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·Review·

Regulation of mitophagy in ischemic brain injury

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The selective degradation of damaged or excessive mitochondria by autophagy is termed mitophagy. Mitophagy is crucial for mitochondrial quality control and has been implicated in several neurodegenerative disorders as well as in ischemic brain injury. Emerging evidence suggested that the role of mitophagy in cerebral ischemia may depend on different pathological processes. In particular, a neuroprotective role of mitophagy has been proposed, and the regulation of mitophagy seems to be important in cell survival. For these reasons, extensive investigations aimed to profile the mitophagy process and its underlying molecular mechanisms have been executed in recent years. In this review, we summarize the current knowledge regarding the mitophagy process and its role in cerebral ischemia, and focus on the pathological events and molecules that regulate mitophagy in ischemic brain injury.

Keywords: mitophagy; ischemic brain injury; mitochondria

Introduction

One of the first autophagy investigations was performed by Keith R. Porter, who in 1962 discovered an increased number of vacuoles in rat liver cells treated with glucagon[1]. Autophagy is a pivotal intracellular process for the bulk degradation and recycling of unnecessary or dysfunctional proteins and organelles by lysosomes[2]. There are three forms: microautophagy, chaperone-mediated autophagy, and macroautophagy[3]. Macroautophagy is the most prevalent form and here is referred to as autophagy. It is critical for maintaining cell functions and deciding cell fate, and its intracellular processes have been elucidated in recent decades[3, 4]. Generally, double-membrane vesicles termed autophagosomes sequester target intracellular cargoes and then fuse with endosomes or directly with lysosomes for degradation. Autophagy is canonically activated under starvation[5], hypoxia[6] and intracellular stress[7, 8]. The mammalian target of rapamycin (mTOR) kinase is a critical regulator of autophagy induction: activation of mTOR by AMPK-p53 signaling promotes it[9], while inhibition of mTOR *via* PI3K-Akt signaling is suppressive[10]. The current knowledge of cell signaling on the regulation of autophagy has been summarized in several comprehensive review articles[11-13].

Autophagy is widely implicated in central nervous system (CNS) disorders[2]. A well-supported theory is that dysfunctional autophagy leads to aberrant accumulations of toxic protein aggregates in specific sites within the CNS and thus causes neurodegenerative diseases[14]. Given that autophagy can promptly respond to insufficient nutrients and energy supply, it is not surprising that it is extensively observed in ischemic brain tissues[6, 15, 16], where it was primarily postulated to promote cell death[17], whereas lines of evidence indicated that it is required for neuronal survival during development and neurodegeneration, as well as in several models of cerebral ischemia[15, 18, 19]. Unlike apoptosis and necrosis, which certainly contribute to ischemic brain injury, autophagy differs in that it may serve as a potential therapeutic target against ischemia[20-22]. Therefore, although they remain controversial, these emerging data suggest that autophagy may play a vital role in neuroprotection against ischemic brain injury, but the underlying mechanisms await clarification.

Selective elimination of mitochondria by autophagy, termed mitophagy, may be a responsible mechanism. Mitophagy is highly evolutionarily conserved; many of its essential proteins in yeast have homologs in mammals[23]. As the powerhouse of cells, mitochondria have a much shorter average lifespan of 10–25 days, compared with neurons, which are postmitotic cells with high energy demands. As a result, mitochondrial turnover by mitophagy is extremely important for neuronal survival. Dysfunctional mitophagy has been implicated in several human CNS diseases, including Alzheimer's disease[24] and Parkinson's disease[25]. Intriguingly, it has also been reported in ischemic brain injury by several independent groups[15, 26-28]. By clearing dysfunctional mitochondria and promoting mitochondrial turnover, cell death resulting from ischemic insult is attenuated[15]. Thus, mitophagy seems to be involved in ischemic brain injury, but questions remain. In this review, we summarize the current progresses on the regulation of mitophagy and its specific role in ischemic brain injury.

