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STATE-OF-THE-ART PAPER

Mitochondria as a Therapeutic Target in Heart Failure

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Heart failure is a pressing public health problem with no curative treatment currently available. The existing therapies provide symptomatic relief, but are unable to reverse molecular changes that occur in cardiomyocytes. The mechanisms of heart failure are complex and multiple, but mitochondrial dysfunction appears to be a critical factor in the development of this disease. Thus, it is important to focus research efforts on targeting mitochondrial dysfunction in the failing heart to revive the myocardium and its contractile function. This review highlights the 3 promising areas for the development of heart failure therapies, including mitochondrial biogenesis, mitochondrial oxidative stress, and mitochondrial iron handling. Moreover, the translational potential of compounds targeting these pathways is discussed. (J Am Coll Cardiol 2013;61:599–610) © 2013 by the American College of Cardiology Foundation

The 20th century has witnessed a dramatic improvement in patients' survival after adverse cardiovascular events. However, heart disease still remains the number one cause of death in the industrialized world, affecting >27 million people in the United States alone. With $40 billion in annual costs and 1 of every 5 patients dying within 1 year of diagnosis (1), HF has become a major public health problem. Although significant progress has been made in the outpatient management of chronic heart failure (HF), post-discharge mortality and rehospitalization rates within 60 to 90 days can be as high as 15% and 30%, respectively (2).

The primary goal of treating HF patients is restoration of cardiac function. Recent studies show that heart function can be successfully recovered in patients with HF, even after structural alterations have occurred. Thus, failing myocardium is "viable but dysfunctional" rather than irreversibly damaged, independent of the presence or absence of coronary artery disease. This finding opens up an avenue for rational design of treatments that target the cardiomyocyte itself, not the indirect pathways that suppress neurohormonal axis or induce vasodilation.

The mechanisms underlying the development of HF are multiple, complex, and not well understood. Although virtually all aspects of myocyte physiology are altered in HF, the past decade of research provided convincing evidence that mitochondrial dysfunction may be an important event in the development of hypertrophy and HF. First, genetic mutations that disrupt mitochondrial function are associated with cardiac dysfunction in mice (3) and humans (4,5). Second, currently available therapies for HF, such as angiotensin-converting enzyme inhibitors and angiotensin receptor II (ATII) blockers significantly improve survival in ischemic and nonischemic HF, and their administration also correlates with improved mitochondrial function (6,7). Finally, it is important to note that cardiomyocytes in the failing heart remain viable, although metabolically stunned, and their function can potentially be rescued (8). Thus, therapeutic efforts should venture beyond symptomatic relief and focus on reviving the dormant myocardium by targeting the underlying molecular defects in HF. In this review, we discuss the changes that occur in the mitochondria of failing myocardium, followed by an overview of the pertinent therapeutic targets and approaches that can potentially reverse these changes and preserve cardiac health. Of note, we refrain from discussing the changes in glucose and fatty acid utilization, as these topics have recently been reviewed in detail elsewhere (8). Instead, we focus on *mitochondrial biogenesis*, production of *reactive oxygen species*

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Abbreviations and Acronyms

AMPK = adenosine monophosphate kinase

ATII = angiotensin receptor II

ATP = adenosine triphosphate

cGMP = cyclic guanosine monophosphate

DFP = deferiprone

eNOS = endothelial nitric oxide synthase

Fe/S = iron/sulfur

FRDA = Friedrich's ataxia

HF = heart failure

I/R = ischemia/reperfusion

MI = myocardial infarction

MnSOD = manganese superoxide dismutase

mtDNA = mitochondrial DNA

NO = nitric oxide

Nox = nicotinamide adenine dinucleotide phosphate (reduced form) oxidase

NRF = nuclear respiratory factor

PDE = phosphodiesterase

PDE5I = phosphodiesterase type 5 inhibitor

PGC1α = peroxisome proliferator–activated receptor gamma coactivator 1α

ROS = reactive oxygen species

SOD = superoxide dismutase

SS = Szeto-Schiller

Tfam = transcription factor A

TPP = triphenylphosphonium

(ROS) and maintenance of cellular *iron homeostasis* as promising novel therapies for HF.

Mitochondrial Biogenesis

Pathophysiology. One of the ways to augment energy production in the setting of increased contractile demand is to stimulate production of new mitochondria, termed mitochondrial biogenesis. Mitochondria contain ~16.5 kilobase of circular double-stranded DNA that encodes 13 protein components of the electron transport chain and needs to be replicated before the division. In addition, as many as 1000 nuclear-encoded proteins must be imported into the newly formed mitochondria to make a fully functional organelle (9). Thus, generation of new mitochondria requires a coordinated transcription of mitochondrial and nuclear genomes orchestrated by peroxisome proliferator–activated receptor gamma coactivator 1α (PGC1α) (10). PGC1α, a nuclear-encoded protein, is induced in the states of enhanced energy demand, such as increased cardiac workload, high adenosine diphosphate/adenosine triphosphate (ATP) ratio, cold, exercise, and fasting (for review, see references 11 and 12). High PGC1α activity is associated with increased mitochondrial content, as exemplified by cardiac-specific PGC1α transgenic mice, which exhibit uncontrolled mitochondrial proliferation and increase in markers of mitochondrial biogenesis (13,14). PGC1α stimulates mitochondrial proliferation through its interaction with several transcription factors. First, PGC1α binds to and coactivates nuclear respiratory factors (NRFs) 1 and 2, which in turn promote transcription of nuclear-encoded genes targeted to mitochondria (15). Second, PGC1α activates estrogen-related nuclear orphan receptors ERRα and γ, which induce expression of genes involved in glucose and fatty acid uptake, energy production, and ATP transport (16,17). Finally, PGC1α promotes replication of mitochondrial genome through NRF1/2-mediated induction of mitochondrial transcription factor A (Tfam) (12). Cardiac-specific deletion of NRF1 (18), ERRα (19), and Tfam (20) are all associated with decreased mitochondrial content or function, confirming their role in mitochondrial biogenesis.

