Mitochondrial quality control mechanisms as molecular targets in cardiac ischemia–reperfusion injury

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Abstract
Mitochondrial damage is a critical contributor to cardiac ischemia/reperfusion (I/R) injury. Mitochondrial quality control (MQC) mechanisms, a series of adaptive responses that preserve mitochondrial structure and function, ensure cardiomyocyte survival and cardiac function after I/R injury. MQC includes mitochondrial fission, mitochondrial fusion, mitophagy and mitochondria-dependent cell death. The interplay among these responses is linked to pathological changes such as redox imbalance, calcium overload, energy metabolism disorder, signal transduction arrest, the mitochondrial unfolded protein response and endoplasmic reticulum stress. Excessive mitochondrial fission is an early marker of mitochondrial damage and cardiomyocyte death. Reduced mitochondrial fusion has been observed in stressed cardiomyocytes and correlates with mitochondrial dysfunction and cardiac depression. Mitophagy allows autophagosomes to selectively degrade poorly structured mitochondria, thus maintaining mitochondrial network fitness. Nevertheless, abnormal mitophagy is maladaptive and has been linked to cell death. Although mitochondria serve as the fuel source of the heart by continuously producing adenosine triphosphate, they also stimulate cardiomyocyte death by inducing apoptosis or necroptosis in the reperfused myocardium. Therefore, defects in MQC may determine the fate of cardiomyocytes. In this review, we summarize the regulatory mechanisms and pathological effects of MQC in myocardial I/R injury, highlighting potential targets for the clinical management of reperfusion.

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1. Introduction

The heart is a strong muscular pump that enables tissue and organ perfusion. Therefore, a continuous supply of fresh blood is vital for cardiac function. In coronary artery disease, plaques or thrombi induce rapid occlusion, which restricts blood flow to the heart. The primary effect of coronary artery disease is substantial cardiomyocyte death, which prevents the heart from effectively pumping blood to vital organs. Emergency coronary recanalization through a coronary artery bypass graft or percutaneous transluminal coronary intervention can limit cardiomyocyte death. However, laboratory experiments have revealed that a significant proportion of cardiomyocyte death occurs during the first few minutes of reperfusion, in what is known as myocardial ischemia/reperfusion (I/R) injury.

Several molecular mechanisms have been proposed to explain the pathological alterations in cardiac I/R injury, including rapid reactive oxygen species (ROS) release, calcium overloading, energy depletion, mitochondrial dysfunction and programmed cell death activation. Mitochondria have been recognized as key triggers of cardiac I/R injury. First, mitochondria are abundant in cardiomyocytes and determine more than 90% of their energy supply. Second, mitochondria can promote cardiomyocyte death by inducing apoptosis or necroptosis in the reperfused myocardium. Third, other pathological situations such as calcium overload, oxidative stress, endoplasmic reticulum stress and immune responses are triggered by, integrated with or augmented by mitochondrial dysfunction. Therefore, it is highly important to understand the regulatory mechanisms and biochemical contributions of mitochondrial dysfunction in cardiac I/R injury.

In response to stressful conditions, the mitochondria can activate mitochondrial quality control (MQC) to preserve mitochondrial structure and function. MQC is a group of adaptive responses that regulate mitochondrial protein turnover, mitochondrial fusion, mitochondrial fission and mitophagy (Fig. 1). The main consequences of MQC are the rapid removal of defective mitochondrial debris and the timely replenishment of the mitochondrial network. These biophysical processes protect the mitochondria from damage and therefore attenuate the vulnerability of cardiomyocytes to I/R injury.

This review summarizes how MQC protects the myocardium from I/R injury, focusing on mitochondrial fission, fusion, mitophagy and mitochondria-dependent programmed cell death (Fig. 1). Recent findings on the contribution of mitochondrial

![Figure 1](image-url)
mitochondria and thus is indispensable for cardiomyocyte viability during cardiac I/R injury. We hope the information presented here will provide new insights into the molecular pathways underlying mitochondria-related myocardial damage in I/R, and will offer useful targets for cardioprotection.

2. Mitochondrial fission

Although mitochondria were originally believed to be static, it is now well accepted that mitochondria are dynamic organelles that are constantly reshaped by fusion and fission. Mitochondrial fission can remove dysfunctional mitochondria from cardiomyocytes, and the extent of mitochondrial fission is largely determined by the metabolic needs of the cells. Proper mitochondrial fission generates many offspring and thus provides the opportunity for MQC and determines cardiovascular mitochondrial homeostasis.

Mitochondrial fission is regulated by dynamin-related protein 1 (DRP1) and its receptors (Fig. 2), including mitochondrial fission factor (MFF), mitochondrial fission one protein (FIS1), mitochondrial dynamics protein of 49 kDa (MID49) and mitochondrial dynamics protein of 51 kDa (MID51). Under physiological conditions, DRP1 is primarily free in the cytoplasm in an inactive form that cannot bind to its receptors, which are anchored to the outer mitochondrial membrane (OMM). Therefore, mitochondrial fission is relatively low under normal conditions. Interestingly, under stressful conditions, DRP1 undergoes conformational changes through post-transcriptional modifications including ubiquitination, acetylation and phosphorylation.

Multiple post-transcriptional modifications of DRP1 have been discovered through mass spectrometry-based proteomics, as shown in the online open database PhosphoSitePlus and a thorough recent review by Jhun et al. Two post-transcriptional modification sites on DRP1 have been well explored: the phosphorylation sites at Ser616 and Ser637. Ser616 phosphorylation promotes DRP1 oligomerization around the OMM, a prerequisite for the formation of a potential mitochondrial fission ring. Phosphorylation at Ser637 has the opposite effect of impairing DRP1 oligomerization and therefore preventing mitochondrial fission.

Post-transcriptional modification also occurs on DRP1 receptors, including MFF, FIS1, MID49 and MID51. Phosphorylation of MFF at Ser146 enhances its affinity for DRP1, and this alteration has been reported in cardiac microvascular I/R injury. Interestingly, the N-terminal arm of FIS1 auto-inhibits its access to DRP1, whereas phosphorylation of this N-terminal arm enhances the binding of FIS1 to DRP1. The effects of MID49/MID51 phosphorylation on DRP1-induced mitochondrial fission in cardiac I/R injury have not been described.

In the context of cardiac I/R injury, mitochondrial fission is associated with mitochondrial damage and cardiomyocyte death (Fig. 2). Following cardiac I/R injury, DRP1 phosphorylation at Ser637 decreases, so the mitochondrial localization of DRP1 increases. Consequently, excessive mitochondrial fission occurs, which induces cytosolic calcium overload and thus promotes cardiomyocyte death and myocardial contractile dysfunction. In contrast, DRP1 phosphorylation at Ser616 increases after myocardial I/R injury, and ROS production and cardiomyocyte oxidative stress are elevated. The expression of MFF and its post-transcriptional phosphorylation at Ser146 are found to be augmented in a mouse model of cardiac microvascular I/R injury, and genetic ablation of Mff is reported to attenuate mitochondrial DNA (mtDNA) breaks, restore mtDNA copying and transcription, improve mitochondrial respiration and enhance endothelial viability.

The increased fission under cardiac I/R injury is known to induce other pathological alterations, including the reduction of ATP levels, the translocation of cytochrome c (Cyt-c) from the mitochondria to the cytoplasm, the opening of the mitochondrial permeability transition pore (mPTP) and the dissipation of the mitochondrial membrane potential; these effects are coupled with...
caspase-3 activation and cardiomyocyte apoptosis. Of note, the cardiomyocyte antioxidant capacity, as reflected by the levels of superoxide dismutase two and heme oxygenase 1, is also found to be altered by mitochondrial fission, although the mechanism is unknown. Moreover, autophagy, a procedure that degrades damaged intracellular components, is reported to be drastically repressed by mitochondrial fission, as demonstrated by the reduced LC3II/I ratio, beclin-1 expression and ATG5/7 expression. In vivo, the extent of mitochondrial fission is found to correlate positively with the size of the myocardial infarction and negatively with cardiac function measures such as the left ventricular ejection fraction and left ventricular fractional shortening. These results illustrate the sufficiency of mitochondrial fission to promote myocardial I/R injury.

On the other hand, genetic or pharmacologic blockades of mitochondrial fission can protect the reperfused heart. Mdivi-1 pharmacologically inhibits mitochondrial fission by preventing DRP1 translocation to the mitochondria, and thus reduces serum cardiac troponin I levels and lactate dehydrogenase activity. Mdivi-1 treatment primarily improves mitochondrial function by blocking mPTP opening and stabilizing the mitochondrial membrane potential. Mdivi-1 treatment can also partly reverse mitochondria-induced apoptosis by suppressing Cyt-c release and caspase-9 activation. Ding and coworkers observed that Mdivi-1 treatment increases the activity of the antioxidant enzyme manganese superoxide dismutase and reduces the content of malondialdehyde, indicating that mitochondrial fission is also associated with the redox status.

