microRNA-30c promotes myocardial ischemia reperfusion injury by activating SIRT1 mediating NF-κB pathway

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Research article

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Abstract

Background This study aimed to investigate the effect of miR-30c on myocardial ischemia reperfusion (IR) injury and its underlying molecular mechanisms.Methods In our study, rat myocardial IR injury model was established and hemodynamic examination was performed. Moreover, the myocardial infarct size was detected by TTC staining. The pathologic change of myocardial tissues was measured by HE staining. The myocardial cell apoptosis was measured by TUNEL staining and flow cytometry. The expression of miR-30c and Sirtuin 1 (SIRT1) was detected by qRT-PCR. The levels of IL-1β, IL-6 and TNF-α were detected by ELISA. The expressions of Bax, Bcl-2, caspase-3, p-IκBα, IκBα, p-NF-κB p65, NF-κB p65 and SIRT1 were detected by western blot. The luciferase activity was measured by dual luciferase reporter gene assay. Interaction between miR-30c and SIRT1 were analyzed by RNA immunoprecipitation assay.Results Our results showed that rat myocardial IR injury model was successfully established and IR injury induced myocardial injury in rats. miR-30c increased the levels of IL-1β, IL-6 and TNF-α and myocardial cell apoptosis by activating NF-κB pathway. In addition, we also confirmed that SIRT1 was the target gene of miR-30c. SIRT1 could reverse the effect of miR-30c on the process of inflammation and apoptosis, as well as the activation of NF-κB pathway in myocardial cells.Conclusions Our study demonstrated that miR-30c could promote myocardial ischemia reperfusion injury through activating SIRT1 mediating NF-κB pathway.

Background

Ischemic heart disease refers to a series of diseases characterized by myocardial ischemia, such as angina pectoris and myocardial infarction [1]. Recently, the most common treatment strategies for ischemic heart disease has been considered to be the reperfusion to ischemic myocardium [2]. Although timely restoring blood flow can relieve myocardial infarction to a large extent, the prognosis of patients is still poor due to their worsened heart function [3]. Therefore, it is urgent to find new therapeutic methods and targets for myocardial infarction treatment.

microRNAs (miRNAs) are identified as a class of small endogenous noncoding RNAs with 19-25 nucleotides in length, which modulate gene expression in the post-transcriptional level [4,5]. Recently, more and more studies have indicated that miRNAs is connection with many cardiovascular diseases, such as myocardial ischemia reperfusion (IR) injury [6]. For example, inhibiting miR-181b-5p could protect cardiomyocytes against ischemia/reperfusion injury through targeting AKT3 and PI3KR3 [7]. Zhao et al. [8] have reported that miR-374a could protects against myocardial IR injury in mice via targeting MAPK6 pathway. Nevertheless, the function of miR-30c on myocardial IR injury and its related mechanism have been rarely studies.

Nuclear factor κB (NF-κB) is reported to play important roles in multiple biological functions including innate immunity, inflammation, cell proliferation and apoptosis [9,10]. Accumulating researches have revealed that myocardial IR injury is associated with the activation of NF-κB [11]. Moreover, emerging

evidences have indicated that various miRNAs exert vital roles by regulating NF-κB pathway in myocardial IR injury. For instance, miR-146a overexpression reduces myocardial IR injury via inhibiting the activation of NF-κB pathway [12]. However, whether miR-30c affects myocardial IR injury through regulating NF-κB pathway is unknown.

In this research, we explored the function of miR-146a on myocardial IR injury and its related molecular mechanisms in rat. Our results indicated that miR-30c could promote myocardial IR injury through activating SIRT1 mediating NF-κB pathway. Findings of our study may provide new theoretical foundation for deeply exploring the treatment of myocardial IR injury.

Methods

Animals

Sixty male Sprague-Dawley (SD) rats (weighting 180-200 g) were supplied by Peking University Laboratory Animal Center. All rats were kept at 22-24°C and 55-60% humidity on a 12 h light-dark cycles. Water and food were free to access. All animal experiments were conducted strictly in accordance with the National Institutes of Health guide for the care and use of Laboratory animals.

After the study, all rats were euthanized. The right hand held the rat tail and pull it back, and the left thumb and forefinger pressed down firmly on the mouse head at the same time. The external force was used to dislocate the cervical spine of the rat, and the spine and the brain were disconnected.

Establishment of the rat myocardial ischemia-reperfusion (IR) model

The left anterior descending (LAD) coronary artery was ligated to establish IR rat model as described in the preivious study [13,14]. Firstly, rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). LAD of coronary artery in IR group was occluded by using 6-0 silk suture slipknot for 30 min and then reperfused for 2 h. For the Sham-operation group, an identical procedure was performed without the LAD ligation.

Hemodynamic examination

To evaluate the cardiac function, changes of hemodynamic parameters including left ventricular ejection fraction (LVEF), left ventricular systolic pressure (LVSP), left ventricular end-diastolic volume (LVEDV), Left ventricular end-systolic volume (lvesv), left ventricular end-diastolic pressure (LVEDP), the maximum up rate of left ventricular pressure ($+dP/dt_{max}$) and the maximum down rate of left ventricular pressure (-dP/dtmax) were measured and recorded after a week of IR model by using an 8-channel polygraph system.

