**Powerful Homeostatic Control of Oligodendroglial Lineage by PDGFR\(\alpha\) in Adult Brain**

**Graphical Abstract**

**Highlights**
- Oligodendrocyte progenitor cells (OPCs) disappeared and then repopulated in CAGG-iKO mice
- Repopulated OPCs are partly derived from pericyte and/or mesenchymal cell population (PC/MC)
- PC/MC-derived OPCs differentiate into MBP-expressing mature oligodendrocytes

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**In Brief**
Đặng et al. show that oligodendrocyte progenitor cells (OPCs) are repopulated from pericyte and/or mesenchymal cell population (PC/MC) and from OPCs that escape Pdgfra inactivation. PC/MC-derived OPCs can differentiate into MBP-expressing mature oligodendrocytes. Our findings reveal a mechanism of homeostatic control of adult OPCs engaging dual cellular sources of adult OPC formation.
Powerful Homeostatic Control of Oligodendroglial Lineage by PDGFRα in Adult Brain

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SUMMARY

Oligodendrocyte progenitor cells (OPCs) are widely distributed cells of ramified morphology in adult brain that express PDGFRα and NG2. They retain mitotic activities in adulthood and contribute to oligodendrogenesis and myelin turnover; however, the regulatory mechanisms of their cell dynamics in adult brain largely remain unknown. Here, we found that global Pdgfra inactivation in adult mice rapidly led to elimination of OPCs due to synchronous maturation toward oligodendrocytes. Surprisingly, OPC densities were robustly reconstituted by the active expansion of Nestin+ immature cells activated in meninges and brain parenchyma, as well as a few OPCs that escaped from Pdgfra inactivation. The multipotent immature cells were induced in the meninges of Pdgfra-inactivated mice, but not of control mice. Our findings revealed powerful homeostatic control of adult OPCs, engaging dual cellular sources of adult OPC formation. These properties of the adult oligodendrocyte lineage and the alternative OPC source may be exploited in regenerative medicine.

INTRODUCTION

Oligodendrogenesis and myelination are largely completed by an early postnatal age and are relatively limited thereafter (Kessaris et al., 2006; Simon et al., 2011). Oligodendrocyte progenitor cells (OPCs) retain mitogenicity and can differentiate to generate new myelin-forming oligodendrocytes that contribute to the plasticity and repair of myelin in adulthood (Gibson et al., 2014; Young et al., 2013; Zawadzka et al., 2010). However, repetitive myelin damages and subsequent impaired myelin repair by either OPCs or oligodendrocytes cause incurable chronic demyelinating diseases (Franklin and Ffrench-Constant, 2008). Oligodendrocytes and myelin may also be among the earliest targets in the pathogenesis of diseases such as amyotrophic lateral sclerosis, as well as in the white matter injury that develops in ischemic stroke (Kang et al., 2013; Wu et al., 2016). Thus, OPCs or equivalent progenitors are likely critically involved in several severe neurological diseases for which no, or only limited, therapeutic options exist.

Nestin+/NG2+ perivascular mesenchymal stem cells or periocytes, with the potential to differentiate into neural cells including OPCs in vitro, have been identified within many organs, including the adult human brain (Crisan et al., 2008; da Silva Meirelles et al., 2008; Dore-Duffy et al., 2006; Méndez-Ferrer et al., 2010; Paul et al., 2012). Similarly, Nestin+ neural stem/progenitor cells (NSPCs) in the subventricular zone (SVZ) of the lateral ventricle are recruited and differentiate into OPCs to repair demyelinating lesions in the corpus callosum and adjacent white matter (Menn et al., 2006; Xing et al., 2014). These adult stem cells, as well as OPCs or equivalent progenitors, are the targets of attempts at regenerative therapies that aim to induce endogenous myelin recovery in demyelinating and other neurological diseases (Akkermann et al., 2016; Franklin and Ffrench-Constant, 2008; Kang et al., 2013). However, the cellular kinetics and regulatory mechanisms of these cells in adult brain largely remain unknown.

Platelet-derived growth factor receptor-alpha (PDGFRα) is highly expressed in OPCs and is, together with its ligand platelet-derived growth factor-A (PDGF-A), of critical importance for oligodendrocyte formation in the developing mouse via the promotion of proliferation and survival and via the inhibition of...
Figure 1. PDGFRα+/NG2+ OPCs Depleted due to a Rapid Differentiation to Oligodendrocytes after Pdgfra Inactivation in CAGG-iKO Mice

(A and B) Schematic representation of the transgenic and mutated alleles. Pdgfra inactivation was induced by tamoxifen (TM) in CAGG-CreER;Pdgfraflox/flox mice to obtain mice with global inactivation of Pdgfra (CAGG-iKO mice) (A). Identically treated Pdgfraflox/flox mice were used as controls (A). TM was given orally (legend continued on next page)
premature maturation of these cells (Barres et al., 1992; Nishi-
yama et al., 2009; Noble et al., 1988; Pringle et al., 1992). Pdgfra
and Pdgfa null mutants showed defective oligodendrocyte
development and severe hypomyelination (Fruttiger et al.,
1999; McKinnon et al., 2005); however, due to the early lethality
of these mutants, the role of PDGFRα signaling largely remains
to be explored in adults.

In the present study, to understand the regulatory mechan-
isms controlling the adult oligodendrocyte lineage, we inac-
ivated Pdgfra in the adult mouse using the tamoxifen
(TM)-inducible Cre-loxP system. This brought about the ex-
tensive cell dynamics of OPCs, comprising a rapid induction
of transient and near-complete depletion of OPCs by synchronous
terminal cell differentiation and a subsequent robust repopula-
tion to reestablish the typical even distribution of OPCs at
near-normal density over the following 1–3 weeks. This repop-
ulation occurred via active expansion of OPCs originating from
immature precursor cells and from preexisting OPCs, both of
which had escaped Pdgfra inactivation. This dual mode of
OPC repopulation resembles liver regeneration, which engages a
dual mechanism: recruitment from resident stem cells and
proliferation of mature hepatocytes, referred to as alternative
and classical regeneration (Khodolenko and Yarygin, 2017).
Our findings reveal powerful homeostatic control of adult
OPCs, in which the controlling mechanism, PDGFRα signaling,
was found to be critically involved. These discovered cell kinetics
and their control mechanism could be the relevant targets of
regenerative medicine of CNS diseases.

RESULTS

PDGFRα Ablation Triggers Differentiation of Adult OPCs

To genetically ablate PDGFRα in adult mice, we generated
Pdgfra<sup>fl<sub>ox</sub>/fl<sub>ox</sub></sup> mice (Horikawa et al., 2015) and crossed them
with CAGG-CreER mice, which express TM-inducible Cre re-
combinase (CreER) under the ubiquitous CAGG promoter
(Hayashi and McMahon, 2002) (Figure 1A). TM was adminis-
tered to CAGG-CreER:Pdgfra<sup>fl<sub>ox</sub>/fl<sub>ox</sub></sup> mice to induce global inactivation
(inducible knockout [iKO]) of Pdgfra (CAGG-iKO mice) (Figures
1A and 1B). TM-treated Pdgfra<sup>fl<sub>ox</sub>/fl<sub>ox</sub></sup> mice displayed normal
PDGFRα protein expression and were used as controls.

OPCs were identified through their characteristic morphology
and the expression of PDGFRα and NG2 (Nishiyama et al., 2009).
PDGFRα protein expression in these cells was not affected in
control mice (Figure 1C), but it was rapidly depleted in CAGG-
KO mice after TM ingestion; it was significantly reduced after
just 2 days (Figures 1Bi and 1D) and fully eliminated after
5 days of TM treatment (Figures 1Bi, 1E, and S1A–S1E). In par-
allel, NG2 expression in cells with OPC morphology disappeared
with a slight delay compared to PDGFRα in CAGG-iKO mice; it
was still observed after 2 days (Figures 1Bi and 1D). NG2<sup>+</sup>
OPCs were fully eliminated after 5 days of TM treatment (Figures
1Bi and 1E), whereas NG2<sup>+</sup> vascular pericytes remained,
judging from their perivascular location and typical morphology
(da Silva Meirelles et al., 2008; Murray et al., 2014) (Figures 1E,
S1F, and S1G).

