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Epigenetic regulation of phosphodiesterase 4d in restrictive cardiomyopathy mice with cTnI mutations

Weian Zhao[1], Xiaoqi Wu[1], Zhiyuan Wang[1], Bo Pan[1], Lifei Liu[1,2], Lingjuan Liu[1], Xupei Huang[3] & Jie Tian[1*]

[1]*Department of Cardiology, Heart Centre, Children's Hospital of Chongqing Medical University; Ministry of Education Key Laboratory of Child Development and Disorders, Key Laboratory of Pediatrics in Chongqing, Chongqing International Science and Technology Cooperation Center for Child Development and Disorders, Chongqing 400014, China;*
[2]*Department of anesthesiology, The Children's Hospital of Chongqing Medical University, Chongqing 400014, China;*
[3]*Department of Biomedical Science, Charlie E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton 33414, USA*

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Epigenetic regulations play an important role in disease development. In this study, we have investigated epigenetic regulations in restrictive cardiomyopathy mice with cTnI 193His mutation. Our results demonstrated that phosphodiesterase (PDEs) 4d was down-regulated in the heart of these mice. Further studies showed that the epigenetic modifications were associated with enhanced acetylation of histone 3 lysine 4 and lysines 9, whereas tri-methylation of histone 3 lysine 4, were decreased in histones near the PDE4d gene promoter regions. The binding levels of histone transmethylase SMYD1 and histone deacetylase HDAC1 were increased in the gene promoter regions in cTnI193His transgenic hearts. Using immune-fluorescent labeling we found an evidence of cTnI existence in the nucleus of cardiomyocytes and Western blotting further confirmed that both wild type and mutated cTnI could be detected in the cell nucleus of the hearts. Furthermore, an interaction between cTnI and SMYD1, or cTnI and HDAC1 was observed. Overexpression of the mutated cTnI in cultured cardiomyocytes reduced the expression of PDE4d. Our data suggest that the decrease of PDE4d expression in RCM mice caused by cTnI mutations may be related to epigenetic regulation, i.e., histone acetylation and methylation, and cTnI might be involved in this procedure via an interaction with HDAC1 and SMAD1 in the hearts.

phosphodiesterase, epigenetics, histone modifications, restrictive cardiomyopathy, cardiac troponin I

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INTRODUCTION

Cardiac troponin I (cTnI) is a sarcomeric protein in thin filament of cardiomyocytes, which regulates muscle contraction by binding to actin-tropomyosin and inhibiting the troponin complex (Pan et al., 2016; Tobacman, 1996). Mutations of cTnI caused by a single nucleotide change of the gene can result in various cardiomyopathy phenotypes including dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), and restrictive cardiomyopathy (RCM) in humans (Florescu et al., 2016; Kimura, 2015; Morimoto, 2008). HCM and RCM share a common pathophysiology, i.e., diastolic dysfunction, whereas the pathophysiology of DCM is a systolic dysfunction. However, the underlying mechanisms that cause cardiac dysfunction remain unknown. Since most inherited cardiomyopathies are monogenic diseases, it is hard to explain the different clinical phenotypes of cardiomyopathies caused by the same cTnI mutation (Monserrat et al., 2015). Emerging evidence has revealed that epigenetic regulation is related to the devel-

*Corresponding author (email: jietian@cqmu.edu.cn)

opment of cardiomyopathies, including HCM, DCM, and diabetic cardiomyopathy (Asrih and Steffens, 2013; Haas et al., 2013; Wu et al., 2015). PDEs in hearts are enzymes that hydrolyze the second messengers to regulate heart contractility and Ca^{2+} transient. One study in 2015 has reported that an increase in the expression of PDEs through epigenetic regulation was proven to be associated with myofibril hyposensitivity to Ca^{2+} in DCM cardiomyocytes (Wu et al., 2015). Since the clinical phenotype and pathophysiology in RCM, i.e., a hypersensitivity to Ca^{2+}, is opposite to that in DCM, we hypothesized that PDEs expression might be decreased in RCM mice through the epigenetic regulations. In this study, we have carried out the experiments with RCM mouse model carrying cTnI 193His mutation to test our hypothesis. From this study, we observed decrease in expression of cardiac specific PDE4d in RCM hearts compared to wild type mice. Further investigation showed that cTnI193His transgenic hearts had reduced acH3K4, acH3K9, and H3K4me3 in histones near the PDE4d gene promoter regions, which could cause down-regulation of this gene. We also found an increased binding of SMYD1 and HDAC1 to the PDE4d gene promoters in cTnI transgenic hearts, which could alter the levels of histone methylation and acetylation.

