Original Paper

Activation of Peroxisome Proliferator-Activated Receptor γ (PPAR γ) Through NF- κ B/Brg1 and TGF- β_1 Pathways Attenuates Cardiac Remodeling in Pressure-Overloaded Rat Hearts

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Key Words

Peroxisome proliferator-activated receptor γ • Cardiac remodeling • Brg1 • TGF- β_1 • NF- κ B

Abstract

Background/Aims: Cardiac remodeling is a common pathophysiological change along with chronic hypertension and myocardial infarction. Recent evidence indicated that cardiac tissue expressed peroxisome proliferator-activated receptor γ (PPAR γ). However, the functional role of PPAR γ in cardiac remodeling remained unclear. The present study was designed to investigate the relationship between PPAR γ activation and pressure overload-induced cardiac remodeling. **Methods:** Cardiac remodeling model was successfully established by abdominal aorta ligation. Cardiac fibrosis and cardiomyocyte hypertrophy were simulated by 100 nM angiotensin II (Ang II) *in vitro*. Haemodynamic parameters, the expressions of Brg1, α -MHC, β -MHC, transforming growth factor beta 1 (TGF- β_1), collagen-I, collagen-III and NF- κ B were examined. **Results:** Morphological and haemodynamic measurements showed that the activation of PPAR γ improved the impaired cardiac function and decreased interstitial fibrosis in cardiac remodeling rats. Further results also showed that the activation of PPAR γ inhibited the expressions of Brg1 and TGF- β_1 in the cardiac remodeling hearts. The activation of PPAR γ also inhibited the proliferation and collagen production of cardiac fibroblasts, and down-regulated the activity of Brg1 and the expression of TGF- β_1 induced by Ang II in cultured neonatal rat cardiomyocytes and cardiac fibroblasts, respectively, through NF- κ B pathway. **Conclusions:** These results suggested that PPAR γ activation effectively inhibited cardiac remodeling processes by suppression of Brg1 and TGF- β_1 expressions through NF- κ B pathway in the pressure-overloaded hearts induced by abdominal aorta ligation in rats.

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Introduction

Cardiac remodeling is typically seen in patients with chronic hypertension, myocardial infarction and cardiomyopathy, and there is particularly high prevalence in elderly patients. With the development of diseases, the heart eventually undergoes hypertrophic and fibrotic alternation. Myocyte hypertrophy, when accompanied by fibrosis during remodeling, is a pathophysiological process leading to a progressive decrease in cardiac function. This cardiac hypertrophy and inappropriate interstitial collagen formation contribute largely to the increase in wall stiffness and diastolic dysfunction [1]. Therefore, an appropriate therapy to alleviate or reverse the cardiac remodeling is desirable, but such ideal therapy is not available to ameliorate cardiac remodeling at present.

Peroxisome proliferators-activated receptor γ (PPAR γ) belongs to a member of the nuclear receptor family that includes 48 human transcription factors whose activity is regulated by natural ligands, including prostaglandin D₂ derivatives, linoleic acid, and components of oxidized LDL, as well as by synthetic antidiabetic drugs, the so-called glitazones or thiazolidinediones (TZDs), such as rosiglitazone and pioglitazone. The function of PPAR γ in the heart and their roles in cardiovascular diseases, such as chronic heart failure (CHF), are far less defined [2]. In experimental studies, the activation of PPAR γ reduces the production of endothelin [3] and proinflammatory cytokines [4, 5]. Since these mediators are involved in the progression of heart failure, a cardioprotective effect of PPAR γ on heart failure would be highly expected [6].

Transforming growth factor beta 1 (TGF- β ₁), a locally generated cytokine, has been known as a major stimulator of tissue fibroinflammatory changes. TGF- β ₁ plays a key role in fibrotic remodeling of the heart, and modulates cardiomyocyte growth, fibroblast activation and extracellular matrix (ECM) deposition. The increased gene expression of ECM components, such as collagen-I, collagen-III and TGF- β ₁, causes cardiac fibrosis and promotes cardiac stiffness, eventually leads to diastolic dysfunction [7]. TGF- β ₁ also modulates the phenotypic conversion of fibroblasts to myofibroblasts that express α -smooth muscle actin (SMA). Myofibroblasts play important roles in a wide range of pathological conditions associated with fibrosis and organ remodeling by producing extracellular matrix components as well as profibrotic mediators. Recent studies in humans and animal models have shown that myocardial TGF- β ₁ expression was upregulated during cardiac fibrosis [8-10].

The Brg1/Brm-associated-factor (BAF) complex, consisting of 12 protein subunits, is a major type of ATP-dependent chromatin-remodeling complex in vertebrates [11]. Some evidence indicated that Brg1, the essential ATPase subunit of the BAF complex [12], interacted with two other classes of chromatin-modifying enzymes, HDAC [13] and PARP [14], to regulate gene expression during cardiac growth, differentiation and hypertrophy in mice. Intriguingly, as potential therapeutic targets, cardiac hypertrophy is dramatically reduced by genetic mutations of class I HDACs and PARP1 in mice or pharmacological inhibition of their activities [15-18]. Furthermore, Brg1 can be activated by cardiac stresses with formation of HDAC and PARP complex, which then results in cardiac hypertrophy. Therefore, reduction of cardiac hypertrophy by inhibiting Brg1 expression would be a promising novel therapeutic target of clinical management for cardiac hypertrophy.