Studies of rodents (21–23), dogs (24), and humans (25) suggest that disruption of mitochondrial biogenesis represents an early event in the pathophysiology of HF, the timely reversal of which is cardioprotective. Grossly, mitochondrial content and mitochondrial DNA (mtDNA) copy number are significantly reduced in rodent and human failing myocardium, and downregulation of the PGC1α pathway has been observed in various models of HF in mice and rats (21,22,26,27). However, the role of PGC1α in human HF remains controversial, and contradictory results have also been reported (28–30). Because PGC1α is extensively regulated on the post-translational level by phosphorylation (31), acetylation (32), and protein stabilization (33), it is not clear whether PGC1α activity is reduced in the failing hearts and whether the reduction in mitochondrial number in HF in humans is due to deregulation of PGC1α signaling.

A defect in mtDNA replication was proposed as an alternative mechanism for the reduction in mitochondrial biogenesis (30,34). Importantly, changes in mtDNA replication machinery represented a very early event detected in hypertrophied hearts that have not yet transitioned to failure (30). The actual trigger for reducing mtDNA replication in a setting of increased workload is unknown, and it would be of interest to replicate these studies in animal models and/or HF patients.

Therapeutic strategies. Despite the controversy about the role of PGC1α in human HF, boosting mitochondrial biogenesis in failing myocardium appears to be beneficial (35). In fact, the angiotensin-converting enzyme inhibitor captopril was shown to increase mitochondrial content in the hearts of dogs after coronary ligation (36), suggesting that some of its beneficial effects may be due to the stimulation of mitochondrial biogenesis. Although currently no drugs that specifically target mitochondrial biogenesis in HF are available, acceleration of this process through adenosine monophosphate–activated kinase (AMPK), endothelial nitric oxide synthase (eNOS), and other pathways may represent a promising therapeutic approach (Fig. 1).

ADENOSINE MONOPHOSPHATE–ACTIVATED KINASE. AMPK exhibits very low baseline activity in the heart, but is upregulated in response to a variety of stressors (37,38). Importantly, activation of AMPK is thought to be the mechanism driving the increase in mitochondrial biogenesis in exercise-induced adaptive hypertrophy of the athlete's heart (39), which, unlike the pathological hypertrophy discussed earlier, does not lead to HF. AMPK induces PGC1α function (39,40), activates NRF1, Tfam (41), and ERRα (28), establishing this kinase as an important modulator of mitochondrial biogenesis.

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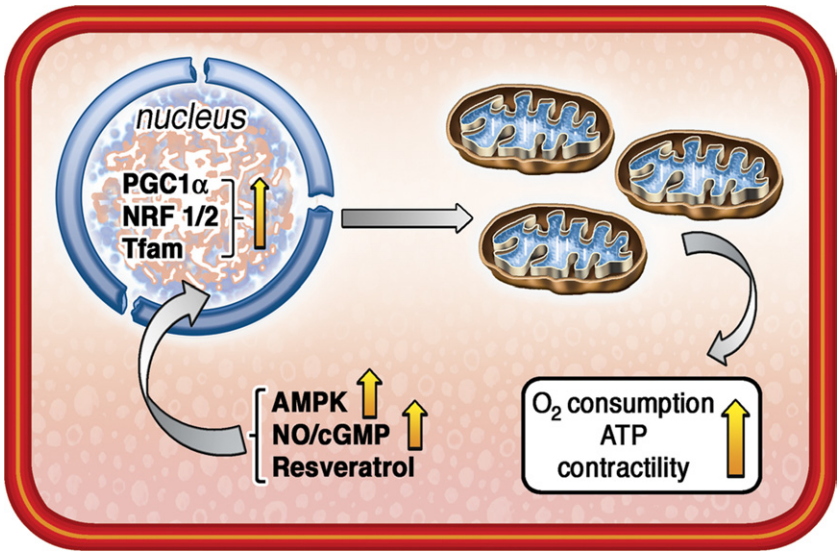

Figure 1 **Mitochondrial Biogenesis**

Mitochondrial biogenesis impairment is an early event in the development of heart failure, and reversal of this process is cardioprotective. Mitochondrial biogenesis can be enhanced therapeutically with the use of adenosine monophosphate kinase (AMPK) agonists, stimulants of nitric oxide/cyclic guanosine monophosphate (NO/cGMP) pathway (including phosphodiesteraes type 5 inhibitors), or resveratrol. All of these approaches stimulate nuclear-encoded proteins peroxisome proliferator–activated receptor gamma coactivator 1α (PGC1α), nuclear respiratory factor (NRF)1/2, and transcription factor A (Tfam), which, in turn, facilitate production of new mitochondria in the heart. ATP = adenosine triphosphate.

