Zeb2 directs EMT-like processes that underlies the glial response to injury

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In both the peripheral and central nervous systems (PNS and CNS, respectively), glial cells are critical for the wound healing response to injury. However, the glial cell types that orchestrate the response and the extent to which the repair process is successful are different between the PNS and CNS. Nevertheless, there are several cellular features shared by PNS and CNS glia that suggest there are also mutual molecular mechanisms that underlie the injury responses. Establishing these shared molecular mechanisms not only expands our fundamental understanding of how the nervous system responds to injury, but it also facilitates the development of strategies to manipulate the glial responses in order to better protect and restore neurological function.

Astrogliosis after CNS injury: A key component of the glial injury response in the CNS is the activation of astrocytes (astrogliosis). This response is characterized by extensive changes to astrocytic gene expression and cellular morphology, although the extent to which astrocytes undergo astrogliosis depends on injury severity and distance from the lesion (reviewed in Sofroniew, 2014). After severe injuries, reactive astrocytes adjacent to the lesion organize to form a dense boundary that isolates the lesion and protects the surrounding tissue from further damage. The formation of this boundary includes astrocytes generated by injury-induced proliferation. Additionally, reactive astrocytes have several additional beneficial roles in the sub-acute and repair stages of injury progression, facilitating blood-brain barrier repair, attenuating osmotic and oxidative stresses, as well as releasing factors to regulate interactions with several other cell types, including immune cells. Impairing the astrocytic response to injury by either genetically modifying or ablating astrocytes increases the lesion size and worsens the recovery of neurological function. Reactive astrocytes that undergo mild to moderate astrogliosis can return to their pre-injury physiology, but boundary-forming astrocytes adjacent to the lesion that experience strong levels of astrogliosis maintain their reactive phenotype in the chronic phase of injury. Taken together, astrocyte activation is an important component of wound healing and tissue repair, but astrocyte behavior is complex and reactive astrocytes can also contribute to an environment that is unfavorable to axon regeneration. The balance between fostering repair and impeding regeneration involves a complicated time-dependent interplay between reactive astrocytes and other cell types at the injury site as well as the extracellular cues generated by all of the cell types. Identifying the critical molecular regulators of astrogliosis will advance the understanding of the factors that mediate the complicated response of astrocytes.

Schwann cell activation after PNS injury: After injury in the PNS, myelinating and Remak (non-myelinating) Schwann cells undergo adaptive reprogramming in order to facilitate axonal regeneration and restore function. This adaptive reprogramming involves extensive gene expression and cellular changes that correspond to de-differentiation or activation (reviewed in Jessen and Mirsky, 2016). This injury response generates a repair Schwann cell phenotype that is characterized by elevated expression levels of growth factors and cell surface adhesion proteins to promote neuron survival and axon regrowth, as well as cytokine expression to direct the immune response. In addition to clearing myelin debris from the injury site, repair Schwann cells proliferate and interact with each other to form columns that provide substrate and guidance for regenerating axons. The repair phenotype is transient, however, and Schwann cells will differentiate back to either myelinating or non-myelinating phenotypes.

At the cellular level, the responses of astrocytes and Schwann cells to injury have several common features. Both cell types undergo substantial phenotypic changes that include cytoskeletal and morphological reorganization as well as altered cell adhesion. Both cell types also proliferate and reorient themselves to facilitate interactions with neighboring reactive astrocytes or repair Schwann cells to form barriers or columns, respectively. The cellular similarities suggest that there are injury-induced molecular mechanisms that direct the gene expression programs that underlie these shared cellular processes associated with wound healing.

***Zeb2:* a common molecular regulator of CNS and PNS injury response:** Recent studies have identified *Zeb2* (Zinc finger E-box binding homeobox 2; *Sip1*, *Zhfx1b*) as a key molecular component of the injury responses in reactive astrocytes and Schwann cells (**Figure 1**). *Zeb2* encodes a zinc-finger homeodomain transcription factor protein that regulates cell mobility, cell adhesion, and cytoskeletal reorganization in non-neural wound healing, development, and cancer. In CNS development, *Zeb2* regulates several processes, including neuroectoderm formation, alternative cell fate decisions, proliferation, and differentiation (reviewed in Epifanova et al., 2019). This regulation includes a key role in directing the differentiation of oligodendrocytes and Bergman glia (He et al., 2018), but whether *Zeb2* also has a role in astrocyte maturation has yet to be established. In the PNS, *Zeb2* is also required for Schwann cell differentiation and myelination (Quintes et al., 2016; Wu et al., 2016). Despite these roles in development, *Zeb2* is not critical for maintaining neural phenotypes and ZEB2 protein levels are low or undetectable in either Schwann cells or most astrocytes in the adult nervous system (Quintes et al., 2016; Wu et al., 2016; Vivinetto et al., 2020). Following injury, however, ZEB2 is re-expressed and it is integral to the glial response.

