PERSPECTIVE

Neuregulin-1: a novel regulator of glial response in spinal cord injury

Spinal cord injury (SCI) results in a dysregulated microenvironment that is largely driven by the immediate and robust response of resident astrocytes and microglia (Filous and Silver, 2016). Activated glial cells initiate a complex innate and adaptive immune response that regulates secondary injury mechanisms with both destructive and supportive impact on the repair processes after SCI (Karimi-Abdolrezaee and Billakanti, 2012; Filous and Silver, 2016). Profound changes in the activity of astrocytes through their interactions with immune cells also result in matrix remodeling and formation of a glial scar within the injured spinal cord (Karimi-Abdolrezaee and Billakanti, 2012; Silver et al., 2014). Astrogliosis is a critical process in SCI that limits the extent of neuroinflammation and supports re-construction of the blood-spinal cord barrier and angiogenesis by secreting growth factors and cytokines (Silver et al., 2014). However, activated astrocytes also release a plethora of inhibitory factors into the extracellular matrix including chondroitin sulphate proteoglycans (CSPGs), which contribute to the impermissible microenvironment of SCI. This matrix modification and upregulation of CSPGs are known to notoriously limit axonal regeneration and cell replacement after SCI (Karimi-Abdolrezaee and Billakanti, 2012; Silver et al., 2014).

Growing evidence suggests that the cross-talk between astrocytes and immune cells through cytokines and chemokines determines glial function and their role in repair process following SCI (Silver et al., 2014; Dyck and Karimi-Abdolrezaee, 2015). Immune mediators can act either as initial molecular inducers [*e.g.*, interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ)] or repressors [*e.g.*, interleukin-10 (IL-10)] of astrocyte proliferation and glia scar formation (Karimi-Abdolrezaee and Billakanti, 2012). Upon activation, reactive astrocytes also participate in neuroinflammation and actively modulate the response of microglia and infiltrating immune cells after injury (Silver et al., 2014). The outcomes of these reciprocal interactions generate an imbalanced inflammatory microenvironment in the injured spinal cord that restricts the endogenous machinery of the injured spinal cord for tissue reconstruction (Dyck and Karimi-Abdolrezaee, 2015).

Degeneration of oligodendrocytes, and consequently demyelination and loss of axons are hallmarks of acute SCI. Studies by our group and others indicate that the ability of endogenous and transplanted precursor cells for regeneration of damaged oligodendrocytes and remyelination is challenged in the post-SCI milieu largely due to the magnitude of glial-derived inhibitory signals, and dysregulation of key supportive factors following injury. Oligodendrocytes and myelin are critical for axonal integrity. Thus, identification of key extrinsic factors that promote oligodendrogenesis at the early stage of SCI can attenuate axonal degeneration and functional deficits. Emerging evidence shows that modulation of activated glia is a critical step to promote white matter repair after SCI (Filous and Silver, 2016).

We have previously identified that the neuronally-derived growth factor neuregulin-1 (Nrg-1) is acutely and permanently dysregulated after compressive SCI in rats (Gauthier et al., 2013). Nrg-1 is a member of the neuregulin family, which plays a pivotal role in the normal development and physiology of oligodendrocytes, axons and myelin in the central nervous system (CNS) (Mei and Nave, 2014). Evidence from our group and others also shows that Nrg-1 regulates oligodendrocyte differentiation in adult neural precursor cells (NPCs) and downregulation of Nrg-1 or deficiency in ErbB receptors impairs oligodendrocyte maturation (Gauthier et al., 2013; Mei and Nave, 2014). In the spinal cord, majority of cell types including NPCs, oligodendrocyte precursor cells (OPCs), glial cells and neurons express Nrg-1 receptors, ErbB2, ErbB3, and ErbB4, suggesting that the decreased levels of Nrg-1 after SCI may have serious ramifications on several aspects of secondary injury mechanisms. Our studies and others have shown that administration of Nrg-1 in the injured spinal cord is sufficient to promote endogenous oligodendrogenesis, and enhance oligodendrocyte and axonal preservation (Whittaker et al., 2012; Gauthier et al., 2013). A recent study by Bartus et al. (2016) has shown that Nrg-1 is essential for endogenous remyelination after SCI. We have shown that astrocytes and microglia express ErbB receptors and therefore, they can respond to changes in Nrg-1 levels (Gauthier et al., 2013). However, the role and mechanisms of Nrg-1 in modulating their response have not been studied in SCI.

