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Mitochondrial apoptosis in response to cardiac ischemia-reperfusion injury

Kaixin Wang[1,2†], Qing Zhu[1,2†], Wen Liu[1,2], Linyuan Wang[1,2], Xinxin Li[1,2], Cuiting Zhao[1,2], Nan Wu[3] and Chunyan Ma[1,2*]

Abstract

In patients with acute myocardial infarction (AMI), thrombolytic therapy and revascularization strategies allow complete recanalization of occluded epicardial coronary arteries. However, approximately 35% of patients still experience myocardial ischemia/reperfusion (I/R) injury, which contributing to increased AMI mortality. Therefore, an accurate understanding of myocardial I/R injury is important for preventing and treating AMI. The death of each cell (cardiomyocytes, endothelial cells, vascular smooth muscle cells, cardiac fibroblasts, and mesenchymal stem cells) after myocardial ischemia/reperfusion is associated with apoptosis due to mitochondrial dysfunction. Abnormal opening of the mitochondrial permeability transition pore, aberrant mitochondrial membrane potential, Ca^{2+} overload, mitochondrial fission, and mitophagy can lead to mitochondrial dysfunction, thereby inducing mitochondrial apoptosis. The manifestation of mitochondrial apoptosis varies according to cell type. Here, we reviewed the characteristics of mitochondrial apoptosis in cardiomyocytes, endothelial cells, vascular smooth muscle cells, cardiac fibroblasts, and mesenchymal stem cells following myocardial ischemia/reperfusion.

Keywords Ischemia/reperfusion injury, Mitochondrial apoptosis, Cardiomyocyte, Endothelial cell

Introduction

Percutaneous coronary intervention or coronary artery bypass grafting is an effective method for treating acute myocardial infarction [1]. However, this strategy of reintroducing fresh blood is often accompanied by damage to cardiomyocytes (CMs) and endothelial cells (ECs), a phenomenon known as myocardial ischemia/reperfusion injury (I/R) injury [1]. It can lead to myocardial contractile depression and arrhythmia, which further increase AMI morbidity and mortality. Therefore, studying the mechanism of myocardial I/R injury is essential in improving its diagnosis and treatment.

Apoptosis is one of the main forms of cell death in myocardial I/R injury and participates in the entire process of myocardial I/R injury development [2]. Mitochondrial apoptosis plays an important role in cell injury and is the main executor of apoptotic events [2, 3]. Therefore, studying the mechanism of cellular mitochondrial apoptosis can help prevent and treat myocardial I/R injury.

We conducted a thorough review of the literature (with detailed materials and methods in Supplement) and discovered that current studies on the mechanism of mitochondrial apoptosis have observed that activation of the mitochondrial apoptotic pathway is associated with oxidative stress, Ca^{2+} overload, and adenosine triphosphate (ATP) depletion [4]. During reperfusion, mitochondria are damaged, with a considerable accumulation of reactive oxygen species (ROS) and an increase in Ca^{2+} in

†Kaixin Wang and Qing Zhu share first authorship.

*Correspondence:
Chunyan Ma
cmu1h_mcy@126.com
[1]Department of Cardiovascular Ultrasound, The First Hospital of China Medical University, Shenyang, China
[2]Clinical Medical Research Center of Imaging in Liaoning Province, Shenyang, China
[3]The Central Laboratory of The First Affiliated Hospital of China Medical University, Shenyang, China

the intracellular matrix, which prompts the opening of the mitochondrial permeability transition pore (mPTP), followed by a decrease in the mitochondrial membrane potential (MMP), leading to the release of mitochondrial contents into the cytoplasm, such as cytochrome C (Cyt-C). Cyt-c interacts with apoptosis protease activator 1 (apaf-1) and binds to ATP, deoxyadenosine triphosphate (dATP), and caspase-9 to form apoptotic vesicles, which further activate the cascade family and initiate the mitochondria-dependent apoptosis [4, 5]. In CMs, mitochondria undergoing sustained dysfunction and altered mPTP initiate the mitochondrial apoptotic pathway, as described above. In contrast to CMs, ECs can sense changes in the microenvironment, and various signaling factors are released in large quantities, such as NO, and Empagliflozin can inhibit EC apoptosis via the Akt/eNOS/NO pathway [6]. Vascular smooth muscle cells (VSMCs), a cellular component of the middle layer of the arterial wall, control vasoconstriction and diastole [7]. NO pretreatment inhibits the mitochondrial pathway of B cell lymphoma /leukemia-2 (Bcl-2) to suppress VSMC apoptosis [8]. Cardiac fibroblasts (CFs) are characterized by an anti-apoptotic state of I/R injury, mainly with hyperplasia and fibrosis, so as to repair the myocardium and eventually lead to myocardial remodeling [9]. Mesenchymal stem cells (MSCs) possess fibroblastic clonal potential and can differentiate into CMs and ECs [10]. The core mechanism involves the PI3K/Akt pathway, which regulates the expression of Bcl-2 family proteins to reduce apoptosis [11].

In summary, mitochondrial apoptosis between cell types is the most critical aspect of myocardial I/R injury; it is different and potentially related. Studying the mechanisms of mitochondrial apoptosis in different cells can help develop targeted preventive and therapeutic programs in the clinic to improve prognosis. Here, we review myocardial mitochondrial apoptosis (Fig. 1) in CMs, ECs, VSMCs, CFs and MSCs after I/R injury to provide a theoretical basis for preventing and treating myocardial I/R injury (as summarized in Table 1).

Initiation of CM apoptosis

The morphological and functional integrity of mitochondria is essential for maintaining normal cellular physiology. In CMs, more than 90% of the ATP is produced by the mitochondria, accounting for approximately one-third of the cell volume [12]. Therefore, cardiac mitochondrial dysfunction severely affects the vital activities of CMs. Consequently, myocardial injury is a major cause of I/R injury. CMs die during the ischemic period due to hypoxia, and although reperfusion is necessary to save them, it can trigger subsequent cell death. During myocardial infarction, 50–65% of CMs die due to apoptosis [13]. While sustained apoptosis of CMs occurs later after myocardial infarction, this cell death is reversible during reperfusion; therefore, it is critical to understand the pathways involved in CM mitochondrial apoptosis.

