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Silence of lncRNA CHRF protects H9c2 cells against lipopolysaccharide-induced injury via up-regulating microRNA-221

Lijuan Zhang[1], Li Wang[1], Enyu Guo[1], Yuefeng Qi*

Department of Pediatrics, Jining No.1 People's Hospital, Jining 272011, China

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ABSTRACT

Background: Cardiac hypertrophy related factor (CHRF), a newly recognized long non-coding RNA (lncRNA), is a central regulator in cardiac hypertrophy responses. This study attempted to show the potential role of lncRNA CHRF in bacterial infection caused myocarditis.

Methods: H9c2 cells were transfected with small interfering RNAs (siRNAs) specific for lncRNA CHRF alone or in combination with miR-221 inhibitor, and then subjected to lipopolysaccharide (LPS). The following parameters were measured: cell viability, apoptosis, reactive oxygen species (ROS) generation, pro-inflammatory cytokines release, microRNA (miR)-221 expression and the activation of nuclear factor-κB (NF-κB) and c-Jun N-terminal kinase (JNK) pathways.

Results: Silence of lncRNA CHRF impeded the LPS injury to H9c2 cells, as cell viability was increased ($p < .05$), apoptosis was inhibited ($p < .05$), ROS generation was decreased ($p < .01$), and the expression of interleukin (IL)-6 and tumor necrosis factor (TNF)-α was suppressed ($p < .05$). However, silence of lncRNA CHRF had no impacts on normal H9c2 cells growth ($p > .05$). miR-221 was negatively regulated by lncRNA CHRF ($p < .01$). LncRNA CHRF silence did not protect H9c2 cells against LPS when miR-221 was suppressed ($p < .05$ or $p < .01$). Also, the inhibitory effects of lncRNA CHRF silence on the activation of NF-κB and JNK pathways were flattened by miR-221 suppression ($p < .05$ or $p < .01$).

Conclusion: These in vitro data collectively demonstrated that lncRNA CHRF silence protected H9c2 cells against LPS-induced injury via up-regulating miR-221 and modulating NF-κB and JNK pathways.

1. Introduction

Myocarditis refers to myocardial inflammatory lesions caused by various factors, like infection, radiotherapy for chest tumor, antibiotics, chemotherapeutics as well as systemic autoimmune diseases. Most often, myocarditis is caused by viral infection, but some bacteria can also trigger heart-specific autoimmunity and inflammation (Whitton and Feuer, 2004). In the heart, myocarditis induces arrhythmias, chest pain, dyspnea, significant cardiomyocyte loss (Baksi et al., 2015), and even sudden death (Theleman et al., 2001). At present, drugs like digoxin and diuretics, as well as bridge therapy are always recommended to treat this disease. However, these treating methods are all supportive and aimed at relieving symptoms (Kang and An, 2018). A better understanding of myocarditis is required, which will be helpful for improving the treatment of this disease.

Long non-coding RNAs (lncRNAs) are a kind of transcripts longer than 200 nt, without protein-coding capacities. Recent decades, scientists around the world have noticed that lncRNAs have important functions in many aspects of cellular biological process (Goff and Rinn, 2015; Rafiee et al., 2018). In regard of myocarditis, several lncRNAs have been recognized as key regulators. For instance, lncRNA taurine up-regulated gene 1 (TUG1) exerted anti-apoptotic and anti-inflammatory functions in lipopolysaccharide (LPS)-injured H9c2 cells (Zhang et al., 2018). LncRNA metastasis associated lung adenocarcinoma transcript 1 (MALAT1) aggravates cardiac inflammation and dysfunction induced by sepsis (Chen et al., 2018a). These previous studies linked lncRNAs to myocarditis. Cardiac hypertrophy related factor (CHRF) is an lncRNA highly expressed in angiotensin II-stimulated cardiomyocytes and acts as a pivotal regulator in cardiac hypertrophy responses (Wang et al., 2014; Wo et al., 2018). Apart from cardiac hypertrophy, the importance of lncRNA CHRF in heart failure is also revealed recently. LncRNA CHRF was up-regulated in mice with heart failure, and silence of lncRNA CHRF exhibited cardioprotective effects (Chen et al., 2018b). It seems that lncRNA CHRF is a key

* Corresponding author at: Department of Pediatrics, Jining No.1 People's Hospital, No.6, Jiankang Road, Jining 272011, China.
E-mail address: qiyuefeng26@sina.com (Y. Qi).
[1] These authors contributed equally to this work.

