EDITORIAL COMMENTARY

Reactive astrocyte scar and axon regeneration: suppressor or facilitator?

Several major factors are known to contribute to CNS axon regenerative failure after injury, including reduced intrinsic growth capacity of developed neurons and extrinsic factors mediating axon outgrowth. For the latter, a non-permissive environment around the lesion and the lack of sufficient neurotrophic support within the adult CNS play important roles (Silver et al., 2015). In addition to generation of various inhibitory substrates by oligodendrocytes, fibrotic tissues, inflammatory cells and other cell types, reactive astrocytes surrounding lesions are thought to highly suppress regeneration of injured CNS axons (Silver and Miller, 2004; Ohtake and Li, 2014). A great number of studies suggest that reactive astrocytic scars form one of the major barriers preventing axon regeneration after CNS injuries, including spinal cord injury (SCI). However, reactive astrocytes were reported to provide a beneficial role by reducing infiltrating immunoreactive cells into adjacent domains, protecting bordering neural tissue from damage and generating numerous supportive extracellular matrix (ECM) components to promote cell survival and growth (Bush et al., 1999). Previous data showed that ablation of reactive astrocytes increased inflammation and secondary tissue damage, prevented blood-brain barrier formation and increased local neurite growth. Interestingly, a recent study by Anderson et al (2016) provides evidence that reactive astrocytes around the lesioned spinal cord support axon regeneration after SCI, rather than block regrowth (Anderson et al., 2016).

By using transgenic mouse models that reduced generation of reactive astrocytes and ablated scar forming astrocytes at a chronic stage, Anderson et al. (2016) studied the roles of reactive astrocytes in preventing regeneration of descending and ascending axons across the lesioned spinal cord in adult mice. In this paper, the authors prevented astrocyte formation by deleting STAT3, an important regulator of astrocyte reactivity (Okada et al., 2006), in conditional knockout (CKO) mice, while ablating reactive astrocytes by treatment with ganciclovir (GCV) in Herpes Simplex virus thymidine kinase (HSV-TK) transgenic mice driven by glial fibrillary acidic protein promoter. Following suppression of reactive astrocyte formation after a T10 mouse spinal cord crush, they focused on evaluating regrowth of descending corticospinal tract (CST), serotonergic (5-HT) and ascending sensory axons, expression of various chondroitin sulfate proteoglycan (CSPG) levels, gene expression profile of astrocytes versus non-astrocytic cells, and axon regeneration following local delivery of neurotrophins (NT3 and BDNF) plus conditioning lesions. Repression of astrocyte reactivity suppressed spontaneous regrowth of these axons through the scar and increased dieback of CST and sensory axons from the lesion site. Moreover, ablation of 5 weeks old chronic astrocytic scar tissue using transgenically targeted diphtheria toxin receptor did not increase spontaneous regrowth of CST, 5-HT and sensory axons, but induced further dieback of lesioned CST and sensory axons. These findings contradict the results of many previous studies describing the functions of reactive astrocytes, but indicate that reactive astrocytes are important for sustaining tissue integrity and increasing regenerative ability at acute and chronic stages after SCI.

Reactive astrocytes are thought to inhibit axon extension primarily due to their expression of high levels of various negative molecules, especially CSPGs (Busch and Silver, 2007). Although other cell types are known to generate CSPGs, reactive astrocytes have long been thought as the major source of CSPGs around the lesion. This study demonstrated that traumatic injury significantly upregulated levels of CSPGs at the spinal cord injury site as reported previously by others, but surprisingly, removal of reactive astrocytes did not significantly attenuate the overall expression levels of CSPGs. Using RNA analysis and immunohistochemistry approach, these authors provide further evidence that both reactive astrocytes and non-astrocyte cells express a number of inhibitory CSPGs, including brevican, neurocan, versican and phosphacan. Aggrecan, one of the lectican CSPGs known to suppress axon growth, was not expressed by reactive astrocytes after lesion. Therefore, these results suggest that other diverse cell types at the spinal cord lesion site, such as fibroblasts, inflammatory cells and pericytes, are able to maintain extremely high levels of CSPGs in the absence of reactive astrocytes. This is not surprising because these cells have been shown to produce a number of extracellular matrices, including CSPGs. However, future studies are required to determine whether cell types other than reactive astrocytes are the major sources of CSPG expression or whether ablation of reactive astrocytes results in compensatory upregulation of CSPGs by other cell types.