ZEB2 expression is induced after either ischemic stroke or spinal cord injury (Vivinetto et al., 2020). Unlike its role in generating neurons and oligodendrocytes during development, ZEB2 is selectively expressed in astrocytes following injury. This induced expression peaks between 7 and 14 days after injury before diminishing, but it remains detectable at the lesion border region for at least 30 days after injury. At the mRNA level, *Zeb2* expression also increases after injury, but its expression is also detectable in uninjured astrocytes. This differential expression between mRNA and protein expression highlights the pivotal role of *Zeb2os*, a lncRNA expressed from the genomic strand opposite *Zeb2* that facilitates efficient translation of *Zeb2* mRNA into protein (Beltran et al., 2008). *Zeb2os*

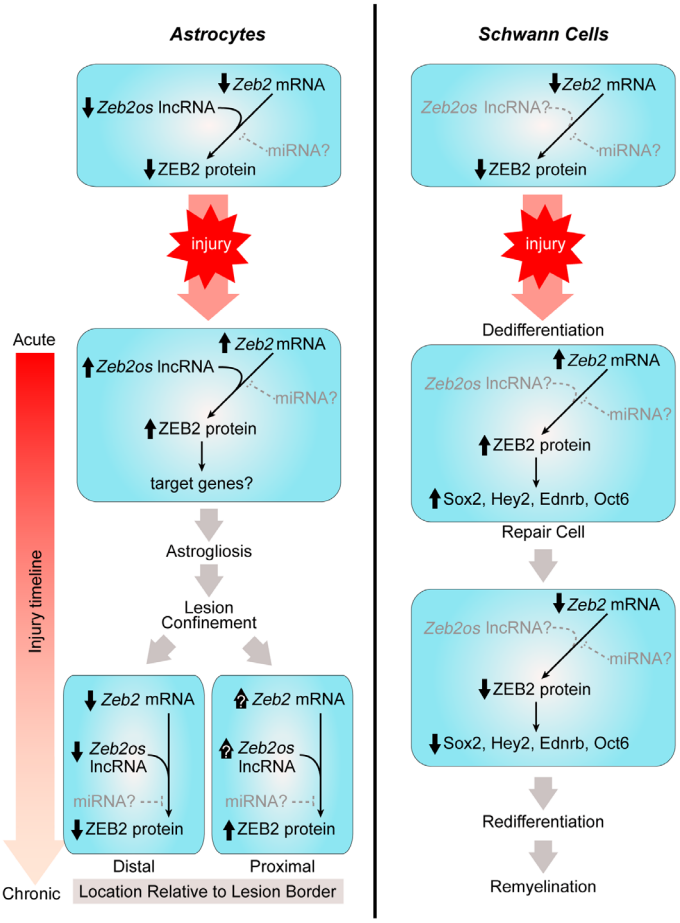

Figure 1 | ***Zeb2* signaling in the injury response of adult astrocytes and Schwann cells.**
In the uninjured adult CNS and PNS, *Zeb2* mRNA levels are detectable in astrocytes and Schwann cells, but ZEB2 protein levels are low. After injury, both astrocytes and Schwann cells increase *Zeb2* mRNA and protein levels. In astrocytes, injury induced up-regulation of *Zeb2os* lncRNA facilitates the increase in ZEB2 protein levels. The role of *Zeb2os* in Schwann cells, however, has not been explored. Several key ZEB2 target genes in Schwann cells have been identified, but the target genes in astrocytes remain to be identified. As injury in the CNS progresses to chronic time points, *Zeb2*, *Zeb2os*, and ZEB2 levels diminish as astrogliosis subsides in astrocytes that are distal to the lesion. Many reactive astrocytes either proximal or adjacent to the lesion remain activated with elevated ZEB2 protein levels, but whether *Zeb2* and *Zeb2os* levels in these cells also remain elevated has not been established. In Schwann cells, *Zeb2* and ZEB2 levels also decrease as the cells re-differentiate into myelinating and non-myelinating (Remak) Schwann cells. *Zeb2* is targeted by miRNA in other types of cells, but whether miRNA also has a role in regulating *Zeb2* levels in either astrocytes and Schwann cells remains to be established.

expression is induced by injury in a *Stat3*-dependent manner and its expression levels follow a pattern that mirrors ZEB2 protein expression. The conditional knockout of *Zeb2* in adult astrocytes attenuates astrogliosis, generates larger lesions, and delays recovery of motor function following either stroke or spinal cord injury (Vivinetto et al., 2020). These findings establish *Zeb2* as an important astrocyte-specific regulator of the injury response in the CNS.

