Overexpression of Mitochondrial Transcription Factor A Ameliorates Mitochondrial Deficiencies and Cardiac Failure After Myocardial Infarction

Masaki Ikeuchi, MD; Hidenori Matsusaka, MD; Dongchon Kang, MD, PhD; Shouji Matsushima, MD; Tomomi Ide, MD, PhD; Toru Kubota, MD, PhD; Toshiyuki Fujiwara, MD, PhD; Naozuke Hamasaki, MD, PhD; Akira Takeshita, MD, PhD; Kenji Sunagawa, MD, PhD; Hiroyuki Tsutsui, MD, PhD

Background—Mitochondrial DNA (mtDNA) copy number is decreased not only in mtDNA-mutation diseases but also in a wide variety of acquired degenerative and ischemic diseases. Mitochondrial transcription factor A (TFAM) is essential for mtDNA transcription and replication. Myocardial mtDNA copy number and TFAM expression both decreased in cardiac failure. However, the functional significance of TFAM has not been established in this disease state.

Methods and Results—We have now addressed this question by creating transgenic (Tg) mice that overexpress human TFAM gene and examined whether TFAM could protect the heart from mtDNA deficiencies and attenuate left ventricular (LV) remodeling and failure after myocardial infarction (MI) created by ligating the left coronary artery. TFAM overexpression could ameliorate the decrease in mtDNA copy number and mitochondrial complex enzyme activities in post-MI hearts. Survival rate during 4 weeks of MI was significantly higher in Tg-MI than in wild-type (WT) littermates (WT-MI), although infarct size was comparable. LV cavity dilatation and dysfunction were significantly attenuated in Tg-MI. LV end-diastolic pressure was increased in WT-MI, and it was also reduced in Tg-MI. Improvement of LV function in Tg-MI was accompanied by a decrease in myocyte hypertrophy, apoptosis, and interstitial fibrosis as well as oxidative stress in the noninfarcted LV.

Conclusions—Overexpression of TFAM inhibited LV remodeling after MI. TFAM may provide a novel therapeutic strategy of cardiac failure. (Circulation. 2005;112:683-690.)

Key Words: free radicals • genes • heart failure • myocardial infarction • remodeling

Myocardial infarction (MI) leads to complex structural alterations (remodeling) involving both the infarcted and noninfarcted left ventricular (LV) myocardium. Early remodeling is LV cavity dilatation occurring during the early phase of MI, which is likely due to wall thinning of the infarct region. During the first several days, LV enlargement follows, and thereafter a progressive dilatation of the noninfarcted LV associated with myocyte hypertrophy and interstitial fibrosis occurs over weeks. These progressive changes in LV geometry contribute to the development of depressed cardiac function, clinical heart failure, and increased mortality. Accordingly, it is of critical importance to explore the mechanisms and to develop therapeutic strategies that will effectively inhibit this deleterious process.

Mitochondria have their own genomic system, mitochondrial DNA (mtDNA), a closed-circular double-stranded DNA molecule. 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 system, ribosomal and transfer RNAs.1,2 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.3 This raises the possibility that mitochondrial gene replication and thus the mitochondrial DNA 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.4–7 Recently, we demonstrated that the decline in mitochondrial function and mtDNA copy number plays a major role in the development of heart failure that occurs after MI.8,9

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From the Department of Cardiovascular Medicine (M.I., H.M., S.M., T.I., T.K., A.T., K.S.) and Clinical Chemistry and Laboratory Medicine (D.K., N.H.), Graduate School of Medical Sciences, Kyushu University, Fukuoka; Department of Biochemistry, Fukuoka University School of Medicine, Fukuoka (T.F.); and Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Sapporo (H.T.), Japan.

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Correspondence to Hiroyuki Tsutsui, MD, PhD, Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Kita-15, Nishi-7, Kita-ku, Sapporo 060-8638, Japan. E-mail htsutsui@med.hokudai.ac.jp

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Mitochondrial transcription factor A (TFAM) is a nucleus-encoded protein that binds upstream of the LSP and HSP of mtDNA and promotes transcription of mtDNA. It also plays an important role in regulating mtDNA copy number.

