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Heart Failure and Mitochondrial Dysfunction: The Role of Mitochondrial Fission/Fusion Abnormalities and New Therapeutic Strategies

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Abstract

The treatment of heart failure has evolved during the last thirty years with recognition of neurohormonal activation and the effectiveness of its inhibition in improving quality of life and survival. Over the last twenty years there has been a revolution in the investigation of the mitochondrion with the development of new techniques and the finding that mitochondria are connected in networks and undergo constant division (fission) and fusion, even in cardiac myocytes. This has led to new molecular and cellular discoveries in heart failure, which offer the potential for the development of new molecular-based therapies. Reactive oxygen species (ROS) are an important cause of mitochondrial and cellular injury in heart failure, but there are other abnormalities, such as depressed mitochondrial fusion, that may eventually become targets of at least episodic treatment. The overall need for mitochondrial fission/fusion balance may preclude sustained change in either fission or fusion. In this review we will discuss current heart failure therapy and its impact on the mitochondria. In addition we will review some of the new drug targets under development. There is potential for effective, novel therapies for heart failure to arise from new molecular understanding.

Keywords

mitochondria; ACE inhibitor; metformin; ss-31; mitochondrial fission; mitochondrial fusion; heart; cardiovascular

Introduction

It is thought that mitochondria arose millions of years ago as a result of the incorporation of unicellular organisms by other unicellular organisms, and this is known as the endosymbiotic theory.¹ Consistent with this theory, mitochondria have lipid bilayers as membranes. The mitochondria contain a circular DNA, like bacteria, and this codes for 13

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essential mitochondrial proteins along with tRNAs. The vast majority of mitochondrial proteins are encoded by nuclear DNA.

Mitochondria, ROS and Heart Failure

Reactive oxygen species (ROS), such as superoxide ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2), play an important role in regulating the cell growth and death of cardiac myocytes. Mitochondria are thought to be both a major source of ROS, as well as a major target for ROS damage. Accumulation of ROS is significantly enhanced in the failing myocardium, as has been showed in numerous experimental and clinical studies.²⁻⁶ The majority of ROS in the heart appear to come from uncoupling of mitochondrial electron transport chain at the level of complexes I and III, although the view of mitochondria as a major source of intracellular ROS has been recently challenged.^{7, 8} Studies with isolated cardiac myocytes have implicated pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF), in the generation of ROS in mitochondria, contributing to the process of heart failure.^{9, 10} Inhibition of ETC complex I and mitochondrial uncouplers have been shown to reduce ischemia-reperfusion injury; it has been proposed that this protection is mediated by reduction in ROS during ischemia.¹¹

The family of NADPH oxidases (NOXs) is the important source of ROS in the heart, whose sole biological function is to produce $O_2^{\cdot -}$ or H_2O_2 , by transferring electrons from NADPH to molecular oxygen.^{12, 13} Five isoforms of Nox have been described, while NOX2 and NOX4 are the major NOX isoforms in the heart.¹² Nox activity is high in human failing hearts.¹⁴ NOX2 is involved in cardiac remodeling after myocardial infarction, whereas NOX4 is a critical mediator of mitochondrial oxidative stress and mitochondrial dysfunction during heart failure.^{15, 16}

Whether there is increased ROS vs. decreased antioxidant defenses leading to cardiac injury, and whether this contributes to the development and progression of HF remains a subject of debate. Tsutsui et al. demonstrated that the activity of SOD, catalase, and GSHPx were not decreased in failing hearts, indicating that oxidative stress in heart failure is primarily due to increased generation rather than to a decline in antioxidant defenses.¹⁷ Certainly decreasing antioxidants can lead to HF. In a transgenic Mn-SOD-deficient mice model loss of manganese superoxide dismutase led to progressive HF with specific defects in mitochondrial respiration.¹⁸ In isolated cardiac myocytes, a subtle increase in ROS caused by partial inhibition of SOD resulted in a phenotype characterized by hypertrophy and apoptosis.¹⁹ Mitochondrial ROS may be particularly prone to trigger apoptosis through activation of the mitochondrial permeability pore transition, release of cytochrome *c*, and activation of effector enzymes in apoptotic signaling.²⁰ Given that ROS affect virtually all aspects of cardiac myocyte physiology and that overall the evidence supports that ROS are increased in HF and have a detrimental effect, ROS are an important potential therapeutic target for treating HF.

Heart Failure

Systolic heart failure has increased in prevalence over the last decades, in part due to the successes in treating coronary disease. Mitochondrial dysfunction is a key feature of systolic

heart failure and is thought to contribute to heart failure progression through both impaired high energy phosphates production and increased production of reactive oxygen species (ROS). In the last decade there have been many new discoveries with regards to mitochondria that provide new insights into mitochondria and their role in disease. In this review we will discuss mitochondrial fission and fusion, essential processes by which the mitochondria divide and fuse repeatedly, and the role of fission and fusion abnormalities in cardiovascular disease. Mouse genetic models have been essential to elucidating molecular mechanisms underlying mitochondrial abnormalities in heart failure, and an overview of these is summarized in Table I. In the second part of the review we will discuss current therapeutics as well as potential new therapies in development and how these agents may directly target mitochondrial abnormalities.

