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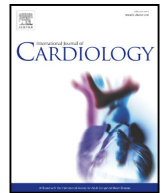

Inhibition of prolyl hydroxylases alters cell metabolism and reverses pre-existing diastolic dysfunction in mice

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ABSTRACT

Background: Diastolic dysfunction is emerging as a leading cause of heart failure in aging population. Induction of hypoxia tolerance and reprogrammed cell metabolism have emerged as novel therapeutic strategies for the treatment of cardiovascular diseases.
Methods and results: In the present study, we showed that deletion of sirtuin 3 (SIRT3) resulted in a diastolic dysfunction together with a significant increase in the expression of prolyl hydroxylases (PHD) 1 and 2. We further investigated the involvement of PHD in the development of diastolic dysfunction by treating the 12–14 months old mice with a PHD inhibitor, dimethyloxalylglycine (DMOG) for 2 weeks. DMOG treatment increased the expression of hypoxia-inducible factor (HIF)-1α in the endothelium of coronary arteries. This was accompanied by a significant improvement of coronary flow reserve and diastolic function. Inhibition of PHD altered endothelial metabolism by increasing glycolysis and reducing oxygen consumption. Most importantly, treatment with DMOG completely reversed the pre-existing diastolic dysfunction in the endothelial-specific SIRT3 deficient mice.
Conclusions: Our findings demonstrate that inhibition of PHD and reprogrammed cell metabolism can reverse the pre-existed diastolic dysfunction in SIRT3 deficient mice. Our study provides a potential therapeutic strategy of induction of hypoxia tolerance for patients with diastolic dysfunction associated with coronary microvascular dysfunction, especially in the aging population with reduced SIRT3.

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1. Introduction

Diastolic dysfunction is one of the major characteristics of heart failure with preserved ejection fraction (HFpEF), as well as in some population of asymptomatic patients and patients with reduced EF (HFrEF) [1]. More than half of the HF patients are diagnosed with diastolic dysfunction [2–5]. Diastolic dysfunction is commonly associated with cardiovascular, metabolic, and inflammatory comorbidities [6]. For instance, age, hypertension, diabetes mellitus, obesity, chronic renal failure, and LV hypertrophy are the major risk factors for diastolic dysfunction [7,8]. Recent studies demonstrate that persistent or progression of diastolic dysfunction, especially with co-existing comorbidities, promotes the development of heart failure in aging population [9,10]. However, currently available and effective treatment for HFrEF have failed to show promising results in patients with diastolic dysfunction [11]. Clinical studies reveal that patients with HFpEF have coronary microvascular rarefaction and more cardiac hypertrophy than age-matched patients without clinical diagnosis of coronary artery disease and heart failure [6]. Despite the clinical importance of HFpEF, our understanding of its pathophysiology and molecular mechanism is incomplete.

Sirtuins are a family of Class III histone deacetylases (HDACs) that require NAD^+ for their lysine residue deacetylase activity [12,13]. Sirtuins regulate cellular homeostasis, including energy metabolism and reactive oxygen species (ROS) [14,15]. Of the Sirtuin family, SIRT3 is primarily localized to the mitochondria in metabolic active organs, including liver, adipose tissue, and heart, where it regulates mitochondrial function and cellular metabolism [15–18]. Increased SIRT3 expression protects cardiomyocytes, pancreatic cells, and neurons from inflammation and apoptosis by reducing oxidative stress [19–22]. SIRT3 levels have been shown to decrease in human cardiac fibroblasts isolated from controls and patients with HF [23]. Hirschey and colleagues report that ablation of SIRT3 in mice impairs glucose tolerance and develops hepatic steatosis and metabolic syndrome [24,25]. In our previous study, we found that ablation of SIRT3 causes coronary microvascular dysfunction and increases ischemic injury in the heart [26]. Moreover, specific deletion of endothelial Sirt3 impairs glycolysis and causes a diastolic dysfunction in mice [27]. Koentges and colleagues report that SIRT3 deficiency causes mitochondrial and contractile dysfunction in the heart [28]. These studies indicate a critical role of SIRT3 in the development of cardiac dysfunction.

