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Implications of Olig2 silencing in oligodendrocyte precursor cells

Li-Pao Fang, Xianshu Bai*

Oligodendrocytes (OLs) are the only myelin-forming cells in the central nervous system. Their differentiation from OL precursor cells (OPCs) occurs throughout life and is mediated by numerous intrinsic and extrinsic factors. OL transcription factor 2 (Olig2), a basic helix-loop-helix transcription factor, is one of the intrinsic factors that specify the OL lineage. It is expressed by both OPCs and OLs, and no variant of Olig2 has yet been identified in rodents. Although the function of Olig2 in OL maturation and myelination is still under debate, Olig2 is essential for OPC differentiation in health and disease. Because of its broad expression throughout the OL lineage, Olig2 is often used as a lineage marker. However, in the healthy perinatal and adult brain, a small population of NG2-positive ($NG2^{pos}$) cells were found to be Olig2-negative ($Olig2^{neg}$), and stab wound injury increased the population of $NG2^{pos}Olig2^{neg}$ cells. NG2 is a protein specifically expressed by OPCs and pericytes in the healthy brain and additionally by microglia after acute brain injury. Therefore, it remained unclear whether these $NG2^{pos}Olig2^{neg}$ cells are OPCs or other cell types, such as pericytes or microglia? If these cells are OPCs, are they functionally different from the $Olig2^{pos}$ OPCs? By immunostaining for platelet-derived growth factor receptor alpha (PDGFRα), the established marker of OPCs, we confirmed that a subset of OPCs does indeed not express Olig2. This population of OPCs could be detected throughout life, from the embryonic stage (embryonic day 14.5) to the aged mouse (44 weeks old). Fate mapping studies provided strong evidence that $Olig2^{neg}$ OPCs are derived from pre-existing $Olig2^{pos}$ OPCs (Fang et al., 2023). Therefore, it is conceivable that $Olig2^{neg}$ OPCs do not represent a separate cell type, but rather a distinct functional stage of OPCs in which Olig2 expression is transiently downregulated in response to microenvironmental changes. In other words, OPCs may dynamically up- and downregulate Olig2 expression in response to changes in brain activity.

Integration of OPCs into local neural circuits: However, the main question remains: why do OPCs downregulate Olig2 upon changes in brain activity? Are they functionally and physiologically different from $Olig2^{pos}$ OPCs? Olig2 is known to be a pro-differentiating transcription factor for OPCs, accompanied by an increase in morphological complexity with more processes and branches. Indeed, $Olig2^{neg}$ OPCs are morphologically simpler than $Olig2^{pos}$ OPCs. Therefore, it is tempting to speculate that $Olig2^{neg}$ OPCs may have less differentiation potential. Olig2 ensures the differentiation of OPCs into OLs by repressing a number of genes involved in general pathways of neuronal differentiation and brain development (Zhang et al., 2022). OPCs could downregulate or turn off Olig2 expression to maintain their progenitor status. In the adult brain, OPCs are also referred to as NG2 glia (based on the specific expression of the *cspg4* gene encoding NG2), particularly when their acute physiological function is addressed. OPCs receive synaptic inputs and are active components of neural circuits. Notably, $Olig2^{neg}$ OPCs were enriched in the juvenile brain (approximately 10–26% of total OPCs), but decreased in the adult brain (approximately 1–2% of OPCs). The appearance of $Olig2^{neg}$ OPCs in the postnatal brain coincided with the development of neuron-OPC connectivity, starting at postnatal days 4–5 and peaking at postnatal day 10 for cortical interneurons (Orduz et al., 2015). In addition, the size of this cell population could be increased by modulation of brain activity, such as acute brain injury or complex motor learning tasks. These observations link neuron-OPC communication to Olig2 silencing in OPCs. Apparently, when neurons are challenged, they increase their firing rate, which could keep OPCs in a progenitor state and feed back to the innervating neuron (**Figure 1**). Indeed, OPCs could tune the activity of neuronal circuits through several pathways. First, OPCs receive direct γ-aminobutyric acid (GABA)-ergic and glutamatergic input that induces differentiation into OLs and subsequent myelination. As OPCs differentiate, they lose their synapses and reprogram their transcriptional profile, including changes in α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid-type and $GABA_A$ receptors. The $GABA_A$ receptor γ2 subunit is expressed exclusively in OPCs, not in OLs, and is essential for interneuron-OPC communication. Its genetic inactivation in OPCs during early postnatal weeks results in attenuated activity and myelination of parvalbumin interneurons (Benamer et al., 2020). Similarly, the L-type voltage-gated calcium channels Cav1.2 and Cav1.3 of OPCs, which facilitate long-term potentiation and N-methyl-D-aspartic acid-mediated long-term depression in the hippocampus, are downregulated in mature OLs (Zhao et al., 2021). In addition, OPCs can set the inhibitory tone in the medial prefrontal cortex by adjusting interneuron density, activity, and myelination. In the first two postnatal weeks, depending on interneuronal GABA release and activation of $GABA_B$ receptors on OPCs, OPCs adjust interneuron density by releasing the weak apoptosis factor TWEAK (tumor necrosis factor-like weak inducer of apoptosis) (Fang et al., 2022). Reduced TWEAK release from $GABA_B$ receptor-deficient OPCs induces an increase in interneuron density but a decrease in interneuron activity and myelination. In addition, OPCs form synapse-like inputs on inhibitory neurons and modulate the network. Using optogenetic stimulation in the juvenile mouse hippocampus, OPCs could be induced to release GABA in a synaptobrevin 2/vascular associated membrane protein 2-dependent mechanism, further demonstrating another pathway of potent OPC-to-interneuron communication (Zhang et al., 2021). Third, OPCs can modulate axonal and cortical development. In the zebrafish optic tectum, ablation of OPCs impairs the arborization of retinal ganglion cell axons (Xiao et al., 2022). In the developing mouse visual cortex, numerous phagolysosomes containing axonal fragments were found in OPC processes, suggesting an involvement of OPCs in axonal pruning (Buchanan et al., 2022). More recently, Auguste et al. (2022) reported

