# Insight into Crosstalk Between Mitophagy and Apoptosis/Necroptosis: Mechanisms and Clinical Applications in Ischemic Stroke*

Yan-di YANG[1], Zi-xin LI[2], Xi-min HU[3], Hao WAN[1], Qi ZHANG[1], Rui XIAO[4#], Kun XIONG[1, 5, 6#]
[1]*Department of Anatomy and Neurobiology, School of Basic Medical Science, Central South University, Changsha 410013, China*
[2]*Clinical Medicine Eight-year Program, 03 Class, 18 Grade, Xiangya School of Medicine, Central South University, Changsha 410013, China*
[3]*Clinical Medicine Eight-Year Program, 02 Class, 17 Grade, Xiangya School of Medicine, Central South University, Changsha 410013, China*
[4]*Administrative Office, the Third Xiangya Hospital, Central South University, Changsha 410013, China*
[5]*Hunan Key Laboratory of Ophthalmology, Changsha 410008, China*
[6]*Key Laboratory of Emergency and Trauma, Ministry of Education, College of Emergency and Trauma, Hainan Medical University, Haikou 571199, China*



**[Abstract]** Ischemic stroke is a serious cerebrovascular disease with high morbidity and mortality. As a result of ischemia-reperfusion, a cascade of pathophysiological responses is triggered by the imbalance in metabolic supply and demand, resulting in cell loss. These cellular injuries follow various molecular mechanisms solely or in combination with this disorder. Mitochondria play a driving role in the pathophysiological processes of ischemic stroke. Once ischemic stroke occurs, damaged cells would respond to such stress through mitophagy. Mitophagy is known as a conservatively selective autophagy, contributing to the removal of excessive protein aggregates and damaged intracellular components, as well as aging mitochondria. Moderate mitophagy may exert neuroprotection against stroke. Several pathways associated with the mitochondrial network collectively contribute to recovering the homeostasis of the neurovascular unit. However, excessive mitophagy would also promote ischemia-reperfusion injury. Therefore, mitophagy is a double-edged sword, which suggests that maximizing the benefits of mitophagy is one of the direction of future efforts. This review emphasized the role of mitophagy in ischemic stroke, and highlighted the crosstalk between mitophagy and apoptosis/necroptosis.
**Key words:** mitophagy; ischemic stroke; apoptosis; necroptosis; clinical application; crosstalk

Due to its increasing incidence, stroke has become one of the leading causes of disability and death worldwide[1, 2]. To date, it has been reported that ischemic stroke is the main type of stroke, which accounts for 70%–80% of total stroke events, seriously lowering the quality of life[3]. Thus, a number of researchers have contributed to the field of ischemic stroke, targeting the pathophysiology and various molecular mechanisms for the treatment of ischemic stroke[4–6]. The initial event of ischemic stroke is the sudden decrease in blood flow and oxygen supply to the cerebrum[7]. Insufficient blood flow deprives brain neurons of glucose and oxygen, and destroys cell homeostasis, leading to excitotoxicity, oxidative stress and inflammation, and neuronal cell death extensively occurs[8]. In order to minimize the irreversible neuronal death, a lot of therapeutic strategies have been provided, which include intravenous thrombolysis, tissue-type plasminogen activator (tPA), and endovascular therapy[9, 10]. Furthermore, the expansion of the time window for reperfusion treatment or emergency reperfusion appears to be an effective intervention for acute ischemic stroke, to date[11]. However, limited by the short treatment window (intravenous thrombolysis within 4.5 h), these strategies also appear to be less effective, and a large number of neurons undergo function loss or even death. For example, it has been reported that the reperfusion process after thrombolysis can cause brain tissue infarction[12]. Thus, clearer

Yan-di YANG, E-mail: yangyandi2018@163.com
#Correspondence authors, Kun XIONG, E-mail: xiongkun2001@163.com; Rui XIAO, E-mail: 272883007@qq.com
*This work was supported by grants from the National Natural Science Foundation of China (No. 81971891, No. 82172196, No. 81772134 and No. 81571939), Key Laboratory of Emergency and Trauma (Hainan Medical University) of Ministry of Education (No. KLET-202108), and the College Students' Innovation and Entrepreneurship Project (No. S20210026020013).

pathophysiology of ischemic stroke may provide a means to establish therapeutic guidelines.

As the "energy factory" of cells, mitochondria play an important role in almost all cells for their energy homeostasis[13]. Some studies have revealed that ischemia-reperfusion (I/R) injury can cause mitochondrial dysfunction, which induces the progressive deterioration of neurons[14]. That is, mitochondrial dysfunction works as a decisive step in I/R injury, and this can be described, as follows: (1) breakdown of mitochondrial dynamics (including ATP/ADP decrease, the production of active oxygen increases, and the clearance decreases); (2) the mitochondrial membrane permeability transition pore (mPTP) continues to open [including cytochrome c (cyt c) and mitochondrial DNA, these are released into the cytoplasm][15, 16]. All these can lead to neuronal death. Therefore, increasing attention has been given to mitochondria, and it has been suggested that the effective treatment prospect of ischemic stroke is to target mitochondria.

For mitochondrial dysfunction, a self-regulatory mechanism would be activated to remove the dysfunctional mitochondria, which is termed as "mitophagy" (also known as mitochondrial autophagy)[17, 18]. After mitochondrial damage, this will be triggered by the initiator, such as parkin[19]. Therefore, when neurons are exposed to external stress, mitophagy is initiated to remove damaged mitochondria, and maintain cellular homeostasis. Notably, mitophagy is not a single signaling pathway, but a complex network between mitophagy and apoptosis/necroptosis, and this has received more attention than one crucial pathway in the field of ischemic stroke[20]. The present review focuses on the crosstalk between mitophagy and apoptosis/necroptosis after ischemic stroke, with emphasis on the improvement of mitochondrial-based ischemic stroke treatment.

## 1 THE MECHANISM OF MITOPHAGY AND ITS FUNCTION IN SCHEMIC STROKE

Mitophagy is a type of selective autophagy that can remove dysfunctional mitochondria and maintain cell homeostasis[21, 22]. It is known that mitochondria are important organelles, which are mainly responsible for providing energy for cells[23]. Once the mitochondria are damaged, there would be some harmful factors released into the cytoplasm, such as reactive oxygen species (ROS) and other oxidants, resulting in an awful case in cells. Even worse, cyt c, a mitochondrial intermembrane space protein, would be released under severe mitochondrial damage[6, 15, 24]. Cyt c triggers the caspase cascade, leading to apoptosis[25]. Interestingly, mitophagy can contribute to the rapid degradation of damaged mitochondria, which in turn can prevent cell apoptosis[26–28]. Mitophagy starts with the formation of the phagophore, which is a membrane structure isolated from the endoplasmic reticulum (ER)[29]. The recognition of target mitochondria by the phagophore occurs through microtubule-associated protein 1 light chain 3 α (LC3) adapters, in an ubiquitin-dependent and independent pathway, and through the direct interaction of LC3 with its receptors[30]. Overall, the use of mitophagy can be divided into two major pathways: ubiquitin-mediated pathway and receptor-mediated pathway.

### 1.1 Molecular Pathways Involved in Mitophagy

**1.1.1 Ubiquitin-mediated Mitophagy** Ubiquitin-mediated mitophagy is considered as the canonical signaling pathway, which is regulated by two proteins, PTEN-induced kinase 1 (PINK1) and parkin[31]. In 2008, a study revealed that loss of mitochondrial membrane potential can trigger the recruitment of parkin to mitochondria. In addition, it was also found that parkin can promote the degradation of damaged mitochondria through autophagy[32]. Furthermore, PINK1, a serine/threonine kinase, has been reported to be able to regulate parkin[33]. Under normal physiological conditions, PINK1 is imported into the mitochondria through the translocase of the outer mitochondrial membrane complex of the outer mitochondrial membrane (OMM), and into the translocase complex of the inner mitochondrial membrane (IMM), where this is cleaved by the mitochondrial processing peptidase and rhomboid protease presenilin-associated rhomboid-like protein[34, 35]. The normal expression level of PINK1 is relatively low, but this will accumulate in the OMM during mitochondrial damage, increasing the mitochondrial ROS, and inducing the depolarization and accumulation of misfolded proteins[36]. The accumulated PINK1 is activated and autophosphorylated, and in turn, this phosphorylates ubiquitin on serine 65, thereby recruiting parkin from the cytoplasm to the mitochondrial membrane. Studies have shown that PINK1 Ser228 and Ser402 residues are autophosphorylated upon decrease in mitochondrial membrane potential, and that this autophosphorylation is essential for parkin recruitment onto damaged mitochondria[37].

