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Review

Review

Targeting neuronal mitophagy in ischemic stroke: an update

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Abstract

Cerebral ischemia is a neurological disorder associated with complex pathological mechanisms, including autophagic degradation of neuronal mitochondria, or termed mitophagy, following ischemic events. Despite being well-documented, the cellular and molecular mechanisms underlying the regulation of neuronal mitophagy remain unknown. So far, the evidence suggests neuronal autophagy and mitophagy are separately regulated in ischemic neurons, the latter being more likely activated by reperfusional injury. Specifically, given the polarized morphology of neurons, mitophagy is regulated by different neuronal compartments, with axonal mitochondria being degraded by autophagy in the cell body following ischemia–reperfusion insult. A variety of molecules have been associated with neuronal adaptation to ischemia, including PTEN-induced kinase 1, Parkin, BCL2 and adenovirus E1B 19-kDa-interacting protein 3 (Bnip3), Bnip3-like (Bnip3l) and FUN14 domain-containing 1. Moreover, it is still controversial whether mitophagy protects against or instead aggravates ischemic brain injury. Here, we review recent studies on this topic and provide an updated overview of the role and regulation of mitophagy during ischemic events.

Key words: Mitophagy, Cerebral ischemia, Neuroprotection, PTEN-induced kinase 1, Parkin, BCL2 and adenovirus E1B 19-kDa-interacting protein 3, Bnip3-like, FUN14 domain-containing 1

Highlights

- Neuronal mitophagy can be activated by ischemia–reperfusional insults.
- A neuroprotective role for mitophagy is supported by recent studies.
- Diverse pathways are involved in neuronal mitophagy following ischemic injury; however, the associated pathways and regulation remain unclear.

Background

Cerebral ischemia is a severe neurological disorder caused by the sudden disruption of blood supply to the brain. Unfortunately, despite extensive efforts, so far only a few therapies are clinically available. This difficulty can be largely attributed to the complex nature of cerebral ischemia. Blood supply interruption promptly leads to the loss of ATP, a compound essential for the maintenance of the neuronal membrane potential.

The depolarized neurons release significant amounts of glutamate and cause excitotoxicity [1,2]. Neurons and other cell types undergo programmed cell death, including apoptosis, necroptosis and, potentially, autophagic cell death (despite the latter remaining controversial) [3]. Neuronal death can be documented along with stroke progression, from minutes to days. Due to a disrupted blood–brain barrier, the brain-resident microglia and infiltrated peripheral cells amplify neuroinflammation, which is closely associated with secondary neurological dysfunction [4]. Moreover, the broader region of ischemic injury, also known as the penumbra, shows remarkable neuronal and vascular remodeling in the late phases following stroke [5]. Restoration of the blood supply remains the most widely used therapeutic strategy for stroke events. However, either thrombolysis or mechanical recanalization treatment lead to reperfusion injury characterized by extensive oxidative stress [6]. Overall, the physiopathological mechanisms underlying stroke remain far from being fully understood, thus compromising the development of effective therapies.

In spite of the aforementioned challenges, it is commonly accepted that mitochondrial dysfunction plays a central role in ischemic brain injury. For example, it is plausible that mitochondria fail to provide adequate levels of energy, resulting in neuronal death. Moreover, mitochondria are also involved in a variety of biological processes beyond supplying energy [7]. In neurons under ischemic stress, damaged mitochondria act as a source of reactive oxygen species (ROS) and trigger apoptosis and necroptosis. Alternatively, it is also possible that mitochondria serve as damage-associated molecular patterns (DAMPs) to induce the activation of the inflammasome and pyroptosis [8,9]. Neuronal cells have a variety of mechanisms to monitor mitochondria quality, primarily by eliminating damaged mitochondria via the autophagosome–lysosome pathway, a process commonly referred to as mitophagy.

Mitophagy has been widely documented in ischemic neuronal cells [10–12]. Despite the specific contributions of mitophagy during brain ischemia remaining controversial, emerging data support the benefits of proper mitophagy for maintaining neuronal homeostasis [13–22]. Multiple signaling pathways are involved in mitophagy activation, including the extensively investigated PTEN-induced kinase 1 (PINK1)–Parkin pathway and various mitophagy receptors involved in mitophagy regulation [12,23–25]. This knowledge provides the rationale for developing strategies to rescue ischemic stroke, with several pharmaceutical compounds proposed to rescue ischemic brain injury by modulating mitophagic activity [19,26–30].