Lesioned spinal cord contains diverse cell types that generate a great number of secreted molecules after acute and chronic SCI. Some of them are inhibitory to block axon growth, but others are growth-promotive to stimulate regeneration. Because STAT3 has been shown to modulate astrocyte reactivity, these authors further evaluated axon growth regulating molecules produced by astrocytes or non-astrocytic cells from lesioned spinal cord derived from either wildtype or STAT3 CKO mice. Analyses of 59 molecules known to modulate axon growth demonstrated that both astrocytes and non-astrocytic cells expressed a mixture of multiple axon growth inhibiting (such as CSPGs, ephrins, netrins, neuropillins, plexins, and slits) and growth promoting (laminins, syndecans, glypicans and decorin) proteins. Notably, NG2 (CSPG4) and neuroglycan C (CSPG5), two CSPGs shown to promote axonal growth, are also upregulated by scar tissues. Preventing formation of reactive astrocytes by deleting STAT3 in CKO mice did not create a more permissive molecular environment for axonal growth because overall CSPG levels remained unaltered and the mRNAs for certain growth-promoting proteins were downregulated. Therefore, this study confirms and extends previous reports indicating that both astrocytes and non-astrocytic cells after SCI express a diverse mixture of inhibitors and axon growth-promoting molecules, including multiple permissive ECM molecules. The overall functions of upregulated CSPGs and many other molecules by scar tissues need to be further studied at different injury stages, especially because the cellular structures and molecular components of scars are highly dynamic with time and types of injury. Any cellular/molecular changes, such as presence/absence of certain ECM proteins (*e.g.*, heparan sulphate proteoglycan and Sema3) and gradual increase in astrocytic density at later stages after injury, may modify the functional properties of other molecules (including CSPGs) and cell types.

To stimulate significant regrowth of severed axons, multiple investigators have attempted to create a more permissive environment and to increase the intrinsic growth capacity of axons with application of various neurotrophic factors and conditioning lesions. Anderson et al then determined the roles of reactive astrocytes in regulating regeneration of lesioned sensory axons by increasing the growth state of the neurons and environment

using neurotrophins NT3 and BDNF with/without conditioning lesion of sciatic nerve. These strategies, especially delivery of NT3 and BDNF plus conditioning lesion, promoted regeneration of injured ascending axons directly through condensed astrocyte scar tissue. Consistent with recent reports by others (Zukor et al., 2013; Xu et al., 2015), injured axons regenerated along the processes of reactive astrocytes. In contrast, neurotrophins alone or in combination with conditioning lesions exhibited a significant reduction in axon regrowth in the absence of reactive astrocytic scar tissues around the lesion in either TK-GCV or STAT3 CKO mice. Therefore, these experiments with a compression SCI model provide further evidence that reactive astrocytes support growth of regenerating axons probably by guiding their elongation and bridging the rostral and caudal spinal cord stumps, rather than blocking regrowth of injured axons. Because the regenerative strategies were applied either before SCI (conditioning lesions) or two days after injury (NT3 and BDNF), it will be interesting to determine whether these and other strategies applied at the delayed stage of SCI, especially after scar formation and maturation, are still effective in supporting regrowth of injured CNS axons. Given that the molecular and cellular structures and function of scar tissues are highly dynamic with injury time and types, validation of an effective strategy applied after scar formation is extremely important not only for dissecting scar tissue function, but also for developing a practical therapy for chronic CNS injuries.