Parkin is an E3 ubiquitin ligase. When parkin is recruited to the OMM and activated, this drives OMM proteins to undergo ubiquitination and degradation, thereby driving mitophagy[38]. Importantly, although activated PINK1 can recruit parkin to the OMM, studies have shown that even without PINK1, parkin can be recruited to depolarized mitochondria to drive the mitophagy[39]. Parkin transfers to mitochondria, and ubiquitinates the proteins on the OMM, such as mitofusin 1/2 and voltage-dependent anion channel 1 (VDAC1)[39] (fig. 1). However, for the *Dmn/Drp1* knockout model, Yamade *et al* reported that the

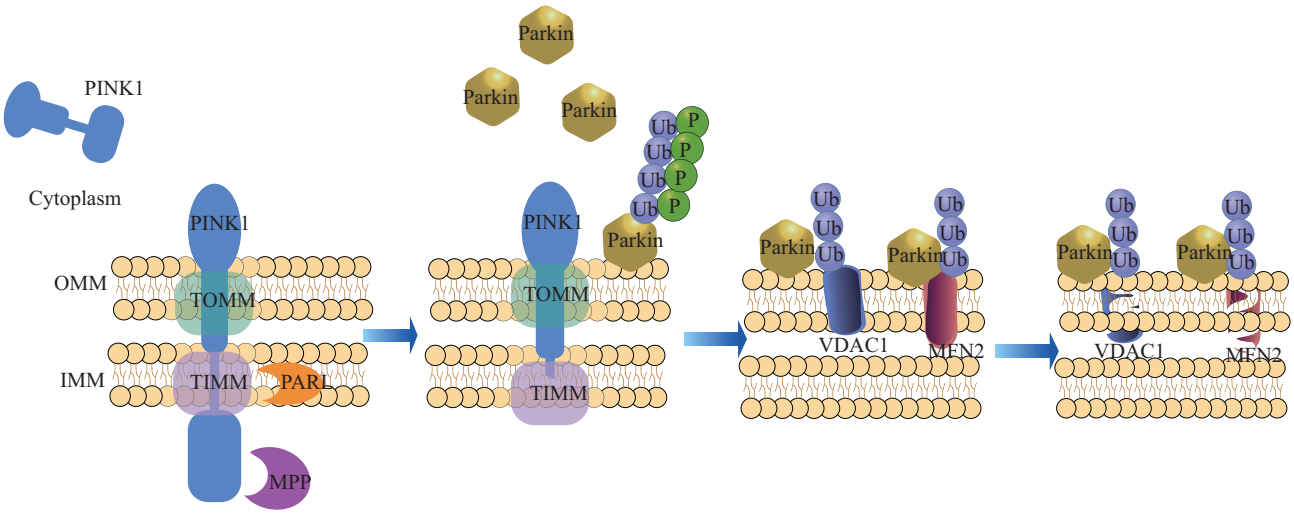


**Fig. 1** Ubiquitin-mediated mitophagy
PTEN-induced kinase 1 (PINK1) translocated to the mitochondria, and was cleaved by presenilin-associated rhomboid-like protein (PARL) and mitochondrial processing peptidase (MPP). This step activated PINK1. The activated PINK1 recruited parkin to the outer mitochondrial membrane (OMM), inducing it to be phosphorylated and ubiquitinated. The ubiquitinated proteins in the OMM include mitofusin 2 (MFN2) and voltage-dependent anion channel 1 (VDAC1). IMM: inner mitochondrial membrane

ubiquitination of mitochondrial proteins required SQSTM1/p62, but not the ubiquitin E3 ligase PRKN/parkin, during mitophagy[40].

PINK1-mediated phosphorylation leads to parkin activation and the ubiquitination of substrates on damaged mitochondria, which is the mitophagy signaling pathway[41,42]. Mitofusin, Miro and VDAC have been identified as parkin substrates[43, 44]. Other OMM proteins that undergo parkin-mediated ubiquitylation have been identified by mass spectrometry, suggesting that parkin can ubiquitylate a large number of proteins on the surface of mitochondria[45]. Hence, parkin appears to have low substrate selectivity. Such a unique specificity appears to be optimal for parkin to achieve the efficient and quick ubiquitylation of dysfunctional mitochondria[46]. Even under steady state, a small amount of ubiquitin is attached to proteins on the surface of mitochondria. When PINK1 phosphorylates the ubiquitin, the resultant phosphor ubiquitin recruits parkin from the cytosol, and activates it on depolarized mitochondria to generate more ubiquitin chains and ubiquitin substrates[47].

**1.1.2 Receptor-mediated Mitophagy** An alternative pathway of mitophagy is through mitophagy receptor signaling. These receptors generally contain a LC3 interaction region (LIR) domain, which is used to directly bind to LC3, and phagocytosed by autophagosomes[48].

At present, a series of receptors have been identified in mitophagy in mammals, such as BCL2 interacting protein 3 (BNIP3), BINIP-like (BNIP3L, also known as NIX) and FUN 14 domain-containing 1 (FUNDC1). BNIP3L is a homology of BNIP3, and has 55% identical amino acid sequence[49]. The C-terminal transmembrane domain of NIX and BNIP3 is inserted into the OMM, while the N-terminal is exposed to the cytoplasm[49]. A number of studies have revealed that NIX and BNIP3 play an important role in mitophagy. NIX and BNIP3 can increase the production of ROS and trigger mitophagy. Furthermore, NIX or BNIP3 competitively binds to Bcl2 to dissociate the Bcl2-Beclin1 complex, and activate the autophagy or mitophagy[50]. A study reported that the glucocorticoid downregulation of BNIP3L/NIX directly guides the glucocorticoid receptor to bind to the PGC1α promoter, and downregulate its expression and nuclear translocation, thereby selectively reducing the NIX-dependent mitophagy[51].

Another important mitophagy receptor, FUN 14 domain-containing 1, is an OMM protein. FUNDC1 contains a C-terminal domain inserted into the IMM and an N-terminal domain exposed to the cytoplasm, as well as a typical LIR sequence. The LIR sequence is located in the 50 amino acid residues exposed to the N-terminus of the cytoplasm[52, 53]. FUNDC1 is mainly recruited to mitochondria-mediated ischemia-induced mitophagy by interacting with LC3[54]. The mass spectrometry results revealed that FUNDC1 has two states: phosphorylation and dephosphorylation. The phosphorylation and dephosphorylation are respectively mediated by protein kinase and phosphatase, and the process is reversible[55]. FUNDC1 controls the interaction with LC3 by regulating its phosphorylation level. Under physiological conditions, Src and casein kinase 2 (CK2) are responsible for the phosphorylation of FUNDC1 at Tyr18 and Ser13, respectively. This phosphorylation event inhibits the interaction of FUNDC1 and LC3[56, 57]. Phosphoglycerate mutase

family member 5 (PGAM5) is a serine/threonine phosphatase located in the mitochondria. After hypoxia stimulation, PGAM5 dephosphorylates FUNDC1 at Ser 13 to promote FUNDC1 interaction with LC3 (fig. 2)[58].

**1.2 Molecular Mechanism and Function of Mitophagy in Ischemic Stroke**

**1.2.1 Diverse Roles of Mitochondria in Mitochondrial Dynamics** Mitophagy plays a vital role in the balance of mitochondrial dynamics and the balance of mitochondrial fission, and fusion is closely correlated to maintaining mitochondrial homeostasis, cell stability and cell survival[59]. The mitochondrial fission process includes mitochondrial contraction and division, while the mitochondrial fusion process includes mitochondrial joining and tethering. Fission can remove damaged mitochondria, such as damaged proteins, unstable membranes, and mutated or damaged mitochondrial DNA (mtNDA)[21, 60, 61]. Conversely, fusion helps maintain the integrity of matrix metabolites and mtDNA, and balance membrane components[62]. The balance of mitochondrial division and fusion is critical for the survival of neurons. Studies have revealed that Drp1-mediated mitophagy is triggered after hypoxic/ischemic stress, and that the inhibition of mitophagy can rescue the loss of neurons[63].

