European Review for Medical and Pharmacological Sciences 2020; 24: 2062-2069

LINC00511 accelerates the proliferation of cardiomyocytes after myocardial ischemia/reperfusion injury by absorbing miRNA-515-5p

X. ZHANG[1], M.-M. LIU[1], M.-X. GUAN[2], T.-T. LI[3], L.-L. MEI[1], H.-L. CONG[3]

[1]Department of Cardiology, Tianjin Baodi Hospital, Baodi College of Clinical Medicine, Tianjin Medical University, Tianjin, China
[2]Department of Clinical Laboratory, Tianjin Baodi Hospital, Baodi College of Clinical Medicine, Tianjin Medical University, Tianjin, China
[3]Department of Cardiovascular Medicine, Tianjin Chest Hospital, Tianjin, China

Xia Zhang and Minmin Liu contributed equally to this work

Abstract. – OBJECTIVE: The aim of this study was to clarify the role of LINC00511 in regulating the proliferative ability of cardiomyocytes undergoing ischemia/reperfusion (I/R) injury by absorbing miRNA-515-5p.

MATERIALS AND METHODS: Adult male C57BL/6 mice were subjected to I/R injury, and I/R model was constructed *in vivo*. Primary cardiomyocytes were isolated from 1-2 days-old male mice and treated with H_2O_2 to establish the I/R model *in vitro*. The relative expression level of LINC00511 was determined after ligation of the anterior descending coronary artery (LAD) in mice or H_2O_2 induction in primary cardiomyocytes for different time points, respectively. The regulatory effect of LINC00511 on the viability of H_2O_2-treated cardiomyocytes was assessed. Subsequently, the interaction between LINC00511 and miRNA-515-5p was evaluated by Dual-Luciferase Reporter Gene Assay. Furthermore, the viability and 5-Ethynyl-2'-deoxyuridine (EdU)-positive rate influenced by LINC00511/miRNA-515-5p were examined.

RESULTS: LINC00511 was gradually downregulated with the prolongation of I/R procedures in mice or H_2O_2 treatment in primary cardiomyocytes. The overexpression of LINC00511 significantly elevated the viability and EdU-positive rate in H_2O_2-treated cardiomyocytes. LINC00511 could bind to miRNA-515-5p. Meanwhile, there was a negative correlation between the levels of LINC00511 and miRNA-515-5p. In addition, the overexpression of miRNA-515-5p reversed the promoting effect of LINC00511 on the proliferative ability of H_2O_2-treated cardiomyocytes.

CONCLUSIONS: LINC00511 accelerates the proliferation of cardiomyocytes after I/R by targeting miRNA-515-5p.

Key Words:
NC00511, MiRNA-515-5p, Ardiomyocytes, I/R, Proliferation.

Introduction

Cardiomyocyte apoptosis is associated with the development of heart diseases[1]. Heart failure (HF) is a cardiac systolic and/or diastolic dysfunction[2]. HF is not an independent disease, but the end stage of heart disease development[3]. The changes in the myocardial structure and function caused by cardiomyocyte apoptosis may eventually lead to HF[4].

Long non-coding RNAs (LncRNAs) are a type of non-coding RNAs with over 200 nucleotides in length[5]. Currently, lncRNAs have been found involved in various cellular processes, such as epigenetics and cellular behaviors[6]. Dys-regulated lncRNAs are closely related to the occurrence and progression of diseases. In addition, lncRNAs can also participate in the pathogenesis of cardiovascular diseases[7,8]. Some studies[9-11] have shown that lncRNAs regulate the gene expressions by absorbing miRNAs.

