The Overexpression of Twinkle Helicase Ameliorates the Progression of Cardiac Fibrosis and Heart Failure in Pressure Overload Model in Mice

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Abstract

Myocardial mitochondrial DNA (mtDNA) copy number decreases in heart failure. In post-myocardial infarction mice, increasing mtDNA copy number by overexpressing mitochondrial transcription factor attenuates mtDNA deficiency and ameliorates pathological remodeling thereby markedly improving survival. However, the functional significance of increased mtDNA copy number in hypertensive heart disease remains unknown. We addressed this question using transgenic mice that overexpress Twinkle helicase (Twinkle; Tg), the mtDNA helicase, and examined whether Twinkle overexpression protects the heart from left ventricular (LV) remodeling and failure after pressure overload created by transverse aortic constriction (TAC). Twinkle overexpression increased mtDNA copy number by 2.2 ± 0.1-fold. Heart weight, LV diastolic volume and wall thickness were comparable between Tg and wild type littersmates (WT) at 28 days after TAC operation. LV end-diastolic pressure increased in WT after TAC (8.6 ± 2.8 mmHg), and this increase was attenuated in Tg (4.6 ± 2.6 mmHg). Impaired LV fractional shortening after TAC operation was also suppressed in Tg, as measured by echocardiography (WT: 16.2 ± 7.2% vs Tg: 20.7 ± 6.2%). These LV functional improvements were accompanied by a decrease in interstitial fibrosis (WT: 10.6 ± 1.1% vs Tg: 3.0 ± 0.6%). In vitro studies, overexpressing Twinkle using an adenovirus vector in cultured cardiac fibroblasts significantly suppressed mRNA of collagen 1α, collagen 3α and connective tissue growth factor, and angiotensin II-induced transforming growth factor β1 expression. The findings suggest that Twinkle overexpression prevents LV function deterioration. In conclusion, Twinkle overexpression increases mtDNA copy number and ameliorates the progression of LV fibrosis and heart failure in a mouse pressure overload model. Increasing mtDNA copy number by Twinkle overexpression could be a novel therapeutic strategy for hypertensive heart disease.

Introduction

Heart failure is the end-stage of various heart conditions and diseases, and has become a major public health problem in most countries. Hypertension is a common risk factor for heart failure, followed closely by antecedent myocardial infarction. Seventy-five percent of heart failure cases have antecedent hypertension [1]. Hypertension affects approximately one billion people worldwide [3]. Sustained cardiac pressure overload induces cellular, molecular and morphologic remodeling and maladaptations contributing to progressive cardiac dysfunction and heart failure [4]. However, except antihypertensive drugs, there is no known effective medical treatment to attenuate pressure overload-induced cardiac remodeling. New therapeutic strategies to prevent maladaptive remodeling and subsequent progression to heart failure in hypertensive heart disease are highly desirable.

Mitochondrial dysfunction has been reported in various forms of heart failure. Especially, mitochondrial DNA (mtDNA) copy number is decreased in the heart of post-myocardial infarction mice [5] and pressure overload models [6]. In humans, Karamanlidis et al. [7] demonstrated that mitochondrial biogenesis is severely impaired in myocardial tissues collected from patients with end-stage heart failure of various etiologies. In a mouse post-myocardial infarction model, overexpression of mitochondrial transcription factor A by a transgenic approach ameliorated the decrease in mtDNA copy number and pathological remodeling dramatically improving survival [8]. These findings indicate that increasing mtDNA copy number attenuates cardiac pathological remodeling and failure. However, the functional significance of increased mtDNA copy number under pressure overload condition has not been established.

In this study, we addressed this question using transgenic mice that overexpress Twinkle, the mtDNA helicase. Previous study showed that systemic overexpression of Twinkle increases mtDNA copy number in muscle and heart up to 3-fold of control levels, more than any other factors reported to date [9]. Twinkle is known to co-localize with mtDNA in mitochondrial nucleoids that are stable assemblies of nucleoproteins and mtDNA. Twinkle...
displays 5’ to 3’ DNA helicase activity in vitro, supporting its role in unwinding the mtDNA replication fork [10]. Dominant mutations of Twinkle are associated with progressive external ophthalmoplegia with multiple mtDNA deletions [11]. Reduced Twinkle expression by RNA interference also mediates a rapid drop in mtDNA copy number, supporting the in vitro results [9]. These data demonstrate that Twinkle is essential for mtDNA maintenance, and that it may be a key regulator of mtDNA copy number in mammals [9,12].

