Superfood for axons: Glial exosomes boost axonal energetics by delivery of SIRT2

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Summary

Axon integrity depends on support by glia facilitating axonal maintenance and energy homeostasis, but the molecular mechanisms are not understood well. In this issue of Neuron, Chamberlain et al. (2021) provide evidence that oligodendrocyte-to-axon transfer of SIRT2 via extracellular vesicles (exosomes) enables deacetylation of mitochondrial proteins, enhancing axonal energy production.

Axons are highly vulnerable to degeneration and energetic breakdown attributed to their specialized architecture and high energy demands. The longer an axon, the more it may require specific mechanisms to maintain homeostasis and energy supply in distal segments. Axonal maintenance is achieved by neuronal functions such as axonal transport but also critically depends on non-cell-autonomous support by glial cells. Myelinating oligodendrocytes enhance axonal conduction speed and play an essential role for the maintenance of axonal integrity by providing local support (Saab et al., 2013). Glial support of axons was originally discovered in oligodendroglial mouse mutants developing secondary axonal degeneration starting with axonal swellings, which also appear in several neurodegenerative diseases including multiple sclerosis (Saab et al., 2013). Axonal degeneration in these mutants has been linked to the transfer of energy metabolites such as lactate via monocarboxylate transporters and, more recently, to the delivery of exosomes. Indeed, oligodendrocyte-derived exosomes support axonal maintenance by promoting the neuronal metabolism and fast axonal transport (Frühbeis et al., 2020). Importantly, oligodendroglial exosomes derived from mutants with axonal defects lacked this activity due to impaired exosome release and aberrant exosome composition. However, how exosomes may support axonal metabolism remained largely unclear. A deeper insight into these processes will thus facilitate better understanding of a spectrum of neurodegenerative diseases with axonal involvement and may lead to novel therapeutic strategies.

Exosomes are a class of membranous extracellular vesicles that are secreted from cells and implicated in neural cell homeostasis as well as neurodegeneration (Budnik et al., 2016, Schnatz et al 2021). Exosomes are generated in the late endosomal system and released from multivesicular bodies (MVBs) into the extracellular space. MVBs indeed are highly prevalent in myelinating oligodendrocytes, in which they localize adjacent to the myelinated axon (Figure 1). Strikingly, oligodendroglial MVBs are triggered to release exosomes upon neuronal activity in response to glutamate activating glial NMDA receptors. Furthermore, glial exosomes can be taken up by axons and the molecular cargo exhibits functional activity within axons (Frühbeis et al 2013).

In this issue of Neuron, Chamberlain et al. (2021) report that the NAD^+-dependent deacetylase sirtuin 2 (SIRT2), a known myelin protein (Werner et al., 2007), is also cargo of oligodendroglial exosomes that enhances energy production in axonal mitochondria by deacetylating adenine nucleotide translocases 1 and 2 (ANT1/2). By ATP FRET-sensor imaging, cortical neurons cocultured with oligodendrocytes in microfluidic devices or treated with oligodendrocyte-conditioned medium displayed increased ATP levels in the axonal compartment. Seahorse experiments confirmed this to reflect elevated mitochondrial respiration. The authors considered the energy metabolite lactate a potential mediator but could not mimic the effect by adding lactate nor interfered with it by inhibiting lactate transporters. However, lactate increased axonal ATP in the absence of glucose, implying that lactate may serve as energy substrate when glucose is depleted, at least under culture conditions.

Next, the study focused on oligodendroglial exosomes, which were characterized before and implicated in axonal maintenance (Frühbeis et al 2020). Addition of highly purified oligodendrocyte-derived exosomes to cortical neurons resulted in their uptake and reproduced the effects on axonal energetics seen in the oligodendrocyte coculture, including increased ATP levels, enhanced respiration parameters and FRET in the axonal compartment. Axonal ATP levels were markedly increased within 2-3 h upon exosome addition. Consistently, inhibition of oligodendroglial exosome release led to reduced axonal ATP levels, supporting the concept that exosomes are both sufficient and required to promote axonal bioenergetics. Furthermore, FRET-imaging of ATP specifically in axonal mitochondria confirmed that treatment of neurons with purified oligodendroglial exosomes indeed increased mitochondrial ATP-levels.

