MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2020; 26: e924016
DOI: 10.12659/MSM.924016

Received: 2020.03.04
Accepted: 2020.04.15
Available online: 2020.06.12
Published: 2020.08.09

Long Non-Coding RNA SNHG8 Plays a Key Role in Myocardial Infarction Through Affecting Hypoxia-Induced Cardiomyocyte Injury

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEFG 1 Yue Zhang
ABCDEG 2 Yunfei Bian

1 Shanxi Medical University, Taiyuan, Shanxi, P.R. China
2 Department of Cardiology, The Second Hospital of Shanxi Medical University, Taiyuan, Shanxi, P.R. China

Corresponding Author: Yunfei Bian, e-mail: tre543546@163.com
Source of support: This study was supported by Mechanisms of HDL-induced Macrophage Pyroptosis Promoting Atherosclerosis Progression in Patients with Coronary Heart Disease (grant no. 201803D31073)

Background: The objective of the study was to explore the role of long non-coding RNA SNHG8 (lncRNA SNHG8) in myocardial infarction (MI) and the related mechanism of action.

Material/Methods: *In vitro* model of MI was established by hypoxia induction in cardiomyocyte line H9c2 cells. H9c2 cells were transfected with control-plasmid, SNHG8-plasmid, control-shRNA and SNHG8-shRNA. Quantitative real-time polymerase chain reaction (qRT-PCR) assay was performed to measure transfection efficiency. Creatine kinase-muscle/brain (CK-MB) release, cardiac troponin 1 (cTnI) release and mitochondria viability were detected by using related detection kits. MTT (3-(45)-dimethylthiahiazo (-z-y 1)-35-diphenytetrazoliumromide) assay was used to detect cell viability and flow cytometry analysis was used to detect cell apoptosis. Western blot assay was performed to measure protein expression of cleaved-Caspase3, p-p65 and p65. Enzyme-linked immunosorbent assay (ELISA) and qRT-PCR assay were performed to detect expression of interleukin (IL)-1β, tumor necrosis factor (TNF)-α and IL-6.

Results: LncRNA SNHG8 was overexpressed in hypoxia-induced cardiomyocytes. SNHG8-plasmid increased lncRNA SNHG8 expression, CK-MB release, cTnI release, and mitochondria viability in hypoxia-induced H9c2 cells. In addition, SNHG8-plasmid reduced cell viability, induced cell apoptosis, and increased expression of cleaved-caspase3, IL-1β, TNF-α, IL-6, and p-p65 in hypoxia-induced H9c2 cells, while the effects of SNHG8-shRNA were opposite.

Conclusions: We demonstrated that lncRNA SNHG8 affected myocardial infarction by affecting hypoxia-induced cardiomyocyte injury via regulation of the NF-κB pathway.

MeSH Keywords: Myocardial Infarction • NF-kappa B • RNA, Long Noncoding

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/924016

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Myocardial infarction (MI) refers to ischemic necrosis of the myocardium. Clinically, there are severe and long-lasting sternum pains, accompanied by increased serum myocardial enzyme activity, and progressive electrocardiogram changes, which can be complicated by arrhythmia, shock, or heart failure and ultimately threaten life [1–3]. At present, MI pathogenesis is not fully understood, but aging, overwork, overeating, and smoking are major pathogenic factors [4]. MI is most common in Europe and the United States, with about 1.5 million people suffering from it each year in the United States. China has shown a clear upward trend in recent years, with at least 500,000 new issues each year and a current incidence of at least 2 million [5,6]. The primary objective of treatment of MI is clearance of the blocked coronary artery and restoration of function of ischemic and hypoxic myocardium [7]. Treatment is usually done by dilating the coronary arteries and removing the pathogenic blockages. In addition, reduction of cardiac load, such as oxygen absorption, absolute bedrest, and avoidance of emotional fluctuations is also conducive to recovery of patients. MI is known to be harmful, but there is no effective treatment for it so far. Therefore, new, safe, and more effective treatments for MI are desperately needed. Previous reports have used an *in vitro* model of MI created by inducting hypoxia in cardiomyocytes to study the related mechanism of MI, and we used hypoxia-induced a similar model to further study MI [8,9].

Long non-coding RNAs (lncRNAs) are usually defined as RNA molecules longer than 200 nucleotides and that lack biological functions [10,11]. To date, lncRNAs have been shown to play an extensive regulatory role in life activities and have been implicated in various diseases, such as cervical, gastric, and esophageal cancers; Parkinson's disease; and myocarditis and MI [12–14]. LncRNA SNHG8, known as a novel small nucleolar guide RNA, is located on 4q26 [15]. LncRNA SNHG8 has been well studied in various types of cancers, including cervical, gastric and colorectal cancers and esophageal squamous cell carcinomas [16–20]. A recent study indicated that lncRNA SNHG8 was significantly overexpressed in patients with MIMI, and found that lncRNA SNHG8 was a risk factor for acute MI, but the specific mechanism of action still needed further study [21].

In this study, we explored whether lncRNA SNHG8 played an important role in hypoxia-induced myocardial cell injury *in vitro*, and further studied its molecular mechanism. Our results provide more theoretical basis and new strategies for treatment of MI.

Material and Methods

Cell culture

Rat cardiomyocyte line H9c2 cells were purchased from the American Type Culture Collection (ATCC, Manassas, Virginia, United States). The cells were cultured in DMEM medium (Gibco, United States) containing 10% fetal bovine serum (FBS, Gibco), 1% penicillin/streptomycin and incubated in 5% CO_2 at 37°C.

Establishment of *in vitro* MI model

We established an *in vitro* MI model by inducting hypoxia in cardiomyocytes. Briefly, rat cardiomyocyte line H9c2 cells were cultured under hypoxic conditions (94% N_2, 5% CO_2, and 1% O_2) for 48 h [22].

Cell transfection assay

Hypoxia-induced cardiomyocytes were seeded at a concentration of 5×10^4 cells/mL in six-well plates and incubated overnight. According to the instructions, control-plasmid or SNHG8-plasmid (GenePharma, Shanghai, China), control-shRNA (CATAGCGGTGTAGTAAAGCATAATA) or SNHG8-shRNA (ATTACGATGGATGATGGAAACATA) (GenePharma, Shanghai, China) were transfected into hypoxia-induced cardiomyocytes using Lipofectamine 2000 reagent (Invitrogen, United States). After transfection for 48 h, we collected the cells for further experiments. Cell transfection efficiency was detected through quantitative real-time polymerase chain reaction (qRT-PCR) assay.

RNA extraction and qRT-PCR

Total RNA was isolated from cells using an RNA isolation kit (Invitrogen, United States), and then reverse transcribed into first-strand cDNA by using the cDNA Synthesis Kit (Invitrogen) following the manufacturer's protocol. All the reactions were performed to quantify miRNA expression of relative genes using a Prism 7000 Real-Time PCR system with SYBR qPCR Master Mix (Vazyme, Nanjing) in accordance with the manufacturer's instruction. Amplification conditions included the following steps: 5 min at 95 ˚C followed by 40 cycles of 95°C for 10 sec and 60°C for 30 sec. GAPDH was used as the internal control. Relative expression of lncRNA SNHG8, IL-1β, TNF-α, and IL-6 mRNA were calculated by $2^{-\Delta\Delta Cq}$ method [23]. All primers were provided by Sangon Biotech (Shanghai, China) and primer sequences were listed as follows:
lncRNA SNHG8 forward, 5'-AAGTTTACAAGCATGCGCGG-3';
reverse, 5'-TCAAACTGACGGTTCTCGGG-3';
IL-1β forward, 5'-TGTGAAATGCCACCTTTTGA-3';
reverse, 5'-TGAGTGATACTGCCTGCCTG-3';
TNF-α forward, 5'-GAACTGGCAGAAGAGGCACT-3';

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

reverse, 5'-GGTCTGGGCCATAGAACTGA-3';
IL-6 forward, 5'-CCGGAGAGGAGACTTCACAG-3';
reverse, 5'-CAGAATTGCCATTGCACA-3';
GAPDH forward, 5'-CTTTGGTATCGTGGAAGGACTC-3';
reverse, 5'-GTAGAGGCAGGGATGATGTTCT-3'. All the experiments were repeated for 3 times.

