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Research Advances in Mitophagy in Hepatic Ischemia-Reperfusion Injury

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Abstract: Hepatic ischemia-reperfusion injury (IRI) is a prevalent pathophysiological phenomenon encountered during liver surgeries and transplantation, leading to hepatocyte damage and liver dysfunction, which significantly affects patient prognosis. In recent years, the role of mitophagy in hepatic IRI has garnered considerable attention. Mitochondria, known as the "powerhouses" of the cell, are crucial for maintaining normal cellular physiological functions. During the ischemia-reperfusion process, mitochondria are susceptible to damage, generating excessive harmful substances, such as reactive oxygen species (ROS), which further exacerbate cellular injury. Mitophagy is a selective cellular self-protection mechanism that maintains the quality and quantity balance of mitochondria within cells by clearing damaged or dysfunctional mitochondria. In the context of liver IRI, the activation of mitophagy is of significant importance. On one hand, mitophagy can rapidly remove damaged mitochondria, thereby reducing the release of harmful products and alleviating oxidative stress and cellular damage. Research has indicated that under ischemia-reperfusion conditions, mitophagy-related pathways are activated, promoting the clearance of damaged mitochondria. On the other hand, mitophagy also regulates cellular energy metabolism, providing essential energy support for cells under stress. With the continuous advancement of research, the understanding of the role of mitophagy in hepatic IRI has become increasingly clear. Numerous studies are dedicated to exploring the specific molecular mechanisms of mitophagy and its regulation, aiming to develop new therapeutic strategies to alleviate hepatic IRI. Although studies have demonstrated that mitophagy has a protective effect in hepatic ischemia-reperfusion injury, many issues still require further investigation. First, it is essential to further elucidate the mechanisms underlying the role of mitophagy in ischemia-reperfusion. Additionally, understanding how to mitigate liver ischemia-reperfusion injury through the modulation of mitophagy represents a key focus for future research. Future studies may encompass drug development, gene therapy, and cell therapy approaches aimed at improving the prognosis of patients affected by liver ischemia-reperfusion.

Keywords: Ischemia-reperfusion; Mitophagy; Oxidative stress; Autophagy pathway; Phosphorylation

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1. Introduction

Liver ischemia inhibits aerobic respiration in mitochondria. Mitochondria, as bilateral lipid membrane energy-producing organelles within cells, provide energy for cellular renewal through aerobic respiration. The production of ATP via mitochondrial respiration necessitates adequate oxygen, a normal cytoplasmic environment, stable mitochondrial structure, and homeostasis within the mitochondrial environment. Notably, as the primary oxygen-consuming organelles, mitochondria exhibit high sensitivity to hypoxia. When the body experiences shock due to various factors or when blood flow is interrupted for surgical purposes, the oxygen supply to cells significantly decreases, leading to damage of the cytochrome oxidase system within mitochondria and the generation of excessive reactive oxygen species. Mitophagy is a highly regulated process involving multiple signaling pathways and proteins that collectively coordinate the selective autophagy of mitochondria. This process enhances the cell's ability to recognize, engulf, degrade, and regenerate damaged mitochondria, thereby maintaining mitochondrial health within the cell.

2. mTOR Pathway

In the context of mitophagy, the mechanistic target of rapamycin (mTOR) pathway serves as a crucial regulatory element. mTOR is a serine/threonine protein kinase with a molecular weight of 289 kDa, classified within the phosphatidylinositol 3-kinase-related kinase (PIKK) family. This protein comprises a catalytic kinase domain, an FRB (FKBP12-rapamycin binding) domain, a predicted autoinhibitory domain (inhibitor domain) located near the C-terminus, as well as up to 20 HEAT motif repeats at the N-terminus, in addition to FAT (FRAP-ATM-TRRAP) and FATC (FAT C-terminal) domains []. In mitochondrial autophagy, the mTOR (mammalian target of rapamycin) pathway plays a crucial regulatory role [2]. mTOR is a serine/threonine protein kinase with a molecular weight of 289 kDa, belonging to the phosphatidylinositol 3-kinase-related kinases (PIKK) family. This protein comprises a catalytic kinase domain, a FRB (FKBP12-rapamycin-binding) domain, a predicted self-inhibitory domain (inhibitory subdomain) near the C-terminus, up to 20 repeats of the HEAT motif at the amino terminus, and the FAT (FRAP-ATM-TRRAP) and FATC (FAT C-terminal) domains, which form two distinct complexes: mTORC1 and mTORC2. mTORC1 consists of mTOR, Raptor, mLST8, and additional components, whose kinase activity can be inhibited by rapamycin [3].

