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Forum Review

Mitochondrial Oxidative Stress, DNA Damage, and Heart Failure

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ABSTRACT

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, whereas antioxidant enzyme activities are preserved as normal. Mitochondrial electron transport is an enzymatic source of oxygen radical generation and also a target of oxidant-induced damage. 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, mitochondrial oxidative stress and mtDNA damage are good therapeutic targets. Overexpression of mitochondrial transcription factor A (TFAM) could ameliorate the decline in mtDNA copy number and preserve it at a normal level in failing hearts. Consistent with alterations in mtDNA, the decrease in oxidative capacities was also prevented. Therefore, the activation of TFAM expression could ameliorate the pathophysiologic processes seen in myocardial failure. Inhibition of mitochondrial oxidative stress and mtDNA damage could be novel and potentially very effective treatment strategies for heart failure. *Antioxid. Redox Signal.* 8, 1737–1744.

INTRODUCTION

HEART FAILURE IS A LEADING CAUSE of morbidity and mortality in industrialized countries (19). 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. 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 ($\bullet O_2^-$) and hydroxy radicals (•OH) cause the oxidation of membrane phospholipids, proteins, and DNAs (34) and have been implicated in a wide range of pathologic conditions including ischemia–reperfusion injury (8), neurodegenerative diseases (35), and aging (51). Under physiologic 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 nonenzymatic antioxidants. However, when the production of ROS becomes excessive, oxidative stress might have a harmful effect on the functional and structural integrity of biologic tissue.

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

DIRECT EVIDENCE OF OXIDATIVE STRESS IN HEART FAILURE

Recent experimental and clinical studies have suggested the generation of ROS to increase in heart failure (7, 17, 18,

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33). Lipid peroxides and 8-iso-prostaglandin F2α, which are the major biochemical markers of ROS generation, have been shown to be elevated in plasma and pericardial fluid of patients with heart failure and also positively correlated to its severity (7, 33).

Using electron spin resonance (ESR) spectroscopy combined with the nitroxide radical, 4-hydroxy-2,2,6,6-tetramethyl-piperidine-*N*-oxyl (hydroxy-TEMPO), a definitive and direct demonstration of enhanced generation of ROS was obtained in the failing myocardium (22). $\bullet O_2^-$ is a primary radical that could lead to the formation of other ROS, such as H_2O_2 and •OH, in the failing myocardium. •OH could arise from electron exchange between $\bullet O_2^-$ and H_2O_2 via the Harber–Weiss reaction. In addition, •OH is also generated by the reduction of H_2O_2 in the presence of endogenous iron by means of the Fenton reaction. The generation of •OH implies a pathophysiologic significance of ROS in heart failure because •OH radicals are the predominant oxidant species causing cellular injury.

The decreased antioxidant capacity could further aggravate the ROS accumulation in heart failure. However, the activities of SOD, catalase, and GSHPx were not decreased in the failing hearts (52), indicating that oxidative stress in heart failure is primarily due to the enhancement of prooxidant generation rather than to the decline in antioxidant defenses.

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. For the demand for synthesis of ATP by oxidative metabolism, cardiac myocytes have the highest volume density of mitochondria. Mitochondria produce ROS through one-electron carriers in the respiratory chain. Under physiologic conditions, small quantities of ROS are formed during mitochondrial respiration, which, however, can be detoxified by the endogenous scavenging mechanisms of myocytes.

