

● PERSPECTIVE

Perspectives on “the biology of spinal cord regeneration success and failure”

In our recently co-authored *Physiological Reviews* manuscript entitled “The biology of regeneration failure and success after spinal cord injury” (Tran et al., 2018b), we sought to provide a comprehensive and up-to-date description of how the glial scar develops following spinal cord injury (SCI) to chronically inhibit axon regeneration. Our additional intention was to clarify some of the confusion in the field relating to an oversimplified view of the glial scar. We would like to take this opportunity to reiterate how the current body of literature, expounding details of the glial scar, has progressed beyond a simplified and outdated understanding of this structure as a mono-cellular arrangement consisting only of astrocytes that solely limit axon regeneration. Instead, our perception of the glial scar has evolved to acknowledge the nuances of this multi-cellular structure to one that is able to limit the expansion of inflammatory processes shortly following SCI and that also persists chronically to limit axon regeneration. Further, we would like to expand upon some of the details presented in the review by offering an outlook on the current state of the field and avenues for progression. This includes the specific role of chondroitin sulphate proteoglycan components in axonal growth and plasticity, and the current assessment of SCI at different stages post injury. As such, this perspective should be considered a companion piece to our original work, adding new insights from across the field from recent publications.

A multicellular view of the glial scar: While astrocytes have been the most prevalently studied component of the glial scar, and are undoubtedly an important functional component, this tissue additionally comprises many other glial and cellular constituents including oligodendrocyte progenitor cells, microglia, macrophages, and fibroblasts/pericytes. Together, these various cell types respond to the inflammatory milieu initiated by injury by becoming “reactive.” Often, this stereotyped response includes such cellular changes as hypertrophy, increased motility, self-proliferation, and a great production of pro-inflammatory factors. Reactive astrocytes, for example, exhibit increased upregulation of intermediate filament proteins such as glial fibrillary acidic protein (GFAP), hypertrophy, and other changes in morphology when exposed to a pro-inflammatory environment (Hara et al., 2017). It is this reactive glial response that provides an inhibitory environment to axonal regeneration chronically. This is further contrasted by the remarkable regenerative properties of invertebrates such as zebrafish which, in addition to displaying extensive neurogenesis in adulthood, show limited inflammatory responses that avoid scar formation chronically (Baumgart et al., 2012; Becker et al., 2018). The capacity for regeneration following SCI in the adult has been lost in warm-blooded species during the processes of development and evolution. Subsequently, the difference in cellular and molecular responses to trauma between mammals, anurans, birds, and marsupials requires substantial investigation to inform novel treatment strategies.

The ultimate effect of reactive glia is to produce an orchestrated restructuring of the tissue at the lesion site to culminate in a complex arrangement of cells and extracellular components we call the glial scar. Along with astrocytes overlaid to form a wall-like formation at the scar border, the glial scar penumbra also includes neural/glial antigen 2 positive (NG2⁺) oligodendrocyte progenitor cells. The extracellular components emanating from the lesion penumbra include chondroitin sulfate proteoglycans (CSPGs) including NG2 and other lecticans that contribute to potentially inhibiting axon regeneration chronically. Further cellular dissection of the mature glial scar will reveal a stereotyped cellular organization beginning with a fibrotic epicenter consisting of fibroblasts, macrophages, and extracellular matrix proteins such as laminin, collagen type I and fibronectin. Arising from this organization is the reformation of a glial limitans initiated through cell-to-cell contact from fibroblasts/pericytes and astrocytes of the surrounding penumbra. Importantly, this structure serves to segregate inflammatory cells and other elements (e.g., newly forming blood vessels) at the lesion epicenter. Recent studies are beginning to highlight the potentially pro-inflammatory effects of the fibrotic components of the scar. Collagen type I alone has, for example, been shown to activate astrocytes, propelling them to form a tight, wall-like scar (Hara et al., 2017). Work from Dias et al. (2018) additionally highlights the contribution of type-A pericytes, which differentiate into fibroblasts to further restrict axon outgrowth following injury to the cord. These recent findings further emphasize that more work will be needed to fully understand the impact of the fibrotic component of the glial scar and whether targeting this structure, along with the astroglial wall, will provide functional recovery following SCI.