LINC00511 is a 2.265-kb lncRNA that is located on chromosome 17q24.3. It is a newly identified carcinogenic lncRNA[12,13]. Sun et al[14] have demonstrated that LINC00511 is widely involved in the regulation of tumorigenesis by regulating the activity of miRNAs. In fact, acting as a competitive endogenous RNA, LINC00511 regulates the expression of VEGFA by adsorbing hsa-miR-29b-3p in pancreatic ductal adenocarcinoma[15]. LINC00511 interacts with miR-765 and regulates the progression of tongue squamous cell carcinoma by targeting LAMC2[16]. LINC00511 promotes the development of breast cancer by inducing the miR-185-3p/E2F1/Nanog axis[17]. Furthermore, LINC00511 promotes the proliferative and mi-

Corresponding Author: Hongliang Cong, MD; e-mail: zxtj000@163.com

gratory capacities of osteosarcoma cells by absorbing miR-765[18]. However, the specific role of LINC00511 in regulating cardiomyocytes has not been fully elucidated. In this paper, both *in vivo* and *in vitro* I/R models were constructed. The aim of this study was to further uncover the regulatory effects of LINC00511 and miRNA-515-5p on cardiomyocytes after ischemia/reperfusion (I/R) injury.

Materials and Methods

Cell Isolation and Culture

Primary cardiomyocytes were isolated from male C57BL/6 mice with 1-2 days old. Briefly, mouse heart tissues were cut into small pieces and digested with trypsin at 37°C. After digestion termination and rapid centrifugation, the cardiomyocytes were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Hyclone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS; Hyclone, South Logan, UT, USA), 1% penicillin, streptomycin. Primary cardiomyocytes were treated with H_2O_2 for different time points.

I/R Procedures in Mice

Male C57BL/6 mice with 8-week-old were provided by Tianjin Medical University Experimental Animal Center. All animal procedures were conducted in accordance with the protocols approved by the Tianjin Medical University Animal Care Committee. The mice were anesthetized, and a longitudinal incision was cut to expose the chest. Ligation of anterior descending coronary artery (LAD) was performed using a snare. The coronary artery was blocked by tightening the snare and secured with a hemostat. Subsequently, the ligation was loosened to allow reperfusion. Meanwhile, the mice in the sham group received the same procedures, except for ligation loosening.

Construction of Overexpression Vectors and Transfection

The plasmid complementary deoxyribonucleic acid 3.0 (pcDNA3.0)-LINC00511 vector was constructed based on amplification with specific primers. Subsequently, the complementary deoxyribonucleic acids (cDNAs) of LINC00511 was cloned into mammalian expression vector pcDNA3.0 (Invitrogen, Carlsbad, CA, USA). Cardiomyocytes were cultured until 60% of confluence. Cell transfection was performed according to the instructions of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). 6 h later, complete medium was replaced. The transfected cells for 24-48 h were harvested for the following *in vitro* experiments.

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The total RNA in cells was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The concentration of RNA was detected by an ultraviolet spectrophotometer (Hitachi, Tokyo, Japan). Subsequently, the extracted RNA was reversely transcribed into cDNA, followed by PCR using the SYBR Green method (TaKaRa, Otsu, Shiga, Japan). Specific procedures were as follow: 94°C for 5 min, and 40 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s. The relative expression of miRNAs was calculated using the $2^{-\Delta\Delta CT}$ method. U6 was used as an internal reference. The primer sequences used in this study were as follows: LINC00511, F: 5'-CGGCTTAACTAACTGTTACTC-3', R: 5'-CAGTACCGATGTCAGACACGGA-3'; miRNA-515-5p, F: 5'-GAGCGTAGTAACCATACTCGACATCG-3', R: 5'-ACCTTGCATGTCTGAAGTAG-3'; miR-126-5p, F: 5'-GAGAACGCGCATATGCCACA-3', R: 5'-CGGTTGCAGAATAGACGACTTCT-3'; miR-15b-3p: F: 5'-CAGTGACTGACACTGACGAAC-3', R: 5'-GCTGCAACGATAGCTTCCACCGA-3'; miR-483-3p: F: 5'-CGCATTGTCTTATACACAGCTTACAAC-3', R: 5'-GTCATACCAATGACGCTGGTGA-3'; U6: F: 5'-GCTTCGGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3'.