Nuclear factor-kappa B (NF- κ B) is a transcription factor governing the gene expression involved in the immune response, embryo or cell lineage development, cell apoptosis, cell cycle progression, inflammation, and oncogenesis. Cumulative evidences indicate that NF- κ B is a potential downstream target for PPAR γ . Chawla et al. confirmed that activation of PPAR γ leads to the inhibition of monocyte and macrophage inflammatory responses by preventing the activation of NF- κ B [19]. Furthermore, several recent reports have also demonstrated that inhibition of NF- κ B blocks the binding of Brg1 to appropriate regulatory elements and further inhibit gene expression and remodeling of the cytokine locus [20]. In addition, Jose et al. indicated that inhibition of NF- κ B activity suppresses TGF- β ₁ mRNA expression in K-BALB cells [21]. Therefore, in our study, we speculated that activation of PPAR γ may prevent cardiac remodeling through inhibition of NF- κ B activity, resulting in the suppression of TGF- β ₁ and Brg1 expression.

Materials and Methods

Ethics Statement

All experimental protocols were pre-approved by the Experimental Animal Ethic Committee of Harbin Medical University, China (Animal Experimental Ethical Inspection Protocol No. 2009104). Use of animals was confirmed with 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).

Experimental Model for Pressure-overloaded Cardiac Hypertrophy

The operation of abdominal aorta ligation was carried out according to the method described by Jouannot [22]. Healthy male Wistar rats (body weights 250-300 g) were anaesthetized with sodium pentobarbital (40 mg/kg, i.p.) and the abdominal aorta was exposed through a midline abdominal incision. To ensure the constant narrowness of the aorta during ligation, stainless steel wire (diameter 0.2 mm) was shaped into a coil (inside diameter 0.75 mm, one pitch 1.15 mm, length of the coil 3.45 mm) by means of an instrument (Coil-95, Yamashita-Giken, Tokushima, Japan), and aseptically placed around the abdominal aorta above the renal arteries by the method originally described by Kudo et al. [23]. Sham-operated control rats underwent an identical procedures using a coil with the inside diameter greater than that of the aorta. The cardiac remodeling rats were randomly divided into three groups at four weeks after ligation: model group, rosiglitazone group, and GW9662 group. In the rosiglitazone group, cardiac remodeling rats (model rats) were administered rosiglitazone (agonist of PPAR γ , Sigma, St. Louis, MO, USA) at 6 g/kg/d for 1 w by intraperitoneal injection. In the GW9662 group, 0.2 g/kg/d GW9662 (antagonist of PPAR γ , Sigma, St. Louis, MO, USA) was added to the model rats by intraperitoneal injection at 2 h prior to the administration of rosiglitazone 6 g/kg/d for 1 w.

Hemodynamic Measurements

After 1 w of drug treatment, the rat was anaesthetized with pentobarbital sodium (40 mg/kg, i.p.), a heparin-filled cannula was inserted into the right carotid artery and then advanced into the left ventricle, where the hemodynamic parameters reflecting cardiac performances, such as left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), and maximum rate of left ventricular pressure rise and fall (+dp/dtmax and -dp/dtmax) were measured by a pressure transducer interfaced with BL-420F organism function experiment system (Chengdu Tai Meng, China). After the hemodynamic data collection, the rat was sacrificed by cervical dislocation and the heart was removed rapidly and washed in ice-cold 0.9% saline. Parts of the heart were frozen in liquid nitrogen or fixed in 4% paraformalin for later use.

Histological Analysis of Collagen Deposition and Myocardial Hypertrophy

The fixed heart tissues were cross-sectioned into 5 mm thick slices. Haematoxylin and eosin (H&E) staining and Masson's trichrome were performed to detect or evaluate the myocardial hypertrophy and collagen deposition, respectively. Sections were imaged 200 $\times$ magnification by bright-field microscopy (IX71, Olympus, Tokyo, Japan). Each observer randomly examined a minimum of five (to eight) separate images from different (non-overlapping) regions. All quantitative evaluations were carried out by Image Pro Plus software (version 6.0, Media Cybernetics, Bethesda, MD, USA).

Isolation and Culture of Neonatal Rat Cardiomyocytes and Cardiac Fibroblasts

The cultured myocytes and cardiac fibroblasts used in these experiments were acutely isolated from 1-3 days old Wistar rats using procedures as previously described [24] with slight modifications. Briefly, after being treated with 0.25% trypsin solution, cardiac fibroblasts were isolated by the removal of myocytes through selective adhesion of non-myocytes at a 1.5 h pre-plating interval. Cardiac fibroblasts were maintained in DMEM supplemented with penicillin and streptomycin (1% v/v) and fetal bovine serum (10% v/v). Cardiac fibroblasts at the 3rd or 4th passage were used in the experiments. Both cardiomyocytes and cardiac fibroblasts were randomly divided into four groups, respectively: (1) control group; (2) model group: cells were incubated with Ang II (100 nM, Sigma, St. Louis, MO, USA) for 24 h; (3) rosiglitazone group: cells were given 10 μ M rosiglitazone for 4 h following by treatment with 100 nM Ang II for 24 h; (4) GW9662 group: cells were treated with 1 μ M GW9662 for 2 h following 100 nM Ang II for 24 h and finally exposed to 10 μ M rosiglitazone for 4 h.

