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Administration of ubiquitin-activating enzyme UBA1 inhibitor PYR-41 attenuates angiotensin II-induced cardiac remodeling in mice

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ABSTRACT

Pathological cardiac hypertrophy is the main risk factor for heart diseases. The ubiquitin-proteasome system (UPS) is the major intracellular protein degradation system involved in the development of cardiac hypertrophic remodeling. Ubiquitin-activating enzyme E1, a key component of the UPS, catalyzes the first step in ubiquitin conjugation to mark cellular proteins for degradation via proteasome. However, the functional role of E1 (UBA1) in regulation of hypertrophic remodeling in angiotensin II (Ang II)-infused mice remains unknown. In this study, male wild-type mice were treated with UBA1 inhibitor PYR-41 at two doses of 5 and 10 mg and infused with Ang II (1000 ng/kg/min) for 14 days. Systolic blood pressure was detected by using tail-cuff system. Cardiac function was assessed by echocardiography. Hypertrophic remodeling was analyzed examined by histological examinations. The expressions of genes and proteins were detected by quantitative real-time PCR and immunoblotting analysis. After 14 days, Ang II infusion significantly increased UBA1 expression at both mRNA and protein levels in the hearts. Furthermore, Ang II-infused mice showed a significant increase in systolic blood pressure compensatory cardiac function, hypertrophy, interstitial fibrosis, inflammation and oxidative stress compared with saline-treated controls, whereas these effects were dose-dependently attenuated in PYR-41-treated mice. These beneficial actions were associated mainly with inhibition of PTEN degradation and multiple downstream mediators (AKT, ERK1/2, STAT3, TGF-β/Smad2/3 and NF-kB(p65)). In conclusion, these results indicate that inhibition of UBA1 suppresses Ang II-induced hypertrophic remodeling, and suggest that administration of low dose PYR-41 may be a new potential therapeutic approach for treating hypertensive heart diseases.

1. Introduction

Pathological cardiac hypertrophy is a major cause of heart failure and sudden death worldwide [1]. Cardiac hypertrophic remodeling is characterized by increased myocyte size, protein accumulation, myocardial interstitial cell proliferation, collagen deposition and infiltration of inflammatory cells, which are regulated by multiple signaling pathways [2–5]. Accumulating evidence demonstrates that angiotensin II (Ang II), the major effector hormone of the renin-angiotensin system (RAS), plays a key role in the regulation of myocyte hypertrophy, fibrosis, inflammation and oxidative stress, which are main pathologic changes in cardiac remodeling. It is well documented that Ang II exerts these actions mainly through angiotensin II type 1 receptor (AT1R) and the downstream mediators such as AKT/mTOR, TGF-β/Smad2/3, NADPH oxidase and NF-kB(p65) signals. In contrast, inhibition of AT1R reduces these responses [6]. (see Table 1)

The normal development and maintenance of the heart depend on the balance between protein synthesis and degradation, while over 90% of the intracellular proteins in the heart are degraded by the ubiquitin proteasome system (UPS) pathway. Thus, the UPS may play a critical role in the development of cardiac hypertrophy [7].

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Table 1
Primers used for quantitative real-time PCR analysis.

Gene	Forward Primer	Reverse Primer
UBA1	5′-GCATCGTCAGTCAGGTCAAAGT-3′	5′-CATCACAAAGTCAGGCTCTACC-3″
ANF	5′-CACAGATCTGATGGATTTCAAA-3′	5′-CCTCATCTTCTACCGGCATC-3′
BNP	5′-GAAGGTGCTGTCCCAGATGA-3′	5′-CCAGCAGCTGCATCTTGAAT-3′
β-MHC	5′-TTGGCACGGACTGCGTCATC-3′	5′-GAGCCTCCAGAGTTTGCTGAAGGA-3′
Collagen I	5′-AGTCGATGGCTGCTCCAAAA-3′	5′-AGCACCACCAATGTCCAGAG-3′
Collagen III	5′-TCCTGGTGGTCCTGGTACTG-3′	5′-AGGAGAACCACTGTTGCCTG-3′
IL-1β	5′-CTTCCCCAGGGCATGTTAAG-3′	5′-ACCCTGAGCGACCTGTCTTG-3′
IL-6	5′-TTCCATCCAGTTGCCTTCTTG-3′	5′-TTGGGAGTGGTATCCTCTGTGA-3′
TNF-α	5′-ATGGCCTCCCTCTCATCAGT-3′	5′-CTTGGTGGTTTGCTACGACG-3′
NOX1	5′-CCCATCCAGTCTCCAAACATGAC-3′	5′- ACCAAAGCTACAGTGGCAATCAC-3′
NOX2	5′-CTTCTTGGGTCAGCACTGGC-3′	5′-GCAGCAAGATCAGCATGCAG-3′
NOX4	5′-CTTGGTGAATGCCCTCAACT-3′	5′-TTCTGGGATCCTCATTCTGG-3′
GAPDH	5′-GGTTGTCTCCTGCGACTTCA-3′	5′-GGTGGTCCAGGGTTTCTTACTC-3′

