Int J Clin Exp Med 2016;9(4):7511-7517
www.ijcem.com /ISSN:1940-5901/IJCEM0022174

Original Article

Impaired mtDNA replication precedes mtDNA depletion in pressure overload-induced cardiac hypertrophy and heart failure

Jing Shi*, Hui Liu*, Hui Wang, Xinli Li, Xiangqing Kong

*Department of Cardiology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu, China. *Equal contributors.*

Received December 17, 2015; Accepted March 19, 2016; Epub April 15, 2016; Published April 30, 2016

Abstract: Accumulating evidence indicates that mitochondrial dysfunction participates in the development of heart failure. However, there is limited knowledge about mtDNA changes during the progression from cardiac hypertrophy to heart failure. Transverse aortic constriction or sham operation was performed on 8-week-old mice to induce cardiac hypertrophy and heart failure as determined by echocardiographic analysis. Only the heart failure group exhibited a significant decrease in succinate dehydrogenase activity, mtDNA content normalized to nDNA, and the expression of mtDNA-encoded genes. However, down-regulation of the genes involved in the mtDNA replication fork exhibited an impairment of mtDNA replication in both stages following pressure overload. Significantly decreased expression levels of mRNA and protein of the PGC-1 pathway suggested that attenuation of this pathway might contribute to the impaired mitochondrial biogenesis and dysfunction in cardiac hypertrophy and heart failure. In conclusion, in our two-stage mouse model, depletion of mitochondrial mass and DNA content was only detected in the heart failure stage. The mtDNA replication and transcriptional regulation of mitochondrial biogenesis was determined to be down-regulated from an early stage following pressure overload.

Keywords: mtDNA, pressure overload, PGC-1

Introduction

Heart failure (HF) is a consequence of exposure to pressure overload caused by various cardiovascular pathologies, such as hypertension and ischemia [1]. After pressure overload, the heart initially performs a compensatory response to the additional load by increasing myocyte size and mass with a relatively normal cardiovascular function, which is typically referred to as cardiac hypertrophy [1]. However, when pressure overload persists, cardiomyocyte apoptosis and interstitial fibrosis lead to a failing heart, which is characterized by ventricular dilation and a decrease in contractile function [1]. HF has become a major public health problem and affects over 23 million patients worldwide [2]. Understanding the molecular and cellular processes that contribute to this transition of cardiac hypertrophy to heart failure could help develop novel therapies for HF [1].

Mitochondrial dysfunction has been widely considered to play an important role in the development of HF [3-6], and this may actually start during cardiomyopathy and contribute to the pathogenesis of subsequent HF [7-9]. But there is still a lack of evidence for the alterations of mtDNA content and mitochondrial biogenesis in the transition of cardiac hypertrophy to HF in the rodent model. In this study, we established an animal model that can accurately reflect the development from cardiac hypertrophy to HF after pressure overload. Depletion of mitochondrial mass and DNA (mtDNA) content was only detected in the heart failure stage. We measured mtDNA replication and transcriptional regulation of mitochondrial biogenesis at an early time point after TAC, in order to determine if this is a possible underlying mechanism for the transition from hypertrophy to heart failure.

Methods

Experimental animals

Male C57BL6/J mice, aged 10-12 weeks old, were purchased from the Model Animal Re-