The activity of AMPK is increased in failing hearts (42); however, pharmacological activation of this pathway appears to exert additional cardiac protection. For example, metformin, a commonly used antidiabetic drug that activates AMPK signaling, reduced infarct size and preserved cardiac function in a long-term post-myocardial infarction (MI) rat model in the absence of diabetes (43) and exhibited cardioprotective properties in a rapid ventricular pacing canine model (44). Gundewar et al. (45) found increased PGC1α levels and preservation of heart function in wild-type metformin-treated mice subjected to MI or ischemia/reperfusion (I/R), but not in a AMPK knockout mouse model (45). Although metformin was also shown to reduce gluconeogenesis through direct inhibition of mitochondrial complex I, the total cellular ATP content in the livers of metformin-treated rats remained unchanged, consistent with the maintenance of the cumulative mitochondrial bioenergetic capacity possibly due to increased biogenesis (46).

Accumulating evidence of the cardioprotective properties of AMPK in the setting of hypertrophy and cardiac failure calls for the development of pharmacological agonists of AMPK in the heart. Several clinically available compounds have been shown to activate AMPK, including metformin, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranosyl 5′-monophosphate (AICAR), thiazolidinediones, and statins. Moreover, the ATII receptor blocker telmisartan was shown to increase phosphorylated AMPKα levels in cultured myotybes (7), suggesting that the beneficial effects of this drug may partially be due to stimulation of mitochondrial biogenesis. However, the effect of current HF therapies on the AMPK pathway is likely indirect via an increase in the adenosine monophosphate/ATP ratio, inhibition of mitochondrial respiration, or other cellular and systemic effects (47). Compounds targeting AMPK itself are currently in development. Of note, compound A769662 by Abbott Laboratories (Abbott Park, Illinois) is a specific allosteric activator of AMPK complexes on the β subunit (48). A769662 reduced infarct size in rats fed low-fat and high-fat diets (49), providing proof-of-principle that a direct activation of AMPK is beneficial to the heart. Unfortunately, the compound also inhibited 26S proteasome and caused cell-cycle arrest through an AMPK-independent mechanism, limiting its clinical prospects (50). Another small molecule, PT1, appears to activate AMPKα1 and AMPKα2 isoforms by removing autoinhibition in the catalytic subunits of the kinase (51). Thus, PT1 potentially targets a vast array of AMPK complexes, although its effects on the heart remain to be studied.

Several points must be considered when developing an AMPK agonist for the treatment of HF. First, AMPK activation may only be suitable for treatment of HF due to defined etiologies. Although AMPK activation was protective in the mouse models of MI and I/R, it failed to preserve cardiac function in rats with chronic volume overload (52). Second, AMPK is ubiquitously expressed and regulates an array of processes, and the subunit composition of AMPK heterodimeric complex differs widely among tissues (53). Thus, to maximize clinical benefits and minimize toxicity,

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specific isoforms of AMPK must be targeted. This principle is exemplified by a finding that a gain-of-function mutation in the AMPKγ2 subunit induces glycogen accumulation and progressive hypertrophic cardiomyopathy in humans (54). Studies examining expression patterns and functions of different AMPK isoforms will lay a foundation for the development of novel therapeutic agonists for the treatment of HF.

ENOS/NITRIC OXIDE/CYCLIC GUANOSINE MONOPHOSPHATE PATHWAY. Nitric oxide (NO) is a diffusible signaling molecule released by endothelial cells through the action of eNOS, which enhances production of cyclic guanosine monophosphate (cGMP) and leads to smooth muscle relaxation. This enzyme is also expressed in the heart where NO and cGMP activate many potentially cardioprotective pathways (55,56). Recent evidence suggests that the eNOS/NO/cGMP pathway is an important activator of mitochondrial biogenesis (57,58). Mice transgenic for cGMP-dependent protein kinase displayed a significant increase in mitochondrial content, size, and upregulation of PGC1α, NRF1, and Tfam in skeletal muscle (59). Treatment of primary brown adipocytes with NO donor also induced mitochondrial biogenesis through cGMP and PGC1α pathways and an increase in mean mitochondrial volume density (60). Activation of the eNOS cascade also increased mitochondrial content in several established cell lines (61), whereas mtDNA and mitochondrial size were reduced in the heart, liver, brain, kidney, and skeletal muscle of mice with global eNOS knockout (61). The mechanism by which the eNOS/NO/cGMP pathway induces mitochondrial biogenesis is unknown, although increase in calcium currents and interaction with AMPK may play a role.

The eNOS/NO/cGMP pathway can be modulated pharmacologically through inhibition of phosphodiesterases (PDEs), which catalyze degradation of cGMP. PDE type 5 inhibitors (PDE5Is) were originally developed as a therapy for cardiac angina, but are currently used in the treatment of erectile dysfunction and pulmonary hypertension. Importantly, PDE5Is stimulate mitochondrial biogenesis through upregulation of PGC1α and a subsequent increase in mtDNA content (62). Compelling evidence exists that PDE5Is can delay the progression of HF and reverse cardiac remodeling in animal models and humans. Several small clinical trials found improvement in hemodynamic and overall clinical measures in patients with congestive HF (for review, see reference 63), although larger samples sizes are needed to confirm the effect. A literature review from 1980 through 2011 found an improvement in cardiac index, ejection fraction, and other markers of heart function in patients with the New York Heart Association functional class II or III HF treated with PDE5Is (64).