Mitochondrial fission is mainly regulated by Drp1, Fis1, Mff and Mid49/51[64]. Drp1 is mainly located in the cytoplasmic matrix, and cannot directly bind to the mitochondrial membrane. Receptors, such as Fis1, Mff and Mid49/51, are needed to help Drp1 translocate to the OMM, and play its role. As a key molecule in mitochondrial fission, Drp1 has been given attention by most researchers[65]. Studies have revealed that the use of Drp1 inhibitor mdivi-1 can effectively improve nerve function damage, reduce mitochondrial damage, and protect neurons[66]. However, the effectiveness and specificity of mdivi-1 remain to be questioned by researchers[67]. Therefore, the identification of molecules that target Drp1 remains promising for the discovery of effective intervention targets. Flippo *et al* reported that in *AKAP1*$^{-/-}$ mice, the mitochondrial fission increased, but the total expression level of Drp1 did not change[68]. On the other hand, the phosphorylation level of Drp1 pS637 decreased, while the phosphorylation level of Drp1 pS616 increased. Furthermore, the phosphorylation of S616 increases the activity of Drp1, while the phosphorylation of S637 reduces the activity of Drp1, indicating that AKAP1 deletion controls the survival of neurons through the inhibition of the levels of Drp1 and pS637[68].

The fusion of mitochondria is mainly mediated by mitofusin 1 (MFN1), mitofusin 2 (MFN2), and optic atrophy 1 (OPA1). MFN1 and MNF2 are located on the OMM, and mediates the fusion of the OMM through homotypic or heterotypic interactions, or GTPase hydrolysis, while the fusion of the IMM is mediated by OPA1. After ischemic stroke, OPA1 would be cleaved into two isoforms: long optic atrophy 1 (L-OPA1) would decrease, and short optic atrophy 1 would increase. Lai *et*

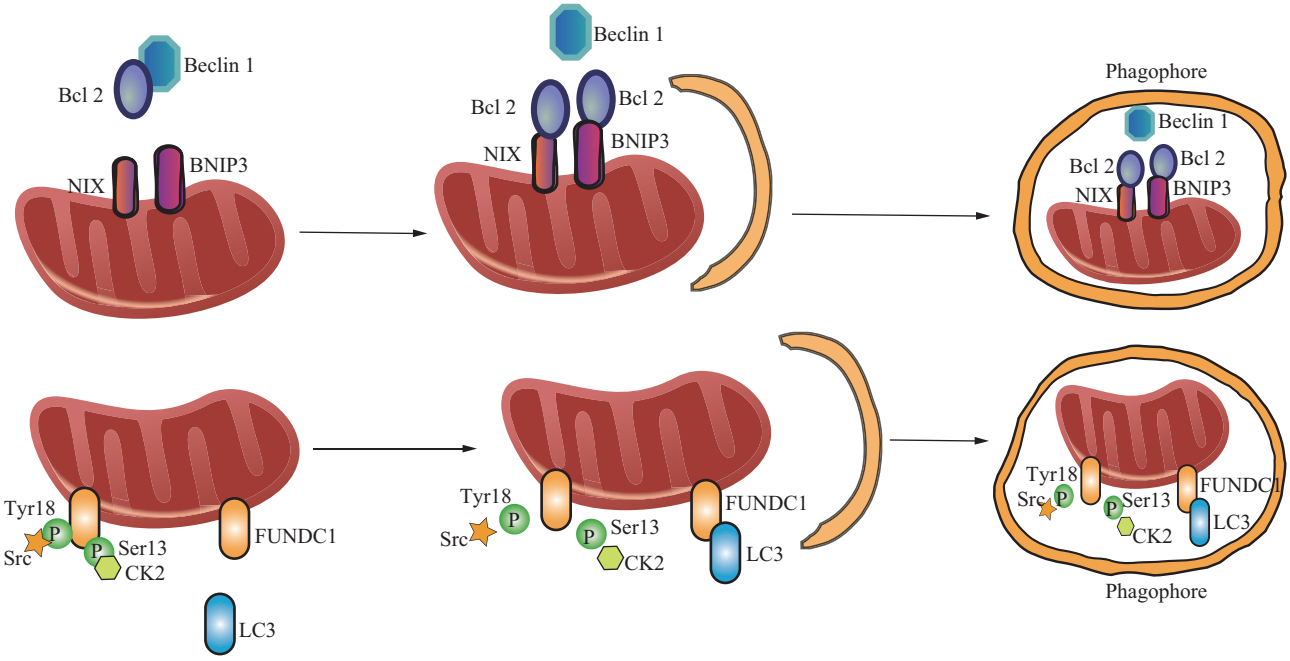


**Fig. 2** Receptor-mediated mitophagy
The receptors that mediated the mitophagy were BNIP3, NIX and FUN 14 domain-containing 1 (FUNDC1). BNIP3 competitively bound to BCL2 apoptosis regulator (Bcl 2), causing the binding of Bcl2 and Beclin 1 to be interfered, and thereby promoting mitophagy. NIX is a homolog of BNIP3. When there is a lack of BNIP3, NIX can compensate for the effect of BNIP3, but this is different, when compared to BNIP3. The post-translational modification of FUNDC1 can regulate the binding of FUNDC1 and light chain 3 α (LC3). When FUNDC1 is dephosphorylated, the binding effect with LC3 is enhanced to promote mitophagy. Then, this could be swallowed by phagosomes, and finally degraded by lysosomes.

*al* reported that after I/R, the overexpression of L-OPA1 significantly reduced OGD/R-induced neuronal death and mitochondrial morphological damage[69]. Recent studies have also revealed that the small molecule echinacoside promotes the process of mitochondrial fusion by targeting CK2 to promote the transcription of *MFN2*[70]. The small molecule echinacoside, as a new molecule that targets CK2, provides a target for the future treatment of ischemic stroke. It has been reported that under stress conditions, mitochondrial ubiquitin ligase 1 deficiency can increase the activity of MFN2, trigger mitochondrial hyperfusion, and act as an ER-Mito tethering antagonist. Decreased ER-mito coupling leads to the increase in cytoplasmic $Ca^{2+}$ activation of calcineurin, and the induction of Drp1-dependent mitochondrial fission and mitophagy[71].

**1.2.2 Diverse Roles of Mitochondria in Ischemic Stroke** Increasing evidence has shown that mitophagy is beneficial to the pathological process after ischemic stroke, suggesting that this is an important therapeutic target[72]. As mentioned above, mitophagy is mainly mediated by the PINK1/parkin signaling pathway after ischemic stroke[36]. Specifically, after ischemic stroke, the mitochondria depolarizes and the membrane potential is lost, and these produce some substances that are harmful to cells, such as ROS[52].

As an E3 ubiquitin ligase, parkin is known to ubiquitinate various mitochondrial outer membrane proteins, and regulate mitophagy by ubiquitinating mitochondrial outer membrane proteins. Necroptosis was found to be involved in cardiac I/R, and it was revealed that parkin attenuates myocardial injury by inhibiting the mPTP opening through catalyzing the Cyclophilin D (CypD) ubiquitination in the necrotic cascade reaction[73]. In addition, studies have revealed that parkin deficiency increases hepatic I/R injury[74], suggesting that parkin plays an important role in I/R injury.

Phosphorylation promotes the activation of PINK1, and activated PINK1 recruits parkin to induce it to transfer to the mitochondria, thereby initiating mitophagy[33]. Studies have revealed that electroacupuncture is effective in treating ischemic stroke. In the middle cerebral artery occlusion (MCAO) model of rats, it was found that the electroacupuncture treatment of cerebral I/R injury can protect brain neurons through PINK1/parkin-mediated mitophagy[75]. Sphingosine kinase 2 protects brain neurons by activating the BCL2 interacting protein 3 (BNIP3) signal, and activating mitophagy[76]. tPA is the most commonly used thrombolytic drug in clinical practice, but its therapy mechanism remains unclear[77, 78]. Recently, a report clarified that the lack of endogenous tPA can significantly aggravate brain damage, apoptosis and mitochondrial damage. Furthermore, it was found that the exposure of neurons to tPA can reduce the severity of damage and protect mitochondria[79]. Moreover, a further study revealed that the protective effect of tPA can be accomplished through the regulation of FUNDC1-mediated mitophagy[54]. Peroxynitrite ($ONOO^-$)-mediated mitophagy represents an important pathogenic mechanism of ischemic stroke. $ONOO^-$-mediated mitophagy is accomplished by recruiting Drp1 to mitochondria, and activating PINK1/parkin[80]. Studies have reported that rehmapicroside, a natural compound of medicinal plants, can reduce the infarct area of MCAO in rats[81]. *In vivo* and *in vitro* experiments have also revealed that rehmapicroside reduces $O_2^-$ and $ONOO^-$, upregulates Bcl2, and downregulates BCL associated X (Bax), caspase 3 and cleaved caspase 3, as well as PINK1, parkin and p62. Rehmapicroside prevents PINK1, parkin and Drp1 from entering the mitochondria to activate the mitophagy induced by cerebral I/R injury in rats[81].

