Inhibition of central axon regeneration: perspective from chondroitin sulfate proteoglycans in lamprey spinal cord injury

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Background: Failure of axon regeneration after spinal cord injury (SCI) underlies the paralysis that so profoundly affects patients’ quality of life. Many factors are involved in the regeneration failure. Chondroitin sulfate proteoglycans (CSPGs), normal constituents of the perineuronal nets in central nervous system (CNS), are sequested at the injury site and initially were thought to act as a purely physical barrier. In the past decade, the receptor-like protein tyrosine phosphatases, protein tyrosine phosphatase sigma (PTPσ), and leukocyte common antigen-related phosphatase (LAR), have been identified as transmembrane receptors for CSPGs. The two receptors for myelin-associated growth inhibitors, Nogo receptors 1 and 3 (NgR1 and NgR3) also have been found to bind with CSPGs (Sharma et al., 2012). These findings suggest that CSPGs inhibit regeneration by interacting with these receptors, initiating downstream inhibitory signaling (Figure 1).

Modulations of CSPG levels or CSPG receptor activities have been intensively studied to determine their roles in axon regeneration after SCI. Many studies have shown that removing the polysaccharide side chains of CSPGs with chondroitinase ABC (ChABC) reduce their axon growth inhibitory effects. In rats with bilateral dorsal column lesions, ChABC treatment promoted growth of spinal axons and functional recovery (Bradbury and Carter, 2011). Digestion of CSPGs with ChABC enhanced sensory recovery after unilateral cervical rhizotomy, which was accomplished via reorganization of intact C7 primary afferent terminals – not by regeneration of severed afferents back into the spinal cord (Bradbury and Carter, 2011). Chondroitin sulphate N-acetylgalactosaminyl-transferase-1 knockout mice recover more completely from SCI than wild-type mice and even ChABC treated mice. Further investigation showed that the knockout mice have upregulated synthesis of heparan sulphate, which promotes axonal growth. ChABC treatment removes CSPGs, but does not increase heparan sulphate (Takeuchi et al., 2013), which can explain the limited efficacy of ChABC treatment in SCI. Taken together, these studies suggest that digestion of CSPGs with ChABC enhances axon sprouting and functional recovery after SCI. Besides the extracellular matrix CSPGs, there are proteoglycans integrated into the cell membrane, of which Neurol Rea Res | Vol 17 | No. 9 | September 2022 | 1955
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site of injury, e.g., glia or infiltrating immune cells, might have indirect effects on RS axon regeneration. The digestion of CSPGs with ChABC at the site of SCI significantly reduced PTPo mRNA expression in the perikarya of the axotomized RS neurons in brain, suggesting that the upregulation of PTPo mRNA observed in RS neurons after SCI is partly due to the actions of elevated CSPGs (Hu et al., 2021). This finding indicates that the locally elevated CSPG levels at the site of injury affect the ability of the RS to form new functional synapses.

Similarity between CSPGs and CSPG receptors has been suggested in mammals. PTPo and LAR are concentrated in dystrophic, stabilized growth cones in vitro, and PTPo levels are elevated in the lesion penumbra following SCI (Lang et al., 2015). Although these studies focused mainly on CSPG receptor levels locally at the site of injury, they are consistent with our findings in lampreys.

Retrograde apoptosis: We previously found that axotomized neurons known to be bad regenerators in lamprey brain eventually die by a very delayed form of TUNEL-positive apoptosis. To dissect the mechanisms involved in this delayed apoptosis, we adapted the use of fluorescently-labeled inhibitors of caspase activation (FLICA) to image apoptotic signaling as early as 1 week after SCI. The number of identified RS neurons containing activated caspases increased significantly post-TX, compared to controls. Digestion of CSPGs with ChABC significantly reduced the number of caspase-positive RS neurons post-TX, and this was accompanied by a reduction in PTPo mRNA expression in the perikarya of the axotomized RS neurons (Hu et al., 2021). This had been suggested previously by the selective extinction of bad regenerators, a bad survivor RS neurons, which became FLICA-positive after SCI. Thus, PTPo might play a role in retrograde neuronal apoptosis.