The role of PDE5I in the maintenance of mitochondrial mass and function in failing hearts warrants further investigation because the connection between eNOS/NO/cGMP pathway and mitochondrial biogenesis mostly comes from studying noncardiac tissues and cell lines. Moreover, other PDE isoforms must be examined in relation to mitochondrial biogenesis specifically in the heart. In addition to inhibition of PDE, NO/cGMP synthesis may be activated directly through maintenance of high eNOS activity, especially during increased cardiac workload. Hemodynamic stress is known to uncouple eNOS, leading to its loss of activity and increased generation of ROS (65). BH4 (tetrahydrobiopterin) supplementation can prevent eNOS uncoupling and was found to reduce left ventricular hypertrophy, cardiac dysfunction, and fibrosis in mice with heart disease due to pressure overload (65,66). Importantly, folic acid is known to replenish reduced BH4 and has been shown to protect the heart through increased eNOS activity. Both folate deficiency and inhibition of BH4 synthesis were associated with reduced mitochondrial number and function (67,68), whereas folate administration to rats subjected to I/R preserved cardiac function (69). Finally, the potential damaging effects of NO should be considered, as this molecule was found to inhibit mitochondrial energy production through reversible binding to cytochrome c and displacement of oxygen, which enhanced production of ROS and reactive nitrogen species (70). NO was also shown to activate mitochondrial permeability transition pore opening and to induce apoptosis (71).

RESVERATROL. Resveratrol, a polyphenol compound responsible for the cardioprotective properties of red wine, was recently identified as a potent stimulator of mitochondrial biogenesis (72). Resveratrol activated both eNOS (73) and AMPK (74,75), and enhanced mitochondrial biogenesis through upregulation of PGC1α, NRFs, and Tfam (76). Treatment of mice with resveratrol increased mitochondrial size and density, mtDNA content, mitochondrial enzyme activity, and oxidative capacity in skeletal muscle. Functionally, these changes were associated with improved motor function and a reduction in resting heart rate (72).

The beneficial effects of resveratrol in the heart are well documented. Two groups have independently shown that resveratrol treatment prevents cardiac dysfunction in hypertensive rats without reduction in blood pressure (75,77), indicating the direct influence of this compound on the heart. Importantly, mitochondrial mass, biogenesis, and function were preserved by resveratrol in salt-sensitive hypertensive rats (77) and rats transgenic for human renin and angiotensin genes (76). A small human clinical trial found significant improvement in diastolic heart function in 40 MI patients receiving 10 mg resveratrol daily during the 3-month trial period. In addition, an improvement in endothelial function and reduction in low-density lipoprotein levels were noted in the treatment group, but mitochondrial biogenesis and function were not assessed (78). Important to note, however, is that resveratrol treatment was found to be ineffective in reversing cardiac hypertrophy induced by volume overload in rats with an aortocaval shunt

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(79). Thus, the beneficial effects of this compound may be limited to select clinical scenarios, such as hypertensive and post-MI patients.

No significant toxicity of this compound was noted in healthy human volunteers in phase I study during the 4-week trial period (80). Unfortunately, resveratrol has a short half-life of 8 to 14 min and is extensively metabolized in the body (81). Thus, an effective human dose cannot be easily extrapolated from animal studies. Development of more potent analogs with longer half-lives may help to overcome these limitations.

OTHER STRATEGIES. The cardioprotective effects of estrogen are well documented in various animal models. Moreover, epidemiological studies reveal a reduced risk of cardiovascular disease in premenopausal, but not post-menopausal, women compared with men. Estrogen-like compounds were shown to stimulate mitochondrial biogenesis through induction of NRF1 expression and increase in mtDNA content (82). Unfortunately, estrogen replacement therapy not only failed to reduce, but actually increased, the number of cardiac events in post-menopausal women (83). The likely reasons for that are discussed by Mendelsohn et al. (84). Thus, although the estrogen pathway represents a promising therapeutic target, more research is needed to understand its risks, benefits, and target patient population.

The approaches discussed here are aimed at the production of new mitochondria via induction of PGC1α, NRFs, and Tfam signaling. However, it is also important to note that mitochondria are dynamic organelles that constantly undergo fusion and fission. Although the significance of these processes in HF is not well understood, both fusion and fission are essential for maintenance of mitochondrial function (for review, see references 85 and 86). Constant exchange of mitochondrial metabolites, proteins, DNA, and Ca^{2+} throughout the mitochondrial network by fusion/fission may protect these organelles in the setting of an insult. On the other hand, selective elimination of damaged mitochondria by the process of autophagy is also critical in cardiac physiology, as disruption of autophagy in a pressure-overloaded heart facilitates its transition into HF. Thus, mitochondrial biogenesis should be re-examined and targeted in a broader context of preserving mitochondrial number, network organization, size, and quality.

Mitochondrial Oxidative Stress

Pathophysiology. Generation of ROS is significantly enhanced in the failing myocardium, as has been unequivocally shown by studies of animal models and human patients (for review, see references 87 and 88). 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 (89), although the view of mitochondria as a major source of intracellular ROS has been challenged (90). The activities of mitochondrial electron transport chain complexes are suppressed in HF, and disruption of mitochondrial bioenergetic function was found to increase ROS and oxidative DNA damage (91), providing a possible pathophysiological link between mitochondrial dysfunction and ROS (88,92).

