The role of lncRNAKCNQ1OT1/miR-301b/Tcf7 Axis in Cardiac Hypertrophy

mingyao e
Baicheng Medical College https://orcid.org/0000-0003-3201-6691
Feifei Ren (1094686866@qq.com)
Baicheng Hospital
Guohong Liu
Baicheng Medical College
Yanhua Yu
Baicheng Medical College
Haiyan Li
Baicheng Medical College
Chao Shen
Baicheng Medical College
Chunyan Sha
Baicheng Medical College

Research

Keywords: cardiac hypertrophy, lncRNAKCNQ1OT1, miR-301b, Tcf7, c-myc

Posted Date: October 14th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-965129/v1

Abstract

Cardiac hypertrophy, acting as a pathologic progress of chronic hypertension and coronary disease. Increasing evidence indicates that lncRNA potassium voltage-gated channel subfamily q member 1 overlapping transcript 1 (KCNQ1OT1) may led to MI. Recent studies showed, miR-301b level was evidently decreased in heart failure patients. To explore the regulate effect of lncRNAKCNQ1OT1 and miR-301b in cardiac hypertrophy, gain-and-lose function assays were tested. Notably, lncRNAKCNQ1OT1 overexpression causes a significant result as cardiac hypertrophy. Furthermore, lncRNAKCNQ1OT1 sponge microRNA-301b. The results showed that miR-301b exhibited lower expression in cardiac hypertrophy model, indicating that it may exert anti-hypertrophic role. Furthermore, the BNP and β-MHC expression increased, as well as cardiomyocyte surface area, with Ang II treatment, while the effect was repealed by miR-301b. Moreover, the protein expression of Tcf7 was down-regulated or up-regulated by miR-301b or AMO-301b. As displayed by luciferase reporter assay, miR-301b binds to 3'UTR of Tcf7. Briefly, we aimed to elucidate a down-regulate role of miR-301b in cardiac hypertrophy, as to provide potential therapeutic strategies.

Introduction

Cardiac hypertrophy, considered as a compensatory mechanism, through which the heart maintains its function under mechanical stress or neurohormonal stress[1]. Pathological cardiac hypertrophy may led to various cardiac diseases, such as myocarditis, coronary heart disease and endocarditis[2], induce heart failure eventually[3]. Great efforts have been made to identify genes and signaling pathways associated with this disease. However, new regulatory mechanisms for cardiac hypertrophy should be identified.

Long non-coding RNAs (lncRNAs), which act as a member of transcribed RNA molecules with more than 200 nucleotides. More and more studies showed that lncRNAs take part in multiple cardiovascular diseases[4], including cardiac hypertrophy[5]. LncRNAKCNQ1OT1 is reported to regulate MI progression[6]. Nevertheless, lncRNAKCNQ1OT1 effect on cardiac hypertrophy is not clear.

MicroRNAs (miRNAs), acting as small non-coding RNAs, could led to the degradation of mRNA [7]. MiRNAs are critical to varieties of basic biological processes, such as cell proliferation and apoptosis[8] [9][10]. A number of studies have suggested miRNAs taking part in cardiac hypertrophy. MiR-1, for instance, can mitigate cardiac hypertrophy induced by thyroid hormone. MiR-146a plays as a suppressor in cardiac hypertrophy, as well as miR-206[11][12][13]. However, miR-301b has been identified took part in the development of multiple diseases. According to reports, miR-301b regulates the proliferation of bladder cancer cells by targeting its targets[14]. Isoliquiritigenin inhibits miR-301b and thus inhibits human melanoma growth[15]. Recent studies showed that miR-301b participates in the regulation of diabetic heart diseases by inhibiting AMP deaminase (AMPD)[16]. Nevertheless, the miR-301b potential functional role in cardiac hypertrophy have not be explained clearly yet.

With targetscan software, this study has predicted that Tcf7 may be the target gene of miR-301b, Tcf7, a member of the T cell factor family. Based on existing studies, Tcf7 has been indicated to extensively participate in numerous cancers[17][18]. Furthermore, it has been confirmed to participate in cardiomyocyte cell cycle control[19]. But in fact, the impact of Tcf7 on cardiac hypertrophy has been rarely reported. Wnt signaling pathway, which Tcf7 pertains to, has been found regulating the end-stage of hypertrophic cardiomyocytes, leading to variations in cardiac hypertrophy and left ventricular structure and function[20]. Other studies have reported that c-myc, a downstream target gene of Tcf7, was activated during cardiac hypertrophy[21]. Collectively, it was speculated that Tcf7 may also take part in the regulation of cardiac hypertrophy as a member of Wnt signaling pathway.

In summary, this study aimed to confirm whether lncRNAKCNQ1OT1, miR-301b and Tcf7 impact cardiac hypertrophy and to delve into the latent relationship between lncRNAKCNQ1OT1, miR-301b, Tcf7 and cardiac hypertrophy. Thus, the mentioned findings may be conducive to providing potential therapeutic targets and strategies for cardiac treatment.

Materials And Methods

Cardiac hypertrophy animal model

Male C57BL/6 mice of 8-10 weeks were anesthetized by intraperitoneal injection of 1% sodium pentobarbital 22-25 g in weight. After the anesthesia, tracheal intubation was performed in mice, and breathing was regulated by ventilator (frequency 90-100 times/min, tidal volume 0.4-0.5 mL). The supine position of mice was fixed on the thermostat pad of the operating table (30°C). The operation area was cleaned. The skin was cut in the middle of the chest, up to the superior sternal fossa and down to 0.3 cm after the right carotid artery was separated. 26G pad needle (0.4 mm in diameter, self-made) was employed to ligate the aorta and pad needle (causing nearly 70% stenosis). After the ligation, the pad needle was pulled out, and the aortic arch was examined. The fluctuation of proximal end was facilitated, the chest was closed layer by layer, while the skin was sutured. After 4 weeks, cardiac hypertrophy was evaluated by echocardiography, covering thickness of anterior and posterior walls of the left ventricular (LVAWs and LVPWs), fractional shortening and ejection fraction (LVFS and LVEF). The ratios of heart weight to body weight were also measured. The guideline issued by the US NIH was rigorously followed. And approved by the Experimental Animal Ethics Committee of Baicheng Medical College.

Hematoxylin-Eosin staining

Male C57BL/6 mouse's left ventricle was incubated into 4 g/L paraformaldehyde solution overnight and subsequently transferred to 30% sucrose solution. The tissue was knifed and placed on slides. Next, the Hematoxylin-eosin (HE) staining was employed to stain these sections and the pathological variations of myocardial tissue were observed. Afterwards, Olympus BX60 microscope was used to capture photographs.

