Effect of TFAM on ATP content in tachypacing primary cultured cardiomyocytes and atrial fibrillation patients

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Abstract. Atrial fibrillation (AF) is one of the most common types of arrhythmia worldwide; although a number of theories have been proposed to explain the mechanisms of AF, the treatment of AF is far from satisfactory. Energy metabolism is associated with the development of AF. Mitochondrial transcription factor A (TFAM) serves a role in the maintenance and transcription of mitochondrial DNA. The present study aimed to investigate the association between TFAM and AF and the effect of TFAM on ATP content in cardiomyocytes. Left atrial appendage tissues were collected from 20 patients with normal sinus rhythm (SR) and 20 patients with AF, and the expression levels of TFAM in SR and AF tissues were evaluated. In addition, a tachypacing model of primary cultured cardiomyocytes was constructed to assess ATP content, cell viability and expression levels of TFAM, mitochondrially encoded (MT)-NADH dehydrogenase 1 (ND1), MT-cytochrome *c* oxidase 1 (CO1), NADH ubiquinone oxidoreductase core subunit 1 (NDUFS1) and cytochrome *c* oxidase subunit 6C (COX6C). Finally, the effects of overexpression and inhibition of TFAM on ATP content, cell viability and the expression levels of MT-ND1 and MT-CO1 were investigated. The expression levels of TFAM were decreased in AF tissues compared with SR tissues ($P<0.05$). The ATP content, cell viability and expression levels of TFAM, MT-ND1 and MT-CO1 were decreased in tachypacing cardiomyocytes compared with non-pacing cardiomyocytes ($P<0.05$), whereas the expression levels of NDUFS1 and COX6C were not changed ($P>0.05$). Overexpression of TFAM increased ATP content, cell viability and expression levels of MT-ND1 and MT-CO1 ($P<0.05$). The inhibition of TFAM decreased ATP content, cell viability and expression levels of MT-ND1 and MT-CO1 ($P<0.05$). In summary, the results of the present study demonstrated that the expression levels of TFAM were decreased in AF tissues and tachypacing cardiomyocytes and that the restoration of TFAM increased the ATP content by upregulating the expression levels of MT-ND1 and MT-CO1 in tachypacing cardiomyocytes. Thus, TFAM may be a novel beneficial target for treatment of patients with AF.

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Introduction

Atrial fibrillation (AF) is one of the most common types of arrhythmia globally, affecting ~33 million individuals worldwide. The incidence of AF increases with age (1-5). AF induces palpitation, heart failure and thrombus formation (6,7), which may impair quality of life (8) and increase the risk of mortality. In fact, one study revealed that at 10 years of follow-up, 61.5% of men with AF had died compared with 30% of men without AF, whereas in women, 57.6% of those with AF had died compared with 20.9% women without AF, which is an approximate doubling of the mortality rate in both sexes (9,10). Since the etiology of AF is complex, it is important to determine the underlying pathogenic mechanisms.

Numerous studies have demonstrated that AF is associated with unbalanced energy supply and consumption (11-13); the downregulation of mitochondrial electron transport chain activity has been found in AF (14,15). Therefore, alterations in energy metabolism, particularly mitochondrial dysfunction, may contribute to the pathogenesis of AF. It has been reported that the development of AF is commonly accompanied by alterations in gene expression levels, such as potassium voltage-gated channel subfamily Q member 1, potassium inwardly rectifying channel subfamily J member 3, collagen type XV α1 chain and matrix metalloproteinase, thus resulting in abnormal protein expression levels (16,17). Mitochondrial transcription factor A (TFAM) is essential for the maintenance of mitochondrial DNA (mtDNA) and regulates mtDNA transcription (18). A number of reports have demonstrated that TFAM dysfunction leads to mitochondrial impairment, which causes cardiovascular diseases, such as heart failure and cardiomyopathy (19-21); however, its role in AF is unknown. The aim of the present study was to investigate the association between TFAM and AF and the effect of TFAM on ATP content in cardiomyocytes.

Materials and methods

Ethics statement. The present study was approved by the Ethics Committee of the First Affiliated Hospital of China Medical University (Shenyang, China; approval no. 2017-69-2) and performed in accordance with Declaration of Helsinki (22). Written informed consent was obtained from all patients prior to tissue collection.

Specimen collection. Between June 2017 and May 2019, left atrial appendage (LAA) tissues were collected from 20 patients with AF who underwent mitral valve repair and maze IV procedure, and from 20 patients with sinus rhythm (SR) and left atrial thrombus who underwent mitral valve repair, LAA resection and thrombectomy. The samples were divided into two parts: One part was quickly frozen in liquid nitrogen and then stored at -80˚C; the other part was fixed with 10% formalin at 4˚C for 48 h for further use. AF was diagnosed using a 12-lead electrocardiogram and 72 h-holter.

