Biochemical and Biophysical Research Communications 464 (2015) 679–684

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The RNA-binding protein PCBP2 inhibits Ang II-induced hypertrophy of cardiomyocytes though promoting GPR56 mRNA degeneration

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ARTICLE INFO

Article history:
Received 18 June 2015
Accepted 21 June 2015
Available online 23 June 2015

Keywords:
Angiotensin II
Cardiomyocyte hypertrophy
GRP56
PCBP2

ABSTRACT

Poly(C)-binding proteins (PCBPs) are known as RNA-binding proteins that interact in a sequence-specific fashion with single-stranded poly(C). This family can be divided into two groups: hnRNP K and PCBP1-4. PCBPs are expressed broadly in human and mouse tissues and all members of the PCBP family are related evolutionarily. However, their physiological or pathological functions in the hearts remain unknown. Here we reported that PCBP2 is an anti-hypertrophic factor by inhibiting GPR56 mRNA stability. We found the downregulation of PCBP2 in human failing hearts and mouse hypertrophic hearts. PCBP2 knockdown promoted angiotensin II (Ang II)-induced hypertrophy (increase in cell size, protein synthesis and activation of fetal genes) of neonatal cardiomyocytes and H9C2 cells, while PCBP2 overexpression obtained oppose effects. Furthermore, PCBP2 was shown to inhibit GPR56 expression by promoting its mRNA degeneration in cardiomyocytes. Finally, we knocked down GPR56 in cardiomyocytes and found that GPR56 promoted Ang II-induced cardiomyocyte hypertrophy and it contributed to PCBP2 effects on cardiomyocyte hypertrophy.

1. Introduction

Heart failure is now one of the leading causes of morbidity and mortality worldwide. Heart failure is featured by systolic-diastolic dysfunction and remodeling of the myocardium. Cardiac hypertrophy is one of the fundamental pathological feature of heart failure [1]. In the general population, one in 500 suffers cardiac hypertrophy. For the reason that the overwhelming major mammalian cardiomyocytes fail to divide soon after birth, hypertrophy is the only way for them to response to the increased workload [2]. Hypertension, myocardial ischemia and neurohumoral activation can lead to pathological hypertrophy. During cardiac hypertrophy, not only the cell size of cardiomyocytes is increased, but the sarcomeres are also added and reorganized [3]. Moreover, a group of genes that are usually expressed during fetal heart development are re-expressed. These changes may be compensatory initially to manage the increased workload on the heart. However, sustained hypertrophy results in congestive heart failure and sudden death due to arrhythmias [4]. Clinical therapeutic strategy for treatment of cardiac hypertrophy and failure remains limited, which prompts us to have better understanding of the underlying mechanism of cardiac hypertrophy and failure.

PCBP2 is a member of the poly(C)-binding protein (PCBP) family, which plays an important role in posttranscriptional and translational regulation by interacting with single-stranded poly(C) motifs in target mRNAs [5]. Most of the reports on PCBP2 have focused on its posttranscriptional and translational controls in RNA viruses [6,7]. Recent findings have implicated that PCBP2 may be an orchestrator of tumor development [8–10]. PCBP2 also expresses during myocardial development [11], indicating its important role in the heart.

GPR56 (G protein-coupled receptor 56, or TM7XN1) is one member of the G protein-coupled receptor (GPR) family that ubiquitously expresses in mammal tissues. Previous reports show that GPR56 critically participates in carcinogenesis [12–14], and rostral cerebellar development [15–18]. Two recent works demonstrated that GPR56 is a regulator for mechanical overload-induced muscle hypertrophy and infusion [19,20], implicating that GPR56 may also participate in hypertrophy of cardiomyocytes.

Herein we identified PCBP2 and GPR56 as critical regulators for hypertrophy of cardiomyocytes. PCBP2 is down-regulated during hypertrophy *in vivo* and *in vitro*. PCBP2 inhibits while GPR56

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http://dx.doi.org/10.1016/j.bbrc.2015.06.139
0006-291X/

promotes Ang II-induced hypertrophic responses in cardiomyocytes. We discover that PCBP2 decreases GRP56 level by regulating the stability of its mRNA, and GRP56 may account for PCBP2 effects on cardiomyocyte hypertrophy.

2. Materials and methods

2.1. Human samples

Human heart samples were obtained from the cardiac transplant program of Xinhua Hospital, Shanghai Jiao Tong University School of Medicine (Shanghai, China). Informed consent was obtained from all patients involved in this study. All procedures were approved by the Institutional Review Board of Shanghai Jiao Tong University School of Medicine. Control samples were obtained intraoperatively from non-failing hearts undergoing ventricular corrective surgery or from donor dysfunctional hearts. Failing heart specimens were obtained from diseased hearts that were removed during orthotopic heart transplantation.

2.2. Pathological hypertrophy by transaortic constriction (TAC)

Pressure overload hypertrophy was induced by TAC of the ascending aorta of 8~10 weeks old male C57BL/6 mice, as described elsewhere [21].

2.3. Primary cultures of neonatal rat cardiomyocyte (NRCM), transfection/infection

Primary neonatal rat cardiomyocytes (NRCMs) were prepared in according to the following protocol. Briefly, PBS containing 0.03% trypsin and 0.04% collagenase type II was used to isolate cardiomyocytes from 1- to 2-day-old Sprague–Dawley rats. NRCMs were seeded at culture plates coated with gelatin in plating medium consisting of DMEM/F12 medium supplemented with 10% fetal calf serum, 0.1 μM BrdU and penicillin/streptomycin. After 48 h, the culture medium was replaced with serum-free DMEM/F12 for another 24 h prior to treatment with angiotensin II (Ang II, 1 μM) isoproterenol (ISO, 10 μM) or phenylephrine (PE, 100 μM).

