Mitochondria as a therapeutic target for cardiac ischemia-reperfusion injury (Review)

WENWEN MARIN¹, DENNIS MARIN², XIANG AO³ and YING LIU¹,³

¹Institute for Translational Medicine, The Affiliated Hospital of Qingdao University, College of Medicine, Qingdao University, Qingdao, Shandong 266071; ²Qingdao University of Science and Technology, Qingdao, Shandong 266061; ³School of Basic Medical Sciences, College of Medicine, Qingdao University, Qingdao, Shandong 266071, P.R. China

Received June 8, 2020; Accepted November 18, 2020

DOI: 10.3892/ijmm.2020.4823

Abstract. Acute myocardial infarction is the leading cause of cardiovascular-related mortality and chronic heart failure worldwide. As regards treatment, the reperfusion of ischemic tissue generates irreversible damage to the myocardium, which is termed ‘cardiac ischemia-reperfusion (IR) injury’. Due to the large number of mitochondria in cardiomyocytes, an increasing number of studies have focused on the roles of mitochondria in IR injury. The primary causes of IR injury are reduced oxidative phosphorylation during hypoxia and the increased production of reactive oxygen species (ROS), together with the insufficient elimination of these oxidative species following reperfusion. IR injury includes the oxidation of DNA, incorrect modifications of proteins, the disruption of the mitochondrial membrane and respiratory chain, the loss of mitochondrial membrane potential (ΔΨm), Ca²⁺ overload, mitochondrial permeability transition pore formation, swelling of the mitochondria, and ultimately, cardiomyocyte necrosis. The present review article discusses the molecular mechanisms of IR injury, and summarizes the metabolic and dynamic changes occurring in the mitochondria in response to IR stress. The mitochondria are strongly recommended as a target for the development of therapeutic agents; however, the appropriate use of agents remains a challenge.

Contents

1. Introduction
2. Molecular mechanisms underlying IR injury
3. Alterations in mitochondrial metabolism during cardiac IR injury
4. Mitochondrial dynamics in response to IR stress
5. Current developments in mitochondria-targeted agents with cardioprotective effects against IR injury
6. Future perspectives
7. Conclusions

1. Introduction

Ischemia is the disruption of blood flow to tissues, which also results in the reduction of delivery of oxygen and metabolites to cells (1). Pathological ischemia in the heart results in oxidative damage, apoptosis and cardiomyocyte death, which is termed myocardial infarction (MI) (2). The standard approach for the management of a patient with MI is to restore blood flow to the ischemic tissue as quickly as possible. However, this restoration of oxygenated blood causes extensive tissue damage, termed ischemia-reperfusion (IR) injury or hypoxia-reoxygenation injury (3,4), which may result in morbidity and mortality in the cardiac intervention following a heart attack or stroke (5,6), as well as in other pathological situations, such as acute kidney injury, muscle injury, organ transplantation, hypovolemic shock and elective surgery (5,7). MI followed by reperfusion is the major stressor leading to chronic heart failure (8). However, reducing the irreversible ischemic damage due to MI and the inevitable IR injury that occurs during the reperfusion of ischemic tissues remains a challenge for cardiac therapy.

The mitochondria occupy >30% of the cardiomyocyte volume, provide 95% of adenosine triphosphate (ATP) generated through oxidative phosphorylation (OXPHOS) for the beating of the heart, and serve as the metabolic hub for the citric acid cycle and fatty acid β-oxidation (3,8,9). IR damages the mitochondrial respiratory chain, and accompanied by ATP depletion, results in the accumulation of mitochondrial reactive oxygen species (ROS; mtROS; ROS produced by mitochondria), and oxidative damage on mitochondrial DNA (mtDNA) or proteins, ultimately leading to necrotic and apoptotic myocardial cell death. The extent of mitochondrial damage is a key determinant in the progression of myocardial IR injury towards to heart failure (10).
2. Molecular mechanisms underlying IR injury

In the normoxic heart (Fig. 1A), glucose and fatty acids are oxidized through glycolysis, β-oxidation and the tricarboxylic acid (TCA) cycle, respectively. The electrons from the final metabolites nicotinamide adenine dinucleotide reduced form (NADH; NADH + H+ + 2e− ↔ NA DH) and flavin adenine dinucleotide hydroquinone form (FADH2; FAD + 2H+ + 2e− ↔ FADH2) reduce oxygen (O2) into water through the electron transport chain in the mitochondrial cristae membrane and generate ATP through OXPHOS driven by the protonmotive force (Δp) (11). During the conventional forward electron transport, 2e− are transferred from NADH to reduce coenzyme Q (CoQ, ubiquinone) into CoQH2 (the fully reduced form of CoQ, ubiquinol), following pumping of 4 protons (4H+) into the mitochondrial intermembrane space to maintain the Δp for the latter ATP synthesis. If complex I is inhibited by the CoQ inhibitor rotenone, the flavin mononucleotide (FMN) can accept the electrons from NADH and reduce O2 to generate superoxide (O2−) at complex I (5,12).

Under hypoxic conditions (Fig. 1B), the levels of the metabolites lactate and succinate are significantly increased in ischemic tissues. The accumulation of succinate is a primary metabolic signature of ischemia (13,14), and is considered to be a consequence of the inhibition of the TCA cycle during ischemia or anoxia. However, recent studies have proposed that it may also be due to the reversal of succinate dehydrogenase (SDH), driven by the electrons transported from the accumulated CoQH2 to fumarate (14,15). Moreover, succinate cannot be further converted into succinyl-coenzyme A (succinyl-CoA) by succinyl-CoA synthetase due to the depletion of GTP and CoA during ischemia (13,14).

During the reperfusion process (Fig. 1C), the accumulated succinate is rapidly re-oxidized by the SDH in the mitochondrial respiratory chain complex II, resulting in a burst of superoxide production from complex I by reverse electron transport (RET). A CoQH2 pool and a high Δp are required to drive RET. The accumulation of CoQH2 is formed during hypoxia and the high Δp is restored from proton pumping by complexes III and IV within seconds of reoxygenation. RET occurs when electrons are forced to flow backward from a reduced CoQ pool through complex I and onto the FMN to drive superoxide formation. The superoxide generated at complex I by RET upon reperfusion is considered as the major source of ROS during IR injury (5,14,15). In addition to the classic theory described above, there are also other sources of mitochondrial damage which contribute to IR injury, which have been elucidated in recent years, such as the ROS formed by monoamine oxidases, p66Shc protein and NADH oxidases (16,17).

The non-enzymatic acylation of lysine residues by acyl-CoA causes matrix protein dysfunction and aggregation (28-30).

Depletion of NAD+. A damaged mitochondrial respiratory chain also causes the depletion of NAD+. Since NAD+ is required for ATP synthesis and also for the deacylation of Lys by sirtuins, NAD+ depletion disrupts the pathways for bioenergetics and also the pathways altered by carbon stress (30,31).

Bioenergetic insufficiency (ATP). Mitochondria generate ATP through OXPHOS within the electron transport chain at the mitochondrial inner membrane. The lack of oxygen and respiratory substrates impairs OXPHOS during ischemia (3). IR injury markedly increases mitochondrial permeability, and dissipates electron and proton gradients. Furthermore, mitochondrial electron transport chain subunits lose their integrity and are degraded under conditions of IR. OXPHOS is markedly diminished in the abnormal mitochondria, leading to bioenergetic insufficiency during MI (23).

Formation of mitochondrial permeability transition pore (mPTP). mPTP is a large conductance channel in the inner membrane, which is activated by Ca2+ accumulation (32,33).
Figure 1. Continued.
Ischemic oxidative damage results in the marked reduction of mitochondrial membrane potential (ΔΨm), mitochondrial Ca²⁺ bursts and the opening of the non-selective mPTP in the inner membrane. Accompanied by the release of matrix proteins and mtDNA into the cytosol, mPTP opening increases colloid-osmotic pressure in the matrix, induces the innate immune response, and initiates the activation of proteases and lipases, leading to mitochondrial swelling and cardiomyocyte death through necrosis (34,35). The excessive activation of the mitochondrial fission protein, dynamin-related protein 1 (drp1), also facilitates mPTP opening and accelerates cell death. The genetic or pharmacological inhibition of drp1 has been shown to decrease the frequency of transient opening of mPTP during cardiac IR and reduce the infarct size following MI (8,36,37).

