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Review

Dynamin-related protein 1 (Drp1) mediating mitophagy contributes to the pathophysiology of nervous system diseases and brain injury

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Summary. As the main source of energy (celluar ATP) in eukaryotic cells, mitochondria are involved in cellular physiology and pathology. The balance of mitochondrial dynamic, fission and fusion regulated by quality control mechanisms, provides a guarantee for maintaining mitochondrial function, even celluar function. Worn out mitochondria would be removed through mitophagy which is regulated by autophagy related proteins and mitochondrial membrane proteins. Drp1, dynamic-related protein 1, is regarded as one of the most important proteins to evaluate mitochondrial fission mediating mitophagy in neurodegenerative diseases (eg. Alzheimer's, Parkinson's, Huntington's, amyotrophic lateral sclerosis) and heart failure. Recent studies have focused on the roles of Drp1 in ischemia-induced mitophagy in the hippocampal CA3 region, and traumatic brain injury (TBI)-induced cell death together with functional deficits. However, the exact mechanisms have not been well characterized. In this review, we will discuss and clarify the role of Drp1 and mitophagy in nervous system diseases and brain injury therein, with a special emphasis on their molecular mechanisms mediating mitochondrial dynamics and mitophagy.

Key words: Mitochondria, Mitochondrial dynamic, Drp1, Mitophagy, Nervous system diseases, Brain injury

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Introduction

Mitochondria, one of the major ancient endomembrane systems, are well-known as a unique and irreplaceable organelle in eukaryotic cells (Friedman and Nunnari, 2014). Now it is widely recognized that mitochondria act as essential organelles which produce energy (cellular ATP), nevertheless, they are also the major intracellular sources for oxidative stress and participate in fatty acid oxidation, heme synthesis, maintenance of calcium homeostasis, and initiation of mitochondrial dysfunction and cell death (Gray et al., 1998). In the presence of severe stress, damaged mitochondria produce excessive ROS and release cytochrome c (cyt-c) to cytosol resulting in mitochondrial membrane potential (MMP) changes, mitochondrion fragmentation and dysfunction, even apoptosis (Ikeda et al., 2015a,b). Mitochondrial dysfunction is involved in a number of neurodegenerative and cardiovascular diseases, such as Parkinson's, Alzheimer's, Huntington's, amyotrophic lateral sclerosis, brain ischemic stroke and cardiac hypertrophy, and also leads to severe maternally inherited diseases (Bratic and Larsson, 2013).

In order to maintain normal function, disrupted mitochondria will be eliminated specifically by the process named mitochondria autophagy or mitophagy, a special pattern of autophagy. Thus, it is important to find molecules which are related with mitochondrial homeostasis and mitophagy. Based on our and other's previous publications, the aims of this review were to (1) introduce mitochondrial homeostasis (structure and function), and the balance of mitochondrial dynamics,

(2) clarify the role of mitophagy in the damaged mitochondrial clearance mainly associated with nervous system diseases and brain injury, and (3) elucidate the molecular mechanisms of mitophagy with a special focus on the function of Drp1, a molecule involved in fission.

Mitochondrial structure

Mitochondria, semi-autonomous cellular organelles with two layers of membrane, have their own genetic system, which uses a distinct DNA code that differs both from that of their bacterial ancestors and their eukaryotic hosts (Gray et al., 1998). In mammals, mitochondria are transmitted to the offspring solely through the maternal lineage, lacking the benefits of recombination arising from sexual reproduction. Thus, when mtDNA suffers significant mutations it will cause maternally inherited diseases characterized by reduced mitochondrial function (Reddy et al., 2011). Outer mitochondrial membrane (OMM) separates mitochondria from the cytoplasm and surrounds the inner mitochondrial membrane (IMM), which separates the inner-membrane space from the protein-dense central matrix. The difference between the outer and inner membrane is that ions and small uncharged molecules can traverse the outer membrane freely through pore-forming membrane proteins, while they get across the inner membrane, a tight diffusion barrier, with the aid of specific membrane transport proteins, which are selective for a certain ion or molecule. Thus, there is an electrochemical membrane potential across the inner membrane, where oxidative phosphorylation takes place. The inner membrane is differentiated into the inner boundary membrane and the cristae. The gap between outer membrane and inner boundary membrane is called inter-membrane space. The inner membrane invaginate forming cristae, the innermost compartment of mitochondria extending more or less deeply into matrix. The shallow proton gradient between the inter-membrane space and the matrix drives ATP production by the ATP synthase in the membranes of the cristae (Bayrhuber et al., 2008; Kuhlbrandt, 2015). Thus, the special structure of mitochondria is essential for the bioenergetics function of mitochondria, and mitochondria dynamics.

