REVIEW

Rescue axonal defects by targeting mitochondrial dynamics in hereditary spastic paraplegias

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Abstract
Impaired axonal development and degeneration underlie debilitating neurodegenerative diseases including hereditary spastic paraplegia, a large group of inherited diseases. Hereditary spastic paraplegia is caused by retrograde degeneration of the long corticospinal tract axons, leading to progressive spasticity and weakness of leg and hip muscles. There are over 70 subtypes with various underlying pathophysiological processes, such as defective vesicular trafficking, lipid metabolism, organelle shaping, axonal transport, and mitochondrial dysfunction. Although hereditary spastic paraplegia consists of various subtypes with different pathological characteristics, defects in mitochondrial morphology and function emerge as one of the common cellular themes in hereditary spastic paraplegia. Mitochondrial morphology and function are remodeled by mitochondrial dynamics regulated by several key fission and fusion mediators. However, the role of mitochondrial dynamics in axonal defects of hereditary spastic paraplegia remains largely unknown. Recently, studies reported perturbed mitochondrial morphology in hereditary spastic paraplegia neurons. Moreover, downregulation of mitochondrial fission regulator dynamin-related protein 1, both pharmacologically and genetically, could rescue axonal outgrowth defects in hereditary spastic paraplegia neurons, providing a potential therapeutic target for treating these hereditary spastic paraplegia. This mini-review will describe the regulation of mitochondrial fission/fusion, the link between mitochondrial dynamics and axonal defects, and the recent progress on the role of mitochondrial dynamics in axonal defects of hereditary spastic paraplegia.

Key Words: *hereditary spastic paraplegia; axonal degeneration; mitochondrial dynamics; fission; fusion; dynamin-related protein 1; mitochondrial dysfunction; induced pluripotent stem cells*

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Introduction
Hereditary spastic paraplegia (HSP) is a heterogeneous group of neural degenerative disease characterized by spasticity of muscle and progressive weakness of lower limbs (Blackstone, 2012). Over 70 distinct genetic loci associated with HSP have been identified (spastic paraplegia 1 (SPG1) to 70 subtypes), which can be classified into autosomal dominant types and autosomal recessive types based on the inheritance. Despite the divergent function of HSP proteins, they are classified into several common cellular themes including mitochondrial dysfunction (Blackstone, 2012). Mitochondria are highly dynamic organelles that fuse, divide and move along axons and dendrites of neurons and contribute to neuronal health by modulating ATP production, apoptosis, Ca^{2+} and redox signaling, *etc*. Mitochondrial fission and fusion are tightly regulated by several fission and fusion mediators including dynamin-related protein 1 (Drp1). The imbalanced mitochondrial fission and fusion could result in abnormal mitochondrial morphology that will disrupt mitochondrial and neuronal function. However, whether mitochondrial fission-fusion is involved in axonal defects and whether these defects can be rescued in HSP by targeting fission-fusion are not known. Our recent study addressed these questions and demonstrated that abnormal mitochondrial morphology caused by imbalanced fission-fusion underlies axonal defects in HSP. Moreover, axonal defects in SPG15 and SPG48 neurons could be mitigated by targeting mitochondrial fission regulator Drp1, suggesting a novel target for rescuing axonal phenotypes in these HSPs (Denton et al., 2018).

Regulation of Mitochondrial Fission and Fusion
Mitochondrial network is regulated by dynamic mitochondrial fission and fusion whose defects result in fragmented or extreme elongated mitochondria. A variety of important molecules regulate mitochondrial fission and fusion to maintain normal mitochondrial morphology and function. Mitochondrial fission is required for increased mitochondrial number, motility of mitochondria to other regions in cells, autophagy of damaged mitochondria (mitophagy) and apoptosis. The regulation of mitochondrial fission is controlled by several key mitochondrial outer membrane (MOM) proteins including Drp1 and Drp1 receptors. Drp1 is an 80-kDa mechanochemical dynamin-like GTPase that is concentrated in MOM to induce membrane tubulation and promote membrane tethering in presence of GTP (Smirnova et al., 2001). Drp1 mainly resides in cytosol as oligomers (dimers/tetramers) and can self-assembly to larger structures on the surface of MOM with recruitment by Drp1 receptors. A variety of cellular events regulate the activity of Drp1 in mitochondrial fission, such as posttranslational modifica-

tion, alternative splicing, and recruitment of Drp1 by Drp1 receptors.