In a pilot study, we have confirmed that overexpressing Twinkle in mice by a transgenic approach inhibits cardiac remodeling and improves survival after experimental myocardial infarction (unpublished data). However, the functional significance of increased Twinkle in pressure overload-induced cardiac remodeling remains unclear. In this study, we examined whether Twinkle overexpression protects the heart from left ventricular (LV) remodeling and failure in a mouse pressure overload model created by transverse aortic constriction (TAC).

Materials and Methods

Ethics Statement

All procedures and animal care were approved by the Committee on Ethics of Animal Experiment, Kyushu University Graduate School of Medical and Pharmaceutical Sciences ( Permit number: A22-075), and performed in accordance with the Guideline for Animal Experiment of Kyushu University, and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Animal Experiments

We utilized transgenic mice that overexpress mouse Twinkle helicase (Tg) as described previously [9]. The animals were kept in 12-hour light-dark cycle and had access to food and water ad libitum. Ten week-old male Tg and wild type littermates (WT) underwent TAC as described previously with a slight modification [13]. Briefly, mice were anesthetized with sodium pentobarbital (35 mg/kg intraperitoneally) and intubated endotracheally. The chest was opened and the aortic arch was identified after blunt dissection through the intercostal muscles. A 8-0 silk suture was tied the suture as tightly as possible to create similar degree of plegia with multiple mtDNA deletions [11]. Reduced Twinkle expression by RNA interference also mediates a rapid drop in mtDNA copy number, supporting its role in displacing the mtDNA replication fork [10]. Dominant mutations of Twinkle and Pressure Overload

We performed in vivo analyses of mice as described previously [8,19]. On day 28 after TAC surgery, echocardiographic studies were performed under anesthesia with a mixture of medetomidine (0.3 mg/kg), midazolam (4 mg/kg) and butorphanol tartrate (5 mg/kg), and spontaneous respiration. A 2D parasternal short-axis view of the LV was obtained at the level of the papillary muscles by applying the transducer lightly to the mid-upper left.
anterior chest wall. After ensuring that the image 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. Anterior, posterior end diastolic wall thickness and LV internal dimensions were measured. While under anesthesia, a 1.4 Fr micromanometer-tipped catheter (Millar Instruments) was inserted into the right carotid artery and advanced into the LV to measure pressures for the assessment of aortic pressure and LV end diastolic pressure.

Histopathological Studies
After in vivo echocardiographic and hemodynamic studies, the heart was excised and weighed, and dissected into the right and left ventricles, including the septum. The heart tissues were fixed in 6% formaldehyde, embedded in paraffin, and cut into 5 μm thick sections. Sections were stained with hematoxylin-and-eosin and Masson’s trichrome for assessments of myocyte cross-sectional area and collagen volume fraction [13]. To measure the myocyte cross-sectional area, each section was photographed under a microscope (DMD108, Leica Microsystems) at a final magnification of 200×. The profiles of 30 to 40 myocytes cut in cross-sections were traced manually and digitized. The digitized profiles were transferred to a personal computer that calculated the area. Three to 4 fields were randomly selected from 3 coronal sections of each heart. Thus, 100 to 200 myocytes were measured for each animal, and the mean myocyte cross-sectional area was calculated. Collagen volume fraction was measured in 6 fields randomly selected from each coronal section (basal, mid and apical sections) in each animal. Each field was photographed under a microscope at a final magnification of 200×, and subjected to color threshold analysis. Collagen volume fraction for the heart was calculated as the sum of all connective tissue areas divided by the sum of all connective tissue and muscle areas in all fields. Collagen surrounding intramycocardial coronary arteries was excluded from analysis.