Since the roles and regulation of autophagy in cerebral ischemia have been elegantly reviewed elsewhere [31–34], here we instead focus on the current knowledge regarding the cellular and molecular regulation of mitophagy in ischemic brains. In particular, we discuss several recent controversial findings in this field.

Review

Mitophagy activation in the ischemic brain

Neurons are the predominant cell type observed with extensive mitophagy activation after ischemic stress. Compared to chemical-induced mitophagy, the most widely used mitophagy induction paradigm which takes hours or days to activate, ischemia can induce mitophagy within minutes [10,35,36]. This may be due to neurons attempting to eliminate a relatively larger number of damaged mitochondria in a short time. Neurons have evolved different strategies to perform mitophagy efficiently. In the case of intact neurons, autophagosomes are generated in the axonal tips, transported along with axons and fused with acidic vesicles for maturation [37]. Axonal mitochondria, which are more prone to be impaired, can be recognized and engulfed by these autophagosomes undergoing maturation in the axons. Although this strategy may accomplish mitophagy under physiological conditions, massive mitophagy has been documented directly in neuronal cell bodies [36]. Neurons are able to generate autophagosomes in cell soma under stress, but it remains unclear whether the spatial-specific biogenesis of autophagosomes share similar mechanisms. The majority of functional mitochondria are distributed in the axon and show retrograde movement and mitophagy in the neuronal soma after ischemic insult [35]. This somatic mitophagy strategy can effectively take advantage of the perinuclear enrichment of lysosomes [38]. Overall, neuronal mitophagy occurs and is regulated distinctively in different neuronal compartments.

Spontaneous blood restoration may occur in part of the ischemic territory even without thrombolysis or recanalization treatment [39]. Ischemia and reperfusion have distinct pathological mechanisms. Specifically, reperfusional stress increases mitophagy compared to ischemia in neurons, but both reperfusion and ischemia similarly induce autophagy, suggesting discrepant regulation of autophagy and mitophagy in ischemic neurons [10,40]. Although it remains unclear how mitophagy is specifically activated by reperfusion, it is likely to involve oxidative stress. The sudden recovery of glucose and oxygen levels after ischemia causes the re-oxidation of accumulated succinate by the succinate dehydrogenase enzyme (complex II of mitochondria respiratory chain), which results in excessive mitochondrial ROS production [6]. The redox regulation of mitophagy in ischemic neurons was supported by recent studies. For example: the deletion of peroxiredoxin 6, an antioxidant protein, exacerbates neuronal mitophagy after ischemic insult [41]; the silencing of ShcA, a protein that regulates ROS, reduces mitophagy activation in a photothrombosis mice model [42]; and the administration of FeTMPyP, a peroxynitrite catalyst, attenuates mitophagy induction in the ischemic brain [43]. Despite these observations, it is still unclear how ischemic neurons sense the redox status to activate mitophagy. It has been hypothesized that superoxide drives Parkin-mediated mitophagy by depolarizing the mitochondrial inner

