Fate mapping of oligodendrocyte progenitor cells (OPC) in adult demyelinating and remyelinating mice

成体マウスの脱髄および再髄鞘化におけるオリゴデンドロサイト前駆細胞の増殖と文化

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Submission: Jan. 26, 2009

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バイオサイエンス研究科　博士論文要旨

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題目	Fate mapping of Oligodendrocyte progenitor cells (OPC) in adult demyelinating and remyelinating mice 成体マウスの脱髄および再髄鞘化におけるオリゴデンドロサイト前駆細胞の増殖と文化		

要旨 Despite the presence of neural stem cells in the adult brain, repair of brain damage caused by injury or disease is still a challenge. It has been shown that oligodendrocyte progenitor cells (OPCs) respond to various kinds of damage in the adult central nervous system (CNS). Indeed, many OPCs are found in acute or chronic demyelinated areas, but not all of them differentiate efficiently into mature oligodendrocytes in the demyelinated CNS. Recent studies have also shown that OPCs marked by Olig2, a basic helix-loop-helix transcription factor, which stimulates OPCs to differentiate into oligodendrocytes, is strongly upregulated in many pathological conditions including acute or chronic demyelinating lesions in the adult CNS. Despite their potential role in the treatment of demyelinating diseases, the long-term cellular fate of these Olig2-upregulated OPCs has not been identified due to the lack of stable labeling methods. To identify the cellular fate of these OPCs in the damaged area of the CNS, I have used double-transgenic mice, in which I was able to label Olig2-expressing OPCs conditionally with green fluorescent protein (GFP). Demyelination was induced in the CNS in these mice by feeding them with cuprizone, a copper chelator. After six weeks of cuprizone induction, Olig2 marked

GFP-positive cells were processed for a second labeling with the major neural cell markers APC (mature oligodendrocyte marker), GFAP (astrocyte marker), NeuN (neuronal marker), Iba1 (microglia marker) and NG2 proteoglycan (OPC marker). More than half of the Olig2 marked GFP-positive cells in the corpus callosum in the CNS showed colocalization with NG2 (55% in the controls and 67% in the cuprizone treated group). Colocalization of APC with Olig2 marked GFP-positive cell was significantly higher in the cuprizone-treated group (38%) than in the controls (25%). While significant difference in oligodendrocytic lineage cell differentiation was found between the groups, no significant difference was observed in astrocytic lineage cell differentiation. No neuronal or microglial lineage cells were found in this experimental condition. Although Olig2 marked GFP-positive cells still showed NG2 colocalization throughout the experimentation period but did not showed any cell lineage differentiation, suggesting that OPCs tend to keep progenitor-like characteristics. My study has identified the fate of the Olig2-expressing progenitors directly by and endogenous genetic labeling approach in vivo in the demyelinated lesions.

Introduction

Neural components relating to neurotransmission:

The the central nervous system (CNS) and the peripheral nervous system (PNS) consist of a huge network of specialized cells that communicate information about an animal's surroundings and themselves. They process neural information from sensory organs, integrate it and induce complex behavioral reactions in all parts of the body. Brain cells comprise neurons and a variety of glial cells, such as oligodendroglia (oligodendrocytes), astroglia (astrocytes), and microglia/macrophages. As shown in Fig. 1, neurons and glial cells interact with each other via their processes and may function interdependently. Neurons are specialized for electrical signaling over long distances for information processing (Fig. 2, A). To support the information transmission by neuronal processes, oligodendrocytes make envelopes which surround axons derived from neuronal cell bodies. These specialized membrane coats are called myelin sheaths (Fig. 2; A, B). The myelin sheath provides the axon with insulation that allows electrical signals to propagate more efficiently. Myelinated axons (bundles of axons) in the CNS

comprise the major white matter tract (i.e. corpus callosum that connects the left and right cerebral hemispheres), which connects various gray matter areas (containing neuronal cell bodies) in the brain. Nerve impulses arise at neurons and travel through the axon bundles. The white matter tract, a major myelinated axon bundles, is easily visible after staining with dyes such as luxol fast blue, and the gray matter is visisble by Cresyl violet (Nissl) staining (fig. 3).

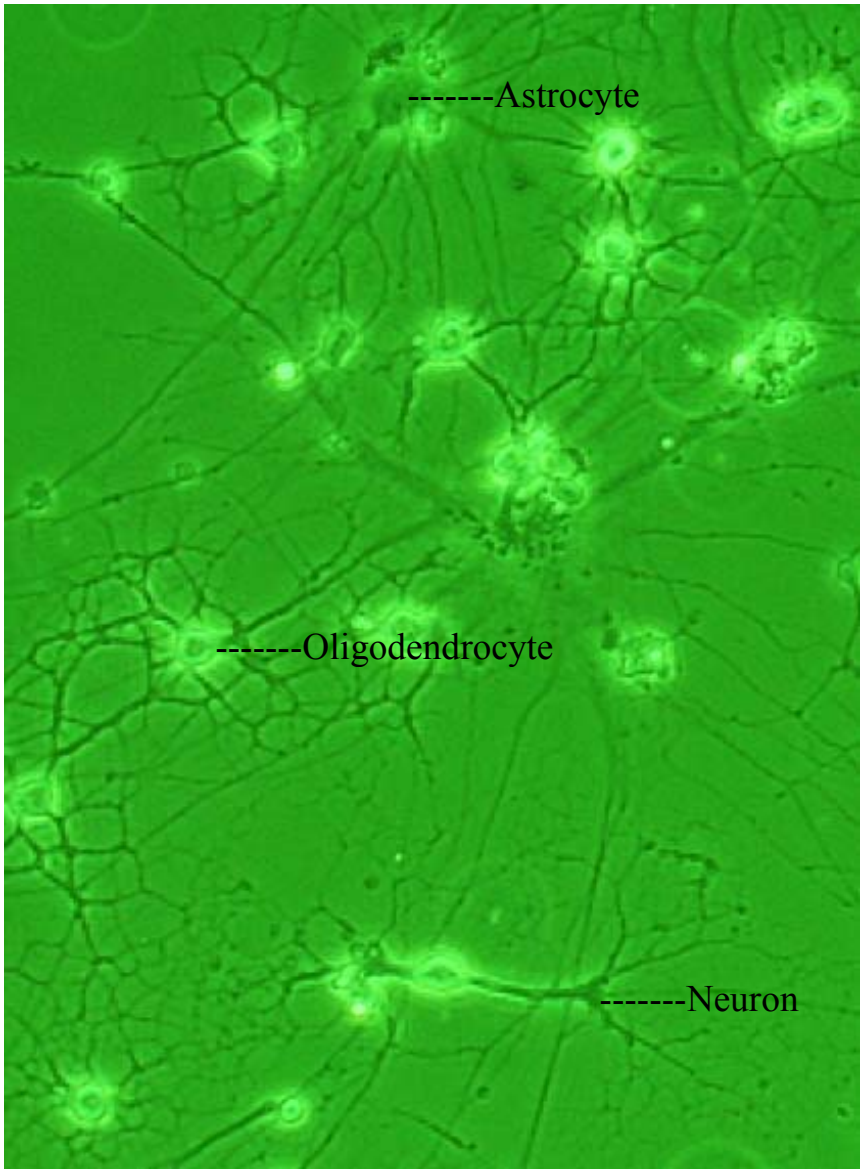
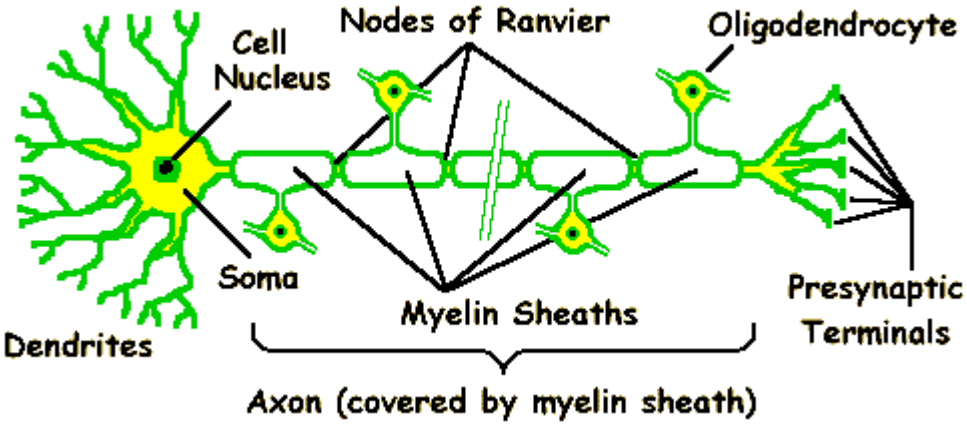


Fig. 1. Close interactions of neurons and glial cells in culture. Cells of the cerebral cortex of a mouse brain were cultured for 10 days and photographed with a light microscope equipped with phase contrast condenser. Structural characteristics define different types of cells.

A



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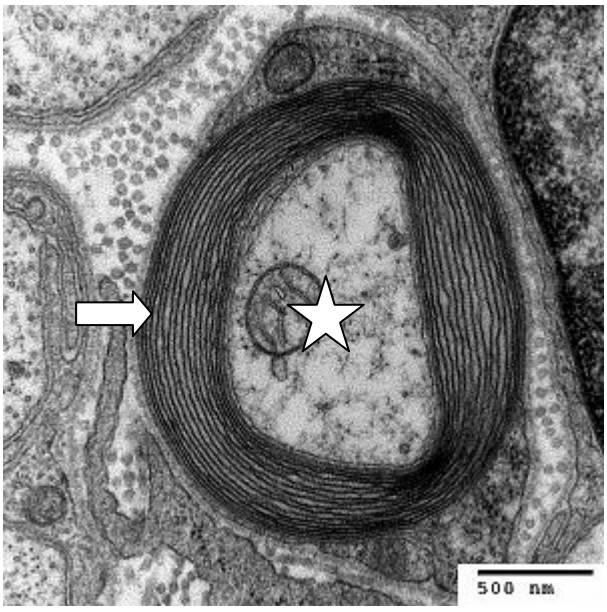

Fig. 2. Schematic diagram of a typical neuron with Oligodendrocyte provided myelin sheath (A). Electron micrograph of myelinated axon (star shows axon and arrow shows myelin sheath provided by oligodendrocyte) in CNS (B). The image in (B) was released by Microscopy Facility at Trinity College, Hartford, CT.

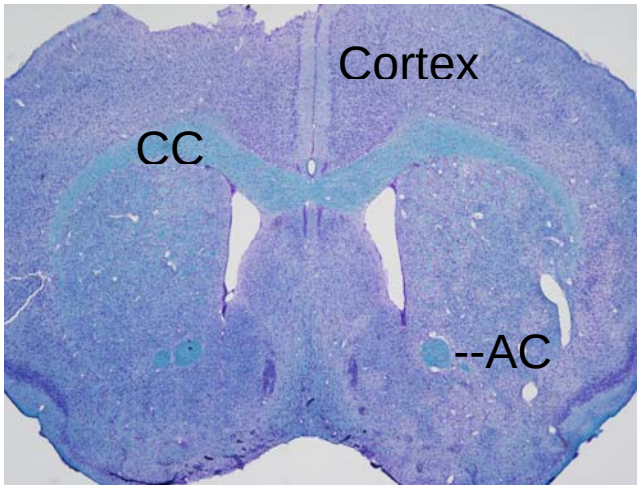

Fig. 3. Luxol fast blue stained section showing major white matter tract of myelinated axons in corpus callosum (CC) and anterior commisure (AC). Section was also processed for Cresyl violet (Nissl) staining which makes visible the Gray matter, localization of neuronal cell bodies.

Developmental differentiation into neurons and oligodendroglia:

After fertilization in mammals, progressing through morula to blastocyst, the embryo begins as a flat disk with three distinct layers of cells called the endoderm, mesoderm, and ectoderm. The nervous system arises from the ectoderm. Specific changes in part of the ectoderm, called the neural plate, produce the neural tube and neural crest. The CNS develops from the neural tube and the PNS develops from the neural crest. Development of mammalian neural properties occurs very early during embryonic stage. Nerve cells become specialized in embryonic life. At mid-gestation, young differentiated neurons migrate above the germinal ventricular zone. After the neurogenic period, stem cells in the mammalian CNS generate glial progenitors that proliferate in the subventricular zone during the perinatal period (Table 1). In general, CNS and PNS neurons arise before glial maturation.

