PERSPECTIVE

Protein deacetylases and axonal regeneration

A neuron with injured or severed axon responds with attempts at axonal regrowth. In this regard, axonal regeneration of peripheral nerves occurs far more efficiently compared to central nervous system (CNS) neurons. The latter typically could not form a proper growth cone, and any axonal regeneration *in vivo* is very limited. The adult CNS environment is not conducive for axonal regrowth. An extensive body of work has revealed mechanisms whereby the myelin-associated inhibitors and extracellular matrix chondroitin sulfate proteoglycans promote collapse of axonal growth cones or repel their advances (Lee and Zheng, 2012). The intrinsic axonal regeneration capacity of an injured neuron is, however, grounded on a combination of factors, including responses to a myriad of survival signaling molecules and pathways, axonal mRNA transport and local protein synthesis, as well as proper axonal growth cone formation and stabilization. Interestingly, recent findings have linked several aspects of axonal regenerative capacity to protein acetylation and deacetylation.

Nuclear histone and a myriad of nuclear and cytoplasmic proteins are post-translationally modified by lysine acetylation, mediated by a range of histone acetyltransferases (HATs). Four classes of histone/protein deacetylases could reverse this modification. There are eleven histone deacetylases (HDACs 1–11), which are grouped into classes 1, II and IV (Yang and Seto, 2008). The class III deacetylases, the sirtuin family with seven members in the mammalian system, stands on its own because of their obligatory cofactor dependence on nicotinamide adenine dinucleotide (NAD^+) (Haigis and Sinclair, 2010). HDACs and sirtuins have a wide range of targets, including histones, transcription factors and cytoplasmic components such as tubulin (Yang and Seto, 2008; Haigis and Sinclair, 2010). In mammals, HDACs and sirtuins-mediated deacetylation are known to have diverse regulatory roles in the nervous system, including cognition and memory. At the cellular level, these deacetylases modulate neuronal activity and survival in a multitude of ways. In principle, protein deacetylases could have direct or indirect role in axonal regeneration. A direct or acute action may pertain to the deacetylases' regulation of growth cone stabilization through tubulin modification. More indirect and chronic influences by the deacetylases would involve changes in transcriptional and epigenetic profiles that would modulate growth cone formation and axonal outgrowth. Some examples of how HDACs and sirtuins may influence axonal regeneration are highlighted below.

HDACs and their roles in neuronal regeneration: Logically, any significant axonal regeneration could only be possible if the injured neuron survives. It should also be noted that the survival response and axonal regeneration share signaling components and pathways, and it is therefore not surprising that these two processes are often intertwined. A general observation made from several studies is that pan-HDAC inhibitors aid the survival of injured neurons, and promote axonal regeneration. One key cellular factor affected by a general HDAC inhibition is the tumor suppressor and apoptosis regulator p53. As a transcription factor, p53 acetylation appeared to result in a transcriptional profile that is conducive for neuronal outgrowth (Gaub et al., 2010). For example, p53 regulates the expression of the small GTPase Rab13 and the actin binding protein coronin 1b, thus influencing neuritic protrusion. p53 also complexes with the acetyltransferase Creb binding protein (CBP)/p300 to transcriptionally enhance the expression of growth associated protein 43 (GAP43), a key factor in neuritogenesis and plasticity (Di Giovanni et al., 2006). Inhibition of HDAC activities induces the transcription of regeneration-associated genes (RAGs) in dorsal root ganglion neurons, and Smad1 (downstream of BMP signaling), in complex with HDAC1, acts in regulating promoter histone acetylation that modulates induction of RAGs (Finelli et al., 2013).

pan-HDAC inhibition was shown also to promote neurite outgrowth on non-permissive CNS myelin substrates, and the main target in this regard appears to HDAC6 (Rivieccio et al., 2009). HDAC6 is mainly found in neurons in the mammalian brain, and it is largely cytoplasmic, associating with microtubules. Its role as a tubulin deacetylase could be gleaned from the effect of HDAC6 knockout in mice, which causes global tubulin hyperacetylation. HDAC6 deficiency resulted only in subtle changes, but it has an important role in modulation of neurodegenerative disease pathology associated with the formation and clearance of cellular aggregates, such as Parkinson's disease and Huntington's disease. HDAC6 binds to both heavily ubiquitinated proteins and dynein, thus facilitating transport to aggresomes. HDAC6 also modulates aggresome autophagy by controlling the fusion of autophagosomes to lysosomes (Lee et al., 2010).

