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Dysregulation of Mfn2 and Drp-1 proteins in heart failure[1]

Srikanth Givvimani, **Sathnur Pushpakumar***, **Sudhakar Veeranki***, and **Suresh C. Tyagi**
Department of Physiology & Biophysics, School of Medicine, University of Louisville, KY 40202, USA

Abstract

Therapeutic approaches for cardiac regenerative mechanisms have been explored over the past decade to target various cardiovascular diseases (CVD). Structural and functional aberrations of mitochondria have been observed in CVD. The significance of mitochondrial maturation and function in cardiomyocytes is distinguished by their attribution to embryonic stem cell differentiation into adult cardiomyocytes. An abnormal fission process has been implicated in heart failure, and treatment with mitochondrial division inhibitor 1 (Mdivi-1), a specific inhibitor of dynamin related protein-1 (Drp-1), has been shown to improve cardiac function. We recently observed that the ratio of mitofusin 2 (Mfn2; a fusion protein) and Drp-1 (a fission protein) was decreased during heart failure, suggesting increased mitophagy. Treatment with Mdivi-1 improved cardiac function by normalizing this ratio. Aberrant mitophagy and enhanced oxidative stress in the mitochondria contribute to abnormal activation of MMP-9, leading to degradation of the important gap junction protein connexin-43 (Cx-43) in the ventricular myocardium. Reduced Cx-43 levels were associated with increased fibrosis and ventricular dysfunction in heart failure. Treatment with Mdivi-1 restored MMP-9 and Cx-43 expression towards normal. In this review, we discuss mitochondrial dynamics, its relation to MMP-9 and Cx-43, and the therapeutic role of fission inhibition in heart failure.

Keywords

mitochondrial fission; fusion; Drp-1; Mfn2; Cx-43; MMP-9; pressure overload heart failure

Introduction

Cardiovascular disease remains the leading cause of morbidity and mortality worldwide, despite extensive research and clinical trials. The heart is a dynamic organ equipped with abundant mitochondria (Hom and Sheu 2009) to meet its continuous energy demands; consequently, current studies are exploring mitochondrial dynamics as a potential target for cardiovascular disease conditions. The structure of the mitochondrion consists of an outer membrane, an inner membrane that forms cristae, and an intermembrane space (Palade 1953). Mitochondria provide 90% of the body's ATP and occupy 30% of the cell volume,

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Corresponding author: Srikanth Givvimani (s0givv01@louisville.edu).
*These authors contributed equally.

thus they are an important organelle in the adult cardiomyocytes. Mitochondria are dynamic organelles that play a critical role in cell growth, cell signaling mechanisms, and cell death. Mitochondrial damage results in disruption of the oxidative phosphorylation reaction, generating excess reactive oxygen and nitrogen species, a reduction in ATP production, disruption of Ca^{2+} homeostasis, and also triggers apoptosis (Diaz and Moraes 2008; Figueira et al. 2013). Accumulating evidence suggests that mitochondrial DNA (mDNA) sustains injury in the aging process and in atherosclerosis (Corral-Debrinski et al. 1992; Ballinger et al. 2002; Puddu et al. 2005), and as a result, protein synthesis and function can be defective. In addition, oxidation of proteins and lipids on the inner and outer mitochondrial membranes can initiate the pathways of cell death (Karbowski and Youle 2011). It is therefore essential for cell survival to sequester and eliminate such dysfunctional mitochondria. Mitochondrial dynamics have been extensively studied and have gained importance with respect to understanding the pathogenesis and molecular mechanisms of heart disease. Mitochondrial dynamics refers to fusion and fission processes during mitochondrial movement (Szabadkai et al. 2006; Detmer and Chan 2007; Suen et al. 2008). Both of these processes are balanced under basal conditions but disrupted during pathological states. The intrinsic mechanism of selective sequestration and fragmentation of damaged mitochondria is termed mitophagy. Although mitophagy is a normal cytoprotective process, abnormal mitophagy owing to an increase in mitochondrial fission leads to cell death, which plays a pathological role in diabetes mellitus, ischemia– reperfusion injury, and heart failure (Fig. 1). Previously, we and others have demonstrated abnormal mitophagy in cardiovascular diseases, and that the regulation of fission process was cardioprotective (Brooks et al. 2009; Ong et al. 2010;Givvimani et al. 2012; Gharanei et al. 2014; Sharp et al. 2014). In addition, studies from our group and from others have shown that the administration of the selective Drp-1 inhibitor mitochondrial division inhibitor (Mdivi-1) in a mouse model of cardiovascular disease reversed pathological remodeling (Ong et al. 2010; Givvimani et al. 2012; Gharanei et al. 2014; Sharp et al. 2014). Although cardiac regenerative mechanisms have been widely explored over the last decade as a therapeutic target for CVD, simultaneous restorative mechanistic studies are also essential. Deciphering the molecular mechanism that stimulates the differentiation and maturation of cardiac progenitor cells is necessary for exploring the pathogenesis of cardiac disease, and also for regenerative therapeutic approaches for damaged myocardium. The significance of normal mitochondrial function is established in embryonic stem cell proliferation and differentiation (Mandal et al. 2011). It has also been shown that fusion protein, especially mitochondrial fusion protein 2 (Mfn2), is required for normal cardiac development and differentiation of the embryonic stem cells (Kasahara et al. 2013). Abnormal mitophagy and enhanced oxidative stress have been implicated in the activation of MMP-9 and degradation of the ventricular gap junction protein Cx-43. We and others have reported that during pressure overload induced heart failure, increased expression of MMP-9 and reduced expression of Cx-43 leads to excessive collagen and fibrosis deposition and ventricular dysfunction (Givvimani et al. 2010, Givvimani et al. 2012; Jansen et al. 2012).

