Review Article

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Mitochondrial Dynamics in the Heart as a Novel Therapeutic Target for Cardioprotection

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Traditionally, mitochondria have been regarded solely as energy generators for cells; however, accumulating data have demonstrated that these complex organelles play a variety of roles within the cardiomyocyte that extend beyond this classic function. Mitochondrial dynamics involves mitochondrial movements and morphologic alterations by tethering, fusion, and fission, which depend on cellular energy requirements and metabolic status. Many studies have indicated that mitochondrial dynamics may be a fundamental component of the maintenance of normal cellular homeostasis and cardiac function. Mitochondrial dynamics is controlled by the protein machinery responsible for mitochondrial fusion and fission, but cardiomyocytes are densely packed as part of an intricate cytoarchitecture for efficient and imbalanced contraction; thus, mitochondrial dynamics in the adult heart are restricted and occur more slowly than in other organs. Cardiac mitochondrial dynamics is important for cardiac physiology in diseased conditions such as ischemia-reperfusion (IR) injury. Changes in mitochondrial morphology through modulation of the expression of proteins regulating mitochondrial dynamics demonstrates the beneficial effects on cardiac performance after IR injury. Thus, accurately defining the roles of mitochondrial dynamics in the adult heart can guide the identification and development of novel therapeutic targets for cardioprotection. Further studies should be performed to establish the exact mechanisms of mitochondrial dynamics.

Key Words: *Mitochondrial dynamics; Myocardial reperfusion injury; Myocytes, cardiac*

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INTRODUCTION

Mitochondria are typically regarded as energy generators, but the latest data demonstrate other divergent functions such as oxygen free radical production, control of cell ion homeostasis, and regulation of cell apoptosis and necrosis.[1] Clearly, ATP generation is the most important role of mitochondria, especially in the heart. Because the heart requires a continuous supply of energy throughout life, cardiomyocytic mitochondria are densely packed to form a complex structure accounting for ~35% of cardiac muscle cell volume.[2] The cardiomyocytic mitochondria maintain the largest relative density of any organ in the body and build a highly organized and solid cytoarchitecture with other organelles. Their regulatory pathway is not fully understood, but many studies have suggested that ADP/ATP/inorganic phosphorus and cytosolic calcium (Ca^{2+}) are essential to regulate oxidative phosphorylation and energy generation.[3-6] Most cytosolic Ca^{2+} is released from the sarcoplasmic reticulum (SR) and acts as a crucial element for contraction of myocytes. Thus, its harmonic import and export via the T-tubules are essential for normal excitation-contraction coupling.[7]

The term "mitochondrial dynamics" connotes the mitochondrial movements, morphologic and distributional alterations, mitophagy, and tethering/fusion/fission that depend on energy requirements and metabolic status.[8-10] It is finely controlled by intricate protein machinery[11] and exhibits high organ specificity. For example, mitochondria in neurons, fibroblasts, and other cells change their locations actively, whereas in adult cardiomyocytes, they are not able to move easily owing to the peculiar subcellular en-

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vironment, i.e., rigid cytoskeleton and abundance of myofilaments.[12] Accumulating data, however, suggest that the proteins involved in cardiac mitochondrial dynamics are important for cardiac physiology in normal and diseased conditions. Here, we review new concepts and evidence for cardiac mitochondrial dynamics.

MITOCHONDRIAL DYNAMICS IN THE HEART

Many early experimental studies investigating mitochondrial dynamics were carried out by use of noncardiac cells. In recent years, however, there have also been studies on mitochondrial dynamics in the heart. Because of their unfettered movement and the comparatively homogeneous reticular distribution pattern of mitochondria, the cells employed in the majority of studies were neonatal cardiomyocytes, immortal cardiac cell lines, or vascular cells.[13-23] Adult cardiomyocytes are organized by a complex cytoarchitecture for efficient and finely controlled contraction so that mitochondrial movements are significantly restricted. But more recent studies have proposed that regulation of mitochondrial dynamics is also present in adult cardiomyocytes, indicating that adult cardiac mitochondria have preserved their fusion, fission, and mitophagy abilities.