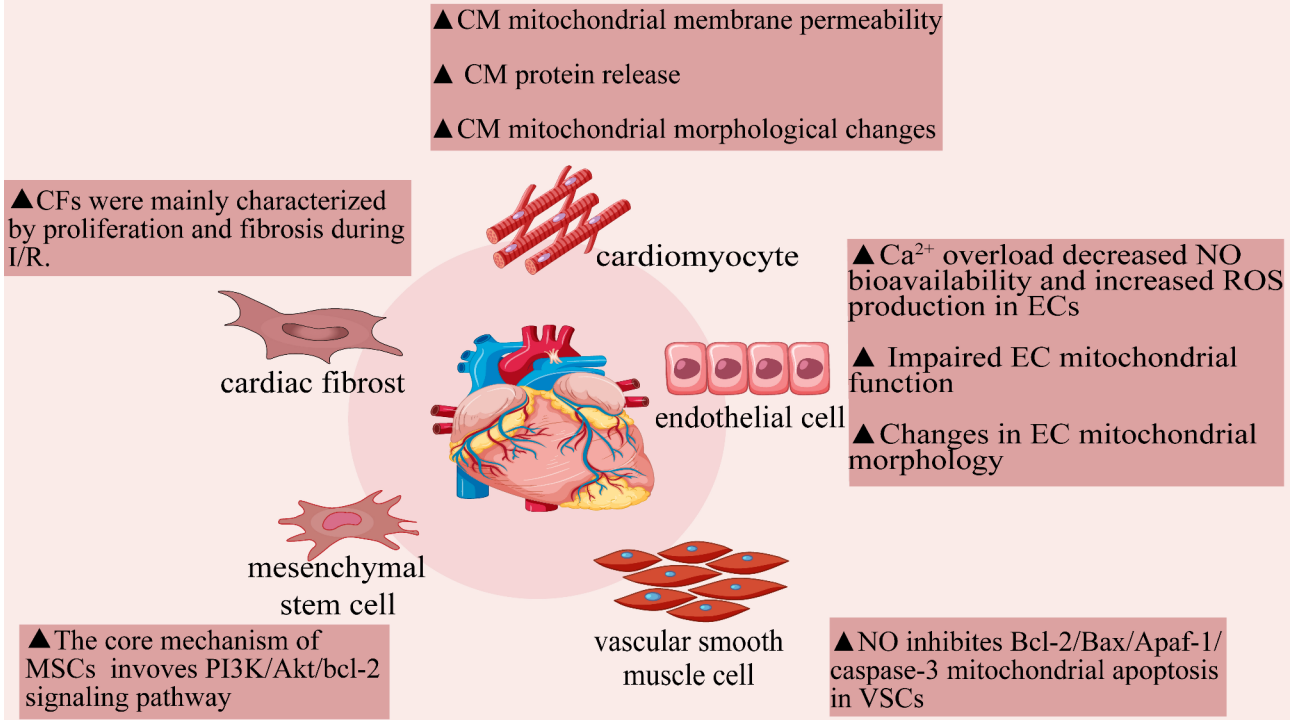

Fig. 1 An overview of this article

Table 1 The main findings of this article

Cell type	Main findings	Reference
CMs	The imbalance of Bax / Bcl2 ratio leads to the permeability of the mitochondrial outer membrane. Some studies have focused on how Bax and Bcl2 change and maintain dynamic balance to protect cardiomyocytes.	[16–24]
	The opening of the mPTP is the prerequisite of the integrity of IMM and plays a decisive role in releasing mitochondrial apoptotic proteins. Studying its opening can reduce mitochondrial apoptosis and protect cardiomyocytes.	[27–31]
	Mitochondrial apoptotic proteins (e.g. Cyt-C, caspase-3, caspase- 9) are increased in I/R injury, and the death of CMs can be reduced by inhibiting the increase of these proteins.	[33–35]
	Mitochondrial morphological changes play an important role in mitochondrial apoptosis. Inhibition of excessive mitochondrial fission and appropriate increase of mitophagy can reduce mitochondrial apoptosis.	[37, 38, 39, 41–42]
ECs	Increased levels of ROS, elevated intracellular Ca^{2+}, and decreased NO bioavailability lead to altered MMP, disruption of the balance between anti-apoptotic and pro-apoptotic proteins, the opening of the mPTP, and mitochondrial swelling and deformation; all of these initiate apoptosis via the mitochondrial pathway	[6, 44, 49] [54–55]
	The decrease in EC membrane stability leads to the release of mitochondrial-related apoptotic proteins, which leads to mitochondrial apoptosis.	[56–59]
	Although the number of mitochondria in ECs is small, they are of great significance. Abnormal mitochondrial morphology can lead to mitochondrial apoptosis.	[60–63]
VSMCs	The NO signaling pathway is important. NO preconditioning can inhibit Bcl-2/Bax/Apaf-1/caspase-3 mitochondrial apoptotic pathway.	[64]
CFs	CFs mainly play a repair and cardiac remodeling role during myocardial I/R, therefore, inhibiting mitochondrial apoptosis can promote fibrosis.	[66–70]
MSCs	The core mechanism of action of MSCs involves the PI3K/Akt signaling pathway.	[11, 71–73]