Detection of CK-MB release, cTnI release and mitochondria viability

To explore the role of lncRNA SNHG8 in cellular damage of hypoxia-induced cardiomyocytes, CK-MB release, cTnI release, and mitochondria viability were detected by the CK-MB Detection Kit, cTnI Detection Kit (ReLIA, California, United States) and mitochondria activity assay kit (Jiancheng Bioengineering Institute, China), respectively, after the cells were transfected for 48 h.

3-(4,5-dimethylthiazol-2-yl)-2,5-dipheyltetrazolium bromide (MTT) assay

After specific treatment, the cells were incubated with 10 μL MTT solution (Beyotime, Shanghai, China) for 4 h. Subsequently, 100 μL dimethyl sulfoxide was added into each well to solubilize the formazan product after the solution was removed. Finally, absorbance at 490 nm was recorded with a microplate reader (Bio-Rad, Hercules, California, United States). Relative cell viability was normalized with the control group using optical density values.

Apoptosis analysis

Detection of cell apoptosis was performed by flow cytometric (FCM) analysis. The cells were transferred into six-well plates for 48 h, and collected by trypsinization after transfection, washed once with phosphate-buffered saline (PBS) and then resuspended in 1×binding buffer. A 100-μL cell suspension was transferred to a 5-mL tube, and then the cells were incubated with 5 μL Annexin V PE and 7-Aminoactinomycin D (7-AAD) (BD Biosciences, San Diego, California, United States), respectively, according to specifications. The stained cells were analyzed by with a FACSCalibur flow cytometer (BD Biosciences, United States) within 1 hr. FlowJo software (version 7.2.4; FlowJo LLC) was used to analyze the data and calculate early and late stage apoptosis rates.

Western blot analysis

Protein expression levels of cleaved-Caspase3, p-p65 and p65 in cells were investigated with Western blot analysis. Briefly, we extracted the protein using a total protein extraction kit (Beyotime, Shanghai, China), and determined protein concentrations using a BCA protein kit (Pierce, United States). The total protein extracted was resolved with SDS-PAGE using a 10% polyacrylamide gel under reduced conditions, and then transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% nonfat milk PBS Tween (PBST) solution at room temperature for 1 h. Afterwards, we incubated cleaved-Caspase3 (cat. no. ab2302; 1: 1,000; Abcam), p-p65 (cat no. 3033; 1: 1,000; Cell Signaling Technology, Inc.) and p65 (cat no. 8242; 1: 1,000; Cell Signaling Technology, Inc.) or GAPDH (cat. no. 5174; 1: 1,000; Cell Signaling Technology, Inc.) at 4°C overnight. Afterwards, the membranes were washed three times with PBST and incubated with horseradish peroxidase-conjugated secondary antibody (cat. no. 7074; 1: 2,000; Cell Signaling Technology, Inc.) at room temperature for 1 h. Protein bands were detected by enhanced chemiluminescent reagent (ECL Advance Western Blotting Detection Kit; GE Healthcare Life Sciences) according to the protocol and protein levels were quantified with Image J software. In addition, we calculated the value of Pp65/p65.

ELISA assay

Forty-eight hours after cell transfection, levels of IL-1β (cat. no. PI303), TNF-α (cat no. PT516), and IL-6 (cat. no. PI328) in hypoxia-induced cardiomyocytes were detected using the enzyme-linked immunosorbent assay (ELISA) kit (Beyotime Institute of Biotechnology, Shanghai, China) following the protocol.

Statistical analysis

Data were displayed as the mean±standard deviation (SD) of a least three independent experiments. Statistical analyses between groups were estimated by Student's *t*-test or one-way ANOVA followed by Tukey's post-hoc test by using SPSS 18.0 software package (SPSS Inc, United States). $P<0.05$ was considered a statistically significant difference.

Results

Hypoxia induced lncRNA SNHG8 accumulation in H9c2 cells

For preliminary assessment of the role of lncRNA SNHG8 in MI, we detected expression of lncRNA SNHG8 in H9c2 cells. As shown in Figure 1, expression of lncRNA SNHG8 was upregulated in hypoxia-induced H9c2 cells significantly compared with the control group.

Effects of high expression of lncRNA SNHG8 on cell injury in hypoxia-induced cardiomyocytes

To study the effect of high expression of lncRNA SNHG8 on cell injury, control-plasmid or SNHG8-plasmid was transfected into hypoxia-induced cardiomyocytes. After transfection,

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

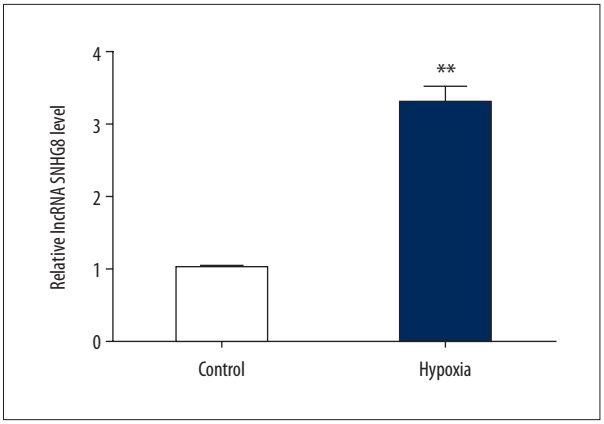

Figure 1. Expression of lncRNA SNHG8 in hypoxia-induced cardiomyocytes. mRNA expression of lncRNA SNHG8 in hypoxia-induced cardiomyocytes was detected by qRT-PCR analysis. Control: cells without any treatment; Hypoxia: H9c2 cells were cultured under hypoxic conditions (94% N_2, 5% CO_2, and 1% O_2) for 48 h. ** P<0.01 *vs.* Control group.