4. Mitochondrial dynamics in response to IR stress

Biogenesis. There are a large number of transcription factors and regulatory proteins to enhance mitochondrial biogenesis, such as nuclear respiratory factors 1 and 2, transcription factor A mitochondrial, peroxisome proliferator-activated receptors (PPARs), estrogen-related receptors, cAMP response element-binding proteins and Forkhead box-O. Transcription factors can be regulated by coactivators and corepressors,
such as PPARγ coactivator-1α (PGC-1α) and nuclear receptor corepressor 1. PGC-1α can be activated by post-translational modifications, such as through phosphorylation by AMP-activated protein kinase (AMPK) and deacetylation by Sirtuin 1 (Sirt1) (41). Under oxygen insufficiency, an energetic stress results in the downregulation of mitochondrial biogenesis by the hypoxia-inducible factor-mediated suppression of PGC-1α (42).

Fission and fusion. Continuous mitochondrial fission and fusion enables a dynamic distribution of mitochondrial proteins and mtDNA through the cellular mitochondrial network (23). Mitochondrial fission involves division into two daughter mitochondria, one with a markedly higher mitochondrial membrane potential (∆Ψm) and fusion affinity than the other one. Subsequently, the active mitochondrion is further regenerated and the defective one is targeted for degradation via mitophagy to maintain mitochondrial homeostasis (3,8,43). Fission and fusion proteins impact mitochondrial morphology and respiration, therefore, a balanced dynamic is essential to adjust mitochondrial metabolism to meet the energy demands in cardiomyocytes (8,44,45). The alteration of proteins involved in mitochondrial fission and fusion is tightly associated with the pathophysiology of several cardiac diseases, such as IR stress and heart failure (10,46).

The mitochondrial fission protein, Drp1, regulates mitochondrial dynamics on the mitochondrial outer membrane (47). Drp1 acts with Bcl-2 family proteins (such as the pro-apoptotic proteins Bax and Bak) to accelerate mitochondrial fragmentation and apoptosis (48). The phosphorylation at Ser637 residue of Drp1 by protein kinase A (PKA) inhibits its GTPase activity and its translocation to the mitochondria (49). Drp1 is enriched in the heart and its inhibition exhibits protective effects during myocardial IR by preventing excessive fission at the beginning of reperfusion (36,40). The overexpression of the cardiac proto-oncogene Ser/Thr kinase, Pim-1, causes the downregulation of Drp1 and an increase in the levels of Ser637 phosphorylation, and also protects against cardiomyocyte ischemia (37). Drp1 can be reactivated via the dephosphorylation of Ser637 by the calmodulin-dependent kinase CaN (50). Under normoxic conditions, the actions of PKA and CaN on Drp1 are well regulated by mitochondrial scaffolding protein the A-kinase anchoring protein 1 (AKAP1) (50). However, in ischemic myocytes, AKAP1 is ubiquitinylated by the hypoxia-induced E3-ubiquitin ligase Siah2 and rapidly degraded via the ubiquitin-proteasome system, which impairs PKA-mediated Drp1 inhibition and initiates mitochondrial fission and cardiomyocyte apoptosis (39,50). In addition, cardiac IR injury may be reduced when the transient suppression of Drp1 has been achieved by small molecular disruptors or by shRNA/siRNA-mediated knockdown (36,40,51). Chronic Drp1 deficiency, such as the heterozygous knockout (Drp1+/−), results in enlarged infarct sizes after IR stress (52).

The mitochondrial fission factor (Mff) is important for Drp1 recruitment in mitochondria during the process of fission (53). The inhibition of Mff has been shown to enhance mitochondrial membrane potential (∆Ψm) and decrease apoptosis under hypoxia/reoxygenation (54).

The dynamin-related GTPases mitofusin (Mfn) 1 and 2 are responsible for the fusion of the mitochondrial outer membrane between two mitochondria (19), whereas, the optic atrophy protein 1 (OPA1) mediates mitochondrial inner membrane fusion via regulation of the cristae structure (8,19). The down-regulation of Mfn1 under conditions of ROS-induced stress during IR disturbs mitochondrial fusion and induces cardiomyocyte apoptosis (55). Conversely, Mfn2 is upregulated in response to the ROS burst during IR and induces the apoptosis of cardiomyocytes by inhibiting Akt (also known as protein kinase B) and activating caspase-9 (39). In the heart, Mfn2 is involved in the regulation of mitophagy, altering mitochondrial morphology and forming the ER/SR-mitochondrial contact sites (8). OPA1 plays a protective role in IR injury. Opa1−/− mice showed larger infarct sizes compared with wild type, and Opa1 overexpression results in decreased damage in cardiac function (17). Bax not only directly interacts with Mfn2 to physiologically regulate its assembly and distribution in the normal heart, but also co-localizes with Drp1 to facilitate mitochondrial fission during early apoptosis (39). The Bcl-2 family proteins, Bax, Bak and Bcl2-interacting protein 3 (BNIP3), promote OPA1 cleavage and mitochondrial cristae remodeling following reperfusion, which in turn exacerbate IR injury (39,56).

Degradation and mitophagy. Mitochondrial dynamics facilitate mitochondrial quality control, which targets aberrant mitochondrial proteins or entirely damaged mitochondria for degradation to maintain myocardial mitochondrial homeostasis (57). The ubiquitin-proteasome system (UPS) and the autophagy-lysosomal system, termed mitophagy, are the major proteolytic pathways (58). The clearance of misfolded proteins usually occurs via UPS, whereas the entire damaged mitochondrion is degraded selectively by mitophagy (59). There are interactions and crosstalk between UPS and mitophagy in several regards, for example, the inhibition of proteasome induces the activation of mitophagy (58,60).

Defective mitochondrial proteins are labeled with ubiquitin proteins by a series of E1, E2 and E3 enzymes and targeted into the proteasome for elimination (61). Impaired UPS proteolytic function has been observed in the heart tissue of patients with cardiomyopathy and heart failure (62); however, the enhancement of proteasome-dependent degradation plays a cardioprotective role against proteinopathy and IR injury in mouse models (63). However, the effects of proteasome inhibitors remain controversial, which could be both beneficial and detrimental on cardiac function during myocardial ischemia and reperfusion (64).

Autophagy is induced by the deprivation of oxygen and nutrition, and refers to the lysosomal degradation of unecessary cellular components, such as protein aggregates, waste lipids and dysfunctional organelles (4). During selective mitochondrial autophagy (mitophagy), the dysfunctional mitochondria are tagged with polyubiquitin, embedded into autophagosomes and delivered to the lysosome for degradation (23,65).

PTEN-induced putative kinase 1 (PINK1) and ubiquitin ligase Parkin-mediated mitophagy. Kinase PINK1 is a Ser/Thr protein kinase that phosphorylates ubiquitin, Parkin and the mitochondrial outer membrane protein Mfn2. The phosphorylation events activate Parkin to translocate from the cytosol to the mitochondria (4,66). Parkin is an E3 ubiquitin

INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE 47: 485-499, 2021
ligase that polyubiquitimates the phosphorylated Mfn2 (67), in-turn promoting the binding of mitophagy proteins (seques-
tosome protein p62/SQSTM1 and autophagy-related protein 8 mammalian homologue LC3) and the cargo sequestration
within the autophagosome for the subsequent degradation in lysosomes (4,66).

The loss of PINK1 has been shown to increase the myocardial infarct size and generate excess ROS during stimulated IR
injury, whereas the overexpression of PINK1 reduces cardiac cell death following stimulated IR injury (68). On the one hand,
Parkin-deficiency in the hearts of mice reduces mitophagy, resulting in the accumulation of dysfunctional mitochondria
and enlarged infarcts following MI (69). However, excessive Parkin-mediated mitophagy also initiates the induction of
apoptotic pathways in cardiac microcirculation endothelial cells and exacerbates microvascular injury, as well as cardiac
reperfusion injury (70).