RESULTS

PDE4d is reduced in transgenic mice

PDE4d is primarily expressed in cardiomyocytes (Beavo, 1995; Fischmeister et al., 2006; Lugnier, 2006; Omori and Kotera, 2007; Zaccolo and Movsesian, 2007; Zhao et al., 2015). We measured PDE4d expression using qPCR assays. Expression of PDE4d mRNA is decreased in cTnI193His mice carrying a heterozygous deletion of cTnI (cTnI193His/cTnI$^{+/-}$) compared to the wild type mice (Figure 1A). Similarly, Western blot assays showed decreased PDE4d protein levels (Figure 1B).

Histone acetylation and trimethylation in the promoter regions of PDE4d gene

To uncover the underlying causes for down-regulation of PDE4d in transgenic mice, we next examined epigenetic modulation of PDE4d using ChIP assays. We observed a significant decrease in histone markers associated with transcription activation (H3K4me3, H3K4ac, and H3K9ac) in PDE4d promoter region in cTnI193His/cTnI$^{+/-}$ mice, relative to wild-type mice and cTnI193His mice (Figure 2A). These findings support the hypothesis that changes in underlying epigenetic modifications lead to altered expression levels of PDE4d in cTnI193His/cTnI$^{+/-}$ mice.

HDAC1 and SMYD1 binding to PDE4d promoter is increased in cTnI mutant mice

To determine how histone markers at PDE4d promoters change, we measured the binding of HDAC1 and SMYD1 to

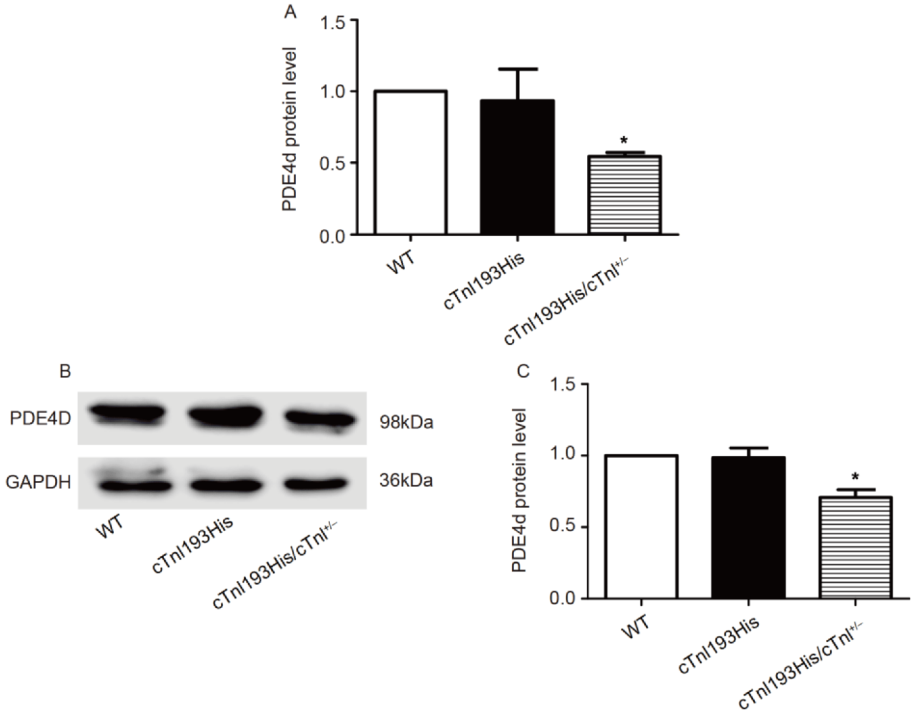

Figure 1 The mRNA and protein levels of PDE4d in WT, cTnI193His, and cTnI193His/cTnI$^{+/-}$ mice. A, PDE4d mRNA levels. B, Western blotting analysis of PDE4dand quantification of Western blotting results. The results are expressed as mean±SD. The WT mean was set as 1 for comparison with other groups. Statistical significance was determined by ANOVA followed by least-significant difference (LSD) tests. *, P<0.05 relative to WT; n=8 per group.