Mitochondrial Fission and Fusion

Mitochondrial fission and fusion were first described in yeast in the 1990s.²¹ Rather than being just static high energy phosphate producing organelles, it was found that mitochondria exist as interconnected networks, and that they continually divide (fission) and fuse.^{22, 23} Furthermore, it was found that continual fission and fusion are critical for the maintenance of normal mitochondrial function. Why fission and fusion are necessary for mitochondrial health remains a subject of debate.^{22, 24} In the simple yeast, fission and fusion are very rapid processes and are thought to occur every two minutes. In contrast in mammalian cells fission and fusion occur over a much longer period of time taking hours or longer.^{21, 25} Given the complexity of the cardiac myocyte with its highly organized sarcomeres many doubted that cardiac myocytes would undergo fission and fusion. However, it is clear that fission and fusion are necessary mitochondrial functions in all cells.²⁶

In mammals three proteins are involved in mitochondrial fusion. Mitofusin (Mfn) 1 and 2 fuse the outer mitochondrial membrane, while optic atrophy (OPA)1 fuses the inner mitochondrial membrane. These proteins have been found to have other critical functions. Mfn2, which is also known as hyperplasia suppressor gene (HSG), inhibits vascular smooth muscle cell proliferation *in vitro* as well as in a rat model of carotid injury.²⁷ Mfn2 is decreased in diabetes and obesity.^{28, 29} There is also work indicating that Mfn2 has a regulatory role in metabolism.³⁰ The fusion and fission proteins also are involved in apoptosis.³¹⁻³⁴

OPA1 is critical for effective mitochondrial function, beyond its role in inner mitochondrial membrane fusion, through key properties essential for maintenance of mitochondrial function. A 10 kDa peptide generated by cleavage of OPA1 is necessary for initiation of mitochondrial DNA replication, an indispensable process for maintenance of robust mitochondria.³⁵ In addition, OPA1 has been found to have a key role in maintaining cristae tight junctions and preventing cytochrome c release.³⁶

Proteins controlling mitochondrial fission in mammalian cells include dynamin related protein (Drp) 1 and fission (Fis)1.^{37, 38} Drp1 is primarily a cytoplasmic protein, however it forms complexes at fission sites on the outer mitochondrial membrane.³⁸ Human Fis1 encircles the outer mitochondria, but does not accumulate at scission sites.^{37, 39} Fis1 contains a tetratricopeptide repeat motif that creates a scaffold, which promotes the

assembly of protein complexes on the outer mitochondrial membrane.³⁹ In mammals, mitochondrial fission protein 1 (Fis1), mitochondrial fission factor (Mff), and mitochondrial dynamics proteins of 49 and 51 kDa (MiD49 and MiD51, respectively)—have been proposed to act as receptors that recruit Drp1 to the mitochondrial surface.^{40, 40-42}

Mitochondrial Energetics, Respiration and Mfn1 and Mfn2

Overall there is a direct correlation between mitochondrial fusion and the oxidative phosphorylation capacity.⁴³ Inhibition of mitochondrial fusion leads to reduced oxygen consumption.²² Similarly, inhibition of Mfn2 expression significantly reduces pyruvate, glucose, and fatty acid oxidation. Interestingly, skeletal muscle from obese humans and animal models has markedly decreased Mfn2 levels.⁴⁴ Mfn2 reduction in fibroblasts diminished oxygen consumption and glucose oxidation.⁴⁵ Consistent with these findings, MEFs with double knockout of Mfn1/2 had loss of mitochondrial membrane potential, reduced respiration, and decreased maximal respiration.⁴⁶ In contrast, Mfn2 over-expression leads to increased respiratory complex activity, increased glycolysis and mitochondrial biogenesis.⁴⁴ Correspondingly, Mfn2 is up-regulated in conditions of high energy demand, such as exercise, and in response to pro-apoptotic stimuli.⁴⁶ Thus, mitochondrial fusion and Mfn1/2 have important affects on mitochondrial energetics and respiration.

Mitochondrial Energetics, Respiration and OPA1

OPA1 is required for normal mitochondrial metabolism. Depletion of OPA1 by RNAi in MEFs leads to reduction in basal respiration and inability to enhance oxygen consumption in the presence of the uncoupler 2,4-dinitrophenol (maximal respiration).⁴⁶ Studies of fibroblasts from patients with autosomal dominant optic atrophy (ADOA) and defined OPA1 mutations revealed impaired complex I substrate driven ATP synthesis and decreased mitochondrial fusion.⁴³ In contrast, other OPA1 mutations did not affect mitochondrial activity and bioenergetics.⁴⁷ These results indicate that there are key OPA1 domains for mitochondrial function. Greater understanding of post-translational modifications and identification of proteins that interact with OPA1 will provide further insight into the mechanism(s) by which some ADOA OPA1 mutations can cause disease, and yet not effect measured mitochondrial function.

