

Critical hubs of renal ischemia-reperfusion injury: endoplasmic reticulum-mitochondria tethering complexes

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Abstract

Mitochondrial injury and endoplasmic reticulum (ER) stress are considered to be the key mechanisms of renal ischemia-reperfusion (I/R) injury. Mitochondria are membrane-bound organelles that form close physical contact with a specific domain of the ER, known as mitochondrial-associated membranes. The close physical contact between them is mainly restrained by ER-mitochondria tethering complexes, which can play an important role in mitochondrial damage, ER stress, lipid homeostasis, and cell death. Several ER-mitochondria tethering complex components are involved in the process of renal I/R injury. A better understanding of the physical and functional interaction between ER and mitochondria is helpful to further clarify the mechanism of renal I/R injury and provide potential therapeutic targets. In this review, we aim to describe the structure of the tethering complex and elucidate its pivotal role in renal I/R injury by summarizing its role in many important mechanisms, such as mitophagy, mitochondrial fission, mitochondrial fusion, apoptosis and necrosis, ER stress, mitochondrial substance transport, and lipid metabolism.

Keywords: Endoplasmic reticulum; Mitochondria tethering complexes; Renal I/R injury; Mitophagy; Mitochondrial fission; ER stress

Introduction

Renal ischemia-reperfusion (I/R) injury is a serious complication after organ transplantation, heart operation, and other major operations.^[1] It is the most common cause of acute kidney injury (AKI), and the mortality rate in the intensive care unit is as high as 50%.^[2] During operation, the blood supply of the kidney is limited, and then the kidney recovers perfusion accompanied by reoxygenation, which will cause inevitable damage to the kidney.^[3] Recognized mechanisms of renal I/R injury include mitochondrial dysfunction, endoplasmic reticulum (ER) stress, apoptosis and necrosis, oxidation, and stress.^[4] Among them, mitochondrial dysfunction and ER stress play an important role. Increasing evidence shows that ER and mitochondrial functions are highly physiologically and pathologically related.^[5] Communication and cooperation between the mitochondria and the ER depend on effective crosstalk, and one way to achieve this is through direct membrane contact. Therefore, proper ER-mito-

chondrial communication requires the formation of specialized membrane microdomains at the contact site, which limit the short distance between membranes and connect them.^[6] It can be observed under the microscope that the mitochondria and ER are bound by some proteins, which are named ER-mitochondria tethering complexes. These tethering proteins are indispensable for mitochondrial and ER interactions. As structures that physically connect the two organelles, ER-mitochondria tethering complexes provide a close functional connection between the ER and mitochondria. These complexes are a platform for many important cellular processes and contain protein molecules such as mitofusin-2 (MFN2), mitochondrial fission 1 protein (Fis1), phosphofurin acidic cluster sorting protein 2 (PACS-2), and so on. In addition, these complexes have an important impact on mitochondrial morphological changes, biogenesis, and mitophagy. For the ER, the ER pressure sensor protein kinase R-like endoplasmic reticulum kinase (PERK) initiates signal transduction in response to stress, thereby maintaining

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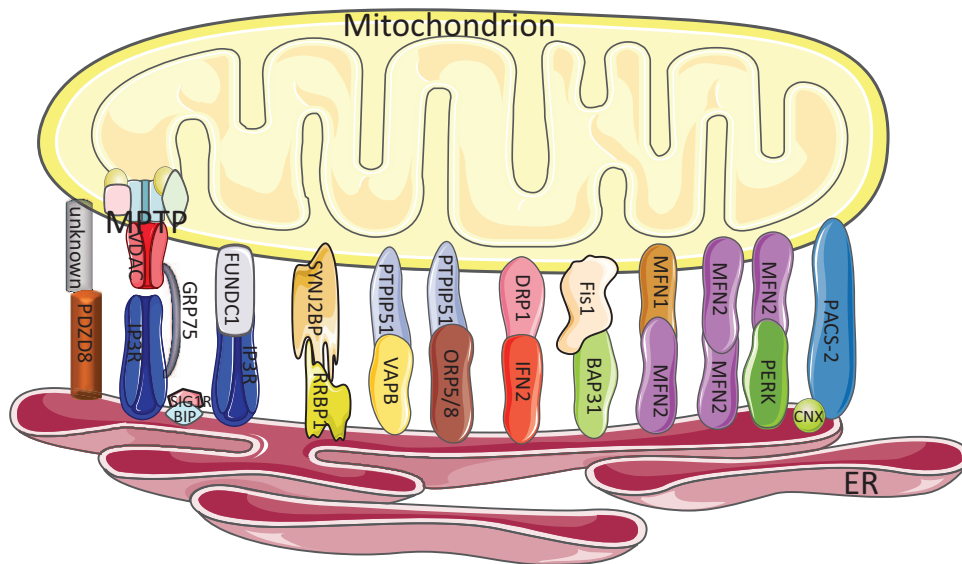