To knockdown PCBP2 or GPR56 expression, three rat shPCBP2 or shGPR56 constructs were obtained from SABiosciences. Ad-sh-Ctrl was the non-targeting control. To overexpress PCBP2, the entire coding region of the human PCBP2 gene, under control of the cytomegalovirus (CMV) promoter, was encompassed by replication-defective adenoviral vectors. A similar adenoviral vector encoding the green fluorescent protein gene was used as a control. NCRMs were infected with adenovirus in diluted media at a multiplicity of infection (MOI) of 100 for 24 h. The sequences of rat PCBP2- or GPR56-targeted shRNAs were listed in supplementary table 1.

2.4. Western blot

Fresh heart tissues and cardiomyocytes were lysed with cell lysis buffer (Beyotime). 40 μg total proteins were subjected to SDS–polyacrylamide gel. After electrophoresis, the proteins were transferred to PVDF membranes, followed by blocking in 5% milk. The membranes were then probed with indicated antibodies overnight, and then washed and incubated with HRP-conjugated secondary antibodies (Beyotime) for 1.5 h and finally visualized using Chemiluminescent ECL reagent (Vigorous Biotechnology). The following antibodies were used: Anti-GAPDH (Cell Signaling Technology), anti-PCBP2 (Abcam), anti-Nppa (Santa), anti-Myh7 (Sigma), anti-GPR56 (Abcam).

2.5. Quantitative real-time PCR (q-PCR)

Total RNA was extracted from fresh hearts and cardiomyocytes with TRIzol and cDNA was synthesized from 1 μg of RNA with One Step RT-PCR Kit (TaKaRa). q-PCR was performed using the SYBR Green (TaKaRa) detection method on an ABI-7500 RT-PCR system (Applied Biosystems). The primers used were listed in supplementary table 2.

2.6. Luciferase assay

For luciferase assay, NRCMs were seeded onto 24-well plates at a density of 1×10^5 per well. 24 h later, either Ad-sh-Ctrl or Ad-sh-PCBP2 with Ad-Nppa-Luc/Myh7-Luc was co-infected into cardiomyocytes. After 24 h of Ang II (1uM) treatment, the cells were harvested, washed three times with PBS and lysed in 100 ml passive lysis buffer (Roche) in according to the guidance. Cell debris was removed by centrifugation, and the supernatant was used for the luciferase assay with a Single-Mode SpectraMax Microplate Reader empty vector.

2.7. Quantitative analysis of cardiomyocyte size

For determining the cross-sectional area of NRCMs and H9C2 cells, the cells were stained with α-actinin antibody (Sigma). Briefly, cardiomyocytes were stimulated with 1 μM Ang II for 48 h after infection with different adenoviruses for 24 h. The cells were then fixed with 4% formaldehyde in PBS for 15 min at room temperature, permeabilized with 0.1% PBST (Triton X-100 in PBS) for 40 min and stained with αa-actinin (1:100 dilution) using standard immunofluorescence staining techniques. Finally, the nucleus was stained with Hoechst (Sigma). The images were obtained by confocal microscopy (Leica). The surface area was quantified using Image J software (NIH).

2.8. GPR56 mRNA degeneration assay

NRCMs cells were infected with the control Ad-sh-Ctrl, Ad-sh-PCBP2, Ad-Ctrl or Ad-PCBP2 adenoviruses. After 48 h, actinomycin D (Sigma, 5 μg/ml) was added. Total RNA was collected at 0, 2, 4, 6 and 8 h after actinomycin D treatment. q-PCR was performed to evaluate the mRNA level of GPR56.

2.9. Statistical analysis

All values are expressed as the mean ± SEM. All experiments were repeated at least three times. Statistical differences between two groups were determined using Student's *t* test. Two-way analysis of variance (ANOVA) with general linear model procedures using a univariate approach was applied for more than two groups. *P* values of less than 0.05 were considered statistically significant.

3. Results

3.1. PCBP2 is downregulated upon hypertrophic stress

The role of the RNA-binding protein PCBP2 in cardiovascular system remains unknown, which prompted us to investigate the pathological role of PCBP2 in cardiovascular system. We found here that PCBP2 was down-regulated upon hypertrophic stress. We first showed the downregulation of PCBP2 mRNA and protein levels in human failing (or hypertrophic) hearts compared to normal non-failing (or non-hypertrophic) hearts (Fig. 1A–B). We then performed TAC surgery in C57Bl/6 mouse to induce pressure overload-