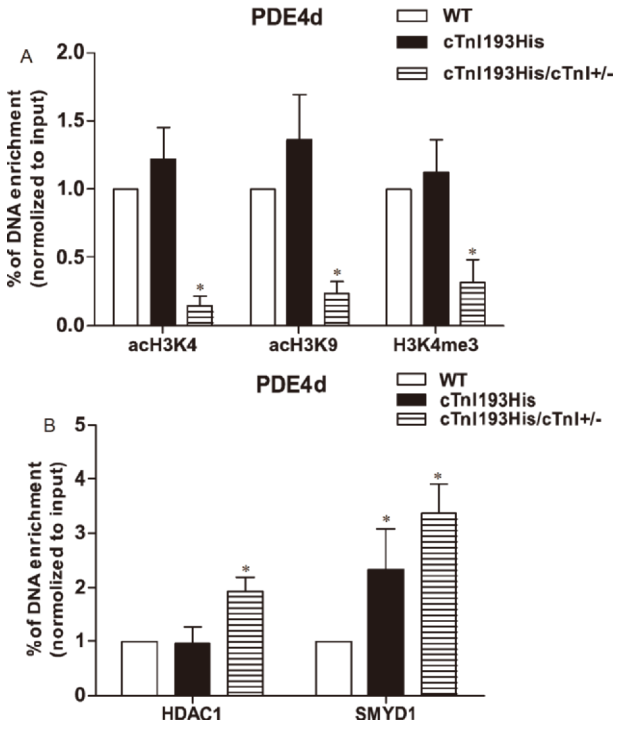

Figure 2 Epigenetic modifications associated with activation and binding of SMYD1 and HDAC1 to the proximal promoter of PDE4d are altered in cTnI193His/cTnI$^{+/-}$ mice. A, Levels of acH3K4, acH3K9, and H3K4me3 detected near the promoter of PDE4d in three groups. B, Binding levels of HDAC1 and SMYD1 to the PDE4d promoter. Input without antibody was used as a negative control. Values are expressed as mean±SD. The WT mean was set as 1 for comparison with other groups. Statistical significance was determined by ANOVA followed by LSD tests. *, P<0.05 relative to WT; n=8 per group.

the promoter regions of PDE4d genes. We found an increase in the binding of HDAC1 and SMYD1 to the PDE4d promoter in both cTnI193His and cTnI193His/cTnI$^{+/-}$ mice (Figure 2B), suggesting the increased binding of these two proteins is related to the gene expression.

Detection of cTnI in nucleus

Fluorescence microscopy was used to determine whether cTnI is present in the nuclei of cardiomyocytes. Our data showed that cTnI was detected in both cytoplasm and nuclei in cultured cardiomyocytes (Figure 3A). This result is consistent with Bergmann's data in human cells and Faizal's observation in rats (Asumda and Chase, 2012; Bergmann et al., 2011). In the hearts of cTnI193His transgenic mice, mutant cTnI is about 20% of the total cTnI in myofilaments. In the heart of cTnI193His/cTnI$^{+/-}$ mice, the mutant cTnI is about 35%–40% of the total cTnI in myofilaments (Li et al., 2013). Nuclear proteins were extracted from wild-type, cTnI193His and cTnI193His/cTnI$^{+/-}$ mice and the total cTnI and wild type cTnI were compared. We found no significant change in the amount of total cTnI in the nucleus, but nuclear wild-type cTnI was decreased in cTnI193His/cTnI$^{+/-}$ mice, suggesting an increase of mutant cTnI (Figure 3B). The amount of wild type cTnI and total cTnI in the cytoplasm showed a similar pattern, indicating there is more mutant cTnI in the heart of the cTnI193His/cTnI$^{+/-}$ mice (Figure 3C).

cTnI interacts with HDAC1 and SMYD1

We used co-IP experiments to identify binding partners of cTnI. We used a cTnI-specific antibody to pull down potential cTnI-interacting proteins, and Western blotting assays further to probe for HDAC1 and SMYD1. The reciprocal experiment was also performed using HDAC1-specific and SMYD1-specific antibodies to pull down their interacting proteins followed by Western blot to further probe for cTnI. We found that immunoprecipitation with cTnI antibodies pulled down HDAC1 and SMYD1, and that immunoprecipitation with HDAC1 or SMYD1 pulled down cTnI (Figure 4A and B). These data indicate that cTnI protein interacts with HDAC1 and SMYD1. SMYD1 contains a MYND domain, which could interact with HDACs that repress gene expression. An interaction between SMYD1 and HDAC1 was also confirmed by co-IP assays (Figure 4C).