Plasmid Construction
Small-interfering RNA (siRNA) targeting rat Twinkle helicase (si-rTwinkle) was synthesized by Takara (Shiga, Japan). The si-rTwinkle gene was sub-cloned into unique BamHI and HindIII sites of pBAsiDNA Vector (Takara, pBAsi-sTwinkle). The full length human Twinkle helicase complimentary DNA (cDNA, hTwinkle) was amplified by PCR with primers containing XbaI and HindIII sites extracted from the placenta human cDNA library. The cDNA library was provided by the Department of Clinical Chemistry and Laboratory Medicine, Graduate School of Medical Sciences, Kyushu University. The PCR product was sub-cloned into distinctive XbaI and HindIII sites of the pcDNA3.1 Expression Vector (Invitrogen, pcDNAhTwinkle). The pBAsi-rTwinkle and pcDNAhTwinkle were amplified, sequenced and used for constructing adenovirus [20].

Adenovirus Transduction
Replication-deficient recombinant adenovirus vectors containing hTwinkle (AxCAhTwinkle), si-rTwinkle (AxCAsi-rTwinkle) or E. coli LacZ cDNA (AxCA LacZ) were constructed using Adenovirus Expression Vector Kit Ver. 2 (Takara) according to manufacturer’s protocol. Adenoviruses were amplified in human embryonic kidney cell line (HEK-293, RIKEN BIORESOURCE CENTER, Cell No. RCB1637) purified with the Adeno-X Maxi purification Kit (Clontech) and then titrated with the Adeno-X Rapid Titer kit (Clontech). The efficiency of virus infection was >95%, as measured by β-gal staining.

In vitro Experiments
Primary culture of neonatal rat cardiac fibroblasts was prepared from the ventricles of neonatal Sprague-Dawley rats as described previously [15,21]. Briefly, neonatal rats were euthanized by decapitation under anesthesia with isoflurane, after which the hearts were rapidly excised and digested. Anesthesia depth was monitored by limb withdrawal in response to toe pinching. After digesting the myocardial tissue with trypsin (Wako) and collagenase type 2 (Worthington), cells were suspended in Dulbecco’s Modified Eagle’s Medium (DMEM, Sigma-Aldrich) containing 10% fetal bovine serum (Thermo SCIENTIFIC), penicillin (Invitrogen) and streptomycin (Invitrogen), and plated in 100 mm culture dishes for 70 minutes to remove non-adherent cardiac myocytes. Adherent cardiac fibroblasts were maintained at 37°C in humidified air with 5% CO2. Considering the possibility that cardiac fibroblasts may lose the original characteristics after prolonged culture, cells were used within 2 passages in all experiments. Cells were infected with AxCAhTwinkle, AxCAsi-rTwinkle or AxCA LacZ (multiplicity of infection; 1) in serum-free DMEM for 1 hour, and cultured for another 72 hours in DMEM containing 5% fetal bovine serum. Then the cells were stimulated with angiotensin II (AngII, Sigma-Aldrich, 1 μM) for 24 hours, and collected for mRNA analyses.

Statistical Analysis
All data were expressed as mean ± SEM. Between-group comparison of means was performed by one-way analysis of variance followed by Bonferroni’s post-hoc test. A P-value less than 0.05 was considered to be statistically significant.

Results
mtDNA Copy Number and Mitochondrial Enzyme Activity
We first examined the mitochondrial characteristics in Tg and WT that were sham-operated or underwent TAC. mtDNA copy number increased significantly in Tg compared to WT, both in sham-operated (2.2-fold, P<0.01) and TAC groups (2.9-fold, P<0.01), mtDNA copy number tended to decrease on day 28 after TAC in both WT (P=0.07 WT-TAC vs. WT-sham) and Tg (P=0.11, Tg-TAC vs. Tg-sham), although the differences were not significant in both groups (Figure 1A). Mitochondrial complexes I and III protein levels and mitochondrial complex I activity were normalized against those of complex II which is entirely encoded by the nucleus [16]. Both mitochondrial protein levels and activities were not affected by Twinkle overexpression, consistent with previous report [16], and were not altered by TAC (Figure 1B and C).

Cardiac Function and Structure
Table 1 shows the hemodynamic data and Table 2 shows the organ weights on day 28 after TAC operation. TAC increased heart weight and LV weight in both WT and Tg, although there was no significant differences between Tg-TAC and WT-TAC. TAC also increased aortic pressure, again with no difference between Tg-TAC and WT-TAC. Importantly, Twinkle overexpression significantly inhibited the increase in LV end-diastolic pressure caused by TAC-induced pressure overload (P<0.05, Tg-TAC vs. WT-TAC).