## 2 THE CROSSTALK BETWEEN MITOPHAGY AND APOPTOSIS/NECROPTOSIS IN I/R INJURY

### 2.1 The Co-network in Mitophagy-apoptosis in I/R Injury

The mitophagy-mediated elimination of mitochondria play a driving role in a series of cases, such as cell differentiation, embryonic development, inflammation, and various cell deaths (including apoptosis, necroptosis, *etc.*)[16, 20, 62]. For apoptosis, mitochondria mediated apoptosis has been regarded as a late event of ischemic stroke[82]. The cyt c and other substances released from the mitochondria to the cytoplasm initiate endogenous apoptosis[83]. Mitochondrial apoptosis is correlated to the continuous opening of the mPTP. If the mPTP remains open, cyt c would be released from the mitochondrial membrane inner space to the cytoplasm, and cyt c would bind to the pro-apoptotic protease activator 1 (APAF-1), and promote its oligomerization. The caspase recruitment domain (CARD) of APAF-1 combines with the CARD domain of pro-caspase 9 to form a complex[84, 85]. Pro-caspase 9 is activated as caspase 9 to cleave pro-caspase 3 and pro-caspase 7, in order to execute their apoptotic function[84–87] (fig. 3).

Mitophagy is a selective autophagy, and an important form of mitochondrial quality control. Cells can escape from death by clearing the damaged mitochondria away through mitophagy[88]. Abundant evidence has shown that proper mitophagy can help neurons survive, while excessive mitophagy can aggravate I/R injury[89]. For example, in the MCAO model of SD rats, the knockdown of peroxiredoxin 6 (PRDX6, an antioxidant protein) aggravated the I/R injury, and increased the expression of mitochondrial autophagy-related proteins and apoptosis-related proteins[90]. However, this injury can be reduced by the

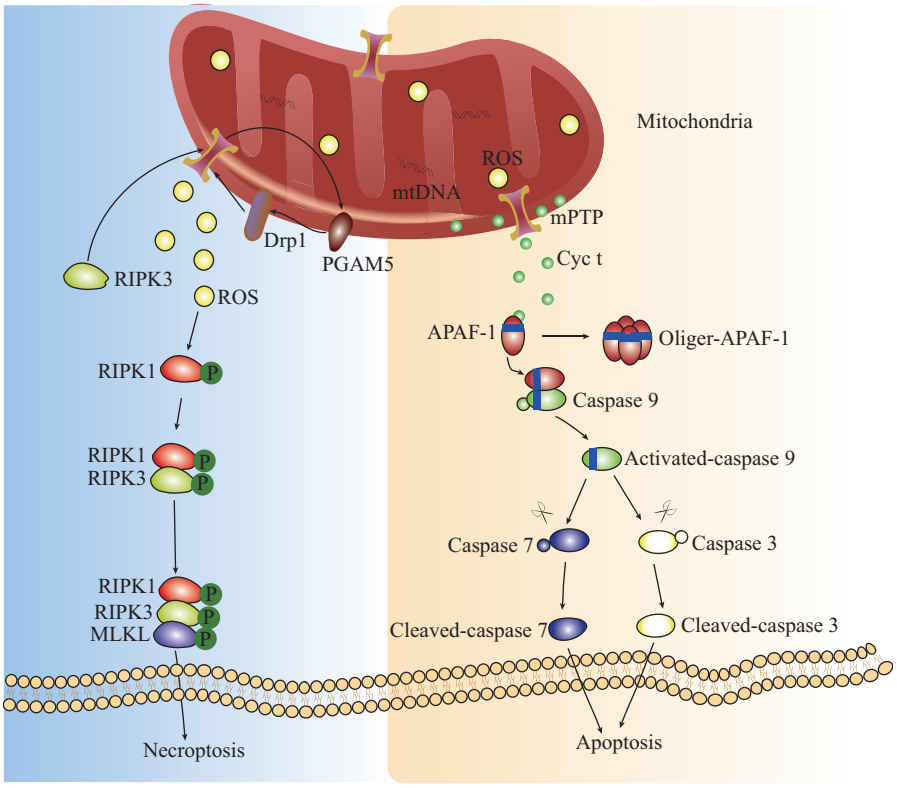


**Fig. 3** The crosstalk between mitophagy and apoptosis/necroptosis in I/R injury

I/R injury leads to the depolarization of the mitochondrial membrane, and the membrane permeability transition pore (mPTP) would remain open. When the mPTP remains open, cyt c is released from the mitochondrial membrane inner space to the cytoplasm, and cyt c binds to the pro-apoptotic protease activator 1 (APAF-1) and promotes its oligomerization. The caspase recruitment domain (CARD) of APAF-1 combines with the CARD domain of pro-caspase 9 to form a complex. Pro-caspase 9 is activated as caspase 9 to cleave pro-caspase 3 and pro-caspase 7, and execute their apoptotic function. The RIPK3-mediated mPTP opening caused phosphoglycerate mutase family member 5 (PGAM5) to be activated. The activated PGAM5 further activated Drp1, inducing the Drp1-miedated mitophagy and mPTP to open, which in turn, caused reactive oxygen species (ROS) to be released into the cytoplasm. ROS can lead to RIPK1 phosphorylation, and promote necroptosis. RIPK1: receptor-interacting protein kinase 1; RIPK3: receptor-interacting protein kinase 3; MLKL: mix lineage kinase domain-like protein

knockdown of PINK1, indicating that the knockdown of PRDX6 can aggravate cerebral I/R injury by enhancing the mitophagy mediated by the PINK1/parkin signaling pathway[90]. Stilbene glycoside promotes mitophagy and inhibits the apoptosis of ischemic neurons by promoting the expression of sirtuin (SIRT) and adenosine monophosphate-activated protein kinase (AMPK)[91]. Uncoupling protein (UCP2) is a member of the IMM protein. The deletion of *UCP2* enlarges the area of brain congestion, increases the number of necrotic and TUNEL-positive cells, and upregulates the expression of PINK1, Beclin 1 and LC3. At the same time, this downregulates the expression of p62. It has been shown that *UCP2* can aggravate I/R injury by promoting mitophagy and apoptosis[92]. In addition to brain I/R injury, in acute kidney injury induced by I/R, the phosphorylation level of AMPKα at Thr172 is significantly reduced. The use of AMPKα activator C24 can protect cells from apoptosis[93]. Consistent with the results reported by this article, by activating AMPK and promoting mitophagy, the damage of ischemic stroke can be reduced. This shows that the protection of ischemic stroke by activating AMPK is a possibility for future treatments[94].

When parkin is activated by PINK1, this ubiquitinates many substrates, such as VDAC1. According to research reports, parkin ubiquitinates VDAC1 in two different ways to control the autophagy and apoptosis process of cells, respectively. Monoubiquitinated VDAC1 inhibits cell apoptosis, and polyubiquitinated VDAC1 promotes mitochondrial autophagy[95]. This means that the effect of the PINK1-parkin pathway on apoptosis and mitophagy occurs through the VDAC1 antagonistic pathway[62]. This is the first evidence that verifies that different types of VDAC1 ubiquitination mediated by parkin can lead to different cellular outcomes. It is known that parkin can ubiquitinate various OMM proteins, except for VDAC1. However, it remains to be determined whether the phenomenon of other proteins is similar to VDAC1, and whether this mechanism exists in ischemic stroke. In addition, a study revealed that the expression of

VDAC1 is reduced in the vulnerable hippocampal CA1 subfield of rats after global ischemia[96]. This suggests that the reduction of VDAC1 may have been caused by the parkin ubiquitination.

BNIP3 is a pro-apoptotic BH3 protein, and has been considered as a regulator of mitophagy. When the BNIP3 gene was silenced, the interaction of BNIP3 with LC3 was reduced, thereby inhibiting mitophagy and reducing cell apoptosis. Furthermore, autophagy markers, such as Beclin 1 and lysosome-associated membrane glycoprotein 2 (LAMP2), and the ratio of LC3-Ⅱ/Ⅰ increased, indicating that the decrease in apoptosis may be correlated to the increase in general autophagy[97]. According to previous studies, I/R injury can lead to a significant increase in the expression of BINP3 and its homologue NIX, indicating that I/R injury induces mitophagy. Dexamethasone (DXMS) is the most widely used glucocorticoid in clinical practice. Higher doses of DXMS can cause cell apoptosis[98]. A recent study reported that under hypoxic conditions, mitophagy-related protein hypoxia-inducible factor-1α (HIF-1α) and BNIP3 protein levels increases. After using DXMS, the expression of BNIP3 was downregulated and the cell apoptosis increased, while the overexpression of HIF-1α significantly increased the expression of BNIP3 and reduced the apoptosis induced by DXMS[99].

