Archives of Biochemistry and Biophysics 603 (2016) 20–28

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

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

Diastolic dysfunction and cardiac troponin I decrease in aging hearts

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ARTICLE INFO

Article history:
Received 27 February 2016
Received in revised form
7 May 2016
Accepted 11 May 2016
Available online 13 May 2016

Keywords:
Epigenetics
Histone acetylation
Gene expression
Troponin
Diastolic dysfunction

ABSTRACT

Cardiac tropnoin I (cTnI) plays a critical role in the regulation of diastolic function, and its low expression may result in cardiac diastolic dysfunction, which is the most common form of cardiovascular disorders in older adults. In this study, cTnI expression levels were determined in mice at various ages and cardiac function was measured and compared between young adult mice (3 and 10 months) and older mice (18 months). The data indicated that the cTnI levels reached a peak high in young adult hearts (3 months), but decreased in older hearts (18 months). Furthermore, the older hearts showed a significant diastolic dysfunction observed by P–V loop and echocardiography measurements. To further define the mechanism underlying the cTnI decrease in aging hearts, we tested DNA methylation and histone acetylation modifications of cTnI gene. We found that acetylation of histone near the promoter region of cTnI gene played an important role in regulation of cTnI expression in the heart at different ages. Our study indicates that epigenetic modification caused cTnI expression decrease is one of the possible causes that result in a reduced cTnI level and diastolic dysfunction in the older hearts.

1. Introduction

Cardiac troponin I (cTnI) is an inhibitory subunit in troponin complex that binds to actin-tropomyosin and regulates muscle contraction and actin-activated myosin (actomyosin) ATPase activities [1]. cTnI plays a critical role in cardiac contraction and relaxation, especially the diastolic function. Our previous studies have demonstrated that deficiency or mutations of cTnI can result in an impaired relaxation in myocardial cells and diastolic dysfunction in the heart [2–9]. Diastolic dysfunction refers to an impaired relaxation and an abnormality in heart filling during diastole while left ventricular systolic function is preserved [10,11]. There is a critical need to understand the mechanisms that cause diastolic dysfunction in an aging heart and to develop target-based medications to treat the disorders.

Because of the difficulty in obtaining human cardiac tissues, the data of cTnI concentrations in the heart are very limited although the cTnI concentration in peripheral blood samples is used as an indicator for diagnosis of heart attack. At least one study has been reported that the content of cTnI in left ventricular myocardial cells decreased in older men with or without cardiac disease [12]. In our previous study, we have demonstrated that the cTnI expression is lower in fetal and new born hearts and up-regulated a week after birth and then reaches a peak high in adult hearts [2]. However, no follow-up studies have been performed to observe the cTnI levels in aging mouse hearts (18 months or older).

Several studies have shown that cTnI gene expression is controlled by the interaction of cis-elements and transcription factors [13]. Our previous studies on mouse cTnI gene have shown that 230 bp of proximal promoters are sufficient to drive cardiac-specific expression in transgenic animals [14]. Two kinds of cis-elements of this promoter, A/T rich and GATA elements have been demonstrated to play considerable roles in the regulation of cTnI

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http://dx.doi.org/10.1016/j.abb.2016.05.008
0003-9861/

gene transcription [15,16]. Mutation of each of these elements markedly reduces gene activation [14]. Cardiac core transcription factors, such as Mef2c and GATA4, can specifically bind to the elements in cardiac genes to initiate the expression. However, the mechanisms underlying the cTnI expression and the interaction between these regulatory cis-elements and transcription factors are still unclear.

Furthermore, a variety of epigenetic alterations affect all cells and tissues throughout life [17]. Epigenetic modifications involve the alteration in DNA methylation patterns, post translational modification of histones, and chromatin remodeling. DNA methylation of cardiac genes or acetylation of histone H3 near the gene promoter region can regulate the cardiac gene expression [18–21].

In this study, cTnI expression levels were determined in mice at various ages (new born, 2 weeks, 3, 10 and 18 months) and cardiac function was measured and compared between young adult mice (3 and 10 months) and older mice (18 months). The experimental data indicated that the cTnI levels were low in new born and reached a peak high in young adult hearts (3 months), but decreased in older hearts (18 months). The patterns were similar in both protein and mRNA levels. Compared to young adult hearts, the older hearts showed a significant diastolic dysfunction observed by P–V loop and echocardiography measurements. Furthermore, we found that epigenetic modifications such as acetylation of histone near the promoter region of cTnI gene played an important role in regulation of cTnI expression in the heart at different ages. Our study indicates that epigenetic modification caused cTnI expression decrease is one of the causes that result in a reduced cTnI level and diastolic dysfunction in the older hearts.