Then we examined whether the rapid loss of PDGFRα<sup>+</sup>/NG2<sup>+</sup>
OPCs in CAGG-iKO mice was due to cell death. We labeled
proliferating OPCs using bromodeoxyuridine (BrdU) administra-
tion before TM treatment (hereafter referred to as preexisting
OPCs) following previously published methods (Figure 1Biii)
(Simon et al., 2011) and confirmed that BrdU immediately
labeled similar numbers of PDGFRα<sup>+</sup> OPCs and Olig2<sup>+</sup>
and Sox10<sup>+</sup> oligodendrocyte-lineage cells (Nishiyama et al., 2009)
in CAGG-CreER:Pdgfra<sup>fl<sub>ox</sub>/fl<sub>ox</sub></sup> and control mice (Figure S2). We
found that immediately after TM treatment lasting 5 days, most
BrdU<sup>+</sup> preexisting OPCs were PDGFRα<sup>+</sup> in controls but PDGFRα
negative (PDGFRα<sup>−</sup>) in CAGG-iKO mice (Figures 1F and 1G).
Moreover, the total number of BrdU<sup>+</sup> cells remained comparable
in Pdgfra<sup>fl<sub>ox</sub>/fl<sub>ox</sub></sup> and CAGG-iKO mice for up to 37 days, with a ten-
dency toward a decrease in CAGG-iKO only at late time points
after TM treatment (Figures 1L–1N). These data suggest that
following PDGFRα ablation, OPCs survive and differentiate to-
ward more mature PDGFRα<sup>−</sup>/NG2-negative stages (Nishiyama
et al., 2009). To test this hypothesis, we first examined the expression of CC1 and GST<sub>τ</sub>, two markers of mature oligoden-
drocytes (Nishiyama et al., 2009; Tansey and Cammer, 1991),
in BrdU<sup>+</sup> preexisting OPCs (Figure S3). Two days after TM

following the protocol (Bi–Bii). The box indicates BrdU labeling before TM treatment, in which mice were given BrdU supplemented in drinking water (1 mg·mL<sup>−1</sup>) for 2 weeks (Biii).

Immunofluorescence labeling of PDGFRα (green) and NG2 (red) in the corpus callosum. PDGFRα<sup>−</sup>/NG2<sup>−</sup> oligodendrocyte progenitor cells (OPCs) in control
Pdgfra<sup>fl<sub>ox</sub>/fl<sub>ox</sub></sup> mice 1 day after TM treatment for 5 days (Bi and C). PDGFRα-negative but NG2<sup>−</sup> OPCs in CAGG-iKO mice 1 day after TM treatment for 2 days (Bi and D).

Depletion of PDGFRα<sup>−</sup>/NG2<sup>−</sup> OPCs in CAGG-iKO mice 1 day after TM treatment for 5 days, leaving NG2<sup>−</sup> vascular pericytes (Biii and E).

Following PDGFRα<sup>−</sup>/NG2<sup>−</sup> OPCs in control mice (Figure S2). We
found that immediately after TM treatment lasting 5 days, most
BrdU<sup>+</sup> preexisting OPCs were PDGFRα<sup>−</sup> in controls but PDGFRα
negative (PDGFRα<sup>−</sup>) in CAGG-iKO mice (Figures 1F and 1G).
Moreover, the total number of BrdU<sup>+</sup> cells remained comparable
in Pdgfra<sup>fl<sub>ox</sub>/fl<sub>ox</sub></sup> and CAGG-iKO mice for up to 37 days, with a ten-
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drocytes (Nishiyama et al., 2009; Tansey and Cammer, 1991),
in BrdU<sup>+</sup> preexisting OPCs (Figure S3). Two days after TM

(... Continued)
A: Tamoxifen ingestion

B: Tamoxifen

C: mCherry

D: MBP

E: PDGFRα

F: Merge & DAPI

G: CAGG-iKO, 1d

H: CAGG-iKO, 7d

I: CAGG-iKO, 14 d

J: NG2 DAPI

(legend on next page)
treatment of 5 days (Figure 1Bii), the proportion of CC1+/BrdU+ cells was around one-third (25%–45%, depending on the brain region) in controls, whereas it was 90%–100% in all regions examined in CAGG-iKO mice (Figures 1H, 1I, and 1O). The proportion of GST-π+/BrdU+ cells was very low in both genotypes before TM treatment (~5 days) but quickly increased and was significantly higher in CAGG-iKO than in controls in all examined areas after TM treatment (Figures 1J, 1K, and 1P–1R). These observations suggested that the rapid loss of PDGFRα+/NG2+ OPCs in CAGG-iKO mice was due to rapid differentiation toward mature (CC1+/GST-π+/NG2+) oligodendrocytes rather than due to cell death.

To confirm the fate of OPCs that were rendered Pdgfra inactive, we determined the fate of Pdgfra-inactivated and mCherry+ cells in mice harboring a Pdgfra-promoter-driven CreER gene, together with Pdgfrafl/fox and an inducible Rosa26R26R-H2B-mCherry+/ reporter allele (PRA-iKO-mCherry mice, after TM treatment) (Figure 2A) (Abe et al., 2011). One week after TM treatment of these mice (Figure 2B), we observed PDGFRα+/mCherry+/myelin basic protein (MBP)+ cells in the corpus callosum, striatum, and cortex, demonstrating that Pdgfra-inactivated OPCs rapidly differentiated into myelin-forming oligodendrocytes (Figures 2C, 2D, and S4). When compared with identically treated control mice harboring Pdgfra-promoter-driven CreER and Rosa26R26R-H2B-mCherry+/ reporter genes and the wild Pdgfra gene, the fraction of mCherry+/MBP+ cells was significantly higher in PRA-iKO-mCherry versus control mice; cortex, 31.5% ± 4.86% versus 5.56% ± 3.29%, p < 0.001; corpus callosum, 79.4% ± 10.5% versus 39.5% ± 3.73%, p < 0.01). Furthermore, these Pdgfra-inactivated and mCherry+ cells in PRA-iKO-mCherry mice were negative for TUNEL or cleaved caspase-3 staining, markers of apoptosis, at 10 and 20 days after TM administration (Figure S5). Collectively, these observations that the rapid loss of PDGFRα+/NG2+ OPCs in CAGG-iKO mice was due to the accelerated differentiation of OPCs toward myelin-forming oligodendrocytes. Premyelinating oligodendrocytes need to have contact with the axon for survival (Trapp et al., 1997). Therefore, the MBP+ oligodendrocyte with the inactivated Pdgfra gene might have survived through contact with unmyelinated fibers that remain in adult brain (Sturrock, 1980; Tomassy et al., 2014) or through participation in activity dependent myelination genesis (Mount and Monje, 2017).

**PDGFRα⁺ OPCs Rapidly Repopulate through Active Expansion from Small Numbers of Cells after Pdgfra Inactivation**

The loss of PDGFRα+/NG2+ OPCs in CAGG-iKO mice was followed by rapid OPC repopulation. One day after TM treatment (Figure 2E), PDGFRα+ cells remained at normal densities in control mice (Figure 2F) but were virtually undetectable in CAGG-iKO mice (Figure 2G). At 7 days, PDGFRα+ OPCs had reappeared but were unevenly distributed in CAGG-iKO brains (Figure 2H). They subsequently expanded, and by 14 days, they were randomly distributed in conspicuous clusters near the meninges, as well as within the brain parenchyma (Figure 2I). These PDGFRα+ cells carried the morphological hallmarks of OPCs and were NG2+ (Figures 2Ii and 2IIi). At 21 days, the repopulated OPCs had resumed typical even distribution and near-normal density (Figure 2J; Figure S6A). Similar to PDGFRα protein, Pdgfa mRNA expression in the brain was strongly suppressed in CAGG-iKO mice at 1 week after TM treatment and subsequently increased to reach 50%–90% of control levels by 4 weeks (Figure S6B). The expression of Olig2 and Sox10 (two markers of oligodendrocyte-lineage cells), Gfap (an astrocyte marker), and Cd11a (a microglial marker) was not significantly altered in CAGG-iKO mice, suggesting that the effects of Pdgfa ablation were specific to OPCs without affecting other glial cell populations (Figures S6C–S6F).