The purpose of this review is to highlight the importance of mitochondrial dynamics in cardiovascular disorders and the role of fission and fusion proteins. We also discuss the roles

of connexin-43, a gap junction protein that is present in cardiac mitochondria and the involvement of matrix metalloproteinase-9 (MMP-9) during cardiac pathology.

Mitochondria in heart disease

The molecular and structural changes that occur during the remodeling in heart failure include oxidative stress, cardiac hypertrophy, and myocyte loss through autophagy, including mitophagy, apoptosis, and fibrosis. At the functional level, there is disturbed excitation and contraction (E-C) coupling, as well as systolic and diastolic dysfunction (Bers 2006; Maack and O'Rourke 2007; Givvimani et al. 2012; Neef and Maier 2013). During heart failure, an imbalance in MMPs and tissue inhibitors of metalloproteinases (TIMPs) causes extracellular matrix (ECM) remodeling in the left ventricle. These changes are compounded by diminished angiogenesis and up-regulated anti-angiogenic factors (Givvimani et al. 2010, 2012). Currently, mitochondria have been implicated in the pathogenesis of heart failure (Ducharme et al. 2000; Peterson et al. 2001; Moshal et al. 2005; Friehs et al. 2006; Givvimani et al. 2010, 2011). Three different types of mitochondria have been identified in the adult heart: (*i*) subsarcolemmal mitochondria present beneath the sarcolemma; (*ii*) interfibrillar mitochondria present among the myofibrils and sarcomeres; and (*iii*) perinuclear mitochondria surrounding the nucleus (Ong and Hausenloy 2010). Interfibrillar mitochondria are more affected, and are decreased during heart failure, owing to cardiac structural remodeling (Lukyanenko et al. 2009). Mitochondria utilize cytosolic Ca^{2+} to regulate metabolic activity, including ATP generation, to meet the energy demands of the relentlessly beating heart (McCormack and Denton 1989). Recent studies have demonstrated a significant role for calcium in the regulation of mitochondrial motility as well as fission and fusion mechanisms (Hom et al. 2007; Saotome et al. 2008; Wang and Schwarz 2009). It is interesting that mitochondria play a dual role; on one hand serving as the main source of myocyte ATP production, while on the other serving as a primary source of reactive oxygen species (ROS), which can trigger several signaling pathways and lead to cardiomyocyte damage (Gudbjarnason et al. 1970; Orrenius et al. 2007). Mitophagy is a quality control mechanism aimed at preventing cell damage during excessive ROS production. But, aberrant mitophagy due to increased fragmentation (fission) of the mitochondria results in cell death and tissue necrosis under disease conditions. Mitophagy involves 2 distinct processes termed mitochondrial fusion and mitochondrial fission.

Mitochondrial fusion

The process of mitochondrial fusion requires 2 outer membrane proteins: (*i*) mitofusin 1 (Mfn1) and (*ii*) mitofusin 2 (Mfn2), which are 85 kD GTPase isoforms (Santel and Fuller 2001); and an inner membrane protein, optic atrophy protein (Opa-1), which is a 100 kD GTPase (Alexander et al. 2000; Olichon et al. 2002; Meeusen et al. 2004, 2006). Both the mitofusin proteins and Opa-1 share strong structural and functional similarities (Piquereau et al. 2013). Opa1, the inner membrane fusion protein, is responsible for the formation of cristae (Frezza et al. 2006) and is resistant to reactive oxygen free radicals (Piquereau et al. 2013). The fusion of outer and inner mitochondrial membranes is regulated by the proteolytic cleavage of OPA. Cytosolic OPA1 is imported into the mitochondria for this purpose, to produce the long and short isoforms, OPA1L and OPA1S, respectively (Alavi