Rapamycin, a macrolide compound first isolated from *Streptomyces* colonies in the soil of Easter Island, acts as an autophagy activator by binding to the FKBP-12 receptor within cells. This interaction forms a complex that directly interacts with the FRB domain of mTOR, thereby inhibiting its protein activity. Additionally, rapamycin can block protein synthesis and induce cell cycle progression, causing arrest in the G1 phase [4]. The human mouse experimental model was utilized for research conducted by Wen Ji'an Zheng [5]. It has been demonstrated that rapamycin can activate mitochondrial autophagy, which in turn improves the mitochondrial function of mice. Rapamycin is a core regulator of cell growth, and its abnormalities are often associated with various diseases, including tumorigenesis, aging, and diabetes, making it an important therapeutic target [6]. Under normal physiological conditions, mTORC1 remains in a relatively active state. A series of signaling pathways can sense the nutritional levels (such as amino acids) and growth factors within cells. When nutrients are abundant and growth factor signals are active, mTORC1 is activated, which subsequently inhibits mitochondrial autophagy.

The specific mechanism is as follows:mTORC1 phosphorylates ULK1, a component of the unc-51-like autophagy activating kinase 1 (ULK1) complex, along with related proteins, thereby inhibiting ULK1 activity.

ULK1 is a crucial kinase in the initiation stage of mitochondrial autophagy; its inhibition obstructs the formation of pre-autophagosome structures, thus preventing the activation of mitochondrial autophagy [7]. However, when cells experience energy depletion, nutrient deficiency, or other stressors, mTORC1 activity is inhibited [8, 9]. This inhibition removal activates the ULK1 complex. The activated ULK1 complex translocates to the vicinity of mitochondria and initiates mitochondrial autophagy by phosphorylating other related proteins. Additionally, mTORC2 also influences mitochondrial autophagy, although its role is relatively complex and may vary by cell type and environmental conditions, as illustrated in **Figure 1** [9]. In summary, the mTOR pathway is finely regulated through the phosphorylation of key proteins, and the occurrence of mitochondrial autophagy plays a vital role in maintaining intracellular stability and adapting to various physiological and stress states.