Primary culture of rat atrial cardiomyocytes. A total of 60 male Sprague Dawley neonatal rats (age, 1 day old; weight, 5.5±0.8 g) were procured from Liaoning Changsheng Biotechnology Co., Ltd. The animal protocol was approved by the Ethics Review Committee for Animal Experimentation of China Medical University. All animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

Neonatal rats were sterilized and sacrificed by cervical dislocation. The whole hearts were excised from the body and atria were isolated from the ventricles. Cardiomyocytes were dissociated with 0.08% trypsin and 0.1% type II collagenase. The cells were resuspended in DMEM (Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (HyClone; Cytiva) and cultured in 6-well plate at a density of $1x10^6$ cells/ml, then 5-BrdU was added at a final concentration of 0.1 mmol/l. The medium was replenished after 24 h and thereafter every 48 h for up to 2 weeks. Beating myocardial cells were observed with an inverted light microscope (DFC295; Leica Microsystems GmbH), and video graphs of beating cells were recorded with a digital camera (Coolpix5400; Nikon Corporation). Detailed procedures are presented in Data S1.

Tachypacing of cardiomyocytes. The spontaneous firing rate of primary cultured cardiomyocytes was ~1 Hz, which was measured by counting the beating frequency of cardiomyocytes with a light microscope (magnification, x200). The cardiomyocytes were cultured in 6-well plates and subjected to electrical field stimulation using a YC-2 stimulator (Cheng Yi). The cardiomyocytes were stimulated at 6 Hz for 24 h as previously described (23,24) using the following parameters: 1.5 V/cm field strength, square-wave, 5-ms pulses. Non-pacing cells were used as a control.

Cell transfection. The TFAM overexpression plasmid pEXP-RB-Mam-TFAM, pEXP-RB-Mam-negative control (NC), three small interfering (si)RNAs for the inhibition of the expression levels of TFAM and siRNA-NC (cat. no. siN0000001-1-5) were commercially constructed by Guangzhou RiboBio Co., Ltd. The siRNA sequences were as follows: siRNA 1, forward 5'-GGAAGAGCAAAUGGCUGAA-3', reverse 5'-CCUUCUCGUUUACCGACUU-3'; siRNA 2, forward 5'-GGCAGAAACGCCUAAAGAA-3', reverse 5'-CCGUCUUUGCGGAUUUCUU-3'; and siRNA 3, forward 5'-CCUGUCAGCCUUAUCUGTA-3', reverse 5'-GGACAGUCGGAAUAGACAU-3'.

Cells were seeded in a 6-well plate at a density of $1x10^5$ cells/well and transfected (2,500 ng pEXP-RB-Mam-TFAM and pEXP-RB-Mam-NC or 50 nM siRNA) with Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) and serum-free Opti-MEM (Gibco; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. After 6 h transfection, the medium was replaced with DMEM containing 10% FBS. After 48 h, cells transfected with pEXP-RB-Mam-TFAM were used for tachypacing, and cells transfected with siRNAs were cultured in non-paced conditions.

Cell viability assay. Cell viability was assessed by Cell Counting Kit 8 (CCK-8) assay (Vazyme Biotech Co., Ltd.), according to the manufacturer's protocol. Cells were seeded into a 96-well plate at a density of $1x10^4$ cells/well in a final volume of 100 μl. Cells were cultured in 5% CO_2 at 37˚C for 12 h and then stimulated at 6 Hz for 8, 16 and 24 h. A total of 10 μl CCK-8 solution was added to each well and cultured for an additional 2 h at 37˚C. Absorbance was measured at 450 nm using an Infinite F200 PRO multimode reader (Tecan Group, Ltd.) and the viability was calculated using the following equation: $Absorbance_{8/16/24\ h}/Absorbance_{0\ h}$. Each experiment was repeated at least three times.

Measurement of ATP content. ATP content was assessed using an ATP Assay kit (Beyotime Institute of Biotechnology) according to the manufacturer's instructions. Luciferase/luciferin chemiluminescence method was used for measurement (25,26). Cells were homogenized in lysis buffer (provided in kit) on ice. Lysates were centrifuged at 12,000 x g for 10 min at 4˚C. The supernatants were collected and used to test protein concentration and ATP content, separately. Proteins were quantified using a bicinchoninic acid protein assay kit (Beyotime Institute of Biotechnology). Each experiment was repeated at least three times.

Reverse transcription-quantitative (RT-q)PCR. Total RNA was isolated from LAA tissues and primary cultured cardiomyocytes using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Total RNA was reverse transcribed into cDNA using the PrimeScript™ RT reagent kit (Takara Bio, Inc.) at 37˚C for 15 min, 85˚C for 5 sec and 4˚C for 1 min. RT-qPCR was performed using a TB Green™ Premix Ex II reagent kit (Takara Bio, Inc.). β-actin was used as the internal control. The data were analyzed using a LightCycler® 480 Real-Time PCR System (Roche Diagnostics) and the relative changes in mRNA expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (27). The following thermocycling conditions were used for the qPCR: Initial denaturation at 95˚C for 30 sec; followed by 50 cycles of 95˚C for 5 sec and 60˚C for 30 sec; 1 cycle of 95˚C for 5 sec and 60˚C for 1 min; and a final cycle at 50˚C for 30 sec.

