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Oxidative Stress and Mitochondrial DNA Damage in Heart Failure

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Recent experimental and clinical studies have suggested that oxidative stress is enhanced in heart failure. The production of oxygen radicals is increased in the failing heart while antioxidant enzyme activities are preserved. Mitochondrial electron transport is an enzymatic source of oxygen radical generation and also a target against oxidant-induced damage in the failing myocardium. Chronic increases in oxygen radical production in the mitochondria can lead to a catastrophic cycle of mitochondrial DNA (mtDNA) damage, as well as functional decline, further oxygen radical generation, and cellular injury. Reactive oxygen species induce myocyte hypertrophy, apoptosis, and interstitial fibrosis by activating matrix metalloproteinases. These cellular events play an important role in the development and progression of maladaptive cardiac remodeling and failure. Therefore, oxidative stress and mtDNA damage are good therapeutic targets. Overexpression of peroxiredoxin-3 (Prx-3), mitochondrial antioxidant, or mitochondrial transcription factor A (TFAM) could ameliorate the decline in mtDNA copy number in failing hearts. Consistent with alterations in mtDNA, the decrease in oxidative capacities is also prevented. Therefore, the activation of Prx-3 or TFAM expression could ameliorate the pathophysiological processes seen in myocardial failure. Inhibition of oxidative stress and mtDNA damage could be novel and potentially effective treatment strategies for heart failure. (*Circ J* 2008; **Suppl A:** A-31 – A-37)

Key Words: DNA; Heart failure; Mitochondria; Oxidative stress; Remodeling

Heart failure is a leading cause of morbidity and mortality in industrialized countries[1] It is also a growing public health problem, mainly because of aging of the population and the increase in the prevalence of heart failure in the elderly. Previous basic, clinical, and population sciences have advanced the modern treatment of heart failure, but despite extensive studies, the fundamental mechanisms responsible for the development and progression of left ventricular (LV) failure have not yet been fully elucidated.

Reactive oxygen species (ROS), such as superoxide anions ($\cdot O_2^-$) and hydroxy radicals ($\cdot OH$), cause the oxidation of membrane phospholipids, proteins, and DNA[2] and have been implicated in a wide range of pathological conditions including ischemia–reperfusion injury[3] neurodegenerative diseases[4] and aging[5] Under physiological conditions, their toxic effects can be prevented by such scavenging enzymes as superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase, as well as by other non-enzymatic antioxidants. However, when the production of ROS becomes excessive, oxidative stress might have a harmful effect on the functional and structural integrity of biological tissue.

ROS cause contractile failure and structural damage in the myocardium. The importance of oxidative stress is increasingly emerging, with respect to a pathophysiological mechanism of the LV remodeling responsible for heart failure progression.

Direct Evidence of Oxidative Stress in Heart Failure

Recent experimental and clinical studies have suggested that the generation of ROS increases in heart failure[6–9] Lipid peroxides and 8-iso-prostaglandin F2α, which are the major biochemical markers of ROS generation, have been shown to be elevated in the plasma and pericardial fluid of patients with heart failure and also positively correlated with severity[6,9]

Using electron spin resonance (ESR) spectroscopy combined with the nitroxide radical, 4-hydroxy-2,2,6,6-tetramethyl-piperidine-N-oxyl, a definitive and direct demonstration of enhanced generation of ROS in the failing myocardium has been obtained[10] The $\cdot O_2^-$ is a primary radical that could lead to the formation of other ROS, such as H_2O_2 and $\cdot O_2^-$, in the failing myocardium. The $\cdot OH$ could arise from electron exchange between $\cdot O_2^-$ and H_2O_2 via the Harber-Weiss reaction. In addition, $\cdot OH$ is generated by the reduction of H_2O_2 in the presence of endogenous iron by means of the Fenton reaction. The generation of $\cdot OH$ implies a pathophysiological significance of ROS in heart failure, because $\cdot OH$ radicals are the predominant oxidant species causing cellular injury.

Decreased antioxidant capacity could further aggravate ROS accumulation in heart failure; however, the activities of SOD, catalase, and GSHPx are not decreased in the failing heart[11] indicating that oxidative stress in heart failure is primarily related to enhancement of pro-oxidant generation rather than to a decline in antioxidant defenses.