Myocardial infarct size determination

Myocardial infarct size was assessed using triphenyltetrazolium chloride (TTC, Sangon, China) staining. At one week after IR, rats were killed, and the hearts were obtained. Subsequently, the ventricular

tissue was sliced into 5 pieces of equal thickness. The slices were then incubated in 2% TTC for 15 min in dark and fixed in 10% formaldehyde for 10 min. The area of infarction tussues was determined by an image analyser. Infarct size was calculated as the ratio of the ventricular infarction areato ventricular total area × 100%.

Hematoxylin-Eosin (HE) Staining

After a week of IR, the hearts were collected and fixed in 4% formaldehyde overnight at 4°C. The paraffin-embedded tissues were cut into 5 μm thick slices after dehydration and vitrification. The paraffin sections were deparaffined in xylene and rehydrated in gradient ethanol. Subsequently, the tissue sections were stained with HE dye. Finally, tissues histopathological changes were analyzed by a light microscope (400 ×) and images were acquired.

Terminal dexynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay

At one week after IR, the heart was collected from rat. After washing twice time, the heart was embedded in paraffin and then heart sections were stained. Apoptotic cells were stained brown due to the binding of dUTP enzyme to their fragmented DNA. Apoptotic cells were counted for five fields per section under a magnification (400×) by a blinded manner.

Cardiacmyocyte cell culture

The myocardial tissues of the ischemic part were collected. The myocardial tissue was minced and then centrifuged for 5 min. Then, the tissue pieces were digested by using collagenase IV (0.45 mg/ml), 0.1% trypsin and 15 μg/ml DNase I. After centrifugal, myocardial cells were collected. Cardiomyocytes in Sham and IR groups were cultured in RPMI 1640 medium (Gibco, USA) containing 15% FBS. The myocardial cells (2.5×10^7 cells/well) were planted into 6-well plates and cultivated in CO_2 incubator.

Cell transfection and grouping

Myocardial cells (1×10^5) of IR group were planted into 24-well plates. After 24 hours of cell adherence, medium was removed. The miR-30c mimics, miR-30c inhibitor, SIRT1 siRNA and the negative control (NC mimics, NC inhibitor and control siRNA) were supplied by Genepharma (Shanghai, China). To further investigate whether SIRT1 is involved in miR-30c function on myocardial cells, SIRT1 siRNA1, SIRT1 siRNA2, SIRT1 siRNA3 and control siRNA were separately transfected into myocardial cells by using Lipofectamine® 3000 reagent. And the transfected myocardial cells were randomly assigned to 5 groups: Control group (no-treated group), si-NC (treated with control siRNA), siRNA1 group (treated with SIRT1 siRNA1), siRNA2 group (treated with SIRT1 siRNA2) and siRNA3 group (treated with SIRT1 siRNA3). SIRT1 siRNA2 with the best transfection effect was selected for subsequent experiments.

The miR-30c mimics, miR-30c inhibitor, SIRT1 siRNA2 and their corresponding negative control (NC mimics, NC inhibitor and control siRNA2) were transfected into myocardial cells by Lipofectamine® 3000

reagent. The transfected myocardial cells were assigned to 9 groups: IR group (no-treated group), inhibitor NC group (treated with NC inhibitor), miR-30c inhibitor group (treated with miR-30c inhibitor), mimics NC group (treated with NC mimics), miR-30c mimics group (treated with miR-30c mimics), siRNA2 NC + miR-30c inhibitor NC group (treated with control siRNA2 and NC inhibitor), siRNA2 + miR-30c inhibitor NC group (treated with SIRT1 siRNA2 and NC inhibitor), siRNA2 + miR-30c inhibitor group (treated with SIRT1 siRNA2 and miR-30c inhibitor) and siRNA2 NC + miR-30c inhibitor group (treated with control siRNA2 and miR-30c inhibitor).

Apoptosis assay

After 48 h of transfection, the myocardial cells were collected. Myocardial cells were washed twice with PBS and then stained by using Annexin V-FITC and propidium iodide (PI) for 15 min in dark. Finally, myocardial cells apoptosis was detected by a flow cytometer (Beckman Coulter, USA).

Enzyme linked immunosorbent assay (ELISA)

The protein samples from myocardial cells and tissues were extracted and immediately placed on ice. The levels of inflammatory factors including TNF-α, IL-1β and IL-6 were detected using their correaponding ELISA kits (Thermo Fisher Scientific, USA).

Real-Time fluorogenic PCR assays

Total RNA was extracted from myocardial cells and tissues by using TRIZOL (Invitrogen, USA). Then, total RNA was reverse-transcribed into cDNA by a Reverse Transcription Kit (ThermoScientific, USA) and measured by using qRT-PCR (Bio-Rad, USA) with SYBR Green Mixture (Roche, Switzerland). Primers used for qRT-PCR analysis were as follows: miR-30c F: 5'-GGGGTGTAAACATCCTACAC-3', R: 5'-GTGGAGTCGGCAATTGCACT-3'; U6 F: 5'-GCTTCGGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAC GAATTTG CGTGTCAT-3'; SIRT1 F: 5'-AAGGAGCAGATTAGTAAGC-3', R: 5'-TAGAGGATAAGGCGTCAT-3'; GAPDH F: 5'-GACGGCCGCATCTTCTTGT-3', R: 5'-CACACCGACCTTCACCATTTT-3'. GAPDH and U6 were respectively used as internal controls of SIRT1 and miR-30c.