Nicotinamide adenine dinucleotide phosphate (reduced form) oxidase (Nox) is another important source of ROS in the heart. Five isoforms of Nox have been described, with Nox4 being the most abundant in cardiomyocytes, endothelial cells, and fibroblasts. Nox4 localizes primarily to the mitochondria, does not require cytosolic subunits for its activation, and is implicated in enhanced ROS production in pressure overload and aging models of HF (93–95). Nox activity is induced by pathways that are also active in dysfunctional myocardium, including ATII stimulation, tumor necrosis factor-α, and mechanical stretch (88). Nox activity is high in human failing hearts (96), whereas genetic deletion of this enzyme protects against cardiac dysfunction and remodeling in the MI mouse model (97).

The exact contribution of ROS to the development of HF is complex and remains a subject of intense debate. One likely mechanism is physical damage of cellular and mitochondrial structures, such as sarcomeric and excitation-contraction coupling proteins, which would impair the mechanical properties of the heart (98). Because the majority of ROS in HF comes from mitochondria, these organelles are the primary target of oxidative damage. mtDNA is particularly sensitive to ROS due to the lack of protective histones and less efficient DNA repair, and mutations in mtDNA-encoded genes are known to cause cardiomyopathy. Moreover, reduction of PGC1α in failing hearts can exacerbate oxidative stress and mitochondrial damage, as this protein was found to maintain mitochondrial, but not cytosolic, antioxidant defenses (99). In addition to damaging cellular components, ROS regulate several signaling cascades, including the known hypertrophic pathways such as protein kinase C, mitogen-activated protein kinase, Jun N-terminal kinase, and Ras (100). Finally, ROS facilitate the remodeling of the extracellular matrix by inducing matrix metalloproteinases through direct post-translational modifications or indirectly through the nuclear factor κB pathway (101). Given that ROS affect virtually all aspects of cardiomyocyte physiology, they represent an important therapeutic target for treating HF. In support of this claim, cardioprotective therapies such as angiotensin-converting enzyme inhibitors and ATII receptor blockers were shown to possess antioxidant properties, although it is not known whether they target mitochondrial ROS directly or indirectly (102,103).

Therapeutic strategies. Several trials have assessed the efficacy of antioxidants in the treatment of HF, but the results were disappointing. Long-term supplementation with α-tocopherol (vitamin E) was actually associated with an increased risk of the development of HF (104). Evidence from animal studies suggests that preferential inhibition of ROS *inside* the mitochondria, rather than global antioxidant treatment, may be cardioprotective. Overexpression of the mitochondria-specific antioxidant peroxiredoxin-3 pro-

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tected the heart against failure and remodeling in the mouse model of MI (105). Similarly, overexpression of mitochondria-targeted catalase attenuated hypertrophy in pressure-overload (106) and hypertensive (107) mouse models. Thus, scavenging ROS within the mitochondria may protect the heart against the development of HF and make it more resistant to stressful stimuli. Several approaches for targeting antioxidant compounds to the mitochondria are being explored and hold promise (Fig. 2).

MITOQ. The best characterized mitochondria-targeted antioxidant to date is MitoQ, a quinol ROS scavenging moiety linked to triphenylphosphonium (TPP), a lipophilic compound that easily crosses membranes and accumulates in the mitochondrial matrix as a function of membrane potential. Scavenging of ROS is achieved through oxidation of MitoQ into its quinone form, which is then recycled back into the active quinol by the action of mitochondrial complex II (108). MitoQ is bioavailable orally with no toxicity detected when administered to mice at an ~20-mg/kg dose. Tracer studies found the compound to be rapidly taken up into the heart, liver, brain, kidney, and muscle, with highest accumulation in the heart and liver (109). Long-term administration of MitoQ had no effect on plasma glucose, insulin, free fatty acid, or cholesterol levels, but was associated with significantly reduced triglycerides. Affymetrix chip analysis of the heart and liver tissue of mice receiving MitoQ revealed no significant differences in gene expression profile between the treatment and control groups (110). Thus, MitoQ is a safe, orally available small molecule that does not significantly alter baseline physiology.

In WT mice, MitoQ does not lead to a significant reduction in oxidative stress at baseline. However, administration of MitoQ to rats for 2 weeks reduced oxidative stress and protected the heart against I/R injury using an ex vivo Langendorff setup. These effects were specifically due to the inhibition of ROS inside the mitochondria, as no protection was observed in control groups receiving either methyl-TPP, which can enter the mitochondria but does not scavenge ROS, or short-chain antioxidant quinol, which is impermeable to the mitochondrial membrane (111). These findings were later confirmed in a mouse model of I/R and in an established cardiac cell line (112). MitoQ also preserved cardiac function in a spontaneously hypertensive rat model of HF. However, this favorable outcome may also be attributed to the reduction in blood pressure and improvement in endothelial function observed in the MitoQ group (113). Finally, MitoQ was found to be protective in other models of mitochondrial oxidative stress, including cardiac damage by doxorubicin (114), liver damage by lipopolysaccharide (115), and protection of substantia nigra from 1-methyl-4-phenyl-1,2,3,6-tetrahydropryridine (mitochondrial permeability transition pore) toxicity (116).

