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The TIR/BB-loop mimetic AS-1 attenuates mechanical stress-induced cardiac fibroblast activation and paracrine secretion via modulation of large tumor suppressor kinase 1

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ABSTRACT

The TIR/BB-loop mimetic AS-1 has been reported to prevent cardiac hypertrophy by inhibiting interleukin-1 receptor (IL-1R)-mediated myeloid differentiation primary response gene 88 (MyD88)-dependent signaling. To date, it remains unknown whether and if so how AS-1 contributes to mechanical stress (MS)-induced cardiac fibroblast activation, a key process in pressure overload-induced cardiac remodeling and heart failure. Here, we show that phosphorylation and expression of large tumor suppressor kinase 1 (LATS1), a key molecule in the Hippo-Yes associated protein (YAP) signaling pathway, were down-regulated in primary neonatal rat cardiac fibroblasts (NRCFs) in response to MS and in the hearts of mice subjected to transverse aortic constriction (TAC) procedure; AS-1 treatment was able to restore LATS1 phosphorylation and expression both in vitro and in vivo. AS-1 treatment suppressed the induction of proliferation, differentiation and collagen synthesis in response to MS in NRCFs. AS-1 also ameliorated cardiomyocyte hypertrophy and apoptosis through dampening paracrine secretion of stretched cardiac fibroblasts. In mice, AS-1 treatment could protect against TAC-induced cardiac hypertrophy, myocardial fibrosis and heart failure. Of note, LATS1 depletion using siRNA completely abrogated the inhibitory effects of AS-1 on NRCFs under MS including accelerated proliferation, differentiation, enhanced ability to produce collagen and augmented paracrine secretion of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) to induce cardiomyocyte hypertrophy. Therefore, our results delineate a previously unrecognized role for LATS1 in cardiac fibroblast to mediate the beneficial effects of AS-1 in preventing pressure overload-induced cardiac remodeling and heart failure.

1. Introduction

Cardiac remodeling and heart failure in response to mechanical stress (MS) as a result of persistent pressure overload (e.g., hypertension) encompass a series of inter-connected pathophysiological processes including cardiac fibroblast hyper-activation, cardiomyocyte hypertrophy and cardiomyocyte apoptosis [1–5]. Excessive mechanical stress could provoke the augmentation of cardiac fibroblast proliferation, differentiation and collagen synthesis [6–8]. Recently, several lines of evidence show that under the influence of mechanical stretch, cardiac fibroblasts are capable of inducing cardiomyocyte hypertrophy and apoptosis by paracrine secretion of cytokines and growth factors such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) [9–11], highlighting a fibroblast–myocyte crosstalk that contributes to pressure overload-induced heart failure. Nevertheless, the underlying molecular mechanism remains unclear. AS-1 (hydrocinnamoyl-L-valylpyrrolidine) is a synthetic low

Abbreviations: AS-1, hydrocinnamoyl-L-valylpyrrolidine; TIR, Toll/interleukin-1 receptor; IL-1R, interleukin-1 receptor; MyD88, myeloid differentiation primary response gene 88; MS, mechanical stress; LATS1, large tumor suppressor kinase 1; YAP, Yes associated protein; NRCFs, neonatal rat cardiac fibroblasts; NRVMs, neonatal rat ventricular myocytes; TAC, transverse aortic constriction; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; Sav, salvador; MST1/2, Mammalian sterile 20-like kinase 1/2; Mob1, mps one binder 1; JNK, c-Jun N-terminal kinase; α-SMA, α-smooth muscle antibody; HW, heart weight; BW, body weight; LVW, left ventricle weight; TL, tibia length; LW, lung weight; IVSd, interventricular septum diastolic dimension; LVPWd, left ventricular posterior wall diastolic dimension; LVIDd, left ventricular internal dimension diastole; %EF, percent ejection fraction; %FS, percent fractional shortening; FSP-1, fibroblast specific protein-1.

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molecular-weight Toll/interleukin-1 receptor (TIR)/BB-Loop mimetic that blocks the interaction of myeloid differentiation primary response gene 88 (MyD88)-TIR with interleukin-1 receptor (IL-1R)-TIR via modeling a tripeptide sequence of the MyD88-TIR-BB loop [12,13]. Our laboratory has recently reported that AS-1 administration prevents cardiac hypertrophy by inhibiting IL-1R-mediated MyD88-dependent signaling and ameliorates IL-1β-induced cardiomyocyte hypertrophy [14]. Additionally, we have demonstrated that AS-1 protects the myocardium from ischemia/reperfusion injury by inhibiting IL-1β-induced expression of IL-1 receptor-associated kinase (IRAK-1) and p38 phosphorylation [15]. Based on these findings, we propose that AS-1 may participate in the pathogenesis of cardiovascular diseases by suppressing IL-1R-mediated MyD88-dependent signaling pathway, resulting in decreased nuclear factor-κB (NF-κB) binding activity and decreased mitogen-activated protein kinase (MAPK) activation. Yet, it is not clear whether and if so how AS-1 might contribute to pressure overload-induced cardiac remodeling and in particular MS-induced activation of cardiac fibroblasts by modulating the fibroblast–myocyte conversation.