Receptor-mediated mitophagy. The mitophagy receptors, such as BNIP3, Nip3-like protein X (NIX) (also known as
Bcl2-interacting protein 3 like) and FUN14 domain containing 1 (FUNDC1), localize to the mitochondrial outer
membrane and serve a role in acute IR injury by regulating mitophagy (4,23,62,65,66). For example, BNIP3 binds to
stabilized PINK1, which facilitates the recruitment of Parkin on the mitochondrial outer membrane (71). NIX is ubiquiti-
nated by Parkin, enhancing the recruitment of the autophagy receptor NBR1 and LC3-coated vesicles to the mitochon-
dria (66). Under hypoxic conditions, the heart-enriched FUNDC1 interacts with LC3 to recruit autophagosomes to
mitochondria and enhances mitophagy (72). The phosphorylation at Ser13 of FUNDC1 by casein kinase 2 (CK2) inhibits the
interaction between FUNDC1 and Drp1, ultimately decreasing mitophagy (66). CK2α has been reported to be upregulated
during IR stress. As a consequence, FUNDC1 is deactivated and suppresses FUNDC1-mediated mitophagy (4). Ischemic
preconditioning has been reported to induce extensive FUNDC1- or Parkin-mediated mitophagy in platelets and
reduces secondary ischemic injury (22). In preclinical studies, myocardial conditioning interventions, including ischemic
pre-, post-conditioning or remote pre-/per-conditioning, are widely used strategies to reduce infarct sizes following
sustained IR. Several signaling pathways activated in response to conditioning stimuli end with the preservation of mitochon-
drial function (17).

Bcl2-associated athanogene (BAG) family molecular chaperone regulator 3 (BAG3)-mediated non-canonical
mitophagy. BAG3 is highly expressed in cardiac muscles and functions as a chaperone to target misfolded proteins for
lysosomal-mediated degradation (73). BAG3-Pro209Leu mutation causes the dysregulation of autophagy, which is asso-
ciated with the rapid progression of restrictive cardiomyopathy in myofibrillary myopathies (74).

Mitochondrial heterogeneity and subpopulations. In neonatal cardiac myocytes, the mitochondria are distributed throughout
the cytosol; however, in the adult cardiac cells, the mitochondria are small, round, hypodynamic and relatively static. In
particular, they are heterogeneous and are clustered into sub-sarcolemmal mitochondria (SSM), inter-myofibril mito-
dochondria (IFM) and perinuclear mitochondria subsets with
distinct morphologies, localizations and functions (8,17,75). These three mitochondrial subpopulations also possess
different sensitivities to pathological processes, such as IR injury. For example, SSM exhibit greater oxidative damage and
increased reduction in complex I-mediated respiration than IFM, whereas IFM consume less oxygen than SSM in
complex II following IR (17,76). Therefore, the mitochondrial defects in ischemic cells are in a heterogeneously distributed
manner amongst distinct subpopulations (76,77). A significant heterogeneity of redox states, Ca2+ and ∆Ψm has also been shown in myocardial cells following IR injury (76).

5. Current developments in mitochondria-targeted agents with cardioprotective effects against IR injury

The defective mitochondria display several different types of abnormalities and disturbances in metabolism and morphology,
which are often reciprocally coupled (41) and contribute to acute, irreversible cell death and potentially, MI (77). The
prevalence of ischemic heart disease is statistically the highest in Europe and North America. An estimated 8.4 million
American adults (≥20 years old) suffered an MI between 2013 and 2016. The mortality related to MI in 2017 was ~0.1
million in the USA. Additionally, ischemic heart diseases have a notable financial impact. For example, the arithmetic
mean cost for each discharge due to acute coronary syndrome (ACS) was $63,578 in 2009. Productivity losses connected to
ACS were ~$8,000 (short-term) and $50,000 (long-term) per disability claim between 2007 and 2010 according to medical,
pharmacy and disability insurance data (78). Therefore, drugs designed to directly act on the mitochondria for IR injury
therapy would have a substantial impact on both medical and societal costs.

With a growing understanding of the molecular mecha-
nisms of IR injury, several mitochondrion-targeted agents are under research and development, which are planned to be
used for cardiac IR injury treatment (Table I). These agents have been designed to improve mitochondrial function via
different underlying strategies, for example: i) Decreasing the accumulation of succinate during ischemia; ii) reducing
succinate oxidation following reperfusion; iii) inhibiting RET at complex I; iv) counteracting the superoxide produc-
tion; v) reducing ROS damage; vi) increasing Na+ levels; vii) blocking the mPTP; viii) maintaining Ca2+ homeostasis;
and ix) regulating mitochondrial dynamics and quality control.

Inhibitors of respiratory complex II (SDH). Malonate is a cell membrane-permeable form of malonate and
its protective effect against IR injury have been investigated in purified mitochondria, mouse hearts, and in murine and rabbit
models of stroke (14,79,80).

Chloramphenicol succinate (CAPS) is a prodrug of chlor-
amphenicol (CAP), which is already approved for clinical use as a human antibiotic, but with a risk of hemotoxicity,
including reversible and irreversible anemia. CAP-induced reversible anemia with reticulocytopenia, often in conjunction
with leucopenia and thrombocytopenia, develops with the increasing dose of CAP during treatment. Daily dose levels
of CAPS between 500-3,500 mg/kg have been observed to induce significant reversible myelotoxicity in different mouse strains. CAPS-induced irreversible anemia is a non-dose-related aplastic anemia with a severe pancytopenia, which is fatal and unpredictable with an incidence of 20-40 cases per million following therapies (81). CAPS can be hydrolyzed by esterase or oxidized by SDH into succinate and CAP, indicating that CAPS may be a competitive substrate of SDH. The active metabolite, CAP, in turn inhibits SDH reductive function, underlying the potential myelotoxicity induced by CAPS (82). The cardioprotective effect of CAPS has been shown in a pig model of MI, where reduced infarct sizes were observed following pre-ischemic in vivo administration of CAPS, as well as in the delayed treatment, where CAPS was administered prior to reperfusion. CAPS may also induce autophagy, indicated by the upregulated specific autophagy markers Beclin-1 and microtubule-associated protein light chain (LC3-II). However, the specific molecular mechanisms underlying its action remain unclear (83).

**Inhibitors of respiratory complex I.** MitoSNO is a mitochondria-targeted S-nitrosating agent, temporarily inhibiting complex I by reversible S-nitrosation of ND3 Cys39 residue during ischemia. The capability of MitoSNO as a potential drug has been assessed through incubation of mitochondria from rat and mouse hearts, ex vivo heart treatment and in vivo administration in mice models of MI (84).

Amobarbital is also a rapidly reversible inhibitor of complex I, which can improve mitochondrial function and protect cell survival during cardiac IR injury possibly via reducing the release of apoptosis inducing factor from the mitochondrial intermembrane space into cytosol. The protective effects of amobarbital against cardiac IR damage has been assessed in buffer perfused rats and Harlequin (Hq) mouse hearts (85).

Following high-throughput screening and validation, performed by Brand et al (86), two families of the cell-permeant compounds synthesized from commercial companies were identified as effective and selective suppressors of ubiquinone (Q)-binding site of respiratory complex I (site L3). Suppressors of site L3 electron leak (SIQELs), SIQELs 1.1-1.6 and SIQELs 2.2-2.4, can inhibit ROS production at complex I during reverse electron transport without altering basal respiration or the resting OXPHOS. SIQEL1.1 is an outstanding candidate, which strongly suppresses tunicamycin-triggered endoplasmic reticulum stress and caspase signaling pathways that leads to apoptosis in cardiomyocyte H9c2 cells and protects Langendorff-perfused mouse heart models of IR injury from endogenous oxidative damage, including enhancement of cardiac function in post-ischemic recovery and the decrease in infarct size. This highlights SIQEL1.1 as a novel therapeutic agent which can be used at the time of reperfusion. The suppression of ROS generation and oxidative stress signaling by SIQELs have also been assessed in isolated rat skeletal muscle mitochondria, 293 cells, primary astrocytes and live Drosophila melanogaster (86).

