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REVIEW ARTICLE

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Mitochondrial Dynamics: An Attractive Therapeutic Target for Ischemia-Reperfusion Injury in the Heart

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Myocardial infarction is one of the leading causes of death worldwide. Current treatments do not compensate for the loss of cardiomyocytes, thus progression to heart failure is often inevitable. In myocardial infarction, the occlusion of coronary arteries and sudden restoration of blood flow give rise to ischemia-reperfusion injury, which leads to cardiomyocyte death. Mitochondria are not only involved in the bioenergetic aspect of the heart but also play a pivotal role in cell death during ischemia-reperfusion injury. Their morphology dynamically changes via fusion and fission in a balanced manner to maintain cellular health. However, ischemia-reperfusion injury triggers excessive mitochondrial fission, which is pathological to the myocardium. This review article discusses the association between myocardial ischemia-reperfusion injury and mitochondrial dynamics, serving as a rationale for a novel therapeutic strategy for myocardial infarction. Strategic modulation of mitochondrial dynamics under this pathological setting has been shown to be effective for cardioprotection. Increasing mitochondrial fusion or reducing excessive mitochondrial fission in the myocardial tissue could prevent cardiomyocyte death, thereby reducing infarct size. Proof-of-concept studies have utilized small molecules and peptides to implement this strategy into *in vivo* myocardial ischemia-reperfusion injury models. However, there remains a need to address the issues of specificity, bioavailability, and potency of these pharmacological agents before future application in cardiovascular therapeutics. Nevertheless, there has been growing interest in this therapeutic strategy in recent years, rendering it an attractive approach for ischemia-reperfusion injury in the heart.

Keywords: *mitochondria, heart, ischemia-reperfusion, cardioprotection*

Introduction

Ischemic heart disease can manifest as myocardial infarction and is responsible for approximately a two-year reduction in life expectancy.[1] Survivors of myocardial infarction are at an increased risk of developing heart failure, a life-threatening disease with a poor prognosis.[2] The prevalence of heart failure is projected to increase by 46% in 2030, with

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approximately 20% associated with ischemic heart disease.[3] Despite improvements in current treatment strategies, the global mortality and morbidity rate of myocardial infarction remains high, imposing a high socioeconomic burden on healthcare systems worldwide.[4] Therefore, there remains a clinical need to discover novel therapeutic strategies that can reduce myocardial infarct damage, prevent the development of heart failure, and improve survival rates among patients. This review elaborates on the pathophysiology of myocardial ischemia-reperfusion injury and its association with mitochondria. The therapeutic implications of modulating mitochondrial dynamics for cardioprotection are also discussed.

Pathophysiology of myocardial infarction and the involvement of mitochondria

Myocardial infarction is a consequence of an imbalance between cardiac supply and demand for oxygen and nutrients, and is often caused by occlusion of the coronary arteries which results in myocardial ischemia.[5] Consequently, the metabolic machinery of cardiomyocytes becomes impaired and leads to the death of cardiomyocytes. This cellular impairment is aggravated by reperfusion, whereby the sudden restoration of metabolic state at the onset of reperfusion causes a series of detrimental cellular alterations further compromising cardiomyocyte survival.[5,6] The cascade of cellular events triggered by ischemia and sudden restoration of blood flow in myocardial infarction is collectively termed as 'ischemia-reperfusion injury' (Figure 1).[5]

The heart depends on a continuous supply of energy in the form of adenosine triphosphate (ATP) to maintain its functions. Cardiomyocytes, through membrane ion channels (transporters, symporters, and exchangers), function to maintain intracellular ion homeostasis. Ion transporters, symporters and exchangers are proteins mediating translocation of ions across cell membranes (Figure 1).[5] Ion transporters depend on energy in the form of ATP to move one type of ion against its concentration gradient, often at the expense of another ion. For instance, the Na^+-K^+