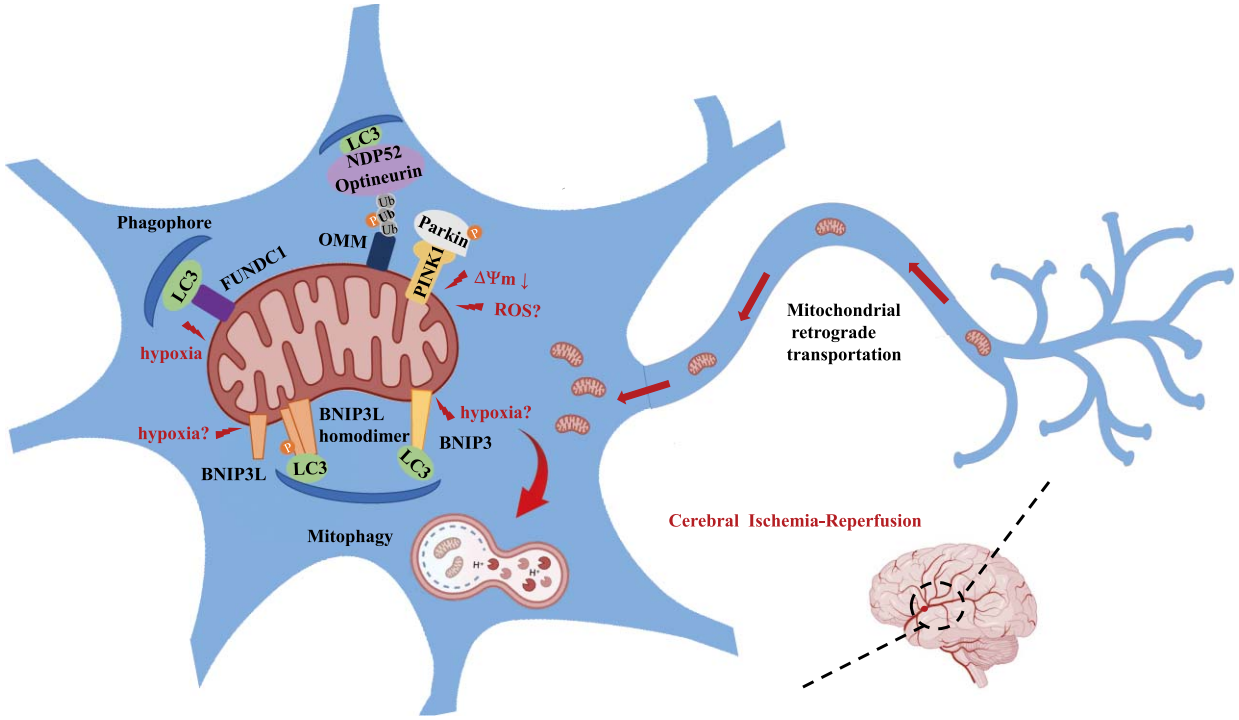

Figure 1. An overview of mitophagy in ischemic neurons. Neuronal mitochondria can be eliminated via the autophagosome–lysosome pathway after ischemic injury. Although autophagy is activated solely by ischemia, mitophagy is selectively activated by reperfusion following ischemia. Axonal mitochondria undergo retrograde transportation and are recognized by the autophagosome in the neuronal soma. There are multiple molecular pathways involved in mitophagy execution in ischemic neurons. PINK1–Parkin senses the loss of mitochondrial transmembrane potential (ΔΨm) and ubiquitinates the outer mitochondrial membrane protein, which is further phosphorylated by PINK1 to amplify the mitophagy signaling. The reactive oxygen species formed by reperfusional insult may also participate in mitophagy activation via the PINK1–Parkin pathway. Bnip3 and Bnip3l serve as mitophagy receptors to recruit autophagosomes by binding with LC3s through the LC3-interacting region (LIR) motif. The expression of these receptors can be upregulated by hypoxia. The mitophagic activity of Bnip3l can be activated by forming a homodimer and/or phosphorylation. FUNDC1 is a hypoxia-response mitophagy receptor that is involved in ischemia-induced mitophagy in neurons. However, it remains unknown how these mitophagy pathways sense ischemic stress and how these signals are integrated. Created with MedPeer (www.medpeer.cn). *LC3* microtubule associated protein 1 light chain 3, *Ub* ubiquitin, *PINK1* PTEN-induced kinase 1, *NDP52* nuclear dot protein 52 kDa, *FUNDC1* FUN14 domain-containing 1, *BNIP3* BCL2 and adenovirus E1B 19-kDa-interacting protein 3, *BNIP3L* BCL2 and adenovirus E1B 19-kDa-interacting protein 3-like, *ROS* reactive oxygen species

membrane [44,45]. In response to the oxidative stress, Nrf2 transcriptionally activates the expression of antioxidant enzymes [46] and upregulates *PINK1* [47]. A recent study proposed that p62 sequesters Keap1 from Nrf2 and promotes mitochondria ubiquitination, which in turn triggers mitophagy [48]. The neuronal TP53-inducible regulator of glycolysis and apoptosis (TIGAR) inhibits glycolysis by switching glucose metabolism to the pentose phosphorylate pathway, generating reductive NADPH to neutralize oxidation caused by ischemia [49]. Moreover, we recently reported that TIGAR switches from generating NADPH to promoting autophagy in ischemic brains via a Nrf2-related mechanism [50]. This finding implies that a prompt mitophagy activation mechanism exists following redox sensing in ischemic neurons.

In line with the idea that reperfusion induces mitophagy specifically, it has been demonstrated that long periods of ischemia alone can lead to excessive degradation of BCL2 and adenovirus E1B 19-kDa-interacting protein 3-like (Bnip3l), a mitophagy receptor, and thus cause mitophagy defect [40]. In addition, extended duration of ischemia halts retrograde traffic of axonal mitochondria, which compromises mitophagy efficiency. These studies may partly explain why ischemia alone induces observable autophagy but not mitophagy in neurons [35]. Moreover, mitophagy activation in ischemic neurons may not be in accordance with autophagy induction, as genes associated with the former but not the latter are upregulated in the ischemic hippocampus [51]. In addition, post-stroke hyperglycemia activates neuronal autophagy but inhibits mitophagy in rats [52].