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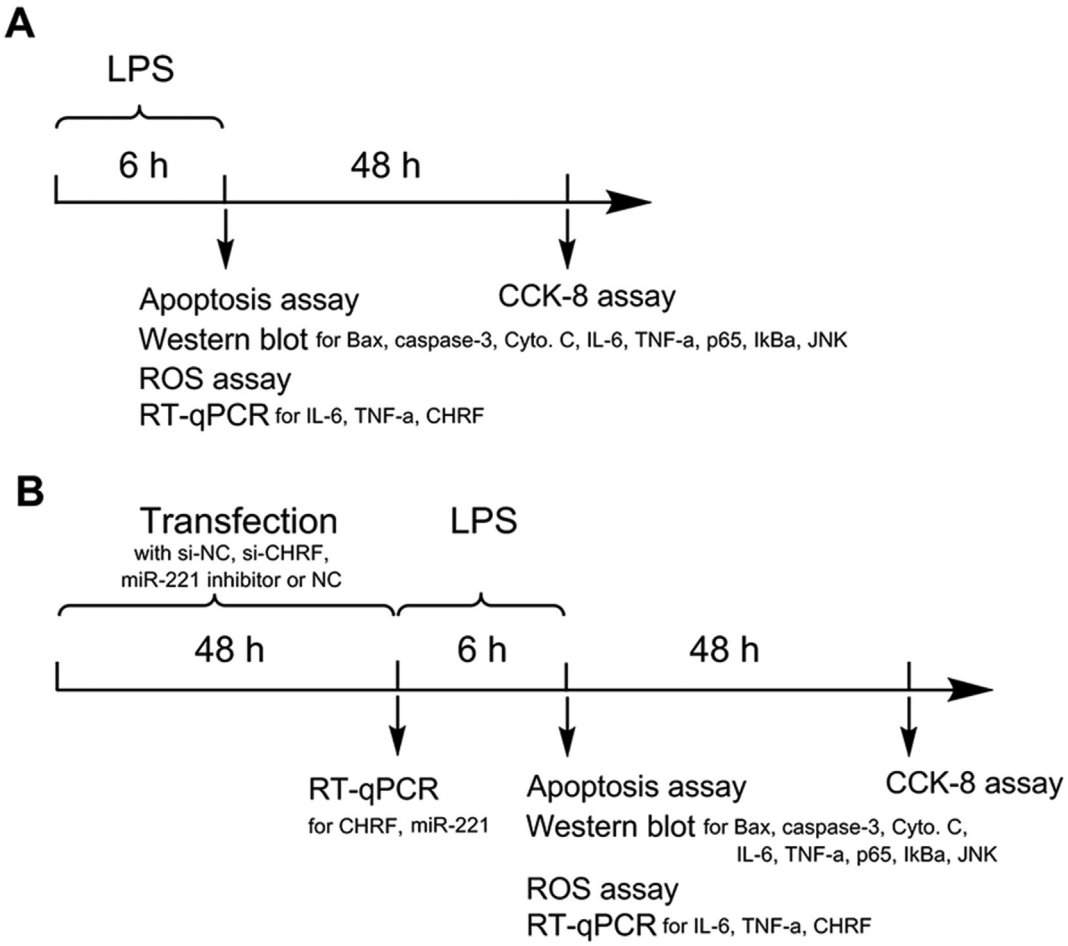

Fig. 1. Schematic representation of the experimental design on a time-dependent axis. (A) Time axis without transfection. (B) Time axis with transfection. LPS, lipopolysaccharide; RT-qPCR, real-time quantitative polymerase chain reaction; ROS, reactive oxygen species; CCK-8, cell counting kit 8; IL-6, interleukin 6; TNF-α, tumor necrosis factor α; CHRF, cardiac hypertrophy related factor; JNK, c-Jun N-terminal kinase; miR-221, microRNA-221; IκBα, inhibitor of NF-κB α; NC, negative control.

regulator in heart-related diseases. However, the function of lncRNA CHRF in myocarditis has not been investigated yet.

microRNAs (miRNAs) are another kind of non-coding transcripts with length approximately 22 nt. A number of new findings over the past decade have revealed an interesting cross-talk between lncRNAs and miRNAs (Cao et al., 2017; Yoon et al., 2014). In some cases, miRNAs can be sponged by lncRNAs, showing the negative regulation between them. miR-221 is a multifunctional miRNA involved in the regulation of many physiological and pathological events, like epithelial-mesenchymal transition process (Liang et al., 2018), neurite outgrowth (Oh et al., 2018), cardiac hypertrophy (Kakimoto et al., 2018), inflammatory response (Ye et al., 2018), and cardiac hypoxia/reoxygenation injury (Chen et al., 2016). Besides, miR-221 has been reported to exert antiviral and anti-inflammatory functions in viral myocarditis (Corsten et al., 2015), which highlighted miR-221 as a regulator of cardiac response to myocarditis.

The present work aimed to reveal the function of lncRNA CHRF on myocarditis by using an in vitro cell model made by LPS and H9c2 cells. The regulatory relationship between lncRNA CHRF, miR-221 as well as nuclear factor-κB (NF-κB) and c-Jun N-terminal kinase (JNK) pathways was studied to explain lncRNA CHRF's function.

2. Materials and methods

2.1. Cell culture

H9c2 cells were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). The complete growth medium of H9c2 cells contains 90% high-glucose dulbecco's modified eagle medium (DMEM, Invitrogen, Carlsbad, CA) and 10% fetal bovine serum (Hyclone, Logan, UT). The cells were cultured under the following conditions: 95% air, 5% CO_2, at 37 °C. The 5th to the 10th passages of H9c2 cells were used throughout the study.

2.2. Study design

The cells were transfected and treated as shown in Fig. 1. For LPS stimulation, the cells were treated by 10 μg/mL LPS for 6 h (Hwang et al., 2018). LPS from *Escherichia coli 055:B5* with purity ≥99% was purchased from Solarbio (Beijing, China).

Two sequences of small interfering RNAs (siRNAs) specific for CHRF (#1 and #2) were synthesized by RioBio (Guangzhou, China). miR-221 inhibitor and the scrambled control (NC) were purchased from GenePharma Co. (Shanghai, China). Lipofectamine 3000 reagent (Invitrogen) was used to help siRNAs and oligonucleotides to transfect into H9c2 cells. Transfection procedure was performed for 48 h.