Echocardiographic study showed enlarged LV end-diastolic dimension after TAC operation in both Tg and WT, with no significant differences between Tg-TAC and WT-TAC (Figure 2A and B). There was also no difference in LV wall thickness between Tg and WT (Figure 2C). However, fractional shortening decreased
by approximately 60% in WT-TAC compared with WT-sham but by only 49% in Tg-TAC ($P < 0.05$, Tg-TAC vs. WT-TAC). Similar results were observed for ejection fraction. These results suggest relatively preserved LV function in Tg-TAC (Figure 2D and E). We also found a tendency of LV dysfunction attenuation in Tg-TAC mice on day 14 after TAC operation (Figure S1).

In histological analyses, we assessed the cross-sectional area of cardiac myocyte as an index of hypertrophy. Consistent with the data of LV weight and echocardiographic wall thickness, the cross-sectional area increased markedly after TAC operation in both Tg and WT, although there was no significant difference between

**Table 1. Hemodynamic data.**

|                | WT-sham (n = 8) | Tg-sham (n = 3) | WT-TAC (n = 11) | Tg-TAC (n = 11) |
|----------------|----------------|----------------|----------------|----------------|
| HR (bpm)       | 522±21         | 506±10         | 521±32         | 535±37         |
| Peak BP (mmHg) | 101±7          | 111±10         | 172±24**       | 167±21**       |
| Mean BP (mmHg) | 86±3           | 77±12          | 116±6**        | 111±11**       |
| LVEDP (mmHg)   | 1.4±1.4        | 1.0±0.5        | 8.6±2.8**      | 4.6±2.6**      |

HR, heart rate; BP, blood pressure; LVEDP, LV end-diastolic pressure. **: $P < 0.01$, *: $P < 0.05$ vs WT-sham.

**Table 2. Organ weight data.**

|                | WT-sham (n = 17) | Tg-sham (n = 12) | WT-TAC (n = 17) | Tg-TAC (n = 16) |
|----------------|----------------|----------------|----------------|----------------|
| Body wt (g)    | 29.0±2.9       | 28.3±1.8       | 27.3±1.6*      | 27.5±1.5*      |
| Heart wt/body wt (mg/g) | 4.9±0.4 | 4.9±0.7       | 6.8±0.9*      | 6.4±0.7*      |
| LV wt/body wt (mg/g) | 3.2±0.3 | 3.2±0.3       | 5.0±0.9*      | 4.5±0.6*      |

*: $P < 0.05$ vs WT-sham.

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WT-TAC and Tg-TAC (Figure 3A and B). Next we investigated the progression of fibrosis in myocardium. We found marked interstitial fibrosis in the myocardium, on day 28 after TAC operation. Twinkle overexpression significantly suppressed the TAC-induced increase in fibrosis \( (P, 0.01, \text{Tg-TAC vs. WT-TAC}; \text{Figure 3C and D}) \). Meanwhile, expressions of COL1a, COL3a and CTGF, which are commonly used markers of fibrosis, tended to increase in WT-TAC on day 28 after TAC operation, but Twinkle overexpression tended to inhibit these increase (Figure S2), supporting our histological result of the inhibition of cardiac fibrosis.

**In vitro Experiments**

In order to confirm the alteration of mRNA in fibroblast specifically, we checked profibrogenic signals in cardiac fibroblast isolated from neonatal rat heart. We found significant suppression in all 3 mRNAs, COL1a, COL3a, and CTGF (Figure 4). To examine the mechanism by which Twinkle overexpression inhibits cardiac fibrosis *in vitro*, we prepared rat neonatal cardiac fibroblasts and stimulated with AngII for 24 hours. AngII increased TGF-\( \beta \)1 mRNA expression, which is a key regulator of fibrosis [22,23]. Twinkle overexpression by adenovirus vector suppressed TGF-\( \beta \)1 expression in cardiac fibroblasts, compared with LacZ overexpression. In contrast, downregulation of Twinkle by siRNA, which inhibited Twinkle mRNA by 35% (Figure S3), exacerbated TGF-\( \beta \)1 expression (Figure 5). These findings suggest that Twinkle overexpression inhibits cardiac fibrosis by suppressing profibrogenic signals in the myocardium.

