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Loss of P53 regresses cardiac remodeling induced by pressure overload partially through inhibiting HIF1α signaling in mice

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ABSTRACT

The tumor suppressor p53 is recognized as the guardian of the genome in cell cycle and cell death. P53 expression increases as cardiac hypertrophy worsens to heart failure, suggesting that p53 may play important role in cardiac remodeling. In the present study, deletion of p53 in the mice heart would ameliorate cardiac hypertrophy induced by pressure overload. The role of p53 on heart was investigated using in vivo models. Cardiac hypertrophy in mice was induced by transverse aortic banding surgery. The extent of cardiac hypertrophy was examined by echocardiography, as well as pathological and molecular analyses of heart tissue. Global knockout of p53 in the mice reduced the hypertrophic response and markedly reduced cardiac apoptosis, and fibrosis. Ejection fraction of heart was also improved in hearts without p53 in response to pressure overload. Protein determination further suggested loss of p53 expression markedly increased Hypoxia-inducible factor 1-alpha (HIF1α) and vascular endothelial growth factor (VEGF) expression. The study indicated p53 deteriorated cardiac functions and cardiac hypertrophy, apoptosis, and fibrosis by partially inhibition of HIF1α and VEGF.

1. Introduction

The epidemiology of chronic heart failure, specifically its morbidity and mortality, is insufficiently known [1,2]. Cardiac hypertrophy is a common precursor to many forms of heart failure, of which molecular and cellular mechanisms remain largely unclear [2,3]. After a period of compensatory adaptation, hypertrophy in the stage of decompensation is developing with cardiac dysfunction, and alteration of tissue and cardiac gene expression [4,5]. P53 pathway plays an important role in the development of heart failure [6,7]. Therefore, the identification of p53 for cardiac hypertrophy is crucial for the development of effective treatment strategies for heart failure after heart hypertrophy.

The tumor suppressor p53 has been involved in a growing number of biological responses including cell cycle, senescence, apoptosis, autophagy, metabolism, and aging [6–8]. In normal condition of cells, p53 expression is kept at low levels by the E3 ubiquitin ligase Mdm2, which targets p53 for proteasomal degradation. In response to acute stress, Mdm2 is inactivated and increased p53 levels block cell division and induce apoptosis [8,9]. Upregulation of p53 in response to various oncogenic stress enhanced ablation of cancer cells by apoptosis or senescence [8]. Activation of p53 is controlled by posttranslational modifications such as phosphorylation and acetylation [6,10].

Endothelial p53 deficiency enhanced angiogenesis and attenuated cardiac fibrosis and heart failure induced by pressure overload in mice [11]. P53 knockout mice exhibit apoptosis-independent prolongation of survival [12]. Our data showed that knockout of p53 in the mice markedly improved cardiac function, reduced apoptosis of cardiomyocyte, and diminished interstitial fibrosis after aortic banding. Despite the potentially significant roles of p53

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in attenuating apoptotic, inflammatory, and hypertrophic signaling in cardiomyocytes and endothelial cells, it has remained unknown how p53 could regulate cardiac remodeling through angiogenesis, which is implicated with HIF1α and VEGF [7,11,13]. Thus, in the present study, we investigate the role of p53 in cardiac remodeling induced by pressure overload and to define the underlying molecular mechanisms.

2. Methods and materials

2.1. Materials

The antibodies against HIF1α, VEGF, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), β-myosin heavy chain (β-MHC), Collagen I, connective tissue growth factor (CTGF), and transforming growth factor (TGF)-activated kinase β3 (TGF β3) were purchased from Cell Signaling Company. All other reagents including PVDF membrane and enhanced chemiluminescence were purchased from Amersham. All the primers were synthesized by Invitrogen Co. Ltd.

2.2. Animals, aortic banding surgery, blood pressure and echocardiography

All protocols were approved by Tongji University review board. Aortic banding (AB) were produced on 8-week-old male mice with p53 knockout (KO) and their control sham mice. Genotyping was performed by polymerase chain reaction (PCR) analysis of tail genomic DNA as described previously [13]. AB was produced as described previously [14]. Briefly, wild-type (WT) and p53 KO mice were anaesthetized with isoflurane. A 7.0 nylon suture ligature was tied against a 27-gauge needle at the transverse aorta to yield a narrowing of 0.4 mm in diameter after removal of the needle. Pulsed Wave Doppler examination ensures that physiologic binding of the transverse aorta was performed successfully. Pressure gradients (PW: mmHg) were calculated from the peak blood velocity (V_{max}) (m/s) detected by Doppler across the aortic constriction, which was equivalent in all groups of AB-subjected mice. A microtip catheter transducer (SPR-839, Millar Instruments, and Houston, Tex) was inserted into the right carotid artery and advanced into the left ventricle to confirm the blood pressure with PW dada.