Akt: Knockout of phosphatase and tensin homolog (PTEN) promotes potent CNS axon regeneration after injury, and the signaling molecules downstream of PTEN that mediate this effect have been studied extensively. Akt is a critical pro-survival molecule and its activation is sufficient to promote optic nerve regeneration, but the regeneration is not as robust as that with PTEN deletion. Akt plays similar roles in the regeneration of lamprey CNS axons after SCI. ChABC treatment greatly promoted axonal regeneration after SCI, and this was accompanied by widespread enhancement of Akt activation (pAkt-308) in individual identified RS neurons (Hu et al., 2021) (Figure 1). ChABC treatment protected identified RS neurons from retrograde apoptosis. The Akt activation pattern in RS neurons post-TX correlated with the observed effect of ChABC treatment on neuronal survival. Thus, Akt activation probably contributes to the beneficial effect of ChABC treatment in protecting RS neurons from retrograde apoptosis after SCI.

Erk: In vitro neuronal cultures, extracellular regulated kinase (Erk) is one of the signaling molecules downstream of CSPGs and their receptors PTPo and LAR. CSPG application reduced Erk activity in neurons, but deletion of either PTPo or LAR alone did not eliminate the Erk inactivation by CSPGs, indicating that Erk signaling mediates actions of both of these receptors in neurons (Ohtake et al., 2016) (and perhaps other unidentified receptors that may mediate CSPG-induced inhibition of axon growth). Deletion of both PTPo and LAR resulted in greater enhancement of axon growth than either alone, indicating that although they both inactivate Erk, other elements of their downstream signalling diverge (Figure 1). Systemic inhibition of PTPo with small peptides showed that Erk is involved in peripheral axon regeneration. Studies on Drosophila larvae showed that specifically activating the Erk and Akt signalling pathways enhanced axon regeneration of the sensory neurons in both PNS and CNS (Wang et al., 2020a). These reports strongly suggest that Erk might be involved in the downstream signalling pathway after ChABC treatment (Hu et al., 2021). We also examined the expression pattern of Erk after SCI in the lamprey (Jin et al., 2020). Erk activation increased rapidly within axons and local cell bodies, most heavily within the 1–2 mm closest to the TX site at between 3 and 6 hours post-TX. Activated Erk colocalized with the retrograde transport motor protein dynein in membranous granules distributed along the axons, indicating that activated Erk may function as a retrograde signal in the lamprey CNS. But unlike descriptions in mammalian neurons in vitro and in PNS, these granules did not stain for vimentin in axotomized lamprey RS neurons. The detailed roles played by Erk and other signalling molecules in SCI, and how they are affected by ChABC treatment remain to be worked out.

Future perspectives: The ChABC itself has the characteristics of thermo-in-stability, which limits its long-lasting efficacy inside the human body (temperature is about 37°C). In the past few years, different methods of delivery, such as nanoparticles or synthetic scaffolds, have been explored and shown promising results in animal SCI models, but these methods still need further optimization to achieve better efficacy (Muir et al., 2019). ChABC treatment for SCI is not yet in clinical trials, while the enzyme has been assessed in a phase III trial in Japan as an alternative to surgical intervention for lumbar disc herniation, in which the ChABC is injected into the lumbar disc to digest the nucleus pulposus (Muir et al., 2019). This shed light for the potential use of ChABC for SCI treatment in humans.

In summary, a successful approach to promoting CNS axon regeneration should combine manipulations of both intracellular and extracellular mechanisms, for example, to remove extracellular inhibitors, enhance expression of intracellular pro-regeneration molecules, and promote the formation of functional synapses by newly-regenerating axons (Figure 1). A better understanding of the mechanisms that bridge the environmental and intraneuronal influences on axon regeneration will allow us to design better treatments that enhance recovery from SCI.

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