Mitochondrial Fission Proteins and Mitochondrial Energetics

Alterations in mitochondrial fission proteins can also affect mitochondrial metabolism. RNAi mediated reduction of Drp1 lowered the basal rate of oxygen consumption, reduced coupled respiration, and lessened the rate of ATP synthesis.⁴⁸ Similarly, a dominant negative Drp1 caused a striking reduction in respiratory capacity in INS1 cells.⁴⁹ Hyperglycemia induced fission and cell death, but inhibition of this using a dominant negative Drp1 markedly impaired the mitochondria's ability to increase respiration.^{50, 51} Similarly Fis1 depletion also decreased the maximal respiratory activity, and Fis1 overexpression restored the phenotype.⁴⁹ Accordingly, both DRP1 and Fis1 have roles in mitochondrial metabolism.

Fission and Fusion in Disease

There are a number of inherited neuropathies, such as Charcot-Marie-Tooth disease and ADOA, that are associated with mutation of genes encoding mitochondrial proteins. Two genes involved in mitochondrial fusion, OPA1 and Mfn2, have been implicated in inherited optic neuropathies.^{52, 53} Abnormal expression of Mfn2 has been reported in Parkinson's disease and type 2 diabetes.^{54, 24}

Fission and Fusion in Heart Failure

A growing literature supports the role of abnormal fission and fusion in heart failure. OPA1 is decreased in ischemic heart failure in both rats and explanted human hearts, while Mfn1/2 and Drp1 are increased in human nonischemic dilated heart failure.⁵⁵ Mutation of OPA1 to model inherited optic atrophy (ADOA) caused late onset of cardiomyopathy, the onset of which coincided with the onset of blindness.⁵⁶ The OPA1 mutant heart has increased ROS, decreased anti-oxidant gene expression, decreased mitochondrial DNA copy number, abnormal calcium transients and impaired mitochondrial respiration (state III and uncoupled) as well as impaired complex I and IV activity.⁵⁶ Despite these abnormalities, expression of TFAM, PGC-1 α , Mfn1/2, Bax, Bak and Nrf2 were unchanged.⁵⁶ However, these findings raise the possibility that the decrease in OPA1 in human ischemic heart failure is accompanied by similar changes, given the known functions of OPA1 discussed above.

It is increasingly evident that abnormal fission and fusion contribute to cardiovascular disease.⁵⁷ OPA1 has been found to be decreased in ischemic cardiomyopathy, while Mfn1/2 were increased in both ischemic and nonischemic heart failure.⁵⁵ Inhibition of mitochondrial fission protected the heart against ischemia/reperfusion injury.⁵⁸ Fission and fusion proteins also have a role in apoptosis.⁵⁹ Thus, these proteins have functions in the cell beyond mitochondrial fission and fusion.

In cultured neonatal ventricular myocytes, inhibition of mitochondrial fission with a dominant negative mutant of Drp1, Drp1-K38A, prevents increased ROS, mitochondrial permeability transition pore formation and eventual cell death under high glucose conditions.⁵⁰ Cytosolic Ca²⁺ overload, with thapsigargin or potassium chloride treatment, caused a quick up-tick in mitochondrial fragmentation in both neonatal and adult cardiac myocytes.⁶⁰ Calcium overload is frequently found in HF, which could lead to increased mitochondrial fission. Consistent with this is the finding that mitochondria in systolic heart failure are small and fragmented, suggesting an imbalance of fission and fusion.⁵⁵ This would exacerbate the energetic abnormalities in HF.⁶¹ Dorn et. al. demonstrated that silencing of OPA1 and mitochondrial assembly regulatory factor (MARF) in the *Drosophila* heart leads to greater mitochondrial heterogeneity and heart tube dilation accompanied by marked contractile impairment. In this model human Mfn1/2 rescued MARF RNAi induced cardiomyopathy.⁶²

In adult mammalian cardiac myocytes the mitochondria are complexly organized in three subsets. The overwhelming majority of mitochondria are located between the contractile filaments (interfibrillar) with a minority of mitochondria next to the sarcolemma

(subsarcolemmal) and the remaining mitochondria perinuclear. In heart failure the interfibrillar mitochondria fail to maintain their normal organization.⁵⁵ In addition there is a decrease in the size and denseness of interfibrillar mitochondria in rodent heart failure models.⁶³ OPA1 is reduced in both human and rat ischemic HF.⁵⁵ In H9c2 cells OPA1 reduction increased apoptosis at both baseline and after ischemia, through cytochrome c release, consistent with OPA1's role in maintaining cristae structure^{36, 55, 64} Electron microscopic evaluation demonstrated more numerous, but small mitochondria in a rat model of ischemic HF.⁵⁵ In contrast, Javadov et al. observed less Mfn2, increased Fis1 and decreased OPA1 expression at 18 weeks in rat hearts in a simple myocardial infarction model.⁶⁵ Mfn2 knockout results in pleiomorphic mitochondria with some increase in size in cardiac myocytes.⁶⁶ *In vivo* conditional cardiac Mfn2 knockout mice have limited cardiac hypertrophy and minor depression of heart function.⁶⁶ It is thought that Mfn1 and Mfn2 can each compensate to a degree for the loss of the other, but neither Mfn can compensate for loss of OPA1, knockout of which is embryonic lethal.^{53, 67}

Cardiomyopathy and Inherited Neuropathies

Mutations of the fusion proteins are associated with inherited optic neuropathies, most of which have a gradual onset over time. Mfn2 mutation accounts for 20% of type II Charcot-Tooth-Marie(CTM) disease cases, and OPA1 mutation less frequently is a cause of CTM.²⁴ OPA1 mutations are more commonly associated with ADOA, characterized by the onset of visual impairment and blindness.⁶⁷ The OPA1 mutation associated with the disease optic atrophy had been reported to be homozygous embryonic lethal, but to not cause cardiac abnormalities in the heterozygote, but this was based on simple parameters such as heart weight.⁶⁷ More intensive investigation with advanced cardiac imaging and functional studies demonstrated that the OPA1 heterozygous mutant mouse develops cardiomyopathy at 12 months.⁵⁶ Multiple mitochondrial abnormalities were present as discussed above.