and Fuhrmann 2013). Further processing of OPA1 isoforms by matrix and intermembrane space associated ATPases and presenilin-associated rhomboid-like protease (PARL) leads to mitochondrial fusion in response to various stimuli (Song et al. 2007). OPA1 mutation has been reported to increase susceptibility to oxidative stress owing to reduced levels of superoxide dismutase 2 (SOD2) (Nguyen et al. 2011). More recently, mutant mice with reduced OPA1 expression in cardiomyocytes showed increased calcium accumulation, which delayed permeability transition pore opening and also increased their sensitivity to hemodynamic stress (Piquereau et al. 2012). In other studies, loss of OPA1 resulting in impaired mitochondrial fusion and thus fragmentation has been shown to initiate mitochondrial autophagy and cell death (Song et al. 2007; Merkwirth et al. 2008; White et al. 2009).

Mfn1 and Mfn2 can substitute for each other in times of deficiency, suggesting an overlap in their function (Chen et al. 2005). However, conditional cardiac genetic ablation of both Mfn1 and Mfn2 is lethal in the embryonic stage itself (Chen et al. 2003). Mfn1 knockout mice (*Mfn1*-KO) display fragmented mitochondria with a normal mitochondrial and cardiac function, and provides protection against ROS-induced mitochondrial dysfunction (Papanicolaou et al. 2012). In a separate study, genetic ablation of Mfn2 did not show any significant mitochondrial and cardiac dysfunction and, furthermore, the cells were protected from ischemia–reperfusion injury (Papanicolaou et al. 2011). Mfn2 regulates mitochondrial structure and metabolism. The expression of Mfn2 is decreased in diabetes and obesity, and increases with weight loss and exercise (Bach et al. 2005; Cartoni et al. 2005).

Significance of Mfn2 in the heart

Mitofusins have been extensively studied in cardiovascular diseases. Mfn2 is reported to have additional mitochondrial functions to Mfn1. Also, Mfn2 is localized to the endoplasmic reticulum (ER) where it regulates structure, function, and calcium uptake in the ER (de Brito and Scorrano 2008). Studies have shown that ER stress up-regulates Mfn2, and genetic ablation of Mfn2 increases cell death during ER stress (Ngoh et al. 2012). Mfn2 tethers the ER and mitochondria, and regulates mitochondrial calcium uptake released by the ER (de Brito and Scorrano 2008; Dorn and Maack 2013). Mfn2 expression is down-regulated in various rat models of cardiac hypertrophy, including spontaneously hypertensive rats, transverse aortic banding, and myocardial infarction (Fang et al. 2007), which contribute to cardiomyocyte remodeling. Interestingly, up-regulation of Mfn2 ameliorated the cardiac hypertrophy induced by angiotensin II (Yu et al. 2011). Further, Mfn2 is crucial for the cardiac differentiation in the embryonic stem cells (Chung et al. 2007; Kasahara et al. 2013). However, results from mitofusin knockout models are equivocal with regards to their role under disease conditions (Chen et al. 2011; Papanicolaou et al. 2011, 2012).

Mitochondrial fission

Fission or fragmentation of the mitochondria can be either physiological or pathological. In mammals, mitochondrial fission occurs by recruitment of cytosolic dynamin like protein (DLP-1; 80–85 kD), also called dynamin related protein (Drp-1), to the outer membrane by human fission1 (hFis1) protein (17 kD) (Yoon et al. 2003; Yu et al. 2005). The structural and functional characterization of mammalian Drp-1 was first reported in 1998 (Imoto et al.

1998) and is the most studied fission protein in cardiovascular pathology. Translocation of cytosolic Drp-1 to the mitochondria is an early step involved in the mitochondrial fission process (Smirnova et al. 1998; Labrousse et al. 1999). Inhibition of mitochondrial fission proteins either delays or suppresses apoptosis (Frank et al. 2001; Breckenridge et al. 2003;Yoon et al. 2003). Conversely, excessive mitochondrial fragmentation promotes apoptotic processes and cell death (Yu et al. 2005). A study in cultured neonatal ventricular myocytes has reported that inhibition of mitochondrial fission prevented ROS production, mitochondrial permeability transition, and the ensuing cell death under hyperglycemic conditions (Yu et al. 2008). Calcium plays an important role in the activation of Drp-1 through the calcineurin pathway, which is of more significance in cardiomyocyte function (Hom et al. 2010).

