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Original Paper

Overexpression of SARAF Ameliorates Pressure Overload–Induced Cardiac Hypertrophy Through Suppressing STIM1-Orai1 in Mice

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Key Words
Transmembrane protein 66 • Store-operated Ca^{2+} entry-associated regulatory factor • Store-operated calcium entry

Abstract
Background/Aims: Activation of stromal interaction molecule 1 (STIM1) and Orai1 participates in the development of cardiac hypertrophy. Store-operated Ca^{2+} entry-associated regulatory factor (SARAF) is an intrinsic inhibitor of STIM1-Orai1 interaction. Thus, we hypothesized that SARAF could prevent cardiac hypertrophy. ***Methods:*** Male C57BL/6 mice, aged 8 weeks, were randomly divided into sham and abdominal aortic constriction surgery groups and were infected with lentiviruses expressing SARAF and GFP (Lenti-SARAF) or GFP alone (Lenti-GFP) via intramyocardial injection. At 4 weeks after aortic constriction, left ventricular structure and function were assessed by echocardiography and hemodynamic assays. The gene and protein expressions of SARAF, STIM1, and Orai1 were measured by quantitative PCR and Western blot, respectively. ***Results:*** Gene and protein expressions of SARAF were significantly decreased, while STIM1 and Orai1 were increased in the heart tissue compared with sham group. Overexpression of SARAF in the heart prevented the upregulation of STIM1 and Orai1, and importantly, attenuated aortic constriction-induced decrease in maximal rate of left ventricular pressure decay and increases in thickness of interventricular septum and left ventricular posterior wall, heart weight/body weight ratio, and size of cardiomyocytes. Blood pressure detected through the carotid artery and left ventricular systolic function were not affected by SARAF overexpression. In addition, overexpression of SARAF also attenuated angiotensin II-induced upregulation of STIM1 and Orai1 and hypertrophy of cultured cardiomyocytes. ***Conclusion:*** Overexpression of SARAF in the heart prevents cardiac hypertrophy, probably through suppressing the upregulation of STIM1/Orai1.

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Introduction

Cardiac hypertrophy develops as an adaptive growth response to stressors such as pressure overload in hypertension [1]. Abnormal hypertrophic remodeling–characterized as increased wall thickness–frequently results in congestive heart failure, which is a leading global cause of death [2]. Due to the limited proliferative capacity of adult cardiomyocytes, cardiac hypertrophy is mainly attributed to an increase in cardiomyocyte size.

A number of intracellular signal pathways participate in the hypertrophy of cardiomyocytes. Cytosolic Ca^{2+} shows dramatic spatial and temporal fluctuations associated with cycles of excitation, contraction, and relaxation. In addition to couple excitation to contraction, Ca^{2+} is an essential intracellular signal for the process of cardiomyocyte hypertrophy to a variety of stimuli [3]. Store-operated Ca^{2+} entry (SOCE) is a major mechanism of intracellular calcium signaling [4]. SOCE refers to a special Ca^{2+} entry route that is activated in response to depletion of endoplasmic reticulum (ER) calcium stores [5]. In this process, inositol 1, 4,5-trisphosphate (IP3)–a second messenger–activates its receptors on the ER and evokes a rapid release of Ca^{2+} into cytosol. The depletion of ER activates stromal interaction molecule 1 (STIM1), a calcium sensor within ER; then the activated STIM1 interacts with and stimulates Ca^{2+} entry through plasma membrane Orai1 channels [6, 7]. Thus, STIM1 and Orai1 are two key molecules that mediate SOCE in cardiomyocytes. There is strong evidence that STIM1 activation is required for pathological cardiac hypertrophy [8], while knocking down STIM1 protected rats from pressure overload–induced cardiac hypertrophy [9]. Increased expression of Orai1 has been found in human failing heart [10], and knocking down Orai1 attenuated phenylephrine-induced hypertrophy of cardiomyocytes [11]. These findings suggest that suppressing STIM1/Orai1 could be a therapeutic strategy for preventing cardiac hypertrophy. Basal levels of STIM1/Orai1 may be essential for adaptive cardiac hypertrophy and maintain normal heart function, as loss of STIM1 or Orai1 promoted the development of dilated cardiomyopathy and heart failure [12-14]. It is difficult to find a balance between the anti-hypertrophic and the detrimental effects of STIM1/Orai1 knockdown. An ideal strategy could be suppressing the activated STIM1/Orai1 rather than their basal activities.