Multiple posttranslational modifications of Drp1, including phosphorylation, ubiquitination, S-nitrosylation, and conjugation of small ubiquitin-like modifier proteins, play an important role in regulating Drp1 subcellular localization, degradation, interaction with receptors, and Drp1 associated signaling pathways. The most extensively reported modification is phosphorylation of Drp1, which occurs on several residues: Ser-637 that inhibits activity of Drp1, Ser-616 that activates its activity in general, Ser-579 that promotes mitochondrial fragmentation under oxidative stress, and Ser-693 that leads to the decrease of GTPase activity of Drp1. The impaired phosphorylation of Drp1 could result in the defects in the recruitment of Drp1 to the MOM, mitochondrial cristae remodeling, cytochrome c release, and cell apoptosis. Several Drp1 receptors recruit Drp1 from cytosol to MOM independently, such as Fis1, Mff, MiD49, and MiD51. Fis1, an adaptor protein of Drp1, is also involved in mitophagy and important for controlling autophagosome morphogenesis during mitophagy (Yamano et al., 2014). Once recruited by adaptor proteins, Drp1 assembles spirals around mitochondria to constrict and divide the mitochondrion in a GTP-dependent manner. Increased Drp1 activity, caused by upregulation or posttranslational modification of Drp1 and its related receptors, could induce fragmentations of mitochondria that fail to regulate neural activity in some neurodegenerative diseases. For example, the increased S-nitrosylated Drp1 that promotes its activity results in abnormal mitochondrial fission/fusion and increased mitochondrial fragmentation in Huntington's disease neurons (Haun et al., 2013). Thus, targeting Drp1 expression or posttranslational modification might be a potential strategy to rescue abnormal neural activity in these neurons.

It is very important to note that mitochondrial fusion is another critical event, and the balance between mitochondrial fission and fusion are critical for neuronal function. Up to now, some GTPase proteins (Opa1, Mfn1 and Mfn2) have been reported to regulate mitochondrial fusion. Mfn1 and Mfn2 are involved in the fusion of MOM, whereas Opa1 is associated with subsequent mitochondrial inner membrane fusion. Mutations or knock-down of *Opa1*, *Mfn1*, or *Mfn2* block mitochondrial fusion resulting in segmented damaged mitochondria that cause cellular defects. Interestingly, there are several Opa1 isoforms playing different roles in the regulation of mitochondrial fusion. Exon 4 of *Opa1* is conserved in alternative splicing of this gene for the maintenance of mitochondrial membrane potential and network, whereas exon 4b and 5b are related to the function of *Opa1* in cytochrome c release (Olichon et al., 2007). Mfn2 partially localizes to both mitochondria and endoplasmic reticulum (ER) to regulate the Ca^{2+} signaling and link the interactions between these two organelles (Merkwirth and Langer, 2008). These multiple regulatory pathways modulate and maintain the balance of fission-fusion and subsequent normal mitochondrial morphology and function.

Mitochondrial Dynamics Link with Axonal Defects

Imbalanced mitochondrial dynamics are an emerging pathological character for neurodegenerative diseases and contribute to axonal degeneration. Neurons are highly polarized cells that can extend extreme long axons to connect with their targets. Maintaining local energy supplying in axonal terminal is required for mitochondrial transport from cell body along microtubules within axons, correct spatial mitochondrial distribution and functioning in axons. The defects in mitochondrial fission and fusion dynamics affect mitochondrial morphology, mobility and subsequent positioning. The mutations or dysregulations of *Drp1*, *Opa1*, *Mfn1*, or *Mfn2* are observed in several neurodegenerative diseases, such as amyotrophic lateral sclerosis, Huntington's disease, and HSP. Imbalanced fission-fusion in these diseases results in impaired mitochondrial dynamics that may cause the dysfunction of oxidative phosphorylation, deletion of mitochondrial DNA, apoptosis and degeneration of axons. Defects in mitochondrial dynamics can induce deficits in mitochondrial motility along microtubules, and mitochondrial accumulation is observed in axonal swellings due to ATP deprivation or impaired cytoskeletons. Moreover, apoptosis and excess radioactive oxygen species production in neurons are initiated by dysregulated mitochondrial fission and fusion that results in the release of cytochrome C and abnormal Ca^{2+} signaling.