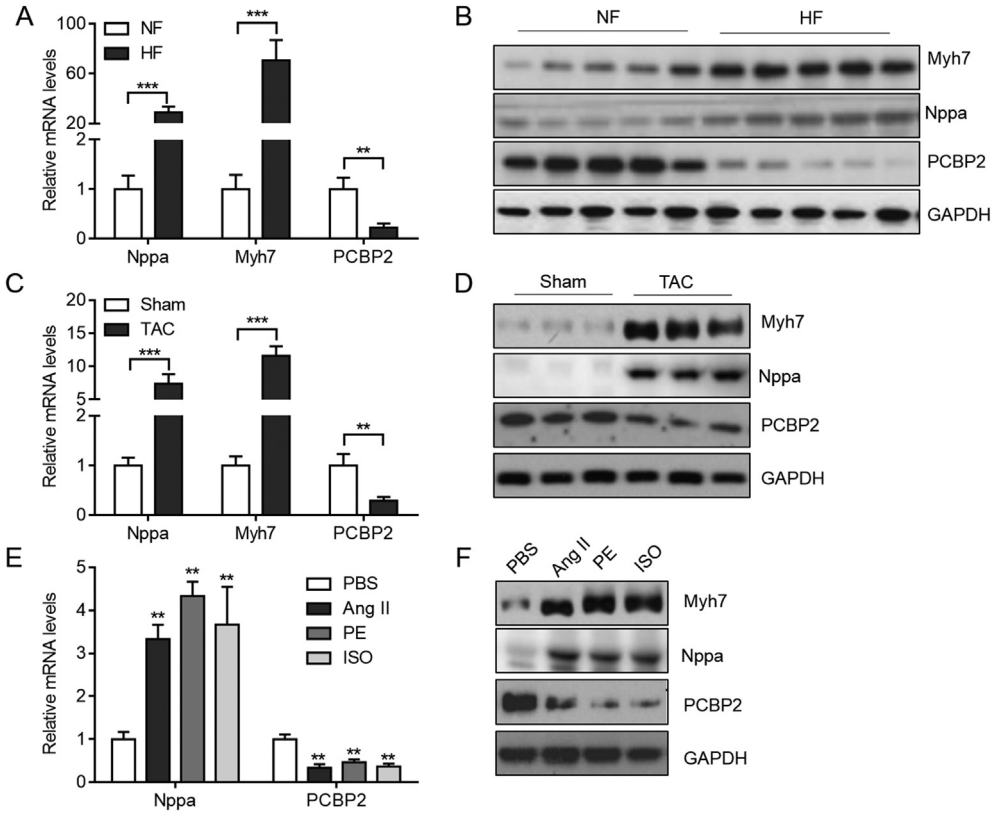

Fig. 1. PCBP2 expression is downregulated by hypertrophic stress. (A) Relative mRNA levels of Nppa, Myh7 and PCBP2 in non-failing (NF) normal human hearts or failing human hearts (HF). N = 5 in each group. **p < 0.01 and ***p < 0.001. (B) Protein levels of Nppa, Mhy7 and PCBP2 in NF and HF hearts. (C) Relative mRNA levels of Nppa, Myh7 and PCBP2 in mouse hearts undergoing sham or TAC surgery for four weeks. N = 5 in each group. **p < 0.01 and ***p < 0.001. (D) Protein levels of Nppa, Myh7 and PCBP2 in mouse hearts undergoing sham or TAC surgery for four weeks. (E) Relative mRNA levels of Nppa and PCBP2 in neonatal rat cardiomyocytes (NRCMs) treated with 10 μM ISO, 100 μM PE or 1 μM Ang II for 48 h. The experiments were repeated for three times. **p < 0.01 vs. PBS. (F) Protein levels of Nppa, Myh7 and PCBP2 in NRCMs treated with 10 μM ISO, 100 μM PE or 1 μM Ang II for 48 h.

mediated cardiac hypertrophy, and found that PCBP2 mRNA and protein levels were also down-regulated in the hearts of these mice compared to that of mice undergoing sham surgery (Fig. 1C–D). We wondered whether the down-regulation of PCBP2 level occurred in cardiomyocytes, therefore we isolated neonatal rat cardiomyocytes (NRCMs) and treated the cells with angiotensin II (Ang II, 1 μM) isoproterenol (ISO, 10 μM) or phenylephrine (PE, 100 μM) to induce hypertrophy. The results showed that both mRNA and protein levels were decreased in cardiomyocytes by hypertrophic stress (Fig. 1E–F). Similar results were found in the rat cardiomyoblast cell line H9C2 cells (data not shown). These findings implicate that PCBP2 may participate in cardiomyocyte hypertrophy.

3.2. PCBP2 inhibits Ang II-induced cardiomyocyte hypertrophy

Since PCBP2 was down-regulated in cardiomyocytes and hearts under hypertrophic stress, we proposed that PCBP2 may participate in hypertrophy of cardiomyocyte. Therefore, we investigated whether PCBP2 regulates hypertrophy in NRCMs and H9C2 cells. We first generated three adenovirus-mediated short hairpin RNAs targeting rat PCBP2 (Fig. 2A), and the first one was chosen for further study because of its high knockdown effect. We infected NRCMs with Ad-sh-Ctrl or Ad-sh-PCBP2 and followed by Ang II treatment. The results showed that PCBP2 knockdown significantly promoted Ang II-induced increase in cardiomyocyte size (Fig. 2B). We tested the hypertrophic fetal genes and found that PCBP2 knockdown could also facilitate Ang II-induced expression of Nppa, Myh7 and Nppb (Fig. 2C). Ang II induced protein synthesis in NRCMs, which was enhanced by PCBP2 knockdown (Fig. 2D). Similar effects of PCBP2 knockdown on cardiomyocyte hypertrophy, expression of fetal genes and protein synthesis were observed in H9C2 cells (Supplementary Fig. 1A–C). We then studied the effects of PCBP2 knockdown on the activity of the promoters of hypertrophic genes. To this end, luciferase reporter assay was performed in NRCMs. In consistent with the above findings, PCBP2 knockdown promoted Ang II-induced activation of Myh7 and Nppb promoters (Fig. 2E–F). These results indicated that PCBP2 negatively regulated hypertrophy of cardiomyocytes. To further make this conclusion more solid, we overexpressed human PCBP2 in NRCMs (Supplementary Fig. 2A) and studied the effects of PCBP2 overexpression on hypertrophy, fetal gene expression and protein synthesis. The results showed that PCBP2 overexpression inhibited hypertrophic growth, fetal gene expression and protein synthesis in NRCMs (Fig. 2G and Supplementary Fig. 2B–C). Taken together, these findings demonstrated that PCBP2 inhibited Ang II-induced hypertrophy of cardiomyocytes.