Neonatal mouse cardiomyocyte culture

According to the study, the hearts of C57BL/6 mice aged 1-3 days were quickly removed and washed with 0.9 % cold phosphate buffer saline (PBS). Then, the ventricular tissue was crushed and hydrolyzed by 0.25% trypsin (Beyotime, China)[22]. The collected cell suspension was centrifuged and then suspended in DMEM supplemented by 10 % fetal bovine serum. Next, myocardial cells were purified by selective adhesion of non-cardiac cells with 90 min pre-plating interval. Cells, which attached weakly, were regarded as cardiomyocytes. Afterwards, 5-bromo-2-deoxydriuine (Brdu,0.1 mmol/L, Sigma, America) was used to inhibit myocardial fibroblasts proliferation. To induce hypertrophy, 100 nmol/L Angiotensin II (Ang II) was added to the cardiomyocyte for 48 hours.

Transfection procedures

According to the manufacturer's protocol, cells were washed with serum-free medium and then incubated in 4 mL of serum-free medium for 4-6 h. The constructs and X-tremeGENE transfection reagent were transfected to myocardial cells. miR-301b complied with the sequence of 5'-GCUCUGACUAGGUUGCAGUACU-3'. AMO-301b: 5'-AGUAGUGCAACCUAGUCAGAGC-3'. N.C: (5'-UUCUCCGAACGUGUCACGUTT-3'), AMO-N.C: 5'-CAGUACUUUUGUGUAGUACAA-3'.

Tcf7 silencing

Cells were transferred to 6-well plates and incubated for 48 h. According to the instruction, siRNA was used to silence Tcf7. Tcf7-siRNA sequence was 5'-GGAAGAGAGGACAAGGAAUTT-3'. Tcf7-sc:5'-UUCUCCGAACGUGUCACGUTT-3'. The sequences were synthesized by GenePharma Co.

Western blot

RIPA buffer was used to lyse cardiac tissues and myocardial cells. SDS-polyacrylamide gel was used to fractionate protein sample of with 60 ug. Following by electrophoretic, the protein transfer onto a nitrocellulose blotting membrane. β-actin was regarded as an internal control. β-MHC antibodies (1:2000) and BNP antibodies (1:500) were respectively purchased by Sigma and Santa Cruz Biotechnology. Tcf7 antibody (1:200) and c-myc antibody (1:500) were offered by Abcam. Membranes were incubated at 4°C overnight. After that, membranes were cleaned by Tris Buffered Saline with Tween-20 (TBST). Subsequently, secondary antibodies were used to incubate membranes for 1 h at ambient temperature. Lastly, the immunoreactivity was ascertained with an Odyssey Imaging System. Membranes were quantified by measuring the intensity.

Immunofluorescence staining

PBS was used to wash myocardial cells. After that 4% paraformaldehyde was used to fix cells for 10 min. Next, the cells were permeabilized with 0.2% TritonX-100. α-SMA antibody was used to incubate cells at 4°C overnight. The nonspecific binding of fixed cells was hatched at ambient temperature for 30 min with 5% BSA solution and then with anti-α-actin antibody (1:200; Beyotime, China) at 4°C overnight. Subsequently, Alexa Fluor™ 568-conjugated goat anti-mouse antibody (1:1000; Molecular Probes, Eugene, OR) was used to administrate cells at 37°C for 1 h. Lastly, DAPI was adopted to affirm the nucleus. Fluorescence microscope was used to capture the photographs.

Quantitative RT-PCR

Trizol (Invitrogen, Carlsbad, CA) was employed to extract total RNA samples. To quantify the expression of miRNAs, the mirVana qRT-PCR miRNA Detection Kit with SYBR Green I was used. Following by 40 cycles reaction, threshold cycles (CT) were measured. Relative levels of microRNA were calculated in line with CT values. Meanwhile, GAPDH levels were normalized. Lastly, the $2^{-\Delta\Delta CT}$ method was employed for date analysis.

Luciferase assay

According to gene sequence and vector characteristics, the 3'UTR of Tcf7 gene that containing the restriction sites of Hind III and SPI enzymes were synthesized. Next, the pLG-3 plasmid and the 3'UTR of Tcf7 were cutted by Hind III and Spe I enzymes, these two were connected by T4 ligase to form a new plasmid (pLG-3-promoter3'UTR). Next, the constructed plasmids were transfected into HEK293 cells what grouped. After 24 h, the DMEM was discarded and the culture dishes were washed with PBS. Then, culture dishes were shaken at room temperature for 15 min with 600 μL lysis solution added, these cell lysates were transferred to centrifugal tubes and putted into high speed centrifuge for 12 000 r/min, and after 30 seconds, the supernatant was transferred to other centrifugal tubes. In the light of established grouping, 100 μL LAR II and 20 μL cell lysates were added to measuring dishes and mixed evenly, then placed in spectrophotometer to obtain data. After that, add Stop & Glo 100 μL and mix evenly, read values and record data.

Statistical analysis

Date are represented as mean ± SEM for a minimum of six independent experiments. Variance analysis (ANOVA) was used for multiple groups. Two groups were compared by student's t test. Meanwhile, $p < 0.05$ was regarded with statistical significance. SPSS 19.0 software and GraphPad Prism 7.0 was used to analyze the date.

Results

LncRNAKCNQ1OT1 is highly elevated in cardiac hypertrophy.

Transverse aortic constricted (TAC) and Ang II were used to build cardiac hypertrophy. As indicated in Fig. 1A-F, LVPWs, LVAWs and HW/BW were increased, whereas LVEF and LVFS were down-regulated in the model group, compared with control group. Subsequently, HE and immunofluorescence staining were used to detected the cell area, and found that cell surface area was increased markedly (Fig. 1G-H). Moreover, BNP and β-MHC, which considered as hypertrophy marker proteins, were significantly increased in hypertrophic models (Fig. 1I-J). The mentioned results showed that we have built cardiac hypertrophy models successfully. Real - time PCR results indicated that the lncRNAKCNQ1OT1 level significantly increased in heart tissue and myocardial cell relative to cardiac hypertrophy (Figure 1K-L).

LncRNAKCNQ1OT1 effects on cardiac hypertrophy.

In order to study whether KCNQ1OT1 affects the development of cardiac hypertrophy, we first use siRNAs to repress lncRNAA02Rik expression. As displayed in Figure 2A, silncRNAKCNQ1OT1 alleviated the Ang-Ⅱ induced the increased of cell surface area. Western blot results showed silncRNAKCNQ1OT1 could repealed the increase expression of BNP and β-MHC. Meanwhile, lncRNAKCNQ1OT1 overexpression increased the surface area of cardiomyocytes (Figure 2C), increased the protein level of β-MHC and BNP (Figure 2D). Collectively, the above results indicating lncRNAKCNQ1OT1 acts as a contributor to cardiac hypertrophy.

