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Original article

Constitutive SIRT1 overexpression impairs mitochondria and reduces cardiac function in mice

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ABSTRACT

Heart failure is associated with a change in cardiac energy metabolism. SIRT1 is a NAD^+-dependent protein deacetylase, and important in the regulation of cellular energy metabolism. To examine the role of SIRT1 in cardiac energy metabolism, we created transgenic mice overexpressing SIRT1 in a cardiac-specific manner, and investigated cardiac functional reserve, energy reserve, substrate uptake, and markers of mitochondrial function. High overexpression of SIRT1 caused dilated cardiomyopathy. Moderate overexpression of SIRT1 impaired cardiac diastolic function, but did not cause heart failure. Fatty acid uptake was decreased and the number of degenerated mitochondria was increased dependent on SIRT1 gene dosage. Markers of reactive oxygen species were decreased. Changes in morphology and reactive oxygen species were associated with the reduced expression of genes related to mitochondrial function and autophagy. In addition, the respiration of isolated mitochondria was decreased. Cardiac function was normal in transgenic mice expressing a low level of SIRT1 at baseline, but the mice developed cardiac dysfunction upon pressure overload. In summary, the constitutive overexpression of SIRT1 reduced cardiac function associated with impaired mitochondria in mice.

1. Introduction

Congestive heart failure (CHF) is associated with a significant change in the energy metabolism of the heart, and the altered energetics is hypothesized to play an important role in the progression of CHF [1]. The alteration includes changes in substrate utilization, mitochondrial function, and energy transfer by the creatine shuttle system. Despite previous intense efforts, the mechanism by which these changes are induced, and the roles of the changes in the progression of CHF are still not clear.

Silent mating type information regulation 2 homolog 1 (SIRT1) is a nicotinamide adenine dinucleotide (NAD^+)-dependent protein deacetylase and a homolog of the yeast silencing information regulator 2 (sir2) [2]. Sir2 plays a critical role in lifespan extension caused by caloric restriction [2]. As expected from its important role in caloric restriction, SIRT1 has been shown to be important in the energy metabolism of cells [3]. SIRT1 deacetylates peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) and increases mitochondrial function in adipocytes [4]. Transient transfection of SIRT1 also increased fatty acid oxidation in a skeletal myocyte cell line [5]. However, cellular respiration was decreased in skeletal muscle cells stably transfected with SIRT1 [6], and SIRT1 overexpression in

Abbreviations: ANF, atrial natriuretic factor; Atg, autophagy-related protein; Bmal1, brain and muscle aryl hydrocarbon receptor nuclear translocator-like1; β-MHC, β-myosin heavy chain; BNP, brain natriuretic peptide; CD36/FAT, CD36/fatty acid translocase; CHF, congestive heart failure; Clock, circadian locomotor output cycles kaput; COX, cytochrome c oxidase; CPT-1b, carnitine palmitoyltransferase-1b; Cyt, cytochrome; ERRα, estrogen-related receptor α; FOXO, forkhead box O; Gabarapl1, γ-aminobutyric acid [GABA] receptor-associated protein-related protein-like 1; GLUT, glucose transporter; IDH3α, isocitrate dehydrogenase 3α; LC, microtubule-associated protein1 light chain; MCAD, medium-chain acyl-coenzyme A dehydrogenase; NAD^+, nicotinamide adenine dinucleotide; NAM, nicotinamide; NAMPT, nicotinamide phosphoribosyltransferase; ND4, NADH dehydrogenase 4; NMN, nicotinamide mononucleotide; NMNAT, nicotinamide mononucleotide adenylyltransferase; NRF-1, nuclear respiratory factor-1; NTg, non-transgenic; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; PPARα, peroxisome proliferator-activated receptor α; ROS, reactive oxygen species; Sir2, silencing information regulator 2; SIRT1, silent mating type information regulation 2 homolog 1; SOD, superoxide dismutase; TAC, transverse aortic constriction; Vps, vacuolar protein sorting.

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skeletal muscle tissue decreased mitochondrial function in animals [7]. These results indicate the role of SIRT1 in energy metabolism to be dependent on the tissue or duration of the activation.

SIRT1 has been implicated in the development of cardiac hypertrophy and failure [8–12]. The amount of SIRT1 protein was increased in hypertrophied or failing hearts of animals [8,10]. SIRT1 attenuated hypertrophy and cell death in cultured cardiac myocytes [8]. In addition, mild (2.5-fold) and moderate (7.5-fold) overexpression of SIRT1 in a heart-specific manner attenuated some of the age-associated changes of the heart, whereas marked (12.5-fold) overexpression caused dilated cardiomyopathy in mice and moderate overexpression protected the heart against oxidative stress [10]. Moderate overexpression of SIRT1 also protects heart tissue against ischemia reperfusion injury [12]. However, the role of SIRT1 in cardiac energy metabolism is not well characterized [8–11]. Here, we created transgenic mice overexpressing SIRT1 in a cardiac-specific manner, and examined cardiac functional reserve, energy reserve, substrate uptake, and markers of mitochondrial function.

2. Methods

See the online-only Data Supplement for additional details.

2.1. Animals

Transgenic lines overexpressing mouse SIRT1 in a heart-specific manner were generated on a C57BL6 background using the α-myosin heavy chain promoter [13]. SIRT1 mice and non-transgenic (NTg) littermates were sacrificed at 3 months of age after overnight fasting and analyzed. The investigation conformed to 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). All animal experiments and methods were approved by the Animal Care and Use Committees of Kyoto University Graduate School of Medicine.

