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Role of mitochondrial fission and fusion in cardiomyocyte contractility ☆

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ABSTRACT

Background: Mitochondria constitute 30% of cell volume and are engaged in two dynamic processes called fission and fusion, regulated by Drp-1 (dynamin related protein) and mitofusin 2 (Mfn2). Previously, we showed that Drp-1 inhibition attenuates cardiovascular dysfunction following pressure overload in aortic banding model and myocardial infarction. As dynamic organelles, mitochondria are capable of changing their morphology in response to stress. However, whether such changes can alter their function and in turn cellular function is unknown. Further, a direct role of fission and fusion in cardiomyocyte contractility has not yet been studied. In this study, we hypothesize that disrupted fission and fusion balance by increased Drp-1 and decreased Mfn2 expression in cardiomyocytes affects their contractility through alterations in the calcium and potassium concentrations.

Methods: To verify this, we used freshly isolated ventricular myocytes from wild type mouse and transfected them with either siRNA to Drp-1 or Mfn2. Myocyte contractility studies were performed by IonOptix using a myopacer. Intracellular calcium and potassium measurements were done using flow cytometry. Immunocytochemistry (ICC) was done to evaluate live cell mitochondria and its membrane potential. Protein expression was done by western blot and immunocytochemistry.

Results: We found that silencing mitochondrial fission increased the myocyte contractility, while fusion inhibition decreased contractility with simultaneous changes in calcium and potassium. Also, we observed that increase in fission prompted decrease in Serca-2a and increase in cytochrome c leakage leading to mitophagy.

Conclusion: Our results suggested that regulating mitochondrial fission and fusion have direct effects on overall cardiomyocyte contractility and thus function.

1. Introduction

Cardiovascular disease (CVD) persists as the leading cause of death despite extensive research. The heart is a dynamic organ with abundant mitochondria to meet its continuous energy demands [1]. Mitochondria provide 90% of ATP and occupy 30% of cell volume, thus becoming an important organelle in adult cardiomyocyte. Rigorous research on cardiac mitochondria has strongly confirmed that structural and functional alterations, termed mitochondrial dynamics play an essential role in maintaining basal cardiac function and any disturbance in these functional changes may lead to various cardiac diseases including myocardial ischemia, infarction and heart failure [2–6]. Mitochondrial dynamics include fission and fusion processes which are balanced under normal physiological status. Aberrant or increased fission will lead to increased mitochondrial fragmentation leading to mitochondrial death or mitophagy. Mitophagy is essential in preventing cell damage during excessive reactive oxygen species (ROS) production. But aberrant mitophagy due to increased mitochondrial fragmentation (fission) leads to cell death and tissue necrosis during disease conditions. We and others have demonstrated abnormal mitophagy in various mice models of cardiovascular disease and regulation of fission process was cardioprotective [4,7–10]. Although 3 fusion proteins and 2 fission proteins are known so far, the predominant among them that are involved in fission and fusion mechanisms are dynamin related protein-1 (Drp-1) and mitofusin 2 (Mfn2) respectively. Several studies have reported that mitochondrial fission by Drp-1 mediates myocardial cell death during ischemia–reperfusion, pressure overload and myocardial infarction [4,8,9,11]. Inhibition of Drp-1 prevented opening of mitochondrial transition pore and reduced infarct size in mouse coronary artery ligation [9]. It was also shown that Drp-1 induced mitochondrial fragmentation precedes ROS production in ventricular myocytes during increased cytosolic calcium [12]. Preclinical studies have effectively demonstrated that Mdivi-1 (mitochondrial division inhibitor), a Drp-1 specific inhibitor is protective in various cardiac diseases. Mdivi-1, by inhibiting Drp-1 preserved the mitochondrial morphology, reduced cytosolic calcium and prevented cell death during ischemia reperfusion [10]. It was also reported that Drp-1 mediates cardiac hypertrophy during pressure overload conditions and treatment with Mdivi-1

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attenuates this process [4,13]. Mitochondrial fusion protein (Mfn2) regulates mitochondrial structure and metabolism, while its expression is decreased in diabetes and obesity, it is improved with weight loss and exercise [14,15]. Mfn2 is primarily involved in mitochondrial calcium reuptake mechanism necessary for ATP production. Mfn2 is localized in endoplasmic reticulum (ER) and regulates ER structure function and calcium uptake [16]. Studies have reported that Mfn2 expression is decreased in various rat models of cardiac hypertrophy including spontaneous hypertensive rats, transverse aortic banding and myocardial infarction [17].

Although, it was known that disrupted mitochondrial fission–fusion balance with increased fragmentation is detrimental to the cell leading to mitophagy its direct effect on cardiomyocyte contractility is unclear. Thus in this study we hypothesize that modulating mitochondrial fission–fusion balance will interfere directly in the cardiomyocyte contractility through alterations in the cytosolic calcium and potassium concentrations.

2. Methods

2.1. Isolation of ventricular cardiomyocytes

Fresh perfusion buffer (120.4 mM NaCl, 14.7 mM KCl, 0.6 mM KH_2PO_4, 0.6 mM Na_2HPO_4, 10 mM Na-HEPES, 1.2 mM $MgSO_4 + -7H_2O$, 4.6 mM $NaHCO_3$, 30 mM Taurine, 10 mM BDM, 5.5 mM Glucose; pH 7.0) was prepared daily. The perfusion system is prepared as follows: the temperature of the circulation water bath is set so that the temperature of the outflow liquid at the tip of the cannula (20-g needle with the nub filed flat and smooth) is 37 °C. Flow rate is set at 4.0 ml/min prior to the cardiomyocyte isolation, about 100 ml of distilled water is run through the system. Then the system is perfused with perfusion buffer for at least 5 min.