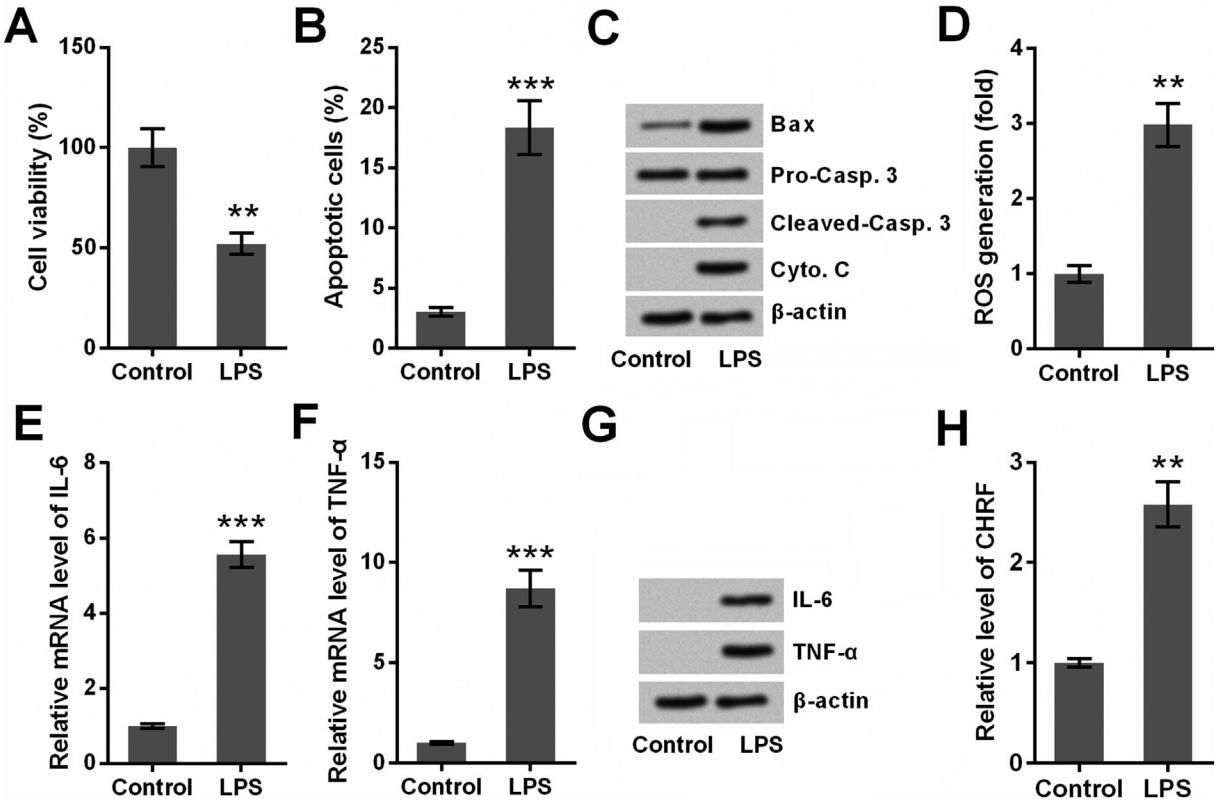

Fig. 2. LPS injured H9c2 cells. H9c2 cells were treated by 10 μg/mL LPS for 6 h. Non-treated cells served as control. (A) Cell viability, (B) apoptotic cell rate, (C) expression of apoptosis-related proteins, (D) ROS generation, (E) mRNA level of IL-6, (F) mRNA level of TNF-α, (G) protein levels of IL-6 and TNF-α, and (H) relative expression of lncRNA CHRF were assessed by CCK-8 assay, flow cytometry, ROS assay, RT-qPCR and Western blot. LPS, lipopolysaccharide; ROS, reactive oxygen species; IL-6, interleukin 6; TNF-α, tumor necrosis factor α; lncRNA, long non-coding RNA; CHRF, cardiac hypertrophy related factor; CCK-8, cell counting kit 8; RT-qPCR, real-time quantitative polymerase chain reaction. ** $p < .01$; *** $p < .001$.

2.3. Cell counting kit (CCK)-8 assay

The transfected H9c2 cells were collected and seeded in 96-well plates (5×10^3 cells/well). After treating with LPS, and another 48 h of incubation in fresh medium, 20 μL CCK-8 solution (Dojindo Molecular Technologies, Gaithersburg, MD) was added for testing cell viability. The optical density (OD) value of each well was measured by an enzyme linked immunosorbent assay (ELISA) reader (Bio-Rad, Hercules, CA) at 450 nm.

2.4. Apoptosis assay

The transfected H9c2 cells in 6-well plates (5×10^5 cells/well) were treated by LPS for 6 h. The numbers of apoptotic cells were then measured by using the AnnexinV Alexa Fluor 647/PI Apoptosis Detection Kit (Solarbio). In brief, at least 1×10^5 H9c2 cells were collected and resuspended in 100 μL Binding Buffer. 5 μL Annexin V/Alexa Fluor 647 and 10 μL propidium iodide (PI, 20 μg/mL) were added into each sample. The samples were incubated at room temperature for 15 min in the dark. Finally, 400 μL phosphate buffered solution (PBS) was added, and the flow cytometry analysis was carried out by using a FACS can (Beckman Coulter, Fullerton, CA, USA).

2.5. Western blot

After the indicated transfection and LPS stimulation, the cells were washed twice with cold PBS and then the cellular protein was extracted by Radio Immunoprecipitation Assay Lysis Buffer (Beyotime, Shanghai, China). Cytoplasmic and nuclear proteins were extracted by using Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, Shanghai, China). The purity of protein in whole-cell extracts was tested by BCA™ Protein Assay Kit (Pierce, Appleton, WI). Then, the protein was denatured by sodium dodecyl sulfate (SDS) loading buffer and heating in water bath. 20 μg protein was separated by SDS-polyacrylamide gel electrophoresis (PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes (Beyotime). After incubation with Blocking Buffer (Beyotime), primary antibodies specific for Bax (sc-20067, Santa Cruz Biotechnology, Santa Cruz, CA), Cytochrome *c* (sc-65396), interleukin (IL)-6 (sc-28343), tumor necrosis factor (TNF)-α (sc-130349), phospho (p)-p65 (sc-71677), p65 (sc-71677), inhibitor of NF-κB α (IκBα, sc-1643), p-IκBα (sc-8404), p-JNK (sc-293136), JNK (sc-7345), β-actin (sc-130065), Lamin A (sc-56137), pro caspase-3 (ab32150, Abcam, Cambridge, MA) and cleaved caspase-3 (ab2302) were used to interact with the proteins in membranes. The membranes were then incubated with the secondary antibodies and positive bands were developed by enhanced chemiluminescence method. The intensity of bands was quantified by Image Lab™ Software (Bio-Rad, Hercules, CA).

