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DUSP14 knockout accelerates cardiac ischemia reperfusion (IR) injury through activating NF-κB and MAPKs signaling pathways modulated by ROS generation

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ABSTRACT

Inflammation and oxidative stress are significantly involved in the progression of a variety of diseases, including myocardial ischemia/reperfusion (IR). In the present study, we hypothesized a protective role of dual-specificity phosphatase 14 (DUSP14) in myocardial IR, as well as the underlying molecular mechanism. The results indicated that DUSP14 was down-regulated following cardiac IR injury. Subsequently, the wild type (WT) and DUSP14-knockout (KO) mice were included to further reveal the potential role of DUSP14 in cardiac IR injury progression. DUSP14-KO mice exhibited increased infarction area and elevated apoptosis, as evidenced by the increased TUNEL-positive cells in ischemia heart following reperfusion compared to WT mice. Further, DUSP14-KO significantly aggregated cardiac dysfunction of mice after IR injury. Cardiac IR injury to DUSP14-KO mice led to markedly increased expression of pro-inflammatory cytokines and activated nuclear factor-κB (NF-κB) pathway in the heart in comparison to WT mice. Meanwhile, mitogen-activated protein kinases (MAPKs), including p38, ERK1/2 and JNK, were significantly activated by DUSO14-KO in mice after IR injury. Compared to WT mice, DUSP14-KO mice showed markedly increased oxidative stress markers in cardiac tissues, including malondialdehyde (MDA), NADPH oxidase-4 (NOX4) and p47, while decreased activities or expressions of anti-oxidants, such as glutathione (GSH), glutathione peroxidase (GPx), glutathion reductases (GR), superoxide dismutase (SOD) and hemeoxygenase-1 (HO-1). DUSP14-knockdown (KD) in primary cardiomyocytes using its specific siRNA sequence elevated hypoxia and reoxygenation (HR)-induced activation of NF-κB and MAPKs signaling pathways, and reactive oxygen species (ROS) generation. Intriguingly, pre-treatment of ROS scavenger, *N*-acetylcysteine (NAC), markedly abolished DUSP14-KD-augmented NF-κB and MAPKs activation in HR-stimulated primary cardiomyocytes. Together, the results above indicated that DUSP14 might be served as a positive regulator to attenuate cardiac IR injury. Suppressing DUSP14 exacerbated cardiac injury through activating NF-κB and MAPKs signaling pathways regulated by ROS production. Thus, DUSP14 could be a valuable target for developing treatments for myocardial IR injury.

1. Introduction

Myocardial ischemia-reperfusion (IR) injury is frequently observed in percutaneous coronary intervention and several cardiac surgeries in clinical practice, which is the primary factor, leading to the morbidity and mortality of coronary artery diseases [1,2]. Thus, management of IR injury is essential for improving the outcome of these patients with cardiac heart injury. The development of myocardial IR injury includes multiple mechanisms. Inflammation and oxidative stress are importantly involved in the development of myocardial I/R [3].

Dual-specificity phosphatases (DUSPs) are critical modulators in various biological processes, including immune regulation, T-cell development, and tumorigenesis [4]. DUSP14, also known as MKP6, is a member of the atypical DUSP family, and contains the consensus C-terminal catalytic domain but lacks the *N*-terminal CH2 domain [5]. DUSP14 dephosphorylates JNK MAPK, ERK MAPK, and p38 MAPK in vitro [6]. DUSP14 suppresses β-cells proliferation through interfering ERK activation [7]. Additionally, DUSP14

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negatively modulate the interleukin-1β (IL-1β)- and tumor necrosis factor-α (TNF-α)-induced NF-κB activation [8]. Both NF-κB and MAPKs signaling pathways play critical roles in IR injury [9]. Therefore, we hypothesized that DUSP14 might be involved in the regulation of cardiac IR injury, and little is understood presently.

This study revealed the potential role and molecular mechanism of DUSP14 in cardiac IR injury and the inflammatory response. The wild type (WT) and DUSP14-knockout (KO) mice were included in our study, and performed cardiac IR surgery to evaluate the effects of DUSP14 on IR-induced heart damage. Our results indicated that DUSP14 expression was down-regulated in the heart of mice after IR injury, and its knockout accelerated myocardial IR injury, indicating that DUSP14 might function as a novel protective modulator in cardiac heart injury.