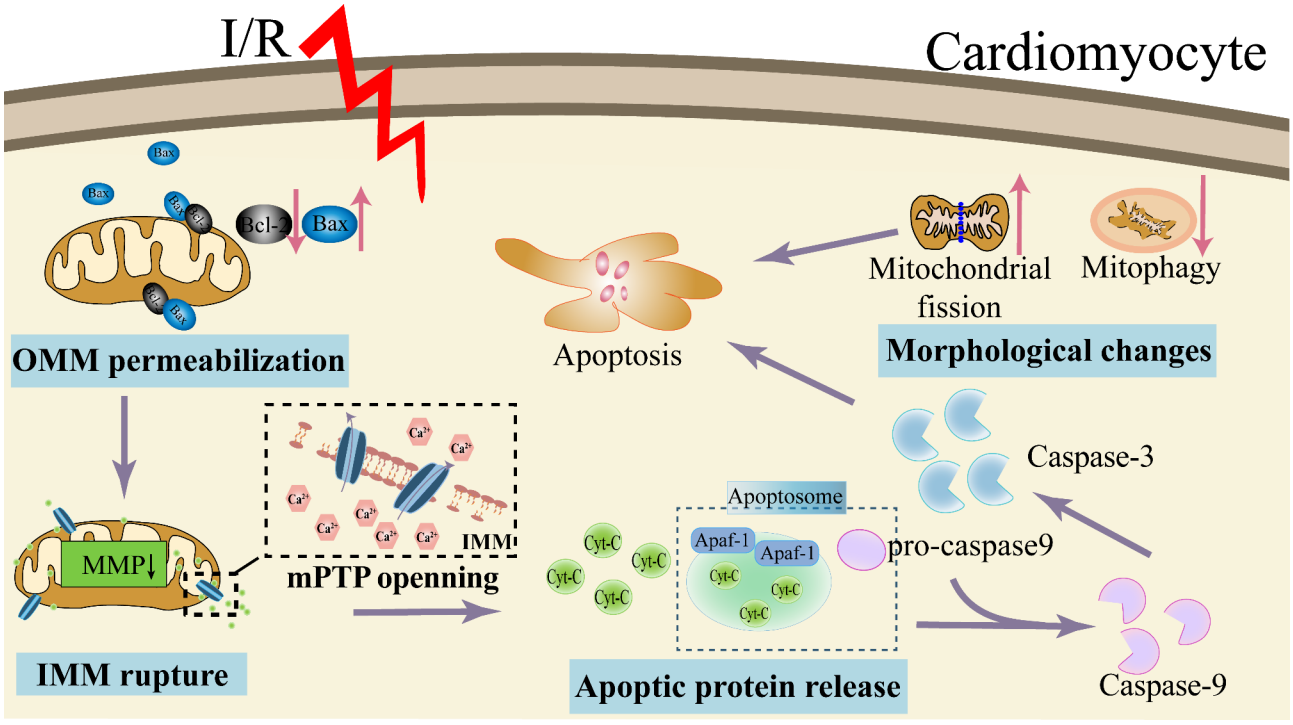

Fig. 2 An overview of CM mitochondrial apoptosis. Mitochondrial apoptosis involves increased mitochondrial membrane permeability, release of pro-apoptotic proteins, and mitochondrial morphological changes

Mitochondrial apoptosis involves three steps: increased mitochondrial membrane permeability, release of mitochondrial pro-apoptotic proteins, and activation of caspase-9 in the cytosol [5]. Mitochondrial apoptosis occurs when mitochondrial morphological changes (fission and autophagy) occur above a certain threshold, causing excessive cell fragmentation (Fig.2).

CM mitochondrial membrane permeability

Altered mitochondrial membrane permeability involves Bcl-2 associated X protein (Bax)-mediated

permeabilization of the outer mitochondrial membrane(OMM) and mPTP-induced rupture of the inner mitochondrial membrane (IMM) [14].

Mitochondrial Outer Membrane Permeabilization (MOMP)

The pro-apoptotic proteins Bax and the anti-apoptotic protein Bcl-2 belong to the Bcl-2 family. They are important regulators of apoptotic mitochondrial membrane permeabilization and are involved in MOMP. Bcl-2 is located in the OMM, endoplasmic reticulum membrane, and outer nuclear membrane, whereas Bax exists mainly in the cytoplasm as an inactive monomer. Only a small proportion of Cyt-C traverses between the mitochondria and cytoplasm, directly regulating mPTP opening and thereby promoting the release of Cyt-C into the cytoplasm. In vivo, Bax and Bcl-2 expression is tightly regulated in a state of dynamic equilibrium [15]. Hu et al. reported that Tanshinone IIA and cryptotanshinone directly inhibit mitochondrial membrane hyperpolarization and Cyt-C translocation by balancing Bcl2 and Bax [16]. Lahnwong et al. found that Dapagliflozin (DAPA), a sodium-glucose cotransporter type 2 inhibitor, upregulates the level of Bcl-2 before ischemia in cardiac I/R rats to protect CMs from apoptosis [17]. Yamazaki et al. showed that, in healthy cells, Bcl-2 prevents mitochondrial outer membrane permeabilization by binding to Bax on the mitochondria and transferring it back to the cytoplasm [18]. In contrast, during I/R injury, Bcl-2 expression is repressed, Bax expression is increased, leading to the accumulation of Bax in the OMM, This accumulation induces conformational changes in BH3-only proteins such as Bcl2/adenovirus E1B 19 kDa-interacting protein 3 (BNIP3), resulting in increased OMM permeability [18]. Therefore, the Bcl-2/Bax ratio is commonly used as a proxy for apoptosis. MicroRNAs (miRNAs) are important regulators of gene expression at the transcriptional and post-transcriptional levels. They have been reported to be upregulated or downregulated in I/R. For example, miRNA-195 represses Bcl-2 expression by targeting the regulatory 3'-untranslated region of the mRNA encoding it [19].

In the early phase of cardiac reperfusion, endogenous protein kinases, such as protein kinase B (Akt), provide potent protection. Previous studies have shown that activation of phosphatidylinositol 3-kinase (PI3K)/Akt signaling reduces Bax expression, stimulates the release of Bcl-2, attenuates mitochondrial apoptosis, and limits myocardial infarction size. Honokiol [20], ginsenoside Rd [21], and vascular endothelial growth factor [22] play cardioprotective roles by activating this pathway.

Akt can phosphorylate the Ser9 site of glycogen synthase kinase-3β (GSK-3β); this phosphorylation protects from myocardial I/R injury. Study have reported that p85α deficiency inhibited mitochondria-mediated CM apoptosis by activating Akt/GSK-3β signaling [23]. Another study showed that B-type natriuretic peptide (BNP) directly inhibits mPTP opening during reperfusion, depending on the PI3K/Akt pathway [24]. BNP has been shown to block calcium transport from the cytoplasm to the mitochondria via mitochondrial calcium uniporter complex, preventing the mitochondrial accumulation of calcium ions, which is beneficial to the heart during reperfusion.

Mitochondrial inner membrane rupture

Unlike the OMM, the IMM is the core mitochondrial location [5]. The mPTP is a non-selective channel in the IMM that mediates the release of excess intracellular ROS and calcium release. Its opening is a prerequisite for losing IMM integrity and plays a decisive role in releasing mitochondrial pro-apoptotic factors [5]. Under physiological conditions, IMM is impenetrable to almost all metabolites and ions. However, after myocardial I/R injury, the uncontrolled and reversible opening of the mPTP leads to edematous IMM rupture, making it easier for Cyt-C, which is otherwise segregated in the inner folds of the IMM, to close the OMM, triggering a response.