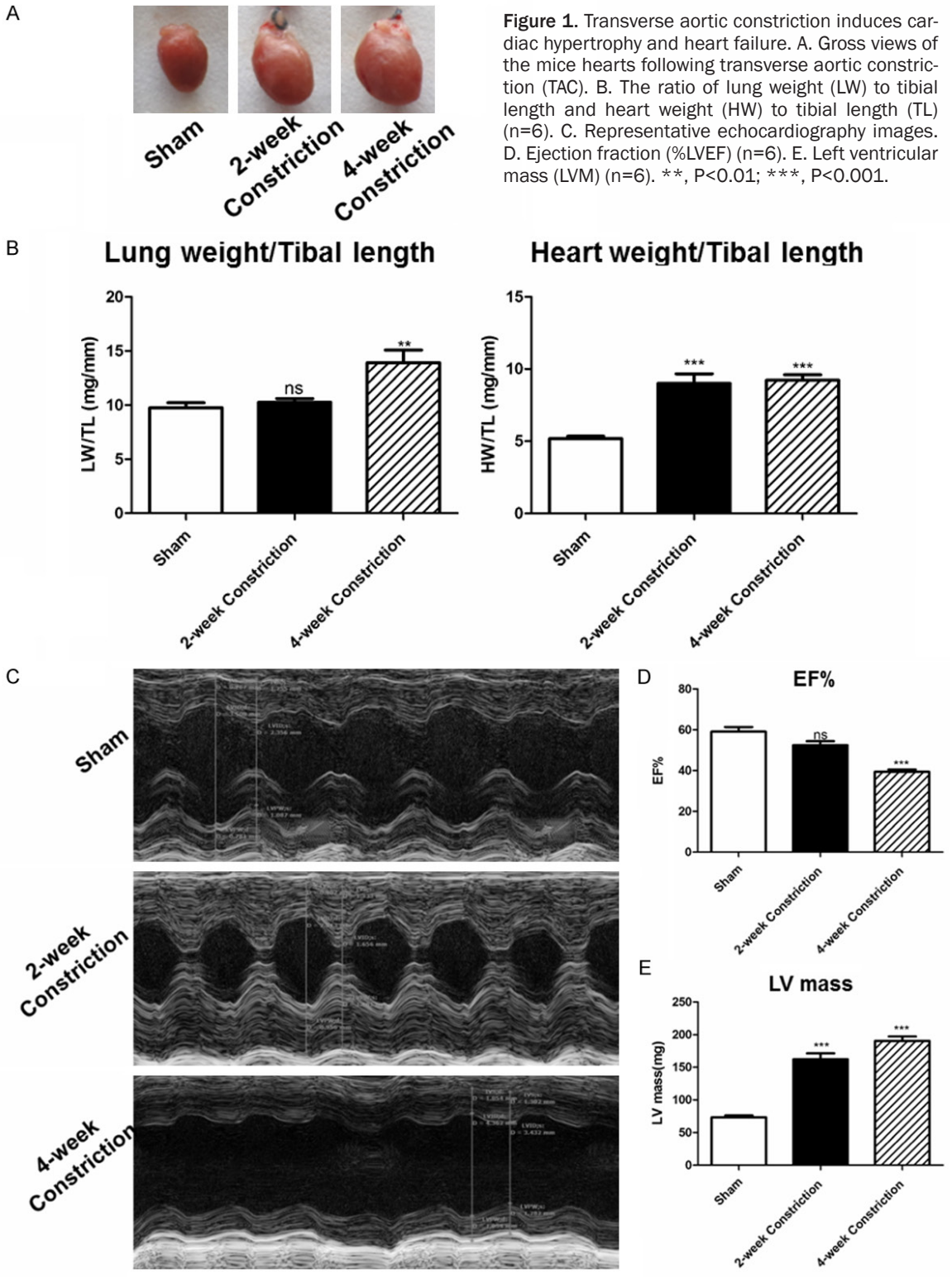

Figure 1. Transverse aortic constriction induces cardiac hypertrophy and heart failure. A. Gross views of the mice hearts following transverse aortic constriction (TAC). B. The ratio of lung weight (LW) to tibial length and heart weight (HW) to tibial length (TL) (n=6). C. Representative echocardiography images. D. Ejection fraction (%LVEF) (n=6). E. Left ventricular mass (LVM) (n=6). **, P<0.01; ***, P<0.001.

search Center of Nanjing University. Mice were maintained in autoclaved cages under a 12 h/12 h light/dark cycle and had free access to standard chow and water. This study was approved by the ethical committees of Nanjing Medical University and all animal experiments were conducted under the established guidelines on the use and care of laboratory animals

for biomedical research published by the National Institutes of Health (No. 85-23, revised 1996). Transverse aortic constriction (TAC) was performed on 8-week-old male C57BL/6J mice purchased and raised at the Experimental Animal Center of Nanjing Medical University (Nanjing, China) as previously described [10].

Echocardiography

Echocardiography was performed in mice 2 weeks or 4 weeks after TAC operation using a Vevo 2100 (VisualSonics Inc, Toronto, Ontario, Canada) with a 30 MHz central frequency scan head. M-mode LV end-systolic and end-diastolic dimensions were averaged over 3 cardiac cycles. Ejection fraction (%LVEF) and left ventricular mass (LVM) were calculated as described previously [11]. Following this, mice were sacrificed, and heart weight (HW), lung weight (LW), and tibial length (TL) were determined.