3.3. PCBP2 promotes the degeneration of GPR56 mRNA in cardiomyocytes

We next wanted to investigate the underlying mechanism by which PCBP2 regulated cardiomyocyte hypertrophy. PCBP2 is a well-known RNA-binding protein. In a previous work, Han et al. [8] identified 35 mRNA targets of PCBP2 in glioma. Among these targets, GPR56 (G-protein coupled receptor 56) attracted our attention. The expression of GPR56 is ubiquitous [22]. GPR56 promotes

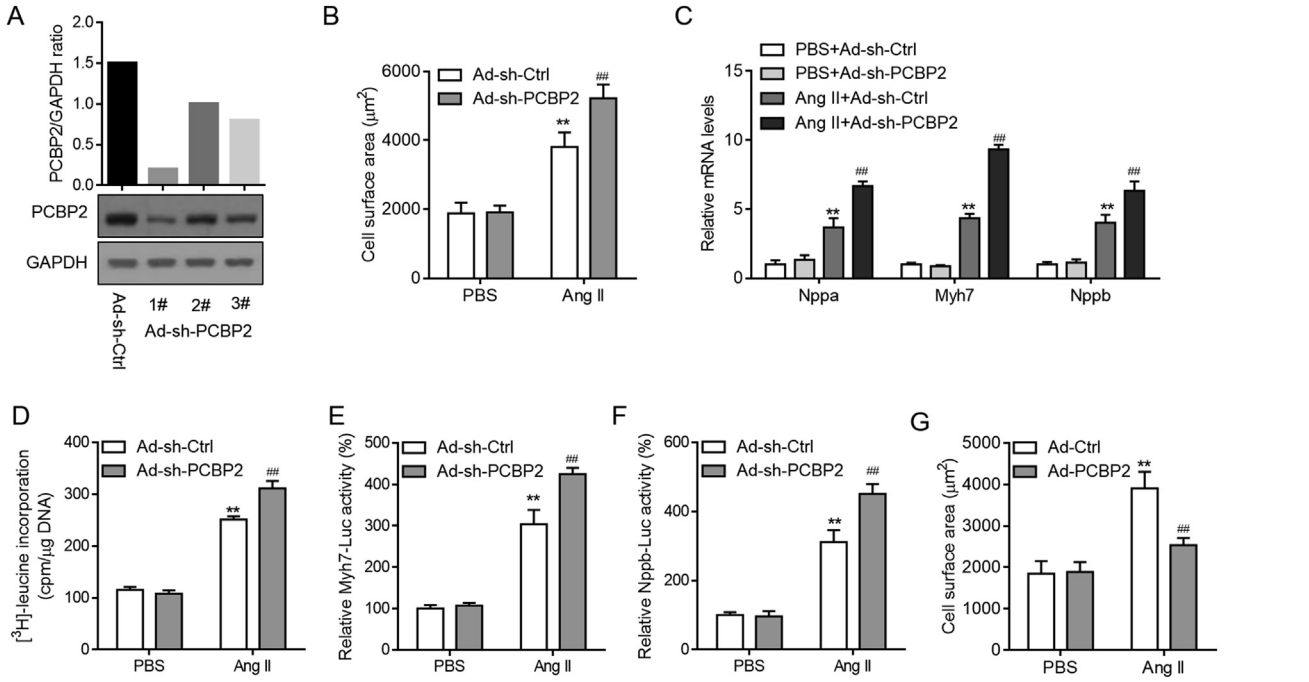

Fig. 2. PCBP2 inhibits Ang II-induced hypertrophic response of cardiomyocytes (A) Knockdown of PCBP2 with adenovirus mediated short hairpin RNAs in NRCMs. Representative western blot and quantitative data are shown. (B) PCBP2 knockdown promotes Ang II-induced hypertrophic growth of NRCMs. **p < 0.01 vs. PBS + Ad-sh-Ctrl; ##p < 0.01 vs. Ang II + Ad-sh-Ctrl. (C) PCBP2 knockdown facilitates Ang II-induced expression of hypertrophic fetal genes (Nppa, Nppb, and Myh7) in NRCMs. **p < 0.01 vs. PBS + Ad-sh-Ctrl; ##p < 0.01 vs. Ang II + Ad-sh-Ctrl. (D) PCBP2 knockdown enhances Ang II-induced protein synthesis in NRCMs. **p < 0.01 vs. PBS + Ad-sh-Ctrl; ##p < 0.01 vs. Ang II + Ad-sh-Ctrl. (E–F) Luciferase assay showing PCBP2 knockdown promotes Ang II induced Myh7 and Nppa promoter activation in NRCMs. **p < 0.01 vs. PBS + Ad-sh-Ctrl; ##p < 0.01 vs. Ang II + Ad-sh-Ctrl. (G) PCBP2 overexpression inhibits Ang II-induced hypertrophy in NRCMs. **p < 0.01 vs. PBS + Ad-Ctrl; ##p < 0.01 vs. Ang II + Ad-Ctrl.

myoblast fusion through SRE-and NFAT-mediated signaling [19], which is activated during cardiac hypertrophy [23]. Recently, GPR56 was reported to regulate mechanical overload-induced muscle hypertrophy [20], strongly implicating the role of GPR56 during hypertrophy of cardiomyocyte. Indeed, our current work has demonstrated the overexpression of GPR56 during cardiac hypertrophy and it acts as a promoter for cardiomyocyte hypertrophy. Therefore, we studied whether PCBP2 regulates GPR56 in cardiomyocyte and if so, whether GPR56 contributes to PCBP2 function in cardiomyocyte during cardiac hypertrophy. In NRCMs, we found that PCBP2 knockdown increased while PCBP2 overexpression decreased the protein and mRNA levels of GPR56 (Fig. 3A–B), indicating that GPR56 is regulated by PCBP2 at transcriptional or post-transcriptional level. Several examples existed in the literature demonstrated that PCBP2 regulate mRNA stability [8,24,25]. Therefore, we designed an assay to assess the degeneration rate of GPR56 mRNA to understand how PCBP2 affects the mRNA level of GPR56. The results showed that PCBP2 knockdown significantly slowed the degeneration rate of GPR56 mRNA (Fig. 3C), while PCBP2 overexpression promoted the degeneration rate of GPR56 mRNA (Fig. 3D). These findings demonstrated the notion that PCBP2 inhibits GPR56 mRNA by regulating its degeneration rate.