Mitochondrial dynamics

As is known to all, mitochondria exist as a highly dynamic tubular network and their morphology is administrated by interactions with the cytoskeleton as well as frequent fusion and fission events (Elgass et al., 2013).

Mitochondrial movement

It has been reported that the cytoskeletal system played an important role in regulating mitochondrial movement, mitochondrial morphology as well as mitochondrial function. Acting in concert with microtubules (MT), microfilaments and intermediate filaments (IF) expedites the complex movement of mitochondria (Anesti and Scorrano, 2006). Microtubule-associated proteins (MAPs) hold a significant position in the interplay of mitochondria with MT and their movement along the MT tracks (Nogales, 2000). Members of kinesin families, a type of MT-associated motors, are responsible for mitochondrial movement (Hollenbeck and Saxton, 2005). Moreover the movement is affected by second messengers generated in signaling events, such as phosphatidyl inositol 4,5-bisphosphate (PtdIns(4,5) P2), Ca^{2+} (De Vos et al., 2003; Rintoul et al., 2003). Microfilaments which are composed by a tight helix of oriented globular (G-) actin monomers perhaps are needed in short-distance movement of mitochondria with the assistance of MT (Hollenbeck and Saxton, 2005). Whereas the role of IF and IF-associated protein in mitochondrial movement is unclear, they appear to be crucial in positioning of mitochondria (Anesti and Scorrano, 2006).

Mitochondrial fission and fusion

Mitochondria, highly dynamic organelles, form a tubular network in the cells that constantly changes by fission and fusion to regulate morphology, distribution, and activity (Chan, 2006). Fusion connects neighboring depolarized mitochondria and mixes their contents to maintain membrane potential. On the contrary, fission segregates damaged mitochondria from intact ones, then the damaged part of mitochondria go through mitophagy to remove (See Fig. 1), a specialized form of autophagy, whereas the intact part is subjected to fusion (Ikeda et al., 2015a,b). Both processes are accomplished by multi-component molecular machineries including a number of dynamin-related GTPases (Detmer and Chan, 2007; Hoppins et al., 2007).

As shown in Fig. 1, mitochondrial fission is mediated by large GTPase dynamin-related proteins (Drp1) and fission 1 protein (Fis1). Fis1 is a small protein that is located on the outer mitochondrial membrane (OMM), whereas Drp1 is predominantly localized in the cytosol. Mitochondrial fission is primarily commanded by the activity of Drp1. In the presence of various stress, Drp1 translocates from the cytosol to the OMM and divides a mitochondrion into two pieces via GTP hydrolysis. In addition to this, endoplasmic reticulum (ER) wrap around mitochondria at prospective fission sites mediating constriction of mitochondrial membranes and acting as a scaffold for Drp1 recruitment (Friedman et al., 2011; Kasahara and Scorrano, 2014). Mitochondrial fusion (Fig. 2), a two-step processes, is critically regulated by mitofusin 1 and mitofusin 2 (Mfn1 and Mfn2), specialized proteins localized on the OMM and involved in OMM fusion, and by optic atrophy 1 (Opa1), a protein localized on the IMM and is required for IMM fusion (Okamoto and Shaw, 2005; Otera and Mihara, 2011).

The imbalance between mitochondrial fusion and fission affects cellular pathology and physiology. As is known to all, mitochondria are highly abundant in the brain and heart which consume more energy. Indeed, mitochondrial morphology regulation has been linked to a number of neurodegenerative and cardiovascular diseases, such as Parkinson's, Alzheimer's, Huntington's, amyotrophic lateral sclerosis, and cardiac hypertrophy, and also cause severe maternally inherited disease termed mitochondrial encephalomyopathies (Nunnari and Suomalainen, 2012; Schon et al., 2012; Itoh et al., 2013; Ong et al., 2013).