Real-time reverse transcription polymerase chain reaction (RT-PCR)

Left ventricles (LVs) harvested from subject mice were snap frozen in liquid nitrogen. RNA was isolated with the RNeasy Mini Kit (Qiagen, Hilden, Germany) and then reverse-transcribed into cDNA with an iScript cDNA synthesis kit (Bio-Rad Laboratories, CA, USA). Each PCR was performed with specific primers using iQ SYBR Green Supermix (Bio-Rad Laboratories, CA, USA) following standard procedures (7900 HT, Applied Biosystems, CA, USA). All mRNA levels were standardized to endogenous control (18s) and expressed as fold changes. The mtDNA was measured in DNA isolated with the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) by qRT-PCR, using primers amplifying the Cytochrome C Oxidase subunit I (COI) region, and then normalized to nDNA (18S) [12].

Western blot

Protein content from all LVs was assessed using a BCA-kit (Thermo Scientific, MA, USA), followed by protein concentration normalization prior to all western blot experiments. Western blot was carried out following a standard procedure. Antibody against PGC-1α (1:1000, cat. NBP1-04676, NOVUS) was used as primary antibody and GAPDH (1:10000, Kangchen, Nanjing, China) was used as loading control. The blots were developed with enhanced chemiluminescence reagent (Thermo Scientific, MA, USA) and exposed on a Chemi-Doc MP imager (Bio-Rad Laboratories, CA, USA). Image Lab™ software was used to quantify band density. All blots were carried out independently three times.

Enzyme activity determination

Succinate dehydrogenase (SDH) enzyme activities were measured in tissue homogenate spectrophotometrically at room temperature (400 nm) using saturating concentrations of substrates and cofactors with an A022 kit (JianChen, Nanjing, China) as previously described [13]. Succinate dehydrogenase activity was calculated as units per microgram of protein.

Statistical analyses

Results are presented as mean ± SEM. Unpaired t-tests were used for comparisons between two groups, with a significant level of 0.05. One-way ANOVA was used to compare three or more groups, followed by Bonferroni's tests to compare the differences between every two groups. All analyses were performed using GraphPad Prism 5.0. Differences were considered significant at $P<0.05$.

Results

TAC induced cardiac hypertrophy and HF

Tissue weight and echocardiography analysis were used to evaluate sham or TAC mice. By gross inspection, mice subjected to TAC surgery exhibited a significant enlargement in the hearts compared to the sham-operated mice (**Figure 1A**). Similarly, heart weight (HW) to tibial length (TL) ratios were significantly greater in TAC mice compared to sham-operated mice (**Figure 1C**), while the ratio of lung weight (LW) to tibial length (TL) was significantly higher only in later stages (4 weeks) but stayed unchanged in early stages (2 weeks) following TAC (**Figure 1B**). Cardiac systolic function was evaluated by echocardiography. Both groups (2 weeks and 4 weeks after TAC) had an attenuated ejection fraction (%LVEF) from 59% to 52% or 39%, respectively, but only the 4-week group reached statistical significance (**Figure 1D**). The left ventricular mass (LVM) was significantly increased by 221% in early stages (2 weeks) and 259% in