The pattern of repopulation of OPCs (Figures 2H and 2I) suggests a process of rapid expansion through cell proliferation, a scenario supported by BrdU labeling that was conducted after TM treatment (Figure 3Ai). Whereas PDGFRα+ OPCs were often BrdU negative in controls, close to 100% of the PDGFRα+ cells were BrdU+ in CAGG-iKO mice after the first week of BrdU administration and decreased somewhat when BrdU was administered during the second and third weeks (Figures 3Bi–3Ei and S7). The percentage of BrdU+ OPCs was significantly higher in CAGG-iKO mice than in controls in the cortex and striatum throughout the periods of observation, whereas the corpus callosum also showed high BrdU incorporation in OPCs in controls as previously reported (Simon et al., 2011) (Figures 3Ci–3Ei). These data show that after near-complete elimination, near-normal density of OPCs was reestablished in CAGG-iKO mice through active expansion from small numbers of cells that escaped Pdgfra inactivation (hereafter referred to as Pdgfra-preserving cells).

**Figure 2. PDGFRα⁺ OPCs Repopulated after Differentiation and Depletion in CAGG-iKO Mice**

Genetic fate mapping of Pdgfra gene-inactivated OPCs.

(A and B) Schematic representation of the transgenic and mutated alleles. Pdgfra-inactivated OPCs were marked with mCherry by tamoxifen (TM) treatment of Pdgfra-CreER;Pdgfrafl/fox; mCherry (PRA-iKO-mCherry) mice (A). Seven days after TM, the fate of mCherry+ Pdgfra-inactivated OPCs was determined (B).

(C and D) Native fluorescence of mCherry (red) and immunofluorescence of MBP (green) and PDGFRα (cyan) in the corpus callosum (C) and in the striatum (D). Arrows indicate mCherry+/MBP+/PDGFRα+ cells. Nuclei were counterstained with DAPI (blue). Scale bars, 5 μm.

(E) Active repopulation of PDGFRα+ OPCs in CAGG-iKO mice. The timed sequences of PDGFRα+ OPC repopulation were examined after TM-induced depletion. (F–J) Immunofluorescence of PDGFRα, shown by grayscale images of the entire plane from the coronally cut mouse brain, except for (Ii) and (IIIi). White dots represent PDGFRα staining. PDGFRα staining of a control Pdgfrafl/fox mouse at 1 day after 5 days of TM treatment (F). Many PDGFRα+ cells were distributed in both brain parenchyma and meninges. Depletion of PDGFRα staining of CAGG-iKO mice at 1 day after 5 days of TM treatment (G), in which meninges are traced by a yellow dotted line. Repopulation of PDGFRα+ cells of CAGG-iKO at 7 days (H) and 14 days (I) after TM. High-magnification views of the boxed area in (I) indicate PDGFRα+ (magenta)/NG2+ (red) OPCs with ramified morphology that repopulated from the vicinity of the meningeal membrane are indicated by a dotted line (Ii) and (IIIi). Repopulated PDGFRα+ OPCs diffusely distributed in CAGG-iKO at 21 days (J). Asterisks indicate the lateral ventricle. No nuclear counterstaining with DAPI (blue) was conducted, except for (Ii) and (IIIi). Scale bars, 500 μm in (F–J) and 20 μm in (Ii) and (IIIi).

See also Figures S4–S6.
OPCs Repopulate from the Meninges and Brain Parenchyma

At 3 days after TM treatment of 5 days, repopulating PDGFRα+/NG2+ OPCs were found to appear as randomly distributed small clusters in close association with meninges, as well as within the brain parenchyma (Figures 3F–3H). These cells displayed the typical immature OPC morphology, including short studded cytoplasmatic processes (Figures 3F–3Fii). They often showed intra-meningeal and perivascular localizations (Figures 3G–3Hii). These repopulating OPCs were highly proliferating, judging from their labeling with 5-ethyl-2'-(deoxyuridin (EdU) that was administered intraperitoneally 8 h before sacrifice (Figure 3Aii). Some of these cells showed EdU labeling in a paired distribution indicative of a recent cell division (Boda et al., 2015) (blue arrows in Figures 3G and 3H–3Hii). Based on these observations, we speculated that OPCs might locally repopulate from both the meninges and the parenchyma of the mouse brain.

To better characterize the site of repopulation origin, we employed lineage-tracing approaches involving stereotaxic administration of GFP-expressing retroviruses into the arachnoid layer of the meninges (Figure 4A). The localized viral infection limited to meningeal tissue was confirmed using GFP-expressing lentivirus or 2-[(1E,3E)-3-(3,3-Dimethyl-1-octadecyl-1,3-dihydro-2H-indol-2-yldene)-1-propen-1-yl]-3,3-dimethyl-1H-indolium perchlorate (DiI) infusion (Figure S8). Three days after a GFP retrovirus infection of the meninges of CAGG-iKO mice, small numbers of GFP+/PDGFRα+ cells with the typical immature OPC morphology were found in the cerebral cortex near the meningeal application site (Figures 4B–4Bii). By 14 days, GFP+/PDGFRα+ cells had increased in number, were distributed into the deeper parts of the cortex, and showed the highly ramiﬁed morphology characteristic of OPCs (Figures 4C–4Cii). In parallel with this increase of GFP+/PDGFRα+ OPCs, GFP+/PDGFRα+ OPCs not infected with GFP retrovirus had increased in number from 3 to 14 days (Figures 4B and 4C). Hence, the kinetics of the appearance and spreading of the meningeal-derived virus-labeled GFP+/PDGFRα+ cells visualized, at least partly, the kinetics of the repopulating processes of OPCs in CAGG-iKO mice. GFP+/PDGFRα+ OPCs were also detected near the injection sites following GFP retrovirus administration into the cerebral cortex (Figures 4D–4Dii) and striatum (data not shown) of CAGG-iKO mice. Not many, but a certain number of GFP+ cells in the cortex expressed MBP in CAGG-iKO mice at 2 weeks (Figure S9A), suggesting that the meningeal-derived cells can differentiate into oligodendrocytes through the stage of OPC for contribution as myelin-forming cells in the brain. No GFP+ cells were found in Pdgfrafloxfloxflox controls following meningeal GFP retrovirus injection, despite extensive searches (n = 3 mice) at 3, 7, and 14 days after injection (data not shown). Whereas previous studies indicate that adult OPCs originate from the SVZ (Menn et al., 2006; Rafalski et al., 2013; Xing et al., 2014; Zawadzka et al., 2010), we were not able to trace detectable recruitment of OPCs from SVZ by GFP-expressing lentivirus labeling (Figures S9B–S9Div).

Because repopulating cells are Pdgfra preserving, we mapped the prevalence of cells that had escaped recombination in CAGG-iKO mice at different locations in the brain, including the meninges. Using the Rosa26R-DsRed-H2B-mCherry+/+ reporter allele as a marker for CAGG-CreER-mediated Pdgfra inactivation in CAGG-iKO mice (CAGG-iKO mice were mCherry+/CD13+ pericyte and/or mesenchymal cell (PC/MC) population in the meninges and perivascular regions (Figures 4E and 4F), raising the possibility that repopulation occurs from these cells. We conﬁrmed that mCherry− meningeal cells, labeled by meningeal GFP retrovirus infection, gave rise to OPCs (Figure 4G). In addition, most repopulated Sox10+/EdU+ oligodendrocyte-lineage cells in CAGG-iKO mice were mCherry negative (Figures 4H and 4I), conﬁrming that repopulation of the oligodendrocyte lineage occurs from Pdgfra-preserving cells. Collectively, these observations are consistent with a scenario in which Pdgfra-preserving cells residing in meningeal and/or perivascular locations take part in OPC repopulation in CAGG-iKO mice.