Role of Drp-1 in the heart

Several studies have attributed mitochondrial fission by Drp-1 as an important mediator of myocardial cell death during ischemia–reperfusion, pressure overload, and myocardial infarction (Ong et al. 2010; Givvimani et al. 2012; Disatnik et al. 2013; Gharanei et al. 2014). Increase in cytosolic calcium in ventricular myocytes promotes mitochondrial fragmentation through the involvement of Drp-1 and is also associated with increased ROS generation (Hom et al. 2010) suggesting the role of calcium in Drp-1 dependent fission and ROS generation in cardiomyocytes. The mechanism of Drp-1 recruitment from cytosol to mitochondria during fission remains unclear; however, studies on yeast suggest the requirement of Fis-1 protein for assembly (Suen et al. 2008). Other proteins such as actin filaments and microtubules have been reported to play a role in Drp-1 recruitment in mammalian cells (De Vos et al. 2005; Varadi et al. 2004). Following translocation into mitochondria, Drp-1 undergoes post-translational modification by cyclin dependent kinase 1/cyclinB phosphorylation of Ser618 leading to fragmentation (Taguchi et al. 2007). Drp-1 inhibition confers cardiac protection from ischemia–reperfusion injury and myocardial infarction by decreasing mitochondrial metabolism and fragmentation (Disatnik et al. 2013; Zepeda et al. 2014). Similarly, the commercially available Drp-1 inhibitor Mdivi-1 has been shown to attenuate doxorubicin-induced cardiomyopathy without altering its anti-cancer properties (Gharanei et al. 2014). Our lab has also demonstrated that Mdivi-1 inhibited MMP-9 induced anti-angiogenic factors and abnormal mitophagy, thus reversing adverse remodeling in pressure overload heart failure (Givvimani et al. 2012). Currently, micro RNAs (miR) are being investigated as therapeutic targets in cardiovascular diseases. Interestingly, miR 499 provides cardiac protection in myocardial infarction by suppressing the Drp-1 mediated mitochondrial fission (Wang et al. 2011). In the same pressure overload cardiac injury mouse model, we found an increase in fission protein Drp-1, with a concomitant decrease in fusion protein Mfn2, suggesting aberrant mitochondrial fragmentation; treatment with Mdivi-1 reversed their expression and restored mitochondrial homeostasis (Fig. 2).

Role of connexin-43 in heart failure

Gap junction proteins are essential myocyte–myocyte couplers that provide a path of least resistance for conductance of depolarization waves, enabling efficient synchronization of metabolites and electrolytes in cardiomyocytes. Connexin-43 (Cx-43) is the major

constituent of cardiac gap junctions in mammals and is localized to ventricular myocytes (Fromaget et al. 1992) and atrial myocytes in vertebrates (Verheule et al. 1997). The following studies noticed unequivocal influence of Cx-43 in conferring protection against heart failure in multiple scenarios. There is recent evidence suggesting that fibroblasts play a crucial role in cardiac remodeling by forming injury-mediated bridges between cardiac myocytes (Zhang et al. 2008). However, the conductance rate in such myocyte–fibroblast–myocyte coupling is slower. This is due to relatively higher levels of Cx-45 than native Cx-43 in the ventricular myocytes. In a rodent model of non-ischemic pacing induced heart failure, part of the observed beneficial influence of G-CSF administration was due to Cx-43 expression enhancement (Milberg et al. 2011). The beneficial influence of triiodothyronine also involves Cx-43-based enhancement of the gap junction formation in cultured neonatal myocytes (Tribulova et al. 2004). These observations imply that Cx-43 is capable of forming gap junctions that permeate higher velocity depolarization waves to preserve proper heart function.