The transmission speed depends on the synaptic junction between nerve cells and the myelin sheath surrounding axons. The ontogeny of the myelination process is rather later than the development of neurons. Classical postmortem studies of the human brain suggest that axon diameters and myelin sheaths continue to grow during the first

two years after birth, and postembryonic development of myelin is crucial for natural brain development in various mammals (Skoff et al., 1976a, b; Dangata and Kaufman, 1997). In adult brain, also, degeneration and regeneration of myelin may happen by incidental axonal damage caused by injury or diseases such as multiple sclerosis, and reorganization of damaged neural networks might depend on the increase of the glial cell population, and on maturation and remyelination of axons.

Forebrain oligodendrocytes originate first from progenitors in the embryonic ventral telencephalon (Spassky et al., 1998) and then from dorsal telencephalon (Kessaries et al., 2005). Oligodendrocyte progenitor cells (OPCs) migrate from their sites of origin to localize in the gray and white matter of the forebrain. It is known that oligodendrocytes continue to be produced in the adult brain (Blakemore, 1972) and these cells are considered to participate in myelin repair (Gensert and Goldman, 1997). Recent studies have demonstrated that stem cells in the subventricular zone also generate oligodendrocytes *in vitro* (Menn et al., 2006), but it is still unclear whether adult OPCs can generate oligodendrocytes in vivo in the remyelination process.

Table 1

Developmental expression of some oligodendro-glial markers in rodent

Developmental stage				
Marker	Cell specification	Early embryonic E9 ~ E14	Mid embryonic E15~ E18	Perinatal to adult E19~ adult
Olig2 (1)	OPC	+++	++	+
PDGFR-α (2, 3)		+++	++	+
A2B5 (4)		++	+	
GD3 (5)		+	++	
NG2 (6,7)		+++	++	+
CNP (8)	Immature oligodendrocyte	+	+++	+
O4 (9)		+	+++	+ (Perinatal) a few (adult)
O1 (5)			+++	+
APC	Mature oligodendrocyte		+	+++
MBP (5)				+++
MOG (5)				+++
PLP (10)				+++

Numbers in parentheses indicate references: (1) Zhou et al., 2000; (2) Pringle and Richardson, 1993; (3) Nishiyama et al., 1996; (4) Rao et al., 1998; (5) Stangel and Hartung, 2002; (6) Stallcup and Beasley, 1987; (7) Levine and Stallcup, 1987; (8). Yu et al., 1994; (9) Ono et al., 1995; (10) Timsit et al., 1995. Abbreviation: Olig2,

oligodendrocyte limneage marker 2; PDGFR-α, Platelet-derived growth factor receptor-α; CNP, 2, 3-cyclic nucleotide 3-phosphodiesterase; APC, adenomatous polyposis coli; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; PLP, myelin proteolipid protein. Note, + indicates degree of abundance.

Early attempts to define origins of oligodendrocytes in the mature brain:

Earlier studies gave rise to a controversy over the identification of progenitors of various types of neuroglial cells (Ramon-Moliner, 1958; Smart and Leblond, 1961; Skoff et al., 1976a, b; Privat et al., 1981), concerning whether the different glial cell types, i.e., oligodendrocytes and astrocytes, originate from different progenitors or from the same progenitor simultaneously or successively (Wood and Bunge, 1984; Fedoroff, 1985). These issues could not be resolved due to the unavailability of cell-specific markers that can now be used to identify progenitors, possible intermediate forms, and terminally differentiated neuroglial cell types, and also due to the limited technical capacity to trace cell lineages. Some conventional approaches that were used in early attempts to trace cell lineage are depicted in Fig. 4. However, the ability of the same progenitor to express different antigen markers at different stages leads to the assumption that they may yield different progeny at different stages. For example, O-2A progenitors, which can differentiate into either oligodendrocytes or type 2 astrocytes in vitro depending on the composition of the culture medium, in adult brains express O4 antigen, which is not expressed on O-2A progenitors in developing brain (Table 1), and they may have a much longer cell cycle time and slower rate of migration than do

progenitors in developing brain (Wolswijk and Noble, 1989; Sommer and Schachner, 1981). Glial cells are produced and die throughout life, and indeed glial progenitors retain the ability to undergo cell division in adulthood.

In the mature brain, resident OPCs marked by NG2 (a chondroitin sulfate proteoglycan), Olig2 (a basic helix-loop-helix transcription factor), and PDGFR-α (platelet-derived growth factor receptor α) seem to keep this ability. The progeny from these OPCs in adult brain may contribute to the repair of brain damage (Table 1).

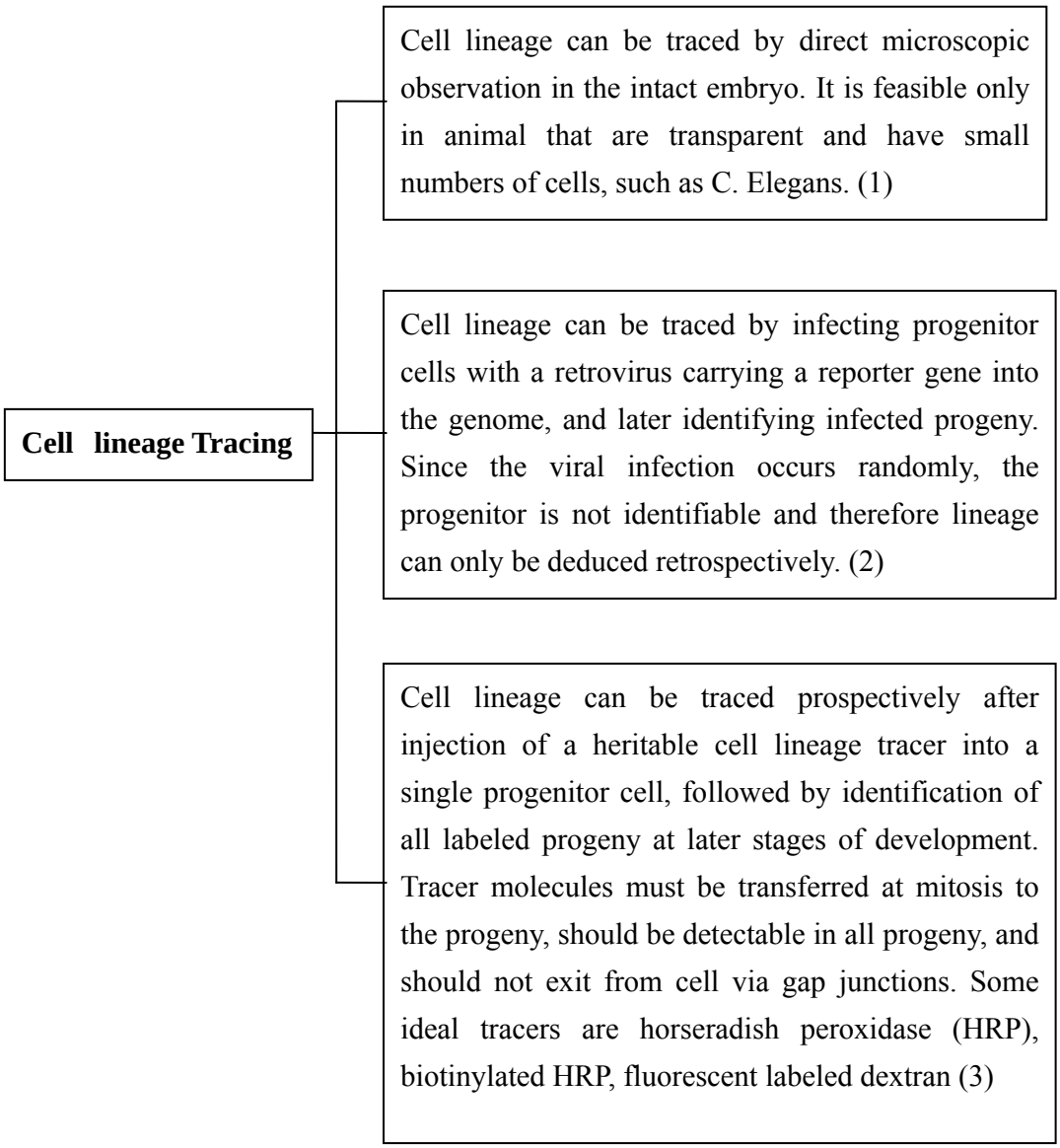

Fig. 4. Common approaches to cell lineage tracing used in earlier studies.

Numbers in parentheses indicate references. (1) Sulston and Horvitz, 1977; Sulston et al., 1983. (2) Turner and Cepko, 1987; Luskin et al., 1988; Price and Thurlow, 1989; Galileo et al., 1990. (3) Jacobson and Hirose, 1978; Moody and Jacobson, 1983; Jacobson and Moody, 1984; Gimlich and Cooke, 1983.

Origin of oligodendrocytes in the mature brain:

Studies concerning the origin of oligodendrocytes in the mature brain have been controversial. Myelination is essential for the proper function and maintenance of neurons, not only in the developing brain but also in the adult brain, as described in the preceding section. Destruction of the myelin sheath leads to a conduction deficit in affected neurons. The myelin sheath is impaired in many demyelinating diseases such as multiple sclerosis, and causes severe disruption of nervous system function (De Groot and Woodroofe, 2001; Lassmann, 2001). Prolonged myelin damage can also potentially result in axonal degeneration and therefore induce a variety of neurological disabilities in adults (Raine, 1997). The remyelination process, in contrast, repairs myelin sheaths and restores demyelinated axons. Only a limited remyelination occurs in multiple sclerosis lesions (Prineas et al., 1993; Raine and Wu, 1993; Bruck et al., 2003) but is typically insufficient to prevent long-term neurological disability. In demyelinating disease lesions, oligodendrocytes are lost either by apoptosis or by necrosis and never contribute to remyelination of axons (Keirstead and Blakemore, 1997; Carroll et al., 1998; Cenci Di Bello et al., 1999; Redwine and Armstrong,1998). A similar demyelination process is observed during acute nerve injury and in animal models of

demyelinating disease. In acute demyelination, endogenous glial progenitors or transplanted myelinating cells are supplied to the lesion area to promote functional recovery (Jeffery et al., 1999; Chari et al., 2002; Blakemore et al., 2002). Previous studies have reported that OPCs marked by NG2 and PDGFR α are activated in response to demyelination and are recruited to the demyelinated lesions (Redwine and Armstrong, 1998; Cenci Di Bello et al., 1999; Chang et al., 2000; Reynolds et al., 2001; Sim et al., 2002), and that Olig2 is strongly upregulated in OPCs in many pathological conditions including acute and chronic brain damage and demyelinated lesions in the adult CNS (Fancy et al., 2004; Watanabe et al., 2004; Buffo et al., 2005; Talbott et al., 2005; Tatsumi et al., 2008). Indeed, Olig2-expressing OPCs were originally regarded as a specific precursor population for oligodendrocytes in development (Zhou et al., 2000, 2001; Lu et al., 2002; Takebayashi et al., 2002; see also Fig. 5, and Table 1), but recent studies have revealed their wider potential to differentiate not only into oligodendrocytes, but also into astrocytes and even into neurons in acute injury-induced lesion of the adult brain (Buffo et al., 2005; Tatsumi et al., 2005; Marshall et al., 2005; Furusho et al., 2006; Magnus et al., 2007). Although these studies suggest that OPCs expressing NG2 and Olig2 in the adult brain may be effective in repairing the damaged CNS, the long-term fate of these OPCs in demyelinated lesions of the adult CNS has not

been clarified. One of the reasons for the lack of exact lineage tracing for Olig2-expressing OPCs is that Olig2 protein disappears rapidly from differentiated cells, and a method for stably labeling Olig2 progenitor cells has not yet been established.

Methodology to define glial lineage in the present study :

An appropriate approach is essential for identification of a progenitor and its progeny. Current approaches have been greatly facilitated by the availability of cell-specific markers that can be used to identify progenitors, possible intermediate forms, and terminally differentiated cell types (Table 1; Fig. 5), as well as the availability of different transgenic animals. Table 1 and figure 5 show some of the currently available neuroglial cell-specific markers.