Involvement of Nestin+ Cells in OPC Repopulation

Nestin, in combination with CD13 and NG2, is a useful marker of immature cells, including those of PC/MC origin (Armulkik et al., 2010; da Silva Meirelles et al., 2008; Murray et al., 2014). The PC/MC has been considered the putative source for OPC repopulation, so we examined the involvement of Nestin+ cells in OPC repopulation in CAGG-iKO mice. Many Nestin+ and Nestin+/CD13+ cells were found to proliferate in meninges and perivascular regions in CAGG-iKO mice during the early days after TM treatment, but not in control mice, through EdU labeling and Ki67 immunostaining; in these experiments, mice were sacrificed at 7 days after TM and EdU was administered immediately before sacrifice (Ai and B–E) BrdU labeling of the repopulating OPCs. Repopulating OPCs were labeled by BrdU that was administrated after tamoxifen (TM) treatment in 3 groups of animals (Ai). Immunofluorescence of BrdU (green) and PDGFRα (red) in the cerebral cortex of Pdgfrafloxfloxflox and CAGG-iKO (B) after BrdU labeling during the second week after TM. White arrows and yellow arrowheads indicate PDGFRα+ cells with and without BrdU labeling, respectively. The percentage of BrdU+ cells within PDGFRα+ cells in Pdgfrafloxfloxflox and CAGG-iKO mice (C–E) (n = 3 at each time point). ***p < 0.001 versus Pdgfrafloxfloxflox mice at the same time point in each brain region.

(Ai and F–H) Repopulation of PDGFRα+ OPCs at 3 days after TM treatment for 5 days in CAGG-iKO mice. EdU was given intraperitoneally 4 times with 2 h intervals until 2 h before sacrifice (Ai). Immunofluorescence of PDGFRα (green) and chemical fluorescence of EdU (magenta) in (F)–(H), NG2 (red) in (F), and CD31 (red) in (G) and (H). High-magnification views of the boxed areas within (F)–(H) are shown in the corresponding (i)–(iii), respectively. Repopulated PDGFRα+ OPCs in close association with the meninges of the cerebral cortex (F and G). Immature ramified PDGFRα+/NG2+ OPCs included EdU+ (F, white arrows, and Fi–IIIi) and EdU− cells (yellow arrows, F). Yellow arrowheads indicate spindle-shaped NG2+/PDGFRα+ pericytes (F). Arrows indicate PDGFRα+/EdU+ OPCs within the meninges and parenchyma of the cerebral cortex (G–IIIi). White and yellow arrows indicate PDGFRα+/EDU+ and PDGFRα+/EDU− repopulated OPCs, respectively, in a deep part of the cerebral cortex (H). PDGFRα+/EdU+ repopulated OPCs are often distributed near blood vessels (G–IIIi) and arranged in paired form (G and H–IIIi, blue arrows). Dotted lines indicate the meninges in (F) and (G).

Nuclei were counterstained with DAPI (blue). Scale bars, 50 μm in (F)–(H) and 20 μm in (B), (IIIi), and (GIIi). See also Figure S7.
A

Meninges of CAGG-iKO, 3d

Meninges of CAGG-iKO, 14d

Cortex of CAGG-iKO, 14d

B

GFP, PDGFRα, DAPI

C

D

E

F

G

H

I

CAGG-iKO-mCherry with GFP-retrovirus infection in Meninges

Meninges of CAGG-iKO-mCherry with GFP-retrovirus infection in Upper cortex

Meninges of CAGG-iKO-mCherry

Meninges of CAGG-iKO-mCherry

Meninges of CAGG-iKO-mCherry

Sox10, EdU, mCherry

Merge & DAPI

Merge & DAPI

Merge & DAPI

Merge & DAPI

Merge & DAPI

Cortex of CAGG-iKO, 14d

2nd week : EdU      : Tamoxifen

: Sacrifice

: Tamoxifen oral ingestion

: GFP-retrovirus infection

: Sacrifice
before sacrifice (Figures 5A–5Biv and S10). Nestin+/CD13+ cells were identified within these sites by genetic mapping in Nestin-nlsCre-mCherry mice harboring a Nestin-promoter and enhancer-driven, constitutively active form of Cre (nlsCre, Cre with a nuclear localization signal) (Tronche et al., 1999) and Rosa26

reporter (Figure S11A). The enhancer encoded in the second intron plays an important role to induce the Nestin gene specific to CNS neural stem cells (Zimmerman et al., 1994). Nestin and CD13 were expressed in early repopulating OPCs in CAGG-iKO mice, but they were not detectable in OPCs in control Pdgfraflox/fox mice (Figures 5C–5G and S12A–S12E). We found Nestin expression in early GFP+ OPCs labeled by GFP retrovirus infection of both meninges and brain parenchyma (Figures 5H and 5I). CD13 and Nestin staining in Pdgfraflox+ cells became negative, but it tended to remain positive when these cells were in contact with blood vessels and meninges after 2 weeks of TM treatment in CAGG-iKO mice (Figures S12F–S12H). Altogether, these data are consistent with repopulating OPCs being at least partly mobilized from activated Nestin+ and possibly CD13+ immature cells residing in meninges and perivascular areas.

To address whether repopulation depended on Pdgfraflox+ cells in Nestin+ cells, we crossbred CAGG-CreER;Pdgfraflox/fox mice with Nestin-CreER mice harboring Nestin-promoter and enhancer-driven CreER gene (Figure 6A) (Lagace et al., 2007). The resulting Nestin-CreER;CAGG-CreER;Pdgfraflox/fox mice were treated with TM (Dual-iKO) 14 times over 23 days and analyzed at 28 days (Figure 6B). In comparison with identically treated Pdgfraflox/fox control mice (Figure 6C), OPC repopulation was not abolished in CAGG-iKO mice despite this intense schedule of TM treatment (Figure 6D). However, the dual-CreER approach had a significantly additive effect: repopulating Pdgfraflox+ OPCs were fewer and more unevenly distributed in TM-treated Dual-iKO mice at 28 days, with near-complete regional elimination of Pdgfraflox+ OPCs (Figures 6E, asterisks, and 6H). These results strengthen the conclusion that OPCs repopulate from the rare cells escaping Pdgfraflox inactivation and that Nestin+ cells significantly contribute to repopulation. However, because interpretations of the results are slightly confounded by Pdgfraflox+ being both a driver of OPC repopulation and a marker that is eliminated by gene inactivation, we also administered a Pdgfraflox-neutralizing antibody into the lateral ventricle of CAGG-iKO mice (Figure 6Bii). Compared with vehicle-treated control CAGG-iKO mice (Figure 6F), this treatment significantly suppressed the number of repopulating OPCs in both cortex and striatum (Figures 6G and 6I), again leaving areas with almost no detectable OPCs (Figure 6G, asterisks). This demonstrates that OPC repopulation from Pdgfraflox-preserving cells in CAGG-iKO mice remains Pdgfraflox dependent.

To obtain direct evidence for the meningeal presence of an OPC repopulating progenitor, meninges were dissected from the brain surface of CAGG-iKO-mCherry mice at 2 weeks after TM treatment, dissociated, and cultured. In these cultures, both Sox10+ oligodendroglial and MAP2+ neuronal lineage cells formed, both being mCherry-negative and Pdgfraflox-preserving cells (Figures S13A and S13B). Similar cultures established from TM-treated Pdgfraflox/fox cultures did not generate Sox10+ or MAP2+ cells (data not shown). These multipotent immature cells likely correspond to the meningeal sources of OPC repopulation in CAGG-iKO mice. Because Dual-iKO strategy still could not suppress OPC repopulation (Figures 6E and 6H), we hypothesized that preexisting OPCs that escaped gene inactivation were an additional source of OPC repopulation. To test this, in addition to the BrdU labeling of preexisting OPCs, the repopulating OPCs with high mitotic activities were labeled with EdU immediately before sacrifice in CAGG-iKO mice (Figure S13C). At 3 days after TM, similar numbers of BrdU+ and BrdU– cells were detected in the early foci of Pdgfraflox+ OPC repopulation with frequent EdU labeling (BrdU+ versus BrdU– cells; 5.21 ± 1.04 versus 5.63 ± 0.95 adjacent to meninges in Figure S13D; 5.63 ± 1.08 versus 5.42 ± 1.37 within parenchyma in Figure S13E; means ± SEM obtained from 3 foci found in 3 mice, respectively). These data show that OPCs can repopulate from recruited Nestin+ immature cells and from preexisting OPCs, both of which had escaped CreER-mediated Pdgfraflox inactivation.