Human Heart Failure

Mitochondrial Changes in Human Heart Failure

Investigating changes in mitochondrial function in human heart failure is challenging given the limitations in obtaining tissue to study, the difficulties of obtaining fresh tissue for functional studies, and donor to donor variability, among other problems. Nonetheless a number of studies have provided correlation between animal studies and the human disease. Oxidative stress, ROS and oxidative damage are increased in the failing human heart.⁶⁸⁻⁷¹ Overall, mitochondrial oxidative capacity is reduced.^{71, 72} As in animal models, mitochondrial complex function is impaired.^{68, 71, 72} Most studies have found no change in antioxidant proteins.⁶⁹⁻⁷¹ However, MnSOD activity has been found to be increased by some and decreased by others.⁶⁹⁻⁷¹ Catalase/GPx activity were also found to be increased in one study.⁶⁹ NADPH oxidase activity is increased in the failing heart, and may be at least one source of increased ROS, as has recently been reviewed.^{70, 73}

AMPK

functions as a cellular energy sensor in the heart and has an important role in energy homeostasis and cell survival. A number of basic studies, including those showing a benefit

from metformin treatment (discussed below), suggest that increasing AMPK in heart failure can improve cardiac function and survival. Investigations of AMPK in the failing human heart are extremely limited. In human HF there was a change in isoform expression with increased $\alpha 1$, $\beta 1$ and $\gamma 2$ isoforms.⁷⁴ There was also increased $\alpha 1$ and $\alpha 2$ activity (phosphorylation increases activity).⁷⁴ Parallel studies of mouse HF showed an increase in the $\alpha 2$, $\beta 2$ and $\gamma 2$ isoforms.⁷⁴ Thus both mouse and human HF had increased $\gamma 2$ isoforms, but there were differences in the changes in expression of α and β isoforms.

Mitochondrial Biogenesis, Fusion and Fission

PGC-1 α is important in biogenesis, and it has been found to be decreased in a number of studies of human heart failure,^{68, 72, 75, 76} while others found it unchanged⁷⁷ or increased.^{68, 71} ERR α is increasingly recognized to have an important role in mitochondrial biogenesis, and this downstream target of PGC-1 α has been found to be decreased in heart failure.^{76, 78} A number of investigators have shown that mtDNA copy number, very important for mitochondrial “viability”, is also decreased in HF.^{68, 71, 79} However, one study found increased mtDNA copy number in DCM (dilated, nonischemic cardiomyopathy).⁷¹ Decreased mtDNA copy number correlated with decreased expression of mtDNA encoded proteins.^{68, 69} MtDNA replication was found to be defective in HF.^{68, 79} This is particularly interesting, as a 10 kDa fragment of the fusion protein, OPA1, has been shown to be essential for initiation of human mtDNA replication, as discussed above, and OPA1 has been found to be decreased in human ischemic cardiomyopathy (ICM, heart failure).^{2, 55} Mitochondrial fusion and fission are essential for maintaining normal mitochondrial function. OPA1 is decreased in ICM, but unchanged in DCM, where mtDNA copy number has been found to be increased.^{55, 71} MFN 1/ 2 were increased in both DCM and ICM in one study, while MFN2 was reported to be decreased in ICM by others.^{55, 71}

There is accumulating evidence that ICM and DCM, two common types of heart failure, differ from one another, even though clinically the two diseases have many similarities. Nevertheless, some of the above studies show clear differences between the two. Many studies combine DCM and ICM samples for study and differences in this mix may explain some of the end-point differences noted above. Most compelling is a recent work by Ahuja et al. identifying clear differences in ICM and DCM mitochondria, discussed above, including increased mtDNA copy number and increased mtDNA deletion mutations, in DCM.⁷¹

Implications of these changes for treatment of heart failure

The heart, like the brain, has a high demand for ATP and is dependent on mitochondrial function. Despite intensive studies of mitochondrial dynamics in other fields, much remains to be understood in the heart, where fusion and fission occur likely at a much slower rate than in yeast. Disorders in mitochondrial organization and the presence of abnormally small and fragmented mitochondria have been observed in end-stage dilated cardiomyopathy, myocardial hibernation and ventricular-associated congenital heart disease supporting an important role for mitochondrial fission/fusion in heart disease.⁶⁰ The contribution of asymmetric mitochondrial fission/fusion in heart failure as a cause vs. a consequence of myocardial injury remains to be determined. Nevertheless, the restoration of mitochondrial

fusion/fission symmetry may help the rescue the failing heart. Thus, the mitochondria represent a new target for development of targeted therapeutics for heart failure.