Western blot analysis

Total proteins from myocardial cells and tissues were extracted using RIPA lysis buffer (Beyotime, China) according to themanufacturer's protocol. 50 μg of protein samples were separated by 10% SDS-PAGE and then transferred onto polyvinylidene difluoride membrane. After blocked with 5% non-fat milk for 2 h, the membranes were incubated with the primary antibody (Bax, 1:1000, 14796; Bcl-2, 1:1000, 4228s; IκBα, 1:500, #4814; p-IκBα, 1:500, #2859; SIRT1, 1:1000, #2310, Cell signal, USA; NF-κB p65, 1:1000, SAB4502610; p-NF-κB p65, 1:1000, SAB4301496, Sigma Aldrich, USA; caspase-3, 1:1000, sc-271759; β-actin, 1:1000, sc-517582, Santa Cruz, USA) with light shaking overnight at 4°C. After washing three times, peroxidase-labeled secondary antibody was used to incubate membranes for 2 h at room temperature. Finnally, the protein bands were visualized with ECL system (Thermo, USA).

Dual luciferase reporter gene assay

TargetScan was used to predict the targeted relationship between SIRT1 and miR-30c. We amplified 3′-untranslated region (3′-UTR), containing miR-30c binding site of SIRT1 and then cloned 3′-UTR fragment into pmirGLO luciferase vector (Promega, USA) to construct wild pmirGLO-WT-SIRT1-3′-UTR (SIRT1-wt) and mutant pmirGLO-MUT-SIRT1-3′-UTR (SIRT1-mut). For luciferase assay, the reporter vectors with miR-30c mimics and miR-30c mimics NC were respectively co-transfected into myocardial cells by Lipofectamine 3000 Reagent. Then, the myocardial were grouped as follows: MT + miR-30c mimics group (treated with SIRT1-mut and miR-30c mimics), MT + NC group (treated with SIRT1-mut and miR-30c mimics NC), WT + miR-30c mimics group (treated with SIRT1-wt and miR-30c mimics) and WT + NC group (treated with SIRT1-wt and miR-30c mimics NC). After 48 h of transfection, the luciferase activity was measured using a dual luciferase kit (Promega).

RNA immunoprecipitation (RIP) assay

The RIP assay was used to isolate target RNA-protein complexes by Magna RIP Kit (Millipore, USA). Breifly, myocardial cells were lysed in lysis buffer. Then, anti-Ago2 and IgG bound beads were added into cell lysates at 4°C for 2 h. After washed with PBS, the RNA-protein-beads complexes were isolated using Trizol reagent. Finally, the expressions of miR-30c and SIRT1 were measured by q-PCR.

Statistical analysis

All data were analyzed using SPSS 22.0 Statistical Software (Chicago, IL). Quantitative results were expressed as mean ± SD. Statistical differences were determined by using Student's t test for two groups, or a one-way ANOVA followed by Tukey's post hoc test. The significant level of the statistical analysis was set at p<0.05.

Results

IR induces myocardial injury in rats

As shown in Figure 1A, the value of LVEF, LVSP, +dP/dt$_{max}$ and -dP/dtmax in IR group was lower than that in Sham group (P < 0.05), while LVEDV, LVESV and LVEDP value was increased (P < 0.05). Moreover, the infarct size of IR rat was significantly increased (P < 0.05) (Figure 1B). Furthermore, HE staining results showed that myocardial fibers were neatly arranged without inflammatory cell infiltration in Sham group. On the contrary, myocardial fibers were disorganized with significant inflammatory cell infiltration in IR group (Figure 1C). The levels of IL-6, IL-1β and TNF-α in Sham group were higher than those in IR group (P < 0.05) (Figure 1D). Moreover, TUNEL assay indicated that myocardial IR injury markedly caused cell apoptosis (P < 0.05) (Figure 1E). The expressions of Bax, caspase-3, p-NF-κB p65/ NF-κB p65 were increased in IR group in comparison to Sham group (P < 0.05), while the expressions of Bcl-2 and p-IκBα/ IκBα were decreased (P < 0.05) (Figure 1F-H). All the results suggested that IR could induce myocardial injury in rats.

miR-30c promotes the process of inflammation and apoptosis by activating NF-κB pathway in myocardial cells

As shown in Figure 2A, miR-30c expression in miR-30c inhibitor group was reduced compared with IR and inhibitor NC group ($P < 0.05$). In comparison to IR and mimics NC group, miR-30c expression of miR-30c mimics group was increased ($P < 0.05$), suggesting the transfection was successful. The level of IL-6, IL-1β and TNF-α in miR-30c inhibitor group was lower than that in IR and inhibitor NC group ($P < 0.05$), while their level of miR-30c mimics group was higher than that of IR and mimics NC group ($P < 0.05$) (Figure 2B). Figure 2C results indicated that cell apoptosis of miR-30c inhibitor group was lowerthan that of IR and inhibitor NC group ($P < 0.05$). Meanwhile, cell apoptosis was increased in miR-30c mimics group compared with IR and mimics NC group ($P < 0.05$). The expressions of Bax, caspase-3, p-NF-κB p65/ NF-κB p65 were decreased in miR-30c inhibitor group in comparison to IR and inhibitor NC ($P < 0.05$), while Bcl-2 and p-IκBα/ IκBα were increased ($P < 0.05$) (Figure 2D and E). In comparison to IR and mimics NC group, Bax, caspase-3, p-NF-κB p65/ NF-κB p65 expressions were increased in miR-30c mimics group ($P < 0.05$), while Bcl-2 and p-IκBα/ IκBα were decreased ($P < 0.05$) (Figure 2D and E). The results above indicated miR-30c might facilitate the process of inflammation and apoptosis by activating NF-κB pathway in myocardial cells.