Figure 1: Schematic diagram of endoplasmic reticulum (ER)-mitochondria tethering complexes. (1) PDZD8 interacts with an undefined protein in mitochondria. (2) Inositol 1,4,5-triphosphate receptor (IP3R), a voltage-dependent anion channel (VDAC), and glucose regulatory protein 75 (GRP75) form a ternary binding complex. VDACS can also form a mitochondrial permeability transition pore with mitochondrial proteins. (3) IP3R interacts with FUN14 domain-containing protein 1 (FUNDC1). (4) Synuclein 2 binding protein (SYNJ2BP) interacts with ribosome binding protein 1 (RRBP1). (5) Vesicle-associated membrane protein-related protein B (VAPB) interacts with protein tyrosine phosphatase-interacting protein 51 (PTPIP51). (6) PTPIP51 can also interact with the oxysterol binding protein-related proteins ORP5 and ORP8. (7) The ER-bound protein inverted formin-2 (INF2) interacts with dynamin-related protein 1 (DRP1). (8) B cell receptor-associated protein 31 (BAP31) and mitochondrial fission 1 protein (Fis1) interact with each other. (9) Mitofusin-2 (MFN2) interacts with mitochondrial fusion protein 1 (MFN1). (10) MFN2 interacts with MFN2. (11) MFN2 can also interact with protein kinase R-like endoplasmic reticulum kinase (PERK). (12) Phosphofurin acidic cluster sorting protein 2 (PACS-2).

ER and cell homeostasis. Recently, the structure and functions of ER-mitochondria tethering complexes have become a research hotspot. It has been proven in models of renal I/R injury that changes in ER-mitochondria tethering complex components impair cell functions. Under stress conditions, such as renal I/R, ER-mitochondria tethering complexes transmit stress signals from the ER to mitochondria and regulate mitochondrial quality control. In this review, we describe the structure of ER-mitochondria tethering complexes and summarize the pathophysiological mechanism of renal I/R injury in which tethering complexes are involved under recent evidence.

ER-mitochondria tethering complexes

The physical interaction of the ER and mitochondria has been evaluated by electron microscopy. A study using electron tomography showed that the minimum distance between the outer mitochondrial membrane (OMM) and ER was only 10 nm for the smooth ER and 25 nm for the rough ER. Studies in yeast revealed the presence of a protein complex known as ER-mitochondria encounter structure (ERMES).^[7] However, no mammalian orthologs of ERMES proteins have been identified yet, but several different protein complexes have been proposed as ER-mitochondria tethers.^[8] ER-mitochondria tethering complexes are composed of many proteins that connect the ER to mitochondria and play an important role in cellular processes.^[9] These complexes are special protein structures connecting the outer membrane of mitochondria and ER, but the specific composition and structure of ER-mitochondria tethering complexes are still not precisely understood. It is generally believed that they include a

variety of proteins, such as voltage-dependent anion channels (VDACs), inositol trisphosphate receptor (IP3R), and ER protein B cell receptor-associated protein 31 (Bap31). The interactions of these proteins binding mitochondria and the ER are shown in Figure 1.

In Table 1, we summarize the components of the tethering complexes and their respective functions. The formation of ER-mitochondria tethering complexes depends on the ternary complex consisting of the Ca^{2+} channel IP3R located in the ER, a VDAC and the mitochondrial chaperone glucose regulatory protein 75.^[30] IP3R (inositol 1,4,5-triphosphate receptor) can interact with FUN14 domain-containing protein 1 (FUNDC1) to adjust mitochondrial calcium homeostasis. In addition, Bap31 located in the ER interacts with Fis1,^[31] which can regulate apoptosis and mitochondrial fission; and ER-bound protein inverted form-2 can interact with mitochondrial protein dynamin-related protein 1 (DRP1),^[17] which regulates mitochondrial fission. Recently, scientists found a candidate protein, the tail-anchored OMM protein SYNJ2BP. It can greatly increase the contact between mitochondria and the rough ER when overexpressed. Ribosomal binding protein 1 (RRBP1) is a binding partner of SYNJ2BP that was identified by immunoprecipitation mass spectrometry.^[20] It has been reported that SYNJ2BP participates in mitophagy.^[32] Whether the combination of RRBP1 and SYNJ2BP can regulate mitophagy is unclear, and their specific functions still need to be explored. Additionally, it has been reported that vesicle-associated membrane protein-related protein B (VAPB) can interact with many proteins to connect the ER to various organelles, including mitochondria. Studies have shown

Table 1: Proteins of endoplasmic reticulum-mitochondria tethering complexes.