The authors then followed the hypothesis that SIRT2, a known oligodendroglial exosome cargo (Frühbeis et al., 2013), is responsible for mediating the effects of oligodendroglial exosomes on axonal energy metabolism. SIRT2 is a largely cytosolic protein, and its expression in the CNS is greatly enriched in myelinating oligodendrocytes (Werner et al., 2007). Furthermore, SIRT2 was previously demonstrated to localize to mitochondria and to mediate deacetylation of mitochondrial proteins (Liu et al., 2017). Indeed, a significant proportion of proteins involved

in energy metabolism is regulated by deacetylation of lysine residues, and ATP levels are reduced in mice lacking *Sirt2* (Liu et al., 2017, Fourcade et al., 2017). The hypothesis was supported by experiments manipulating SIRT2 levels, involving its overexpression in cortical neurons or its depletion in oligodendrocytes by siRNA knockdown or *Sirt2*-deficiency. Together, these experiments demonstrated that ATP FRET-sensor signals in axons were respectively increased or unaffected, depending on SIRT2 expression. Mass spectrometric analysis of purified neuronal mitochondria revealed 9 acetylated candidate target proteins, among which ANT1 and ANT2 were specifically deacetylated in response to treating neurons with oligodendrocyte-conditioned medium. When *Sirt2* was lacking in oligodendrocytes, deacetylation of neuronal ANT1/2 could not be observed. Although the contribution of exosomes was not further controlled in these experiments, e.g. by depleting exosomes, the results provide evidence that ANT1/2 in neuronal mitochondria are targets of SIRT2. Together, the picture emerges that oligodendrocyte-to-axon transfer of SIRT2 via exosomes and subsequent SIRT2-dependent deacetylation of ANT1/2 enhances mitochondrial ATP production (Figure 1). Because the mitochondrial-localized sirtuins, SIRT3-5, are not abundant in the brain, the external supply of axons with glial SIRT2 may provide axons with the opportunity to modulate mitochondrial functions.

Finally, the authors injected exosomes into the dorsal spinal cord of *Sirt2*-deficient mice followed by assessment of the mitochondrial membrane potential (Ψm) via the co-injected dye CMTMRos. *Sirt2*-deficient mice exhibited decreased Ψm in the dorsal spinal cord white matter compared to wild-type mice. Importantly, injection of exosomes derived from wild-type oligodendrocytes rescued the decreased Ψm in *Sirt2*-deficient mice, while *Sirt2*-deficient exosomes did not. These experiments are quite demanding and remarkably were performed with highly purified exosomes isolated via density gradient centrifugation. However, normalization of injected exosomes and the efficiency of delivery is virtually impossible to control. Although neuronal uptake of injected exosomes was verified using a less pure, dye-labelled exosome fraction, it is not straightforward to envision how myelinated axons may incorporate exosomes, which in the physiological situation would be released directly at the axon-myelin interface and thus underneath the myelin sheath. Moreover, it is not entirely clear to which degree the Ψm measurements reflect axonal mitochondria, considering that oligodendroglial cell bodies are also present in the vicinity. Yet, the study provides immuno-electron micrographs that indicate SIRT2-immunopositive membrane structures reminiscent of exosomes in the periaxonal space, which are interpreted as undergoing internalization in myelinated spinal cord axons. Furthermore, SIRT2-immunolabeling was associated with mitochondria in *Sirt2*-deficient axons following injection of wild-type exosomes, providing proof of exosomal delivery of SIRT2 to axons. To obtain a biochemical readout of ANT1/2 acetylation levels in the tissue, immunoprecipitation of acetylated proteins from spinal cord injected with

wild-type or *Sirt2*-deficient exosomes was performed, revealing reduced acetylation of ANT1/2 in spinal cords injected with wild-type but not with *Sirt2*-deficient exosomes.

The study by Chamberlain et al. (2021) implies a molecular mechanism of local axonal support important for axonal energetics and stability. It provides a continuous line of evidence for the oligodendrocyte to neuron delivery of SIRT2 via exosomes that enhances the axonal energy metabolism by mitochondrial protein deacetylation. The observed effects on axonal ATP content may appear small at first glance; however, the effect size is expected to be underestimated due to technical reasons of FRET sensor imaging. Probably, the effect size may increase if neurons were exposed to starvation or other types of stress. In line with the present findings, *Sirt2*-deficient mice develop late-onset moderate axonal degeneration and locomotor deficits in conjunction with redox imbalance and reduced ATP levels (Fourcade et al., 2017). Intriguingly, SIRT2 is also depleted in oligodendroglial exosomes derived from *Plp1*- and *Cnp*-deficient mice, which are affected by early-onset progressive axonal degeneration. P*lp1*- and *Cnp*-deficient exosomes lack the ability of wild-type exosomes to increase the neuronal reduction potential and to promote axonal transport (Frühbeis et al., 2020). While these findings may underscore the relevance of exosomal SIRT2 delivery for axonal energetics, it is likely that other exosomal cargo plays additional roles in supporting axonal functions. For example, oligodendrocyte-to-neuron delivery of ferritin heavy chain (FTH) via exosomes facilitates antioxidant defense (Mukherjee et al., 2020). Taken together, these recent studies highlight the importance of exosome delivery from myelinating cells to axons for local supply that strengthens axonal energetics and maintains homeostasis.