Fibroblast Proliferation Assay

Fibroblasts proliferation was measured by counting cell number. Cardiac fibroblasts were seeded in 24-well tissue culture plates (2×10^4 cells per well) in DMEM supplemented with 10% fetal bovine serum (FBS). After cardiac fibroblasts were deprived of serum by being placed in serum-free DMEM culture medium along with Ang II (100 nM) for 24 h, the fibroblasts were then incubated with rosiglitazone (10 μ M) or in combination with GW9662 (1 μ M) and rosiglitazone (10 μ M), trypsinized, and counted by using a haemocytometer.

Bromodeoxyuridine Incorporation

We measured BrdU incorporation using BrdU proliferation assay kits according to the manufacturer's protocol. Briefly, the cells were labeled with 10 ng/ml of BrdU during the incubation, washed three times with cold Wash Buffer, fixed, air-dried and incubated with mouse anti-BrdU monoclonal antibody (diluted 1: 200, millipore, Darmstadt, Germany) at room temperature for 1 h. The antibody was removed. The cells were washed three times and then incubated with peroxidase goat anti-mouse IgG (1: 2000) at room temperature for 30 min. The cells were washed three times, and 100 μ l substrates were added to each well and incubated for 30 min in darkness. Thereafter, absorbance at dual-wave lengths of 450 to 540 nm was measured.

Measurement of Cell Surface Area

Cultured cardiomyocytes were fixed with 4% paraformaldehyde for 0.5 h. Then the cell membrane was penetrated by 0.4% Triton X-100 for 1 h and blocked by goat serum for 1 h. After that, the cells were incubated with anti-sarcomeric actin antibody (1:200, Sigma, St. Louis, MO, USA) at 4°C overnight, subsequently with Cy3-conjugated goat anti-mouse antibody (1:1000, Sigma, St. Louis, MO, USA) for 1 h. Immunofluorescence was analyzed under a fluorescence microscope (Nikon, 80i, Japan). Cell surface area was quantified and averaged by measuring 60 random cells from three experiments with Image-Pro Plus Data Analysis Software.

Protein Isolation and Western Blot Analysis

The total amount of protein was extracted from cardiac tissue. Briefly, the protein content was determined by use of a bicinchoninic acid protein assay kit using bovine serum albumin as the standard. Protein samples (100 mg) were subjected to 10% SDS-PAGE and blotted to nitrocellulose. The blots were blocked by 5% non-fat milk and dissolved in phosphate buffered solution (PBS) for 1 h, then probed with anti-Brg1 (Santa Cruz Biotech, Santa Cruz, CA, USA), anti-collagen-I (Santa Cruz Biotech, Santa Cruz, CA, USA), anti-collagen-III (Santa Cruz Biotech, Santa Cruz, CA, USA), anti-TGF- β_1 (Cell Signaling Tech, Danvers, MA, USA), anti-NF- κ B (Cell Signaling Tech, Danvers, MA, USA), anti- α -MHC (Sigma-Aldrich, St. Louis, MO, USA), anti- β -MHC (Sigma, St. Louis, MO, USA), anti-Histone-H1 (Santa Cruz Biotech, Santa Cruz, CA, USA), anti- β -actin (Santa Cruz Biotech, Santa Cruz, CA, USA) and anti-GAPDH (Kangcheng Inc., Shanghai, China) antibodies in PBS and incubated at 4°C overnight. Membranes were washed three times, 15 min each time, with PBS containing 0.5% Tween 20 (PBS-T) and incubated with secondary antibody (Alexa Fluor, Mol Probes, Eugene, OR, USA) for 1 h. Western blot bands were collected by using Imaging System (LI-COR Biosci, Lincoln, NE, USA) and quantified with Odyssey v1.2 software (LI-COR Biosci, Lincoln, NE, USA) by measuring the band intensity (area $\times$ OD) in each group and normalizing this to that obtained with internal control.

ATPase Assays

ATPase assays were carried out as described [25] with modifications. Samples were lysed in RIPA buffer and protease inhibitor tablet (Roche, Basel, Switzerland), then sonicated, and clarified by centrifugation. Protein concentration of lysates was quantified by Bradford assay. Lysates were incubated with 2 μ g of Brg1-specific antibodies at 4°C overnight and then with protein G agarose beads (Zymed, San Diego California, USA). The precipitates were washed with buffer containing (in mM) 20 HEPES, 10% glycerol, 12.5 MgCl $_2$, 0.1 EDTA, 0.2% NP-40, 0.1 DTT, and 0.6 M KOAc and then resuspended in assay buffer containing (in mM) 10 Tris, 50 NaCl, 5 MgCl $_2$, 20% glycerol in the presence of 1 mg/ml BSA, 0.5 PMSF, 1 DTT, 0.5 ATP, 20 nM plasmid DNA, and 1 μ l Ci [32P] ATP. The reaction was carried out at 37°C for 1 h and stopped with the addition of 1 μ l 0.5 μ M EDTA per 20 μ l reaction volumes. Immediately, 1 μ l reaction was spotted on a polyethyleneimine cellulose TLC plate and separated using 1 M formic acid and 0.5 M LiCl. The ratio of inorganic phosphate to ATP was quantified using Scion Image Software.