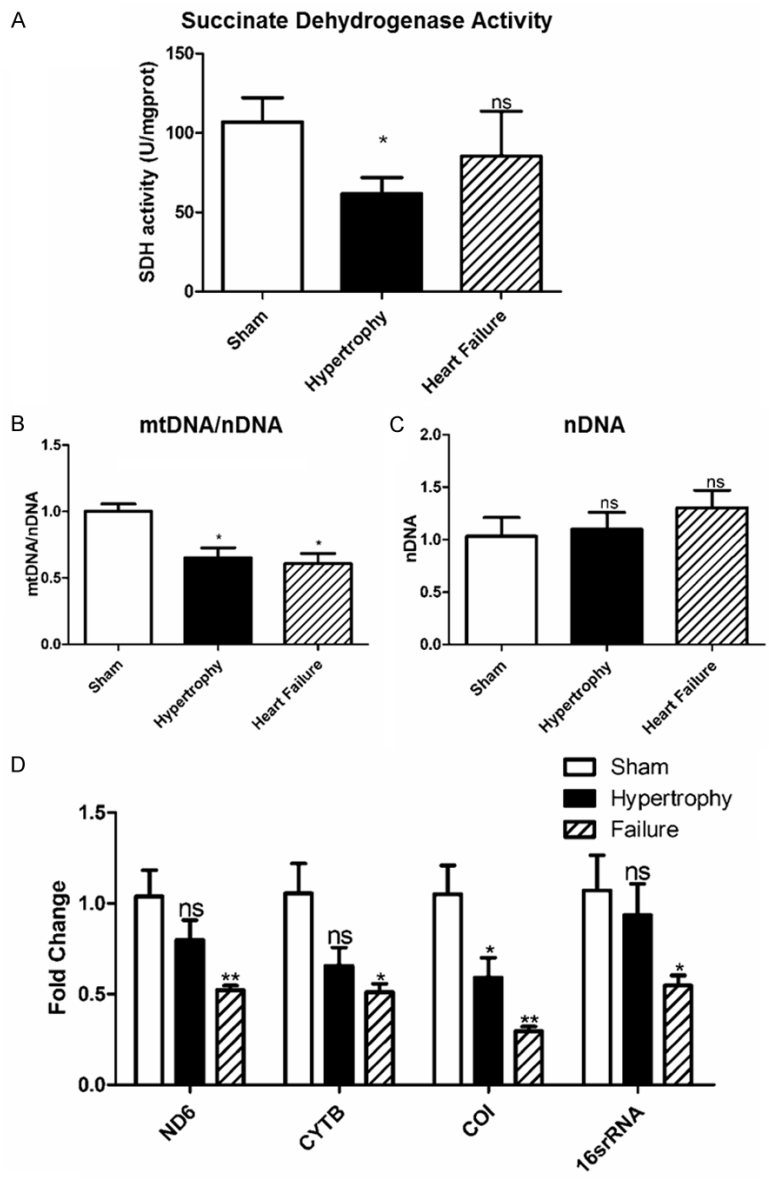

Figure 2. Depletion of mtDNA following transverse aortic constriction. (A) Succinate dehydrogenase (SDH) activity of the sham (n=4), hypertrophy (n=5), and failure group (n=5). (B) mtDNA per nDNA and (C) nDNA per total DNA in the sham (n=3), hypertrophy (n=4), and failure groups (n=4). (D) Relative mRNA expression of ND6, CYTB, COI and 6S rRNA normalized to 18s (n=5). *, P<0.05; **, P<0.01.

later stages (4 weeks) following TAC (**Figure 1E**). These data implicate that the pressure overload by TAC could induce cardiac hypertrophy (CH) in the early stage and heart failure (HF) in later stages.

TAC-induced mtDNA depletion

Succinate dehydrogenase (SDH) is a key enzyme of the tricarboxylic acid cycle and an important marker of mitochondrial mass. In this study, an SDH activity decrease of 42% was observed in CH groups, but not in HF groups (**Figure 2A**). Additionally, the mtDNA copy number has been widely used as an indicator of impaired mitochondrial biogenesis. MtDNA normalized to nDNA was decreased at CH and HF stages by 35% and 39%, respectively, with no change in the nDNA content (**Figure 2B**, **2C**). Further, the mtDNA-encoded genes, NADH-ubiquinone oxidoreductase chain 6 (ND6), cytochrome b (CYTB), cytochrome c oxidase I (COI), and 16S ribosomal RNA (16S rRNA), showed a significantly lower expression in the HF group compared to the sham group. Among the 4 genes, only COI levels were down-regulated at the stage of CH. Only mRNA expression of COI and ND6 showed a further decrease during the progression from CH to HF. These results suggest that mtDNA depletion occurred when subjected to pressure overload in mouse hearts.

Impaired mtDNA replication occurred early after TAC

Impairment of mtDNA replication appeared to account for mtDNA depletion in this pressure overload model. We investigated the expression of several genes involved in the mtDNA replication fork: DNA polymerase gamma (POLG); single-stranded DNA binding protein 1 (SSBP1); and Twinkle and DNA topoisomerase I, mitochondrial (TOP1MT). POLG and TOP1MT were down-regulated at the stage of CH and persisted during HF; Twinkle mRNA expression had a progressive decrease in the HF group, while the downregulation of SSBP1 was statistically significant only in the HF group (**Figure 3**). These findings implicate that impaired mtDNA replication is an early event that accounts for mtDNA depletion in CH and HF mouse hearts.