1. Mitochondrial distribution and subpopulations in adult cardiomyocytes

Mitochondria are an indispensable part of the cardiac cytoarchitecture and are arranged in three distinct subpopulations in the adult heart: intermyofibrillar mitochondria, subsarcolemmal mitochondria, and perinuclear mitochondria.[24] Intermyofibrillar mitochondria are aligned along the myofibrils and function as a main producer of ATP. They are actively in contact with the surrounding SR and myofibrils.[12,25] Subsarcolemmal mitochondria are arranged just beneath the sarcolemma and are possibly involved in ion homeostasis or signaling pathways.[26,27] Last, perinuclear mitochondria are adjacent to the nucleus and probably participate in the nuclear transcription and translocation processes, but the exact mechanism has not yet been elucidated (Fig. 1).

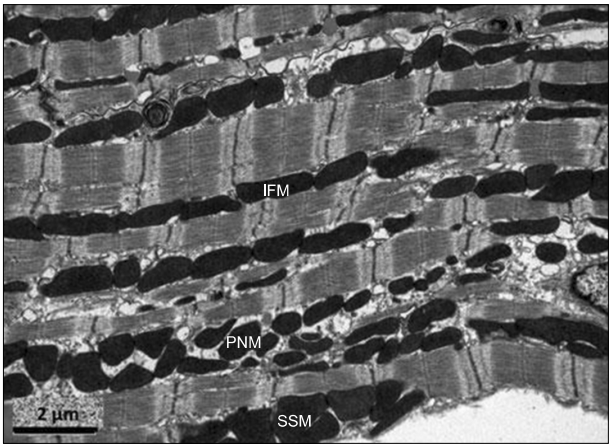

FIG. 1. The three subpopulations of mitochondria: interfibrillar mitochondria (IFM), perinuclear mitochondria (PNM), and subsarcolemmal mitochondria (SSM). Reprinted with permission from Ong et al.[84]

2. Mitochondrial fusion

Mitochondria have a double-membrane structure including the outer mitochondrial membrane (OMM) and inner mitochondrial membrane (IMM). The OMM encloses the entire organelle, isolating the contents from the cytoplasm, and contains numerous porins for passive and active transport of molecules. The IMM divides the internal substance into a matrix core and an intermembranous space, which is compartmentalized into an abundance of cristae. This double-membrane structure is an essential part of distinguishing between the enzymes required for ATP generation, but hinders mitochondrial tethering and fusion.

Mitochondrial fusion of the OMM and IMM occurs individually and sequentially and is mediated by distinct proteins containing GTPase and coiled-coil domains (heptad-repeat domains, HR1 and HR2). Fusion of the OMM is regulated by mitofusins (Mfn1, Mfn2) located in the outer membrane,[28,29] whereas fusion of the IMM is arbitrated by optic atrophy 1 (Opa1) present in the inner membrane.[30] Structurally, the proteins share an extracellular amino terminal GTPase domain mediating GTP hydrolysis, a transmembrane (TM) domain allowing the anchorage of the proteins to the membranes, and HR domains involved in homotypic (e.g., Mfn1-Mfn1, Mfn2-Mfn2, and Opa1-Opa1) and heterotypic (e.g., Mfn1-Mfn2) interactions.[23,31]

Human mitofusins were first discovered by the Santel group in 2001.[28] Mitofusins reside in the OMM and act as a starter for the mitochondrial fusion process. The HR2 domains of mitofusins on one mitochondrion are assumed to develop complexes with mitofusins on other mitochondria, and these complexes initiate tethering and fusion between neighboring mitochondria.[32] Mfn1 has higher GTPase activity than does Mfn2, so it is reported that Mfn1 tethers mitochondria more efficiently than does Mfn2.[33] As mentioned, mitofusins can interact both homotypically and heterotypically; thus, the fusion process has adaptability. Mfn1 or Mfn2 single knockout cells or tissues do not prevent fusion, but Mfn1 and Mfn2 double knockout cells or tissues demonstrate marked mitochondrial fragmentation.[34-36]