In fact, disruption of the Tfam gene in mice causes depletion of mtDNA, loss of mitochondrial transcripts, loss of mtDNA-encoded polypeptides, and severe respiratory chain deficiency. Moreover, targeted disruption of Tfam in cardiac myocytes induced deletion of mtDNA and dilated cardiomyopathy. 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 physiological function of the heart in vivo. In addition, a reduction in TFAM expression has been demonstrated in several forms of cardiac failure. Therefore, an increase in TFAM expression may exert beneficial effects on cardiac remodeling after MI. However, it has not yet been analyzed whether an increase in TFAM expression can ameliorate mitochondrial dysfunction in heart failure and whether this protein may have therapeutic potential. To address these questions, we created transgenic (Tg) mice containing human TFAM gene. Accordingly, human TFAM Tg mice and their wild-type (WT) littermates were randomized to have either a large transmural MI induced by coronary artery ligation or sham operation.

Methods

Generation of Tg Mice

Human TFAM cDNA was inserted into the unique EcoRI site between the CAG (modified chicken β-actin promoter with CMV-IE enhancer) promoter and 3′-flanking sequence of the rabbit β-globin gene of the pCAGGS expression vector and used to generate Tg mice (Figure 1A). The pronuclei of fertilized eggs from hyperovulated C57BL/6 mice were microinjected with this DNA construct. The presence of the TFAM transgene was confirmed by polymerase chain reaction (PCR) before the experiments. Four independent founder lines were identified and mated to C57BL/6 WT mice to generate pure C57BL/6 genetic background WT and Tg offspring. Heterozygous Tg mice were used at 10 to 13 weeks of age. The study was approved by our Institutional Animal Research Committee and conformed to the animal care guidelines of the American Physiological Society.

Western Blotting

The protein levels human TFAM and mouse Tfam were analyzed in cardiac tissue homogenates by Western blot analysis with a polyclonal antiserum against human TFAM and mouse Tfam, respectively. In brief, the LV tissues were homogenized with the lysis buffer (1% SDS, 1.0 mmol/L sodium orthovanadate, 10 mmol/L Tris; pH 7.4). After centrifugation, equal amounts of protein (5 μg protein per lane), estimated by the Bradford method with the use of a protein assay (Bio-Rad), were electrophoresed on a 12.5% SDS–polyacrylamide gel and then electrophoretically transferred to a nitrocellulose membrane (Millipore). After blocking with 5% nonfat milk in PBS containing 0.05% Tween-20 at 4°C overnight, the membrane was incubated with the first antibody and then with the peroxidase-linked second antibody (Amersham Pharmacia). Chemiluminescence was detected with an ECL Western blot detection kit (Amersham Pharmacia) according to the manufacturer’s recommendation.

Immunohistochemistry

Frozen sections of cardiac tissues were incubated in the presence of 100 nmol/L Mitotracker Red CMXRos (Molecular Probes) at 37°C for 20 minutes. We did not repeat freezing-thawing to avoid the loss of mitochondrial integrity. After they were washed, the fixed sections were incubated with 100-fold diluted anti-TFAM affinity purified antibodies (10 μg/mL) in PBS at 4°C overnight. Fluorescence images were taken with a confocal laser scanning microscope (Bio-Rad MRC 1000) with laser beams of 488 and 568 nm for excitation.

Creation of MI

We created MI in mice by ligating the left coronary artery. Sham operation without coronary artery ligation was also performed. Tail clips were applied, and a PCR protocol was performed to confirm the genotype by a group of investigators. Next, MI was induced in these mice by another subset of investigators, who were not informed of the genotyping results. This assignment procedure was performed with numeric codes to identify the animals.