**Mitochondria-permeable -targeted antioxidants.** Coenzyme Q₁₀ (CoQ₁₀) is an endogenous lipid, with an oxidized-form (ubiquinone) and fully reduced-form (ubiquinol), which is the most common form of CoQ in humans. In addition to its primary role in electron- and proton-transport in mitochondria for production of ATP (87), CoQ₁₀ is also a mitochondria-permeable antioxidant (88). CoQ₁₀ deficiency is associated with several diseases, such as cardiovascular diseases, muscular dystrophy and neurodegenerative disorders (87). Administration of CoQ₁₀ as a supplement along with standard clinical therapy shows positive effects in patients with hypertension (87) and heart failure (89). Higher plasma CoQ₁₀ concentrations in patients with MI lead to improved left ventricular function. An intravenous CoQ₁₀ injection in rat models of coronary artery blockage has been shown to reduce cardiomyocyte necrosis and limit post-infarction hypertrophy of the left ventricle (87). A significant improvement in patients with chronic heart failure was shown in the Q-SYMBIO study (a randomized double-blind trial of 420 international patients) (90).

### Table I. Research and development status of the current mitochondria-targeted agents with cardioprotective effects against ischemia-reperfusion injury.

| Development stage | Mitochondria-targeted agent |
|-------------------|-----------------------------|
| Discovery: Natural source, high-throughput screening and drug design | MitoGSH (96) |
| Pre-clinical: Tested in cells, isolated tissues and animals to determine efficacy, toxicity and pharmacokinetic properties | Dimethyl malonate (80), chloramphenicol succinate (83), MitoSNO (84), amobarbital (85), SIQELs (86), SkQ (88), Euk-8 (97), CMX-2043 (α-lipoic acid) (98), NIM811 (105), sanglifehrin A (106), 5-aminoimidazole-4-carboxamide ribonucleotide (115,116), resveratrol (123), mitochondrial division inhibitor-1 (125), P110 (51), rapamycin (126), miR-499 (127) |
| Clinical trials: Tested in humans | Bendavia (NCT01572909), nicotinamide mononucleotide (UMIN000021309), Coenzym Q₁₀ (ISRCTN94506234), cyclosporine A (NCT01650662), bezafibrate (https://www.acc.org/Latest-in-cardiology/clinical-Trials/2013/04/13/21/02/BIP), pioglitazone (NCT00091949), metformin (NCT01217307) |
| Marketing, approved | None |
MitoQ is a bioactive ubiquinone covalent compound linked with a lipophilic cation triphenylphosphonium (TPP), which is reduced into the antioxidant ubiquinol in the mitochondria, and protects against oxidative injury. MitoQ is a promising mitochondria-targeted antioxidant, which has shown positive results in an intravenous and oral toxicity study in mice, in long-term oral administration studies in mice and rats, and in human Phase I and II trials (no significant difference in the NCT00329056 study and a potential benefit in the NCT00433108 study) (91,92).

10-(6'-Plastoquinonyl) decyltriphenyl-phosphonium (SkQ) is a lipophilic cation attached to a plastoquinone, which is a mitochondria-targeted antioxidant that protects cells from age-related diseases and ROS-burst-mediated acute diseases, such as IR. SkQ has not yet been approved by the US Food and Drug Administration as the safety and the clinical application of SkQ requires further study (88).

Bendavia (also known as SS31 and MTP-131) is a Szeto–Schiller (SS) peptide that can penetrate the mitochondria and ameliorate IR injury by interacting with the phospholipid cardiolipin and protecting mitochondrial cristae membranes (93). Positive results have been indicated in animal studies (93,94); however, Bendavia was unsuccessful in the EMBRACE STEMI study (Phase 2a trial). Therefore, MTP-131 is considered a potential pharmacological compound that protects mitochondria, but has not yet successfully translated into clinical use for the patients with ST-elevation MI (95).

MitoGSH is a group of synthetic forms of reduced glutathione (GSH) tagged with SS peptides or TPP ions that can target the mitochondria. MitoGSH is a class of novel drugs that may be highly valuable compounds for treatment of heart diseases by effectively restoring mitochondrial GSH levels. However, the limitations of TPP and SS peptides should be taken into consideration (96).

Euk-8 is a SOD/catalase mimetic antioxidant that is capable of potently scavenging oxyradicals. The positive cardiac-protective effects of Euk-8 include the reduction of both oxidative stress and pressure overload-triggered heart failure in the X-linked Hq mutant and WT mice (97).

α-lipoic acid is an important mitochondria-permeable antioxidant (88). α-lipoic acid is a natural dithiol that exerts protective effects against IR injury in several organs (98) and is used as a treatment of diabetic neuropathy and eye-disorders clinically, but has several side effects (88). A novel analogue of α-lipoic acid, which has been shown to achieve efficacious reduction of infarct damage in a rat model of myocardial IR injury (98).

**Precursors of NAD⁺.** Nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN) are the natural precursors of NAD⁺. The supplementation of NR or NMN can increase cellular NAD⁺ levels, without any noticeable side-effects (99,100). Bioactive NMN is synthesized from nicotinamide, a phosphate group and a ribonucleoside by nicotinamide phosphoribosyltransferase (100). Both NR and NMN have been shown to exert beneficial effects on cardiovascular functions under IR conditions. The administration of NMN has been shown to exhibit timing-dependent patterns on the reduction of infarct size (before ischemia or before reperfusion) (100). Several clinical studies regarding the safety and effects of NR on human physiology are currently underway in Europe and the USA (NCT03432871, NCT03423342 and NCT02812238). NMN is available on the market as a dietary supplement. A Phase I human clinical study for NMN has commenced in Japan and has evaluated the safety and the bioavailability of NMN in humans, with the aim of developing NMN into an anti-aging nutraceutical (99,101).

**Inhibitors of mPTP.** Cyclosporine A (CsA) is widely used as an immunosuppressive drug following organ transplantation and autoimmune disorders clinically, due to its ability to suppress the transcription of cytokines in activated T cells via blocking the CaN/nuclear factor of activated T cells pathway (95,102). However, CsA is not only an inhibitor of cytosolic CaN, but also an inhibitor of mPTP, binding to mitochondrial cis-trans prolyl isomerase cyclophilin D (CyD) (95). CyD is a positive regulator of mPTP with a chaperone localized in the mitochondrial matrix (3,8). The cardioprotective effects of CsA have been assessed in several species of animals using reperfused MI models, with inconsistent results in reduction of infarct size (95). Patients with ST-elevation MI (STEMI) administered CsA have exhibited promising results in a Phase II trial; however, CsA was unsuccessful in the Phase III CIRCUS and CYCLE trials (95,103,104).

NIM811 is a CsA derivative without immunosuppressive capabilities, which can also specifically inhibit mPTP opening in the mitochondria from the reperfused myocardium (95). Adult male New Zealand white rabbits injected with NIM811 at the time of reperfusion have been shown to exhibit preserved OXPHOS in the heart mitochondria, reduced infarct sizes and reduced myocardial damage (105).

Sanglifehrin A (SfA) is a specific inhibitor of cyclophilin D, similar to CsA, although it cannot suppress CaN activity. The specific inhibition of CyD by SfA protects against cardiac IR injury by limiting mPTP opening (3,106). The beneficial effects of SfA have been examined in MI female rat hearts. Significantly improved cardiac function was only shown in reperfused hearts treated with one intravenous bolus of SfA in the acute phase (2 days) of post-MI remodeling (106).