Dual-Luciferase Reporter Gene Assay

Cardiomyocytes were co-transfected with LINC00511-MT/LINC00511-WT and miRNA-515-5p mimics/NC, respectively. 48 h after transfection, the cells were lysed. Finally, the relative Luciferase activity was determined by the Dual-Luciferase Reporter Gene Assay (Promega, Madison, WI, USA).

Cell Counting Kit-8 (CCK-8) Assay

The cardiomyocytes were first seeded into 96-well plates at a density of 5×10^3 cells per well and cultured overnight. Absorbance (A) at 450 nm was recorded at appointed time points using the CCK-8 kit (Dojindo Laboratories, Kumamoto, Japan).

5-Ethynyl-2'-Deoxyuridine (EdU) Assay

The cardiomyocytes were first seeded into 96-well plates, with 300 cells per well. ECs were labeled with 50 μmol/L EdU at 37°C for 2 h. After fixation with 4% paraformaldehyde for 30 min, the cells were incubated with Phosphate-Buffered Saline (PBS) containing 0.5% Triton-100 for 20 min. Then, the cells were washed with PBS containing 3% bovine serum albumin (BSA), followed by incubation with 100 μL of dying solution for 1h in the dark. Subsequently, the cells were counter-stained with 100 μL of 4',6-diamidino-2-phenylindole (DAPI; 5 μg/ mL; Sigma-Aldrich, St. Louis, MO, USA) for 30 min. Finally, the EdU-positive cells, DAPI-labeled nucleus, and merged images were captured under a microscope (magnification 100×).

Statistical Analysis

The Statistical Product and Service Solutions (SPSS) 18.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. GraphPad Prism (Version X; La Jolla, CA, USA) was applied for image editing. The experimental data were expressed as mean ± standard deviation. The intergroup differences were analyzed by the *t*-test. $p<0.05$ was considered statistically significant.

Results

LINC00511 Was Downregulated After I/R Injury

I/R injury in mice was successfully established by performing LAD. With the prolongation of ischemia, the relative level of LINC00511 was gradually down-regulated (Figure 1A). Meanwhile, the primary cardiomyocytes were isolated from newborn mice and subjected to H_2O_2 treatment for 0, 6, 12, and 24 h, respectively. The results demonstrated that LINC00511 was down-regulated in a time-dependent manner following I/R injury *in vitro* (Figure 1B).

Overexpression of LINC00511 Protected H_2O_2-Induced Proliferative Inhibition in Cardiomyocytes

LINC00511 level was significantly reduced in the H_2O_2-treated cardiomyocytes. The transfection of pcDNA-LINC00511 remarkably up-regulated the expression of LINC00511 in cardiomyocytes following I/R injury. This suggested an effective transfection efficacy (Figure 2A). CCK-8 assay revealed that the viability of H_2O_2-treated cardiomyocytes was remarkably reduced. However, it was markedly elevated after the overexpression of LINC00511 (Figure 2B). EdU assay obtained similar results. The transfection of pcDNA-LINC00511 markedly increased the EdU-positive rate in cardiomyocytes treated with H_2O_2 (Figure 2C). All these findings suggested that LINC00511 protected the inhibitory effect of H_2O_2 on the proliferative ability of cardiomyocytes.

LINC00511 Could Bind to MiRNA-515-5p

Through online prediction, several miRNAs have been screened out to bind to LINC00511, including miRNA-515-5p, miR-126-5p, miR-15b-3p, and miR-483-3p. In mice received 120-min of I/R injury, only the expression level of miRNA-515-5p was remarkably up-regulated (Figure 3A). Identically, miRNA-515-5p was the only miRNA significantly up-regulated after 24h of H_2O_2 treatment in cardiomyocytes (Figure 3B). To further clarify the biological function of miRNA-515-