Survival

To perform the survival analysis, cages were inspected for deceased animals during the study period of 4 weeks. All deceased mice were examined for the presence of MI as well as pleural effusion and cardiac rupture. We performed the subsequent molecular (mtDNA copy number and mtRNA), biochemical (mitochondrial enzyme activity and apoptosis), and histopathological (myocyte cross-sectional area, collagen volume fraction, and mitochondrial ultrastructure) analysis by using the LV from sham-operated mice and the noninfarcted LV from MI mice.

Figure 1. Characterization of human TFAM Tg mice. A, Diagram of the human TFAM transgenic construct. Plasmid was constructed by inserting a human TFAM cDNA (0.74 kb) into the unique EcoRI site between the CAG promoter and 3′-flanking sequence of the rabbit β-globin gene of the pCAGGS expression vector. Tg mice harboring human TFAM cDNA were identified by PCR with genomic DNA prepared from tail biopsies. CMV indicates cytomegalovirus; IE, immediate early; SV40, simian virus; and Ori, origin of DNA replication. B, Western blot analysis of human TFAM protein in various tissues from Tg mice. Total protein extracts from heart, lung, liver, kidney, and skeletal muscle were probed with a polyclonal antiserum against human TFAM. The antibody recognized TFAM as a single band of 24 kDa. C, Western blot analysis of human TFAM and mouse Tfam protein levels in the heart from Tg and WT mice.
Southern Blot Analysis
DNA was extracted from cardiac tissues, and a Southern blot analysis was performed to measure the mtDNA copy number as described earlier.® Primers for the mtDNA probe corresponded to nucleotides 2424 to 3605 of the mouse mitochondrial genome, and those for the nuclear-encoded mouse 18S rRNA probe corresponded to nucleotides 435 to 1951 of the human 18S rRNA genome. The mtDNA levels were normalized to the abundance of the 18S rRNA gene run on the same gel.

RNA Isolation and Northern Blot Analysis
Total RNA was isolated from frozen LV by the guanidinium method, and a Northern hybridization analysis was performed according to methods described previously.® Probes for mtRNA analysis were prepared by amplification of nucleotides 1209 to 2606 (probe 1), nucleotides 3351 to 7570 (probe 2), nucleotides 8861 to 14549 (probe 3), and nucleotides 14729 to 15837 (probe 4) of mtDNA from mouse genomic DNA.

Mitochondrial Enzyme Activity
The specific activity of complex I, complex II, complex III, and complex IV was measured in the myocardial tissues according to methods described previously.® The specific enzymatic activity of rotenone-sensitive NADH-ubiquinone oxidoreductase (complex I) was measured by a reduction of the ubiquinone analogue decyubiquinone. For the activity of succinate-ubiquinone oxidoreductase (complex II), the reduction of 2,6-dichlorophenolindophenol, when coupled to complex II–catalyzed reduction of decyubiquinone, was measured. For the specific activity of ubiquinol/cytochrome c oxidoreductase (complex III), the reduction of cytochrome c catalyzed by complex III in the presence of reduced decyubiquinone was monitored. The specific activity of cytochrome c oxidase (complex IV) was measured by following the oxidation of reduced cytochrome c, which had been prepared in the presence of dithionite. All enzymatic activities were expressed as nanomoles per minute per milligram protein.

Echocardiographic and Hemodynamic Measurements
After 4 weeks of surgery, echocardiographic studies were performed under light anesthesia with tribromoethanol/amylene hydrate (Avertin; 2.5% wt/vol, 8 µL/g IP) and spontaneous respiration. A 2D parasternal short-axis view of the LV was obtained at the level of the papillary muscles. In general, the best views were obtained with the transducer lightly applied to the mid upper left anterior chest wall. The transducer was then gently moved cephalad or caudal and angled until the best images were obtained. After it was ensured that the imaging was on axis (based on roundness of the LV cavity), 2D targeted M-mode tracings were recorded at a paper speed of 50 mm/s. Our previous study has shown that the intraobserver and interobserver variabilities of our echocardiographic measurements for LV dimensions were small, and measurements made in the same animals on separate days were highly reproducible.® Then, under the same anesthesia with Avertin, a 1.4F micromanometer-tipped catheter (Millar Instruments) was inserted into the right carotid artery and then advanced into the LV to measure pressures.®