Mitophagy in Ischemic Brain Injury

Stroke is the third leading cause of death and disability worldwide. Cerebral ischemia makes up 65%–80% of total stroke events. Ischemic brain injury is generally caused by the sudden block of blood supply, leading to decreased oxygen and glucose supply to the brain tissue. The pathological mechanisms include, but are not limited to, neuro-excitotoxicity, brain inflammation, microglial activation, and endothelial injury[29-32]. In general, apoptosis is primarily found in striatal and cortical neurons after ischemic reperfusion, followed by necrotic neuronal death in the ischemic core area[33]. Autophagy, however, is widely involved in ischemic brain injury[15-18], and the autophagosome marker LC3-II is strongly elevated at the onset of ischemia[15], indicating the robust activation of autophagy, which might represent a cell defense mechanism against ischemic insult. Further, within 6 h after reperfusion, electron microscopy has shown damaged mitochondria surrounded by autophagosomes in the ischemic penumbra[15], and the numbers of mitochondria assessed by the constitutively-expressed mitochondrial proteins TOMM20 and COX4I1 are significantly reduced[15], suggesting that mitochondria are degraded by autophagy. In primary cultured neurons with oxygen and glucose deprivation (OGD) treatment, mitophagy is observed within 1 h, revealed by the co-localization of Mito-DsRed-labeled mitochondria and GFP-LC3-labeled autophagosomes (Fig. 1). The data indicated that mitophagy may reach a maximum within 3 h of OGD. Mitophagy is mainly evident

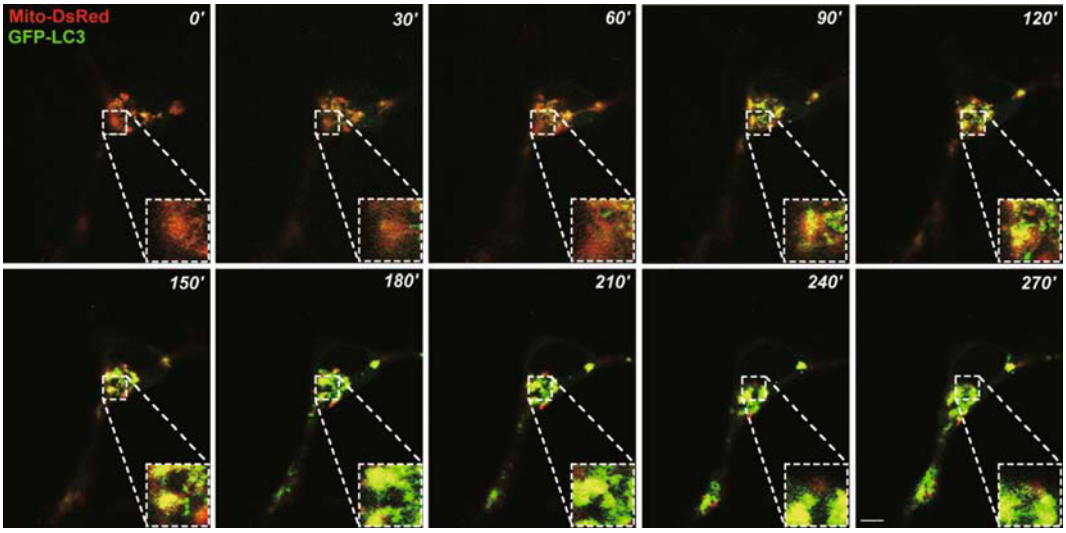

Fig. 1. Time-lapse imaging of mitophagy. Primary cultured mouse cortical neurons expressing Mito-DsRed and GFP-LC3 were exposed to oxygen and glucose deprivation for 2 h, and imaged at 1 frame/30 min. Mitophagy was determined by the co-localization of Mito-DsRed-labeled mitochondria and GFP-LC3-labeled autophagosomes. The boxed regions were amplified 4 times in the bottom right boxes. Scale bar, 10 μm.

in neurons undergoing brain ischemia[15, 19, 34], although astrocytes have also been documented[35, 36].