Clearly, the glial scar does not exist to only limit axon regeneration

chronically. Inherent in this complicated meshwork of cells is the effective ability to stabilize rampant inflammatory processes shortly following injury as seen in the reformation of the glia limitans as the scar matures. Ablating the ability of astrocytes to become reactive, for example, through genetically ablating GFAP or Vimentin has been reported to increase lesion volume and exacerbate inflammation-induced injury in some cases. Work from the Sofroniew lab has additionally shown the importance of a reactive astrocyte response following stab injuries to the spinal cord. Genetically ablating signal transducer and activator of transcription 3 (STAT3), for example, and preventing astrocytes from forming a wall around the injury epicenter induced worse functional recovery than wild type controls (Wanner et al., 2013). Our current understanding of the glial scar should, therefore, resist oversimplification of this structure to encompass a more nuanced understanding of the remarkable plasticity of astrocytes based on the extent of inflammation within the injured environment. This is in contrast to interpretations made by Anderson et al. (2016), which claim that reactive astrocytes have only one state and that “contrary to the prevailing dogma, astrocyte scar formation aids rather than prevents central nervous system axon regeneration.” The influence of the inflammatory context in which astrocytes find themselves can be further exemplified by recent work from the Okada group, which highlights the malleability of reactive astrocytes by implanting them in naive or injured spinal cords. Reactive astrocytes, for example, implanted into naive cords became non-reactive while those implanted into injured cords adopted scar-like morphological changes (Hara et al., 2017). Inhibiting the wall-like state sub-acutely rather than immediately after injury promoted axonal regeneration (Hara et al., 2017). Thus, the extent of the inflammatory milieu following injury induces cellular reactivity to beneficially limit the spread of inflammation. The chronic persistence of the glial scar including its fibrotic and CSPG components, however, ultimately hinders axon regeneration and restoration of function. While there are several strategies that are in pre-clinical assessment including intracellular sigma peptide (ISP; a CSPG receptor blocking molecule) and viral delivery of chondroitinase ABC (Bartus et al., 2014; Lang et al., 2015), there currently exists no treatment for SCI scar modification that is ready to be assessed in the human patient. This may limit the success of strategies for SCI treatment currently under clinical trial, such as the implantation of autologous Schwann cells. Continued development and assessment of such new treatments may be key for future success in clinical trials.

CSPGs and their component structure: Within our review, we concentrated on the traditional role of CSPGs following SCI to inhibit growth, regeneration and plasticity. These data are well established in the literature, with numerous *in vitro* and *in vivo* studies showing how they are upregulated following SCI within the glial scar initially to aid neuroprotection but ultimately act to limit growth and functional recovery. However, while this class of large macromolecules, in general, upregulate following spinal trauma, there is substantial evidence to show that the different CSPG lecticans up- and down-regulate both mRNA and protein expression at different time points following trauma. This creates a well characterized pattern of CSPG changes that, to some extent, varies depending on the size, location and type of injury (Andrews et al., 2012). Studies have shown that the different CSPG lecticans broadly perform the same functions. However, recent evidence has demonstrated that brevicin alone mediates cellular activity through the gating of parvalbumin positive interneurons within the structure of the perineuronal net (Favuzzi et al., 2017). Indeed, this CSPG acts to control α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor input and potassium channel localization, regulating cellular excitability. This, in turn, was shown to regulate brevicin levels. These data are of substantial significance as they demonstrate that the individual CSPGs may act independently and dynamically to modify specific cellular responses in precise ways. These data may signify how alterations in specific CSPG levels and expression within the central nervous system are associated with the wide variety of neurological disorders and diseases mentioned within our review (Tran et al., 2018b). As such, it may be of exceptional importance for the success of specific SCI treatment strategies, and the recovery of function following injury, to assess which CSPGs are expressed at different times and precisely when we act to modify their inhibitory influence upon regeneration.

The specific function of CSPGs following SCI may be further complicated. Within our *Physiological Reviews* article, we briefly touched upon the notion that the glycosaminoglycan (GAG) chains of the upregulated CSPGs are differently sulfated after SCI with chondroitin sulphate (CS)-A, -C, and -E predominating. Indeed, Brown et al. (2012) suggest the consequence of CS-E upregulation following SCI is of particular importance as this residue not only inhibits axon growth post injury, but also acts to bind with the CSPG receptor protein tyrosine phosphatase sigma (RPTP σ). However, this is of exceptional importance to the function of these macromolecules, where subtle differences in sulphation may cause opposing effects on neuronal activity. For example, an increase in the ratio of 4-sul-