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Hypoxia triggers the activation of hypoxia-inducible factors (HIFs) and the expression of many genes involving in glucose uptake, glycolysis, erythropoiesis, and angiogenesis [29–31]. Prolyl hydroxylases (PHDs) play an important role in the regulation of HIFs [32,33]. Deactivation of PHD1 reduces oxygen consumption and mitochondrial oxidative stress and protects against muscle ischemic necrosis [34]. However, the consequence of administering PHD inhibitor on the diastolic dysfunction is unclear. In the present study, we hypothesized that inhibition of PHDs that mimics induction of hypoxia tolerance is protective against the diastolic dysfunction in the SIRT3 deficient mice. Our study reveals that the expression of PHD1 and PHD2 is significantly upregulated in the SIRT3 deficient mice. Moreover, treatment with PHD inhibitor DMOG reprograms endothelial metabolism, improves coronary microvascular function and diastolic function in global SIRT3 knockout (KO) mice and endothelial-specific SIRT3 KO mice.

2. Methods

See Online Data Supplement for detailed methods and materials.

2.1. Animals

All animals were fed with laboratory standard chow and water and housed in individually ventilated cages in the Laboratory Animal Facilities at the University of Mississippi Medical Center. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Mississippi Medical Center (Protocol ID: 1280B) and were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996).

2.2. DMOG treatment

Endothelial SIRT3 knockout (SIRT3 ECKO) mice were obtained by crossbreeding SIRT3$^{flox/flox}$ mice with VE-Cadherin-Cre (Cdh5-Cre) transgenic mice [B6.FVB-Tg(Cdh5-cre)7Mlia/J from Jackson Laboratories] expressing Cre recombinase in vascular endothelial cells, as described in previous study [27]. Male SIRT3 global knockout (SIRT3 KO) or SIRT3 ECKO mice at age of 12–14 months were injected with DMOG (25 μg/g/day) intraperitoneally for 14 days as shown in Supplemental Fig. S1. The number of mice used for each experiment was indicated in the figure legends. This dosage used in this study was chose based on previous studies with minor change [35–37]. Diastolic function and coronary flow reserve was monitored using echocardiography before the treatment and every 7 days. After 14 days of treatment, the mice were sacrificed, and heart tissue were harvested for Western blot and immunofluorescence staining.

2.3. Statistical analysis

Data are presented as mean ± S.D. All data were tested for normality and passed the test. Statistical significance was determined using Student's *t*-test (two-tailed) between means of two groups, or one-way ANOVA with repeated measures as indicated, followed by Bonferroni's *post hoc* test (Sigmaplot v12.5). $p < 0.05$ is considered as statistically significant.

3. Results

3.1. SIRT3 KO mice develops diastolic dysfunction

We examined whether SIRT3 KO mice developed a diastolic dysfunction in the presence of impaired CFR. Pulse-wave (PW) Doppler measurements indicated that the isovolumic relaxation time (IVRT) was significantly increased in SIRT3 KO mice (Fig. 1A and B). In addition, the calculated myocardial performance index was significantly elevated (Fig. 1B). The mitral valve inflow velocity during early diastolic (E) phase was similar between WT and SIRT3 KO mice, but it was associated with a significant decrease in late diastolic (A) phase (Supplemental Table 1, Fig. 1A and B). However, the E/A ratio was not significantly different between the two groups (Supplemental Table 1). Moreover, Tissue Doppler of mitral annulus indicated that the peak velocity of E′ and A′ was significantly decreased, resulting in a significant elevation in the E/E′ ratio (Fig. 1C and Supplemental Table 1). These data indicate that loss of SIRT3 is associated with impaired left ventricle (LV) relaxation and filling pressure. SIRT3 KO mice also exhibited a systolic dysfunction compared to the age-matched WT mice as shown in Supplemental Table 2.

