

Mitochondrial integrity in neuronal injury and repair

Qi Han, Xiao-Ming Xu*

The mitochondrion is the powerhouse of a cell. As the principal subcellular organelles that mediate adenosine triphosphate (ATP) production and calcium buffering, mitochondria actively distribute to areas of high energy demand and calcium flux. The highly polarized nerve cells in the central nervous system (CNS), which have unparalleled size and complexity and long-projection axons, are cells with high-energy requirements. Mitochondria are regionally organized within these neurons, with higher accumulations in the soma, the hillock, the nodes of Ranvier, and the axon terminals. In the synaptic region, mitochondria regulate calcium and ATP levels, thereby maintaining synaptic transmission and structure. Defects in mitochondrial dynamics can cause deficits in neuronal transport, transmission, and metabolism (Misgeld and Schwarz, 2017).

In normal conditions, neuronal mitochondria are transported along the axons for considerable distances to meet local needs for ATP and calcium buffering. Whereas in injury conditions, axotomy ruptures the axon plasma membrane, causing unwanted ion influx, disassembly of the cytoskeleton, and depolarization of mitochondria (Patrón and Zinsmaier, 2016). The production of reactive oxygen species by stress mitochondria can cause further mitochondrial damage in the rest of the cells, leading to further impairment in these processes such as impaired energy supply, Ca²⁺ buffering, and enhanced apoptosis. These impaired processes contribute to neurodegeneration rather than neuroregeneration (Murphy, 2009).

For successful axon regeneration, injured neurons face even greater difficulties in meeting the energy demand than healthy neurons do. Higher energies are needed in regenerating axons to reseal injured terminals, reconstruct cytoskeleton, synthesize and transport building materials, and form growth cones (Sheng, 2017). Therefore, how functional mitochondria and energy supply are maintained when cells experience metabolic or environmental stresses is critical for neuronal survival, repair, and regrowth. Because the diffusion capacity of intracellular ATP through long axons is rather limited, the mitochondrial transport toward an active growth cone may be important for regulating axonal and neuronal injury responses. However, the axonal mitochondrial transport becomes far from motile during maturation and aging. In this regard, constrained mitochondrial transport associated with limited ATP diffusion may explain the observations that the rate of axon tips for regeneration is different after injuring the nerve at different distances from the nerve cell body and that CNS axons are refractory to regenerate after injury in adult mammals.

Previous work by Zhou et al. (2016) points to the possibility that the mitochondrial transport decline could be associated with the progressively increased expression of an anchor protein, syntaphilin, which can dock the mitochondria on the axonal microtubule (Kang et al., 2008). Zhou et al. (2016) then knocked

out the gene encoding syntaphilin to enhance mitochondrial mobility and observed an abrogation of the energy deficit, and enhanced axon regrowth *in vitro* and following peripheral nervous system crush. Notably, two other independent studies published in the journal *Neuron*, in 2016 by Han et al. and Cartoni et al. concurred with the idea that ATP generation by axonal mitochondria is critical to support regeneration. For example, Han et al. found that axon injury and activation of DLK-1 MAP kinase increase axonal mitochondria density. Robust mitochondria density is required to produce adequate ATP for axon regeneration. While Cartoni et al. (2016) demonstrated that the mammalian-specific mitochondrial protein *Armcx1* mobilizes mitochondria and promotes neuronal survival and axonal regeneration in an optic nerve injury model. These significant studies demonstrate a critical role of mitochondrial transport and energy supply for axon regeneration in various models with different injury regimes. Future work will be needed to explore how DLK-1 and *Armcx1* mobilize mitochondrial transport into axons following neuronal injury (Patrón and Zinsmaier, 2016).