Apart from the Cx-43 levels, Cx-43 distribution is also altered in cardiomyopathies. The inverse relationship between Cx-43 levels and fibroblast proliferation has been proposed for mouse hearts, and enhancing Cx-43 levels increased the conduction coupling. In hypertension-induced hypertrophy and cardiac fibrosis, maladaptive localization of Cx-43 into the lateral surfaces of cardiomyocytes was observed to promote fatal arrhythmias (Fialova et al. 2008). In addition, in ischemic and dilated cardiomyopathies, focal areas of disorganized and (or) reduced Cx-43 levels were associated with the heart failure (Kostin 2007). Moreover, the protective effects of melatonin on ventricular arrhythmias were attributed in part to its enhancement of Cx-43 levels and distribution in hypertensive rat models (Benova et al. 2013). Furthermore, in a diabetic rat model, reduction in Cx-43 expression and hyper phosphorylation of Cx-43 was associated with diminished myocyte coupling and cardiac conductivity (Lin et al. 2006). Decreased levels of Cx-43 have been observed in septicemia (Fernandez-Cobo et al. 1999) and electrocution induced cardiac dysfunction (Huang et al. 2012). Whereas decreases in Cx-43 expression and abnormal distribution are associated with aberrant heart function and failure, enhancement of Cx-43 by treatment with programmed Sca-1+ stem cells in rat myocardial infarction was shown to attenuate infarct zones (Lu et al. 2009). The ability of Cx-43 to mediate synchronous contractions and efficient coupling has been further demonstrated by successful engraftment of skeletal myoblasts expressing Cx-43 in end-stage heart failure (Suzuki et al. 2001). Besides reduction in Cx-43 levels, post-translational modifications have also been observed in heart failure, compromising myocyte coupling and cardiac conduction (Ai and Pogwizd 2005). Vagus nerve stimulation protects against ventricular arrhythmias during myocardial infarction (MI) by prevention of Cx-43 dephosphorylation. In support of this, the administration of ghrelin, which mimics vagal stimulation, mediated protection against ventricular arrhythmias in MI, through preservation of the phosphorylated Cx-43 levels (Soeki et al. 2013). Further, fibroblast growth factor (FGF) induced protection against ischemic injury also comprises Cx-43 phosphorylation (Srisakuldee et al. 2006). The abovementioned studies underscore the significance of Cx-43 phosphorylation status in the modulation of ventricular conduction (Ando et al. 2005). Similarly, acetylation of Cx-43 has also been implicated in dystrophic heart phenotypes (Colussi et al. 2011).

Recent findings have demonstrated yet another unexplored function of Cx-43 in the mitochondria in amelioration of heart failure: Cx-43 expression in the mitochondria is indispensable for ischemic-preconditioning-based protection and increased survival of implanted stem cells in infarcted areas of the heart (Boengler et al. 2007; Ruiz-Meana et al. 2008, 2014; Lu et al. 2012). Mitochondrial Cx-43 (mCx-43) is essential for normal mitochondrial respiration and ATP production (Boengler et al. 2012). Other beneficial effects include mitochondrial K^+ influx (Miro-Casas et al. 2009) and inhibition of apoptosis (Goubaeva et al. 2007). In addition, we observed decreased expression of Cx-43 in a mouse model of pressure overload heart failure, and treatment with mitochondrial fission (Drp-1) inhibitor Mdivi-1 enhanced its expression (Fig. 3). Together, the abovementioned findings emphasize the importance of mCx-43 in the course of a variety of cardiovascular diseases.

The abovementioned studies describe various scenarios for Cx-43 level modulation and the consequences of alterations in Cx-43 post-translational modifications. Therefore, drugs that enhance mCx-43 levels and inhibit dephosphorylation have the potential to improve survival rates in end-stage heart failure.

Role of MMP-9 in heart failure

Matrix metalloproteinases (MMPs) are proteases that degrade ECM components such as collagen and play a crucial role in cardiac remodeling. The role of cardiac fibroblasts and tissue plasminogen activator in the enhancement of MMP-9 levels have been proposed in heart failure (Tyagi et al. 1996a, 1996b). Enhanced levels of MMP-9 in decompensatory heart failure reduces angiogenesis (Givvimani et al. 2010) and also causes endocardial apoptosis and endothelium–myocyte uncoupling (Ovechkin et al. 2005). MMP-2, MMP-9, TIMP-1, and TIMP-2 were found to contribute to ECM remodeling in valvular heart diseases (Soini et al. 2001). Serum levels of MMP-9 following MI were higher in decompensatory heart failure than in the compensatory stage. (Jong et al. 2006). Although the mechanism of increase in plasma MMP-9 has not been established, the deteriorating heart could be one possible source. The above study and others proposed MMP-9 as therapeutic target in ischemic and non-ischemic heart damage and also suggest MMP-9 as a diagnostic marker in failing hearts. (Creemers et al. 2001; Wilson et al. 2002; Sundstrom et al. 2004; Wagner et al. 2006; Yan et al. 2006; Moe et al. 2008; Zhao et al. 2009; Buralli et al. 2010; Dini et al. 2010; Yang et al. 2010). However, recent studies have found no significant association between plasma levels of MMP-9 and increased risk for myocardial infarction as well as other forms of heart failure (Vorovich et al. 2008; Welsh et al. 2008; Eldrup et al. 2012). The apparent difference could be due to variations in the study population and (or) gene polymorphisms (Mizon-Gerard et al. 2004; Shevchenko et al. 2010). Nonetheless, in chronic heart failure patients, it has been reported that cardiac resynchronization therapy mediated improvement in biventricular function is associated with down-regulation of MMP-9 (Szulik et al. 2011).