2. Materials and methods

2.1. Animals

The present study was approved by the First Affiliated Hospital of Zhengzhou University Institutional Animal Care and Use Committee (Henan, China). DUSP14$^{-/-}$ mice with a C57BL/6 background were generated as previously described and obtained from Animal Model Research Center, Nanjing University (Jiangsu, China) [10]. IR surgery was carried to wild type (WT) and DUSP14$^{-/-}$ male mice (12-wk-old, n = 20/group) in line with previous study [11]. All mice were anaesthetized with isoflurane (Wako Pure Chemical Industries, Ltd., Japan), and the heart was exposed through a left thoracotomy. Then, a 7-0 silk suture was tied around to the left anterior descending coronary artery using a slipknot and left for 45 min to induce ischemia, followed by 0–24 h reperfusion. Upon the completion of reperfusion, the hearts were stained with 2% Evans Blue (Sigma-Aldrich, USA) and 1% 2,3,5-triphenyltetrazolium chloride (Sigma-Aldrich). The infarct size was expressed as a percent of the risk zone (n = 6/group in each experiment).

2.2. Cells isolation and treatments

The primary cardiomyocytes were isolated from WT mice according to previous study [12]. After isolation and purification, cells were maintained in DMEM/F-12 medium (Gibco, USA). 1% Penicillin/Streptomycin (Gibco) and 10% fetal bovine serum (FBS) (Hyclone, Thermo scientific, USA) were added to the DMEM. As for the suppression of DUSP14, Scrambled siRNA and siRNA against DUSP14 (Shanghai Generay Biotech, Shanghai, China) was transfected to cardiomyocytes isolated from WT mice with Lipofectamine RNAiMAX (Invitrogen, USA) according to manufacturer's instructions. The cells were transfected with 30 pmol siRNA and incubated for 24 h, and subsequent experiments were preformed after transfection efficiency analyzed by RT-qPCR and Western blot.

2.3. Western blot analysis

To calculate the protein expression levels, western blot was performed as previously described [13]. Protein expression levels were quantified using ImageJ software (USA) and normalized to GAPDH. The antibodies that were used in the present study were shown in Supplementary Table 1.

2.4. RT-qPCR analysis

Total mRNA was extracted from heart tissues and cultured cells and reverse-transcribed into cDNA (Takara, Dalian, China) following the manufacturer's instructions. Quantitative RT-PCR was performed using SYBR Green PCR Master Mix (Roche, USA) as described previously [13]. mRNA expression was normalized to GAPDH expression. The primer sequences that were used in the present study were shown in Supplementary Table 2.

2.5. Cell viability analysis

Cells were collected for viability analysis using MTT assays (MTT Cell Viability Assay Kit, KeyGen Biotech, Nanjing, China) following the manufacturers' instructions.

2.6. Biochemical measurements

Serum cytokines (IL-1β, TNF-α, IL-6, IL-4 and IL-2) and Troponin T were measured using commercial ELISA kits (R&D Systems, USA) following the manufacturers' instructions. The content of lactate dehydrogenase (LDH), creatine kinase-MB (CK-MB), MDA, GSH, GPx, GR and SOD levels or activities in serum or heart tissue samples were determined using commercial kits (Jiancheng Biotech Co., Ltd, Nanjing, China) following the manufacturers' protocols.

2.7. The analysis of apoptosis cells

Cell apoptosis was detected using the Annexin V-FITC Apoptosis Detection Kit with propidium iodide (PI) (BD Bioscience, USA) according to the manufacturer's protocol. All the cells stained positively for Annexin V-FITC were considered apoptotic cells.

2.8. ROS production determination

The ROS was measured with 2′,7′-dichlorofluorescein-diacetate (DCFHDA, Beyotime Institute of Biotechnology, Jiangsu, China) and dihydroethidium (DHE, KeyGen Biotech, China) staining following the manufacturers' protocols, and observed using a microscope or analyzed by flow cytometry (BD Bioscience) [14].

2.9. Cardiac function analysis

Electron microscopy was conducted to determine the cardiac function as previously described [15]. Ejection fraction (EF)%, fraction shortening (FS)%, left ventricular contraction volume (LVVl; s), and LV internal diameter at the end-systole (LVID; s) were calculated using Vevo Analysis software (version 2.2.3, USA) as previously described [16].

2.10. Determination of cardiomyocyte properties

The mechanical properties of cardiomyocyte were calculated using a SoftEdge Myocam system (IonOptix Corporation, USA). SoftEdge software was used to capture changes in cardiomyocyte length during shortening and re-lengthening. Cell shortening and re-lengthening were evaluated as previously described [17]. The indices of peak shortening (PS), time-to-PS (TPS), time-to-90% relengthening (TR_{90}), maximal velocity of shortening (+dL/dt) and relengthening (-dL/dt) were assessed.

2.11. Immunohistochemical analysis

Heart samples were embedded in paraffin for sectioning (4 μm thickness) and stained with hematoxylin and eosin (H&E). Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) (KeyGen Biotech) was performed to assess cell death as previously described [18]. The immunohistochemical analysis of DUSP14 of heart tissue sections was performed as previously described [19].

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