Journal Club

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# Canonical Wnt Signalling in PDGFRα-Expressing Cells is a Critical Regulator of Astrogliosis and Axon Regeneration following CNS Injury

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Review of Rodriguez et al.

Spinal cord injury (SCI) in the mammalian CNS results in the formation of a glial scar around the lesion site (Fig. 1*A*). The scar limits axon regeneration but it also serves a protective role by sequestering inflammatory cells to the lesion center, reducing tissue damage (Herrmann et al., 2008). Although astrocytes have been thought to be the primary cellular source of the glial scar, recent work suggests the scar is comprised of a complex milieu of interacting cell types, including perivascular fibroblasts, stromal cells, inflammatory cells, and oligodendrocyte precursor cells (OPCs) (Cregg et al., 2014). OPCs, distinguishable from mature oligodendrocytes by their expression of NG2 and PDGFRα, rapidly proliferate and form new oligodendrocytes in the spared parenchyma after injury, likely mediating myelin repair (Tripathi and McTigue, 2007). However, proximal to the lesion core, OPCs are found at very high densities, often in close association with the dystrophic end-bulbs of axons that have failed to regenerate (Cregg et al., 2014). Given the interaction of NG2 cells with dystrophic end-bulbs of axons in the lesion, it seems plausible that reducing their contribution to scarring might enhance regeneration.

Wnt signaling is a likely candidate to regulate the OPC response after SCI. Oligodendrocyte development and OPC differentiation in demyelinating lesions are regulated by Wnt signaling (Fancy et al., 2011) and following SCI, Wnt ligand expression is increased (Fernández-Martos et al., 2011). β-catenin is an intracellular signal transducer in the Wnt signaling pathway (Fig. 1*A′*) and its deletion effectively halts canonical Wnt target gene expression (Fig. 1*B′*). In a recent study, Rodriguez et al. (2014) modulated the OPC response to CNS trauma by deleting β-catenin from PDGFRα-expressing cells, a population that includes OPCs. The authors crossed PDGFRαcreER$^{T2}$ mice with floxed β-catenin mice to excise β-catenin when tamoxifen is administered, just before SCI. Inducible expression of yellow fluorescent protein allowed tracking of the fate of recombined cells.

Rodriguez et al. (2014) first examined the effect of inducible deletion of β-catenin from PDGFRα-expressing cells on OPC density and proliferation in both the uninjured and injured CNS. The removal of β-catenin did not affect the percentage of cells that remained as OPCs (defined by the authors as expressing NG2) in the uninjured CNS 25 d later (Rodriguez et al., 2014, their Fig. 1*C*). This suggests that β-catenin deletion does not alter OPC differentiation in the uninjured CNS during this timeframe. However, after SCI, β-catenin inducible conditional knock-out (ICKO) mice had reduced NG2 cell density and proliferation, such that these cells failed to accumulate around the lesion (Fig. 1*B*) (Rodriguez et al., 2014, their Fig. 3). Because inhibiting canonical Wnt signaling promotes differentiation of OPCs in chemical demyelinating models (Fancy et al., 2011), accelerated differentiation of OPCs could explain the reduction in NG2 cell density observed by Rodriguez et al. (2014). The authors did not assess whether β-catenin deletion was sufficient to enhance OPC differentiation into mature oligodendrocytes after injury, however. Doing so may have yielded insight into the fate of these cells and their capacity to remyelinate.

Rodriguez et al. (2014) next examined astrogliosis and inflammation in β-catenin ICKO mice subjected to SCI. Astrogliosis was reduced in β-catenin ICKO mice; specifically, there were reductions in astrocyte hypertrophy, chondroitin sulfate proteoglycan (CSPG) expression, and formation of the compact glial fibrillary acidic protein (GFAP) lesion border typical of the glial scar (Fig. 1*B*) (Rodriguez et al., 2014, their Figs. 5

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and 6). Interestingly, the level of microglia/macrophage inflammation was also sharply reduced in the penumbra of β-catenin ICKO mice (Rodriguez et al., 2014, their Fig. 4). This comes as a surprise, as a failure of astroglial scar formation has previously been implicated in exacerbated microglia/macrophage spread and accumulation (Herrmann et al., 2008). However, in Rodriguez et al. (2014), changes in microglia/macrophage density occurred concurrently with changes in NG2 density but before the formation of a mature glial scar, indicating that OPCs may directly affect inflammation independently of subsequent astrogliosis.

Recent studies (Kang et al., 2013; Yuen et al., 2014) shed light on how OPCs might regulate inflammation following SCI. The amassing of inflammatory microglia/macrophages after SCI results from both local microglia proliferation/migration as well as the accumulation of blood-derived macrophages (Cregg et al., 2014). Importantly, Rodriguez et al. (2014) demonstrated that microglia/macrophage cell proliferation was not disrupted following β-catenin deletion (Rodriguez et al., 2014, their Fig. 4*C*), implying that β-catenin deletion from OPCs may be affecting the accumulation of blood-derived macrophages. Macrophage accumulation occurs concurrently with revascularization of the tissue (Casella et al., 2002). Recent work by Yuen et al. (2014) suggests that OPCs promote brain angiogenesis by directly inducing endothelial cell proliferation and blood vessel formation during development. Furthermore, OPCs regulate inflammation in experimental autoimmune encephalomyelitis by proinflammatory cytokine production and by promoting blood–brain barrier permeability (Kang et al., 2013). Thus, an intriguing possibility is that dampened inflammation in β-catenin ICKO mice following SCI is the result of an impairment in OPC-induced proinflammatory signals and angiogenesis, which is required for macrophage infiltration. Future studies should examine the role of OPCs in the formation of new vasculature after traumatic injury and the subsequent impact on the ability of monocytes to enter damaged tissue.