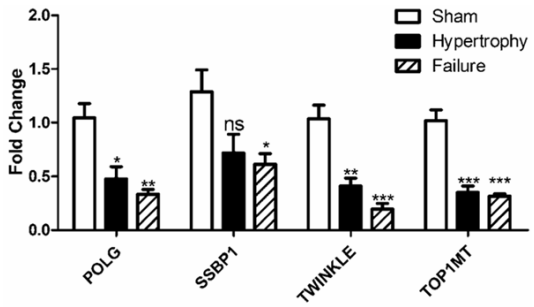

Figure 3. Impaired mtDNA replication after pressure overload. Relative mRNA expression of POLG, SSBP1, Twinkle and TOP1MT normalized to 18s (n=5). *, P<0.05; **, P<0.01; ***, P<0.001.

The PGC-1 pathway was attenuated following TAC

We examined the expression and activity of some regulators within the PCG-1 pathway, which is an important component of mitochondrial biogenesis. PGC-1α and PGC-1β mRNA were significantly decreased in both the CH and HF groups (**Figure 4A**), which was in compliance with the changes in nuclear respiratory factor 1 (NRF1), nuclear respiratory factor 2 alpha subunit (NRF2A), and nuclear respiratory factor 2 beta subunit (NRF2B2) that are well known as interaction partners of PGC-1 (**Figure 4A**). There was also a decrease of PCG-1α protein level in CH and HF groups (**Figure 4A**). Additionally, the expression of Transcription factor A, mitochondrial (TFAM), Transcription factor B1, mitochondrial (TFB1M), and Transcription factor B2, mitochondrial (TFB2M), which are regulated by NRF1/2 and important regulators of mtDNA transcription as well as mtDNA maintenance, were significantly lower at both stages following TAC operation (**Figure 4A**). These data implicate that the expression and function of the PGC-1 pathway is attenuated in the early stage following TAC and may contribute to impaired mitochondrial biogenesis and dysfunction.

Discussion

In our study, we established a TAC mouse model with two stages following pressure overload: cardiac hypertrophy (CH) in an early stage and HF in a late stage. By detecting enzyme activity, mtDNA mass and mtDNA-encoded genes, only the heart failure stage showed significant depletion in mtDNA compared to the sham group. However, impaired mtDNA replication occurred early after TAC, as the genes involved in the mtDNA replication fork decreased in both the cardiac hypertrophy and the HF stages. Additionally, attenuated expression and function of the PGC-1 pathway following pressure overload may contribute to the impaired mitochondrial biogenesis and dysfunction in our mouse model.

Accumulating evidence indicated that mitochondrial dysfunction occurred in the development of HF in both rodents [3] and human beings [4-6], and this could be a cause of cardiomyopathy and HF [7-9]. However, there is limited knowledge about mtDNA change during progression from cardiac hypertrophy to HF after pressure overload in a mouse model. For the first time, in this study, we showed that rather than the mtDNA content, the mtDNA replication was depleted in the early stage after pressure overload, and that was a consequence of down-regulation of the PGC-1 pathway.

Although our results showed that in both early and late stage following TAC there was a dysfunction in mtDNA replication and that further contributed to mtDNA depletion in the HF stage, it still needs to be determined at which time point mtDNA replication starts to fade and at what time point mtDNA depletion occurs.

As a therapeutic strategy, increasing mtDNA copy number by overexpression of TFAM could ameliorate the pathophysiological processes seen in HF [14]. However, previous work in a rodent and a canine model of heart failure had shown that the defects in energy metabolism in HF were not due to a decrease in the mitochondrial DNA content [15, 16]. Interestingly, our data suggested that mtDNA depletion occurred early after TAC (2 weeks) and persisted in the HF stage, which is in accordance with the latest three studies in human failing hearts [4-6].