Axonal defects including decreased axonal outgrowth, disturbed axonal transport and increased axonal swellings are characteristic pathological changes in several cellular and animal models of neurodegenerative diseases including HSP (Denton et al., 2016). Since neurons are highly polarized organelles with long axons, sufficient energy supply and efficient axonal transport are critical to maintain normal functions of neurons. The axonal defects of some HSP types are indicated to be associated with mitochondrial dynamics recently, such as SPG15, SPG48 and SPG31 due to impaired mitochondrial dynamics, abnormal contacts between ER and mitochondria (Lavie et al., 2017; Denton et al., 2018). Impaired mitochondrial morphology and network have also been reported in other mutations of HSP including SPG7 and SPG61 (Fowler and O'Sullivan, 2016; Cooper et al., 2017; Magri et al., 2018), suggesting the importance to maintain the homeostasis of mitochondrial dynamics. Another important cellular event in the regulation of axonal defects is mitophagy that is linked to mitochondrial dynamics and is required to maintain normal mitochondrial morphology and function. Drp1, Opa1, Mfn1 and Mfn2 proteins are involved in regulating the interaction between mitochondrial dynamics and mitophagy (Twig et al., 2008; Tanaka et al., 2010). There are some subtypes of HSP related to mitophagy including SPG11, SPG15 and SPG48. SPG11, SPG15 and SPG48 are autosomal recessive forms caused by the mutations in *SPG11*, *ZFYVE26* and *AP5Z1* genes encoding protein of spatacsin, spastizin and AP5Z1, respectively. These three proteins could bind with each other and are also

reported to affect autophagy. A recent elegant study further showed that spatacsin and spastizin are required for autophagic lysosome reformation, a process important for autophagy (Chang et al., 2014). Thus, mutation in these proteins could induce dysfunction of endosome/lysosome system leading to defects in mitophagy. The impaired mitophagy results in abnormal mitochondrial networks and contributes to the axonal defects. The detailed link between these two important events in SPG11, SPG15 and SPG48, as well as other types of HSP, awaits further investigation.

Targeting Drp1 to Rescue Axonal Defects in HSP by Improving Mitochondrial Function

Stem cell technology has provided researchers unique systems to generate different types of neurons to study various neurodegenerative diseases. Our group successfully recapitulates axonal defects in different types of HSP using patient-specific induced pluripotent stem cells, such as SPG3A, SPG4, SPG15 and SPG48. SPG15 and SPG48 with symptoms of juvenile-onset parkinsonism can be improved with dopaminergic therapy, suggesting that the midbrain dopaminergic neurons might also be affected by these mutant HSP proteins. In our recent study, induced pluripotent stem cells derived from SPG15 and SPG48 patients were differentiated to telencephalic glutamatergic neurons, spinal neurons and midbrain dopaminergic neurons. We observed impaired mitochondrial morphology, including reduced mitochondrial length and densities within axons in forebrain and midbrain neurons (affected in patients), but not in spinal neurons (not affected in patients) (Denton et al., 2018). These findings using patient induced pluripotent stem cells recapitulate disease-specific defects, suggesting that impaired mitochondrial dynamics and function contribute to the axonal defects and apoptosis of SPG15 and SPG48 neurons (**Figure 1**). More importantly, we further revealed that targeting mitochondrial fission with Drp1 inhibitor mdivi-1 or by genetically knocking down of *Drp1* rescued the axonal defects in these HSP neurons (**Figure 1**) (Denton et al., 2018). Our study not only demonstrates that mitochondrial dysfunction underlies axonal defects in SPG15 and SPG48 neurons, but also suggests that restoring mitochondrial morphology by targeting fission-fusion is a novel therapeutic strategy for HSP. In our study, we were focused on the mitochondrial fission mediator Drp1 whose expression was increased in HSP. It would be interesting to examine mitochondrial fusion-associated factors (*e.g.*, Opa1, Mfn1, and Mfn2) in HSP and whether regulating these factors (*e.g.*, overexpression) would restore mitochondrial morphology and rescue axonal defects in the future. In addition, mdivi-1 was shown to restore the reduced mitochondrial density in HSP neurons, and the detailed mechanisms underlying this (*e.g.*, alteration in mitophagy and/or axonal transport) await further investigation.

Recently, rescue of neural activity by restoring mitochondrial function is also reported in another type of HSP, SPG31, an autosomal dominant HSP (Lavie et al., 2017).