Store-operated Ca^{2+} entry-associated regulatory factor (SARAF), also known as transmembrane protein 66, has recently been identified as an inhibitor of SOCE by interaction with the STIM1 region responsible for Orai1 activation [15, 16]. SARAF, an ER resident protein, is a highly conserved protein across the animal kingdom [17]. It has been reported that SARAF inhibited spontaneous activation of STIM1 and prevented excess calcium refilling [17]. The function of SARAF in the heart is largely unknown. Based on the currently available evidence, we hypothesized that SARAF could attenuate cardiac hypertrophy via inhibiting the activation of SOCE. We tested the effects of SARAF overexpression on cardiac hypertrophy in a pressure-overloaded mouse model and on angiotensin (Ang) II-induced hypertrophy of cultured cardiomyocytes.

Materials and Methods

Animals and surgeries

Eight-week-old male C57BL/6 mice were purchased from the Animal Center of Dashuo Biotechnology (Chengdu, Sichuan, China) and used for experiments. Mice were housed under a 12h/12h day/night cycle, with *ad libitum* food and water. All experimental procedures were approved by Institutional Animal Care and Use Committee and the Ethic Committee of Chengdu Military General Hospital. *In vivo* gene transfer was performed as previously described [18, 19]. Mice were anesthetized by intraperitoneal injection of a cocktail of ketamine (150 mg/kg) and xylazine (5 mg/kg), orally intubated and connected to a rodent ventilator, and placed in the supine position. A left thoracotomy was performed at the third intercostal space. Lentiviruses expressing full-length mouse Saraf and GFP cDNA (Lenti-SARAF) and lentiviruses carrying GFP alone (Lenti-GFP) were constructed by Shanghai R&S Biotechnology (Shanghai, China) and injected into the myocardium. Intramyocardial injections were made in 5 sites within left ventricular free wall proximal

to the apex, with 6μL of virus at 1 x 10^9 TU/mL for each site. After injection, the chest was sutured and the abdominal aortic constriction (AAC) mouse model was then established as previously described [20]. Briefly, suprarenal abdominal aorta was banded against a 27-gauge blunt needle; the needle was removed latter. A sham procedure was performed in control group without banding. Then mice were allowed to recover. Four weeks later, echocardiography and hemodynamic analysis were performed; then mice were euthanized with pentobarbital (200 mg/kg) by an intraperitoneal injection, the hearts were harvested and weighed to calculate the heart weight/body weight ratio (HW/BW; mg/g).

Echocardiography

After anesthetized with 1.5% isoflurane, echocardiography was performed using a Visualsonics Vevo 2100 (Visualsonics Inc., Toronto, Ontario, Canada) ultrasound system with a 40-MHz transducer. The parameters, including interventricular septum end diastole (IVSd), left ventricular posterior wall end diastole (LVPWd), left ventricle end-diastolic diameter (LVEDD), left ventricle end-systolic diameter (LVESD), left ventricular ejection fraction (EF), and fractional shortening (FS), were measured [21].

Blood pressure and hemodynamics

A 1.4-F microconductance pressure catheter (ARIA SPR-853; Millar Instruments, Houston, TX) was inserted into the right common carotid artery; systolic (SBP) and diastolic (DBP) blood pressure and mean arterial pressure (MAP) were measured. Then, the catheter was introduced into the ascending aorta and then advanced into the left ventricle as described previously [21]. The hemodynamic parameters, including left ventricular end-systolic pressure (LVESP), left ventricular end-diastolic pressure (LVEDP), and minimum (-dp/dt) and maximum (+dp/dt) rate of pressure change in the ventricle, were collected on Chart via PowerLab (ADInstruments Pty Ltd, Castle Hill, Australia).

Histology

Hearts were fixed with 10% formalin and embedded in paraffin. Paraffin sections (5μm in thickness) of interventricular septum were stained with hematoxylin & eosin. The cross-sectional area of cardiomyocyte was calculated using ImageJ (NIH) [22]. A group of sections were subjected to immunohistochemistry staining. After being blocked, sections were incubated overnight at 4°C with primary antibodies against SARAF, STIM1, and Orai1. Sections were then incubated with horseradish peroxidase–conjugated secondary antibody and visualized with diaminobenzidine.