Although not firmly implicated in axonal regeneration, HDAC6 is known to modulate several aspects of neuritogenesis. For example, HDAC6 is known to promote ubiquitination of Cdc20, which in turn stimulates the anaphase promoting complex/cyclosome in postmitotic neurons to drive dendritic outgrowth. Tubulin deacetylation destabilizes microtubules, but microtubule stabilization is critical for formation of growth cone in lesioned axonal tips. A microtubule-stabilizing drug, epothilone B, was recently shown to induce concerted microtubule polymerization into the axon tip, thus promoting axon regeneration (Ruschel et al., 2015). HDAC6 inhibition could therefore aid regeneration. Indeed, HDAC6 levels are elevated by injury, and both HDAC6-silencing and specific pharmacological inhibition promoted neurite outgrowth (Rivieccio et al., 2009). HDAC6's interaction with the scaffolding septins could also negatively regulate microtubule stability during axonal and dendritic outgrowth during development. However, HDAC6's role in neurite outgrowth is likely to be complex and context dependent, as inhibition of HDAC6 activity or reduction in HDAC6 expression was also known to alter kinesin motor distribution and reduce axonal growth (Tapia et al., 2010).

Recent findings have implicated the involvement of HDAC5 in promoting axonal regeneration of peripheral neurons *via* two

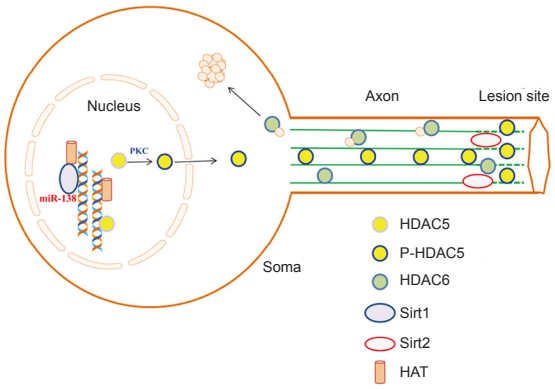

Figure 1 A schematic diagram illustrating the possible roles and site of action of histone deacetylases (HDACs) and sirtuins during axonal injury. Nuclear HDAC5 is phosphorylated by protein kinase C (PKC) activated by calcium flux from the site of injury. Phosphorylation of HDAC5 (p-HDAC5) facilitates both its nuclear exit and engagement of kinesin, and is transported to the distal tip of the injured axon. Nuclear exit of HDAC5 changes the transcription profile to one that is pro-regeneration. Cytoplasmic HDAC6 may facilitate transport of cargos and injury-induced protein aggregates to aggresomes *via* dynein, and regulates autophagy. It may thus be beneficial to injured axon that is trafficking-impaired. Sirt1 acts largely at the nucleus, and its activity is pro-survival and regeneration, in reciprocal feedbacks with HATs and miR-138. Sirt2 is a major tubulin deacetylase and may have a role in microtubule dynamics at the injury site. HAT: Histone acetyltransferase.

