

● PERSPECTIVE

Collagen 1 signaling at the central nervous system injury site and astrogliosis

Central nervous system (CNS) injuries are often devastating as functional recovery *via* axonal regrowth over the lesion site is very minimal. Failure of regeneration by injured CNS neurons is known to be due to both a reduced intrinsic regenerative capacity of adult neurons, as well as a non-permissive environment for axonal regrowth. In particular, the induction of astrogliosis and glial scar formation, which are prominently observed in brain and spinal cord injury (SCI) models, are widely assumed to contribute to both neuronal demise, as well as an inhibition of axonal extension past the lesion site (Cregg et al., 2014). Over the years the process of astrogliosis, and the phenotypic as well as transcriptional profile changes that drive a dormant naïve astrocyte into gliosis, characterized by morphological changes and cell replication, have been extensively documented. Several recent reports have contributed significantly to advances in glia-based neuropathology in the CNS. The Barres lab, for example, has identified a subtype of reactive astrocytes (the A1 astrocytes) that through the influence of secreted factors from reactive M1 microglia in the injured or diseased CNS environment, promotes the demise of oligodendrocytes and neurons (Liddelow et al., 2017). However, the injured CNS environment has more to offer than secreted proinflammatory factors in terms of astrogliosis induction. In this regard, Hara et al. (2017) have now shown that upregulation of a common extracellular matrix (ECM) component in the injured spinal cord, and its signaling through cell adhesion molecules, may serve as a major driver of astrocyte activation and glia scar formation.

SCI lesion site ECM and its influence on astrogliosis/scar formation: To better characterize subpopulations of reactive astrocytes in the injured CNS, Hara et al. (2017) isolated morphological variants of these by laser microdissection at the lesion site of a mouse contusion SCI model. The authors confirmed a number of genes that are previously known to be upregulated during astrocyte activation, and noted that some of these, such as *Gfap*, *Nes*, *Vim*, *Ctnnb1*, *Plaur*, *Mmp2*, *Mmp13* and *Axin2*, could serve well to differentiate reactive astrocytes from naïve astrocytes. Although the sample sizes were not large, the expression levels of the prototypical astrocytic marker glial fibrillary acidic protein (GFAP) could be clearly differentiated between naïve, reactive and scar-forming astrocytes. Importantly, the authors found significant elevated expression of *Cdh2* (encoding N-cadherin), *Sox9*, *Slit2* as well as genes encoding a range of axonal growth inhibitory chondroitin sulfate proteoglycans (CSPGs) to be significantly upregulated in scar-forming astrocytes. These markers would serve as expression profile identifiers for the different subclasses of astrocytes. With these markers, the authors went on to show that, in line with previous findings, enhanced green fluorescent protein (EGFP)-marked naïve astrocytes remained phenotypically naïve when transplanted into the spinal cord of uninjured mice, but became activated and expressed reactive astrocyte markers after transplantation into injured spinal cord. More interestingly, by inducing SCI in mice bearing a *Nes-EGFP* transgene and isolating EGFP-positive reactive astrocytes by flow cytometry, the authors showed that while reactive astrocytes grafted into injured spinal cord form astrocytic scars, those grafted into an uninjured spinal cord reverted into a histologically naïve phenotype. The observations made provided not only unequivocal support for the notion that the injured CNS environment critically influences astrogliosis and scar formation, but also illustrated the rather amazing plasticity of astrocytes as they switch bidirectionally from the naïve to the scar-forming end of the spectrum, apparently in full dependence on the graft environment.

What factor and condition in the injured CNS environment are actively changing the reactive phenotype and scar-forming propen-