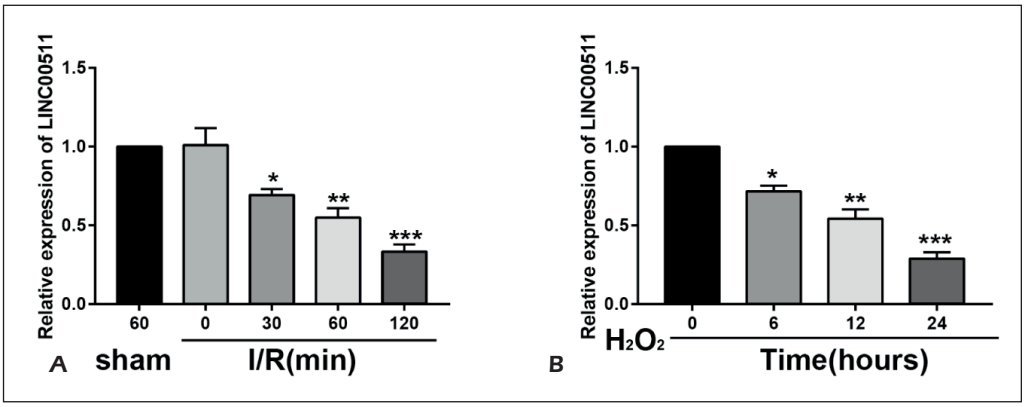

Figure 1. LINC00511 was downregulated following I/R injury. **A**, Relative level of LINC00511 in mice undergoing sham operations for 60 min, and I/R injury for 0, 30, 60, and 120 min. **B**, Relative level of LINC00511 in primary cardiomyocytes treated with H_2O_2 for 0, 6, 12, and 24 h, respectively.