Protein	Localization	Function
PACS-2	ER	Interacts with calnexin, regulates ER-mitochondrial communication ^[10]
PERK	ER	Binds to Bip, regulates the UPR ^[11]
MFN2	ER, MAM, mitochondria	Regulates mitochondrial fusion and mitochondrial fission, regulates Ca ²⁺ homeostasis ^[12,13]
PDZD8	MAM	Tethers the ER with mitochondria, regulates Ca ²⁺ dynamics ^[14]
BAP31	ER, MAM	Promotes Ca ²⁺ release from the ER, promotes apoptosis ^[15]
INF2	ER	Interacts with DRP1, regulates mitochondrial fission ^[16,17]
VAPB	ER MAM	Interacts with PTPIP51, regulates Ca ²⁺ homeostasis ^[18]
ORP5/8	ER	Interacts with PTPIP51 to tether the ER with mitochondria ^[19]
RRBP1	ER	Interacts with the ER protein SYNJ2BP, increases mitochondrial contacts with the rough ER ^[20]
IP ₃ R	ER, MAM	Ca ²⁺ channel, regulates Ca ²⁺ in mitochondria ^[21]
FIS1	MAM, mitochondria	Participates in mitochondrial fission, promotes apoptosis, bridges mitochondria and the ER ^[15]
DRP1	MAM, mitochondria	Regulates mitochondrial fission ^[16,22]
VDACs	MAM, OMM	Major mitochondrial Ca ²⁺ transport channel ^[21]
GRP75	ER, MAM, cytosol	Chaperone, forms link between IP3R and VDAC and promotes Ca ²⁺ transfer from ER to mitochondria ^[23]
FUNDC1	MAM, mitochondria	Activator of hypoxia-induced mitophagy, participates in mitochondrial quality control ^[24]
PTPIP51	MAM, OMM	Interacts with VAPB and ORP5/8, regulate Ca ²⁺ homeostasis, tether ER with mitochondria ^[25]
SYNJ2BP	MAM, OMM	Interacts with the ER protein RRBP1, increases mitochondrial contacts with rough endoplasmic reticulum ^[20]
MFN1	MAM, mitochondria	Participates in mitochondrial fusion and mediates mitochondrial tethering ^[26]
SIGMAR1	ER, MAM	Chaperone, stabilizes IP3Rs at the MAM ^[27]
CNX	ER, MAM	Chaperone, Ca ²⁺ sensing proteins ^[10,28]
Bip	ER, MAM	Chaperone, participates in ER stress ^[29]

PACS-2: Phosphofurin acidic cluster sorting protein 2; PERK: Protein kinase R-like endoplasmic reticulum kinase; MFN2: Mitofusin-2; BAP31: B cell receptor-associated protein 31; VAPB: Vesicle-associated membrane protein-related protein B; DRP1: Dynamin-related protein 1; VDAC: Voltage-dependent anion channel; GRP75: Glucose regulatory protein 75; FUNDC1: FUN14 domain-containing protein 1; PTPIP51: Protein tyrosine phosphatase-interacting protein 51; SYNJ2BP: Synuclein 2 binding protein; MFN1: Mitochondrial fusion protein 1; CNX: Chaperone protein calmodulin; BiP: Binding immunoglobulin protein; ER: Endoplasmic reticulum; MAM: Mitochondrial-associated membranes; OMM: Outer mitochondrial membrane; UPR: Unfolded protein response.

that VAPB binds mitochondria and the ER mainly to interact with protein tyrosine phosphatase-interacting protein 51 (PTPIP51).^[33] Changes in these proteins are related to disease, and mutations or disrupted VAPB-PTPIP51 tethers have been reported in amyotrophic lateral sclerosis.^[34] PTPIP51 can also bind mitochondria to the ER by interacting with the oxysterol-binding proteins ORP5 and ORP8.^[35] MFN2 located in the ER can form molecular chaperone complexes with other molecules in mitochondria, such as MFN1 and MFN2. PERK knock-down can disrupt ER morphology and reduce ER-mitochondrial contact points, proving that MFN2 can also interact with the ER transmembrane protein PERK to connect the ER and mitochondria.^[36] However, as recent studies in different laboratories have shown that the loss of MFN2 does not reduce ER-mitochondrial exposure, whether MFN2 is a component of the complex is questioned.^[12,37-39] A recent study found that a novel protein candidate located at the rough ER-mitochondrial contact site, PACS-2, which is a multifunctional sorting protein in the cytoplasm, also plays a role in binding mitochondria and the ER.^[40] It sometimes interacts with

the chaperone protein calmodulin (CNX) to form complexes. In addition, according to Yusuke Hirabayashi's research, PDZD8 represents a new ER-mitochondria tethering protein in mammalian cells that is involved in the regulation of dendritic Ca²⁺ dynamics.^[41] There are also some chaperone proteins, such as sigma-1 receptor (SIG1R) and CNX, that are directly or indirectly involved in the process of closely linking the ER and mitochondria. SIG1R can form a complex with the chaperone protein binding immunoglobulin protein (BiP) to participate in Ca²⁺ regulation and cell survival.^[42] In short, the contact between mitochondria and the ER depends on the interaction of many proteins, and the components of the binding complex are constantly updated as research progresses.

Mechanisms related to ER-mitochondria tethering complexes during renal I/R injury

ER-mitochondria tethering complexes are platforms upon which many reactions occur. Components of these complexes are involved in many physiological processes,