Besides these findings, we still need to know much more, such as whether enhancing mitochondrial transport would promote the regeneration of corticospinal tract (CST), which has the most refractory regenerative capacity of all CNS axons, whether these regenerating axons would also reconnect with their targets to the extent and in the manner required for functional recovery, and whether we would find a safe and effective means, such as small molecule compounds, to increase mitochondrial mobility and the energy supply local to axonal injury. We address these remaining questions with findings from our recent paper published in *Cell Metabolism* (Han et al., 2020) (Figure 1). Using three CNS injury mouse models, we demonstrate that *SnpH* knockout mice display enhanced CST regeneration passing through a spinal cord lesion, accelerated regrowth of monoaminergic axons across a transection gap, and increased compensatory sprouting of uninjured CST axons following a unilateral hemisection. Notably, regenerated CST axons form functional synapses and promote motor functional recovery. To further test our hypothesis of the “injury-induced energy crisis”, we aimed to directly target energy metabolism with creatine, an FDA approved blood-brain-barrier permeable ergogenic compound that rapidly regenerates ATP from adenosine diphosphate independent of mitochondrial transport (Figure 1). We found that the administration of the bioenergetic compound creatine also facilitates regeneration. Our study establishes that injury-induced energy crisis contributes to CNS regeneration failure. Recovering local energy by either enhancing mitochondrial transport or increasing energy metabolism promotes axonal sprouting and regeneration after spinal cord injury. Our study suggests that mitochondrial dynamics and quality control play critical roles for CNS neurons in reconstructing their damaged components when cells experience

environmental stresses.

In addition to the enhancement of mitochondrial transport, the combined actions of fission and fusion also contribute to mitochondrial quality control and the responses of neurons to stress (Youle and van der Bliek, 2012). Purposeful segregation and disposal of damaged mitochondria through changes in fission and fusion pathways are involved in intrinsic regulation of mitochondria in the distal axons and growth cones of retinal ganglion cells. Removal of the mitochondrial fission mediator, dynamin-related protein 1 gene (*Drp1*) after nerve injury, impaired membrane potential and axonal transport velocity, and eventually resulted in neuronal death (Kiryu-Seo et al., 2016). However, an abnormal increase of nitrosylation of *Drp1* can trigger excessive mitochondrial fission and fragmentation, contributing to the pathogenesis of Alzheimer’s disease (Cho et al., 2009). Mitophagy is another level of quality control that entails the wholesale elimination of damaged mitochondria after neuronal injury. A recent study reported that overexpression of an anchoring protein, syntaphilin, blocked mitophagy under oxygen-glucose-deprivation-induced ischemia, leading to the aggravation of dysfunctional mitochondrial and neuronal injury (Zheng et al., 2019). This finding consistently suggests the important role of mitochondrial quality in pathological conditions.

During axonal regrowth after injury, neurons not only require an increased amount of energy but also require replacing the damaged mitochondria and replenishing with healthy ones. To meet such considerable energy demands, neurons need to generate new mitochondria because the preexisting mitochondria are unlikely to provide sufficient amount of ATP production. Vaarmann et al. (2016) found that activating the CaMKK β , LKB1-STRAD or TAK1 pathways as well as coactivating the AMPK-PGC-1 α -NRF1 axis leads to the generation of new mitochondria to ensure energy for upcoming growth. Because neurons are capable of signaling for upcoming energy requirements by regulating mitochondrial biogenesis and mitochondrial transport, further studies of interest would be to focus on the reciprocal interactions between these dynamic processes, such as whether increased mitochondrial transport would promote mitochondrial biogenesis or vice versa, and whether the strategies that upregulate the rate of both mitochondrial transport and biogenesis would synergistically promote neural axonal regrowth. Beside mitochondrial biogenesis, another strategy to repair and replenish damaged mitochondria, termed mitochondrial transplantation, has been developed in which mitochondria harvested from the healthy tissue are transplanted to injured tissue to restore mitochondrial function and viability. A recent study showed that live-isolated mitochondrial transplantation increased cell survival of injured retinal ganglion cells and facilitated axon extension after optic nerve crush injury (Nascimento-dos-Santos et al., 2020). The mitotherapy has also been applied to pediatric patients who have myocardial ischemia-reperfusion injury to supply ATP for myofilament contraction (Emani et al., 2017). Despite rapid translation of this method into clinic, many important questions still need to be adequately addressed to maximum its effects and minimize its risks, such as how mitochondria survive after transplantation in high Ca²⁺ concentrations, how mitochondria enter the targeted cells in the injured