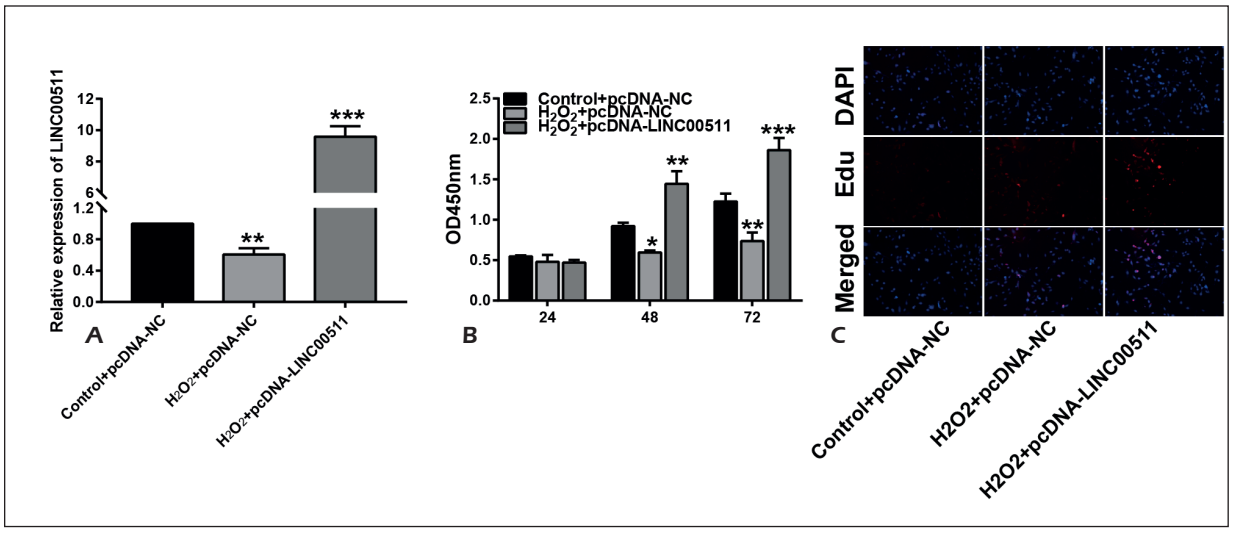

Figure 2. Overexpression of LINC00511 protected H_2O_2-induced proliferative inhibition in cardiomyocytes. **A**, Relative level of LINC00511 in primary cardiomyocytes transfected with control + pcDNA-NC, H_2O_2 + pcDNA-NC or H_2O_2 + pcDNA-LINC00511. **B**, CCK-8 assay showed the viability of primary cardiomyocytes transfected with control + pcDNA-NC, H_2O_2 + pcDNA-NC or H_2O_2 + pcDNA-LINC00511. ***C,*** DAPI-labeled, EdU-labeled and merged images of primary cardiomyocytes transfected with control + pcDNA-NC, H_2O_2 + pcDNA-NC or H_2O_2 + pcDNA-LINC00511 (magnification × 100).

5p, we constructed miRNA-515-5p inhibitor and miRNA-515-5p mimics (Figure 3C). Meanwhile, the wild-type and mutant-type LINC00511 vectors were constructed for Dual-Luciferase Reporter Gene Assay (Figure 3D). The results indicated that the relative Luciferase activity markedly decreased after the co-transfection of LINC00511-WT and miRNA-515-5p mimics, verifying the binding relationship between LINC00511 and miRNA-515-5p (Figure 3E). The transfection of miRNA-515-5p inhibitor significantly upregulated LINC00511 level. However, the opposite trend was obtained after transfection of miRNA-515-5p mimics (Figure 3F). Wild-type and mutant miR-515-5p were further constructed for luciferase reporter gene experiment (Figure 3G), and the results again confirmed that LINC00511 regulated mir-515-5p (Figure 3H). In cardiomyocytes, the transfection of LINC00511 overexpressed plasmid significantly reduced the level of miR-515-5p (Figure 3I). In summary: LINC00511 specifically regulates the expression of miR-515-5p.

LINC00511 Influenced Cardiomyocyte Proliferation by Targeting MiRNA-515-5p

Thereafter, we speculated whether miRNA-515-5p was involved in LINC00511-mediated proliferation of cardiomyocytes following I/R injury. Firstly, the results found that LINC00511 level in cardiomyocytes overexpressing LINC00511 was remarkably down-regulated by the co-transfection of miRNA-515-5p mimics (Figure 4A). Interestingly, pcDNA-LINC00511 transfection markedly increased the number of EdU-positive cells in cardiomyocytes treated with H_2O_2. However, this could be further reversed by the overexpression of miRNA-515-5p (Figure 4B). To sum up, LINC00511 accelerated cardiomyocyte proliferation after H_2O_2 treatment by negatively regulating miRNA-515-5p level (Figure 4B).

Discussion

Currently, HF poses a huge threat to human health[19]. It has been identified that cardiomyocyte apoptosis leads to the occurrence and progression of myocardial infarction and HF[20]. Therefore, it is of great significance to clarify the molecular mechanism of the cardiomyocyte apoptosis[21]. HF is a syndrome in which the cardiac output is reduced due to primary cardiac damage. Under pathological circumstances, cardia output is insufficient to the metabolic needs of tissues[4]. Reduction in cardiomyocyte number and systolic or diastolic overload eventually lead to HF[22]. Meanwhile, the decreased blood output of the heart cannot satisfy the metabolic needs of the body[23,24]. Previous experimental trails[25,26] have demonstrated that LAD procedures and H_2O_2 treatment can mimic *in vivo* and *in vitro* I/R injury in cardiomyocytes, respectively.