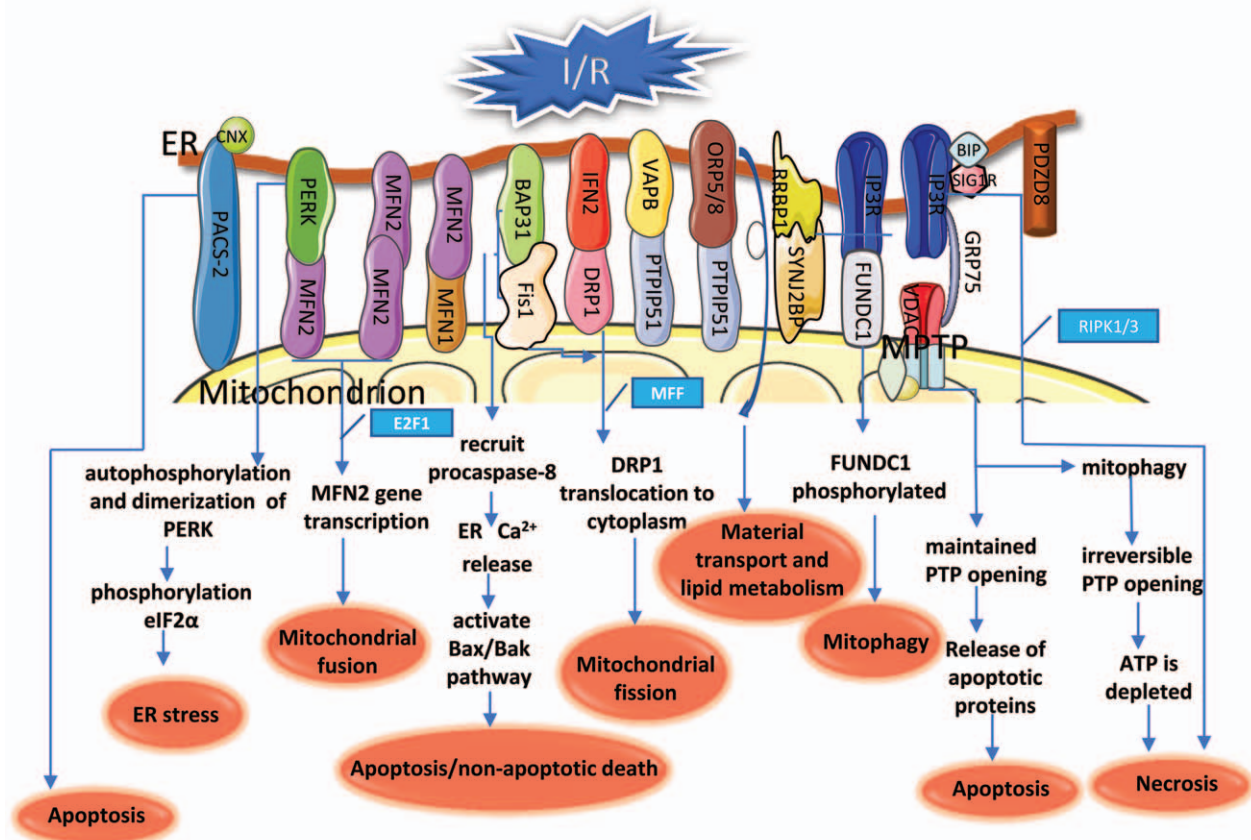


Figure 2: Role of endoplasmic reticulum (ER)-mitochondria tethering complexes in renal ischemia-reperfusion (I/R) injury. ER-mitochondria tethering complexes are involved in many mechanisms in renal I/R injury, such as mitochondrial dynamics, mitophagy, ER stress, and cell death. ATP: Adenosine triphosphate; eIF2 α : Eukaryotic initiation factor 2 α ; E2F1: E2F transcription factor 1; Bax/Bak: Members of the Bcl-2 family and core regulators of the intrinsic pathway of apoptosis; MFF: Mitochondrial fission factor; PTP: Mitochondrial permeability transition pore; RIPK1/3: Receptor-interacting protein kinase 1/3.

such as cell death and mitophagy.^[43] In this section, we illustrate the prime functionalities of ER-mitochondria tethering complexes in renal I/R injury [Figure 2].

Mitophagy

Autophagy has long been considered a non-selective, massive degradation pathway.^[44,45] Mitophagy is a selective form of autophagy that specifically eliminates excess or damaged mitochondria. To date, mitophagy defects have been associated with a variety of human diseases, such as neurodegenerative diseases, metabolic diseases, I/R injury, and so on.^[46-48] ER-mitochondria tethering complexes have been discovered to be a platform for mitophagy. Many reports have revealed that autophagosome membranes may mainly come from the ER.^[49,50] Imaging data reveal that autophagosomes form at ER-mitochondria contact sites.^[51] Hamasaki *et al*^[51] used green fluorescent protein to label the autophagosome marker ATG14 and then used three-color imaging to detect the site of autophagosome formation. Under fed conditions, the ATG14 complex seems to diffuse within the ER membrane; after induction of autophagy by starvation, the complex assembles at several specific points before autophagosome formation. Gelmetti *et al*^[52] found the mitochondrial quality control protein PTEN-induced

kinase 1 (PINK1) and the preautophagic protein Beclin-1 were in close contact. Both of them could increase ER-mitochondrial coupling and promote autophagosome formation after induction. Additionally, related studies have shown that components of binding complexes such as DRP1, Fis1, MFN, and so on can interact with PINK1 and parkin molecules to affect mitochondrial fission and mitophagy.

At present, the recognized mechanisms of mitophagy can be divided into two categories according to whether they depend on ubiquitin (Ub). The Ub-dependent mechanism is clearer and plays a major role in diseases.