Mitochondrial fission influences a variety of cardiac protective pathways, including the protein kinase B (PKB), extracellular-signal-regulated kinase (ERK), 5’-adenosine monophosphate-activated protein kinase (AMPK) and nitric oxide pathways. Several of these proteins have been identified as upstream regulators of the post-transcriptional modifications of DRP1 and its receptors. For instance, ERK and AMPK can attenuate DRP1 phosphorylation at Ser616, whereas PKB can promote DRP1 phosphorylation at Ser637. The above data indicate that mitochondrial fission is a complex and progressive process involving either positive or negative feedback signals between various signaling pathways. However, several critical events should be emphasized. First, Mdivi-1 can improve cardiac function when it is given during ischemia and at the onset of reperfusion, but to a lesser extent than when it is administered before ischemia. One possibility is that inhibiting physiological mitochondrial fission impairs cardiac function, whereas pathological mitochondrial fission primarily takes place after ischemia or reperfusion. Second, the inhibition of fission attenuates apoptosis but exacerbates necroptosis in cardiomyocytes. This unexpected phenomenon seems to be present in Mdivi-1-injected mice, but not in Drp1-deleted or Mff-depleted mice. Thus, it is possible that Mdivi-1 is not a specific blocker of mitochondrial fission. Actually, several findings have suggested that Mdivi-1 can repress mitophagy, a protective pathway that prevents cell death by impeding apoptosis or necroptosis. Lastly, Mdivi-1 treatment following ischemia primarily seems to reverse cardiac diastolic dysfunction, as evidenced by the improved left ventricular developed pressure and lower left ventricular end diastolic pressure after such treatment. Thus, careful attention is needed when interpreting studies in which Mdivi-1 has been used to inhibit mitochondrial fission in myocardial I/R injury.

3. Mitochondrial fusion

In contrast to mitochondrial fission, fusion is a process that integrates several mitochondrial fractions into long filamentous mitochondria. Mitochondrial fusion can be divided into three distinct steps: tethering, outer membrane fusion and inner membrane fusion. Like mitochondrial fission, mitochondrial fusion is regulated by large guanosine triphosphatases (GTPases). The transmembrane GTPases mitofusin one and 2 (MFN1/2) are OMM-localized proteins, whereas the dynamin-like GTPase optic atrophy 1 (OPA1) promotes IMM intermingling. Structurally, MFN1 and MFN2 on two physically contacting mitochondria promote homotypic or heterotypic coordination to stimulate OMM fusion. The long isoform of OPA1 (L-OPA1) triggers IMM interactions between two mitochondria, resulting in the formation of the short isoform of Opa1 (S-OPA1) with the help of the proteases yeast mitochondrial escape one like one ATPase (YME1L1) and OMA1 zinc metalloproteinase (OMA1).

Most experimental evidence indicates that mitochondrial fusion protects cells during stress by two independent mechanisms. First, fusion offsets the effects of excessive mitochondrial fission and thus limits fission-initiated mitochondrial apoptosis. Second, fusion generates a long, shared electrochemical potential within the mitochondrial network, enhancing the timely detection of damaged parts in the mitochondrial mass. Fusion also equilibrates mitochondrial proteins, lipids, metabolites and mtDNA, which is thought to alleviate the local stress response and restore mitochondrial homeostasis.

Figure 3 Mitochondrial fusion is controlled by outer mitochondrial membrane (OMM)-localized mitofusin 2 (MFN2) and inner mitochondrial membrane (IMM)-localized optic atrophy 1 (OPA1). Increased mitochondrial fusion inhibits mitochondrial fission, sustains mitochondrial potential, promotes mitochondrial bioenergetics and suppresses mitochondrial apoptosis.
While fused mitochondria may be protective under physiological conditions, the involvement of fusion-related factors in cardiac I/R injury is the subject of hot debate. First, Mfn1-null mice are healthy and fertile, whereas Mfn2-null mice die soon after birth. Mfn1 deletion seems to have little influence on cardiac function under either physiological or pathological conditions, whereas Mfn2-deficient hearts exhibit extensive mtDNA breaks and mitochondrial damage. More surprisingly, cardiomyocyte-specific Mfn1-knockout mice display a normal respiratory repertoire and are protected from mitochondrial depolarization. In addition, Mfn1-knockout cardiomyocytes exhibit improved viability in a hydrogen-peroxide-induced oxidative stress microenvironment due to their reduced mPTP opening rate, suggesting that Mfn1 deletion may protect cardiomyocytes from oxidative-stress-induced injury. In contrast, Mfn2 deficiency in cardiomyocytes promotes mPTP opening, augments ROS production and triggers cell death. In a hypoxia/reoxygenation-mimicked I/R injury model in vitro, Mfn2 silencing sensitizes H9C2 cells to apoptosis, and this process could be partly reversed through the inhibition of caspase-9 or the overexpression of BCL-x(L). In accordance with the effects of cardiomyocyte-specific Mfn2 ablation, Mfn1/ Mfn2 double deletion causes defective mitochondria to accumulate and exhibit an unfolded protein response. These data may reflect an additional function of Mfn1 that has not yet been documented. However, relatively low levels of Mfn1 have been detected in many tissues, especially the brain, which may explain why Mfn1 loss is not so detrimental to cardiac function. This concept requires additional studies for verification. Mfn2 and Mfn1 may exert completely different effects on cardiomyocyte viability upon cardiac I/R injury, despite their similar functionality in promoting mitochondrial fusion.

Unlike the effects of Mfn1 and Mfn2, the effects of OPA1 on cardiomyocyte fate and mitochondrial function have been well established. The heart-specific knockdown of Opa1 increases mitochondrial morphometric heterogeneity and ultimately induces ventricular dilation with irreversible contractile dysfunction. In myocardial I/R injury, OPA1 expression is found to be reduced, while the genetic activation of OPA1 suppresses mitochondrial fission and cardiomyocyte death. Knocking out Opa1 expands the infarction size and induces cardiac dysfunction in reperfused hearts. Reperfusion induces the self-cleavage and activation of OMA1, and cleaved OMA1 accelerates the conversion of L-OPA1 to S-OPA1, leading to mitochondrial fragmentation, Cyt-c release and apoptosis. OPA1 dysregulation impairs mitochondrial bioenergetics and exacerbates oxidative stress. Of note, other molecular mechanisms may account for OPA1-induced cardioprotection. For example, OPA1 is found to enhance myocardial fatty acid utilization and thus attenuate ROS generation and sustain the mitochondrial morphology in failing hearts. However, this finding has not been validated in the process of cardiac I/R injury.

Like DRP1, Mfn1/2 can be post-transcriptionally phosphorylated by a number of kinases. However, the phosphorylation of Mfn1/2 partially reduces their GTPase activities and thus abolishes their ability to induce mitochondrial fusion. Mfn1 phosphorylation at Ser86 by beta II protein kinase C (bIIPKC) leads to a buildup of mitochondrial fragments in heart failure. In addition, mitogen-activated protein kinase/ERK phosphorylates Mfn1 at T562, thereby reducing its efficiency in oligomerization and mitochondrial tethering but increasing the susceptibility to BAX-induced mitochondrial apoptosis. Similarly, MFN2 phosphorylation by PTEN-induced putative kinase protein 1 (PINK1) facilitates depolarization-induced PARKIN translocation onto mitochondria, thus promoting mitophagy and reducing the accumulation of morphologically and functionally abnormal mitochondria. In contrast, after exposure to stress, MFN2 is primarily phosphorylated and then degraded by c-Jun N-terminal kinase, which impairs mitochondrial fusion and enhances cell death. In the context of cardiac I/R injury, although post-transcriptional modifications of Mfn1/2 have not been confirmed, Mfn1/2 protein levels are significantly downregulated.

Of note, unlike Mfn1/2, OPA1 is primarily regulated at the protein level by two mechanisms: redox status and mitochondrial proteolytic enzyme activity. OMA1 and YME1L1, which are mainly upregulated by stress or the mitochondrial unfolded protein response, have been acknowledged as upstream inducers of OPA1 degradation during cardiac I/R injury. In addition, mitochondrial ROS levels are found to correlate with the extent of proteolytic processing of OPA1, while the scavenging of mitochondrial ROS is reported to prolong the protein stability of OPA1 in cardiomyocytes. OPA1 transcription in cardiomyocytes is activated by STAT3 and RelA, which form a supercomplex that binds to the promoter region of OPA1. Although STAT3 has not been observed to transcriptionally modify OPA1 in cardiac I/R injury, STAT3 activity is significantly downregulated in the reperfused heart. This downregulation, together with OMA1/YME1L1-induced OPA1 degradation, may further reduce OPA1 expression during myocardial I/R injury. Additionally, in hearts under pressure overload or hyperglycemic conditions, OPA1 is found to be hyperacetylated and O-GlcNAcylated, respectively. These structural modifications reduce the GTPase activity of OPA1, leading to mitochondrial morphological disorder and cardiomyocyte death. It would be interesting to explore the post-transcriptional hyperacetylation and O-GlcNAcylation of OPA1 in myocardial I/R injury.