Overexpression of mutant cTnI reduces PDE4d mRNA expression *in vitro*

To detect the direct effect of WT cTnI and mutant cTnI on PDE4d gene, we over-expressed the genes using expression plasmid. The primers used could detect both WT cTnI and cTnI193His. cTnI vectors and cTnI193His vectors could induce a significant increase of cTnI and cTnI193His mRNA level 48 h after transfection. Meanwhile, the relative expression of PDE4d mRNA was up-regulated after transfection with WT cTnI vectors (Figure 5A). Oppositely, PDE4d was down-regulated after transfection with the mutant cTnI193 vectors (Figure 5B).

cTnI-interacting proteins and pathway enrichment analysis

To isolate potential cTnI-interacting proteins from cardiomyocyte extracts, we used a cTnI-specific antibody to perform immunoprecipitation. Samples were then subjected to mass spectrometry analysis. The identified proteins and their localization patterns are presented in Table S1 in Supporting Information. Pathway enrichment analysis was performed using the KEGG database (Figure S1 in Supporting Information). Our results indicate that cTnI may function both in the cytoplasm and in the nucleus of cardiomyocytes, and that cTnI may be involved in multiple physiological and pathological processes. Several of the cTnI-interacting proteins have previously described roles in epigenetic regulation

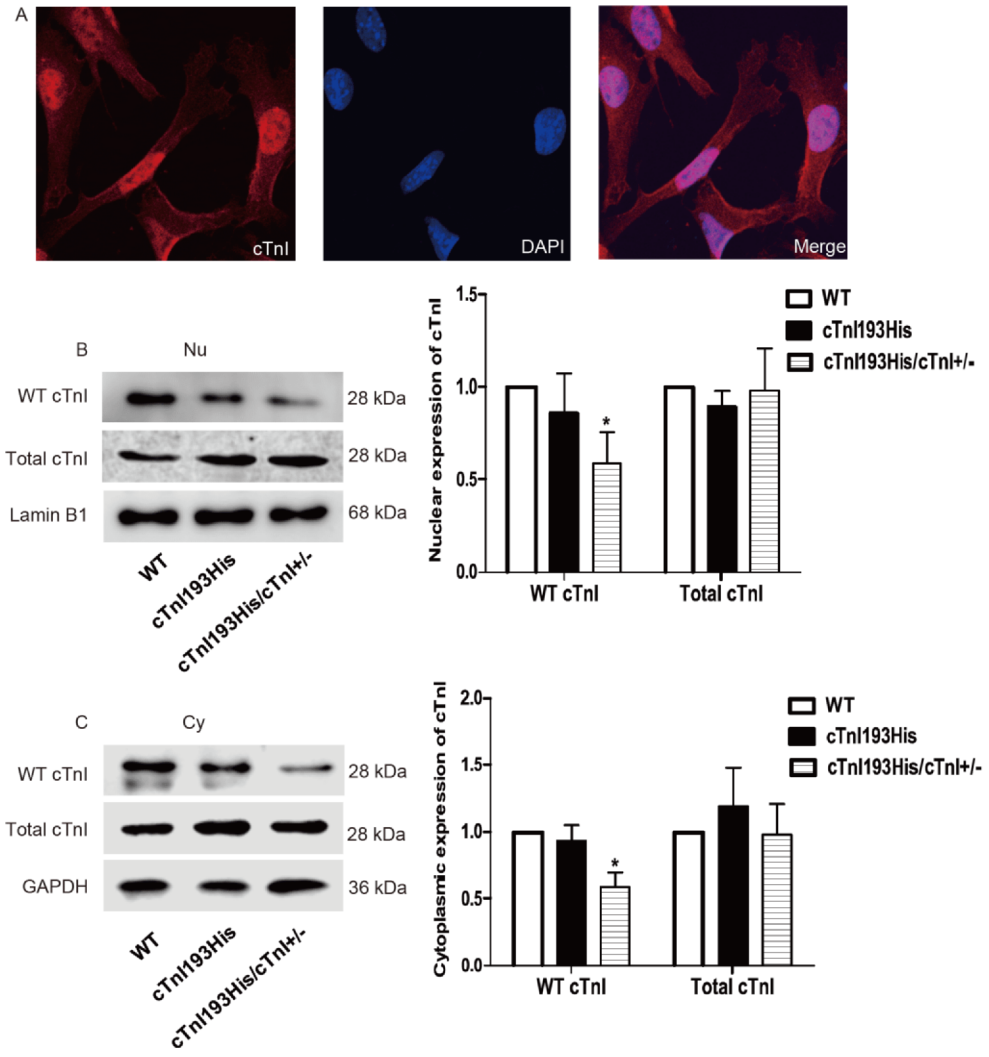

Figure 3 Detection of cTnI in cardiac myocyte nuclei. A, Cultured neonatal ventricular cardiomyocytes were fixed, permeabilized, and stained with 4H6 antibodies to detect cTnI. Nuclei were stained with DAPI. cTnI could be detected in both the nucleus and cytoplasm. B, Western blot analysis using TnI-1 and 4H6 antibodies to detect WT cTnI and total cTnI respectively in the nucleus (Nu), and summary of Western blot results for WT cTnI and total cTnI. C, WT cTnI and total cTnI in cytoplasm (Cy). The results are expressed as mean±SD. The WT mean was set as 1 for comparison with other groups. Statistical significance was determined by ANOVA followed by LSD tests. *, $P<0.05$ as compared with WT; $n=6$ per group.