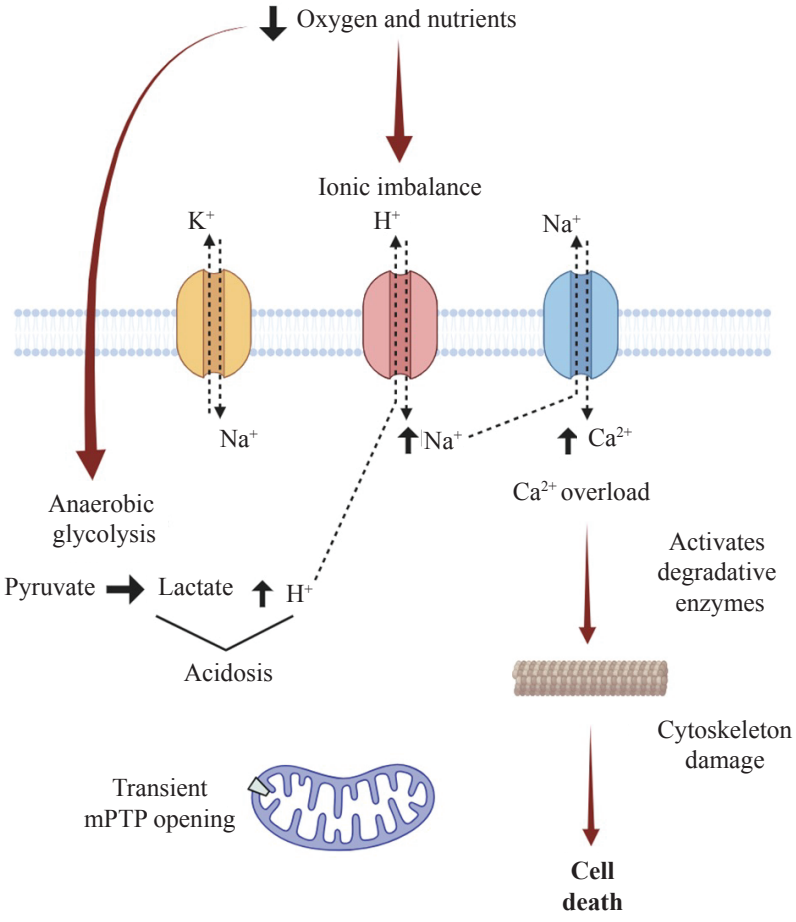

Figure 1. Pathophysiology of ischemic injury. Pathological events during myocardial ischemia, including intracellular acidosis, ionic imbalance, cytoskeleton damage and mitochondrial impairment. Depicted ion channels are Na^+-K^+ ATPase transporter, Na^+-H^+ exchanger and Na^+-Ca^{2+} exchanger. Ca^{2+}: Calcium; H^+: Hydrogen; K^+: Potassium; Na^+: Sodium; mPTP: Mitochondrial permeability transition pore. This figure was created with BioRender.com.

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ATPase transporter transports sodium ions out of the cell and potassium ions into the cell, utilizing ATP as energy. Ion symporters also require ATP, but transport two ionic species without the expense of other ions, for example the HCO_3-Na^+ symporter (Figure 2).[7] In contrast, ion exchangers do not require ATP, but rather, depend on the concentration gradient between two ion species, for example the Na^+-H^+ and Na^+-Ca^{2+} exchanger (Figure 1).[5] These ion channels play a pivotal role in the ionic imbalance occurring in ischemia and reperfusion injury, which ultimately lead to calcium overload and change of intracellular pH that triggers the opening of the mitochondrial permeabilization transition pore in the mitochondria (Figure 1, Figure 2).[5] This pore is a transmembrane protein in the inner mitochondrial membrane that is typically closed under physiological conditions.[8] The opening of this pore initiates mitochondrial permeabilization, a process where the inner mitochondrial membrane becomes disintegrated and releases the pro-apoptotic factor cytochrome *c* which activates the apoptotic pathway.[8]

Ischemic injury

Reduced ATP supply due to oxygen and glucose deprivation during ischemia will impede cardiomyocyte survival which results in calcium overload, oxidative stress and mitochondrial impairment.[5,6] During ischemia, the myocardium depends on anaerobic glycolysis to continuously produce ATP. The major waste products of anaerobic glycolysis are H^+ ions and lactate (Figure 1).[9] Intracellular accumulation of these products results in acidosis (low pH; pH <7.0) in cardiomyocytes (Figure 1).[5,6] To restore intracellular pH, the cell extrudes H^+ ions via the Na^+-H^+ exchanger, which increases intracellular Na^+ concentration (Figure 1).[5] This Na^+ increase is also compounded by the declined activity of Na^+-K^+ ATPase transporter, which is unable to pump Na^+ out of the cell due to the lack of ATP during ischemia (Figure 1). The influx of Na^+ is followed by entry of water, which increases the intracellular osmotic load that leads to cell swelling.[5,10]