Cell culture

Mouse cardiomyocytes were purchased from National Infrastructure of Cell Line Resource (Beijing, China) and cultured in Dulbecco modified Eagle medium with 10% fetal bovine serum. Cardiomyocytes were infected with 6μL of Lenti-SARAF or Lenti-GFP viruses at 1 x 10^8 TU/mL. The medium was changed after 24 hours, and the transfected cardiomyocytes were cultured for another 24 hours. Next, serum-free medium was applied, and the cardiomyocytes were stimulated with 1 μM of Ang II (Sigma, St. Louis, MO, USA) for 72 hours [23]. Cardiomyocytes on glass coverslips were washed in PBS and 4% formaldehyde fixed at room temperature. Slides were blocked with 10% normal donkey serum for 1 hour at room temperature and then incubated with anti-cardiac α-actin (1:1000, Abcam, Cambridge, MA, USA) overnight at 4°C. After being washed in PBS for three times, slides were incubated with Alexa Fluor 594-conjugated goat anti-rabbit IgG secondary antibody (1:500, Invitrogen, Carlsbad, CA, USA). Cell nuclei were stained with DAPI. Cell size was measured via cross-sectional area calculation using ImageJ (NIH). For each group, 100 individual cardiomyocytes were measured, and average value was calculated [24].

Real-time polymerase chain reaction (RT-PCR)

The RNA was extracted from the heart tissue and cardiomyocytes using the RNAiso Plus reagent. The RT-PCR was performed using One Step SYBR Prime Scrip RT-PCR Kit II (RR086A, TaKaRa). The relative amount of mRNA was calculated by $2^{-\Delta\Delta CT}$ and was normalized to a housekeeping gene β-actin. Each sample was run and analyzed in triplicate. PCR primer sequence is listed as follow: Saraf: F, 5'-GGA CTC CTG TGG CTT GGT TA-3'; R, 5'-TGC TCT GTG GTC CTG TGA AG-3'; Stim1: F, 5'-AGC TCC TGG TAT GCT CCT GA-3'; R, 5'-ACC TCA CTC AGT GCC TGC TT -3'; Orai1: F, 5'-GCT CTG CTG GGT GAA GTT CT -3'; R, 5'-GGA ACT GTC GGT CCG TCT TA -3'; brain natriuretic peptide (BNP): F, 5′-AAG CTG CTG GAG CTG ATA AGA-3'; R, 5′-GTT ACA

GCC CAA ACG ACT GAC-3'; atrial natriuretic peptide (ANP): F, 5'-GAA CCT GCT AGA CCA CCT-3'; R, 5'-CCT AGT CCA CTC TGG GCT-3'; beta-myosin heavy chain beta (β-MHC): F, 5'-CAC CAG CTG CGA TGC AAT G-3'; R' 5'-AGG ATG CGA TAC CTC TGC CG-3'; β-actin: F, 5'-CGC GGT TCT ATT TTG TTG GTT T-3'; R, 5'-GCG CCG GTC CAA GAA GAA TTT-3'.

Western blotting

Total proteins were extracted from heart tissue and cardiomyocytes using a protein extraction kit (Keygen Biotech, Nanjing, China) and quantified using an enhanced BCA Protein Assay Kit (Beyotime, Jiangsu, China). Protein were loaded onto 8-12% SDS polyacrylamide gels. The separated proteins were then transferred to PVDF membranes (Millipore, Bedford, MA, USA). Membranes were blocked with 5% bovine serum albumin in TBS-T (Tris-buffered saline, 0.1% Tween 20) for 1 hour and then incubated with anti-SARAF (1:1000, Abcam), anti-STIM1 (1:1000, Abcam), anti-Orai1 (1:1000, Abcam), and anti-GAPDH (1:1000, Abcam) antibodies overnight. Membranes were rinsed three times with TBS-T and incubated with horseradish peroxidase-conjugated secondary antibodies for 1 hour. Membranes were rinsed three times with TBS-T. Chemiluminescence detection reagent (BeyoECL Plus, Beyotime, Jiangsu, China) were dropwise added on the membranes. The luminescent signal was detected by exposure to x-ray film.

Statistical analysis

Continuous data are presented as mean ± SEM. Comparisons among groups were determined by analysis of variance with post hoc Tukey HSD test (SPSS Inc, Chicago, IL, USA). $P<0.05$ was considered statistically significant.