The contributions of autophagy and/or mitophagy to ischemic brain injury have been controversial for years[6, 16]. In long-term ischemia, for example, permanent middle cerebral artery occlusion (MCAO) models, the excessive activation of autophagy has been proposed to be associated with increased injury by inducing autophagic cell death, and inhibition of autophagy by 3-MA is neuroprotective[18]. Similarly, excessive induction of mitophagy leads to apoptosis in a neonatal ischemia/hypoxia model[37], in which the restoration of circulation is deficient due to intravascular coagulation. However, in the reperfusion phase after ischemia, mitophagy plays beneficial roles[15]. Inhibition of autophagy either pharmacologically or genetically rescues the ischemic brain[15] and conversely, injection of rapamycin, an autophagy inducer, has the opposite effects in rats with transient MCAO[28]. More interestingly, inhibition of autophagy immediately after reperfusion aggravates ischemic brain injury by reducing mitochondria-based apoptosis[15], while delayed inhibition of autophagy by 3-MA after reperfusion (3 h later) does not significantly increase brain damage (unpublished data), indicating that the protective action of autophagy occurs only at the initial step. In another study, rapamycin treatment was shown to reduce necrotic cell death in neonatal hypoxia-ischemia-induced brain injury[38]. Increasing evidence implies that autophagy may switch its contributions to ischemic stroke along with the pathological stages, but the reason is not clear. Energy restoration upon reperfusion[39] is perhaps the most plausible since autophagy/mitophagy is an energy-consuming process. But how the energy state may exquisitely regulate autophagy is far from clear. Alternation of mitochondrial dynamics during ischemia and reperfusion is an intriguing speculation[26, 40]. Upon reperfusion, mitochondria are fragmented by Drp1, a mitofission protein[26]. It is hypothesized that mitofission allows mitochondria to be engulfed by autophagosomes much more easily. The distinct mechanisms of permanent and transient ischemia may be another factor. In the reperfusion phase after ischemia, apoptosis is prone to be induced otherwise the prolonged ischemia normally results in necrosis[15, 41-44]. This may provide a chance for mitophagy to confer neuroprotection by clearing the damaged mitochondria. Subsequently, cytochrome *c* released from mitochondria is reduced and thus mitochondria-dependent apoptosis can be inhibited[15]. Although remaining enigmatic, the simplistic tagging of autophagy as 'bad' or 'good' in ischemic brains deserves to be reconsidered.

Pathological Events in Mitophagy Activation in Ischemic Brain

Although lines of evidence have implicated mitophagy in ischemic brains, the specific events initiating it and their integrated mechanisms remain largely unsolved. Recent work on endoplasmic reticulum (ER) stress, oxidative stress, and excitotoxicity may offer a solution to these questions.

Endoplasmic Reticulum Stress

ER stress has been reported to be widely involved in ischemic injury[45, 46]. However, consensus has not been reached on its role in this process. ER stress leads to apoptosis[47] and inhibition of the ER-associated pro-apoptotic factor CHOP confers remarkable neuroprotection in the mouse bilateral common carotid arteries occlusion model[48]. Consistently, several reagents that relieve ER stress protect against brain ischemia-reperfusion injury[49, 50], indicating that ER stress may be responsible for it. However, ER stress also triggers the unfolded protein response to restore ER functions by activating the ER transmembrane receptors PERK, IRE1, and ATF6[51]. It is well-accepted that increased ER stress leads to autophagy, and correspondingly autophagy helps to suppress ER stress[7, 52]. In the context of ischemic myocardial injury, treatment with ER stressors before operation induces autophagy[53]. Furthermore, mild induction of ER stress selectively reinforces ischemia-reperfusion-induced mitophagy *via* the PERK-EIF2S1-ATF4-Parkin signaling pathway[54]. Notably, the autophagic machinery does not accordingly increase with regard to the remarkable activation of mitophagy, as revealed by the numbers of autophagic vacuoles on mitochondria[54]. Since the stimulation of ER stress has been reported to result in mitochondrial dysfunction and thus impaired mitophagy in lung epithelial cells[55], it is of interest to explore why moderate ER stress reinforces mitophagy. The ER-mitochondria contact site (MAM) has recently been reported to be the location of autophagosome generation[56]. It is likely that mitochondria have priority to