The state of the mPTP is closely related to that of the MMP. The supply of cellular energy depends on the MMP, and maintaining its potentiation is essential for cell survival. Studies have suggested that the opening of the mPTP leads to MMP breakdown, which in turn leads to the release of caspase-3 [25]. However, other studies have suggested that the collapse of MMP, caused by excess intracellular ROS production, leads to the opening of the mPTP and rapid release of Cyt-C into the cytoplasm [26]. Despite these different perceptions of the causal relationship between the collapse of MMP and the opening of mPTP, the alteration of MMP and mPTP is undoubtedly a prerequisite for releasing Cyt-C from the mitochondria to the cytoplasm.

Intramitochondrial Ca^{2+} overload is a key factor in inducing the opening of the mPTP. the PLCδ1 protein regulates Ca^{2+} homeostasis for cardioprotective effects. Voltage-dependent anion channel 1 (VDAC1) is localized to the OMM, and its multimerization causes it to lose its affinity for hexokinase-2 (HK2), thereby enhancing mPTP opening and increases Cyt-C release. Pentoxifylline hydrochloride (PHC) pretreatment inhibited this pathway [27].

Translocator protein (TPO), also known as the peripheral benzodiazepine receptor, is an OMM protein upregulated during I/R injury and causes membrane electrolysis [28]. BNIP3 is another OMM protein that serves as a mitochondrial sensor of cytoplasmic oxidative stress and an effector of mitochondrial apoptosis. It plays an important role in apoptosis by forming homodimers

through its transmembrane structural domain. Overexpression of miRNA-210 directly targets BNIP3 [29] to rescue myocardial apoptosis.

The soluble receptor for advanced glycosylation end products (sRAGE) inhibits the Fox03a-BNIP3 pathway [30], thus inhibiting mitochondrial apoptosis by suppressing the loss of mitochondrial membrane potential and the aberrant opening of mPTP. It has been reported that members of the nuclear hormone receptor superfamily, such as peroxisome proliferator-activated receptors (PPARα, β, and γ), regulate CM apoptosis [31].

CM protein release

Mitochondria-associated apoptotic protein release involves the release of mitochondria-localized pro-apoptotic proteins and caspase-9 activation in the cytosol. Cyt-C is commonly used to assess I/R injury. High-temperature demand protein A2 (HtrA2) is another pro-apoptotic protein localized in the CM mitochondrial intermembrane space. Following cardiac I/R injury, these proteins are released into the cytosol and activate caspase-mediated apoptosis. The calcium-sensing receptor [32], a G protein-coupled receptor, activates intracellular effectors to stimulate intracellular Ca^{2+} release and protein kinase C activation.

Following I/R injury, the intracellular calcium concentration ($[Ca^{2+}]_i$) increases rapidly, and the administration of the calcium-sensing receptor agonist CaGdCl3, which acts on protein kinase C, leads to the release of Cyt-C. Caspases-9 and −3 are downstream proteins involved in CM apoptosis. In the adult heart, their expression levels are low; therefore, caspase-dependent apoptotic mechanisms are largely silenced. Once released during I/R injury, Cyt-C binds to the C-terminal domain of apoptotic protease activator-1 (Apaf-1), inducing a conformational change. Cyt-C, Apaf-1, and caspase-9 precursors form an apoptotic complex that activates the caspase-3 pathway, leading to apoptosis [5]. Toll-like receptor 4 (TLR4) is a potent inducer of these apoptotic cascades. Overexpression of the radioprotective 105-kDa protein (RP105) is a specific negative regulator of TLR4 signaling. Myocardial overexpression of RP105 leads to the inactivation of TLR4, inhibition of the apoptotic cascade, and injury [33]. Additionally, miRNAs directly target caspases. One study reported that miRNA-140 directly targets the 3'-UTR of YES1 mRNA, decreasing YES1 expression, reducing caspase-9 and −3 levels, and inhibiting mitochondrial apoptosis [34].Another recent study reported that mild therapeutic hypothermia suppresses mitochondrial apoptosis by modulating SLC25A10 expression [35].

CM mitochondrial morphological changes

Morphological changes in mitochondria play an important role in apoptosis. Physiologically, mitochondrial fission is activated primarily in response to increased energy demand, rapidly increase the number of mitochondria, separating damaged mitochondrial fragments, and maintaining overall mitochondrial health [36]. Fan et al. found that DAPA can normalize mitochondrial division and reduce apoptosis by activating the PGAM5/Drp1 signaling pathway [37]. However, growing evidence suggests that excessive mitochondrial fission promotes apoptosis, as smaller mitochondria are more capable of generating ROS. Fission is primarily controlled by the mitochondrial dynaminrelated protein 1(Drp-1). Upon I/R injury, the Drp-1 GTPase inhibitor Mdivi-1 and claudin-5 overexpression [38] downregulate Drp-1, whereas the KLF4-MARCH-Drp1 pathway, activated by Baicalein attenuates Drp1 expression [39], which in turn decreases mitochondrial swelling and fission and protects the myocardium from I/R injury.

Mitophagy is a protective mechanism that selectively eliminates damaged mitochondria by phagocytosing ruptured mitochondria, neutralizing pro-apoptotic factors, maintaining mitochondrial function, and attenuating mitochondrial apoptosis [40]. However, dopamine receptor 1 agonists have been shown to promote the release of pro-apoptotic proteins (e.g., Cyt-C) during I/R, leading to mitochondrial apoptosis [41].

FUNDC1 plays a major role in mitophagy. Under ischemic conditions, its activation promotes mitochondrial phagocytosis and selectively degrades mitochondria to reduce the leakage of pro-apoptotic factor Cyt-C, ultimately inhibiting apoptosis via caspase-9. One study reported that the FUNDC1 upstream factor Mst1 inhibits FUNDC1 expression by inhibiting MAPK/ERK-CREB signaling [42] and that FUNDC1 depletion failed to initiate protective mitophagy, leading to delayed mitophagy and mitochondria-mediated cell death. BNIP3/NIX, a classical mitophagy pathway, is distinct from its apoptotic pathway.

Endothelial cells

Compared to CMs, vascular ECs better tolerate myocardial ischemia, but multiple abnormalities occur rapidly during reperfusion [43]. In other words, compared to ischemia alone, ECs are more sensitive to reperfusion injury than CMs and are key mediators of cardiac I/R injury [44]. One of the important functions of vascular ECs is the nutritional support of CMs and the release of paracrine signaling peptides and signaling molecules (e.g., ROS, NO, and adenosine), which play important roles in the regulation of vascular tone [45]. Under pathological conditions, it is a potent regulator of CMs metabolism and survival.