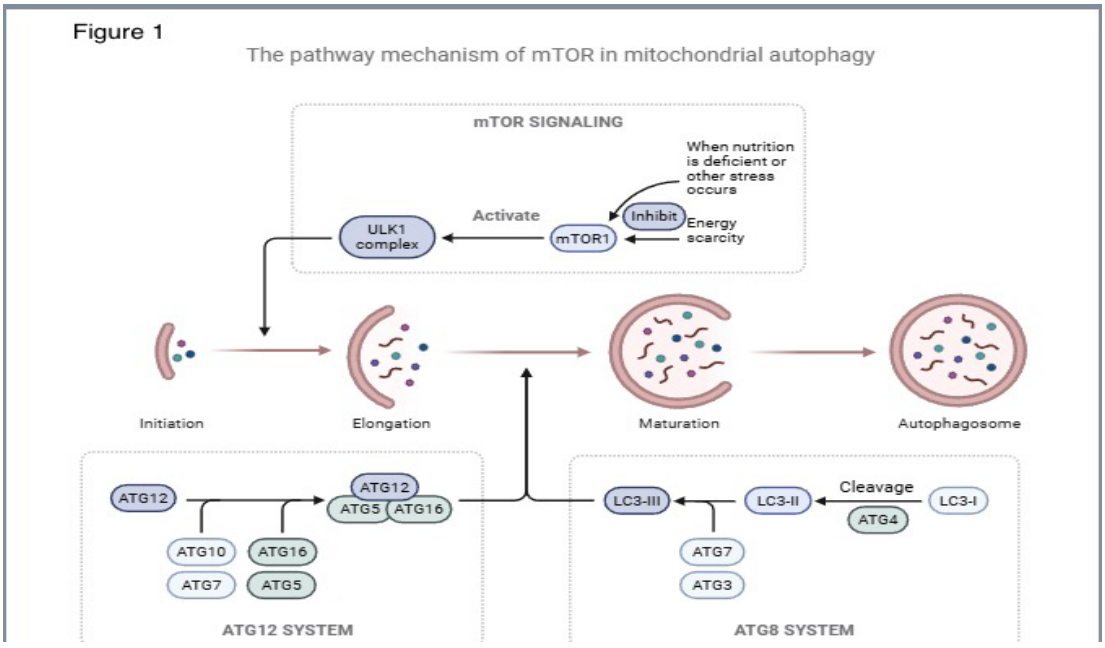

Figure 1. The pathway mechanism of mTOR in mitochondrial autophagy

3. PARKIN pathway

PARKIN is a key mitochondrial receptor protein and a ubiquitin ligase. It consists of an N-terminal ubiquitin-like domain (UbL), RING0 (also known as the unique Parkin structure domain, UPD), RING1, an intermediate structure domain (IBR), a Parkin repressor element (REP), and a C-terminal RING2 [10–13]. Typically, PARKIN is located in the cytoplasm in a self-restraining 'closed' state. Its formation occurs during hepatic ischemia-reperfusion, although PARKIN is not substrate-specific. It plays a crucial role in recognizing and labeling damaged mitochondria to promote their autophagy [11]. The activation of PARKIN usually depends on the rupture of the mitochondrial membrane and changes in membrane potential. The PARKIN pathway is a significant mechanism in mitochondrial autophagy, primarily involving two proteins: PARKIN and PINK1 [12, 13].

During liver ischemia, mitochondria are subjected to ischemia-induced oxidative stress and damage, leading to mitochondrial membrane rupture, changes in membrane potential, and leakage of internal mitochondrial components. The PINK1 protein accumulates on the outer mitochondrial membrane, where it phosphorylates proteins on the mitochondrial membrane [12]. Concurrently, PINK1 activates PARKIN, facilitating its transport to the damaged mitochondria. Once activated, PARKIN ubiquitinates the surface of the damaged mitochondria,

prompting the identification and degradation of the target mitochondria that require removal [13, 14]. Later, the marked mitochondria are associated with autophagy. The mitochondria fuse into a structure enveloped by autophagosomes, which can be cleared and restored in a timely manner through the PARKIN pathway, thereby helping to reduce oxidative stress and inflammation. This reaction protects hepatocytes from further damage and plays a crucial role in hepatic ischemia-reperfusion injury. Furthermore, the abnormal function of this pathway is associated with the onset of neurodegenerative diseases, such as Parkinson's disease, which is significant for understanding the pathophysiology of these conditions. Thus, the theoretical basis for this research is established [14].

4. AMPK pathway

Under hypoxic stress, cells are activated through various pathways, one of which is the AMPK (AMP-activated protein kinase) pathway, leading to mitochondrial autophagy (mitophagy) [15]. AMP is a crucial intracellular signaling molecule that regulates energy balance, metabolism, and cell survival. AMPK is a heterotrimeric protein composed of three subunits: α, β, and γ. The α subunit contains the N-terminal kinase domain and the C-terminal affinity binding region, while the β subunit includes the nucleotide-binding region and the basic nuclear cleavage region. The γ subunit also contains a nucleotide-binding region [16–18]. The N-terminal kinase domain of the α subunit catalyzes the phosphorylation reaction that activates AMPK.

Furthermore, the C-terminal affinity binding region of the α subunit is essential for its interaction with the other subunits (β and γ). This region interacts with the carbohydrate-binding module (CBM) of the β subunit, thereby regulating AMPK activity and the stability between subunits [17, 18]. Notably, AMPK function can be modulated even in states of liver ischemia, enabling cells to adapt to changes in energy metabolism. The β subunit of AMPK contains a nucleotide-binding region responsible for binding AMP and ATP, which regulates AMPK activity. The basic nucleolytic region may play a role in substrate-mediated AMPK phosphorylation reactions. The γ subunit also includes a nucleotide-binding region that is highly sensitive to fluctuations in intracellular AMP and ATP concentrations.

When intracellular ATP levels decrease and AMP levels increase, AMP binds to the γ subunit of AMPK [18]. This binding induces a conformational change in AMPK, facilitating the phosphorylation of the α subunit and inhibiting the suppression of AMPK by ATP. Consequently, the binding of AMP enhances AMPK's kinase activity, promoting ATP generation by activating intracellular pathways, such as increasing glucose uptake and glycolysis, which further elevates ATP levels [19, 20]. Additionally, AMPK activation inhibits various ATP-consuming processes in the cell, including fatty acid and protein synthesis.

