PERSPECTIVE

Casein kinase signaling in axon regeneration

Recent studies suggest that cell cycle pathways may contain therapeutic targets important for neurotrauma. An example of this is the finding that the vertebrate cell cycle exploits proteolysis pathways, yet these activities persist in fully differentiated cells that have exited the cell cycle such as neurons. We have known for some time that a ubiquitin ligase, the anaphase promoting complex (APC/C) required for progression through the M and G1 phases of the cell cycle, is also active in fully differentiated neurons that are no longer dividing (Penas et al., 2011). Several studies demonstrated roles for APC/C in restraining neurite outgrowth in fully differentiated neurons (Stegmuller and Bonni, 2005). Depleting the APC/C activator Cdh1 increased neurite outgrowth in cerebellar granule cells (Stegmuller and Bonni, 2005). Similarly, overexpression of nondegradable APC/C substrates such as SnoN and Id2 promoted neurite outgrowth in cerebellar granule cells (Lasorella et al., 2006; Stegmuller et al., 2006). Interestingly, nondegradable Id2 overexpression increased axonal growth after spinal cord injury (Yu et al., 2011), suggesting that modulating protein levels by affecting degradation rates may be therapeutically attractive in preclinical models of neurotrauma.

One means of modulating protein levels in neurotrauma is by inhibiting protein turnover *via* small molecule inhibitors. Inhibition of the protein destruction machine, or proteasome, in cells is one therapeutic strategy for increasing protein levels in neurons. Indeed, proteasome inhibitors are used for treatment of some cancers and thus potentially useful therapeutically in treating spinal cord injury (SCI) or traumatic brain injury (TBI). However, proteasome inhibitors suffer from toxicities in patients, thereby prompting the need for identifying more selective inhibitors in the ubiquitin proteasome pathway.

A potentially better tolerated means of inhibiting the ubiquitin proteasome pathway, and thereby upregulating proteins in neurite outgrowth, could be to inhibit a specific ubiquitin ligase, such as the APC/C. Small molecule inhibitors of APC/C interaction with its activators have been recently described (Zeng et al., 2010; Zeng and King, 2012; Sackton et al., 2014), and thus testing APC/C inhibitors in nerve regeneration assays *in vitro* and *in vivo* is technically feasible. Interestingly, APC/C^{Cdh1} interacts with PTEN (phosphatase and tensin homolog) (Song et al., 2011), whose genetic deletion promotes axon regeneration after SCI (Park et al., 2010). However, these inhibitors inhibit both the APC/C^{Cdc20} and the APC/C^{Cdh1} forms of APC/C, which also function during the cell cycle. Thus, potential off-targets in rapidly dividing cells will likely lead to toxicities, thereby limiting their use at therapeutic doses that would induce neurite outgrowth *in vivo*.

One means of avoiding such toxicities is by developing inhibitors that target a specific ligase-substrate interaction, while leaving global ligase activity unperturbed. Precedence for developing such an inhibitor comes from studies on the MDM2 ubiquitin ligase, which controls p53 degradation. Small molecule inhibitors of the MDM2-p53 interaction have been developed that only disrupt the protein-protein interaction and leave the intrinsic MDM2 ligase activity intact (Gessier et al., 2015). Thus, it should be technically feasible to identify small molecules, which disrupt interactions of the APC/C with any protein required for neurite outgrowth.

One newly discovered APC/C substrate implicated in neurite outgrowth is casein kinase 1δ (CK1δ). The Ayad laboratory recently reported that CK1δ is unique among Casein kinase family members in being targeted by APC/C^{Cdh1} (Penas et al., 2015). CK1δ is a serine-threonine kinase required for various biological processes including cell proliferation, circadian rhythm, and neurite outgrowth. Conditional deletion of Cdh1 in cerebellar granule cell progenitors (GCPs) increased CK1δ levels, which was associated with increased proliferation in GCPs as they are still dividing (Penas et al., 2015). It will be interesting to determine what is the consequence of increasing CK1δ levels in cells that have exited the cell cycle. One prediction in neurons is that CK1δ upregulation or inhibition of degradation should stimulate neurite outgrowth in cultured neurons. Another prediction is that small molecule CK1δ inhibitors should reduce neurite outgrowth in primary neurons as was reported for cell lines. However, CK1δ inhibitors almost always inhibit the related kinase CK1ε and thus assessing the contribution of these two CK1 isoforms to neurite outgrowth is difficult within this context. Perhaps a means of identifying the relative roles of CK1δ and CK1ε in neurite outgrowth is to utilize a machine-learning algorithm to identify targets and anti-targets in this biological process (Al-Ali et al., 2015). The Lemmon and Bixby laboratory recently generated an algorithm to identify kinases that are important for neurite outgrowth by combining *in vitro* kinome profiling of individual kinase inhibitors with neurite extension assays. For instance, Rho kinase inhibitors are known to promote neurite outgrowth and thus it would potentially be attractive to inhibit Rho kinase while not inhibiting kinases that could potentially be required for neurite outgrowth. Future studies will determine whether kinase inhibitors that target Rho kinase yet do not inhibit CK1δ are better at promoting neurite outgrowth than those inhibitors who inhibit both kinases.

Given the reported role for CK1δ in neurite outgrowth *in vitro*, the prediction is that CK1δ inhibition should reduce axonal growth after neurotrauma. However, extrinsic cues from the glial scar is an important regulator of axon regeneration. While the role of CK1 in astrocytes is virtually unknown, overexpression of Cdh1 in astrocytes has been reported to reduce reactive astrocyte proliferation (Qiu et al., 2013). In addition, CK1δ inhibition reduces neuropathic pain after SCI, possibly *via* reducing inflammation (Kurihara et al., 2014). Future studies will delineate the role of APC/C and CK1δ in glial scar formation and axon regeneration after neurotrauma. Furthermore, the contribution of

APC/C dependent degradation should be analyzed to determine whether it is feasible to simultaneously stimulate APC/C-dependent degradation of CK1δ in scar-forming cells while inhibiting the APC/C-CK1δ interaction in neurons.

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