The Ub-dependent mechanism can be divided into parkin-mediated mitophagy and parkin-independent mitophagy according to whether parkin is involved. Parkin-mediated mitophagy occurs as follows: in healthy mitochondria with active membrane potential (\uparrow mt $\Delta\Psi$), PINK1 is introduced into the inner mitochondrial membrane and is flipped constitutively by proteolysis. However, after impaired mitochondrial activity and loss of membrane potential (\downarrow mt $\Delta\Psi$), PINK1 becomes stabilized on the OMM, allowing the recruitment of the E3-Ub ligase parkin. Parkin activation results in ubiquitination of OMM proteins, which serves as an “eat me” signal for neonatal

autophagosome recognition. Whether MFN2 works during this process is controversial. Chen and Dorn found that PINK1 can phosphorylate the Thr111 and Ser442 sites of MFN2 to bind MFN2 to parkin, and mutations in these phosphorylation sites can prevent the two from binding.^[53] If PINK1 on the outer membrane of the mitochondria can phosphorylate MFN2 stably, it can convert MFN2 into a receptor that parkin can bind, allowing parkin to interact with many mitochondrial ubiquitinated substrates. Bhatia *et al* demonstrated that PINK1-mediated downstream MFN2 phosphorylation promotes parkin recruitment to damaged mitochondria in the kidney.^[54] However, Pickrell and Youle found that parkin can also translocate to mitochondria in *MFN1/MFN2* knockout cells, suggesting that *MFN2* does not participate in the translocation.^[55] However, in mice, the loss of cardiomyocytes and neurons and macrophage-specific depletion of *MFN2* leads to defects in mitochondrial parkin localization. There is no parkin translocation in *MFN2*-deleted neurons,^[56] and macrophage-specific *MFN2* deletion exacerbates renal fibrosis.^[54] Therefore, the role played by *MFN2* in mitophagy cannot be negated, but as for the specific translocation mechanism of parkin, further exploration is needed.

On the other hand, parkin-independent mitophagy can be divided into receptor-mediated mitophagy, lipid-mediated mitophagy, and Ub-mediated mitophagy. (1) In receptor-mediated mitophagy, receptors localize on the mitochondrial outer membrane and contain an LIR domain that binds directly to the LC3 protein, including BCL2 interacting protein 3 (BNIP3), BNIP3-like (BNIP3L/NIX), and FUNDC1, allowing phagocytes to recruit damaged mitochondria and cause them to be degraded.^[57,58] (2) In lipid-mediated mitophagy,^[59] IMM cardiolipin (CL) can be translocated to the OMM through the action of PLS3. Once it reaches the OMM, CL binds to LC3A to recruit phagocytic molecules and remove damaged mitochondria. (3) In Ub-mediated mitophagy,^[59] E3 ligases that can localize ubiquitinated OMM proteins on damaged mitochondria for recruitment of phagocytic cells engulf and degrade damaged mitochondria. Alternatively, Ub binding proteins bind to K63 Ub chains through their UBA (Ub binding-associated) domain and induce mitophagy of damaged mitochondria. Currently, receptor-mediated mitophagy has become another hotspot in the study of mitophagy. The role of mitophagy in renal I/R injury has been validated.

Regarding renal I/R injury, Dong *et al* found that ischemic AKI was exacerbated in PINK1 and PARK2 single- and double-knockout mice. Mechanistically, PINK1 and PARK2 deficiency can enhance mitochondrial damage, reactive oxygen species production, and inflammatory responses. Also, PINK1-parkin-mediated mitophagy can play a protective role in septic AKI.^[60] These results indicate that PINK1-PARK2-mediated mitophagy plays an important role in mitochondrial quality control, renal tubular cell survival, and renal function during AKI.^[61] Tang *et al*^[62] demonstrated that functionally silencing *Bnip3* by specific short hairpin RNAs in cultured renal tubular cells could reduce oxygen-glucose deprivation/reoxygenation (OGD-R)-induced mitochondrial phagocytosis and enhance OGD-R-induced cell death.

In vivo, BNIP3 gene knockout aggravated renal I/R damage in the form of more severe renal insufficiency and tissue damage, indicating that BNIP3-mediated mitochondrial phagocytosis plays a vital role in mitochondrial quality control and renal tubular cell survival. In addition, FUNDC1 becomes enriched on MAMs by interacting with the ER-resident protein CANX (cadherin) under hypoxic conditions. As mitophagy proceeds, FUNDC1 is separated from CANX and preferably binds with DRP1 to drive mitochondrial fission in response to hypoxic stress.^[63] Related studies show that FUNDC1-mediated mitophagy is mainly activated through ischemic preconditioning and provides protection against reperfusion injury.^[64-66]

Recently, it has been demonstrated that FUNDC1 can combine with IP3Rs to form ER-mitochondrial microdomains, thereby regulating ER-mitochondrial Ca²⁺ exchange, mitochondrial fission, and mitophagy.^[67,68] These studies were the first in which the mitochondrial-related protein FUNDC1 was identified as a component of ER-mitochondrial tethering complexes. At the same time, FUNDC1 can also activate mitophagy through phosphorylation. Other related studies have further found that the autophagy proteins BECN1/beclin 1, which are closely related to autophagy, are components of the complexes and enhance the interaction of the ER and mitochondria and increase the activity of mitochondria.^[52]

In conclusion, ER-mitochondrial tethering complexes can serve as a platform for autophagosome formation and autophagy survival mechanisms. They can also help relieve renal I/R injury by regulating mitophagy. The ER-mitochondria tethering complexes play important direct and indirect roles in mitophagy.

