Original Paper

MicroRNA-223 Displays a Protective Role Against Cardiomyocyte Hypertrophy by Targeting Cardiac Troponin I-Interacting Kinase

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Key Words

MicroRNA-223 • TNNI3K • Cardiomyocyte • Hypertrophy

Abstract

Background/Aims: MicroRNAs play regulatory role in cardiovascular disease. MicroRNA-223 (miR-223) was found to be expressed abundantly in myocardium. TNNI3K, a novel cardiac troponin I (cTnI)-interacting and cardiac hypertrophy related kinase, is computationally predicted as a potential target of miR-223. This study was designed to investigate the cellular and molecular effects of miR-223 on cardiomyocyte hypertrophy, focusing on the role of TNNI3K. **Methods:** Neonatal rat cardiomyocytes (CMs) were cultured, and CMs hypertrophy was induced by endothelin-1 (ET-1). *In vivo* cardiac hypertrophy was induced by transverse aorta constriction (TAC) in rats. Expression of miR-223 in CMs and myocardium was detected by real-time PCR (RT-PCR). MiR-223 and TNNI3K were overexpressed in CMs via chemically modified sense RNA (miR-223 mimic) transfection or recombinant adenovirus infection, respectively. Cell size was measured by surface area calculation using fluorescence microscopy after anti- α -actinin staining. Expression of hypertrophy-related genes was detected by RT-PCR. The protein expression of TNNI3K and cTnI was determined by Western blots. Luciferase assay was employed to confirm the direct binding of miR-223 to the 3'UTR of TNNI3K mRNA. Intracellular calcium was measured by sensitive fluorescent indicator (Furo-2). Video-based edge detection system was employed to measure cardiomyocyte contractility. **Results:** MiR-223 was downregulated in ET-1 induced hypertrophic CMs and in hypertrophic myocardium compared with respective controls. MiR-223 overexpression in CMs alleviated ET-1 induced hypertrophy, evidenced by smaller cell surface area and downregulated ANP, α -actinin, Myh6 and Myh7 expression. Luciferase reporter gene assay showed that TNNI3K serves as a direct target gene of miR-223. In miR-223-overexpressed CMs, the protein expression of TNNI3K was significantly downregulated. MiR-223 overexpression also rescued the upregulated TNNI3K

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expression in hypertrophic CMs. Furthermore, cTnI phosphorylation was downregulated post miR-223 overexpression. Ad.rTNNT3K increased intracellular Ca^{2+} concentrations and cell shortening in CMs, while miR-223 overexpression significantly rescued these hypertrophic effects. **Conclusion:** By direct targeting TNNT3K, miR-223 could suppress CMs hypertrophy via downregulating cTnI phosphorylation, reducing intracellular Ca^{2+} and contractility of CMs. miR-223 / TNNT3K axis may thus be major players of CMs hypertrophy.

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Introduction

Cardiac hypertrophy is characterized by an increase in the size of individual cardiomyocyte. The pathologic process of cardiomyocyte hypertrophy is highly related to complex alteration in cardiac gene expression pattern, and changes in molecular pathways [1]. Elucidation of signaling cascades involved in cardiomyocyte hypertrophy will be of critical importance for the design of target molecular therapy against cardiac hypertrophy.

MicroRNAs (miRNAs) are small RNAs approximately 22 nucleotides in length that bind the 3' untranslated region (3'-UTR) of messenger RNAs to post-transcriptionally regulate the translation of target genes [2]. Accumulating evidence suggests that miRNAs may play important roles in the pathology of cardiovascular system, especially in the cardiac hypertrophy [3, 4].

As one of miRNAs with multiple biologic functions, miR-223 has been studied widely in recent years. It is now clearly established that miR-223 plays an essential role in the proliferation of granulocytes, gastric cancer cells, venous smooth muscle cells, and hematopoietic cells [5-8], and it is also a key factor during osteoclast differentiation [9]. Recent report demonstrated a role for miR-223 in cardiac function and tissue metabolism [10], and in addition, circulating levels of miR-223 are responsive to therapeutic interventions and change during the progression of hypertension-induced heart disease [11]. These data attract special interest to researchers in the cardiovascular field and hint a potential role of miR-223 in pathologic process of cardiac hypertrophy. However, the mechanisms by which miR-223 regulates the biological characteristics of cardiomyocyte (CMs) hypertrophy are not fully understood.