Using ESR spectroscopy with 5,5′-dimethyl-1-pyrroline-*N*-oxide (DMPO) as a spin trap, the inhibition of electron transport at the sites of complex I and complex III in the normal submitochondrial particles resulted in a significant production of $\bullet O_2^-$ (23). Mitochondria from heart failure produced more $\bullet O_2^-$ than normal mitochondria in the presence of NADH, indicating that mitochondrial electron transport could be the predominant source of such $\bullet O_2^-$ production. Furthermore, heart failure mitochondria were associated with a decrease in complex enzyme activity. Therefore, mitochondria are an important source of ROS in failing hearts, indicating a pathophysiologic link between mitochondrial dysfunction and oxidative stress (42), 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 the ROS production in heart failure, we could 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) could also contribute to oxidative stress (37). Bauersachs *et al*. (6) demonstrated that vascular NAD(P)H oxidase was activated in heart failure. This enzyme system is the major source of ROS in both the endothelium and vascular smooth muscle. It is able to generate ROS in response to angiotensin II, which stimulates the expression of NAD(P)H oxidase. Plasma renin activity as well as tissue angiotensin-converting enzyme (ACE) activity is activated in heart failure. Therefore, an 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 ~16.5 kb. MtDNA contains two 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 seven subunits (ND1, ND2, ND3, ND4, ND4L, ND5, and ND6) of rotenone-sensitive NADH-ubiquinone oxidoreductase (complex I), one subunit (cytochrome *b*) of ubiquinol-cytochrome *c* oxidoreductase (complex III) , three subunits (COI, COII, and COIII) of cytochrome *c* oxidase (complex IV), and two subunits (ATPases 6 and 8) of complex V along with 22 tRNAs and two rRNA (12S and 16S) subunits (4, 44). 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 (9). 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 (26, 31, 38, 54). Recently, the decline in mitochondrial function and mtDNA copy number has been shown to play a major role in the development of heart failure that occurs after myocardial infarction (MI) (21, 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 a complex chromatin organization consisting of histone proteins, which may serve as a protective barrier against ROS. Second, mtDNA has a limited repair activity against DNA damage. Third, a large part of $\bullet O_2^-$, which is formed inside the mitochondria, cannot pass through the membranes and, hence, ROS damage may be

contained largely within the mitochondria. mtDNA accumulates significantly higher levels of the DNA oxidation product, 8-hydroxydeoxyguanosine, than does nuclear DNA (15). As opposed to nuclear-encoded genes, mitochondrial-encoded gene expression is largely regulated by the copy number of mtDNA (57). 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 (5, 56). We have shown that the increased generation of ROS is associated with mitochondrial damage and a dysfunction in the failing hearts, which were characterized by an increased lipid peroxidation in the mitochondria, a decreased mtDNA copy number, a decrease in the number of mtRNA transcripts, and a reduced oxidative capacity due to low complex enzyme activities (21). Chronic increases in ROS production are associated with mitochondrial damage and dysfunction, which thus can lead to a catastrophic cycle of mitochondrial functional decline, further ROS generation, and cellular injury (Fig. 1). 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 (54). An accumulation of the deleted forms of mtDNA in the myocardium frequently results in either cardiac hypertrophy, conduction block, or heart failure (2). Furthermore, now a consensus view exists that mutations in mtDNA and abnormalities in mitochondrial function are associated with common forms of cardiac diseases such as ischemic heart disease (11) and dilated cardiomyopathy (3). In these conditions, however, the strict causal relations between abnormalities in mtDNA and cardiac dysfunction have yet to be fully elucidated (10). Even though the mechanisms by which mtDNA damage arises in these conditions have not been clarified, ROS have been proposed to be 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 heart failure phenotype occurring after MI.

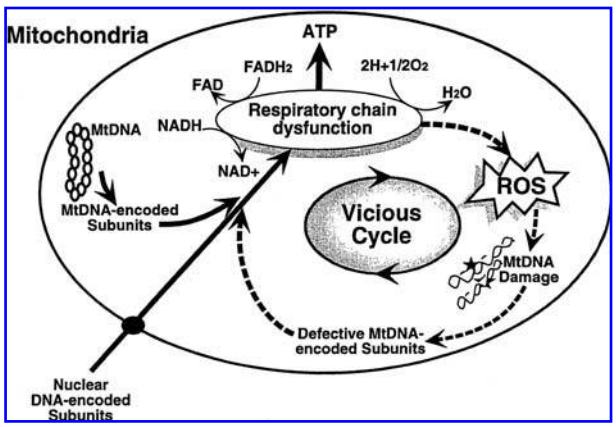

FIG. 1. Schematic representation of an intimate link between ROS, mtDNA damage, and respiratory chain dysfunction in the mitochondria. Mitochondrial ROS generation may lead to a catastrophic cycle of mitochondrial functional decline, further ROS generation, and cellular injury. (Reproduced with permission from Ide T et al. Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts following myocardial infarction. *Circ Res* 88: 529–535, 2001.)

