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SIRT2 regulates microtubule stabilization in diabetic cardiomyopathy

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ABSTRACT

Stable microtubules (MTs) is involved the mechanism of diabetic cardiomyopathy (DCM), which is induced by acetylation of α-tubulin. The present study investigated whether SIRT2, a deacetylase, regulates MT stability through α-tubulin deacetylation in DCM and whether the receptor of advanced glycation end products (AGEs) signaling pathway is involved in this effect. Type 1 diabetic mellitus (T1DM) rats model was established by a single intraperitoneal injection of streptozotocin (STZ, 65 mg/kg), and neonatal rat cardiomyocytes were also cultured. Heart function was detected by Doppler. MT stability was elevated by β-tubulin expression density. The protein expression of SIRT2, acetylated α-tubulin and AGEs receptor were detected by immunohistochemistry or Western blots. The interaction of SIRT2 and acetylated α-tubulin was detected by Co-immunoprecipitation. In an animal model of T1DM, Western blots and immunohistochemistry revealed downregulation of SIRT2 but upregulation of the acetylated α-tubulin protein. These effects were reduced by treatment of aminoguanidine, an inhibitor of AGEs production. HDAC6 expression did not regulated in heart. In primary cultures of neonatal rat cardiomyocytes, the AGEs treatment impaired the SIRT2/acetylated α-tubulin signaling pathway, and SIRT2-overexpression reversed the function of AGEs on cardiomyocytes. In addition, gene silencing of AGEs receptor alleviated the impairment effect of AGEs on cardiomyocytes. In conclusion, these data demonstrate that AGEs/AGEs receptor promote MT stabilization via the suppression of the SIRT2/acetylated α-tubulin signaling pathway in DCM development.

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1. Introduction

Diabetic cardiomyopathy (DCM) is characterized by myocardial left ventricular (LV) dysfunction shifting from diastolic to systolic dysfunction with disease progression, which is independent of atherosclerosis and coronary artery disease(Pepe et al., 2013; Luo et al., 2014). However, the molecular mechanism underlying these defects remains controversial, and there is currently no effective treatment for DCM.

Microtubules (MTs) are cytoskeletal filaments that play essential roles in diverse cellular functions. In large mammals with adult-onset pressure-overload-induced cardiac hypertrophy, an abnormally high density of the MT network in cardiocytes was identified as an important factor in the development of ventricular contractile dysfunction (Koide et al., 2000). Previous studies found that stable MTs contribute to cardiac dysfunction in streptozotocin (STZ)-induced model of type 1 diabetes in rats (T1DM) (Shiels et al., 2007). MTs are composed of heterodimers containing α- and β-tubulin subunits diversified through post-translational modifications such as polyglycylation, detyrosination, polyglutamylation, and acetylation (Conacci-Sorrell et al., 2010; Hammond et al., 2008). Among these modifications, tubulin acetylation induces MT stabilization, which can be achieved by treatment with taxol, and is therefore regarded as a marker of long-lived MTs (Piperno et al., 1987). In fact, tubulin acetylation also modulates the ability of MTs to bind to microtubule-associated proteins (MAPs) and motor proteins, thus affecting MTs stability and functions (Reed et al., 2006; Sudo and Baas, 2010). Accordingly, tubulin acetylation reportedly promotes cardiac myocyte hypertrophy and dysfunction of myocardial contraction (Ng et al., 2011). Thus, aberrant tubulin acetylation and MT stabilization may directly contribute to DCM.

SIRT2 belongs to a highly conserved NAD^+-dependent deacetylase family comprising seven members (SIRT1–SIRT7), which are

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found in different subcellular localizations, and their substrates range from histones to transcription factors and enzymes (Houtkooper et al., 2012; Imai and Guarente, 2014). SIRT2 co-localizes with MTs and functions as an α-tubulin deacetylase by deacetylating the lysine-40 residue of α-tubulin both in vitro and in vivo (North et al., 2003) and its association with histone deacetylases (HDAC6) (Southwood et al., 2007). On the other hand, SIRT2 knockdown by siRNA results in tubulin hyperacetylation (Donmez, 2012). These data suggest that changes in the activity and/or expression of SIRT2 affect cytoskeletal stability through changes in the α-tubulin acetylation level and are involved in the development of many pathological conditions. Therefore, we hypothesized that downregulated expression of SIRT2 contributes to excess MT stability in DCM by α-tubulin deacetylation.

Protein glycation reactions leading to advanced glycation end products (AGEs) accumulated in the myocardium are thought to be the major causes of DCM (Terashima and Goto, 2002). These intracellular AGEs play important roles as stimuli for activating intracellular signaling pathways as well as modifying the function of intracellular proteins (Brownlee, 1995). AGEs can cause intracellular changes in vascular and myocardial tissue via interaction with the receptor for advanced glycation end products expressed on the surface of cardiomyocytes (Bodiga et al., 2014). But no evidence showed the effect of AGEs/AGEs receptor on SIRT2 mediated deacetylation of α-tubulin in the progression of DCM.