Using bioinformatics predictions [12], complementary binding sites have been identified in both miR-223 and the 3'-UTR of the cardiac troponin I-interacting kinase (TNNT3K) mRNA. TNNT3K, also known as CARK, is a novel cardiac-specific kinase. It contains a central kinase domain, flanking by an ankyrin repeat domain in the amino terminus and a serine-rich domain in the carboxyl terminus [13]. Studies have revealed that TNNT3K is a functional kinase which could directly interact with cardiac troponin I (cTnI), and is actively involved in regulating cardiac remodeling [14]. TNNT3K induced CMs hypertrophy was already demonstrated *in vitro* studies [15]. Based on these data, we hypothesized that miR-223 might act as an upstream regulator of TNNT3K, thereby modulating the CMs hypertrophy process.

To confirm and further investigate the potential role of miR-223 / TNNT3K pathway in pathologic process of CMs hypertrophy, endothelin-1 (ET-1, a peptide hormone) was routinely employed to establish model of CMs hypertrophy *in vitro* [16], and expression of miR-223 was detected in ET-1 induced hypertrophic CMs. Myocardial expression of miR-223 was also detected in rats post transverse aorta constriction (TAC). In addition, the cellular and molecular effects of miR-223 overexpression on CMs, and direct targeting of TNNT3K by miR-223 were explored in this study.

Materials and Methods

Animals

One to two days old neonatal Sprague-Dawley rats were used to isolate CMs for this study. Adult rats (250g-300g, without gender restriction) were used for TAC surgery. Animals used in these experiments were

handled in accordance with the Guiding Principles in the Care and Use of Laboratory Animals, approved by Animal Care and Use Committee.

CMs culture

As we described previously [17], neonatal rat were anesthetized and sacrificed by immersing in 70% alcohol. The ventricles were removed and washed three times in warm PBS solution, then minced and incubated with 0.25% trypsinase. Dulbecco's modified Eagle's medium (DMEM, Gibco, CA, USA) containing 10% FBS (HyClone, UT, USA) was used to terminate the digestion. Digestion step was repeated four times. Cells in the supernatant were isolated by centrifugation for 10 min at 1500 rpm at room temperature. CMs were cultured as monolayer at 5×10^4 cells/cm² and incubated with BrdU (0.1 mmol/L) for 24h. Then the culture medium was changed, and cells were used for experiment.

Pressure overload rat model

Pressure overload model was created by TAC surgery. Rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and the suprarenal area of the descending aorta was isolated through a midline laparotomy. Then, abdominal aortic constriction surgery was performed around the abdominal aorta between the renal and superior mesenteric arteries. The sham-operated rat underwent the same surgical procedures without aorta constriction. After surgery, animals were placed in warm environment and remained connected with a rodent respirator until restoration of spontaneous breathing. Four weeks after TAC, the survived animals were sacrificed, and heart hypertrophy was confirmed by increased heart weight (HW) / body weight (BW) and left ventricular weight (LVW) / body weight (BW) ratios. Heart tissue was rapidly frozen in liquid nitrogen and stored in -80°C freezer for subsequent examinations.

Real-time PCR

Total RNA was isolated from cells or tissue by using Trizol reagent (Sigma, MO, USA). Expression of miR-223 was evaluated using the mirVana™ qRT-PCR miRNA Detection Kit (Ambion, CA, USA). Primer sets for miR-223 were purchased from Applied Biosystems (CA, USA). cDNA was synthesized from 2 µg total RNA (SuperScript II, GIBCO, CA, USA). Specific primers for ANP, α-actinin, Myh6, Myh7 were listed in Table 1. GAPDH was set as internal control. Gene transcript levels were measured using SYBR green quantitative real-time PCR. Electrophoresis of reverse transcription PCR products was used as evidence for effectiveness of primers. BioEasy™ SYBR Green I kit (Bioer, Hangzhou, China) was used for quantitative measure. Reactions were prepared in triplicate for each of the two biological replicates, and the conditions were set as: 50°C for 2 min; 95°C for 10 min; 45 cycles of 95°C for 2 min, 62°C for 30 s, and 72°C for 30 s. The relative quantitative expression was determined using method $2^{-\Delta\Delta Ct}$.