Infarct Size
To measure the infarct size after 28 days of MI, the heart was excised, and the LVs were cut from apex to base into 3 transverse sections. Five-micrometer sections were cut and stained with Masson’s trichrome. Infarct length was measured along the endocardial and epicardial surfaces from each of the cardiac sections, and the values from all specimens were summed. Infarct size (in percentage) was calculated as total infarct circumference divided by total cardiac circumference.®

In addition, to measure infarct size after 24 hours when most animals were still alive, a separate group of animals including WT-MI (n=6) and Tg-MI (n=6) was created. After 24 hours of coronary artery ligation, Evans blue dye (1%) was perfused into the aorta and coronary arteries, and tissue sections were weighed and then incubated with a 1.5% triphenyltetrazolium chloride solution. The infarct area (pale), the area at risk (not blue), and the total LV area from each section were measured.® In our preliminary study, we confirmed excellent reliability of infarct size measurements, in which a morphometric methodology similar to that used in this study was used. The intraobserver and interobserver variabilities between 2 measurements divided by these means, expressed as a percentage, were <5%.

Histopathology
After in vivo hemodynamic studies, the heart was excised and dissected into the right and left ventricles, including the septum. Five-micrometer sections were cut and stained with Masson’s trichrome. Myocyte cross-sectional area and collagen volume fraction were determined by the quantitative morphometry of LV tissue sections.®

For assessment of mitochondrial ultrastructure by electron microscopy, LV tissues were fixed in a mixture of 1% glutaraldehyde and 4% paraformaldehyde in 0.1 mol/L phosphate buffer at pH 7.4 for 2 hours at room temperature. After they were washed in 0.1 mol/L phosphate buffer containing 0.25 mol/L sucrose, they were postfixed with 1% osmium tetroxide for 2 hours, dehydrated in a graded series of ethanol, and embedded in Epon. Ultrathin sections were double stained with uranyl acetate and lead citrate and then were observed under an electron microscope (Hitachi H7000). For quantitative morphometric analysis, the number and size of the mitochondria were examined according to methods described previously.® The number of mitochondria and the cross-sectional area (size) of each mitochondrion were measured within a sampling region of 100 square sarcomeres (sm²). Eighteen regions were selected at random for each specimen, and for all regions the averages of mitochondrial number and cross-sectional area were calculated.

Apoptosis
To detect apoptosis, LV tissue sections were stained with terminal deoxynucleotidyl transferase–mediated diUTP nick end-labeling (TUNEL) staining. The number of TUNEL-positive cardiac myocyte nuclei was counted, and the data were normalized per 10⁵ total nuclei identified by hematoxylin-positive staining in the same sections. We further examined whether apoptosis is present by the more sensitive ligation-mediated PCR fragmentation assays (Maxim Biotech Inc).®

Statistical Analysis
Data are expressed as mean±SEM. Survival analysis was performed by the Kaplan-Meier method, and between-group difference in survival was tested by the log-rank test. Between-group comparison of means was performed by 1-way ANOVA, followed by t tests. The Bonferroni correction was done for multiple comparisons of means. P<0.05 was considered statistically significant.

Results
Characterization of Human TFAM Tg Mice
Human TFAM cDNA was used to generate Tg mice (Figure 1A). Four lines of Tg mice were confirmed by PCR. These lines were viable and fertile, and there were no detectable differences in cardiac size and structure between Tg and WT mice either macroscopically or microscopically.