As we understand more of the distinct abnormalities occurring in the mitochondria with heart failure, the goal becomes to identify targeted therapy to reverse or ameliorate the mitochondrial abnormalities. Current therapy for heart failure with ACE inhibitors or angiotensin receptor blockers, beta blockers and inhibition of aldosterone have improved the course of heart failure and prolonged survival; however, there is much need for improved therapies. Most of the therapies for systolic heart failure have some impact, often indirect, on ROS. First we will review specific currently used drug therapy with significant impact on the mitochondria. Then we will discuss new drug therapy that is in development.

Established Pharmacologic Therapy and the Mitochondria in Heart Failure

There are a number of well-established treatments for HF based on many studies in both experimental models and humans. Table II provides an overview of current heart failure pharmacologic treatments, as well as new treatments under development, and their effects on the heart and mitochondria.

Renin-Angiotensin System Inhibition and Mitochondrial Function in Heart Failure

Although inhibition of the renin-angiotensin system (RAS) is highly beneficial in heart failure, there is only limited data supporting that angiotensin converting enzyme inhibitors (ACE) or angiotensin receptor blockers (ARBs) have a beneficial effect on mitochondrial function. Trandopril, an ACE, in a rat model of heart failure (high LAD ligation) attenuated the increase in HSP60, the decrease in oxygen consumption rate (OCR), and the increase in mitochondrial thiobarbiturate reacting substance (TBARs) that occurred with HF.⁸⁰ There was some improvement in hemodynamics with a lower LVEDP with trandopril treatment. In addition LV weight, lung weight and the LV/body weight ratio and lung/body weight ratio were all lower with trandopril treatment.⁸⁰ In an aortic stenosis model of heart failure in male rats 10 weeks of treatment with perindopril, an ACE inhibitor, decreased plasma angiotensin II levels, but did not improve the reduction in enzymes involved in energy metabolism, including citrate synthase and cytochrome C oxidase in either heart or skeletal muscle. Perindopril did increase basal respiration, but had no effect on the maximal respiration rate.⁸¹ Others have found that telmisartan, an ARB, improves skeletal muscle endurance in normal mice, and this is mediated through activation of the PPAR- δ /AMPK pathway.⁸² This pathway is involved in mitochondrial biogenesis, and thus angiotensin receptor blockers might increase in mitochondrial biogenesis in HF, but this remains to be proved.

Beta Blocker

Beta blockers are a mainstay of therapy in heart failure. Primarily this treatment addresses the upregulation in the beta adrenergic system that occurs in heart failure with increased beta adrenergic receptor expression in the heart. Activation of the beta adrenergic receptors has

been shown to increase ROS production.⁸³ Furthermore, carvedilol, but not metoprolol, reduced oxygen consumption and superoxide production by mitochondria.⁸⁴ Carvedilol is a third generation beta blocker with nonselective beta blocking plus α_1 blocking. On the other hand metoprolol is a selective beta 1 blocker. It may be that beta 2 or α_1 inhibition are contributing to this difference. Overall beta blockers have not been shown to have mitochondrial specific effects, though studies such as these suggest there may be specific beneficial effects on the mitochondria, possibly only with a subset of beta blockers.

Spironolactone

Investigation of the role of spironolactone, a competitive antagonist of aldosterone, in protecting mitochondria and decreasing ROS generation during HF has been very limited. Mulder et al. reported that aldosterone synthase inhibitor FAD286, but not long-term spironolactone, reduced the CHF-related enhancements in LV ROS, reduced-oxidized glutathione ratio, and aortic NADPH oxidase activity.⁸⁵ However, Bauersachs et al. found that spironolactone added to an ACE inhibitor normalized NO-mediated relaxation in HF by beneficially modulating the balance of NO and ROS generation.⁸⁶ More recently, It was shown that spironolactone provides additional beneficial effects to olmesartan, an angiotensin II receptor blocker, in reducing oxidative stress in postinfarct failing hearts.⁸⁷ These experiments suggested synergistic effects on attenuation of cardiac oxidative stress, leading to an improvement in cardiac function in postinfarct failing hearts through antagonism of aldosterone action.

Other Drugs Commonly Used in Heart Failure

Metformin

Metformin, a biguanide, is a commonly used drug for treatment of diabetes, and many patients with heart failure have diabetes. The guanides were originally used in herbal therapies and derived from the lilac. Metformin is the only guanide used for treatment of diabetes. There had been concerns that metformin could cause lactic acidosis if patients had renal impairment or received iodinated contrast, but these concerns have turned out to be unjustified.⁸⁸ Interestingly, there is growing data supporting a decreased risk of cancer in patients taking metformin, possibly secondary to inhibition of the inflammatory response, including activation of NF κ B, associated with cell transformation and cancer cell proliferation.^{44, 89}

Human Studies—Anecdotal reports showed better survival in diabetic heart failure patients taking metformin. Masoudi et al. reviewed the records of over 16,000 diabetic Medicare beneficiaries following hospitalization for heart failure.⁹⁰ Overall mortality was strikingly decreased in the metformin treated group. Furthermore, metformin treatment was associated with a significantly reduced risk for rehospitalization for heart failure. In an observational study of a cohort of over 6,000 veterans with diabetes and heart failure, the use of metformin (25.2% of patients) was associated with significantly lower mortality after two years compared to patients not taking metformin.⁹¹ There have been no randomized trials of metformin in heart failure, likely because of the widespread use of metformin in

patients with cardiovascular disease, making recruitment and randomization of patients difficult.