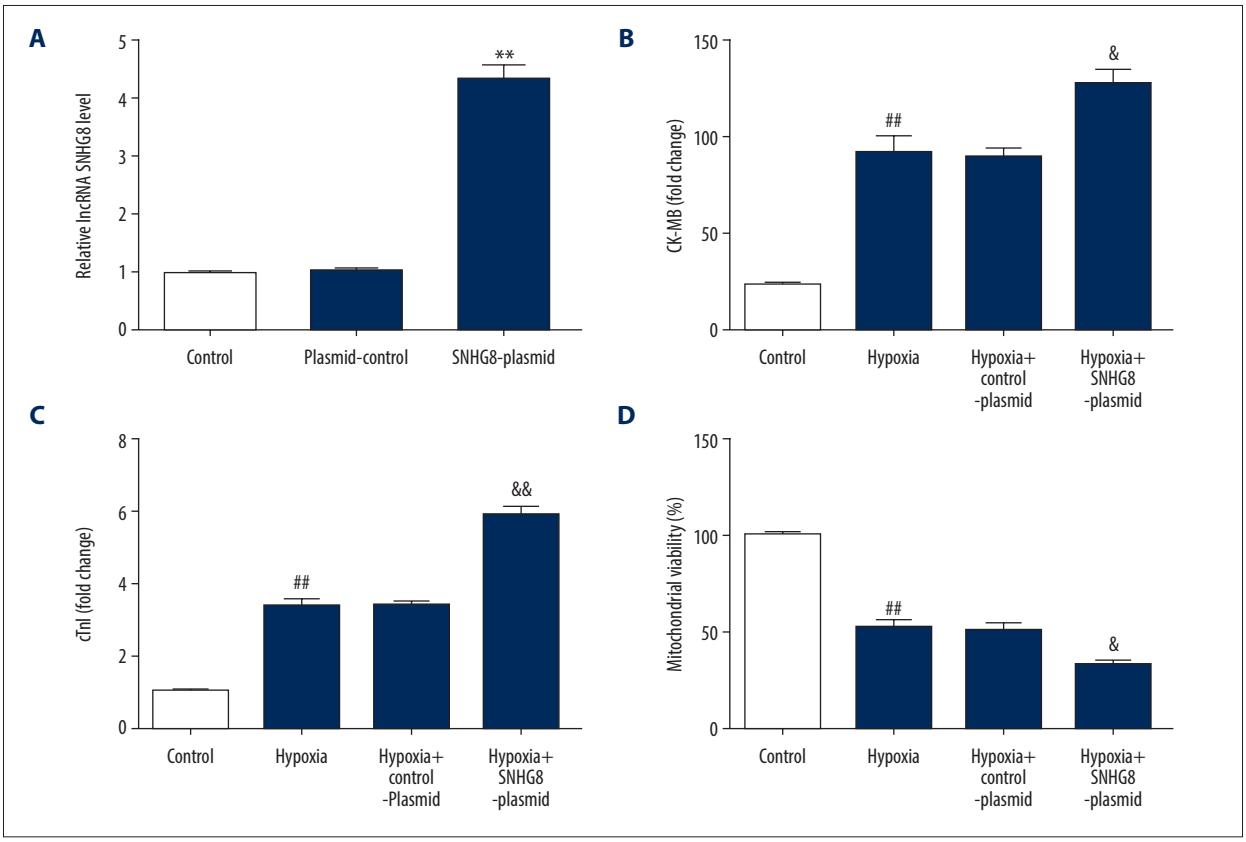

Figure 2. Effects of SNHG8-plasmid on cell injury in hypoxia-induced cardiomyocytes. (**A**) Transfection efficiency was evaluated by qRT-PCR assay. (**B**) Release of CK-MB was measured by using a CK-MB Detection Kit. (**C**) Release of cTnI was measured by using a cTnI Detection Kit. (**D**) A mitochondrial activity assay kit was used to detect mitochondrial viability of hypoxia-induced cardiomyocytes. Control: cells without any treatment; plasmid-control: H9c2 cells were transfected with control-plasmid for 48 h; SNHG8-plasmid: H9c2 cells were transfected with SNHG8-plasmid for 48 h; Hypoxia: H9c2 cells were cultured under hypoxic conditions (94% N_2, 5% CO_2, and 1% O_2) for 48 h; Hypoxia+control-plasmid: H9c2 cells were transfected with control-plasmid for 48 h under hypoxic conditions; Hypoxia+SNHG8-plasmid: H9c2 cells were transfected with SNHG8-plasmid for 48 h under hypoxic conditions. ** P<0.01 *vs.* control-plasmid; ## P<0.01 *vs.* Control group; &, && P<0.5, 0.01 *vs.* Hypoxia+control-plasmid.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

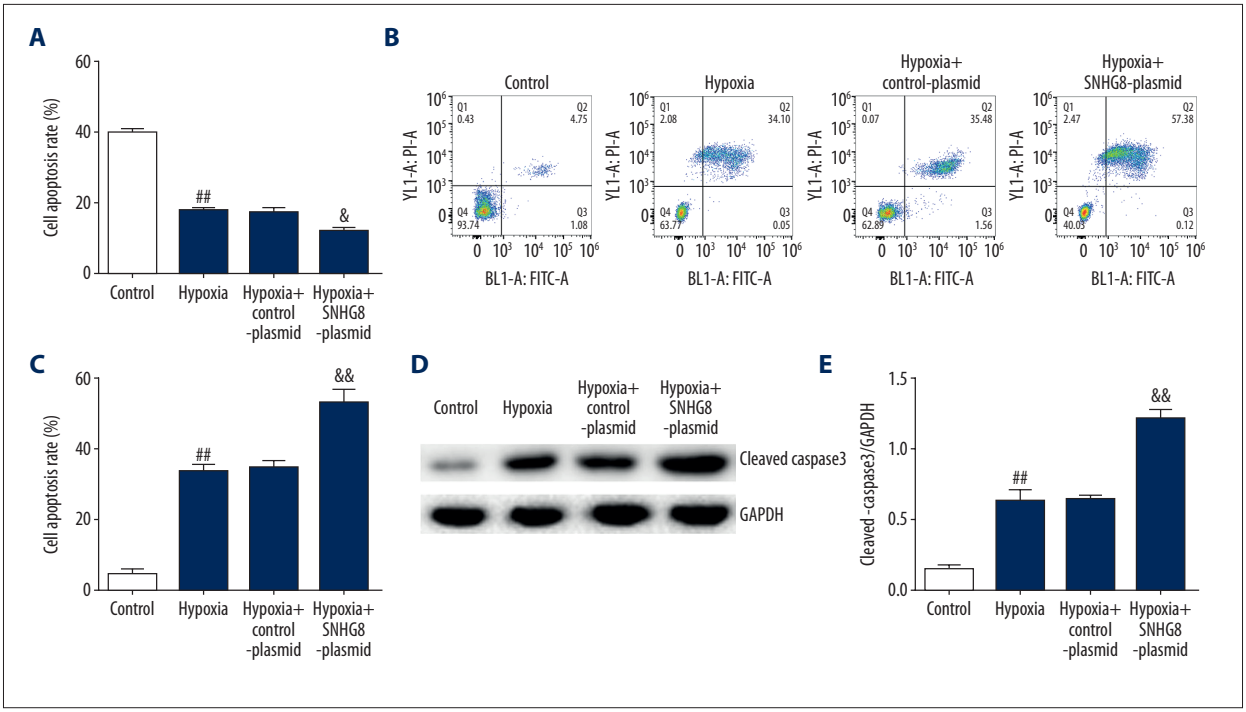

Figure 3. Effects of SNHG8-plasmid on the cell viability and apoptosis in hypoxia-induced cardiomyocytes. (**A**) Cell viability in hypoxia-induced cardiomyocytes following transfection was measured by MTT assay. (**B, C**) Flow cytometry analysis was used to determine the apoptotic rate of hypoxia-induced cardiomyocytes. (**D, E**) Western blotting analysis was performed to detect relative expression of cleaved-Caspase3 protein. Control: cells without any treatment; Hypoxia: H9c2 cells were cultured under hypoxic conditions (94% N_2, 5% CO_2, and 1% O_2) for 48 h; Hypoxia+control-plasmid: H9c2 cells were transfected with control-plasmid for 48 h under hypoxic conditions; Hypoxia+SNHG8-plasmid: H9c2 cells were transfected with SNHG8-plasmid for 48 h under hypoxic conditions. ## P<0.01 *vs.* Control group; &, && P<0.5, 0.01 *vs.* hypoxia+control-plasmid.

the cells were separated into four groups: control, hypoxia, hypoxia+control-plasmid and hypoxia+SNHG8-plasmid group. Transfection efficiency was evaluated by qRT-PCR assay. Compared with the control-plasmid group, expression of lncRNA SNHG8 was dramatically increased after cells were transfected with SNHG8-plasmid (Figure 2A). In comparison with the control group, hypoxia significantly induced release of creatine kinase isoenzyme-MB (CK-MB) and cardiac troponin I (cTnI), while SNHG8-plasmid further promoted release of CK-MB and cTnI (Figures 2B, 2C). Meanwhile, we detected viability of mitochondria in H9c2 cells. The results showed that hypoxia reduced mitochondrial viability remarkably compared to the control group, and SNHG8-plasmid further reduced mitochondrial viability (Figure 2D).