siRNA Transfection

The siRNA sequences against rat I κ B α (5'-AGGCCAGCGTCTGACATTA-3') was purchased from Gene Pharma (Shanghai, China), annealed according to manufacturer's instruction, and then stored at -20°C. Cardiomyocytes and cardiac fibroblasts were transfected with siRNA (1.0 mg/ml) using lipofectamine 2000 according to the manufacturer's instruction and incubated at 37°C for 48 h. The efficiency for successful transfection was determined by counting the number of positive cells with FITC labeled Oligo under the fluorescent microscopy. Over 80% of successfully transfected cells were randomly divided into three groups: (1) siRNA I κ B α group: after 48 h siRNA transfection, cardiomyocytes and cardiac fibroblasts were treated with Ang II 100 nM for 24 h; (2) rosiglitazone + siRNA I κ B α group: after 48 h siRNA transfection, cardiomyocytes and cardiac fibroblasts were treated with Ang II 100 nM for 24 h and then by 4 h incubation with 10 μ M rosiglitazone; (3) model + nRNA group: cardiomyocytes and cardiac fibroblasts were transfected with 1.0 mg/ml of non-silencing siRNA (5'-CCUGAGGCUAUAACUCGCCUAGUGA-3', Gene Pharma, Shanghai, China) for 48 h, which is ineffective in rat since it has no mammalian target, then the cells were incubated with Ang II 100 nM for another 24 h.

Statistical Analysis

All data were expressed as mean $\pm$ SEM. Statistical analysis was performed using one-way analysis of variance followed by Dunnett's *t*-test. Differences were considered to be statistically significant when $P < 0.05$.

Results

Effects of PPAR γ on Hemodynamics in Cardiac Remodeling Rat Hearts

To examine the effects of PPAR γ on cardiac function, ventricular hemodynamics were monitored in cardiac remodeling rats. As shown in Fig. 1, there were a significant increase in LVEDP as well as reduction in $\pm dp/dt_{max}$ and LVSP in model group compared with control group ($P < 0.05$), indicating that the cardiac dysfunction was developed in all tested model rats. In the rosiglitazone group, the abnormal hemodynamic parameters were improved compared with model group ($P < 0.05$), but improved cardiac dysfunction was not observed in GW9662 group by co-treatment with rosiglitazone and GW9662 compared with rosiglitazone alone ($P < 0.05$).

Fig. 1. Effects of PPAR γ on hemodynamics in myocardial remodeling rat hearts induced by abdominal aorta ligation. The hemodynamic parameters, LVSP (A), LVEDP (B), $+dp/dt_{max}$ and $-dp/dt_{max}$ (C) were measured throughout the experiment. LVSP: left ventricular systolic pressure; LVEDP: left ventricular end-diastolic pressure; $+dp/dt_{max}$ and $-dp/dt_{max}$: maximum rate of left ventricular pressure rise and fall. Data were represented by mean $\pm$ SEM ($n = 8$ per group). * $P < 0.05$ vs. control group; @ $P < 0.05$ vs. model group; # $P < 0.05$ vs. rosiglitazone group.

Fig. 2. Effects of PPAR γ on cardiac hypertrophy in myocardial remodeling rat hearts induced by abdominal aorta ligation. (A) Histological sections were stained with haematoxylin and eosin (H&E) to detect hypertrophy ($\times 200$). (B) Quantitative data of myocyte area. (C) Quantitative data of heart-to-body weight ratio. * $P < 0.05$ vs. control group; @ $P < 0.05$ vs. model group; # $P < 0.05$ vs. rosiglitazone group ($n = 6$).

Effects of PPAR γ on Myocardial Hypertrophy and Interstitial Fibrosis in Cardiac Remodeling Rat Hearts

After 5 w ligation was performed at the abdominal aorta, left ventricular hypertrophy was developed in model rats, the heart-to-body weight ratio was increased by 63% (Fig. 2C), and cell size revealed by analysis of the cross-sectional cardiomyocyte areas also increased compared with control rats ($P < 0.05$) (Fig. 2A and 2B). By the treatment with rosiglitazone, the heart-to-body weight ratio and cardiomyocyte size were both significantly reduced compared with model rats ($P < 0.05$), and these effects of rosiglitazone were reversed in the presence of GW9662 ($P < 0.05$).

ECM deposition in the cardiac remodeling region was assessed by using Masson's trichrome staining of the histological sections of the hearts. The interstitial fibrotic area in model rats was significantly increased compared with control animals ($P < 0.05$). Treatment with rosiglitazone resulted in the reduction of ECM deposition, while GW9662 increased it (Fig. 3).

Effects of PPAR γ on Hypertrophy of Cardiomyocytes and the Proliferation of Cardiac Fibroblasts in vitro

Myocardial hypertrophy was induced by exposing neonatal cardiomyocytes to 100 nM Ang II for 24 h. Hypertrophic growth was assayed by the staining of α -sarcomeric actinin protein in cultured cardiomyocytes. As shown in Fig. 4, Ang II significantly increased cell surface area of cardiomyocytes. Whereas, cell surface area in Ang II-treated cardiomyocytes was markedly inhibited in the presence of rosiglitazone, and this effect of rosiglitazone was reversed by GW9662.