Thus, Drp1 may play a vital role not only in regulating mitochondrial morphology and mitochondrial division, but also in cellular pathology and physiology.

Mitophagy and mechanisms of mitophagy

When stimulated by a variety of factors such as ROS, lack of nutrition, even damaged mitochondrial DNA (Suen et al., 2010), mitochondrion will become depolarized and damage itself. The damaged mitochondria are recognized by autophagosome and fused with lysosomes, so as to complete the degradation process, this process is termed mitophagy (Song et al., 2015), as is shown in Fig. 2. This progress includes a series of events, such as stabilization of Pink1 on depolarized mitochondria, phosphorylation of Mfn2, recruitment of Parkin to mitochondria and so on (Youle and Narendra, 2011; Saito and Sadoshima, 2015). Two main signals are shown as follows.

PINK1–Parkin Signaling

The most thoroughly explored mechanism for homeostatic mitochondrial quality control is PINK1–Parkin mediated mitophagy. In normal mitochondria, PINK is proteolytically degraded when it is imported, leading to a low level of PINK protein and PINK kinase activity (Shirihai et al., 2015). During mitochondrial depolarization, PINK1 is accumulated and phosphorylates its substrate proteins (Jin et al., 2010), and Parkin, a member of E3 ubiquitin ligase complex encoded by the Park2 gene in humans (Shimura et al., 2000), is recruited to the damaged mitochondria where PINK1 is accumulated. Then Parkin's E3 ligase activity is activated through PINK1 induced phosphorylation of Ser65 within Parkin's ubiquitin-like (UBL) domain (Kondapalli et al., 2012; Shiba-Fukushima et al., 2012; Iguchi et al., 2013). In addition, ubiquitin are also phosphorylated at Ser65 so as to enhance Parkin-induced ubiquitination of substrate proteins which are located on the outer mitochondrial membrane (Shiba-Fukushima et al., 2012; Kane et al., 2014; Koyano et al., 2014; Ordureau et al., 2014). Before recruiting autophagy receptor proteins such as p62/SQSTM1, the progress of mitochondrial outer membrane proteins ubiquitination is finished. Then the autophagy receptor proteins firsthand interact with the LC3 protein to recruit autophagosomes to encircle the receptor decorated mitochondria and transport the damaged mitochondria to lysosomes for their degradation (Kawajiri et al., 2010; Matsuda et al., 2010; Narendra et al., 2010).

There are three most studied substrates for mitophagy initiation, the mitochondrial fusion proteins Mfn1 and Mfn2, the mitochondrial trafficking protein Miro1, and voltage-dependent anion channel (VDAC) (Williams and Ding, 2015). As is mentioned above, Mfn1 and Mfn2 manage mitochondrial dynamic via regulating mitochondrial fusion, whereas ubiquitinated Mfn1 and Mfn2, degraded by proteasome, lose their original function leading to mitochondrial fission and