be targeted by autophagosomes generated in the MAM, and appropriate stimulation of ER stress may accelerate the mitophagy originated by the ER. The mechanisms underlying ER stress-induced mitophagy, however, are not fully understood. Further, the extent to which ER stress favors mitophagy, and how to target mitophagy for therapy through ER stress activation, need to be further addressed.

Reactive Oxygen Species

Reactive oxygen species (ROS) are byproducts of oxygen metabolism, extensively and promptly generated in ischemic tissues after reperfusion[57-60]. ROS accumulation results in oxidative damage, opening of the mitochondrial permeability transition pore, and mitochondria-dependent cell death. Mitochondria produce most of the cellular ROS *via* the mitochondrial electron-transport chain[61], and play an important role in the pathogenesis of ischemic brain injury. ROS initiate autophagosome formation[62], and excessive ROS formation triggers bulk autophagy[63], while conversely, autophagy helps to reduce ROS levels by removing damaged organelles and abnormal proteins[64]. Interestingly, moderate ROS levels specifically induce mitophagy but not general autophagy to protect against cell death in a Drp1-dependent manner[63], indicating the ability of ROS to stimulate mitophagy. In support of this, in another study, increased ROS levels were shown to lower the mitochondrial membrane potential and activate Parkin-dependent mitophagy. Conversely, overexpression of superoxide dismutase-2, a mitochondrial antioxidant protein, blocks mitophagy by clearing photosensitizer-induced mitochondrial ROS in HeLa cells[62]. However, the association of ROS extension with the selective activation of mitophagy is still not clear.

In the context of ischemia, like ER stress, ROS may also be a double-edged sword with regard to cell survival (Fig. 2). On one hand, antioxidants that relieve ROS have been reported to help minimize ischemic injury[65-67]; on the other hand, ROS-induced mitophagy protects against ischemic myocardial injury. In p53-deficient mice, a p53-TIGAR-mediated decrease in the ROS signal reduces Bnip3-dependent mitophagy and enlarges the infarcted myocardium, while these are reversed by injection of the antioxidant N-acetylcysteine[68]. Therefore, it is likely that ROS are also responsible for brain ischemia-reperfusion-induced mitophagy, and serve as a potential target for clinical therapy.

Excitotoxicity

Excitotoxicity is evoked by excessive activation of neurotransmitters such as glutamate, which is the main neurotransmitter in the CNS. Excitatory neurotransmitters are widely involved in neurodegenerative diseases like Alzheimer's disease[24], Parkinson's disease[69], and stroke[59, 60, 70]. Excitotoxicity is induced by high levels of glutamate caused by over-activation of N-methyl-D-aspartate receptors (NMDARs) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors in ischemic brain injury. NMDA antagonists such as memantine are neuroprotective both *in vivo* and it *in vitro* in brain ischemia models[70, 71]. Excitotoxicity has been postulated to be associated with autophagy/mitophagy in mammals. In rats treated with the NMDAR agonist quinolinic acid, excitotoxicity is induced and it aggravates the neuronal injury by p53-activated autophagy[9]. A more recent study indicated that glutamate exposure results in the translocation of Parkin to mitochondria in a NMDAR-dependent manner, but is not sufficient to activate mitophagy. However, when co-treated with the antioxidant N-acetylcysteine, mitophagy is promoted[72], indicating that excitotoxicity can induce autophagy, but is insufficient to activate it alone. Therefore, excitotoxicity may not always be deleterious, since it facilitates the translocation of Parkin to mitochondria, which favors mitophagy, especially in the complex pathological process of ischemic brain injury.