Taken together, these results suggest that neuronal mitophagy can be activated promptly by ischemia, particularly ischemia–reperfusional insult, and that the redox status determines mitophagy induction. Mitochondria in different neuronal compartments can be eliminated in different ways by the autophagy machinery (Figure 1).

Molecular regulation of mitophagy in ischemic stroke

While an increasing number of mitophagy genes have recently been unmasked in mammalian cells, only a few showed associations with cerebral ischemia. This raises important questions, such as how many genes are involved in mitophagy in ischemic brains, how are these mitophagy genes regulated by sensing pathological stress and how are redundant mitophagy pathways integrated to control neuronal mitophagy.

PINK1 and Parkin

PINK1 and Parkin are the most extensively studied genes in mitophagy regulation, and both are involved in various physiological and pathological processes. PINK1 senses the drop in mitochondrial membrane potential ($\Delta\Psi m$) and accumulates on the mitochondrial surface, where it recruits Parkin and phosphorylates target proteins. Parkin, an E3 ubiquitin ligase, ubiquitinates proteins of the outer mitochondrial membrane, which is further phosphorylated by PINK1 and amplifies mitophagy signaling [53–56]. Both PINK1 and Parkin are upregulated in ischemic mice brains [57]. A recent study indicated that Parkin upregulation in plasma from neonatal infants experienced cerebral hypoxia–ischemia, suggesting its promising role as a biomarker [58]. Parkin is immediately recruited to mitochondria after oxygen and glucose deprivation–reperfusion (OGD/R) in primary cultured neurons [10] and is able to reduce mitochondrial turnover following middle cerebral artery occlusion (MCAO) [23]. Conversely, PINK1 overexpression significantly improves mitochondrial integrity in OGD/R-treated neurons, even though mitophagy activity was not determined [59]. Overall, PINK1–Parkin signaling is triggered to activate mitophagy in ischemic neurons.

PINK1–Parkin signaling is activated by the drop in $\Delta\Psi m$, a process that is well-documented in various ischemic models both *in vivo* and *in vitro*. In fact, the knockdown of the ATPase inhibitory factor IF_1 reversed $\Delta\Psi m$ loss and prevented Parkin-mediated mitophagy in ischemic neurons [60]. However, the sensing of $\Delta\Psi m$ by PINK1–Parkin signaling was challenged by recent studies. The deletion of the tissue type plasminogen activator (tPA) gene in mice further reduced neuronal $\Delta\Psi m$ but failed to promote Parkin translocation to mitochondria [25]. Previous studies described the neuroprotective effects of acidosis or hypoxia post-conditioning that prevent $\Delta\Psi m$ loss in ischemic neurons but reinforce Parkin-mediated mitophagy [14,23,61]. These observations suggest the involvement of other factors besides $\Delta\Psi m$ loss in provoking PINK1–Parkin signaling. As discussed above, Parkin may sense the redox status to activate mitophagy in ischemic neurons [41,43]. The S-nitrosylation (a form of oxidative modification) of the Cys323 residue in Parkin induces mitophagy activity in SH-SY5Y cells [62], and S-nitrosylated PINK1 attenuates mitophagic activity in iPSC cells [63]. Nevertheless, it is still unclear whether and how PINK1–Parkin signaling senses the redox balance and regulates mitophagic activity during cerebral ischemia.

Parkin recruitment to mitochondria is essential for mitophagy induction. Regardless of the complicated molecular network involved, mitofusin 2 (Mfn2) seems to translocate Parkin to mitochondria in ischemic neurons and act as a mitochondria outer membrane target protein responsible for Parkin recruitment [64]. In OGD/R-treated neuronal cells, Mfn2 is degraded by the proteasomes [65], which may be linked with mitophagy insufficiency. Knockdown of Mfn2 leads to reduced mitochondrial distribution of Parkin and delayed mitophagosome generation in primary cultured neurons subjected to OGD/R [66]. Mfn2 is sufficient to prevent mitochondria fragmentation, a prerequisite for mitophagy due to the length limitation of the autophagosomes. Hence, it cannot be excluded that Mfn2 regulates mitophagy in a Parkin-independent manner.

Current studies emphasize the predominant role of PINK1–Parkin signaling in controlling mitophagy. Although the molecular regulation of this pathway has received extensive attention elsewhere, it is not yet fully understood how neuronal PINK1 and Parkin sense ischemic stress and thus trigger mitophagy in ischemic neurons.