**PPAR agonists for the activation of mitochondrial biogenesis.** Fibrates have been discovered to activate PPARs, particularly PPARα, which regulate glucose and lipid metabolism in the heart. Fibrates have been widely used as a class of hypolipidemic drugs for treatment of heart attacks and strokes (107,108). Bezafibrate (marketed as Bezalip) is a fibrate drug widely used for the treatment of metabolic syndrome and hyperlipidemias. Bezafibrate is an agonist of PPARα and partially activates PPARγ and PPARβ, upregulating mitochondrial biogenesis by increasing PGC-1α expression (109). A small clinical trial including patients with acute STEMI and hyperfibrinogenemia showed bezafibrate treatment resulted in lower fibrinogen levels and lower frequency of major cardiovascular events compared with conventional therapy (110). The clinical trial Bezafibrate Infarction Prevention (BIP) study with 16 years of follow-up has shown that bezafibrate treatment reduces the risk of cardiac death and the non-fatal MI as well as mortality in patients with coronary arterial diseases (107).
Thiazolidinediones (TZDs) are structurally and pharmacologically similar to fibrate and more specifically act on PPARγ. TZD (also known as glitazone) is a class of hypoglycemic drugs used for treatment of type 2 diabetes clinically and protects against cardiovascular diseases. Pioglitazone and rosiglitazone are TZDs, which are also PPARγ agonists and are used as anti-diabetic drugs (108). With the risk of bias and uncertain adverse events, pioglitazone was shown to also reduce non-fatal MI and fatal or non-fatal stroke in four clinical studies (IRIS, NCT00099149; J-SPRINT, UMIN000013499; Kernan et al (111); and PROActive, NCT00174993) (112). Rosiglitazone has exhibited opposite results in pre-clinical and clinical trials. Rosiglitazone administration to pig and rat with IR-affected hearts has been shown to significantly decrease the infarct size, but without improvement of cardiac mitochondrial function (113). From the data pooled from 42 clinical studies, a significant increase in MI and severe adverse cardiovascular causes were observed with the use of rosiglitazone for patients with type 2 diabetes (114). Only one phase II clinical trial (NCT00064727) has been completed to investigate the therapeutic efficiency of rosiglitazone in patients with congestive heart failure (107).

Activators of mitochondrial biogenesis. 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR) is an AMPK agonist, activating PGC-1α by mimicking AMP (109). Serum-starved rat cardiomyocytes stimulated with AICAR have exhibited the full activation of AMPK downstream targets, such as Sirt1 and PGC-1α, which may contribute to mitochondrial homeostasis and cardioprotective effects against ischemic stress (115). Dogs treated with AICAR during cardiac bypass surgery followed by reperfusion have exhibited an improved recovery of myocardial glucose uptake and utilization (116).

Metformin is also an AMPK activator which exerts protective effects against cardiac ischemia, MI and heart failure. Additionally, metformin is a hypoglycemic drug widely used for the treatment of diabetes. Metformin has been proposed to reduce myocardial IR injury via increasing PGC-1α levels and mitochondrial biogenesis (117). Even though both preclinical and clinical studies (such as the DIGAMI 2 trial) have suggested the cardioprotective effects of metformin against IR injury (118-121), 4 months of metformin treatment in the GIPS-III trial did not result in improved left ventricular function in patients without diabetes presenting with STEMI. The 2-year follow-up results of the GIPS-III trial also revealed no beneficial long-term effects (122).

Resveratrol is a natural polyphenol (stilbenoid) found in red wine, that functions as a Sirt1 and PGc-1 activator, and an inhibitor of mitochondrial ATPase (109). The protective effects of resveratrol have been shown in animal models of cardiovascular, neurodegenerative and metabolic disorders. Five clinical trials of resveratrol for treatment of pre-diabetes and pulmonary diseases are currently in progress (NCT02502253; NCT02565979; NCT03762096; NCT02245932; NCT03819517). However, resveratrol has limitations in human therapeutic application due to its low potency and poor pharmacokinetics. Resveratrol and several other polyphenols are often used as dietary antioxidants (123).

Inhibitors of mitochondrial fission. Mitochondrial division inhibitor-1 (Mdivi-1) is a derivative of quinazolinone, which can inhibit mitochondrial fission by decreasing Drp1 activity, thereby reducing short term ischemic stress and myocardial IR injury. Mdivi-1 has been shown to exert cardioprotective effects in pre-treated cardiomyocytes (HL-1 cells), primary isolated neonatal mice ventricular myocytes, hearts from Langendorff perfusion in a rat model of IR, a murine coronary artery ligation model and a pressure overload mouse model with induced heart failure (40,124,125).

P110 is a small peptide (7-amino acids) which blocks the contact between Drp1 and mitochondrial fission 1 protein, ameliorating MI and long-term heart remodeling. P110 peptide has been demonstrated to inhibit excessive mitochondrial fission in primary neonatal rat cardiomyocytes, decreasing the infarct size and improving cardiac function in an ex vivo Langendorff rat heart model and an in vivo rat model of MI (51).

Regulator of mitophagy. Rapamycin has been found to promote mitophagy by inhibiting the mammalian target of rapamycin (mTOR) pathway and reducing infarct size in MI and cardiomyocyte apoptosis. As an allosteric inhibitor of mTOR complex I (mTORC1), rapamycin plays a role in the suppression of cell growth and division, and has been used as an immunosuppressant drug, or for the treatment of cancer and in-stent restenosis in clinical practice. Rapamycin has also been shown to reduce cardiomyocyte hypertrophy in a mouse model of chronic ischemic injury and to inhibit cardiomyocyte apoptosis in vitro (angiotensin II-induced myocyte apoptosis model) and in vivo (MI-induced chronic heart failure rat model) (126).

MicroRNA (miRNA/miR) regulation. miR-499 is an intronic miRNA identified to be involved in inhibiting apoptosis and MI induced by ischemia. miR-499 suppresses the expression of CaN and reduces cell apoptosis following cardiac ischemia, protecting against the MI. miR-499 transgenic mice were previously created, and when they were intravenously injected with the antagonim of miR-499 following IR induction via surgery, they were used for the in vivo evaluation of cardiac function, and hearts harvested from these mice were used for histological and western blot analysis (127).

6. Future perspectives

Limiting early mtROS production in IR injury. Superoxide production driven by succinate oxidation upon reperfusion through RET at complex I in the mitochondrial respiratory chain is considered the major source of ROS in IR injury (5). In vivo experiments have indicated that the succinate accumulated during ischemia undergoes rapid oxidation and returns to normoxic levels within 5 min following reperfusion (14). Therefore, it could be an effective therapeutic strategy for the treatment of IR injury by inhibiting RET at complex I for at least 5 min, until the succinate is no longer functional as an electron sink (Fig. 2).

The electron transport chain complex I undergoes a structural change, which is termed ‘active/deactive transition’ during IR (128). Cys39 in complex I is the critical switch of this conformational transition (Fig. 2), which is exposed in the deactive state of complex I under ischemic conditions and
Several thiol-reactive agents can be used to lock complex I in the deactive state by thiol modification on Cys39, including S-nitrosation (-SH into -SNO) by S-nitrosothiols (MitoSNO) or sodium nitrite (NaNO2) (84,130), S-sulfenylation (-SH into -SOH) by H2O2 (131), S-glutathionylation (-SH into -SSG) by glutathione disulfide (GSSG) (132) and S-sulfhydration/persulfidation (-SH into -SSH) by hydrogen sulfide (H2S) (133). These post-translational modifications can later be reversed by the internal mitochondrial GSH and thioredoxin systems (84).

However, the appropriate onset and duration of administration of thiol-reactive agents remains uncertain, otherwise this intervention based on the structural interconversion of complex I may be an ideal therapeutic method for preventing the burst of ROS production via RET and to return complex I activity in time.

Mitochondrial quality control. A poor quality of mitochondrial network generates and accumulates excess ROS (35). The burst of mtROS results in a series of downstream oxidative damages in cardiomyocytes after acute MI or during cardiac senescence (134,135), which may lead to sudden cardiac death or heart failure (136).

Mitophagy plays an important role in maintaining mitochondrial quality control, particularly in adult cardiomyocytes (43,137). However, cardiac mitophagy plays both beneficial and detrimental roles in response to IR stress (22,66,138). On the one hand, the clearance of the defective mitochondria via mitophagy may reduce the risk of ROS release and cell apoptosis, which exerts protective effects by decreasing myocardial oxidative stress and infarct size following MI (23,137,138). During IR, additional mitochondrial mass causes excess oxygen consumption and ATP depletion, which exacerbates the energetic stress. When energy is insufficient, active mitophagy can enhance cell survival (19,23). However, excessive mitophagy causes a drastic loss of mitochondrial mass and ATP supply in cardiomyocytes, leading to cell death and heart failure (54,66). When mitophagy is insufficient, the accumulation of damaged mitochondria and mtROS weakens the adaptiveness of cardiomyocytes towards cellular stress and apoptosis (4,66). Therefore, intervention with properly modulated mitophagy can serve as an ideal, yet difficult to achieve, therapeutic method for cardiac IR stress and other diseases (19,66,138).

