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SPOTLIGHT

cADPR induced calcium influx mediates axonal degeneration caused by paclitaxel

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Activation of the NAD hydrolase domain of Sarm1 mediates axonal degeneration caused by chemotherapy drugs, but the downstream events are unknown. In this issue, Li and colleagues (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202106080) demonstrate that cADPR, a breakdown product of NAD, mediates paclitaxel-induced axonal degeneration by promoting influx of calcium into the axons.

Chemotherapy-induced peripheral neuropathy (CIPN) is a common and often disabling side effect of many traditional chemotherapy drugs. Currently the interventions used to treat other painful neuropathic conditions are less effective at relieving CIPN-induced pain. Chemotherapy drugs from different classes often present in a similar manner with a stocking-and-glove distribution of painful paresthesias and sensory loss. Furthermore, the underlying pathological process is shared among the different chemotherapy drugs and is characterized as a dying-back of distal axons, starting at the most distal ends of the sensory axons in the epidermis. Although diverse chemotherapy drugs have different mechanisms of action in terms of their anti-neoplastic efficacy and neurotoxicity, recent advances in the field of programmed axon degeneration (PAD) have shown that they converge on a key molecule, Sterile α and Toll/Interleukin-1 Receptor (TIR) motif-containing 1 (Sarm1; reviewed in 1). Sarm1 was identified as a key mediator of Wallerian degeneration of distal axons after traumatic injury using genetic screening in flies (2) and mammalian sensory neurons (3). Flies and mice lacking the *Sarm1* gene show markedly delayed axon degeneration after axotomy. More importantly, and relevant to the clinical populations, mice lacking the Sarm1 gene are resistant to development of peripheral neuropathy when exposed to chemotherapy drugs such as vincristine (4), paclitaxel (5), bortezomib (6), or cisplatin (7). These observations raised the possibility that pharmacological and genetic inhibition of Sarm1 could be developed as a therapeutic option for CIPN. Among the neurodegenerative diseases, CIPN is unique in that it is the ideal platform to evaluate "neuroprotection" because the timing of the neuronal injury is known and therefore an intervention could potentially be made before the injury occurs.

Recent advances in the field have shed light onto how Sarm1 is activated, but how this leads to the actual axon degeneration is unknown. Activation of Sarm1 leads to dimerization of the TIR domain and enabling of nicotinamide adenine dinucleotide (NAD) hydrolase activity (8), which in turn converts NAD into nicotinamide, cyclic-ADP Ribose (cADPR), and ADP Ribose (ADPR). Although the dimerization of TIR domain and activation of the NAD hydrolase activity is sufficient and necessary to cause axonal degeneration, it is unclear how this was achieved.

The manuscript by Li and colleagues fills this gap in the PAD pathway (9). They hypothesize that cADPR-driven calcium influx in the axons may mediate Sarm1-dependent axonal degeneration induced by paclitaxel. The rationale for this hypothesis is sound, given that axonal calcium influx is a critical component of Wallerian degeneration and that cADPR is known to mobilize calcium in a wide range of cells. First, they demonstrate that paclitaxel treatment of sensory neurons in compartmentalized Campenot chambers leads to elevation of cADPR in axons and that shRNA depletion of Sarm1, but not CD38 (another NAD hydrolase), prevents axonal degeneration and elevation of cADPR. They also show that there is an increase in intra-axonal calcium in response to paclitaxel and that this process is Sarm1-dependent in compartmentalized microfluidic cultures. Interestingly, though, the authors find that the concentration of paclitaxel needed to induce the calcium flux in microfluidics chambers is 20-fold higher than at 24 h when they observe clear axonal degeneration. This is likely to be due to a culture artifact, as the substrate in Campenot chambers is Matrigel but in microfluidic cultures it is laminin, which may alter the balance between growth-promoting versus axon-degenerating intracellular signals (10). A critical experiment in this paper was to use 8-Br–cADPR, a cell-permeable antagonist of cADPR, which blocks cADPR-induced calcium release in many cell types. The authors show that 8-Br–cADPR partially blocks the paclitaxel-induced rise in intra-axonal calcium, but this is sufficient to block axon degeneration fully. It is interesting to note