Several drugs and gene-modifying technologies have been created to restore mitochondrial fusion. Sevoflurane-induced anesthetic postconditioning has been demonstrated to reduce cardiac I/R injury in basic research and clinical surgery. Interestingly, the benefits of sevoflurane are attributed to the upregulation of OPA1 and Mfn2 in hypoxia/reoxygenation-treated neonatal rat cardiomyocytes. OPA1 and Mfn2 levels are also found to be induced by vagal nerve stimulation, which improves mitochondrial dynamics in the ischemic myocardium. Epigallocatechin gallate effectively inhibits OPA1 degradation by OMA1, and therefore maintains mitochondrial morphological homeostasis in reperfused hearts. Melatonin transcriptionally upregulates OPA1 expression through the AMPK pathway and thus increases the resistance of mitochondria and cardiomyocytes to I/R injury. Based on this information, preserving mitochondrial fusion through Mfn2 activation or OPA1 stabilization is critical when designing cardioprotective therapies for myocardial I/R injury.

4. Mitophagy

Mitochondrial components are eventually recycled through a specialized autophagic pathway known as mitophagy. Mitophagy is a kind of selective organelle autophagy that prevents the accumulation of abnormal mitochondria that might otherwise trigger cardiomyocyte dysfunction or death. Proper mitophagy
Mitochondrial quality control mechanisms in cardiac ischemia—reperfusion injury

also recycles metabolic substrates that are vital for cardiomyocyte metabolism under stressful conditions. BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3), FUN14 domain containing 1 (FUNDC1) and NIX are expressed on the OMM and therefore elicit receptor-dependent mitophagy (Fig. 4). However, PARKIN is mainly localized in the cytoplasm and translocates onto the mitochondria with lower membrane potential to initiate receptor-independent mitophagy after stimulation. Mechanistically, the targeted mitochondria are then engulfed by the pre-autophagosome, forming an autophagosome. Subsequently, microtubule-associated protein 1A/1B-light chain 3 (LC3) binds to phosphatidylethanolamine, generating the LC3-phosphatidylethanolamine conjugate (LC3II). Finally, the lysosome induces the proteolytic degradation of the autophagosomal proteins, nucleic acids, carbohydrates and lipids, which are recycled by the cell to restore homeostasis.

Since mitophagy is a “self-eating” process, excessive mitophagy is maladaptive and has been linked to cell death. Accordingly, genetically or pharmacologically blocking mitophagy can attenuate cell death. Three molecular mechanisms have been proposed to explain mitophagic cell death. First, many stimuli can trigger both mitophagy and cell death, so the cell fate is mainly determined by the degree and duration of stress. Under mild stress, when parts of the mitochondria are damaged, the selective removal of mitochondria via mitophagy is protective for the cell. However, once stress becomes severe, the number of damaged mitochondria increases and may overwhelm the capacity of mitophagy, leading to cell death. Thus, mitophagy is pro-survival, and cell death occurs when mitophagy cannot preserve mitochondrial homeostasis. Second, excessive mitophagy significantly reduces the mitochondrial mass and therefore causes ATP exhaustion. Cells with low ATP levels have a pronounced susceptibility to stress-induced death via necroptosis rather than apoptosis, because apoptosis is ATP-dependent programmed cell death. Therefore, mitophagy is fatal when it “eats” too many mitochondria.

Third, mitophagy is stimulated by many proteins that also induce apoptosis, such as the BCL2-family proteins NIX and BNIP3. Although BNIP3 initiates mitophagy by promoting the binding between LC3 and mitochondria, BNIP3 overexpression sensitizes cells to the intrinsic apoptotic cell death pathway. Thus, the death-suppressing or -promoting actions of mitophagy are determined by upstream adaptors. Lastly, we must emphasize that cell fate management via mitophagy may also depend on the tissue and cell type.

In the setting of cardiac I/R injury, numerous experiments have investigated how mitophagy influences myocardial function and cardiomyocyte viability. The effects of mitophagy (i.e., suppressing or promoting cell death) mainly seem to depend on the adaptors involved. For example, reperfusion-induced cardiomyocyte death through calcium overload is followed by OPA1-induced mitophagy; however, the pharmacologic activation of OPA1-induced mitophagy is found to protect the heart against I/R injury. Similarly, an early study indicated that the genetic ablation of Opa1 impairs mitophagy and augments I/R-induced myocardial damage. In addition, Saito et al. demonstrated that protective mitophagy during myocardial ischemia is mediated by RAB9. Phosphorylated RAB9 at Ser179 promotes the assembly of the ULK1–RAB9–RIP1–DRP1 complex, and then activates mitophagy to protect myocardium against ischemia. In contrast, PARKIN-induced mitophagy is found to be fatal for reperfused hearts because it enhances cyclophilin D (CypD)-induced mPTP opening, a feature of necroptosis. In a cardiac microvascular I/R injury model, PARKIN-induced mitophagy causes excessive mitochondrial elimination and an ATP undersupply, thus conveying death-promoting signals to cardiac microvascular endothelial cells. BNIP3-induced mitophagy is also demonstrated to be lethal in cardiac I/R injury. Abrogating BNIP3 activity not only prevents mitophagy, but also suppresses necrotic cell death in cardiomyocytes.

Unlike PARKIN- and BNIP3-induced mitophagy, cardiolipin-induced mitophagy is a cardioprotective process that attenuates mitochondrial oxidative stress, reduces calcium overload and promotes cardiomyocyte survival during I/R injury. Protective mitophagy can also be triggered by FUNDC1, an OMM protein that is regulated through post-transcriptional modification. At the stage of ischemia, FUNDC1 is found to be activated (dephosphorylated) and to foster mitophagy, thus reducing reperfusion-induced myocardial damage. FUNDC1-induced mitophagy has been reported to reverse the mitochondrial membrane potential, reduce mitochondrial ROS production and prevent mitochondria-induced apoptosis. TNF-receptor-associated factor 2 (TRAF2), an E3 ubiquitin ligase, also has been found to trigger protective mitophagy and reduce mitochondrial fragmentation in reperfused hearts.
Although the induction of mitophagy by different adaptors can have distinct effects on cell fate, ranging from survival to death, little is known about the molecular crosstalk among these adaptors. Thus, the net effect of mitophagy on cardiac I/R injury remains unclear. Of note, some studies have found that mitophagy is activated during I/R injury\textsuperscript{13,15}, whereas others have found that it is inhibited\textsuperscript{12,25}. This may be due to the different time points of reperfusion evaluated after ischemia. There is no doubt that ischemic/hypoxic stress induces autophagy (mitophagy)\textsuperscript{126,127}. During reperfusion, autophagy flux is reduced in the early phase (0–24 h after I/R injury), but is augmented at the later recovery stage (1–3 days after I/R injury)\textsuperscript{128,129}. In a careful recent study\textsuperscript{30}, autophagy reporter (CAG-RFP-EGFP-LC3) mice are generated and subjected to renal I/R injury. Autophagy is altered in the first 4 h after reperfusion, but autophagosome formation is overtly reduced from four to 24 h post-reperfusion\textsuperscript{30}. This conclusion is also highlighted in a number of high-profile thematic reviews\textsuperscript{131–133}.

Actually, the early inactivation and late activation of mitophagy may be an adaptive and protective response. From a pathophysiological perspective, cardiomyocyte death mainly occurs within the early period of reperfusion due to ROS overproduction and calcium overload\textsuperscript{134,135}. Under these conditions, cellular damage overwhelms the defense and/or repair systems of cardiomyocytes, including their anti-oxidative, anti-apoptotic and metabolism-remodeling capacities. Hence, either apoptosis or necroptosis is somewhat inevitable, so mitophagy is inhibited. Cardiomyocytes may thus avoid the possible activation of mitophagic cell death, which would otherwise accelerate or aggravate cardiomyocyte loss and myocardial dysfunction. However, in the late phase of reperfusion, the myocardium requires mitophagy to repair damaged mitochondria and restore cardiomyocyte viability, so the net result of mitophagy increases at this stage. Although mitophagy is inhibited or activated at different phases of reperfusion, we cannot conclude that various adaptors are inhibited or activated within a similar window. For example, in the early stage of reperfusion, PARKIN\textsuperscript{136} seems to be upregulated, whereas FUNDC1\textsuperscript{121} is rapidly inactivated. Thus, the ultimate effect of mitophagy results from the crosstalk among various mitophagy adaptors, although the time mapping of these adaptors has not yet been reported.