Since the multitude of lineages suggested for Olig2-expressing OPCs highlights the need for appropriate fate mapping techniques to elucidate their lineage in the adult demyelinated brain lesion, I have taken advantage of recent advances involving a technique with which it has now become possible, by tamoxifen-inducible Cre-mediated recombination targeted to adult progenitors, to follow progeny arising from Olig2-expressing OPCs in the adult brain (Novak et al., 2000; Masahira et al., 2006). Briefly, to achieve long-term lineage tracing in vivo of Olig2-positive progenitor cells, I have employed double-transgenic mice which express tamoxifen-inducible Cre-ER under the control of the Olig2 promoter, together with the ROSA-EGFP reporter (Fig.

6）. Demyelination was induced in these transgenic mice by feeding them cuprizone, a copper chelator. Cuprizone exposure can induce significant myelin loss in the corpus callosum. By injecting tamoxifen during the second week of cuprizone exposure, activated Olig2-positive cells in this early demyelination phase became labelled with a long-term lineage tracing marker, GFP, and I was able to observe that these Olig2-marked GFP-positive Olig2 lineage cells showed proliferative activity. The majority of these progenitor cells showed colocalization with NG2, which is a marker for pro-oligodendrocytes (Fig. 5).

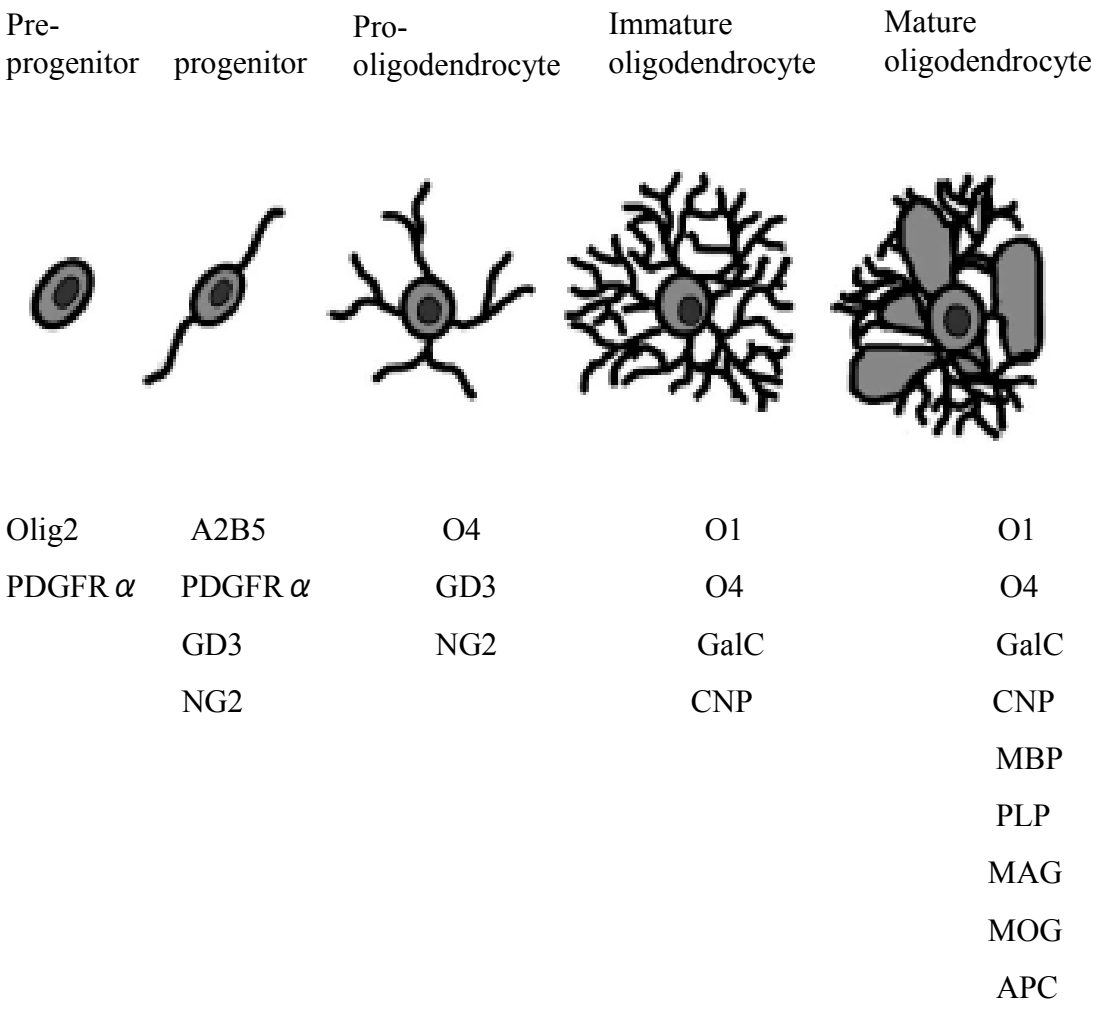

Fig. 5. The oligodendroglial lineage and its specific marker (source:Stangel and Hartung, 2002).

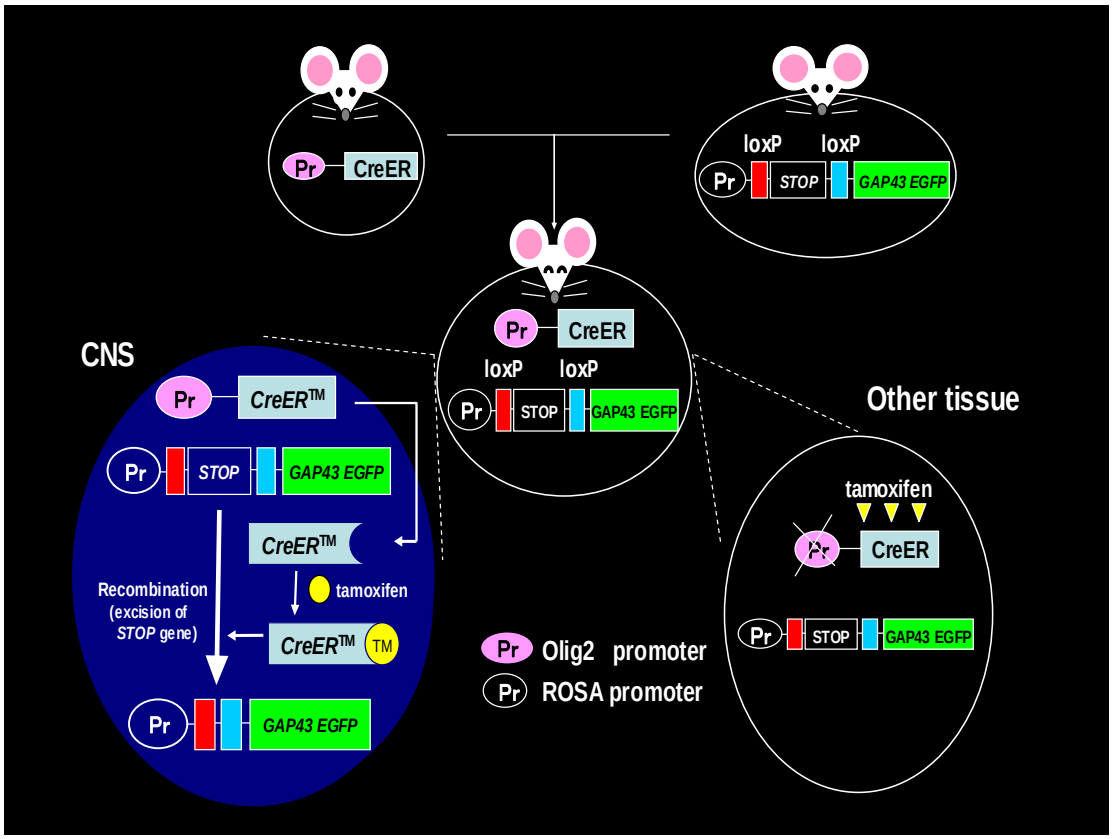

Fig.6. Construction of a double transgenic mouse strain for lineage tracing. Transgenic mice that express tamoxifen–inducible Cre-ER in oligodendrocyte progenitors under the control of the Olig2 promoter were crossed with a ROSA-EGFP reporter line. This double transgenic mouse line enabled a lineage tracing experiment of genetically labeled Olig2-expressing OPC by EGFP *in vivo* after tamoxifen treatment.The ROSA-GAP43-EGFP reporter mice were provided by Dr. Martin Goulding, and Olig2 knock-in mice (Olig2^{KICreER}) were provided by Drs. Hirohide Takebayashi and Kuzuhiro Ikenaka.

Experimental procedures

Animals:

Olig2 knock-in mice ($Olig2^{KICreER}$) and the ROSA-GAP43-EGFP reporter line have been described previously (Takebayashi et al., 2002; Masahira et al., 2006; Tatsumi et al., 2008). Heterozygous $Olig2^{KICreER/WT}$ mice are normal and were crossed with the reporter line to obtain $Olig2^{KICreER/WT}$: ROSA-GAP43-EGFP mice. Genotypes were determined by genomic polymerase chain reaction (PCR), as described previously (Takebayashi et al., 2002; Masahira et al., 2006). All animal protocols were approved by the Animal Care Committee of Nara Medical University in accordance with the policies established in the NIH Guide for the Care and Use of Laboratory Animals.

Cuprizone, tamoxifen and BrdU treatment:

Young adult mice (eight weeks old) were fed ad libitum with 0.2% cuprizone (Oxalic bis cyclohexylidenehydrazide; Sigma-Aldrich) mixed into pellet chow. Mice were exposed to continuous cuprizone feeding for two or six weeks (Fig. 7) until perfusion. Untreated control mice were fed with normal pellet chow.

Tamoxifen treatment methods have been described previously (Danielian et al., 1998; Masahira et al., 2006). Briefly, to activate Cre-ER, adult mice were injected intraperitoneally with 1.5 mg of 4-hydroxytamoxifen (Sigma) dissolved in a dimethyl sulphoxide/ethanol/sesame oil (4:6:90) mixture at a concentration of 10 mg/ml. To label cells whose Olig2 promoter was activated during cuprizone-induced demyelination events, injections of TM were performed for five consecutive days from the 1st to the 5th day of the second week of cuprizone feeding (Fig. 7). To check the proliferative activity of Olig2 cells in response to 2 weeks of cuprizone-induced early demyelination, mice received 1mg/ml BrdU in water ad libitum for 2 weeks (until perfusion, Fig. 7).

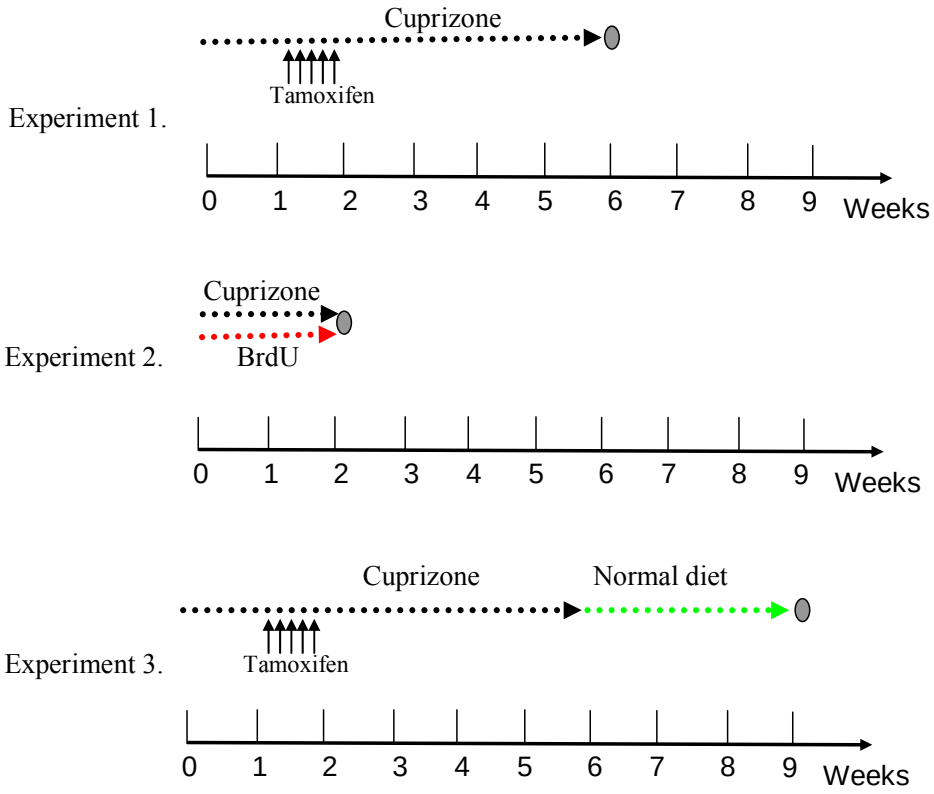

Fig. 7. Schedule of animal treatment has been depicted. Eight-week-old double transgenic mice were used in each experiment.

