Notch Signaling Controls Oligodendrocyte Regeneration in the Injured Telencephalon of Adult Zebrafish

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The myelination of axons in the vertebrate nervous system through oligodendrocytes promotes efficient axonal conduction, which is required for the normal function of neurons. The central nervous system (CNS) can regenerate damaged myelin sheaths through the process of remyelination, but the failure of remyelination causes neurological disorders such as multiple sclerosis. In mammals, parenchymal oligodendrocyte progenitor cells (OPCs) are known to be the principal cell type responsible for remyelination in demyelinating diseases and traumatic injuries to the adult CNS. However, growing evidence suggests that neural stem cells (NSCs) are implicated in remyelination in animal models of demyelination. We have previously shown that olig2⁺ radial glia (RG) have the potential to function as NSCs to produce oligodendrocytes in adult zebrafish. In this study, we developed a zebrafish model of adult telencephalic injury to investigate cellular and molecular mechanisms underlying the regeneration of oligodendrocytes. Using this model, we showed that telencephalic injury induced the proliferation of olig2⁺ RG and parenchymal OPCs shortly after injury, which was followed by the regeneration of new oligodendrocytes in the adult zebrafish. We also showed that blocking Notch signaling promoted the proliferation of olig2⁺ RG and OPCs in the normal and injured telencephalon of adult zebrafish. Taken together, our data suggest that Notch-regulated proliferation of olig2⁺ RG and parenchymal OPCs is responsible for the regeneration of oligodendrocytes in the injured telencephalon of adult zebrafish.

Key words: Neural stem cells, Oligodendroglia, Regeneration, Telencephalon, Wounds and injuries, Zebrafish

INTRODUCTION

Oligodendrocytes are glial cells that myelinate axons in the central nervous system (CNS) to provide electrical insulation for axonal conduction. Oligodendrocytes also play a crucial role in promoting neuronal survival by providing metabolic support, including the supply of lactate to neurons [1]. Various factors including genetics, immune system dysfunction, and traumatic injury can induce demyelination, which can be restored through oligodendrocytes. However, persistent demyelination and the failure of remyelination may cause several neurological disorders, such as multiple sclerosis [2].

Severe traumatic injury to the adult mammalian CNS has catastrophic effects, after which significant regeneration does not occur. In contrast, adult zebrafish possess a remarkable ability to regenerate neurons in the injured CNS. Previous studies have shown that radial glia (RG) detected using markers such as BLBP, GFAP, and S100B exist in the ventricular zone (VZ) and function as neural precursors to generate neurons in the telencephalon of adult zebrafish [3-7]. Injury of the telencephalon induces the proliferation of RGs in the VZ of the injured hemisphere following
active neurogenesis for neuronal regeneration [8-11]. In addition, lineage-tracing studies of RG using retroviral and lentiviral vectors and the Cre-loxP system in the normal and injured telencephalon, respectively, have provided direct evidence that RG can act as neural stem cells to generate new neurons in adult zebrafish [8, 12]. However, the cellular and molecular mechanisms underlying the regeneration of oligodendrocytes in the injured brain are largely unknown.

We previously reported that regulatory DNA from the olig2 locus in transgenic zebrafish embryos drives the expression of enhanced green fluorescent protein (EGFP) in RGs of the pMN precursor domain of the ventral spinal cord and their descendant motor neurons and oligodendrocyte progenitor cells (OPCs), which migrate and divide to produce cells of the oligodendrocyte lineage only [13-15]. Additionally, we showed that oligodendrocytes are continuously generated from the post-embryonic phase to the adult spinal cord and that olig2+ RG have the potential to function as neural stem cells to produce oligodendrocytes in the post-embryonic spinal cord [16]. The current study aimed to develop a zebrafish model of adult telencephalic injury to investigate the cellular and molecular mechanisms underlying the regeneration of oligodendrocytes. Using this model, we aimed to reveal regenerative processes of oligodendrocytes from olig2+ RG and underlying mechanisms in the injured telencephalon. The study findings would provide a novel zebrafish disease model for studying oligodendrocyte regeneration.