2.6. Reactive oxygen species (ROS) assay

After the indicated transfection and LPS stimulation, H9c2 cells in 6-well plates were washed twice with PBS, and then incubated with 10 μM 2′,7′-dichlorofluorescin diacetate (DCFH-DA, Sigma-Aldrich, St. Louis, MO) for 30 min at 37 °C. Subsequently, the cells were washed three times with serum-free culture medium, and collected by trypsin digestion. The cells resuspended in PBS were analyzed by the FACS can.

2.7. Real-time quantitative polymerase chain reaction (RT-qPCR)

After the indicated transfection and LPS stimulation, H9c2 cells in

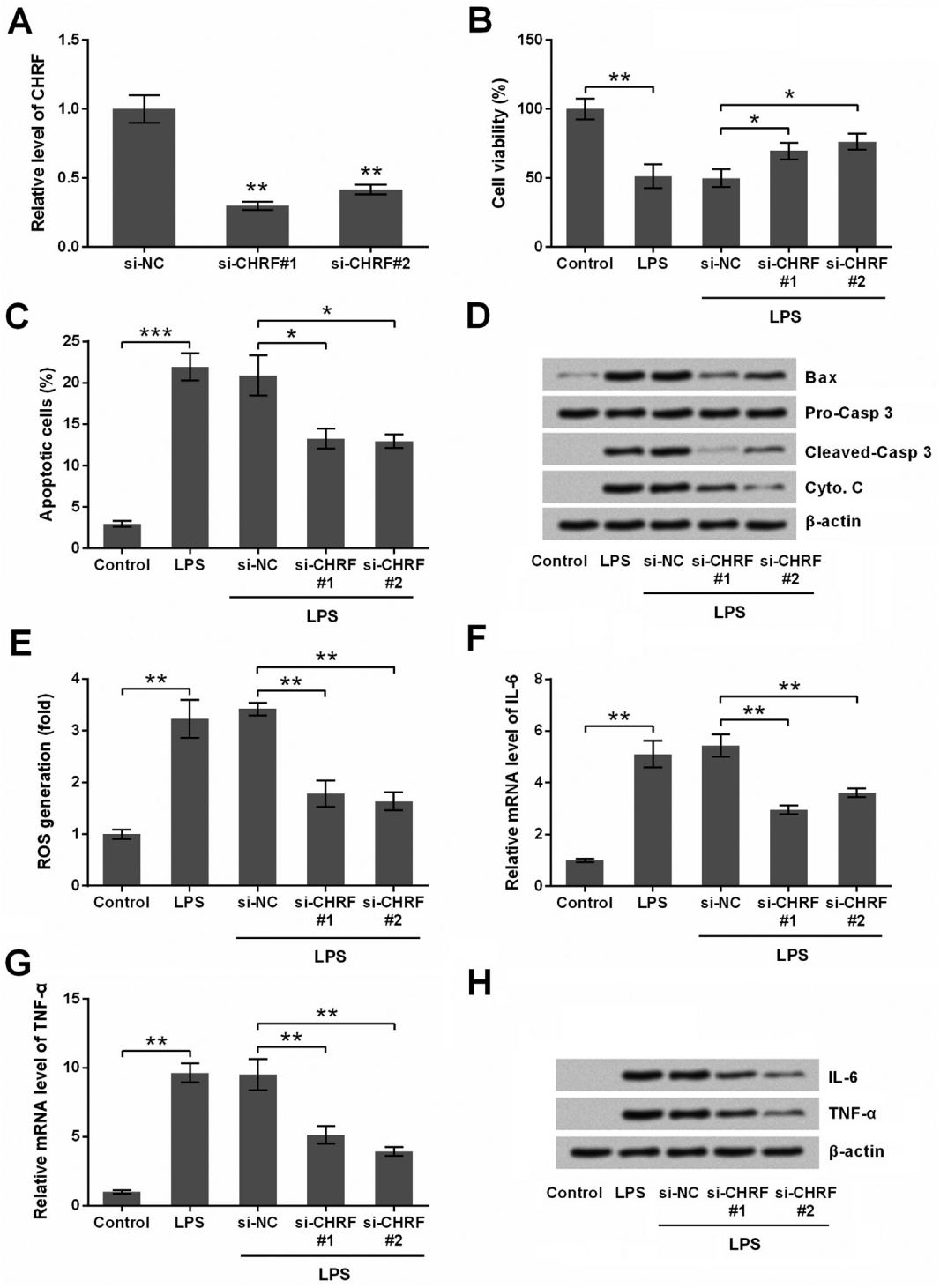

Fig. 3. Silence of lncRNA CHRF protected H9c2 cells against LPS-induced injury. (A) Relative expression of lncRNA CHRF in H9c2 cells transfected with siRNAs (#1 or #2) specific for lncRNA CHRF was measured by RT-qPCR. Vectors inserted with non-targeting sequences were transfected as negative control (si-NC). Thereafter, the transfected cells were treated by LPS. Non-treated cells acted as control. (B) Cell viability, (C) apoptotic cell rate, (D) expression of apoptosis-related proteins, (E) ROS generation, (F) mRNA level of IL-6, (G) mRNA level of TNF-α, and (H) protein levels of IL-6 and TNF-α were assessed by CCK-8 assay, flow cytometry, ROS assay, RT-qPCR and Western blot. lncRNA, long non-coding RNA; CHRF, cardiac hypertrophy related factor; LPS, lipopolysaccharide; siRNA, small interfering RNA; RT-qPCR, real-time quantitative polymerase chain reaction; ROS, reactive oxygen species; IL-6, interleukin 6; TNF-α, tumor necrosis factor α; CCK-8, cell counting kit 8. * $p < .05$; ** $p < .01$; *** $p < .001$.