SIRT1 is the target gene of miR-30c

As shown in Figure 3A, miR-30c expression in IR group was dramatically decreased in comparison to Sham group ($P < 0.05$). Moreover, Figure 3B indicated miR-30c expression was negatively correlated with the expression of SIRT1 ($P < 0.05$). Figure 3C showed that SIRT1 mRNA and protein expressions of myocardial cells in miR-30c inhibitor group were increased in comparison to IR and inhibitor NC group ($P < 0.05$). Meanwhile, SIRT1 expressions in miR-30c mimics group were decreased in comparison to IR and mimics NC group ($P < 0.05$) (Figure 3C). TargetScan predicted that the binding site of SIRT1 to miR-30c was the 3'-UTR region (Figure 3D). In addition, miR-30c mimics significantly reduced the intensity of luciferase activity in the SIRT1-Wt plasmid group ($P < 0.05$), but it had no significant effect on luciferase activity intensity in the SIRT1-Mut plasmid group ($P > 0.05$) (Figure 3E). Furthermore, RIP assay indicated the expressions of SIRT1 and miR-30c were increased in Input and Anti-Ago2 group compared with Anti-IgG group ($P < 0.05$) (Figure 3E). All those results suggested that SIRT1 might be the target gene of miR-30c.

miR-30c promotes the process of inflammation and apoptosis by activating SIRT1 mediating NF-κB pathway in myocardial cells

Figure 4A indiated SIRT1 expression was decreased in siRNA1, siRNA2 and siRNA3 group in comparison to Control and si-NC group ($P < 0.05$) (Figure 4A). It was worth noting that SIRT1 expression was the lowest in siRNA2 group. Therefore, siRNA2 was selected for subsequent experiments. As shown in Figure 4C, when compared with siRNA2 NC + miR-30c inhibitor NC group, cell apoptosis was significantly increased in siRNA2 + miR-30c inhibitor NC group ($P < 0.05$), while cell apoptosis was decreased in siRNA2 NC + miR-30c inhibitor group ($P < 0.05$). Meanwhile, cell apoptosis was decreased in

siRNA2 + miR-30c inhibitor and siRNA2 NC + miR-30c inhibitor group compared with siRNA2 + miR-30c inhibitor NC group (P < 0.05). Moreover, Bax, caspase-3, p-NF-κB p65/ NF-κB p65 expressions were significantly increased in siRNA2 + miR-30c inhibitor NC group (P < 0.05), while their expressions were markedly decreased in siRNA2 NC + miR-30c inhibitor group (P < 0.05) in comparison to siRNA2 NC + miR-30c inhibitor NC group (Figure 4B and D). When compared with siRNA2 NC + miR-30c inhibitor NC group, Bcl-2 and p-IκBα/ IκBα expressions were dramatically decreased in siRNA2 + miR-30c inhibitor NC group (P < 0.05), while their expressions were markedly increased in siRNA2 NC + miR-30c inhibitor group (P < 0.05) (Figure 4B and D). Meanwhile, when compared to siRNA2 + miR-30c inhibitor NC group, Bax, caspase-3, p-NF-κB p65/ NF-κB p65 expressions were decreased in siRNA2 + miR-30c inhibitor and siRNA2 NC + miR-30c inhibitor group (P < 0.05), while Bcl-2 and p-IκBα/ IκBα were increased in siRNA2 + miR-30c inhibitor and siRNA2 NC + miR-30c inhibitor group (P < 0.05) (Figure 4B and D). All those results suggested miR-30c could promote the process of inflammation and apoptosis by activating SIRT1 mediating NF-κB pathway in myocardial cells.

Discussion

Myocardial infarction is one of the most common cause of death worldwide [15]. UP to now, more and more researches have been devoted to exploring new therapeutic targets of myocardial infarction, but the effect is not so ideal. Therefore, it is urgent to explore and discover new molecular approaches to better understand this disease and identify new therapeutic targets. In this research, we demonstrated that miR-30c could protect myocardial IR injury through activating NF-κB pathway in rats.

It is reported that myocardial IR injury process often leads to the inflammatory cascade and further myocardial cell apoptosis in the heart [16,17]. miRNAs exert important roles in biological processes of various diseases, such as cell proliferation and apoptosis [18,19]. In the past years, researches have revealed that abnormal expression of miRNAs is related to various cardiovascular diseases, such as myocardial I/R injury [20,21]. For example, miR-146a overexpression has been reported to reduce myocardial IR injury [12]. Chen et al. [22] have confirmed that miR-322 could mediate cardioprotection against myocardial IR injury through FBXW7/notch signaling pathway. Previous studies have indicated that miR-144 could attenuate myocardial IR injury via targeting forkhead box protein O1 [23]. Tan et al. [24] have reported that miR-24-3p could relieve myocardial IR injury through inhibiting RIPK1 expression in mice. Our results confirmed miR-30c expression was significantly upregulated in myocardial IR injury model rats. In addition, we also found miR-30c could facilitate the process of inflammation response and apoptosis in myocardial cells, which was consistent with the previous researches.