Experiment 1. Mice were exposed to 0.2% cuprizone for six weeks (black dotted line) and were injected with 1.5 mg tamoxifen once per day (arrows, 24 hours apart) for five consecutive days after seven days of cuprizone treatment and were sacrificed (gray oval).

Experiment 2. Mice were fed cuprizone and simultaneously received BrdU water (red dotted line) ad libitum for 2 weeks and were sacrificed (gray oval).

Experiment 3. Mice were treated as in experiment 1, except that these mice were returned to normal diet for 3 weeks after 6 weeks of cuprizone treatment (green dotted line) and were sacrificed (gray oval).

For control mice with the same genetic background and age, all the treatments were the same except that they were fed normal diet throughout the experiment.

Tissue preparation and immunohistochemistry:

Mice were deeply anesthetized with an intraperitoneal injection of 10% ethyl-carbamate (10 μl/g body weight) and were perfused transcardially with 10 ml saline followed by 4% paraformaldehyde in phosphate-buffered saline (PBS). Following perfusion, mice were decapitated and brains were removed from the skull as soon as possible, and were postfixed overnight in the same fixative solutions at 4℃. The brains were cryoprotected in 30% sucrose-PBS solution to prevent freezing artifacts, snap-frozen with powdered dry ice, and stored at -80℃ until use. Frozen brain sections were cut at 20-μm thickness between bregma 0.98mm and -1.82mm using a cryostat vibrating microtome (Bright Instruments, Huntingdon, England). All histological procedures were performed as described previously (Tatsumi et al., 2005). Briefly, immunohistochemistry of Olig2, GFP and ssDNA by the diaminobenzidine staining method was as follows: brain sections were washed in Saline contained 0.1M PB 3 times at room temperature for 10 minutes. The sections were pre-incubated with 5% bovine serum albumin-PBS for 1h at room temperature, and then incubated with respective primary antibody separately for 2 over nights. Following incubation, sections were washed and incubated with respective biotinylated secondary antibody. To

inactivate endogenous peroxidase activity, the sections were incubated in PBS containing 3% H_2O_2 for 30 min at room temperature. After several washes, bound antibody was detected by staining with ABC vectastatin kit (Vector Laboratory, USA) for 2 hr at room temperature with a subsequent staining with 0.05% 3,3-diaminobenzidine tetrahydrochlodire in 50 mM Tris-HCl, pH 7.5, containing 0.01% H_2O_2 for 5 min. Sections were then washed, dried, dehydrated and covered with Entellan (Merck, Germany). For double and triple-labelling with immunofluorescence antibody in which BrdU staining was a common component, staining was performed by simultaneous incubation of relevant primary antibodies alone (double labeling) or together (triple labeling) as stated above. Following several washes, sections were incubated with relevant secondary antibody conjugated with Alexa-488 or -546. In all cases, BrdU staining was done last, as follows. After immunohistochemical detection of other partners, sectiones were denatured with 2N HCl for 15 min at 37℃、neutralized with 0.1M sodium borate for 10 min, and then washed with PBS. Successive treatments were then done with relevant primary and secondary antibodies. BrdU was labeled with either Alexa 546- or Alexa 633- conjugated secondary antibody. Sections were then covered with cover-slips in anti fade mount solution (Molecular Probes). Antibodies and dilutions used were as follows: anti-GFP (1:5000, rabbit polyclonal, Invitrogen; 1:1000,

rat monoclonal IgG2a, Nacalai Tesque); anti-Olig2 (1:200, rabbit polyclonal, IBL); anti-glial fibrillary acidic protein (GFAP) (1:10,000, rabbit polyclonal, DAKO; 1:500, mouse monoclonal IgG1, Chemicon); anti-NG2 chondroitin sulfate proteoglycan (1:100, rabbit polyclonal, Chemicon); anti-adenomatous polyposis coli (APC) (1:400, mouse monoclonal IgG2b, Calbiochem); anti-Iba1 (1:200, rabbit polyclonal, Wako); anti-myelin basic protein (MBP) (1:400; rabbit), and anti-neuronal nuclei (NeuN) (1:100, mouse monoclonal IgG1, Chemicon). To assay for cell death, anti- ssDNA antibody (1:1000, rabbit polyclonal, DAKO) was used. Goat anti-rabbit, anti-rat and anti-mouse secondary antibodies conjugated to Alexa-488 or Alexa-546 or Alexa-633(1:1000, Invitrogen) were used for double-immunofluorescence staining. A light and confocal laser scanning microscope (Olympus Fluoview FV1000) was used for histological analysis.

To determine the extent of cuprizone-induced demyelination, sections were processed for Sudan Black B (Wako) staining (Fig. 8). Briefly, sections were incubated with 0.01% Sudan Black B solution in 70% ethanol at 37℃ for 40 min, rinsed briefly in 70% ethanol and washed in de-ionized water, and finally covered with 50% glycerine in PBS. In addition, demyelination was also assessed by immunohistochemistry using anti-MBP.

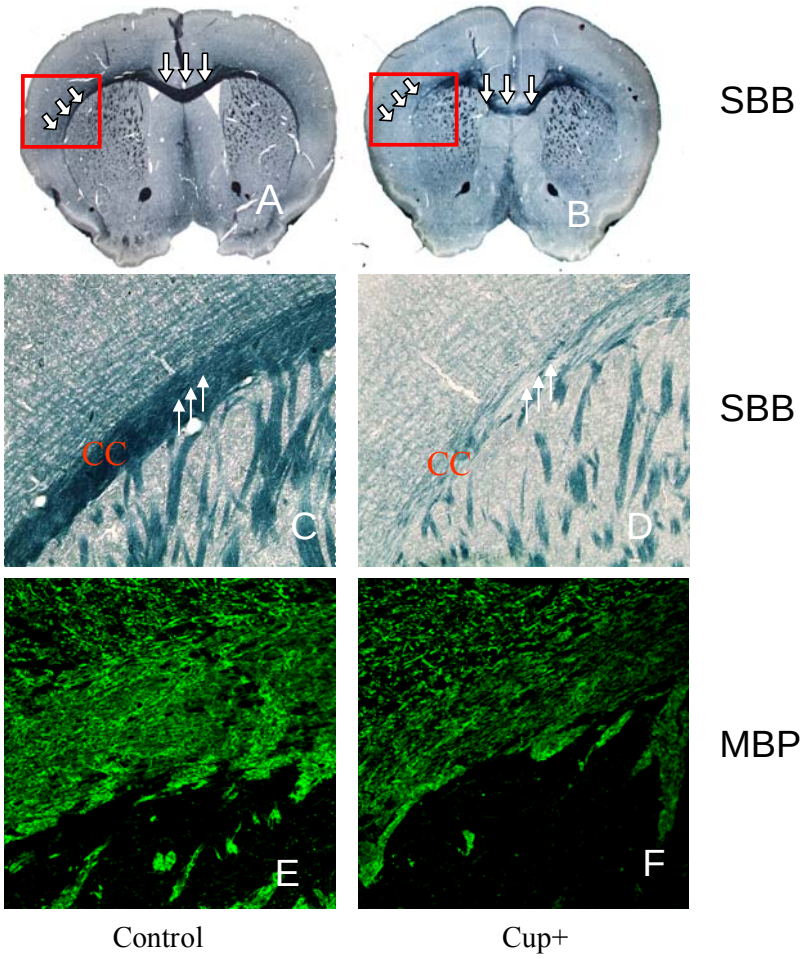

Fig. 8. Cuprizone treatment induced extensive demyelination. Coronal brain sections were stained with Sudan Black B (SBB) to assess the myelin level in mice treated with cuprizone for 6 weeks (B) and in the control group (A). C, D: Higher magnification of the red-framed areas in A and B, respectively. Huge myelin loss (demyelination) was observed in the corpus callosum of the cuprizone-treated mice (Cup+, arrows in B, D) relative to control mice (A, C). Demyelination was also observed when sections were processed for myelin basic protein (MBP) in cuprizone-treated mice (F) compared to controls (E). CC: Corpus callosum.

Cell counting:

To monitor cellular activity in demyelinated lesions induced by cuprizone intoxication, the number of Olig2 and GFP cells in the corpus callosum to the external capsule was counted in randomly selected areas. The number of active proliferative cells (double-positive for Olig2 and BrdU) in demyelinated lesions was also counted in the same way in the above-mentioned area. To characterize the differentiation of Olig2-positive progenitors in demyelinated and remyelinated lesions, cells that were double-positive for a cell marker and GFP in the corpus callosum were counted after six weeks' cuprizone exposure and after 3 weeks of recovery on normal diet. Care was taken to minimize counting bias; serial sections between bregma 0.98 mm and -1.82 mm were cut and picked up at regular intervals, and then subjected to double-labelling immunohistochemistry. In the picked-up sections, we defined lateral borders of the cell-counting area of the corpus callosum as being at 3.0 mm from the midline. This defined area was reproducibly affected by the cuprizone treatment (Fig. 8). One-way ANOVA was used to analyze significant statistical differences between the two groups. Values are expressed as mean ±S.E.M.

At least three brains were used in each experiment group unless otherwise stated.

Results

Assessment of demyelination induced by cuprizone:

Cuprizone induces recurrent demyelination and remyelination in the CNS (Johnson and Ludwin, 1981) and has been established as an experimental tool in demyelinating animal models (Hiremath et al., 1998; Mason et al., 2001a; Matsushima and Morell, 2001; Armstrong et al., 2002, 2006; Song et al., 2005). I used the cuprizone model to take advantage of the ability to stop active demyelination simply by returning the mice to a normal diet. In my experiments, when coronal brain sections were stained with Sudan Black B, I found that mice fed with chow containing 0.2% cuprizone for six weeks showed extensive demyelination in the major white matter tract from the corpus callosum (CC) to the external capsule (EC) (Fig 7, experiment. 1; Fig. 8, B, D, arrows), compared to control mice fed with normal diet (fig. 8, A, B). The same result was observed when sections were immunostaind for MBP, a widely used marker for myelin (Fig. 8, E, F). These results are thus consisted with previous studies that showed acute and extensive demyelination by cuprizone intoxication (Hiremath et al., 1998; Mason et al., 2001a; Matsushima and Morell, 2001; Armstrong et al., 2002, 2006; Song et al., 2005).