24-well plates were lysed by TRIzol reagent (Invitrogen) to extract total RNAs from cell. PrimeScript RT Master Mix and TB Green *Premix Ex Taq* II both purchased from Takara (Dalian, China) were used for the reverse transcription and qPCR steps respectively for detection of IL-6, TNF-α and CHRF. Mir-X™ miRNA First-Strand Synthesis Kit and Mir-X™ miRNA qRT-PCR TB Green™ Kit (both from Takara) were used for miR-221 quantitation. The qPCR conditions were 50 °C for 2 min, 95 °C for 10 min, and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Data were calculated according to the $2^{-\Delta\Delta Ct}$ method. Internal control for IL-6, TNF-α and CHRF is β-actin, and internal control for miR-221 is U6. The

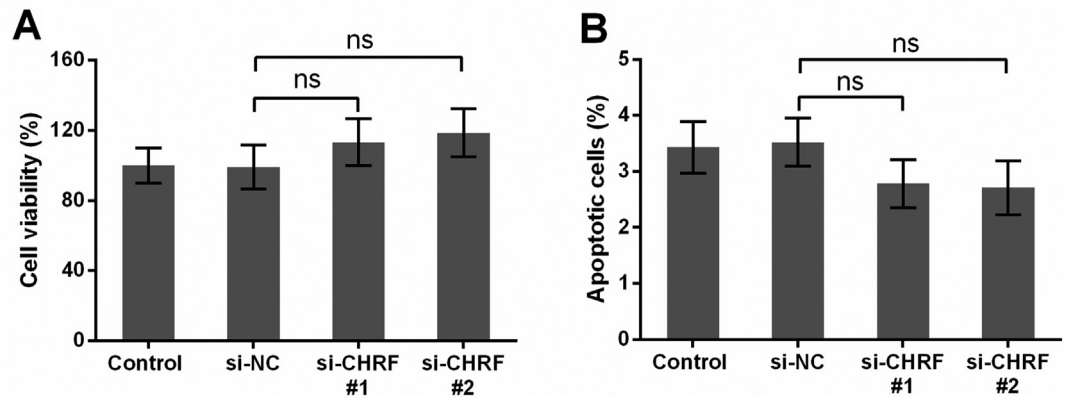

Fig. 4. Silence of lncRNA CHRF had no impacts on the viability and apoptosis of normal H9c2 cells. H9c2 cells were transfected with siRNAs (#1 or #2) specific for lncRNA CHRF. Vectors inserted with non-targeting sequences were transfected as negative control (si-NC), and cells without any transfection were considered as control. (A) Cell viability, and (B) apoptotic cell rate were respectively assessed by CCK-8 assay and flow cytometry. lncRNA, long non-coding RNA; CHRF, cardiac hypertrophy related factor; siRNA, small interfering RNA; CCK-8, cell counting kit 8. ns, $p > .05$.

primary sequences were listed as follows. CHRF: 5′-AGA TTC ACC TGG TGT CCT GAA C-3′ (F), 5'-TAC TCT GAC CAC ATA TTG TCT C-3′ (R); IL-6: 5′-GTT GCC TTC TTG GGA CTG ATG-3′ (F), 5′-ATA CTG GTC TGT TGT GGG TGG T-3′ (R); TNF-α: 5′-AGC ATG ATC CGA GAT GTG GAA-3′ (F), 5′-TAG ACA GAA GAG CGT GGT GGC-3′ (R); β-actin: 5′-GAG ACC TTC AAC ACC CCA GCC-3′ (F), 5′-AAT GTC ACG CAC GAT TTC CC-3′ (R); miR-221: 5′-TGG TCG ACA TTT CCT TAT CTG TAC TTC-3′ (F), 5'-TCG CTC GAG GCA TGT GAG ACT GTT TTA G-3′ (R); U6: 5′-TGG CAC CCA GCA CAA TGA A-3′ (F), 5'-CTA AGT CAT AGT CCG CCT AGA AGC A-3′ (R).

2.8. Statistical analysis

Data presented as mean ± standard deviation (SD). SPSS 18.0 software (SPSS Inc., Chicago, IL) was used for statistical analysis. Student *t*-test and one-way analysis of variance (ANOVA) were used for analyzing the statistical difference between two or more groups. *p*-values of < 0.05 were considered as statistically significant results.

3. Results

3.1. LPS injured H9c2 cells

To start with, H9c2 cells were treated by 10 μg/mL LPS for 6 h. As a result, cell viability was significantly reduced ($p < .01$, Fig. 2A) in LPS-treated group than the control group. On the contrary, the apoptosis was significantly induced by LPS. As compared to control group, apoptotic cell rate was significantly increased ($p < .001$, Fig. 2B), Bax, cleaved capsase-3 and Cytochrome *c* were remarkably up-regulated (Fig. 2C), and ROS generation was increased ($p < .01$, Fig. 2D) in LPS group. Besides, the mRNA ($p < .001$, Fig. 2E-F) and protein (Fig. 2G) levels of IL-6 and TNF-α were highly expressed in LPS group than the control group. These data suggested that LPS stimulation induced a significant apoptotic and inflammatory injury in H9c2 cells.

Next, RT-qPCR analysis was carried out to assess the expression changes of lncRNA CHRF in response to LPS stimulation. Results in Fig. 2H showed that lncRNA CHRF was significantly up-regulated in LPS group when compared to control group.

3.2. Silence of lncRNA CHRF protected H9c2 cells against LPS-induced injury

To study whether lncRNA CHRF plays a role in LPS injury to H9c2 cells, siRNAs specific for lncRNA CHRF were transfected into cells. As shown in Fig. 3A, lncRNA CHRF expression was significantly silenced by siRNA transfection as compared to si-NC transfection ($p < .01$). The transfection efficiency of siRNA #1 and siRNA #2 was 39.8% and 36.5%, respectively. Moreover, we found that transfection of cells with si-CHRF significantly attenuated LPS-induced injury. As compared to LPS + si-NC group, cell viability was increased ($p < .05$, Fig. 3B), apoptotic cell rate ($p < .05$, Fig. 3C) and ROS generation ($p < .01$, Fig. 3E) were decreased, and Bax, cleaved caspase-3 and Cytochrome *c* were remarkably down-regulated (Fig. 3D) in LPS + si-CHRF group. Also, the mRNA ($p < .01$, Fig. 3F-G) and protein (Fig. 3H) levels of IL-6 and TNF-α were down-regulated in LPS + si-CHRF group, as compared to LPS + si-NC group. Altogether, these data suggested that silence of lncRNA CHRF could protect H9c2 cells against LPS-induced apoptotic and inflammatory injury.