MiR-301b inhibits cardiac hypertrophy

To ascertain the role of miR-301b on cardiac hypertrophy, real-time PCR was used to test the expression of miR-301b in mice myocardial tissues and neonatal cardiomyocytes. As shown in Fig. 3A and B, the level of miR-301b was lower in model group compared with control group considerably, suggesting that miR-301b may have a role in the development of myocardial hypertrophy. In order to detect miR-301b role in cardiac hypertrophy, this study overexpressed miR-301b in sham and model mice, respectively. Five-week-old mice were infected with AAV9–miR-301b or with an equivalent volume of PBS (Fig. 3D), after 3 weeks, they were subjected to TAC or Sham (Fig. 3D). Subsequently, HW/BW, LVPWs and LVAWs were decreased, whereas LVEF and LVFS were up-regulated in the model with AAV-miR-301b group (Figure 3E-H). Meanwhile, miR-301b overexpression significantly narrowed the cell surface area induced by TAC (Fig. 3I), and decreased the expression of BNP and βMHC, as compared with model group (Fig. 3J). Consistent with these findings, the overexpression of miR-301b was used on neonatal mouse primary cardiomyocytes (Fig. 3K). MiR-301b overexpression diminished the enhancement of cell surface areas and β-MHC and BNP level, induced by Ang-Ⅱ (Fig. 3L-N), although these works were restored to control levels by AMO-301b. The mentioned results demonstrated that miR-301b exert an anti-hypertrophic role.

Tcf7 effects on cardiac hypertrophy

First, this study would identify whether Tcf7 take part in the progress of cardiac hypertrophy. *In vivo*, it was revealed from the results that Tcf7 expression was up-regulated obviously in the model group. Consistent with this finding, the expression of c-myc protein also markedly higher in model group, compared with normal group (Fig. 4A). Similar results have been achieved *in* vitro experiments (Fig. 4B). Secondly, in order to verify the effect of Tcf7 in cardiac hypertrophy, the expression of Tcf7 was successfully inhibited by targeting Tcf7 with siRNA (Fig. 4C-D). Thirdly, it was found that silencing Tcf7 distinctly narrowed cell surface area (Fig. 4E). Meanwhile, BNP and β-MHC level were also decreased on the effect of siTcf7, compared with the model group. Moreover, c-myc expression was also down-regulated (Fig. 4F). In brief, the results suggested activation of Tcf7 might facilitate cardiac hypertrophy by activating c-myc.

Tcf7 acts as a target of miR301b.

Targetscan software results indicated Tcf7 was a target of miR-301b. Accordingly, the role of miR-301b on Tcf7 with the AAV-miR-301b in vivo was studied. As suggested from the results, the protein expression

of Tcf7 and c-myc was down-regulated in model with AAV-miR-301b group, compared with model group (Fig. 5A). Meanwhile, Tcf7 and c-myc level was decreased by miR-301b over-expressing, and it was up-regulated when miR-301b was inhibited, in contrast to the control group (Fig. 5B-C). Likewise, in order to detect whether Tcf7 was the target of miR-301b, the luciferase activities were performed (Fig. 5D). As shown from the results, when wild-type Tcf7 3’-UTR and miR-301b were over-expressed, the luciferase activity of Tcf7 3'-UTR was decreased. While, the activity of the mutant Tcf7 3'-UTR remained almost unchanged on miR-301b over-expression unchanged Tcf7 luciferase activity, demonstrating miR-301b regulate Tcf7 negatively. Collectively, these data indicated that miR-301b can directly bind to the 3'-UTR of Tcf7 to suppress its activity.

LncRNAKCNQ1OT1 contributes to cardiac hypertrophy via miR-301b mediated regulation of Tcf7

To investigated the relationship between lncRNA KCNQ1OT1 and miR-301b, the luciferase activity was tested. As shown in Fig. 6A, miR-301b luciferase activity was decreased in cardiomyocytes, transfected with lncRNAKCNQ1OT1. However, miR-301b silencing up-regulated the luciferase activity of lncRNAKCNQ1OT1. These results indicating that lncRNAKCNQ1OT1 bound miR-301b to negatively regulate miR-301b. Moreover, as displayed as Fig. 6B, lncRNAKCNQ1OT1 decreased the luciferase activity of Tcf7, while this effect could be reversed by miR-301b knockdown, indicating that lncRNAKCNQ1OT1 by negatively modulating miR-301b to regulated Tcf7. Collectively, these data suggest that lncRNAKCNQ1OT1 contributes to cardiac hypertrophy via miR-301b mediated regulation of Tcf7.

Discussion

Cardiac hypertrophy, a process of adapting to cellular stress, is manifested in the increase of myocardial cell volume at the cellular level, the increase in cell diameter and length, as well as the increase in ventricular wall mass at the tissue level. Pathological cardiac hypertrophy acts as a reaction to disease or stress, resulting in elevated cell volume and myocardial protein synthesis,which is different from athlete's heart. Long-term stress induced pathological cardiac hypertrophy may led to heart failure. The latest research has revealed ceRNA play an important role in cardiac hypertrophy. However, the mechanism of which have not been clearly defined. Briefly, the research has indicated lncRNAKCNQ1OT1 was able to regulate cardiac hypertrophy by sponging miR-301b via suppression of Tcf7.

Tcf7 has been identified as a vital participant in lots of diseases, for instance: pancreatic cancer and colorectal cancer[23][24]. Meanwhile, as a member of Wnt signaling pathway, Tcf7 is associated with cardiac developmental processes[25]. Our research further revealed that Tcf7 protein level and its downstream target gene c-myc protein was significantly up-regulated in cardiac hypertrophy. In order to ascertain the effect of Tcf7 on cardiac hypertrophy, Tcf7 was silenced by siRNA technology. Myocardial cell surface area was decreased by siTcf7. Consistent with this finding, BNP and β-MHC level, which regarded as hypertrophic markers, were also significantly down-regulated. Likewise, results displayed c-

myc level was also down-regulated after Tcf7 knockdown. It was speculated that the activation of Tcf7 can increase c-myc level and eventually cause cardiac hypertrophy.

MiRNAs could induce messenger RNA (mRNA) degradation by binding to the 3'-untranslated region (UTR) of mRNAs. The study has displayed important effect of microRNAs (miRNAs) on cardiac disease, such as myocardial ischemia, arrhythmia, and myocardial fibrosis. For instance, miR-133 was decreased in cardiac hypertrophy[26]. MiR-22 and miR-27b also have effects on cardiac hypertrophy[27][28]. However, whether miR-301b is critical to cardiac hypertrophy has not been reported. MiR-301b was predicted to be increased in the cardiac hypertrophy model by bioinformatic approach-a finding that triggered the present study. MiR-301b overexpression down-regulated myocardial cell area as well as BNP and β-MHC level. In contrast, the effect can be reversed by AMO-301b. These findings suggested that miR-301b inhibits cardiac hypertrophy. Furthermore, the relationship of miR-301b and Tcf7 was assessed by targetscan software. Western blot results displayed Tcf7 level was inhibited when overexpressing miR-301b, and promoted by AMO-301b, compared with the control group. Subsequently, luciferase reporter assay displayed that Tcf7 was a target gene of miR-301b, indicating that miR-301b bind to Tcf7 3’UTR and repress its activity.