We analyzed TFAM protein levels in various tissues by Western blot analysis using anti-human TFAM antibody. We found a robust expression of human TFAM protein in the heart and skeletal muscle, but it was barely detected in the lung, liver, and kidney (Figure 1B). Among 4 established lines of Tg mice, 1 line that expressed the highest level of the human TFAM protein in the heart was used for further
experiments. The endogenous expression level of the mouse Tfam protein was not modified or downregulated by the overexpression of human TFAM gene (Figure 1C). Immunohistochemical studies showed homogeneous human TFAM distribution in cardiac myocytes and colocalized with the mouse mitochondria (Figure 2). Human TFAM staining showed a relatively spotty staining pattern. With higher magnification, its expression appeared not to be restricted to a specific site of mitochondria (Figure 2, inset). These results suggest that the human TFAM exerts an expression pattern similar to that observed for the endogenous mouse Tfam and may function in the mouse heart.

**MtDNA Copy Number and Mitochondrial Enzymes**

We created MI in male Tg mice (Tg-MI) and nontransgenic wild-type littermates (WT-MI). Sham operation without coronary artery ligation was also performed in WT (WT-sham) and Tg (Tg-sham) mice. After 4 weeks of surgery, we measured mtDNA copy number, expressed as the ratio of mtDNA to nuclear DNA (18S rRNA), in the myocardial tissue by a Southern blot analysis. In parallel to an increase in TFAM protein, mtDNA copy number increased in the heart from Tg animals compared with WT controls (Figure 3A). In WT-MI animals, mtDNA copy number in the noninfarcted LV showed a 41% decrease (P<0.01) compared with sham mice, which was significantly prevented and preserved at a normal level in Tg-MI mice (Figure 3A).

To determine the effects of mtDNA copy number alterations on mtRNA, mtRNA transcript levels were measured by Northern blot analysis. As previously reported,9 mtRNA transcript levels, including ND1+ND2, ND4, ND4L, ND5, cytochrome b, COI, COII, and COIII transcripts as well as 16S rRNA, were lower in WT-MI than those in WT-sham. However, overexpression of human TFAM did not increase, and even decreased, these mRNA levels in Tg-sham as well as in Tg-MI (online-only Data Supplement I). These results indicate that the regulation of mtRNA transcripts is dissociated from that of mtDNA copy number.

We next measured the respiratory chain enzyme activities. Despite the significant increase in mtDNA copy number in the heart from Tg, complex I, complex II, complex III, and complex IV demonstrated no significant changes in the enzymatic activity in comparison with WT controls (Figure 3B). Consistent with mtDNA copy number, the enzymatic activities of complex I, complex III, and complex IV were significantly lower in the noninfarcted LV from WT-MI than those from WT-sham. Most importantly, there was no such decrease observed in Tg-MI (Figure 3B). The enzymatic activity of complex II, exclusively encoded by nuclear DNA, was not altered in either group. These results indicate that mtDNA and mitochondrial enzymatic activities are downregulated in the hearts after MI, and human TFAM gene overexpression efficiently counteracts these mitochondrial deficiencies.

The overall number of mitochondria and the overall average size of the mitochondria demonstrated no significant changes in Tg-sham in comparison with WT controls. In contrast, the mitochondrial number was significantly increased and their size was decreased in WT-MI, both of which were attenuated in Tg-MI (online-only Data Supplement II).

**Survival**

The survival analysis was performed in 4 groups of mice during the study period of 4 weeks; WT-sham (n=20), WT-MI (n=21), Tg-sham (n=29), and Tg-MI (n=29). There were no deaths in sham-operated groups. The survival rate was significantly higher in Tg-MI compared with WT-MI (100% versus 66%; P<0.01; Figure 4A).

**Infarct Size**

We determined the infarct size by morphometric analysis in the surviving mice 28 days after MI, and it was comparable between WT-MI and Tg-MI (Figure 4B). To further confirm that overexpression of TFAM gene did not alter the infarct size, both area at risk and infarct area were measured in a separate group of mice 24 hours after coronary artery ligation.
The infarct size (infarct/risk area) was also comparable between WT-MI and Tg-MI mice (84.5 ± 0.4% for n=6 versus 83.2 ± 1.1% for n=6; P=NS; Figure 4C).