5. PINK1-PRKN pathway

PINK1, or PTEN-induced kinase 1, is a serine-threonine kinase that specifically accumulates on damaged mitochondria. It comprises multiple domains, including a mitochondrial targeting sequence, an auxiliary domain, a kinase domain, and a phosphorylated kinase domain. PINK1 is primarily associated with the pathogenesis of Parkinson's disease and represents the most thoroughly studied ubiquitin-dependent pathway, playing a crucial role in mitochondrial dynamics [20–22]. The main factor for degradation is linked to PRKN, an E3 ubiquitin ligase responsible for the conjugation of ubiquitin molecules to substrate proteins. In normal mitochondria, PINK1 is directed into the inner mitochondrial membrane via its mitochondrial-targeted sequence, where it is cleaved by

proteases located in the mitochondrial matrix and inner membrane before being released into the cytoplasm for ubiquitination and proteasomal degradation.

Conversely, in damaged mitochondria, the mitochondrial membrane becomes depolarized, resulting in a reduced membrane potential that leads to PINK1 aggregation on the mitochondrial membrane surface. This aggregation activates the E3 ligase activity of PRKN through phosphorylation, which subsequently ubiquitinates numerous outer mitochondrial membrane (OMM) proteins, thereby triggering mitochondrial autophagy. The regulation of the PINK1-PRKN pathway primarily depends on changes in mitochondrial membrane potential; when the potential decreases, PINK1 stabilizes on the outer mitochondrial membrane and undergoes trans-autophosphorylation. This oxidation activates PRKN, leading to the ubiquitination of a significant number of OMM proteins, which form ubiquitin chains. These chains are recognized and bound by autophagy adapters, such as OPTN and NDP52, thereby promoting the formation of autophagosomes and initiating lysosomal autophagy [21–23].

Autophagy-related connexins, such as p62, play a significant role in mitochondrial autophagy. They facilitate the recognition and clearance of damaged mitochondria by autophagosomes through their binding to ubiquitin chains. On one hand, this process triggers the initiation of mitochondrial autophagy by promoting the ubiquitination of mitochondrial surface proteins; on the other hand, it interacts with autophagy adapters. The interaction of these proteins enhances the recognition and clearance of damaged mitochondria by autophagosomes [22, 23]. This process is crucial for maintaining the stability of the intracellular environment and ensuring normal cellular function. Increasing research indicates that abnormalities in the PINK1-PRKN pathway are closely associated with various human diseases. For instance, Parkinson's disease (PD), a neurodegenerative disorder, is closely linked to mitochondrial dysfunction and involves the PINK1 and PRKN genes.

Mutations in these genes are considered major genetic factors for PD [23, 24]. These mutations lead to impaired function of the PINK1-PRKN pathway, resulting in mitochondrial autophagy impairment and mitochondrial dysfunction, which ultimately leads to neuronal death and PD. Furthermore, the PINK1-PRKN pathway is also closely related to many other diseases, such as tumors and metabolic disorders [24]. Excessive accumulation of reactive oxygen species (ROS) during ischemia-reperfusion of the liver can lead to mitochondrial damage [25]. Moreover, high levels of ROS may adversely affect the PINK1-PRKN pathway, causing oxidative damage to PINK1 and PRKN, which leads to their injury and inactivation [26].

6. FUNDC1 pathway

FUNDC1 is a protein located on the outer membrane of mitochondria, playing a crucial role in regulating the interaction between mitochondria and the endoplasmic reticulum during mitochondrial autophagy. Its structure comprises an N-terminal transmembrane domain and a C-terminal cytoplasmic domain. The cytoplasmic domain contains a microtubule-associated protein light chain 3 (LC3) interaction region (LIR) and a casein kinase 2 (CK2) phosphorylation site, which are essential for the induction of mitochondrial autophagy initiation [27]. Upon mitochondrial damage, FUNDC1 recognizes the impaired mitochondria and binds to LC3 through its BH3 domain, forming a complex. This binding facilitates the recognition of damaged mitochondria by autophagosomes for packaging [28]. However, the expression and activity of FUNDC1 are regulated by various factors, including oxygen concentration and cellular energy state. Under hypoxic conditions, the expression of FUNDC1 increases, and its activity is enhanced through phosphorylation and other modifications, thereby promoting mitochondrial self-cleansing [29].

The specific mechanism of the FUNDC1 pathway in hepatic ischemia-reperfusion injury remains to be explored, as there is currently no direct research evidence or detailed elaboration. The FUNDC1 pathway has primarily been studied in the context of mitochondrial autophagy and nervous system function, particularly in brain development [30, 31]. This is based on general work in FUNDC1 mitochondrial autophagy. Using this, it can speculate on the potential mechanisms it may play in hepatic ischemia-reperfusion injury. First, FUNDC1, as a regulator of mitochondrial autophagy, may be involved in the clearance and renewal of mitochondria following hepatic ischemia-reperfusion injury. In such injuries, mitochondria can sustain damage, leading to functional abnormalities and disruptions in cellular energy metabolism. At this juncture, FUNDC1 could facilitate the formation of autophagosomes and the clearance of damaged mitochondria by recognizing and labeling them, thereby assisting in the restoration of normal liver cell function.