CMs hypertrophy induction and cell size measurement

CMs were incubated in the presence or absence of the hypertrophic stimulus endothelin-1 (ET-1, Sigma, MO, USA, 60 nmol/L). Cell size was measured via surface area calculation under fluorescence microscopy after anti-α-actinin (Sigma, MO, USA) staining. Briefly, cells on glass coverslips were washed in PBS and 4% formaldehyde fixed at room temperature. Slide glasses were treated with 10% goat serum for 1 h at room temperature. Samples were stained with anti-α-actinin (1:1000) overnight at 4°C. After being washed in PBS, samples were incubated with fluorescein-labeled IgG and DAPI. Images were analyzed using

Table 1. Primers for RT-PCR detection of cardiac hypertrophy related marker genes

mRNA	Primers (5' - 3')	Annealing temperature (°C)	Expected product length (bp)
ANP	F: AGAAACCAGAGAGTGAGCCG R: GGTGGTCTAGCAGGTTCTTGAAG	55	195
α-actinin	F: GGCTGTGTTCCTCCATCCATCGT R: CCCGGTTAGCTTTGGGGTTCA	57	266
Myh6	F: AGAGTGACAGGATGACGGAT R: TCCTTCACAGTCACCGTCTTG	57	229
Myh7	F: CAGTCATGGCGGATCGAGAG R: CGGATTCTCCGGTGATGAGG	55	546

image analysis software (NIS-Elements BR, Nikon, JPN), with a photographed scale of the 10× objective lens for calibration. For each group, 50 individual CMs were measured and average value was calculated (μm^2), and 6 measurements in different field of microscope were performed.

MiR-223 mimics transfection

Chemically modified sense RNA (miR-223 mimic) was synthesized by Qiagen (Germany). The sequence was 5' - CGUGUAUUUGACAAGCUGAGUUG - 3' (Syn-rno-miR-223). Transfection with the miR-223 mimics was performed using HiPerFect Transfection Reagent (Qiagen, DEU). Briefly, 5 nM of miR-223 mimics was mixed with 20 μL HiPerFect in 100 μL serum-free culture medium for 10 min at room temperature to form transfection complexes. The cells were incubated with the transfection complexes for 48 h. Scrambled miRNA (scr-miR) that commercially synthesized by Qiagen was used as control.

Recombinant Adenovirus and adenoviral infection

Adenovirus encoding full-length rat TNNT3K (Ad.rTNNT3K) was constructed by using AdEasy Adenoviral Vector System (Agilent Technologies, CA, USA). NCBI reference sequence of TNNT3K is NM_181769.1. For adenoviral infection, cardiomyocytes were incubated for 2 hours with Ad.rTNNT3K or Ad.Vector at an approximate multiplicity of infection of 100. 48 hours following infection, 95% of the cells were GFP positive.

Western Blots

Total protein was extracted from cells and heart tissue in lysis buffer for 1 h on ice. After centrifugation at 10000 $\times g$ for 5 min, the supernatant was collected and protein concentration was determined using a Bio-Rad DC Protein Assay kit (BIORAD, CA, USA). Subsequently, 50 μg of lysate was fractionated through a 4% stacking and 10% running SDS-PAGE gel, and the fractionated proteins were transferred to PVDF membrane. Blots were blocked for 1 h at room temperature with blocking buffer. The antibodies (rabbit anti-TNNT3K, mouse anti-p-TroponinI, rabbit anti-TroponinI, mouse anti-GAPDH, SANTA CRUZ, CA, USA) were reacted with the blots overnight at 4 °C. After washing (3 $\times$ 5 min in 1 $\times$ TBS-0.1% Tween 20), the blots were incubated with horseradish peroxidase-conjugated secondary antibody at 1:2000 dilution for 1 h at room temperature. Visualization was performed with enhanced chemiluminescence. For quantification, densitometry of the target bands was divided by the corresponding densitometry of the GAPDH band using graph software (Image J 2.1.7.4, NIH, USA).