Cardiac Function and Structure
The echocardiographic studies of surviving mice at 4 weeks showed that cardiac diameters were significantly increased in WT-MI over the values in WT-sham or Tg-sham. Tg-MI showed less cavity dilatation and improved contractile function compared with WT-MI (Figure 5).

There was no significant difference in heart rate and aortic blood pressure among 4 groups of mice (Table). LV end-diastolic pressure increased in WT-MI and was significantly attenuated in Tg-MI. Coinciding with increased LV end-diastolic pressure, lung weight/body weight increased in WT-MI and was also attenuated in Tg-MI (Table). The prevalence of pleural effusion, a clinical sign of heart failure, was significantly lower in Tg-MI than that in WT-MI (Table).
Cross-sectional area of cardiac myocytes, an index of cellular hypertrophy, increased in the noninfarcted LV from WT-MI and was significantly attenuated in Tg-MI (Figure 6A). Collagen volume fraction, an index of myocardial interstitial fibrosis, also increased in the noninfarcted LV from WT-MI and was significantly smaller in Tg-MI (Figure 6B). These results indicate that TFAM efficiently counteracts structural and functional deterioration in post-MI hearts.

**Apoptosis**

To detect apoptosis, myocardial tissue sections were stained with TUNEL staining. TUNEL-positive nuclei were rarely seen in control mice, whereas their number increased in the noninfarcted LV from WT-MI and was significantly decreased in Tg-MI (Figure 7A). In addition, DNA ladder appeared faint in the noninfarcted LV from Tg-MI compared with that from WT-MI, suggesting the attenuation of apoptosis by TFAM overexpression (Figure 7B).

**Discussion**

The present study provides the first direct evidence that the overexpression of TFAM can prevent the decline in mtDNA as well as mitochondrial respiratory defects in post-MI hearts. TFAM significantly attenuated cardiac chamber dilatation and dysfunction as well as histopathological changes such as myocyte hypertrophy, interstitial fibrosis, and apoptosis. The apparent beneficial effects of TFAM overexpression were not due to its MI size–sparing effect, but they occurred secondary to more adaptive remodeling. All of these beneficial effects could contribute to the improved survival in Tg mice after MI.

Previous studies have suggested an intimate link between mtDNA damage, increased lipid peroxidation, and a decrease in mitochondrial electron transport complex enzyme activities. A growing body of evidence suggests that mtDNA deficiencies and mitochondrial dysfunction play a major role in the development and progression of cardiac failure. A recent study from our laboratory demonstrated a decline in TFAM and mtDNA copy number in a murine heart failure model after MI. These studies imply a relationship between TFAM, mtDNA copy

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**Characteristics of Animal Models**

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Values are mean±SEM. EDP indicates end-diastolic pressure; RV, right ventricle.

**Figure 6.** Summary data for histopathological analysis of LV tissue sections in 4 groups of animals (n=6 for each). Myocyte cross-sectional area (A) and collagen volume fraction (B) are shown. Values are mean±SEM. **P<0.01 vs WT-sham; †P<0.05, ‡P<0.01 vs WT-MI.

**Figure 7.** A, Number of TUNEL-positive myocytes in noninfarcted area of LV from 4 groups of animals (n=8 each). Values are mean±SEM. **P<0.01 for difference from WT-sham values. ††P<0.01 for difference from WT-MI values. B, DNA ladder indicative of apoptosis in genomic DNA from LV.

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number, and mitochondrial function because the magnitude of the mtDNA defects is parallel to quantitative deficiencies in electron transport function. We thus proposed a direct relationship between TFAM content and electron transport chain activity during the post-MI remodeling process, ignoring the possibility of direct ischemic damage to the electron transport chain complexes. The downregulation of TFAM gene expression and a concurrent decrease in mitochondrial genes have been also shown in heart failure induced by aortic banding. In addition, mtDNA depletion has been reported in mitochondrial myopathy and respiratory defects. On the basis of these studies, mtDNA defects are considered to be involved not only in the pathogenesis of the diseases caused by inherited defects of mtDNA but also in those secondary to ischemia or mechanical overload.