Mitochondrial fission and fusion

We observed mitochondrial fission and fusion in renal I/R injury through microscopy. Brooks *et al*^[69] observed mitochondrial fragmentation in mouse proximal renal tubular cells in a renal ischemia/reperfusion model. The purpose of fission is to produce more mitochondrial mitochondria to meet the needs of renal tubular epithelial cells during the ischemic and/or reperfusion stage. The ER-mitochondria tethering complexes are also rich in proteins related to controlling mitochondrial fission^[70] and dynamics.^[71] ER-mitochondrial contact is a site of fusion and fission processes and autophagosome formation. Therefore, in addition to participating in mitophagy, tethering complexes are also involved in the regulation of mitochondrial morphology and biogenesis and are maintained through a balance between fission and fusion events.^[72]

Members of the dynein family of guanosine triphosphatases (GTPases) mediate mitochondrial fission.^[73] In yeast, the major regulator is dynein-like protein 1, and in mammalian cells, the major regulator is DRP1 located on the OMM. DRP1 has a GTPase domain, interacts with three mitochondrial binding proteins (Mid49, Mid51, and mitochondrial fission factor [MFF]) at the cleavage site and acts as a mechanical enzyme that contracts and cuts mitochondria.^[74-76] DRP1 is a key mitochondrial fission

protein that translocates to mitochondria in the early stages of renal tubular injury, and knockdown of DRP1 by siRNA as well as the newly identified DRP1 pharmacological inhibitor mdivi-1 can inhibit mitochondrial fragmentation and weaken apoptosis of renal tubular cells and acute renal injury. These results suggest the importance of DRP1 and mitochondrial fission in the development of renal I/R injury. The fission activation signal results in the oligomerization of DRP1 on the outer membrane of mitochondria. DRP1 binds to Fis1 and MFF to form a ring structure and mediates the isolation of dielectric mitochondria. Fission factors may cause DRP1 changes through four pathways, including calmodulin-dependent kinase (CamK) phosphorylation, cyclin B1-CDK1 phosphorylation, nitric oxide nitrosylation, and PKA phosphorylation; in addition, MARCH5 and parkin Ub ligase regulates DRP1 activation. In renal I/R injury, Bhargava P *et al* found that inhibition of the fission protein DRP1 may improve ischemic kidney injury by blocking mitochondrial fission.^[11] Heather M. Perry *et al* found in a genetic mouse model suffering from renal I/R that proximal tubule-specific DRP1 deletion inhibited renal damage and inflammation and promoted the recovery of epithelial cells.^[77] Related studies show that DRP1 preferentially accumulates at sites where the ER and mitochondria are in contact.^[78] Indeed, mitochondrial fission occurs at positions where ER tubules contact and constrict mitochondria.^[70] These constrictions facilitate the recruitment of DRP1, a major player in mitochondrial fission.^[79] This suggests that ER-mitochondria tethering complexes may play an active role in the early stages of mitochondrial fission by defining fission sites. Therefore, the positive effects of ER-mitochondria tethering complexes early in mitochondrial fission may aggravate renal I/R injury through DRP1 recruitment and contraction. In addition, mitochondrial fission may also be regulated by MFN in tethering complexes. However, MFN mainly plays a role in mitochondrial fusion. Mitofusin 1 (MFN1), mitofusin 2 (MFN2), and OPA1 are three key GTPases involved in mitochondrial fusion.^[80] The outer membrane fusion protein MFN2 and the ER derivatives of MFN1 and MFN2 stop at specific ER-mitochondrial contact sites, and under the regulation of E2F1, MFN2 gene transcription is enhanced, which in turn induces mitochondrial fusion. The interaction of MFN1 and MFN2 in the ER and mitochondria is essential for ER-mitochondrial binding.^[81] Therefore, inhibiting abnormal changes in key components of tethering complexes seems to reduce fatal mitochondrial fission to reduce kidney I/R damage.

Apoptosis and necrosis

Previously, acute tubular necrosis was considered the leading cause of AKI-related renal tubular epithelial cell death, as many experiments have shown that most cell death can be prevented by suppressing necrosis. However, recent experimental studies have shown that apoptosis is also involved in the pathogenesis of AKI. The renal I/R model shows a large number of apoptotic cells in the damaged renal tubules, in addition to activation of caspases.^[82,83] Stefan Grimm and his colleagues^[84] found that the outer membrane fission protein Fis1, which is related to apoptosis, can make physical contact with ER-