Opa1 is expressed throughout the body, and it mediates the IMM fusion process and modulates apoptotic cristae remodeling.[37] Mutations in the *Opa1* gene are related to autosomal dominant optic atrophy.[38] Opa1 has 8 human isoforms and is cleaved by mitochondrial protease. As a result, short forms of Opa1 (S-Opa1) and long forms of Opa1 (L-Opa1) are generated. The former is water-soluble and is located in the intermembranous space, whereas the latter has a TM domain and is anchored in the IMM.[11] Genetic ablation of *Opa1* also prevents mitochondrial fusion and

produces mitochondrial fragmentation.[39,40]

3. Mitochondrial fission

Mitochondrial fusion mechanisms are thought to be relatively well established, but mitochondrial fission processes have not been fully clarified. Fission processes are probably divided into several stages: mitochondrial constriction, dynamin-related peptide 1 (Drp1) recruitment, fission complex assembly on the OMM, de facto mitochondrial fission, and fission complex dismantling.[11]

Drp1, also called dynamin-like protein 1 (Dlp-1), is a cytosolic protein that has a GTPase domain and a GTPase effector (assembly) domain.[41] Drp1 translocates to the fission site on the OMM of the mitochondria via cytosolic dynein or the actin network.[42,43] After proper translocation, Drp1 oligomerizes to form a ring and constricts the mitochondrial fission site in a GTP-dependent manner. Drp1 has no TM domain for anchoring to the mitochondrial membrane, so it requires a docking receptor on the OMM. Human mitochondrial fission protein 1 (Fis1) was the first protein regarded as a mitochondrial receptor for Drp1.[44] It is a small protein (17 kDa) anchoring to the OMM, and its amino-terminus contains five α-helices that allow interaction with Drp1.[45] Fis1 is thought to conduct suborganelle localization of activated Drp1 oligomer to the constrictive site of mitochondria and facilitates the mitochondrial fission process.[46]

Some studies have suggested that Fis1 deficiency does not influence the recruitment of Drp1.[47,48] Some investigators found three other proteins involved in mitochondrial fission located on the OMM: mitochondrial fission factor (Mff) and mitochondrial dynamics proteins of 49 kDA or 51 kDA (MiD49 and MiD51). However, the exact mechanisms underlying the fission process remain unclear.

4. Mitochondrial trafficking

Mitochondrial trafficking is controlled by specific proteins such as mitochondrial Miro1 and Miro2 and cytosolic Grif-1 and OIP106 in a calcium-dependent manner. Miro proteins are located on the OMM and comprise two Ras-GTPase domains, a TM domain, and calcium-sensitive EF motifs.[49] Cytosolic Grif-1 and OIP106 bind Miro proteins and motor molecules (dynein, kinesin), inducing mitochondrial trafficking along microtubules.[50] Miro proteins are reported to influence mitochondrial morphology in immortalized cardiac cells (H9c2 cells). Their overexpression leads to mitochondrial elongation; on the other hand, genetic ablation of Miro genes induces mitochondrial fragmentation.[13] However, the roles of mitochondrial motility proteins are restricted in adult cardiomyocytes owing to the complex and dense cytoarchitecture.

ALTERED MITOCHONDRIAL DYNAMICS IN HEART DISEASE

Mitochondria modulate cardiomyocyte contractility by supplying ATP and participating in calcium homeostasis. Some studies have suggested that altered mitochondrial morphology is directly involved in the detriment to cardiac function under stress.[51,52] However, the precise mechanisms by which mitochondria interact with cardiac myofibrils are not fully understood.