With the progress made so far, multiple pathways could be involved in mitophagy in ischemic brain injury (Fig. 2). And more pathological events might be involved in mitophagy activation in the ischemic brain that need to be explored. How mitophagy occurs and how to promote it require further studies.

PINK1- and Parkin-Mediated Mitophagy

PINK1 (PTEN-induced kinase 1)- and Parkin-mediated mitophagy is perhaps the most extensively-studied mechanism underlying mitophagy. Both having loss-of-function mutations in familial Parkinson's disease, PINK1 and Parkin physically interact and work together in the same pathway in *Drosophila*[73]. In dysfunctional mitochondria, due to depolarization of the mitochondrial membrane, PINK1 is concentrated in the outer mitochondrial membrane, and then Parkin, an E3 ubiquitin

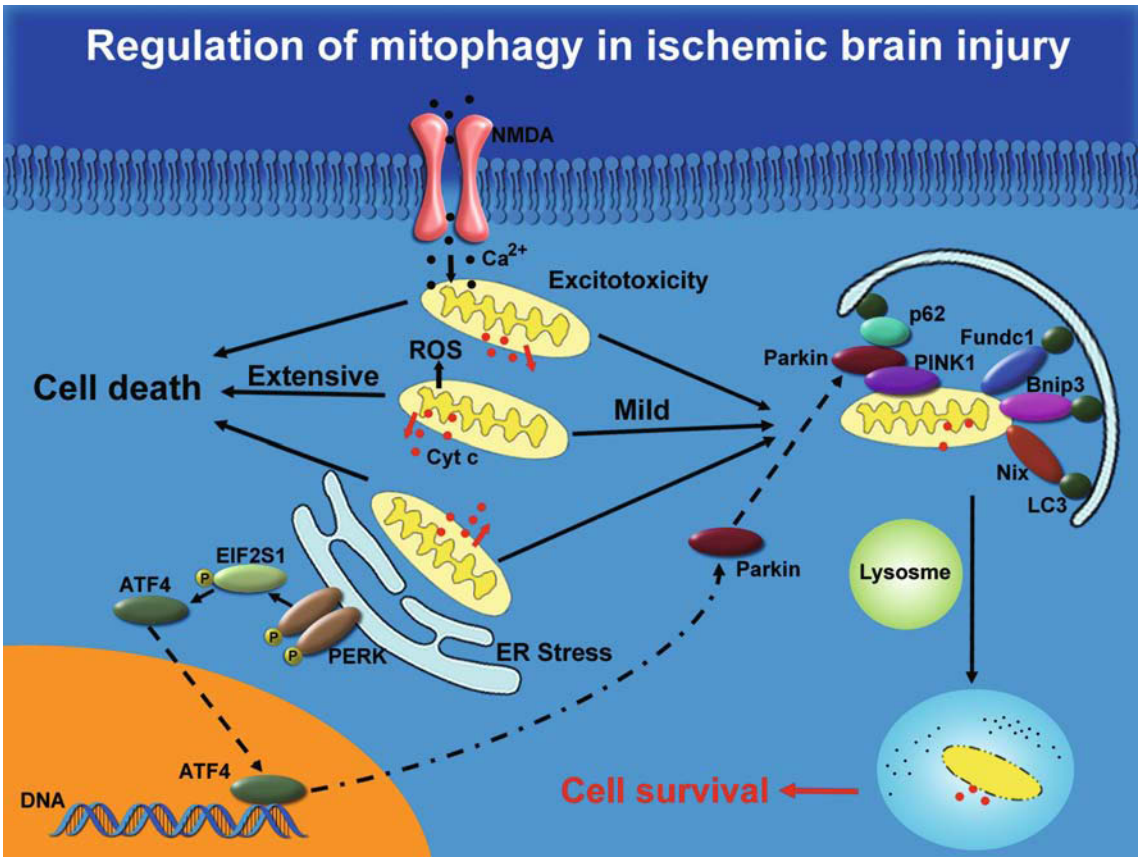

Fig. 2. Regulation of mitophagy in ischemic brain injury. Neuronal mitochondria are damaged by ER stress, the activation of oxidative stress, and/or excitotoxicity upon brain ischemia. Subsequently, the release of mitochondrial proteins such as cytochrome *c* ultimately leads to apoptotic cell death. However, on the other hand, mild ER stress, ROS generation, and excitotoxicity trigger the degradation of damaged mitochondria by mitophagy and therefore protect against ischemic brain injury. PINK1/Parkin, Bnip3, Nix, and Fundc1 are critical molecules responsible for mitophagy.

ligase, is phosphorylated by PINK1, and is further recruited to the damaged mitochondria[74] (for review, see [75]).

Lines of evidence have suggested that PINK1-Parkin are involved in ischemic brain injury. Parkin is markedly down-regulated after reperfusion for 3 h in a mouse model of transient MCAO[39]. This is temporally consistent with the peak of autophagic flux[15], implying that Parkin-labeled mitochondria can be eliminated by autophagy. Besides, ubiquitinated proteins are also present in the same model[76], regardless of whether Parkin is responsible for the ubiquitination or not, and this is now under discussion. Parkin is known to ubiquitylate several important substrates in cardiac injury[77], indicating that it may participate in ischemic brain injury by activating mitophagy. In ischemic preconditioning (IPC) of the rat heart, Parkin is