It is reported that NF-κB regulates multiple biological effects including innate immunity, inflammation, cell proliferation and apoptosis [9,10]. Under normal physiological condition, inactive NF-κB stays in cell cytoplasm and bounds to inhibitor of κB (IκB) proteins, of which the typical member is IκBα [25]. When stimulated, activated IκB kinase complex (IKK) promotes the phosphorylation and subsequent degradation of IκBα [25]. Subsequently, NF-κB translocates to cell nucleus where it regulates targeted gene

expression [26]. More and more evidences have indicated that various miRNAs exert vital roles by regulating NF-κB pathway in myocardial IR injury [12]. Li et al. [6] have confirmed that miR-340-5p could suppress hypoxia/reoxygenation-induced apoptosis via modulating Act1/NF-κB signaling in myocardial cells. Liu et al. [27] have reported that the inhibition of miR-27a could induce high thoracic epidural block to protect against myocardial IR injury via regulating ABCA1 and NF-κB pathway in mice. In this study, miR-30c could promote the process of inflammation and apoptosis by activating NF-κB pathway in myocardial cells.

Sirtuin 1 (SIRT1), a member of the sirtuin family, is considered to be a provotal inducer in cell proliferation, apoptosis and autophagy through miRNAs targeting in different diseases [28,29]. In addition, more and more researches have indicated that SIRT1 is related to the inflammation and cell death induced by myocardial IR injury [30]. Therefore, we investigate the association between miR-30c and SIRT1 and found SIRT1 was the target gene of miR-30c. It is worth noting that SIRT1 interacts with NF-κB and inhibits transcription through the deacetylation of NF-κB [31,32]. Subsequently, the deacetylation of NF-κB regulated by SIRT1 can suppress the activation of its downstream pathways and then relieve the inflammatory response [33]. In this research, SIRT1 could reverse the function of miR-30c on the process of inflammation and apoptosis, as well as the activation of NF-κB pathway in myocardial cells.

Conclusions

left anterior descending (LAD)

microRNAs (miRNAs)

ischemia reperfusion (IR)

Declarations

Ethics approval and consent to participate: This study was conducted after obtaining Luoyang Central Hospital Affiliated to Zhengzhou University's ethical committee approval .

Consent for publication: Not applicable.

Availability of data and material: All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Competing interests: The authors declare that they have no competing interests.

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Author Contributions: JFC and SFX: conception, design and analysis of data, performed the data analyses and wrote the manuscript;

MMJ and JLW: contributed to the conception of the study;

SYZ: contributed significantly to analysis and manuscript preparation;

All authors have read and approved the manuscript.

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Figures
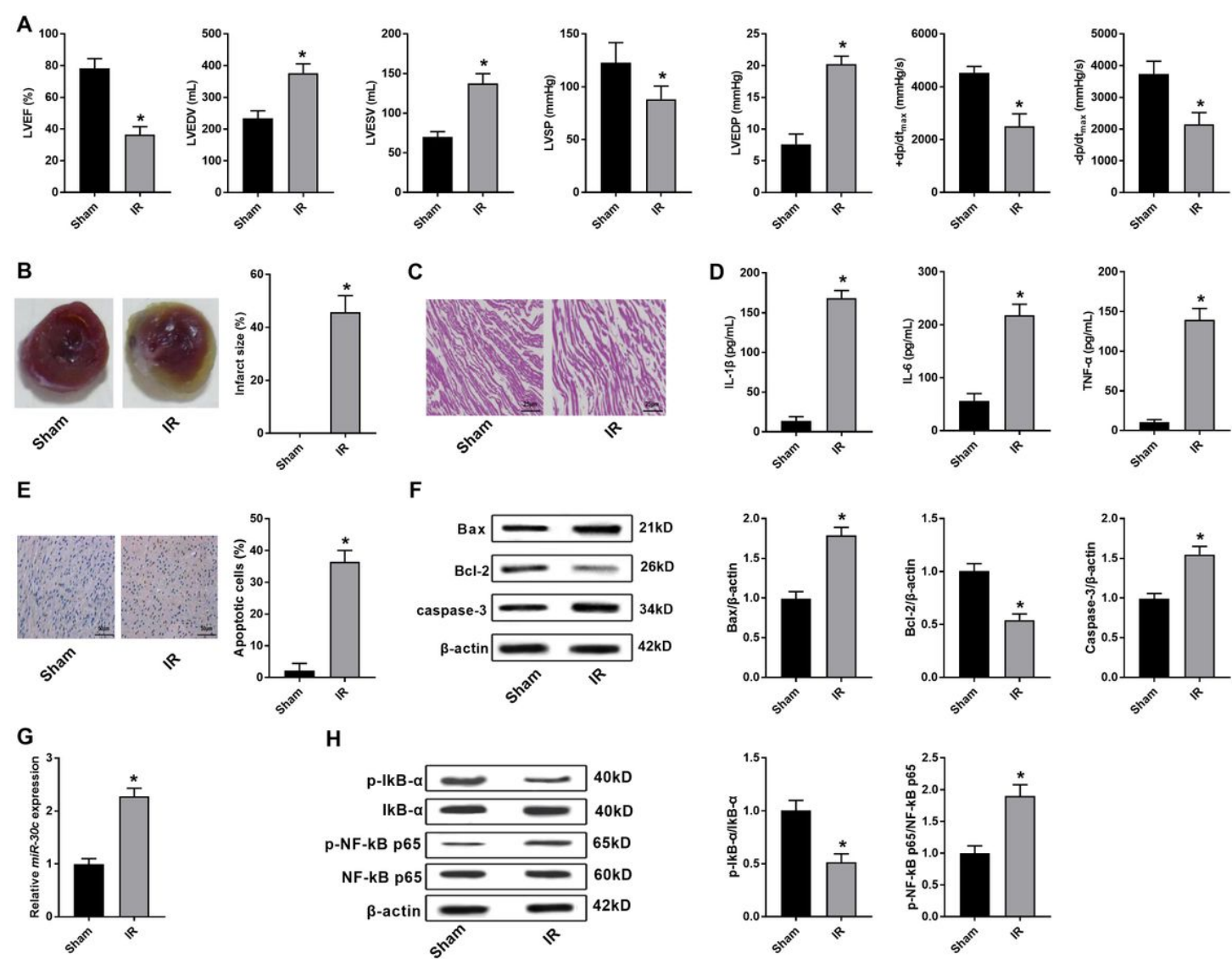
Figure 1