To develop highly effective strategies to repair the injured CNS after traumatic injuries and in neurological disorders, it is extremely important to better understand the cellular and molecular mechanisms underlying axon regenerative failure mediated by reactive scar tissue and other factors. Although previous reports indicate a positive role for reactive scar tissue after CNS injury, Anderson et al. employed various transgenic approaches and provided further direct evidence that reactive astrocytes essentially support and stimulate axon regrowth rather than suppress regeneration. Because numerous previous studies support the inhibitory features of glial scars, which induce both chemical and physical barriers for axon elongation, further studies are required to integrate the findings of this study with previous observations. Given the complicated cellular and molecular components, interactions and functions of CNS scar tissues, it is very important to further dissect functional interactions of individual cell types (even subtypes) and molecules for their overall roles in regulating neural damage and repair after CNS injury. Recent advancements in research technologies, including CRISPR-Cas9, target gene deletion, RNA sequencing and cell ablation approaches, will be helpful in this pursuit. In addition to the reactive astrocytes, many other cell types contribute to the formation of glial scars and may modulate axonal growth and CNS repair after lesions, including fibroblasts, pericytes, oligodendrocyte progenitor cells, inflammatory cells, migrated Schwann's cells, and other cell types (Goritz et al., 2011). It is important to dissect the functions of these cell types and their integration as scar components. It is also critical to determine why reactive glial cells generate diverse types of proteins, including repulsive and growth promoting ECM molecules as well as many guidance cues.

In summary, the study by Anderson et al. (2016) further dissected function of reactive astrocytes after SCI using different cell ablation models and provided strong and direct evidence for the beneficial effects of reactive astrocytes. Many research labs have attempted to repair injured CNS and to stimulate regeneration by suppressing reactive scar formation with various molecular and cellular approaches. Although multiple cell types ultimately contribute to the inhibitory role of glial scars, one central issue is whether we should switch to facilitate formation of reactive astrocytes in order to create a more permissive environment for CNS repair and axon regeneration. Obviously, to design highly effective therapeutic strategies that target scar tissue, a better understanding of the molecular and cellular mechanisms underlying scar formation is required, including signaling control of generating various cell types and multiple mixed molecules, functions of individual cell types and molecules, dynamic changes of scar tissue and their expressed molecules, interactions among different cell types and molecules, and communication between axons and scar-related cells and molecules. Addressing these and other related issues is critical for future development of potent strategies for axon regeneration and neuronal repair.

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References

Anderson MA, Burda JE, Ren Y, Ao Y, O'Shea TM, Kawaguchi R, Coppola G, Khakh BS, Deming TJ, Sofroniew MV (2016) Astrocyte scar formation aids central nervous system axon regeneration. Nature 532:195-200.

Busch SA, Silver J (2007) The role of extracellular matrix in CNS regeneration. Curr Opin Neurobiol 17:120-127.

Bush TG, Puvanachandra N, Horner CH, Polito A, Ostenfeld T, Svendsen CN, Mucke L, Johnson MH, Sofroniew MV (1999) Leukocyte infiltration, neuronal degeneration, and neurite outgrowth after ablation of scar-forming, reactive astrocytes in adult transgenic mice. Neuron 23:297-308.

Goritz C, Dias DO, Tomilin N, Barbacid M, Shupliakov O, Frisen J (2011) A pericyte origin of spinal cord scar tissue. Science 333:238-242.

Ohtake Y, Li S (2014) Molecular mechanisms of scar-sourced axon growth inhibitors. Brain research.

Okada S, Nakamura M, Katoh H, Miyao T, Shimazaki T, Ishii K, Yamane J, Yoshimura A, Iwamoto Y, Toyama Y, Okano H (2006) Conditional ablation of Stat3 or Socs3 discloses a dual role for reactive astrocytes after spinal cord injury. Nat Med 12:829-834.

Silver J, Miller JH (2004) Regeneration beyond the glial scar. Nat Rev Neurosci 5:146-156.

Silver J, Schwab ME, Popovich PG (2015) Central nervous system regenerative failure: role of oligodendrocytes, astrocytes, and microglia. Cold Spring Harb Perspect Biol 7:a020602.

Xu B, Park D, Ohtake Y, Li H, Hayat U, Liu J, Selzer ME, Longo FM, Li S (2015) Role of CSPG receptor LAR phosphatase in restricting axon regeneration after CNS injury. Neurobiol Dis 73:36-48.

Zukor K, Belin S, Wang C, Keelan N, Wang X, He Z (2013) Short hairpin RNA against PTEN enhances regenerative growth of corticospinal tract axons after spinal cord injury. J Neurosci 33:15350-15361.