Fig. 3. Effects of PPAR γ on interstitial fibrosis in myocardial remodeling rat hearts induced by abdominal aorta ligation. (A) Masson's trichrome staining was used to detect fibrosis ($\times 200$). The interstitial fibrosis was in blue staining. (B) Quantification of fibrosis. Average data were represented by mean $\pm$ SEM ($n = 6$). * $P < 0.05$ vs. control group; @ $P < 0.05$ vs. model group; # $P < 0.05$ vs. rosiglitazone group.

The effect of PPAR γ on the proliferation of cardiac fibroblasts was assessed by counting the number of cells and 5-bromodeoxyuridine incorporation assay. Treatment of cardiac fibroblasts with 100 nM Ang II led to the increase in the number of cardiac fibroblasts and 5-bromodeoxyuridine incorporation, and the concomitant administration of rosiglitazone inhibited the proliferation of cardiac fibroblasts and this effect of rosiglitazone was blocked by GW9662 (Fig. 5). The above results indicated that the activation of PPAR γ plays protective roles in cardiac hypertrophy and cardiac fibroblast proliferation induced by Ang II.

Effects of PPAR γ on the Expressions of Brg1, α -MHC, β -MHC, TGF- β_1 , Collagen-I and Collagen-III Protein

Pressure-overload and Ang II constant infusion induced significant hypertrophy and cardiac fibrosis *in vivo* and *in vitro*, respectively. So, we further investigated the protein expression related to hypertrophy and cardiac fibrosis. In terms of cardiac hypertrophy, Brg1 and α -MHC/ β -MHC were determined in cardiac remodeling tissue and cultured cardiomyocytes challenged by Ang II. We found that the expression of Brg1 was remarkably increased in model groups compared with control group ($P < 0.05$), but this action was partially reduced in rosiglitazone group ($P < 0.05$) and rosiglitazone's effect was attenuated by GW9662 ($P < 0.05$) (Fig. 6A and 6B). Interestingly, the opposite trends for the expression of α -MHC/ β -MHC were confirmed in both cardiac tissue and cardiomyocytes ($P < 0.05$) (Fig. 6C and 6D).

In aspects of myocardial fibrosis, TGF- β_1 (the pro-fibrotic cytokine), collagen-I and collagen-III (major component of the ECM), were measured in model hearts and cultured cardiac fibroblasts challenged with Ang II. The results showed that TGF- β_1 (Fig. 6E and 6F), collagen-I (Fig. 6G and 6H) and collagen-III (Fig. 6I and 6J) were significantly increased in press-overloaded hearts and Ang II-treated cardiac fibroblasts compared with control group

Fig. 4. Inhibitory effects of PPAR γ on Ang II-induced cardiac hypertrophy in cultured neonatal rat myocytes. Hypertrophic growth was evaluated by the visualization of sarcomere organization. Ang II-induced the enlargement of cell surface area was inhibited by rosiglitazone. (A) Representative photographs of cardiomyocytes of cell area. Cardiomyocytes were identified ($\times 200$) with sarcomeric α -actinin antibody (red signal). (B) Summarized data of cell area. Average data were represented by mean $\pm$ SEM ($n = 6$). * $P < 0.05$ vs. control group; @ $P < 0.05$ vs. model group; # $P < 0.05$ vs. rosiglitazone group.

Fig. 5. Inhibitory effects of PPAR γ on Ang II-induced cardiac fibroblast proliferation *in vitro*. (A) Cell number counting. (B) BrdU incorporation assay. Data are expressed as mean $\pm$ SEM ($n = 6$). * $P < 0.05$ vs. control group; @ $P < 0.05$ vs. model group; # $P < 0.05$ vs. rosiglitazone group.

($P < 0.05$), which were down-regulated by rosiglitazone treatment ($P < 0.05$). However, the effects of rosiglitazone were reversed by PPAR γ -selective antagonist GW9662 ($P < 0.05$).

Fig. 6. Detection of Brg1, α -MHC, β -MHC, TGF- β_1 , collagen-I and collagen-III protein expression by western blot analysis. The expressions of Brg1 (A and B), α -MHC and β -MHC (C and D) protein were measured in myocardial remodeling rat hearts induced by ligating abdominal aorta and cultured cardiomyocytes challenged by Ang II 100 nM. TGF- β_1 (E and F), collagen I (G and H) and collagen III (I and J) protein expressions were measured in myocardial remodeling rat hearts induced by ligating abdominal aorta and cultured cardiac fibroblasts challenged by Ang II 100 nM. Average data were represented by mean $\pm$ SEM ($n = 6$). * $P < 0.05$ vs. control group; @ $P < 0.05$ vs. model group; # $P < 0.05$ vs. rosiglitazone group.

Effects of PPAR γ on the Activity of Nuclear NF- κ B in Neonatal Rat Cardiomyocytes and Cardiac Fibroblasts

To understand the effects of PPAR γ on NF- κ B in neonatal cardiomyocyte and cardiac fibroblasts, the protein of nuclear NF- κ B was assessed by western blot. Treatment of neonatal cardiomyocyte and cardiac fibroblasts with 100 nM Ang II up-regulated the protein levels of NF- κ B. Rosiglitazone significantly inhibited Ang II-mediated NF- κ B expression, whereas, this inhibitory effect of rosiglitazone on NF- κ B expression was abolished by co-application of GW9662 on top of rosiglitazone in neonatal cardiomyocyte (Fig. 7A) and cardiac fibroblasts (Fig. 7B), respectively.