### 2.2 The Co-network in Mitophagy-necroptosis in I/R Injury

Necroptosis is the regulated form of cell death, and this is mainly regulated by receptor-interacting protein kinase 1 (RIPK1), receptor-interacting protein kinase 3 (RIPK3), and mix lineage kinase domain-like protein (MLKL)[100–102]. In I/R injury, RIPK1 is phosphorylated, and the downstream molecule RIPK3 is phosphorylated, which promotes MLKL activation. Then, the activated MLKL executes death commands[103–105]. Recently, studies have shown crosstalk between I/R injury-induced necroptosis and mitochondrial dysfunction[105, 106]. For example, the RIPK3-mediated activation of $Ca^{2+}$/Calmoduline-dependent protein kinase Ⅱ (CaMKⅡ) promotes mPTP opening[107] and RIP3-PGAM5-Drp1 mediates the mitochondria fission[108]. In addition, studies have shown that in an *in vitro* model, cardiomyocytes and brain cells were more severely damaged after I/R injury in *PGAM5*-knockout mice[20]. As a mitochondrial membrane protein, PGAM5 is an important protective gene in ischemic injury, and it is also the anchor of the RIP1-RIP3-MLKL complex in mitochondria. Furthermore, research has revealed that PGAM5 is essential for PINK1-dependent mitophagy. PGAM5 independently promotes mitophagy to protect cells from necroptosis[20]. The continuous opening of the mPTP mediated by RIPK3 is the key to regulate the necroptosis under different stimuli[98, 109]. Mounting evidence has revealed that the activation of mitophagy can inhibit the opening of the mPTP[110]. It has been reported that RIPK3 can reduce the level of parkin phosphorylation, and reduce the interaction between parkin and LC3 induced by hypoxia damage, thereby inhibiting mitophagy and promoting mPTP opening. The knockout of RIPK3 can reverse this phenomenon via the AMPK-parkin-mitophagy signal axis[110] (fig. 3).

## 3 THERAPEUTIC TARGET FOR MITOPHAGY-DEPENDENT ISCHEMIC STROKE

As mentioned above, mitophagy exerts a vital role in the mitochondria and ischemic stroke. A number of studies have contributed in providing a wide range of strategies to improve neuronal protection during ischemic stroke, based on the molecular mechanism, which includes intravenous thrombolysis, tPA, and endovascular therapy[111, 112].

For example, tPA is the most important clinical thrombolytic drug for ischemic stroke, and has a neuroprotective effect[113]. The knockout of endogenous tPA can significantly aggravate the brain damage, and increase the neuronal apoptosis and mitochondrial damage. Mitochondrial damage in I/R injury is correlated to the endogenous reduction of tPA. Cyt c is released into the cytoplasm, triggering a caspase cascade reaction and neuronal apoptosis. At the same time, the mitochondrial damage would lead to the degradation of FUNDC1, preventing FUNDC1 from binding to free LC3, thereby inhibiting mitophagy. After adding exogenous tPA, the AMPK phosphorylation level and FUNDC1 both increase, and FUNDC1 binds to LC3 through the LIR domain, leading to the autophagy double-layer membrane wrapped mitochondria, and inducing mitophagy[54]. Although this is the only clinical thrombolytic drug approved by the U.S. FDA, a large number of problems need to be solved, such as determining how tPA responds to I/R injury and the mechanism, including how tPA enters into neurons and the downstream mechanism of FUNDC1. Ligustilide (3-butylidene-4, 5-dihydroisodenzofuranone; LIG) is the main active ingredient in the traditional medicine *Angelica sinensis*. DL-3-n-butylphthalide, which is the first innovative drug with independent intellectual property rights in China, has been widely used in the treatment of ischemic stroke in clinic, and has a similar structure to LIG[94]. This study revealed that LIG attenuates the injury of ischemic stroke *via* the activation of AMPK, and the promotion Drp1 to mediate mitochondria fission[94]. In addition, ligustilide has a wide range of pharmacological properties, including anticancer, anti-inflammatory, anti-oxidant and neuroprotective activities[114]. Rehmapicroside ameliorates cerebral I/R injury by attenuating the peroxynitrite-

**Table 1 The potential therapeutic targets for mitophagy-dependent ischemic stroke**

| Inhibitor/Therapy | Pathway | Molecules | Effects | References |
|---|---|---|---|---|
| tPA | FUNDC1/apoptosis | Drp1, Bax, Bcl 2, caspase 3, caspase 9 | Upregulated mitophagy, reduced ischemic stroke | 54 |
| Ligustilide | AMPK/Drp1/apoptosis | AMPK, Drp1, cleaved caspase 3 | | 94 |
| Rehmapicroside | $ONNO^-$/apoptosis | $ONNO^-$, Bax, caspase 3 | Downregulated mitophagy, reduced ischemic stroke | 81 |
| Electroacupuncture | PINK1/parkin | PINK1/parkin | Upregulated mitophagy, reduced ischemic stroke | 75 |
| CsA | mPTP | CypD | | 75 |
| Mdivi-1 | Drp1 | Drp1 | Downregulated mitophagy, increased ischemic stroke | 75 |
| FeTMPyP | $ONNO^-$ | $ONNO^-$ | Downregulated mitophagy, reduced ischemic stroke | 75 |
| Tetrahydrocurcumin | N/A | N/A | Reduced ischemic stroke | 115 |
| Methylene blue | mPTP | MMP, RIPK1, RIPK3 | Upregulated mitophagy, reduced ischemic stroke | 116 |

tPA: tissue-type plasminogen activator; FUNDC1: FUN 14 domain-containing 1; AMPK: AMP-activated protein kinase; Drp1: dynamin-related protein 1; Bax: BCL2 associated X, apoptosis regulator; Bcl 2: BCL2 apoptosis regulator; $ONNO^-$: peroxynitrite; CypD: cyclophilin D, peptidylprolyl isomerase; mPTP: membrane permeability transition pore; MMP: mitochondrial membrane potential; RIPK1: receptor-interacting protein kinase 1; RIPK3: receptor-interacting protein kinase 3

mediated mitophagy activation[81]. Tetrahydrocurcumin (THC) epigenetically ameliorates the mitochondrial dysfunction in brain vasculature during ischemic stroke[115]. Methylene blue promotes mitophagy by maintaining the mitochondrial membrane potential (MMP) at a relatively high level. This contributes to the decrease in necrosis, and the improvement in neurological function, thereby protecting against acute cerebral ischemic injury[116] (table 1). It has been found that some traditional Chinese medicine ingredients and compounds can treat ischemic stroke through mitophagy. However, it remains a challenge to find the target of traditional Chinese medicine, and the research at this stage remains on the phenotype[75].

## 4 CONCLUSION AND PERSPECTIVES

Mitochondria plays a driving role on tissue homeostasis to provide energy for the normal life activities of cells through the production of ATP. A vital process, known as mitophagy, can work as an emergency strategy after a series of stimuli damage. To date, a fuller molecular mechanism of mitophagy has been performed, which is closely correlated to the control of the quality of mitochondria involved in various diseases. Recent studies have revealed that mitophagy can aggravate neuronal death[81]. Among these, focus has been given on the field of ischemic stroke, in which the mitophagy was significantly downregulated, while the apoptosis and necroptosis were upregulated. Promoting mitophagy can alleviate I/R injury, and reduce neuronal apoptosis and necroptosis. In addition, promoting mitophagy can reduce apoptosis to a certain extent, but this may promote its general autophagy process, indicating that mitophagy and general autophagy also have a crosstalk. Thus, the mitophagy pathway is a complex, but practical, integrated network, and there is a necessity to focus on this overall crosstalk.