Effects of high expression of lncRNA SNHG8 on cell viability and apoptosis of hypoxia-induced cardiomyocytes

To assess whether lncRNA SNHG8 interfered with cell viability and apoptosis of hypoxia-induced cardiomyocytes, cell viability, apoptosis, and cleaved-Caspase3 protein expression were measured following transfection. Compared with the control group, hypoxia markedly reduced the cell viability of cardiomyocytes (Figure 3A), induced cell apoptosis (Figure 3B, 3C), and promoted cleaved-Caspase3 protein expression (Figure 3D, 3E), while SNHG8-plasmid deepened these effects.

Effect of high expression of lncRNA SNHG8 on secretion of inflammatory factors in hypoxia-induced cardiomyocytes

To study the mechanism of lncRNA SNHG8 in cardiomyocytes, expression of related inflammatory factors was detected. As shown in Figure 4A–4C, in contrast with the control group, hypoxia markedly increased secretion of TNF-α, L-1β, and IL-6, while SNHG8-plasmid further induced secretion of TNF-α, L-1β and IL-6. Similarly, hypoxia increased mRNA expression of TNF-α, L-1β and IL-6 remarkably compared with the control group, and all these effects were further improved by SNHG8-plasmid (Figure 4D–4F).

Effects of knockdown of lncRNA SNHG8 on cell damage in hypoxia-induced cardiomyocytes

Rat cardiomyocyte line H9c2 cells were transfected with control-shRNA or SNHG8-shRNA in anoxic condition and the cells were

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

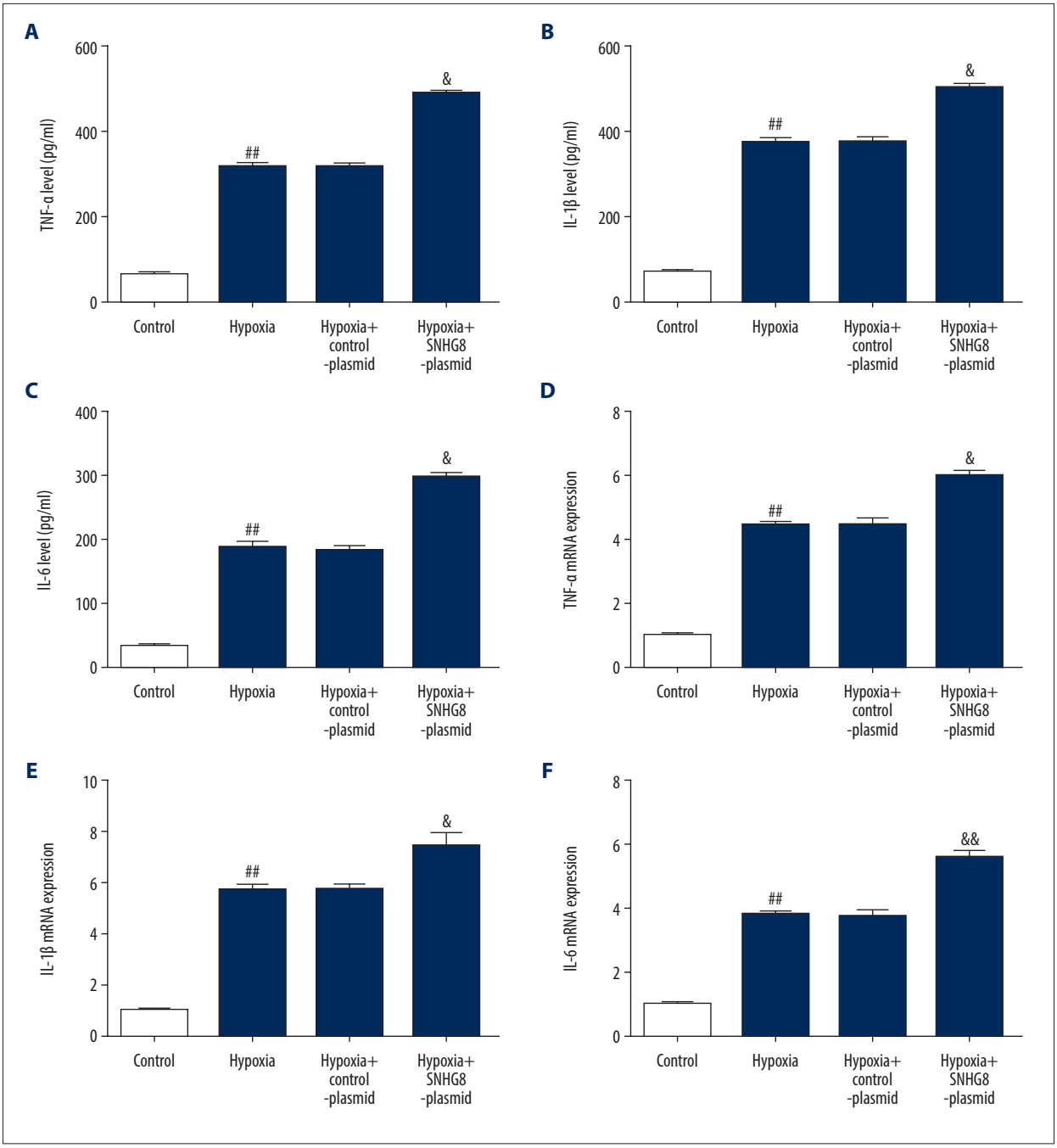

Figure 4. Effects of up-regulating lncRNA SNHG8 on the secretion of inflammatory factors in hypoxia-induced cardiomyocytes. (**A–C**) Secretion of IL-1β, TNF-α, and IL-6 in transfected hypoxia-induced cardiomyocytes were measured by ELISA. (**D–F**) Relative expression levels of IL-1β, TNF-α, and IL-6 in transfected hypoxia-induced cardiomyocytes were determined by qRT-PCR analysis. Control: cells without any treatment; Hypoxia: H9c2 cells were cultured under hypoxic conditions (94% N_2, 5% CO_2, and 1% O_2) for 48 h; Hypoxia+control-plasmid: H9c2 cells were transfected with control-plasmid for 48 h under hypoxic conditions; Hypoxia+SNHG8-plasmid: H9c2 cells were transfected with SNHG8-plasmid for 48 h under hypoxic conditions. $^{\#\#}$ P<0.01 *vs.* Control group; $^{\&, \&\&}$ P<0.5, 0.01 *vs.* Hypoxia+control-plasmid.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