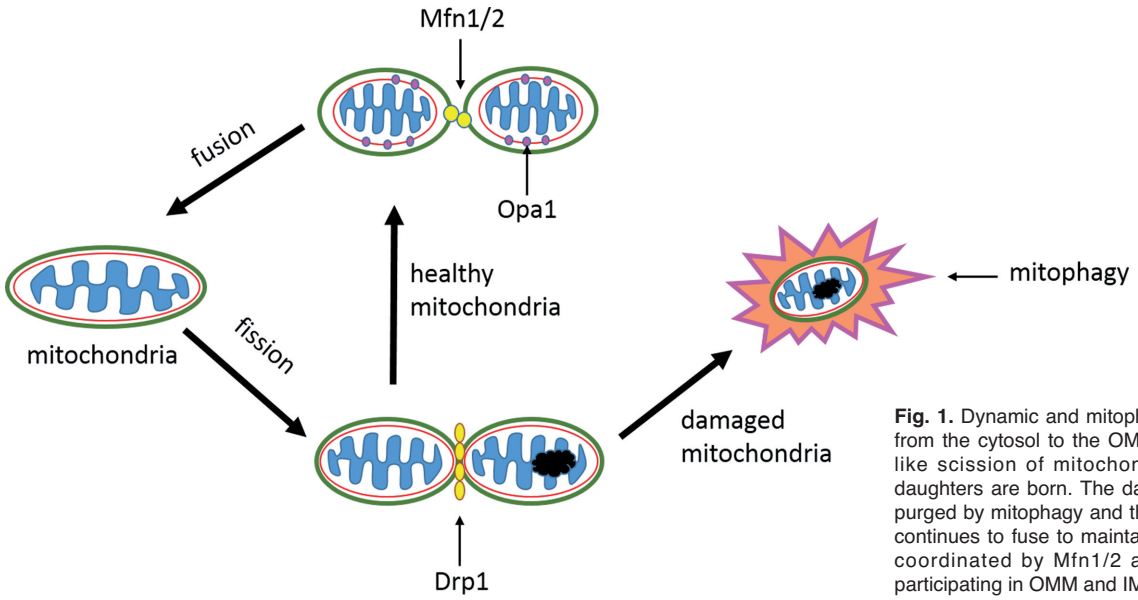

Fig. 1. Dynamic and mitophagy. Drp1 translocates from the cytosol to the OMM and drives dynamin-like scission of mitochondria, so two unequal daughters are born. The damaged mitochondria is purged by mitophagy and the healthy mitochondria continues to fuse to maintain the network which is coordinated by Mfn1/2 and Opa1, separately participating in OMM and IMM fusion.

fragmentation (Gegg et al., 2010; Geisler et al., 2010; Poole et al., 2010; Chan et al., 2011). Mitochondrial fission and fragmentation make great contribution to mitophagy. It makes mitochondria easier to be engulfed by autophagosomes by segregating damaged mitochondria from healthy mitochondria and making smaller pieces of mitochondria (Twig et al., 2008; Kim and Lemasters, 2011; Yoshii et al., 2011; Ding and Yin, 2012; Ordureau et al., 2014). Moreover, Mfn2 may also take part in mediating Parkin recruitment to the damaged mitochondria in addition to PINK1 (Chen and Dorn, 2013). Miro is a subfamily of the Mitochondrial Rho GTPase having two EF hand Ca^{2+}-binding domains. Parkin-induced ubiquitination of Miro1 initiates mitochondrial arrest, or inhibition of mitochondrial motility to isolate a depolarized mitochondrion from the healthy mitochondria population. Thus, it facilitates damaged mitochondria separating from healthy mitochondria population to make it more easily targeted and engulfed by autophagosomes (Wang et al., 2011).

Parkin-induced ubiquitination of VDAC results in recruitment of the autophagy adaptor protein p62/SQSTM1 to the mitochondria (Geisler et al., 2010). p62/SQSTM1 is a conductor between LC3, located on autophagosome membrane, and ubiquitinated proteins and organelles because it contains both a microtubule-associated protein light chain 3 (LC3)-interacting region (LIR) and ubiquitin-associated domain (UBA). The compound ubiquitinated protein-p62-LC3 can be transported to autophagosomes for degradation (Manley et al., 2013). But beyond that, there are several Parkin-independent pathways for mitophagy induction (Williams and Ding, 2015).

These findings suggest PINK1-Parkin signaling plays an important role in mitophagy by Parkin-induced ubiquitination of substrate proteins and PINK1-induced phosphorylation. However, further research is needed to confirm the relationship between Parkin-dependent and Parkin-independent pathways.

NIX and Bnip3 signaling

Both Nip3-like protein X (NIX) and Bcl-2/adenovirus E1B 19-kDa–interacting protein-3 (Bnip3) are pro-apoptotic BH3-only proteins that activate Bax/Bak to permeabilize mitochondrial membrane and facilitate opening of the mitochondrial permeability transition pore (mPTP) (Kubli et al., 2007; Novak et al., 2010). NIX, which situates in the outer mitochondrial membrane, contributes to programmed mitochondrial elimination via interacts with Atg8 mammalian homologs termed LC3/GABARAP directly, an important formation of autophagosomes forming new membranes (Novak et al., 2010). Furthermore, Bnip3, another potent inducer of mitochondrial autophagy, is high sequence to NIX (Quinsay et al., 2010). In addition to direct interaction, NIX and Binp3 can disrupt the interaction between Bcl-2/Bcl-XL and Beclin1 to set free Beclin1 to

