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JIP3 deficiency attenuates cardiac hypertrophy by suppression of JNK pathway

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ABSTRACT

Pathological cardiac hypertrophy is a leading cause of morbidity and mortality worldwide; however, our understanding of the molecular mechanisms revealing the disease is still unclear. In the present study, we suggested that *c*-Jun N-terminal kinase (JNK)-interacting protein 3 (JIP3), involved in various cellular processes, played an essential role in regulating pathological cardiac hypertrophy through in vivo and in vitro studies. JIP3 was highly expressed in human hearts with hypertrophic cardiomyopathy (HCM), and in mouse hypertrophic hearts. Following, the wild type (WT) and JIP3-knockout (KO) mice subjected to aortic banding (AB) challenge were used as animal models with cardiac hypertrophy. The results showed that JIP3-KO mice after AB operation exhibited attenuated cardiac function, reduced fibrosis levels and decreased hypertrophic marker proteins, including atrial natriuretic peptides (Anp) and brain/B-type natriuretic peptides (Bnp) and β-myosin heavy chain (β-Mhc). Loss of JIP3 also ameliorated oxidative stress, inflammatory response, apoptosis and endoplasmic reticulum (ER) stress in hearts of mice after AB surgery. Consistently, the expressions of ER stress-related molecules, such as phosphorylated-α-subunit of the eukaryotic initiation factor-2 (eIF2α), glucose-regulated protein (GRP) 78 and C/-EBP homologous protein (CHOP), were markedly decreased by JIP3-deficiency in hearts of AB-operated mice. JNK and its down-streaming signal of p90rsk was highly activated by AB operation in WT mice, while being significantly reversed by JIP3-ablation. Intriguingly, the in vitro results showed that promoting JNK activation by using its activator of anisomycin enhanced AngII-stimulated ER stress, oxidative stress, apoptosis and inflammatory response in cardiomyocytes isolated from WT mice. However, JIP3-KO-attenuated these pathologies was rescued by anisomycin treatment in AngII-incubated cardiomyocytes. Together, the findings indicated that blockage of JIP3 could alleviate cardiac hypertrophy via inactivating JNK pathway, and thus might be a promising strategy to prevent pathological cardiac hypertrophy.

1. Introduction

Cardiac hypertrophy presents a leading cause of morbidity and mortality in the world [1]. Pathological cardiac hypertrophy is characterized by an increase in cardiomyocyte size, elevated protein synthesis, and enhanced sarcomere organization, linked to reactivation of the fetal gene program [2,3]. Advanced pathological hypertrophy is deleterious and could result in ventricular dilatation and heart failure eventually and sudden death [4]. Increasing studies have indicated that the pathological hypertrophy response is associated with various pathologies, including fibrosis accumulation, inflammation, oxidative stress and ER stress [5–7]. However, much less is understood that how cardiac hypertrophy is inhibited.

JIP3, also known as JSAP1, is first identified as scaffold proteins for specific JNK mitogen-activated protein kinase (MAPK) signaling modules [8,9]. JIP3 functions as an adaptor protein to modulate the trafficking of cargo through bridging them to kinesin [10,11]. Many studies have focused on the effects of JIP3 on brain progression. JIP3 meditates neuronal axon elongation and branching in cultured neurons [12,13]. Additionally, JIP3 knockout was suggested to attenuate inflammatory response through inactivating toll like receptors (TLRs) pathway. And oxidative stress was suppressed by

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JIP3 inhibition in animals with dyslipidemia [14,15]. JNK plays an essential role in promoting fibrosis formation, contributing to renal and hepatic injury [14,16]. Phenotypic changes of the extracellular matrix (ECM) is tightly involved in the pathogenesis heart failure [17]. Therefore, we supposed that JIP3 might be of potential in modulating cardiac hypertrophy. ER stress is an evolutionarily conserved cell stress response and has been linked to numerous diseases, including cardiovascular diseases [18]. However, if JIP3 alteration could modulate ER stress, and subsequently influence cardiac hypertrophy, little is to be known.

In the present study, we investigated the physiological role of JIP3 in heart using WT and JIP3-KO mice and uncovered a novel role of JIP3 in the modulation of cardiac hypertrophy. JIP3 deficiency suppressed inflammation, oxidative stress, fibrosis accumulation and ER stress in AB-operated hypertrophic hearts or in AngII-induced cardiac cell hypertrophy. The protective effects of JIP3-knockout on cardiac hypertrophy might be, largely, linked to the inactivation of JNK pathway.