translocated to mitochondria, and its ablation abolishes the cardioprotective effects of IPC[27], suggesting that Parkin-mediated mitophagy is required in this process. In support, we also found translocation of Parkin to mitochondria in primary cultured neurons exposed to OGD-reperfusion, and knockdown of Parkin impairs OGD-reperfusion-induced mitophagy[54]. Interestingly, the translocation of Parkin to mitochondria is accelerated by moderate activation of ER stress, ROS, and excitotoxicity as noted above, indicating that multiple stimulations may trigger Parkin-mediated mitophagy, which, from another perspective, ensures the integrity of execution of Parkin-mediated mitophagy in ischemic brain injury. Above all, Parkin-dependent mitophagy could be a target for ischemic brain injury therapy.

Bnip3- and Nix/Bnip3L-Mediated Mitophagy

Both Bnip3 and Nix (Bnip3L) are BH3-only proteins implicated in apoptosis and autophagy[78-80]. Bnip3 has been reported to participate in hypoxia-induced mitophagy in rat heart. Nix was first found to be essential for the induction of mitophagy during the maturation of red blood cells[79]. Both Bnip3 and Nix are primarily localized on the outer mitochondrial membrane, and share a similar mechanism to induce mitophagy (for reviews, see[80, 81]). The difference between the two proteins during the induction of mitophagy is of interest. Nix up-regulation cannot compensate for the loss of Bnip3-induced mitophagy[37], indicating clear differences between Bnip3- and Nix-dependent mitophagy. Bnip3 exclusively activates excessive mitophagy by interacting with LC3 in a mouse model of neonatal ischemia/hypoxia, and loss of Bnip3 significantly decreases mitophagy and reduces neuronal apoptosis. However, Nix has been proposed to function only under physiological conditions[37]. Nevertheless, a recent investigation found that Nix overexpression promotes carbonylcyanide-3-chlorophenylhydrazone (CCCP)-induced mitophagy in human embryonic kidney cells[82], suggesting that Nix also participates in stress-induced mitophagy. A possible hypothesis is that Nix may act as a substrate of Parkin, through which it senses damage to mitochondria[82]. Nevertheless, whether Nix-mediated mitophagy is involved in ischemic brain injury needs further investigation.