(red frames), suggesting that cTnI may also function in epigenetic regulation.

DISCUSSION

Hereditary cardiomyopathies caused by genetic mutations are a heterogeneous group of disorders (Varian and Tang, 2017). As two subunits of cardiac troponin, cTnI and cTnT are well characterized thin filament components that regulate cardiac systolic/diastolic function. Mutations in these two genes could cause HCM, DCM or RCM (Kimura, 2015). Although it is well known that cardiomyopathies belong to a group of single-filament gene disorders (Marian et al., 2016), how a single gene mutation could cause such different phenotypes remains largely unknown. Even within families with a known genetic cause of cardiomyopathy, there is remarkable variability in the disease phenotype, age of onset, and severity. This phenotypic variation may suggest the presence of alterations in multiple downstream genetic/epigenetic pathways. Increase in PDEs' expression via histone epigenetic regulation has been observed in DCM caused by mutation of cTnT. In the present study, we have measured PDE4d expression in RCM mice with cTnI193His and cTnI193His/cTnI$^{+/-}$ mutations. Our data have demonstrated that expression of PDE4d was decreased at both the mRNA and protein levels. As PDEs are reported to be regulated by epigenetic modifications, including histone methylation and acetylation (Paige et al., 2012), we further investigated histone modification markers near the gene's promoter regions. Our results showed that markers for activation, including H3K4me3, acH3K4, and acH3K9, near the promoter of

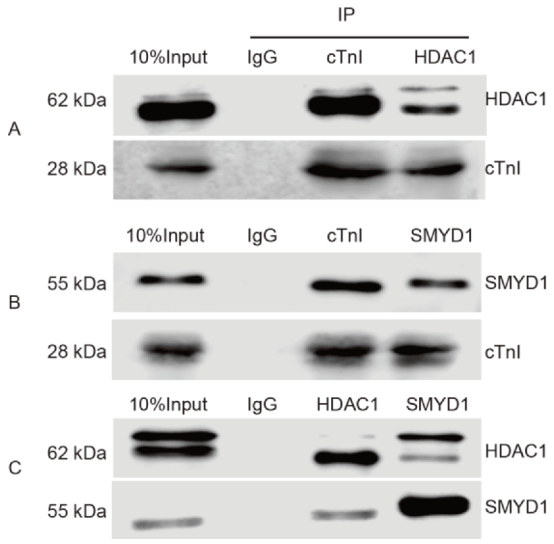

Figure 4 Protein-protein interactions between cTnI and HDAC1/SMYD1. An IP-grade cTnI antibody was used for co-IP analysis of the cTnI-interacting proteins. Co-IP analysis to probe for interaction between cTnI/HDAC1 (A), cTnI/SMYD1 (B) and HDAC1/SMYD1 (C). 10% input was used as the positive control and IgG was used as the negative control. The experiments were performed at least three biological triplicates.