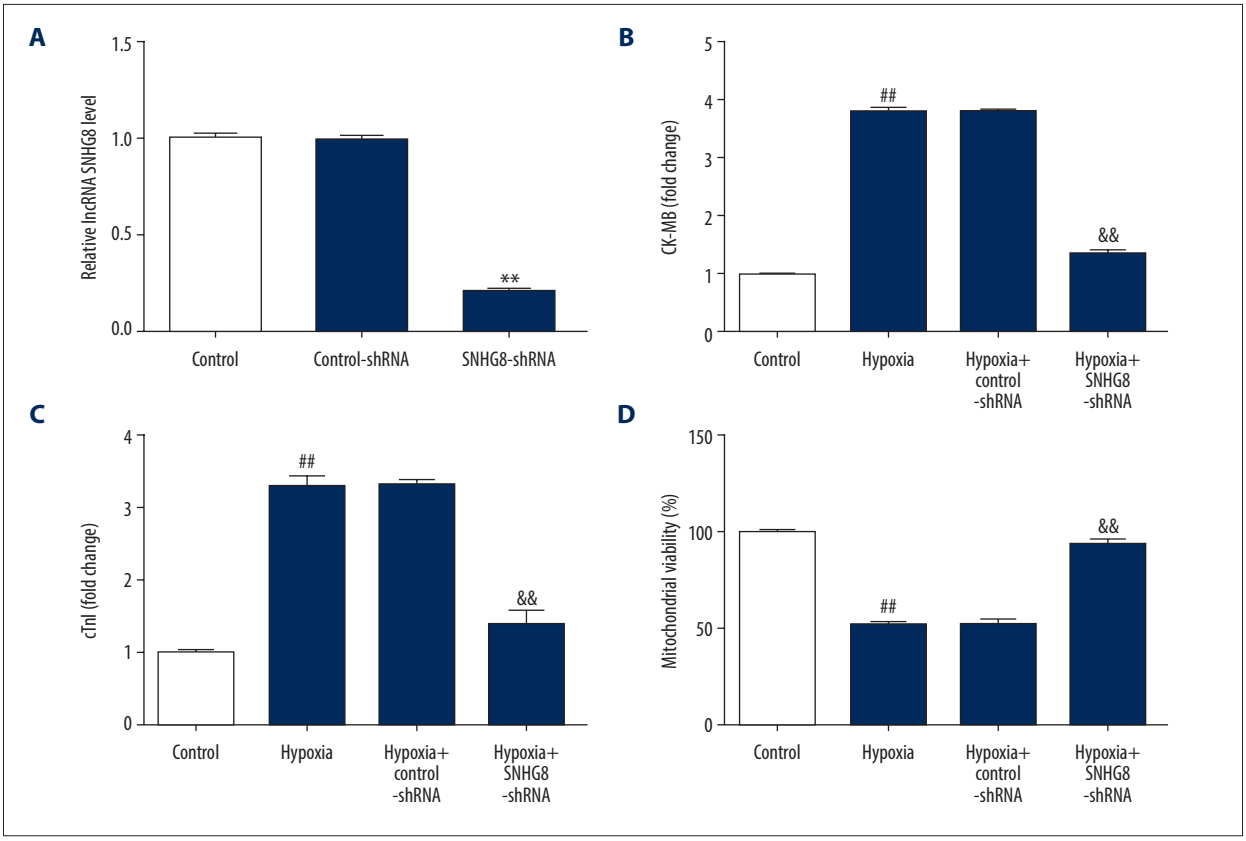

Figure 5. Effects of down-regulating lncRNA SNHG8 on cell damage in hypoxia-induced cardiomyocytes. (**A**) qRT-PCR assay was performed to measure transfection efficiency. (**B–D**) Release of CK-MB and cTnI and mitochondrial viability in hypoxia-induced cardiomyocytes were measured by using a K-MB Detection Kit, a cTnI Detection Kit, and a mitochondrial activity assay kit, respectively. Control: cells without any treatment; control-shRNA: H9c2 cells were transfected with control-shRNA for 48 h; SNHG8-shRNA: H9c2 cells were transfected with SNHG8-shRNA for 48 h; Hypoxia: H9c2 cells were cultured under hypoxic conditions (94% N_2, 5% CO_2, and 1% O_2) for 48 h; Hypoxia+control-shRNA: H9c2 cells were transfected with control-shRNA for 48 h under hypoxic conditions; Hypoxia+SNHG8-shRNA: H9c2 cells were transfected with SNHG8-shRNA for 48 h under hypoxic conditions. ** P<0.01 *vs.* control-shRNA; ## P<0.01 *vs.* Control group; && P<0.01 *vs.* Hypoxia+control-shRNA.

separated into four groups: control, hypoxia, hypoxia+control-shRNA group, and hypoxia+SNHG8-shRNA group. Results of transfection efficiency detection showed that the expression of lncRNA SNHG8 was lower in the SNHG8-shRNA group than that in the control-shRNA group (Figure 5A). In contrast with the control group, hypoxia significantly increased release of CK-MB and cTnI, while SNHG8-shRNA significantly reduced release of CK-MB and cTnI (Figure 5B, 5C). As shown in Figure 5D, hypoxia remarkably reduced mitochondrial viability compared with the control group, while SNHG8-shRNA improved mitochondrial viability.

In addition, cell viability, apoptosis, and cleaved-Caspase expression were detected after transfection. Compared with the control group, hypoxia reduced cell activity of cardiomyocytes (Figure 6A), induced cell apoptosis (Figure 6B, C), and promoted cleaved-Caspase3 protein expression (Figures 6D, 6E), while SNHG8-shRNA reversed these effects.

Effects of knockdown of lncRNA SNHG8 on secretion of inflammatory factors in hypoxia-induced cardiomyocytes

As shown in Figure 7A–7C, hypoxia markedly promoted secretion of TNF-α, L-1β, and IL-6, while SNHG8-shRNA decreased secretion of TNF-α, L-1β, and IL-6. Similarly, hypoxia significantly increased mRNA expression of TNF-α, L-1β, and IL-6, while SNHG8-shRNA decreased mRNA expression levels of TNF-α, L-1β, and IL-6 (Figure 7D–7F).

Effects of lncRNA SNHG8 on NF-κB signal pathway in hypoxia-induced cardiomyocytes

To investigate the signal pathway underlying the role of lncRNA SNHG8 in hypoxia-induced cardiomyocytes, we detected expression of p-p65 and p65 in H9c2 cells using Western blot assay. The results showed that hypoxia increased p-p65 protein expression and p-p65/p65 ratio remarkably in comparison with

