were prepared by transcription of linearized plasmids using T7, T3, or Sp6 RNA polymerase and the DIG RNA labeling kit (Roche, Indianapolis, IN).

Immunohistochemistry was performed as previously described (Takebayashi et al., 2002) using the following primary antibodies: rabbit polyclonal anti-GFP antibody (1:3000; Molecular Probes, Eugene, OR), rabbit polyclonal anti-Cre antibody (1:3000; Novagen, San Diego, CA), rat monoclonal anti-GFP