Bnip3 and Bnip3l

Bnip3 and Bnip3l are highly conserved homologues that were initially identified as BH3-only pro-apoptotic proteins in tumor cells [67,68]. A decade later both were recognized as mitophagy receptors that directly bind with Atg8 family proteins via their microtubule-associated protein 1 light-chain 3 interacting region motif [69]. *Bnip3* knockdown significantly reduces ischemia-induced mitophagy in mouse brain [12], and its transcription or translation is considered a biomarker for mitophagy induction in ischemic brain and neurons [51,70,71]. However, we note that like many other pro-apoptotic proteins, Bnip3 upregulation is designed to induce programmed neuronal cell death.

Bnip3l shows a weaker capacity to induce apoptosis than Bnip3 [72,73] and is abundantly expressed in a variety of tissues, including the brain. Our previous study identified Bnip3l-mediated mitophagy by employing a MCAO model in Bnip3l knockout (KO) mice. We demonstrated that Bnip3l induces mitophagy in ischemic brains in the absence of Parkin [24], in a mechanism distinct from that of Parkin activation, which is initiated by translocation to mitochondria. In the case of Bnip3l, the protein is located on the outer membrane of mitochondria in intact neurons, and its mitophagic activation depends on a Ser81-mediated phosphorylation under ischemia in stroke models [24]. We thus postulate that ischemia–reperfusion insult activates Bnip3l phosphorylation and serves as a recognition signal for mitochondria degradation. However, while the kinases and phosphatases involved in this process remain unknown, they likely serve as potential targets for the regulation of Bnip3l-induced mitophagy.

The transmembrane domain of Bnip3l determines the distribution of this protein in mitochondria. In addition, the transmembrane domain is required for the formation of the Bnip3l homodimer [72], whose biological function remains elusive. Emerging data demonstrates that this dimer is essential for mitophagy activity of Bnip3l [40,74], as mutant Bnip3l failing to form the dimer cannot induce mitophagy in ischemic neurons, regardless of its mitochondrial distribution. Moreover, the Bnip3l dimer is more prone to degration by proteasomes, leading to mitophagy defects in brains that experienced permanent ischemia [40]. These observations indicate that Bnip3l dimer formation may serve as a regulatory mechanism for mitophagic activity, but how phosphorylation and dimerization of Bnip31 regulate mitophagy in ischemic brains remains unclear. Both Bnip3 and Bnip3l