It is well-established that PGC-1, as the master regulator of mitochondrial biogenesis, plays a critical role in the development of heart failure [17]. Down-regulation of PGC-1α was observed in heart failure [18-20], while TAC in mice genetically engineered to lack PGC-1α leads to accelerated HF [21]. Our findings suggest that the expression and function of the PGC-1 cascade is attenuated in the early stage following TAC and remains down-regulated at the HF stage.

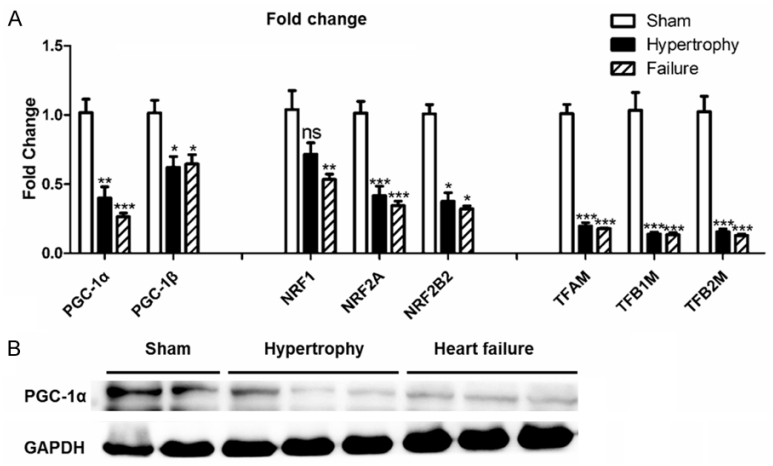

Figure 4. Transcriptional down-regulation of mitochondrial biogenesis. A. Relative mRNA expression of PGC-1, NRF1/2, TFAM and TFBM normalized to 18s (n=5). B. Relative protein expression of PGC-1α. *, P<0.05; **, P<0.01; ***, P<0.001.

However, another study suggested normal PGC-1 in human failing hearts [4] and other showed that the expression of PGC-1 returned to normal at the stage of HF [5]. These divergent observations of expression of PGC-1 in failing hearts could be the result of different severities or different causes of HF.

In conclusion, depletion of mitochondrial mass and DNA content was only detected in the HF stage. The mtDNA replication and transcriptional regulation of mitochondrial biogenesis was determined to be down-regulated from an early stage following pressure overload.

Acknowledgements

This work was supported by the grants from National Natural Science Foundation of China (81370332 and 81170201 to XL Li), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD20102013 to XL Li). Dr. XQ Kong is a Fellow and Dr. XL Li is an Associate Fellow at the Collaborative Innovation Center For Cardiovascular Disease Translational Medicine.

Disclosure of conflict of interest

None.

Address correspondence to: Xiangqing Kong and Xinli Li, Department of Cardiology, The First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, Jiangsu, China. Tel: 0086-25-84352775; Fax: 0086-25-84352775; E-mail: xiangqingkong_nj@163.com (XQK); xinli3267_nj@hotmail.com (XLL)

References

[1] Heusch G, Libby P, Gersh B, Yellon D, Böhm M, Lopaschuk G and Opie L. Cardiovascular remodeling in coronary artery disease and heart failure. Lancet 2014; 383: 1933-1943.

[2] Liu X, Xiao J, Zhu H, Wei X, Platt C, Damilano F, Xiao C, Bezzerides V, Boström P, Che L, Zhang C, Spiegelman BM and Rosenzweig A. miR-222 is necessary for exercise-induced cardiac growth and protects against pathological cardiac remodeling. Cell Metab 2015; 21: 584-595.

[3] Ide T, Tsutsui H, Hayashidani S, Kang D, Suematsu N, Nakamura K, Utsumi H, Hamasaki N and Takeshita A. Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction. Circ Res 2001; 88: 529-535.

[4] Karamanlidis G, Nascimben L, Couper GS, Shekar PS, del Monte F and Tian R. Defective DNA replication impairs mitochondrial biogenesis in human failing hearts. Circ Res 2010; 106: 1541-1548.

[5] Karamanlidis G, Bautista-Hernandez V, Fynn-Thompson F, Del Nido P and Tian R. Impaired mitochondrial biogenesis precedes heart failure in right ventricular hypertrophy in congenital heart disease. Circ Heart Fail 2011; 4: 707-713.