However, silence of lncRNA CHRF had no impacts on normal H9c2 cells growth. As compared to si-NC group, transfection of cells with siRNAs specific for lncRNA CHRF did not alter the viability and apoptosis of H9c2 cells ($p > .05$, Fig. 4A-B).

3.3. Silence of lncRNA CHRF protected H9c2 cells against LPS-induced injury through up-regulating miR-221

Next, the expression changes of miR-221 in response to lncRNA CHRF silence were tested to assess the involvement of miR-221 in lncRNA CHRF's function. RT-qPCR data in Fig. 5A showed that, miR-221 level was significantly up-regulated in si-CHRF group compared to si-NC group ($p < .01$), indicating miR-221 was negatively regulated by lncRNA CHRF. Subsequently, miR-221 inhibitor was transfected into cell to knock down the expression of miR-221. As shown in Fig. 5B, miR-221 level was significantly down-regulated by miR-221 inhibitor ($p < .01$). The transfection efficiency was 37.6%. Rescue assay results in Fig. 5C-I showed that, the protective functions of si-CHRF in H9c2 cells were all abolished when miR-221 was silenced. In particular, cell viability was decreased, apoptosis and ROS generation were promoted, and the expression of pro-inflammatory cytokines was increased in LPS + si-CHRF + miR-221 inhibitor group, as compared to LPS + si-CHRF + NC group ($p < .05$ or $p < .01$).

3.4. Silence of lncRNA CHRF deactivated NF-κB and JNK signaling pathways through up-regulating miR-221

Finally, we studied the regulatory roles of lncRNA CHRF and miR-221 in NF-κB and JNK signaling pathways to reveal the underlying mechanisms of which lncRNA CHRF silence protected H9c2 cells. Western blot analytical results in Fig. 6A showed that LPS promoted the export of p65 from the cytoplasm to the nucleus. The impacts of LPS on the localization of p65 were partially recovered by lncRNA CHRF silence. More interestingly, silence of lncRNA CHRF became hard to