Unlike CMs, however, ECs have fewer mitochondria (2–6% of cell volume). Endothelial mitochondria play a limited role in energy production, but they transmit

extracellular and intracellular signals in response to environmental signals, thereby regulating ECs activities. In addition to assisting in ATP production, endothelial mitochondria regulate the homeostasis of NO, ROS, and Ca^{2+} under normal conditions [46]. An imbalance in NO, ROS, or Ca^{2+} signaling can result in a loss of normal mitochondrial function, mitochondrial signaling machinery perturbation, or damage to the vascular endothelium (Fig. 3).

Ca2+ overload decreased NO bioavailability and increased ROS production in ECs

Numerous studies have shown that myocardial perfusion limitation results in decreased energy metabolism in myocardial microvascular ECs, increased ROS levels, increased intracellular Ca^{2+}, and decreased NO bioavailability, leading to altered mitochondrial membrane permeability, disruption of the balance between anti-apoptotic and pro-apoptotic proteins, the opening of the mitochondrial permeability transition pore, and mitochondrial swelling and deformation; all of these initiate apoptosis via the mitochondrial pathway [47].

ROS, negatively charged and short-lived oxides, are continuously produced in cells. In normal cells, ROS constitute a routine component of multiple signaling pathways; however, in I/R injury, ROS are important mediators of EC damage [48]. ROS production by human umbilical vein endothelial cells (HUVECs) increases under prolonged extreme ischemia; however, the same result was observed in HUVECs after 90 min of ischemia, followed by reoxygenation. The key sites of ROS production in ECs are complexes I and III in the mitochondrial electron transport chain [49]. Although ROS produced in the mitochondria are not affected by prolonged diffusion of cytoplasmic ROS, they rapidly alter mitochondrial function by increasing mitochondrial membrane permeability and facilitating Cyt-C release. Additionally, mitochondrial fission factor (Mff) activates mitochondrial apoptosis by triggering Cyt-C release via ROS overproduction [49].

NO, a key regulator of biological systems, is involved in the mitochondrial apoptotic pathway and plays a role in cardioprotection [50]. The three members of the NO synthase (NOS) family are neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). In a normal heart, the largest producer of NO is eNOS, which is mainly (but not exclusively) observed in ECs, localizes to the plasma membranes and exerts potent anti-inflammatory and anti-apoptotic effects on myocardial I/R injury [51]. Although the half-life of NO is short, the close proximity of ECs and CMs allows NO to diffuse into CMs and regulate their contractility, electrical activity, and oxygen consumption [52]. After reperfusion, cellular production of ROS increases, NO bioavailability is disrupted, and Ca^{2+} homeostasis is imbalanced, leading to apoptotic

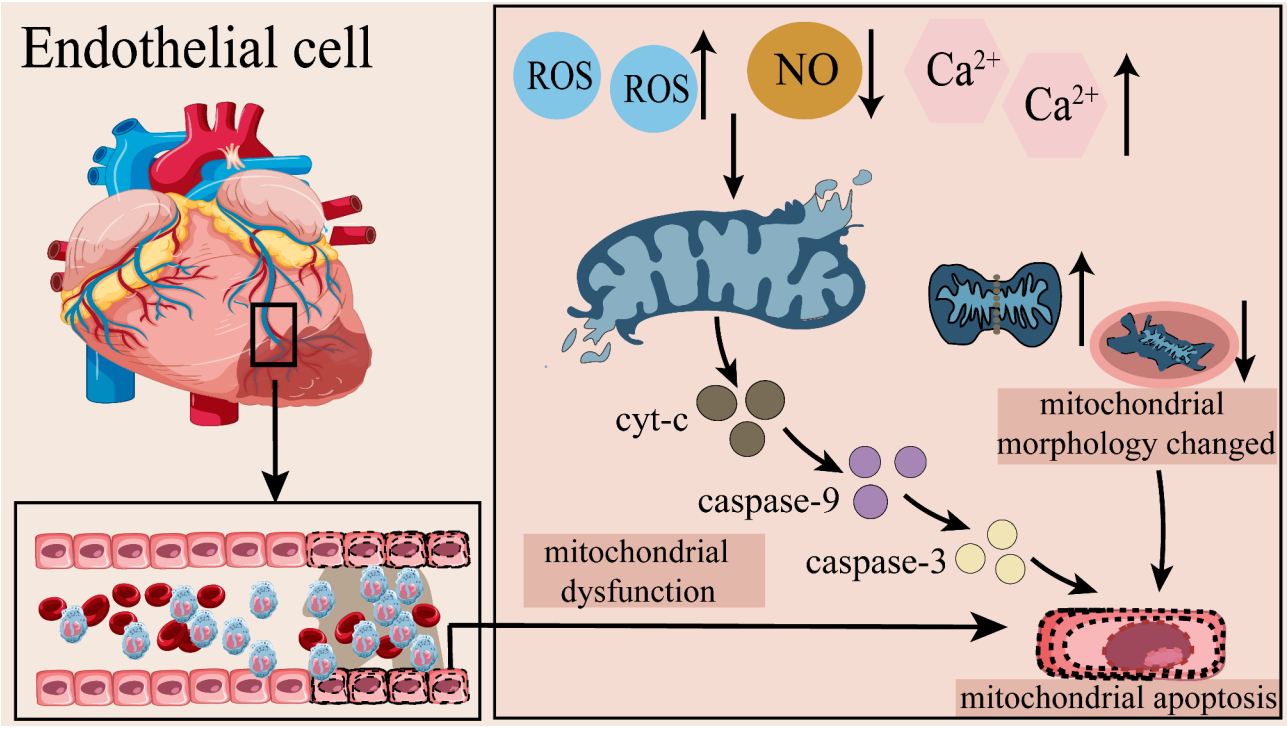

Fig. 3 myocardial I/R injury results in increased ROS levels, increased intracellular Ca^{2+}, and decreased NO bioavailability in myocardial microvascular ECs, leading to altered permeability of mitochondrial membranes, release of apoptotic proteins, and mitochondrial swelling and deformation; all of these initiate mitochondrial apoptosis

cell death. Zinnia flavonoid pretreatment of the rat myocardium significantly increased the levels of eNOS and increased NO content, preventing the opening of the mPTP and inhibiting apoptosis. Other study have shown that engeletin inhibits endothelial cell apoptosis via the AKT/eNOS/NO pathway, thereby maintaining cardiac function [6].