Apart from coding genes, lncRNAs take important roles in cardiac diseases. A key finding of our research showed that lncRNAKCNQ1OT1 was highly expressed in hypertrophic mice. The significantly higher expression level of lncRNAKCNQ1OT1 was also demonstrated to induce cardiac hypertrophy by gain-and-lose function assay. Moreover, lncRNAKCNQ1OT1 has been proven to regulate miR-301b expression in cardiac hypertrophy via recruitment of Tcf7.

In summary, lncRNAKCNQ1OT1 sponging miR-301b via suppressing the expression of Tcf7, thereby leading to the reduction of c-myc activity and eventually induce cardiac hypertrophy. Our research revealed a lncRNAKCNQ1OT1/miR-301b/Tcf7/c-myc axis as a new regulator, capable of provide new treatment to prevent cardiac hypertrophy by manipulating such signaling axis.

Declarations

COMPETING INTEREST

All the data are true and reliable. The authors declare that they have no conflict of interest. There is no objection to the content of the text.

ETHICS APPROVAL

All animal experiments were approved by the Ethics Committee of Baicheng Medical College

Author contribution statement

Mingyao E was responsible for design of the subject and the writing of the paper; Yanhua Yu, Guohong Liu and Haiyan Li are responsible for data collection; Feifei Ren, Chao Shen and Chunyan Sha were in

charge of molecular biology experiments.

FUNDING

This work was supported by The Education Department of Jilin Province, China [grant numbers 1564557149790]

ACKNOWLEDGEMENTS

Thanks to the Education Department of Jilin Province and Baicheng Medical College for providing funds and laboratory for this study.

References

[1] R. Gélinas, F. Mailleux, J. Dontaine, et al. AMPK activation counteracts cardiac hypertrophy by reducing O-GlcNAcylation, Nat. Commun. 9 (2018) 374. https://doi.org/10.1038/s41467-017-02795-4.

[2] J.N. Cohn, Structural changes in cardiovascular disease, Am. J. Cardiol. 76 (1995) 34E-37E. https://doi.org/10.1016/S0002-9149(99)80501-3.

[3] K.-Q. Deng, G.-N. Zhao, Z. Wang, et al. Targeting TMBIM1 Alleviates Pathological Cardiac Hypertrophy., Circulation. (2017). https://doi.org/10.1161/CIRCULATIONAHA.117.031659.

[4] M.T. Piccoli, S.K. Gupta, J. Viereck, et al. Inhibition of the cardiac fibroblast-enriched lncRNA Meg3 prevents cardiac fibrosis and diastolic dysfunction, Circ. Res. 121 (2017) 575–583. https://doi.org/10.1161/CIRCRESAHA.117.310624.

[5] K. Wang, F. Liu, L.Y. Zhou, et al. The long noncoding RNA CHRF regulates cardiac hypertrophy by targeting miR-489, Circ. Res. 114 (2014) 1377–1388. https://doi.org/10.1161/CIRCRESAHA.114.302476.

[6] X. Li, Y. Dai, S. Yan, et al. Down-regulation of lncRNA KCNQ1OT1 protects against myocardial ischemia/reperfusion injury following acute myocardial infarction, Biochem. Biophys. Res. Commun. 491 (2017) 1026–1033. https://doi.org/10.1016/j.bbrc.2017.08.005.

[7] W. Ying, M. Riopel, G. Bandyopadhyay, et al. Adipose Tissue Macrophage-Derived Exosomal miRNAs Can Modulate in Vivo and in Vitro Insulin Sensitivity, Cell. 171 (2017) 372-384. https://doi.org/10.1016/j.cell.2017.08.035.

[8] C. Liu, M. Jian, H. Qi, et al. Microrna 495 inhibits proliferation and metastasis and promotes apoptosis by targeting twist1 in gastric cancer cells, Oncol. Res. 27 (2019) 389–397. https://doi.org/10.3727/096504018X15223159811838.

[9] K. Xu, J. Xiao, K. Zheng, et al. MiR-21/STAT3 Signal Is Involved in Odontoblast Differentiation of Human Dental Pulp Stem Cells Mediated by TNF-α, Cell. Reprogram. 20 (2018) 107–116.

https://doi.org/10.1089/cell.2017.0042.

[10] X. Yu, L. Zhong. Pioglitazone/microRNA-141/FOXA2: A novel axis in pancreatic β-cells proliferation and insulin secretion, Mol. Med. Rep. 17 (2018) 7931-7938. https://doi.org/10.3892/mmr.2018.8813.

[11] G.P. Diniz, C.A. Lino, C.R. Moreno, et al. MicroRNA-1 overexpression blunts cardiomyocyte hypertrophy elicited by thyroid hormone, J. Cell. Physiol. (2017). https://doi.org/10.1002/jcp.25781.

[12] W.A. Heggermont, A.P. Papageorgiou, A. Quaegebeur, et al. Inhibition of MicroRNA-146a and overexpression of its target dihydrolipoyl succinyltransferase protect against pressure overload-induced cardiac hypertrophy and dysfunction, Circulation. 136 (2017) 747-761. https://doi.org/10.1161/CIRCULATIONAHA.116.024171.

[13] Y. Yang, D.P. Del Re, N. Nakano, et al. MIR-206 Mediates YAP-Induced Cardiac Hypertrophy and Survival, Circ. Res. 117 (2015) 891-904. https://doi.org/10.1161/CIRCRESAHA.115.306624.

[14] S. Yang, P. He, J. Wang, et al. Abstract 4363: Macrophage migration inhibitory factor (MIF) and miR-301b interactively enhance disease aggressiveness by targeting NR3C2 in human pancreatic cancer, Cancer Res, 74 (2014) 4363-4363. https://doi.org/10.1158/1538-7445.am2014-4363.

[15] S. Xiang, H. Chen, X. Luo, et al. Isoliquiritigenin suppresses human melanoma growth by targeting miR-301b/LRIG1 signaling, J. Exp. Clin. Cancer Res. 37 (2018) 184. https://doi.org/10.1186/s13046-018-0844-x.

[16] Y. Tatekoshi, M. Tanno, H. Kouzu, et al. Translational regulation by miR-301b upregulates AMP deaminase in diabetic hearts, J. Mol. Cell. Cardiol. 119 (2018) 138-146. https://doi.org/10.1016/j.yjmcc.2018.05.003.