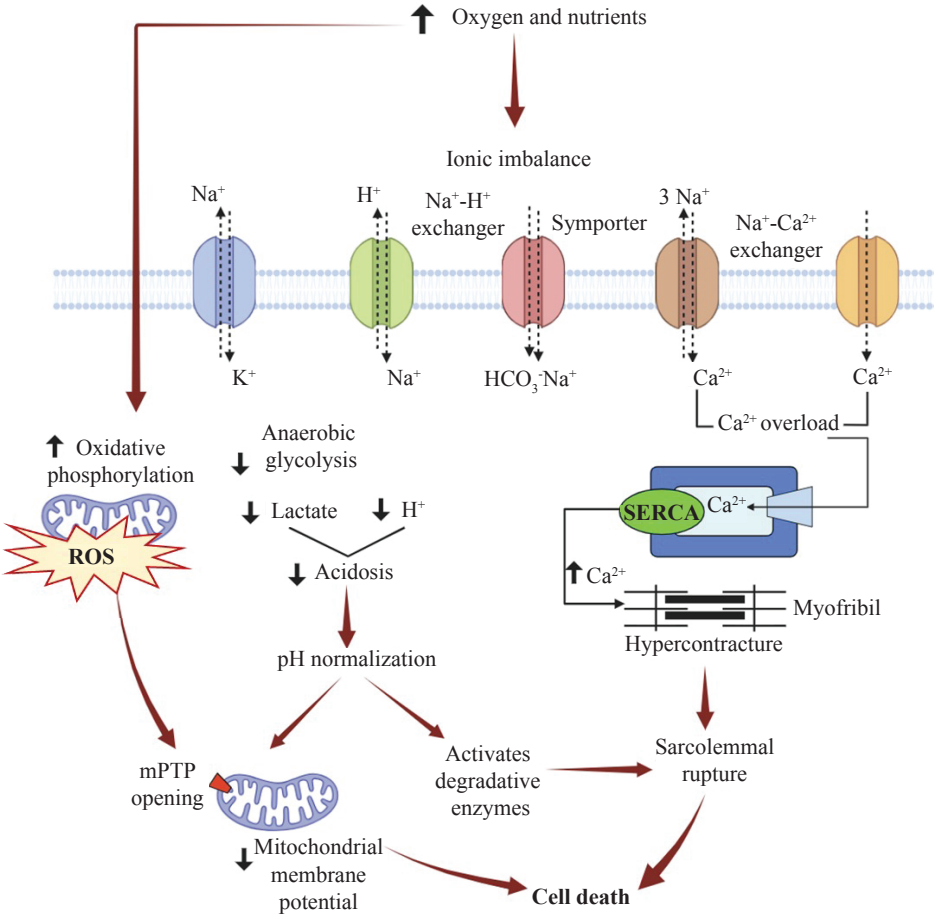

Figure 2. Pathophysiology of reperfusion injury. Pathological events during myocardial reperfusion, including ionic imbalance, hypercontracture, sarcolemmal rupture, oxidative stress and mitochondrial impairment. Depicted ion channels are Na^+-K^+ ATPase transporter, Na^+-H^+ exchanger, Na^+-Ca^{2+} exchanger, HCO_3-Na^+ symporter and Ca^{2+} uniporter. Ca^{2+}: Calcium; H^+: Hydrogen; K^+: Potassium; Na^+: Sodium; mPTP: Mitochondrial permeability transition pore; ROS: Reactive oxygen species; SERCA: Sarco/endoplasmic reticulum Ca^{2+}-ATPase transporter. This figure was created with BioRender.com.

To restore the ionic balance, the Na^+-Ca^{2+} exchanger is activated in reverse mode (normally, it extrudes Ca^{2+} in expense of Na^+, but is now reversed during ischemia to extrude excess intracellular Na^+ ions), which subsequently develops a Ca^{2+} overload in the cytosol (Figure 1).[5] Excessive intracellular Ca^{2+} accumulation can eventually lead to deleterious consequences, including activation of degradative enzymes that weaken the cytoskeleton and ultimately lead to cell death (Figure 1).[5] While elevated levels of cytosolic Ca^{2+} can also trigger the opening of the mitochondrial permeability transition pore, the acidotic environment during ischemia prevents this pore from opening, because its opening requires a normal pH.[5,9] However this view has been debatable, as recent studies observed transient opening of the mitochondrial permeabilization transition pore during ischemia due to the presence of other stimuli such as increasing levels of superoxide in the mitochondria.[9,10] This transient opening of the mitochondrial permeabilization transition pore during ischemia is exacerbated and sustained in the reperfusion phase.[11]

Reperfusion injury

Despite the aim to reduce ischemic injury, the restoration of blood flow during reperfusion can paradoxically lead to further cell death, known as 'reperfusion injury'. Upon reperfusion, the pH in the interstitial space is rapidly normalized (physiological pH is 7.0-7.4), but still leaves the cytosol with a high H^+ concentration, thereby creating a trans-sarcolemmal pH gradient.[5] This will activate the Na^+-H^+ exchanger and the Na^+-HCO_3^- symporter to extrude excess intracellular H^+ and restore the cytosolic pH at the expense of Na^+ (Figure 2).[5] The increase in cytosolic Na^+ concentration leads to activation of the reverse mode of the Na^+-Ca^{2+} exchanger to remove intracellular Na^+ and increase Ca^{2+}, further aggravating the pre-existing Ca^{2+} overload in the cell (Figure 2).[5] Consequently, the same vicious cycle of ionic imbalance that occurs during ischemia is exacerbated, but in a more rapid manner.[5]

During reperfusion, energy is re-supplied to the myofibrillar contractile elements causing rapid cyclic uptake and release of Ca^{2+} by the transporter sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) (Figure 2), causing oscillations of Ca^{2+} release into the cytosol.[10] The ATP-dependent activation of myofibrils in the presence of high cytosolic Ca^{2+} generates an uncontrolled and excessive force, which favors the development of hypercontracture and can lead to sarcolemmal rupture (Figure 2). This injury can spread to adjacent cells via gap junction communication.[12] It occurs mechanically via exchange of forces exerted by tight intercellular junctions, resulting in hypercontracture, thereby leading to sarcolemma rupture of adjacent cells. Another way to spread the injury is via diffusion of Ca^{2+} and other secondary messengers through gap junctions to transmit the trigger for hypercontracture. In addition, normalization of intracellular pH during reperfusion also induces calpain-mediated proteolysis of sarcolemma and cytoskeleton proteins (Figure 2). This renders the already-stressed cardiomyocytes more fragile and susceptible to rupture.[12]