The primers were as follows: Hsa-TFAM, forward 5'-TTCCAAGAAGCTAAGGGTGATT-3', reverse 5'-AGAAGATCCTTTCGTCCAACTT-3'; hsa-β-actin, forward 5'-CCTGGCACCCAGCACAAT-3', reverse 5'-GGGCCGGACTCGTCATAC-3'; rno-TFAM, forward 5'-GTGATCTCATCCGTCGCAGTGTG-3', reverse 5'-TGCCCAATCCCAATGACAACTCTG-3'; and rno-β-actin, forward, 5'-TGTCACCAACTGGGACGATA-3', reverse 5'-GGGGTGTTGAAGGTCTCAAA-3'.

Western blotting. Western blotting analysis was performed as previously described (28). Tissues or cells were homogenized on ice in RIPA lysis buffer supplemented with phenylmethylsulfonyl fluoride (Beijing Solarbio Science & Technology Co. Ltd.). Lysates were centrifuged at 12,000 x g for 10 min at 4˚C. The supernatants were collected, and protein concentrations were quantified using an enhanced BCA protein assay kit (Beyotime Institute of Biotechnology. Proteins (40 μg/lane) were separated by 10% SDS-PAGE and transferred onto polyvinylidene fluoride membranes by electroblotting. The membranes were blocked in 5% skim milk dissolved in TBS-0.1% Tween-20 for 2 h at room temperature. Membranes were incubated at 4˚C overnight with primary antibodies against TFAM (cat. no. ab131607; Abcam; 1:2,000), mitochondrially-encoded (MT)-NADH dehydrogenase 1 (ND1) (cat. no. ab181848; Abcam; 1:10,000), MT-cytochrome *c* oxidase 1 (CO1) (cat. no. ab203912; Abcam; 1:1,000), NADH ubiquinone oxidoreductase core subunit 1 (NDUFS1; cat. no. ab169540; Abcam; 1:20,000) and cytochrome *c* oxidase subunit 6C (COX6C) (cat. no. ab150422; Abcam; 1:5,000). β-actin (ProteinTech Group, Inc.; 1:10,000) was used as a loading control and for normalization. Membranes were subsequently incubated with horseradish peroxidase-conjugated Affinipure goat anti-rabbit secondary antibodies (ProteinTech Group, Inc.; cat. no. SA00001-2; 1:10,000) at room temperature for 2 h. Protein bands were visualized by exposure to ECL buffer (Beyotime Institute of Biotechnology) and the signals were captured by MicroChemi system (DNR Bio-Imaging Systems, Ltd.). The expression levels were analyzed using ImageJ software (version 1.52a, National Institutes of Health).

Immunohistochemistry and immunocytochemistry. Tissues were fixed with 10% formalin at 4˚C for 48 h and then dehydrated with an increasing series of alcohol at room temperature, made transparent with dimethylbenzene at room temperature and paraffin embedded. Sections were cut to a thickness of 5-μm and blocked with 5% goat serum (Fuzhou Maixin Biotech Co., Ltd.) for 10 min at room temperature. Meanwhile, the cells were fixed with 4% paraformaldehyde at 4˚C for 15 min, permeated with 0.5% Triton X-100 and blocked with 5% goat serum for 30 min at room temperature. UltraSensitive™ S-P kit (Fuzhou Maixin Biotech Co., Ltd.) was used for immunohistochemical staining. Slides were incubated with the primary antibody to TFAM (cat. no. ab176558; Abcam; 1:300) at 4˚C overnight. Cells (10^6 cells/ml) were incubated with primary antibody against α-sarcomeric actin (cat. no. BM0001; Wuhan Boster Biological Technology, Ltd.; 1:500) at 4˚C overnight. PBS without primary antibodies was used as a negative control. Following the primary antibody incubation, the membranes were incubated with biotin-labeled goat anti-mouse/rabbit IgG secondary antibodies (solution C in S-P kit) for 10 min at room temperature, followed by a further incubation with anti-biotin-peroxidase complex (solution D in S-P kit) for 10 min at room temperature. 3,3-diaminobenzidine was used as a chromogen (Fuzhou Maixin Biotech Co., Ltd.). Subsequently, the slides or cells were counterstained with 4% hematoxylin for 2 min at room temperature (Beijing Solarbio Science & Technology Co., Ltd.), blued in 1% ammonium hydroxide for 3 min at room temperature, dehydrated and mounted. All sections were observed, and images were captured with an Olympus BX51 light microscope (magnification, x400; Olympus Corporation). Average optical density (AOD) of TFAM protein was calculated by ImageJ software (version 1.52a, National Institutes of Health).

Statistical analysis. Data analysis was performed using SPSS software (version 22.0; IBM Corp.). All continuous data are presented as the mean ± standard deviation of at least three independent repeats. Unpaired Student's t-test was used for two-group comparisons. ANOVA was used for comparison of multiple groups followed by post hoc Bonferroni's correction. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Expression levels of TFAM in SR and AF tissues. RT-qPCR results indicated that the mRNA expression levels of TFAM were decreased in AF compared with SR tissue ($P<0.05$; Fig. 1A). Western blotting demonstrated that the protein expression levels of TFAM were also decreased in AF compared with SR tissue ($P<0.05$) (Fig. 1B and C). Immunohistochemical staining indicated that the TFAM protein was expressed in the cytoplasm, and the AOD of TFAM protein in SR tissue was 63.96±2.89%, whereas that in AF tissue was 26.04±3.52% ($P<0.05$; Fig. 1D-F).