Ca^{2+} homeostasis plays an important role in the regulation of EC I/R injury. Ca^{2+} overload is considered an early marker of CM apoptosis after I/R injury [43]. Like CMs, apoptosis in ECs is associated with intracellular Ca^{2+} overload; however, the mechanisms responsible for Ca^{2+} overload may differ depending on the cell type. Mitochondria controls the vital activities of ECs and stimulates mitochondrial metabolism by Ca^{2+}. In particular, the discovery that Ca^{2+} regulates key mitochondrial enzymes in the mitochondria has led to the realization that an increase in $[Ca^{2+}]_m$ increases mitochondrial ATP production in ECs under physiological conditions. However, under pathological conditions, Ca^{2+} overload triggers the opening of the mPTP, which results in the release of mitochondrial contents (e.g., Cyt-C), induction of apoptosis, or loss of membrane potential (a consequence of prolonged mPTP opening), leading to ATP deprivation and necrosis [53]. Inward mitochondrial Ca^{2+} flow is mediated by the mitochondrial calcium uniporter (MCU). Li et al. reported that HINT2 overexpression modulated the MCU complex in ECs, inhibited $[Ca^{2+}]_m$ overload, and reduced mitochondrial apoptosis [44]. Calpain 1 (CPN1) and calpain 2 (CPN2) are ubiquitous calpains that exist in cytosol and mitochondria, and they are activated by cleaving cytosolic and mitochondrial proteins. Ca^{2+} overload is positive feedback to the activation of CPN1 and CPN2, which is conducive to the opening of mPTP during I/R [54]. In ECs, Ca^{2+} overload causes mitochondrial calpain1 to cut the Na^+/Ca^{2+} exchanger, leading to mitochondrial Ca^{2+} accumulation. Activated calpain1 can cleaved Bid and induce Cyt-C release and apoptosis [55].

In summary, compared to CMs during reperfusion, ECs show an increase in ROS production, disruption of NO bioavailability, and an imbalance in Ca^{2+} homeostasis, leading to subsequent mitochondrial dysfunction and morphology change, resulting in apoptotic cell death.

Impaired EC mitochondrial function

Similar to CMs, the mitochondria in ECs undergo persistent dysfunction, with altered permeability of the inner and outer mitochondrial membranes, releasing apoptotic proteins into the cytoplasm. This triggers a caspase cascade reaction within the cytoplasm, causing irreversible apoptosis.

Altered mitochondrial membrane permeability

Similar to the CMs, altered mitochondrial membrane permeability in ECs is associated with Bax-mediated OMM hyperpermeability and mPTP-induced IMM rupture. Under steady-state conditions, the mPTP is closed and maintains the mitochondrial membrane potential by oxidative phosphorylation to produce ATP. HK2 is the rate-limiting enzyme for cellular anaerobic respiration in myocardial ECs and is anchored to the OMM by interacting with VDAC1 to inhibit the opening of excess mPTP [56]. PI3K/Akt inhibits apoptosis by activating eNOS and/or NO production to prevent the opening of the mPTP. Studies have shown that ischemic preconditioning [57], ischemic postconditioning [57], Bauhinia championii flavone (BCF) [58], diazoxide (DZ) [59], and mitochondrial ATP-sensitive potassium channels (Mito K_{ATP}) play protective roles in the vasculature by activating PI3K/Akt signaling. Changes in MMP are signs of mPTP opening. MMP disturbances may be caused by alterations in mitochondrial proton transport in the electron transport chain, manifesting as depolarization and hyperpolarization.

Mitochondria-associated apoptotic protein release

Under physiological conditions, Cyt-C is tightly anchored to the inner mitochondrial membrane by cardiolipin (CL). Peroxidation of the CL contributes to the detachment of Cyt-C from the outer surface of the IMM and its subsequent release into the cytoplasm via mPTP. In I/R, moderate mPTP opening causes swelling of the IMM and oxidation of the CL itself, which promotes the release of Cyt-C from the mitochondria via the mPTP, leading to the ensuing caspase cascade of apoptosis. Other pro-apoptotic factors, including a second mitochondria-derived activator of caspases, mitochondria-derived second caspase activator Omi/HtrA2 (SMAC or DIABLO), and apoptosis-inducing factor (AIF), are released from the IMM into the cytoplasm via the uncontrolled opening of the mPTP [5]. Once in the cytoplasm, these factors interact with Apaf-1 to form apoptotic factors that activate caspase-9, which cleaves caspase-3 [49].

Changes in EC mitochondrial morphology

Fission and mitophagy are critical for keeping mitochondrial quantity and quality, and mitochondrial fission is a prerequisite for mitophagy, which removes defective or fissioned mitochondria to maintain their quantity and quality, thereby exerting antioxidant and/or anti-apoptotic effects on ECs mitophagy is regulated by various receptors, including Parkin, FUN14 structural domain containing 1 (FUNDC1), and BNIP3 [5]. Dual-specific protein phosphatase 1 (DUSP1) [60] and nuclear receptor superfamily 4A1 (NR4A1) [61] are upstream regulators of Mff-associated mitochondrial fission. They not

only phosphorylate Mff factors, thereby increasing fission, but are also responsible for mitophagy in ECs during cardiac I/R injury. Although phosphorylation of DUSP1 and NR4A1 have entirely different roles in mitochondrial activation, as previously described, DUSP1 phosphorylation activates mitophagy through activation of BNIP3 phosphorylation; NR4A1 phosphorylation, which activates the serine/threonine kinase tyrosine kinase 2α(CK2α) and leads to a decrease in the activity of FUNDC1, which inhibits mitophagy, disrupts mitochondrial homeostasis and promotes EC apoptosis. Liu et al. reported that Shuangshen Ningxin attenuates ECs I/R injury by inhibiting the NR4A1/Mff/Drp1 pathway [62]. Finally, Cai et al. reported that Empagliflozin activates FUNDC1-dependent mitophagy via the AMPKα1/ULK1 pathway, inhibiting mitochondrial apoptosis and protecting against I/R injury [63].