Second, the FUNDC1 pathway may influence the inflammatory response and oxidative stress that follow hepatic ischemia-reperfusion injury by regulating mitochondrial autophagy. During hepatic ischemia-reperfusion, a significant production of oxygen free radicals and other inflammatory mediators occurs, which can damage liver cells. The FUNDC1 pathway may mitigate the production of oxygen free radicals and the release of inflammatory mediators by clearing damaged mitochondria, thereby alleviating the inflammatory response and oxidative stress, and promoting liver recovery. Finally, the FUNDC1 pathway may be associated with cell survival and apoptosis following ischemia-reperfusion injury in the liver.

During ischemia-reperfusion, liver cells may experience severe damage, resulting in cell apoptosis or necrosis. The FUNDC1 pathway may influence cell survival and apoptosis processes by regulating mitochondrial autophagy. For instance, it may reduce cell apoptosis by clearing damaged mitochondria or promote cell survival and recovery by facilitating the clearance of these organelles. However, it is crucial to emphasize that the aforementioned content is based on speculations regarding the general role of the FUNDC1 pathway in mitochondrial autophagy and ischemia-reperfusion injury in the nervous system and heart [31]. Notably, there is a lack of specific research evidence supporting the involvement of the FUNDC1 pathway in liver ischemia-reperfusion injury and the precise mechanisms underlying mitochondrial injury. Therefore, future research must further investigate the role and mechanisms of the FUNDC1 pathway in liver ischemia-reperfusion injury. Additionally, FUNDC1-mediated mitochondrial autophagy occurs independently of Parkin protein involvement; it facilitates the clearance of damaged mitochondria by directly binding to LC3, which induces the formation of autophagosomes and initiates mitochondrial autophagy.

7. NIX pathway

NIX is a protein associated with mitochondrial autophagy that promotes the degradation of damaged mitochondria. Also known as NIP3-like protein X (BNIP3L), it plays a crucial role as one of the mitochondrial autophagy receptors [32]. Research indicates that ferroptosis can be mitigated by reducing levels of mitochondrial reactive oxygen species (ROS). This can be achieved by maintaining mitochondrial stability through enhanced autophagy. In cells with a double knockout of BNIP3 and NIX, the complete loss of mitochondrial autophagy results in elevated mitochondrial ROS levels. This observation suggests that BNIP3 and NIX are pivotal in regulating mitochondrial ROS levels and protecting cells from ferroptosis [33]. BNIP3L contains a mitochondrial targeting sequence at its N-terminus, which is essential for directing proteins to the mitochondrial membrane. This localization signal enables BNIP3L to accurately position itself within mitochondria, thereby participating in the

regulation of mitochondrial function and autophagy processes. The C-terminus of BNIP3L features a covalently linked domain rich in helical structures, facilitating interactions with other proteins, thus regulating the process of mitochondrial autophagy [34].

The steps involved are as follows: firstly, under hypoxic conditions, a signaling pathway is activated, leading to the translocation of NIX from the outer mitochondrial membrane to the cytoplasm. Secondly, in the cytoplasm, NIX binds with LC3 to form the LC3-NIX complex, which recruits additional autophagy-related proteins to form autophagosomes. Finally, the autophagosome envelops the thread. The mitochondria fuse with lysosomes to form autophagolysosomes, which are responsible for degrading mitochondrial components and the F-box protein FBXL4 [35]. This F-box protein interacts with SKP1 and Cullin1 to form the SCF-FBXL4 E3 ubiquitin ligase complex. This complex facilitates the ubiquitination and subsequent degradation of mitochondrial autophagy receptors BNIP3 and NIX, thereby inhibiting mitochondrial autophagy. When FBXL4 is mutated, the assembly of the SCF-FBXL4 E3 ubiquitin ligase complex is disrupted, leading to the accumulation of BNIP3 and NIX, which in turn overactivates mitochondrial autophagy. This suggests that FBXL4 plays a crucial role in regulating mitochondrial autophagy mediated by BNIP3 and NIX [36]. Notably, NIX can also bind to Bcl-2 through its BH3 domain, inhibiting Bcl-2's suppressive effect on Beclin-1. This interaction releases Beclin-1, thereby activating the autophagy process. In addition to its interactions with LC3 and Bcl-2, NIX also engages with other molecules, as illustrated in **Figure 2**.