In the study by Alizadeh et al. (2017), we investigated the hypothesis that dysregulation of Nrg-1 in acute SCI is an underlying cause of the imbalanced glial response and poor recovery after SCI. On this line, we extensively characterized the functional impact of Nrg-1 on astrogliosis and neuroinflammation. Utilizing clinically relevant SCI and *in vitro* models, we demonstrated that intrathecal administration of Nrg-1 positively modulates several aspects of glial activity following SCI. Nrg-1 treatment remarkably attenuated the production of key pro-inflammatory mediators including nitric oxide, interleukin-1 beta (IL-1β) and TNF-α as well as matrix metalloproteinase (MMP) 2 and 9 following SCI. These cytokines and MMPs are associated with an M1 pro-inflammatory response in SCI and trigger cell death and tissue degeneration (Donnelly and Popovich, 2008). Importantly, our work identified that availability of Nrg-1 can promote a pro-regenerative inflammatory response in SCI characterized by upregulation of arginase-1 (Arg1) and IL-10. These mediators are well-known for their beneficial immunomodulatory effects on attenuating pro-inflammatory cytokine production by activated microglia/macrophages following CNS injury (Thompson et al., 2013). Studies in CNS demyelinating lesions have also unraveled a direct role for IL-10 produced by M2 polarized microglia in enhancing oligodendrocyte preservation (Thompson et al., 2013). Moreover, the positive role for Nrg-1 in oligodendrogenesis and oligodendrocytes preservation in rat SCI (Whittaker et al., 2012; Gauthier et al., 2013) appears to be closely correlated with Nrg-1 induced upregulation of IL-10 (Alizadeh et al., 2017).

Another important outcome of our study was the discovery of a novel role for Nrg-1 in moderating glial scarring and notably production of CSPGs in SCI. CSPGs play a multifaceted inhibitory role in spinal cord regeneration including their inhibition of axon regeneration and sprouting (Karimi-Abdolrezaee and Billakanti, 2012; Dyck and Karimi-Abdolrezaee, 2015; Filous and Silver, 2016). Our group and others have also identified an inhibitory role for CSPGs in regulating the regenerative response of NPCs and OPCs in SCI and demyelinating lesions (Dyck and Karimi-Abdolrezaee, 2015). Therefore, the ability of Nrg-1 to modulate CSPGs production has a great impact on cell replacement process in SCI. Indeed, the positive impact of the Nrg-1 on modulation of microenvironment was established when we observed that Nrg-1 treatment led to improved recovery of locomotion without any adverse effects on allodynia following SCI.

On a mechanistic point of view, we found that Nrg-1 exerts its immunomodulatory effects by activating ErbB2/ErbB3 receptor complex and modulating multiple intracellular pathways involved in inflammatory response. Nrg-1 treatment resulted in increased extracellular signal-regulated kinase 1/2 (Erk1/2) and signal transducer and activator of transcription 3 (STAT3) phosphorylation while supressing myeloid differentiation primary