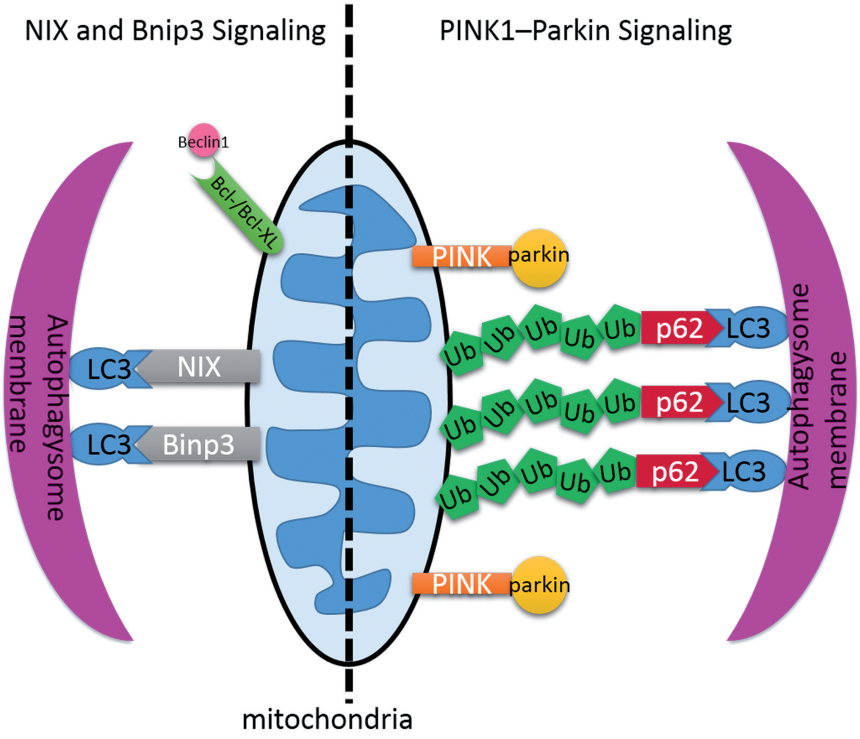

Fig. 2. Mechanisms of mitophagy. Two signals are shown in the picture. The left one indicates NIX and Bnip3 signaling showing how NIX and Binp3 play a part in mitophagy. NIX and Binp3 directly interact with LC3 and disrupt the interaction between Bcl-2/Bcl-XL and Beclin1 to induce autophagy. The right side of the photo shows PINK1-Parkin signaling. PINK is accumulated and stabilized on OMM leading to translocation of parkin to mitochondria, which ubiquitinates damaged mitochondria. Ubiquitination of OMM proteins recruit autophagy receptor proteins p62/SQSTM1 which directly interact with the LC3 protein to initiate mitophagy.

induce autophagy (Pattingre et al., 2005).

However, further research is needed to understand the role of Drp1 in NIX and Bnip3 Signaling, particularly in nervous system diseases and brain injury.

The role of mitophagy in pathophysiological process

As essential organelles, mitochondria are also involved in a series of cellular processes: calcium homeostasis, heme and iron-sulfur cluster protein biosynthesis, cellular differentiation, control of the cell cycle and cell death regulation (Osellame et al., 2012). Thus, they are linked to many pathophysiological processes and diseases. In this paper we will make a simple introduction.