**The Oligodendrocyte Lineage Originates from the Meninges in the Normal Adult Mouse Brain**

Lineage tracing using GFP retrovirus uncovered the meninges as the origin of OPC repopulation in CAGG-iKO mice, but not in control mice (e.g., Figures 4 and 5). This could be explained by
Figure 5. Nestin and CD13 Were Expressed in Activated Cells in Meninges and in Early Repopulated OPCs in the Brain Parenchyma of CAGG-iKO

(A–Biv) Chemical fluorescence of EdU (magenta) and immunofluorescence of Nestin (red) and CD13 (green) in the meninges of Pdgfra<sup>fl<sup/>fox</sup> and CAGG-iKO mice at 7 days after tamoxifen (TM) treatment for 5 days. EdU was given intraperitoneally 4 times with 2 h intervals until 2 h before sacrifice. EdU<sup>+</sup> cells were rare in the meninges of Pdgfra<sup>fl<sup/>fox</sup> (A) but were common in that of CAGG-iKO (arrows, B). High-magnification views of the boxed area in (B) indicate EdU+/Nestin+/CD13+ cells (arrows, Bi–Biv).

(C–E) Double immunofluorescence of PDGFR<sup>α</sup> (green) and Nestin (red) in the septum of Pdgfra<sup>fl<sup/>fox</sup> mice at 3 days (C) and CAGG-iKO mice at 3 days (D) and 28 days (E) after TM for 5 days. Arrowheads and arrows indicate PDGFR<sup>α</sup>+/Nestin<sup>−</sup> cells and PDGFR<sup>α</sup>+/Nestin<sup>+</sup> cells, respectively.

(F and G) Double immunofluorescence of PDGFR<sup>α</sup> (magenta) and CD13 (red) at 3 days after TM for 5 days in the cortex of Pdgfra<sup>fl<sup/>fox</sup> (F) and CAGG-iKO (G) mice. Arrowheads and arrows indicate PDGFR<sup>α</sup>+/CD13<sup>−</sup> cells and PDGFR<sup>α</sup>+/CD13<sup>+</sup> cells, respectively.

(legend continued on next page)
the activation of immature meningeal cells in CAGG-iKO (e.g., Figure 5) making these cells susceptible to retrovirus infection. Therefore, the recruitment of OPCs from the meninges was examined by lineage tracing in Pdgfra-preserving mice using a GFP lentivirus, which can infect nondividing cells (Freed and Martin, 1994) (Figure 7A). Fourteen days after infection of the meninges, GFP+/Pdgfrα− or GFP+/NG2− cells with a ramified morphology typical of OPCs were identified within the cerebral cortex adjacent to the meninges (Figures 7B and S8E–S8Evi), indicating that meningeal cells were recruited into the brain parenchyma and differentiated into OPCs in the normal adult mouse brain. In another experiment, seven days after GFP lentivirus infection of the meninges of Pdgfraflx/flx mice (Figure 7C), GFP+/mCherry−/PDGFRα− cells were found in the cortex adjacent to the meninges (Figure 7D, arrows). This indicated that some Nestin+ cells were recruited from the meninges in control mice. In the same experiment, GFP+/mCherry+/PDGFRα−/NG2− cells were detected (Figure 7D, arrowheads), which may correspond to immature cells that have not differentiated toward OPCs, because they mainly reside within the meninges.

We next examined the role of PDGFRα in the recruitment of OPCs from the meninges in the normal mouse brain. The lentivirus expressing codon-improved CreER (iCre) (Shimshek et al., 2002), a TM-independent active form of Cre, was injected into the meninges of Pdgfraflx/flx mice with Rosa26R26R-H2B-mCherry+/+ reporter mouse (Pdgfraflx/flx, mCherry). In this procedure, mCherry+ cells should represent the cells subjected to iCre lentivirus-mediated Pdgfra inactivation within meninges (Figure 7E). Most mCherry+ cells recruited from meninges (26 of 32 mCherry+ cells) corresponded to Olig2+ or Sox10− non-oligodendrocyte-lineage cells, with a few exceptional mCherry+ cells with Olig2− or Sox10+ (Figures 7F and 7G). Therefore, it was indicated that PDGFRα is important for the differentiation of recruited immature cells toward the oligodendrocyte lineage in normal mouse brain.

**DISCUSSION**

The oligodendrogenesis is a highly dynamic cellular process that had been assumed to largely terminate before the early postnatal period. The functionally redundant OPCs with ventral and dorsal origins are compensatory to each other in developing brain: when one population of OPCs is genetically eliminated, the remaining cells take over and the mice survive and behave normally with a normal complement of oligodendrocytes and myelin (Kessaris et al., 2006; Richardson et al., 2006). A plasticity in adult mouse brain has been shown that maintains the OPCs in developing immature brain. Therefore, it was indicated that PDGFRα is important for the differentiation of recruited immature cells toward the oligodendrocyte lineage in normal mouse brain.

(Pdgfra inactivation, in which after near-complete elimination of OPCs by rapid differentiation toward mature oligodendrocyte stages, new OPCs of even distribution and of near-normal density repopulated from PDGFRα-low/negative, Nestin+ cells residing in meninges and brain parenchyma or from residual pre-existing OPCs, both of which had escaped Cre-mediated inactivation of the Pdgfra gene (summarized in Figure S14). Similarly, but at a very low rate, OPC recruitment from meninges was detected in normal adult mouse. Therefore, the findings in Pdgfra-inactivated mouse suggest that rapid OPC depletion accelerates and thereby unmask a process that normally occurs at a very low rate. Our study revealed unexpectedly potent reparative mechanisms of adult OPCs that had only been demonstrated in developing immature brain.

The stage at which an OPC exits the cell cycle and differentiates is one of the most important points of regulation of oligodendrocyte lineage. This stage is regulated by many extrinsic and intrinsic mechanisms, including Notch signaling, the Wnt pathway, the Sox family of transcription factors, and microRNA such as miR-219 (Emery and Lu, 2015). Among them, ablation of both Sox5 and Sox6 genes in the oligodendroglial lineage results in marked precocious differentiation of OPCs in the developing spinal cord (Stolt et al., 2006). Within the many factors reported so far, our study directly indicated that a PDGFRα signal is a crucially important effector to suppress precocious maturation of OPCs in vivo, because OPCs with Pdgfra inactivation quickly matured to express maturation markers including CC1, GSTπ, and MBP. Along this line, the most recent network-based genomic analysis of human OPC differentiation identified a useful molecule downstream of the PDGFRα signal that improves the impaired OPC maturation (Pol et al., 2017), an impairment that has been an assumed central pathogenesis of multiple sclerosis (Franklin and French-Constant, 2008).

The remyelination and oligodendrocyte density improved significantly in human PDGFA transgenic mice compared with wild-type mice after cuprizone-induced chronic demyelination (Vana et al., 2007). Transplantation of OPCs with PDGF-AA overexpression improved the repair of spinal cord injury in adult rat (Yao et al., 2017). We showed that OPC repopulation after Pdgfra inactivation-induced depletion was an actively proliferating and migrating process of Nestin+ immature cells and preexisting OPCs, both of which retained the intact Pdgfra gene. When Pdgfra deletion was induced by repeated TM administration in double CAGG- and Nestin-driven CreER genes, or when PDGFRα was inhibited by the application of neutralizing antibodies, OPC repopulation became incomplete, resulting in areas in the brain that were devoid of OPCs. Therefore, the newly repopulated OPCs depended on PDGFRα for active expansion by proliferation, eventually leading to a reestablishment of near-normal OPC density and distribution. Altogether, the PDGF-A/PDGFRα signal axis was suggested to endow a potent reparative nature upon the adult OPCs for the regeneration of brain.
Tamoxifen            Sacrifice

CCCtx Str

7d 14d 21d 0d 28d

CAGG-iKO –V
CAGG-iKO –N

CCCtx N

16 14 12 10 8 6 4 2

C CreER

Nestin promotor

CreER

poly A

Exon 4

Exon 5

Tamoxifen ingestion

Pdgfra locus

Inactivation of Pdgfra

LoxP

C

LV

LV

F

LV

LV

Neutralizing antibody against PDGFRα

H

I

PDGFRα cells

Pdgfra<sup>lox/lox</sup>
Pdgfra<sup>iKO</sup> CAGG-iKO Dual-iKO

LoxP

PDGFRα<sup>α</sup><sup>+</sup> cells

Pdgfra<sup>α</sup><sup>+</sup> cells

Neutralizing antibody against PDGFRα

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insult, being supported by PDGFRα effects on OPCs that had been obtained mainly from studies of in vitro or developing immature CNS (Barres et al., 1992; Nishiyama et al., 2009; Noble et al., 1988; Pringle et al., 1992; Fruttiger et al., 1999; McKinnon et al., 2005). Further studies are needed to elucidate the mechanism by which PDGFRα induces OPC repopulation.