Although mitochondrial fission is reportedly to induce cardiomyocyte reperfusion damage through activating apoptosis, fission is supposed to occur prior to mitophagy at the stage of ischemia. A recent study\textsuperscript{137} reported that Unc-51 like autophagy activating kinase-1 (ULK1) phosphorylates RAB9 at Ser179, which promotes association between RAB9 and RIP1, followed by phosphorylation of DRP1 at Ser616 and its activation. Then, DRP1-induced fission sequesters damaged mitochondria and facilitates mitophagy to attenuate myocardial ischemic injury. Besides, DRP1 SUMOylation also contributes to mitophagy activation in hypoxia-treated cardiomyocyte, which is followed by sustained mitochondrial potential and decreased cardiomyocyte apoptosis\textsuperscript{138}. These observations demonstrate that mitophagy is induced by moderate fission under ischemia/hypoxia conditions. In fact, an early study has proposed that PARKIN-independent mitophagy requires DRP1 to maintain the integrity of mammalian heart\textsuperscript{139}. When DRP1 is absent, PARKIN becomes necessary to sustain mitochondrial function and structural integrity\textsuperscript{139}. This notion is also supported by several following studies that FUNDC1 requires DRP1-dependent fission to control mitophagy\textsuperscript{139,140}. Interestingly, at the stage of reperfusion, fission is significantly upregulated whereas protective mitophagy is largely inhibited. Re-introduction of mitophagy has been found to stop fatal fission\textsuperscript{139,140}, resulting into mitochondrial potential stabilization and cardiomyocyte survival. These data suggest that mitophagy may in turn restrict abnormal mitochondrial fission. However, inhibition of DRP1-mediated mitochondrial fragmentation seems to impair autophagosome recognition and engulfing of damaged mitochondria\textsuperscript{141}, reconfirming that fission is the prerequisite for mitophagy induction. Overall, although abnormal mitochondrial fission is followed by cardiomyocyte death in cardiac I/R injury, mitophagy requires moderate fission to sequester damaged mitochondria at the ischemic stage whereas reperfusion-induced excessive fission could be in turn corrected by mitophagy.

Notably, mitophagy is regulated by various adaptors, when one adaptor is inhibited, another may be induced in compensation. For example, germline Parkin ablation in mice has proven not to be an ideal experimental model for mitophagy depletion, in part due to the compensation from mitochondrial E3 ubiquitin protein ligase 1 (MULTI)-induced mitophagy in the physiological state\textsuperscript{142}. Further, although ATG32 is the primary mitophagy receptor in yeast, its mammalian homologue BCL2 like 13 (BCL2L13) compensates somewhat for basal mitophagy activity in Atg32-null yeast\textsuperscript{143}. Moreover, under normal conditions, nonselective autophagy compensates for the lack of mitophagy in Mfn2-knockout mice\textsuperscript{144}. Since compensatory mechanisms ensure that mitophagy occurs under various conditions, the next key question is how the various mitophagy adaptors interact with and compensate for one another in cardiac I/R injury.

5. Mitochondria-dependent cell death

Cardiac I/R injury involves the rapid loss of functional cardiomyocytes through programmed cell death, the final step of MQC. Mitochondria induce or inhibit cardiomyocyte death by two routes (Fig. 5). The first approach is the hyper-permeabilization of

![Figure 5](image-url)
the OMM, followed by the leakage of Cyt-c from the mitochon-
dria into the cytoplasm. There, Cyt-c activates caspase-9, which
subsequently cleaves caspase-3\(^{146,147}\). This classical
mitochondria-induced apoptotic pathway is also characterized by
mitochondrial membrane potential reduction, ROS overload, BAX
upregulation and BCL2 downregulation\(^{148,149}\). The second death
pathway is induced by the protracted opening of the mPTP due to
voltage-dependent anion-selective channel multimerization, CypD
phosphorylation and adenine nucleotide translocator upregulation,
although the primary constituents of the mPTP complex are being
intensely debated\(^{150,151}\). The mPTP induces the opening of the
IMM by forming a non-specific pore, leading to mitochondrial
swelling, mitochondrial electron transport chain dysfunction and
tricarboxylic acid cycle termination\(^{152,153}\). Subsequently, due to
ATP exhaustion, the cell undergoes cytoplasmic swelling, mem-
brane rupture and organelle breakdown, which lead to cell death
through necroptosis\(^{154}\). In contrast to apoptosis, necroptotic cell
death does not require energy, and exhibits features such as cell/
organelle swelling, extensive mitochondrial disruption, blebbing
and irreversible plasma membrane disintegration\(^{155,156}\).

Several regulators of mitochondrial apoptosis or necroptosis
should be highlighted to illustrate the signal transduction path-
ways underlying mitochondria-induced cell death in cardiac I/R
injury. First, with respect to apoptosis, BAX is an important
inducer of OMM permeabilization, whereas BCL2 guards against
BAX-induced OMM damage\(^{157,158}\). Under normal conditions,
BCL2 heterodimerizes with BAX to inhibit its pro-apoptotic ac-
tivity. However, certain stimuli upregulate the transcription of
BAX, ultimately increasing the abundance of BAX proteins in the
cytoplasm and enabling their homodimerization\(^{159}\). Subsequently,
BAX homodimers migrate to and insert themselves into the
OMM, thus permeabilizing it\(^{160,161}\). Accordingly, the levels of
BCL2 and BAX, as well as the mitochondrial membrane potential,
are usually used to monitor mitochondrial apoptosis\(^{162,163}\).

Regarding necroptosis, the initial signals include receptor
interacting serine/threonine kinase 3 (RIPK3), phosphoglycerate

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**Table 1** Compounds or drugs targeting mitochondrial quality control (MQC) in cardiac I/R injury.

| Name                          | Target                                                                 | Ref.       |
|-------------------------------|------------------------------------------------------------------------|------------|
| Mdivi-1                       | Mitochondrial fission (DRP1)                                           | 179,180    |
| Propofol                      | Mitochondrial fission (DRP1)                                           | 181        |
| Dapagliflozin                 | Mitochondrial fission (DRP1)                                           | 182        |
| Dynasore                      | Mitochondrial fission (DRP1)                                           | 183        |
| Isosteviol sodium             | Mitochondrial fission (DRP1/FIS1)                                      | 184        |
| Tetrahydrocurcumin            | Mitochondrial fission and fusion (DRP1 and MFN2)                       | 185        |
| Pravastatin                   | Mitochondrial fission and fusion (DRP1 and MFN1)                       | 186        |
| Vildagliptin                  | Mitochondrial fusion (MFN2)                                           | 64         |
| Mitochondrial fusion promoter M1 | Mitochondrial fusion (MFN1/2 and OPA1)                              | 187        |
| Melatonin                     | Mitochondrial fusion and mitophagy (OPA1 and FUNDC1)                   | 79,122     |
| Tongxinluo                    | Mitophagy (PARKIN)                                                    | 188        |
| Bicarbonate                   | Mitophagy (PARKIN)                                                    | 189        |
| Simvastatin                   | Mitophagy (PARKIN)                                                    | 190        |
| Ellagic acid                  | Mitophagy (BNIP3)                                                     | 117        |
| Hydrogen-rich saline           | Mitophagy (PARKIN)                                                    | 191        |
| Metformin                     | Necroptosis (RIPK1 and RIPK3)                                          | 192        |
| Dexametetomidine              | Necroptosis (RIPK3)                                                   | 193        |
| Necrostatin-1                 | Necroptosis (RIPK1)                                                   | 194        |
| Basilicin                     | Necroptosis (RIPK3 and MLKL)                                          | 195        |
| Ciclosporin A                 | Necroptosis (mPTP opening)                                            | 167        |
| Tanshinone IIA                | Apoptosis (BAX/BCL2)                                                  | 196        |
| Taxifolin                     | Apoptosis (BAX/BCL2)                                                  | 197        |
| Glutamine                     | Apoptosis (Cyt-c)                                                     | 198        |
| Febuxostat                    | Apoptosis (Cyt-c)                                                     | 199        |
| PD150606                      | Apoptosis (Cyt-c)                                                     | 42         |
| Ru360                         | Apoptosis (BAX)                                                       | 200        |
genes such as caspase inhibitor zVAD. In contrast, the depletion of necroptotic cell death during I/R injury could be reversed by the pan-
apoptosis fills the ischemic region16,169,170. Different zones: the inner area of the infarcted myocardium (the umbra), and the surrounding ischemic penumbra. Necroptosis is induced apoptosis in myocardial infarction171,172, but activated caspase-8 can degrade RIPK3 and thus inhibit necroptosis173,174. These studies suggest that RIPK3 may promote mitochondrial fission and subsequent mitochondrial apoptosis. Interestingly, the deletion of Ripk3 reverses FUNDC1-induced mitophagy and thus sends an anti-apoptotic signal to reperfused hearts170,172. On the other hand, the suppression of autophagy flux is found to trigger cardiomyocyte death via necroptosis173. These studies suggest that there is reciprocity between necroptosis and mitochondrial dynamics. Although necroptosis and apoptosis are regulated by completely different upstream signaling pathways, there is a striking pattern of overlap in their downstream events. Accordingly, when cardioprotective drugs are designed to reduce myocardial I/R injury, both anti-apoptotic and anti-necroptotic actions should be considered.