Both mitophagy and mitochondrial biogenesis are upregulated following heart surgery (139). Thus, it is essential to maintain a balance between mitochondrial degradation and biogenesis to maintain mitochondrial homeostasis and myocardial protection under stress (138,140).

Attention must be paid to mitophagy, which is a complex process and intersects with several other cellular events. Some mediators in mitophagy signaling pathways, such as mTOR and AMPK, also play roles in mitochondrial biogenesis (19,66), intrinsic fatty acid metabolism (141), glucose-modulated

Figure 2. Schematic diagram showing the strategy of using the characteristics of complex I active/deactive transition to reduce ROS production upon reperfusion by temporarily locking complex I in the deactive state with reversible modifications on residue Cys39. ROS, reactive oxygen species; GSH, glutathione; TRX, thioredoxin; GSSG, glutathione disulfide.
of ischemic pre-conditioning, triggering several signaling pathways and finally inhibiting the formation of mPTP. As discussed above in Chapter 5 entitled ‘Current developments in mitochondria-targeted agents with cardioprotective effects against IR injury’, even though several agents have shown promising cardioprotective effects in pre-clinical studies, notable clinical trials in the past have highlighted the limitations or translational failures. The administration of certain autacoids, such as adenosine, bradykinin and opioids, can mimic the cardioprotective state of ischemic pre-conditioning, triggering several signaling pathways and finally inhibiting the formation of mPTP. As adjunctive therapies, a common limitation of these pre-conditional mimetics is the prerequisite administration before lethal ischemia, which makes them suitable for the planned cardiac surgeries but not effective in the clinical practice of acute MI. Two large-scale clinical trials, AMISTA I and II, which evaluated the effects of adenosine during acute STEMI therapy, exhibited restrictive infarct size-reduction and a number of adverse events, such as hypotension, bradycardia, heart block and ventricular tachycardia, especially in the adenosine patients with nonanterior MI (145).

Bradykinin, as the vasodilatory peptide, plays a role in decreasing blood pressure and increasing vascular permeability (146). Bradykinin signaling deficiency may be responsible for the vascular contraction and the high epithelial Na⁺ reabsorption observed in patients with hypertension and cardiovascular diseases. Bradykinin signaling has also been demonstrated to reduce inflammation and endothelial apoptosis in animal models of MI and cardiac IR injury (147). However, an increasing bradykinin concentration either inherited or acquired, which is a main mediator of hyperpermeability of capillaries and accumulation of local edema fluid, results in a rare disease termed bradykinin-mediated angioedema (146,148).

Opioids, such as morphine have been widely and frequently used as analgesics for the patients with STEMI (149). First of all, STEMI patients often have intense chest pain and anxiety in the acute phase of the treatment. As a traditional analgesia, opioids are effective in symptomatic relief. In addition, the reduction of pain and stress also results in vasodilation, an increase in blood flow, a decrease in arterial blood pressure and heart rate, equilibrating the supply and demand of oxygen and nutrients in cardiomyocytes (149,150). However, increased severe side-effects of opioids in STEMI patients have been revealed in recent clinical studies. For example, the low myocardial contractility, hypotension and fainting due to morphine-induced peripheral vasodilation are the main hemodynamic adverse effects for the patients with ventricular failure. A long-term use of opioids raises the risk for respiratory and gastrointestinal adverse effects, such as suppression of breathing, gut motility and absorption (150). Therefore, opioid administration may disrupt the absorption of certain essential oral medicaments, such as platelet inhibitors, and consequently delay the action of antiplatelet agents and prolong the platelet reactivity in the treatment of STEMI (149).

One of the major reasons for the limited clinical success is incomplete or insufficient pre-clinical investigation that does not offer accurate, reproducible and systematic examination of results from multiple animal models before large-scale clinical trials. The multicenter CAESAR (Consortium for Preclinical Assessment of Cardioprotective Therapies) initiative supported by the National Heart Lung and Blood Institute was created to improve translation of cardioprotective therapies into clinical use (151). CAESAR promotes integrated, standardized, solid and rigorous preclinical evaluations to examine potential drugs or combined therapies in centralized laboratories.

7. Conclusions

In conclusion, MI is one of the leading causes of mortality and economic burden on health systems. Cardiac IR injury following MI is due to the oxidative damage from mtROS, which leads to severe mitochondrial damage and cardiomyocyte death. Therefore, the mitochondria are strongly recommended to be considered as a therapeutic target to prevent cardiac IR injury. Several promising mitochondria-targeted agents are under research and development with significant focus on protecting against cardiac IR injury by counteracting the changes in mitochondrial metabolism and/or morphology during IR. However, none of these have exhibited sufficient efficacy and safety for clinical use. For future studies, some theoretical and practical points are suggested to be taken into consideration: Limiting ROS production by the reversible and temporary interference on the complex I structure is an interesting novel potential strategy. In addition, the side-effects and limitations of drug use should be thoroughly examined. A beneficial short-term outcome could lead to undesirable long-term outcomes over time, such as mitophagy, which is a double-edged sword for the mitochondrial quality and cell survival. Finally, an innovative multicenter approach is suggested, such as that advocated by CAESAR, in order to improve the translation of drugs from the bench to the bedside.

Acknowledgements

Not applicable.

Funding

The present study was funded by grants from the National Natural Science Foundation of China (nos. 81702785 and 81802822) and supported by the postdoctoral programme of Qingdao University.

Availability of data and materials

Not applicable.