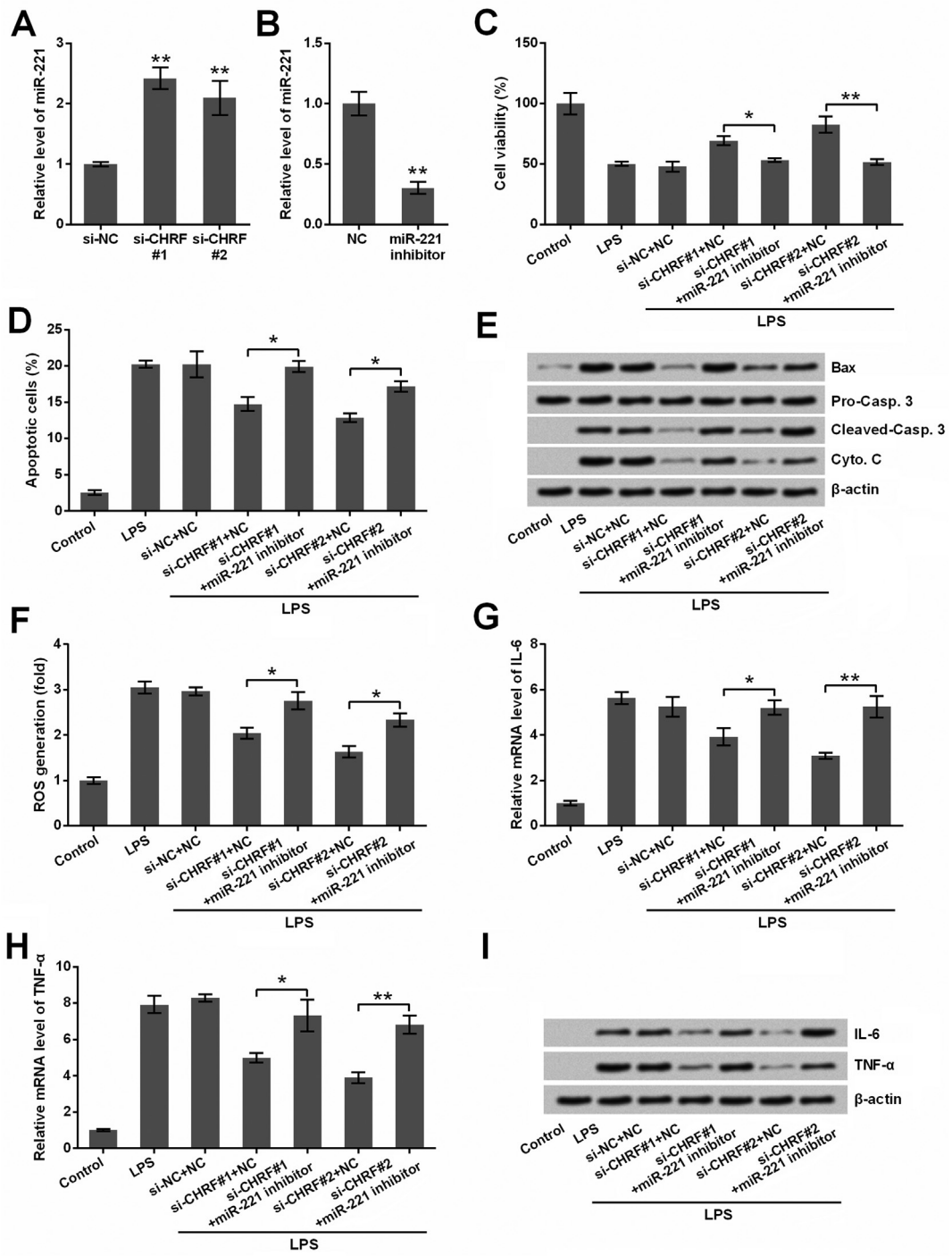

Fig. 5. Silence of lncRNA CHRF protected H9c2 cells against LPS-induced injury through up-regulating miR-221. (A) Relative expression of miR-221 in H9c2 cells transfected with siRNAs (#1 or #2) specific for lncRNA CHRF was measured by RT-qPCR. Vectors inserted with non-targeting sequences were transfected as negative control (si-NC). (B) Relative expression of miR-221 in H9c2 cells transfected with miR-221 inhibitor or its scrambled negative control (NC) was measured by RT-qPCR. H9c2 cells co-transfected with si-CHRF (#1 or #2) and miR-221 inhibitor were treated by LPS. Non-treated cells acted as control. (C) Cell viability, (D) apoptotic cell rate, (E) expression of apoptosis-related proteins, (F) ROS generation, (G) mRNA level of IL-6, (H) mRNA level of TNF-α, and (I) protein levels of IL-6 and TNF-α were assessed by CCK-8 assay, flow cytometry, ROS assay, RT-qPCR and Western blot. lncRNA, long non-coding RNA; CHRF, cardiac hypertrophy related factor; LPS, lipopolysaccharide; miR-221, microRNA-221; siRNA, small interfering RNA; RT-qPCR, real-time quantitative polymerase chain reaction; ROS, reactive oxygen species; IL-6, interleukin 6; TNF-α, tumor necrosis factor α; CCK-8, cell counting kit 8. * $p < .05$; ** $p < .01$.

recover LPS-induced alteration in p65 localization when miR-221 was suppressed. Additionally, Fig. 6B-E showed that LPS stimulation induced significant phosphorylation levels of IκBα, p65 and JNK ($p < .001$), indicating LPS induced the activation of NF-κB and JNK signaling pathways. However, the effects of LPS on these two signaling were attenuated by si-CHRF transfection ($p < .05$, $p < .01$ or $p < .001$). More interestingly, si-CHRF did not reverse the effects of LPS on these two signaling when miR-221 was silenced by inhibitor transfection.