complementary mechanisms. A gradient of microtubule deacetylation, which signifies an increase in dynamic microtubules proximal to the lesion tip, is induced by axonal injury, and formation of this gradient could be attenuated by HDAC inhibitors. Interestingly, silencing of HDAC5 affected tubulin deacetylation, and markedly suppressed axon regeneration and growth cone dynamics. Injury increased calcium influx and protein kinase C (PKC) activation, which phosphorylates HDAC5. Although acetylated tubulin may not be acted upon by HDAC5 under basal conditions, phosphorylated HDAC5 could become a major tubulin deacetylase in injured axons (Cho and Cavalli, 2012). PKC phosphorylated HDAC5 has apparently an increased tubulin deacetylase activity, as well as increased interaction with kinesin 1, which would facilitate its accumulation at the lesion tip. Furthermore, PKC phosphorylation of HDAC5 appears to promote its nuclear-cytoplasmic translocation, and its subsequent transport from the cell soma to the axon tip. Other than availing itself for tubulin deacetylation, another important consequence of HDAC5 nuclear exit is a change in the expression profile of HDAC5-dependent genes, some of which have known roles in neuronal regeneration and stress response (Cho et al., 2013). Exit of HDAC5 from the nucleus therefore elicits a change in gene expression profile that results in the enhancement of regenerative pathways. In fact, promotion of HDAC5 nuclear exit *in vivo* with a PKC activator generates a gene expression profile that partially mimics an injury preconditioning effect, which was known to be associated with greater regenerative capacity.

Sirtuins and axonal regeneration: Sirtuins are key sensors and regulators of cellular energetic and metabolism, and are therefore likely to play some part in injured neurons with altered energy status. The best studied sirtuin, SIRT1, has a myriad of demonstrated roles in the nervous system, which include the modulation of axonal and dendritic outgrowth. SIRT1 could be found at the axonal growth cone and its activation could promote axogenesis through deacetylation and consequential activation of AKT, a key regulator of neuronal survival and axonal growth. AKT activation in turns inactivates GSK3, which inhibits axonal growth (Li et al., 2013). A particularly interesting recent finding pertained to the description of a mutual negative feedback loop between Sirt1 and the micro RNA miR-138 in the regulation of axonal regeneration (Liu et al., 2013). miR-138 is highly expressed in the nervous system and is important for modulating axonal growth during development as well as during peripheral nerve injury. The down-regulation of miR-138 after sciatic nerve injury was apparently necessary for axonal regeneration of dorsal root ganglion neurons. Sirt1 is a target of miR-138 mediated transcriptional repression, but it turns out that Sirt1 could also in turn suppress the expression of miR-138. The interplay between the two could be important in determining the outcome of axonal regrowth.

Other than HDAC6 described above, Sirt2 is another major tubulin deacetylase in the cytoplasm of several cell types. It is therefore conceivable that Sirt2 activities or levels in an injured axon would have a role in axonal regrowth. Sirt2 over-expression has been shown to inhibit neurite outgrowth of mouse hippocampal neurons (Pandithage et al., 2008). Interestingly, over-expression of Sirt2 also stabilized the growth cone against collapse induced by sphrinA5-EphA receptor. As Sirt2's activity, like that of Sirt1 and other sirtuins is very dependent on the status of NAD^+, energy and redox states of the injured axon would likely have be limiting the roles of sirtuins in axonal regeneration.

Epilogue: The paragraphs above provide a brief outline of the involvement of HDACs and sirtuins in axonal regeneration (summarized schematically in **Figure 1**). Both cytoplasmic HDAC5 and HDAC6 could have a direct effect on the growth cone through tubulin deacetylation. Cytoplasmic Sirt2 may act likewise. On the other hand, nuclear HDAC5 and Sirt1 could impact on axonal regeneration *via* mediation of transcriptional profiles that could favor regeneration, or otherwise. A more detail understanding between the interplay of protein deacetylases and their substrates would certainly be useful in the design of regeneration strategies or therapeutics. It should be noted that the findings on the involvement of protein deacetylases in axonal regeneration were largely made in peripheral nerves injury models. An interesting and important direction in future work would be to see how manipulation of HDAC and sirtuin levels and activities could aid axonal regeneration of recalcitrant CNS neurons. Also, epigenetic modulations of HDAC and sirtuins with regards to axonal regeneration may go beyond direct effects on the neuron itself, but also transcriptional changes on the part of glial cells that may indirectly affect the axonal regeneration process (Wong and Zou, 2014). A better understanding of the systemic effects of protein deacetylases would aid therapeutic discoveries.

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