Diabetic Cardiomyopathy—In the diabetic OVE26 mouse model of diabetic cardiomyopathy⁹² EM revealed loss of the orderly array of mitochondria along the myofibrils. Increased polyubiquitination and apoptosis were also found. Inhibition of AMPK with a dominant negative-AMPK led to a decrease in autophagy, increased cardiac dysfunction and increased mortality. Chronic metformin treatment increased autophagy and preserved cardiac function, but this could be prevented by overexpression of DN-AMPK.⁹² Metformin activated AMPK and ameliorated the decrease in LC3-II seen in the diabetic hearts. Treatment of HL-1 cells, a cardiac model cell line, with metformin for 16 h increased AMPK phosphorylation and LC3-II levels.

Ischemic Heart Failure—In nondiabetic mice metformin (125 ug/kg daily) begun after coronary occlusion improved survival, ejection fraction, LV size and heart/body wt. ratio at 4 weeks.⁹³ Metformin treatment was associated with increased AMPK, increased eNOS phosphorylation, and increased PGC-1 α . Knockdown of AMPK or eNOS prevented the improvements seen with metformin treatment. The investigators also assessed mitochondrial respiration, which was significantly improved as was ATP synthesis.⁹³ Similarly in an ischemic, nondiabetic rat model of heart failure, metformin treatment begun 48 h before ligation improved cardiac function, reduced LV dilatation, and decreased ANF levels.⁹⁴ Phosphorylated AMPK levels increased with metformin treatment. Interestingly, there was no effect on fasting glucose levels.⁹⁴ Metformin has also been reported to decrease infarct size in the absence of diabetes.⁴⁵ In contrast, others observed no improvement with treatment of volume overload heart failure with metformin.⁹⁵ Many of the abnormalities in ischemic HF that responded to metformin were not present in this study, likely accounting for this lack of benefit.

Additional Underlying Mechanisms—Quentin et al. investigated the effect of metformin on the endoplasmic reticulum (ER) stress response using primary rat cardiac myocytes.⁹⁶ Metformin activated PERK-ATF4, but neither the ATF6 or IRE1-XBP1 pathway in the ER stress response. PERK-ATF4 activation was followed by upregulation of CHOP, but this did not lead to apoptosis.⁹⁶ Although the ER stress response (unfolded protein response) had not been viewed to have broader benefits beyond the ER, there is now some evidence that the ER stress response and the mitochondrial stress response can be associated with longevity and other positive properties.⁹⁷ Thus, metformin may provide additional benefit via activation of the ER stress response, but much more research is needed in this area. Overall there are clear benefits with metformin treatment in both diabetic and nondiabetic models, and no hypoglycemia occurred in the nondiabetic models.

Future Therapeutic Targets

Mitochondrial Fission and Fusion

As discussed, loss of mitochondrial fission/fusion balance is associated with ischemic/reperfusion injury and heart failure.^{55, 56, 98, 99} Furthermore, mitochondrial fission/fusion proteins have a role in apoptosis.^{32, 33} Inhibition of mitochondrial fission has been

considered to be a new therapeutic approach to rescue MI/HF. In 2010, Ong et al.⁵⁸ reported that dominant negative mutation of mitochondrial fission protein Drp1 reduced HL-1 cell death after simulated ischemia/reperfusion injury. More interestingly, the authors evaluated the effect of mitochondrial division inhibitor-1, a pharmacological inhibitor of Drp1 in both HL-1 cells and cardiac myocytes. They found the convincing beneficial effects in HL-1 cells subject to IR injury. Furthermore, treatment of adult murine cardiac myocytes with mitochondrial division inhibitor-1 reduced cell death and inhibited mitochondrial permeability transition pore opening after simulated ischemia/reperfusion injury.⁵⁸ Importantly, *in vivo* treatment with mitochondrial division inhibitor-1 reduced myocardial infarct size in mice with coronary artery occlusion and reperfusion.⁵⁸ Short term inhibition of Drp1 might be feasible, such as in acute MI, but long term inhibition would be expected to cause significant mitochondrial abnormalities secondary to fission/fusion imbalance. In another study, Wang et al. found that cardiac microRNA miR-499 could reduce myocardial infarction by inhibiting calcineurin-dependent trafficking of Drp1 to mitochondria, preventing mitochondrial fission and cell death.¹⁰⁰ These findings identified the critical role of mitochondrial fission in MI/HF and highlighted the therapeutic potential of inhibiting mitochondrial fission to rescue MI/HF. These results suggest a novel pharmacological strategy for cardioprotection.