1. Mitochondrial permeability transition pore (MPTP)

The mitochondrial permeability transition pore (MPTP) is a nonselective channel located on the IMM. It is permeable to solutes up to 1.5 kDa and mediates the degree of mitochondrial permeability by alteration of mitochondrial membrane potential. Excessive production of reactive oxygen species (ROS) and calcium overload in the mitochondrial matrix dissipate the proton electrochemical gradient ($\Delta\Psi$m) and open the MPTP. This leads to uncoupling of oxidative phosphorylation and further production of ROS, resulting in ATP depletion and mitochondrial rupture.[53,54] This in turn releases proapoptotic proteins such as cytochrome c, Smac/DIABLO, and endonuclease-G (endoG), which activate the caspase protease system and ultimately induce apoptosis or necrosis of the cell.[1,54-56]

Numerous heart diseases are related to increases in MPTP activators such as calcium and oxidative stress and reductions in MPTP inhibitors such as ATP/ADP. Several studies have demonstrated that inhibition of the MPTP pore lessens the cardiomyocyte loss that underlies some cardiac pathologies including myocardial ischemia/reperfusion (IR) injury,[57,58] calcium-induced cardiomyopathy,[59] diabetic cardiomyopathy,[60] and the cardiotoxicity of anti-cancer drugs.[61]

There have been a few studies of the relationships between mitochondrial fusion proteins and the MPTP opening. In accordance with these studies, lack of Opa1 and Mfns was associated with delayed MPTP opening,[18,51,52] which suggested that mitochondrial fusion proteins expedite MPTP opening under normal conditions. However, Neuspiel et al.[62] demonstrated that the overexpression of Mfn2 protects against MPTP opening. Further investigations are required to elucidate the exact mechanisms involved in these phenomena.

2. Mitochondrial dynamics and apoptosis in cardiac cells

Studies on the relationships between mitochondrial dynamics and apoptosis are at an early stage even now. Mfn2 is being highlighted as a regulator of the apoptotic process, but its primary actions remain controversial. Guo et al.[63] and Shen et al.[64] suggested that Mfn2 may function as a pro-apoptotic modulator by suppressing the phosophoinositide 3-kinase (PI3K)-Akt pathway under oxidative stress in vascular smooth muscle cells and H9c2 cells, respectively. On the other hand, Parra et al. reported that ablation of Mfn2 exacerbated the ceramide-induced pro-apoptotic processes such as mitochondrial fragmentation, Drp1 localization, and release of cytochrome *c* in neonatal cardiomyocytes.[21]

3. Mitochondrial dynamics and myocardial ischemia-reperfusion injury

In coronary artery disease, the most effective ther-

apeutic option for reducing ischemic myocardial injury and infarct size is prompt and efficient myocardial reperfusion, but the myocardial reperfusion process can induce further myocardial cell death. This phenomenon is called myocardial IR injury.[65,66] The outcomes of IR injury are reversible (arrhythmia, myocardial stunning) or irreversible (microvascular obstruction, cardiomyocytic death). Oxidative stress, intracellular calcium overload, MTPT opening, and hypercontracture of myofibrils are important contributory factors in lethal myocardial IR injury.[66]

Mitochondrial fission and fragmentation are commonly observed in cells with IR injury. Chen et al.[67] found that the deficiency of OPA1 is closely associated with IR-induced mitochondrial fragmentation in H9c2 cells. Additionally, Ong et al.[68] elucidated that mitochondrial fragmentation during myocardial ischemia turns on the actions of Drp1 in HL-1 cell lines. The exact activation process of Drp1 under ischemic conditions is not fully understood, but calcineurin-induced dephosphorylation of Drp1 by mitochondrial calcium overload has been observed in some studies.[69,70]

4. Mitochondrial dynamics and other heart diseases

Until recently, mitochondrial fusion proteins were relatively disregarded in studies on human heart disease, and in fact, Charcot-Marie-Tooth syndrome and autosomal dominant optic atrophy caused by functional loss of Mfn2 and Opa1, respectively, show no cardiac manifestations.[71] However, there has been some evidence that cardiomyopathies are the result of an imbalance in mitochondrial dynamics, and that heart failure is related to altered cardiac mitochondrial morphology.