**Discussion**

Hypertension is a major public health problem, affecting approximately one billion people worldwide [3]. Sustained pressure overload causes hypertrophic changes in the myocardium. While these changes may represent adaptive remodeling in the early phase, they eventually progress to maladaptive remodeling and exacerbate heart failure. No therapeutic options are currently available to prevent the progression to maladaptive remodeling for hypertensive heart disease. We report for the first time that Twinkle overexpression ameliorates cardiac fibrosis and heart failure in a mouse pressure overload model. In this study, Twinkle overexpression did not inhibit myocardial hypertrophy.
**Figure 3. Histopathological analyses.** A. Representative photomicrographs of hematoxylin-eosin-stained LV cross-sections obtained from 4 groups of animals. Scale bar = 1 mm (upper sections) and 50 μm (lower sections). B. Myocyte cross-sectional area in WT-sham, Tg-sham, WT-TAC and Tg-TAC. C. Representative photomicrographs of Masson’s trichrome-stained LV cross-sections obtained from 4 groups of animals. Scale bar = 50 μm. D. Collagen volume fraction in WT-sham, Tg-sham, WT-TAC, and Tg-TAC. Values are mean ± SEM. **; P<0.01 vs WT-sham. †; P<0.01 vs WT-TAC. doi:10.1371/journal.pone.0067642.g003

**Figure 4. Effects of the upregulation of Twinkle on fibrosis signaling.** A-C. mRNA expression of COL1α (A), COL3α (B), and CTGF (C), quantified by real-time PCR relative to housekeeping gene (18S gene) in neonatal rat cardiac fibroblast. Cells were preinfected with AxCAhTwinkle (Twinkle) or AxCALacZ (LacZ) for 72 hours. Values are mean ± SEM. Data are presented as ratio to LacZ. **; P<0.01, *; P<0.05 vs LacZ. doi:10.1371/journal.pone.0067642.g004
Cardiac Hypertrophy, Function, and Fibrosis

Twinkle overexpression ameliorated TAC-induced decreases in LV fractional shortening and ejection fraction, as well as increase in LV end-diastolic pressure (Table 1 and Figure 2). These changes were significant although the magnitudes were small. As mentioned earlier, the relatively mild pressure overload produced in our model may partially explain the small amelioration of cardiac function by Twinkle overexpression. Nevertheless, the significant improvements in cardiac function indicate the benefit of Twinkle overexpression.

The mechanism by which increased Twinkle expression prevents heart failure under pressure overload condition remains unknown. In this study we showed that Twinkle overexpression prevented cardiac fibrosis in vivo and in vitro (to be discussed in detail below). We therefore speculate that Twinkle overexpression somehow inhibits cardiac profibrogenic signals. We need to conduct further investigation about the mechanism.

Mitochondrial Characteristics

In the present study, mtDNA copy number tended to decrease ($P=0.07$) in TAC compared to sham on day 28 after operation, but the difference was not significant (Figure 1A). However, a previous study showed that mtDNA copy number decreased in a similar animal model of aortic banding [6]. This discrepancy may be due to the difference in severity of pressure overload between the two studies, judging from the hypertrophy data. Increasing the pressure overload intensity in our model may result in a significant decrease in mtDNA copy number in TAC. In the present study, we used a 26-gauge needle to induce pressure overload, which produced stable hypertrophy but rather mild effect on heart failure. On the other hand, using a 27- or 28-gauge needle as in previous study [13] resulted in higher surgical mortality but produces greater pressure overload in our preliminary experiments.

We found that increasing mtDNA copy by Twinkle overexpression did not affect mitochondrial enzyme activity, which is consistent with a previous report [16]. Furthermore, TAC also did not affect mitochondrial enzyme activity (Figure 1B and C). These results suggest that mitochondrial electron transport complex activity is not directly related to the cardioprotective effect of Twinkle overexpression.

The mechanism by which increased Twinkle expression prevents heart failure under pressure overload condition remains unknown. In this study we showed that Twinkle overexpression prevented cardiac fibrosis in vivo and in vitro (to be discussed in detail below). We therefore speculate that Twinkle overexpression somehow inhibits cardiac profibrogenic signals. We need to conduct further investigation about the mechanism.