localized Bap31, which brings mitochondria and the ER near each other. This mechanism provides a theoretical basis for the role of Fis1 in cell death. Under stress, Fis1 is ectopically expressed on the outer membrane of the mitochondria. The preformed Fis1-Bap31 complex recruits procaspase-8 in response to ectopic expression of Fis1, and then caspase-8 cleaves Bap31 and stimulates ER Ca²⁺ release; Ca²⁺ activates cytochrome C release, activates Bax/Bak or stimulates non-apoptotic death pathways. There is no doubt that the dynamic structural and functional connections between mitochondria and the ER support a wide range of cellular functions, including the ability of cells to kill themselves through apoptosis or necrosis. It has been reported that one of the components of ER-mitochondria tethering complexes, the multifunctional sorting protein PACS-2, can also play a role in apoptosis.^[40] Under stress, such as the stress caused by renal I/R, PACS-2 translocates from the ER/cytosol to the mitochondrial-containing membrane portion, triggering mitochondrial cutting/truncating BH3 interaction domain death agonist (Bid) (tBid), releasing cytochromes c and f and ultimately leading to apoptosis through subsequent activation of caspase-3. The chaperone protein SIG1R can also regulate apoptosis. Related studies have shown that the reduction of SIG1R can promote apoptosis.^[42] As mentioned earlier, SIG1R can form complexes with the chaperone protein BiP in MAMs. When ER Ca²⁺ is depleted, SIG1R dissociates from BiP, which causes prolonged Ca²⁺ signals to enter mitochondria through IP3Rs, thereby offsetting ER stress.

Cyclophilin (Cyp) D-mediated mitochondrial permeability transition and receptor-interacting protein kinase (RIPK) 1/3-mediated necroptosis are the major pathways that regulate parenchymal cell death during renal I/R injury.^[85] Mitochondria are considered to be an integral part of necrotic and apoptotic processes and are the basis for renal tubular injury and cell death after I/R injury.^[86] For apoptosis and necrosis caused by mitochondrial permeability transition, mitochondrial permeability transition pore (mPTP) opening is induced by activation of CypD, ATP synthase complexes in the mitochondrial membrane, and the high cellular calcium concentration during renal I/R, which lead to mitochondrial permeability transition.^[87,88] Continuous opening will cause mitochondria to release catabolic hydrolases and activators of such enzymes. The release of these catabolic enzymes and the loss of mitochondrial bioenergy and redox functions eventually lead to cell necrosis and apoptosis and other forms of death.^[89,90] The mPTP is a macromolecular complex located on the mitochondrial membrane. VDAC, a component of ER-mitochondria tethering complexes, is a key protein of the mPTP that was identified previously. For RIPK1/3-mediated necroptosis,^[91] it has been shown that I/R mouse models lacking RIPK3 are protected from I/R injury in.^[85] Recent evidence demonstrates that *Ripk3* gene ablation eliminates reperfusion-induced up-regulation of IP3R and ER stress.^[92] A large number of studies^[93] on the myocardium have demonstrated that IP3R expression is up-regulated in response to I/R stress, leading to mitochondrial calcium overload. Subsequently, calcium overload will activate necrotic signaling in the reperfused heart via the CaMKII-mPTP^[94] or XO-ROS-mPTP^[92]

pathway. As mentioned earlier, IP3R is located on the ER membrane and interacts with other proteins to bind the ER and mitochondria together. These findings confirm that ER-mitochondria tethering complexes are required for Ripk3-induced necrosis in I/R injury. Additionally, mitochondrial CL is widely believed to prevent I/R injury by inhibiting apoptosis and necrosis.^[95,96] Because mitochondria cannot synthesize lipids, they rely on the ER for synthesis, and their transport relies on close ER-mitochondrial contact.

In conclusion, ER-mitochondria tethering complexes are important in the regulation of apoptosis and necrosis, and maintaining a balance of tethering complexes may become a treatment strategy for renal I/R injury.

ER stress

After acute ischemia or poisoning,^[97-100] renal epithelial cells can induce ER stress both *in vivo* and *in vitro*. In response to epithelial stress, unfolded or misfolded proteins accumulate in the ER, triggering the unfolded protein response (UPR).^[101-104] The UPR has three main pathways: the PERK, IRE1, and ATF6 pathways. It first restores normal cell function by stopping protein translation, degrading misfolded proteins, and activating signaling pathways that increase the production of chaperone proteins involved in protein folding. If these goals are not reached within a certain time frame or the interruption time is extended, then the UPR directly induces apoptosis. For example, as mentioned earlier,^[40] during renal I/R injury, prolonged ER stress causes PACS-2 to translocate to the ER and mitochondria and then activate caspase-3, leading to apoptosis.

