

● PERSPECTIVE

The expression of histone deacetylases and the regenerative abilities of spinal-projecting neurons after injury

Epigenetic control of regeneration after spinal cord injury: Complete spinal cord injury (SCI) in humans and other mammals leads to irreversible paralysis below the level of injury, due to failure of axonal regeneration in the central nervous system (CNS). Previous work has shown that successful axon regeneration is dependent upon transcription of a large number of regeneration-associated genes (RAGs) and transcription factors (TFs) (Van Kesteren et al., 2011). A prominent theory in the field of axon regeneration is that the large differences in regenerative potential between peripheral nervous system (PNS) neurons, which regenerate well, and CNS neurons, which do not, reflect differences in intrinsic transcriptional networks, rather than individual genes (Van Kesteren et al., 2011). These injury-inducible TFs are presumed to control hundreds of transcriptional targets of multiple regeneration-associated signaling pathways (Van Kesteren et al., 2011). Thus the seeming intractability of CNS axon regeneration might be due to the need to simultaneously turn on or off multiple regeneration-associated signaling pathways. One strategy to promote axon regeneration after SCI is to activate this TF “master switch” and enhance the axon growth capacity in adult neurons. Thus far, no such TF “master switch” has been found and it is possible that epigenetic modifications function as “master switches” that regulate transcription of RAGs after SCI, and thus activate or suppress entire regeneration-promoting pathways.

Gene expression in eukaryotes is governed by a cell's transcriptional machinery (RNA polymerases, transcription factors, and chromatin remodeling enzymes). Genomic DNA in eukaryotic cells is packaged with histones to form protein/DNA chromatin complexes. Histones pack DNA into nucleosomes, the building blocks of chromatin. Every nucleosome contains two subunits each of histones H2A, H2B, H3 and H4, known as the core histones. Epigenetic mechanisms - DNA methylation and histone modifications - result in changes in the chromatin structure, which in turn influence gene transcription. The amino-terminal tails of core histones undergo various post-translational modifications, including acetylation, phosphorylation, methylation, and ubiquitination, which serve to divide the genome into euchromatin, active regions where DNA is accessible for transcription, and heterochromatin, inactive regions, where DNA is more compact and therefore less accessible for transcription.

Acetylation is one of the most widely studied histone modifications, as it was one of the first described and linked to transcriptional regulation (Roth et al., 2001). Acetylation on lysine residues leads to relaxation of the chromatin structure, which allows the binding of transcription factors and significantly increases gene expression. The enzymes responsible for regulating the acetylation of histone tails are lysine acetyltransferases (KATs), which add acetyl groups to lysine residues, and histone deacetylases (HDAC), which remove the acetyl groups. Removing acetyl groups from the lysines of histones HDACs induces formation of a compact, transcriptionally repressed chromatin structure. Eighteen different mammalian HDACs have been identified and divided into four classes (I-IV). Class I includes HDACs 1, 2, 3 and 8, which are localized in the nucleus and expressed in all mammalian tissues. They are involved in regulating gene-specific transcription. Class IIa-HDACs 4, 5, 7 and 9 - shuttle between the nucleus and the cytoplasm. They have histone deacetylase activity only by interacting with HDAC3.

Recent studies have implicated epigenetic mechanisms in axonal regeneration (for review see Lindner et al., 2013). Histone acetylation appears to play an important role in PNS and CNS regeneration. Treatment with HDAC inhibitors (HDACi) increases global histone acetylation levels, and *in vitro* studies showed that HDAC