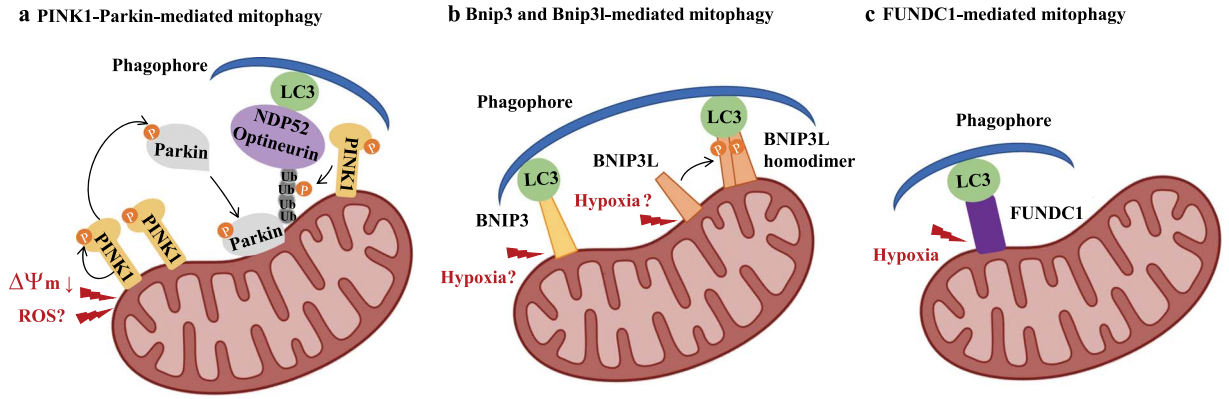

Figure 2. An overview of the molecular mechanisms of mitophagy. (**a**) After losing mitochondria membrane potential (ΔΨm) or under oxidative stress, PINK1 stabilizes outer mitochondrial membrane (OMM) proteins and phosphorylates itself. PINK1 next recruits Parkin and phosphorylates target proteins. Parkin further ubiquitinates OMM proteins, leading to the recruitment of receptor proteins for the autophagosome and subsequent degradation of the mitochondrion. (**b**) Under ischemic injury, BNIP3 directly binds with Atg8 family proteins via their LIR motif to induce mitophagy. Ischemic injury activates the phosphorylation of Bnip3l and forms a Bnip3l homodimer to degrade mitochondria. The BNIP3L LIR motif interacts with LC3s to induce mitophagy. (**c**) FUNDC1 binds LC3 proteins and then targets the mitochondria autophagy machinery by sensing the hypoxic environment. *LC3* microtubule associated protein 1 light chain 3, *Ub* ubiquitin, *PINK1* PTEN-induced kinase 1, *NDP52* nuclear dot protein 52 kDa, *FUNDC1* FUN14 domain-containing 1, *BNIP3* BCL2 and adenovirus E1B 19-kDa-interacting protein 3, *BNIP3L* BCL2 and adenovirus E1B 19-kDa-interacting protein 3-like, *ROS* reactive oxygen species

respond to hypoxia and are upregulated by HIF-1α in tumor cells [75]. Paradoxically, their transcription is not significantly upregulated in ischemic brains, implying distinct regulatory mechanisms from cancer cells.

These results illustrate how Bnip3 and Bnip3l serve as receptors to induce mitophagy in ischemic brains and promote apoptosis in some immortal cells. Neuronal Bnip3l undergoes phosphorylation and dimerization to regulate mitophagic activity under ischemia, but the underlying mechanisms need further study.

FUN14 domain-containing 1

FUN14 domain-containing 1 (FUNDC1) is a mitophagy receptor that senses the hypoxia environment [76], but whose potential involvement in cerebral ischemia remains uncertain. A recent study found a deletion in the tPA gene that further aggravates mitochondrial dysfunction and, conversely, that tPA treatment activates mitophagy in ischemic neurons. This tPA deletion reduces the expression of FUNDC1 either in ischemic brains or neurons, and FUNDC1 silencing stops mitophagic activation [25]. It should also be noted that FUNDC1 activates mitophagy in a Parkin-independent manner and may instead be regulated by AMPK signaling, mTOR and ULK1. At present, a paucity of data prevents clarification of the significance of FUNDC1-mediated mitophagy in stroke brain, but the recently uncovered regulatory mechanisms of FUNDC1 in other models [77–79] may be extended to ischemic brain injury in the future.

In summary, multiple molecules participate in mitophagy induction in the context of cerebral ischemia (Figure 2). Besides the involvement of PINK1–Parkin, Bnip3, Bnip3l and FUNDC1, several more mitophagy receptors have been discovered in a variety of biological models. The diversity of mitophagy pathways may enable neurons to sense distinct environmental stresses and provide the redundancy to ensure mitochondria elimination. Although some studies showed potential connections between these mitophagy pathways [80], it is still unclear whether and how they regulate mitochondrial quality in ischemic neurons.

Lysosomal dysfunction in cerebral ischemia

Despite the extensive attention to autophagy and mitophagy, the significance of lysosomes in cerebral ischemia has been underestimated. Recent studies indicate lysosomal dysfunction in ischemic models *in vitro* or *in vivo*, in particular in association with reperfusion [81]. The insufficiency of lysosomal activity in cerebral ischemia was attributed to either aberrant nucleus-derived signaling, including TFEB and mTOR or, alternatively, to the dysfunction of lysosomal proteins, such as cathepsin D and TMEM175 [82–84]. The role of lysosomal dysfunction in impairing mitophagy efficiency in stroke brain has not been addressed.

Contributions of mitophagy to ischemic brain

Autophagy seems to play a ‘double-edged sword’ role during ischemia, as it protects the ischemic brain but, in the case of ‘excessive autophagy’, can accelerate ischemic brain injury [12,71]. The latter hypothesis lacks a clear definition in ischemic stroke and the roles of mitophagy in cerebral ischemia may vary across experimental models (Table 1). In fact, previous studies propose that different compounds or gene manipulations confer neuroprotection with reduced mitophagy in ischemic brains [65,66,85]. Bnip3 knockout attenuates ischemic brain injury and mitophagy induction [12], but the gene plays a more complex role in regulating apoptosis and lysosomal function [86,87]. Similarly, blocking the mitochondrial calcium uniporter (MCU) confers protection and reduces mitophagy in OGD/R-treated SH-SY5Y cells [88], but MCU also participates in