Ischemia reperfusion (IR) induced myocardial injury in rats. (A) The value of LVEF, LVEDV, LVESV, LVSP, LVEDP, +dP/dtmax and -dP/dtmax was measured by hemodynamic examination. (B) Myocardial infarct size. (C) Hematoxylin-eosin staining (×400). (D) The levels of IL-1β, IL-6 and TNF-α were detected by ELISA. (E) The myocardial cell apoptosis was measured by TUNEL staining. (F) The expressions of Bax, Bcl-2 and caspase-3 were detected by western blot. (G) The expression of miR-30c was detected by qRT-PCR. (H) The expressions of p-IκBα, IκBα, p-NF-κB p65 and NF-κB p65 were detected by western blot. Data were presented as mean ± standard deviation with repeated for three times. *P < 0.05, vs. Sham group.
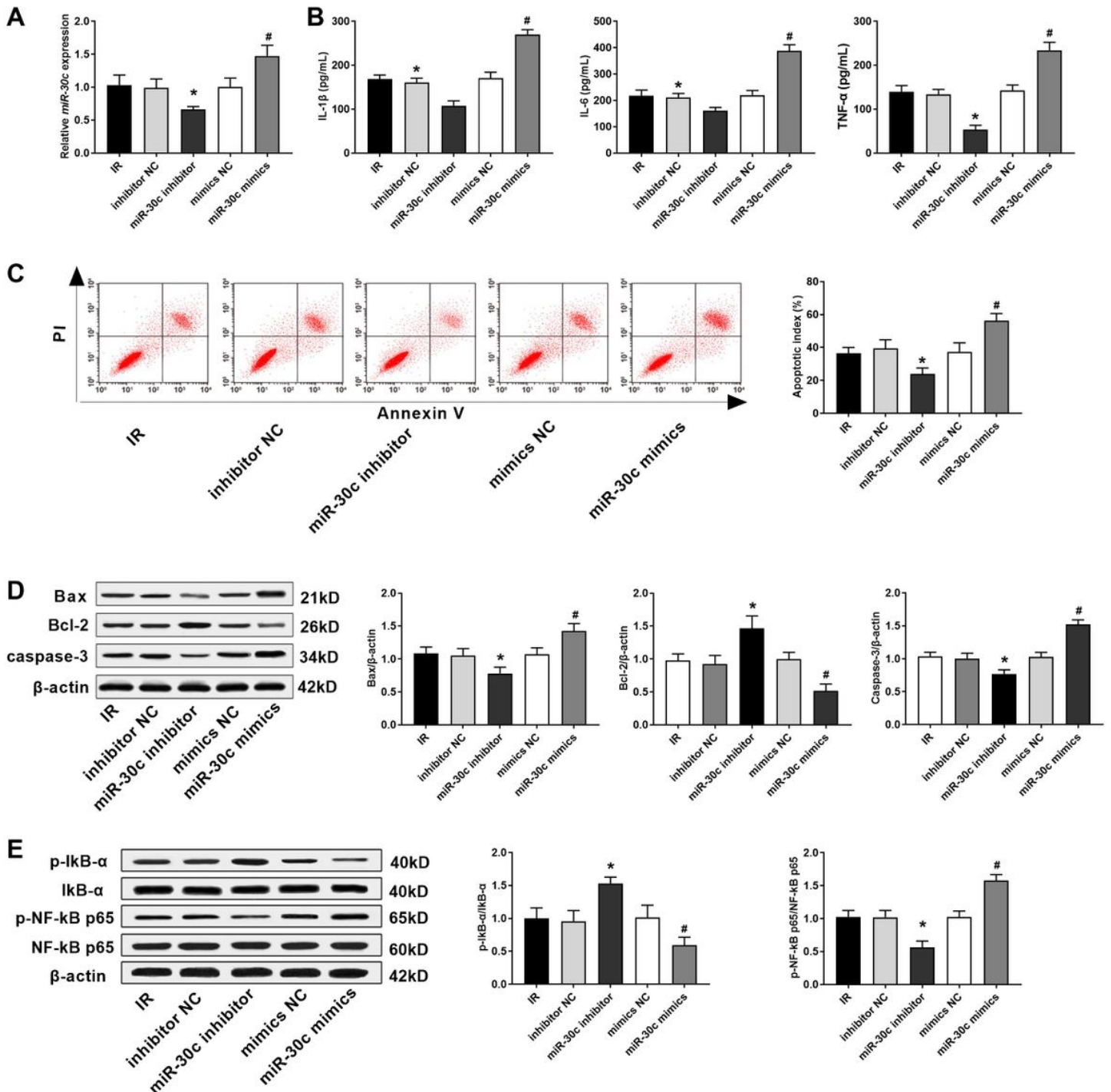
Figure 2