2. Materials and methods

2.1. Animals

Healthy and newborn, 2w, 3 m, and 18 m SPF class c57bl/6 mice were purchased from the Experimental Animal Center in Chongqing Medical University (Chongqing, China). All procedures on experimental animals were approved by the Animal Care and Use Committee at the Chongqing Medical University. Animal experiments were performed conform the NIH guidelines (Guide for the care and use of laboratory animals) [22]. All the mice we used in this study are female mice. And in our animal facilities, we have a strict regulation for animal breeding and a complete breeding recording. The range for animals aging over 3 months is ±1 day. The mice were killed by carbon dioxide asphyxia, and cardiac tissues were collected.

2.2. Morphological observations

Transmission Electron Microscope observation of cardiac ultrastructure was performed as well in mice at different ages. The mice were killed by carbon dioxide asphyxia, and heart samples of 3 m mouse and 18 m mouse were collected, then clipped a piece of left ventricle tissue and pruned it immediately into 1 mm [3] pieces and fixed in 2.5% glutaraldehyde for at least 2 h. Fixed heart samples were embedded after dehydration. Samples were sliced by ultra microtome (Leica, Solms, German) and stained negative using lead citrate. For EM morphological assays, 5 young adult mice (3 months) and 5 old mice (18 months) were used to take the cardiac samples for examination using a transmission electron microscope (Hitachi, Tokyo, Japan). In each group, 30 fields under EM were observed from each mouse by an expert in the EM laboratory who was blinded to the animal ages. The field with abnormal morphological changes was counted and compared finally between two age groups.

2.3. Echocardiography

Echocardiography studies were performed in our laboratory by using a Vevo 770 High-Resolution In Vivo Imaging System (VisualSonics, Toronto, ON, Canada) as described previously [6,7]. To decrease experimental bias, the echocardiography measurements were performed in the study by an examiner blinded to the genotype. All data and images were saved and analyzed with the Advanced Cardiovascular Package Software (VisualSonics, Toronto, ON, Canada) using an automated analysis or semi-automated analysis to evaluate the cardiac function.

2.4. In vivo pressure–volume loop analysis

Mouse left ventricular pressure and volume were simultaneously measured in intact experimental animals. Briefly, mice were anesthetized with 3% isoflurane and were supported by a ventilator with a maintenance dose of 2% isoflurane after tracheostomy. A 1F P–V catheter was inserted into the left ventricle through the right carotid artery. Signals of pressure and volume were continually recorded by using a P–V conductance system (MPVS Ultra, AD Instruments, INC. Houston, TX) coupled to a digital converter (PowerLab, AD Instruments). Hemodynamic parameters were measured under different preloads, which were elicited by transiently compressing the abdominal inferior vena cava. 5–8 mice were measured in each experimental group.

2.5. Western blotting

The quantities of cardiac proteins, especially troponins, were determined using Western blotting assays as described previously [2]. The immunoreactive bands were detected with a Chemiluminescence Analyzer, scanned and analyzed using Quantity One Version 4.4 software (Bio-Rad, Richmond, CA).

2.6. Total RNA extraction and quantitative real-time PCR analysis

Total RNA was extracted using a RNA Extraction kit (Bioteck, Beijing, China). Single-strand cDNA was reverse transcribed from 500 to 1000 ng RNA by using oligo dT-Adaptor primers and AMV reverse transcriptase kit (TaKaRa, Otsu, Japan). cDNA was detected using quantitative RT-PCR assay with a SYBR Green RealMasterMix kit (Tiangen, Beijing, China). The mRNA expression levels of cTnI were quantified. β-actin was used as the endogenous "housekeeping" gene to normalize the RNA sample levels. The primer sequences of cardiac-specific genes and controls were designed as follows:

The analyses of relative mRNA expression were carried out using $2^{-\Delta\Delta Ct}$ method [23].

2.7. Chromatin immunopreciptitation (ChIP) assay

The heart tissues of each group were collected and prepared for ChIP assay following the manufacturer's protocol using a ChIP assay kit (Millipore, MA, USA). Proteins and DNA were crossed link after adding formaldehyde (Sigma-Aldrich, St. Louis, USA) into heart

cTnI,	5′-gcaggtgaagaaggaggaca-3′ (forward)
	5′-cgatattcttgcgccagtc-3′ (reverse).
β-actin,	5′-CACACCCGCCACCAGTTCG-3′(forward)
	5′-GTCCTTCTGACCCATTCCCACC-3′ (reverse).