Therefore, the present study used STZ-induced T1DM rats and isolated cardiomyocytes to determine the importance of SIRT2-mediated deacetylation of α-tubulin in the progression of DCM and to determine the involvement of the AGEs/AGEs receptor signaling pathway.

2. Materials and methods

2.1. Induction of type 1 diabetes and experimental design

Thirty male Sprague Dawley (SD) rats (180–200 g, 8 weeks) were purchased from the Laboratory Animal Center (Hubei, China). All rats were treated in accordance with 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 experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Wuhan University of Technology and Science. These rats were fed a basal diet (BD) for 1 week before the experiments. STZ (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 0.1-M sodium citrate buffer (pH 4.3). After 1 week of acclimation to the vivarium, diabetes was induced in rats by a single intraperitoneal injection of STZ (65 mg/kg). The control group received a single intraperitoneal injection of sodium citrate buffer of the same volume. After 72 h, the rats with a fasting blood glucose level of >11.1 mmol/l were included in the T1DM group. And the rats including normal and T1DM rats were separated into four treatment groups ($n=5$–7 per group): (1) the control group (no diabetes), (2) T1DM group, (3) T1DM+insulin (T1DM rats were treated by subcutaneous injection of insulin 4 U/kg/day) (Semaming et al., 2014), (4) T1DM+aminoguanidine (T1DM rats were treated by aminoguanidine, the inhibitor of AGE production, 50 mg/kg body weight once a day orally for 12 weeks) (Kim et al., 2014). 12 weeks after the induction of T1DM, all rats were killed for tissue harvesting.

2.2. Echocardiography

The cardiac function of rats and morphological were detected by the echocardiographic system (GE logiq5 pro) equipped with a 12-MHz linear probe as as described previously (Yuan et al., 2014). The parameters recorded were heart rate, left ventricular end-systolic diameter (LVESD), and ejection fraction (EF).

2.3. Metabolic analysis and morphological analysis

The blood glucose level was measured using an automated analyzer (Wako, Japan). Glycated hemoglobin was determined using a commercial kit (Unimate HbA1c, Roche Diagnostics, Mannheim, Germany). Plasma levels of AGEs were detected using a commercial ELISA kit (OxiSelect™ Advanced Glycation End Products, Cell Biolabs Inc., San Diego, USA). Two days after echocardiographic evaluation, rats were anesthetized to remove the hearts. Transverse tissue sections were fixed in 4% neutral buffered formalin and paraffin-embedded for light microscopic evaluation. Sections were fixed in acetone (-20 °C) and stained with hematoxylin–eosin (H&E)

2.4. Primary cultures of neonatal rat cardiomyocytes and in vitro experimental protocols

Primary cultures of neonatal rat cardiomyocytes from 1-old SD rats were prepared as reported previously (Yue et al., 2000).

2.5. Visualization and quantification of microtubules

Heart tissues were fixed and labeled with antibodies to rabbit monoclonal antibody against β-tubulin (1:200 dilution; Sigma Chemical Co.) using the protocol described by previous (Howarth et al., 1999). Secondary antibody used was FITC-labeled goat anti-rabbit (1:200 dilution; Jackson ImmunoResearch Laboratories Inc., USA). Sections of approximately 5 μM thickness were obtained by fluorescence microscopy (Nikon, Tokyo, Japan). Excitation wavelengths were 488 nm for FITC conjugates. Images used for quantification were collected using the same settings and analyzed offline using NIS-Elements (Nikon, Tokyo, Japan) to calculate the mean fluorescent intensity, which was used as a measure of microtubule density.

2.6. Immunofluorescence

Formalin-fixed myocardial sections were deparaffinized, rehydrated, and incubated with mouse monoclonal antibody against β-tubulin (Santa Cruz, CA, USA; 1:200 dilution). Then, they were incubated with a green fluorescent protein (GFP) labeled anti-rabbit antibody (Vector Laboratories Inc., CA, USA; 1:200). The mean density of β-tubulin expression was analysised from six separate fields of transverse LV tissue sections observed using light microscopy (400×).

2.7. Immunohistochemistry

Formalin-fixed myocardial sections were deparaffinized, rehydrated, and incubated with one of the following primary antibodies: rabbit polyclonal antibody against SIRT2 (Santa Cruz, CA, USA; 1:200 dilution), rabbit monoclonal antibody against HDAC6 (Biosynthesis Biotechnology, Beijing, China; 1:500 dilution) or rabbit polyclonal antibody against acetylated α-tubulin (Biosynthesis Biotechnology, Beijing, China; 1:500 dilution). Then, they were incubated with a biotinylated secondary anti-rabbit antibody (Vector Laboratories Inc., CA, USA; 1:2000). The mean percentage of $SIRT2^{+}$, $HDAC6^{+}$ or acetylation $\alpha\text{-tubulin}^{+}$ cardiomyocytes were calculated from six separate fields of transverse LV tissue sections observed using light microscopy (400×).

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