3.4. GRP56 contributes to PCBP2 effects on cardiac hypertrophy

Finally, we asked whether PCBP2-mediated GPR56 down-regulation contributes to its anti-hypertrophy effects. We knocked down GPR56 in NRCMs (Fig. 4A), and the first one shRNA for GPR56 targeting was chosen for further investigation. We found that GPR56 knockdown inhibited Ang II-induced cardiac hypertrophy, fetal gene expression and protein synthesis as well as promoter activation of Myh7 and Nppb (Fig. 4B–F). Interestingly, when

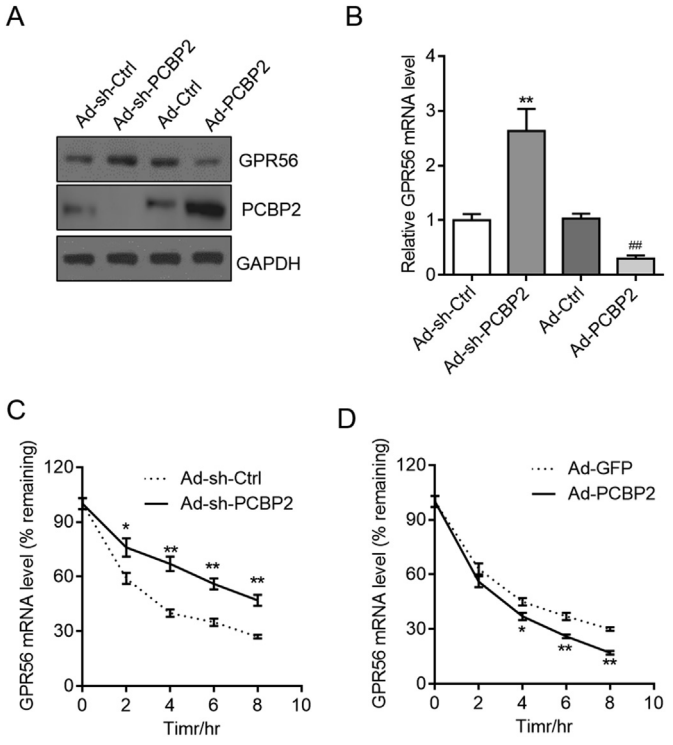

Fig. 3. PCBP2 promotes GPR56 mRNA degeneration in cardiomyocytes (A) PCBP2 reduces the protein level of GPR56 in NRCMs. (B) PCBP2 reduces the mRNA level of GPR56 in NRCMs. **p < 0.01 vs. Ad-sh-Ctrl; ##p < 0.01 vs. Ad-Ctrl. (C) PCBP2 knockdown delays the degeneration of GPR56 mRNA. **p < 0.01 vs. Ad-sh-Ctrl of the same time point. (D) PCBP2 overexpression promotes the degeneration of GPR56 mRNA. *p < 0.05 and **p < 0.01 vs. Ad-Ctrl of the same time point.