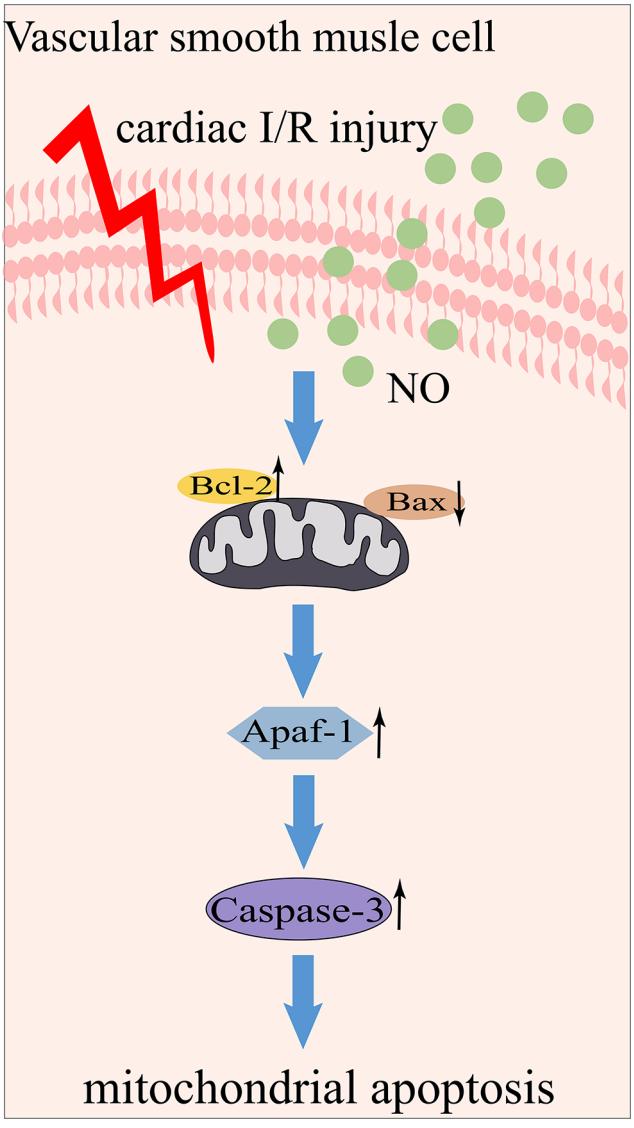

Fig. 4 NO inhibits the Bcl-2/Bax/Apaf-1/caspase-3 mitochondrial apoptotic pathway during cardiac I/R injury

Vascular smooth muscle cells

VSMCs are a major component of the arterial medial layer and are essential for vascular function.

Unlike the CMs, these cells are highly plastic and undergo reversible phenotypic changes in response to environmental stimuli. They act as biosynthetic agents, proliferators, and contractors in vessel walls [8]. During I/R, VSMCs undergo apoptosis due to ischemia and hypoxia. As mentioned earlier, the NO signaling pathway is important for maintaining vascular homeostasis and disease development. NO preconditioning inhibits VSMC apoptosis, and low concentrations of sodium nitroprusside increase heme oxygenase-1 expression, thereby protecting smooth muscles against I/R injury by inactivating p38 kinase and inhibiting the Bcl-2/Bax/Apaf-1/caspase-3 apoptotic pathway [64] (Fig. 4).

Cardiac fibroblasts

Different from the previous three types of cells, apoptosis is the main cause of I/R injury, while CFs were mainly characterized by proliferation and fibrosis [9]. CFs are the most abundant cells in myocardial tissue, accounting for 60-70% of the total number of cells. They are crucial for maintaining the extracellular matrix and for maintaining the structural integrity of connective tissue [65]. During the left ventricular remodeling process after cardiac I/R, CFs play a key role in maintaining the structural integrity of connective tissue. They achieve this by proliferating, depositing collagen and other extracellular matrix proteins, and replacing cardiomyocytes with fibrotic scars, thereby contributing to pathological structural changes [65] (Fig. 5). The StaR protein in CFs has anti-apoptotic activity after infarction, preventing the mitochondria division and Cyt-C release, and participates in tissue repair and remodeling in the infarction area [66]. Vivar et al. found that angiotensin II induced a decline in mitochondrial membrane potential, an increase in the Bax/Bcl-2 ratio, the activation of caspase-3 and the DNA breakdown, thus leading to the apoptosis of CFs [67]. However, trichostatin A (TSA) [68] can reverse the decreased mitochondrial membrane potential of CFs, and the increased expression of osteopontin (OPN) [69] may inhibit mitochondrial apoptosis by inhibiting caspase-3, protect CFs, and promote fibrosis. Lysophosphatidic acid (LPA) induces dose-dependent proliferation and collagen synthesis, mediated by LPA3 at low doses, and induces mitochondrial dysfunction and activation of caspase-3 at high doses [70].

