Biochemical and Biophysical Research Communications 409 (2011) 125–130

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

The δA isoform of calmodulin kinase II mediates pathological cardiac hypertrophy by interfering with the HDAC4-MEF2 signaling pathway

Changlin Li [a], Xiangyu Cai [a], Haili Sun [a], Ting Bai [a], Xilong Zheng [a,e], Xing Wang Zhou [b], Xiongwen Chen [c], Donald L. Gill [d], Jing Li [a,*], Xiang D. Tang [a,e,*]

[a] Department of Pharmacology, Nankai University School of Medicine, Nankai, Tianjin 300071, China
[b] Department of Biochemistry, Sun Yat-Sen University Zhongshan School of Medicine, Guangzhou, Guangdong 510080, China
[c] Cardiovascular Research Center and Department of Physiology, Temple University School of Medicine, Philadelphia, PA 19140, USA
[d] Cardiovascular Research Center and Department of Biochemistry, Temple University School of Medicine, Philadelphia, PA 19140, USA
[e] MOE Key Laboratory of Bioactive Materials, Nankai, Tianjin 300071, China

ARTICLE INFO

Article history:
Received 25 April 2011
Available online 3 May 2011

Keywords:
Cardiac hypertrophy
Nuclear factor of activated T-cell (NFAT)
Histone deacetylase (HDAC)
Atrial natriuretic factor (ANF)
β-Myosin heavy chain (βMHC)

ABSTRACT

Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is a new promising target for prevention and treatment of cardiac hypertrophy and heart failure. There are three δ isoforms of CaMKII in the heart and previous studies focused primarily on δB and δC types. Here we report the δA isoform of CaMKII is also critically involved in cardiac hypertrophy. We found that δA was significantly upregulated in pathological cardiac hypertrophy in both neonatal and adult models. Upregulation of δA was accompanied by cell enlargement, sarcomere reorganization and reactivation of various hypertrophic cardiac genes including atrial natriuretic factor (ANF) and β-myocin heavy chain (β-MHC). Studies further indicated the pathological changes were largely blunted by silencing the δA gene and an underlying mechanism indicated selective interference with the HDAC4-MEF2 signaling pathway. These results provide new evidence for selective interfering cardiac hypertrophy and heart failure when CaMKII is considered as a therapeutic target.

1. Introduction

Heart failure remains a leading cause of death and disability in industrialized society and cardiac hypertrophy is a promising target for prevention and treatment of heart failure. A number of signaling pathways have been identified mediating pathological cardiac hypertrophy [1,2]. Among others, dysregulation of various protein kinases and phosphatases is known to be central to hypertrophic remodeling followed by cell death, heart failure and cardiac arrhythmias [3,4]. Many prohypertrophic kinases such as Ca^{2+}/calmodulin-dependent kinase II (CaMKII), protein kinase D1 (PKD1), and Ca^{2+}-activated phosphatase calcineurin (CaN), are involved in cardiac hypertrophy by sensing increased intracellular Ca^{2+}. Downstream, CaN dephosphorylates the nuclear factor of activated T-cell (NFAT), followed by its nuclear entry. In parallel, the class II histone deacetylases (HDACs) were phosphorylated by CaMKII and PKD1, and the phosphorylated HDAC45 disinhibits the inert cardiac genome and activates myocyte enhancer factor-2 (MEF2) [1,2,4]. Activation of the CaN-NFAT and/or HDAC45-MEF2 signaling pathways initiates hypertrophic genomic reprogramming.

The critical role of CaMKII δ-isoforms in cardiac hypertrophy and failure has been well documented, but the major isoforms extensively investigated are δB and δC [5–7]. These two isoforms have a broad spectrum of substrates including phospholamban (PLB), ryanodine receptors, HDACs, and the L-type calcium channels [3,4]. However, CaMKIIδ has an additional isoform termed δA, which is mainly expressed in neonatal cardiac myocytes. Whether δA plays any role in adult cardiac hypertrophy and heart failure remains elusive. A recent study indicated CaMKIIδA is significantly over-expressed along plasma membranes and T-tubules and is required for cardiac hypertrophy in ASF/SF2-deficient animals [8]. These results suggest CaMKIIδA could play an important role in adult cardiac hypertrophy. Here we report CaMKIIδA was significantly upregulated in cardiac hypertrophy in both neonatal and adult models. Upregulation of CaMKIIδA was accompanied by reactivation of fetal cardiac genes such as ANF and β-MHC.