Re-oxygenation of the mitochondria during reperfusion also results in overproduction of reactive oxygen species (ROS) such as superoxide and hydroxyl radicals. During ischemia, oxidative phosphorylation in the mitochondria is impeded due to the lack of oxygen, and thus limits the production of ROS, a byproduct of oxidative phosphorylation.[13] At the onset of reperfusion, the abrupt availability of oxygen causes a major increase of oxidative phosphorylation activity, which generates abundant ROS that exceeds the intracellular antioxidative defense mechanism.[13] This leads to oxidative stress that can damage cellular membranes by causing peroxidation of its lipid constituents and degrades the myocardial contractile machinery by activating proteolytic enzymes and inducing non-enzymatic protein modifications.[5,6,10]

Furthermore, excessive ROS, high cytosolic Ca^{2+} and normalization of pH during reperfusion also trigger the opening of the mitochondrial permeability transition pore, which permeates the inner mitochondrial membrane and depolarizes the mitochondrial membrane potential.[9,10] This permeabilization induces mitochondrial swelling and ultimately rupture of the outer mitochondrial membrane, resulting in apoptotic cell death due to release of cytochrome *c* from the mitochondrial intermembrane space.[10,11] In summary, both ischemia and reperfusion cause deleterious effects to the myocardium. Therapeutic strategies that can effectively counteract this damage has been difficult to achieve due to the complex multifaceted pathology of myocardial ischemia-reperfusion injury.

Current treatments for myocardial infarction

Most pharmacological and surgical interventions for myocardial infarction have a common goal to achieve timely

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reperfusion.[6] The longer time elapsed since occlusion of coronary artery, the larger the extent of myocardial necrosis.[14] Therefore, timely reperfusion is crucial for the salvage of myocardial tissues.[15] Pharmacological interventions involve administration of vasodilators (*e.g.* nitroglycerin and beta blockers) and thrombolytic agents (*e.g.* aspirin) to promote reperfusion.[14] Anticoagulant and antiplatelet agents are also administered to prevent thrombosis. However, surgical procedures such as percutaneous coronary intervention and coronary artery bypass graft surgery remain the preferred methods for patent revascularization. These procedures yield superior results over pharmacological reperfusion using thrombolytics.[14] However, these therapies are costly and time-dependent. Specifically, the timing of these procedures affects the outcomes for myocardial salvage and patient survival. For example, percutaneous coronary intervention, a procedure to open obstructed coronary artery using a stent or balloon, is recommended for myocardial infarction that occurs <12 hours and must be performed within 90 minutes.[16-18] This window period is recommended because delayed or prolonged percutaneous coronary intervention has been associated with increased patient mortality.[19] Not all patients have access to a resourceful coronary unit, thus these surgical interventions are not always feasible. Most importantly, they do not compensate for the loss of cardiomyocytes, thus progression to heart failure is often inevitable.[20] Therefore, myocardial salvage with novel cardioprotective strategies is an attractive approach to limit myocardial ischemic-reperfusion injury.

Mitochondria in the heart

About 90% of ATP utilized by the heart is generated by the mitochondria.[21] This signifies the importance of mitochondria in maintaining cardiac health and function.[21] Based on specific localizations related to the energy demand adjacent to cell and tissue structures of the adult heart, there are three populations of mitochondria, namely perinuclear, interfibrillar and subsarcolemmal mitochondria (Table 1). Perinuclear mitochondria generates ATP for metabolism occurring near the nucleus, interfibrillar mitochondria for contraction of sarcomere, and subsarcolemmal mitochondria for active transportation across the sarcolemma.[22] Distinct cristae structure of these mitochondria populations also reflect their different bioenergetic capacity. Most interfibrillar mitochondria have tubular cristae with smaller intra-cristae space compared to subsarcolemmal mitochondria which is mainly comprised of broad and flat cristae (lamelliform). This prominent feature allows interfibrillar mitochondria to generate relatively higher proton gradient across the inner mitochondrial membrane, thus enhancing the activity of ATP synthase to support the high energy demand of the contractile elements of cardiomyocytes.[22,23] In addition to a higher respiratory rate, interfibrillar mitochondria also show better resistance to ischemia.[22,24]

Mitochondrial dynamics

The term 'mitochondrial dynamism' refers to the dynamic process of mitochondria undergoing changes in shape,

Table 1. Phenotypes of mitochondrial populations in the heart.