2. Materials and methods

2.1. Human heart tissues

Samples of human failing hearts were collected from the LVs of HCM patients undergoing heart transplants. The control segments were obtained from the left ventricles (LVs) of normal heart donors. The segments were obtained after informed consent and were approved by the local Human Research Ethics Committee of Shandong Provincial Hospital (Jinan, China). All studies that involved human samples conformed with the Declaration of Helsinki.

2.2. Animals and culture

All animals used in the study were received humane care in line with principles stated in the Guide for the Care and Use of Laboratory Animals, NIH Publication, 1996 edition. All protocols were approved by the Animal Care Committee of Shandong Provincial Hospital. Male, 6-8 week-old, weighed 18–20 g C57BL/6J mice, including the wild type (WT) and JIP3-knockout (KO), were obtained from Jackson Laboratory (Bar Harbor, ME), and housed for 1 week as an acclimatization period before the experiment. All mice were then subjected to aortic banding (AB) or a sham operation as described previously [19]. Finally, after AB surgery for 8 weeks, all hearts were harvested for further analysis.

2.3. Cardiomyocytes isolation and treatment

Primary cardiomyocytes were prepared from the hearts of WT and JIP3-KO mice as previously described [20]. The obtained cardiomyocytes were cultured in DMEM/F12 (Gibco, USA) supplemented with 20% fetal calf serum, 0.1 mM 5-bromodeoxyuridine to suppress fibroblast proliferation and penicillin/streptomycin. Cardiomyocyte were subsequently stimulated with 1 μM Ang II (Sigma Aldrich, USA) for 24 h. JNK activator of anisomycin (10 μM) (Sigma Aldrich) was treated to cells for 24 h for subsequent experiments.

2.4. Echocardiographic measurement

Echocardiography was carried out on the anaesthetized (1.5% isoflurane) mice, using ultrasound system with a 17L5 transducer (Sequoia 512, Siemens, Germany) as previously described [21]. Measurements of fractional shorting (FS), end-diastolic posterior wall thickness (dPW), left ventricular end-diastolic diameter (LVEDd) and left ventricular end-systolic diameter (LVESd) were obtained through M-mode images as described previously [22].

2.5. Cardiac MDA and SOD measurements

Malonaldehyde (MDA) levels and superoxide dismutase (SOD) activity in hearts of mice was measured using commercially available kits (Nanjing Jiancheng Biotechnology, Nanjing, China).

2.6. DCF staining

Intracellular reactive oxygen species (ROS) production was measured using the fluorogenic probe 2′,7′-dichlorodihydrofluorescein diacetate (DCFH2-DA, KeyGen Biotech).

2.7. Flow cytometry analysis

Apoptosis of cardiomyocytes was measured using the Annexin V-FITC Apoptosis Detection Kit with propidium iodide (PI) (KeyGen Biotech, Nanjing, China) according to the manufacturer's protocol. All the cells stained positively for Annexin V-FITC were considered apoptotic cells and quantified.

2.8. RT-qPCR analysis

Total RNA was extracted from mouse hearts or cultured cells using Trizol (Invitrogen, USA) following the manufacturer's instruction. RT-qPCR analysis was then performed using the SYBR Green Master (Invitrogen) and a 7900 HT Fast Real-Time PCR System (Thermo, USA) as described previously [23]. Primer sequences were exhibited in Supplementary Table 1. GAPDH mRNA served as an internal standard.

2.9. Western blot analysis

Heart tissue samples or cells were homogenized in modified RIPA buffer (KeyGen Biotech). The western blot protocol was performed as previously described [23]. Primary antibodies used in the study were listed in Supplementary Table 2.

2.10. Immunohistochemistry (IHC) analysis

Freshly dissected heart sample was embedded in OCT embedding matrix (Thermo Fisher Scientific, USA). Sections were cut at 7 μm thickness and stored in −80 °C freezer for H&E, wheat germ agglutinin (WGA), Masson's trichrome and Sirius Red staining were performed on heart sections [24,25]. IHC staining of α-SMA or JIP3 (Abcam, USA) was performed on mouse heart tissue sections as previously described [26].

2.11. TUNEL staining

TUNEL assay was performed on cardiac sections using the in situ Cell Death Detection Kit (Roche, Switzerland) following the manufacturer's protocols.

2.12. Data analysis

Data are expressed as mean ± SEM. Statistical analyses were performed using GraphPad PRISM (Version 6.0, Graph Pad Software, USA). Differences among groups were determined by two-way ANOVA followed by a post hoc Tukey test. Comparisons between two groups were performed using an unpaired Student's t-test. A value of $P < 0.05$ was considered significant.

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