Although a number of studies have proved that mitophagy can exert a neuroprotective effect on ischemic stroke, in the complex molecular network of the human body, it remains unclear how mitophagy works on neuron protection. For example, a more comprehensive explanation should be provided in promoting PINK1/parkin and BNIP3/NIX, and Drp1-mediated mitophagy could alleviate I/R injury, while promoting $ONNO^-$-mediated mitophagy, which promotes I/R injury. More research is needed to improve the molecular network diagram of mitophagy. In addition, after I/R injury, it remains to be determined how these molecules work at different time points after ischemia, such as AMPK, FUNDC1 and BNIP3. Promoting mitophagy can reduce the apoptosis or necroptotic pathways, and determine whether other cell apoptosis pathways are involved. The intersection of mitochondria and cell death is also a problem worth exploring. Answering these questions would not only be of great significance to our understanding of the protection of neurons through mitophagy in ischemic stroke, but also provide new theoretical support for the treatment of ischemic stroke.

**Conflict of Interest Statement**

The authors declare no conflict of interest.

Author Kun XIONG is a member of the Young Editorial Board for Current Medical Science. The paper was handled by the other editor and has undergone rigorous peer review process. Author Kun XIONG was not involved in the journal's review of, or decision related to, this manuscript.

## REFERENCES

1 Benjamin EJ, Muntner P, Alonso A, *et al*. Heart Disease and Stroke Statistics-2019 Update: A Report From the American Heart Association. Circulation, 2019, 139(10):e56-e528
2 Lakhan SE, Kirchgessner A, Hofer M. Inflammatory mechanisms in ischemic stroke: therapeutic approaches. J Transl Med, 2009,7:97
3 Barthels D, Das H. Current advances in ischemic stroke research and therapies. Biochimi Biophys Acta Mol Basis Dis, 2020,1866(4):165260
4 National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. N Engl J Med, 1995,333(24):1581-1587
5 Goyal M, Demchuk AM, Menon BK, *et al*. Randomized Assessment of Rapid Endovascular Treatment of Ischemic Stroke. N Engl J Med, 2015,372(11):1019-1030
6 Chen W, Huang JJ, Hu YQ, *et al*. Mitochondrial Transfer as a Therapeutic Strategy Against Ischemic Stroke. Transl Stroke Res, 2020,11(6):1214-1228
7 Mayevsky A, Kutai-Asis H, Tolmasov M. Mitochondrial function and brain Metabolic Score (BMS) in ischemic Stroke: Evaluation of "neuroprotectants" safety and efficacy. Mitochondrion, 2020,50:170-194
8 Hu HZ, Feng XB, Shao ZW, *et al*. Application and Prospect of Mixed Reality Technology in Medical Field. Curr Med Sci, 2019,39(1):1-6
9 Lip GYH, Nieuwlaat R, Pisters R, *et al*. Refining Clinical Risk Stratification for Predicting Stroke and Thromboembolism in Atrial Fibrillation Using a Novel Risk Factor-Based Approach The Euro Heart Survey on Atrial Fibrillation. Chest 2010,137(2):263-272
10 Hacke W, Kaste M, Bluhmki E, *et al*. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med, 2008,359(13):1317-1329
11 Rabinstein AA. Update on Treatment of Acute Ischemic Stroke. Continuum (Minneap Minn), 2020,26(2):268-286
12 Zhang D, Zou X, Sy C, *et al*. Thrombolysis and reperfusion: advanced understanding of early management strategies in acute ischemic stroke. Neurol Res, 2014,36(5):391-396
13 Cagalinec M, Safiulina D, Liiv M, *et al*. Principles of the mitochondrial fusion and fission cycle in neurons. J Cell Sci, 2013,126(Pt 10):2187-2197
14 Chan PH. Mitochondrial dysfunction and oxidative stress as determinants of cell death/survival in stroke. Ann N Y Acad Sci, 2005,1042:203-209
15 Correa F, Soto V, Zazueta C. Mitochondrial permeability transition relevance for apoptotic triggering in the post-ischemic heart. Int J Biochem Cell Biol, 2007,39(4):787-798
16 Carinci M, Vezzani B, Patergnani S, *et al*. Different Roles of Mitochondria in Cell Death and Inflammation: Focusing on Mitochondrial Quality Control in Ischemic Stroke and Reperfusion. Biomedicines, 2021,9(2):169
17 Wang L, Qi H, Tang Y, *et al*. Post-translational Modifications of Key Machinery in the Control of Mitophagy. Trends Biochem Sci, 2020,45(1):58-75
18 Li X, Huang L, Lan J, *et al*. Molecular mechanisms of mitophagy and its roles in neurodegenerative diseases. Pharmacol Res, 2021,163:105240
19 Bingol B, Sheng M. Mechanisms of mitophagy: PINK1, Parkin, USP30 and beyond. Free Radic Biol Med, 2016,100:210-222
20 Lu W, Sun J, Yoon JS, *et al*. Mitochondrial Protein PGAM5 Regulates Mitophagic Protection against Cell Necroptosis. PLoS One, 2016,11(1):e0147792
21 Shen L, Gan Q, Yang Y, *et al*. Mitophagy in Cerebral Ischemia and Ischemia/Reperfusion Injury. Front Aging Neurosci, 2021,13:687246
22 Kapadia M, De Snoo ML, Kalia LV, *et al*. Regulation of Parkin-dependent mitophagy by Bcl-2-associated athanogene (BAG) family members. Neural Regen Res, 2021,16(4):684-685
23 Filippov MA, Tatarnikova OG, Pozdnyakova NV, *et al*. Inflammation/bioenergetics-associated neurodegenerative pathologies and concomitant diseases: a role of mitochondria targeted catalase and xanthophylls. Neural Regen Res, 2021,16(2):223-233
24 Park HA, Broman K, Jonas EA. Oxidative stress battles neuronal Bcl-xL in a fight to the death. Neural Regen Res, 2021,16(1):12-15
25 Ott M, Robertson JD, Gogvadze V, *et al*. Cytochrome c release from mitochondria proceeds by a two-step process. Proc Natl Acad Sci USA, 2002,99(3):1259-1263
26 Baechler BL, Bloemberg D, Quadrilatero J. Mitophagy regulates mitochondrial network signaling, oxidative stress, and apoptosis during myoblast differentiation. Autophagy, 2019,15(9):1606-1619
27 Cen X, Chen Y, Xu X, *et al*. Pharmacological targeting of MCL-1 promotes mitophagy and improves disease pathologies in an Alzheimer's disease mouse model. Nat Commun, 2020,11(1):5731
28 Kang L, Liu S, Li J, *et al*. Parkin and Nrf2 prevent oxidative stress-induced apoptosis in intervertebral endplate chondrocytes via inducing mitophagy and anti-oxidant defenses. Life Sci, 2020,243:117244
29 Erustes AG, D'Eletto M, Guarache GC, *et al*. Overexpression of alpha-synuclein inhibits mitochondrial Ca(2+) trafficking between the endoplasmic reticulum and mitochondria through MAMs by altering the GRP75-IP3R interaction. J Neurosci Res,