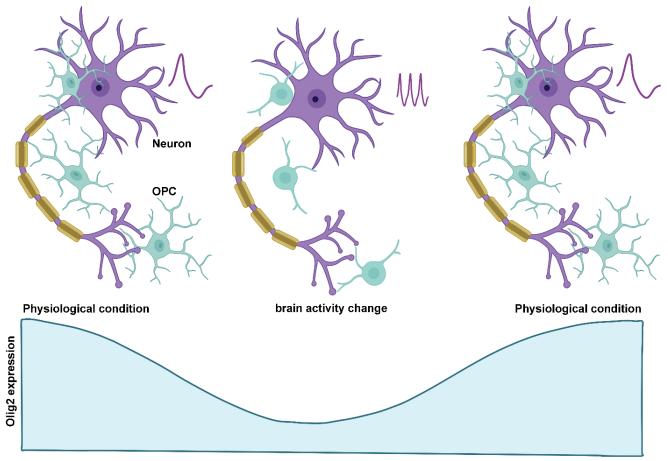

Figure 1 | **Dynamic expression of Olig2 in OPCs.**
OPCs are innervated into neural circuits by forming pre- and postsynaptic structures with neurons. Changes in brain activity induced by acute brain injury or complex motor learning tasks transiently suppress Olig2 expression in OPCs. Thus, OPCs remain in their progenitor state. Olig2: Oligodendrocyte transcription factor 2; OPC: oligodendrocyte precursor cell. Created with BioRender.com.

that OPCs engulf and eliminate thalamocortical synapses in the developing and adult mouse visual cortex, with a beneficial effect on sensory experience during neural circuit refinement. Taken together, OPCs may temporally shut down Olig2 and regulate various circuit functions as required by brain development and neural network activity. This hypothesis can be tested in the double transgenic Olig2-DsRed × PDGFRα-EGFP mouse line by *in vivo* imaging of DsRed^{pos}EGFP^{pos} cells (indicating Olig2^{pos} OPCs) using two-photon laser scanning microscopy. Accordingly, after acute brain injury, a DsRed^{pos}EGFP^{pos} cell would lose its red fluorescence and transform into a cell expressing only EGFP, i.e., an Olig2^{neg} OPC that could eventually reexpress DsRed and EGFP.

It appears that Olig2^{neg} OPCs are not restricted to the mouse brain. Olig2^{neg} OPCs rarely express Ki67 and exhibit less proliferative properties than Olig2^{pos} cells. Interestingly, single cell transcriptomic studies of zebrafish spinal cord and mouse brain suggest a subset of PDGFRα^{pos} OPCs that appear to lack expression of Olig2 as well as the mitotic marker Ki67. In the zebrafish spinal cord, 'cluster #1' OPCs were also found to be negative for Ki67 and considered to be 'quiescent' OPCs as they lacked proliferation and differentiation-related markers (Marisca et al., 2020). Instead, these cells were enriched for mRNAs involved in axon guidance and synaptic communication and preferentially remained in the progenitor stage. If these are the same cell types, then Olig2^{neg} OPCs may represent these quiescent cells in the mouse brain. However, further studies are needed to determine how Olig2^{neg} OPCs are functionally different from Olig2^{pos} cells, for example by patch-clamp recordings of DsRed^{neg}EGFP^{pos} in Olig2-DsRed x PDGFRα-EGFP mice. The basic electrophysiological properties as well as the postsynaptic current of Olig2^{neg} (DsRed^{neg}EGFP^{pos}) and Olig2^{pos} OPCs (DsRed^{pos}EGFP^{pos}) will be compared after stimulation of neighboring neurons. Furthermore, single cell Patch-Seq analysis of these two cell types will be performed to compare their transcriptomic profiles.

Prospects: Two major questions remain: (1) how do OPCs regulate Olig2 expression, and (2) what is the underlying molecular mechanism? *In silico* analysis of published single-cell RNA sequencing data revealed several differentially expressed genes in Olig2^{pos} and Olig2^{neg} OPCs. In Olig2^{pos} cells, myelination-facilitating factors such as *Mbp* or *Egr2* genes were enriched, while Olig2^{neg} cells were found to express higher levels of the adenosine A1 receptor (*Adora1*), suggesting a putative purinergic signaling pathway involved in Olig2 suppression. Notably, elevated levels of extracellular adenosine has been observed in many neuropathologic context. Alternatively, suppression of the Olig2 gene may be due to upregulation of bone morphogenetic protein 4 during development and after brain injury. Not only is bone morphogenetic protein 4 known to repress Olig2, but it is also involved in synaptic plasticity. Further studies are needed to fully elucidate the molecular mechanisms by which OPCs regulate Olig2 expression.

Olig2 may participate in the progress of many neurological disorders. Overexpression of Olig2 is observed in disorders of neural development such as Down syndrome and autism spectrum disorder. However, clinical treatment of these disorders targets neurons and the therapeutic effect of such perturbation is still quite limited. The molecular mechanism of OPC function in controlling neural circuits is just beginning to be understood. Additional studies are needed to further elucidate the function and molecular mechanism of Olig2 involved in OPC control of neural circuits. Such studies focusing on myelinating glia would not only provide new insights into the regulation of neural circuits, but also offer alternative ways to modify central brain functions without targeting neurons. In the future, promising therapeutic approaches for neurodevelopmental disorders may be on the horizon.

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