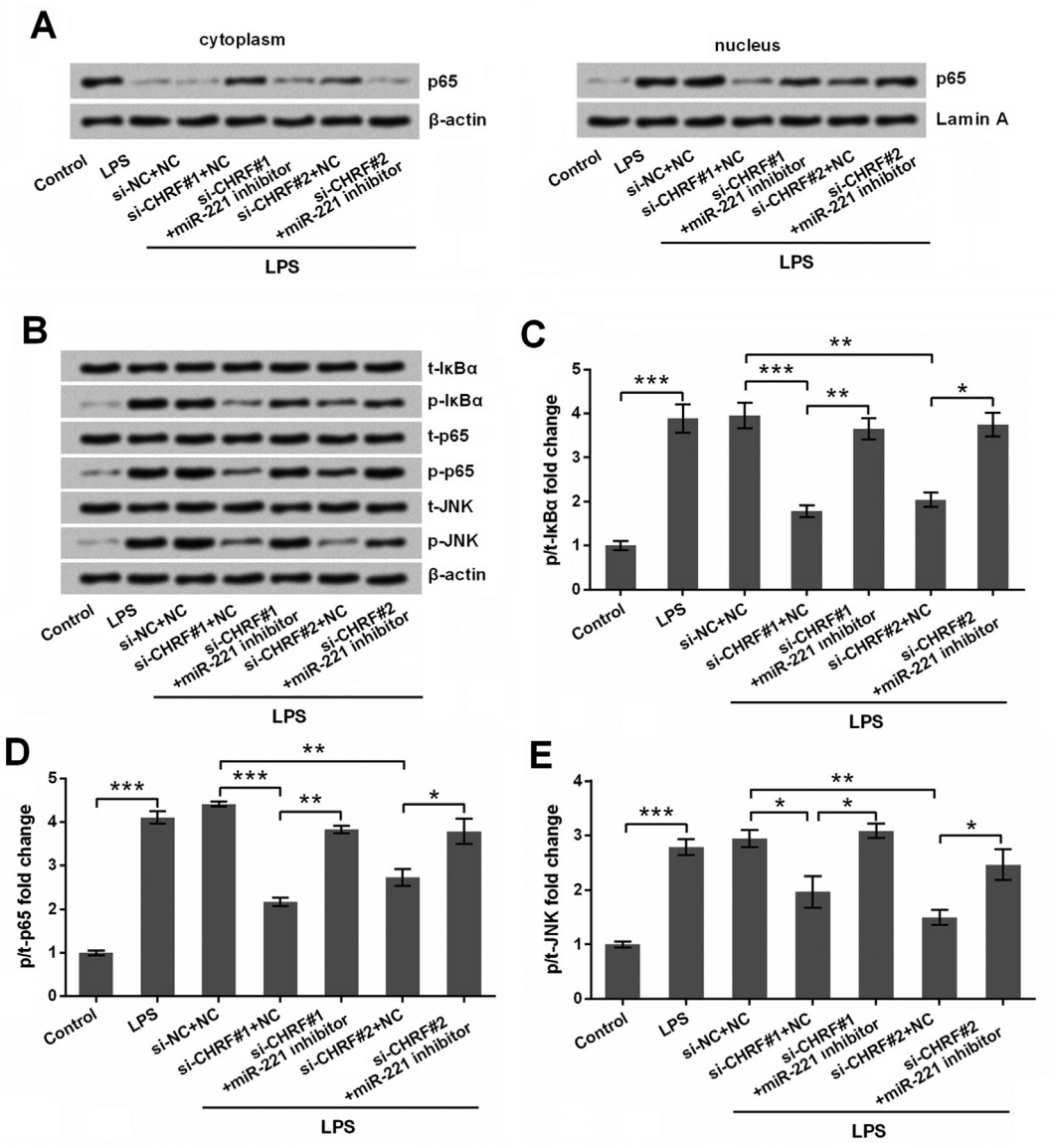

Fig. 6. Silence of lncRNA CHRF deactivated NF-κB and JNK signaling pathways through up-regulating miR-221. H9c2 cells co-transfected with siRNAs (#1 or #2) specific for lncRNA CHRF and miR-221 inhibitor were treated by LPS. Vectors inserting with non-targeting sequences (si-NC) and scrambled oligonucleotides (NC) were transfected as negative control. Non-treated cells served as control. (A) The expression changes of p65 in cytoplasm and nucleus were tested by Western blot. (B) The phosphorylation of IκBα, p65 and JNK were detected by Western blot. The fold changes of (C) phospho-(p) IκBα to total-(t) IκBα, (D) p-p65 to t-p65, and (E) p-JNK to t-JNK were quantified. lncRNA, long non-coding RNA; CHRF, cardiac hypertrophy related factor; NF-κB, nuclear factor-κB; JNK, c-Jun N-terminal kinase; miR-221, microRNA-221; siRNA, small interfering RNA; LPS, lipopolysaccharide; IκBα, inhibitor of NF-κB α. * $p < .05$; ** $p < .01$; *** $p < .001$.

4. Discussion

Myocarditis is an inflammatory disease which can be caused by injection of vital, bacteria, fungi, protozoa and metazoa, and leads to the injury of myocardium. LPS is the main component of the cell wall of gram-negative bacteria. It can cause damage in different types of cells, showing cell loss, ROS generation and overproduction of pro-inflammatory cytokines. This phenomenal was also observed in this study which was performed in H9c2 cells, indicating a cell model of myocarditis was constructed successfully. We then tested the functional effects of lncRNA CHRF on H9c2 cells stimulated by LPS. Data indicated that silence of lncRNA CHRF prevented LPS injury to H9c2 cell. miR-221 might be one of the downstream effectors of lncRNA CHRF, as miR-221 was negatively regulated by lncRNA CHRF. Besides, miR-221 inhibitor flattened the protective effects of lncRNA CHRF silence on H9c2 cells. What's more, the inhibitory effects of lncRNA CHRF silence on NF-κB and JNK signaling pathways were also impeded by miR-221 inhibitor.

Apoptosis is a non-inflammatory programmed cell death caused by local physiological or pathological conditions. Typically, mitochondrial-dependent apoptosis is the most common type of apoptosis, and is mediated by a series of genes, like Bax, Bcl-2, Caspases and Cytochrome *c*. Recent evidence suggests that myocardial cells undergo excessive apoptosis under LPS stimulation (Wang et al., 2016; Xu et al., 2018). Besides, cell-mediated immune responses can lead to apoptosis (Huber, 2000). The present work suggested that silence of lncRNA CHRF could prevent LPS-triggered mitochondrial apoptosis and inflammation, as cell viability was increased, apoptosis rate was decreased, Bax, activated Caspases-3 and Cytochrome *c* were all down-regulated, and the expression of IL-6 and TNF-α was inhibited. These findings were consistence with previous studies (Chen et al., 2018b; Wu et al., 2016), suggesting the cardioprotective effects of lncRNA CHRF silence in heart.

ROS is a key regulator in apoptosis induction (Simon et al., 2000). Mitochondrial permeabili transition pore (MPTP) can be changed by

ROS directly or indirectly, that promotes Cytochrome *c* release from mitochondria to cytoplasm, and leads to caspase reaction. Apart from apoptosis, ROS also triggers inflammation (Kim et al., 2014). It has been reported that TNF influences immune cells by regulating the generation of ROS (Blaser et al., 2016). In the present work, ROS generation was inhibited by lncRNA CHRF silence, further confirming the anti-apoptotic and anti-inflammatory effects of lncRNA CHRF silence.

LncRNAs have recently been reported to work as molecular sponges for miRNAs, which bind with miRNAs and having them exhausted (Yoon et al., 2014). Several previous studies have shown that lncRNA CHRF confers its function via sponging miR-489 (Tao et al., 2017; Wang et al., 2014; Wu et al., 2016). Another recent literature demonstrated that lncRNA CHRF induced cardiac hypertrophy partially through sequestering miR-93 from RAC-γ serine/threonine-protein kinase (AKT3) (Wo et al., 2018), indicating miR-93 was a downstream effector of lncRNA CHRF. In this study, we found that miR-221 was negatively regulated by lncRNA CHRF. Moreover, rescue assay results showed that the effects of lncRNA CHRF on H9c2 cells were remarkably flattened by miR-221 suppression. Altogether, this study suggested that miR-221 is a downstream molecule of lncRNA CHRF, and anti-LPS properties of lncRNA CHRF silence were possibly through up-regulating miR-221.

It has been shown that NF-κB and JNK pathways are associated with the development of myocarditis (Kim et al., 2004; Watanabe et al., 2013). Indeed, NF-κB and JNK are two drivers of cardiomyocytes death and inflammation. Inhibition of these two signaling is capable of protecting cardiomyocytes against various stimulations, like high glucose (Pan et al., 2014), streptozotocin (Zuo et al., 2018), H_2O_2 (Wei et al., 2014) and LPS (Meng et al., 2018). In the present study, NF-κB and JNK signaling was significantly activated by LPS, and lncRNA CHRF silence impeded it. More interestingly, the impacts of lncRNA CHRF silence on these two signaling were flattened by miR-221 suppression. These findings preliminarily suggested that lncRNA CHRF silence deactivated NF-κB and JNK pathways also via a miR-221-dependent fashion. Thus, it seems that silence of lncRNA CHRF exhibited cardioprotective effects through up-regulating miR-221 and thus blocking the activation of NF-κB and JNK pathways.

5. Conclusion

To conclude, our in vitro data collectively demonstrated that lncRNA CHRF silence protected H9c2 cells against LPS injury via up-regulating miR-221 and modulating NF-κB and JNK pathways. These data for the first time suggested lncRNA CHRF as a potential target for treating myocarditis. This study suggests that targeting lncRNA CHRF may be an effective strategy to protect cardiomyocyte against myocarditis induced by bacteria infection.

Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this article.

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