ER-mitochondria tethering complexes, as physical structures that tightly bind mitochondria and the ER, provide a platform for crosstalk between them. Therefore, the tethering complexes are closely related to ER stress.^[105] In the early stages of ER stress, the number of binding proteins increases, promoting the transport of Ca^{2+} between the ER and mitochondria, which increases mitochondrial energy synthesis and provides energy for adaptive responses.^[22] PERK can gather on the ER and interact with MFN2 on the OMM to form a stent that supports close contact. Under stress, PERK activates the PERK/eIF2 α /ATF4 pathway through autophosphorylation and dimerization and regulates renal I/R injury by affecting autophagy and apoptosis. Similarly, related studies have shown that elimination of MFN2 can inhibit PERK activation and lead to ER stress.^[106] In addition, IRE1 can be enriched on mitochondria and ER-related membranes and promote cell survival by stabilizing mitochondrial Ca^{2+} concentration by inhibiting IP3R in tethering complexes.^[107] Under the action of tethering proteins, the ER and mitochondria can be in close contact. These contacts are rich in chaperone proteins such as SIG1R, CNX, and calreticulin. The transduction of stress signals depends on them.^[108,109] SIG1R can affect PERK, eIF2 α , and ATF4, stabilize the binding protein IP3R, reduce ER Ca^{2+} release, and stabilize the Ca^{2+} concentration at the mitochondrial ER contact site.^[110] Therefore, it can protect against ER stress.^[108]

Mitochondria and the ER can communicate in both directions due to the close contact between the two organelles. When adverse factors act on cells, the two organelles regulate each other to resist risks and maintain the homeostasis of the intracellular environment. ER stress maintains mitochondrial functional integrity through mitochondrial fission and fusion and clearance of damaged mitochondria. During renal I/R injury, Ca^{2+} overload results in ROS production, and ROS can phosphorylate the serine of DRP1, which causes DRP1 to accumulate on the OMM and promote mitochondrial fission. However, when the ER is overstressed, the ER can transmit stress signals to the mitochondria through close contact and cause apoptosis. Persistent ER stress causes a large amount of Ca^{2+} release from the ER. The released Ca^{2+} causes mitochondrial Ca^{2+} overload through the IP3R-VDAC1 channel between the two organelles, resulting in Bax and Bak oligomerization at the OMM. Then, the mPTP opens, inducing the release and activation of pro-apoptotic factors and ultimately apoptosis.^[111]

In summary, ER stress and the transmission of stress signals rely on the participation of ER-mitochondria tethering complexes. At present, many related studies have reported that inhibition of ER stress *in vivo* or *in vitro* can reduce renal I/R injury.^[112-114] This suggests that inhibiting ER stress may be a way to treat I/R injury. ER-mitochondria tethering complexes are non-negligible targets for improving injury by suppressing ER stress.

Mitochondrial material transport and lipid metabolism

The main feature of AKI in the human body is renal tubular epithelial cell damage.^[115] Recent experimental progress has shown that mitochondrial biogenesis in the injured environment can increase the production of ATP by generating new and functional mitochondria to continuously respond to the body's increased energy requirements during I/R,^[11] thereby reducing kidney damage and/or accelerating the recovery of AKI.^[116] Mitochondrial biogenesis is a complex process that requires the synthesis and input of proteins and lipids. There are many kinds of lipids and more than 1000 kinds of proteins in mitochondria. Mitochondria themselves can synthesize lipids such as phosphatidylethanolamine (PE), phosphatidylglycerol, and CL, but other lipids must be transported from the ER to mitochondria. Most mitochondrial proteins are encoded by nuclear genes, synthesized by nuclear ribosomes, folded via the ER, and shuttled into the mitochondria from the ER membrane. Therefore, mitochondrial material transport relies on the ER. In other words, ER-mitochondria tethering complexes tightly connect the ER and mitochondria to make material transport between the two organelles a reality. In addition, lipid synthesis, including synthesis of triacylglycerol, phosphatidylcholine (PC), and PE, requires enzymes related to the ER and mitochondria, and most of these key enzymes are present in the ER-mitochondria contact sites. For example, phosphatidylserine synthase is the key enzyme in PS synthesis, is located in the ER and can be converted into PE by phosphatidylserine decarboxylase in mitochondria. ER-mitochondria contact sites are the sites at which phosphatidylserine (PSS1) and PC are produced by PS. Likewise, one of the final enzymes

involved in PC synthesis, phosphatidylethanolamine N-methyltransferase 2,^[117,118] was found to be limited to ER-mitochondria contact sites.^[119] In addition, acyl-CoA/diacylglycerol acyltransferase 2, which catalyzes the synthesis of triacylglycerols and promotes the formation of lipid droplets, is also located at ER-mitochondria contact sites.^[120] ACAT1/SOAT1, as a multi-membrane transferase, is most abundant at this specific cellular location.^[71,121] Therefore, ER-mitochondria tethering complexes bind mitochondria and the ER and bring them into close contact, which is essential for material transport and lipid metabolism.

Concluding remarks and prospects

In conclusion, ER-mitochondria tethering complexes and the close contact between mitochondria and the ER are involved in many key pathophysiological mechanisms of renal I/R injury and are undoubtedly a key component in these processes. However, we cannot deny the complexity of the relationship between the ER and mitochondria. At present, scientists have put forward new views on the connection between the ER and mitochondria. For example, in the new field of microproteins, the Saghatelian group led by Dr. Uri Manor discovered a mitochondrial microprotein, the human 54-amino acid PIGB opposite strand 1 (PIGBOS) microprotein, that can regulate the ER stress response.^[122] In addition, an urgent issue is that we need to fully understand the binding between mitochondria and the ER and the specific interaction mechanisms of tethering proteins, especially in the context of renal I/R injury. Similarly, a better understanding of the functional regulation of ER-mitochondria tethering complexes is critical for advancing drug development.

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Conflicts of interest

None.

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