Coenzyme Q—There is a single case report of partial reversal of visual symptoms in a patient with hereditary motor sensory neuropathy type VI (HMSN VI), who was treated with high dose coenzyme Q10 (200 mg/day) for eight months.¹⁰¹ Although there is an extensive literature on coenzyme Q in heart failure, it has never been conclusively shown to improve symptoms or outcomes.¹⁰² HMSN VI involves a mutation of Mfn2. In CMT 2A, where Mfn2 is also mutated, expression of nuclear components of complex I, II, III and IV are depressed.^{103, 104} However, mitochondrial function was not investigated in the HMSN VI case report. It is possible that coenzyme Q would be of benefit in a subset of patients with specific mitochondrial abnormalities, but more investigation would be needed.

SS-31 and Mitochondrial ROS

Mitochondria, ROS and Heart Failure

The mechanisms underlying the development of heart failure are multiple, complex, and not well understood but evidence showed that mitochondria are key players in the progression of heart failure (HF) and hypertrophy.¹⁰⁵ Reactive oxygen species (ROS) from multiple cardiac sources are thought to be an important factor in heart failure.^{56, 106, 107} Mitochondria are a major source of ROS in heart failure and at the same time are particularly vulnerable to oxidative stress.^{107, 108} Damage to mitochondria, particularly to the electron transport chain increases ROS production. Therefore, protecting mitochondria from oxidative damage could be an effective therapeutic strategy.

Currently available treatment options with antioxidants have shown limited therapeutic success.^{109, 110} Many different interventions have been tried including alpha tocopherol, ascorbic acid, and allopurinol to target xanthine oxidase. The results of clinical trials have been mixed with no convincing evidence that outcomes are improved in heart failure.¹⁰⁹ A likely issue is the systemic nature of the treatments, when targeted reduction in ROS in

cardiac cells is needed. Thus, even though there is substantial evidence that an increase in ROS is an important factor in heart failure, disappointingly, treatment strategies to date have been inconclusive to ineffective.

Catalase

Catalase is found in nearly all living organisms and detoxifies hydrogen peroxide produced by the peroxisomal aerobic dehydrogenase reaction. Surprisingly, the heart actually has weak antioxidant capacity, with particularly low catalase activity. Thus catalase activity in the human heart is only about 2% of that in the liver.^{111, 112} This low catalase activity likely adds to the sensitivity of the heart to oxidative stress. Kang et al made a transgenic mouse model with cardiac specific over expression of catalase and found that this increased catalase protected against oxidative cardiotoxicity from doxorubicin treatment.¹¹¹ In a second study the same investigators demonstrated that cardiac catalase overexpression protected against ischemia/reperfusion injury.¹¹² Thus this work provide rationale for increasing catalase activity in the heart as potentially cardioprotective.

Targeted Mitochondrial Catalase Overexpression

Research to date supports that preferential scavenging of ROS within the mitochondria, rather than global antioxidant treatment, will be more beneficial and could protect the heart against the development of HF and provide resistance to stressful stimuli.¹⁰⁵ Mice overexpressing catalase targeted to the mitochondria (mCAT) have better protection from ROS injury to mitochondria and have an approximately 20% extension of median and maximum lifespan.¹¹³ These beneficial effects did not occur in mice with overexpression of catalase targeted to peroxisomes (pCAT, the natural location of catalase). In a second study the same group demonstrated in an AngII model of pathologic hypertrophy/failure that mitochondria are the primary target of oxidative damage and that angiotensin II increases cardiac mitochondrial reactive oxygen species (ROS) production.¹¹⁴ Again the protective effects of overexpression of pCAT versus mCAT was compared. Ang II-induced cardiac hypertrophy, fibrosis and mitochondrial damage is reduced in mCAT but not in pCAT mice. The investigators argue that NADPH oxidase are also an important source of ROS in angiotensin II induced hypertrophy and that ROS generated by NADPH oxidase lead to mitochondrial damage, which further magnifies ROS generation. Breaking the ROS vicious cycle within mitochondria by mCAT is effective to attenuate mitochondrial damage, cardiac hypertrophy and failure caused by angiotensin II. However, targeted gene therapy in humans is experimental, and a different approach was needed.

Pharmacologic Strategies to Target Mitochondrial Peroxide

Recently a new series of small mitochondria-targeting tetrapeptides called SS peptides (Szeto-Schiller peptides) have been developed. SS peptides selectively accumulate in the mitochondrial matrix independent of membrane potential manner and scavenge ROS. Among them the dimethyltyrosine-containing SS-31 variant have shown therapeutic promise in targeted delivery of antioxidants to mitochondria. The structural motif of SS-31 consists of an alternating aromatic residues dimethyl tyrosine and basic amino acids enabling SS-31 bind to the inner membrane of mitochondria, and scavenge various ROS including, hydrogen peroxide [H₂O₂], superoxide [O₂⁻], and hydroxyl radicals [[•]OH].^{115, 116}

Moreover, due to its positive charge, SS-31 accumulates >1,000-fold in negatively charged mitochondria.¹¹⁶ SS-31 has excellent pharmacokinetic properties and great potential to be used for therapeutic purposes for the treatment of HF. SS-31 can be delivered intravenously, intraperitoneally, or subcutaneously to the heart, kidney, lung, and brain.¹¹⁷ It is water soluble due to the 3+ net charge, for 6 months at 37C. Moreover, SS peptides have low toxicity with no side effects were observed after 5 months of daily treatments in mice.¹¹⁸ Studies of SS-31 in several *in vivo* models of chronic cardiac dysfunction showed that administration of SS-31 before ischemia and reperfusion reduced MI size, lipid peroxidation indexes, and increased ATP content in the rat heart, as compared to the non-targeted antioxidant N-acetylcysteine.^{119,120} Mitochondrial calcium overload is known to stimulate ROS production, and the scavenging peptide SS-31 may protect mitochondria by scavenging ROS through targeted delivery to the mitochondrial inner membrane. Therefore, SS-31 has great potential to be used for the treatment of myocardial ischemia injury which is at least partially mediated by oxygen-derived free radicals.