Authors’ contributions

WM conceived the idea for the review, drafted and revised the manuscript. DM contributed to the revision, the provision of some of the data and databases, and also contributed to the proofreading and language correction of the manuscript.
WM and XA performed the literature search and prepared the figures. YL critically discussed and revised the manuscript, and provided some updates on the literature and clinical trials. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References
1. Carlucci A, Adornetto A, Scorziello A, Viggiano D, Foca M, Cuomo O, Annunziato L, Gottesman M and Feliciello A: Proteolysis of AKAP121 regulates mitochondrial activity during cellular hypoxia and brain ischemia. EMBO J 27: 1073-1084, 2008.
2. Haidarali S, Patil R, Ojha S, Mohanraj R, Arya S and Goyal SN: Targeting apoptotic pathways in myocardial infarction: Attenuated by phytochemicals. Cardiovasc Hematol Agents Med Chem 12: 72-85, 2014.
3. Kuznetsov AV, Javadov S, Margreiter R, Grimm M, Hagenbuchner J and Ausserlechner MJ: The role of mitochondria in the mechanisms of cardiac ischemia-reperfusion injury. Antioxidants (Basel) 8: 454, 2019.
4. Yang M, Linn BS, Zhang Y and Ren J: Mitophagy and mitochondrial integrity in cardiac ischemia-reperfusion injury. Biochem Biophys Acta Mol Basis Dis 1865: 2293-2302, 2019.
5. Chouchani ET, Pell VR, James AM, Work LM, Saeb-Parsy K, Frezza C, Krieg T and Murphy MP: A unifying mechanism for mitochondrial superoxide production during ischemia-reperfusion injury. Cell Metab 23: 254-263, 2016.
6. Lenesfedy EJ, Chen Q, Tandler B and Hoppel CL: Mitochondrial dysfunction and myocardial ischemia-reperfusion: Implications for novel therapies. Annu Rev Pharmacol Toxicol 57: 535-565, 2017.
7. Eltzschig HK and Eckle T: Ischemia and reperfusion—from mechanism to translation. Nat Med 17: 1391-1401, 2011.
8. Wang W, Fernandez-Sanz C and Sheu SS: Regulation of mitochondrial bioenergetics by the non-canonical roles of mitochondrial dynamics proteins in the heart. Biochim Biophys Acta Mol Basis Dis 1864: 1991-2001, 2018.
9. Spinelli JB and Havig MC: The multifaceted contributions of mitochondria to cellular metabolism. Nat Cell Biol 20: 745-754, 2018.
10. Niemann B, Schwarzer M and Rohrbach S: Heart and mitochondria: Pathophysiology and implications for cardiac surgeons. Thorac Cardiovasc Surg 66: 11-19, 2018.
11. Bender DA: Oxidative phosphorylation. In: Encyclopedia of food sciences and nutrition. Caballero B (ed): 2nd edition. Academic Press, Oxford, pp4295-4301, 2003.
12. Murphy MP: How mitochondria produce reactive oxygen species. Biochem J 417: 1-13, 2009.
13. Guzy RD, Sharma B, Bell E, Chandel NS and Schumacker PT: Loss of the SdhB, but not the SdhA, subunit of complex II triggers reactive oxygen species-dependent hypoxia-inducible factor activation and tumorigenesis. Mol Cell Biol 28: 718-731, 2008.
14. Chouchani ET, Pell VR, Gaude E, Akseintijevic D, Sundler SY, Robb EL, Logan A, Nadtochiy SM, Ond ENJ, Smith AC, et al: Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature 515: 431-435, 2014.
15. Pell VR, Chouchani ET, Frezza C, Murphy MP and Krieg T: Succinate metabolism: A new therapeutic target for myocardial reperfusion injury. Cardiovasc Res 111: 134-141, 2016.
16. Bagger H and Pleil K: Mitochondrial ROS in myocardial ischemia reperfusion and remodeling. Biochim Biophys Acta Mol Basis Dis 1866: 165768, 2020.
17. Boengler K, Lochnit G and Schulz R: Mitochondria ‘THE’ target of myocardial conditioning. Am J Physiol Heart Circ Physiol 315: H1215-H1231, 2018.
18. Bugger H and Beal MF: Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443: 787-795, 2006.
19. Marzetti E, Csiszar A, Dutta D, Balagopal G, Calvani R and Leeuwenburgh C: Role of mitochondrial dysfunction and altered autophagy in cardiovascular aging and disease: From mechanisms to therapeutics. Am J Physiol Heart Circ Physiol 305: H459-H476, 2013.
20. Nordberg J and Arner ES: Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic Biol Med 31: 1287-1312, 2001.
21. Judge S and Leeuwenburgh C: Mitochondrial bioenergetics, oxidative stress, and aging. Am J Physiol Cell Physiol 292: C1983-C1992, 2007.
22. Zhang W, Chen C, Wang J, Liu L, He Y and Chen Q: Mitophagy in cardiomyocytes and in platelets: A major mechanism of cardioprotection against ischemia/reperfusion injury. Physiology (Bethesda) 33: 86-98, 2018.
23. Tahir FG, Langford D, Amini S, Mohseni Ahoyti Y and Khalili K: Mitochondrial quality control in cardiac cells: Mechanisms and role in cardiac cell injury and disease. J Cell Physiol 234: 8122-8133, 2019.
24. Paradies G, Petrohilos G, Paradies V and Ruggiero FM: Role of cardioprotein peroxidation and Ca2+ in mitochondrial dysfunction and disease. Cell Calcium 45: 643-650, 2009.
25. Kay L, Nicolay K, Wieringa B, Saks V and Wallimann T: Direct evidence for the control of mitochondrial respiration by mitochondrial creatine kinase in oxidative muscle cells in situ. J Biol Chem 275: 6937-6944, 2000.
26. Dolder M, Wendt S and Wallimann T: Mitochondrial creatine kinase in contact sites: Interaction with porin and adenine nucleotide translocase, role in permeability transition and sensitivity to oxidative damage. Biol Signals Recept 10: 93-111, 2001.
27. Arslan F, de Klein DP and Pasterkamp G: Innate immune signaling in cardiac ischemia. Nat Rev Cardiol 8: 292-300, 2011.
28. James AM, Hoogewijs K, Logan A, Hall AR, Ding S, Fearnley IM and Murphy MP: Non-enzymatic N-acetylation of lysine residues by AcetylCoA often occurs via a proximal S-acetylated thiol intermediate sensitive to glyoxalase II. Cell Rep 18: 2015-2021, 2017.
29. Wagner GR, Bhatt DP, O’Connell TM, Thompson JW, Dubois LG, Backos DS, Yang H, Mitchell GA, Ilkayeva OR, Stevens RD, et al: A class of reactive Acyl-CoA species reveals the non-enzymatic origins of protein acylation. Cell Metab 25: 823-837.e8, 2017.
30. Wagner GR and Hirsche MD: Nonenzymatic protein acylation as a carbon stress regulated by sirtuin deacetylases. Mol Cell 54: 55-65, 2014.
31. Yang SJ, Choi JM, Kim L, Park SE, Rhee EJ, Lee WY, Oh KW, Park SW and Park CY: Nicotinamide improves glucose metabolism and the non-enzymatic origins of protein acylation. Cell Metab 25: 55-65, 2017.
32. Carraro M and Bernardi P: Calcium and reactive oxygen species in regulation of the mitochondrial permeability transition and of programmed cell death in yeast. Cell Calcium 60: 102-107, 2016.
33. Giorgio V, Guo L, Bassot C, Petronilli V and Bernardi P: Calcium and regulation of the mitochondrial permeability transition. Cell Calcium 70: 56-63, 2018.
34. Nakagawa T, Shimizu S, Watanabe T, Yamaguchi O, Otsu K, Kanamata H, Inohara H, Kubo T and Tsujimoto Y: Cypeolin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. Nature 434: 652-658, 2005.
35. Martin JL, Gruszczyk AV, Beach TE, Murphy MP and Saeb-Parsy K: Mitochondrial mechanisms and therapeutics in ischemia reperfusion injury. Pediatr Nephrol 34: 1167-1174, 2019.
36. Ong SB, Subrayan S, Lim SY, Yellon DM, Davidson SM and Hausenloy DJ: Inhibiting mitochondrial fission protects the heart against ischemia/reperfusion injury. Circulation 121: 2012-2022, 2010.
37. Din S, Mason M, Volkers M, Johnson B, Cottage CT, Wang Z, Joyo AY, Quijada P, Erhardt P, Magnuson NS, et al: Pim-1 preserves mitochondrial morphology by inhibiting dynamin-related protein 1 translocation. Proc Natl Acad Sci USA 110: 5969-5974, 2013.
38. Balaban RS: Cardiac energy metabolism homeostasis: Role of cytosolic antioxidants. J Mol Cell Cardiol 34: 1259-1271, 2002.
39. Nan J, Zhu W, Rahman MS, Liu M, Li D, Su S, Zhang N, Hu X, Yu H, Gupta MP and Wang J: Molecular regulation of mitochondrial dynamics in cardiac disease. Biochim Biophys Acta Mol Cell Res 1875: 2699-2723, 2018.

40. Sharp WW, Fang YH, Han M, Zhang HJ, Hong Z, Banathy A, Morrow E, Ryan JJ and Archer SL: Dynamic-related protein 1 (Drp1)-mediated diastolic dysfunction in myocardial ischemia-reperfusion injury: Therapeutic benefits of Drp1 inhibition to reduce mitochondrial fission. FASEB J 28: 316-326, 2014.

41. Murray JA, Perry R and Otsuki Y: Sterile inflammation and degradation systems in heart failure. Circ J 81: 622-628, 2017.

42. Li J, Horak KM, Su H, Sanbe A, Robbins J and Wang X: Enhancement of proteasomal function protects against cardiac proteinopathy and ischemia/reperfusion injury in mice. J Clin Invest 123: 3689-3700, 2011.

43. Yu X and Kem DC: Protein culling during myocardial ischemia. Cardiovasc Res 85: 312-320, 2010.

44. Zhou H and Toan S: Pathological roles of mitochondrial oxidative stress and mitochondrial dynamics in cardiac microvascular ischemia/reperfusion injury. Biomedicine & Molecular Biology 10: 85, 2020.