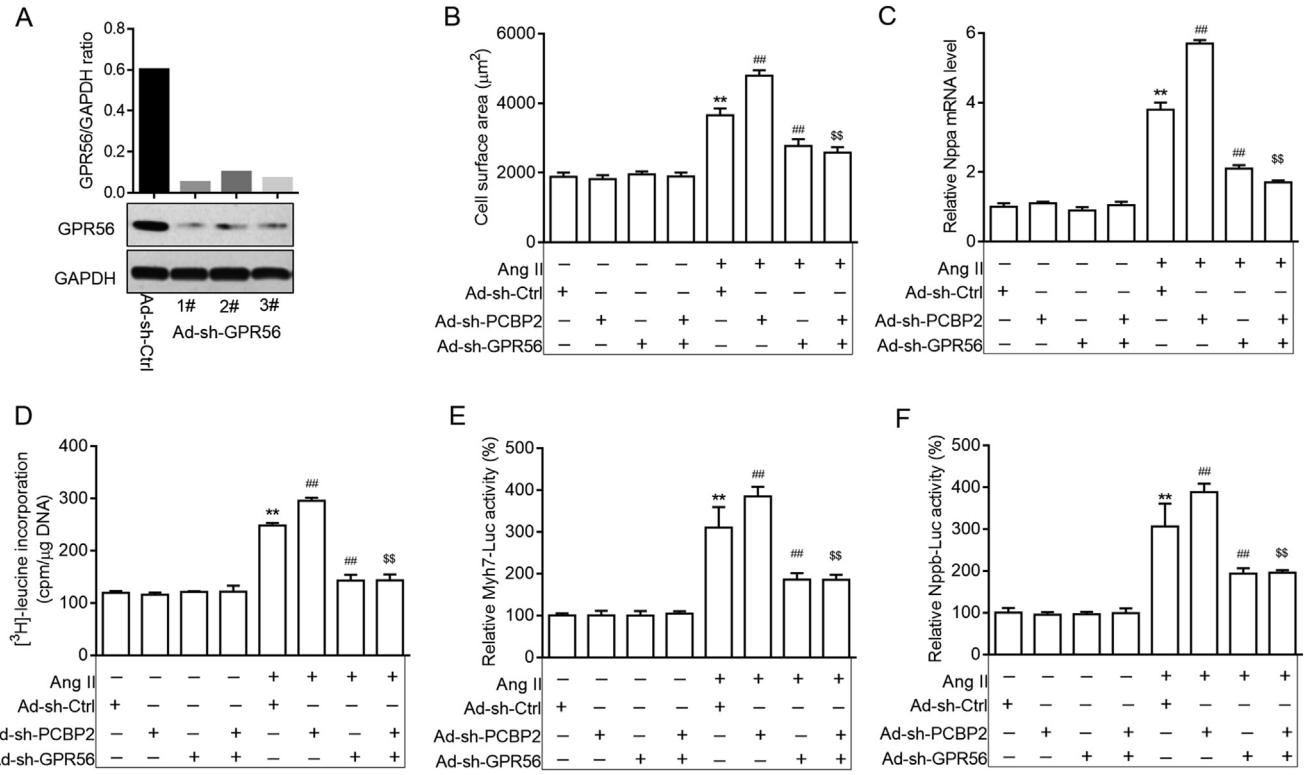

Fig. 4. GPR56 contributes to PCBP2 effects on hypertrophy (A) Knockdown of GRP56 with adenovirus mediated short hairpin RNAs in NRCMs. Representative western blot and quantitative data are shown. (B) Knockdown of GPR56 blocks PCBP2 effects on Ang II induced hypertrophy of NRCMs. **p < 0.01 vs. Ad-sh-Ctrl; ##p < 0.01 vs. Ang II + Ad-sh-Ctrl; $$p < 0.01 vs. Ang II + Ad-sh-PCBP2. (C) Knockdown of GPR56 blocks PCBP2 effects on Ang II induced expression of Nppa. **p < 0.01 vs. Ad-sh-Ctrl; ##p < 0.01 vs. Ang II + Ad-sh-Ctrl; $$p < 0.01 vs. Ang II + Ad-sh-PCBP2. (D) Knockdown of GPR56 blocks PCBP2 effects on protein synthesis in NRCMs. **p < 0.01 vs. Ad-sh-Ctrl; ##p < 0.01 vs. Ang II + Ad-sh-Ctrl; $$p < 0.01 vs. Ang II + Ad-sh-PCBP2. (E–F) Knockdown of GPR56 blocks PCBP2 effects on Ang II induced activation of Myh7 (E) and Nppb (F) promoters. **p < 0.01 vs. Ad-sh-Ctrl; ##p < 0.01 vs. Ang II + Ad-sh-Ctrl; $$p < 0.01 vs. Ang II + Ad-sh-PCBP2.

GPR56 was knocked down, PCBP2 was unable to affect cardiac hypertrophy, fetal gene expression, protein synthesis, and promoter activation of Myh7 and Nppb upon hypertrophic stress (Fig. 4B–F). There results demonstrated that GPR56 was a positive regulator of Ang II-induced cardiomyocyte hypertrophy and GPR56 downregulation contributed to PCBP2 effects on hypertrophy.

4. Discussion

In this work, we identify PCBP2 as a negative factor and GPR56 as a positive factor for hypertrophic growth of cardiomyocytes. GPR56 acts as a downstream of PCBP2 and contributes to PCBP2 effects during cardiac hypertrophy. We first showed the down-regulation of PCBP2 in failing human hearts and hypertrophic mouse hearts as well as in NRCMs treated with hypertrophic agonists. Further, we provided evidence that PCBP2 inhibited Ang II-induced hypertrophy in NRCMs and H9C2 cells by using loss-of-function and gain-of-function strategies. Next, we showed that PCBP2 promoted the degeneration rate of GPR56 mRNA. Finally, we demonstrated the contribution of GPR56 to Ang II-induced hypertrophy and GPR56 is critically essential for PCBP2 functions during cardiac hypertrophy.

The PCBP family proteins regulate gene expression at various levels, including transcription, mRNA processing, mRNA stabilization, and translation. One of the least studied member, PCBP2, functions through posttranscriptional and translational controls in RNA viruses. PCBP2 participates in the replication and translation of many RNA viruses, including poliovirus [6], coxsackievirus [7], and rhinovirus [26]. Interestingly, PCBP2 also binds to the 5′ UTR [27] and 3′ UTR [28] of the HCV gene. PCBP2 is induced after viral infection and interacted with MAVS, which shows that PCBP2 is a negative regulator of MAVS-mediated antiviral signaling [29]. Recent publications reveal that PCBP2 is a regulator for tumor development [9]. Recently, Han et al. [30] showed that PCBP2 modulated glioma growth by targeting 3′-UTR of FHL3 mRNA.

Here we report the roles of PCBP2 in cardiovascular system. We first found the role of PCBP2 during cardiac hypertrophy. PCBP2 transcriptional level was significantly down-regulated in human failing hearts, which were coupled with myocardial hypertrophy. In mouse model of hypertrophy surgically induced by TAC, the protein and mRNA levels of PCBP2 was also down-regulated. The down-regulation of PCBP2 was occurred in the cardiomyocytes, as we demonstrated that PCBP2 level was inhibited by hypertrophic agonists in NRCMs. Further, we showed the anti-hypertrophic role of PCBP2 in NRCMs and H9C2 cells. PCBP2 knockdown promoted Ang II induced increase in cell size, protein synthesis, hypertrophic gene expression, and promoter activation of Myh7 and Nppb genes.

