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 astroglial 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 astroglial 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 astroglial 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 gli 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 Gfair, Nes, Vim, Ctnnb1, Plaur, Mmp2, Mmp13 and Aixin2, 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 Col1a2 (encoding N-cadherin), Sox3, Slt2 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 graft-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 propensity of astrocytes? Previous work may have provided links between this influence with inflammatory factors, cellular energetic 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 I 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 I (Col1) in the lesion is likely produced by pericytes and fibroblasts in response to cytokines like transforming growth factor β (TGFβ), 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 astroglial and astrocytic scar formation. This pivotal role for the ubiquitously found Col1 in astroglial 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 (Jakovcevski 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 to which these cell adhesion molecules may contribute to the processes of astroglial 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, α1β1, α2β1, α10β1, and α11β1, are all present on astrocytes, and an αβ1 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 astroglial were not further delineated in Hara et al. (2017), but are worth deeper consideration here. That β1 integrin signaling could mediate astroglial 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 β1-integrin activity and induces astroglial via NADPH oxidase (Wyssenbach 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 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
Common ECM component upregulated at CNS lesion sites could enhance functional recovery. These observations attested to the more widely accepted negative effect of the astrogial 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 ablating 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 regeneration 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 astrogial activation, but also induces the differentiation of neural progenitor cells (NPCs) towards the astrogial lineage. This may be undesirable in transplantation therapy with neuronal replacement as a main strategy. Expression of β1-integrin is known to be elevated in ependymal stem cells (EZCs) following SCI, and its signaling suppressed astrogial differentiation, while conditional deletion of β1-integrin enhanced EZC differentiation into the astroglial lineage (North et al., 2015). Signaling from β1-integrin may therefore be a double-edged sword in the injured adult CNS with regards to astrogliosis and astrogial scar formation, promoting reactivation of naïve mature astrocytes on one hand, but suppressing astrogial differentiation by EZCs or NPCs on the other hand. It is yet unclear if manipulating Coll-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 of 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 translation- al potential that could be applicable to the treatment of CNS injury.

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