FUNDC1-Mediated Mitophagy

FUNDC1, which is located on the mitochondrial outer membrane, contains three transmembrane domains and has been newly identified as the mitophagy receptor in hypoxia[83]. FUNDC1 directly binds to LC3 *via* its conserved LIR motif. Both knockdown of FUNDC1 and mutation of the LIR motif inhibit mitophagy[83]. This binding is strengthened under hypoxic conditions, and is much stronger than that of Nix, suggesting that FUNDC1 has important implications for ischemia. Further, FUNDC1 can be phosphorylated by ULK1 at serine 17, which is critical for mitophagy induction under hypoxia. In contrast, FUNDC1 promotes the recruitment of ULK1 to damaged mitochondria[84]. Nonetheless, the role of FUNDC1 in brain ischemia has not been discussed.

Interestingly, increasing numbers of studies have indicated that these mitophagy pathways work cooperatively in pathological processes to ensure the effectiveness of mitophagy, especially in mammals. For example, Nix initiates autophagy and promotes the CCCP-induced translocation of Parkin[85]. In addition, Nix has very recently been reported to be the substrate of Parkin in PD[82], suggesting that Nix functions downstream of Parkin. Besides, mitophagy is nevertheless induced in HeLa cells that lack the Parkin protein[82]. Finally, Bnip3, Nix, and FUNDC1 have all been reported to be involved in hypoxia-induced mitophagy, suggesting that these proteins work together in specific mitochondrial diseases. However, how these pathways cooperate and which occupies a dominant role in ischemic brain injury need to be addressed.

Proteins That Potentially Regulate Mitophagy in Ischemic Brain Injury

Many mitophagy-related proteins have been implicated in ischemic brain injury (Table 1). However, whether they all participate in the process by regulating brain injury-induced mitophagy remains unclear. We summarize the features of several frequently-reported proteins below.

Beclin1

Beclin1 is an autophagy regulator and is implicated in mitophagy[86]. In myocardial ischemia, autophagy is activated by an AMPK-dependent mechanism, but in the reperfusion phase, Beclin1 is required[87]. Knockdown of Beclin1 by RNA interference protects against cerebral ischemic injury in rats by activating autophagy[88]. In addition, Beclin1 interacts with PINK1[86], and turns on autophagic flux. Nevertheless, mitophagy can also be triggered in a Beclin1-independent pathway with the parkinsonian neurotoxin MPP^+ treatment of neurons[89], suggesting that Beclin1 may not be as important in ischemic brain injury-induced mitophagy as in myocardial ischemia.

VDAC1

VDAC1 (voltage-dependent anion channel 1) is a poly-ubiquitination substrate of Parkin[90]. It is located on mitochondrial outer membrane, and has recently been reported to promote mitophagy and protect neurons in subarachnoid hemorrhage[34]. While VDAC1 is required for