phated GAGs to 6-sulphated GAGs in perineuronal nets may decrease synaptic plasticity in aged animals (Foscarin et al., 2017), while knockout studies have shown that 6-sulphation of CS-C facilitates growth (Lin et al., 2011). Indeed, the effects of specific CSPG sulphation patterns upon neuronal activity is likely to be of exceptional importance to the growth and recovery of individuals following SCI as these macromolecules are a prominent feature of the extracellular matrix surrounding all cells of the central nervous system. Similarly, sulphation patterns affect the interactions and binding of CSPGs with receptors such as RPTP σ , the Nogo-66 receptors 1 and 3 (NgR1 and 3), and (potentially) semaphorins. Indeed, recent investigations have confirmed the affinity of CS-E for RPTP σ , while CS-A and -E potentially dock with NgR1Ind 3 (Griffith et al., 2017). Further, the GAG sulphation affects CSPG association with guidance molecules (ephrins, Wnts, etc.), growth factors (fibroblast growth factor, glial cell-derived neurotrophic factor, etc.), and other molecules of the extracellular matrix (integrins, tenascins, etc.). It is of exceptional importance to both determine the biological function of these specific sulphotransferases and the exact mechanism and pathways through which CSPGs bind and mediate their specific function to ascertain how they may be best manipulated for the treatment of SCI.

A final complication of CSPG alteration in the treatment of SCI currently includes that the mechanism of receptor-mediated inhibition is still largely unknown. Within our recent *Physiological Reviews* article we described at length the newly discovered conjugate CSPG receptors RPTP σ and LAR (leukocyte common antigen-related phosphatase), their relation to intrinsic and extrinsic neuronal growth properties, and their putative signaling cascades (Tran et al., 2018b). Understanding the pathways downstream of RPTP σ is also critical to regeneration and treatment strategies in light of a recent publication from our laboratory where we show that Cathepsin B characterizes protease activity in peripheral axons following RPTP σ modulation with the synthetic peptide ISP (Tran et al., 2018a). Further, ISP modulation of RPTP σ in a post-injury environment has revealed that local and systemically-infiltrating immune cells also respond to RPTP σ (Dyck et al., 2018). Collectively these data show that CSPG-receptor dynamics are not only limited to the leading edge of axons, but to the many components of the glial scar as well, further contributing to the complexity of the post-injury environment and potential treatment of any SCI.

The stages of SCI: Within our review, we discuss the primary and secondary stages of SCI, paying particular attention to the biological processes involved in this traumatic progression of damage, inflammation and cellular death. We also describe experimental pre-clinical injury models as being at acute or chronic stages following trauma. These definitions are largely subjective. Acute phases of injury are broadly defined as occurring two hours to two weeks following trauma. However, the time point when an injury becomes chronic is typically based upon the idiosyncratic judgement of each investigator grounded upon the literature of the specific discipline in which they are working. As such, ‘chronic’ injuries have been defined as starting as early as two weeks’ to six months’ post trauma. Indeed, even after this time point, spinal injury can still be defined as dynamic with continued scar development and progressive alterations to neural circuitry (Kwon et al., 2002). Respiratory motor function has been shown to have some degree of spontaneous recovery following chronic SCI (Fuller et al., 2008), although the reason for this remains unclear. Although less explored than acute injuries, there are numerous investigations into SCI and treatment application at early chronic time points. However, largely due to ethical and financial concerns, very few papers are written which examine these issues at extended lengths (six months +) post injury. This represents a gap in the current knowledge base, especially when one considers that clinical SCI ‘chronic’ injuries are typically defined as being over one year post trauma, which is when most individuals experience the greatest degree of functional recovery. Chronic injuries are typically considered a difficult environment in which to mediate functional recovery, but this is not necessarily what is observed clinically. However, experimental studies have not traditionally assessed spinal tissue long after the initial insult, and thus, there is little data available to assess why this occurs. Greater assessment of alterations to the spinal cord at extended chronic stages will be essential for the continued understanding of SCI progression and potentially successful treatment, and ultimately recovery, of the current SCI population.

Concluding remarks: Our view of SCI post injury has developed over recent years from a simplistic view of the factors and molecules acting to inhibit growth to something more complex and nuanced involving a large cast of interacting and transforming cells and processes which integrate to prevent functional restoration of activity. However, we believe that the continued understanding and exploration of these processes and the minutia of the changes which occur holds the key to the successful development of combination and stratified treatment strategies that will facilitate

recovery at all stages post injury.

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