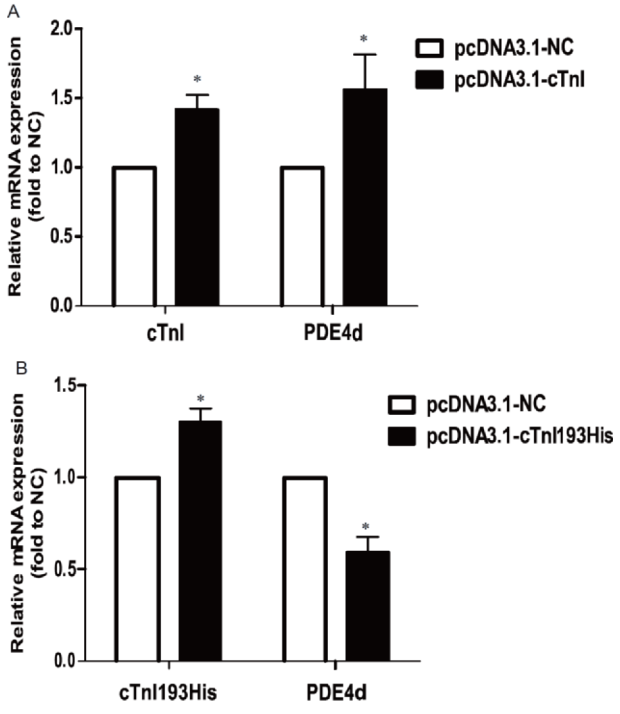

Figure 5 Overexpression of mutant cTnI reduces PDE4d mRNA expression in cardiomyocytes. A, cTnI and PDE4d mRNA expression levels after WT cTnI vectors transfection in cultured cardiomyocytes. B, cTnI193His mRNA expression level after the mutant cTnI193His vectors transfection in cultured cardiomyocytes. The results are expressed as mean ±SD. The NC mean was set as 1 for comparison with other groups. Statistical significance was determined by ANOVA followed by LSD tests. *, $P<0.05$ as compared with WT. The experiments were performed at least three biological triplicates.

PDE4d is decreased in double transgenic mice, indicating that an epigenetic mechanism represses PDE4d expression in cTnI-mutant RCM mice. However, we have not determined the direct roles of PDE4d in RCM in this study and few other studies reported their roles in RCM. Consistent with the fact that the clinical phenotype and pathophysiology of RCM is the opposite to that of DCM in which the gene is up-regulated, we found that PDE4d decreased in RCM. Therefore, we speculate that PDE4d might also play a role in RCM. However, the transgenic mice could still express 20% to 40% wild type cTnI. A mutant knock-in would be more convincing to reach a conclusion.

HDAC1 can decrease histone acetylation while SMYD1 is required for H3K4me3 and increases histone methylation (Stewart et al., 2016). Our results showed that binding of HDAC1 to the PDE4d promoter was increased in cTnI mutant mice. This could explain why acH3K4 and acH3K9 levels are decreased at the gene promoter of PDE4d. We also found an increased SMYD1 binding to the gene's promoter. This could not explain why H3K4me3 was down-regulated near the promoter of PDE4d or why expression of both genes was repressed. However, SMYD1 contains a MYND domain, which is a protein-protein interaction motif that interacts with HDAC-containing co-repressor complexes that repress gene expression (Liu et al., 2007; Masselink and Bernards, 2000; Stewart et al., 2016). The Co-IP assays confirmed the interaction of HDAC1 and SMYD1 (Figure 4C). This might explain why we observe decreased expression of PDE4d with concurrent elevated SMYD1 binding to PDE4d promoter. Further research is needed to determine whether other histone transmethylases are responsible for the decrease of H3K4me3 that we observed. These data demonstrate that histone epigenetic regulation via HDAC1 and SMYD1 represses PDE4d in RCM mice with cTnI193His mutation.

Along with cTnT, cTnI is known as well to localize in the nucleus (Asumda and Chase, 2012; Bergmann et al., 2011; Kajstura et al., 2010; Sahota et al., 2009; Zheng et al., 2016). In this study, we observed nuclear localization of cTnI and found that both wild type and mutant cTnI could exist in the nucleus of cardiomyocytes. However, the function of nuclear cTnI is not well understood. Faizal et al. reported that cTnI could play a role in Ca^{2+}-regulation of nuclear processes such as those controlling protein transport across the nuclear envelope, proliferation, and growth (Asumda and Chase, 2012). Wu et al. demonstrated that nuclear cTnT may interact with some histone demethylases and affect epigenetic modification (Wu et al., 2015). We found HDAC1 and SMYD1 might interact with nuclear cTnI in cardiomyocytes, suggesting an epigenetic role for cTnI.