Type	Localization	Shape	Length	Cristae Structure
Perinuclear mitochondria	Clustered at cell nucleus	Predominantly spherical	0.8-1.4 μm	Curved cristae with comparably little matrix
Interfibrillar mitochondria	Between myofibrils	Elongated	1.5-2.0 μm	Predominantly tubular shaped, some are lamelliform
Subsarcolemmal mitochondria	Underneath sarcolemma	Combination of polygonal and spherical	0.4-3.0 μm	Closely packed, mostly lamelliform, some are tubular

Heart tissue for electron microscopy was obtained from the left ventricle of a non-human primate (*Macaca fuscata*) and mouse (*Mus musculus*).[22,24,77]

quantity and distribution throughout the cell.[25] Collectively, these changes occur through constant transformation of mitochondrial morphology by means of mitochondrial fusion and fission.[25,26] Mitochondrial dynamics, as exemplified by fusion and fission events, have been shown to regulate cellular metabolic programming and determine cell fate.[27-29] Smaller mitochondrial size generated from fission facilitates mitochondrial trafficking and calcium buffering at specific cell regions, enabling regulation of various physiological processes including synaptogenesis, cell division, and T cell activation.[28,30,31] In contrast, elongated mitochondria from fusion allows exchange of mitochondrial matrix of damaged mitochondria and maintenance of proton gradient required for ATP production to maximize oxidative capacity.[32] Mitochondrial fusion is essential for complementation of mitochondrial DNA and distribution of lipids and proteins to other mitochondria.[33]

Regulators of mitochondrial dynamics include fusion proteins mitofusin (Mfn)1, Mfn2 and optic atrophy (Opa)1, and fission proteins dynamin related protein (Drp)1, mitochondrial fission factor (Mff), mitochondrial dynamics proteins of 49 and 51 kDa (MiD49/51) and mitochondrial fission (Fis)1 protein (Figure 3).[27] Mitochondrial fusion is a step-by-step process that involves the integration of the outer mitochondrial membrane, inner mitochondrial membrane and the mitochondrial matrix. The fusion of outer mitochondrial membrane is carried out by Mfn1 and Mfn2, whereas the fusion of inner mitochondrial membrane and matrix as well as maintenance of cristae morphology is regulated by Opa1.[34] The key regulator of mitochondrial fission, Drp1, largely resides in the cytosol and translocates to the outer mitochondrial membrane to polymerize into ring-like structures and actively constrict the organelle to yield two daughter mitochondria.[35] Mitochondrial translocation of Drp1 is regulated by multiple post-translational modifications such as phosphorylation, ubiquitination, SUMOylation and S-nitrosylation. For example, phosphorylation at Ser616 promotes translocation of Drp1 to the mitochondria, whereas Ser637 prevents this event.[36-39]

Mitochondrial morphology can differ in many cell types. Mitochondria are generally considered as 'interconnected' and 'highly mobile' organelles. However, this assumption is less relevant for adult cardiomyocytes. Mitochondria in adult cardiomyocytes are relatively short and lack interconnected networks, as the latter can impose biomechanical restriction during cardiomyocyte contraction.[40] The fission-fusion cycle is approximately 16 days in adult mouse cardiomyocytes, which is relatively slow compared to other cell types, such as INS1 rat

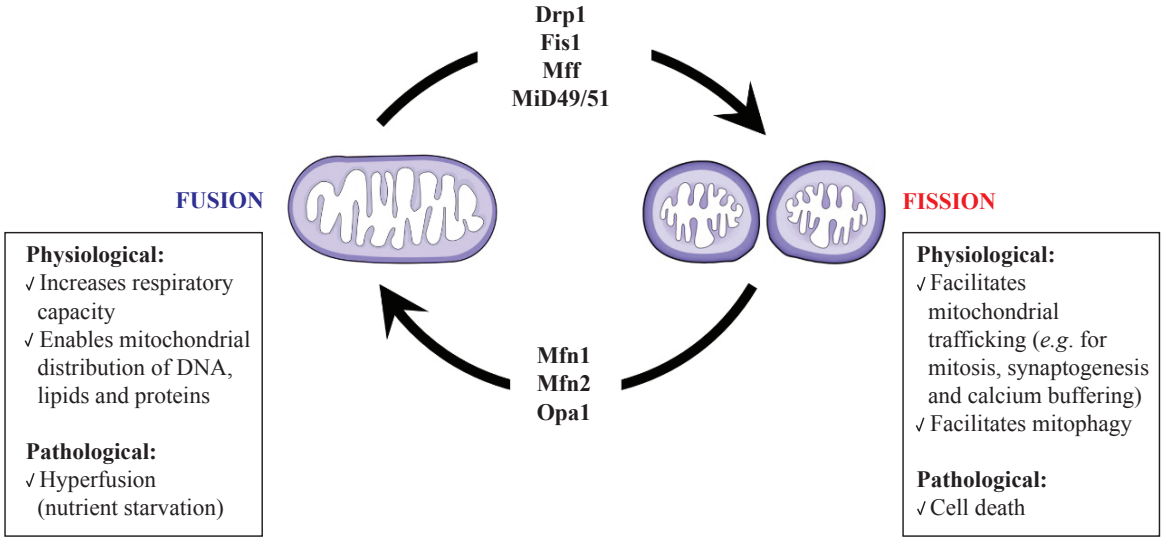

Figure 3. Mitochondrial dynamics. Mitochondrial fission is mainly regulated by Drp1, Fis1, Mff and MiD49/51 proteins, whereas fusion by Mfn1, Mfn2 and Opa1 proteins. Fusion and fission play pivotal roles to maintain cellular homeostasis in physiological settings. However, the excessive can lead to detrimental consequences in pathological settings. Drp1: Dynamin related protein 1; Fis1: Mitochondrial fission 1 protein; Mfn1/2: Mitofusin 1/2; Mff: Mitochondrial fission factor; MiD49/51: Mitochondrial dynamics proteins of 49 and 51 kDa; Opa1: Optic atrophy 1.