Two human trials assessed MitoQ's efficacy in the treatment of Parkinson's disease (PROTECT) and in patients with chronic hepatitis C infection. Although the results of the PROTECT trial were negative, it provided a wealth of data on the safety of the drug administered orally for as long

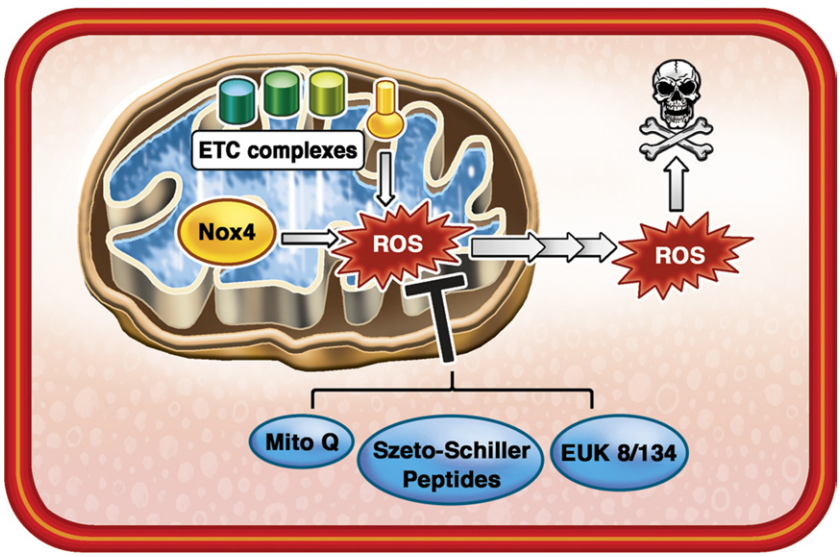

Figure 2 **Targeting Mitochondrial ROS Production**

Mitochondrial electron transport chain (ETC) complexes and nicotinamide adenine dinucleotide phosphate (reduced form) oxidase 4 (Nox4) enzyme generate excessive amounts of reactive oxygen species (ROS) in failing hearts. Moreover, mitochondria are very sensitive to oxidative stress, and their function is severely impaired in heart failure (HF). Although nonspecific antioxidants, such as vitamin E, show no benefit in HF, targeting of ROS-scavenging molecules to mitochondria is protective. Various approaches to targeting antioxidant compounds to mitochondria, including triphenylphosphonium conjugation (MitoQ), Szeto-Schiller peptides, and synthesis of novel manganese superoxide dismutase/catalase mimetics, should be explored in the development of HF treatments.

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as 1 year (117). On the other hand, the patients receiving 40 and 80 mg MitoQ in the chronic hepatitis C trial showed significant improvement in hepatic function (118). Importantly, no severe side effects of the MitoQ regimen were reported in either of the trials.

Despite the significant therapeutic potential of MitoQ and other TPP-conjugated antioxidants, there are limitations. The uptake of these compounds is governed by the mitochondrial membrane potential, which may be severely disrupted in failing hearts. Moreover, accumulation of cationic TPP in the matrix can potentially depolarize mitochondria, leading to unwanted side effects.

SZETO-SCHILLER PEPTIDES. Unlike TPP conjugates, the small (<10 amino acids) Szeto-Schiller (SS) peptides selectively accumulate in the mitochondrial matrix independent of membrane potential. SS peptides are rapidly taken up by the mitochondria, with 1,000- to 5,000-fold accumulation in this organelle compared with the cytosolic compartment (119). Multiple variants of SS molecules have been synthesized to date, and the tyrosine-containing SS-02 and SS-31 peptides hold therapeutic promise due to their antioxidant properties (120). These products reduced mitochondrial ROS production in cells treated with mitochondrial complex I (121) and II and III (119) inhibitors. SS compounds were also shown to be protective in vivo.

Administration of SS-31 before ischemia and before reperfusion reduced MI size, lipid peroxidation indexes, and increased ATP content in the rat heart (122). Recently, SS-31, but not the nontargeted antioxidant *N*-acetylcysteine, was shown to protect the heart from cardiomyopathy due to angiotensin II administration or Gαq overexpression in mice (123), providing the first evidence of SS-31 effectiveness in a more chronic model of cardiac dysfunction.

Although peptides are typically considered poor candidates for drug development due to the issues of solubility, stability, rapid clearance, and inability to cross cellular membranes, studies have revealed excellent pharmacokinetic properties of the SS peptides. These molecules are water soluble due to the 3+ net charge and are stable in an aqueous solution at 37°C for 6 months. They can be delivered via the intravenous, intraperitoneal, or subcutaneous route and are rapidly distributed to highly perfused organs, including the heart, kidney, lung, and brain (120). Moreover, enzymatic degradation of the SS peptides is low, and they remain stable even after 2 h of incubation in whole blood (124). Finally, the toxicity of SS compounds is low at therapeutic doses, with no side effects observed after 5 months of daily treatments in mice (125). Given these favorable pharmacokinetic properties, SS peptides appear to be good candidates for further testing in the treatment of HF. However, additional animal and human studies are required to validate these compounds as therapeutic candidates.