inhibition improved neurite outgrowth of neurons cultured on both permissive and inhibitory substrates (Gaub et al., 2010) and allowed neurons from embryonic spinal cord or hippocampus to partially overcome Nogo-A inhibition of neurite extension (Lv et al., 2012). Furthermore, HDAC5 recently was implicated in dorsal root ganglion (DRG) axon regeneration (Cho et al., 2013). While systemic administration of HDACi to mice caused some ascending DRG sensory fibers to regenerate *in vivo* and grow longer axons than vehicle-treated mice 2 weeks after SCI, direct treatments of dissociated DRG neurons with class I HDAC inhibitor MS-275 did not significantly increase mean axonal length or the percentage of axon bearing neurons over controls (Finelli et al., 2013). Those somehow inconsistent findings could result from a fact that HDAC inhibitors lack specificity, particularly lack of isoform selectivity (Marks et al., 2004; Dokmanovic et al., 2007; Delcuve et al., 2012). HDAC inhibitors can be structurally grouped into at least four classes: hydroxamates (SAHA, TSA, LBH589, PXD101, and tubacin), cyclic peptides (depsipeptide), aliphatic acids (valproic acid and butyrate) and benzamides (MS-275). TSA (and structurally similar to TSA vorinostat) are pan-HDAC inhibitors that inhibit all class I, II, and IV HDACs, whereas MS-275 inhibits only HDAC 1, 2, and 3, and valproic acid inhibits HDAC1, 2, 3, 4, 5, 7, 8, and 9. Therefore, HDACi may selectively inhibit different HDACs that will lead to different functional outcomes. For example, *in vivo* delivering of valproic acid (VPA) reduce microgliosis in lesioned spinal cord, and purinergic P2X4R expression in activated microglia, which is associated with neuropathic pain. VPA treatment *in vitro* seems to down-regulate microglial activation, whereas, in contrast, TSA and sodium butyrate appear to enhance activation (Lu et al., 2013).

HDACs expression in regenerating and non-regenerating neurons after SCI: Unlike in mammalian CNS, axons regenerate in lamprey, and animals recover behaviorally after SCI. Spinal-projecting reticulospinal (RS) neurons in the lamprey brain display great heterogeneity in their regeneration abilities - some neurons are good regenerators (axon regeneration rate > 50%) and others regenerate poorly (regeneration rate < 30%) (Jacobs et al., 1997). We utilized the exceptional advantage of the lamprey CNS, which enables the regenerative abilities of identifiable neurons to be correlated directly with HDACs and KATs expression in brain whole mounts.

In our research we compared the patterns of HDACs and KATs expression in regenerating vs. non-regenerating neurons at the cellular level (Chen et al., 2016). In control animals, both low and high regenerating capacity neurons expressed HDAC1 and HDAC3 and also several KATs (KAT2A, KAT5 and P300) mRNAs. Our data indicated that expression of the KAT2A, KAT5 and P300 did not change after SCI in either high regeneration capacity or low regeneration capacity neurons. However, HDAC1 (but not HDAC3) expression was significantly downregulated in both high and low regenerative capacity neurons 2 and 4 weeks after SCI. Surprisingly, at 10 weeks after SCI, HDAC1 mRNA expression in high regenerative capacity neurons was at pre-lesion level but HDAC1 mRNA expression in low regenerative capacity neurons was downregulated.

In animals that recover for 10 weeks, axons have sufficient time to reach the transection site and grow into the distal stump. Therefore, it is possible to label only regenerated neurons whose axons grew beyond the transection site. Regenerating neurons were retrogradely labeled and used *in situ* hybridization to determine whether expression of HDAC1 and 3 correlated with the regeneration propensities of spinal-projecting neurons. In agreement with our data that only HDAC1 expression was upregulated in high regenerative capacity neurons at 10 weeks after SCI, we found that more regenerating neurons expressed HDAC1 than HDAC3. While approximately 30% of regenerated RS neurons expressed HDAC3, more than 70% expressed HDAC1 mRNA, suggesting that HDAC1 activity is required for spinal-projecting neurons to regenerate their axons after SCI.