3.2. Upregulation of PHD 1 and PHD2 in the hearts of SIRT3 KO mice

To further investigate the molecular basis of diastolic dysfunction in SIRT3 deficient mice, we analyzed the expression of PHDs that induces hypoxia tolerance and reprograms cell metabolism. We found that the expression of PHD1 and PHD2 was significantly upregulated whereas the expression of PHD3 was downregulated in SIRT3 KO mouse heart (Fig. 1D–F). Intriguingly, the expression of atrial natriuretic peptide (ANP) was diminished in the heart of SIRT3 KO mice (Supplemental Fig. S2A). We found that the level of gp91, a subunit of NADPH oxidase, was significantly upregulated in the heart of SIRT3 KO mice (Supplemental Fig. S2B). There was a significant decrease in the expression of ERK1/2 in the heart of SIRT3 KO mice (Supplemental Fig. S2C). Moreover, the expression of caspase-3 and Wnt7b was markedly increased in the heart of SIRT3 KO mice (Supplemental Fig. S2D and E), suggesting cardiomyocytes are under stress and vulnerable to apoptosis.

3.3. Inhibition of PHD improves CFR and reverses pre-existed diastolic dysfunction

We further tested if suppression of PHDs could improve diastolic function. SIRT3 KO mice were injected with DMOG (25 μg/g/day) intraperitoneally for 14 days. First, we analyzed the effect of DMOG on the expression of HIF-1α and HIF-2α by Western blots and found only mild increase in HIF-1α in SIRT3 KO heart and no change in HIF-2α (Supplemental Fig. S3). Intriguingly, HIF-1α and HIF-2α were enriched in the nuclei of both endothelial cells and cardiomyocytes (Supplemental Fig. S4A–D), as well as in the endothelium of coronary arteries (Supplemental Fig. S5). However, capillary density was not significant different between the two groups (Supplemental Fig. S4E). Cardiac diastolic function was measured at day 7 and day 14 by echocardiography after DMOG treatment. As shown in Fig. 2A, DMOG treatment significantly increased CFR at day 14 in SIRT3KO mice. Moreover, DMOG treatment significantly improved diastolic function, as evidenced by gradual decline of IVRT and myocardial performance index (MPI) at day 7 (Fig. 2B–C). Transmitral inflow Doppler showed a significantly increase in peak velocity of E and A, along with recovered peak velocity of E′ and A′ at day 7 (Fig. 2B, D, and Supplemental Table 1). The E/E' ratio was also significantly decreased (Supplemental Table 1). Systolic function was also improved in SIRT3 KO mice treated with DMOG for 2 weeks (Supplemental Table 2). Although we did not observe any cardiac hypertrophy in SIRT3 KO mice compared to WT mice, DMOG decreased heart weight to tibia length ratio (Supplemental Fig. S6A). There was no difference in body weight (Supplemental Fig. S6B). Also, DMOG treatment did not alter blood pressure measured by tail-cuff method nor heart rate (Supplemental Fig. S7). These data suggest PHD may play an important role in coronary microvascular dysfunction in association with diastolic function during SIRT3 deficiency.

3.4. PHD inhibition alters glycolytic metabolism and oxygen consumption in ECs

Using cultured endothelial cell, we further investigated the effect of PHD inhibition on EC metabolism. In WT-ECs, treatment with DMOG (1 mM) for 24 h increased the expression of HIF-2α and glycolytic enzyme, PFKFB3 (Supplemental Figs. S8A and S9A). Whereas, the expression of HIF-1α did not change (Supplemental Fig. S10). This was accompanied by a significant increase in glycolytic metabolism (Fig. 3A). Interestingly, basal oxygen consumption and maximum respiratory capacity were suppressed in WT-ECs by DMOG (Fig. 3B). Similarly, exposure of SIRT3 KO-ECs to DMOG led to a dose-dependent increase in the expression of HIF-2α and PFKFB3 (Supplemental Figs. S8B and S9B) and improvement of glycolysis in SIRT3 KO-ECs (Fig. 3A). Furthermore, exposure of SIRT3 KO ECs to DMOG significantly reduced maximum oxygen consumption (Fig. 3B). To further test the hypothesis that PHD1 plays an important role in endothelial metabolism, we specifically

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