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

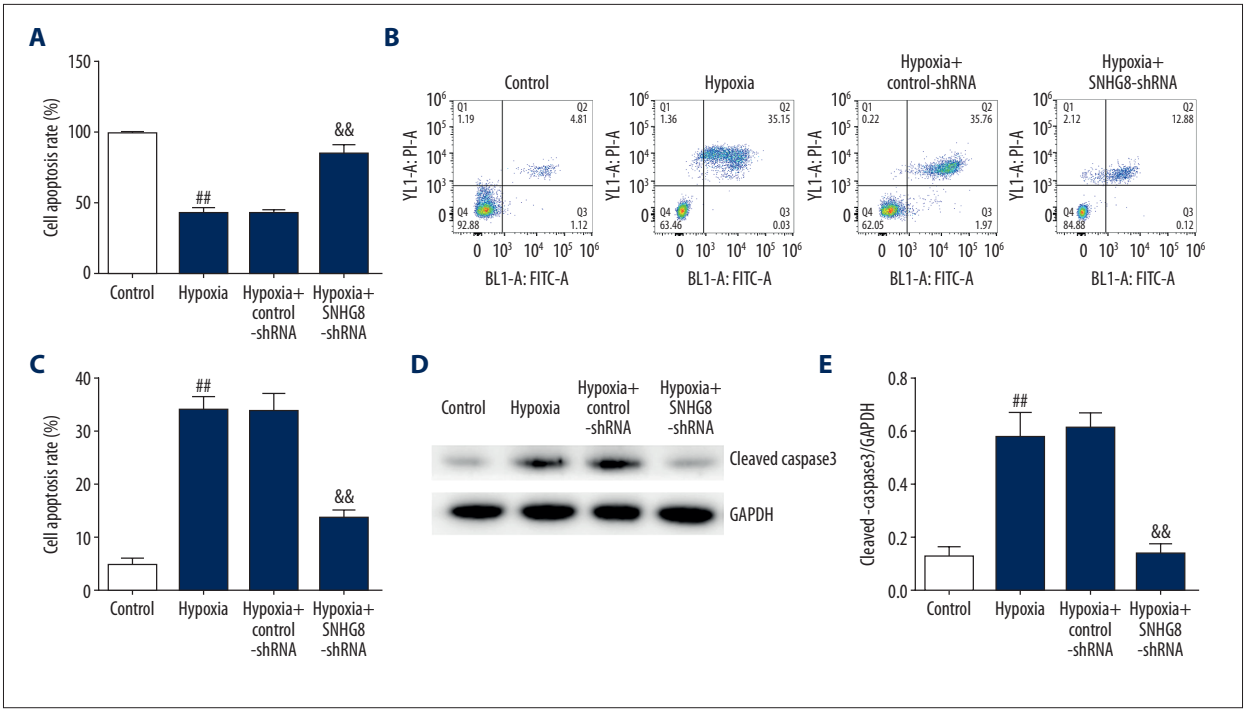

Figure 6. Effects of downregulating lncRNA SNHG8 on the cell viability and apoptosis in hypoxia-induced cardiomyocytes. (**A**) Cell viability in hypoxia-induced cardiomyocytes after transfection was determined by MTT assay. (**B, C**) Flow cytometry analysis was used to detect the apoptotic rate of hypoxia-induced cardiomyocytes. (**D, E**) Western blotting analysis was used to detect relative expression of cleaved-Caspase3 protein. Control: cells without any treatment; Hypoxia: H9c2 cells were cultured under hypoxic conditions (94% N_2, 5% CO_2, and 1% O_2) for 48 h; Hypoxia+control-shRNA: H9c2 cells were transfected with control-shRNA for 48 h under hypoxic conditions; Hypoxia+SNHG8-shRNA: H9c2 cells were transfected with SNHG8-shRNA for 48 h under hypoxic conditions. ## P<0.01 *vs.* Control group; && P<0.01 *vs.* Hypoxia+control-shRNA.

the control group, and SNHG8-plasmid further increased protein expression of p-p65 and p-p65/p65 ratio (Figure 8A, 8B). SNHG8-shRNA markedly decreased expression of p-p65 protein and p-p65/p65 ratio (Figure 8C, 8D).

Discussion

Coronary heart disease (CHD) is a cardiovascular disease with high incidence. Data indicate that the rate of CHD in China continues to increase every year, and it is being seen in younger ande younger patients [24]. MI is a type of CHD that involves myocardial necrosis and often results in malignant arrhythmia, heart failure, other serious complications, or even sudden death [25,26]. In previous studies, we developed an *in vitro* MI model by inducing hypoxia in cardiomyocytes. Studies show that lncRNA SNHG8 is highly expressed in patients with MI. Rat cardiomyocyte line H9c2 cells were cultured under oxygen-deficit conditions for 48 h in one study. We found that expression of lncRNA SNHG8 was obviously higher in hypoxia-induced H9c2 cells which is consistent with previous research. To further study mechanism of action of lncRNA SNHG8, we up-regulated and down-regulated lncRNA SNHG8 by transfection. Creatine kinase, also known as phosphocreatine or creatine phosphokinase, is an important kinase found in a variety of tissues and cells. It consists of three isozyme forms: CK-BB, CK-MM, and CK-MB. CK-MB accounts for 20% of the total creatine kinase in the myocardial tissues. In addition, CK-MB is specific for myocardial tissues, and CK-MB level can be considered an important clinical indicator for diagnosis of MI [27,28]. Cardiac troponin (cTn) is mainly present in myocardial myofibroblasts, including three subunits: troponin I, troponin T, and troponin C. It is an important functional protein that maintains myocardial fiber contraction and relaxation. Cardiac troponin I (cTnI) has myocardial specificity and is diagnostic for myocardium. Combined with CK-MB, it can improve specificity of diagnosis of MI. Mitochondrial dysfunction and resulting oxidative stress also are closely related to myocardial ischemia-reperfusion injury [29–31].

In this study, we found that overexpression of lncRNA SNHG8 increased CCK-MB release, cTnI release, and mitochondria viability. Upregulation of lncRNA SNHG8 also reduced cell viability, induced cell apoptosis, and increased the expression of cleaved-Caspase3, while downregulation of lncRNA SNHG8 had the opposite effect. In addition, we found that lncRNA SNHG8