Results

SARAF attenuated pressure overload-induced diastolic dysfunction

As expected, SBP, DBP, and MAP were significantly increased in all groups with AAC compared with sham group (all $P<0.05$); the increase in blood pressure was not influenced by overexpression of SARAF (Fig. 1A). Similarly, LVESP was increased by AAC but not affected by SARAF overexpression (Fig. 1B). LVEDP and +dp/dt had no difference among four groups (Fig. 1C and 1D). An important parameter of diastolic dysfunction, -dp/dt, was significantly decreased by AAC ($P<0.05$), which was attenuated by SARAF overexpression ($P<0.05$, Fig. 1E).

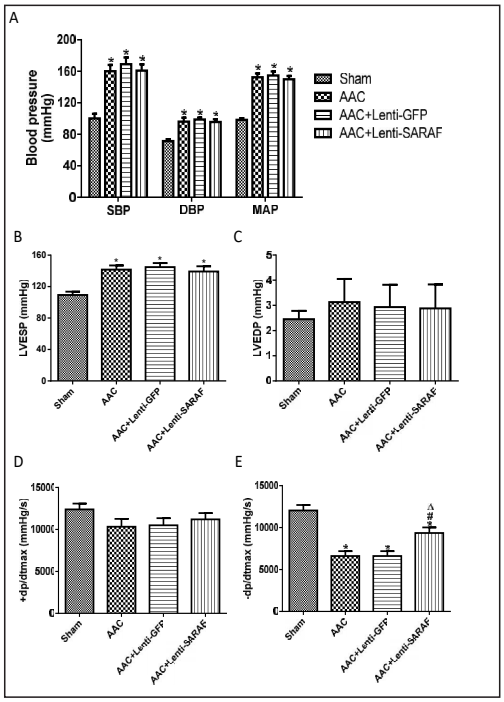

Fig. 1. Blood pressure and hemodynamic parameters. (A) Systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) were measured directly via right common carotid artery in mice at 4 weeks after received sham or abdominal aortic constriction (AAC) surgery and treated with or without Lenti-GFP or Lenti-SARAF. (B) Left ventricular end-systolic pressure (LVESP) (B), left ventricular end-diastolic pressure (LVEDP) (C), and minimum (-dp/dt) (D) and maximum (+dp/dt) (E) rate of pressure change in the ventricle were measured by a catheter inserted in to the left ventricle. Data are means ± SEM from 8 mice in each group. *P<0.05 vs. sham group; #P<0.05 vs. AAC group, ΔP<0.05 vs. AAC+Lenti-GFP group.

SARAF attenuated pressure overload-induced cardiac hypertrophy

Transthoracic echocardiography was performed at 4 weeks after surgery (Fig. 2A). The wall thickness parameters–IVSd and LVPWd–were significantly increased in groups with AAC ($P<0.05$); while these increases were attenuated by SARAF overexpression ($P<0.05$, Fig. 2B and 2C). The chamber size, expressed as LVEDD and LVESD, was not affected by either AAC surgery or SARAF overexpression (Fig. 2D and 2E). The systolic function, in terms of EF and FS, was preserved in mice received AAC

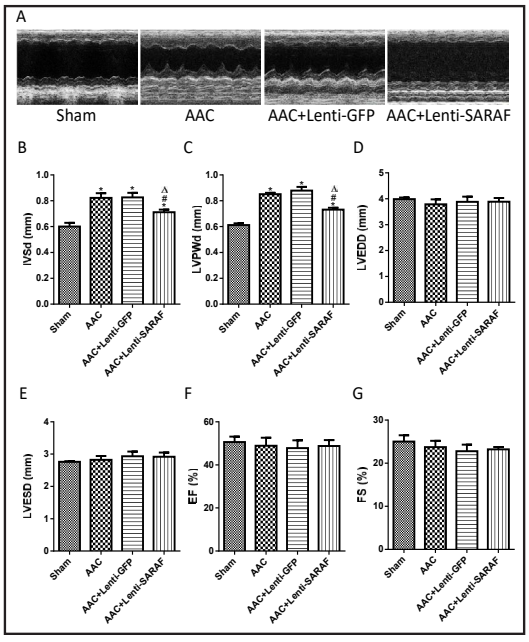

Fig. 2. Transthoracic echocardiography. (A) Representative echocardiography images of mice at 4 weeks after received sham or abdominal aortic constriction (AAC) surgery and treated with or without Lenti-GFP or Lenti-SARAF. The parameters, including interventricular septum end diastole (IVSd) (B), left ventricular posterior wall end diastole (LVPWd) (C), left ventricle end-diastolic diameter (LVEDD) (D), left ventricle end-systolic diameter (LVESD) (E), left ventricular ejection fraction (EF) (F), and fractional shortening (FS) (G), were measured. Data are means ± SEM from 8 mice in each group. *P<0.05 vs. sham group; #P<0.05 vs. AAC group, ΔP<0.05 vs. AAC+Lenti-GFP group.