Several drugs or cell surface receptor binding agents that modulate MMP-9 expression and (or) activity have been observed to confer beneficial influence during heart failure. A parallel study revealed that pharmacological inhibition of adrenoceptors was associated with inhibition of MMP-9 activity, and could prevent tissue damage in heart failure (Song et al.

2006). A recent report noted that pharmacological activation of angiotensin receptor resulted in protection from MI in mouse models through reduced expression of MMP-9 (Lauer et al. 2014). In support of a pathological role for MMP-9 in dilated cardiomyopathies, broad spectrum pharmacological inhibition of MMPs or specific ablation of MMP-9 were observed to enhance the heart function in different heart failure scenarios as well as survival of cardiac stem cells (Mishra et al. 2012). The cardio-protective effects from the naturally occurring flavonol glycoside icariin, involve down-regulation of MMP-9 activity during the amelioration of left ventricular dysfunction (Song et al. 2011). Treatment with atorvastatin protected against volume overload induced heart failure by reducing MMP-9 expression and activity (Cheng et al. 2007). A selective inhibitor of MMP-9, PG-530742, was shown to prevent global left ventricle dysfunction in dogs (Morita et al. 2006).

Interestingly, MMP-9 localization has also been reported in mitochondria. In hyperhomocysteinemia (HHcy), mitochondrial oxidative stress was shown to activate MMP-9 in microvascular endothelial cells (Moshal et al. 2006). Moreover, it was noted that during HHcy, Cx-43 was abnormally translocated to mitochondria and was degraded by the mitochondrial MMP-9 (mMMP-9) (Tyagi et al. 2010). The Cx-43 degradation and MMP-9 activation in the mitochondria were ameliorated upon ablation of cardiac-specific putative homocysteine receptor *N*-methyl-D-aspartate receptor 1 (NMDA-R1). Further, in a mouse model of pressure overload heart failure, it was demonstrated that inhibition of mitochondrial fission through Mdivi-1 (mitochondrial division inhibitor) inhibited MMP-9 up regulation in the heart that was induced by the abnormal hemodynamic stress (Givvimani et al. 2012). In a rabbit model of acute myocardial infarction, the beneficial effect of implanted pretreated bone marrow derived mononuclear cells (BMDMNC) include reduced MMP-9 levels, enhanced Cx-43 levels, and improved ROS management by the mitochondria (Sheu et al. 2010). Similar results were also observed in another cell-based therapy for dilated cardiomyopathy in a rat model (Sun et al. 2009). Invitro studies also suggested that abnormal pacing or homocysteine treatment of isolated myocytes resulted in enhancement of mMMP-9 and myocyte dysfunction (Moshal et al. 2009; Vacek et al. 2011). Together, these studies suggest that abnormal mitochondrial biogenesis or enhanced oxidative stress in the mitochondria contribute to abnormal activation of MMP-9 and lead to degradation of important myocyte couplers such as Cx-43.

The broad spectrum of studies encompassing multiple animal models of heart failure along with the human heart disease suggest that targeting MMP-9 would preserve heart function from various inducers of heart damage. Based on our findings and the accumulating evidence, combination drugs capable of targeting different processes that are vital for proper heart function, such as mitochondrial biogenesis, maintenance of gap junction integrity, and ECM remodeling regulators have the potential to cure different end-stage ailments of the heart.

Summary and conclusions

There is increased fragmentation (fission) of mitochondria leading to mitophagy during various cardiovascular diseases. Increased expression of MMP-9 and decreased Cx-43 is seen during heart failure. Inhibition of aberrant mitochondrial fission by Mdivi-1 (Drp-1

specific inhibitor) ameliorates cardiac function in heart failure by restoring the mitochondrial homeostasis and also by increasing Cx-43 levels in the cardiomyocyte mitochondria.

Mitochondria play a significant role not only in ATP production but also in various other molecular mechanisms that can occur during cardiovascular pathology. The fact of the involvement of mitochondria in embryonic stem cell cardiac differentiation and maturation through mitofusins emphasizes their significance in cardiac regenerative mechanisms. Structural and functional aberrations have been observed in mitochondria during heart failure. The observation of abundant small and fragmented mitochondria during heart failure is consistent with increased fission (Drp-1) and decreased fusion (Mfn2) mechanisms. Regulation of mitochondrial fusion and fission mechanisms under diseased conditions by pharmacological agents that can reestablish the normal mitochondrial structure and function could be a promising therapeutic approach to heart failure.