miR-30c promoted the process of inflammation and apoptosis by activating NF-κB pathway. (A) The expression of miR-30c was detected by qRT-PCR. (B) The levels of IL-1β, IL-6 and TNF-α were detected by ELISA. (C) The myocardial cell apoptosis was measured by flow cytometry. (D) The expressions of Bax, Bcl-2 and caspase-3 were detected by western blot. (E) The expressions of p-IκBα, IκBα, p-NF-κB p65 and NF-κB p65 were detected by western blot. Data were presented as mean ± standard deviation with repeated for three times. *P < 0.05, vs. IR and inhibitor NC group; #P < 0.05, vs. IR and mimics NC group.
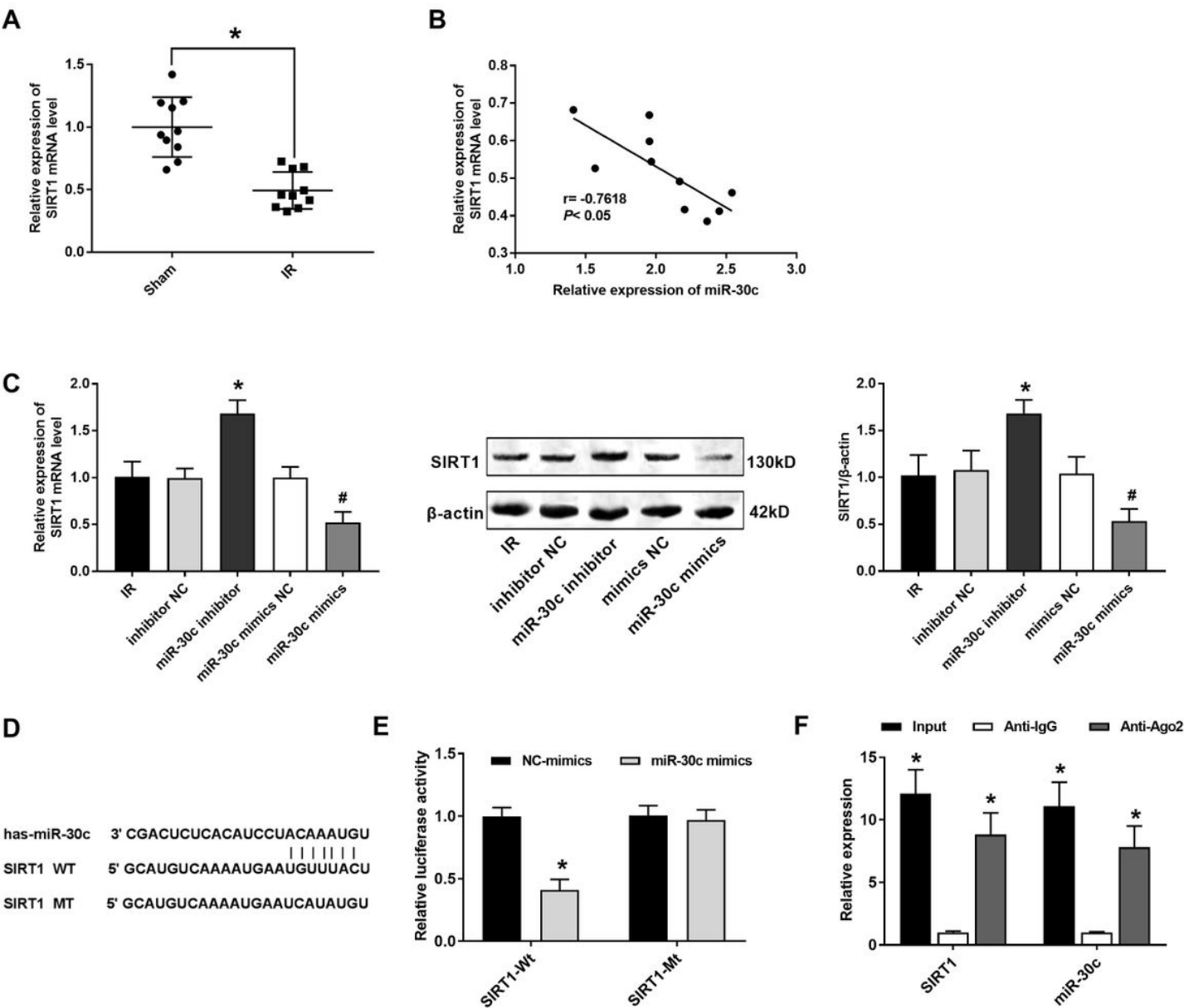
Figure 3