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 (47). ROS have also been shown to activate matrix metalloproteinase (MMP) in cardiac fibroblasts (46). Myocardial MMP activity is increased in the failing hearts (12, 48). Further, an MMP inhibitor has been shown to limit early LV dilatation in a murine model of MI (40). We have shown the significant improvement in the survival after MI in MMP-2 knockout mice, which is mainly attributable to the inhibition of early cardiac rupture and the development of subsequent LV dysfunction (16). Because MMP can be activated by ROS (39), one proposed mechanism of LV remodeling is the 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 •OH scavenger, dimethylthiourea, inhibits the activation of MMP-2 in association with the development of LV remodeling and failure (28). These data raise the interesting possibility that increased ROS after MI can be a stimulus for myocardial MMP activation, which might play an important role in the development of heart failure.

AMELIORATION OF MITOCHONDRIAL OXIDATIVE STRESS, MITOCHONDRIAL DNA DAMAGE, AND HEART FAILURE

GSHPx

The first line of defense mechanism against ROS-mediated cardiac injury comprises several antioxidant enzymes including SOD, catalase, and GSHPx. Among these antioxidants, GSHPx is an important enzyme that performs several vital functions. GSHPx is a key antioxidant that catalyses the reduction of H_2O_2 and hydroperoxides. It not only scavenges H_2O_2 but also prevents the formation of other more-toxic radicals such as •OH. GSHPx possesses a higher affinity for H_2O_2 than catalase. Further, it is present in relatively high amounts within the heart, especially in the cytosolic and mitochondrial compartments (30). These lines of evidence imply the primary importance of GSHPx as a defense mechanism within the heart compared with catalase. Moreover, GSHPx is expected to exert greater protective effects against oxidative damage than SOD because greater dismuta-

tion of $\bullet O_2^-$ by SOD may result in an increase of 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 (50).

GSHPx overexpression inhibited the development of LV remodeling and failure after MI, which might contribute to the improved survival (45). These findings not only extended the previous observation that used antioxidants, but also revealed the major role of ROS in the pathophysiology of post-MI remodeling. These effects were associated with the attenuation of myocyte hypertrophy, apoptosis, and interstitial fibrosis (45). Therefore, therapies designed to interfere with oxidative stress using GSHPx could be beneficial to prevent heart failure.

Mitochondrial transcription factor A (TFAM)

TFAM is a nucleus-encoded protein that binds upstream of the LSP and HSP of mtDNA and promotes transcription of mtDNA. TFAM not only regulates mtDNA transcription and replication (43), but also maintains mtDNA copy number. *Tfam* knockout mice, which had a 50% reduction in their transcript and protein levels, exerted a 34% reduction in the mtDNA copy number, 22% reduction in the mitochondrial transcript levels, and partial reduction in the cytochrome *c* oxidase levels in the heart (29). Moreover, cardiac-specific disruption in the *Tfam* gene in mice exhibited dilated cardiomyopathy in association with a reduced amount of mtDNA and mitochondrial transcripts (55). The transfection of antisense plasmids in culture, designed to reduce the expression of *TFAM*, effectively decreased the levels of mitochondrially encoded transcripts (25). On the contrary, the forced overexpression of *TFAM* could produce the opposite effect (36). These lines of evidence obtained from knockout mice have established a critical role for TFAM in regulation of mtDNA copy number and mitochondrial function as well as maintenance of the physiologic function of the heart *in vivo*. In addition, a reduction in TFAM expression has been demonstrated in several forms of cardiac failure (14, 21, 27, 31).