SS-31 substantially attenuated Ang-induced NOX4 up-regulation and mitochondrial oxidative damage, suggesting its potential clinical application for target organ protection in hypertensive cardiovascular diseases.¹²¹ Dai et al. further showed that SS-31 partially rescued the heart failure phenotype in mice overexpressing Gαq, including a significant amelioration of systolic dysfunction, cardiac hypertrophy and myocardial performance.¹²¹ Thus strategies targeting mitochondria either by the use of synthetic peptides have the potential to effectively reduce ROS and limit cardiac damage.

Research interest in mitochondria had waned, but with the development of new tools and models, along with the discovery of fusion and fission, new avenues of investigation have opened that have the potential to provide fresh insight into cardiac diseases, such as heart failure. Thus, the next decade of mitochondrial research should be an exciting one indeed, and holds the potential for the development of new therapies for the patient with HF.

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Figure 1.

Image summarizes pathways contributing to mitochondrial damage and ROS production, and identifies actions of discussed therapeutic agents for heart failure. X - inhibition, ARB - angiotensin receptor blocker.

Table I
Animal models in which genetic manipulation of mitochondrial proteins may cause heart failure

Gene	Protein	Species	Genetic manipulation	Ref.
SCO2	Cytochrome c oxidase (COX)	mouse	A Sco2 knock-out (KO) allele and a Sco2 knock-in (KI) allele expressing an E-->K mutation at position 129 (E129K)	122
ATP8	ATP synthase 8 (complex V)	mouse	B6-mt(FVB) strain carries a point mutation of the mtDNA-coded Atp8 gene (ATP synthase)	123, 124
Hadhb	Mitochondria trifunctional protein (MTP) beta-subunits	Mouse	a nucleotide T-to-A transversion in exon 14 (c. 1210T>A) of beta-subunit (Hadhb)	125
SLC25A4	Adenine nucleotide translocator 1 (ANT1)	Mouse	'knockout' mice deficient in the heart/muscle isoform of the adenine nucleotide translocator (Ant1)	126
Sod2	Superoxide dismutases (SODs)	Mouse	A conditional Sod2 knockout mouse using a Cre-loxP system	127
Mfn1/Mfn2	Mitofusins (MFN 1 and MFN2)	Mouse	Conditional combined Mfn1/Mfn2 ablation	26
OPA1	Optic atrophy 1 (OPA1)	Mouse	ENU-induced mutant mouse carrying a protein-truncating nonsense mutation in opa1	56
Ndufs6	Mitochondrial complex I (CI) unit	Mouse	a mouse model by knockdown of the Ndufs6 gene using a gene-trap embryonic stem cell line	128
ABCB8	ATP-binding cassette (ABC) transporter	Mouse	Induced genetic deletion of ABCB8 in mouse hearts	129
Txnrd2	Mitochondrial thioredoxin reductase	Mouse	Mouse model with α -MHC-restricted Cre-mediated Txnrd2 deficiency	130
Taz	Mitochondrial transacylase tafazzin	Mouse	Tafazzin-deficient Mice with taz shRNA allele in ROSA26 Locus	131
Tfam	Mitochondrial transcription factor A	Mouse	A loxP-flanked Tfam allele (TfamloxP; 8) in combination with muscle creatinine kinase promoter	132
FXN	Friedreich Ataxia Protein (Frataxin)	Mouse	A striated muscle frataxin-deficient line and a neuron/ cardiac muscle frataxin-deficient line	133

Table II

Summary of current heart failure drugs and new therapeutics under development. Effect of individual drugs on mitochondria and heart failure overall in last column.

Drug	Action	Heart/Mitochondria Effects
ACE/ARB	RAS inhibition	HF improved survival and symptoms; General ROS decrease
Beta Blockers	Beta adrenergic inhibition	Improved survival; Overall decreased ROS
Spirolactone	Aldosterone inhibition	Decreased morbidity and mortality; May provide overall decrease in ROS
Metformin	Reduces hyperglycemia. Does not seem to cause hypoglycemia in non-diabetics	Improved survival; Increased LC3-II and autophagy; Increased AMPK activity; Increased ATP; Improved mitochondrial respiration
Experimental		
Drp1 Inhibition	Fission inhibitor	Decreased infarct size
miR-499	Inhibits calcineurin dependent Drp1 trafficking to mitochondria	Decreased infarct size
SS-31	Mitochondrial-targeted anti-oxidant peptide	Decreased infarct size; Increased ATP content; Ameliorates AngII-induced cardiomyopathy