Echocardiography was performed on anaesthetized (2% isoflurance) mice by a 30-MHz scanhead (VisualSonics Vevo 770, VisualSonics, Canada) [14]. End-systole or end-diastole was defined as the phase during which the smallest or largest area of left ventricle (LV), respectively, was obtained. LV end-systolic diameter (LVESD) and LV end-diastolic diameter (LVEDD) were examined from the LV M-mode at the mid-papillary muscle level. Mice were euthanized by cervical dislocation 4 weeks post-operatively. Hearts and lungs of sacrificed mice were harvested and weighed (in milligrams per gram) in p53 KO and control mice in the different groups.

2.3. Histological analysis and detection of apoptosis

Hearts were harvested, placed immediately in 10% potassium chloride solution to ensure that they were arrested in diastole, washed with pre-cold phosphate-buffered saline (PBS) solution, and put into 10% formalin. Hearts were sliced transversely at the papillary muscle level to expose the left and right ventricles. Several sections (4–5 μm thick) were produced and stained with haematoxylin-eosin (HE) and Trichrome Stain (Masson) Kit for histopathology then visualized by 400× light microscopy. To determine the cross-sectional area of cardiomyocytes (CAC), HE-stained slices were photographed, respectively. The areas of cardiomyocytes were determinated using a DigitalPhoto quantitative system (Image-Pro plus 6.0). The outlines of approximately 150 cardiomyocytes in the LV areas were traced in each group. Apoptosis was detected by TUNEL assay using Roche Diagnostics according to the manual. The quantitative analysis of histological images was performed in a blinded fashion.

2.4. Western Blotting(WB) analysis

WB was performed to detect protein levels of p53, hypertrophic (ANP, BNP, β-MHC) and fibrosis markers (Collagen I, CTGF, TGF β3), and the activation state of HIF1α and VEGF pathway. Heart tissue were homogenized in RIPA buffer containing a proteinase and phosphatase inhibitor cocktail (Amersham). A total of 20 μg of sample sulution per lane was run and transferred to a PVDF membrane. All the proteins were determined with the primary antibodies. The antibody-antigen reaction was detected using a secondary antibody linked to horseradish peroxidase. Quantification of WB images was performed using an automated Photo-analysis system (Image-Pro Plus 5.0, Media Cybernetics, Bethesda, MD, USA). The expressions of protein normalized to tubulin were compared for the total cell lysates.

2.5. Real-time quantitative RT-PCR (qPCR)

qPCR was performed as described in the previous study [15]. Total RNA was harvested from the heart tissues of mice by using TRIzol reagent. 500 ng RNA was reverse-transcribed to obtain the template complemental DNA (cDNA) for the next qPCR analysis. The qPCR program was run on a Bio-Rad IQ5 detection system using 1.2 μg of cDNA. Hypertrophic or fibrosis biomarker of heart were detected using primer below: For ANP: Forward, GGATCTCCTG AAGGTGCTGT, Reverse, CAGCTTCTGCATCTTGAATT; BNP: Forward, GGTTTGGGAAGCGGCAGAGA, Reverse, ATTGGGGAGCCCAGACGTCA; β-MHC: Forward, ATCTATACCT ACTCGGGCCT, Reverse, TGACA GTCTTCCCAGCTCCG; Collagen I: Forward, CCTGGTAAAGATGGTGCC, Reverse, CACCAGGTTCACCTTTCGCACC; CTGF: Forward, GGAGC GTCCA GACACCAACC, Reverse, GCTGCTTCGGCTGCGCA CTG; TGFβ3: Forward, TCCAGAGACAAGTGTGTCCT, Reverse, GAAAGTG AGTAT TTTAAAGA; Tubulin: Forward, AGCTGCGACTGTCTCCAGGG, Reverse, TCTCATCAGTGTTCTCCACC. A double delta comparative analysis was employed to compare the RNA expression of the groups. All the qPCR operations were in triplicate in a blinded fashion.

2.6. Statistical analysis

Data are expressed as mean + SEM. Differences among groups were determined by two-way ANOVA followed by a post hoc Tukey's test. Comparisons between two groups were performed using an unpaired Student's t-test. A value of $P < 0.05$ was considered significant.

3. Results

3.1. P53 deficiency abrogates pathological cardiac hypertrophy in response to pressure overload

To examine the function of p53 in the heart following chronic pressure overload, we established AB or sham surgery model on 8-week-old p53 knockout (Fig. 1B) or WT mice heart. In this mice model, cardiac hypertrophy slowly developed, hit a peak following 14 day of AB and reduced afterwards. As shown in Fig. 1, HW/BW and LW/BW ratios were remarkably decreased in KO mice compared with WT mice, as well as cardiac fibrosis (Fig. 1C–E) after

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