To explore the potential function of cTnI, we used a cTnI-specific antibody to isolate potential cTnI-interacting proteins from cardiomyocyte extracts and performed mass spectrometry analysis. Our data showed that multiple potential cTnI-interacting proteins exist both in nucleus and in

cytoplasm. Although we did not identify SMYD1 and HDAC1 as potential cTnI-interacting proteins from our mass spectrometry data, some of the proteins are histone subtypes, indicating a potential epigenetic role for cTnI in the nucleus. We used the KEGG database to perform pathway enrichment analysis. The data show that cTnI target proteins are involved in several signaling pathways, including the PI3K-AKT pathway and the adrenergic-PKA signaling pathway. Interestingly, genes specifically dysregulated in PI3K-AKT pathway in RCM patients have been reported through analysis of RNA-seq data (Fu et al., 2017; Rindler et al., 2017), which indirectly supports our findings. These data demonstrate that cTnI not only functions as a thin filament protein to regulate muscle contraction, but might also be involved in epigenetic signaling pathways that are essential for physiological or pathological processes.

Our results indicate that PDE4d decreases in RCM mice expressing mutant cTnIs. Overexpression of the mutated cTnI in cultured cardiomyocytes also reduced the expression of PDE4d. The alteration of PDE4d is regulated by HDAC1 and SMYD1 associated histone acetylation and methylation modifications. cTnI may interact with proteins such as HDAC1 and SMYD1. Taken together, cTnI might be involved in the epigenetic regulation of PDE4d via binding with HDAC1 and SMAD1. These data might provide us with a novel clue to elucidate the etiology of the heterogeneous inherited cardiomyopathies caused by single-gene mutations.

MATERIALS AND METHODS

Transgenic animals

cTnI193His and cTnI-KO mice were gifts from Florida Atlantic University (USA). All procedures on experimental animals were approved by the Animal Care and Use Committee in Chongqing Medical University. Animal experiments were performed in accordance with NIH guidelines. According to our previous experiments, the mutant cTnI in transgenic mice is about 20% of the total cTnI in the heart. By crossing cTnI193His with cTnI-KO mice, we produced cTnI193His mice carrying a heterozygous deletion of cTnI (cTnI193His/cTnI$^{+/-}$) in which the mutant cTnI is about 35%–40% of the total cTnI. Wild-type (WT) C57BL/6 mice were used as controls. A PCR based assay and Western blotting assays were performed to determine the genotypes as previously described (Li et al., 2013).

Immunofluorescence microscopy

Primary culture of cardiomyocytes was performed as described previously (Xu et al., 2015). Cells were plated at a density of 1×10^5 mL^{-1} into a 24-well culture plate with coverslips. After 48 h, cells were fixed in ice-cold 4% formaldehyde solution for 20 min prior to incubating for 15 min at room temperature with 0.05% Triton-X. Then the slides of each group cells were blocked with normal goat serum for 30 min. After three times' washes, the slides of cells were incubated for overnight at 4°C temperature with mouse 4H6 antibody (1:300) to detect cTnI. Cells were then incubated for 60 min at room temperature with appropriate secondary Cy-3-conjugated anti-mouse antibodies (Seville, Wuhan, 1:250), and then washed three times. The nuclei of cells were identified using DAPI (Roche, 10236276001, 5 μg mL^{-1}), incubating for 15 min at room temperature. Coverslips were mounted on glass slides using DAPI Fluoromount-GTM (YEASEN, 36308ES11, Shanghai) and examined with confocal microscope (Nikon, Tokyo, Japan). Image analysis was performed with NIHI mageJ software.

Plasmid constructs and transfection into cardiomyocytes

The cTnI and cTnI193His expression vectors were prepared by cloning wild-type mouse cTnI and cTnI193His cDNA into a pcDNA3.1(–) vector respectively. The expression vectors were transfected respectively into primary cultured cardiac myocytes using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. 48 h after transfection, total RNA was extracted for the later RT-PCR assays.

RT-PCR

Real-time PCR was carried out as described previously (Zhao et al., 2015). Total RNA was extracted using an RNA extraction kit (Bioteck, Beijing, China). Singlestrand cDNA was reverse transcribed from 500 to 1,000 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). GAPDH was used as an endogenous 'housekeeping' gene to normalize RNA levels across samples. The primer sequences for genes of interest and controls are listed in Table S2 in Supporting Information.