2021,99(11):2932-2947
30 Yoo SM, Jung YK. A Molecular Approach to Mitophagy and Mitochondrial Dynamics. Mol Cells, 2018,41(1):18-26
31 Roberts RF, Tang MY, Fon EA, *et al*. Defending the mitochondria: The pathways of mitophagy and mitochondrial-derived vesicles. Int J Biochem Cell Biol, 2016,79:427-436
32 Narendra D, Tanaka A, Suen DF, *et al*. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J Cell Biol, 2008,183(5):795-803
33 Narendra DP, Jin SM, Tanaka A, *et al*. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. PLoS Biol, 2010,8(1):e1000298
34 Lazarou M, Jin SM, Kane LA, *et al*. Role of PINK1 binding to the TOM complex and alternate intracellular membranes in recruitment and activation of the E3 ligase Parkin. Dev Cell, 2012,22(2):320-333
35 Deas E, Plun-Favreau H, Gandhi S, *et al*. PINK1 cleavage at position A103 by the mitochondrial protease PARL. Hum Mol Genet, 2011,20(5):867-879
36 Sekine S. PINK1 import regulation at a crossroad of mitochondrial fate: the molecular mechanisms of PINK1 import. J Biochem, 2020,167(3):217-224
37 Okatsu K, Oka T, Iguchi M, *et al*. PINK1 autophosphorylation upon membrane potential dissipation is essential for Parkin recruitment to damaged mitochondria. Nat Commun, 2012,3:1016
38 Zhang T, Liu Q, Gao W, *et al*. The multifaceted regulation of mitophagy by endogenous metabolites. Autophagy, 2021,1-24
39 Ni HM, Williams JA, Ding WX. Mitochondrial dynamics and mitochondrial quality control. Redox Biol, 2015,4:6-13
40 Yamada T, Dawson TM, Yanagawa T, *et al*. SQSTM1/p62 promotes mitochondrial ubiquitination independently of PINK1 and PRKN/parkin in mitophagy. Autophagy, 2019,15(11):2012-2018
41 Yamano K, Matsuda N, Tanaka K. The ubiquitin signal and autophagy: an orchestrated dance leading to mitochondrial degradation. EMBO Rep, 2016,17(3):300-316
42 Yamano K, Queliconi BB, Koyano F, *et al*. Site-specific Interaction Mapping of Phosphorylated Ubiquitin to Uncover Parkin Activation. J Biol Chem, 2015,290(42):25199-25211
43 Poole AC, Thomas RE, Yu S, *et al*. The mitochondrial fusion-promoting factor mitofusin is a substrate of the PINK1/parkin pathway. PLoS One, 2010,5(4):e10054
44 Wang X, Winter D, Ashrafi G, *et al*. PINK1 and Parkin target Miro for phosphorylation and degradation to arrest mitochondrial motility. Cell, 2011,147(4):893-906
45 Sarraf SA, Raman M, Guarani-Pereira V, *et al*. Landscape of the PARKIN-dependent ubiquitylome in response to mitochondrial depolarization. Nature, 2013,496(7445):372-376
46 Koyano F, Yamano K, Kosako H, *et al*. Parkin recruitment to impaired mitochondria for nonselective ubiquitylation is facilitated by MITOL. J Biol Chem, 2019,294(26):10300-10314
47 Matsuda N, Yamano K. Two sides of a coin: Physiological significance and molecular mechanisms for damage-induced mitochondrial localization of PINK1 and Parkin. Neurosci Res, 2020,159:16-24
48 Hamacher-Brady A, Brady NR. Mitophagy programs: mechanisms and physiological implications of mitochondrial targeting by autophagy. Cell Mol Life Sci, 2016,73(4):775-795
49 Chen G, Cizeau J, Vande Velde C, *et al*. Nix and Nip3 form a subfamily of pro-apoptotic mitochondrial proteins. J Biol Chem, 1999,274(1):7-10
50 Bellot G, Garcia-Medina R, Gounon P, *et al*. Hypoxia-induced autophagy is mediated through hypoxia-inducible factor induction of BNIP3 and BNIP3L via their BH3 domains. Mol Cell Biol, 2009,29(10):2570-2581
51 Choi GE, Lee HJ, Chae CW, *et al*. BNIP3L/NIX-mediated mitophagy protects against glucocorticoid-induced synapse defects. Nat Commun, 2021,12(1):487
52 Lei L, Yang S, Lu X, *et al*. Research Progress on the Mechanism of Mitochondrial Autophagy in Cerebral Stroke. Front Aging Neurosci, 2021,13:698601
53 Kuang Y, Ma K, Zhou C, *et al*. Structural basis for the phosphorylation of FUNDC1 LIR as a molecular switch of mitophagy. Autophagy, 2016,12(12):2363-2373
54 Cai Y, Yang E, Yao X, *et al*. FUNDC1-dependent mitophagy induced by tPA protects neurons against cerebral ischemia-reperfusion injury. Redox Biol, 2021,38:101792
55 Liu H, Zang C, Yuan F, *et al*. The role of FUNDC1 in mitophagy, mitochondrial dynamics and human diseases. Biochem Pharmacol, 2022,197:114891
56 Liu L, Feng D, Chen G, *et al*. Mitochondrial outer-membrane protein FUNDC1 mediates hypoxia-induced mitophagy in mammalian cells. Nat Cell Biol, 2012,14(2):177-185
57 Chen G, Han Z, Feng D, *et al*. A regulatory signaling loop comprising the PGAM5 phosphatase and CK2 controls receptor-mediated mitophagy. Mol Cell, 2014,54(3):362-377
58 Sugo M, Kimura H, Arasaki K, *et al*. Syntaxin 17 regulates the localization and function of PGAM5 in mitochondrial division and mitophagy. EMBO J, 2018,37(21):e98899
59 Xin Y, Zhang X, Li J, *et al*. New Insights Into the Role of Mitochondria Quality Control in Ischemic Heart Disease. Front Cardiovasc Med, 2021,8:774619
60 Nan J, Zhu W, Rahman MS, *et al*. Molecular regulation of mitochondrial dynamics in cardiac disease. Biochim Biophys Acta Mol Cell Res, 2017,1864(7):1260-1273
61 Anzell AR, Fogo GM, Gurm Z, *et al*. Mitochondrial fission and mitophagy are independent mechanisms regulating ischemia/reperfusion injury in primary neurons. Cell Death Dis, 2021,12(5):475
62 Jia J, Jin H, Nan D, *et al*. New insights into targeting mitochondria in ischemic injury. Apoptosis, 2021,26(3-4):163-183
63 Zuo W, Zhang S, Xia CY, *et al*. Mitochondria autophagy is induced after hypoxic/ischemic stress in a Drp1 dependent manner: the role of inhibition of Drp1 in ischemic brain damage. Neuropharmacology, 2014,86:103-115
64 Zhou BH, Wei SS, Jia LS, et al. Drp1/Mff signaling