6. Conclusions

MQC is an adaptive response that adjusts the morphology and function of mitochondria during cardiac I/R injury (Fig. 6). After exposure to stress, cardiomyocytes employ anti-oxidative factors to neutralize mitochondrial ROS, reduce oxidative stress damage and ensure mitochondrial homeostasis. Concurrently, mitochondrial fission is activated so that damaged mitochondrial fractions can be removed from the mitochondrial network, with the cooperation of mitophagy. In contrast, healthy, long mitochondria can integrate with several small mitochondrial fragments to enhance the resistance of the entire mitochondrial population to stress. When these adaptive responses fail, programmed cell death by apoptosis or necroptosis is activated, and damaged mitochondria become the inducers of cell death, enabling the sequestration of incurable and dysfunctional cardiomyocytes. During this process, mitochondrial fission and mitophagy serve as a double-edged sword in the reperfused heart: on one hand, they exert pro-survival mechanisms by isolating damaged mitochondria, and on the other hand, if fission and mitophagy persist beyond a certain threshold, they may lead to cellular demise. Therefore, selective, effective, moderate and differential activation of mitophagy and mitophagy are essential for MQC, and could synergistically enhance cardiac function in I/R injury. Necroptosis and apoptosis, although activated by various stimuli, are functionally governed solely by mitochondria. As the final steps of MQC to maintain tissue homeostasis, necroptosis and apoptosis communicate with each other, and offer new targets for therapeutic approaches. The compounds or drugs targeting MQC are summarized in Table 1. More studies are required to further verify the therapeutic effects of these compounds/drugs in clinical practice.

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Author contributions

Jin Wang and Hao Zhou were responsible for original draft and visualization. Hao Zhou was responsible for review and editing, supervision, and funding acquisition.

Conflicts of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analysis or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Heusch G. 25 years of remote ischemic conditioning: from laboratory curiosity to clinical outcome. Basic Res Cardiol 2018;113:15.
2. Heusch G. Coronary microvascular obstruction: the new frontier in cardioprotection. Basic Res Cardiol 2019;114:45.
3. Tai Y, Li L, Peng X, Zhu J, Mao X, Qin N, et al. Mitochondrial uncoupler BAM15 inhibits artery constriction and potently activates AMPK in vascular smooth muscle cells. Acta Pharm Sin B 2018;8:909–18.
4. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P, et al. Molecular mechanisms of cell death: recommendations of the nomenclature committee on cell death 2018. Cell Death Differ 2018;25:486–541.
5. Zhou H, Li N, Yuan Y, Jin YG, Guo H, Deng W, et al. Activating transcription factor 3 in cardiovascular diseases: a potential therapeutic target. Basic Res Cardiol 2018;113:37.
6. Zhao J, Gao JL, Zhu JX, Zhu HB, Peng X, Jiang M, et al. The different responses of cardiomyocytes and cardiac fibroblasts to
Mitochondrial quality control mechanisms in cardiac ischemia–reperfusion injury

11. Wang M, Smith K, Yu Q, Miller C, Singh K, Sen CK. Mitochondrial
20. Forini F, Nicolini G, Kusmic C, Iervasi G. Protective effects of
18. Boyman L, Karbowski M, Lederer WJ. Regulation of mitochondrial
10. Lochner A, Marais E, Huisamen B. Melatonin and cardioprotection
24. Mughal W, Martens M, Field J, Chapman D, Huang J, Rattan S, et al.
26. Wang HH, Wu YJ, Tseng YM, Su CH, Hsieh CL, Yeh HI. Mito-
9. Zhou H, Wang S, Hu S, Chen Y, Ren J. ER
8. Ren J, Zhang Y. Editorial: new therapeutic approaches in the man-
7. Zhou H, Ma Q, Zhu P, Ren J, Reiter RJ, Chen Y. Protective role of melatonin in cardiac ischemia–reperfusion injury: from pathogenesis to targeted therapy. J Pineal Res 2018;64:e12471.

Ren J, Zhang Y. Editorial: new therapeutic approaches in the management of ischemia reperfusion injury and cardiometabolic diseases: opportunities and challenges. Curr Drug Targets 2017;18:1687–8.

Zhou H, Wang S, Hu S, Chen Y, Ren J. ER–mitochondria micro-domains in cardiac ischemia–reperfusion injury: a fresh perspective. Front Physiol 2018;9:755.

Lochner A, Marais E, Huisamen B. Melatonin and cardioprotection against ischaemia/reperfusion injury: what’s new? J Pineal Res 2018;65:e12490.

Wang M, Smith K, Yu Q, Miller C, Singh K, Sen CK. Mitochondrial connexin 43 in sex-dependent myocardial responses and estrogen-mediated cardiac protection following acute ischemia/reperfusion injury. Basic Res Cardiol 2019;115:1.

Zhang HF, Wang YL, Tan YZ, Wang HJ, Tao P, Zhou P. Enhancement of cardiac lymphangiogenesis by transplantation of CD34+ VEGFR-3+ endothelial progenitor cells and sustained release of VEGF-C. Basic Res Cardiol 2019;114:43.

Kuznetsova AV, Javadov S, Margreiter R, Grimm M, Hagenbuchner J, Ausserlechner MJ. The role of mitochondria in the mechanisms of cardiac ischemia–reperfusion injury. Antioxidants (Basel) 2019;8:e454.

Scarabelli TM, Gottlieb RA. Functional and clinical repercussions of myocyte apoptosis in the multifaceted damage by ischemia/reperfusion injury: old and new concepts after 10 years of contributions. Cell Death Differ 2004;11:514–52.

Vela D. Keeping heart homeostasis in check through the balance of iron metabolism. Acta Physiol (Oxf) 2019;228:e13324.

Del Re DP, Angalán D, Linckermann A, Liu Q, Kitsis RN. Fundamental mechanisms of regulated cell death and implications for heart disease. Physiol Rev 2019;99:1765–817.

Maneechote C, Palee S, Chattipakorn SC, Chattipakorn N. Protective role of melatonin in cardiac ischemia. Trends Cell Biol 2020;30:38–44.

Hornbeck PV, Zhang B, Murray B, Kornhauser JM, Latham V, Skrzypek E. PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. Nucleic Acids Res 2015;43:D512–20.

Jhun BS, Ou I, Adaniya SM, Cypress MW, Yoon Y. Adrenergic regulation of Drp1-driven mitochondrial fission in cardiac physiology. Antioxidants 2018;7:e195.

Xu S, Wang P, Zhang H, Gong G, Gutierrez Cortes N, Zhu W, et al. CaMKII induces permeability transition through Drp1 phosphorylation during chronic beta-AR stimulation. Nat Commun 2016;7:13189.

Sharp WW, Fang YH, Han M, Zhang HJ, Hong Z, Banathy A, et al. Dynamin-related protein 1 (Drp1)-mediated diastolic dysfunction in myocardial ischemia–reperfusion injury: therapeutic benefits of Drp1 inhibition to reduce mitochondrial fission. Faseb J 2014;28:316–26.

Trindade F, Vitorino R, Leite-Moreira A, Falcão-Pires I. Pericardial fluid: an undervalued molecular library of heart conditions and a potential vehicle for cardiac therapy. Basic Res Cardiol 2019;114:110.

Zhou H, Wang J, Zhu P, Zhu H, Toan S, Hu S, et al. NR4A1 aggravates the cardiac microvascular ischemia reperfusion injury through suppressing FUND1-mediated mitophagy and promoting MIF-required mitochondrial fission by CK2alpha. Basic Res Cardiol 2018;113:23.

Wells RC, Picton LC, Williams SC, Tan FJ, Hill RB. Direct binding of the dynamin-like GTPase, Dnm1, to mitochondrial dynamin protein Fis1 is negatively regulated by the Fis1 N-terminal arm. J Biol Chem 2007;282:33769–75.

Atkins K, Dasgupta A, Chen KH, Mewburn J, Archer SL. The role of Drp1 adaptor proteins Mdi49 and Mdi51 in mitochondrial fission: implications for human disease. Clin Sci (Lond) 2016;130:1861–74.

Zhang Z, Liu L, Wu S, Xing D, Drp1, Mif, Fis1, and Mdi51 are coordinated to mediate mitochondrial fission during UV irradiation-induced apoptosis. FASEB J 2016;30:466–76.

Zaia J, Bai X, Liu Y, Kikuchi C, Dosenovic S, Yan Y, et al. Cdk1, PKCdelta and calcineurin-mediated Drp1 pathway contributes to mitochondrial fission-induced cardiomyocyte death. Biochem Biophys Res Commun 2014;453:710–21.