**MATERIALS AND METHODS**

**Zebrafish line and ethics**

The following transgenic lines were used: Tg(olig2:EGFP) [13] and Tg(mbp:EGFP) [17]. Adult animals were housed in a 14-h light and 10-h dark cycle. All experimental procedures were approved by the Korea University Institutional Animal Care & Use Committee and performed in accordance with the animal experiment guidelines of the Korea National Veterinary Research and Quarantine Service.

**Drug treatment**

For LY-411575 treatment, the following stock solution was made and stored at -20°C: 10 mM LY-411575 (medchemexpress, HY50752) in dimethyl sulfoxide (DMSO); this was added to the swimming water at a temperature of 28°C and at a final concentration of 10 μM for 4 days. The control fish were placed in water with 0.1% DMSO.

**RESULTS AND DISCUSSION**

**olig2+ RG exist in the telencephalic ventricular zone of adult zebrafish**

Previously, we have shown that olig2+ RG have the hallmarks of stem cells in the post-embryonic spinal cord of zebrafish [16].
We, therefore, hypothesized that olig2+ RG are responsible for the regeneration of oligodendrocytes in the adult CNS and initially investigated the existence of olig2+ RG in the telencephalon of adult Tg(olig2:EGFP) zebrafish, which express EGFP under the control of the olig2 promoter [13]. Immunohistochemical analysis of the adult zebrafish telencephalon with BLBP, S100β, and GFAP antibodies that mark RG [4, 6, 18-20], revealed that olig2+ RG exist in the medial ventricular zone (MVZ) of the adult telencephalon (Fig. 1A–C’). However, we did not detect any olig2 expression in RGs of the lateral ventricular zone (LVZ; A~C, A~C”). Consistent with a previous report that shows that olig2+ cells are oligodendrocyte lineage cells including OPCs and mature oligodendrocytes in the telencephalon [21], olig2:EGFP expression was detected in parenchymal mature oligodendrocytes, which express mbp:EGFP [17] in the telencephalon of the adult Tg(mbp:EGFP);Tg(olig2:Dsred2) zebrafish (Fig. 1D, D’).

**Olig2+ RGs and OPCs are responsible for the regeneration of oligodendrocytes in the injured telencephalon**

To test the response of olig2+ RG and parenchymal OPCs to invasive injury in the telencephalon of adult zebrafish, we inserted a 31-gauge needle from the anterior side of the telencephalon to generate reproducible stab wound injuries (Fig. 2A). The size of the resultant lesion was consistent across the brains that were dissected after the injury (n=15; Fig. 2C). To examine cell proliferation induced by injury to the telencephalon, we analyzed dividing cells 4 days post-lesion (dpl) based on their PCNA expression. In the normal adult zebrafish telencephalon, the proliferation of PCNA+/olig2+ RGs and OPCs was not observed in the ventricular zone or parenchymal area, respectively (Fig. 2B~B”). However, we observed an increased number of PCNA+/olig2+ OPCs near the injury site in the telencephalic parenchyma, which suggests that OPCs in the lesioned hemisphere responded to injury and increased proliferation 4 dpl (Fig. 2C, C”, E). Consistent with previous studies, which have shown that RGs in the LVZ increase proliferation to regenerate new neurons in the lesioned telencephalon [9], we also observed the increased proliferation of S100β+ RGs in the LVZ of the lesioned telencephalon 4 dpl (Fig. 2B, C, yellow arrows). Interestingly, increased proliferation of olig2+ RGs was also detected in the MVZ of the lesioned adult zebrafish telencephalon 4 dpl (Fig. 2C, C’, F). Proliferation of OPCs and olig2+ RGs in the lesioned telencephalon decreased 21 dpl, indicating that the injury response had diminished at this stage (Fig. 2D–F). Altogether, these data suggest that olig2+ RG in the MVZ and parenchymal...
OPCs respond to injury and temporarily increase proliferation.