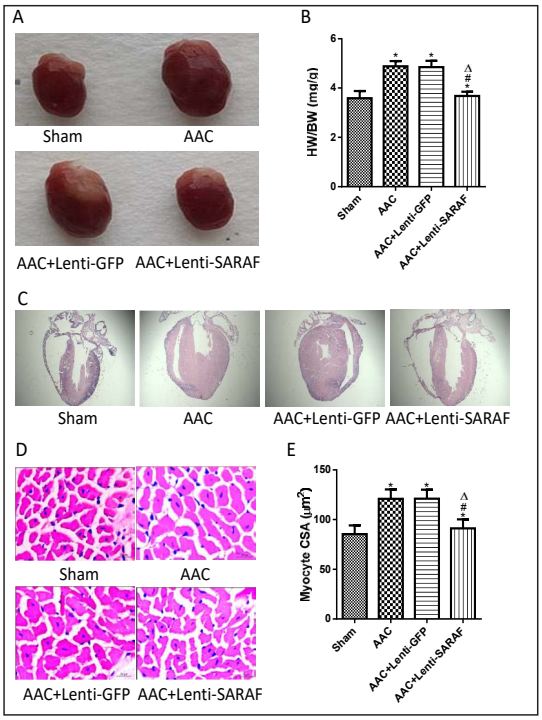

Fig. 3. Heart weight and cardiac hypertrophy. (A) Gross hearts of mice at 4 weeks after received sham or abdominal aortic constriction (AAC) surgery and treated with or without Lenti-GFP or Lenti-SARAF. (B) The heart weight (HW)/body weight (BW) ratio of mice. (C) Representative gross morphology of mouse hearts. (D) Hematoxylin & eosin-stained sections of heart tissues. (E) Cross-sectional areas (CSA) of cardiomyocytes were calculated based on (D). Data are means ± SEM from 8 mice in each group. *P<0.05 vs. sham group; #P<0.05 vs. AAC group, ΔP<0.05 vs. AAC+Lenti-GFP group.

(Fig. 2F and 2G). AAC increased heart weight expressed as HW/BW ratio (P<0.05), which was abolished by SARAF overexpression (P<0.05, Fig. 3A and 3B). The gross sections of the hearts showed similar results (Fig. 3C). In addition, overexpression of SARAF also attenuated AAC-induced increase in cardiomyocyte size (P<0.05, Fig. 3D and 3E).

SARAF suppressed pressure overload-upregulated STIM1/Orai1

The gene expression of SARAF was significantly downregulated (P<0.05) and STIM1 and Orai1 were upregulated (both P<0.05) in heart tissue from mice received AAC when compared with sham group (Fig. 4A). Infection with Lenti-SARAF (but not Lenti-GFP) increased the expression of SARAF (P<0.05) in mouse heart tissue and remarkably attenuated AAC-stimulated increases in STIM1 and Orai1 (both P<0.05, Fig. 4A). We obtained very similar results in measuring protein expression by Western blotting (Fig. 4B and 4C) and immunohistochemistry staining (Fig. 5).

SARAF attenuated Ang II-induced cardiomyocyte hypertrophy

Ang II induced significant hypertrophy of cardiomyocytes (P<0.05), which was significantly attenuated by overexpression of SARAF in cultured cardiomyocytes (P<0.05, Fig. 6A and 6B). In addition, the mRNA levels of BNP, ANP, and β-MHC were upregulated by Ang II, which were attenuated by overexpression of SARAF (Fig. 7).

