PERSPECTIVE

Role of chondroitin sulfate proteoglycan signaling in regulating neuroinflammation following spinal cord injury

Spinal cord injury (SCI) elicits a robust inflammatory response that is a hallmark of the secondary injury mechanisms. Neuroinflammation is orchestrated initially by the response of resident astrocytes and microglia to injury, which subsequently facilitates the recruitment of peripheral immune cells into the SCI lesion (Orr and Gensel, 2018). This inflammatory response contributes to cell death and tissue degeneration through the production of pro-inflammatory cytokines and chemokines, free radicals and proteolytic enzymes. However, neuroinflammatory cells also play beneficial regulatory role in repair mechanisms after SCI by adopting a reparative and wound healing phenotype (Orr and Gensel, 2018; Tran et al., 2018). Hence, understanding the underlying mechanisms by which immune cells are regulated within the microenvironment of injury would aid in harnessing the reparative potential of inflammation following SCI.

Remodeling of the extracellular matrix in the injured spinal cord has critical impact on both injury and repair mechanisms. It is well-established that robust upregulation of chondroitin sulfate proteoglycans (CSPGs) by activated glia including astrocytes, microglia and oligodendrocyte precursor cells dramatically alters the composition of the extracellular matrix within the SCI lesion (Tran et al., 2018). A plethora of data has demonstrated that CSPGs negatively regulate several aspects of the repair process including neuronal survival, synaptogenesis, axonal sprouting, regeneration and conduction, as well as replacement of oligodendrocytes and remyelination (Karimi-Abdolrezaee et al., 2010; Tran et al., 2018). Given the multifaceted inhibitory role of CSPGs in the injured spinal cord, their manipulation has become a promising therapeutic approach for functional and physiological improvements following SCI. Interestingly, despite this wealth of information; the role and mechanisms of CSPGs in modulating neuroinflammation are still emerging.

After SCI, the complex innate and adaptive immune responses can be both beneficial and detrimental, based on the timing and phenotype of immune cells within the injury site (Orr and Gensel, 2018). Resident microglia and infiltrating monocyte-derived macrophages play critical roles in initiation and progression of the inflammatory response as well as the wound healing and regenerative processes in the injured spinal cord. Classically activated M1 microglia and macrophages are thought to promote a pro-inflammatory milieu following injury due to the production of a host of pro-inflammatory cytokines and mediators. On the other hand, alternatively activated M2 microglia and macrophages are involved in resolution of inflammation and wound healing through phagocytosis of myelin debris and their ability to provide anti-inflammatory and growth promoting factors (Orr and Gensel, 2018; Tran et al., 2018). Importantly, an anti-inflammatory M2 phenotype has been associated with improvements in axonal sprouting and regeneration in SCI and oligodendrocyte maturation and remyelination in multiple sclerosis (Miron et al., 2013; Tran et al., 2018). In mice with SCI, there is initially a relatively equal number of M1 and M2 microglia and macrophages in the spinal cord, which over time, shifts to an increasingly more prominent M1 inflammatory response (Kigerl et al., 2009). These findings indicate that the microenvironment of SCI appears to favour an M1 phenotype. This notion was supported when transplantation of M2 macrophages into the injured spinal cord at 7 days post-SCI drove the majority of these cells to adopt an M1 phenotype shortly after transplantation (Kigerl et al., 2009). This evidence collectively suggests the presence of extrinsic mechanisms that regulate the phenotype of microglia and macrophages within the injured spinal cord.

Emerging evidence from our group and others has identified a pro-inflammatory role for CSPGs in the lesions of SCI and multiple sclerosis (Didangelos et al., 2014; Dyck et al., 2018; Stephenson et al., 2018). Didangelos and colleagues demonstrated that degradation of CSPGs with chondroitinase ABC promotes an M2 response after SCI marked by a robust increase in the expression of interleukin (IL)-10 (Didangelos et al., 2014). Studies on multiple sclerosis and experimental autoimmune encephalomyelitis conditions have unraveled that CSPGs are upregulated within inflammatory lesions particularly in "the leucocyte-containing perivascular cuff", where immune cells are accumulated and enter the central nervous system (Stephenson et al., 2018). Abundance of CSPGs in these perivascular cuffs facilitates trafficking of leukocytes to the central nervous system parenchyma and promotes their pro-inflammatory response (Stephenson et al., 2018). While the involvement of CSPGs in immune modulation has been recognized, the cellular and molecular mechanisms of CSPGs have yet to be elucidated.

In recent years, identification and characterization of specific CSPGs signaling receptors including, leukocyte common antigen-related (LAR) and protein tyrosine phosphatase-sigma (PTPσ), has opened a new avenue to unravel CSPG cell-specific mechanisms in SCI (Shen et al., 2009; Dyck et al., 2015; Lang et al., 2015; Tran et al., 2018). Of note, while Nogo receptors NgR1 and NgR3 have also been identified as CSPG receptors, LAR and PTPσ are shown to be primarily involved in CSPG signalling in the injured spinal cord (Tran et al., 2018). Growing evidence shows that genetic and pharmacological manipulation of LAR and PTPσ receptors is sufficient to overcome the inhibitory effects of CSPGs in the injured spinal cord, and in multiple cell types *in vitro* including neurons, neural precursor cells (NPCs) and microglia (Dyck et al., 2015, 2018; Lang et al., 2015). These discoveries have identified LAR and PTPσ receptors as new potential targets for inhibiting the detrimental effects of CSPGs in SCI.