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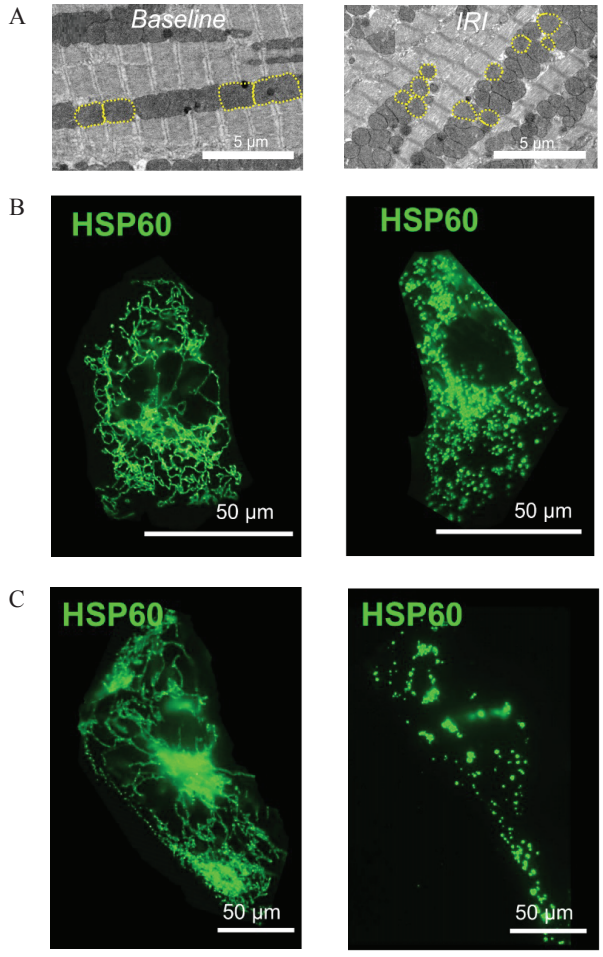

Figure 4. Mitochondrial morphology of cells subjected to ischemia-reperfusion injury. Fused mitochondrial morphology at baseline (left column) and fragmented mitochondrial morphology after ischemia-reperfusion injury (IRI) (right column) in (A) adult mouse cardiomyocytes, (B) mouse atrial HL1 cells and (C) human induced pluripotent stem cells (iPSCs).

insulinoma cells, HeLa human cervical cancer cells, and human umbilical cord vein endothelial cells (HUVECs).[41–44] Physiologically, adult cardiomyocytes display a lattice-like mitochondrial organization (Figure 4A), whereas immature cardiomyocytes from atrial tumor derived HL1 cells and induced pluripotent stem cells exhibit a more interconnected network (Figure 4B, Figure 4C). Meanwhile, non-cardiomyocytes, such as vascular smooth muscle cells, have elongated tubular mitochondrial phenotype with less branching.[45] Under pathological stimuli such as ischemia-reperfusion injury, fragmented mitochondrial morphology is generally represented as punctate/spherical mitochondria in all cells (Figure 4).

Strategic modulation of mitochondrial dynamics as a novel therapeutic approach for myocardial ischemia-reperfusion injury

Physiologically, mitochondrial fusion and fission constantly occur in a balanced manner. Fission segregates dysfunctional mitochondria for mitophagy and fusion allows damage in the counterpart mitochondria to be diluted by fusing with healthy mitochondrial cohort.[33] However, this balance is shifted under pathological insults. Nutrient deprivation triggers hyperfusion of mitochondria as a means to sustain oxidative phosphorylation and energy production (Figure 3).[46] Pathological stimulus, such as ischemia-reperfusion injury, triggers excessive mitochondrial fission which produces dysfunctional mitochondria that are smaller in size and may lead to cellular demise in multiple tissues including the brain, liver and heart (Figure 3).[47-49] Shifting the balance towards mitochondrial fusion either by enhancing mitochondrial fusion or inhibiting mitochondrial fission has been extensively investigated as a therapeutic approach to improve cell survival.[27] For example, overexpression of mitochondrial fusion proteins (Mfn1 and Mfn2) or the dominant negative mutant of Drp1, Drp1K38A, has been demonstrated to protect cardiomyocytes from simulated ischemia-reperfusion injury. Conversely, overexpression of the mitochondrial fission protein Fis1 leads to cardiomyocyte death.[50] In summary, a balanced mitochondrial dynamic is required to maintain cellular health. In the context of myocardial ischemia-reperfusion injury, pharmacological and genetic targeting of mitochondrial fusion (Mfn1, Mfn2, Opa1) and fission proteins (Drp1, Fis1) have been shown to be cardioprotective.[51-53] However, modulation of mitochondrial fusion proteins have been associated with their pleiotropic roles that are unrelated to mitochondrial fusion, such as via the sarcoplasmic reticulum.[51,53] In contrast, directly targeting the Drp1 fission protein has been more consistently shown to induce cardioprotection through its role in mitochondrial fission.[47,54,55] The reason for these mechanistic differences is unclear, but may be related to the more prominent role of the mitochondrial fission protein Drp1 in cardiac diseases compared to the mitochondrial fusion proteins.[56-58]