Compared with other organs, such as skin and liver, regeneration of the CNS is limited. However, in our study, the adult oligodendroglial lineage was found to possess an astonishing regenerative capacity. Our in vivo and in vitro data indicated Nestin+ immature cells that were activated in the meninges, as well as preexisting OPCs, as sources of OPC repopulation. During liver generation, both proliferation of preexisting hepatocytes and new recruitment of hepatocytes from stem and/or progenitor cells contribute to liver regeneration, and it is assumed that the blockage of the former process stimulates the latter process (Itoh and Miyajima, 2003). Further studies are needed to elucidate the mechanisms of regenerative approaches to treat demyelinating diseases (Maki et al., 2013; Menn et al., 2006; Nait-Oumesmar et al., 2007; Xing et al., 2014). Adding to this perspective, the Nestin+ immature cells of the meninges and perivascular origin, uncovered in our study can be exploited for OPC repopulation in such diseases (Ozen et al., 2012), considering that brain damage such as by demyelinating lesions is frequently distributed apart from the SVZ (Ozawa et al., 1994).

**STAR METHODS**

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**SUPPLEMENTAL INFORMATION**

Supplemental Information can be found online at https://doi.org/10.1016/j.celrep.2019.03.084.

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Stop mCherry Rosa26 locus iCre CAG promotor Myc poly A infection Lentivirus Exon 4 Pdgfra locus Exon 5 mCherry expression Inactivation of Pdgfraα Rosa26 locus mCherry activity mCherry

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Lentivirus infection
Sacrifice

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Dotted lines indicate the meninges in (B), (D), (F), and (G). Nuclei were counterstained with DAPI (blue). Scale bars, 20


dendrocyte lineage after

populated OPCs in the brain parenchyma originated from Nestin+ cells in meninges. Yellow arrowheads indicate GFP+/mCherry+/PDGFR

a

imagination of (red) at 14 days after GFP lentivirus infection (B).


REFERENCES


Figure 7. Oligodendrocyte-Lineage Cells Recruited from Nestin+ Cells in the Meninges in Pdgfra-Preserving Mice

(A–D) Nestin+ cells were normally recruited from meninges and differentiated into OPCs. The meninges were infected by a lentivirus that expresses GFP (GFP lentivirus) in Pdgfrafl/fl mice (A), Native fluorescence of GFP (green) and immunofluorescence of PDGFRa (red) at 14 days after GFP lentivirus infection (B). GFP lentivirus was infected into the meninges of mice harboring Nestin-promoter-driven Cre recombinase with the nuclear localization signal (nlsCre) and Rosa26R2iRES-iR2B-mCherry+ reporter, in which mPdgfra alleles are wild type (Nestin-nlsCre-mCherry mice) (C). Native fluorescence of GFP (green) and mCherry (red) and immunofluorescence of Pdgfra (magenta) at 7 days after GFP lentivirus infection (D). GFP+/mCherry+/Pdgfraflox/flox cells (white arrows) indicate repopulated OPCs in the brain parenchyma originated from Nestin+ cells in meninges. Yellow arrowheads indicate GFP+/mCherry+/Pdgfrafl/fl mice (E). Arrows represent mCherry+ cells (red) (F and G) that differentiated into the Pdgfrafl/fl (magenta) or Olig2+ (green) (F) or Sox10+ (magenta) (G) oligodendrocyte lineage after Pdgfra inactivation by Cre lentivirus infection in meninges. Dotted lines indicate the meninges in (B), (D), (F), and (G). Nuclei were counterstained with DAPI (blue). Scale bars, 20 μm.


# STAR METHODS

## KEY RESOURCES TABLE

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Pdgfra conditional inactivation mice and reporter mice

A mutant mouse harboring a genetically mutated Pdgfra gene, in which exons 4–5 of Pdgfra were flanked by two loxP sequences (Pdgfra<sup>lox/lox</sup>) was generated as follows (Horikawa et al., 2015). In brief, a BAC genomic clone (RP24-148N4) originating from the DNA of a C57BL/6 mouse and containing Pdgfra was obtained from the BACPAC Resource Center CHORI. The constructed targeting vector included a DNA fragment containing a loxP sequence and pgk-Neo cassette flanked by two Fip recognition target (frt) sites (Takeuchi et al., 2002) and pMC1DTABGHA with a bovine growth hormone-derived polyadenylation signal sequence (Kitayama et al., 2001). The embryonic stem cell line RENKA, derived from the C57BL/6N strain (Fukaya et al., 2006), was used and the constructed targeting vector was electroporated as described previously (Miya et al., 2008). The obtained male chimeric mouse was crossed with a female CAG-FLPe deleter mouse (Kanki et al., 2006) to remove the pgk-Neo selection cassette and obtain the heterozygous Pdgfra<sup>lox/+</sup> strain. Genotyping was performed by PCR using the following primers: 5′-ATGCCAAACTCTGCCTGATTGA-3′ and 5′-CTCACGGAACCCCCACAAC-3′. Pdgfra<sup>lox/lox</sup> mice were crossed with chicken β-actin-promoter/CMV-enhancer-driven Cre-transgenic mice (Hayashi and McMahon, 2002) (CAGG-CreER; Jackson Laboratories) that harbor the fusion gene Cre recombinase and estrogen receptor (CreER). The resulting offspring were mice harboring CAGG-CreER<sup>+/−</sup>-Pdgfra<sup>lox/lox</sup> or Pdgfra<sup>lox/lox</sup>. Mice with a systemic Pdgfra inactivation (CAGG-iKO mice) were obtained by tamoxifen (TM, Sigma-Aldrich) administration to CAGG-CreER;Pdgfra<sup>lox/lox</sup> mice. In the present study, TM was orally administered at a dose of 225 mg·kg<sup>−1</sup>·day<sup>−1</sup> for the time indicated to induce Cre-mediated DNA recombination in mice with the CreER-transgene. Pdgfra<sup>lox/lox</sup> mice were treated identically and used as controls. To target Pdgfra in nestin-expressing cells in CAGG-iKO mice, mice with CreER gene driven by a nestin promoter (Lagace et al., 2007) (C57BL/6-Tg (Nes-cre/Esr1)1Kuan/J; Jackson Laboratories) were crossed with CAGG-CreER<sup>+/−</sup>-Pdgfra<sup>lox/lox</sup> mice. Within the transgene of Nes-cre/Esr1 mice, the 5′-flanking region of Nestin gene contained the second intron, in which an enhancer is encoded that induces CreER gene expression specific to CNS neural precursor cells (Zimmerman et al., 1994). The resultant offspring, harboring double transgenes of Cre including CAGG-CreER<sup>+/−</sup> and Nestin promoter-CreER<sup>+/−</sup>, and Pdgfra<sup>lox/lox</sup> were administered with TM as described to obtain dual-iKO mice.

To detect Cre-mediated gene recombination, CAGG-CreER;Pdgfra<sup>lox/lox</sup> mice were cross-bred with a reporter line, Rosa26<sup>R26R-H2B-mCherry</sup>/+, which has the fusion cDNAs of H2B-mCherry inserted into the Rosa26 gene next to the stop sequences flanked with loxP sequences (Abe et al., 2011) (termed mCherry mice). The male offspring, harboring CAGG-CreER;Pdgfra<sup>lox/lox</sup>, Rosa26<sup>R26R-H2B-mCherry</sup>/+(CAGG-iKO-mCherry mice) were used to detect gene recombination induced by TM in CAGG-iKO mice. Mice harboring Pdgfra<sup>lox/lox</sup>;Rosa26<sup>R26R-H2B-mCherry</sup>/+(Pdgfra<sup>lox/lox</sup>-mCherry mice) were used for the analyses of iCre-expressing lentiviral infection in the meninges. For lineage tracing of Nestin<sup>+</sup> cells in the meninges and perivascular regions, we crossbred mice expressing the active form of Cre (nlsCre, Cre with nuclear localization signal) under the control of the nestin promoter and enhancer (Tronche et al., 1999) (Nestin-nlsCre mice, B6.Cg-Tg (Nes-cre)1Kln/J, Jackson Laboratories) with the reporter mouse line of Rosa26<sup>R26R-H2B-mCherry</sup>/+. Male offspring expressing Nestin-nlsCre;Rosa26<sup>R26R-H2B-mCherry</sup>/+ (Nestin-nlsCre-mCherry mice) were used in the experiment.