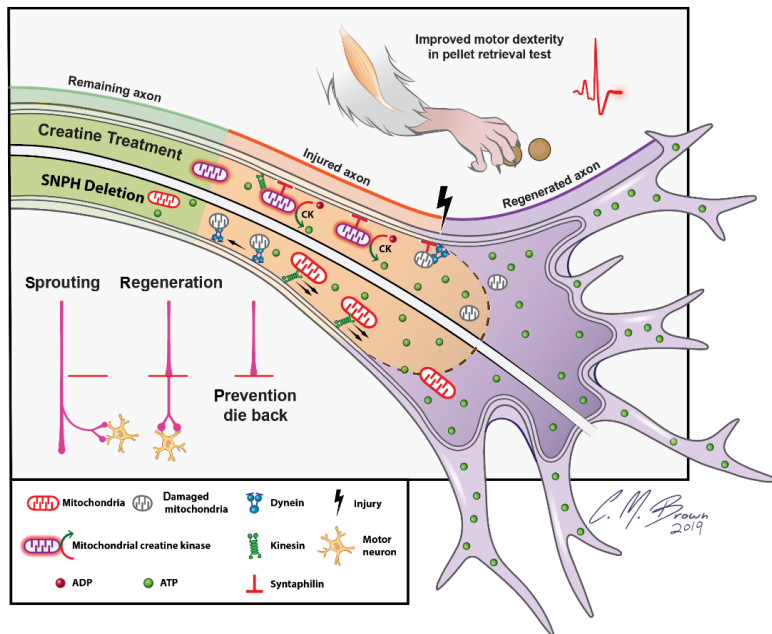


Figure 1 | Restoring neuronal energetics promotes axon regeneration after spinal cord injury. Injury-induced mitochondrial stress causes an energy challenge for regrowth in distal injury axonal terminals. Removal of axonal mitochondrial anchor protein, SNPH, increases mitochondrial transport and turnover in adult CNS axons. Such genetic manipulation improves mitochondrial quality and accelerates the energy supply needed for regenerating axons by removing the damaged mitochondrial from the injury site as well as by replenishing the healthy ones to the regrowth zones. Alternatively, administration of CK supplement facilitates ATP metabolism of injured axons with high-energy demands and promotes regrowth of CNS axons. Reprinted from Han et al. (2020). ADP: Adenosine diphosphate; ATP: adenosine triphosphate; CK: creatine kinase; CNS: central nervous system; SNPH: syntrophin.

tissue, and how mitochondria that enter the cells produce sufficient ATP for functional restoration.

Mitochondrial transport helps mitochondrial dynamics and flow within neuronal axons. The mitochondrial fusion and fission cycle is proposed to balance two competing processes: compensation of damage by fusion and elimination of damage by fission (Youle and van der Bliek, 2012). Mitophagy and autophagy could purify the cellular pool of mitochondria if debris is aggregated and segregated by fission in a subset of mitochondria. Mitochondrial biogenesis could generate new mitochondria to provide sufficient acceleration in ATP production. Under metabolic or environmental stresses, neuronal functions critically depend on the integrity and functionality of mitochondria. The mitochondrial dynamic mechanisms are involved not only in regulating individual mitochondrial fidelity within cells but also in participating in neuronal survival and repair at the whole-cell level.

Current studies have identified multiple novel proteins and pathways that are involved in and contribute to neural injuries. Moving toward *in vivo* studies, it is important to appreciate the molecular overlap that exists among pathways and find the common ground for the roles of proteins and pathways that play among different neural stress conditions, as mitochondrial dysfunction is implicated in multiple neural injury models and diseases. In addition, peripheral nervous system axons can readily regenerate while the CNS has limited regeneration capacity after injury in adult mammals. Misgeld et al. (2007) demonstrated that anterograde mitochondrial transport in the uninjured proximal sciatic nerve increased by more than 80% at 12 hours after nerve cut, which provide a rapid and stimulatory environment for peripheral nervous system

regeneration. In future, it would be interesting to compare the mitochondrial turnover between CNS and peripheral nervous system axons after injury. In this way, disruption to mitochondria may partially explain why CNS environment is inhibitory for axon regeneration after injury. Furthermore, many major human neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, display disruption of intracellular transport processes including mitochondrial transport (De Vos et al., 2008). Future issues include which mechanisms control disrupted mitochondrial transport and how manipulating mitochondrial dynamics through molecules or drugs that regulate mitochondrial axonal transport might have applications in correcting axonal transport defects and attenuating neurodegeneration. Overall, an in-depth understanding of such dynamic processes in different neurophysiological and neuropathological conditions could aid the development of new treatments for axonal regrowth after injury.

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Qi Han, Xiao-Ming Xu*
Spinal Cord and Brain Injury Research Group, Stark Neurosciences Research Institute; Department of Neurological Surgery, Indiana University School of Medicine, Indianapolis, IN, USA

*Correspondence to: Xiao-Ming Xu, MD, PhD, xu26@iupui.edu.
<https://orcid.org/0000-0002-7229-0081>
(Xiao-Ming Xu)

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