Zhou H, Hu S, Jin Q, Shi C, Zhang Y, Zhu P, et al. Mif-dependent mitochondrial fission contributes to the pathogenesis of cardiac microvascular ischemia/reperfusion injury via induction of mROS-mediated cardiopin oxidation and HK2/VDAC1 disassociation-involved mPTP opening. J Am Heart Assoc 2017;6:e005328.

Luo T, Yue R, Hu H, Zhou Z, You KH, Zhang S, et al. PD150606 protects against ischemia/reperfusion injury by preventing calpain-induced mitochondrial apoptosis. Arch Biochem Biophys 2015;586:1–9.

Zhou H, Shi C, Hu S, Zhu H, Ren J, Chen Y. B11 is associated with microvascular protection in cardiac ischemia reperfusion injury via repressing Syk-Nox2-Drp1-mitochondrial fission pathways. Angiogenesis 2018;21:599–615.

Ding M, Ning J, Feng N, Li Z, Liu Z, Wang Y, et al. Dynamin-related protein 1-mediated mitochondrial fission contributes to post-traumatic cardiac dysfunction in rats and the protective effect of melatonin. J Pineal Res 2018;64:e12447.
45. Ter Horst EN, Krijnen PAJ, Hakimzadeh N, Robbers L, Hirsch A, Nijveldt R, et al. Elevated monocyte-specific type 1 interferon signaling correlates positively with cardiac healing in myocardial infarct patients but interferon alpha application deteriorates myocardial healing in rats. Basic Res Cardiol 2018;114:1.

46. Yu P, Zhang J, Yu S, Luo Z, Hua F, Yuan L, et al. Protective effect of sevoflurane postconditioning against cardiac ischemia/reperfusion injury via ameliorating mitochondrial impairment, oxidative stress and rescuing apoptotic clearance. PLoS One 2015;10:e0134666.

47. Su HH, Liao JM, Wang YH, Chen KM, Lin CW, Lee IH, et al. Exogenous GDF11 attenuates non-canonical TGF-beta signaling to protect the heart from acute myocardial ischemia—reperfusion injury. Basic Res Cardiol 2019;114:20.

48. Ding M, Dong Q, Liu Z, Liu Z, Qu Y, Li X, et al. Inhibition of dynamin-related protein 1 protects against myocardial ischemia—reperfusion injury in diabetic mice. Cardiovasc Diabetol 2017;16:19.

49. Yu J, Maimaitili Y, Xie P, Wu JJ, Wang J, Yang YN, et al. High glucose concentration abrogates sevoflurane post-conditioning cardioprotection by advancing mitochondrial fission but dynamin-related protein 1 inhibitor restores these effects. Acta Physiol (Oxf) 2017;220:83–98.

50. Schreiber T, Salhofer L, Quinting T, Fandrey J. Things get broken: the hypoxia-inducible factor prolyl hydroxylases in ischemic heart disease. Basic Res Cardiol 2019;114:16.

51. Gharanei M, Hussain A, Janneh O, Maddock H. Attenuation of Mitofusins in mitochondrial fusion. Cardiol Rev 2018;26:211.

52. Xue RQ, Sun L, Yu XJ, Li DL, Zang WJ. Vagal nerve stimulation and rescuing autophagic clearance. Neuronal nitric oxide synthase and c-Src signaling: a molecular target for cardioprotection: critical importance of mitochondrial dynamics/mitophagy inhibitor. Acta Physiol (Oxf) 2015;211:58–71.

53. Totzeck M, Hendgen-Cotta UB, Fassler T. Nitrite-nitric oxide signaling and cardioprotection. Adv Exp Med Biol 2017;982:335–46.

54. Manechote C, Palee S, Kerdpoo S, Jowdy C, Schneider TG, Csordas N, Wang W, et al. Nitrite-nitric oxide synthase improves mitochondrial dynamics/mitophagy inhibitor. PLoS One 2013;8:e77713.

55. Xue RQ, Sun L, Yu XJ, Li DL, Zhang WJ. Vagal nerve stimulation improves mitochondrial dynamics via an M3 receptor/Calcium/mitochondrial DNA/microRNA-34a/mitochondrial-peroxisome-targeted AMPK pathway in isoproterenol-induced myocardial ischemia. J Cell Mol Med 2017;21:58–71.

56. Totzeck M, Hendgen-Cotta UB, Fassler T. Nitrite-nitric oxide signaling and cardioprotection. Adv Exp Med Biol 2017;982:335–46.

57. Rossello X, Yellon DM. The RISK pathway and beyond. Basic Res Cardiol 2018;113:2.

58. Meyer JN, Leuthner TC, Luz AL. Mitochondrial fusion, fission, and mitochondrial toxicity. Toxicology 2017;391:42–53.

59. Park M, Sandner P, Krieg T. cGMP at the centre of attention: emerging strategies for activating the cardioprotective PKG pathway. Basic Res Cardiol 2018;113:24.

60. Cohen MM, Tareste D. Recent insights into the structure and function of Mitofusins in mitochondrial fusion. F1000Res 2018;7:1983.

61. Yang M, Linn BS, Zhang Y, Ren J. Mitophagy and mitochondrial integrity in cardiac ischemia—reperfusion injury. Biochim Biophys Acta Mol Basis Dis 2019;1865:299–302.

62. MacVicar T, Langer T. OPA1 processing in cell death and disease—the long and short of it. J Cell Sci 2016;129:2297–306.

63. Guan L, Che Z, Meng X, Yu Y, Li M, Yu Z, et al. MCU up-regulation contributes to myocardial ischemia—reperfusion Injury through calpain/OPA1-mediated mitochondrial fusion/mitophagy inhibition. J Cell Mol Med 2019;23:7830–43.

64. Pirzeh L, Babapour V, Badalzadeh R, Panahi N. Pretreatment with vildagliptin boosts ischemic—postconditioning effects on cardioprotection and expression profile of genes regulating autophagy and mitochondrial fusion/fission in diabetic heart with reperfusion injury. Naunyn-Schmiedeberg’s Arch Pharmacol 2019;392:1371–82.

65. Wang Q, Xu J, Li X, Liu Z, Han Y, Xu J, et al. SirT3 modulate renal ischemia—reperfusion injury through enhancing mitochondrial fusion and activating the ERK—OPA1 signaling pathway. J Cell Physiol 2019;234:23495–506.

66. Chen H, Ren S, Clish C, Jain M, Mootha V, McCaffery JM, et al. Titration of mitochondrial fusion rescues Mff-deficient cardiomyopathy. J Cell Biol 2015;201:795–805.

67. Chen Y, Cordsas G, Jowdy T, Schneider TG, Cordsas N, Wang W, et al. Mitofusin 2-containing mitochondrial-reticular microdomains direct rapid cardiomycyte bioenergetic responses via interorganellar Ca++ crosstalk. Circ Res 2012;111:863–75.

68. Chen Y, Sparks M, Bhandari P, Matovich SJ, Dom 2nd GW. Mitochondrial genome linearization is a causative factor for cardiomyopathy in mice and Drosophila. Antioxidants Redox Signal 2014;21:1949–59.

69. Papanicolaou KN, Ngoh GA, Dabkowski ER, O’Connell KA, Ribeiro Ir RF, Stanley WC, et al. Cardiomyocyte deletion of mitofusin-1 leads to mitochondrial fragmentation and improves tolerance to ROS-induced mitochondrial dysfunction and cell death. Am J Physiol Heart Circ Physiol 2012;302:H1167–79.

70. Papanicolaou KN, Khairallah RJ, Ngoh GA, Chikando A, Luptak I, O’Shea KM, et al. Mitofusin-2 maintains mitochondrial structure and contributes to stress-induced permeability transition in cardiac myocytes. Mol Cell Biol 2011;31:1309–28.

71. Ndongson-Dongmo B, Lang GP, Mece O, Hechaichi N, Lajqi T, Hoyer D, et al. Reduced ambient temperature exacerbates SIRS-induced cardiac autonomic dysregulation and myocardial dysfunction in mice. Basic Res Cardiol 2019;114:26.

72. Shen T, Zheng M, Cao C, Chen C, Tang J, Zhang W, et al. Mitofusin-2 is a major determinant of oxidative stress-mediated heart muscle cell apoptosis. J Biol Chem 2007;282:23534–61.

73. Song M, Mihara K, Chen Y, Scorrano L, Dorn 2nd GW. Mitochondrial fusion and fusion factors reciprocally orchestrate mitochondrial culling in mouse hearts and cultured fibroblasts. Cell Metab 2015;21:273–86.

74. Mouton AJ, DeLeon-Pennell KY, Rivera Gonzalez OJ, Flynn ER, Freeman TC, Sauerman JJ, et al. Mapping macrophage polarization over the myocardial infarction time continuum. Basic Res Cardiol 2018;113:26.