As PCBP2 is an RNA-binding protein, Han et al. [20] used RIP-ChIP analysis to fish PCBP2-binding RNAs and they identified preferentially 35 mRNAs that are associated with PCBP2. Among these targets, GPR56 is a current reported factor for hypertrophy of muscle. We currently found that GPR56 was up-regulated during cardiac hypertrophy in human, mouse and NRCMs. Modulation of GPR56 could also regulate Ang II-induced hypertrophic growth, protein synthesis, gene expression in NRCMs. Strictly, we demonstrated that GPR56 was regulated by PCBP2 at the post-transcriptional level. PCBP2 inhibited the mRNA stability of GPR56 in NRCMs. During cardiac hypertrophy, PCBP2 was down-regulated by unclear mechanism, which slowed the degeneration of GPR56 mRNA level and thus contributed to cardiac hypertrophy.

In cultured murine muscle cells anabolic effects of PGC-1α4 are dependent on GPR56 signaling, because knockdown of GPR56 attenuates PGC-1α4–induced muscle hypertrophy *in vitro* [20]. Forced expression of GPR56 results in myotube hypertrophy through the expression of insulin-like growth factor 1, which is dependent on Gα12/13 signaling [20]. Gα12/13 Mediates α1-adrenergic receptor–induced cardiac hypertrophy [31,32]. Activation of Gα12/13 in cardiomyocytes by the extracellular nucleotides-stimulated P2Y6 receptor triggers fibrosis in pressure overload-induced cardiac fibrosis, which works as an upstream mediator of the signaling pathway between Ang II and TGF-β [31]. These facts indicate the GPR56 may contribute to cardiomyocyte hypertrophy by activating Gα12/13 signaling, which remains to be further confirmed.

In conclusion, we identify a novel signal pathway during cardiac hypertrophy. PCBP2 is down-regulated by hypertrophic stress, which results in higher stability of GPR56 mRNA and thus increases GPR56 level, leading to cardiac hypertrophy. This new notion may provide new understanding of cardiac hypertrophy and new therapeutic target for treatment.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.06.139.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.06.139.