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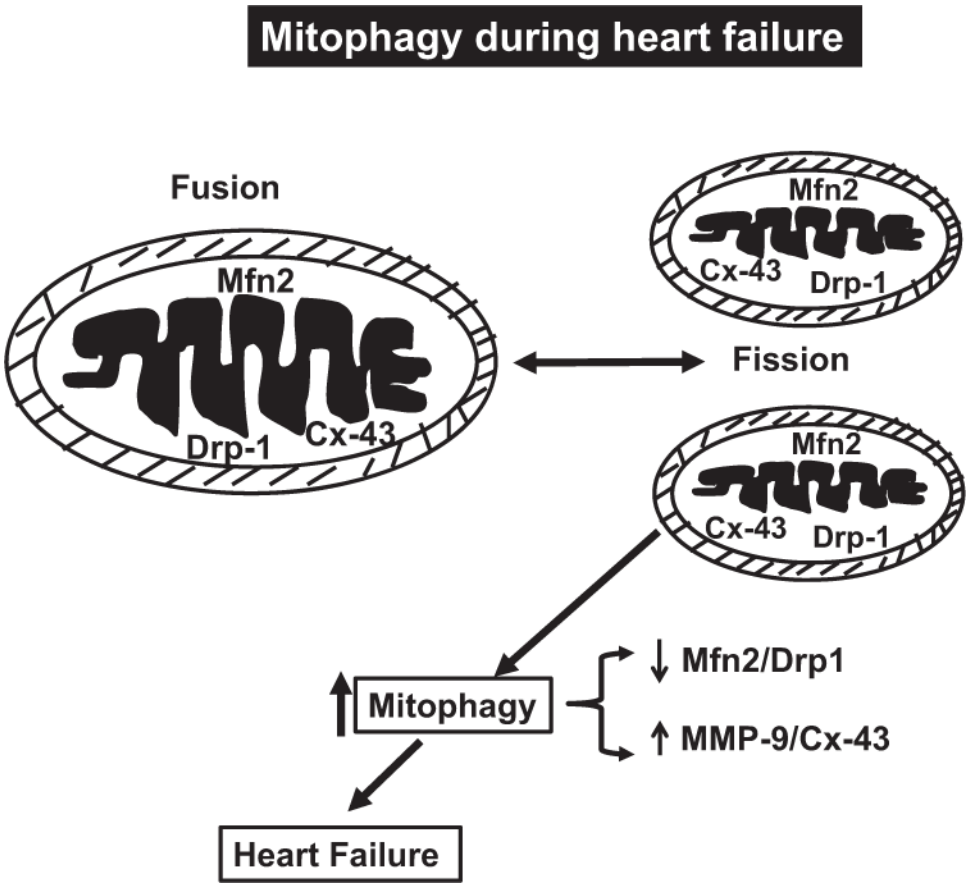

Fig. 1.

Schematic representation of mitochondrial fusion and fission mechanisms including the possible role of Cx-43 and MMP-9 during heart failure. Cx-43, connexin-43; MMP-9, matrix metalloproteinase 9; Mfn2, mitofusin 2; Drp-1, dynamin related protein-1.

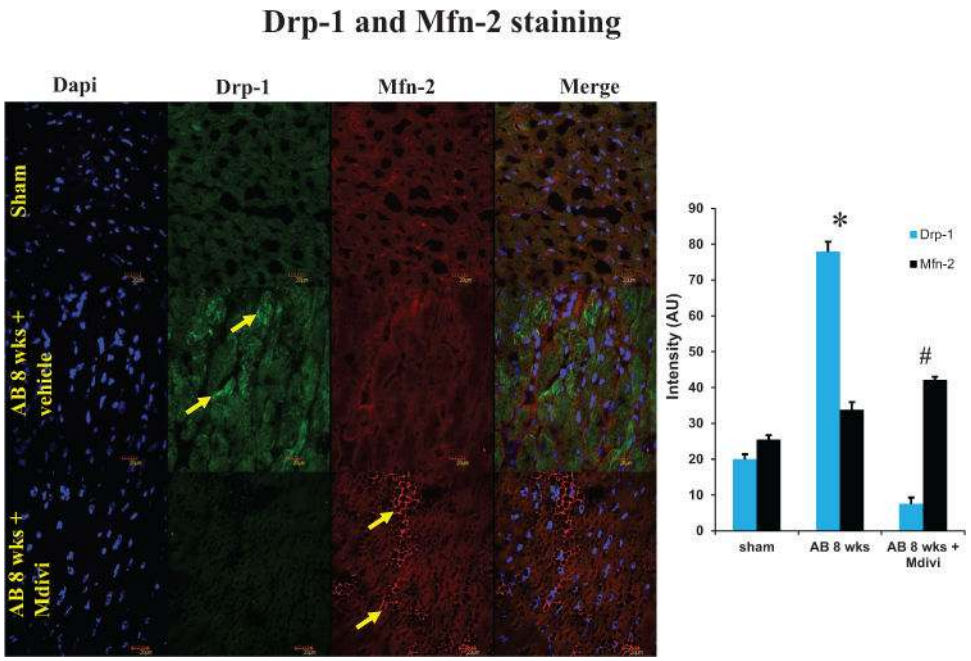

Fig. 2.