75. Detmer SA, Chan DC. Complementation between mouse Mfn1 and Mfn2 protects mitochondrial fusion defects caused by CMT2A disease mutations. J Cell Biol 2007;176:405–14.

76. Chen H, Detmer SA, Ewald AJ, Griffin EE, Fraser SE, Chan DC. Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. J Cell Biol 2003;160:189–200.

77. Morell M, Burgos JI, Gonano LA, Vila Petroff M. AMPK-dependent nitric oxide release provides contractile support during hyperosmotic stress. Basic Res Cardiol 2018;113:7.

78. Dorn 2nd GW, Clark CF, Eschenbacher WH, Kang MY, Engelhardt JT, Warner SJ, et al. MAFR and Opal control mitochondrial and cardiac function in Drosophila. Circ Res 2011;108:12–7.

79. Zhang Y, Wang Y, Xu J, Tian F, Hu S, Chen Y, et al. Melatonin attenuates myocardial ischemia—reperfusion injury via improving mitochondrial fusion/mitophagy and activating the AMPK—OPA1 signaling pathways. J Physiol 2019;596:12542.

80. Nan J, Nan C, Ye J, Qian L, Geng Y, Xing D, et al. EGCG protects cardiomycocytes against hypoxia—reperfusion injury through inhibition of OMA1 activation. J Cell Sci 2019;132:e20871.

81. Ma S, Dong Z. Melatonin attenuates cardiac reperfusion stress by improving OPA1-related mitochondrial fusion in a Yap—Hippo pathway-dependent manner. J Cardiovasc Pharmacol 2019;73:27–39.

82. Guo Y, Wang Z, Qin X, Xu J, Hou Z, Yang H, et al. Enhancing fatty acid utilization ameliorates mitochondrial fragmentation and cardiac dysfunction via rebalancing optic atrophy 1 processing in the failing heart. Cardiovasc Res 2018;114:979–91.
Mitochondrial quality control mechanisms in cardiac ischemia–reperfusion injury

83. Moore JB, Tang XL, Zhao J, Fischer AG, Wu WJ, Uchida S, et al. Epigenetically modified cardiac mesenchymal stromal cells limit myocardial fibrosis and promote functional recovery in a model of chronic ischemic cardiomyopathy. Basic Res Cardiol 2018;114:3.

84. Ferreira JCB, Campos JC, Ovit N, Qi X, Bozi LHM, Bechara LRG, et al. Selective inhibitor of mitofusin 1-betaIPPKC association improves heart failure outcomes in rats. Nat Commun 2019;10:329.

85. Pyakurel A, Savoia C, Hess D, Scorrano L. Extracellular regulated kinase phosphorylates mitofusin 1 to control mitochondrial morphology and apoptosis. Mol Cell 2015;58:244–54.

86. Chen Y, Dorn 2nd GW. PINK1-phosphorylated mitofusin 2 is a Parkin receptor for culling damaged mitochondria. Science 2013;340:471–5.

87. Lebouche GP, Tsai VC, Yang M, Shaw KC, Zhou M, Veenstra TD, et al. Stress-induced phosphorylation and proteasomal degradation of mitofusin 2 facilitates mitochondrial fragmentation and apoptosis. Mol Cell 2012;47:547–57.

88. Zhang Y, Si W, Zhang Q, Xu J, Liu H, Lao J, et al. Glycine protects H9C2 cardiomyocytes from high glucose- and hypoxia/reoxygenation-induced injury via inhibiting PKCbeta2 activation and improving mitochondrial quality. J Diabetes Res 2018;2018:9502895.

89. Tsushima K, Bugger H, Wende AR, Soto J, Jenson GA, Tor AR, et al. Mitochondrial reactive oxygen species in lipotoxic hearts induce post-translational modifications of AKAP121, DRP1, and OPAL1 that promote mitochondrial fission. Circ Res 2018;122:58–73.

90. Nan J, Hu H, Sun Y, Zha L, Wang Y, Zhong Z, et al. TNFFR2 stimulation promotes mitochondrial fusion via Stan-3 and NF-κB-dependent activation of OPAL1 expression. Circ Res 2017;121:392–410.

91. Chen PJ, Shang AQ, Yang JP, Wang WW. microRNA-874 inhibition targeting STAT3 protects the heart from ischemia–reperfusion injury by attenuating cardiomyocyte apoptosis in a mouse model. J Cell Physiol 2019;234:6182–93.

92. Zaurbier CJ, Jong WM, Eerbeek O, Koeman A, Pulskens WP, et al. Deletion of the innate immune NLRP3 receptor for culling damaged mitochondria. Basic Res Cardiol 2018;113:101065.

93. Meyer IS, Leuschner F. The role of Wnt signaling in the healing myocardium: a focus on cell specificity. Basic Res Cardiol 2018;113:44.

94. Samant SA, Zhang HJ, Hong Z, Pilai VB, Sundaresan NR, Wolfgeher D, et al. SIRT3 deacetylases and activates OPAL1 to regulate mitochondrial dynamics during stress. Mol Cell Biol 2014;34:807–19.

95. Makino A, Saaeza J, Gawlowsk T, Han W, Wang H, Scott BT, et al. Regulation of mitochondrial morphology and function by O-GlcNAcylation in neonatal cardiac myocytes. Am J Physiol Regul Integr Comp Physiol 2011;300:R1296–302.

96. Mehra P, Guo Y, Menzies KJ, Burelle Y. Mitochondrial quality control in the cardiac system: an integrative view. Biochim Biophys Acta Mol Cell Res 2019;1867:118519.

97. Kowaltowski AJ. Strategies to detect mitochondrial oxidants. Redox Biol 2019;21:101065.

98. Cadete VJJ, Vasan GM, Menzies KJ, Burelle Y. Mitochondrial quality control in the cardiac system: an integrative view. Biochim Biophys Acta Mol Basis Dis. 2019;1865:782–96.

99. Li J, Cai SX, He Q, Zhang H, Friedberg D, Wang F, et al. Intravascular mir-144 reduces left ventricular remodeling after myocardial infarction. Basic Res Cardiol 2018;113:36.

100. Strappazzon F, Di Rita A, Peschiarioli A, Leoncini PP, Locatelli F, Melino G, et al. HUWE1 controls MCL1 stability to unleash AMBRA1-induced mitophagy. Cell Death Differ 2020;27:1155–68.

101. Landry NM, Cohen S, Dixon IMC. Peritoxin in cardiovascular disease and development: a tale of two distinct roles. Basic Res Cardiol 2018;113:1.

102. Shimizu S, Yoshida T, Tsujikoa M, Arakawa S. Autophagic cell death and cancer. Int J Mol Sci 2014;15:3145–53.

103. Mekala NK, Kurdysh J, Depuydt MM, Vazquez EF, Rosca MG. Apoptosis inducing factor deficiency causes retinal photoreceptor degeneration. The protective role of the redox compound methylene blue. Redox Biol 2019;20:107–17.

104. Button RW, Luo S, Rubinstein DC. Autophagic activity in neuronal cell death. Neurobiol Cell 2015;31:382–94.

105. Liu Y, Levine B. Autosis and autophagic cell death: the dark side of autophagy. Cell Death Differ 2015;22:367–76.

106. Jin Q, Li R, Hu N, Xin T, Zhu P, Hu S, et al. DUSP1 alleviates cardiac ischemia/reperfusion injury by suppressing the Mff-required mitochondrial fission and Bnip3-related mitophagy via the JNK pathways. Redox Biol 2018;14:576–87.

107. Saito T, Nah J, Oka SI, Mukai R, Monden Y, Maejima Y, et al. An alternative mitophagy pathway mediated by Rab9 protects the heart against ischemia. J Clin Invest 2019;129:802–19.

108. Sun T, Ding W, Xu T, Ao X, Yu T, Li M, et al. Parkin regulates programmed necrosis and myocardial ischemia/reperfusion injury by targeting cyclophilin-D. Antioxidants Redox Signal 2019;31:1177–93.

109. Zhou H, Zhang Y, Hu S, Shi C, Zhu P, Ma Q, et al. Melatonin protects cardiac microvasculature against ischemia/reperfusion injury via suppression of mitochondrial fission–VDAC1–HK2–mPTP–mitophagy axis. J Pineal Res 2017;63:e12413.

110. Dhingra A, Jayas R, Afshar P, Guberman M, Maddaford G, Gerstein J, et al. Ellagic acid antagonizes Bnip3-mediated mitochondrial injury and necrotic cell death of cardiac myocytes. Free Radic Biol Med 2017;112:411–22.

111. Paradies G, Paradies V, Ruggiero FM, Petrosillo G. Mitochondrial biogenesis and cardioprotection in myocardial ischemia–reperfusion injury: implications for pharmacological cardioprotection. Am J Physiol Heart Circ Physiol 2018;315:H1341–52.