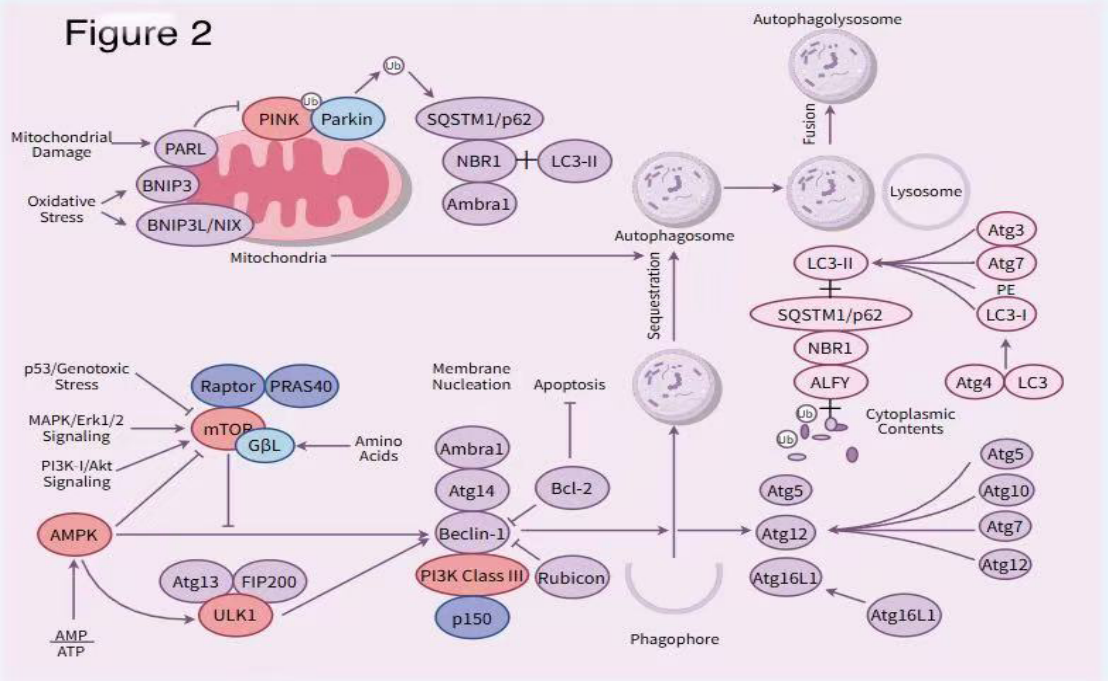

Figure 2. Mitochondrial autophagy mechanism pathway map

NIX can interact with the protein FUNDC1, located in the inner mitochondrial membrane, to regulate mitochondrial autophagy. Additionally, NIX interacts with molecules such as Parkin, thereby participating in the regulation of mitochondrial autophagy [37]. These interactions enable NIX to exert complex and precise regulatory functions in this process. Although the specific mechanisms of the NIX pathway and hepatic ischemia-reperfusion injury (HIRI) remain incompletely understood, the NIX pathway may promote the clearance of damaged

mitochondria through these mechanisms, thereby reducing oxidative stress and inflammatory responses during liver ischemia-reperfusion, and consequently offering some protection to the liver against further damage.

8. BNIP3 pathway

BNIP3 is a member of the Bcl-2 protein family, located at 10q26.3 on chromosome 10. It consists of six exons and has a full-length sequence of 585 bp, encoding 194 amino acids, with a molecular weight of approximately 21 kDa. BNIP3 is a multifunctional protein that localizes to the outer mitochondrial membrane. Its molecular structure includes a transmembrane domain and a cytoplasmic domain. The transmembrane domain anchors BNIP3 to the outer mitochondrial membrane, while the cytoplasmic domain facilitates interactions with other proteins [38, 39]. BNIP3 serves dual functions: promoting apoptosis and mitochondrial autophagy [39]. In apoptosis, BNIP3 activates members of the Bcl-2 family to promote the release of cytochrome c, thereby triggering cell death. When mitochondria are damaged or dysfunctional, BNIP3 expression increases, facilitating mitochondrial autophagy. It binds to LC3 through its cytoplasmic domain, marking damaged mitochondria as autophagy substrates that are then recognized and cleared by autophagosomes.