SIRT1 was the target gene of miR-30c. (A) The expression of miR-30c was detected by qRT-PCR. (B) Correlation analysis between the expression of miR-30c and SIRT1. (C) The mRNA and protein expressions of SIRT1 were detected by qRT-PCR and western blot. (D) The binding target of miR-30c and

SIRT1 was predicted by Target Scan software. (E) The luciferase activity was measured by dual luciferase reporter gene assay. (F) Interaction between miR-30c and SIRT1 were analyzed by RIP assay. Data were presented as mean ± standard deviation with repeated for three times. *P < 0.05, vs. Sham group (A); *P < 0.05, vs. IR and inhibitor NC group, #P < 0.05, vs. IR and mimics NC group (C); *P < 0.05, vs. NC-mimics group (E); *P < 0.05, vs. Anti-IgG group (F).
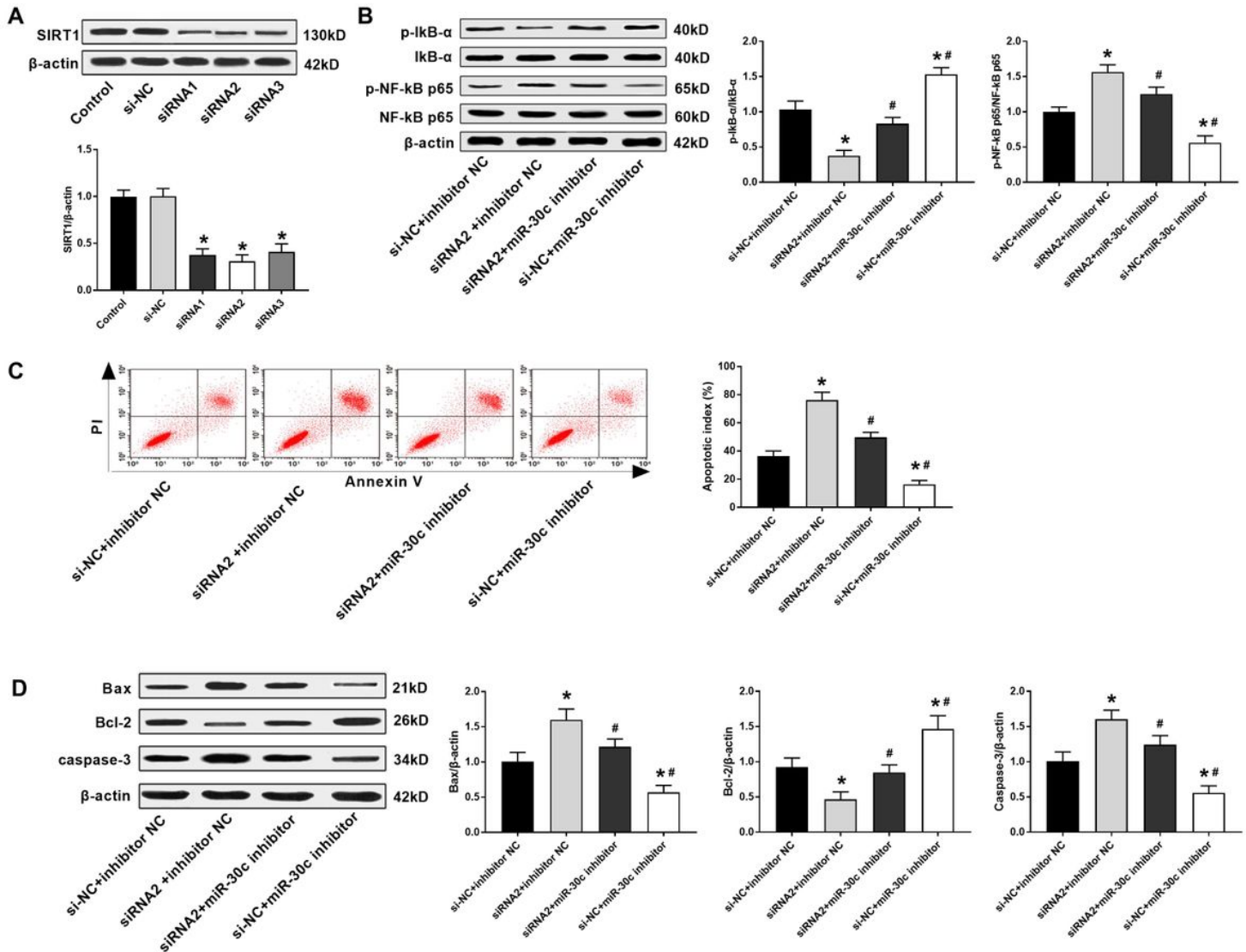
Figure 4

miR-30c promoted the process of inflammation and apoptosis by activating SIRT1 mediating NF-κB pathway. (A) The expressions of SIRT1 were detected by western blot. (B) The expressions of p-IκBα, IκBα, p-NF-κB p65 and NF-κB p65 were detected by western blot. (C) The myocardial cell apoptosis was measured by flow cytometry. (D) The expressions of Bax, Bcl-2 and caspase-3 were detected by western blot. Data were presented as mean ± standard deviation with repeated for three times. *P < 0.05, vs. Control and si-NC group (A); *P < 0.05, vs. siRNA2 NC + miR-30c inhibitor NC group, #P < 0.05, vs. siRNA2 + miR-30c inhibitor NC group (B-D).