Olig2-positive cell numbers increase in demyelinated lesions induced by cuprizone treatment of mice:

Olig2-positive cells increase in both acute and chronic injury of the CNS (Fancy et al., 2004; Buffo et al., 2005; Talbott et al., 2005; Tatsumi et al., 2008). I was therefore curious to see whether Olig2-positive cells also increase in response to cuprizone-induced demyelinated lesions. The CC nad EC of mice in which early demyelination was induced by 2 weeks of cuprizone treatment and then immediately sacrificed, showed apparently more Olig2-positive cells in the demyelinated area than were present in the same area of control mice (Fig. 9, A, B, C, D, arrows; Fig. 1, experiment 2). The same results were obtained when Olig2-positive cells were labeled with fluorescence-conjugated antibody and observed under a fluorescence microscope (Fig. 9, E, F). I counted the Olig2-positive cells per area in the demyelinated lesion in the EC and found approximately 120 Olig2-positive cells per area in the demyelinated region of the cuprizone-treated mice group. In contrast, approximately 60 cells were found per area in the same region of control mice and the difference between these two groups was statistically significant (Fig.10, A; p=0.002). I also checked the extent of cell death in adjacent sections that occurs by cuprizone intoxication at this time point. There were very few dead cells in either group and there was no significant difference in the

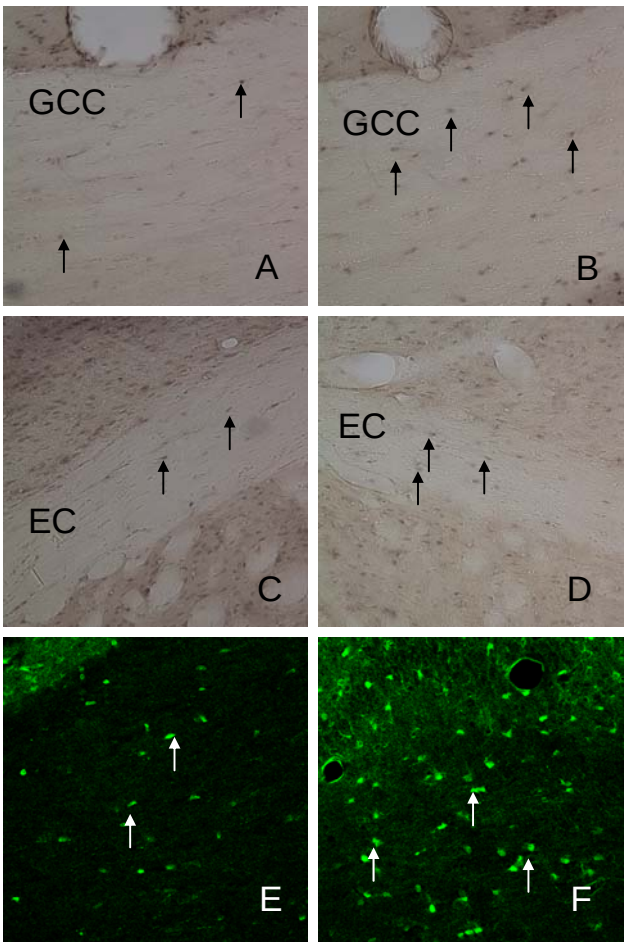

Fig. 9. Immunoreactivity of Olig2 cells in corpus callosum after 2 weeks of cuprizone treatment. Olig2 immunoreactivity was detected by anti-Olig2 antibodies using the DAB method (A-D). Olig2-positive cells also showed green fluorescence when was detected by Alexa 488-conjugated anti-Olig2 antibody (E, F). Note that apparently higher number of Olig2 immunoreactive cells were found in the cuprizone treated-group (Cup+; B, D, F) than in the controls (A, C, E). GCC: Genu of corpus callosum, EC: External capsule. Arrows show Olig2-positive cells.

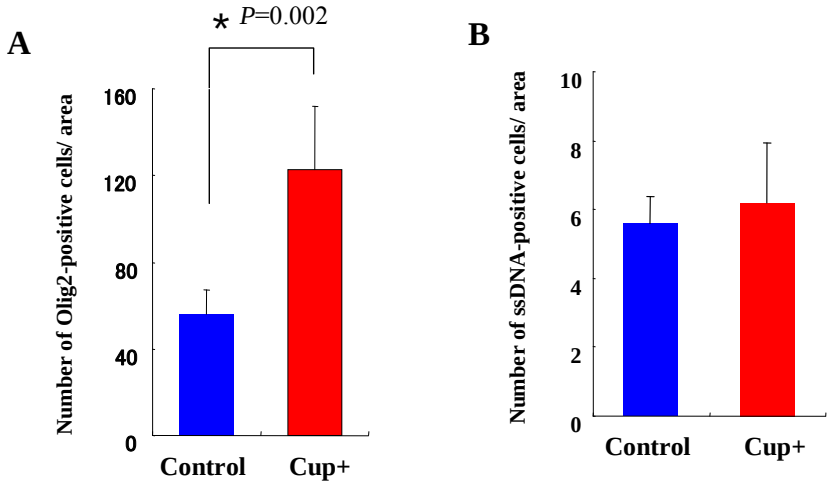

Fig. 10. Olig2 cells increased in response to cuprizone treatment for 2 weeks. A, graph showing the number of Olig2-positive cells per area after 2 weeks of cuprizone treatment. Significantly higher numbers of Olig2-positive cells were found in cuprizone-treated (Cu+) mice than in controls (*P**= 0.002). B, after 2 weeks of cuprizone treatment, very few apoptotic cells were detected per area by anti-ssDNA antibody, and there was no significant difference in the number of apoptotic cells between the two groups (*P**= 0.8).

number of dead cells between the treated and control groups (Fig. 10, B). Since a significant difference was found in the number of Olig2-positive cells between the groups, I then checked how the number of Olig2-positive cells increased: whether by active proliferation or simply by up-regulation of Olig2 protein by resident dormant Olig2-positive cells in response to cuprizone treatment. For this purpose, I examined the proliferative activity of Olig2-positive cells by labelling proliferating cells with BrdU (Fig. 7, experiment 2). After two weeks of cuprizone and BrdU treatment, mice were sacrificed and brain sections were processed for double immunostaining with anti-Olig2 and -BrdU antibodies. BrdU-labelled Olig2-positive cells were observed in the demyelinated white matter area of both cuprizone-treated and control mice (Fig.11, A-F). Double-labeled cells were counted in the same region of the demyelinated area. My quantitative analysis showed that ~75% of the Olig2-positive cells in cuprizone-treated mice were labelled with BrdU (Table 2, Fig 12). On the other hand, ~56% of the Olig2-positive cells were labeled with BrdU in the control group (Table 2, Fig. 12). The proportion of BrdU-labeled Olig2-positive cells was significantly higher in cuprizone-treated mice than in control mice (Fig. 12; $p = 0.001$). These results indicate that proliferation of Olig2-positive cells was stimulated by cuprizone treatment in the adult brain, and that the cells contributing to this increment arose by direct proliferation of resident

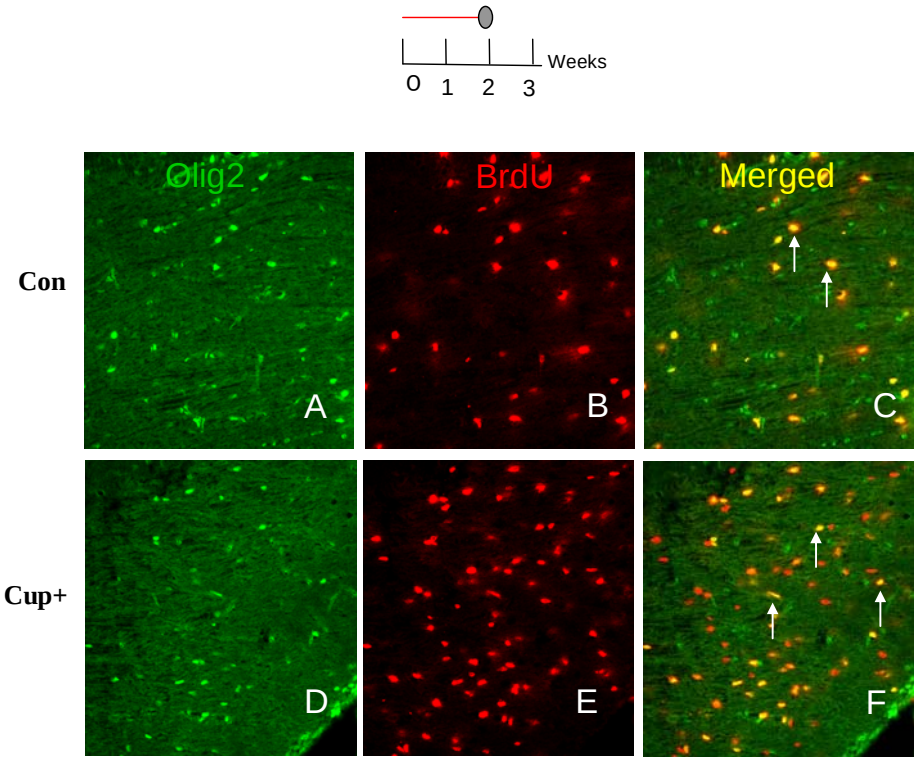

Fig. 11. Increased proliferative activity of Olig2 positive cells after cuprizone treatment for 2 weeks. Confocal images of cells, which were double-stained for Olig2 and BrdU are shown. Olig2-positive cells exhibited green fluorescence (Alexa 488) and BrdU immunoreactivity exhibited red fluorescence (Alexa 546). White arrows (C, F) indicate some of the double-positive cells (Olig2-positive cells that were also positive for BrdU). Upper diagram shows the BrdU treatment for 2 weeks (red line) of both control (Con) and cuprizone-treated (Cu+) mice groups.

Control	56.42%±3.2 (932/1739)
Cup+	74.53%±1.8 (763/1001)

Table 2. Percentage of Olig2-positive cells that co-expressed BrdU. Data are expressed as mean ±SEM. Cup+: Cuprizone-treated group.

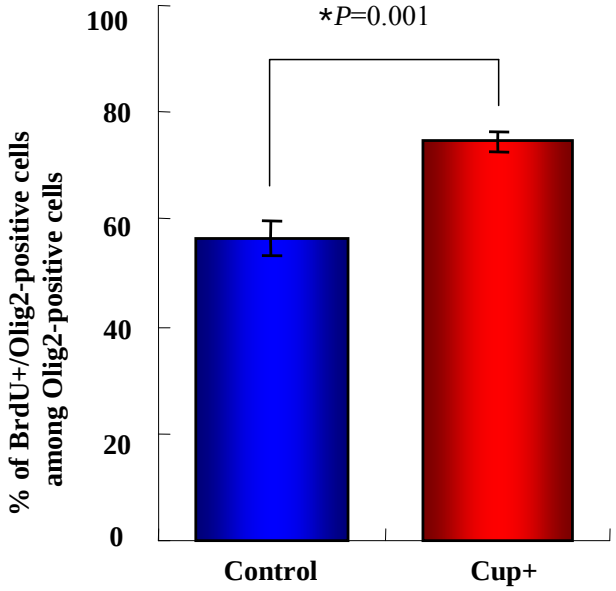

Fig. 12. Increased proliferative activity of Olig2 cells in cuprizone treated-mice group. Percentage of Olig2-positive cells which showed colocalization with BrdU indicated. 56.4%± 3.2 and 74.5% ±1.8 of Olig2-labeled cells showed colocalization with BrdU in control and cuprizone treated mice (Cup+) groups, respectively. Note the significant increase of BrdU/ Olig2 double-labeled cells in the cuprizone treated mice group (*P** =0.001). Data are expressed as mean±SEM.

(local) dormant or migrated (i.e., from the subventricular zone) Olig2-positive cells.

In my experiment, GFP expression was under direct control of the Olig2 promoter and recombination was induced by tamoxifen treatment. I assumed that an Olig2 cell that was activated by early cuprizone intoxication (2 weeks of cuprizone treatment, Fig. 7, experiment 2), undergoes further proliferation in response to 4 more weeks of continuous cuprizone intoxication (Fig. 7, experiment 1), and therefore that the number of Olig2-marked GFP-positive cells will increase. In fact, Olig2-marked GFP-positive cells were apparently more abundant in the white matter tract after 6 weeks of continuous cuprizone-induced demyelination than they were at this location in the control group (Fig 13, A, B). Quantitative analysis showed that ~ 2.5- to 3- fold Olig2-marked GFP-positive cells were found per area in cuprizone-induced demyelinated lesions (Fig. 13, C). Although GFP expression is under Olig2 control in this experiment, and Olig2 expression is very transient in the cell, some cells showed Olig2 and GFP expression simultaneously even at the end of the 6^{th} week (Fig. 14, A) indicating that these cells had not yet acquired their final phenotype. Some cells even showed colocalization for GFP, BrdU and APC (a marker for mature oligodendrocytes) at this time, which indicates that proliferation takes place in Olig2-marked GFP-labeled cells and that these cells are destined to become oligodendrocytic lineage cells (Fig. 14, B).