45. Morales PE, Avalos-Bravo KL, Jimenez R, Petrovsky S, Murphy AN and Gustafsson AB: Parkin protein deficiency exacerbates cardiac injury and reduces survival following myocardial infarction. J Biol Chem 288: 915-926, 2013.

46. Sabbah HN: Targeting the mitochondria in heart failure: A translational perspective. JACC Basic Transl Sci 5: 88-106, 2020.

47. Song M, Mihara K, Chen Y, Scorrano L and Dorn GW II: BNIP3-induced adenosine triphosphatase activity on mitochondrial dynamics and their dysregulation in cardiac disease. J Mol Cell Cardiol 78: 116-122, 2015.

48. Große L, Wurm CA, Bruser C, Neumann D, Jans DC and Joels M: Bax assembles into large ring-like structures remodeling the mitochondrial outer membrane in apoptosis. EMBO J 36: 402-413, 2017.

49. Kim H, Scimia MC, Wilkinson D, Trelles RD, Wood MR, Bowtell D, Dillín L, Mercela M and Ronai ZA: Fine-tuning of Drp1/Drp1 activity by AKAP121/SH3P2 regulates mitochondrial adaptation to hypoxia. Mol Cell 44: 532-544, 2011.

50. Marini W, A-kinase anchoring protein 1 (AKAP1) and its role in some cardiovascular diseases. J Mol Cell Cardiol 138: 99-109, 2020.

51. Disatnik MH, Ferreira JC, Campos JC, Gomes KS, Dourado PM, Qi X and Mochly-Rosen D: Acute inhibition of excessive mitochondrial fission after myocardial infarction protects against energy stress. Circ Res 116: 264-278, 2015.

52. Zhao H, Zhang Y, Hu S, Shi C, Zhu P, Ma Q, Jin Q, Cao F, Tian F and Chen Y: Melatonin protects cardiac microvasculature against ischemia/reperfusion injury via suppression of mitochondrial fission-VDAC1-HK2-mPTP-mitophagy axis. J Physiol Res 63: 12413, 2017.

53. Zhang T, Xue L, Li L, Tang C, Wan Z, Wang R, Tan J, Tan Y, Han H, Tian R, et al: BNIP3 protein suppresses PINK1 kinase proteolytic cleavage to promote mitophagy. J Biol Chem 291: 21616-21629, 2016.

54. Wu W, Wu K, Liu C, Jiang L, Wang X, Li W, Zhan X, Zhu H, Li S, et al: FUNDCL1 regulates mitochondrial dynamics at the ER-mitochondrial contact site under hypoxic conditions. EBIOJ 35: 1368-1384, 2016.

55. Tahiri FG, Knezevic T, Gupta MK, Gordon J, Cheung JY, Feldman AM and Khalili K: Evidence for the role of BAG3 in mitochondrial quality control in cardiomyocytes. J Cell Physiol 232: 797-805, 2017.

56. Schänzer A, Rupp S, Graf S, Zengeler C, Schinitz C, Kuttner U and Jürgens M: Loss of PINK1 increases the heart's vulnerability to ischemia-reperfusion injury. JACC Basic Transl Sci 1: e12413, 2017.

57. Schänzer A, Rupp S, Graf S, Zengeler C, Akin K, Akin Tur K: Sterile inflammation and degradation systems in heart failure. J Mol Cell Cardiol 75: 122-130, 2014.

58. Minoia M, Boncoraggio A, Vinet J, Morelli FF, Brunsting JF, Poliatti A, Krom S, Reits E, Kampinga HH and Carra S: BAG3 induces the sequestration of proteasome clients into cytoplasmic puncta: Implications for a proteasome-to-autophagy switch. Autophagy 10: 1603-1621, 2014.

59. Jakobs P, Geddes CL, Gorevic DP, Cossart Y, Tzagoloff A and Cossart Y: Mitochondrial quality control in the myocardium: Cooperation between protein degradation and mitophagy. J Mol Cell Cardiol 75: 122-130, 2014.

60. Kozatek Z and Gregorc G: Crosstalk between mitochondrial autophagy and the Ubiquitin-Proteasome system. Front Cell Dev Biol 6: 128, 2018.
...
121. Mellbin LG, Malmborg K, Norhammar A, Wedel H and Rydén L: DIGAMI 2 Investigators: The impact of glucose lowering treatment on long-term prognosis in patients with type 2 diabetes and myocardial infarction: A report from the DIGAMI 2 trial. Eur Heart J 29: 166-176, 2008.

122. Hartman MHT, Prins JKB, Schurer RAJ, Lipsic E, Lexis CPH, van der Horst-Schrivers ANA, van Veldhuisen DJ, van der Horst ICC and van der Harst P: Two-year follow-up of 4 months metformin treatment vs. placebo in ST-elevation myocardial infarction: Data from the GIPS-III RCT. Clin Res Cardiol 106: 939-946, 2017.

123. Whitaker RM, Corum D, Beeson CC and Schnellmann RG: Mitochondrial biogenesis as a pharmacological target: A new approach to acute and chronic diseases. Annu Rev Pharmacol Toxicol 56: 229-249, 2016.

124. Kim H, Lee JY, Park KJ, Kim WH and Roh GS: A mitochondrial division inhibitor, Mdivi-1, inhibits mitochondrial fragmentation and attenuates kainic acid-induced hippocampal cell death. BMC Neurosci 17: 33, 2016.

125. Veeranki S and Tyagi SC: Mdivi-1 induced acute changes in the angiogenic profile after ischemia-reperfusion injury in female mice. Physiol Rep 5: e13298, 2017.

126. Gao G, Chen W, Yan M, Liu J, Luo H, Wang C and Yang P: Rapamycin regulates the balance between cardiomyocyte apoptosis and autophagy in chronic heart failure by inhibiting miR-192 signaling. Int J Mol Med 45: 416-425, 2020.

127. Wang JX, Jiao QI, Li Q, Long B, Wang K, Liu JP, Li YR and Li PF: miR-499 regulates mitochondrial dynamics by targeting calcineurin and dynamin-related protein-1. Nat Med 17: 71-78, 2011.

128. Babot M, Birch A, Labarbuta P and Galkin A: Characterisation of the active/de-active transition of mitochondrial complex I. Biochim Biophys Acta 1837: 1083-1092, 2014.

129. Gorenkova N, Robinson E, Grieve DJ and Galkin A: Conformational change of mitochondrial complex I increases ROS sensitivity during ischemia. Antioxid Redox Signal 19: 1459-1469, 2013.

130. Chouchani ET, James AM, Methner C, Pell VR, Prime TA, Erickson BK, Forkink M, Lau GY, Bright TP, Menger KE, et al: Identification and quantification of protein S-nitrosation by nitrite in the mouse heart during ischemia. J Biol Chem 292: 14486-14497, 2017.

131. Pan J and Carroll KS: Light-mediated sulfinic acid generation from photocaged cysteine sulfoxide. Org Lett 17: 6014 -6017, 2015.

132. Ukwela AA, Bush AJ, Wedd AG and Xiao Z: Reduction potentials of protein disulffides and catalysis of glutathionylation by glutaredoxin enzymes. Biochem J 474: 3799-3815, 2017.

133. Kolluru GK, Shen X and Kevil CG: Reactive sulfur species: A new redox player in cardiovascular pathophysiology. Arterioscler Thromb Vase Biol 40: 874-884, 2020.

134. Davalli P, Mitic T, Caporali A, Lauriola A and D’Arca D: ROS, cell senescence, and novel molecular mechanisms in aging and age-related diseases. Oxid Med Cell Longev 2016: 3565127, 2016.

135. Shahzad S, Hasan A, Faizy AF, Mateen S, Fatima N and Moin S: Elevated DNA damage, oxidative stress, and impaired repair defense system inflicted in patients with myocardial infarction. Clin Appl Thromb Hemost 24: 780-789, 2018.

136. Dey S, DeMazumder D, Sidor A, Foster DB and O’Rourke B: Mitochondrial ROS drive sudden cardiac death and chronic proteome remodeling in heart failure. Circ Res 125: 356-371, 2019.