Pharmacologically, there have been few small molecule regulators of mitochondrial dynamics. In general, they either promote mitochondrial fusion or inhibit mitochondrial fission. Small molecules that are reported to promote fusion lack information on their exact protein target. For example, M1, a hydrazone derivative, promotes mitochondrial

fusion in mouse embryonic fibroblasts and protect SH-SY5Y cells, a neuroblastoma cell line, against 1-methyl-4-phenyl-pyridinium-induced cell death. Although the exact binding target is unclear, mechanistically, M1 increases the expression levels of ATP5A/B, one of the proteins which form the mitochondrial complex V (ATP synthase), which in turn promotes mitochondrial fusion.[59] Another example is S3 (15-oxospiramilactone), a diterpenoid derivative, which induces mitochondrial fusion in Mfn1 or Mfn2 knockout mouse embryonic fibroblasts by inhibiting the mitochondrial deubiquitinase protein ubiquitin-specific peptidase 30 (USP30), thereby preventing degradation of Mfn1/2 fusion proteins and promoting mitochondrial fusion.[60] There have also been reports of a small molecule mimicking the protein-protein interface of Mfn2 and an Food and Drug Administration (FDA)-approved rheumatoid arthritis drug, Leflunamide, which also possess pro-fusion activity.[61,62]

Mitochondrial fission protein, Drp1, is essential for the development of organs, as its global knockout has been reported to be lethal in mouse embryos.[63,64] Cardiac-specific conditional Drp1 knockout mice have been shown to exhibit mitochondrial derangements that compromise the development of the heart, leading to dilated cardiomyopathy and heart failure.[57,58,63] These pathologies are associated with diminished mitophagy and increased mitochondrial ubiquitination.[57,58,63]

Inhibition of Drp1 using small molecules or peptide inhibitors has been shown to be cardioprotective in myocardial ischemia-reperfusion injury and other pathological settings.[27] Inhibition of Drp1-mediated mitochondrial fission in cardiomyocytes subjected to oxidative stress-induced injury is associated with increased levels of Akt, suggesting the involvement of Akt-dependent survival pathway (Figure 5).[54] This signaling pathway involves upstream regulators such as sirtuin (Sirt)1, as well as downstream effectors of Akt such as proto-oncogene serine/threonine-protein kinase-1 (Pim1) and glycogen synthase kinase 3 beta (GSK3β) (Figure 5).[65,66] GSK3β has been shown to bind directly to Drp1 at the GTPase effector domain and induce phosphorylation at Ser693 which leads to a reduction of the GTPase activity of Drp1, thereby preventing mitochondrial fission.[65] Furthermore, the cardioprotective effect of Drp1 inhibition has also been shown to involve the mammalian Ste20-like kinase 1 (Mst1)- c-Jun N-terminal kinase (JNK) signaling pathway in a mouse model of myocardial infarction (Figure 5).[67] Collectively, it is evident that mitochondrial fission protein Drp1 is heavily involved in cardiac pathophysiology, and inhibition of Drp1-associated fission has been suggested as an advantageous therapeutic strategy for ischemia-reperfusion injury in the heart.[68]

Dynasore, P110 and Mdivi-1 are suggested to inhibit mitochondrial fission protein Drp1 with varying degrees of