[17] Z. Liu, R. Sun, X. Zhang, et al. Transcription factor 7 promotes the progression of perihilar cholangiocarcinoma by inducing the transcription of c-Myc and FOS-like antigen 1, EBioMedicine. 45 (2019) 181-191. https://doi.org/10.1016/j.ebiom.2019.06.023.

[18] X. Xu, Z. Liu, F. Tian, et al. Clinical significance of transcription factor 7 (TCF7) as a prognostic factor in gastric cancer, Med. Sci. Monit. 25 (2019) 3957-3963. https://doi.org/10.12659/MSM.913913.

[19] B. Ye, L. Li, H. Xu, et al. Opposing roles of TCF7/LEF1 and TCF7L2 in cyclin D2 and Bmp4 expression and cardiomyocyte cell cycle control during late heart development, Lab. Investig. 99 (2019) 807–818. https://doi.org/10.1038/s41374-019-0204-2.

[20] O.H. Cingolani, Cardiac hypertrophy and the Wnt/Frizzled pathway, Hypertension. 49 (2007) 427–428. https://doi.org/10.1161/01.HYP.0000255947.79237.61.

[21] A.K. Olson, D. Ledee, K. Iwamoto, et al. C-Myc induced compensated cardiac hypertrophy increases free fatty acid utilization for the citric acid cycle, J. Mol. Cell. Cardiol. 55 (2013) 156–164. https://doi.org/10.1016/j.yjmcc.2012.07.005.

[22] H. Liang, Z. Pan, X. Zhao, et al. LncRNA PFL contributes to cardiac fibrosis by acting as a competing endogenous RNA of let-7d, Theranostics. 8 (2018) 1180-1194. https://doi.org/10.7150/thno.20846.

[23] J. Xiang, Q. Hu, Y. Qin, et al. TCF7L2 positively regulates aerobic glycolysis via the EGLN2/HIF-1α axis and indicates prognosis in pancreatic cancer, Cell Death Dis. 9 (2018) 321. https://doi.org/10.1038/s41419-018-0367-6.

[24] B.H. Cui, X. Hong, Mir-6852 serves as a prognostic biomarker in colorectal cancer and inhibits tumor growth and metastasis by targeting tcf7, Exp. Ther. Med. 16 (2018) 879-885. https://doi.org/10.3892/etm.2018.6259.

[25] L.M. Iyer, S. Nagarajan, M. Woelfer, et al. A context-specific cardiac-catenin and GATA4 interaction influences TCF7L2 occupancy and remodels chromatin driving disease progression in the adult heart, Nucleic Acids Res. 46 (2018) 2850-2867. https://doi.org/10.1093/nar/gky049.

[26] G.P. Diniz, C.A. Lino, E.C. Guedes, et al. Cardiac microRNA-133 is down-regulated in thyroid hormone-mediated cardiac hypertrophy partially via Type 1 Angiotensin II receptor, Basic Res. Cardiol. 110 (2015) 49. https://doi.org/10.1007/s00395-015-0504-7.

[27] Z.P. Huang, J. Chen, H.Y. Seok, et al. MicroRNA-22 regulates cardiac hypertrophy and remodeling in response to stress, Circ. Res. 112 (2013) 1234-1243. https://doi.org/10.1161/CIRCRESAHA.112.300682.

[28] J. Wang, Y. Song, Y. Zhang, et al. Cardiomyocyte overexpression of miR-27b induces cardiac hypertrophy and dysfunction in mice, Cell Res. 22 (2012) 515-527. https://doi.org/10.1038/cr.2011.132.

Figures

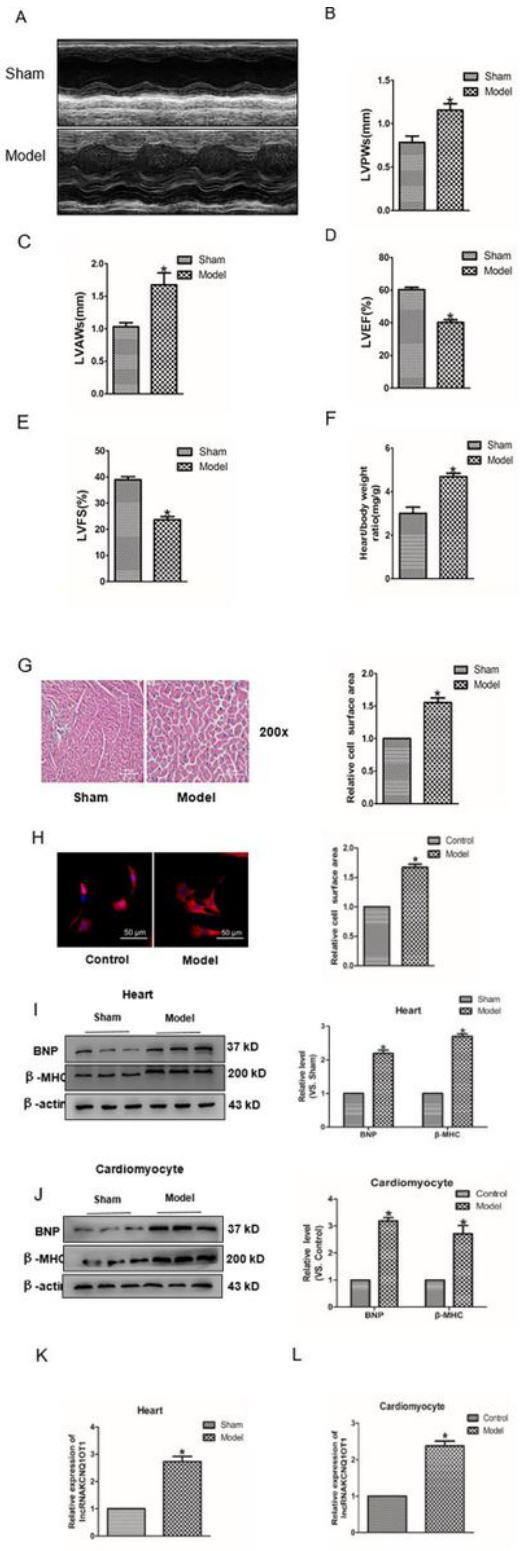

Figure 1

Increased expression of lncRNAKCNQ1OT1 in cardiac hypertrophy mode (A) Representative images of echocardiographic. Four weeks after TAC, echocardiographic imaging showed that level of left ventricular anterior wall thickness (LVPWs) (B), left ventricular posterior wall thickness (LVPWs) (C), left ventricular ejection fraction (LVEF) (D), and left ventricular fraction shortening (LVFS)(E). (F) The ratio of heart weight to body weight. (G) Representative images of HE staining (200×). (H) Immunofluorescence

staining with α-SMA antibody (200×). (I) BNP and β-MHC proteins level were detected in vivo. (J) BNP and β-MHC proteins level were detected in vitro. (K) LncRNAKCNQ1OT1 level was tested by real-time PCR analysis. (L) The expressions of lncRNAKCNQ1OT1 in vitro. Data were represented by mean ± SEM (n = 6). *P < 0.05 vs Sham or Control group.