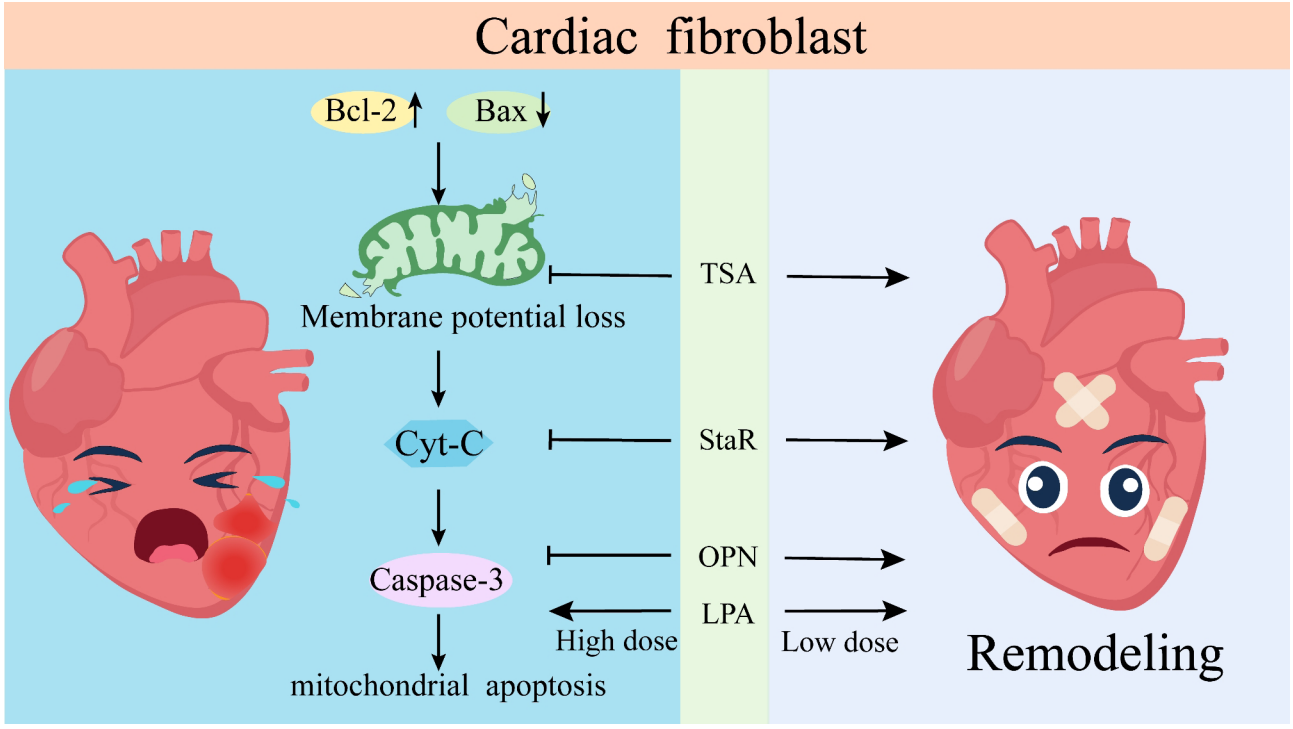

Fig. 5 Mitochondrial apoptosis of CFs and the effect of medicine on it

Mesenchymal stem cells

MSCs are adult stem cells, like CFs, that can treat tissue damage (e.g., myocardial infarction and stroke). They can self-renew and differentiate into multiple lineages, including ECs, neuronal cells, VSMCs, adult skeletal muscle cells, and CMs. Increasing evidence suggests that MSCs primarily repair damaged tissues by secreting paracrine factors (such as cytokines) to promote vasculogenesis and activate endogenous repair responses rather than by direct differentiation to replace missing cell clusters [10]. The core mechanism of MSCs action involves the PI3K/Akt signaling pathway (Fig. 6). Yin et al. reported that omentin-1 increased the level of phospho-Akt in MSCs in a time- and concentration-dependent manner, activating FoxO3a and GSK-3β to further regulate the expression of Bcl-2 family proteins [11]. Additionally, novel anti-apoptotic adipokines, C1q tumor necrosis factor-related proteins (CTRPs) [71], and berberine [72] activate PI3K/Akt signaling to reduce hypoxia-induced apoptosis in MSCs. Moreover, miRNAs are involved in MSC self-renewal and differentiation; for example, miR-16 regulates mitochondrial apoptosis via the APAF-1/caspase-9/ADP-ribose polymerase (PARP) pathway [73].

Summary

Increasing evidence suggests that mitochondrial apoptosis plays an important role in the onset, progression, and pathomechanisms of I/R injury. However, mitochondrial dysfunction involves many complex pathological processes, and mitochondrial apoptosis differs between myocardial cell types. CMs are rich in mitochondria, and the mitochondrial pathway itself is the most classical. One of the important functions of ECs is the release of paracrine signaling peptides and signaling molecules (e.g., ROS, NO, and adenosine), and an imbalance in the signaling of NO, ROS, and Ca^{2+} can lead to the loss of mitochondrial function. As the most abundant cell in myocardial tissue, CFs mainly activates and proliferates during the I/R process. VSMCs, as the inner layer of arteries, are mainly regulated by NO signaling molecules. In MSCs, self-renewing and multi-lineage differentiating cells, the core mechanism lies in the PI3K/Akt-related signaling pathway. Targeted mitochondrial repair of damaged CMs, ECs, VSMCs, CFs and MSCs is expected to be a potential therapeutic target for cardiac I/R injury in the future. Further studies on the mechanisms of mitochondrial apoptosis in these cells will likely provide new insights into treating I/R injury.

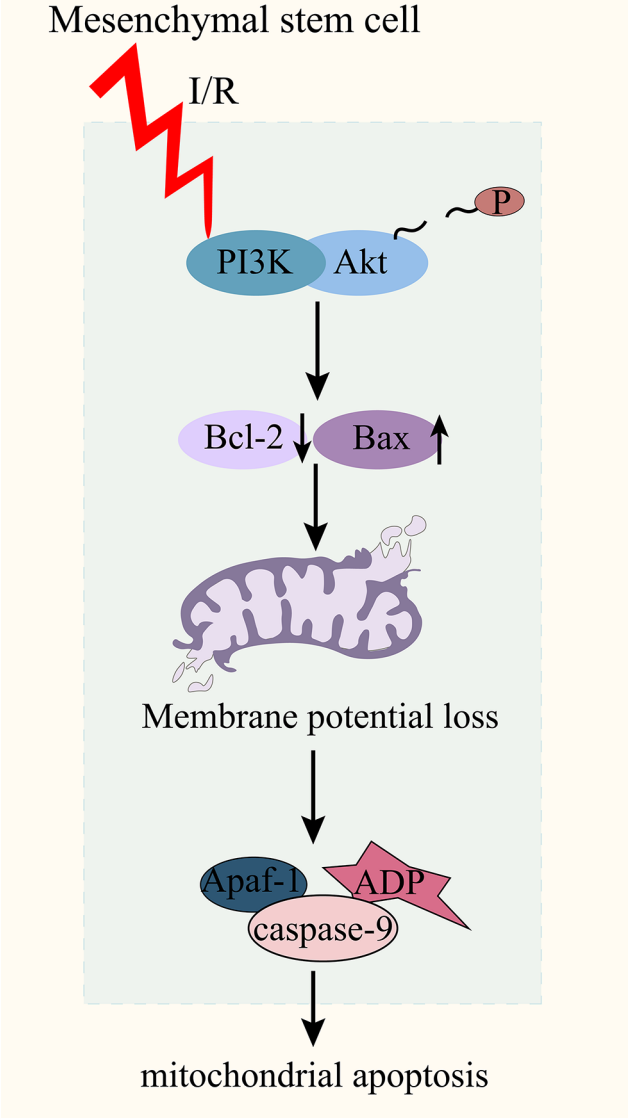

Fig. 6 Dephosphorylation of PI3K/Akt leads to increase of Bax and decrease of Bcl-2, resulting in loss of mitochondrial membrane potential, Apaf-1 entering cytoplasm and forming complex with ADP and caspase-9, therefore, leading to mitochondrial apoptosis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-06136-8 .

Supplementary Material 1

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Not applicable.

Author contributions
CYM conceived the manuscript. KXW and QZ designed and finished the manuscript. NW, KXW, QZ and WL contributed to the critical revision of the manuscript for important intellectual content. KXW, LYW, XXL, and CTZ contributed to perform the literature search. All authors read and approved the final manuscript.

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Declarations

Competing interests
No conflicts.

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