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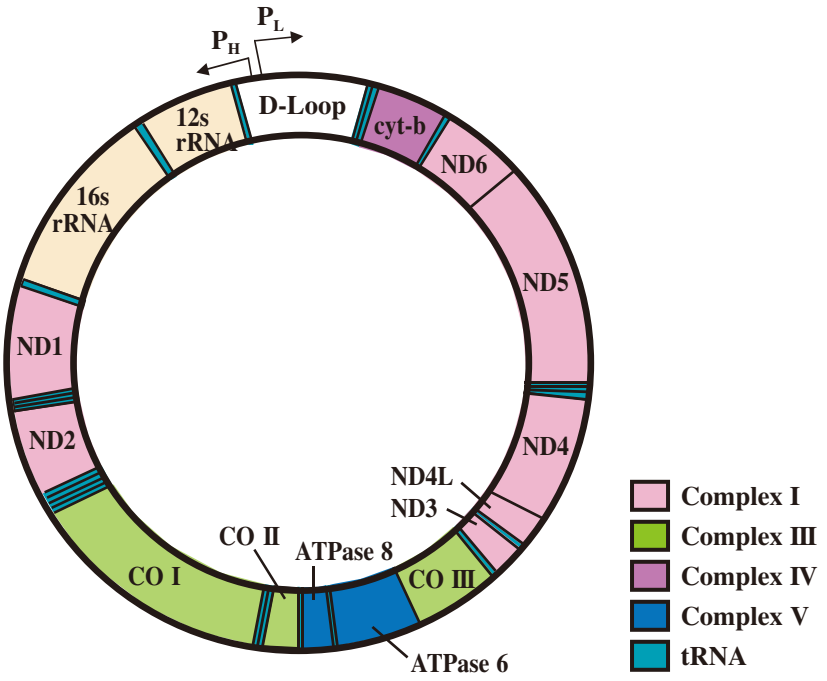

Fig 1. Map of the mitochondrial genome. The 16.3-kb mouse mitochondrial genome is shown with the 13 mRNA, 2 rRNA (12S and 16S), and 21 tRNA coding genes. mRNA genes are the areas labeled with the codes of the corresponding electron transport chain complexes I, III, IV, and V. P_H and P_L are the promoters of heavy (H) and light (L) strand transcription, respectively.

Mitochondria as a Source of Oxidative Stress

The cellular sources of ROS generation within the heart include cardiac myocytes, endothelial cells, and neutrophils. Within cardiac myocytes, ROS can be produced by several mechanisms, including mitochondrial electron transport, NADPH oxidase, and xanthine dehydrogenase/xanthine oxidase.

The heart has the highest oxygen uptake rate within the human body, consuming about 0.1 ml O_2/g min at basal rates. To meet the demand for synthesis of ATP by oxidative metabolism, cardiac myocytes have the highest volume density of mitochondria, which produce ROS through 1-electron carriers in the respiratory chain. Under physiological conditions, the small quantities of ROS formed during mitochondrial respiration can be detoxified by the endogenous scavenging mechanisms of myocytes.

Using ESR spectroscopy with 5,5'-dimethyl-1-pyrroline-N-oxide as a spin trap, the inhibition of electron transport at the sites of complex I and complex III in the normal submitochondrial particles results in a significant production of $\cdot O_2^-$.[12] Mitochondria in heart failure produce more $\cdot O_2^-$ than normal mitochondria in the presence of NADH, indicating that mitochondrial electron transport could be the predominant source of such $\cdot O_2^-$ production. Furthermore, the failing mitochondria are associated with a decrease in complex enzyme activity. Therefore, mitochondria are an important source of ROS in the failing heart, indicating a pathophysiological link between mitochondrial dysfunction and oxidative stress,[13] as has been reported in other disease conditions including aging and neurodegenerative diseases.