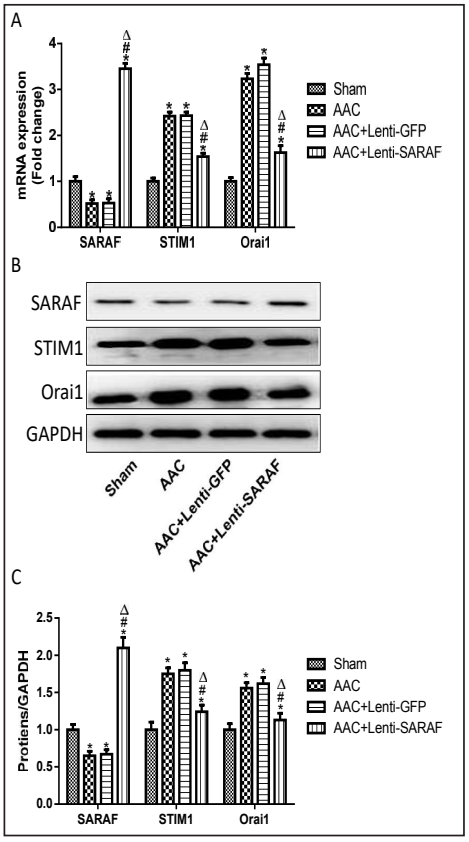

Fig. 4. Gene and protein expression of SARAF, STIM1, and Orai1 in heart tissues. (A) mRNA levels of SARAF, STIM1, and Orai1 were measured in heart tissues from mice at 4 weeks after received sham or abdominal aortic constriction (AAC) surgery and treated with or without Lenti-GFP or Lenti-SARAF. (B) Representative Western blots and (C) quantification of the protein expression of SARAF, STIM1, and Orai1. Data are means ± SEM from 4 mice in each group. *P<0.05 vs. sham group; #P<0.05 vs. AAC group, ΔP<0.05 vs. AAC+Lenti-GFP group.

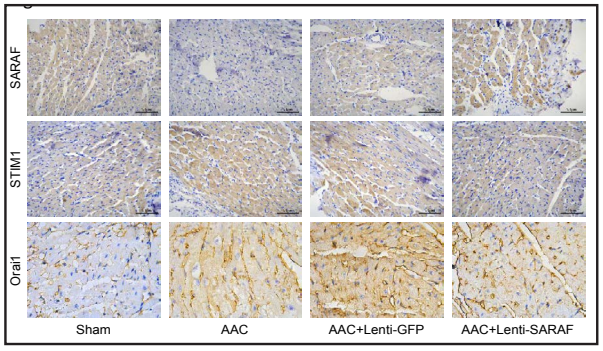

Fig. 5. Distribution of SARAF, STIM1, and Orai1 in heart tissues. Representative immunohistochemistry staining of SARAF, STIM1, and Orai1 in heart sections from mice at 4 weeks after received sham or abdominal aortic constriction (ACC) surgery and treated with or without Lenti-GFP or Lenti-SARAF.

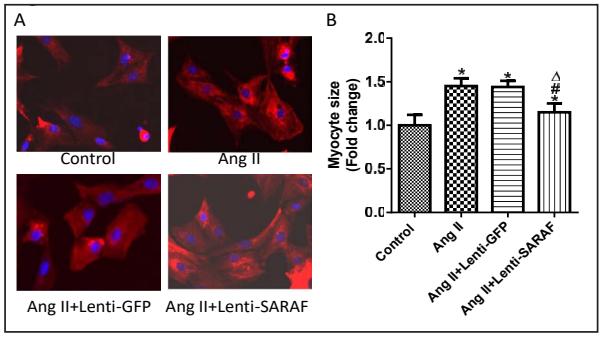

Fig. 6. Angiotensin (Ang) II-induced cardiomyocyte hypertrophy. (A) Representative immunofluorescence images of cultured cardiomyocytes infected with or without Lenti-GFP or Lenti-SARAF and then treated with 1 μM of Ang II for 72 hours and stained with anti-cardiac α-actin. (B) Cross-sectional area of cultured cardiomyocytes were calculated. Data are means ± SEM of 4 independent experiments in each group. *P<0.05 vs. control group; #P<0.05 vs. Ang II group, ΔP<0.05 vs. Ang II+Lenti-GFP group.

SARAF suppressed Ang II-upregulated STIM1/Orai1

Similar to the results from heart tissue, both the gene and protein expressions of SARAF were significantly downregulated (both $P<0.05$) and STIM1 and Orai1 were upregulated (all $P<0.05$) by Ang II in cultured cardiomyocytes when compared with control group (Fig. 8A-8C). Overexpression of SARAF attenuated the increases in STIM1 and Orai1 in cultured mouse cardiomyocytes (all $P<0.05$, Fig. 8A-8C).