Identification of cardiomyocytes. The identification of cardiomyocytes was performed by observation of beating cells and immunocytochemistry. The myocardial cells observed under an inverted microscope are shown in Fig. 2A, and video graphs of beating cells are presented in Video S1. Immunocytochemical staining of cells showed the presence of myocardial cell-specific protein α-sarcomeric actin (Fig. 2B), which demonstrated that the primary cultured cells were cardiomyocytes.

Expression levels of TFAM, ATP content and cell viability of tachypacing cardiomyocytes. RT-qPCR results indicated that the mRNA expression levels of TFAM were decreased in tachypacing cardiomyocytes compared with non-pacing cardiomyocytes ($P<0.05$; Fig. 2C). Western blotting showed that the protein expression levels of TFAM were also decreased in tachypacing cardiomyocytes compared with non-pacing cardiomyocytes ($P<0.05$) (Fig. 2F).

To explore the energy status, ATP content in cardiomyocytes was evaluated. The results indicated that ATP content was significantly lower in tachypacing cardiomyocytes (4.09±0.13 nmol/mg) compared with that in non-pacing cardiomyocytes (10.98±0.37 nmol/mg) ($P<0.05$; Fig. 2D).

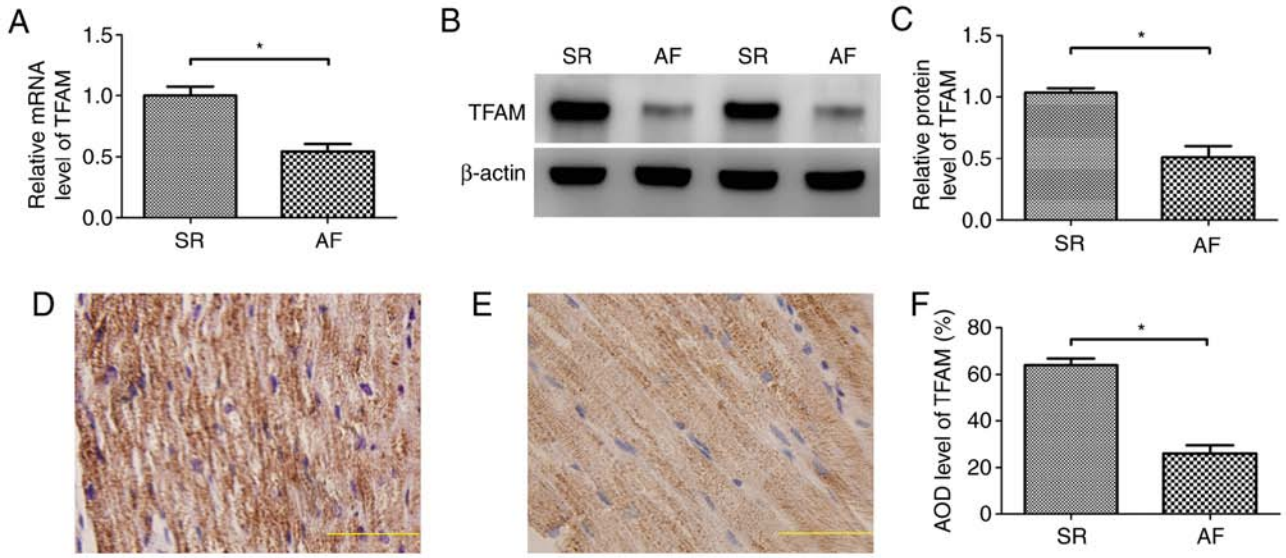

Figure 1. Expression levels of TFAM in SR and AF tissues. (A) mRNA expression levels of TFAM in SR and AF tissues. (B) Representative western blotting images and (C) semi-quantification of protein levels of TFAM in SR and AF tissues; β-actin was used as a loading control. Immunohistochemical analysis of TFAM protein expression in (D) SR and (E) AF tissue; magnification, x400; scale bar, 25 μm. (F) AOD of TFAM protein in SR and AF tissue. Data are presented as the mean ± standard deviation. *P<0.05 vs. SR. AF, atrial fibrillation; AOD, average optical density; SR, sinus rhythm; TFAM, mitochondrial transcription factor A.