Using transgenic mice that overexpress human *TFAM* gene, we examined whether TFAM could protect the heart from mtDNA deficiencies and attenuate LV remodeling and failure after MI (24). *TFAM* overexpression could ameliorate the decline in mtDNA copy number and preserve it at a normal level in post-MI hearts from TFAM transgenic mice (Fig. 2). *TFAM* overexpression might increase the steady-state levels of mtDNA by directly stabilizing mtDNA. Consistent with alterations in mtDNA, the decrease in oxidative capacities seen in MI was also prevented (Fig. 2). Moreover, TFAM played an important role in myocardial protection against remodeling and failure (Figs. 3 and 4).

Several factors may be attributable to the protective effects conferred by TFAM against myocardial remodeling and failure. First, *TFAM* overexpression prevented the decrease in mtDNA copy number and mitochondrial electron-transport function, which may contribute to the decrease in myocardial oxidative stress. The decreased oxidative stress could contribute to the amelioration of cardiac hypertrophy, apoptosis, and interstitial fibrosis (24). A recent study by Ekstrand *et al.* (13) demonstrated that the overexpression of human *TFAM* in the mouse increased mtDNA copy number. These lines of evidence imply the primary importance of TFAM as a regulatory mechanism of mtDNA copy number. TFAM has been shown to interact directly with mtDNA to form nucleoids (1, 49). Therefore, increased TFAM may increase the steady-state levels of mtDNA by directly binding and stabilizing mtDNA in transgenic mice (Fig. 5). Second, *TFAM* overexpression may induce mitochondrial biogenesis, which, however, is thought to be unlikely because the number and size of

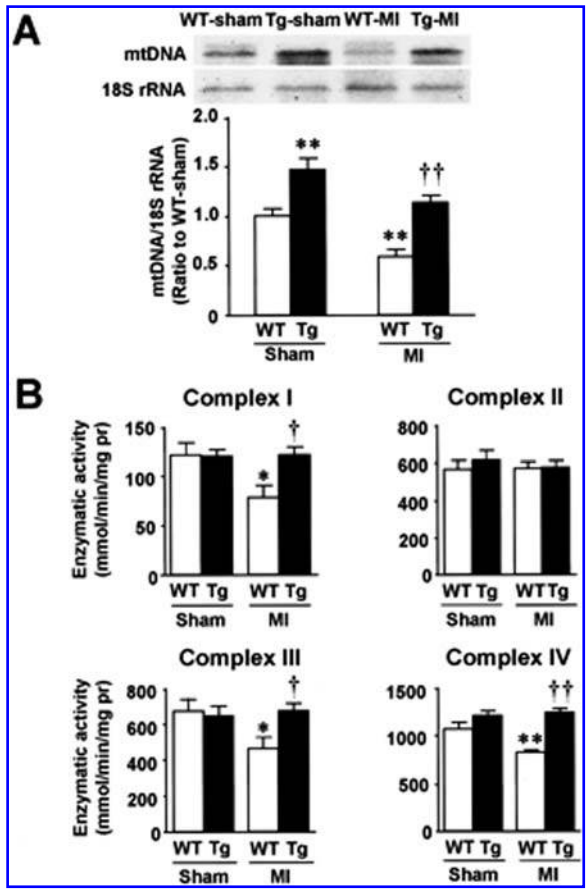

FIG. 2. mtDNA and mitochondrial function. (A) Top, Southern blot analysis of mtDNA copy number in total DNA extracts from the heart from WT-sham, Tg-sham, WT-MI, and Tg-MI mice. Top bands show signals from the mtDNA fragment, and bottom bands show signals from the nuclear DNA fragment containing the 18S rRNA gene. **(A)** Bottom, Summary data for a Southern blot analysis of mtDNA copy number in four groups of animals ($n = 8$ for each). Data were obtained by a densitometric quantification of the Southern blots such as those shown in **A. (B)** Enzymatic activity of respiratory chain complex I, complex II, complex III, and complex IV in isolated mitochondria from four groups of animals ($n = 6$ for each). Each assay was done in triplicate. Values are expressed as mean ± SEM. $*p < 0.05$, $**p < 0.01$ for difference from WT-sham values. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ for difference from WT-MI values. pr, Protein. (Reproduced with permission from Ikeuchi M et al. Overexpression of mitochondrial transcription factor a ameliorates mitochondrial deficiencies and cardiac failure after myocardial infarction. *Circulation* 112: 683–690, 2005.)