Clinical Implication

We speculate that increased mtDNA copy number by Twinkle overexpression is responsible for the cardioprotective effects. Previous studies have proposed various strategies such as resveratrol intake [14], exercise training [27], and caloric restriction [28] to increase mtDNA copy number systematically. We have also reported that exogenously administered recombinant mitochondrial transcription factor A protein increases mtDNA copy number in cardiac myocytes [15]. Increasing mtDNA copy number in clinical situation using these methods would be beneficial for the prevention of heart failure caused by pressure overload. Further investigations, especially in human studies, are anticipated.

Conclusion

Overexpression of Twinkle helicase ameliorated the progression of cardiac fibrosis in a mouse pressure overload model. Increasing mtDNA copy number by Twinkle overexpression could be a novel therapeutic strategy for hypertensive heart disease.

Supporting Information

Figure S1 The time course of LV fractional shortening after TAC. The change of LV fractional shortening over time, after TAC operation. Values are mean ± SEM. *; $P<0.05$ vs day 0, †; $P<0.05$ vs WT-TAC (day 28). FS; fractional shortening. (TIF)
Figure S2 mRNA expressions after TAC operation. A–C. mRNA expression of COL1a (A), COL3a (B), and CTGF (C), 28 days after TAC or sham operation. They were quantified by real-time PCR relative to nuclear genome (HPRT gene). Values are mean ± SEM. Data are presented as ratio to WT-sham. (TIF)

Figure S3 Twinkle mRNA expression in siTwinkle. Rat Twinkle mRNA expression in cultured cardiac fibroblasts were quantified by real-time PCR relative to housekeeping gene (18S gene). Cells were preinfected with AxCALacZ (LacZ). Values were quantified by real-time PCR relative to nuclear genome (HPRT gene). Values are mean ± SEM. Data are presented as ratio to LacZ. **, P<0.01 vs LacZ. (TIF)

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Author Contributions
Conceived and designed the experiments: AT TI KS. Performed the experiments: AT TF KO MI YH TT EY HT AS. Analyzed the data: AT TI TF YH ET AS. Contributed reagents/materials/analysis tools: TI AS KS. Wrote the paper: AT TI TF KS.