The integrity of the mitochondrial network is compromised and the mitochondrial membrane is disrupted in chronically failed hearts. Additionally, reductions in the total mitochondrial number and size have also been observed.[72,73] Sabba et al.[74] and Di Lisa et al.[75] suggested that these phenomena are positively correlated with the heart failure severity index and are capable of determining the destiny of cardiac cells. Furthermore, recent studies have suggested that Opa1 deficiency decreases the expression of nuclear antioxidant genes, induces fragmentation of cardiac mitochondria, and results in late-onset heart failure.[67,76]

Changes in mitochondrial dynamics have also been observed in dilated cardiomyopathy. A heterozygous *Drp1* gene mutation (C452F) gave rise to severe dilated cardiomyopathy after 11 weeks in mice.[77] Selective ablation of Mfn1 and Mfn2 also resulted in severe dilated cardiomyopathy in a mouse model, whereas combined Mfn1/Mfn2 ablation was lethal.[36] Both studies demonstrated defects in mitochondrial respiration and mitochondrial fragmentation.

MITOCHONDRIAL DYNAMICS AS A NOVEL THERAPEUTIC TARGET

Modification of mitochondrial dynamics has emerged as a novel pharmacological strategy for cardioprotection, especially in IR injury. Previous studies reported that trimetazidine reduced infarct size in animal ischemic heart models.[78,79] The exact mechanism is not fully understood, but there has been some evidence that trimetazidine decreases ROS production and delays calcium-mediated MPTP opening via re-coupling of the mitochondrial respiratory chain.[80-82] Ranolazine also demonstrated similar effects on mitochondria and reduced additional IR injury.[83] The proteins modulating mitochondrial dynamics are regarded as an essential component of appropriate mitochondrial and cell survival, and ultimately, normal cardiac function, but their pharmacological use is still not realized.

1. Mitochondrial dynamics as a therapeutic target for cardioprotection

Altering mitochondrial morphology by regulating the expression of the proteins involved in mitochondrial dynamics has demonstrated beneficial effects on cardiac performance after IR injury in some studies. Mdiv-1, a Drp1-specific inhibitor, impeded mitochondrial fragmentation, as determined by electron microscopy; delayed the opening of MPTP; and reduced myocardial infarct size significantly in *in vivo* murine hearts as well as in HL-1 cells.[68] Wang et al.[70] reported that microRNA-499 (miR-499), which inhibits the calcineurin-dependent activation of Drp-1, as mentioned above, could protect against IR injury. Thus, short-term inhibition of Drp1 during IR injury may be advantageous for effective cardioprotection. However, the adult hearts of Mfn2 knockout mice exhibit an enlargement of the subsarcolemmal mitochondria, depolarization of the mitochondrial membrane, delayed MPTP opening, and also improvement of cardiac function following *ex vivo* IR injury compared with mice from the control group.[51] These findings contrast with the results of other studies that demonstrated a beneficial role of Mfn2 in terms of MPTP opening in neurons[62] and HL-1 cells.[68] These findings suggest that the effects of Mfn2 are cell-specific and may change with mitochondrial cell development but have not yet been fully elucidated.[84]

SUMMARY

Numerous studies have described how mitochondrial dynamics may be essential in the maintenance of normal mitochondrial and cellular homeostasis and cardiac function. Mitochondrial dynamics is modulated by complex protein machinery regulating the fusion and fission of mitochondria. In adult heart, cardiomyocytes are organized by an intricate cytoarchitecture for efficient and well-modulated contraction, and thus, mitochondrial movements are markedly restricted and morphological changes of the mitochondria are more slowly controlled than in other organs.

Mitochondrial dynamics is a crucial component for the adaptation of cardiomyocytes under stresses such as IR injury. Rats with genetically manipulated mitochondrial

dynamics genes are especially vulnerable to ischemia, and the proteins encoded by these genes act as key determinants of cellular fates in such environments.

Accordingly, identifying the various roles of mitochondrial dynamics in the adult heart could result in the development of novel therapeutic targets for cardioprotection. Further animal- and human-based studies should be performed to establish the exact mechanisms underlying mitochondrial dynamics.

CONFLICT OF INTEREST STATEMENT

None declared.

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