Abbreviations: CaMKII, Ca^{2+}/calmodulin-activated kinase II; HDAC, histone deacetylase; MEF2, myocyte enhancer factor-2; CaN, calcineurin; NFAT, nuclear factor of activated T-cell; PKD, protein kinase D; ANF, atrial natriuretic factor; βMHC, β-myosin heavy chain; NRVM, neonatal rat ventricular myocytes; ARVM, adult rat ventricular myocytes; siRNA, small RNA interference; qPCR, quantitative real-time polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ISO, isoproterenol.

* Corresponding authors. Address: Department of Pharmacology, Nankai University School of Medicine, Nankai, Tianjin 300071, China. Fax: +86 22 2350 2554 (Xiang D. Tang).

E-mail addresses: stellarli@nankai.edu.cn (J. Li), stang@nankai.edu.cn (X.D. Tang).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.04.128

These pathological changes were largely blunted by silencing the δA gene and an underlying mechanism indicates selective interference with the HDAC4-MEF2 signaling pathway.

2. Materials and methods

2.1. Isolation and culture of ventricular myocytes

Sprague–Dawley rats were used throughout experiments and purchased from the Military Academy of the Medical Science Laboratory Animal Center (Beijing, China). The principles governing the care and treatment of animals as described by the American Physiological Society were followed at all times during this study. Isolation of adult rat ventricular myocytes (ARVM) was performed by Langendorff perfusion with a buffer containing low Ca^{2+}, collagenase and protease as described in our previous papers [9,10]. Isolation and culture of neonatal rat ventricular myocytes (NRVM) were conducted using the overnight trypsin–collagenase digestion method as described [9,10]. All experiments with NRVMs were performed on 2–4 d cultures when synchronously contracting cells were observed. The purity of the cardiomyocytes was confirmed by anti-α-actin antibody.

2.2. Small interfering RNAs (siRNA)

siRNA was performed using the standard method as described in our recent publication [11]. Two siRNA sequences were designed to target the coding region of CaMKIIδA: 5′-AGCCAACGUGGUAACCAGCTT-3′ and 5′-GCUGGUUACCACGUUGGCUTT-3′. The choice of these sequences was based on high silencing efficacy as verified by reverse transcription-polymerase chain reaction (RT-PCR). A scrambled siRNA 5′-UUCUCCGAACGUGUCACGUTT-3′ was used as negative control (NC). All nucleotides were chemically synthesized and 2′ *O*-methyl modified by GenePharma Co. (Shanghai, China). Transfection efficiency was estimated using FAM-conjugated NC siRNA and defined as the percentage of FAM-positive cells of the propidium iodine (PI) positive cells. For transient transfection, cells were incubated for 6 h in transfection medium comprising serum-free DMEM, 2 mM glutamine supplemented with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Cells were retained in incubation medium containing serum-free DMEM, 2 mM glutamine, 1× ITS Liquid Media Supplement (Sigma–Aldrich) and penicillin/streptomycin for 36 h. This was followed by treatment with N.S. vehicle or isoproterenol (ISO, 10 μM) for 24 h.