Additionally, BNIP3 interacts with autophagy-related proteins, such as Beclin1, promoting the formation and maturation of autophagosomes. Therefore, BNIP3 is crucial in the initiation, recognition, and clearance processes of mitochondrial autophagy [40, 41]. The expression and activity of BNIP3 are regulated by various factors, including transcription factors and miRNAs. The activation of HIF-1α under hypoxic conditions promotes the transcriptional expression of BNIP3, subsequently activating mitochondrial autophagy. Furthermore, the activity of BNIP3 is regulated by post-translational modifications, such as phosphorylation and ubiquitination, which can influence its stability. The interaction of BNIP3 with other proteins also affects the occurrence of mitochondrial autophagy. For instance, the interaction between BNIP3 and Parkin enhances the recognition and clearance of damaged mitochondria [41]. During hepatic ischemia-reperfusion, hepatic ischemia induces obstacles in intracellular energy metabolism, leading to mitochondrial dysfunction and potentially inhibiting BNIP3 expression, as cells primarily confront hypoxia and energy crises during this period.

Upon restoration of liver perfusion, the increased oxygen supply within cells generates substantial amounts of reactive oxygen species (ROS), including superoxide radicals, hydroxyl ions, and hydrogen peroxide, which trigger oxidative stress responses. The accumulation of ROS exacerbates mitochondrial damage, consequently activating BNIP3 expression. BNIP3, through its cytoplasmic binding domain, binds to LC3, marking damaged mitochondria as autophagy substrates [42, 43]. Additionally, BNIP3 may interact with other autophagy-related proteins, such as Beclin1, to promote the formation and maturation of autophagosomes. This process of mitochondrial autophagy facilitates the removal of damaged mitochondria, thereby mitigating cellular damage, as illustrated in **Figure 2**.

9. ATG pathway

The ATG protein (Autophagy-related gene proteins) family plays a crucial role in the autophagy process. This family includes proteins such as ATG1-ATG10, ATG12-ATG16, and ATG18, which are integral to autophagy. The formation, expansion, closure, and fusion of phagosomes with lysosomes are specific processes that involve these proteins [43]. The steps of autophagy are as follows: ATG proteins interact with various other proteins to form multiple

complexes, including the ATG1/ULK1 kinase complex, the ATG12-ATG5-ATG16 complex, the ATG9 transport system, and the LC3/ATG8 system. These complexes accumulate on the double membrane of autophagosomes, promoting the extension and bending of membranes, which aids in the formation of autophagosomes. ULK1, a key kinase that initiates autophagy, receives upstream signals. For instance, when ATP levels are low, AMPK can directly phosphorylate ULK1, enhancing its activity and initiating the mitochondrial autophagy program.

Conversely, when cellular nutrients are abundant, mTORC1 inhibits ULK1 activity; however, during nutrient deficiency, mTORC1 activity decreases, relieving the inhibition of ULK1 and enabling its activation to initiate mitochondrial autophagy [44, 45]. Although the ATG1/ULK1 complex does not directly participate in the specific recognition stage of mitochondrial autophagy, it is essential for autophagy induction, as well as for the formation and expansion of autophagosomes during this process. The ATG9 transport system comprises ATG2, WDR45/WIPI4, and lipids from the endoplasmic reticulum channel. During mitochondrial autophagy, autophagosomes play a crucial role in packaging and removing damaged mitochondria. ATG9A, a transmembrane protein, is dynamically transported between the endoplasmic reticulum and autophagosomes. Intermembrane material provides the necessary membrane components for the formation of autophagosomes [45]. This transport may interact with other key proteins involved in the mitochondrial autophagy process, such as PINK1 and Parkin. Such interactions could involve direct binding of ATG9A to mitochondrial autophagy-related proteins or modulation of these proteins' activities, thereby influencing the mitochondrial autophagy process. When the ATG9 transport system functions correctly, autophagosomes can form and expand efficiently, effectively enveloping and clearing damaged mitochondria.

Furthermore, impairment of the ATG9 transport system may hinder the formation and expansion of autophagosomes, leading to reduced or failed mitochondrial autophagy [46]. In the LC3/ATG8 system, LC3 (Microtubule-Associated Protein 1 Light Chain 3) serves as the mammalian homolog of ATG8. LC3 exists in two forms: LC3-I (cytoplasmic form) and LC3-II (membrane-bound form) [47]. LC3-I undergoes a series of modification processes, wherein Phosphatidylethanolamine (PE) is linked to LC3-II. LC3-II is a hallmark protein of autophagosomes, attached to their membranes, and plays a vital role in identifying and marking substances for degradation, including damaged mitochondria [48, 49]. The LC3/ATG8 system is involved in the conformational changes necessary for the construction and expansion of autophagosomes. After binding to PE, LC3-I is transferred to the double membrane of autophagosomes to form the membrane-bound LC3-II.During the degradation process, LC3-II located on the outer membrane of the autophagosome is cleaved and reused, while LC3-II on the inner membrane is degraded along with the enclosed material by lysosomes. LC3-II can bind to mitochondrial autophagy receptors (such as p62 and NDP52) through its LIR (LC3-interacting region) motif, thereby linking damaged mitochondria to autophagy [48, 49].