Injuries in the PNS, such as sciatic nerve crush or transection, induce a transient re-activation of ZEB2 expression in Schwann cells that peaks at about 7 days post-injury before returning to undetectable levels at 28 days post-injury (Quintes et al., 2016; Wu et al., 2016). In mice with *Zeb2* conditionally deleted in adult Schwann cells, de-differentiation and proliferation after injury are not disturbed (Quintes et al., 2016; Wu et al., 2016). At 4 days post-injury, however, differences in toe pinch test responses are apparent and tissue analyses reveal that axon outgrowth is less efficient when compared to control animals. Throughout an 8-week recovery period following sciatic nerve injury, mice lacking *Zeb2* in Schwann cells remain severely impaired with undetectable nerve conduction velocities and significantly fewer remyelinated fibers. At 8 weeks post-injury, Schwann cells in *Zeb2* conditional knock-out mice still express high levels of de-differentiation markers, whereas myelination markers are down-regulated. Thus, in the absence of *Zeb2*, Schwann cells can initiate the normal injury response, but the completion of this process is impaired and Schwann cells are unable to re-differentiate, remyelinate axons, and the promote recovery of function.

***Zeb2* and epithelial-to-mesenchymal transitions (EMT) in the glial response to injury:** Together, these studies in the CNS and PNS show that *Zeb2* is an important component of the glial response to injury. Examining the gene expression changes in reactive astrocytes and Schwann cells further reveals that the injury-induced expression of *Zeb2* is part of a shared molecular response. In reactive astrocytes, *Zeb2* was identified as part of a subset of genes upregulated after injury that also promote EMT in cancer, development, and fibrosis (Vivinetto et al., 2020). EMT in these other processes drives a cellular transition from an epithelial phenotype to a mesenchymal, migratory phenotype. EMT is associated with extensive changes in the expression levels of genes regulating cell adhesion, cytoskeletal, extracellular matrix, cellular junctions, as well as inter- and intracellular signaling proteins (reviewed in Nieto et al., 2016). These types of changes in gene expression also occur in astrocytes and Schwann cells after injury. Correspondingly, studies in Schwann cells have also reported changes in gene expression patterns that resemble those associated with EMT (Arthur-Farraj et al., 2017; Clements et al., 2017). The generation of reactive astrocytes or repair Schwann cells after injury is not a canonical EMT process since neither astrocytes nor Schwann cells are epithelial cells and their injury-induced phenotypes are not mesenchymal cells. Even in the canonical cellular contexts, however, EMT is not a completely binary process and partial EMT phenotypes are common (Nieto et al., 2016). Thus, injury-mediated induction of reactive astrocytes and repair Schwann cells phenotypes are a partial or an EMT-like process.

Establishing that glial responses to injury in the PNS and CNS are EMT-like processes, together with identifying *Zeb2* as a pivotal regulator of these responses, improves our fundamental understanding of the neurobiology of how the nervous system responds to injury. Further studies are required, however, in order to better define the similarities and differences in these glial responses. In astrocytes, for example, the identities of ZEB2 target genes are not known. By contrast, ZEB2 represses expression of negative regulators of Schwann differentiation and

myelination, in part, by directly binding the *Sox2*, *Hey2*, *Ednrb*, *Oct6* promoter regions (Quintes et al., 2016; Wu et al., 2016). *Zeb2* also promotes Schwann cell differentiation by antagonizing the Notch signaling pathway. The Notch pathway also regulates astrocyte proliferation after stroke (LeComte et al., 2015), but further studies are needed to characterize the interplay of *Zeb2* and the Notch pathway in reactive astrocytes.

The role of non-coding RNAs (such as miRNA and lncRNA) in the PNS and CNS glial response is also an important component that requires further exploration. Studies in the CNS point to a pivotal role for *Zeb2os* in facilitating ZEB2 protein expression after injury (Vivinetto et al., 2020), but a role for *Zeb2os* in Schwann cells has been not studied. In addition, there are several miRNAs that modulate *Zeb2* levels in the context of cancer, but whether any of these miRNAs also modify the injury-induced expression of ZEB2 in either reactive astrocytes or Schwann cells remains to be addressed. In general, the roles and mechanisms of action for non-coding RNA (such as miRNA and lncRNA) in directing the response to the injury within the nervous system are understudied, but continuation exploration will likely provide critical details and novel therapeutic targets for treating neurological injury.

Future directions: enhancing repair by targeting *Zeb2*: Identifying *Zeb2* and EMT as regulators of glial responses to injury and reparative cellular adaptations may also inform the development of strategies to manipulate the glial responses after neurological injury. In the PNS, Schwann cells facilitate the restoration of function following injury, but the ability of these cells to provide repair diminishes with age and post-injury duration. Understanding how to prolong the adaptive responses of repair Schwann cells could mitigate several therapeutic challenges in the PNS. In the CNS, astrocytes are essential for restricting the spread of injury and developing interventions that can enhance the ability of reactive astrocytes to isolate an injured region will increase neuroprotection and improve functional recovery. Additionally, manipulating reactive astrocytes and their interactions with other cells in the chronic lesion boundary may improve the axon outgrowth through the lesion and the restoration of function. Studies in wound healing and cancer fields have described approaches to modify *Zeb2* expression and/or EMT processes. Exploring how these approaches can be used to manipulate glial responses after injury has the potential to open new therapeutic direction for improving nervous system repair and the recovery of function.

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