PPAR γ Activation-mediated Inhibition of Brg $_1$ Activity and TGF- β_1 Expression Through NF- κ B Pathway

NF- κ B is a well known downstream transcription factor of PPAR γ signaling pathway. To examine if this was also the case in suppression of Brg1 and TGF- β_1 activation induced by Ang II, siRNA technology was employed to silence I κ B α and the results showed that Brg1 activity and TGF- β_1 expression were dramatically improved (Fig. 8A and Fig. 8B) in cardiomyocytes and cardiac fibroblasts challenged by Ang II 100 nM, respectively. However, the effect of RNAi knockdown of I κ B α was attenuated by rosiglitazone (agonist of PPAR γ). These results indicated that activation of PPAR γ contributed at least to the inhibition of Brg1 activity and TGF- β_1 expression through NF- κ B pathway.

Discussion

Pressure overload stimulates a series of cellular and molecular changes and eventually leads to myocardial remodeling as well as subsequent ventricular dysfunction as result [26, 27]. The underlying molecular mechanism was not fully understood with current knowledge. Here, to our knowledge, we demonstrate, for the first time, the potential effect of PPAR γ activation on cardiac molecular/cellular events and ventricular dysfunction during pressure overload through NF- κ B-Brg1/TGF- β_1 pathway.

Fig. 7. Effects of PPAR γ on NF- κ B expression in cultured neonatal rat cardiomyocytes and cardiac fibroblasts. NF- κ B expression was detected in cardiomyocytes (A) and cardiac fibroblasts (B) challenged by Ang II 100 nM by western blot. Average data were represented by mean $\pm$ SEM (n = 6). *P<0.05 vs. control group; @P<0.05 vs. model group; #P<0.05 vs. rosiglitazone group.

Fig. 8. Effects of PPAR γ on Brg1 activity and TGF- β_1 expression through NF- κ B pathway. The activation of NF- κ B induced by silencing IκB α improved the activity of Brg1 (A) and the expression of TGF- β_1 (B) in cultured cardiomyocytes and cardiac fibroblasts challenged by Ang II 100 nM, in siRNA IκB α groups. These effects were abolished by the activation of PPAR γ in rosiglitazone+siRNA IκB α groups. Average data were represented by mean $\pm$ SEM (n = 6). *P<0.05 vs. control group; @P<0.05 vs. model group; #P<0.05 vs. siRNA IκB α group.

The present study showed that the ligation of abdominal aorta for 5 w significantly led to cardiac remodeling and dysfunction, and the agonist or antagonist of PPAR γ weakened or enhanced the deleterious changes in pressure overload-induced cardiac remodeling rats. These results suggested that activation of PPAR γ may be a key player in cardioprotection against cardiac remodeling induced by pressure overload.

It is well known that cardiac hypertrophy is the feature of cardiac remodeling after constricting the abdominal aorta. Other researches have reported that PPAR γ agonist may

have direct vasodilator action on the vasculature. Further report also suggests rosiglitazone-induced vasorelaxation [28], following a fall in blood pressure. In agreement with this hypothesis, we presumed that activation of PPAR γ plays, at least in part, an important role in cardiac hypertrophy during cardiac remodeling induced by pressure overload. In current study, we found that left ventricular (LV) weight to body weight (LVW/BW), and mean cardiomyocyte diameter were significantly elevated in cardiac remodeling heart induced by pressure overload, but attenuated in rosiglitazone group, and increased in GW9662 group. These data imply that PPAR γ activation might suppress cardiac hypertrophy induced by pressure overload, and eventually ameliorate cardiac remodeling and dysfunction.

Cardiac fibrosis is another major character of cardiac remodeling and leads to cardiac dysfunction and failure [29-31] without proper measures. The molecular mechanism of interstitial fibrosis is complicated tissue remodeling process with significant intracellular signaling cascade. At present study, activation of PPAR γ inhibited the deposition of ECM and the levels of collagen-I and collagen-III, whereas, GW9662 (an antagonist of PPAR γ) weakened the effect of PPAR γ on protecting hearts from fibrosis not only *in vivo* but *in vitro* as well, suggesting the direct role of PPAR γ activation in cardioprotection.

Brg1 is known to control cardiovascular development in a time- and tissue-specific manner. Recent report indicates that Brg1 can be activated by cardiac stresses with a complex formed with its embryonic partners, HDAC and PARP, which then induces a pathological α -MHC to β -MHC shift [18]. Preventing Brg1 expression attenuates hypertrophy and reverses the pathological MHC switch. In this study, we have found that the expression of Brg1 was significantly increased and α -MHC/ β -MHC ratio was significantly decreased in cardiac remodeling heart, and these changes can be reversed by rosiglitazone, whereas, GW9662 abolishes above mentioned effects. These data confirmed that activation of PPAR γ protected hearts from cardiac hypertrophy during cardiac remodeling induced by pressure overload, and PPAR γ activation and consequent attenuation of Brg1 protein expression is the likelihood underlying molecular and cellular mechanisms.