sity of astrocytes? Previous work may have provided links between this influence with inflammatory factors, cellular energetics or even redox status. The authors performed a temporal genome-wide expression analysis and found that amongst the 5% of genes that were considerably elevated at 14 days post-injury, a number of them encode ECM proteins. Of these, those encoding type 1 collagen (*Col1a1* and *Col1a2*) were most highly expressed in the injured spinal cord at day 14. While the CSPGs are upregulated and secreted by astrocytes, collagen 1 (Col1) in the lesion site is likely produced by pericytes and fibroblasts in response to cytokines like transforming growth factor β (TGF β), and elevation of Col1 in fibroblasts and around blood vessels post-SCI has been previously demonstrated. Histologically, the scars were shown to be populated with astrocytes with the scar-forming phenotype of high-GFAP and tight cell clustering (likely a result of increased N-cadherin expression), that are localized to Col1-enriched areas. In Col1-poor or absent areas, astrocytes are phenotypically less reactive and had much lower levels of GFAP. When reactive astrocytes were cultured on Col1-coated substratum, these tend to cluster together tightly and have elevated GFAP and N-cadherin expressions characteristic of scar-forming astrocytes, while those cultured on a surface without collagen had retracted processes and reduced GFAP expression. These observations suggest that Col1 upregulation in the injured ECM environment is at least partly, if not largely, responsible for driving astrogliosis and astrocytic scar formation.

This pivotal role for the ubiquitously found Col1 in astrogliosis was somewhat unexpected. Other astrocyte expressed cell adhesion molecules have been previously investigated with regards to astrogliosis and neuronal regeneration after injury. The immunoglobulin (Ig) superfamily adhesion molecule cell adhesion molecule-like 1 (CHL1), for example, was shown earlier by Schachner's lab to be upregulated in astrocytes at SCI lesion cores, and functional recovery after injury was significantly improved in CHL1^{-/-} mice compared with wild-type mice (Jakovcsevski et al., 2007). On the other hand, another Ig family member, the neural cell adhesion molecule (NCAM), appears to positively affect recovery after SCI. As shown recently, NCAM^{-/-} mice exhibited reduced locomotor recovery after injury in comparison to control mice, with NCAM^{-/-} astrocytes having a reduced migration capacity *in vitro* as illustrated by a wound healing assay (Saini et al., 2016). The extent which these cell adhesion molecules may contribute to the processes of astrogliosis and scar formation, as well as the signaling mechanisms involved, were however unclear.

Signaling mechanisms and caveats: How does Col1 activate astrocytes and drive these towards a scar-forming phenotype? Collagen binds to integrins, the cell surface ECM receptors which are functional heterodimers of a multitude of α and β subunits. The collagen-binding integrin subtypes, $\alpha1\beta1$, $\alpha2\beta1$, $\alpha10\beta1$, and $\alpha11\beta1$, are all present on astrocytes, and an anti- $\beta1$ antibody inhibited the clustering and elevation of GFAP/N-cadherin levels that are characteristic of scar-forming astrocytes when cultured on a Col1 surface. Cadherins are Ca²⁺-dependent cell adhesion molecules that mediate cell-cell adhesion *via* homotypic intercellular interactions. The authors showed that an N-cadherin neutralizing antibody likewise inhibited the phenotype transformation from reactive astrocytes to scar-forming astrocytes. Col1 therefore acts through signaling pathways involving both integrins and N-cadherin to promote the scar-forming phenotype.

The pathways and components downstream of integrin and N-cadherin leading towards astrogliosis were not further delineated in Hara et al. (2017), but are worth deeper consideration here. That $\beta1$ integrin signaling could mediate astrogliosis has also received support from investigations on a different type of CNS insult, as it was recently shown that soluble, potential neurotoxic forms of amyloid β interacts with and modulates $\beta1$ -integrin activity and induces astrogliosis *via* NADPH oxidase (Wyszenbach et al., 2016). At first glance, Col1-integrin interaction-mediated signaling appears to be an event that is separated from N-cadherin mediated cell adhesion. However, it has been previously shown in mammary epithelial cells that Col1-induced scattering of these cells resulted in upregulation of N-cadherin through phosphoinositide 3-kinase (PI3K)-Rac1-c-Jun N-terminal kinase (JNK) signaling (Shintani et al., 2006). Inhibition of the PI3K-Akt-mechanistic target of



rapamycin (mTOR) pathway is also known to attenuate glial scar formation, and N-cadherin's role in astrogliosis has been previously demonstrated. Astrocyte-specific knockout of N-cadherin resulted in impairment of astrogliosis and neuroprotection from Ca²⁺-induced injury (Kanemaru et al., 2013).