Our observations about downregulation of HDAC1 mRNA in lamprey neurons 2 and 4 weeks after SCI and published experimental results (Biermann et al., 2010; Gaub et al., 2010; Lv et al., 2011, 2012; Lin et al., 2015) suggest that inhibition of HDAC class I is

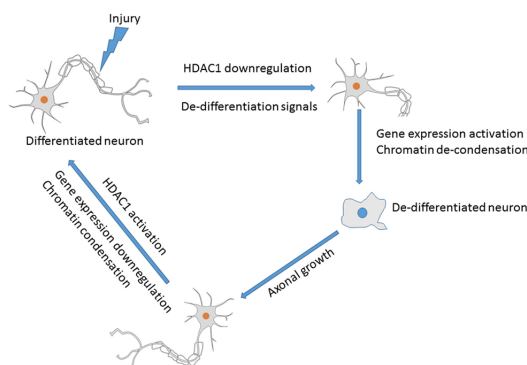


Figure 1 Model of the possible involvement of histone deacetylases 1 (HDAC1) in neuronal regeneration.

After injury insult, retrograde injury signals lead to HDAC1 downregulation and as a result of this, to increase in histone acetylation. Histone acetylation leads to chromatin structure de-condensation, gene expression activation and possible neuronal de-differentiation. De-differentiated neurons returned to an earlier (less mature) state that is more conducive to axonal growth initiation. At the end of axonal growth stage, HDAC1 expression increased, leading to downregulation of pro-axonal growth genes, chromatin condensation and neuron redifferentiation to a mature phenotype.

beneficial in the early stages of recovery after injury. However, at 10 weeks after SCI, HDAC1 expression was upregulated only in high regeneration capacity neurons. Why did HDAC1 mRNA expression follow this multi-phasic pattern?

Probable role of neuronal dedifferentiation in initiation of regeneration process after SCI: Mammalian neurons are unable to regenerate injured axons after complete SCI, but in many non-mammalian vertebrate species CNS axons possess a remarkable capacity to regenerate. One of the mechanisms associated with this natural regeneration is dedifferentiation, in which a terminally differentiated cell reverts back to a less differentiated stage within its own lineage (Tang, 2012). We therefore hypothesize that lamprey neurons de-differentiate after SCI and that this process is necessary to induce neurons to switch to regenerative mode (Figure 1). Downregulation of HDAC1 and continued expression of HATs (Chen et al., 2016) may lead to hyperacetylation of core histones that results in transcriptional reprogramming, which is largely responsible for activating the initial regenerative programs. In the next stage of the regenerative reaction, however, when the axons of good-regenerating neurons extend beyond the injury zone and even reach their original targets in caudal spinal cord, these neurons need to switch from the growth program to a differentiated stable state. We hypothesize that, as a component of this switch, high regeneration capacity neurons upregulate levels of HDAC1. Indeed, HDAC1 and HDAC2 are critical, redundant regulators of neuronal differentiation during neocortical, hippocampal, and cerebellar development. Neuronal precursors lacking HDAC1 and HDAC2 therefore are unable to differentiate specifically into mature neurons and undergo cell death (Montgomery et al., 2009). In addition, HDAC1 has recently been identified as an essential component of the mechanism that assigns neural progenitors to the oligodendrocyte fate; it acts by attenuating expression of a subset of neural progenitor genes (Cunliffe and Casaccia-Bonnel, 2006).

Conclusions: Chromatin-based epigenetic mechanisms underlie important aspects of CNS functions, including axon regeneration. Recent studies illuminated the involvement of the enzymes responsible for regulating the acetylation of core histones – KATs and HDACs – in the epigenetic mechanisms that influence axon regeneration in the adult CNS. Our experiments identified the patterns of HDAC1 and HDAC3 expression in regenerating vs. non-regenerating neurons at the cellular level and indicated that HDAC1 may play a prominent role in initiating axon regeneration after SCI and in maintaining neuronal stability of regenerating neurons. Future experiments will further investigate the epigenetic mechanisms that

influence axon regeneration in the mature, injured CNS. If pharmacological HDAC1 modulation increases the effectiveness of axon regeneration, this could form the basis for novel therapies to promote axon regeneration in patients with SCI and other CNS disorders.

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