Luciferase reporter assay

To confirm that TNNT3K was targeted by miR-223, we constructed a chimeric luciferase reporter system (pMIR-REPORT, Ambion, CA, USA) tagged with the full-length 3'-UTR region of rat TNNT3K harboring the seedmatch sequence with (3'-mUTR, CT $\rightarrow$ GA, position 41 to 42.) or without two nucleotide mutations (3'-UTR). HEK 293 cells were transfected with 200 ng of pMIR-REPORT and 8 ng of pRL-SV40 renilla luciferase control vector in the presence of the miR-223 mimics or scr-miR (as negative control) using Lipofectamine 2000 (Invitrogen, NY, USA). Luciferase activity was assayed after 48 h and expressed as a percentage of the luciferase activity of the vector group.

Measurement of intracellular calcium

CMs were incubated with Furo-2 (Fanbo Biochemicals, Beijing, PR China) for 30 minutes at 37°C, to measure Ca^{2+} concentrations. Detection parameters were set as: excitation at 338 nm, emission at 510 nm. CMs were washed twice with phosphate-buffered saline (PBS), and fluorescence intensity was measured using a spectra Max M5 plate reader. Fluorescence intensity was normalized against control (Furo-2-loaded cells) after subtraction of baseline (Furo-2 without cells).

Measurement of cardiomyocyte contractility

CMs contractility was measured by using video-based edge detection system (Ion Optix Co., NY, USA). Under microscope, CMs were superfused with low flow (1.8 mL/min) of Tyrode solution containing Ca^{2+} (1.8 mM), and electrically stimulated at 1 Hz at room temperature. Cell length was monitored from the bright-field image by optical edge-tracking method. The contraction amplitude was measured as the percentage of shortening of cell length.

Fig. 1. Relationship of miR-223 and cardiomyocyte hypertrophy. A: In hypertrophic CMs which induced by ET-1, expression of miR-223 was measured by using real-time PCR analysis (n=6). B: Comparison of HW/BW and LVW/BW. Sham (n = 6); TAC (n = 5). C: In heart tissue of TAC and Sham group, expression of miR-223 was measured by using of real-time PCR analysis (n=6). D: Cell size was measured via surface area calculation after anti- α -actinin staining (green), under fluorescence microscopy. The nucleus was stained with DAPI (blue). The figure shows a representative stain. Scale bar shows 20 μ m. src-miR shown no effect on change of cell size in ET-1 induced hypertrophic CMs. Data are expressed as mean $\pm$ SD for 6 individual measurements.

Statistical Analysis

Data were provided as means $\pm$ SD; "n" represents the number of independent experiments. All data were tested for significance using ANOVA or Student's t-test as indicated. $P < 0.05$ was considered statistically significant.

Results

Relationship of miR-223 and cardiomyocyte hypertrophy

Expression of miR-223 in normal CMs and ET-1 induced hypertrophic CMs was detected by real-time PCR. MiR-223 mRNA expression was significantly downregulated (about 40% reduction) in hypertrophic CMs compared with control group (Fig. 1A). Accordingly, significantly downregulated miR-223 mRNA expression (about 60% reduction) was evidenced in myocardial tissue of TAC rats compared with sham rats (Fig. 1B and C). As shown via surface area calculation after anti- α -actinin staining (Fig. 1D), overexpression of miR-223 in CMs by miR-223 mimics transfection reduced ET-1 induced CMs size enlargement. These results revealed that there was a negative correlation between miR-223 expression and cardiomyocyte hypertrophy, and miR-223 is an effective regulator against cardiomyocyte hypertrophy.

Effects of miR-223 overexpression on hypertrophy marker genes in CMs

To clarify the effects of miR-223 on CMs hypertrophy, the expression levels of classic hypertrophy marker genes such as ANP, α -actinin, Myh6, Myh7 were measured in CMs with

Fig. 2. Assessment of hypertrophy marker genes in CMs with miR-223 mimics transfection. Relative quantitative expression of ANP, α -actinin, Myh6, Myh7 were detected by Real-time PCR analysis (Bar graphs, n=6). Electrophoresis bands demonstrated products of reverse transcription PCR which confirm the validity of PCR primers.

or without miR-223 mimics transfection by real-time PCR analysis. As presented in Fig. 2, expression of ANP, α -actinin, Myh6, Myh7 were all significantly down-regulated by miR-223 (about 40%, 20%, 50%, 40% reduction, respectively). These results further supported an important role of miR-223 as a negative regulator of CMs hypertrophy.