To test the hypothesis that increased proliferation of OPCs and olig2+ RGs results in the regeneration of new oligodendrocytes, which are marked by mbp:EGFP fluorescence in Tg(mbp:EGFP) zebrafish, we performed a BrdU pulse-chase experiment with the Tg(olig2:Dsred2);Tg(mbp:EGFP) line and traced the newborn progeny of olig2+ proliferative cells in the lesioned telencephalon of adult zebrafish (Fig. 3). After the telencephalic injury, we incorporated BrdU for 4 days (Fig. 3A) and observed that, similar to PCNA-labeled cells, BrdU-labeled cells were also detected throughout the lesioned hemisphere including the LVZ, MVZ, and parenchyma 4 dpl (Fig. 3B, C). However, BrdU+/mbp:EGFP+ new oligodendrocytes were not detected at this stage, indicating that there were no newly regenerated mature oligodendrocytes 4 dpl (Fig. 3C, C'). After the long-term BrdU pulse-chase experiments, a significant number of BrdU+/mbp:EGFP+/olig2:Dsred+ regenerated oligodendrocytes were observed in the parenchyma of the lesioned hemisphere 21 dpl (Fig. 3D, D', E), indicating that newly generated oligodendrocytes differentiated into mature oligodendrocytes.

**Notch signaling is required for the proliferation of OPCs and olig2+ RGs in the lesioned telencephalon**

Previously, Notch signaling has been shown to play a crucial role in adult neurogenesis and injury-induced regeneration of neurons in the zebrafish telencephalon [9, 11]. To study the role of Notch signaling in the injury-induced proliferation of olig2+ RGs and OPCs in adult zebrafish telencephalon, we inhibited Notch signaling in vivo through treatment with LY-411575, a pharmacological inhibitor of r-secretase that impairs the generation of the Notch intracellular domain, thus blocking Notch activation [22].
Regeneration of Oligodendrocytes

Treatment with LY-411575 increased the number of PCNA+ proliferating RG in the LZV and MZV of the telencephalon (Fig. 4A–B', E). We also observed increased numbers of PCNA+/olig2+ RG in the MVZ of the LY-411575-treated zebrafish (Fig. 4A', B', G). These data indicate that Notch signaling inhibits proliferation of RG, including olig2+ RG, in the normal telencephalon of adult zebrafish. Increased proliferation of RGs and olig2+ RGs was consistently observed in lesioned hemispheres of the adult telencephalon following treatment with LY-411575 (Fig. 4C–D', E, G). Interestingly, proliferation of OPCs in normal and lesioned hemispheres of the adult telencephalon were also increased following treatment with LY-411575, suggesting that Notch signaling is also involved in the regulation of parenchymal OPC proliferation in the normal and injured telencephalon of adult zebrafish. A previous study has shown that neuronal regeneration was observed 4 dpl [8], that is, before the timing of oligodendrocyte regeneration observed in our study. This indicates that neuronal regeneration is followed by oligodendrocyte regeneration after telencephalic injury. Together, our data indicate that olig2+ RG are neural stem cells and participate in the regeneration of oligodendrocytes along with parenchymal OPCs in the injured telencephalon of adult zebrafish.

In mammals, although genetic fate-mapping studies have demonstrated that parenchymal OPCs are the principal cell type responsible for oligodendrocyte regeneration in demyelinating diseases in the adult brain [23, 24], NSCs in the subventricular zone (SVZ) also have been shown to have the potential to produce oligodendrocytes after injury [25, 26]. The participation of SVZ-derived progenitors in the remyelination process has been demonstrated in several experimental mouse models of demyelination [27, 28]. Taken together, these data suggest that, like zebrafish, olig2-expressing cells among the NSCs in the MVZ may be responsible for the regeneration of oligodendrocytes in mammals.

Moreover, we showed that blocking Notch signaling promotes
the proliferation of olig2+ RGs and OPCs in the normal and injured telencephalon of adult zebrafish, suggesting that Notch signaling is implicated in the regeneration of oligodendrocytes by regulating the proliferation of olig2+ RGs and pre-existing OPCs. Our data are also supported by a previous study which showed that NSCs transition between the quiescent and proliferating states according to the level of Notch activity and that Notch induction drives NSCs into a quiescence state; in contrast, blocking Notch induces the proliferation of quiescent NSCs and the subsequent generation of neurons in the normal telencephalon of adult zebrafish [29]. Taken together, Notch signaling is suggested to be involved in the regeneration of neurons and oligodendrocytes by regulating the quiescence status of NSCs in the telencephalon of adult zebrafish.

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