Journal Club

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Axotomy-Induced Ganglioside Processing: A Mediator of Axon Regeneration Restricted to the PNS

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Review of Kappagantula et al.

It has long been observed that axons in the CNS have a poor ability to regenerate, while peripheral axons regenerate readily. Characterizing the molecular determinants that allow peripheral axons to regenerate might identify therapeutic strategies that may stimulate nerve regeneration and functional recovery after nerve damage. The permissiveness of the extracellular environment as well as cell-intrinsic molecular programs have been identified as critical variables that differ between the CNS and the PNS and that underlie the disparity in regeneration.

Evidence suggests that gangliosides, a family of plasma membrane glycosphingolipids with one or more sialic acid residues, are important regulators of axon regeneration (Schnaar et al., 2014). Gangliosides play important roles in cell–cell and cell–environment interactions by organizing plasma membrane microdomains as well as acting as receptors for extracellular ligands (Schnaar et al., 2014). For example, the monosialylated GM1 ganglioside is a critical organizer of lipid rafts, which act as hubs for receptors and signaling complexes. GM1 has been shown to promote axon growth and regeneration *in vitro* and *in vivo* (Schnaar et al., 2014). In contrast, the polysialylated gangliosides, GD1a and GT1b, have been shown to act as receptors for myelin-associated inhibitors (Vinson et al., 2001; Vyas et al., 2002) and application of exogenous sialidase, an enzyme that cleaves sialic acid residues from polysialylated gangliosides to generate monosialylated GM1, has been shown to relieve myelin-dependent neuronal outgrowth inhibition (Vyas et al., 2002) as well as promote axon regeneration and functional recovery in rats that have been subjected to spinal cord injury (Mountney et al., 2010).

Although there is evidence to support a role for sialidase in axon regeneration, the underlying mechanisms are unclear and a role for endogenous regulation of gangliosides in axon regeneration has not been clearly defined. However, a recent study has identified an injury-induced engagement of neuronal sialidase activity that is restricted to the PNS and is critical for axon regeneration (Kappagantula et al., 2014). Mechanical axotomy of cultured adult dorsal root ganglion (DRG) neurons resulted in a transient enrichment of axonal GM1 ganglioside, as revealed by comparing the ratios of immunostained GM1 to GD1a and GT1b (Kappagantula et al., 2014, their Fig. 1). Pharmacological inhibition or siRNA-mediated knockdown of the sialidase Neuraminidase-3 (Neu3) blocked this postinjury enrichment of GM1 and significantly reduced the regeneration of adult DRG axons (Kappagantula et al., 2014, their Fig. 2), suggesting that Neu3-mediated ganglioside processing is a critical injury-induced mediator of peripheral axon regeneration. Because axon regeneration was assessed only 1 h after axotomy, however, it is possible that axons may still be regeneration-competent in the context of sialidase antagonism, albeit on a slower timescale. Analysis of injured axons at later time points as well as live imaging of axotomized neurons could provide further insight into the contribution of sialidase to the regeneration of a new growth cone following injury.

To determine whether PNS injury modifies the ganglioside profile *in vivo*, Kappagantula et al. (2014) examined the ratio of GM1/GD1a levels in adult rats after sciatic nerve injury. A transient upregulation of GM1 was observed in immunostained sections of injured sciatic nerve taken 6 h after injury (Kappagantula et al., 2014, their Fig. 3), supporting the hypothesis that injury-induced ganglioside processing is physiologically relevant. However, as the authors point out, it is not possible to rule out the contribution of other cell types to the enhanced periaxonal GM1 staining observed in the sciatic nerve sections. While the conclusion drawn from these *in vivo* data is that peripheral nerve injury stimulates Neu3 activity, it is also possible that injury induces the synthesis of GM1 from precursor gangliosides or suppresses its degradation, re-

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sulting in a transient accumulation of GM1.

To investigate whether injury-induced ganglioside processing is operative in CNS neurons, cultured retinal neurons were axotomized and analyzed for GM1 content. Interestingly, GM1 enrichment was not observed after axotomy (Kappagantula et al., 2014, their Fig. 5*B*), despite the presence of Neu3 (Kappagantula et al., 2014, their Fig. 5*E*). This suggested that Neu3 is inactive in CNS neurons and prompted the authors to determine whether exogenous Neu3 sialidase could improve the regeneration of retinal neurons, of which a significantly smaller proportion of axons regenerate compared with DRG neurons. Indeed, application of Neu3 to retinal axons caused an enrichment of GM1 (Kappagantula et al., 2014, their Fig. 5*C*) and resulted in a significant increase in the proportion of regenerating axons (Kappagantula et al., 2014, their Fig. 5*P*). Sialidase treatment also reduced the average distance retracted by the axons, raising the possibility that GM1 may facilitate axonal adhesion to the substrate. Consistent with this idea, GM1 has been shown to associate with integrins and facilitate focal adhesion kinase signaling (Kato et al., 2006; Wu et al., 2007). Furthermore, ganglioside conversion to GM1 does not seem to be localized to the cut end of the axon, but distributed throughout the injured axon, suggesting a role for GM1 in stabilizing the axotomized axon, potentially by facilitating integrin clustering and substrate engagement.