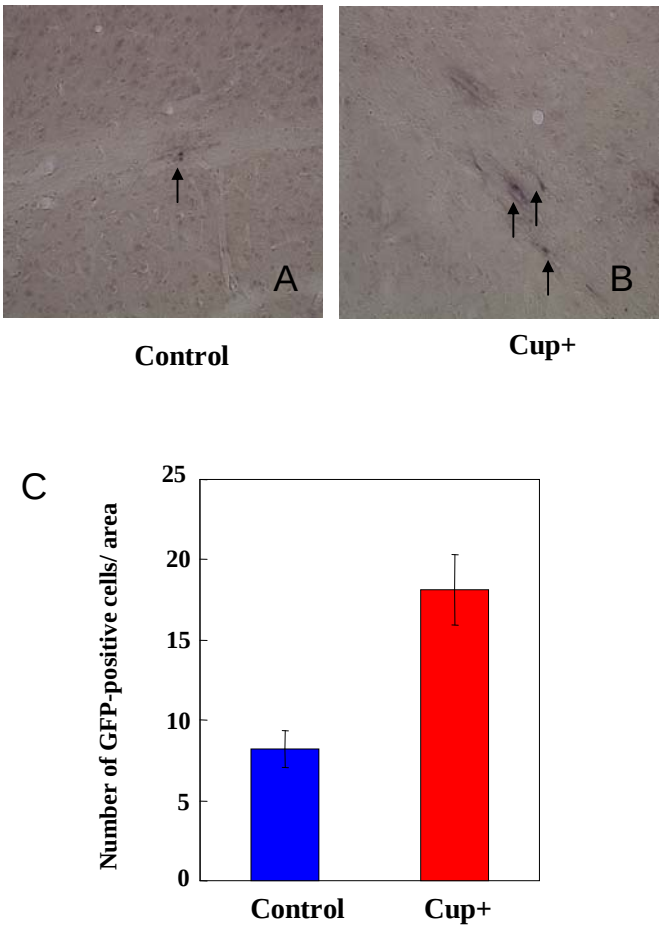

Fig 13. Olig2-marked GFP-positive cells increased in response to 6 weeks of cuprizone treatment. A, B: GFP-positive cells in corpus callosum (arrows). Approximately 8 and 18 cells were found per area in control and cuprizone treated (Cup+) mice, respectively. Note that the number of GFP-positive cells was more than double in cuprizone-treated mice compared to control mice (C).

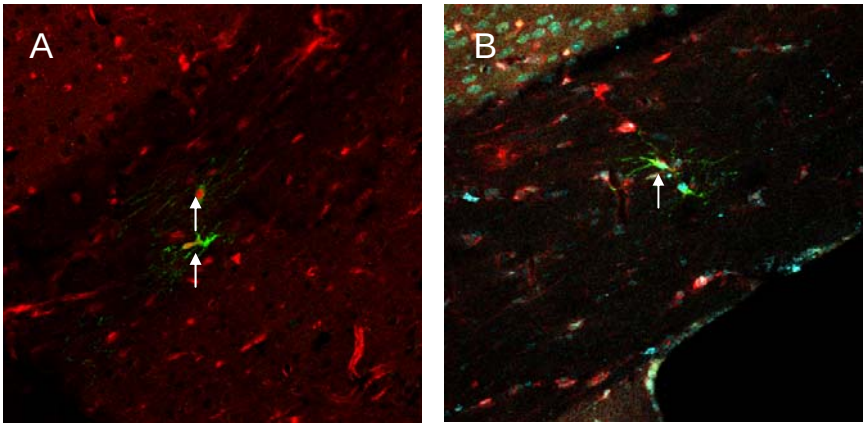

Fig 14. Confocal images of cells have been shown. Double labeled cell showing colocalization for GFP (green) and Olig2 (red), arrows in A. Triple labeled cell showing colocalization for GFP (green), adenomatous polyposis coli (APC, red) and BrdU (blue), arrow in B.

Fate determination of activated Olig2-positive cells in the adult brain corpus callosum:

Since an increased number of proliferative Olig2 cells was found in the early demyelinated lesion after 2 weeks of cuprizone exposure (Figs. 11 and 12; Fig. 7, experiment 2), I next traced the exact cellular fate of these proliferative cells in the demyelinated lesion at a later time point when massive demyelination was observed (Fig. 8; Fig.7, exp.1). I characterized the fate of Olig2-marked GFP-positive cells after six weeks of cuprizone exposure, using the inducible Cre/LoxP system to target the Olig2 locus (Fig. 7, experiment 1). Tamoxifen was administered during the second week of cuprizone exposure, permitting Olig2-activated cells to be labeled with GFP. Although less than 10% of the Olig2-positive cells expressed GFP under these conditions in ROSA-GAP43-EGFP reporter mice, GFP expression was stable for up to one year (K. Tatsumi, unpublished observation). Olig2-positive cells were examined by double-staining with anti-GFP antibody and antibodies to the cell markers NG2, APC, GFAP (for astrocytes), NeuN (for mature neurons), and Iba1 (for microglia) (Fig. 15, A-E). Many Olig2-marked GFP-positive cells showed NG2 immunoreactivity in both control and cuprizone-treated mice (Cup+). Many GFP-positive cells showed colocalization for NG2, APC and GFAP in the corpus callosum area (Fig. 15, A-C, arrows).

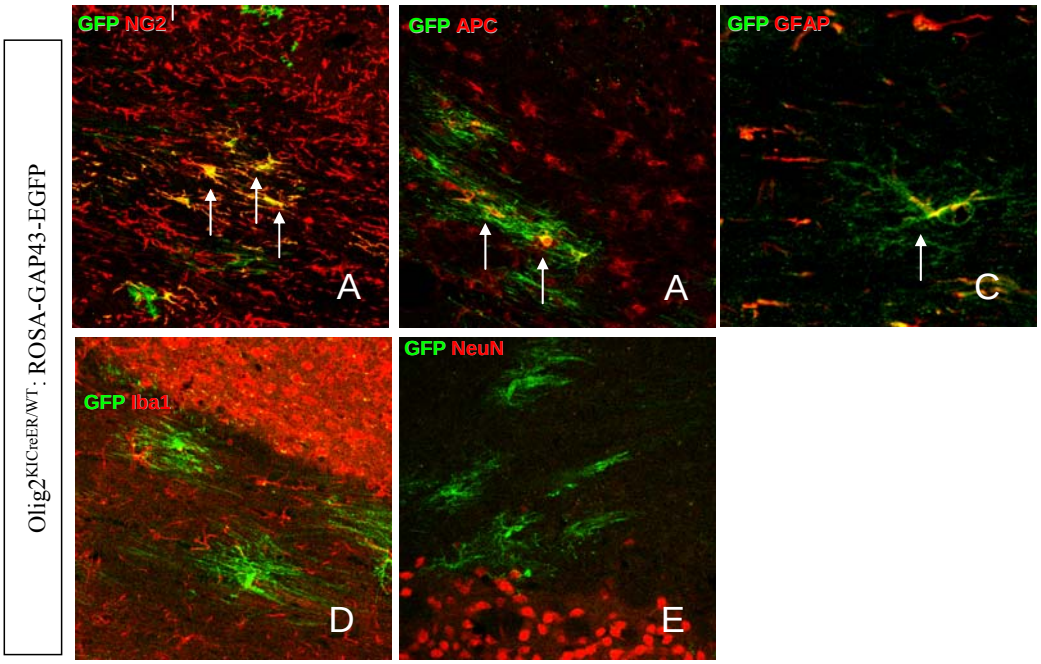

Fig. 15. Confocal images of cells, processed for double-labeling with GFP and cell markers NG2, APC, GFAP, Iba1, and NeuN. GFP-labeled cells showed green fluorescence and cell markers showed red fluorescence. Double labeling cells were observed only for GFP and NG2, APC, GFAP (arrows in A, B, C). Note that no double-labeled cells were found for GFP and Iba1 or NeuN (D, E).

Quantitative analysis showed that 67% of the Olig2-marked GFP-positive cells co-expressed NG2 in the cuprizone-treated group, compared with 55% in the control group (Table 3, Fig. 16). Sub-populations of the Olig2-marked GFP-positive cells also showed APC co-expression in cuprizone-treated (38%) and in control (25%) mice (Table 3, Fig. 16). In contrast, 13% and 14% of the Olig2-marked GFP-positive cells showed co-localization with the astrocytic marker GFAP in cuprizone-treated and control mice, respectively (Table 3, Fig. 16). Statistically significant differences in double colocalization for GFP and cell markers were found only in the cases of NG2 and APC (Fig. 16, p=0.004 and p=0.003, respectively) in the two different treatments. Microglial or neuronal differentiation of the Olig2-marked GFP-positive cells, tested for by second-labelling them with Iba-1 or NeuN, was never observed (Fig. 15, D-E; Table 3).

I was then interested to see what terminal fate the activated Olig2-positive cells would take if the cuprizone intoxication was terminated. For this purpose, mice were returned to normal diet for 3 weeks after 6 weeks of cuprizone treatment (Fig. 7, experiment 3). The mice were then sacrificed and processed for Sudan Black B staining to assess myelin level in this treatment, and for double labeling for Olig2-marked GFP and other cell markers (NG2, APC, GFAP, Iba-1 and NeuN) to detect cell fate.

	NG2	APC	GFAP	Iba1	NeuN
Control	55.28%±5.6 (129/229)	24.92%±4.5 (67/260)	14.44%±2.3 (44/289)	0% (0/86)	0% (0/119)
Cup+	67.38%±3.2 (463/700)	37.88%±4.0 (302/780)	13.21%±2.8 (94/705)	0% (0/143)	0% (0/212)

Table 3. Percentage of GFP positive cells that expressed the indicated phenotypic markers. Data are expressed as mean±SEM. Cup+: Cuprizone treated group.

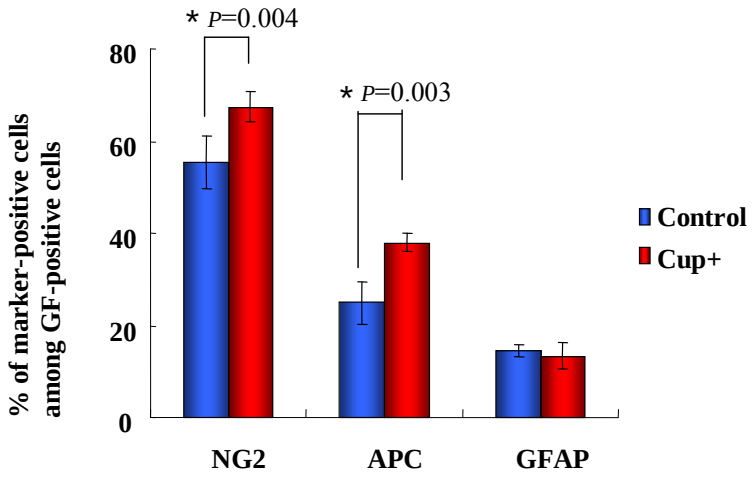

Fig 16. OPCs preferentially differentiated into oligodendrocytes in mice treated for 6 weeks with cuprizone. Percentage of double positive cells (GFP-positive cells that were also positive for the cell marker) of total GFP-positive cells are indicated. 55.3%± 5.6 and 67.4% ±3.2 of GFP-labeled cells colocalized with NG2 in control and cuprizone-treated mice, respectively. Double positive for APC was 24.9%±4.5 , 37.9%±4.0 and for GFAP was 14.4%±2..3, 13.2%±2.8 in control and cuprizone-treated groups, respectively. Note the significant increase ofNG2/GFP and APC/GFP double-labeled cells in cuprizone-treated (Cup+) mice group (*P**= 0.004 and 0.003 respectively). Data are expressed as mean±SEM.