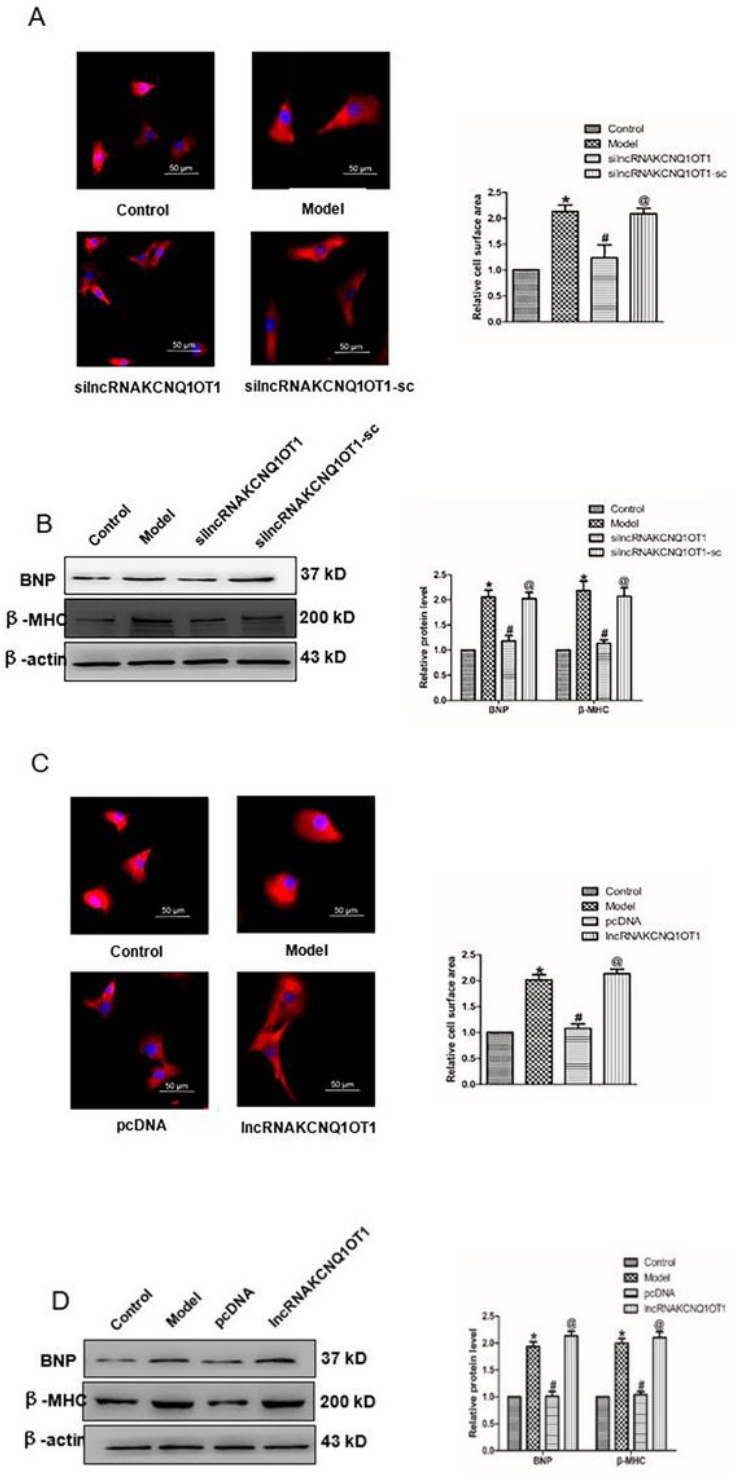

Figure 2

lncRNAKCNQ1OT1 contributes to cardiac hypertrophy (A) Immunostaining with α-SMA in cardiomyocytes displayed that knockdown of lncRNAKCNQ1OT1 decrease the cell surface area. Bar=50 μm. (B) β-MHC and BNP proteins expression by lncRNAKCNQ1OT1 knockdown. (C) Immunostaining of α-SMA in cardiomyocytes demonstrated that enhanced expression of lncRNAKCNQ1OT1 promoted the cell surface area. Bar=50 μm. (D) Western blot results of β-MHC and BNP proteins expression by lncRNAA02Rik overexpression. Mean ± SEM was used to identify the date (n = 6). *P < 0.05 vs Control group; # P < 0.05 vs Model group. @P < 0.05 vs pcDNA group.