MANGANESE SUPEROXIDE DISMUTASE/CATALASE MIMETICS. Superoxide dismutases (SODs) are metal-containing antioxidant enzymes that catalyze the conversion of superoxide radical to hydrogen peroxide and O_2. The mitochondria-specific manganese SOD (MnSOD or SOD2) is located in the matrix, and its overexpression was shown to protect against HF (126). Several inorganic MnSOD mimetics have been synthesized, and many of these compounds exert protection in conditions associated with oxidative stress (for s review, see reference 127). Salen derivatives, such as EUK-8 and EUK-134, possess antioxidant properties of both MnSOD and catalases and appear to be effective in the heart. Although no studies have assessed the ability of these molecules to penetrate mitochondria directly, their chemical properties (small, lipophilic, water soluble) and documented ability to reduce mitochondrial ROS and maintain activities of mitochondrial enzymes (128) support this assumption.

Both EUK-8 and EUK-134 were found to protect mitochondria and the heart against various oxidative insults. In an early study by Pucheu et al. (129), EUK-8 protected iron-overloaded rat hearts from I/R injury, maintained left ventricular diastolic pressure, and preserved mitochondrial integrity. EUK-8 treatment also prevented the development of cardiomyopathy and maintained contractility and ATP content in the heart/muscle-specific MnSOD2 knockout mice, in which HF develops as early as 4 weeks after birth. Importantly, ROS generation by isolated mitochondria was significantly reduced by EUK-8 in these mice, suggesting the ability of this molecule to offset mitochondrial oxidative stress (130). Finally, EUK-8 ameliorated pressure overload-induced cardiac dysfunction in wild-type mice and mice with the deletion of mitochondrial antioxidant, apoptosis-inducing factor (131). EUK-134, a more lipophilic derivative of EUK-8, exhibited similar protective properties in pulmonary arterial hypertension–induced HF (132) and I/R (133) and reduced apoptosis in norepinephrine-stimulated isolated adult rat ventricular myocytes (134).

New SOD/catalase mimetics are being developed and studied, thus opening up an exciting new therapeutic direction. However, most evidence of the mitoprotective and cardioprotective properties of these molecules come from genetic models of increased mitochondrial oxidative stress. The mechanism of cardiac protection conferred by EUK-8/EUK-134 and related compounds must be thoroughly investigated. In particular, cardioprotective properties of these molecules may be independent of their antioxidant effects, as EUK-8 was reported to possess significant vasodilatory properties, which may increase oxygen and nutrient delivery to the heart and indirectly improve heart function (135).

Other strategies. As our understanding of the chemistry behind mitochondrial targeting is increasing, rational design of novel therapies holds promise. A number of antioxidant moieties have been conjugated to TPP and appear to confer protection as well as control the degree of antioxidation and

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the duration of the effect (108). In addition to synthesizing new molecules, it is important to understand various signaling pathways that regulate mitochondrial antioxidant defenses. A recent report by Lu et al. (99) found antioxidant enzyme levels to be significantly decreased in PGC1α knockout mice with pressure-overload hypertrophy, suggesting that enhancement of mitochondrial biogenesis and PGC1α expression by the strategies discussed earlier may have a positive effect on an antioxidant profile as well.

Mitochondrial Iron Homeostasis

Pathophysiology. Although the role of mitochondrial iron in HF has not been explicitly studied, indirect evidence points toward potential therapeutic implications of altering mitochondrial iron homeostasis in the diseased heart. Iron is essential for maintenance of cellular viability and function through its role in oxidative phosphorylation, antioxidant enzyme activities, ribosome biogenesis, oxygen storage and delivery, and more (136). Mitochondria are the key sites of cellular iron processing where synthesis of iron/sulfur (Fe/S) clusters and heme takes place, but are also the place where ROS are generated (137). Being a reactive metal, free iron catalyzes production of highly toxic hydroxyl radicals from less reactive species such as hydrogen peroxide and superoxide anion via the Fenton reaction (138).

Although many studies examined changes in systemic iron homeostasis in animals and humans with HF, very little work has been done to characterize the intrinsic defects in iron regulatory pathways of failing cardiomyocytes. In particular, mitochondrial iron regulation remains understudied. Several lines of evidence suggest that accumulation of iron in mitochondria can cause or exacerbate cardiomyopathy. The best documented example is cardiomyopathy of Friedreich's ataxia (FRDA), a human genetic disease caused by GAA triplet expansion in the frataxin gene. Although the precise function of frataxin is unknown, it likely plays a role in the regulation of mitochondrial iron homeostasis and Fe/S cluster synthesis. In FRDA patients, progressive cardiomyopathy develops that is characterized by extensive mitochondrial dysfunction and oxidative damage (139). Moreover, frataxin deficiency was associated with significant accumulation of iron inside the mitochondria both in patients (140) and in mouse models of the disease (141,142), whereas no difference was detected in the total iron content in FRDA hearts (140). Treatment of patients with a combination of the mitochondria-permeable iron chelator deferiprone and an antioxidant partially reversed the cardiac phenotype observed in FRDA (143), supporting the role of mitochondrial iron in the pathophysiology of cardiac dysfunction. Another example of a cardiomyopathy resulting from preferential iron accumulation in the mitochondria was reported by our group. We showed that deletion of mitochondrial ATP binding cassette transporter B8 reduces iron export from this organelle and leads to mitochondrial iron overload. Mice with inducible knockout of ATP binding cassette transporter B8 in the heart displayed progressive systolic and diastolic dysfunction 8 weeks after gene deletion, which was associated with an increase in oxidative stress, severe disruption of mito-