Western blot analysis

Briefly, total protein was extracted from cardiac tissue using a protein extraction kit (KeyGEN Bio-TECH, China) and then quantified using a BCA assay (BioTeke Biotechnology, China). Nuclear protein was extracted using a Nuclear Extract Kit (KeyGEN Bio-TECH, China) according to the manufacturer's instructions. Total protein (50 μg per lane) was separated on a 12% SDS-PAGE gel and transferred to a PDVF membrane. Non-specific bands were blocked with mixture of Tris-Buffered Saline and Tween 20 (TBST) containing 5% BSA for 1.5 h at room temperature. Then, the

membranes were successively incubated with specific primary antibodies at 4°C overnight. Proteins bound to the PDVF membrane were analyzed by Western blot using primary antibodies against PDE4d (Abcam, USA), GAPDH (Arigo, Taiwan), and lamin B1 (Abcam, USA). TnI-1 antibody and 4H6 antibody were donated from Florida Atlantic University. Anti-TnI-1 antibody was used to detect wild-type cTnI and 4H6 antibody was used to probe for both wild-type and mutant cTnI, as previously described (Li et al., 2013). The next day, PVDF membranes were incubated with corresponding secondary antibodies at room temperature for 1.5 h. Band intensity was analyzed and quantified using a G-BOX imaging system (Syngene, UK).

Chromatin immunoprecipitation (ChIP) assay

ChIP experiments were conducted using a ChIP assay kit (ChIP Kit-one step, Abcam, USA). After homogenization of cardiac tissues, 1% formaldehyde (Sigma-Aldrich, St. Louis, MO, USA) was added to the samples to cross-link proteins to DNA. Cross-linked DNA was then ultrasonically fragmented (Bioruptor UCD-200) into small fragments (500–1000 bp). Protein-DNA complexes were incubated at room temperature for 3 h using a monoclonal antibody either against acH3K4, H3K4me3, HDAC1, or SMYD1 (Abcam, Cst, Abcam, Abcam). Anti-RNA polymerase antibodies were used as a positive control, and mouse IgG was used as a negative control. Total column input also served as a positive control. Next, cross-linked protein-DNA complexes were removed, and the DNA was extracted. Specific quantitative real-time PCR (qPCR) primers were designed to determine the level of acH3K4, acH3K9 and H3K4me3, and the amount of HDAC1 and SMYD1 bound near the –1000 bp upstream regions PDE4d. Primers used for PCRs are listed in Table S2 in Supporting Information.

Co-immunoprecipitation (Co-IP)

Co-IP kits (Touch and Release, Millipore, USA) were used to perform co-immunoprecipitation to identify protein-protein interactions. Trials were conducted following the manufacturer's instructions. Briefly, 100 μL of protein lysis reaction was diluted in 500 μL of IP-incubation buffer and mixed with 5 μg of cTnI (IP grade, Santa Cruz), HDAC1- and SMYD1-specfic antibody (Abcam), or IgG control antibody (santacruz). The sample/antibody mixture was incubated in a spin column overnight at 4°C on a rotator. Afterward, IP incubation buffer was discarded, while potential cTnI-, HDAC1-, and SMYD1-binding proteins were pulled down in the spin column. Columns were washed with IP washing buffer 3 times, and then the denatured protein was eluted from the column. Protein solution (10 uL) was then used in Western blot analysis (described above).

Mass spectrometry

Protein extraction of cultured cardiomyocytes was conducted using Pierce® IP Lysis Buffer (Thermo, USA). Next, we conducted cTnI co-IP assays as described above. Pull-down protein solution was conducted WB assays and silver staining. Protein samples were then reduced with DTT (5 mmol L^{-1}) and alkylated using propionamide (10 mmol L^{-1}). Digestion was performed overnight using trypsin/lysC mix at a 1:50 protease to protein ratio. Following digestion, samples were acidified and peptides cleaned on C18 Stage Tips followed by speed vacuum to dryness. Samples were reconstituted in 0.1% formic acid, 2% acetonitrile, and 97.9% water prior to injection into the LC. The LC was an Eksigent (SCIEX) utilizing an in-house packed C18 reversed phase analytical column. Peptides were injected onto the analytical column and electrosprayed into the mass spectrometer where the source was a NanoSpray Flex NG. The mass spectrometer was an Orbitrap Fusion set in data dependent acquisition mode to acquire the maximum number of fragment ion spectra in the ion trap per 3 s time window. The RAW data was converted and searched using Uniport (Protein Metrics).

Statistical analysis

All data were expressed as mean±SD and analyzed by one-way ANOVA. The differences were considered statistically significant when $P<0.05$.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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SUPPORTING INFORMATION

Table S1 Selected mass spectrometry analysis of potential cTnI-interacting proteins in cardiomyocytes. Red frames: potential proteins indicating epigenetic regulation

Table S2 Primers used in this study

Figure S1 Pathway enrichment analysis.

The supporting information is available online at http://life.scichina.com and https://link.springer.com. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.