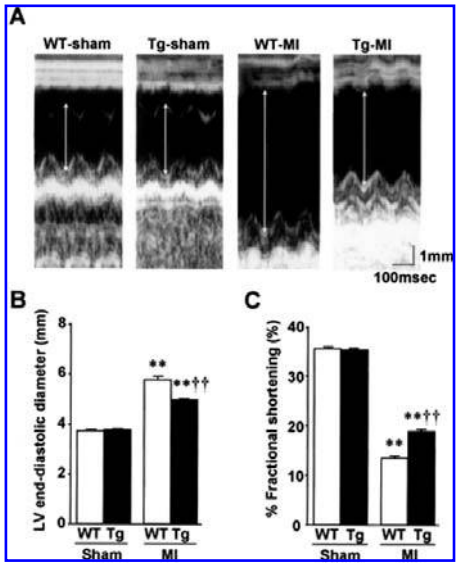

FIG. 3. **(A)** Representative M-mode echocardiograms obtained from WT-sham, Tg-sham, WT-MI, and Tg-MI mice. *Arrows* indicate LV end-diastolic diameter. **(B, C)** Summary data for echocardiographic measurements in four groups of animals ($n = 6$ for each). LV end-diastolic diameter **(B)** and percentage fractional shortening **(C)** are shown. Values are expressed as mean ± SEM. $^{**}p < 0.01$ for difference from WT-sham values; $^{\dagger\dagger}p < 0.01$ for difference from WT-MI values. (Reproduced with permission from Ikeuchi M et al. Overexpression of mitochondrial transcription factor a ameliorates mitochondrial deficiencies and cardiac failure after myocardial infarction. Circulation 112: 683–690, 2005.)

the mitochondria assessed by electron microscopy were not altered.

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 development of reversible cardiomyopathy (41). PGC-1α is the transcriptional coactivator, acts upstream of TFAM, and also has the capacity to increase mtDNA levels as well as mitochondrial mass in cultured cells and in transgenic mice (32, 58). The reason for the discrepant results between PGC-1 and TFAM transgene overexpression remains unsolved, which, however, may be related to the complex regulatory mechanisms of mitochondrial biogenesis and function by PGC-1 and its downstream factors, including nuclear respiratory factors 1 and 2 and TFAM (20, 53).

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 on the mechanisms of TFAM for maintenance of mtDNA copy number and mitochondrial function, it may eventually be possible to develop novel strategies for the treatment of such diseases based on the manipulation of TFAM.

SUMMARY

To improve the prognosis of patients with heart failure, novel therapeutic strategies based on a novel insight into the