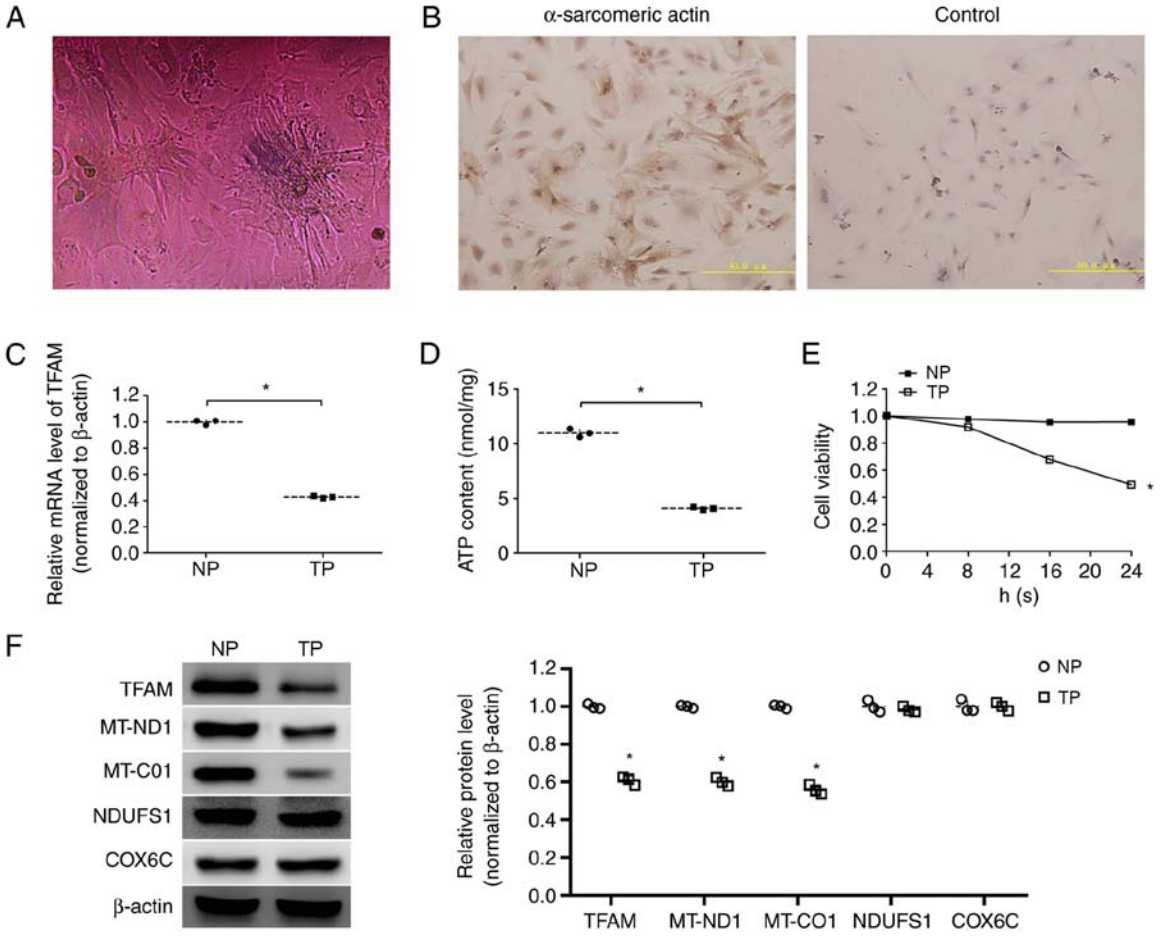

Figure 2. Differential expression of mRNA, proteins, ATP content and cell viability in NP and TP cardiomyocytes. (A) Photomicrograph of primary cultured cardiomyocytes; magnification, x200. (B) Positive staining with anti-α-sarcomeric actin antibody and negative control; magnification, x200; scale bar, 50 μm. (C) mRNA expression levels of TFAM in NP and TP cardiomyocytes. (D) ATP content in NP and TP cardiomyocytes. (E) Viability of primary cultured cardiomyocytes in NP and TP groups. (F) Representative western blotting images and semi-quantitative analysis of protein expression levels of TFAM, MT-ND1, MT-CO1, NDUFS1 and COX6C in NP and TP cardiomyocytes. Data are presented as the mean ± standard deviation. *P<0.05 vs. NP cardiomyocytes. COX6C, cytochrome c oxidase subunit 6C; MT-CO1, mitochondrially encoded-cytochrome c oxidase 1; MT-ND1, mitochondrially encoded-NADH dehydrogenase 1; NDUFS1, NADH ubiquinone oxidoreductase core subunit 1; NP, non-pacing; TFAM, mitochondrial transcription factor A; TP, tachypacing.