112. Morton AB, Smuder AJ, Wiggs MP, Hall SE, Ahn B, Hinkley JM, et al. Increased SOD2 in the diaphragm contributes to exercise-induced protection against ventilator-induced diaphragm dysfunction. Redox Biol 2019;20:402–13.

113. Zhou H, Zhu P, Guo J, Hu N, Wang S, Li D, et al. Ripk3 induces mitochondrial apoptosis via inhibition of FUNDC1 mitophagy in cardiac IR injury. Redox Biol 2017;13:498–507.
121. Zhou H, Zhu P, Wang J, Zhu H, Ren J, Chen Y. Pathogenesis of cardiac ischemia reperfusion injury is associated with CK2alpha-disturbed mitochondrial homeostasis via suppression of FUNDC1-related mitochondria. Cell Death Differ 2018; 25:1080–93.

122. Zhou H, Li D, Zhu P, Hu S, Hu N, Ma S, et al. Melatonin suppresses platelet activation and function against cardiac ischemia/reperfusion injury via PPARg6alpha/FUNDC1 mitochondrial pathways. J Pineal Res 2017, 63:e12438.

123. Yang KC, Ma X, Liu H, Murphy J, Barger PM, Mann DL, et al. Tumor necrosis factor receptor-associated factor 2 mediates mitochondrial autophagy. Circ Heart Fail 2018; 11:175–87.

124. Florido A, Saraiva N, Cerqueira S, Almeida N, Parsons M, Batinic-Haberle I, et al. The manganese(III) porphyrin MnTnHex-2-PyP5+ modulates intracellular ROS and breast cancer cell migration: impact on doxorubicin-treated cells. Redox Biol 2019; 20:367–78.

125. Zhang J, Nadtoychy SM, Urcuolii WR, Brookes PS. The cardioprotective compound cloxyquin uncouples mitochondria and induces autophagy. Am J Physiol Heart Circ Physiol 2016; 310:H29–38.

126. Esposti DD, Domart MC, Sebagh M, Harper F, Pierron G, Brenner C, et al. Autophagy is induced by ischemic preconditioning in human livers formerly treated by chemotherapy to limit necrosis. Autophagy 2010; 6:172–4.

127. Otani H. Ischemic preconditioning: from molecular mechanisms to therapeutic opportunities. Antioxidants Redox Signal 2008; 10:207–47.

128. Ji J, Zhou X, Xu P, Li Y, Shi H, Chen D, et al. Deficiency of apoptosis-stimulating protein two of p53 ameliorates acute kidney injury induced by ischemia reperfusion in mice through upregulation of autophagy. J Cell Mol Med 2019; 23:2457–67.

129. Feng J, Li H, Zhang Y, Wang Q, Zhao S, Meng P, et al. Mammalian STE20-like kinase 1 deletion alleviates renal ischaemia–reperfusion injury via modulating mitochondria and the AMPK–YAP signalling pathway. Cell Physiol Biochem 2018; 81:2359–76.

130. Li L, Wang ZV, Hill JA, Lin F. New autophagy reporter mice reveal dynamics of proximal tubular autophagy. J Am Soc Nephrol 2014; 25:305–35.

131. Lin F. Autophagy in renal tubular injury and repair. Acta Physiol (Oxf) 2017; 220:229–37.

132. Kaushal GP, Shah SV. Autophagy in acute kidney injury. Kidney Int 2016; 89:779–91.

133. Havasi A, Dong Z. Autophagy and tubular cell death in the kidney. Semin Nephrol 2016; 36:174–88.

134. Nybo T, Dieterich S, Gannon LF, Chang CY, Hammer A, Hoeffer G, et al. Chlorination and oxidation of the extracellular matrix protein laminin and basement membrane extracts by hypochlorous acid and myeloperoxidase. Redox Biol 2019; 20:496–513.

135. Narzt MS, Nagelreiter IM, Oskolkova O, Bochkov VN, Laurelle J, Fedorova M, et al. A novel role for NUPR1 in the keratinocyte stress response to UV oxidized phospholipids. Redox Biol 2019; 20:467–82.

136. Bian X, Xu J, Zhao H, Zheng Q, Xiao X, Ma X, et al. Zinc-induced SUMOylation of dynamin-related protein 1 protects the heart against ischemia–reperfusion injury. Oxid Med Cell Longev 2019; 2019:1232146.

137. Kageyama Y, Hoshijima M, Seo K, Bedja D, Sysa-Shah P, Andrabi SA, et al. Parkin-independent mitophagy requires Drp1 and maintains the integrity of mammalian heart and brain. EMBO J 2014; 33:2798–813.

138. Roy M, Kageyama Y, Iijima M, Sasaki H, PARK2/Parkin becomes critical when DNM1L/Drp1 is absent. Autophagy 2015; 11:573–4.

139. Chen M, Chen Z, Wang Y, Tan Z, Zhu C, Li Y, et al. Mitophagy receptor FUNDC1 regulates mitochondrial dynamics and mitophagy. Autophagy 2016; 12:689–702.

140. Wu W, Lin C, Wu K, Jiang L, Wang X, Li W, et al. FUNDC1 regulates mitochondrial dynamics at the ER–mitochondrial contact site under hypoxic conditions. EMBO J 2016; 35:1368–84.

141. Yu J, Li Y, Liu X, Ma Z, Michael S, Orgahnio J, et al. Mitochondrial dynamics modulation as a critical contribution for Shennai injection in attenuating hypoxia/reoxygenation injury. J Ethnopharmacol 2019; 235:9–19.
Mitochondrial quality control mechanisms in cardiac ischemia—reperfusion injury

162. Qi S, Guo L, Yan S, Lee RJ, Yu S, Chen S. Hypocrellin A-based photodynamic action induces apoptosis in A549 cells through ROS-mediated mitochondrial signaling pathway. Acta Pharmac Sin B 2019;9:279–93.

163. Hu C, Zhang X, Wei W, Zhang N, Wu H, Ma Z, et al. Matrine attenuates oxidative stress and cardiomyocyte apoptosis in doxorubicin-induced cardiotoxicity via maintaining AMPK-alpha1/UCP2 pathway. Acta Pharmac Sin B 2019;9:690–701.

164. Quispe RL, Jaramillo ML, Galant LS, Engel D, Dafre AL, Teixeira da Rocha JB, et al. Diphenyl diselenide protects neuronal cells against oxidative stress and mitochondrial dysfunction: involvement of the glutathione-dependent antioxidant system. Redox Biol 2019;20:118–29.

165. Zhou H, Li D, Zhu P, Ma Q, Toan S, Wang J, et al. Inhibitory effect of melatonin on necroptosis via repressing the Ripk3-promotes ER stress-induced necroptosis in cardiac IR injury: a mechanism involving calcium overload/XO/ROS/mdpPT pathway. Redox Biol 2018;16:157–68.

166. Hou H, Li D, Zuo P, Ma Q, Toan S, Wang J, et al. Inhibitory effect of melatonin on necroptosis via repressing the Ripk3-promotes ER stress-induced necroptosis in cardiac IR injury: a mechanism involving calcium overload/XO/ROS/mdpPT pathway. Redox Biol 2018;16:157–68.

167. Hu C, Zhang X, Wei W, Zhang N, Wu H, Ma Z, et al. Matrine attenuates oxidative stress and cardiomyocyte apoptosis in doxorubicin-induced cardiotoxicity via maintaining AMPK-alpha1/UCP2 pathway. Acta Pharmac Sin B 2019;9:279–93.

168. Quispe RL, Jaramillo ML, Galant LS, Engel D, Dafre AL, Teixeira da Rocha JB, et al. Diphenyl diselenide protects neuronal cells against oxidative stress and mitochondrial dysfunction: involvement of the glutathione-dependent antioxidant system. Redox Biol 2019;20:118–29.

169. Zhang T, Zhang Y, Cui M, Jin L, Wang Y, Lv F, et al. CaMKII is a RIP3 substrate mediating ischemia- and oxidative stress-induced myocardial necrosis. Nat Med 2016;22:175–82.

170. Schmidt HM, Kelley EE, Straub AC. The impact of xanthine oxidase inhibitor of mitochondrial fission, on rapidly activating delayed-rectifier K+ current and membrane potential in HL-1 murine atrial cardiomyocytes. Eur J Pharmacol 2012;683:1–9.

171. Onb SB, Kwak SY, Katwadi K, Hernandez-Resendiz S, Crespo-Avila GE, Isnaim NI, et al. Targeting mitochondrial fusion using Mdivi-1 in a clinically relevant large animal model of acute myocardial infarction: a pilot study. Int J Mol Sci 2019;20:e3972.

172. Zhao L, Zhuang J, Wang Y, Zhou D, Zhao D, Zhu S, et al. Propofol ameliorates H9c2 cells apoptosis induced by oxygen glucose deprivation and reperfusion injury via inhibiting high levels of mitochondrial fusion and fission. Front Pharmacol 2019;10:61.