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that axon degeneration was blocked by an even lower dose of 8-Br–cADPR than what was needed to induce a change in calcium levels, suggesting that the effects of 8-Br–cADPR may not be wholly mediated via altering the intra-axonal calcium levels. However, this protection provided by 8-Br–cADPR is not as long-lasting as knockdown of Sarm1. Although 8-Br–cADPR was able to provide full axonal protection against paclitaxel at 24 h, like Sarm1 knockdown, the effect was lost at later time points, indicating that cADPR is unlikely to be the sole mediator of axonal degeneration when Sarm1 is activated.

How cADPR mediates the rise in intra-axonal calcium is unclear. It is possible that cADPR might elicit an increase in calcium in cells from both intracellular and extracellular sources, via ryanodine receptor 3 (RyR3) and Transient Receptor Potential Cation Channel Subfamily M Member 2 (TRPM2), respectively. Li and colleagues found that knockdown of both RyR3 and TRPM2 provide full protection against axon degeneration caused by paclitaxel or direct activation of Sarm1 via Sarm1 TIR domain (sTIR) dimerization, at least for 24 h (9). This is an interesting observation as one would have expected only partial rescue if both intracellular and extracellular calcium played equal roles. Complicating the matters further, knockdown of inositol 1,4,5-trisphosphate receptor (IP_3R) also prevents axon degeneration induced by sTIR dimerization. This is an interesting observation because cADPR-mediated increases in intracellular calcium are not dependent on IP_3R.

These observations suggest that upon activation of Sarm1, there are likely several downstream mediators of axon degeneration and that cADPR is one of them, albeit a likely critical one. This hypothesis is further strengthened by the observation that 8-Br–cADPR does not prevent axon degeneration caused by axotomy or treatment by a mitochondrial inhibitor, carbonyl cyanide m-chlorophenylhydrazone (9). This is one of the critical observations in this paper because it informs us that although Sarm1 activation via various upstream mechanisms (axotomy, mitochondrial toxicity, chemotherapy drugs, etc.) leads to NAD hydrolysis and cADPR production, downstream mechanisms that mediate axon degeneration are also varied and not all depend on cADPR or cADPR-mediated calcium influx.

Similar to the in vitro observations, Li and colleagues also found that in vivo treatment with the cADPR antagonist, 8-Br–cADPR, partially prevented paclitaxel-induced peripheral neuropathy as measured by mechanical allodynia and changes in intraepidermal nerve fiber density in mouse hind paws. Although the effect size was not as strong as genetic deletion of Sarm1, cADPR antagonists can potentially be added to the Sarm1 inhibitors (11) to combat CIPN.

A major challenge in the clinical translation of inhibiting PAD in neurodegeneration is the lack of a reliable in vivo biomarker of Sarm1 activation. Although reduction in NAD and subsequent elevation in cADPR has been demonstrated convincingly in vitro for axotomy and paclitaxel, these in vitro experiments have all been done on a much-accelerated timescale, often lasting less than 24 h. It is unclear if Li and colleagues attempted to measure cADPR levels in peripheral nerves of paclitaxel-treated mice, but it is likely to be technically very challenging because the degeneration occurs very distally and only in a small fraction of epidermal nerve fibers, often 20–30% at the clinically relevant doses of paclitaxel. Although neurofilament light chain (NF-L) is potentially a powerful biomarker of axon degeneration in CIPN (12), it is not a direct biomarker of Sarm1 activation, and therapies aimed at inhibiting Sarm1 may not be reflected in NF-L levels.

Overall, the future of developing truly disease-modifying therapies in neurodegenerative diseases characterized by distal axon degeneration looks promising. The paper by Li and colleagues helps solve a piece of the puzzle of PAD and teaches us that there are likely converging and diverging mediators of axon degeneration caused by different types of insults. Sarm1 inhibitors and antagonists of other players in the pathway are likely to play a significant role in clinical translation of these discoveries over the next 2–5 yr and have a tremendous impact on the quality of life of cancer survivors.

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