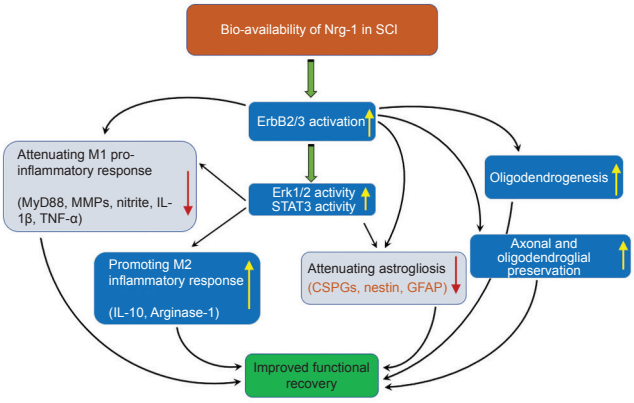

Figure 1 Our current proposed mechanisms of neuregulin-1 (Nrg-1) in spinal cord injury (SCI).
We demonstrate that restoration of Nrg-1 levels in the injured spinal cord has multifaceted beneficial effects that results in better neurological recovery after SCI. Nrg-1 reduces proinflammatory factors including interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), matrix metalloproteinases (MMP-2 and -9) and nitric oxide after injury (NO) while promoting a neuroprotective phenotype in inflammatory cells associated with increased interleukin-10 (IL-10) and arginase-1 expression. Beneficial effects of Nrg-1 are partly through modulation of astrogliosis and scar formation by reducing inhibitory chondroitin sulfate proteoglycans after SCI. Nrg-1 effects on activated glia appear to be mediated through ErbB2 tyrosine phosphorylation in an ErbB2/3 heterodimer complex. The downregulation of myeloid differentiation primary response 88 (MyD88), a downstream adaptor of Toll-like receptors, and increased phosphorylation of extracellular signal-regulated kinase 1/2 (Erk1/2) and signal transducer and activator of transcription 3 (STAT3) were identified as intracellular mechanisms involved in the effects of Nrg-1. Collectively, Nrg-1 treatment promotes oligodendrogenesis, improves tissue preservation and functional recovery following SCI. CSPGs: Chondroitin sulphate proteoglycans; GFAP: glial fibrillay acidic protein.

response 88 (Myd88) protein in subacute phase of SCI. Of note, Myd88 is a key mediator in Toll-like receptors (TLRs) pro-inflammatory cascade (Pineau et al., 2010). Activation of Erk1/2 signaling is an established pathway in glial activation following CNS injuries (Karimi-Abdolrezaee and Billakanti, 2012; Dyck and Karimi-Abdolrezaee, 2015). STAT3 is also a key signaling mechanism implicated in IL-10 mediated anti-inflammatory response in microglia/macrophages (Karimi-Abdolrezaee and Billakanti, 2012; Thompson et al., 2013). Taken together, our data collectively establish a positive role for Nrg-1 in modulating a favorable response by astrocytes and leukocytes in SCI. Further elucidation is needed to dissect the role and mechanisms of Nrg-1 in immune response at various stages of SCI.

In conclusions, our studies have provided novel insight into the impact of Nrg-1 on secondary injury mechanisms after SCI. We have demonstrated that bio-availability of Nrg-1 through intrathecal delivery promotes endogenous repair mechanism after SCI that can be attributed to its remarkable ability to promote IL-10 release while reducing CSPGs production. Importantly, we have identified that restoration of Nrg-1 improves recovery of neurological functions following SCI through multiple putative mechanisms that include: (i) modulation of activated glia and immune cells, (ii) attenuating glial scar formation and CSPGs production, (iii) promoting oligodendrogenesis, and (iv) enhancing preservation of white matter after injury (**Figure 1**). Therefore, owing to the multifaceted beneficial roles of Nrg-1, it demonstrates the potential to serve as a therapeutic target for the treatment of SCI. However, additional work is needed to elucidate the feasibility of systemic Nrg-1 delivery and any associated systemic side effects of Nrg-1 treatment after SCI as this study primarily focussed on restoration of Nrg-1 levels through intrathecal delivery in the injured rat spinal cord. Given Nrg-1 is a growth factor involved in cardiovascular physiology, further investigation into the systemic effects of Nrg-1 after SCI is essential in future therapeutic studies.

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Hardeep Kataria, Soheila Karimi-Abdolrezaee*
Regenerative Medicine Program, Department of Physiology and Pathophysiology, Spinal Cord Research Centre, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada
***Correspondence to:** Soheila Karimi-Abdolrezaee, Ph.D., Soheila.Karimi@umanitoba.ca.*
***orcid:** 0000-0002-0683-2663 (Soheila Karimi-Abdolrezaee)*
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