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 (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 lysine residues leads to histone deacetylation and transcriptional silencing. However, HDACs have been shown to have multiple functions, including roles in transcriptional activation, chromatin remodeling, and non-coding RNA processing.

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 transaction site and grow into the distal stump. Therefore, it is possible to label only regenerated neurons whose axons grew beyond the transaction 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 HDAC1 expression was unchanged 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 its correlation with regeneration propensity implies that HDAC1 might be involved in the process of axon regeneration. However, further studies are needed to confirm this hypothesis. Additional experiments are required to investigate the role of HDAC1 in the process of axon regeneration and to determine whether it plays a direct role in promoting axon regeneration after SCI.
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 significant 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.

This work was supported by grants from Shriners Research Foundation grant SHC-85310.

Jie Chen, Michael I. Shifman∗

Shriners Hospitals Pediatric Research Center (Center for Neural Repair and Rehabilitation), Temple University School of Medicine, Philadelphia, PA, USA (Chen J, Shifman MI)
Department of Neuroscience, Temple University School of Medicine, Philadelphia, PA, USA (Shifman MI)

*Correspondence to: Michael I. Shifman, Ph.D., mishifman@temple.edu. Accepted: 2016-09-28

How to cite this article: Chen J, Shifman MI (2016) The expression of histone deacetylases and the regenerative abilities of spinal-projecting neurons after injury. Neural Regen Res 11(10):1577-1578.

Open access statement: This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported license, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

References

Biermann J, Grieshaber P, Goebel U, Martin G, Thanos S, Giovanni SD, Lagrèze WA (2010) Valproic acid-mediated neuroprotection and regeneration in injured retinal ganglion cells. Invest Ophthalmol Vis Sci 51:526-534.

Chen J, Laramore C, Shifman MI (2016) Differential expression of HDACs and KATs in high and low regeneration capacity neurons during spinal cord regeneration. Exp Neurol 280:50-59.

Cho Y, Sloutsky R, Naegle Kristen M, Cavalli V (2013) Injury-induced HDAC5 nuclear export is essential for axon regeneration. Cell 155:894-908.

Cuniliffe VT, Casaccia-Bonnefil P (2006) Histone deacetylase 1 is essential for oligodendrocyte specification in the zebrafish CNS. Mech Dev 123:24-30.

Delcove G, Khan D, Davie J (2012) Roles of histone deacetylases in epigenetic regulation: emerging paradigms from studies with inhibitors. Clin Epigenet 4:5.

Dokmanovic M, Clarke C, Marks PA (2007) Histone deacetylase inhibitors: overview and perspectives. Mol Cancer Res 5:981-989.

Finelli MJ, Weng JK, Zou H (2012) Epigenetic regulation of sensory axon regeneration after spinal cord injury. J Neuroscience 33:19664-19676.

Gaub P, Tedeschi A, Puttganga R, Nguyen T, Schmandke A, Di Giovanni S (2010) HDAC inhibition promotes neuronal outgrowth and counteracts growth cone collapse through CRMP3 and P/CAD-dependent p53 acetylation. Cell Death Differ 17:1392-1408.

Jacobs AJ, Swain GP, Snedaker JA, Pijak DS, Gladstone LJ, Selzer ME (1997) Recovery of neurotransmitter expression selectively in regenerating retinol spiral ganglion neurons. J Neuroscience 17:5206-5220.

Lin S, Nazif K, Smith A, Baas PW, Smith GM (2015) Histone acetylation inhibitors promote axon growth in adult dorsal root ganglia neurons. J Neuroscience 35:1215-1228.

Lindner R, Puttganga R, Giovanni S (2013) Epigenetic regulation of axon outgrowth and regeneration in CNS injury: the first steps forward. Neurotherapeutics 10:771-781.

Lv L, Han X, Sun Y, Wang X, Dong Q (2012) Valproic acid improves locomotion in vivo after SCI and axonal growth of neurons in vitro. Exp Neurol 233:783-790.

Lv L, Sun Y, Han X, Xu CC, Tang YP, Dong Q (2011) Valproic acid improves outcome after rodent spinal cord injury: Potential roles of histone deacetylase inhibition. Brain Res 1396:56-68.

Marks PA, Richon VM, Miller T, Kelly WK (2004) Histone Deacetylase Inhibitors. Inn: Advances in Cancer Research, pp 157-168. Academic Press.

Montgomery RL, Hsieh J, Barbosa AC, Richardson JA, Olson EN (2009) Histone deacetylases 1 and 2 control the progression of neural precursors to neurons. Cancer Cell 16:461-474.

Montgomery RL, Hsieh J, Barbosa AC, Richardson JA, Olson EN (2009) Histone deacetylases 1 and 2 control the progression of neural precursors to neurons. Exp Cell Res 315:448-461.

Montgomery RL, Hsieh J, Barbosa AC, Richardson JA, Olson EN (2009) Histone deacetylases 1 and 2 control the progression of neural precursors to neurons. J Neuroscience 29:4652-4664.

Zhang X, Wu W, Pan H, Li S, Li Y (2012) Benefits of differentiated stem cells for neural regeneration. Stem Cell Rev 8:201-212.

Van Kesteren RE, Mason MRJ, MacCallum HD, Smit AB, Verhaagen J (2011) A gene network perspective on axonal regeneration. Front Mol Neurosci 4.