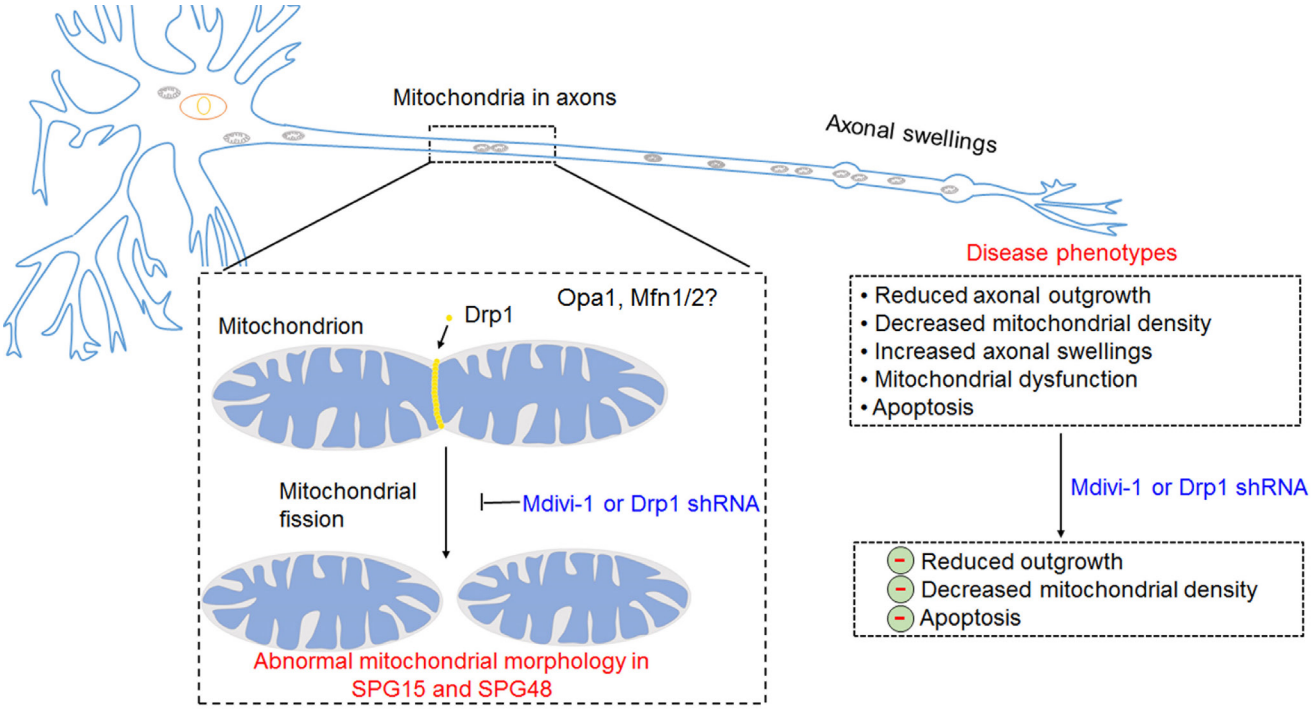

Figure 1 Schematic illustration of impaired mitochondrial dynamics (fission and fusion) in hereditary spastic paraplegia (HSP).
In HSP neuron axons, mitochondrial morphology is impaired (a significant reduction of mitochondrial length and density), leading to impaired axonal outgrowth, accumulated axonal swellings and apoptosis in HSP. Targeting mitochondrial fission-fusion using mdivi-1 or Drp1 shRNA can rescue neural axonal defects by restoring mitochondrial morphology and increasing mitochondrial networks in SPG15 and SPG48. mdivi-1: Drp1 inhibitor; Drp1: dynamin-related protein 1; shRNA: short hairpin RNA.

SPG31 is caused by mutations in receptor expression enhancing protein 1, which may affect ER shaping and ER-mitochondrial contacts. In fibroblasts of SPG31 patients, receptor expression enhancing protein 1 mutations cause Drp1 hyperphosphorylation at Ser637 residue, leading to impaired mitochondrial morphology in these patient cells (Lavie et al., 2017). Moreover, genetically or pharmacologically targeting Drp1, using either mutated Drp1 or a selective PKA inhibitor (H89) to reduce Drp1 hyperphosphorylation, restored mitochondrial morphology and subsequent neural activity (Lavie et al., 2017). These data imply the important role of Drp1 in the pathogenic of SPG31, and bring up an interesting question of what the similarities and differences are in terms of the mechanisms underlying mitochondrial dysfunctions in different types of HSPs.

Conclusions and Future Directions

Given the important role of mitochondrial dynamics in maintaining mitochondrial morphology and neural activity, targeting mitochondrial fission and fusion pharmacologically or genetically emerges as one of the promising therapies in neurodegenerative diseases including HSP. In terms of small molecules targeting Drp1, mdivi-1 and P110 (Qi et al., 2013) have been used to inhibit mitochondrial fission to elongate mitochondrial networks and to improve neural activity. It is important to note that some inhibitors may also affect other cellular processes, and thus confirmation of the effects using genetic approach to directly target Drp1 is required. It is also important in the future to screen more effective and specific small molecules that can regulate mitochondrial fission-fusion pathway including Drp1 and its receptor. In addition to Drp1, Opa1, Mfn1 and Mfn2 and their related mediators are also critical in balancing mitochondrial fission and fusion to regulate mitochondrial dynamics and function. We have just started to study the role of Drp1 in axonal defects in HSP. A better understanding of the role of mitochondrial fission-fusion regulators and the interplay between these factors and other pathways (*e.g.*, mitophagy, ER) in axonal degeneration in HSP will provide important insights into the development of novel therapeutics through regulating mitochondrial dynamics.

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