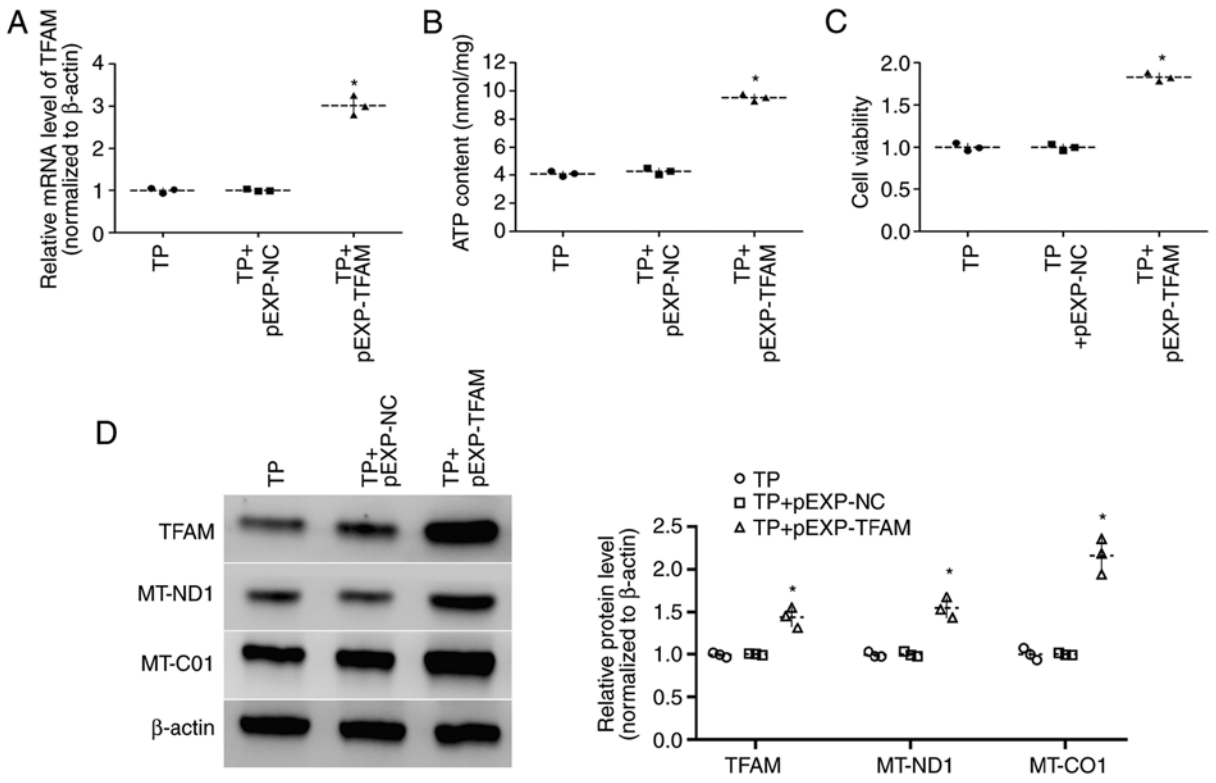

Figure 3. Effects of TFAM overexpression on TP cardiomyocytes. (A) mRNA expression levels of TFAM in TP cardiomyocytes transfected with pEXP-TFAM or pEXP-NC. (B) ATP content and (C) viability of TP cardiomyocytes transfected with pEXP-TFAM or pEXP-NC. (D) Protein expression levels of TFAM, MT-ND1 and MT-CO1 in TP cardiomyocytes transfected with pEXP-TFAM or pEXP-NC. Data are presented as the mean ± standard deviation. *P<0.05 vs. TP + pEXP-NC. MT-CO1, mitochondrially encoded-cytochrome c oxidase 1; MT-ND1, mitochondrially encoded-NADH dehydrogenase 1; pEXP-NC, pEXP-RB-Mam-NC; pEXP-TFAM, pEXP-RB-Mam-TFAM; TFAM, mitochondrial transcription factor A; TP, tachypacing.

The viability of cells in the non-pacing and the tachypacing groups is presented in Fig. 2E. By measuring the viability at different times, it was demonstrated that the cell viability in the non-pacing group remained stable over 24 h; however, that in the tachypacing group decreased gradually. There was a significant difference between two groups following 24 h tachypacing (P<0.05).

Expression levels of subunits of oxidative respiratory chain complexes in tachypacing cardiomyocytes. The protein expression levels of MT-ND1, MT-CO1, NDUFS1 and COX6C were measured by western blotting, which demonstrated that the protein expression levels of MT-ND1 and MT-CO1 were lower in tachypacing cardiomyocytes compared with expression in the non-pacing cardiomyocytes (P<0.05), whereas there was no difference in the protein expression levels of NDUFS1 and COX6C between the two groups (Fig. 2F).

Effect of TFAM on ATP content and viability in cardiomyocytes. To investigate the effect of TFAM on ATP content and viability of cardiomyocytes, cells that were transfected with pEXP-RB-Mam-TFAM were used for tachypacing and cells transfected with TFAM siRNA were cultured in non-pacing conditions. Transfection of pEXP-RB-Mam-TFAM upregulated TFAM expression levels and increased viability and ATP content in tachypacing cells compared with cells transfected with pEXP-RB-Mam-NC (9.52±0.25 nmol/mg vs. 4.25±0.23 nmol/mg, respectively) (all P<0.05; Fig. 3A-D). By contrast, TFAM siRNA transfection downregulated TFAM expression levels and decreased the viability and ATP content of non-pacing cells compared with siRNA-NC transfection (all P<0.05; Fig. 4A-D). These results indicated that TFAM may be involved in the energy synthesis of cardiomyocytes.