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

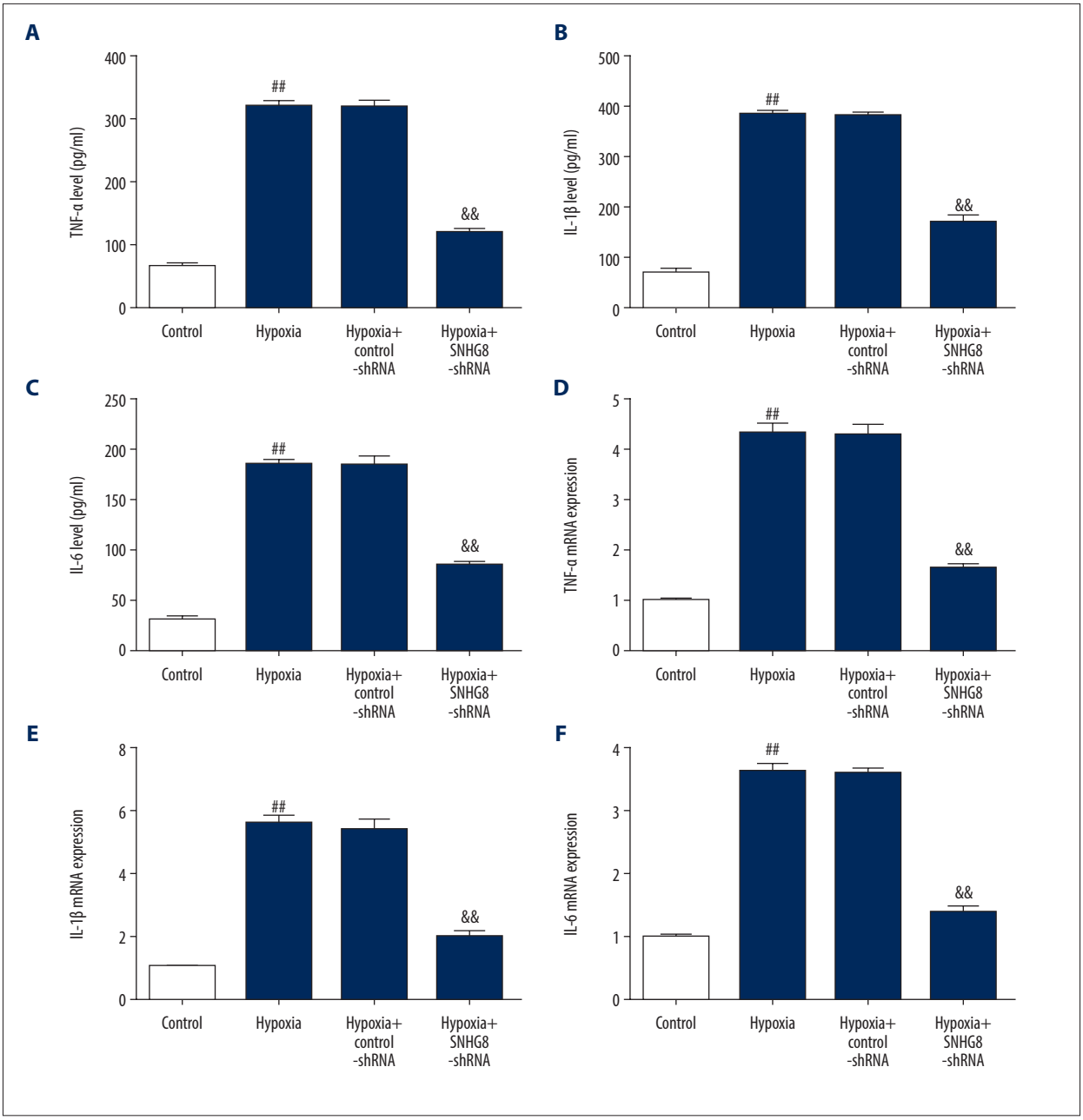

Figure 7. Effects of down-regulating lncRNA SNHG8 on the secretion of inflammatory factors in hypoxia-induced cardiomyocytes. (**A–C**) secretion of IL-1β, TNF-α and IL-6 in transfected hypoxia-induced cardiomyocytes were detected by ELISA. (**D–F**) mRNA expression levels of IL-1β, TNF-α and IL-6 in transfected hypoxia-induced cardiomyocytes were measured by qRT-PCR analysis. Control: cells without any treatment; Hypoxia: H9c2 cells were cultured under hypoxic conditions (94% N_2, 5% CO_2, and 1% O_2) for 48 h; Hypoxia+control-shRNA: H9c2 cells were transfected with control-shRNA for 48 h under hypoxic conditions; Hypoxia+SNHG8-shRNA: H9c2 cells were transfected with SNHG8-shRNA for 48 h under hypoxic conditions. ## P<0.01 *vs.* Control group; && P<0.01 *vs.* Hypoxia+control-shRNA.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

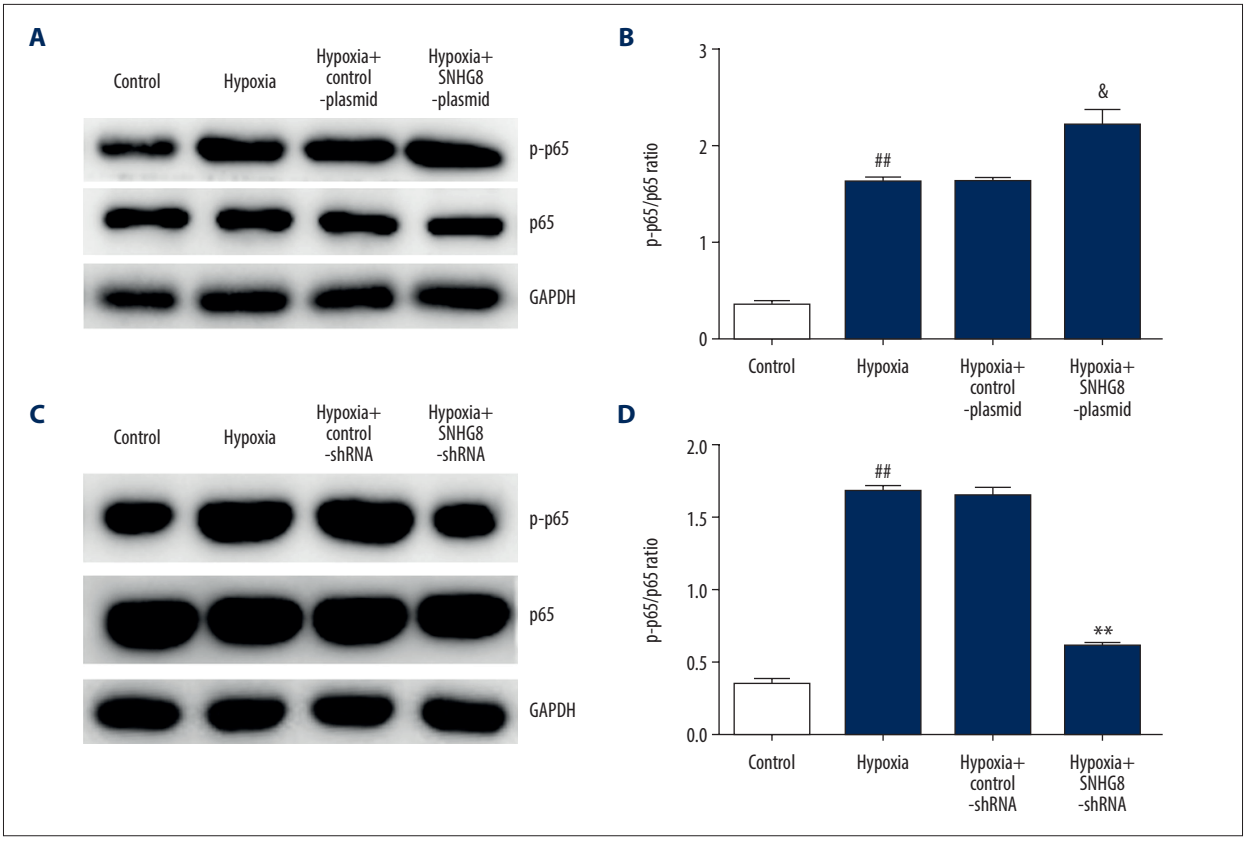

Figure 8. Effects of lncRNA SNHG8 on NF-κB signal pathway in hypoxia-induced cardiomyocytes. (**A, C**) Western blotting analysis was used to determine p-p65 and p65 protein expression in H9c2 cells. (**B, D**) p-p65/p65 ratio in hypoxia-induced cardiomyocytes following transfection. Control: cells without any treatment; Hypoxia: H9c2 cells were cultured under hypoxic conditions (94% N_2, 5% CO_2, and 1% O_2) for 48 h; Hypoxia+control-plasmid: H9c2 cells were transfected with control-plasmid for 48 h under hypoxic conditions; Hypoxia+SNHG8-plasmid: H9c2 cells were transfected with SNHG8-plasmid for 48 h under hypoxic conditions. Hypoxia+control-shRNA: H9c2 cells were transfected with control-shRNA for 48 h under hypoxic conditions; Hypoxia+SNHG8-shRNA: H9c2 cells were transfected with SNHG8-shRNA for 48 h under hypoxic conditions. ## P<0.01 *vs.* Control group; & P<0.05 *vs.* Hypoxia+control-plasmid; ** P<0.01 *vs.* Hypoxia+control-shRNA.

upregulation further promoted L-1β, TNF-α and IL-6 expression, while hypoxia-induced L-1β, TNF-α and IL-6 expression was significantly reduced by lncRNA SNHG8 downregulation.

Several protein kinase pathways were activated in myocardial ischemia and hypoxia [32]. Some studies indicate that pro-inflammatory cytokine levels are elevated in an NF-κB activation-dependent manner during myocardial damage [33]. During treatment of ischemic myocardium, the NF-κB pathway is crucial to preventing myocardial cell death [34]. But the relationship between lncRNA SNHG8 and the NF-κB pathway remains unclear. Therefore, we analyzed whether the NF-κB pathway was involved in regulation of lncRNA SNHG8 in hypoxia-induced myocardial cell injury. Results indicated that lncRNA SNHG8 upregulation further enhanced the NF-κB signal pathway activation in hypoxia-induced cardiomyocytes, while lncRNA SNHG8 downregulation inhibited NF-κB signal pathway activation.