How do exosome-mediated axonal support and the axonal supply of energy metabolites via monocarboxylate transporters cooperate? The experiments performed by Chamberlain et al. (2021) suggest that fast delivery of lactate may be particularly relevant when energy resources become scarce to neurons. External supply with energy metabolites thus may be required when neurons are highly active, rapidly necessitating energy to restore ion gradients. Conversely, exosome-mediated support of axons through delivery of SIRT2 may rather contribute to axonal energetics by tuning of mitochondria to more efficient energy production. Thus, oligodendrocyte-to-axon metabolic support via monocarboxylate transporters and exosome-mediated transfer appears to serve complementary functions in axonal energy homeostasis.

Many neurological disorders share features of metabolic imbalance, axonal degeneration and oligodendrocyte dysfunction. It remains a challenging and promising task to further elucidate the molecular mechanisms employed by glial exosomes to maintain axonal energy homeostasis and integrity.

Figure 1

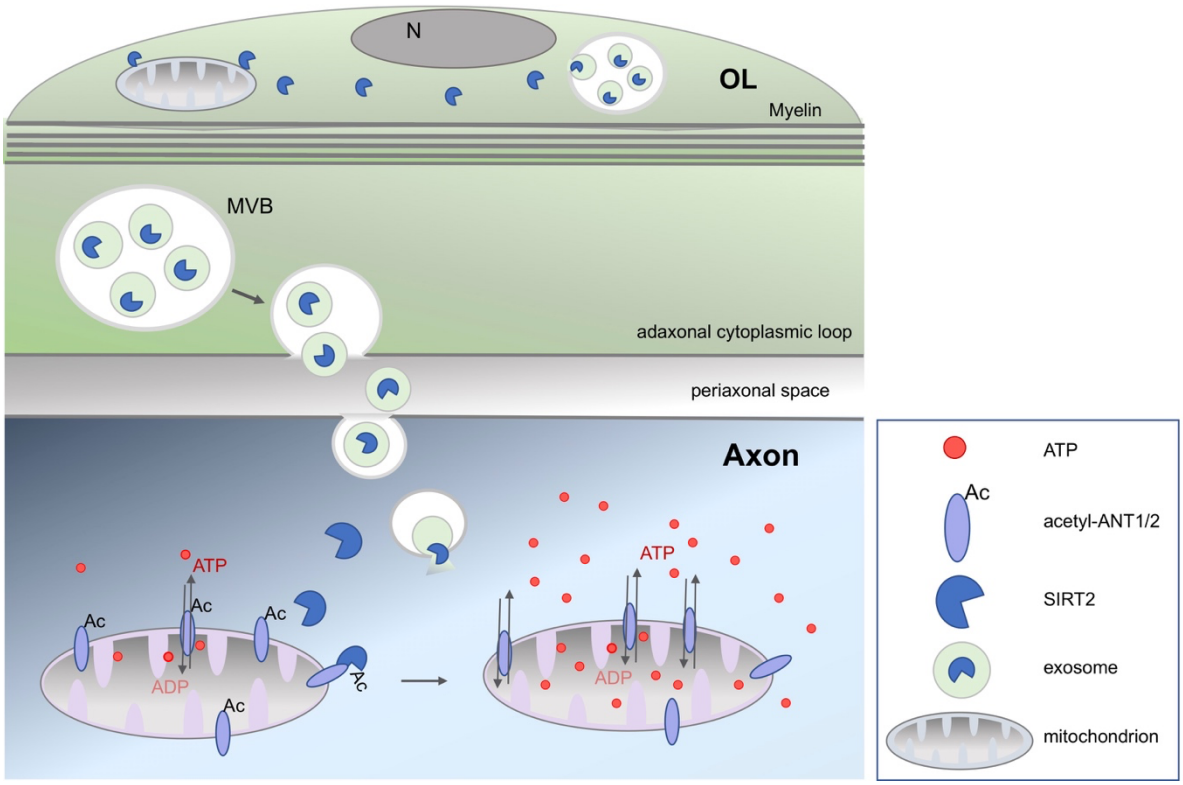

Figure 1: Exosomes released by myelinating oligodendrocytes at the axon-glia interface deliver SIRT2 to axonal mitochondria enhancing ATP production by deacetylation of ANT1/2.

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