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Seiji Yamamoto (seiyama@med.u-toyama.ac.jp, seiyama.flyfishing@gmail.com).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Ethics

All experimental animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Toyama (University of Toyama, Toyama, Japan).

Animal care, sex and age/developmental stage of mice

All mice were housed at 25°C with a 12/12 h light/dark cycle and allowed access to pellet chow and water ad libitum. Eight- to 12-week old male mice were used for all experiments.
For the fate mapping of Pdgfra-inactivated OPCs, transgenic mouse line was generated harboring Pdgfra-promoter driven CreER gene. The plasmid pCAG-CreERT2 was a gift from Connie Cepko (Addgene plasmid # 14797) (Matsuda and Cepko, 2007). We constructed single open reading frame DNA encoding CreERT2, the 2A/furin cleavage sequence (Jostock et al., 2010), and EGFP by PCR and recombinant DNA methods. The resulting DNA fragment was inserted at the translational initiation Met of the mouse PDGFRα gene in the BAC genomic clone (RP24-148N4) by using a Counter-selection BAC modification kit (Gene Bridges, Dresden, Germany) and recombinant DNA methods. The resulting DNA fragment was inserted at the translational initiation Met of the mouse PDGFRα-structed single open reading frame DNA encoding CreERT2, the 2A/furin cleavage sequence (Jostock et al., 2010), and EGFP by PCR. Genotyping was performed by PCR targeting EGFP, by use of the following primers: 5′-GGCAAGCTGACCCTGAAGTTCATCTG-3′ and 5′-ATCGGCTTCTCTGTTGGGTCTTGCTCAG-3′. The established Pdgfra-CreER mouse line was cross-bred with Pdgfra<sup>fox/fox</sup> and Rosa26<sup>ER<sub>a</sub>CreER-R26R-H2B-mCherry<sup>+</sup></sup> mice. The off-springs, Pdgfra-CreER<sup>+/−</sup>;Pdgfra<sup>fox/fox</sup>;Rosa26<sup>R26R-H2B-mCherry<sup>+</sup></sup> mice (PRa-iKO-mCherry mice) were treated with TM, and were examined for the fate of the mCherry<sup>+</sup> cells.

**METHOD DETAILS**

**Immunofluorescence staining of frozen tissue sections**

Mice were deeply anesthetized with sodium pentobarbital (intraperitoneal injection, 100 mg·kg<sup>−1</sup>; Dainippon Sumitomo Pharma) and perfused transcardially with ice-cold phosphate-buffered saline (PBS, 0.01 M, pH 7.4), followed by 4% (w/v) paraformaldehyde (PFA; Sigma-Aldrich) in phosphate-buffered solution (0.1 M, pH 7.4). PFA was used to detect the native-fluorescence of mCherry and GFP. Brains were removed and post-fixed overnight at 4°C using the same fixative as the perfusion-fixation, transferred to 30% (w/v) sucrose (Wako) solution (in PBS, pH 7.4), stored at 4°C for more than 36 h, then frozen in Tissue-Tek® O.C.T.™ compound (Sakura Finetek) on dry ice (Kitahara et al., 2018). To obtain representative coronally cut sections, the brains were coronally cut at 20-μm thickness at the positions between 0.5–1.1 mm relative to bregma with a cryostat, and mounted on glass-slides (Frontier FRC-05, Matsunami). Following this, immunofluorescence was performed. Permeabilization and antigen retrieval were conducted using a permeabilizing solution (0.3% Triton X-100 in PBS pH 7.4 for 30 min at room temperature) and target retrieval solution (Dako; at 98°C for 25 min, following supplier’s protocol) before immuno-staining, where necessary. Non-specific staining was prevented using Protein block serum-free (Dako) for 30 min at room temperature. In addition, tissue sections were treated with Histofine Mouse Stain kit (Nichirei) for 1 h prior to blocking, before using mouse-raised primary antibodies. We used following primary antibodies: rat monoclonal anti-BrdU (1:100, AbD Serotec), mouse monoclonal anti-CC1 (1:200, Millipore), rat monoclonal anti-CD13 (1:400, AbD Serotec), mouse monoclonal anti-CC1 (1:200, Millipore), rat monoclonal anti-CD31 (1:100, Millipore), rabbit polyclonal anti-GFP (1:1000, Frontier FRC-05, Matsunami), goat polyclonal anti-Sox10 (1:100, Santa Cruz). As secondary antibodies, we used Alexa-Fluor488-, Alexa-Fluor568-, or Alexa-Fluor633-conjugated antibodies appropriate for primary antibodies (Life Technologies) at 1:500 dilutions. The primary and secondary antibodies were diluted in Dako REAL™ antibody diluent (Dako) for 30 min at room temperature. As primary antibodies, we used rabbit polyclonal anti-NG2 (1:200, Millipore), rabbit polyclonal anti-Olig2 (1:200, AbD Serotec), rat monoclonal anti-CD31 (1:100, Millipore), rabbit polyclonal anti-γH2AX (1:200, IBL), goat polyclonal anti-PDGFRα (1:1000, Neuromics) and goat polyclonal anti-Sox10 (1:100, Santa Cruz). As secondary antibodies, we used Alexa-Fluor488-, Alexa-Fluor568-, or Alexa-Fluor633-conjugated antibodies appropriate for primary antibodies (Life Technologies) at 1:500 dilutions. The primary and secondary antibodies were diluted in Dako REAL™ antibody diluent (Dako). The primary antibodies were applied to sections overnight at 4°C. The secondary antibody incubation was performed at room temperature for 1 h. EdU was detected using the Click-iT® Edu Alexa Fluor® 647 Imaging Kit (Invitrogen) following the manufacturer’s protocol. All sections were mounted using VECTASHIELD Mounting Media with DAPI (Vector Laboratories).

**Cell Death Assays**

To detect apoptosis in PDGFRα<sup>−</sup> OPCs after Pdgfra-inactivation, the frozen tissue sections of PRa-iKO-mCherry mice prepared at 10 and 20 days after TM treatment for 5 days were assessed by TUNEL staining and by the immuno-detection of Caspase-3 of active form. As for the positive controls of these procedures, the Pdgfra<sup>fox/fox</sup> mouse brain sections with experimentally induced ischemic lesion of 3-day-old were used. TUNEL staining was conducted with use of DeadEnd Fluorometric TUNEL System Kit according to the manufacturer instructions (Promega, G3250, USA). The immuno-detection of active Caspase-3 was conducted with rabbit anti-cleaved caspase-3 (1:200, Cell Signaling Technology) and donkey anti rabbit (1:500, conjugated with Alexa-Fluor488, Life Technologies), as primary and secondary antibodies, respectively. The procedures of this immuno-detection were carried out as described in “Immunofluorescence staining of frozen tissue sections.” Images were taken with Biorevo BZ-9000 microscope (Keyence).

**BrdU and EdU administration**

To trace the fate of OPCs following Pdgfra inactivation, OPCs were labeled with BrdU (Sigma-Aldrich) dissolved in the drinking water (1 mg·mL<sup>−1</sup> supplemented with 1% sucrose) that was administrated for 14 days to CAGG-CreER;Pdgfra<sup>fox/fox</sup> and Pdgfra<sup>fox/fox</sup> mice prior to 5 days of TM treatment. To quantify the proliferation of the repopulating OPCs following Pdgfra inactivation, BrdU supplemented in drinking water was administrated to CAGG-iKO and Pdgfra<sup>fox/fox</sup> mice for 7 days during the first, second and third weeks after TM-induced gene inactivation. Repopulating OPCs in CAGG-iKO-mCherry mice after TM treatment were labeled with EdU.
Mice were anesthetized with sodium pentobarbital (intraperitoneal injection, 50 mg·kg⁻¹) and mice were sacrificed at 2 h after the last injection to determine the proliferation of repopulating cells.