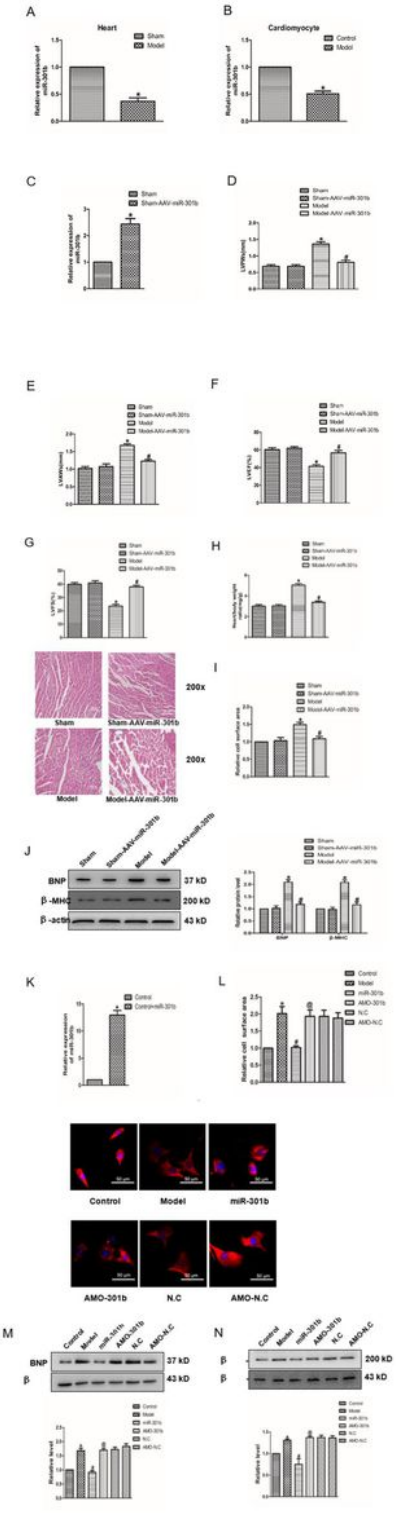

Figure 3

The role of miR-301b in cardiac hypertrophy (A) MiR-301b expression in vivo model. *P < 0.05 vs Sham. (B) MiR-301b expression in vitro model. *P < 0.05 vs Model. (C) miR-301b expression measured by real-time PCR. (D) LVPWs: left ventricular posterior wall depth. (E) LVAWs: left ventricular anterior wall thickness. (F) LVEF: left ventricular ejection fraction. (G) LVFS: left ventricular fractional shortening. (H)The ratio of heart weight to body weight. (I) Representative images of HE staining (200×). (J) BNP and β-MHC proteins expression. *P < 0.05 vs Sham-AAV-miR-301b group; # P < 0.05 vs Model group. (K) miR-301b expression measured by real-time PCR. (L) Immunofluorescence staining with α-SMA antibody. Bar=50 μm. (M) BNP protein expression. (N) β-MHC protein expression. Mean ± SEM was used to analysis the date (n = 6). *P < 0.05 vs Control group; # P < 0.05 vs Model group; @P < 0.05 vs miR-301b group.

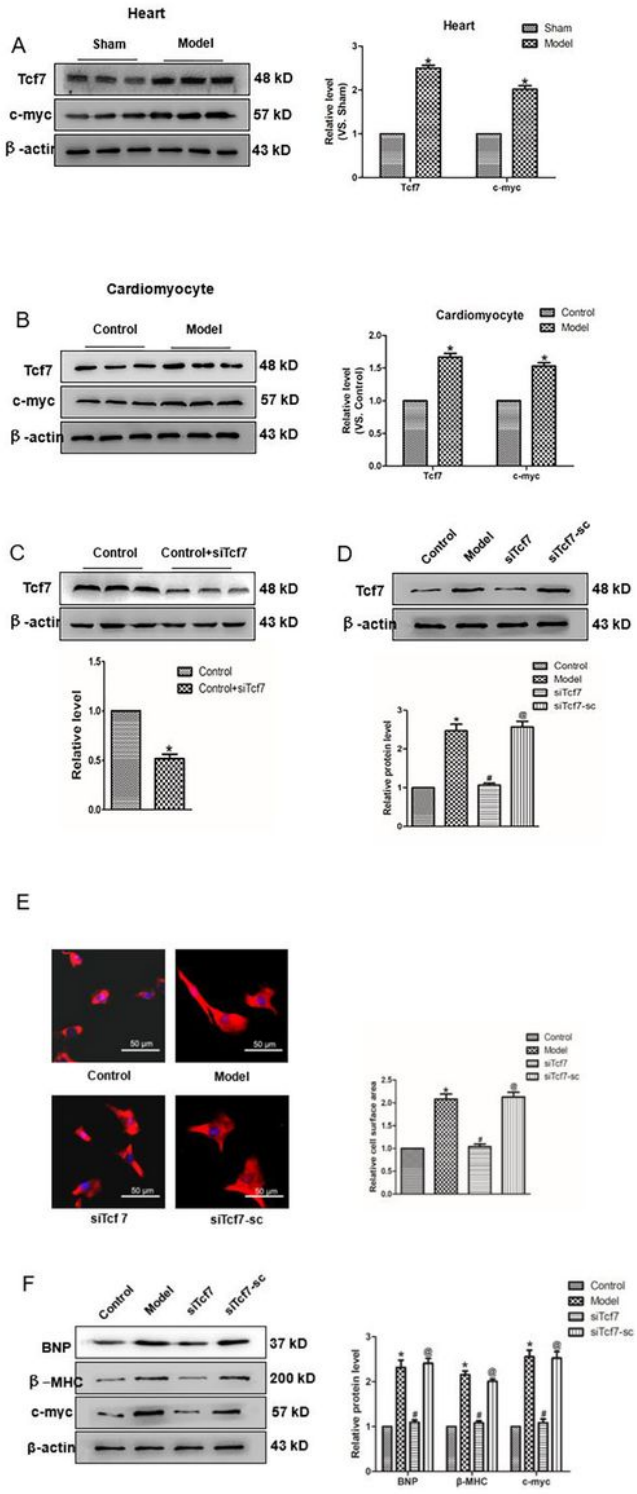

Figure 4

The activation of Tcf7 promoted cardiac hypertrophy (A) Tcf7 and c-myc proteins expression in vivo. (B) The expressions of Tcf7 and c-myc proteins were measured in vitro. (C and D) Tcf7-siRNA successfully silenced Tcf7. (E)Si-Tcf7 decreased cardiomyocytes surface area induced by Ang II (×200). (F) BNP, β-MHC and c-myc proteins expression were decreased by si-Tcf7 induced by Ang II. Mean ± SEM was used to analysis data. (n = 6) *P < 0.05 vs Control group; # P < 0.05 vs Model group; @P < 0.05 vs siTcf7 group.