Direct targeting of TNNT3K by miR-223 mimics transfection in CMs

Bioinformatics approach was employed to predict mRNA targets of miR-223 (www.targets.org). We screened rat genes that predicted to be potential targets of miR-223, narrowed them down to a few targets involved in CMs remodeling, TNNT3K appeared to be a major candidate because the miR-223 seed sequence is reverse complementary to the seed-match sequence in the 3'-UTR region of TNNT3K (Fig. 3A). As shown in Fig. 3A, luciferase activity was significantly repressed by the miR-223 mimics (~ 60% reduction). This result thus provided solid evidence of the direct binding of miR-223 to the 3'-UTR of TNNT3K.

Furthermore, Western blotting was performed to examine the TNNT3K protein expression in CMs with or without miR-223 overexpression. The miR-223 mimics also induced a significant (~ 90%) TNNT3K downregulation of compared to the scr-miR control (Fig. 3B).

Western blotting results further confirmed the regulative link between miR-223 and TNNT3K in hypertrophic CMs, in that there was an about 2 folds higher TNNT3K protein expression in ET-1 induced hypertrophic CMs compared to control CMs, and miR-223 mimics transfection again significantly attenuated the up-regulated TNNT3K expression in ET-1 treated CMs (Fig. 3C).

Effect of miR-223 mimics transfection on cTnI phosphorylation in CMs

As TNNT3K could directly interact with cTnI, and miR-223 is an upstream regulator of TNNT3K, we also evaluated the effect of miR-223 overexpression on cTnI phosphorylation.

Fig. 5. miR-223 reversed the effects of TNNI3K on intracellular Ca²⁺ and cardiomyocyte contractility. A: Expression of recombinant adenoviral transgene in CMs. CMs were infected with adenoviral vectors carrying EGFP as marker gene, after 24 h infection *in vitro* culture (100 multiplicity), with/without miR-223 mimics or scr-miR transfection. B: Ad.rTNNI3K induced elevation of intracellular Ca²⁺ concentrations in CMs. miR-223 reversed the enhanced intracellular Ca²⁺. The quantification of intracellular Ca²⁺ level was measured by using Furo-2 dye staining (n=6). There was no statistic difference between Ad.rTNNI3K group and Ad.rTNNI3K+scr-miR group. C: Effects of miR-223 / TNNI3K on cardiomyocyte contraction. Representative traces of cell shortening from non-infected CMs, CMs infected with Ad.Vector or Ad.rTNNI3K, and Ad.rTNNI3K transgenic CMs with miR-223 mimics or scr-miR transfection were recorded. Ad.Vector and scr-miR exerted no effect on cardiomyocyte contraction.

cellular characteristics of CMs. Changes on Ad.rTNNI3K (Ad.Vector as vector control) infected CMs, with or without miR-223 mimics co-transfection (scr-miR as control) were evaluated (Fig. 5A). Ad.rTNNI3K induced an about 90% increase in intracellular Ca²⁺ concentrations in CMs, while this effect could be significantly blocked by miR-223 mimics co-transfection (Fig. 5B). As shown in Fig. 5C, Ad.rTNNI3K induced a significant increase in cell shortening of CMs (Ad.rTNNI3K group: $2.69 \pm 0.11\%$ vs. blank control group: $1.81 \pm 0.06\%$, $p < 0.05$) while miR-223 mimics significantly blocked this effect (Ad.rTNNI3K plus miR-223 group: $0.98 \pm 0.08\%$ vs. blank control group, $p < 0.01$).

Discussion

Heart undergoes compensatory hypertrophic growth and cardiac remodeling for sustaining cardiac output and these processes will sometimes result in cardiac dysfunction in the long-term [18]. The process of pathological myocardial hypertrophy is a highly complex event, and has been studied in relation to miRNAs to date. Whole arrays in pathological hypertrophy animal models indicated that some particular miRNAs were upregulated or downregulated, such as miR-1, miR-18b, miR-21, miR-133, miR-208 [19-21]. Present study showed that change in endogenous miR-223 expression was related to cardiac hypertrophy, and overexpression of miR-223 by miR-223 mimics transfection reduced the ET-1 induced

hypertrophic response in neonatal rat CMs. MiR-223 overexpression also suppressed activation of hypertrophic marker genes (ANP, α -actinin, Myh6, Myh7). These results revealed that miR-223 appears to be a negative regulator of cardiac hypertrophy.