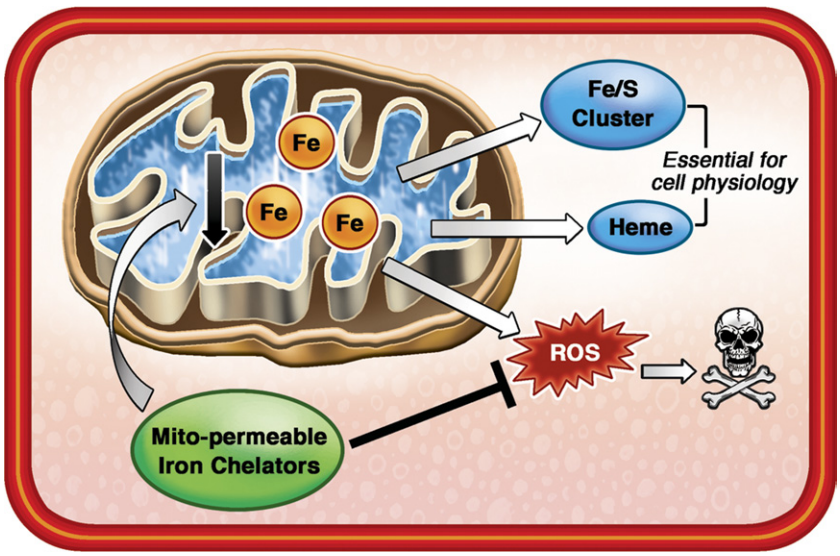

Figure 3 Mitochondrial Iron as a Promising Therapeutic Target

Functional and structural damage to the mitochondria is a prominent feature of heart failure. In addition to generating adenosine triphosphate, mitochondria play a key role in the regulation of cellular iron balance through the synthesis of heme and iron sulfur clusters. However, accumulation of iron in the mitochondria can catalyze generation of reactive oxygen species (ROS) and exacerbate damage. Reducing mitochondrial iron through development of mitochondria-permeable iron chelators can potentially protect failing hearts. Fe/S = iron/sulfur.

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chondrial architecture, and the presence of mitochondrial iron aggregates (144).

Whether iron accumulates in the mitochondria of failing hearts due to common etiologies such as hypertension and MI has not been examined directly. One study used electron paramagnetic resonance spectroscopy to analyze the iron status of the failing hearts in the mouse model of cardiac-specific overexpression of Gαq. Significant accumulation of iron inside cardiomyocytes was noted, although the mitochondria-specific iron pool has not been examined. However, activities of mitochondrial Fe/S cluster proteins were reduced in failing hearts, and the defect in complex III activity was specifically attributed to the lack of a Fe/S cluster center (145). These findings suggest deregulation of mitochondrial iron processing in at least 1 model of chronic HF. Thus, the defects in mitochondrial iron handling may contribute to the development of HF through an ROS-dependent mechanism and through potential disruption of Fe/S cluster biogenesis.

Therapeutic strategies. Extensive characterization of mitochondrial iron homeostasis in various models of HF must be performed to target these pathways in the translational studies. Reducing mitochondrial iron may exert cardioprotection through inhibition of hydroxyl radical formation and alleviation of oxidative stress (Fig. 3). The therapy must be precisely targeted to the mitochondria, as iron homeostasis is often disrupted in HF patients and global iron deficiency is common (146). Instead of chelating iron on a systemic level and exacerbating iron deficiency, the drug must remove free iron from the mitochondria and donate it to other cellular compartments. Such redistribution of iron within a cell was reported for deferiprone (DFP), an orally available reverse siderophore iron chelator. DFP was shown to enter the cells, reduce mitochondrial free iron levels, exit cells as an iron chelate, and transfer chelated iron to apotransferrin in the blood, thus potentially increasing systemic iron availability (147). Oral administration of DFP to FRDA patients for 6 months significantly improved neurological symptoms and reduced iron accumulation in cerebellar dentate nuclei, with no hematological side effects noted (148). Moreover, DFP and idebenone treatment led to a partial reversal of FRDA cardiomyopathy in human patients (143). In addition to DFP, analogs of hydrophobic iron chelator pyridoxal isonicotinoyl hydrazone, such as 2-pyridylcarboxaldehyde isonicotinoyl hydrazone were shown to selectively remove radioactive isotopes of iron from the mitochondria of rabbit reticulocytes (149) and were proposed as a potential therapy for FRDA and other diseases associated with mitochondrial iron overload (150). The effects of 2-pyridylcarboxaldehyde isonicotinoyl hydrazone on systemic iron homeostasis have not yet been examined.

Conclusions

Mitochondria are taking the center stage in our search for novel cardioprotective therapies, as their dysfunction appears early and invariably in the development of hypertrophy and HF. Maintenance of mitochondrial biogenesis against cardiac insults and reduction in mitochondrial ROS production are the 2 promising directions that may soon yield effective treatments. Moreover, exploration and targeting of other vital mitochondrial processes in HF, including regulation of iron homeostasis, should be actively pursued. Importantly, our basic research and translational efforts should focus on targeting the intrinsic processes of viable, but dysfunctional, cardiomyocytes.

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