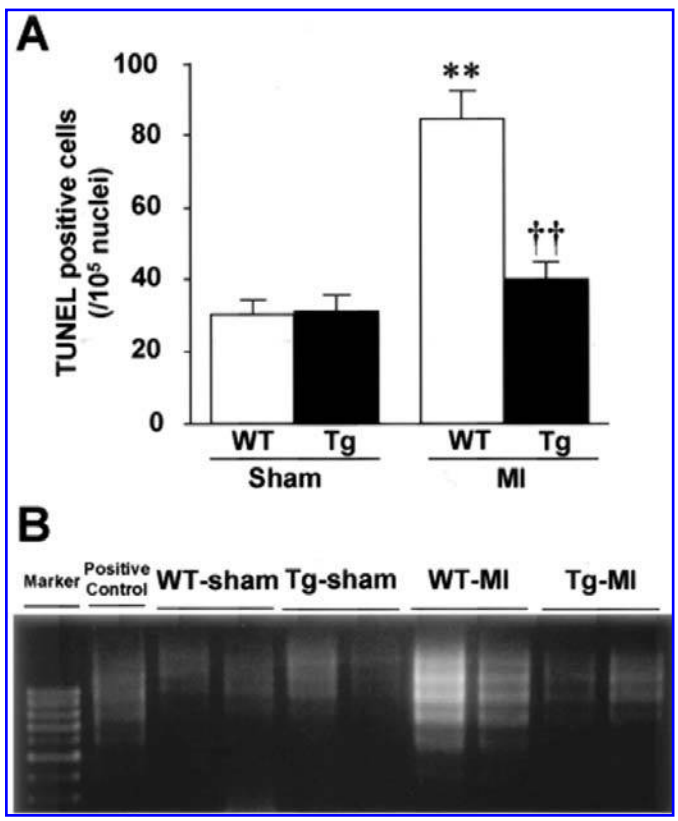

FIG. 4. **(A)** Number of TUNEL-positive myocytes in noninfarcted area of LV from four groups of animals ($n = 8$ each). Values are expressed as mean ± SEM. $^{**}p < 0.01$ for difference from WT-sham values. $^{\dagger\dagger}p < 0.01$ for difference from WT-MI values. (B) DNA ladder indicative of apoptosis in genomic DNA from LV. (Reproduced with permission from Ikeuchi M et al. Overexpression of mitochondrial transcription factor a ameliorates mitochondrial deficiencies and cardiac failure after myocardial infarction. *Circulation* 112: 683–690, 2005.)

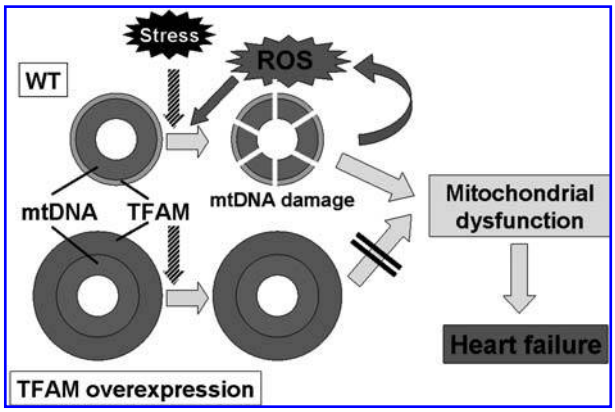

FIG. 5. Proposed mechanisms through which mitochondrial transcription factor A (TFAM) overexpression prevents mitochondrial DNA (mtDNA) damage, oxidative stress, and myocardial remodeling and failure. In wild-type (WT) mice, TFAM directly interacts with mtDNA to form nucleoids. Stress such as ischemia causes mtDNA damage, which increases the production of reactive oxygen species (ROS) 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.

pathophysiology of myocardial remodeling and failure must be developed. The approach by regulating mitochondrial oxidative stress and mtDNA damage may contribute to the establishment of the effective treatment strategies for patients with heart failure. Oxidative stress is involved not only in heart failure, but also in various cardiovascular diseases including atherosclerosis, hypertension, and even aging. Therefore, therapeutic strategies to modulate this maladaptive response should definitely become a target for future extensive investigation and could have a broader application.

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ABBREVIATIONS

DMPO 5,5′-dimethyl-1-pyrroline-*N*-oxide; ESR, electron spin resonance; GSHPx, glutathione peroxidase; HSP, heavy-strand promoter; LSP, light-strand promoter; LV, left ventricular; mtDNA mitochondrial DNA; mtRNA, mitochondrial RNA; MMP, matrix metalloproteinase; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; ROS, reactive oxygen species; SOD, superoxide dismutase; TEMPO, 2,2,6,6-tetramethyl-piperidine-*N*-oxyl.

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