Table 1. Mitophagy-related proteins involved in ischemic brain injury

Protein	Location	Function	Ref
SQSTM1	Cytosol	Autophagy receptor, ubiquitination substrate of Parkin	[98, 99]
ALOX15	Cytosol	Key enzyme in mitophagy induction in reticulocytes	[100]
CDC37	Cytosol	Co-chaperone of HSP90	[101]
P38	Cytosol	Phosphorylates LC3	[102, 103]
HSP90	Cytosol	Regulates ULK1- and ATG13-mediated mitophagy	[101]
TNFα	Cytosol	Induces mitophagy in mouse macrophages	[104]
RIPK2	Cytosol	Regulates mitophagy by phosphorylating ULK1	[105]
PINK1	Mitochondria or Cytosol	Involved in mitophagy by promoting translocation of Parkin	[75, 106]
Parkin	Mitochondria or Cytosol	E3 Ub ligase, ubiquitylates OMM proteins to promote mitophagy	[54, 75]
FUNDC1	Mitochondria	Receptor for hypoxia-induced mitophagy	[83]
VDAC1	Mitochondria	Ubiquitination substrate of Parkin	[34]
Drp1	Mitochondria	Required in mitochondrial division	[97]
Mitofusin1	Mitochondria	Mediates mitochondrial fusion	[107]
Mitofusin2	Mitochondria	Mediates mitochondrial fusion	[107]
PARL	Mitochondria	Prevents release of mitochondrial cytochrome C	[108, 109]
Beclin1	Mitochondria	Induces autophagy	[86, 88]
Bcl-2	Mitochondria	Regulates PINK1-Parkin-mediated mitophagy	[110]
Bcl-2l1	Mitochondria	Suppresses FUNDC1-mediated mitophagy	[111]
TRAF2	Mitochondria	E3 ubiquitin ligase	[112]
Nix	Mitochondria and ER	Mitophagy receptor, interacts with LC3 and GABARAP	[85, 113]
Bnip3	Mitochondria and ER	Mitophagy receptor, interacts with LC3	[78, 80]
HDAC6	Nucleus	Regulates Parkin-induced mitophagy	[114]
HMGB1	Nucleus	Regulates mitochondrial function and morphology	[115]
HSP27	Nucleus	Required for mitochondrial quality control	[116]

GABARAP, GABA receptor-associated protein.

Parkin-dependent mitophagy[90], whether it is dispensable for the induction of mitophagy is still under debate, since it cannot fully account for the mitochondrial K63-linked ubiquitin immunoreactivity after mitochondria depolarization[91].

Drp1

Mitochondrial dynamics and mitophagy are closely related (see reviews[92, 93]). Mitochondrial fission can produce an impaired unit that undergoes autophagic elimination[94]. Dynamin-related protein 1 (Drp1) is required for mitochondrial fission, and it controls the integrity of mitochondrial structure[95]. By interacting with Parkin, Drp1 is ubiquitylated and degraded by proteasomes, leading to mitofusion that blocks mitochondria from removal by autophagy[96]. Parkin and Drp1 may work synergistically, and interestingly when Drp1 is absent, Parkin becomes much more critical[97], suggesting that Parkin may help to initiate a compensatory pathway. Further, selective inhibition of Drp1 by mdivi-1 prevents mitochondrial division and mitophagy in brain ischemia-reperfusion, as well as exacerbating brain infarct volume[15].

Clinical Advances by Targeting Mitophagy

Molecular targets of autophagy have been identified for the discovery of inhibitors or enhancers (see review [117]), and promising patents are emerging[117]. For example, by targeting a functional region of Beclin1, Tat-Beclin1 peptide

interacts with GAPR-1, a negative regulator of autophagy, to induce protective autophagy[118]. Because of the crucial role of mitophagy in promoting cell survival, mitophagy has been considered as a clinical target for conquering diverse diseases[64, 119]. However, the specific selectivity of mitophagy, i.e., that specifically targets damaged mitochondria with autophagosomes, makes it much harder to take advantage of mitophagy for clinical applications. However, several mitophagy-related proteins, such as Parkin and Beclin1, have been proposed to be beneficial targets for ischemic brain injury treatment[54, 88]. Besides, the pathologic processes of ischemic brain injury have become increasingly clearer, and drugs that target mitophagy could be possible in the near future.

Conclusions and Future Directions

Looking deep into mitophagy helps to better understand the pathology of ischemic brain injury. The molecular regulation of mitophagy has achieved great progress, but many questions remain to be solved, for example, the role(s) of mitophagy in different models of brain ischemia, how mitophagy is initiated after ischemia-reperfusion, the detailed underlying mechanisms, and how to take advantage of mitophagy for clinical therapy. These questions still need further investigations in the future.

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