Analysis of ganglioside ratios after optic nerve crush *in vivo* paralleled the lack of GM1 enrichment observed in culture, further suggesting that injury-induced ganglioside processing does not occur in CNS nerves (Kappagantula et al., 2014, their Fig. 5*H*). However, an interesting observation was a prominent increase in GM1 staining in the vicinity of the axons. Astrocytes, oligodendrocytes, or resident microglia may be possible sources of this robust staining observed in injured optic nerves.

Previous work has identified Erk, p38MAPK, and mTOR as signaling molecules that promote axon regeneration in the CNS (Mar et al., 2014). To determine whether these signaling molecules trigger sialidase activity and lead to conversion of membrane GD1a to GM1 after axotomy, the authors treated DRG neurons with a panel of inhibitors of these molecules before axotomy. In these experiments, the absence of calcium, as well as inhibition of Erk and p38MAPK, abrogated injury-induced GM1 enrichment (Kappagantula et al., 2014, their Fig. 7). Additionally, exogenous application of Neu3 sialidase activated the Erk signaling pathway (Kappagantula et al., 2014, their Fig. 8), suggesting that Erk acts in a positive feedback loop to enhance Neu3 sialidase activity. Inhibition of mTOR with Rapamycin had no effect on postinjury GM1 enrichment (Kappagantula et al., 2014, their Fig. 7), despite blocking axon regeneration (Kappagantula et al., 2014, their Fig. 6), indicating that injury-induced GM1 enrichment is not sufficient to overcome mTOR inhibition. This raises the possibility that signaling through mTOR is required for GM1-dependent axon regeneration and suggests a potential role for mTOR downstream of GM1-dependent signaling complexes at the plasma membrane. Accordingly, lipid rafts have been shown to be critically important for mTOR signaling by allowing for local recruitment and activation of its upstream activator, Akt, following phosphatidyl-3-kinase (PI3K)-induced generation of phosphatidyl-inositol-triphosphate (PIP_3), which is known to cluster at lipid rafts (Lasserre et al., 2008, Gao et al., 2011). The ability of enhanced mTOR signaling to promote CNS axon regeneration is underscored by studies demonstrating robust regeneration of injured optic nerve in mice with conditional knock-out of PTEN, a molecule that antagonizes the mTOR pathway by converting PIP_3 to PIP_2 (Park et al., 2008).

Extending the mechanism further, Kappagantula et al. (2014) determined that in the absence of calcium, axotomy failed to induce p38MAPK activity and that forced activation of p38MAPK with anisomycin was sufficient to enhance axotomy-induced GM1 enrichment in the absence of calcium. Together, these data suggest that calcium entry leads to p38MAPK activity, which in turn, stimulates sialidase activity. Anisomycin is not a specific activator of p38MAPK, however, and it is frequently used as a protein-synthesis inhibitor. It is therefore difficult to conclude with certainty that GM1 enrichment in retinal axons depends on p38MAPK. Moreover, how p38MAPK stimulates sialidase activity is uncertain. Finally, the possible contribution of p38MAPK to the end result of axon regeneration remains unclear, because there was no indication of whether forced activation of p38MAPK with anisomycin or by other means promoted regeneration.

Many questions remain to be answered to fully understand the contribution of sialidase to axon regeneration. Several possibilities exist. First, given that the polysialylated gangliosides GD1a and GT1b can act as receptors for myelin-associated inhibitors, processing of these gangliosides may promote regeneration by rendering neurons insensitive to myelin-associated inhibitors. However, given the absence of myelin-associated inhibitors in the neuronal cultures used in these experiments, it is likely that sialidase-mediated GM1 upregulation plays an active role in promoting regeneration. Second, as mentioned above, GM1 is critical for organizing lipid rafts, rigid cholesterol-rich microdomains within the plasma membrane. The importance of GM1 in organizing these microdomains raises the possibility that GM1 could be structurally important for membrane sealing immediately following axotomy. The present study assayed the role of sialidase and signaling molecules in injury-induced ganglioside processing by bath-application of inhibitors to dissociated and explant cultures, but it may be worthwhile to consider the use of compartmentalized cultures to separate the axons from the cell bodies to acutely manipulate axonal signaling to determine whether injury-induced sialidase activity and subsequent GM1 enrichment acts locally at the axon to promote regeneration. Finally, because lipid rafts are important signaling hubs for receptors, it is also possible that GM1 increases the sensitivity of injured axons to growth promoting factors. In one study, for example, GM1 was observed to increase local TrkA activity, accompanied by decreased RhoA signaling and stability of the actin cytoskeleton (Da Silva et al., 2005).

Injured sciatic and optic nerve sections both exhibit a striking increase in GM1 staining after injury (Kappagantula et al., 2014, their Figs. 3 and 5), but while GM1 staining appears to be tightly associated with the axons in the sciatic nerve, the staining in optic nerve is more distant from the axons. This differential distribution of staining between injured PNS and CNS nerves raises the possibility that GM1 enrichment in cell-types closely apposed to the axons in the PNS may locally instruct regeneration. In the sciatic nerve, it is likely that some of the GM1 staining surrounding the axons is derived from Schwann cells, which may have a GM1-dependent growth supportive role. Because an increase in membrane GM1 likely translates into increased cell signaling through clustering of receptors at lipid rafts, this may occur, for example, through local release of neurotrophic

factors. In the optic nerve, however, the robust injury-induced GM1 staining does not occur in close proximity to the axons. Together, these studies have improved our understanding of the fundamental differences between PNS and CNS and support a central role for injury-induced ganglioside processing in axon regeneration.

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