CMs contain arrays of serially aligned and laterally registered sarcomeres that parallel the longitudinal cellular axis. Some specific proteins comprise the sarcomere's thick myofilament, intermediate myofilament, and thin myofilament [22]. The mechanism for cardiac hypertrophy is related to changes in the organization of the sarcomeric structure that induced by altered protein phosphorylation [23]. There is evidence that CMs hypertrophy is provoked by the activation of protein kinase cascades which, in turn, activate MAPK. These kinases are serine-threonine kinases and phosphorylate a number of important cellular substrates for cell growth and differentiation processes that related to cardiac hypertrophy [24]. In recent years, the contribution of some novel protein kinases involved in cardiac hypertrophy has been the subject of fairly detailed study. TNNT3K, as a cardiomyocyte-specific kinase, is known to promote ischemia/reperfusion injury, oxidative stress, and CMs death. TNNT3K-mediated injury is mediated through increased mitochondrial superoxide production and impaired mitochondrial function and is largely dependent on p38 mitogen-activated protein kinase (MAPK) activation [25]. Recent studies have revealed that TNNT3K is associated with hemodynamic overload induced cardiac remodeling. TNNT3K overexpression readily induced CMs hypertrophy and accelerated hypertrophy in hypertrophic CMs [15]. By using bioinformatics approach, we found TNNT3K as a predict target of miR-223, in fact, present study revealed that miR-223 mimics transfection obviously shut down the TNNT3K protein expression and canceled hypertrophy-induction effect of TNNT3K on CMs. Luciferase assay also confirmed the direct targeting of TNNT3K by miR-223 overexpression. These results collectively suggest an important role of miR-223 / TNNT3K pathway in modulating CMs hypertrophy.

It was shown that TNNT3K could promote concentric hypertrophy with enhancement of cardiac function via regulating the phosphorylation of cTnI [26] and thus contributed to the regulation of cardiac myofilament contraction [27]. Troponin I (TnI) is a known inhibitory unit of the troponin complex associated with the thin filament. It links Ca^{2+} -TnC binding with activation of cross bridge reactions with the thin filament, and thus inhibits actomyosin interactions at diastolic levels of intracellular Ca^{2+} . cTnI also represents a key regulatory protein in cardiac muscle contraction and relaxation [28]. In line with above findings, present study revealed the suppressive effect of miR-223/TNNT3K pathway on cTnI phosphorylation. Attention has also been focused on changes in the intracellular Ca^{2+} cycle of CMs, and the contractual capacities of CMs. It is widely accepted that the increase of intracellular Ca^{2+} could activate hypertrophic program. Our results demonstrated the TNNT3K induced elevation of the Ca^{2+} concentration in CMs, and this effect could be abrogated by miR-223 mimic transfection. In addition, the contractility of individual ventricular CMs was enhanced by TNNT3K overexpression, while suppressed by miR-223 mimics. These observations supplied evidence of the modulating role miR-223 / TNNT3K pathway on cTnI phosphorylation and CMs hypertrophy.

It's worth noting that early data suggested TNNT3K had a protective role post myocardial injury, based on findings of increased beating mass and number of α -actinin-positive cells after TNNT3K overexpression [13]. We think these observed results might actually support the "myogenic" but not "protective" function of TNNT3K, and in some ways, were consistent with pro-hypertrophic effect of TNNT3K observed in present study. Indeed, most of recent studies demonstrated that both normal expression and TNNT3K overexpression presented a detrimental effect on cardiac function [14, 15, 25]. Our experimental data also suggested the detrimental pro-hypertrophic role of TNNT3K, and provided a preliminary exploration on TNNT3K's upstream regulatory mechanism. However, further studies are warranted to explore the exact function and mechanism of TNNT3K in regulating cardiac remodeling and function.

In summary, we show that, as negative regulator to CMs hypertrophy, miR-223 expression is downregulated in ET-1 induced CMs and in myocardial tissue of TAC rats

while miR-223 overexpression can suppress ET-1 induced CMs hypertrophy. By direct targeting TNNT3K, miR-223mimic transfection could downregulate cTnI phosphorylation, and reverse the effects of TNNT3K on intracellular Ca^{2+} and contractility of CMs. Therefore, miR-223 appears to be a promising candidate for inhibiting CMs hypertrophy, and miR-223 / TNNT3K axis might be one of interesting targets for the clinical treatment of hypertrophy.

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