On the other hand, it should be noted that the current notion of Col1-integrin-mediated astrogliosis contradicted at least one previous finding. Robel et al. (2009) reported that conditional knockout of β 1-integrin in astrocytes (but not neurons) resulted in a condition of "partial" reactive gliosis, as reactive astrocyte markers such as GFAP and vimentin were upregulated, but the astrocytes did not divide or proliferate. The mechanism underlying this loss of β 1-integrin-induced, albeit incomplete, astrocyte activation is unclear, but does caution against the formulation of a generalized notion that β 1-integrin activation in astrocytes would invariably lead to their activation.

Implications for CNS neuronal regeneration: The findings discussed above have important implications for our fundamental understanding of CNS neuronal regeneration as well as the quest for novel regeneration promoting strategies. Notably, this is an intriguing alternative to the much investigated mechanism of inhibition of axonal growth by astrocytic ECM components that are elevated during CNS injury, particularly the CSPGs. Hara et al. (2017) have shown that blocking Col1-mediated transformation of astrocytes towards the scar-forming phenotype by administration of anti- β 1 antibody or N-cadherin neutralizing antibody could effectively reduce astrocytic scar formation, promote axonal regrowth and enhance functional recovery. These observations attested to the more widely accepted negative effect of the astroglial scar on CNS axonal regeneration, a notion that has been disputed by a recent paper from the Sofroniew lab. Anderson et al. (2016) have shown that ablation of scar forming astrocytes and scars by genetic manipulations not only did not promote regeneration upon injury, but instead reduced neurotrophin-stimulated axonal regrowth. The discrepancies between the main findings and conclusions by the different groups are glaring and perplexing. The effect on axonal regrowth and functional recovery of genetic manipulations that drastically prevents astrogliosis and scar formation would require further mechanistic exploration before the different findings could be explained or reconciled.

The injured CNS environment not only promotes astroglia activation, but also induces the differentiation of neural progenitor cells (NPCs) towards the astroglial lineage. This may be undesirable in transplantation therapy with neuronal replacement as a main strategy. Expression of β 1-integrin is known to be elevated in ependymal stem cells (EZCs) following SCI, and its signaling suppressed astrocytic differentiation, while conditional deletion of β 1-integrin enhanced EZC differentiation into the astroglial lineage (North et al., 2015). Signaling from β 1-integrin may therefore be a double-edged sword in the injured adult CNS with regards to astrogliosis and astroglial scar formation, promoting reactivation of naïve mature astrocytes on one hand, but suppressing astrocytic differentiation by EZCs or NPCs on the other hand. It is yet unclear if manipulating Col1-integrin/N-cadherin signaling might in any way affect EZC or NPC fate, or attenuate neuronal differentiation at CNS lesion sites. Furthermore, it should be noted that expression or activated integrin in axons is known to promote CNS axonal regeneration (Cheah et al., 2016), and any attempt at non-selective suppression of integrin-based signaling might work against neuronal regeneration. However, taken as a whole, the finding that a common ECM component upregulated at CNS lesion sites could promote astrogliosis and scar formation has significant translational potential that could be applicable to the treatment of CNS injury.

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Sin Hui Neo, Bor Luen Tang*

Department of Biochemistry, Yong Loo Lin School of Medicine, National University Health System, Singapore (Neo SH)
NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Medical Drive, Singapore

(Tang BL)

*Correspondence to: Bor Luen Tang, bchtbl@nus.edu.sg

orcid: 0000-0002-1925-636X (Bor Luen Tang)

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Open peer review reports:

Reviewer 1: Ling-Xiao Deng, Indiana University, USA.

Comments to authors: This mini review is well organized and straightforward. It focus on the effect of ECM environment on astrogliosis which is important barrier for the neural plasticity and neural regeneration. The quality of this manuscript is high and can be accepted to publication. The title is too ambitious for a mini review. Since the majority of this review focus on one component of ECM (Col1), I would like to suggest to narrow down their title to be more specific.

Reviewer 2: Andrew Kaplan, McGill University, Canada.

Comments to authors: This is an interesting and generally well-written perspective on the role of the glial scar in axon regeneration after spinal cord injury. The authors have discussed important findings from Hara et al. in the context of recent studies addressing this topic. While the article has thoroughly addressed the detrimental role for astrocytic expression of integrins at the lesion, it could perhaps also be noted that expression of integrins in the axon has been shown to promote axon regeneration (e.g., Cheah et al., 2016), suggesting that broadly targeting integrins at the lesion could also have detrimental effects on axon growth.

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