A mouse of at least 20 g is injected with heparin (1 000 U/kg; i.p.). Then the mouse is anesthetized with TBE (Avertin 100 mg/kg, i.p.). The chest is wiped with 70% ethanol. A skin incision is made revealing the xiphoid process. The rib cage is completely cut starting at the xiphoid process running up the chest cavity. To avoid heart damage the diaphragm is cut as well. The heart is secured with forceps and all vessels are cut. The aorta is cut so as to leave the maximal length, which is important for rapid cannulation. The dissected heart is immediately placed in a Petri dish containing ice-cold calcium free perfusion buffer. To expose the aorta, all the remnant excess tissue is removed and discarded. Holding the aorta with two fine forceps, it is slid onto the vertically mounted cannula until the tip of the needle reaches the aortic valve. The heart is secured on the needle with a small brass clip and is immediately perfused with perfusion buffer at a flow rate of 4.0 ml/min for 4 min or until the outflow from apex is clear from blood. The aorta is tied to the needle with silk thread. Then the perfusion with digestion buffer consisting of 29 ml perfusion buffer and 1.0 ml of 5 mg/ml Liberase TH Research Grade (Roche Diagnostics Corp. Indianapolis, IN) is continued for 7–8 min. At the end of the perfusion the tissue became soft, swollen and light pink.

After the perfusion the heart is cut from the needle just below the atria using sterile fine scissors and is placed in a Petri dish with 10 ml incubation buffer (135 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES, 0.33 mM NaH_2PO_4, 10 mM glucose, 10 mM BDM, 1.2 mM $CaCl_2$). The heart is gently teased into small pieces with fine forceps. The obtained suspension of cardiomiocytes is gently pipetted up and down with a plastic pipette (2 mm tip) several times. Then the cells are transferred to a 60-mm nonstick Valmark dish and 10, 20, 30, 30 and 30 μl of 100 mM $CaCl_2$ solution is added at 5 min intervals. The final content of calcium is 1.2 mM. Isolated myocytes are maintained at room temperature in contractility buffer (Minimum Essential Medium with Hanks Salts (Gibco), Butadione Monoxime (25 mM, Sigma), Bovine Calf Serum (5%, HyClone), Penicillin (100 u/ml, Sigma), L-Glutamine (2 mM, Gibco)).

2.2. Analysis of myocyte contractility

Cardiomyocyte contractility is controlled by electrical stimulation. Mechanical properties of isolated ventricular myocytes are assessed by video-based edge detection. An inverted microscope, a low light-level video camera and a computer-based motion analyzer are used to track the movement of cell edges (6).

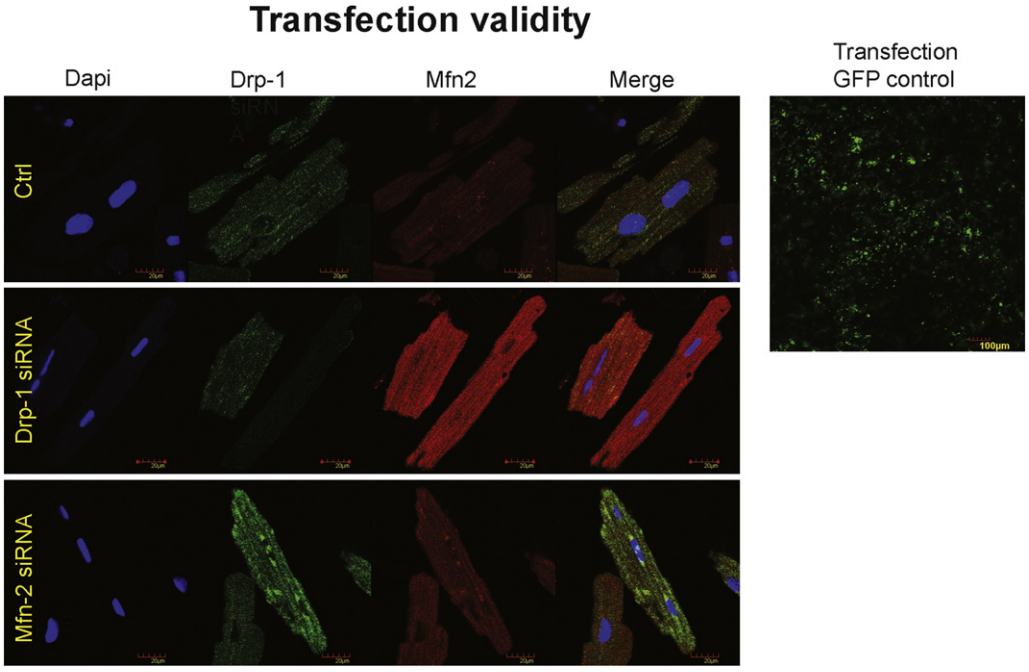

Fig. 1. Transfection after overnight is validated with GFP negative control and followed by the immunocytochemistry staining for the silenced genes Drp-1 (green), Mfn2 (red) and dapi (blue) staining nuclei. We observed that during transfection with Drp-1 siRNA, we noticed least expression of it while we found increased expression of fusion protein Mfn2. Similarly, transfection of cardiomyocytes with Mfn2 siRNA resulted in decreased expression of Mfn2 suggesting decreased fusion process, while that of Drp-1 is increased suggesting increased fission process.