TGF- β_1 is a key pro-fibrotic cytokine that is markedly elevated in remodeling hearts and cardiomyocytes, and anti-TGF gene therapy mitigates cardiac remodeling by affecting cardiac fibrosis [32]. Our results showed that the activation of PPAR γ significantly inhibited the expression of TGF- β_1 in the hearts of cardiac remodeling rats and cardiac fibroblasts. These data suggested that the suppression of TGF- β_1 by PPAR γ activation represents one of the possible mechanisms accounting for its beneficial effects on impaired cardiac fibrosis.

The above results showed that PPAR γ activation reduced cardiac remodeling by repressing the expressions of Brg1 and TGF- β_1 , suggesting the possible connection between PPAR γ activation and the expressions of Brg1 and TGF- β_1 .

NF- κ B is found in almost all cell types and can be activated by proinflammatory cytokines and endotoxins, and then translocated to the nucleus to regulate inducible gene expression under both physiological and pathological conditions [33-36]. Our study indicated that the activation of NF- κ B induced by silencing I κ B α enhanced the activity of Brg1 in cardiomyocyte, as well as the expression of TGF- β_1 in cardiac fibroblasts. In addition, a growing number of studies have highlighted the interaction of PPAR γ and NF- κ B [37, 38], in which PPAR γ could inhibit NF- κ B activation via several mechanisms and then repress NF- κ B-mediated transcription for certain inducible gene. In our study, the activation of PPAR γ by rosiglitazone significantly inhibited the expression of NF- κ B in cardiac fibroblasts and cardiomyocyte, respectively. Overall, PPAR γ suppresses the activity of TGF- β_1 and Brg1 due at least to downregulation of NF- κ B pathway.

Taken all data together, these results demonstrate that activation of PPAR γ significantly improves the cardiac function in cardiac remodeling rats by inhibiting interstitial fibrosis and cardiac hypertrophy due at least in part to the suppression of Brg1 activity and TGF- β_1 expression through NF- κ B pathway. These data suggest the potential target of PPAR γ as a new anti-cardiac remodeling in the future.

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References

- 1 Cohn JN: Structural changes in cardiovascular disease. *Am J Cardiol* 1995;76:34E-37E.
- 2 Bishop-Bailey D, Hla T, Warner TD: Bisphenol A diglycidyl ether (BADGE) is a PPAR gamma agonist in an ECV304 cell line. *Br J Pharmacol* 2000;131:651-654.
- 3 Buchanan GR, Rogers ZR: Conservative versus aggressive transfusion regimens in sickle cell disease. *N Engl J Med* 1995;333:1641-1642.
- 4 Sidhu JS, Kaski JC: Peroxisome proliferator activated receptor gamma: a potential therapeutic target in the management of ischaemic heart disease. *Heart* 2001;86:255-258.
- 5 Chawla A, Boisvert WA, Lee CH, Laffitte BA, Barak Y, Joseph SB, Liao D, Nagy L, Edwards PA, Curtiss LK: A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. *Mol Cell* 2001;7:161-171.
- 6 Wilson-Fritch L, Nicoloso S, Chouinard M, Lazar MA, Chui PC, Leszyk J, Straubhaar J, Czech MP, Corvera S: Mitochondrial remodeling in adipose tissue associated with obesity and treatment with rosiglitazone. *J Clin Invest* 2004;114:1281-1289.
- 7 Sevinig, Bochaton Piallat ML, Ropraz P, Geinoz A, Borsi L, Zardi L, Gabbiani G: The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor-beta1. *J Cell Biol* 1998;142:873-881.
- 8 Kapur NK: Transforming growth factor- β : governing the transition from inflammation to fibrosis in heart failure with preserved left ventricular function. *Circ Heart Fail* 2011;4:5-7.
- 9 Van Setten GB, Blalock TD, Grotendorst G, Schultz GS: Detection of connective tissue growth factor in human aqueous humor. *Ophthalmic Res* 2002;34:306-308.
- 10 Polyakova V, Loeffler I, Hein S, Miyagawa S, Piotrowska I, Dammer S, Risteli J, Schaper J, Kostin S: Fibrosis in endstage human heart failure: severe changes in collagen metabolism and MMP/TIMP profiles. *Int J Cardiol* 2011;151:18-33.
- 11 Martens JA, Winston F: Recent advances in understanding chromatin remodeling by Swi/Snf complexes. *Curr Opin Genet Dev* 2003;13:136-142.
- 12 Dunaief JL, Strober BE, Guha S, Khavari PA, Alin K, Luban J, Begemann M, Crabtree GR, Goff SP: The retinoblastoma protein and BRG1 form a complex and cooperate to induce cell cycle arrest. *Cell* 1994;79: 119-130.
- 13 Xue Y, Wong J, Moreno GT, Young MK, Côté J, Wang W: NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. *Mol Cell* 1998;2:851-861.
- 14 Hang CT, Yang J, Han P, Cheng HL, Shang C, Ashley E, Zhou B, Chang CP: Chromatin regulation by Brg1 underlies heart muscle development and disease. *Nature* 2010;466:62-67.
- 15 Lee D, Kim JW, Seo T, Hwang SG, Choi EJ, Choe J: SWI/SNF complex interacts with tumor suppressor p53 and is necessary for the activation of p53-mediated transcription. *J Biol Chem* 2002; 277: 22330-22337.
- 16 Bochar DA, Wang L, Beniya H, Kinev A, Xue Y, Lane WS, Wang W, Kashanchi F, Shiekhatter R: BRCA1 is associated with a human SWI/SNF Related complex: linking chromatin remodeling to breast cancer. *Cell* 2000;102:257-265.
- 17 Marignani PA, Kanai F, Carpenter CL: LKB 1 associates with Brg1 and is necessary for Brg1-induced growth arrest. *J Biol Chem* 2001;276:32415-32418.
- 18 Hang CT, Yang J, Han P, Cheng HL, Shang C, Ashley E, Zhou B, Chang CP: Chromatin regulation by Brg1 underlies heart muscle development and disease. *Nature* 2010;466:62-67.
- 19 Chawla A, Boisvert WA, Lee CH, Laffitte BA, Barak Y, Joseph SB, Liao D, Nagy L, Edwards PA, Curtiss LK, Evans RM, Tontonoz P: A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. *Mol Cell* 2001;7:161-167.