Activity of LAR and PTPσ can be effectively blocked by specific membrane permeable peptides; Intracellular LAR Peptide (ILP) and Intracellular Sigma Peptide (ISP) (Lang et al., 2015; Dyck et al., 2018; Tran et al., 2018). Importantly,

this strategy has allowed studying the role of CSPG/LAR and CSPG/PTPσ signaling in clinically relevant models of SCI. Recent studies show that inhibition of PTPσ with ISP promotes serotonergic innervation below the level of injury, which is associated with improved neurological recovery in rats with contusive SCI (Lang et al., 2015). Studies on neurons *in vitro* also indicate that CSPGs induce growth cone dystrophy and inhibition of process outgrowth through the activation of PTPσ receptor (Lang et al., 2015; Tran et al., 2018). Our group has also identified a direct negative role for CSPGs in regulating the behaviour of NPCs (Dyck et al., 2015). In primary cultures of spinal cord-derived NPCs, direct exposure to CSPGs inhibits several cellular properties of NPCs including their growth, migration, survival, proliferation, and oligodendrocyte differentiation (Dyck et al., 2015). We uncovered that CSPGs exert their effects on NPCs by signalling through both LAR and PTPσ receptors as well as activation of the Rho/Rho-associated protein kinase (ROCK) pathway. At the intracellular level, activation of CSPGs signaling declines the phosphorylated state of Akt and extracellular-signal-regulated kinase (Erk)1/2 in NPCs, which appears to be downstream mediators of CSPG effects on spinal cord NPCs (Dyck et al., 2015). These findings suggest that the regenerative response of spinal cord NPCs can be influenced by SCI-induced upregulation of CSPGs due to their expression of LAR and PTPσ receptors.

Capitalizing on these findings, we recently investigated whether LAR and PTPσ modulate inflammatory response after SCI (Dyck et al., 2018). In a clinically-relevant model of compressive/contusive SCI in the rat, we blocked LAR and PTPσ with intrathecal delivery of ILP and ISP to the areas surrounding the spinal cord lesion. Our studies identified a novel role for LAR and PTPσ receptors in regulating several components of the immune response. Blockage of LAR and PTPσ allowed a shift from an M1 pro-inflammatory to an M2 pro-regenerative phenotype in microglia and macrophages, which was accompanied by an upregulation in IL-10 and arginase-1 protein expression (Dyck et al., 2018). Of note, increase in the population of IL-10 expressing M2 cells in the injured spinal cord tissue has been associated with a reparative phenotype and better outcomes (Orr and Gensel, 2018). Interestingly, our studies also unraveled a new inhibitory role for LAR and PTPσ in regulating T cell response in SCI (Dyck et al., 2018). While ILP/ISP treatment had no effects on the overall number of T helper cells, there was a significant decrease in the population of T effector cells expressing interferon-gamma and instead an increase in the number of IL-10 and FOXP3 expressing T regulatory cells (Dyck et al., 2018). These findings, for the first time, suggest the involvement of CSPG signaling receptors in regulation of both innate and adaptive immune responses.

To provide mechanistic insights into the role of LAR and PTPσ signaling in neuroinflammation, we studied microglia in culture (Dyck et al., 2018). Interestingly, we found that CSPGs do not induce an M1 phenotype in resting microglia *per se*. However, the presence of CSPGs in the milieu of M1 polarized microglia promoted their pro-inflammatory phenotype while suppressing IL-10 release by M2 polarized microglia (Dyck et al., 2018). Importantly, the effects of CSPGs on M1 and M2 polarization was ameliorated by inhibition of LAR and PTPσ signaling (Dyck et al., 2018). These *in vitro* findings corroborated our SCI assessments uncovering a previously unknown regulatory role for LAR and PTPσ in modulating microglia polarization. Additionally, our studies identified an inhibitory role for CSPGs in regulating microglia mobilization and phagocytosis (Dyck et al., 2018). Migration of activated microglia to the site of central nervous system injury is important for their contribution to the repair process including phagocytosis of debris and wound healing. Our data identified that M1 polarization or the presence of CSPGs restricts microglia mobility and diminishes their ability for phagocytosis. In this regard, our direct *in vitro* systems revealed that the effects of CSPGs on microglia mobility and phagocytosis is mediated through LAR/PTPσ signaling as well as intracellular activation of the Rho/ROCK pathway (Dyck et al., 2018). Interestingly, in M1 microglia, inhibition of LAR and PTPσ also promoted phagocytosis in the absence of CSPGs suggesting that these receptors may interact with other ligands or have other functions (Dyck et al., 2018). Further investigation is required to elucidate the underlying mechanisms of LAR and PTPσ in microglia phagocytosis. Nevertheless, promoting the ability of microglia for phagocytosis is beneficial for the repair process since impaired phagocytosis of myelin debris by microglia has been correlated with limited tissue regeneration in SCI (Orr and Gensel, 2018; Tran et al., 2018). Altogether, these new findings provide evidence suggesting that long-lasting upregulation of CSPGs in the extracellular matrix of SCI may underlie the ineffective and prolonged clearance of debris in the injured spinal cord. To our knowledge, this study is the first to identify the impact of CSPGs, and LAR and PTPσ signaling on microglia polarization, mobilization and phagocytosis.