In other words, SNHG8 played a significant role in myocardial cell injury caused by hypoxia, knockdown of SNHG8 played a protective role in the myocardial cell injury caused by hypoxia, and overexpression of SNHG8 promoted myocardial cell injury caused by hypoxia.

Conclusions

We found that lncRNA SNHG8 affected MI by promoting hypoxia-induced cardiomyocyte injury via regulation of the NF-κB signaling pathway. These findings suggest that SNHG8 may be an effective target for treatment of MI, which could translate into new opportunities for clinical therapies for MI.

Conflict of interests

None.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

References

1. Horne BD, Muhlestein JB, Bhandary D et al: Clinically feasible stratification of 1-year to 3-year post-myocardial infarction risk. Open Heart, 2018; 5: e000723
2. Thygesen K, Alpert JS, Jaffe AS et al: Fourth universal definition of myocardial infarction (2018). Kardiol Pol, 2018; 76: 1383–415
3. Anderson JL, Morrow D: Acute myocardial infarction. N Engl J Med, 2017; 376: 2053–64
4. Dong J, Dang Q: Effect of natriuretic peptide on vascular endothelial function and inflammatory factors of patients with heart failure complicating myocardial infarction. Drug Evaluation Res, 2017; 40: 365–68
5. Sun D, Li S, Zhu CG et al: Prevalence and clinical features of familial hypercholesterolemia in Chinese patients with myocardial infarction. Chinese J Cardiov Dis, 2018; 46: 109–13
6. Wang W, Liang S, Liu W: Opinion on the recent development of injectable biomaterials for treating myocardial infarction. Sci China Technol Sc, 2017; 60: 1278–80
7. Yu L, Zhang M: Transplantation of human umbilical cord blood mononuclear cells for treatment of myocardial infarction with heart failure. J Clin Rehabilitative Tissue Eng Res, 2014; 18: 8103–6
8. Luo Q, Guo D, Liu G et al: Exosomes from MiR-126-overexpressing adscs are therapeutic in relieving acute myocardial ischaemic injury. Cell Physiol Biochem, 2017; 44: 2105–116
9. Alqudah A, Mcnally R, Todd N et al: The role of a novel anti-angiogenic protein, FKBPL, in angiogenesis associated with cardiac dysfunction. Open Heart, 2018; 104: A2
10. Goyal R, Kumar MM: LncRNA as a therapeutic target for angiogenesis. Curr Top Med Chem, 2017; 17: 1750–57
11. Rinn JL, Chang HY: Genome regulation by long noncoding RNAs. Annu Rev Biochem, 2012; 81: 145–66
12. Cao SH, Liu WL, Li F et al: Decreased expression of lncRNA GAS5 predicts a poor prognosis in cervical cancer. Int J Clin Exp Med, 2014; 7: 6776–83
13. Zhang AL, Xu M, Mo YY: Role of the lncRNA-p53 regulatory network in cancer. J Mol Cell Biol, 2014; 6: 181–91
14. Kraus TF, Haider M, Spanner J et al: Altered long noncoding RNA expression precedes the course of Parkinson's disease – a preliminary report. Mol Neurobiol, 2016; 54: 2869–77
15. Yang CH, Zhang XY, Zhou LN et al: LncRNA SNHG8 participates in the development of endometrial carcinoma through regulating c-MET expression by miR-152. Eur Rev Med Pharmacol, Sci 2018; 22: 1629–37
16. Qu X, Li Y, Wang L et al: LncRNA SNHG8 accelerates proliferation and inhibits apoptosis in HPV-induced cervical cancer through recruiting EZH2 to epigenetically silence RECK expression. J Cell Biochem, 2020 [Online ahead of print]
17. Song H, Song J, Lu L, Li S: SNHG8 is upregulated in esophageal squamous cell carcinoma and directly sponges microRNA-411 to increase oncogenicity by upregulating KPNA2. Onco Targets Ther, 2019; 12: 6991–7004
18. Zhang P, Li S, Chen Z et a: LncRNA SNHG8 promotes proliferation and invasion of gastric cancer cells by targeting the miR-491/PDGFRA axis. Hum Cell, 2020; 33: 123–30
19. Zhen Y, Ye Y, Wang H et al: Knockdown of SNHG8 repressed the growth, migration, and invasion of colorectal cancer cells by directly sponging with miR-663. Biomed Pharmacother, 2019; 116: 109000
20. Dong J, Teng F, Guo W et al: lncRNA SNHG8 promotes the tumorigenesis and metastasis by sponging miR-149-5p and predicts tumor recurrence in hepatocellular carcinoma. Cell Physiol Biochem, 2018; 51: 2262–74
21. Zhuo LA, Wen YT, Wang Y et al: LncRNA SNHG8 is identified as a key regulator of acute myocardial infarction by RNA-seq analysis. Lipids Health Dis, 2019; 18: 201
22. Gong L, Chang H, Zhang J et al: Astragaloside IV protects rat cardiomyocytes from hypoxia-Induced injury by down-regulation of miR-23a and miR-92a. Cell Physiol Biochem, 2018; 49: 2240–53
23. Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta c(T)) method. Methods, 2001; 25: 402–8,
24. Ren YP, Yang H, Colette B et al: Prevalence of depression in coronary heart disease in China: A systematic review and meta-analysis. Chinese Med J, 2014; 127: 2991–98
25. Rosamond WD, Chambless L, Heiss G et al: Twenty-two year trends in incidence of myocardial infarction, CHD mortality, and case-fatality in four US communities, 1987 to 2008. Circulation, 2012; 125: 1848–57
26. Zhou M, Liu Y, Xiong L et al: Cardiac sympathetic afferent denervation protects against ventricular arrhythmias by modulating cardiac sympathetic nerve activity during acute myocardial infarction. Med Sci Monit, 2019; 25: 1984–93
27. Wu AH, Clive JM: Impact of CK-MB testing policies on hospital length of stay and laboratory costs for patients with myocardial infarction or chest pain. Clin Chem, 2020; 43: 326–32
28. Venta R, Geijo SA, Sánchez AC et al: IgA-CK-BB complex with CK-MB electrophoretic mobility can lead to erroneous diagnosis of acute myocardial infarction. Clin Chem, 2019; 35: 2003–8
29. Wu AH, Feng YJ, Moore R et al; Characterization of cardiac troponin subunit release into serum after acute myocardial infarction and comparison of assays for troponin T and I. Clin Chem, 2020; 44: 1198–208
30. Wang SY, Chen RF, Liang JD et al: Clinical evaluation of high-sensitive troponin t in acute myocardial infarction. J Pract Med, 2017; 33: 4160–64
31. Huang MK, Luo YF, Huang Y: Application of joint detection of MYO, cTnI, CK-MB, D-D and hs-CRP in acute myocardial infarction. Med Plant, 2015; 6: 42–44
32. Ferdinandy P, Schulz R, Baxter GF: Interaction of cardiovascular risk factors with myocardial ischemia/reperfusion injury, preconditioning, and postconditioning. J Pharmacol Rev, 2007; 59: 418–58
33. Wei H, Li H, Wan SP et al: Cardioprotective effects of Malvidin against isoproterenol-induced myocardial infarction in rats: A mechanistic study. Med Sci Monit, 2017; 23: 2007–16
34. Liu Y, Wang T, Zhang M et al: Down-regulation of myocardial infarction associated transcript 1 improves myocardial ischemia-reperfusion injury in aged diabetic rats by inhibition of activation of NF-κB signaling pathway. Chem Biol Interact, 2019; 300: 111–22

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]