References
1. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, et al. (2012) Heart disease and stroke statistics—2012 update: a report from the American Heart Association. Circulation 125: e2–e220.
2. Heidenreich PA, Trogdon JG, Khazam OA, Butler J, Dracup K, et al. (2011) Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. Circulation 123: 935–944.
3. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, et al. (2005) Global burden of hypertension: analysis of worldwide data. Lancet 365: 217–223.
4. Takimoto E, Champion HC, Li M, Belardi D, Ren S, et al. (2005) Chronic inhibition of cyclic GMP phosphodiesterase 5A prevents and reverses cardiac hypertrophy. Nat Med 11: 214–222.
5. Ide T, Tsutsui H, Hayashidani S, Kang D, Suenatsu N, et al. (2001) Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction. Circ Res 89: 529–535.
6. Kuroda J, Ago T, Matsushima S, Zhai P, Schneider MD, et al. (2010) NADPH oxidase 4 (Nox4) is a major source of oxidative stress in the failing heart. Proc Natl Acad Sci U S A 107: 15565–15570.
7. Karamanlidis G, Nascimben L, Couper GS, Shekar PS, Del Monte F, et al. (2004) Twinkle helicase is essential for mtDNA maintenance and regulates mitochondrial deficiencies and cardiac failure after myocardial infarction. Circulation 110: 1779–1786.
8. Matsuoka H, Yang M, Matsushima S, Ito H, Hattori F, et al. (2006) Overexpression of mitochondrial transcription factor a ameliorates mitochondrial deficiencies and cardiac failure after myocardial infarction. Circulation 112: 683–690.
9. Tynninen H, Sehnbrong H, Bokori-Brown M, Gradycome C, Ashley N, et al. (2004) Twinkle helicase is essential for mtDNA maintenance and regulates mtDNA copy number. Hum Mol Genet 13: 3219–3227.
10. Korhonen JA, Gaspari M, Falkenberg M. (2003) Twinkle Helicase Has 5′–3′ DNA helicase activity and is specifically stimulated by mitochondrial single-stranded DNA-binding protein. J Biol Chem 278: 48627–48632.
11. Spelbrink JN, Li FV, Tiranti V, Nikkilä K, Yuan QP, et al. (2001) Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene-4-like protein localized in mitochondria. Nat Genet 28: 223–231.
12. Tynninen H, Mäejoos KP, Wanaoq S, Lappalainen I, Ylikallio E, et al. (2005) Mutant mitochondrial helicase Twinkle causes multiple mtDNA deletions and a late-onset mitochondrial disease in mice. Proc Natl Acad Sci U S A 102: 17687–17692.
13. Matsusaka H, Ide T, Matsushima S, Ikuchi M, Kubota T, et al. (2006) Targeted deletion of matrix metalloproteinase 2 ameliorates myocardial remodeling in mice with chronic pressure overload. Hypertension 47: 711–717.
14. Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, et al. (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha. Cell 127: 1109–1122.
15. Fujino T, Ide T, Yoshida M, Onitsuka K, Tanaka A, et al. (2012) Recombinant mitochondrial transcription factor A protein inhibits nuclear factor of activated T cells signaling and attenuates pathological hypertrophy of cardiac myocytes. Mitochondrion.
16. Ylikallio E, Tynninen H, Tsutsui H, Ide T, Suomalainen A (2010) High mitochondrial DNA copy number has detrimental effects in mice. Hum Mol Genet 19: 2095–2105.
17. Izly A, Marjavaara SK, Kystala A, Uusi-Rauva K, Lairo K, et al. (2008) Deficiency of the INCL protein Ppil results in changes in ectopic F1-ATP synthase and altered cholesterol metabolism. Hum Mol Genet 17: 1406–1417.
18. Antonicka H, Saasarmen NG, Shoubridge EA (2006) The molecular basis for tissue specificity of the oxidative phosphorylation deficiencies in patients with mutations in the mitochondrial translation factor EFG1. Hum Mol Genet 15: 1835–1846.
19. Matsushima S, Ide T, Yamato M, Matsusaka H, Hattori F, et al. (2006) Overexpression of mitochondrial peroxiredoxin-3 prevents left ventricular remodeling and failure after myocardial infarction in mice. Circulation 113: 1779–1786.
20. Mizuguchi H, Kay MA (1998) Efficient construction of a recombinant adenovirus vector by an improved in vitro ligation method. Hum Gene Ther 9: 2577–2583.
21. Fuji T, Onohara N, Maruyama Y, Tanabe S, Kobayashi H, et al. (2005) Galphad12/13-mediated modulation of reactive oxygen species is critical for angiotensin receptor-induced NEXT activation in cardiac fibroblasts. J Biol Chem 280: 23041–23047.
22. Biernacka A, Dobaczewski M, Frangogiannis NG (2011) TGF-beta signaling in fibrosis. Growth Factors 29: 196–202.
23. Dobaczewski M, Chen W, Frangogiannis NG (2011) Transforming growth factor (TGF)-beta signaling in cardiac remodeling. J Mol Cell Cardiol 51: 600–606.
24. Moncier J, Lindsay MM, Dunn FG (2004) Hypertensive heart disease and fibrosis. Curr Opin Cardiol 19: 326–331.
25. Gonzalez A, Ravassa S, Beaumont J, Lopez B, Diez J (2011) New targets to treat the structural remodeling of the myocardium. J Am Coll Cardiol 58: 1833–1843.
26. Diez J, Quezada L, Lopez B, Gonzalez A, Larman M, et al. (2002) Losartan-dependent regression of myocardial fibrosis is associated with reduction of left ventricular chamber stiffness in hypertensive patients. Circulation 105: 2512–2517.
27. Guo W, Wong S, Li M, Liang W, Liesa M, et al. (2012) Testosterone plus low-intensity physical training in late life improves functional performance, skeletal muscle mitochondrial biogenesis, and mitochondrial quality control in male mice. PLoS One 7: e41100.
28. Lanza IR, Zabihian KL, Klaas PA, Moese DM, Heppelmann GJ, et al. (2012) Chronic caloric restriction preserves mitochondrial function in senescence without increasing mitochondrial biogenesis. Cell Metab 16: 777–788.