CSPGs are shown to limit cell replacement activities in the injured spinal cord (Karimi-Abdolrezaee et al., 2010). Previously, we reported that degradation of CSPGs with chondroitinase ABC promotes endogenous replacement of oligodendrocytes after SCI. We therefore hypothesized that this effect of chondroitinase ABC may be partially due to its beneficial role in modulating microglia response in the injured spinal cord (**Figure 1**) (Didangelos et al., 2014; Dyck et al., 2018). The importance of microglia in regulating endogenous cell replacement is becoming increasingly appreciated in central nervous system injury. For example, pro-inflammatory cytokines such as tumor necrosis factor α and IL-6 inhibit hippocampal neurogenesis in the lipopolysaccharide-treated brain whereas anti-inflammatory cytokines such as insulin-like growth factor-1 and IL-10 promote cell renewal (Miron and Franklin, 2014). Our complimentary *in vitro* assessment demonstrated that M2 microglia promote proliferation and oligodendrocyte differentiation of NPC in a paracrine fashion (Dyck et al., 2018). Although multiple trophic factors likely contributed to these effects, IL-10 was found to play an important part in promoting oligodendrogenesis in NPC culture (Dyck et al., 2018). Similar findings have been shown in OPCs in an animal model of multiple

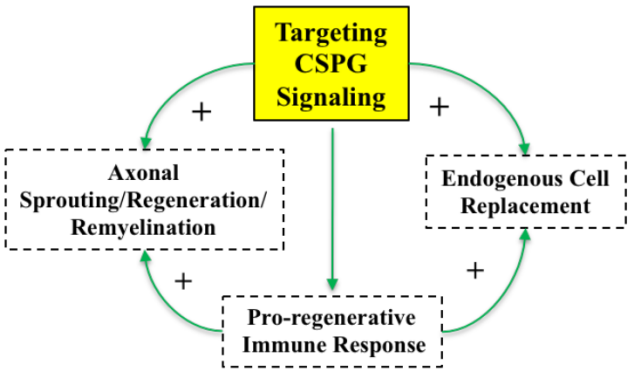

Figure 1 Targeting chondroitin sulfate proteoglycan (CSPG) signaling has a multifaceted beneficial effect on endogenous repair mechanisms after spinal cord injury (SCI).
It has been established that CSPGs negatively modulate axonal sprouting and regeneration as well as endogenous oligodendrocyte replacement and remyelination following SCI. New evidence has uncovered that targeting CSPGs with chondroitinase ABC or inhibition of their receptors, leukocyte common antigen-related and protein tyrosine phosphatase-sigma, can also drive a pro-regenerative inflammatory response after SCI. We therefore propose that the beneficial effects of targeting CSPGs and their signaling receptors after SCI is partially due to modulation of the inflammatory response.

sclerosis (Miron et al., 2013). In this study, Miron et al. (2013) identified M2-derived activin-A as an important mediator of OPC maturation and remyelination *in vitro* and following a lysolecithin-induced demyelination model. Altogether, modulation of the immune response appears to be a viable strategy to promote endogenous cell replacement after SCI and other central nervous system diseases. Importantly, CSPGs also restrict the outcomes of NPC therapies in chronic SCI. We have shown that dramatic upregulation of CSPGs in chronic lesions poses a challenge to transplantation of NPCs into the injured spinal cord (Karimi-Abdolrezaee et al., 2010). Interestingly, pre-treatment of the injured spinal cord with chondroitinase ABC prior to cell transplantation allowed NPCs to survive and integrate into the spinal cord cellular network, indicating a critical role for CSPGs in regulating NPCs (Karimi-Abdolrezaee et al., 2010). Collectively existing findings suggest that CSPGs negatively influence the regenerative potential of NPCs directly through LAR and PTPσ mediated mechanisms (Dyck et al., 2015) or indirectly through their pro-inflammatory effects following SCI (Dyck et al., 2018).

In conclusion, new evidence has identified a pro-inflammatory role for CSPGs and their signaling receptors LAR and PTPσ in the secondary injury mechanisms after SCI. Upregulation of CSPGs is a long-lasting pathology after SCI with a multifaceted inhibitory impact on the repair process. Currently, there is an unmet need to develop clinically-relevant strategies to target CSPGs in SCI. Growing evidence suggests that manipulation of CSPG receptors renders a feasible and targeted strategy to optimize the hostile microenvironment of the injured spinal cord for repair and regeneration.

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