First of all, development. Mitophagy plays an essential role in paternal mitochondria destruction and erythrocyte differentiation and maturation. Nix, a number of mitophagy related protein, is highly expressed during erythroid differentiation joining in the clearance of mitochondria in reticulocytes (Schweers et al., 2007). And during fertilization, paternal mitochondria are labeled by ubiquitin proteasome immediately when they enter the ooplasm indicating degradation after fertilization (Sutovsky et al., 2004). Second, aging. One of the remarkable characteristics of the aging process is the decline of autophagic activity as well as related proteins and mitochondrial dysfunction accelerates the aging process (Lionaki et al., 2013; Schiavi and Ventura, 2014). Thus, it was suggested that mitophagy may contribute to eliminate dysfunctional mitochondria, although the exact mechanisms are not clear (Ding and Yin, 2012). Then, tissue injury. Coordinate with the function of mitochondria, mitophagy can protect cardiomyocytes against ischemia-reperfusion-induced heart injury and reduce alchohol-induced liver damage (Ding et al., 2010; Huang et al., 2011). Finally, neurodegenerative disease. With the deepening of the research, increasing evidence indicates the therapeutic role for mitochondria in neurodegenerative disease, accompanied by decreased mitochondrial function (Zhu et al., 2013). At the early stage of AD process, ultra-structurally changed mitochondria were accumulated in brains inducing Parkin-mediated mitophagy which is essential for us to understand the pathophysiological process in AD brains (Ye et al., 2015). In post-mitotic neurons, unstable mitophagy is harmful in vitro neuronal AD model, while inhibit the excessive mitophagy in neurotoxic neuron do not prevent mitochondrial damage but provide an obvious protection against cell death (Corsetti et al., 2015). Moreover, PINK-Parkin mutations cause familial Parkinson disease which is a failure to trigger mitophagy. It is clear that mitochondrial dysfunction is the major pathological mechanism of neuronal loss, though optimum levels of NO enabled PINK1-null dopaminergic neuronal cells to regain the mitochondrial translocation of Parkin, there are no effective measures to alleviate mitochondrial deficits in PD (Schapira, 2012; Han et al., 2015).

The role of Drp1, a mitophagy regulator, in brain disease

Drp1, a ~80 kDa protein, has a common domain structure including an N-terminal GTP-banding domain, a middle assembly domain, a small insert, and a C-terminal GTPase effector domain (GED) (Praefcke and McMahon, 2004). In general, Drp1 exists primarily as small oligomers (dimers/tetramers) in the cytosol, while it can self-assemble into higher order structures upon binding to OMM (Chang et al., 2010; Smirnova et al., 2001). The dynamics, localization and activity of Drp1 are changed by posttranslational modifications including protein phosphorylation, SUMOylation, ubiquitination and S-nitrosylation (Chang and Blackstone, 2010). There are two widely studied phosphorylation sites Ser616 and Ser637. Phosphorylation at Ser616 by cyclin B dependent kinase, not directly affect GTPase activity, promotes Drp1-dependent mitochondrial fission and facilitates the increasing of mitochondrial fragmentation (Cribbs and Strack, 2007; Taguchi et al., 2007), but phosphorylation at Ser637 by cyclic AMP-dependent protein kinase A (PKA) inhibits mitochondrial fission through a reduction in GTPase activity and/or inhibition of Drp1 translocation to mitochondria (Chang and Blackstone, 2007; Cereghetti et al., 2008). SUMOylation may exert an effect on Drp1 interactions with the OMM or other proteins, Ubiquitination might affect mitochondrial association of Drp1 to alter mitochondrial morphology and S-nitrosylation shares many properties with protein phosphorylation (Karbowski et al., 2007; Chang and Blackstone, 2010).

Despite regulated mitochondrial fission Drp1 is associated with several cellular functions, including peroxisomal fragmentation, SUMOylation, phosphorylation, ubiquitination, and cell death (Reddy et al., 2011). Moreover, Drp1 not only has a crucial role in mediating Parkin-induced mitochondria selective autophagy in mouse embryonic fibroblast (MEF) cells (Tanaka et al., 2010), but also mediates Bnip3-induced autophagy in adult cardiomyocytes (Lee et al., 2011).

Recent research focused on the protein Drp1 to explore its exact status in mitophagy. Ikeda et al. (2015a,b) suggested that endogenous Drp1 is important in mediating autophagy in cardiomyocytes. When Drp1 was downregulated in the presence of chloroquine, an inhibitor of autophagic flux or autophagosome-lysosome fusion, there were fewer GFP-LC3 puncta, indicating that Drp1 controls autophagic flux at least at the level of autophagosome formation. But the effects of Drp1 on mitophagy could not be evaluated because of technical limitations (Ikeda et al., 2015a,b). Moreover, Lin et al. (2015) found that inhibition of Drp1 impaired autophagosomes clearance, thus resulting in accumulation of the autophagosomes without an augmentation in autolysosomes (Lin et al., 2015). Kageyama et al. (2014) demonstrated that in the absence of Drp1, mediating mitochondrial fission, mitophagy was incompletely executed in mammalian brain and