Even though mitochondrial electron transport is considered to play an important role in ROS production in heart failure, we can not completely exclude the possibility that other enzymatic sources of ROS generation, such as vascular endothelial cells (via xanthine oxidase and/or NADPH oxidase) and activated leukocytes (via NADPH oxidase), also contribute to oxidative stress.[14] In fact, Bauersachs et al have demonstrated that vascular NAD(P)H oxidase is activated in heart failure.[15] This enzyme system is the major source of ROS in both the endothelium and vascular smooth muscle, which are able to generate ROS in response to angiotensin II and thus stimulate the expression of NAD(P)H oxidase. Plasma renin activity, as well as tissue angiotensin-converting enzyme activity, is activated in heart failure. Therefore, enhanced formation of angiotensin II may lead to oxidative stress via this enzyme system.

Consequences of Oxidative Stress in Heart Failure

Oxidative Stress and Mitochondrial DNA (mtDNA) Damage

Mitochondria have their own genomic system, mtDNA, a closed-circular double-stranded DNA molecule of approximately 16.5 kb (Fig 1). MtDNA contains 2 promoters, the light-strand and heavy-strand promoters (LSP and HSP, respectively), from which transcripts are produced and then processed to yield the individual mRNAs encoding 13 subunits of the oxidative phosphorylation, including 7 subunits (ND1, ND2, ND3, ND4, ND4L, ND5, and ND6) of rotenone-sensitive NADH-ubiquinone oxidoreductase (complex I), 1 subunit (cytochrome b) of ubiquinol-cytochrome c oxidoreductase (complex III), 3 subunits (COI, COII, and COIII) of cytochrome-c oxidase (complex IV), and 2 subunits (ATPases 6 and 8) of complex V, together with 22 tRNAs and 2 rRNA (12S and 16S) subunits.[16,17] Transcription from the LSP also produces RNA primer, which is necessary for initiating mtDNA replication. Mitochondrial function is controlled by the mtDNA, as well as factors that regulate mtDNA transcription and/or replication.[18] This raises the possibility that mitochondrial gene replication, and thus the mtDNA copy number and/or mitochondrial gene transcription, are impaired in heart failure. Indeed, heart failure is frequently associated with qualitative and quantitative defects in mtDNA.[19–22] Recently, the decline in mitochondrial function and mtDNA copy number was shown to play a major role in the development of the heart failure that

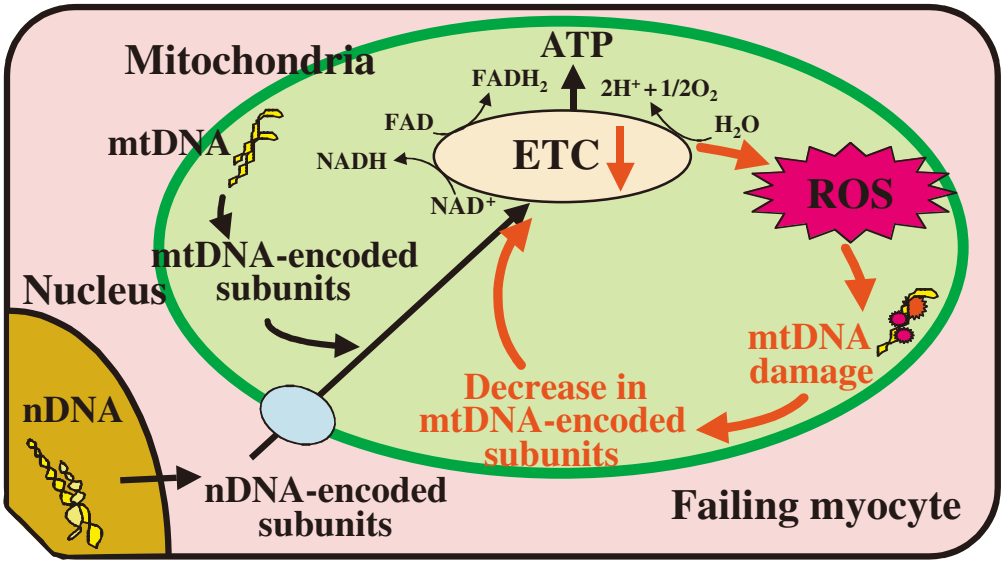

Fig 2. Schematic representation of the intimate link between reactive oxygen species (ROS), mitochondrial DNA (mtDNA) damage, and respiratory chain dysfunction in the mitochondria. Mitochondrial ROS generation may lead to a catastrophic cycle of mitochondrial functional decline, further ROS generation, and cellular injury.