2.2. Transverse aortic constriction (TAC)

Twelve-week-old male mice were subjected to transverse aortic constriction (TAC) [14]. Briefly, the animals were intubated and ventilated with a small-animal respirator (model SN-480-7-10; Shinano Seisakusyo, Tokyo, Japan), at a rate of 110 cycles/min and a tidal volume of 1 ml/100 g body weight. Aortic constriction was performed by tying a 7-0 silk string ligature around a 26-gauge needle and then removing the needle. The chest was then closed and the mice were extubated and allowed to recover.

2.3. Statistical analysis

All data were expressed as the mean ± SEM. Differences between the groups were compared using a one-way ANOVA followed by Fisher's protected least significant difference for multiple comparisons. Survival was analyzed by the standard Kaplan–Meier method with a log-rank test. For the analysis of hemodynamic data, a two-way repeated-measure ANOVA was used to test the differences between groups in the response to dobutamine. In all tests, a value of $p<0.05$ was considered significant.

3. Results

3.1. Generation of transgenic mice overexpressing SIRT1 in the heart

Transgenic mice overexpressing murine SIRT1 in a heart-specific manner were generated using the αMyHC promoter on a C57BL6 background. Twenty independently derived founders were produced from 110 screened mice. Ten of the founders expressed the transgene product as determined by a Western blot analysis. We analyzed 3 lines of SIRT1 mice expressing different levels of the SIRT1 protein in the heart (low: 3.2-fold, moderate: 6.8-fold, high: 20-fold compared with levels in non-transgenic (NTg) mice) (Fig. 1A). Hereafter, the three transgenic lines are designated as SIRT1 (L, low), SIRT1 (M, moderate), and SIRT1 (H, high) mice, according to the level of SIRT1 protein expression. Over a follow-up period of 1 year, SIRT1 (L) and SIRT1 (M) mice survived normally. SIRT1 (H) mice died of heart failure at a mean age of 3–4 months ($p<0.0001$, by log-rank test, Fig. 1B).

3.2. Cardiac function was preserved in SIRT1 (L) mice, and reduced in SIRT1 (M) and SIRT1 (H) mice

Echocardiographic analysis revealed left ventricular diastolic diameter (LVDd), fractional shortening (FS), and diastolic posterior wall thickness (PWTd) to be normal in SIRT1 (L) and SIRT1 (M) mice. However, in SIRT1 (H) mice, LVDd was increased and FS and PWTd were decreased (Table 1). Thus, the systolic function of SIRT1 (H) mice at rest was reduced. Next, we measured cardiac function using cardiac catheterization in mice infused with dobutamine. Cardiac function was normal in SIRT1 (L) mice. In SIRT1 (M) mice, left ventricular pressure (LVP) and maximum dP/dT were preserved, but minimum dP/dT was impaired, suggesting diastolic dysfunction in response to dobutamine. In SIRT1 (H) mice, LVP, maximum dP/dT, and minimum dP/dT were markedly impaired, indicating that both systolic and diastolic functions were affected. Thus, SIRT1 expression impaired cardiac function in a transgene dosage-dependent manner (Fig. 1C).

3.3. Myocardial energy reserve measured by in situ ^{31}P magnetic resonance spectroscopy was preserved in SIRT1 (L) and SIRT1 (M) mice

We measured the energy reserve of the heart by measuring high-energy phosphates using in situ ^{31}P magnetic resonance spectroscopy (MRS). The phosphocreatine (PCr)/ATP ratio was identified as a marker of myocardial energy reserve, and reported to be a prognostic indicator of CHF [15]. Representative cardiac ^{31}P MR spectra from mice are shown in Fig. 2B. The mean cardiac PCr/ATP ratio of NTg mice was 2.0, consistent with a previous report [16]. We analyzed SIRT1 (L) and SIRT1 (M) mice at rest, since it was difficult to do stress tests in the MRS apparatus. The cardiac PCr/ATP ratio was preserved in SIRT1 (L) and SIRT1 (M) mice (Fig. 2C). We could not measure the PCr/ATP ratio of SIRT1 (H) mice since they did not tolerate the anesthesia for the 30 min required for the analysis.

3.4. Fatty acid uptake decreased and glucose uptake increased in SIRT1 (M) and SIRT1 (H) mice

To examine the change in substrate utilization in the SIRT1 mouse heart, we examined the myocardial uptake of fatty acids and glucose using ^{125}I-labeled 15-(*p*-iodophenyl)-9-*R*,*S*-methylpentadecanoic acid (^{125}I-9MPA) and ^{18}fluorodeoxyglucose (^{18}FDG), respectively [17]. ^{125}I-9MPA uptake was decreased by 25% in SIRT1 (M) mice and by 55% in SIRT1 (H) mice compared with the level in NTg control mice (Fig. 2D). ^{18}FDG uptake was increased 3.1-fold in SIRT1 (M) mice and 5.3-fold in SIRT1 (H) mice. The gene expression of glucose transporter (GLUT) 1 and GLUT4 was not increased in SIRT1 (M) mice, and the expression of GLUT4 was decreased and that of GLUT1 was increased in SIRT1 (H) mice (Fig. 2E). Thus, a shift in substrate utilization from fatty acids to glucose, commonly observed in animal models of CHF [18], occurred in a manner that was dependent on the SIRT1 transgene dosage.

3.5. Pathological examination of SIRT1 mice

The heart weight/body weight ratio and lung weight/body weight ratio were increased in SIRT1 (H) mice (Table 2). There was also a