Discussion

The main findings of this study are that preventing the downregulation of SARAF in the heart inhibited pressure overload-induced STIM1/Orai1 upregulation, cardiac hypertrophy, and diastolic dysfunction. Similar results were found in cell culture study that showed

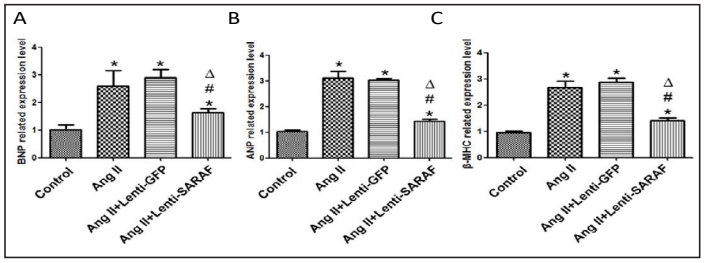

Fig. 7. Gene expression of brain natriuretic peptide (BNP), atrial natriuretic peptide (ANP), and beta-myosin heavy chain beta (β-MHC) in cultured cardiomyocytes. mRNA levels were measured in cultured cardiomyocytes infected with or without Lenti-GFP or Lenti-SARAF and then treated with 1 μM of Ang II for 72 hours. Data are means ± SEM of 4 independent experiments in each group. *P<0.05 vs. control group; #P<0.05 vs. Ang II group, ΔP<0.05 vs. Ang II+Lenti-GFP group.

overexpression of SARAF prevented Ang II-induced STIM1/Orai1 upregulation and cardiomyocyte hypertrophy.

This study provides further understanding about the role of SOCE in cardiac hypertrophy. Previous studies have demonstrated that abnormal activation of SOCE (or upregulation of its key mediators STIM1 and Orai1) contributed to the development of cardiac hypertrophy [8, 10]. However, the mechanism underlying the upregulation of STIM1 and Orai1 in hypertrophic hearts was unknown. In this study, we found that the increases in STIM1 and Orai1 were associated with the decrease of SARAF, an inhibitor of STIM1/Orai1, in pressure overload-induced hypertrophic heart tissue with diastolic dysfunction expressed as decreased -dp/dt. Blood pressure and left ventricular systolic function (evaluated by hemodynamic measurement and echocardiography) were not changed by SARAF overexpression, indicating SARAF is less important in regulating blood pressure and systolic function than in mediating cardiac hypertrophy and diastolic dysfunction. This result suggests that the downregulation of SARAF may be the original factor that triggers STIM1/Orai1 upregulation, SOCE activation, and cardiac hypertrophy. This speculation was further supported by the evidence that overexpression of SARAF abolished the upregulation of STIM1 and Orai1. Therefore, targeting SARAF may be useful to prevent or treat cardiac hypertrophy.