Effect of TFAM on the expression levels of MT-ND1 and MT-CO1 in cardiomyocytes. The expression levels of MT-ND1 and MT-CO1 were measured by western blotting, which indicated that the expression levels of MT-ND1 and MT-CO1 were increased in tachypacing cells transfected with pEXP-RB-Mam-TFAM compared with pEXP-RB-Mam-NC-transfected cells (P<0.05; Fig. 3D). However, compared with siRNA-NC transfection, MT-ND1 and MT-CO1 levels were decreased in non-pacing cells transfected with TFAM siRNAs (P<0.05; Fig. 4D).

Discussion

AF is a notable medical problem presenting a burden to the individual and the society (29). However, current therapeutics and radial frequency ablation used for the treatment of AF are unable to provide a complete cure and may cause unexpected complications (30-32). Thus, the treatment of AF is far from satisfactory owing to insufficient knowledge of the mechanism underlying AF; further research on the pathogenesis of AF and molecular level of dysfunction may provide novel methods for treatment. Combined metabolomic and proteomic analysis

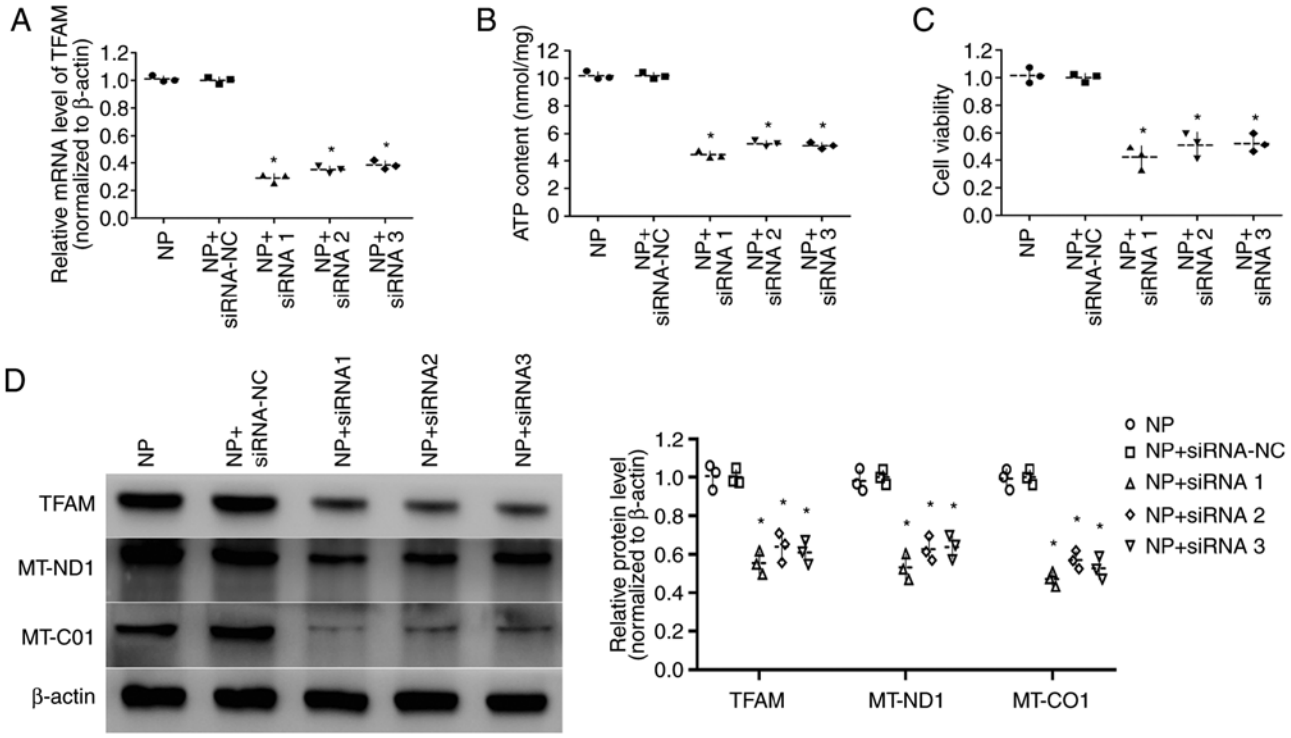

Figure 4. Effects of inhibition of TFAM expression on NP cardiomyocytes. (A) mRNA expression level of TFAM in NP cardiomyocytes transfected with siRNAs. (B) ATP content and (C) cell viability of NP cardiomyocytes transfected with siRNAs. (D) Representative western blotting images and protein expression levels of TFAM, MT-ND1 and MT-CO1 in NP cardiomyocytes transfected with siRNAs. Data are presented as the mean ± standard deviation. *P<0.05 vs. NP + siRNA-NC. MT-CO1, mitochondrially encoded-cytochrome c oxidase 1; MT-ND1, mitochondrially encoded-NADH dehydrogenase 1; NP, non-pacing; siRNA, small interfering RNA; TFAM, mitochondrial transcription factor A.

of AF has demonstrated that energy metabolism-associated proteins are altered in AF tissues (33,34), which indicated that energy metabolism serves a role in the pathophysiology of AF.