β-catenin ICKO from PDGFRα-expressing cells reduces injury-induced astrogliosis and CSPG expression, a known inhibitor of axon regeneration. Therefore, the knock-out may produce an environment permissive to axonal regeneration. To test this hypothesis, Rodriguez et al. (2014) crushed the optic nerve in β-catenin ICKO and control mice. β-catenin ICKO mice had less accumulation of recombined cells at the lesion site and greater axon growth up to 1 mm past the lesion relative to control (Rodriguez et al., 2014, their Fig. 7). Therefore, β-catenin deletion in PDGFRα-expressing cells promoted axon regeneration in the optic nerve. No histological assessment was made of supraspinal axon growth after SCI, which would demonstrate whether Wnt signaling in PDGFRα-expressing cells restricts axon growth following SCI.

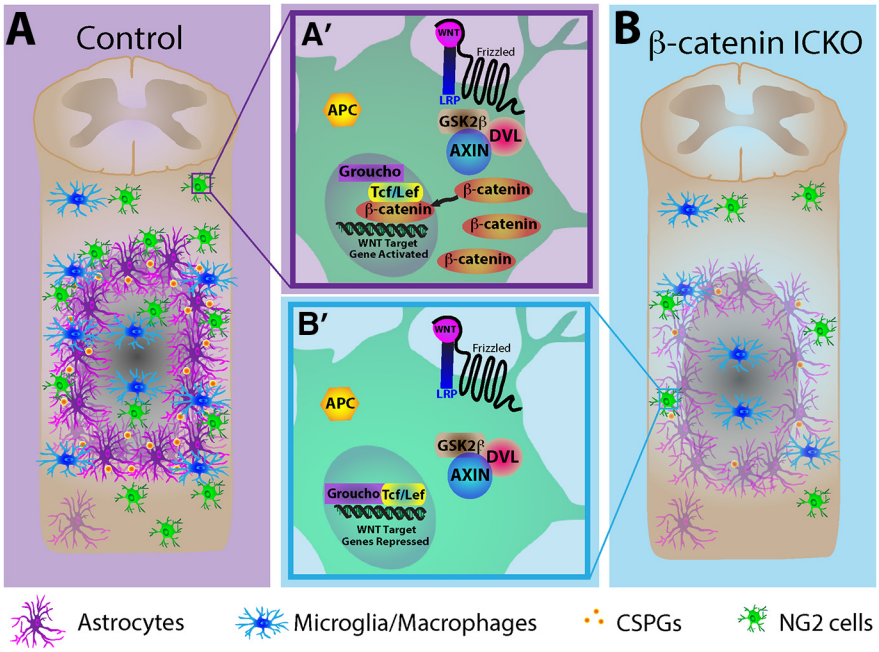


**Figure 1.** Deletion of β-catenin from NG2 glia before SCI reduces astrogliosis and inflammation. ***A***, An overview of the cellular response to SCI by glial and inflammatory cells. A prominent glial scar containing hypertrophic astrocytes (purple) and inflammatory microglia/macrophages (blue) is formed. NG2 glia (green) proliferate and accumulate around the lesion. They express Wnt target genes after SCI. ***A′***, β-catenin is the downstream signal inducer of canonical Wnt signaling. When Wnt ligands bind to the Frizzled receptor, Dishevelled (DVL)-LRP-Frizzled recruits the Axin complex to the receptors, allowing β-catenin to drive Wnt target gene expression in conjunction with the TCF/LEF family of transcription factors. ***B***, ICKO of β-catenin reduces astrogliosis, CSPG expression (yellow), microglial/macrophage accumulation, and NG2 recruitment to the lesion. Lacking hypertrophic astrocytes, the boundary of the glial scar does not form a compact border. ***B′***, β-catenin deletion inhibits Wnt target gene expression in the presence of Wnt ligands.

Although Rodriguez et al. (2014) attribute the changes in astrocytic, inflammatory, and regenerative responses following injury to abrogation of β-catenin in OPCs specifically, it is worth noting that PDGFRα is also expressed in pericytes (Göritz et al., 2011). Indeed, recombination in pericytes of PDGFRαcreER mice occurs after tamoxifen administration (Kang et al., 2010), so the mice used by Rodriguez et al. (2014) (β-catenin$^{fl/fl}$:PDGFRαcreER$^{T2}$) would lack β-catenin in PDGFRα-expressing pericytes. Considering that pericytes in the CNS can also express NG2 (Ozerdem et al., 2001), the marker Rodriguez et al. (2014) use for OPCs, measurements of both NG2 and recombined cell density after SCI in β-catenin ICKO may also reflect changes in pericyte density. Pericytes have a robust role after SCI: they dissociate from the vasculature and contribute extensively to fibrosis within the lesion core (Göritz et al., 2011). In addition, studies on kidney fibrosis suggests that Wnt signaling is active in pericytes and drives their differentiation into myofibroblasts (DiRocco et al., 2013). These myofibroblasts express αSMA like some pericyte-derived cells do after SCI (Göritz et al., 2011). Together, this evidence suggests a role for β-catenin in the accumulation and possibly the differentiation of fibrotic pericyte derived-cells after SCI. Changes in astrogliosis, inflammation, and axon regeneration described by Rodriguez et al. (2014) might result, at least in part, from pericytic β-catenin deletion.

In summary, the work of Rodriguez et al. (2014) highlights a critical role for β-catenin in PDGFRα-expressing cells to promote astrogliosis and inflammation, and attenuate axon regeneration after injury. The pathway has been modulated *in vivo* using small molecule inhibitors (Fancy et al., 2011) and evidence from Rodriguez et al. (2014) strongly suggests that reducing Wnt signaling warrants therapeutic investigation for SCI.

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