When cuprizone intoxication was terminated for 3 weeks after 6 weeks of cuprizone treatment, myelin was restored to almost its normal level in the corpus callosum (Fig. 17, D), comparable to levels in the 6-week control or 9-week control group (Fig. 17; A, C, respectively), while huge myelin loss was observed after 6 weeks of cuprizone treatment (Fig. 17, B). Cell marker colocalization with Olig2-labeled GFP occurred only in the cases of NG2 and APC; no other markers showed colocalization with GFP in this experimental treatment (Fig. 18, Table 4, Fig. 19), although colocalization of GFP and GFAP was observed in the 6-week exposure experiment (Fig. 15, C). Quantitative analysis showed that 64% of Olig2-marked GFP-positive cells co-expressed NG2 in the 3-weeks recovery group, and 48% did so in the 9-week control group (Table 4, Fig 19). Although a higher percentage of double-labeled cells was observed in the 3-week recovery group than in the controls, the difference was not statistically significant. Sub-populations of the Olig2-marked GFP-positive cells also showed expression of APC: 45% and 21% in the 3-weeks recovery group and the 9-weeks control group, respectively (Table 4, Fig. 19), a statisticallysignificant difference (p= 0.001). At all time points tested, most of the progeny of Olig2-expressing progenitors showed mature myelinating oligodendrocytic characteristics, colocalizing with MBP (Fig. 20).

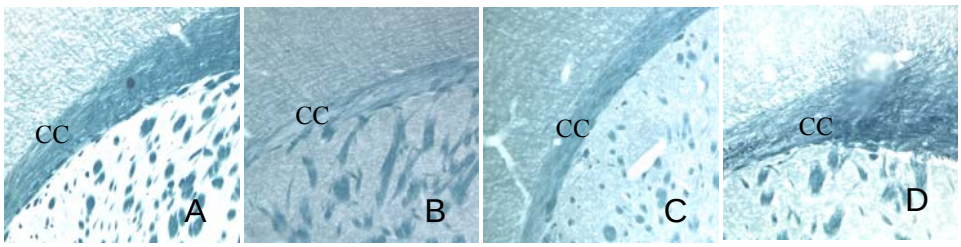

Fig. 17. Three-week recovery on normal diet after 6 weeks of cuprizone treatment yielded complete remyelination in the demyelinated corpus callosum (CC). Coronal brain sections were stained by Sudan Black B. A, 6-week normal control (6 wk con) CC; B, 6-week cuprizone-induced (6 wk Cup+) demyelination at CC; C, 9-week normal control (9 wk con) CC; and D, 6-week cuprizone treatment and 3-week recovery on normal diet (6 wk Cup+ + 3 wk recovery) CC. Note that CC in B showed complete demyelination, whereas that in D showed complete remyelination after 3 weeks of recovery on normal diet.

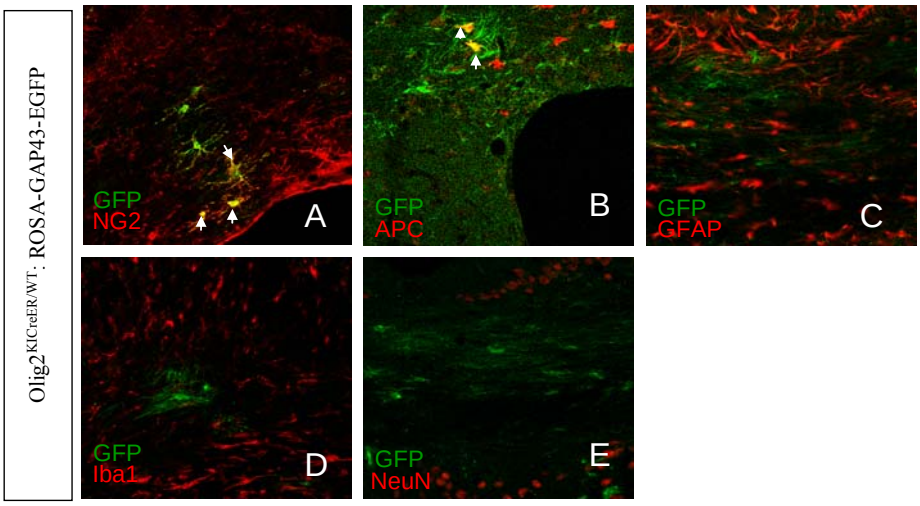

Fig. 18. Fate of OPC after restoring mice on cuprizone-free diet for 3 weeks. Confocal images of cells processed for double-labeling with GFP and cell markers NG2, APC, GFAP, Iba1, NeuN. GFP-labeled cells showed green fluorescence and cell markers showed red fluorescence. Double-labeled cells were observed only for GFP, and NG2, APC (arrows in A and B). Note that no double-labeled cells were found for GFP, and GFAP, Iba1, NeuN (C-E).

	NG2	APC	GFAP	Iba-1	Neu-N
Control	48.39%±5.10 (97/200)	21.39%±6.35 (32/150)	0% (0/147)	0% (0/80)	0% (0/85)
Cuprizone treatment for 6 weeks and then 3 weeks on normal diet	63.93%±3.80 (141/220)	42.81%±6.50 (75/175)	0% (0/165)	0% (0/97)	0% (0/93)

Table 4. Percentage of GFP-positive cells that expressed the indicated phenotypic markers after restoring mice on cuprizone-free diet for 3 weeks. Data are expressed as mean±SEM.

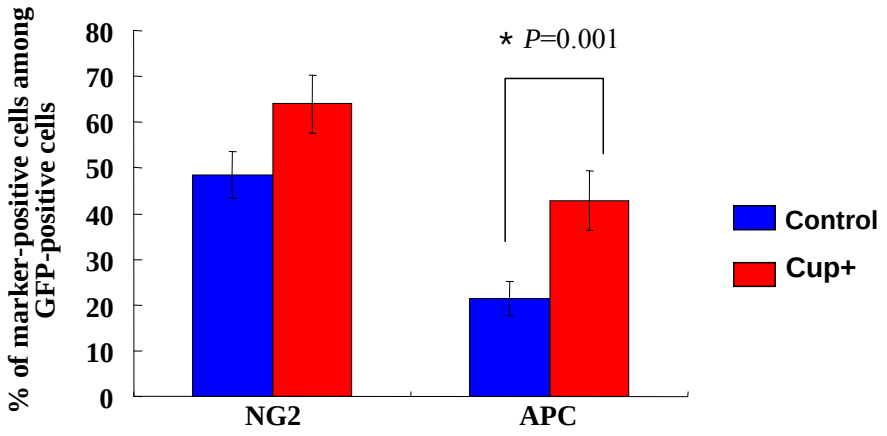

Fig. 19. Percentage of double-positive cells (GFP-positive cells that were also positive for the cell marker) of total GFP-positive cells after 3 weeks of recovery to normal diet. 48.39%± 5.10 and 63.93 ±3.80 of GFP-labeled cells showed co-localized with NG2 in control and cuprizone-treated mice, respectively. Double-positive cells for APC were 21.39%±6.35 and 42.81±6.5 in control and cuprizone-treated groups, respectively. Note the significant increase of APC/GFP double-labeled cells in cuprizone-treated (Cup+) mice (*P** =0.001). Data are expressed as mean±SEM.

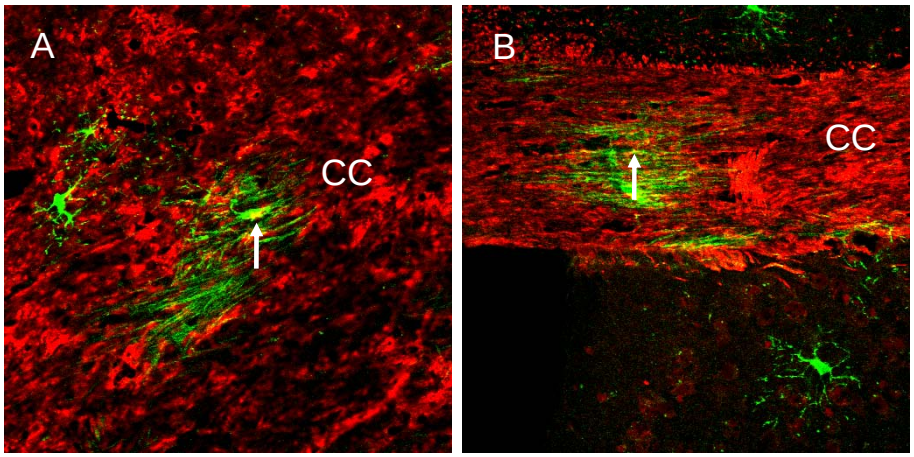

Fig. 20. Progeny of Olig2-expressing progenitors gave rise to myelinating oligodendrocytes. Confocal microscope images showing the colabeling of Olig2-marked GFP-positive cells and myelin sheaths with the myelin marker MBP in the white matter (CC). Arrows show myelin sheath provided by the progeny a nearby axon (A) and a relatively distant axon (B).

Discussion

What are the sources of new oligodendrocytes that participate in the myelination process in adult brain?

Answering this question is a fundamental target for basic neuroscience, and the solution may yield a variety of clinical approaches for treating demyelination diseases and neural injury. An earlier study using a recombinant retrovirus as a reporter gene demonstrated that the ventricular zone contains separate progenitor cells for the two glial cell types, oligodendrocyte and astrocyte. Only a very small number of OPCs, localized in the ventricular zone, contribute to corticogenesis at this early stage (embryonic day 14-16), with astrocyte clones being in the majority (Parnavelas, 1999). Injections of retrovirus at increasingly later stages, from perinatal to early postnatal periods, resulted in progressively more oligodendrocyte clones and fewer astrocyte clones, which predominantly localize in the white mater of both hemispheres and in the corpus callosum. Late postnatal injection (postnatal day 14) of the reporter into the subventricular zone resulted in generation of only oligodendrocyte clones in the white matter of both hemispheres. Parnavelas (1999) also suggested that the subventricular

zone of the postnatal brain might be a mosaic of (1) glial progenitors (OPCs) that give rise to cortical astrocytes and oligodendrocytes, (2) neuronal progenitors that produce a population of olfactory bulb neurons, (3) multipotential progenitors, and (4) a pool of stem cells. These cells have a capacity for self-renewal and may represent a source for neurons and glia in the adult brain.

It is still unclear, as discussed in the introduction section, whether adult resident (local) progenitors which localize in the corpuss callosum or subventricular zone generate new oligodendrocytes *in vivo* in the remyelinating process, although most investigators have reached the consensus that remyelinating cells in the adult brain are likely to come from these progenitors (Franklin, 2002). In agreement with this hypothesis, adult brain also expresses the early pre-progenitor marker Olig2 and a progenitor marker, NG2, as shown in the present study (Fig. 5, Table 1). In addition, Olig2-positive pre-progenitors and OPCs in the subventricular zone seem likely to contribute to adult remyelination. Goal in this study was to clarify the cellular fate of resident OPCs, particularly Olig2-expressing OPCs, in the experimental demyelination and remyelination mouse model.

Animal model of demyelination and remyelination:

Various experimental demyelination animal models have been introduced to date. These were experimental allergic encephalomyelitis (EAE, which shares many features with multiple sclerosis), infection by viruses, physical injury and exposure to a demyelinating toxic agent (Yajima and Suzuki, 1979; Rodriguez et al., 1983; Morel et al., 1998; Woodruff and Franklin, 1999; Gold et al., 2000; Marten et al., 2001). The toxin agent model, systemic delivery of cuprizone by diet, induces demyelination and, when the agent is removed from food, reverses the process to yield remyelination (Ludwin, 1980; Fig. 17, present study). This biphasic process provided by a single agent permits simultaneous investigation of both the demyelination and remyelination step. Cuprizone causes death of oligodendrocytes and results in demyelination without damaging other cells types in early events of demyelination in the CNS (Komoly et al., 1987; Fujita et al., 1990; Cammer and Zhang, 1993). This model does not involve the immune system, and T cells are almost completely absent (Bakker and Ludwin, 1987; Kondo et al., 1987). Activation of T cells might trigger complex immunological events, with production of antibodies which could prevent remyelination (Bornstein and Reine, 1970). Mice treated with 0.2% cuprizone displayed extensive demyelination in the

present study (Fig. 8; B, D, F), consistent with previous studies (Mason et al., 2001a; Matsushima and Morell, 2001; Song et al., 2005, Armstrong et al., 2006). In the early phase of this toxic demyelination (i.e., the second week of cuprizone intoxication), more than 70% of Olig2-positive cells showed active proliferation by incorporating BrdU (Figs. 11, 12 and Table 2). Although BrdU incorporation was also observed in the control group (not exposed to cuprizone), the percentage of BrdU and Olig2 double-positive cells was significantly higher in cuprizone-treated mice (Fig. 12). BrdU and Olig2-positive cells observed in the control mice may represent the progenitor population that is known to participate in the continuous turnover of oligodendrocytes in adulthood (Ffrench-Constant and Raff, 1986; Wolswijk and Noble, 1989). The significant increase in the number of BrdU and Olig2-positive cells in the cuprizone-treated mice can be considered as an endogenous response of Olig2-positive cells to pathological insult in the CNS.