occurs after myocardial infarction (MI)[12,23]

ROS can damage mitochondrial macromolecules either at or near the site of their formation. Therefore, in addition to the role of mitochondria as a source of ROS, the mitochondria themselves can be damaged by ROS. The mtDNA could be a major target for ROS-mediated damage for several reasons. First, mitochondria do not have the complex chromatin organization consisting of histone proteins that may serve as a protective barrier against ROS. Second, mtDNA has limited repair ability against DNA damage. Third, a large part of the $\cdot O_2^-$ formed inside the mitochondria can not pass through the membranes and hence, ROS damage may be contained largely within the mitochondria. In fact, mtDNA accumulates significantly higher levels of the DNA oxidation product, 8-hydroxydeoxyguanosine, than nuclear DNA[24] As opposed to nuclear-encoded genes, mitochondrial-encoded gene expression is largely regulated by the copy number of mtDNA[25] Therefore, mitochondrial injury is reflected by mtDNA damage, as well as by a decline in the mitochondrial RNA (mtRNA) transcripts, protein synthesis, and mitochondrial function[26,27] We have shown that increased generation of ROS is associated with mitochondrial damage and dysfunction in the failing heart, characterized by increased lipid peroxidation in the mitochondria, decreased mtDNA copy number, a decrease in the number of mtRNA transcripts, and reduced oxidative capacity because of low complex enzyme activities[23] A chronic increase in ROS production is associated with mitochondrial damage and dysfunction, which can lead to a catastrophic cycle of mitochondrial functional decline, further ROS generation, and cellular injury (Fig 2). MtDNA defects may thus play an important role in the development and progression of myocardial remodeling and failure.

A number of pathogenic mtDNA base substitution mutations, such as missense mutations and mtDNA rearrangement mutations (deletions and insertions), have been identified in patients with mitochondrial diseases[22] An accumulation of the deleted forms of mtDNA in the myocardium frequently results in cardiac hypertrophy, conduction block, or heart failure[28] Furthermore, there is now a consensus view that mutations in mtDNA and abnormalities in mitochondrial function are associated with common forms of cardiac disease, such as ischemic heart disease[29] and dilated cardiomyopathy[30] In those conditions, however, the strict causal relationships between abnormalities in mtDNA and cardiac dysfunction have yet to be fully elucidated[31] Even though the mechanisms by which mtDNA damage arises have not been clarified, ROS have been proposed as the primary contributing factor. We have provided direct evidence that mtDNA defects occur not only in a limited, small subset of mitochondrial diseases, but also in a more common form of heart failure phenotype occurring after MI.

Oxidative Stress and Myocardial Damage

ROS have direct effects on cellular structure and function, and may be integral signaling molecules in myocardial remodeling and failure. ROS result in a phenotype characterized by hypertrophy and apoptosis in isolated cardiac myocytes[32] ROS have also been shown to activate matrix metalloproteinase (MMP) in cardiac fibroblasts[33] Myocardial MMP activity is increased in the failing heart[32,34] Furthermore, an MMP inhibitor has been shown to limit early LV dilatation in a murine model of MI[35] We have shown significant improvement in survival after MI in MMP-2 knockout mice, which was mainly attributable to inhibition of early cardiac rupture and the development of subsequent LV dysfunction[36] Because MMP can be activated by ROS[37] a proposed mechanism of LV remodeling is activation of MMPs secondary to increased ROS production. Sustained MMP activation might influence the structural properties of the myocardium by providing an abnormal extracellular environment with which the myocytes interact. We have demonstrated that the $\cdot OH$ scavenger, dimethylthiourea, inhibits the activation of MMP-2 in association with the development of LV remodeling and failure[38] These findings raise the interesting possibility that increased ROS after MI may be a stimulus for myocardial MMP activation, which