pathway is involved in fluoride-induced abnormal fission of hepatocyte mitochondria in mice. Sci Total Environ, 2020,725:138192
65 Luan Y, Ren KD, Luan Y, *et al*. Mitochondrial Dynamics: Pathogenesis and Therapeutic Targets of Vascular Diseases. Front Cardiovasc Med, 2021,8:770574
66 Wu P, Li Y, Zhu S, *et al*. Mdivi-1 Alleviates Early Brain Injury After Experimental Subarachnoid Hemorrhage in Rats, Possibly via Inhibition of Drp1-Activated Mitochondrial Fission and Oxidative Stress. Neurochem Res, 2017,42(5):1449-1458
67 Smith G, Gallo G. To mdivi-1 or not to mdivi-1: Is that the question? Dev Neurobiol, 2017,77(11):1260-1268
68 Flippo KH, Gnanasekaran A, Perkins GA, *et al*. AKAP1 Protects from Cerebral Ischemic Stroke by Inhibiting Drp1-Dependent Mitochondrial Fission. J Neurosci, 2018,38(38):8233-8242
69 Lai Y, Lin P, Chen M, *et al*. Restoration of L-OPA1 alleviates acute ischemic stroke injury in rats via inhibiting neuronal apoptosis and preserving mitochondrial function. Redox Biol, 2020,34:101503
70 Zeng KW, Wang JK, Wang LC, *et al*. Small molecule induces mitochondrial fusion for neuroprotection via targeting CK2 without affecting its conventional kinase activity. Signal Transduct Target Ther, 2021,6(1):71
71 Puri R, Cheng XT, Lin MY, *et al*. Mul1 restrains Parkin-mediated mitophagy in mature neurons by maintaining ER-mitochondrial contacts. Nat Commun, 2019,10(1):3645
72 Ham PB 3rd, Raju R. Mitochondrial function in hypoxic ischemic injury and influence of aging. Prog Neurobiol, 2017,157:92-116
73 Sun T, Ding W, Xu T, *et al*. Parkin Regulates Programmed Necrosis and Myocardial Ischemia/Reperfusion Injury by Targeting Cyclophilin-D. Antioxid Redox Signal, 2019,31(16):1177-1193
74 Ning XJ, Yan X, Wang YF, *et al*. Parkin deficiency elevates hepatic ischemia/reperfusion injury accompanying decreased mitochondrial autophagy, increased apoptosis, impaired DNA damage repair and altered cell cycle distribution. Mol Med Rep, 2018,18(6):5663-5668
75 Wang H, Chen S, Zhang Y, *et al*. Electroacupuncture ameliorates neuronal injury by Pink1/Parkin-mediated mitophagy clearance in cerebral ischemia-reperfusion. Nitric Oxide, 2019,91:23-34
76 Chen JL, Wang XX, Chen L, *et al*. A sphingosine kinase 2-mimicking TAT-peptide protects neurons against ischemia-reperfusion injury by activating BNIP3-mediated mitophagy. Neuropharmacology, 2020,181: 108326
77 Yepes M. The Plasminogen Activation System Promotes Neurorepair in the Ischemic Brain. Curr Drug Targets, 2019,20(9):953-959
78 Jeanneret V, Yepes M. Tissue-type plasminogen activator is a homeostatic regulator of synaptic function in the central nervous system. Neural Regen Res, 2017, 12(3):362-365
79 Thiebaut AM, Gauberti M, Ali C, *et al*. The role of plasminogen activators in stroke treatment: fibrinolysis and beyond. Lancet Neurol, 2018,17(12):1121-1132
80 Feng J, Chen X, Guan B, *et al*. Inhibition of Peroxynitrite-Induced Mitophagy Activation Attenuates Cerebral Ischemia-Reperfusion Injury. Mol Neurobiol, 2018,55(8):6369-6386
81 Zhang Y, He Y, Wu M, *et al*. Rehmapicroside ameliorates cerebral ischemia-reperfusion injury via attenuating peroxynitrite-mediated mitophagy activation. Free Radic Biol Med, 2020,160:526-539
82 Shao Z, Dou S, Zhu J, *et al*. The Role of Mitophagy in Ischemic Stroke. Front Neurol, 2020,11:608610
83 Guan R, Zou W, Dai X, *et al*. Mitophagy, a potential therapeutic target for stroke. J Biomed Sci, 2018,25(1):87
84 Cain K, Bratton SB, Langlais C, *et al*. Apaf-1 oligomerizes into biologically active approximately 700-kDa and inactive approximately 1.4-MDa apoptosome complexes. J Biol Chem, 2000,275(9):6067-6070
85 Bratton SB, Walker G, Srinivasula SM, *et al*. Recruitment, activation and retention of caspases-9 and -3 by Apaf-1 apoptosome and associated XIAP complexes. EMBO J, 2001,20(5):998-1009
86 Hu XM, Li ZX, Zhang DY, *et al*. A systematic summary of survival and death signalling during the life of hair follicle stem cells. Stem Cell Res Ther, 2021,12(1):453
87 Hu XM, Li ZX, Lin RH, *et al*. Guidelines for Regulated Cell Death Assays: A Systematic Summary, A Categorical Comparison, A Prospective. Front Cell Dev Biol, 2021, 9:634690
88 Yang L, Ma YM, Shen XL, *et al*. The Involvement of Mitochondrial Biogenesis in Selenium Reduced Hyperglycemia-Aggravated Cerebral Ischemia Injury. Neurochem Res, 2020,45(8):1888-1901
89 Dong G, Xu N, Wang M, *et al*. Anthocyanin Extract from Purple Sweet Potato Exacerbate Mitophagy to Ameliorate Pyroptosis in Klebsiella pneumoniae Infection. Int J Mol Sci, 2021,22(21):11422
90 Hong T, Zhou Y, Peng L, *et al*. Knocking down peroxiredoxin 6 aggravates cerebral ischemia-reperfusion injury by enhancing mitophagy. Neuroscience, 2021, 482:30-42
91 Li Y, Hu K, Liang M, *et al*. Stilbene glycoside upregulates SIRT3/AMPK to promotes neuronal mitochondrial autophagy and inhibit apoptosis in ischemic stroke. Adv Clin Exp Med, 2021,30(2):139-146
92 He M, Zhang T, Fan Y, *et al*. Deletion of mitochondrial uncoupling protein 2 exacerbates mitophagy and cell apoptosis after cerebral ischemia and reperfusion injury in mice. Int J Med Sci, 2020,17(17):2869-2878
93 Ma H, Guo X, Cui S, *et al*. Dephosphorylation of AMP-activated kinase exacerbates ischemia/reperfusion-induced acute kidney injury via mitochondrial dysfunction. Kidney Int, 2022,101(2):315-330
94 Wu Q, Liu J, Mao Z, *et al*. Ligustilide attenuates ischemic stroke injury by promoting Drp1-mediated mitochondrial fission via activation of AMPK. Phytomedicine, 2021,95:153884
95 Geisler S, Holmstrom KM, Skujat D, *et al*. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat Cell Biol, 2010,12(2):119-131
96 Yao GY, Zhu Q, Xia J, *et al*. Ischemic postconditioning confers cerebroprotection by stabilizing VDACs after brain ischemia. Cell Death Dis, 2018,9(10):1033
97 Shi RY, Zhu SH, Li V, *et al*. BNIP3 interacting with LC3 triggers excessive mitophagy in delayed neuronal death

in stroke. CNS Neurosci Ther, 2014,20(12):1045-1055
98 Lim SY, Hausenloy DJ, Arjun S, *et al*. Mitochondrial cyclophilin-D as a potential therapeutic target for post-myocardial infarction heart failure. J Cell Mol Med, 2011,15(11):2443-2451
99 Xu K, Lu C, Ren X, *et al*. Overexpression of HIF-1alpha enhances the protective effect of mitophagy on steroid-induced osteocytes apoptosis. Environ Toxicol, 2021,36(11):2123-2137
100 Shang L, Ding W, Li N, *et al*. The effects and regulatory mechanism of RIP3 on RGC-5 necroptosis following elevated hydrostatic pressure. Acta Bioch Bioph Sin (Shanghai), 2017,49(2):128-137
101 Guo LM, Wang Z, Li SP, *et al*. RIP3/MLKL-mediated neuronal necroptosis induced by methamphetamine at 39°C. Neural Regen Res, 2020,15(5):865-874
102 Wang Z, Guo LM, Zhou HK, *et al*. Using drugs to target necroptosis: dual roles in disease therapy. Histol Histopathol, 2018,33(8):773-789
103 Yan WT, Lu S, Yang YD, *et al*. Research trends, hot spots and prospects for necroptosis in the field of neuroscience. Neural Regen Res, 2021,16(8):1628-1637
104 Zhang Q, Wan XX, Hu XM, *et al*. Targeting Programmed Cell Death to Improve Stem Cell Therapy: Implications for Treating Diabetes and Diabetes-Related Diseases. Front Cell Dev Biol, 2021,9:809656
105 Yan WT, Yang YD, Hu XM, *et al*. Do pyroptosis, apoptosis, and necroptosis (PANoptosis) exist in cerebral ischemia? Evidence from cell and rodent studies. Neural Regen Res, 2022,17(8):1761-1768
106 Horvath C, Young M, Jarabicova I, *et al*. Inhibition of Cardiac RIP3 Mitigates Early Reperfusion Injury and Calcium-Induced Mitochondrial Swelling without Altering Necroptotic Signalling. Int J Mol Sci, 2021, 22(15):7983
107 Zhang T, Zhang Y, Cui M, *et al*. CaMKII is a RIP3 substrate mediating ischemia- and oxidative stress-induced myocardial necroptosis. Nat Med, 2016,22(2): 175-182
108 She L, Tu H, Zhang YZ, *et al*. Inhibition of Phosphoglycerate Mutase 5 Reduces Necroptosis in Rat Hearts Following Ischemia/Reperfusion Through Suppression of Dynamin-Related Protein 1. Cardiovasc Drugs Ther, 2019,33(1):13-23
109 Zhu P, Hu S, Jin Q, *et al*. Ripk3 promotes ER stress-induced necroptosis in cardiac IR injury: A mechanism involving calcium overload/XO/ROS/mPTP pathway. Redox Biol, 2018,16:157-168
110 Zhu P, Wan K, Yin M, *et al*. RIPK3 Induces Cardiomyocyte Necroptosis via Inhibition of AMPK-Parkin-Mitophagy in Cardiac Remodelling after Myocardial Infarction. Oxid Med Cell Longev, 2021, 2021:6635955
111 Berkhemer OA, Fransen PS, Beumer D, *et al*. A randomized trial of intraarterial treatment for acute ischemic stroke. N Engl J Med, 2015,372(1):11-20
112 Jovin TG, Chamorro A, Cobo E, *et al*. Thrombectomy within 8 hours after symptom onset in ischemic stroke. N Engl J Med, 2015,372(24):2296-2306
113 Baker TS, Robeny J, Cruz D, *et al*. Stimulating the Facial Nerve to Treat Ischemic Stroke: A Systematic Review. Front Neurol, 2021,12:753182
114 Xie Q, Zhang L, Xie L, *et al*. Z-ligustilide: A review of its pharmacokinetics and pharmacology. Phytother Res, 2020,34(8):1966-1991
115 Mondal NK, Behera J, Kelly KE, *et al*. Tetrahydrocurcumin epigenetically mitigates mitochondrial dysfunction in brain vasculature during ischemic stroke. Neurochem Int, 2019,122:120-138
116 Di Y, He YL, Zhao T, *et al*. Methylene Blue Reduces Acute Cerebral Ischemic Injury via the Induction of Mitophagy. Mol Med, 2015,21(1):420-429

(Received Jan. 7, 2022; accepted Feb. 14, 2022)