[6] Wang H, Bei Y, Lu Y, Sun W, Liu Q, Wang Y, Cao Y, Chen P, Xiao J and Kong X. Exercise prevents cardiac injury and improves mitochondrial biogenesis in advanced diabetic cardiomyopathy with PGC-1α and akt activation. Cell Physiol Biochem 2015; 35: 2159-2168.

[7] Wang J, Wilhelmsson H, Graff C, Li H, Oldfors A, Rustin P, Brüning JC, Kahn CR, Clayton DA, Barsh GS, Thorén P and Larsson NG. Dilated cardiomyopathy and atrioventricular conduction blocks induced by heart-specific inactivation of mitochondrial DNA gene expression. Nat Genet 1999; 21: 133-137.

[8] Li H, Wang J, Wilhelmsson H, Hansson A, Thoren P, Duffy J, Rustin P, Larsson NG. Genetic modification of survival in tissue-specific knockout mice with mitochondrial cardiomyopathy. Proc Natl Acad Sci U S A 2000; 97: 3467-3472.

[9] L'Ecuyer T, Sanjeev S, Thomas R, Novak R, Das L, Campbell W, Heide RV. DNA damage is an early event in doxorubicin-induced cardiac myocyte death. Am J Physiol Heart Circ Physiol 2006; 291: H1273-1280.

[10] deAlmeida AC, van Oort RJ, Wehrens XH. Transverse aortic constriction in mice. J Vis Exp 2010.

[11] Hudson B, Hidalgo C, Saripalli C, Granzier H. Hyperphosphorylation of mouse cardiac titin contributes to transverse aortic constriction-induced diastolic dysfunction. Circ Res 2011; 109: 858-866.

[12] Mambo E, Chatterjee A, Xing M, Tallini G, Haugen BR, Yeung SC, Sukumar S and Sidransky D. Tumor-specific changes in mtDNA content in human cancer. Int J Cancer 2005; 116: 920-924.

[13] Hoppel C and Cooper C. The action of digitonin on rat liver mitochondria. The effects on enzyme content. Biochem J 1968; 107: 367-375.

[14] Ikeuchi M, Matsusaka H, Kang D, Matsushima S, Ide T, Kubota T, Fujiwara T, Hamasaki N, Takeshita A, Sunagawa K and Tsutsui H. Overexpression of mitochondrial transcription factor a ameliorates mitochondrial deficiencies and cardiac failure after myocardial infarction. Circulation 2005; 112: 683-690.

[15] Garnier A, Fortin D, Deloménie C, Momken I, Veksler V and Ventura-Clapier R. Depressed mitochondrial transcription factors and oxidative capacity in rat failing cardiac and skeletal muscles. J Physiol 2003; 551: 491-501.

[16] Marín-García J, Goldenthal MJ and Moe GW. Abnormal cardiac and skeletal muscle mitochondrial function in pacing-induced cardiac failure. Cardiovasc Res 2001; 52: 103-110.

[17] Ventura-Clapier R, Garnier A and Veksler V. Transcriptional control of mitochondrial biogenesis: the central role of PGC-1alpha. Cardiovasc Res 2008; 79: 208-217.

[18] Sihag S, Cresci S, Li AY, Sucharov CC and Lehman JJ. PGC-1alpha and ERR alpha target gene downregulation is a signature of the failing human heart. J Mol Cell Cardiol 2009; 46: 201-212.

[19] Garnier A, Fortin D, Deloménie C, Momken I, Veksler V and Ventura-Clapier R. Depressed mitochondrial transcription factors and oxidative capacity in rat failing cardiac and skeletal muscles. J Physiol 2003; 551: 491-501.

[20] Garnier A, Zoll J, Fortin D, N'Guessan B, Lefebvre F, Geny B, Mettauer B, Veksler V and Ventura-Clapier R. Control by circulating factors of mitochondrial function and transcription cascade in heart failure: a role for endothelin-1 and angiotensin II. Circ Heart Fail 2009; 2: 342-350.

[21] Arany Z, Novikov M, Chin S, Ma Y, Rosenzweig A and Spiegelman BM. Transverse aortic constriction leads to accelerated heart failure in mice lacking PPAR-gamma coactivator 1alpha. Proc Natl Acad Sci U S A 2006; 103: 10086-10091.