2.3. RT-PCR and real-time PCR (qPCR)

Total RNA was isolated from heart tissue or ventricular myocytes using Trizol reagent (Invitrogen). For cDNA synthesis 1.0 μg RNA was used and reactions were carried out using a reverse transcription system (Promega). PCR was performed in a Genemate thermal cycler (Jinge Instr, Hangzhou, China) with the following primer sets for CaMKIIδA: forward, 5′-CGAGAAATTTTTCAGCAGCC-3′; reverse, 5′-ACAGTAGTTTGGGGCTCCAG-3′. 18-S ribosomal RNA (18-S rRNA) was used as an internal control and the primer sets used are: forward, 5′-ACCGCAGCTAGGAATAATGGA-3′; reverse, 5′-GCCTCAGTTCCGAAAACCA-3′. PCR products were subjected to 1.8% agarose gel electrophoresis, stained with ethidium bromide, and visualized with LAS3000.

qPCR was performed using SYBR Green Master Mix (Takara, Japan) in a Bio-Rad IQ5 detection system as described [12]. The primer sets for each gene are as follows: ANF (forward, 5′-GG GGGTAGGATTGACAGGAT-3′; reverse, 5′-CTCCAGGAGGGTATTCA CCA-3′); β-MHC (forward, 5′-CCTCGCAATATCAAGGGAAA-3′; reverse, 5′-TACAGGTGCATCA GCTCCAG-3′); MEF2 (forward, 5′-GA GCAGAGCCCCCTGCTGGAGGACA-3′; reverse, 5′-TAGCAGGCCGC TGGGGCAGGCCCGG-3′). 18-S rRNA was used as internal standard. The cycle threshold (CT) values corresponding to the PCR cycle number at which fluorescence emission reaches a threshold above the baseline emission were automatically determined in triplicates and averaged. Abundances of each target gene was normalized to that of 18-S rRNA using the formula of $2^{-\Delta CT}$, where ΔCT = CT of target genes – CT of 18-S rRNA. The ratios were expressed as –fold changes when compared with saline controls.

2.4. Western blotting

Cells were lysed in RARI buffer (50 mM Tris, pH 7.4, 1.0 mM EDTA, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1 mM sodium orthovanadate, 1 mM sodium pyrophosphate). Cell lysates were resolved in 10% SDS–PAGE and transferred to PVDF membranes (Millipore) as described previously [9]. HDAC4 and HDAC4-p proteins were reacted with polyclonal anti-HDAC4 or anti-HDAC4-p (Santa cruz) followed by incubation with horseradish peroxidase-conjugated secondary antibody (Invitrogen). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was measured as an internal control with appropriate antibody (Santa cruz). Band density was quantified using ImageJ (The US National Institutes of Health, http://rsb.info.nih.gov/ij/) where required.

2.5. Indirect immunofluorescence

Indirect immunofluorescent staining was conducted essentially the same as described previously [9,11]. Heart cells were grown on laminin-coated glass coverslips and fixed in 4% paraformaldehyde followed by permeabilization with 0.5% Triton X-100. After blocking in 1% BSA-containing PBS, cells were incubated with primary antibody and subsequently with secondary antibodies (Invitrogen). Images were collected and analyzed by TCS-SP confocal laser microscopy (Leica, Germany). For surface area determination ImageJ was used and at least 50 individualized cells were analyzed for each experiment.

2.6. NFAT–luciferase assays

Neonatal myocytes were co-transfected with NFAT reporter plasmid pGL4.30[luc2P/NFAT-RE/Hygro] and control plasmid pGL7.4 expressing *Renilla reniformis* luciferase reporter gene (Promega) in the presence or absence of NC siRNA or CaMKIIδA siRNA. Transient transfection was performed with the electroporation nucleofector kit (AMAXA Biosystems). After 36 h incubation in a 12-well plate, cells were treated with N.S. or 10 μM ISO for further 48 h. Cells were then lysed and a luciferase assay was performed with the dual luciferase kit (Promega) according to the manufacturer's instructions. Luciferase activity was measured using a TR717 microplate luminometer (Applied Biosystems). Data were expressed as fold change (= average relative light units of induced cells/average relative light units of control cells).

2.7. In vivo cardiac hypertrophic model and immunohistochemistry

In vivo cardiac hypertrophy was induced by chronic ISO injection as described [13]. For histology, paraffin-embedded heart tissue sections (4 μm in thickness) were made and stained with standard HE or immunohistochemistry techniques.