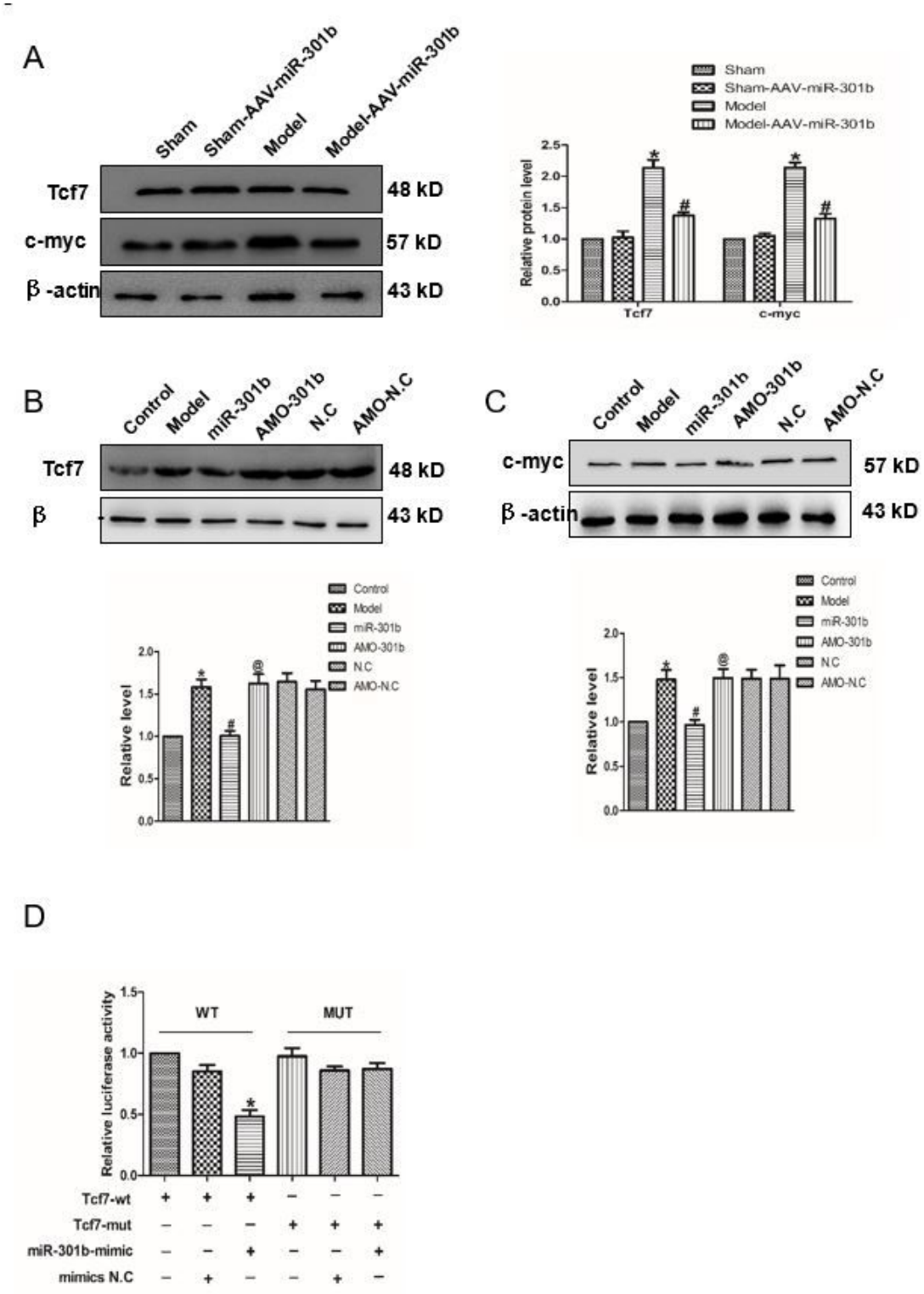

Figure 5

Tcf7 is the target gene of miR-301b in cardiac hypertrophy (A) Western blot results of Tcf7 and c-myc proteins expression by miR-301b mimic in vivo. Data were represented by mean ± SEM (n = 6). *P < 0.05 vs Sham-AAV-miR-301b group; # P < 0.05 vs Model group. (B) and (C) Western blot results of Tcf7 and c-myc proteins expression by miR-301b mimic in vitro. Mean ± SEM was used to analysis the data (n = 6). *P < 0.05 vs Control group; # P < 0.05 vs Model group; @P < 0.05 vs miR-301b group. (D) To further prove

that the miR-301b act directly on Tcf7, luciferase reporter gene was performed. Mean ± SEM was used to analysis the data. *p < 0.05 vs Tcf7-WT group, n=6.

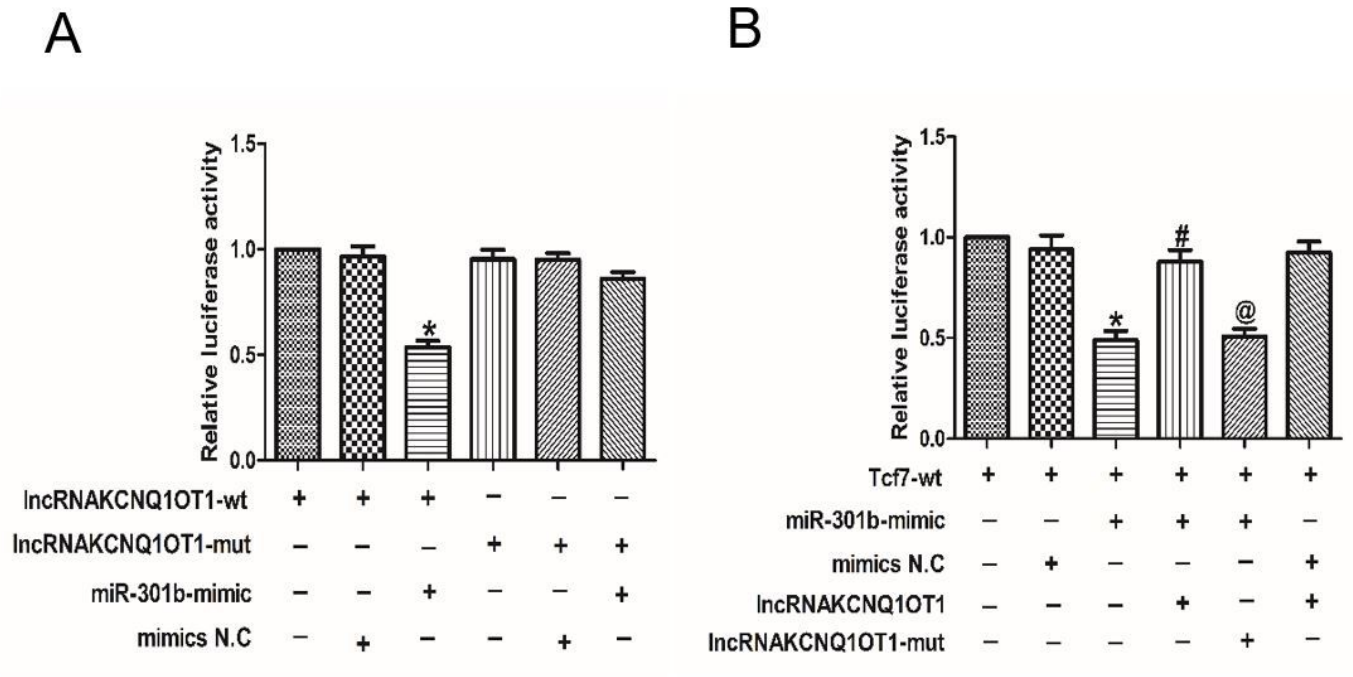

Figure 6

The relationship between lncRNAKCNQ1OT1miR-301b and Tcf7 (A) Luciferase reporter gene of chimeric vectors carrying the luciferase gene and a fragment of lncRNAKCNQ1OT1 containing wild-type or mutated miR-301b binding sites. Mean ± SEM was used to analysis the data (n=3). *p < 0.05 vs lncRNAKCNQ1OT1-WT group. (B) The luciferase reporter gene was performed to validate the regulatory interactions among lncRNAKCNQ1OT1, miR-301b and Tcf7. Mean ± SEM was performed to analysis the data (n=6). *p < 0.05 vs Tcf7-wt group, #p < 0.05 vs Tcf7-wt+miR-301b-mimic group, @p < 0.05 vs Tcf7-wt+miR-301b-mimic+lncRNAKCNQ1OT1 group.