- 20 Wurster AL, Precht P, Pazin MJ: NF- κ B and BRG1 bind a distal regulatory element in the IL-3/GM-CSF locus. *Mol Immunol* 2011;48:2178-2188.
- 21 Perez JR, Higgins-Sochaski KA, Maltese JY, Narayanan R: Regulation of adhesion and growth of fibrosarcoma cells by NF-kappa B RelA involves transforming growth factor beta. *Mol Cell Biol* 1994;14:5326-5332.
- 22 Jouannot P, Crozatier B, Hatt PY: Anomalous ventricular contractile performance in experimental chronic aortic insufficiency in the dog. *Arch Mal Coeur Vaiss* 1976;69:809-817.
- 23 Kudo A: A method for calculating the inbreeding coefficient. *Am J Hum Genet* 1962;14:426-432.
- 24 Yang B, Lin H, Xiao J, Lu Y, Luo X, Li B: Themuscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. *Nat Med* 2007;13:486-491.
- 25 Douglas PS, Katz SE, Weinberg EO, Chen MH, Bishop SP, Lorell BH: Hypertrophic remodeling: gender differences in the early response to left ventricular pressure overload. *JACC* 1998;32:1118-1125.
- 26 McMullen JR, Sherwood MC, Tarnavski O, Zhang L, Dorfman AL, Shioi T, Izumo S: Inhibition of mTOR signaling with rapamycin regresses established cardiac hypertrophy induced by pressure overload. *Circulation* 2004;109:3050-3055.
- 27 Nakamura Y1, Ohya Y, Onaka U, Fujii K, Abe I, Fujishima M: Inhibitory action of insulin-sensitizing agents on calcium channels in smooth muscle cells from resistance arteries of guinea-pig. *Br J Pharmacol* 1998;123: 675-682.
- 28 Bultman SJ, Gebuhr TC, Magnuson T: A Brg1 mutation that uncouples ATPase activity from chromatin remodeling reveals an essential role for SWI/SNF-related complexes in beta-globin expression and erythroid development. *Genes Dev* 2005;19:2849-2861.
- 29 Boluyt MO, O'Neill L, Meredith AL, Bing OH, Brooks WW, Conrad CH, Crow MT, Lakatta EG: Alterations in cardiac gene expression during the transition from stable hypertrophy to heart failure. Marked upregulation of genes encoding extracellular matrix components. *Circ Res* 1994;75:23-32.
- 30 Heling I, Slutzky-Goldberg I, Lustmann J, Ehrlich Y, Becker A: Bone-like tissue growth in the root canal of immature permanent teeth after traumatic injuries. *Endod Dent Traumatol* 2000;16:298-303.
- 31 Murakami A, Koshimizu K, Ohigashi H, Kuwahara S, Kuki W, Takahashi Y, Hosotani K, Kawahara S, Matsuoka Y: Characteristic rat tissue accumulation of nobiletin, a chemopreventive polymethoxyflavonoid, in comparison with luteolin. *Biofactors* 2002;16:73-82.
- 32 Bujak M, Frangogiannis NG: The role of TGF-beta signaling in myocardial infarction and cardiac remodeling. *Cardiovasc Res* 2007;74:184-195.
- 33 Gilmore TD: Introduction to NF- κ B: players, pathways, perspectives. *Oncogene* 2006;25:6680-6684.
- 34 Brasier AR: The NF- κ B regulatory network. *Cardiovasc Toxicol* 2006;6:111-130.
- 35 Perkins ND: Integrating cell-signalling pathways with NF- κ B and IKK function. *Nat Rev Mol Cell Bio* 2007;8:49-62.
- 36 Tian B, Brasier AR: Identification of a nuclear factor kappa B-dependent gene network. *Recent Prog Horm Res* 2003;58:95-130.
- 37 Zhang Y, Hu L, Cui Y, Qi Z, Huang X, Cai L, Zhang T, Yin Y, Lu Z, Xiang J: Roles of PPAR γ /NF- κ B signaling pathway in the pathogenesis of intrahepatic cholestasis of pregnancy. *PLoS One* 2014;9:e87343.
- 38 Remels AH, Langen RC, Gosker HR, Russell AP, Spaapen F, Voncken JW, Schrauwen P, Schols AM: PPAR gamma inhibits NF-kappaB-dependent transcriptional activation in skeletal muscle. *Am J Physiol Endocrinol Metab* 2009;297:E174-E183.