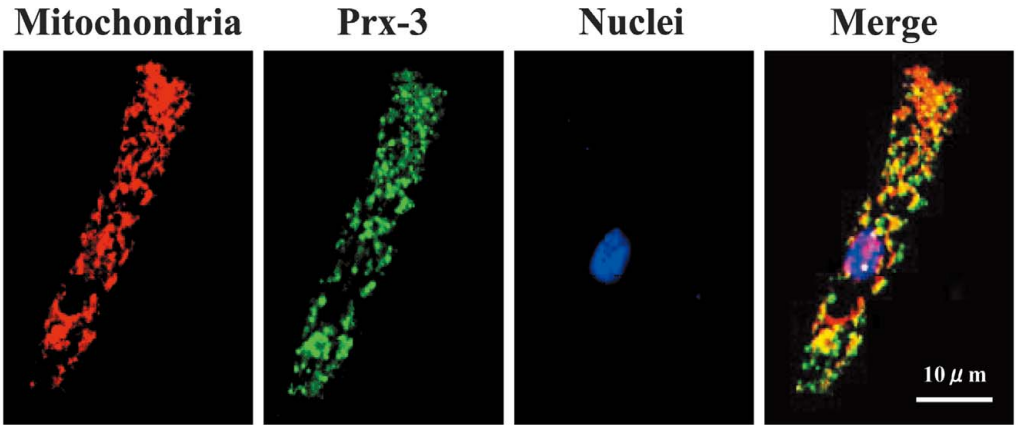

Fig 3. Cardiac myocytes isolated from transgenic mice were doubly stained with MitoTracker dye (red), a rat Prx-3-specific antibody (green), and nucleus (blue). Immunoreactivity for Prx-3 can be seen in the cytoplasm of cardiac myocytes. The merged images show Prx-3 colocalized with the mitochondria (yellow). Scale bar=10 μm.

then play an important role in the development of heart failure.

Oxidative Stress and Skeletal Muscle Damage

Limited exercise capacity is a major symptom in patients with heart failure[39] and is independent of the degree of cardiac dysfunction.[40] Increased oxidative stress has been shown to be related to the limitation of exercise capacity in patients with heart failure.[41] We have demonstrated that ROS are increased in skeletal muscle in patients with heart failure after MI and that they originate from $\cdot O_2^-$ produced by mitochondrial oxidase.[42] Recently, Kinugawa et al clarified the relationship between $\cdot O_2^-$ and the limitation of exercise capacity by using heterozygous manganese superoxide anion dismutase (SOD2) gene–knockout mice, in which SOD2, a family of enzymes that catalyze the dismutation of $\cdot O_2^-$, is reduced by 30–80%, increasing $\cdot O_2^-$ production in the mitochondria, associated with altered mitochondrial function.[43] The whole-body oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) at rest were increased in $SOD2^{+/-}$. The work (vertical distance run × body weight) to exhaustion was decreased in $SOD2^{+/-}$. When the maximum $\dot{V}O_2$ and $\dot{V}CO_2$ were corrected to the per work unit, they were increased in $SOD2^{+/-}$. Tempol normalized the basal $\dot{V}O_2$ and $\dot{V}CO_2$ and improved the work to exhaustion and corrected $\dot{V}O_2$ and $\dot{V}CO_2$ in $SOD2^{+/-}$. There was a decrease in the SOD2 protein level and a concomitant increase in lucigenin-detectable $\cdot O_2^-$ production in skeletal muscle from $SOD2^{+/-}$. Therefore, exercise capacity was reduced in conditions in which $\cdot O_2^-$ was increased, and this was associated with a greater increase in whole-body oxygen consumption.

Amelioration of Oxidative Stress, MtDNA Damage, and Heart Failure

GSHPx

The first line of defense against ROS-mediated cardiac injury comprises several antioxidant enzymes including SOD, catalase, and GSHPx. Among these, GSHPx is an important enzyme that performs several vital functions. It is a key antioxidant that catalyses the reduction of H_2O_2 and hydroperoxides. GSHPx not only scavenges H_2O_2, but also prevents the formation of other more toxic radicals such as $\cdot OH$. GSHPx possesses a higher affinity for H_2O_2 than catalase. Furthermore, it is present in relatively high amounts within the heart, especially in the cytosolic and mitochondrial compartments.[44] These lines of evidence imply the primary importance of GSHPx as a defense mechanism within the heart, compared witho catalase. Moreover, GSHPx is expected to exert greater protective effects against oxidative damage than SOD because greater dismutation of $\cdot O_2^-$ by SOD may result in increased H_2O_2. Therefore, compared with SOD or catalase, GSHPx is thought to be more effective in protecting cells, tissues, and organs against oxidative damage.[45]