**Quantitative real-time PCR**

Total RNA isolation and quantitative real-time PCR analysis were performed as described previously (Yamamoto et al., 2017; Yamamoto et al., 2015). Briefly, real-time PCR was performed with a Thermal Cycler Dice Real Time System TP800 (Takara) using cDNA (1:25) in a reaction mixture consisting of SYBR Premix EX TaqII (Takara). The real-time PCR program consisted of hot start enzyme activation at 95°C for 10 s, 45 cycles of amplification at 95°C for 10 s and 60°C for 40 s. Finally, to obtain the dissociation curve, a cycle was performed at 95°C for 1 min, 60°C for 30 s and 95°C for 10 s. For data analysis, the mouse beta-actin (Actb) housekeeping gene was used as an internal control. Expression levels were calculated using analysis software. Primer sequences are available upon request from the Takara Bio Inc. website (http://www.takara-bio.co.jp).

**Virus constructions expressing GFP and iCre**

To produce the GFP-expression retrovirus (GFP-retrovirus), pMXs-SIN-CAG-GFP (Niwa et al., 1991) was co-transfected with pVSVG-G (Clontech) into PLAT-gp cells, counterparts of PLAT-E cells lacking the transgene for envelope (Morita et al., 2000; Ohkawa et al., 2012), by FuGENE HD (Roche). Retrovirus-containing culture supernatants were collected after 2 days and the retrovirus was concentrated by ultracentrifuge at 5 × 10⁶ g for 90 min at 4°C. The retrovirus pellet was resuspended to 0.5% of its original volume in PBS. The Copepod GFP-expressing lentivirus (GFP-lentivirus) was constructed with pSIH-H1 (System Biosciences) as previously described (Shehata et al., 2012). Improved Cre recombinase expressing lentivirus (iCre-expressing lentivirus) was constructed as follows. The plasmid containing codon-improved Cre recombinase (iCre) sequence (pBlue.iCre) was kindly provided by Dr. Rolf Sprengel (Shimshek et al., 2002). The oligonucleotides extending flexible linkers and double Myc tag sequences (GGGGSGSGSMQKLLSEEDLLGGGSGSMQKLLSEEDL; single letter of amino acids) were used for the construction of the plasmid pLIT-dMyc. The 142 bp DNA fragment was amplified with the primers Upper (5'-CCCCCAGGATGGGACGGCGGTGGCGGATCCGCATCAATGCAAAAGCTGATCTC-3' the containing Xhol site) and Lower (5'-GGGGTACCGAATTCACGCGTCACAGGTCTTCCTCGGAGATC for 3' containing MluI, EcoRI, and KpnI sites) with pLIT-dMyc as the template. The 135-bp XhoI-KpnI fragment was prepared from this amplified DNA fragment and ligated with the 4.0-kbp XhoI-KpnI fragment from pBlue.iCre to yield the plasmid pLIT-dMyc. The 1.2-kbp EcoRI fragment from pBlue.iCreMyc was ligated with EcoRI-digested pCAGGS (Niwa et al., 1991) to yield the plasmid pCAG-iCreMyc. The 2.9-kbp SalI (blunt)-MluI fragment from pCAG.iCreMyc was ligated with the 6.4-kbp BstXI (blunt)-MluI fragment from pLenti6PW TGB to yield the pLenti-iCre plasmid. In this plasmid, the iCredMyc gene is driven with strong CAG promoter.

**Stereotactic injection of viral vectors**

Mice were anesthetized with sodium pentobarbital (intraperitoneal injection, 50 mg·kg⁻¹) and were placed in stereotaxic apparatus (Narishige). Using a small dental drill, a hole was made in the skull at the correct stereotaxic coordinates. Following this, the dura mater was removed with small curved forceps and exposure of the meninges was confirmed by observation of a small amount of cerebrospinal fluid at the exposed position. It is particularly necessary to keep the meninges intact to deliver the virus to meninges. A needle (internal diameter, 0.13 mm) was set in the stereotaxic device (David Kopf Instruments), connected to a Hamilton micro syringe via polyethylene tubes filled with water, and set on a micro syringe pump (CMA 400, Harvard Apparatus). The needle was inserted to the targeted location in the brain at the following coordinates (millimeter, anteroposterior relative to bregma-AP, mediolateral-ML, and dorsoventral-DV from surface of the brain): meninges (0.5, 2.0, 3.0), lateral ventricle (0.5, 0.9, 2.5), cortex (0.5, 1.2, 0.5), striatum (0.5, 2.0, 3.0). One microliter of lentiviral suspension (2.0 × 10⁶ cfu·mL⁻¹) or 3 μL of retroviral suspension (2.0 × 10⁶ cfu·mL⁻¹) was infused at a flow-rate of 0.1 μL·min⁻¹. After the infusion, the needle was retained at the same position for 10 min to ensure no backflow.

**Culture of multipotent immature cells from meninges**

The meninges were dissected from the mouse brains at 14 days after TM-treatment for 5 days under deep anesthesia with sodium pentobarbital (intraperitoneal injection, 50 mg·kg⁻¹). Cells were grown in proliferation culture medium consisting of RHB-A (Takara Bio Inc.), 20ng/mL EGF (Peprotech) and 10ng/mL FGF2 (Peprotech) on collagen-I coated plastic dishes. At 14 days in vitro, cells were plated in 8 chamber-slides coated with poly-D-lysine in RHB-A medium for differentiation without growth factors. Then, 3 days after, cells were fixed with PFA 1% and processed for cytological analyses. The details are given in Supplemental Information.
**Intracerebroventricular infusion of the PDGFRα neutralizing antibody**

PDGFRα neutralizing antibody (Takakura et al., 1996) (3.3 μg μL$^{-1}$ of PBS, 20 μg day$^{-1}$) or PBS (Nacalai tesque) was infused for 28 days into the lateral ventricle with a mini-osmotic pump (Durect Corporation) at a flow rate of 0.25 μL/h to deliver. Forty-eight hours prior to surgery, the osmotic pump was filled with the solution to be delivered and primed at 37°C in sterile saline. Mice were anesthetized, set in stereotaxic apparatus (Narishige) and a midline incision was made at the base of the skull. Stereotaxic coordinates for implantation of the Alzet brain infusion cannula (Brain infusion kit 3.3 mm depth; Durect Corporation) were set at −0.5 mm anteroposterior and −1.1 mm lateral relative to bregma and secured with Loctite adhesive (Henkel Corporation). The cannula was attached to an osmotic pump and the pump was placed subcutaneously below the scapula. Two days after implantation, the mice were subjected to TM administration (225 mg·kg$^{-1}$ for 5 consecutive days). Animals were sacrificed 3 weeks after TM administration.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

**Microscopic analysis and quantification**

The immunofluorescence images were taken by a Biorevo BZ-9000 microscope (Keyence) or TCS-SP5 confocal laser scanning microscope (Leica). Morphometrical analyses were conducted using the sections prepared from approximately 0.8 mm relative to bregma. Ten-micrometre z stacked images were rendered and merged using Biorevo BZ-9000. For cell counting, four fields were randomly taken of the motor cortex and striatum, and three fields of the corpus callosum were examined. In the Dual-CreER mouse tissue sections, the distribution of PDGFRα$^+$ cells was uneven. Accordingly, more than 12 fields from the striatum, 6 fields from the corpus callosum, and 18 fields from the dorsal cerebral cortex were examined in each mouse to fully cover the majority of each examined region. Blinded investigators manually counted the cells by viewing the photomicrographs using Adobe Photoshop software.

**Statistical analysis and software**

Quantitative data are expressed as the mean ± standard error of the mean (SEM). Comparisons between two experimental groups were made using unpaired Student’s t tests. Multivariate analyses were made with ANOVA followed by Turkey’s analyses. A value of $p < 0.05$ was considered statistically significant. Graphs were drawn using GraphPad Prism 6 (GraphPad Software, Inc.).