Table 1. The role of mitophagy in cerebral ischemia

Role of mitophagy	*In vitro* models	*In vivo* models	Interventions	Ref.
Aggravated ischemic neuronal injury	OGD/R in primary cultured neurons	tMCAO/Hypoxia in mice pups	BNIP3 knockout	[12]
	OGD/R in SH-SY5Y cells	tMCAO in rats	Naringin	[16]
		BCCAO in rat	URB597	[17]
	OGD in primary cultured neurons	pMCAO in rats	miR-330 antagomir and antagomir	[18]
		PT in rats	ShcA silence	[42]
	OGD/R in SK-N-BE [2] cells		USP30 overexpression	[65]
	OGD/R in primary cultured neurons		Mfn2 knockdown	[66]
		RA in rats	GRP78 overexpression	[85]
	OGD/R in SH-SY5Y cells		MCU inhibitor	[88]
Ameliorated ischemic neuronal injury		PT in rats	/	[13]
		tGCI in rats	Hypoxic postconditioning	[14]
		tMCAO in rats	ATF4 knockdown	[15]
	OGD in PC12 cells	pMCAO in rats	Methylene Blue	[19]
	OGD/R in PC12 cells	tMCAO in rats	Baicalin	[20]
	OGD/R in N2a cells	tMCAO in rats	HSPB8 overexpression/silence	[21]
	OGD/R in N2a cells	tMCAO in rats	NR4A1 knockout	[22]
	OGD/R in HT22 cells	tMCAO in mice	tPA	[25]
		tMCAO in rats	Rapamycin	[26]
	Primary cultured neurons exposed to excitotoxicity	tMCAO in rats	Resveratrol	[27]
		BCCAO in rats	URB597	[28]
		tMCAO in mice	Garciesculenxanthone B	[29]
	OGD/R in primary cultured neurons, SH-SY5Y, N2a and PC12 cells		Brazilin	[30]
		tMCAO in rats	PRDX6 knockdown	[41]
		pMCAO in rats	/	[52]
	OGD/R in primary cultured neurons and SH-SY5Y cells	tMCAO in rats	IF_1 overexpression	[60]
		tMCAO in mice	PGAM5 knockout	[92]
		Langendorff heart ischemia reperfusion model in mice		
		tMCAO in mice	TAT-SPK2 peptide	[93]
	OGD/R in HT22 cells		Apelin-36	[94]
	OGD/R in BV2 cells	tMCAO in mice	PGC-1α overexpression	[95]
	OGD/R in SH-SY5Y cells	tMCAO in rats	Cx32 silence	[96]
		tMCAO in rats	EA pretreatment	[97]
	Primary cultured neurons and HT22 cells exposed to L-Glu		L-Glu	[98]
	OGD/R in SH-SY5Y cells	tMCAO in mice	CERKL overexpression	[99]
	OGD/R in SH-SY5Y cells	tMCAO in mice	TUG1 knockdown	[100]
		tMCAO in mice	EE	[101]
	OGD/R in primary cultured neurons	CA in rats	Therapeutic hypothermia	[102]

tMCAO Transient middle cerebral artery occlusion, *OGD/R* oxygen and glucose deprivation–reperfusion, *BNIP3* BCL2 and adenovirus E1B 19-kDa-interacting protein 3, *MCU m*itochondrial calcium uniporter, *BCCAO* bilateral common carotid artery occlusion, *pMCAO p*ermanent middle cerebral artery occlusion, *PT* photothrombosis, *RA* spinal root avulsion, *USP30* ubiquitin specific peptidase 30, *GRP78* glucose-regulated protein 78, *Mfn2* mitofusin 2, *IF1* mitochondrial ATPase inhibitory factor 1, *PGM5* phosphoglycerate mutase 5, *HSPB8* heat shock protein family B member 8, *NR4A1 n*uclear receptor subfamily 4 group A member 1, *CA* cardiac arrest, *SPK2* sphingosine kinase 2, *tPA* tissue type plasminogen activator, *PGC-1α* Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, *Cx32* connexins 32, *EA* electroacupuncture pretreatment, *PRDX6* peroxiredoxin 6, *CERKL* ceramide kinase like, *TUG1* taurine upregulated 1, *EE e*nriched environment

mitochondrial energy metabolism [89], ROS production [90] and apoptosis induction [91]. Accordingly, current evidence does not completely support a causal role for mitophagy in promoting ischemic neuronal injury. Conversely, one should also be careful of interpreting the aforementioned observations as evidence supporting the pro-survival role of mitophagy in ischemic stroke [92–102] and instead determine whether the effects of mitophagy can be abolished with autophagy defects (e.g. *Atg7* or *Atg5* knockout). The lack of proper means to modulate mitophagy remains the main obstacle in the field, which requires specific mitophagy modulators to fully circumvent.