GSHPx overexpression inhibits the development of LV remodeling and failure after MI, and so might contribute to improved survival.[46] These findings not only extend the previous observational study that used antioxidants, but also reveal the major role of ROS in the pathophysiology of myocardial remodeling. The effects were associated with attenuation of myocyte hypertrophy, apoptosis, and interstitial fibrosis.[46] Similarly, overexpression of GSHPx attenuates myocardial remodeling and preserves diastolic function in the diabetic heart.[47] Therefore, therapies designed to interfere with oxidative stress by using GSHPx could be beneficial in preventing heart failure.

Peroxiredoxin-3 (Prx-3)

We have recently demonstrated that the overexpression of a mitochondrial antioxidant, Prx-3, a member of peroxiredoxin family that can scavenge H_2O_2 in cooperation with thiol and peroxynitrite (Fig 3), protects the heart against post-MI remodeling and failure in mice. It reduces LV cavity dilatation and dysfunction, as well as myocyte hypertrophy, interstitial fibrosis, and apoptosis of the noninfarcted myocardium. These beneficial effects of Prx-3 gene overexpression are associated with attenuation of oxidative stress, mtDNA decline, and dysfunction.[48] The specific localization of Prx-3 in the mitochondria suggests that mitochondrial oxidative stress plays an important role in the development and progression of heart failure, and that the antioxidant localized specifically within the mitochondria provides a primary line of defense against this disease process.

Mitochondrial Transcription Factor A (TFAM)

TFAM is a nuclear-encoded protein that binds upstream

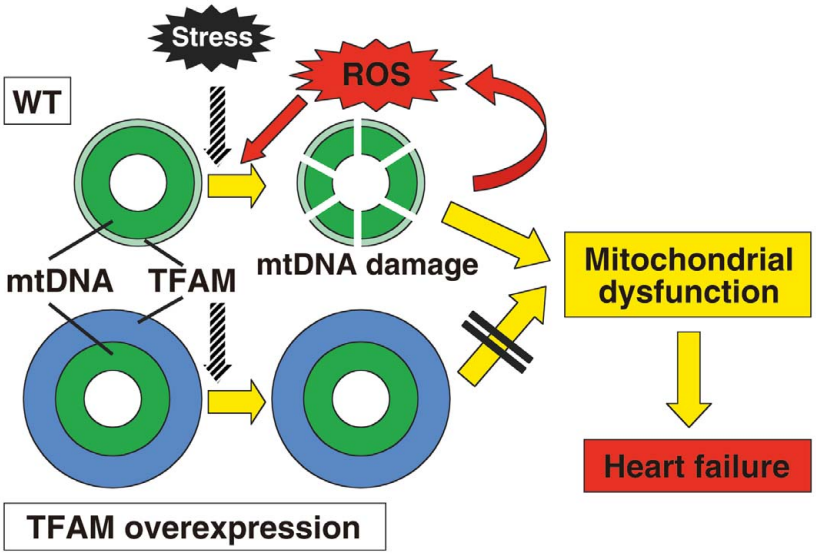

Fig 4. Proposed mechanisms byh which mitochondrial transcription factor A (TFAM) overexpression prevents mitochondrial DNA (mtDNA) damage, oxidative stress, and myocardial remodeling and failure. In wild-type mice, TFAM directly interacts with mtDNA to form nucleoids. Stress such as ischemia causes mtDNA damage, which increases the production of reactive oxygen species and thus leads to a catastrophic cycle of mitochondrial electron transport impairment, further ROS generation, and mitochondrial dysfunction. TFAM overexpression may protect mtDNA from damage by directly binding and stabilizing mtDNA and increase the steady-state levels of mtDNA, which ameliorates mitochondrial dysfunction and thus the development and progression of heart failure.