Validity of the doubl-labeling study for NG2 proteoglycan and GFP, under control of the Olig2 promoter:

In the present study, I investigated the cellular responses and fates of Olig2-positive progenitor cells that were activated during the second week of cuprizone exposure and then underwent four more weeks of continuous cuprizone intoxication. To identify the differentiation (cellular fate) of these actively proliferating Olig2-positive cells in the demyelinated lesions, I used double-transgenic mice expressing tamoxifen-inducible Cre under the control of the Olig2 promoter together with a GFP reporter. This system enabled genetic and stabe labeling of cells having an activated Olig2 promoter during a desired period by means of tamoxifen injection (Robinson et al., 1991; Danielian et al., 1998). At the end of the sixth week of cuprizone treatment, the proportion of cells that were double-positive for NG2 and Olig-marked GFP was significantly higher in the cuprizone-treated groups comparing to than in the control group (Fig. 16), and this result was consistent with previous studies that reported up-regulated expression of NG2 in the injured area after various kinds of brain injury (Alonso, 2005; Tatsumi et al., 2005; Magnus, 2007). Since NG2 is widely accepted as a marker for oligodendrocyte progenitor cells (Fig. 5), their significantly higher coloca-lizaton rate with Olig2-marked GFP further validated the proliferative cells as OPCs.

Oligodendrocytic differentiation of resident and/or migrated OPCs in the mature brain:

I found a significant increase in differentiation to the oligodendrocytic lineage in cuprizone-treated mice (Fig. 10). This indicates that even in the presence of cuprizone, which inhibits oligodendrocytic differentiation of OPCs in vitro (Cammer, 1999), a sub-population of OPCs can spontaneously take an oligodendrocytic lineage. A small percentage of Olig2-marked GFP-positive cells were positive for the astrocyte marker GFAP, with no significant difference between the two groups (14% and 13% in control and cuprizone-treated mice, respectively). When mice were restored to normal diet for 3 weeks, almost complete remyelination was observed (Fig. 17), while the overall percentage of oligodendrocytic lineage cells barely increased compared with the 6-week treatment, a two-fold increase in oliogodendrocytic lineage cell differentiation was observed in cuprizone-treated mice relative to control mice in the 3-week diet restoration group. Despite a slight decrease in the percentage of colocalization of NG2 and Olig2-marked GFP in the 3-week restoration group compared to the 6-week group, the vast majority of GFP-positive cells still showed colocalization with NG2. NG2 proteoglycan expression is known to be down-regulated very rapidly when Olig2-positive cells differentiate either into either oligodendrocytes or astrocytes

(Levine and Stallcup, 1987; Levine et al., 1993; Shi et al., 1998; Tatsumi et al., 2005). The present results therefore suggest that a significant proportion of Olig2-positive cells remain undifferentiated in the cuprizone-treated mice as well as in the untreated mice. Recent reports have provided evidence that NG2 proteoglycan is also expressed in a subset of activated microglia which is recruited to lesions (Bu et al., 2001; Jones et al., 2002). While this prompts caution about assessing the nature of NG2 and GFP-double positive cells, most, if not all, of the NG2 and GFP double-positive cells are likely to be OPCs, because GFP-positive cells never displayed Iba1 immunoreactivity, which is a definitive marker for microglia. Taking these observations together, OPCs in the chronic demyelinating environment either retain their progenitor characteristics or differentiate into oligodendrocytes, but do not differentiate into other lineages such as astrocytes. This finding is consistent with a previous study which showed that human OPCs were recruited to lesions but that their differentiation was hindered by environmental factors (Back et al., 2005).

Differentiation of Olig2-expressing progenitor cells into microglial and neuronal lineage:

Olig2-positive cells displayed neither microglial nor neuronal fates in the present experiments, and the majority of the progeny showed remyelinating oligodendrocytic properties (Fig. 20). Neuronal fate may be inhibited cell-autonomously by Olig2 expression, as has been described previously (Buffo et al., 2005; Hack et al., 2005; Colak et al., 2008).

However, demyelination and remyelination are not absolute events that occur successively; instead, they can occur simultaneously once demyelination has occurred. Eearly demyelination is observed in the cuprizone-induced demyelination model at around four weeks of cuprizone treatment (Woodruff et al., 2004), and indeed is detectable as early as after three weeks of treatment (Mason et al., 2000). When the cellular fate of OPCs was characterized in the 6-week cuprizone-induced demyelination model, I thus assume that demyelination and remyelination events were already taking place simultaneously at this stage. Therefore, a complete recovery of demyelinated lesions in this model is unlikely to occur.

Conclusions:

The present study describes the basic characteristics of Olig2-expressing OPCs in an active demyelinating lesion. In this demyelinating model, the fates of Olig2 progenitors were destined only in the two cell lineages: oligodendrocytes and astrocytes (Fig. 21). It is noted that the genetic and stable marking technique for OPCs, especially for undifferentiated OPCs which still express NG2 after six weeks of cuprizone treatment, employed here should be very useful for monitoring the efficacy of remyelination-inducing agents such as PDGFR-α (Vana et al., 2007), TNF-α (Arnett et al., 2001) and IL-1 β (Mason et al., 2001b).

Finally, these data provide direct evidence that proliferation and differentiation of local and/or recruited Olig2 progenitor cells into corpus callosum contribute to remyelination in demyelinated white matter, at least partially, and highlight the potential of these progenitor cells to be manipulated, by growth factor or cytokine treatment, into oligodendrocytic lineage cells for the purpose of providing better repair strategies in demyelinating diseases such as multiple sclerosis (Fig. 22).

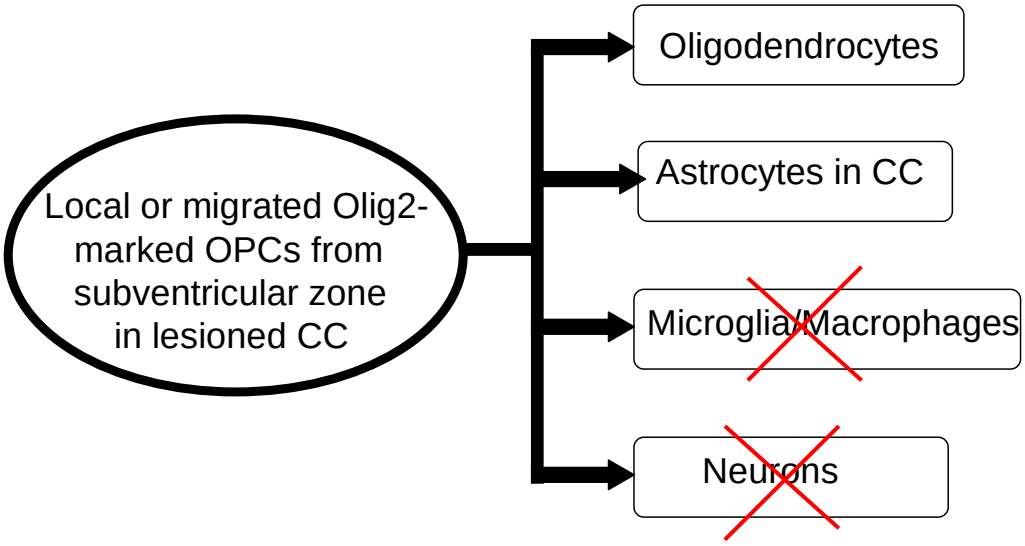

Fig. 21. Diagram showing the fate of Olig2-expressing progenitors in demyelinated lesions in the corpus callosum (CC). Resident Olig2-positive progenitors in CC (local or migrated from the subventricular zone in the CC) differentiate into only two lineages of cells: oligodendrocytes and astrocytes, in the cuprizone-induced acute demyelination and remyelination mouse model. No neuronal or microglial lineage cells were observed for these progenitors.

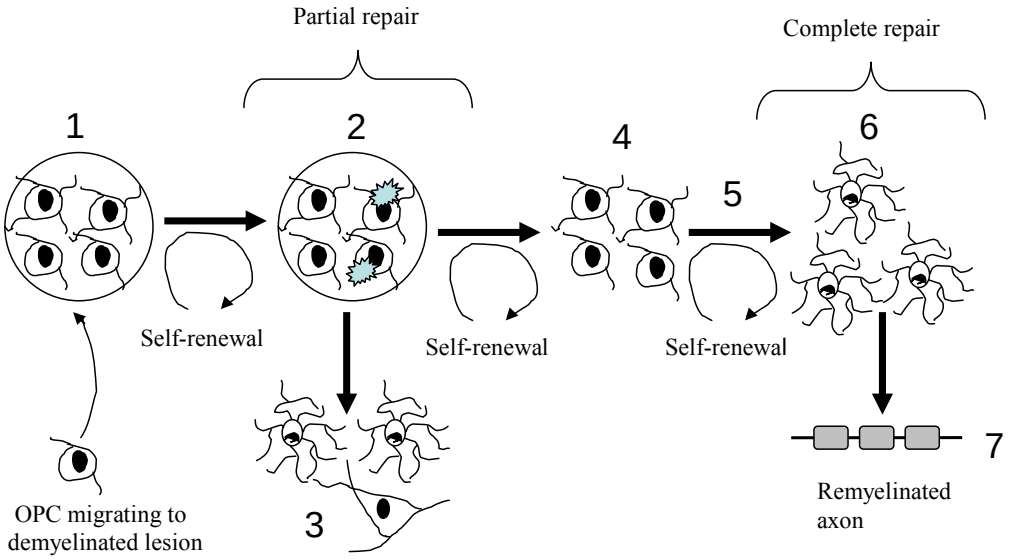

Fig. 22. Diagram depicting the possibility of effective remyelination in a lesioned area by OPC. When demyelination occurs in the normal adult brain, there appear many OPC marked by Olig2, either resident or migrated from the subventricular zone (1). Some of these OPCs become activated (2) and differentiate into oligodendrocytes and astrocytes in the demyelinated lesion, and hence provide partial repair (3), but many preserve their progenitor characteristics without differentiating into any lineage of cells (4). These OPCs can be manipulated into oligodendrocytic lineage differentiation by growth factors or cytokines (5), to yield more oligodendrocytes (6) which eventually take part in repair of demyelinated axons (7).

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Acknowledgements

I would like to take this opportunity to express my sincere gratitude to everyone who has contributed directly or indirectly to this study.

First of all, I would like to express my sincere and deep gratitude to Prof. Sadao Shiosaka, who gave me a golden chance to study and carry out research in his laboratory at NAIST, and to Prof. Akio Wanaka, the head of the laboratory of Anatomy and Neurogenesis at Nara Medical University, for proving me the great opportunity to carry out this entire study in his lab. I also express my deep gratitude to Dr. Kouko Tatsumi, of the same latter lab, without whose active guidance and support I would never have come up to this point with the present study.

I thank Drs. Komai, Ishikawa, Tamura、Hara, Hatanaka and Okuda for their valuable suggestions for this study.

My special thanks to Drs. Nakamura, Horinouchi and Harada and Mr. Ichida for their kind help, in either study or daily life, especially during my initial days at NAIST.

I also thank all the present and past colleagues of the Shiosaka and the Wanaka labs for their friendly behavior and smiling face, which made the lab like a home to me.

I wish to thank my three academic advisory members, Profs. Kinichi Nakashima, Hiroshi Itoh and Kenji Kohno, as well as Dr. Ian Smith, visiting scientific English teacher, for their valuable comments and suggestions on the study.

I express my deep gratitude to my parents and all family members, whose love and affection from far away always inspired me to go ahead with my study.

Finally, I would like to express my deep gratitude to my wife, who passed many lonely late nights during my stay at the lab, for her continuous encouragement and support throughout the study.