heart. In Drp1KO cardiomyocytes, mitochondria showed increased size and connectivity and became deficient in mitophagy. This mitophagy defect generates intermediate structures with accumulations of the autophagy adaptor protein p62/SQSTM1 and ubiquitinated proteins on mitochondria in a parkin-independent manner, without increases in the LC3-II (Kageyama et al., 2014). In addition to this, researchers also considered that downregulation of Drp1 promotes the interaction between Bcl-2/Bcl-xL and Beclin1 and disrupts Drp1 induced mitochondrial elongation inhibited, even abrogated mitochondrial autophagy in neurodegenerative diseases and heart failure (Reddy et al., 2011; Shirakabe et al., 2016). Overall, all these findings revealed Drp1 is essential for general autophagy, mitophagy, mitochondrial division, and cell survival in mammalian brain and heart.

It has been suggested that Drp1 and parkin act at different phases in mitophagy; Drp1-mediated mitochondrial division helps autophagosomes engulf mitochondria while parkin stimulates degradation of mitochondria by ubiquitination of mitochondrial proteins. Both parkin-dependent and Drp1-dependent mechanisms for regulation of mitophagy may contribute to elimination of damaged mitochondria. Drp1 and parkin synergistically maintain the integrity of mitochondrial structure and function in mammalian brain and heart (Youle and Narendra, 2011; Kageyama et al., 2014). Zhang et al. discovered that in MPTP-induced PD mouse models, Drp1 is upregulated and phosphorylated at Ser616 result from Parkin reduced its ability to repress Drp1 expression. Moreover, they also suggested that inhibition of Drp1 reduced the neurotoxicity and dopamine release deficits in vivo (Zhang et al., 2016). In the model transient global ischemia (TGI) causing hippocampal injury, the expressions of PINK and pDrp1 (Ser616) are increased. S. D. Chen et al. consider that PINK1 protect TGI-induced neuronal injury through regulating pDrp1 (Ser616) expression (Chen et al., 2015). In addition, the association between mitochondria and dendritic spines is crucial in neuron cellular physiology. The GTPase, Drp1 and OPA1, regulate the density and plasticity of synapses. During synaptogenesis Drp1 is needed to modify neuronal development and synaptic strength (Drp1 overexpression could cause a higher density of spines and synapses yet overexpression of dominant-negative Drp1 results in a lower density of spines and synapses) (Li et al., 2004; Jahani-Asl et al., 2015). However, it is unclear the mechanistic role of Parkin, PINK and Drp1, particularly in nervous system diseases and brain injury.

Perspectives

It is proved that mitophagy is an important mechanism for mitochondrial function and quality control, moreover, mitochondrial fission is prerequisite and vital for mitophagy activation (Youle and van der Bliek, 2012). Nevertheless, how execution of fission, such as how Drp1 protein coordinates with mitophagy activation, is not fully understood. Thus, further more research is needed to exploit the molecular mechanisms by which Drp1 contributes to mitophagy, as well as the roles of different Drp1 levels.

A recent study reported that Drp1 could inhibit ischemia-induced mitophagy via the recruitment of pro-autophagic factors, eg. LC3 instead of the overall or general autophagy in the hippocampal CA3 region and help contribute to the survival of hippocampal neurons, indicating that Drp1-mediated mitochondrial fission and mitophagy is essential or vital for the rapid removal of the dysfunctional mitochondria after brain ischemic injury (Zuo et al., 2016). Our previous research has proved a novel application of mdivi-1, an inhibitor of Drp1 for neuroprotection in mice after traumatic brain injury (TBI), and described that the use of Mdivi-1 could attenuate TBI-induced behavioral dysfunction and brain edema, and decrease mitochondrial fission assessed using transmission electron microscopy (TEM) evidence, together with apoptotic cell death detected by blot for cytochrome c (cyt-c) and caspase-3 (Wu et al., 2016). However the underlying mechanisms are not discovered. We suspect that the neuroprotection of Mdivi-1 is related to mitophagy. Thus, we will detect the levels of mitophagy and discover the schedule changes of every indicator in further studies in order to provide a potential and novel therapeutic target for brain injury and nervous system diseases.

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