References

[1] B.J. Maron, M.S. Maron, Hypertrophic cardiomyopathy, Lancet 381 (2013) 242–255.
[2] J. Heineke, J.D. Molkentin, Regulation of cardiac hypertrophy by intracellular signalling pathways, Nat. Rev. Mol. Cell. Biol. 7 (2006) 589–600.
[3] J.A. Hill, E.N. Olson, Cardiac Plasticity, N. Engl. J. Med. 358 (2008) 1370–1380.
[4] R.E. Hershberger, D.J. Hedges, A. Morales, Dilated cardiomyopathy: the complexity of a diverse genetic architecture, Nat. Rev. Cardiol. 10 (2013) 531–547.
[5] A.V. Makeyev, S.A. Liebhaber, The poly(C)-binding proteins: a multiplicity of functions and a search for mechanisms, Rna 8 (2002) 265–278.
[6] P. Sean, J.H. Nguyen, B.L. Semler, The linker domain of poly(rC) binding protein 2 is a major determinant in poliovirus cap-independent translation, Virology 378 (2008) 243–253.
[7] P. Sean, J.H. Nguyen, B.L. Semler, Altered interactions between stem-loop IV within the 5' noncoding region of coxsackievirus RNA and poly(rC) binding protein 2: effects on IRES-mediated translation and viral infectivity, Virology 389 (2009) 45–58.
[8] W. Han, Z. Xin, Z. Zhao, W. Bao, X. Lin, B. Yin, J. Zhao, J. Yuan, B. Qiang, X. Peng, RNA-binding protein PCBP2 modulates glioma growth by regulating FHL3, J. Clin. Investigation 123 (2013) 2103–2118.
[9] R.J. Molinaro, B.K. Jha, K. Malathi, S. Varambally, A.M. Chinnaiyan, R.H. Silverman, Selection and cloning of poly(rC)-binding protein 2 and Raf kinase inhibitor protein RNA activators of 2',5'-oligoadenylate synthetase from prostate cancer cells, Nucleic Acids Res. 34 (2006) 6684–6695.
[10] J.S. Chang, R. Santhanam, R. Trotta, P. Neviani, A.M. Eiring, E. Briercheck, M. Ronchetti, D.C. Roy, B. Calabretta, M.A. Caligiuri, D. Perrotti, High levels of the BCR/ABL oncoprotein are required for the MAPK-hnRNP-E2 dependent suppression of C/EBPalpha-driven myeloid differentiation, Blood 110 (2007) 994–1003.
[11] S.Y. Chan, A.K. Chan, B.P. Cheung, Y. Liang, M.P. Leung, Identification of genes expressed during myocardial development, Chin. Med. J. Engl. 116 (2003) 1329–1332.
[12] S. Shashidhar, G. Lorente, U. Nagavarapu, A. Nelson, J. Kuo, J. Cummins, K. Nikolich, R. Urfer, E.D. Foehr, GPR56 is a GPCR that is overexpressed in gliomas and functions in tumor cell adhesion, Oncogene 24 (2005) 1673–1682.
[13] L. Xu, S. Begum, J.D. Hearn, R.O. Hynes, GPR56, an atypical G protein-coupled receptor, binds tissue transglutaminase, TG2, and inhibits melanoma tumor growth and metastasis, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 9023–9028.
[14] L. Yang, S. Friedland, N. Corson, L. Xu, GPR56 inhibits melanoma growth by internalizing and degrading Its Ligand TG2, Cancer Res. 74 (2014) 1022–1031.
[15] S. Koirala, Z. Jin, X. Piao, G. Corfas, GPR56-regulated granule cell adhesion is essential for rostral cerebellar development, J. Neurosci. 29 (2009) 7439–7449.
[16] S. Giera, Y. Deng, R. Luo, S.D. Ackerman, A. Mogha, K.R. Monk, Y. Ying, S.-J. Jeong, M. Makinodan, A.R. Bialas, B.S. Chang, B. Stevens, G. Corfas, X. Piao, The adhesion G protein-coupled receptor GPR56 is a cell-autonomous regulator of oligodendrocyte development, Nat. Commun. 6 (2015).
[17] B.-I. Bae, I. Tietjen, K.D. Atabay, G.D. Evrony, M.B. Johnson, E. Asare, P.P. Wang, A.Y. Murayama, K. Im, S.N. Lisgo, L. Overman, N. Šestan, B.S. Chang, A.J. Barkovich, P.E. Grant, M. Topçu, J. Politsky, H. Okano, X. Piao, C.A. Walsh, Evolutionarily dynamic alternative splicing of GPR56 regulates regional cerebral cortical patterning, Science 343 (2014) 764–768.
[18] S.D. Ackerman, C. Garcia, X. Piao, D.H. Gutmann, K.R. Monk, The adhesion GPCR Gpr56 regulates oligodendrocyte development via interactions with Gα12/13 and RhoA, Nat. Commun. 6 (2015).
[19] M.P. Wu, J.R. Doyle, B. Barry, A. Beauvais, A. Rozkalne, X. Piao, M.W. Lawlor, A.S. Kopin, C.A. Walsh, E. Gussoni, G-protein coupled receptor 56 promotes myoblast fusion through serum response factor- and nuclear factor of activated T-cell-mediated signalling but is not essential for muscle development in vivo, Febs J. 280 (2013) 6097–6113.
[20] J.P. White, C.D. Wrann, R.R. Rao, S.K. Nair, M.P. Jedrychowski, J.S. You, V. Martinez-Redondo, S.P. Gygi, J.L. Ruas, T.A. Hornberger, Z. Wu, D.J. Glass, X. Piao, B.M. Spiegelman, G protein-coupled receptor 56 regulates mechanical overload-induced muscle hypertrophy, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 15756–15761.
[21] N.R. Sundaresan, P. Vasudevan, L. Zhong, G. Kim, S. Samant, V. Parekh, V.B. Pillai, P.V. Ravindra, M. Gupta, V. Jeevanandam, J.M. Cunningham, C.X. Deng, D.B. Lombard, R. Mostoslavsky, M.P. Gupta, The sirtuin SIRT6 blocks IGF-Akt signaling and development of cardiac hypertrophy by targeting c-Jun, Nat. Med. 18 (2012) 1643–1650.
[22] J.B. Regard, I.T. Sato, S.R. Coughlin, Anatomical profiling of G protein-coupled receptor expression, Cell 135 (2008) 561–571.
[23] J.O. Mudd, D.A. Kass, Tackling heart failure in the twenty-first century, Nature 451 (2008) 919–928.
[24] B. Stefanovic, C. Hellerbrand, M. Holcik, M. Briendl, S. Aliebhaber, D.A. Brenner, Posttranscriptional regulation of collagen alpha1(I) mRNA in hepatic stellate cells, Mol. Cell. Biol. 17 (1997) 5201–5209.
[25] W.R. Paulding, M.F. Czyzyk-Krzeska, Regulation of tyrosine hydroxylase mRNA stability by protein-binding, pyrimidine-rich sequence in the 3'-untranslated region, J. Biol. Chem. 274 (1999) 2532–2538.
[26] E. Rieder, W. Xiang, A. Paul, E. Wimmer, Analysis of the cloverleaf element in a human rhinovirus type 14/poliovirus chimera: correlation of subdomain D structure, ternary protein complex formation and virus replication, J. Gen. Virol. 84 (2003) 2203–2216.
[27] S. Fukushi, M. Okada, T. Kageyama, F.B. Hoshino, K. Nagai, K. Katayama, Interaction of poly(rC)-binding protein 2 with the 5'-terminal stem loop of the hepatitis C-virus genome, Virus Res. 73 (2001) 67–79.
[28] P. Tingting, F. Caiyun, Y. Zhigang, Y. Pengyuan, Y. Zhenghong, Subproteomic analysis of the cellular proteins associated with the 3' untranslated region of the hepatitis C virus genome in human liver cells, Biochem. Biophys. Res. Commun. 347 (2006) 683–691.
[29] F. You, H. Sun, X. Zhou, W. Sun, S. Liang, Z. Zhai, Z. Jiang, PCBP2 mediates degradation of the adaptor MAVS via the HECT ubiquitin ligase AIP4, Nat. Immunol. 10 (2009) 1300–1308.
[30] W. Han, Z. Xin, Z. Zhao, W. Bao, X. Lin, B. Yin, J. Zhao, J. Yuan, B. Qiang, X. Peng, RNA-binding protein PCBP2 modulates glioma growth by regulating FHL3, J. Clin. Invest 123 (2013) 2103–2118.
[31] M. Nishida, Y. Sato, A. Uemura, Y. Narita, H. Tozaki-Saitoh, M. Nakaya, T. Ide, K. Suzuki, K. Inoue, T. Nagao, H. Kurose, P2Y6 receptor-Gα12/13 signalling in cardiomyocytes triggers pressure overload-induced cardiac fibrosis, EMBO J. 27 (2008) 3104–3115.
[32] Y. Maruyama, M. Nishida, Y. Sugimoto, S. Tanabe, J.H. Turner, T. Kozasa, T. Wada, T. Nagao, H. Kurose, Gα12/13 mediates α1-Adrenergic receptor–induced cardiac hypertrophy, Circulation Res. 91 (2002) 961–969.