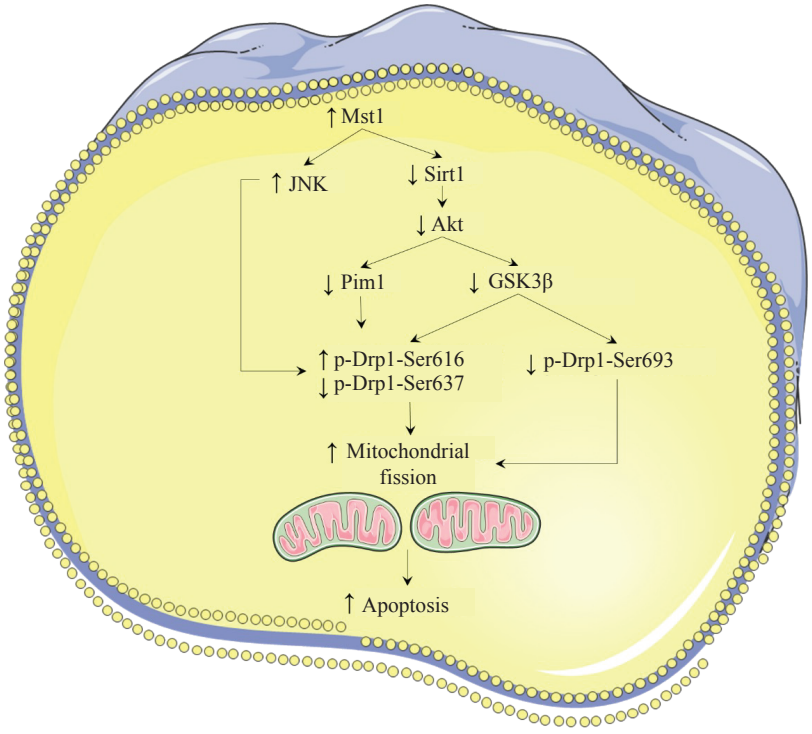

Figure 5. Signaling pathways involving Drp1 in ischemia-reperfusion injury. Akt: Protein kinase B; Drp1: Dynamin related protein 1; GSK3β: Glycogen synthase kinase 3 beta; JNK: c-Jun N-terminal kinase; Mst1: Mammalian Ste20-like kinase 1; Pim1: Proto-oncogene serine/threonine-protein kinase-1; p-Drp1-Ser616/Ser637/Ser639: Phosphorylated Drp1 at Ser616/Ser637/Ser639; Sirt1: Sirtuin 1.

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specificity. Dynasore, originally known as an endocytosis inhibitor, is a non-competitive GTPase inhibitor of dynamin-1, dynamin-2 and Drp1. Dynasore has been shown to promote survival of cardiomyocytes against oxidative stress through the inhibition of mitochondrial fission, as well as exhibit cardioprotective and lusitropic effects in a murine model of myocardial ischemia-reperfusion injury.[69] The Drp1 protein requires other pro-fission docking proteins such as Mff1, MiD49/51 and Fis1 for anchorage to the mitochondria.[25] P110 is a synthetic peptide that inhibits mitochondrial fission by blocking the interaction between Drp1 and Fis1, thereby inhibiting mitochondrial fission. P110 has been shown to improve mitochondrial biogenesis and attenuate oxidative stress in hearts of rats subjected to ischemia-reperfusion injury.[70]

Mdivi-1 is a quinazoline compound widely used to inhibit the mitochondrial fission protein Drp1. In its seminal paper, Mdivi-1 was reported to inhibit the GTPase activity of the Drp1 yeast homolog (IC_{50} 1-10 μM).[71] Since then, several studies have demonstrated the protective effect of Mdivi-1 in various experimental settings, including ischemia-reperfusion injury.[27] However, the specific targeting of this inhibitor towards the Drp1 human homolog has been challenged in the recent years. Studies indicate that Mdivi-1 likely possesses Drp1-independent protective mechanisms involving other cellular targets such as potassium and calcium channels, mitochondrial complex I, and Nrg1, a transcriptional factor for yeast–hypha morphogenesis in *Candida albicans*.[72-74] In addition, despite another study using the same *in vitro* experimental setting, the effects of Mdivi-1 previously reported in its seminal paper was irreproducible.[71,72] A recent paper suggests that the inconsistent finding and non-Drp1 specific effects of Mdivi-1 may be attributed to its tendency to aggregate.[75] Therefore, results on Drp1 inhibition based solely on Mdivi-1 should be interpreted with caution. Efforts have been made to identify more specific inhibitors of the mitochondrial fission protein Drp1 for cardioprotection, but still need further optimization for potency.[75,76]

Conclusion

Mitochondria are directly involved in the pathophysiology of myocardial ischemia-reperfusion injury. Ultimately, the ionic imbalance, oxidative stress and calcium overload in ischemia-reperfusion injury lead to the permeabilization of the inner mitochondrial membrane which allows release of the pro-apoptotic factor cytochrome *c* that activates the apoptotic pathway. As a dynamic organelle, the mitochondria are capable of fusing and dividing (fission) in a balanced manner as a means to regulate various physiological processes and maintain cellular health. In general, mitochondrial fusion allows maintenance of mitochondrial health, thus preserving cellular health, whereas excessive mitochondrial fission often leads to cell death. The latter is often observed in heart tissues subjected to ischemia-reperfusion injury. Shifting the balance of mitochondrial morphology towards fusion has shown beneficial results for cardioprotection. This strategy is often implemented by increasing mitochondrial fusion activity or reducing excessive mitochondrial fission. There has been a considerable number of studies showing proof-of-concept of this therapeutic strategy to salvage the heart from ischemia-reperfusion injury by utilizing small molecules and peptides. However, these pharmacological agents are yet to be investigated in clinical trials, likely due to shortcomings such as specificity and bioavailability. Future research is still required to search for more clinically suitable drug candidate targeting mitochondrial dynamics for ischemia-reperfusion injury in the heart.

Authors contribution

ERR, EFZ and AAR were involved in the conceptualization and revision of the manuscript. ERR and AAR prepared the manuscript draft. ERR and EFZ designed the table and figures.

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