The present study measured the expression levels of TFAM in tissues and cells. TFAM is a transcription factor which is produced in the nucleus and transported to the mitochondria (35,36). TFAM is essential for mtDNA maintenance; it serves a key role in mtDNA stability and modifies mitochondrial gene expression levels (37,38). Previous reports have demonstrated that TFAM dysfunction causes cardiovascular diseases, such as heart failure and cardiomyopathy (39,40). However, it remains unclear whether TFAM is involved in the progression of AF. Results from the present study demonstrated that expression levels of TFAM were decreased in both AF tissue and tachypacing cardiomyocytes. Without the protection of TFAM, mtDNA becomes unstable and degrades (35), resulting in decreased ATP synthesis, which exacerbates the development of AF by decreasing ion pump efficiency and cardiomyocyte contraction.

Primary cultured cardiomyocytes have been used to study cardiac bioenergetics (41). The whole heart contains a mixture of myocytes and non-myocytes; however, primary cultured cardiomyocytes are pure, with minimal contamination of fibroblasts and endothelial cells (42,43). α-sarcomeric actin is specifically expressed in myocardial cells, and was therefore selected to distinguish cardiomyocytes and non-myocytes in primary cultured cells in the present study (44). A tachypacing model of cardiomyocytes was constructed to investigate the pathological changes similar to those in patients with AF. Because the spontaneous frequency of primary cultured cardiomyocytes was 1 Hz, 6 Hz was used to produce a similar frequency increment (6-fold increase) to that which occurs in humans with AF (23,24).

The present study measured the ATP content in cardiomyocytes because in order to sustain cardiac function, cardiomyocytes need a constant supply of energy, primarily in the form of ATP (45). Emelyanova *et al* (14) reported that the overall functional activity of the electron transport chain (ETC) was reduced by 30% in AF tissues compared with non-AF tissues, which was accompanied by reduced ATP production (14). In addition, Schild *et al* (46) concluded that the rapid pacing of cardiomyocytes decreased mitochondrial ATP synthesis (46). In the study, ATP content was decreased in tachypacing cardiomyocytes compared with non-pacing cardiomyocytes. Furthermore, the effect of TFAM on ATP content was investigated. The overexpression of TFAM in tachypacing cardiomyocytes increased the ATP content, whereas inhibition of TFAM in non-pacing cardiomyocytes decreased the ATP content. These findings indicated that the expression levels of TFAM may regulate ATP content in cardiomyocytes. Viability of cardiomyocytes was also assessed; the cell viability decreased gradually in tachypacing cardiomyocytes and was restored by overexpression of TFAM.

The majority (>90%) of the cellular ATP used in the heart is produced through oxidative phosphorylation by the mitochondria (47). To investigate the downstream factors of TFAM that affect ATP synthesis, expression levels of certain subunits [encoded by both nuclear DNA (NDUFS1 and COX6C) and mtDNA (MT-ND1 and MT-CO1)] of oxidative respiratory chain complexes were measured in the present study. Previous

studies revealed that the activities of complex I and II were selectively reduced in AF and the function of ETC was impaired in tachypacing cardiomyocytes (14,46). In the present study, the protein levels of MT-ND1 and MT-CO1 were decreased in tachypacing cardiomyocytes whereas there was no difference in expression levels of NDUFS1 and COX6C between the two groups. Furthermore, the effect of TFAM on the expression of MT-ND1 and MT-CO1 was investigated. The overexpression of TFAM in tachypacing cardiomyocytes increased the expression levels of MT-ND1 and MT-CO1, whereas inhibition of TFAM in non-pacing cardiomyocytes decreased the expression levels of MT-ND1 and MT-CO1. These results indicated that the decrease in ATP content was induced by decreased MT-ND1 and MT-CO1 in tachypacing cardiomyocytes, and that TFAM may regulate ATP content through the expression levels of MT-ND1 and MT-CO1 in cardiomyocytes.

In summary, the present results demonstrated that the expression levels of TFAM were decreased in AF tissue and tachypacing cardiomyocytes and that the restoration of TFAM increased ATP content by upregulating the levels of MT-ND1 and MT-CO1 in tachypacing cardiomyocytes. Thus, TFAM may be a novel beneficial target for treatment of patients with AF. The expression levels of TFAM have an effect on the content of mtDNA, that is, the overexpression of TFAM increased the mtDNA content, whereas the knockdown of TFAM decreased the content; however, further investigation is required to determine if expression levels of TFAM affect the content of mtDNA in tachypacing cardiomyocytes. The present study only measured 2 of the 13 subunits encoded by mtDNA; further research is required to assess the other subunits, and animal models are needed to further elucidate the function of TFAM in AF.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YL and YZ wrote and edited the manuscript and designed the study. RT and XJ performed reverse transcription-quantitative PCR and western blotting. YW cultured the cardiomyocytes and measured the ATP content. TG is the guarantor of this work and analyzed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Human and animal studies were approved by the Ethics Committee of The First Affiliated Hospital of China Medical University (Shenyang, China; approval no. 2017-69-2) and were performed in accordance with Declaration of Helsinki. Written informed consent was obtained from all patients prior to tissue collection.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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