of the LSP and HSP of mtDNA and promotes transcription of mtDNA. TFAM not only regulates mtDNA transcription and replication[49] but also maintains mtDNA copy number. In fact, *Tfam* knockout mice, which have a 50% reduction in their transcript and protein levels, show a 34% reduction in mtDNA copy number, 22% reduction in the mitochondrial transcript levels, and partial reduction in the cytochrome *c* oxidase levels in the heart.[50] Moreover, cardiac-specific disruption in *Tfam* in mice results in dilated cardiomyopathy in association with a reduced amount of mtDNA and mitochondrial transcripts.[51] The transfection of antisense plasmids in culture, designed to reduce the expression of *TFAM*, effectively decreased the levels of mitochondrially encoded transcripts.[19] In contrast, forced overexpression of *TFAM* produced the opposite effect.[52] These lines of evidence obtained from knockout mice have established a critical role for TFAM in the regulation of mtDNA copy number and mitochondrial function, as well as maintenance of the physiological function of the heart in vivo. In addition, a reduction in *TFAM* expression has been demonstrated in several forms of cardiac failure.[20,23, 3,54]

By using transgenic mice that overexpress human *TFAM*, we examined whether TFAM could protect the heart from mtDNA deficiencies and attenuate LV remodeling and failure after MI.[55] *TFAM* overexpression could ameliorate the decline in mtDNA copy number and preserve it at a normal level in post-MI hearts. *TFAM* overexpression might increase the steady-state level of mtDNA by directly stabilizing mtDNA. Consistent with alterations in mtDNA, the decrease in oxidative capacities seen in MI was also prevented. Moreover, TFAM played an important role in myocardial protection against remodeling and failure.

Several factors may be attributed to the protective effects conferred by *TFAM* overexpression against myocardial remodeling and failure. First, *TFAM* overexpression prevented a decrease in mtDNA copy number and mitochondrial electron transport function, which may contribute to decreased myocardial oxidative stress, which in turn could contribute to the amelioration of cardiac hypertrophy, apoptosis, and interstitial fibrosis.[55] A recent study by Ekstrand et al demonstrated that the overexpression of human *TFAM* in the mouse increased mtDNA copy number.[56] These lines of evidence imply the primary importance of TFAM as a regulatory mechanism of mtDNA copy number. TFAM has been shown to directly interact with mtDNA to form nucleoids.[57,58] Therefore, in transgenic mice increased TFAM may increase the steady-state levels of mtDNA by directly binding and stabilizing mtDNA (Fig 4). Second, *TFAM* overexpression may induce mitochondrial biogenesis, although this is thought to be unlikely because the number and size of the mitochondria assessed by electron microscopy were unaltered.

The results obtained from human *TFAM* transgenic mice differ from those from the inducible, cardiac-specific overexpression of peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) transgene in adult mice, which leads to a modest increase in mitochondrial number and the development of reversible cardiomyopathy.[59] PGC-1α is a transcriptional coactivator and acts upstream of TFAM, and also has the capacity to increase mtDNA levels as well as mitochondrial mass in both cultured cells and transgenic mice.[60,61] The reason for the discrepant results between the PGC-1α and TFAM transgene overexpression studies remains unsolved, but may be related to the complex regulatory mechanisms of mitochondrial biogenesis and function by PGC-1α and its downstream factors, including nuclear respiratory factors 1 and 2 and TFAM.[62,63]

MtDNA decline and mitochondrial defects are now well recognized in a variety of diseases, such as neurodegenerative diseases, diabetes mellitus, cancer, and even aging. Therefore, with further knowledge about the mechanisms of TFAM for maintaining mtDNA copy number and mitochondrial function, it may eventually be possible to develop novel strategies for the treatment of such diseases based on manipulation of TFAM.

Conclusions

To improve the prognosis of patients with heart failure, novel therapeutic strategies based on new insights into the pathophysiology of myocardial remodeling and failure need to be developed. The approach to regulating mitochondrial oxidative stress and mtDNA damage may contribute to the establishment of effective treatment strategies for patients with heart failure. Oxidative stress is involved not only in heart failure, but also in various other diseases, including

atherosclerosis, hypertension, and aging. Therefore, therapeutic strategies to modulate this maladaptive response should definitely become a target for future extensive investigation and could have broad application.

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