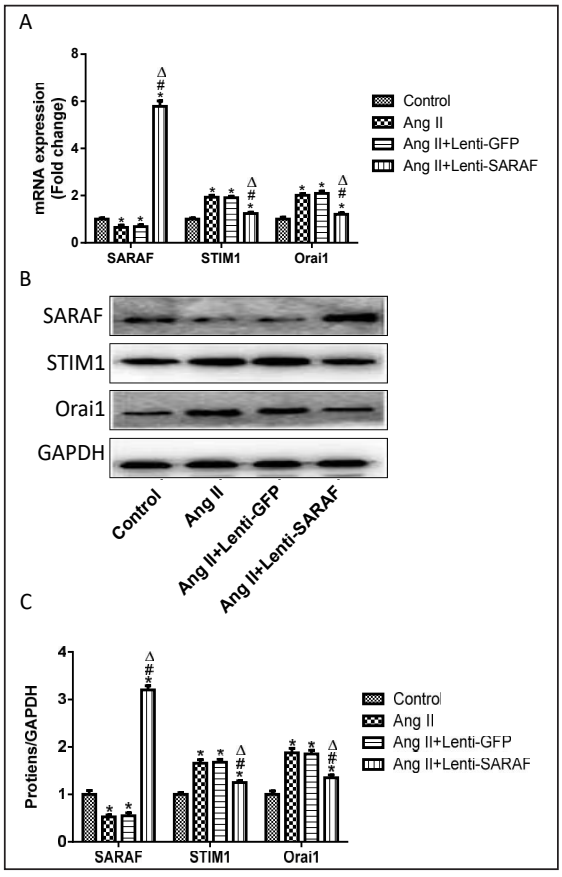

Fig. 8. Gene and protein expression of SARAF, STIM1, and Orai1 in cultured cardiomyocytes. (A) mRNA levels of SARAF, STIM1, and Orai1 were measured in cultured cardiomyocytes infected with or without Lenti-GFP or Lenti-SARAF and then treated with 1 μM of Ang II for 72 hours. (B) Representative Western blots and (C) quantification of the protein expression of SARAF, STIM1, and Orai1. Data are means ± SEM 4 independent experiments in each group. *P<0.05 vs. control group; #P<0.05 vs. Ang II group, ΔP<0.05 vs. Ang II+Lenti-GFP group.

The beneficial effects of SARAF overexpression are most likely due to the decreased expression of STIM1 and Orai1. Previous study showed that SARAF negatively modulated SOCE by preventing the spontaneous activation of STIM1 and inhibiting the formation of the STIM1-Orai1 complex

[17, 25]. The interaction between SARAF and STIM1-Orai1 was not fully understood; and it is still unknown how SARAF affects the expression of STIM1 and Orai1. SARAF likely interacts with Orail's C-terminal in a STIM1-independent manner [16]. It has also been reported that STIM1 can, in turn, affect the translocation of SARAF from ER to plasma membrane [25]. Besides regulating STIM1-Orai1 interaction, SARAF modulates transient receptor potential canonical 1 (TRPC1) channels [26]. The upregulation of TRPC1 has been involved in the development of cardiac hypertrophy [27]. Therefore, it is also possible that SARAF protects against cardiac hypertrophy through suppressing TRPC1. In addition, Albarran *et al*. reported that SARAF negatively regulates store-independent Ca^{2+} entry via arachidonate-regulated calcium channels [15]. Taken together, the exact mechanisms responsible for SARAF-induced protection of cardiac hypertrophy warrant further investigation.

This study provides a better target for the prevention or treatment of pressure overload-induced cardiac hypertrophy. Over the recent years, many efforts have been made to prevent cardiac hypertrophy by targeting STIM1 and Orai1. Although excessive activation of STIM1 and Orai1 is involved in the development of cardiac hypertrophy, basal activity of these two proteins are essential for responding to physiological stimuli and maintaining homeostasis of the myocardium. Gene silencing study demonstrated that basal level of STIM1 is critical for cardiac adaptive hypertrophy under physiological conditions and knocking down STIM1 led to progressive cardiac dilation and dysfunction [12]. Knockout of Orai1 induced dilated cardiomyopathy in pressure overloaded mice [13], suggesting Orai1 is also essential for cardiac hypertrophy to counteract pressure overload. Therefore, directly inhibiting STIM1 and Orai1 may be harmful to the heart and cause ventricular dilation and heart failure. The intrinsic inhibitor SARAF may sophisticatedly regulate STIM1/Orai1 and SOCE. In this study, we found that lentivirus-mediated overexpression of SARAF inhibited the pressure overload- and Ang II-induced upregulation of STIM1 and Orai1, but interestingly did not affect their basal levels as the expression of STIM1/Orai1 was similar between sham and pressure overload plus SARAF overexpression in mouse heart tissue as well as between control and Ang II plus SARAF overexpression in cultured cardiomyocytes. Moreover, dilated cardiomyopathy and heart failure were not observed in mice overexpressed with SARAF in this study. Therefore, SARAF may be a better target for preventing or treating cardiac hypertrophy.

There are limitations in this study. Firstly, Ang II may be not the most important mediator of pressure overload-induced cardiac hypertrophy. Therefore, Ang II-induced cardiomyocyte hypertrophy may be not an ideal model to mimic the *in vivo* experiments. Secondly, intra-myocardial injection may be less practical in clinical application, thus, better delivery strategies are required in further studies.

In summary, this study shows that overexpression of SARAF suppresses the upregulation of STIM1 and Orai1 and attenuates pressure overload- and Ang II-induced cardiac hypertrophy. SARAF could be a therapeutic target for preventing or treating cardiac hypertrophy and diastolic dysfunction.

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Disclosure Statement

The authors declare that there is no conflict of interests regarding the publication of this paper.

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