Ubiquitination of the target proteins is achieved via an enzymatic cascade involving ubiquitin-activating enzymes E1s, ubiquitin-conjugating enzymes E2s, and ubiquitin ligase enzymes E3s [8]. Among these enzymes, the E1 enzyme is responsible for ubiquitin activation, which is the initial step of the ubiquitin reaction [9]. Until now, there are only two human ubiquitin-activating enzymes, including UBA1 (also known as UBE1) and UBA6, and thus UBA1 is largely responsible for protein ubiquitination in humans [10]. UBA1 is a −120 kDa monomeric protein that is essential because the full deletion of this gene is lethal [11–13]. Several studies have shown that UBA1 is the major isoform that initiates the process of protein ubiquitination, and has been implicated in the development of cancer and neurodegenerative diseases [9,14]. However, the role of UBA1 in regulating cardiac remodeling and the possible mechanism remain unclear.

In this study, we explored the effect of UBA1 inhibition by UBE1-specific inhibitor, 4[4-(5-nitro-furan-2-ylmethylene)-3,5-dioxo-pyrazolidin-1-yl]-benzoic acid ethyl ester (PYR-41) in Ang II-induced cardiac remodeling. Our result showed that Ang II infusion significantly upregulated UBA1 expression in the heart. Administration of PYR-41 in mice dose dependently reduced Ang II-induced blood pressure elevation, cardiac hypertrophy, fibrosis, oxidative stress and inflammation, and improved cardiac contractile function. This beneficial effect was possible associated with inhibition of PTEN degradation leading to inhibition of multiple downstream signaling pathways (AKT/ERK1/2, STAT3, Smad2/3 and NF-kB(p65)). In conclusion, these results suggest that UBA1 promotes Ang II induced cardiac remodeling. The UBA1 inhibitor PYR-41 may be a novel therapeutic drug for treating hypertensive heart diseases.

2. Materials and methods

2.1. Antibodies and reagents

Antibodies against UBA1(#4890), AKT (#9272), phospho-AKT (#4060), ERK1/2 (#9102), phospho-ERK1/2 (#9101), STAT3 (#8768), phospho-STAT3 (#9131), p65 (#8242), p-p65 (#3033), TGF-β (#3711), Smad2/3(#8685), and phospho-Smad2/3(#8828) were from Cell Signaling Technology (Danvers, MA); anti-PTEN (sc-7974) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA); α-tublin (11224-1-AP) was from Proteintech (Chicago, IL). Primers including ANF, BNP, β-MHC, collagen I, collagen III, IL-1β, IL-6, TNF-α, NOX1, NOX2, NOX4 and GAPDH were purchased from Sangon Biotech (Shanghai, China). The purified PYR-41 (≥90% purity) was purchased from Selleck Chemicals (Houston, TX, USA), Ang II (Aladdin, A107852), WGA and pentobarbital was purchased from Sigma-Aldrich (St Louis, MO). All other chemicals frequently used in our laboratory were purchased from either Sigma-Aldrich or BD Pharmingen (San Jose, CA). TRizol was obtained from Invitrogen (Carlsbad, CA). All other chemicals frequently used in our laboratory were from Sigma-Aldrich.

2.2. Animal and treatment

Male C57BL/6 mice used were obtained from The Jackson Laboratory (Bar Harbor, ME). All mice had free access to water and standard laboratory diet. This study was approved by the Animal Care and Use Committee of Dalian Medical University. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No.85-23, revised 1996).

Male wild-type (WT) C57BL/6 mice (8–10-week old, n = 8 per group) were randomly assigned to 4 groups: saline control, Ang II, and Ang II + PYR-41(5 or 10 mg/kg). Cardiac remodeling was induced by subcutaneous infusing of saline or Ang II (1000 ng/kg/min) using Alzet mini pump (Model 1002) for 2 weeks as described [15,16]. UBA1 inhibitor PYR-41 was administered intraperitoneally at doses of 5 or 10 mg/kg/alternate day beginning 1 day before angiotensin II infusion and continued during Ang II infusion. All mice were anaesthetized by an overdose of pentobarbital (100 mg/kg, intra-peritoneal injection) [15,16]. The hearts were removed and prepared for further histological and molecular analysis.

2.3. Blood pressure measurement

The blood pressure of all mice was measured before Ang II infusion for 2 days and every other day after Ang II infusion by the tail-cuff system (BP-2010, Softron, Tokyo, Japan) as described previously [15,16].

2.4. Echocardiographic assessment

After 2 weeks of saline or Ang II infusion, cardiac function of all mice was evaluated by echocardiography using a 30 MHz probe (Vevo 770 system; VisualSonics, Toronto, Ontario, Canada) [16]. Left ventricular (LV) ejection fraction (EF%), LV fractional shortening (FS %), LV anterior wall thickness (LVAW) at systole and diastole were calculated as previously described [16].

2.5. Histopathologic examinations

The hearts were fixed in 4% paraformaldehyde for 24 h, embedded in paraffin and then sectioned into 5 μm. The sections were stained with wheat germ agglutinin (WGA, 50 μg/ml) for 60 min to evaluate cross-sectional area of myocytes (15–200 cells

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