Immunohistochemistry staining of frozen heart sections with Drp-1 and Mfn-2 antibodies from the following treatment groups: sham treated; aortic banding for 8 weeks + vehicle (AB 8 wks + vehicle); and, aortic banding for 8 weeks + Mdivi-1 (AB 8 wks + Mdivi). Nuclei are stained with Dapi (blue color, on the Web site only). The expression of Drp-1 and Mfn2 is seen as fluorescence (green and red, respectively, on the Web site only). Scale bar = 20 μm. Arrows point to the fluorescence staining. Quantified intensity data are represented in arbitrary units (AU) in the adjacent bar graphs. Data are the mean ± SE from $n = 4$ measurements per group; *, $p \leq 0.05$ compared with the sham group; #, $p \leq 0.05$ compared with the AB 8 wks + Mdivi group. Drp-1, dynamin related protein-1; Mfn-2, mitofusin 2; Mdivi-1, mitochondrial division inhibitor 1.

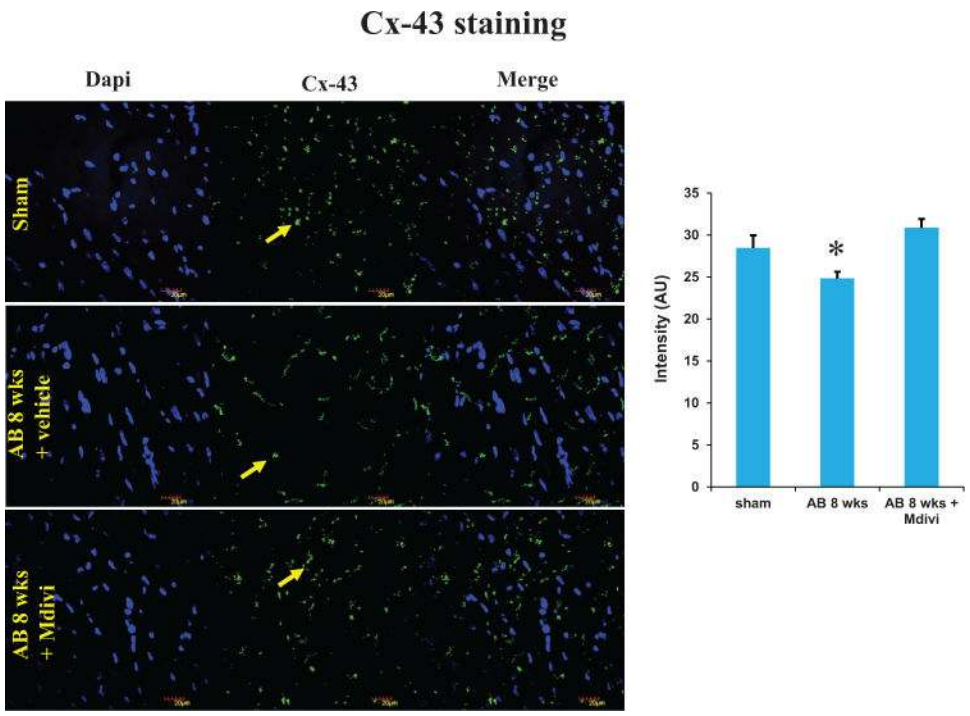

Fig. 3.

Immunohistochemistry staining of frozen heart sections with connexin-43 (Cx-43) from the following treatment groups: sham treatment; aortic banding for 8 weeks + vehicle (AB 8 wks + vehicle); and aortic banding for 8 weeks + Mdivi-1 (AB 8 wks + Mdivi). Nuclei are stained with Dapi (blue fluorescent stain) and Cx-43 is seen as fluorescence (green, on the Web site only). Scale bar = 20 μm. Arrows point to the fluorescing Cx-43. Quantified data are presented in the adjacent graphs. Data is for the mean ± SE from $n = 4$ measurements per group; *, $p \leq 0.05$ compared with the sham group.