Large tumor suppressor kinase 1 (LATS1) is an important component in the Hippo signaling pathway originally identified in organogenesis and carcinogenesis [16]. In mammals, the nuclear mediators of the Hippo signaling pathway include highly conserved salvador (Sav), Mammalian sterile 20-like kinase 1/2 (MST1/2), mps one binder 1 (Mob1), LATS1/2 and the downstream transcription factor Yes associated protein (YAP) [17]. Several recent reports have implicated the Hippo–YAP pathway in cardiac pathophysiology. For instance, Heallen et al. have reported that mice with cardiac conditional Sav gene knockout have enlarged hearts apparently due to hyper-proliferation of cardiomyocytes [18]. There is also indication that MST1 phosphorylation and activation by interacting with Rassf1A protect against pressure overload-induced cardiac fibrosis via negatively regulating the synthesis and excretion of TNF-α [19]. In addition, the up-regulated expression of YAP has been shown in both heart samples of dilated cardiomyopathy patients and transverse aortic constriction (TAC)-induced animal models [20]. Moreover, cardiac specific over-expression of YAP directly contributes to cardiac hypertrophy. These investigations strongly support a model wherein components in the Hippo signaling pathway are closely linked to pressure overload-induced heart diseases. Therefore, in the present study, we investigate the involvement of LATS1 in AS-1 mediated protection against MS-induced cardiac fibroblast activation and paracrine secretion leading up to cardiac remodeling and heart failure.

2. Methods

2.1. Synthesis of TIR/BB-loop mimetics

AS-1 was prepared as described previously [12–14]. AS-1 was obtained as slight yellow oil and dried in vacuo for 24 h. The crystals of AS-1 were dissolved in Dimethyl Sulfoxide (DMSO). There was no cytotoxicity of AS-1 determined by the mono-nuclear cell direct cytotoxicity (MTT) assay (data not shown). Cells were treated with AS-1 or DMSO 30 min before stimulus.

2.2. Experimental animals and surgery

C57BL/6 mice, male, 6–8 weeks old, were acquired from Model Animal Research Center (MARC) of Nanjing University, raised in the Animal Laboratory Resource Facility of Nanjing Medical University and kept under constant environmental conditions with 12 h light/dark cycles and libitum access to food and water. Animal care and investigations are accomplished in accordance with the guidelines of 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).

Mice were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (8 mg/kg) and placed on a temperature-controlled surgical table. TAC was performed as described previously [14,21]. Sham surgically operated mice were served as sham control. 4 weeks after TAC, hearts were harvested and the ratios of heart weight/body weight (HW/BW), left ventricular weight/tibia length (LVW/TL) and lung weight/BW (LW/BW) were calculated. The heart samples were frozen in liquid nitrogen and stored at −80 °C for protein analysis or fixed in 10% formalin for immunohistochemistry analysis. Mice assigned to the treated groups were given AS-1 at 50 mg/kg BW/day for 4 weeks since 3 days before TAC and for 2 weeks since 2 weeks after TAC by intraperitoneal injection. There were 5 experimental groups: sham control (sham); untreated TAC; TAC + DMSO; TAC + AS-1 and TAC2w + AS-1.

2.3. Echocardiography

To measure cardiac function, echocardiography (GE Vivid 7 equipped with a 14-MHz phase array linear transducer, S12, allowing a 150 maximal sweep rate) was performed in anesthetized mice (n = 6 in each group) as described. Mice were anesthetized with a mixture of isoflurane (1.5%) and oxygen (0.5 L/min). The body temperature was maintained at 37 °C using a heating pad. M-mode tracings were used to measure interventricular septum diastolic dimension (IVSd), left ventricular posterior wall diastolic dimension (LVPWd), and left ventricular internal dimension diastole (LVIDd). Percent fractional shortening (%FS) and percent ejection fraction (%EF) were calculated as described in our previous study [3,22]. The ascending and descending aortic flow velocities were measured by Doppler from the suprasternal notch view 3 days after surgery. Mice with less than 20 mm Hg calculated from ascending and descending aortic flow velocities were excluded from the experiment. All measurements were averaged over six consecutive cardiac cycles and were made by the observer who was blinded with respect to the identity of the tracings.

2.4. Histology

Mice hearts acquired from each group were fixed in 10% formalin, embedded in paraffin, cut at 5 mm, and counterstained with wheat germ agglutinin (WGA) staining to determine cardiomyocyte short axis cross-sectional area (CSA) in the LV, or stained with Masson's trichrome to determine the degree of fibrosis. LATS1 and fibroblast specific protein-1 (FSP-1) labels were also done to determine the localization of LATS1. CSA and percent area fraction were measured using ImageJ software (version 1.389, National Institutes of Health). A value from each heart was calculated using the measurements of 40–50 cells from an individual heart.

2.5. Hydroxyproline measurement

The content of hydroxyproline was determined using a commercially available kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) on the basis of the instructions of the manufacturer.

2.6. Cross-linked collagen content measurement

The content of cross-linked collagen was measured as described before [3]. Myocardial collagen was digested with pepsin, and then centrifugated. The supernatant of the pepsin-digested collagen sample was subjected to acid hydrolysis and hydroxyproline determination. This was non-cross-linked collagen. Then, the amount of cross-linked collagen in the myocardium was determined based on the total myocardial collagen amount.

2.7. Cell culture and cell stretch

Neonatal rat ventricular myocytes (NRVMs) and neonatal rat cardiac fibroblasts (NRCFs) were isolated from 1 to 2-day-old Sprague–Dawley