Additionally, the LC3/ATG8 system may also interact with key regulators of autophagy, such as PINK1 and Parkin, to promote mitochondrial autophagy. Consequently, the strength of autophagy can be assessed by detecting changes in the LC3-II/I ratio. For instance, when mitochondrial autophagy is active, the level of LC3-II increases while the level of LC3-I correspondingly decreases [49]. During the formation of the ATG12-ATG5-ATG16 complex, ATG12 first undergoes covalent binding with ATG5 through the ubiquitin-binding system, resulting in the formation of the ATG12-ATG5 complex. As an autophagy-related protein, ATG16L1 can interact with the ATG12-ATG5 complex to form the polymeric ATG12-ATG5-ATG16 complex. This complex binds to specific receptor proteins on the outer membrane of mitochondria, targeting mitochondria that require degradation via autophagy [50]. Under the coordinated action of other autophagy-related proteins, the autophagosome membrane

begins to extend and envelop the mitochondria, forming an autophagosome precursor. As the autophagosome membrane continues to extend and envelop, it gradually matures, ultimately forming a complete autophagosome.

Other members of the ATG protein family, such as ATG4 and ATG7, are involved in the effective fusion of mature autophagosomes with lysosomes, facilitating the degradation and recycling of mitochondrial contents, as illustrated in **Figure 1** [51]. The complex is involved in the closure of the autophagosome precursor membrane, leading to the formation of complete autophagosomes. In hepatic ischemia-reperfusion injury (HIRI), the activity of ATG proteins may be regulated, thereby affecting the efficiency and effectiveness of autophagy. First, ATG proteins influence the clearance and reuse of damaged organelles by regulating the formation and degradation of autophagosomes. Second, ATG proteins may participate in processes such as inflammation and apoptosis, thus impacting the progression and outcome of HIRI [52]. Finally, ATG proteins may interact with other signaling pathways. Multiple studies have demonstrated that ATG proteins play a significant role in HIRI, particularly ATG5 and ATG7. The loss or inhibition of these ATG proteins may exacerbate HIRI [53]. The formation of the ATG12-ATG5 complex may promote the formation of autophagosomes and reduce liver ischemia-reperfusion injury (LIRI). Additionally, some studies have found that the expression levels of ATG proteins may be associated with the severity and prognosis of HIRI.

10. Conclusion

Ischemia-reperfusion injury of the liver can lead to mitochondrial dysfunction and the activation of the mitochondrial autophagy pathway. During ischemia, mitochondria experience oxidative stress and damage, which can result in the rupture of the mitochondrial membrane and the leakage of internal components. Upon restoration of blood flow through reperfusion, damaged mitochondria release pro-inflammatory factors and free radicals, further exacerbating hepatocyte injury. Mitochondrial autophagy plays a critical role in the context of ischemia-reperfusion injury in the liver. By eliminating damaged mitochondria, mitochondrial autophagy assists cells in returning to normal function, reduces responses to oxidative stress and inflammation, and promotes cell survival. However, excessive activation of mitochondrial autophagy may also lead to cell death, thereby worsening the injury. Consequently, in exploring the mechanisms and treatments for ischemia-reperfusion injury in the liver, researchers often focus on the regulation of mitochondrial autophagy. By modulating the extent of mitochondrial autophagy, it is possible to influence the survival and damage of liver cells in a targeted manner, offering new insights for the treatment and prevention of ischemia-reperfusion injury.

Future research will further investigate the specific mechanisms of mitochondrial autophagy in hepatic ischemia-reperfusion injury (HIRI) and will focus on regulating mitochondrial autophagy to develop novel therapeutic strategies. This research will enhance the understanding of the pathogenesis of HIRI and provide innovative ideas and methods for clinical treatment. By examining the roles of autophagy-related proteins, including ATG, PTEN, PARKIN, PINK, AMPK, mTOR, BNIP3, and NIX, further studies aim to formulate effective therapeutic strategies for HIRI.

Disclosure statement

The authors declare no conflict of interest.

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