The role of mitophagy in ischemic stroke was determined by knocking out Parkin, with Parkin KO mice showing a higher infarct volume when subjected to MCAO [24], a widely applied model to mimic ischemic stroke. Conversely, the overexpression of PINK1, a kinase that acts upstream of Parkin to induce mitophagy, reduces OGD/R-induced ROS production and improves mitochondrial quality [103]. The ectopic expression of mutant Parkin lacks the UBL domain that is essential for mitophagy induction and fails to rescue ischemic neuronal cells [104]. Hence, current evidence supports that the PINK1–Parkin dimer protects against ischemic stroke by activating mitophagy. Recent data further showed how mitophagy can be prevented in stroke brains by deleting mitophagy receptors. Bnip3l eliminates mitochondria in reticulocytes [105,106], with previous studies demonstrating its role in mitochondria clearance in ischemic neurons. *Bnip3l* KO mice show larger brain infarct areas and worse neurological defects, which can be reversed by Bnip3l overexpression [24]. Another study showed that FUNDC1 silencing eliminates tPA protection by abolishing mitophagy induction [25]. However, it is unclear whether FUNDC1 can rescue the ischemic brain.

Damaged mitochondria cause neuronal apoptosis in ischemic brains, and mounting evidence indicates that the release of pro-apoptotic proteins from ischemic neurons can be attenuated by mitophagy activation and aggravated by mitophagy inhibition. Ischemia-insulted mitochondria are considered a source of DAMPs, including mtDNA, ROS and lipids, and thus further lead to neuroinflammation. Recent studies showed that mtDNA deletion improves inflammatory response in cancer cells [107], while Parkin and PINK1 knockout increase the amount of inflammatory cytokines released in the circulation after extensive physical exercise [108]. Additionally, the ectopic expression of PINK1 suppresses inflammasome activation in ischemic livers [109]. However, direct evidence demonstrating the anti-inflammatory role of mitophagy in ischemic brains is still lacking. Given the crucial role of excessive neuroinflammation in neurological dysfunction after stroke, it is plausible that reinforced mitophagy reduces mitochondria-derived DAMPs and thus serves as a promising protective strategy.

Overall, the ongoing debate surrounding the advantages and disadvantages of mitophagy in ischemia will last a while longer due to the paucity of specific mitophagy modulators and the complex nature of mitophagy induction. Accumulating evidence from stroke models that selectively knockout mitophagy-related genes support a neuroprotection role for mitophagy, whose deficiency may lead to programmed cell death or neuroinflammation.

Conclusions

There is little debate that cerebral ischemia causes extensive autophagy in affected neuronal cells, but does not necessarily induce mitophagy. Damaged mitochondria are more prone to elimination by reperfusional injury, a process that can either be initiated by thrombolysis or occur spontaneously as a response to oxidative stress. A variety of factors impact the efficiency of mitophagy in ischemic neurons, autophagic flux, mitophagy receptors, lysosome functions and mitochondrial distribution. Importantly, however, it is still unclear how neurons sense distinct stress conditions and trigger mitophagy, but the process likely involves different mitophagy receptors. Although the specific mechanisms underlying the negative regulation of mitophagy have been discovered, it is unknown how mitophagy ceases during ischemia. Regardless of these unknowns, an increasing body of evidence suggests the benefits of correct mitophagy for neuroprotection in the context of ischemic stroke. While various synthetic and natural compounds seemingly protect ischemic neurons by enhancing mitophagy, their promising role for rescuing cerebral ischemia requires further verification.

Abbreviations

ShcA: SH2 domain-containing protein A; FeTMPyP: Fe (III) tetrakis (1-methyl-4-pyridyl) porphyrin pentachlorideporphyrin pentachloride; Nrf2: Nuclear factor erythroid 2-related factor; Keap1: Kelch-like ECH-Associating protein 1; BH3: B cell lymphoma-2 (BCL-2) homology domain 3; HIF1α: Hypoxia-inducible factor 1-alpha; AMPK: Adenosine 5'-monophosphate (AMP)-activated protein kinase; mTOR: Mammalian target of rapamycin; ULK1: UNC-51 like autophagy activating kinase 1; TFEB: Transcription factor EB; TMEM175: Transmembrane protein 175; UBL: Ubiquitin-like; mtDNA: Mitochondria DNA; PTEN: Phosphatase and tensin homolog; Atg8: Autophagy-related protein 8.

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Authors' contributions

X Zhang, JL and JW conceptualized the study. JL wrote the original draft of the manuscript. X Zhou and YG created the figures. All authors collected and reviewed the literature, and wrote the submitted version of the manuscript.

Conflict of interests

None declared.

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