TFAM not only regulates mtDNA transcription and replication but also maintains mtDNA copy number. In fact, 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. Moreover, cardiac-specific disruption in the Tfam gene in mice exhibited dilated cardiomyopathy in association with a reduced amount of mtDNA and mitochondrial transcripts. The transfection of antisense plasmids in culture, designed to reduce the expression of TFAM, effectively decreased the levels of mitochondrially encoded transcripts. On the contrary, the forced overexpression of TFAM could produce the opposite effect. Consistent with the present results (Figure 3A, 3B), a recent study by Ekstrand et al 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 directly interact with mtDNA to form nucleoids. Therefore, increased TFAM may increase the steady-state levels of mtDNA by directly binding and stabilizing mtDNA in Tg-sham mice. Our study also showed that overexpression of human TFAM did not increase the respiratory chain complex enzyme activities in Tfam-sham mice (Figure 3C), suggesting that the regulation of mtDNA copy number is dissociated from that of electron transport function. Furthermore, our proposed association between TFAM, mtDNA copy number, and electron transport chain activity may be weakened by our data that TFAM overexpression did not affect mRNA levels (online-only Data Supplement I). There may be complex regulatory mechanisms responsible for the association of TFAM, mtDNA, and mitochondrial function, and further studies are clearly needed to solve this issue.

The results obtained from human TFAM Tg-sham 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. PGC-1α is the transcriptional coactivator and acts upstream of TFAM and also has the capacity to increase mtDNA levels as well as mitochondrial mass in cultured cells and in Tg mice. The reason for the discrepant results between PGC-1 and TFAM transgene overexpression remains unsolved in this study, 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. This may also be due to the difference in the timing of transgene overexpression. Moreover, even though the present study demonstrated the beneficial effects of TFAM overexpression on post-MI LV remodeling, it could not determine whether it must occur before the ischemic insult or only during the post-MI phase.

The present study clearly demonstrated that TFAM overexpression could ameliorate the decline in mtDNA copy number and preserve it at a normal level in hearts from Tg-MI mice (Figure 3A). 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 (Figure 3B). Moreover, our studies establish an important role of TFAM in myocardial protection against remodeling and failure (Figures 4A and 5). The beneficial effects of TFAM overexpression shown in the present study were not due to its MI size–sparing effect because infarct size was comparable between WT-MI and Tg-MI mice (Figure 4B, 4C). Furthermore, its effects were not due to the effects on hemodynamics because blood pressure and heart rate were not altered (Table).

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 (Figure 3A) and mitochondrial electron transport function (Figure 3B), 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. Second, TFAM overexpression may induce mitochondrial biogenesis, which, however, is thought to be unlikely because the number and size of the mitochondria assessed by electron microscopy were not altered in Tg-sham mice (online-only Data Supplement II). Importantly, the beneficial effects of TFAM overexpression on LV remodeling and failure occurred with the attenuation of increased mitochondrial number seen in MI. Furthermore, an increase in mitochondrial number itself did not necessarily exert beneficial effects in MI.

Several pathogenic mtDNA base substitution mutations, such as missense mutations and mtDNA rearrangement mutations (deletions and insertions), have been identified in patients with mitochondrial diseases. An accumulation of the deleted forms of mtDNA in the myocardium frequently results in either cardiac hypertrophy, conduction block, or heart failure. Furthermore, there is now a consensus view that mutations in mtDNA and abnormalities in mitochondrial function are associated with common forms of cardiac diseases, such as ischemic heart disease and dilated cardiomyopathy. In these conditions, however, the strict causal relationship between abnormalities in mtDNA and cardiac dysfunction has yet to be fully elucidated.

The present study supports our earlier conclusions that the deficiencies of mtDNA contribute to cardiac failure. Furthermore, it confirms that the defects in TFAM are critically involved in mitochondrial dysfunction as well as maladaptive cardiac remodeling and failure. More importantly, the increased
TFAM expression could ameliorate the pathophysiological processes seen in heart failure. 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.

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