MiRNAs are a class of endogenous regulatory non-coding RNAs found in eukaryotes[27]. Mature

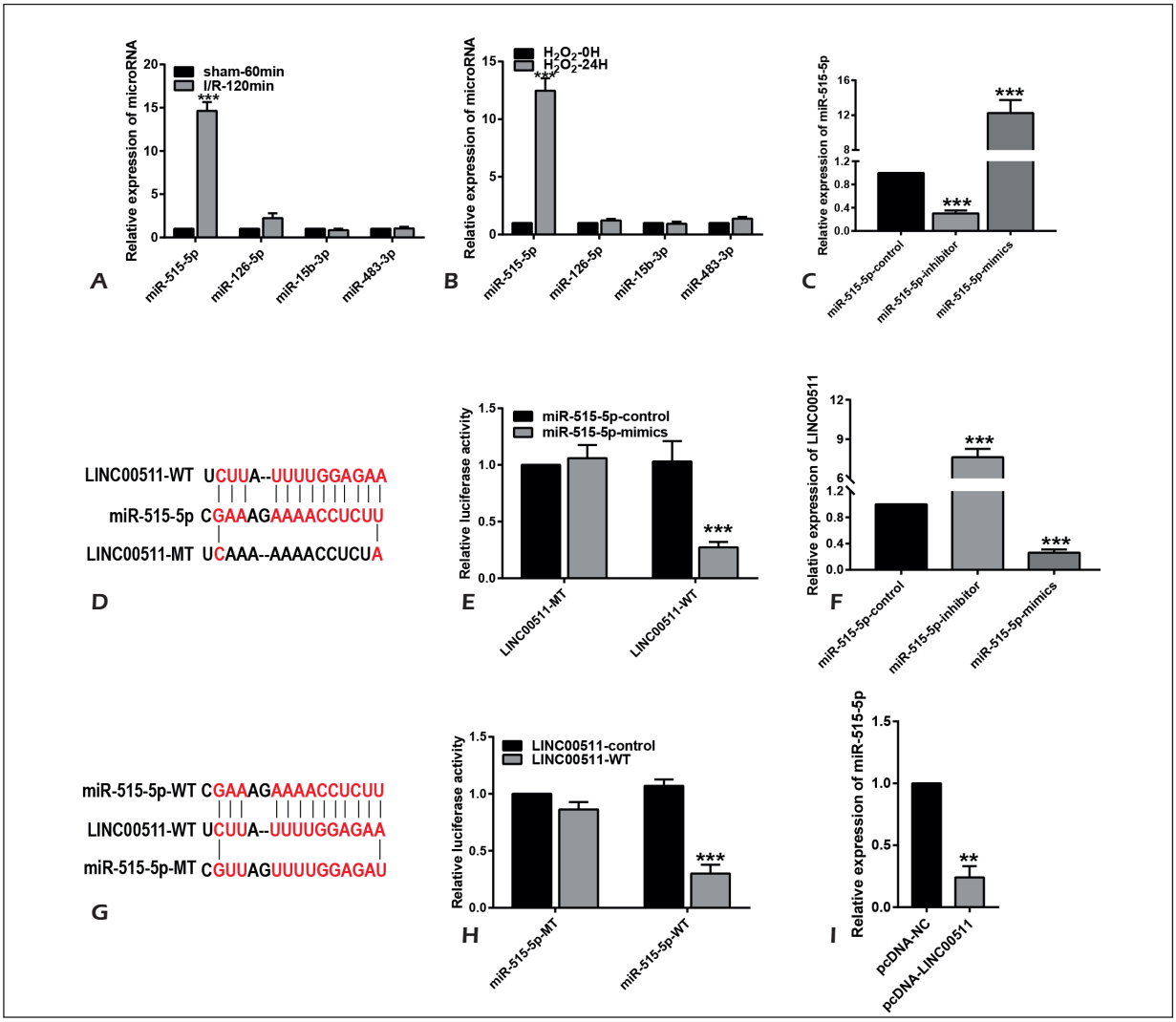

Figure 3. LINC00511 bound to miR-515-5p. **A**, Relative levels of miR-515-5p, miR-126-5p, miR-15b-3p, and miR-483-3p in mice undergoing 60-min of sham operation or 120-min of I/R injury. **B**, Relative levels of miR-515-5p, miR-126-5p, miR-15b-3p, and miR-483-3p in primary cardiomyocytes undergoing 0 or 24-h H_2O_2 treatment. **C**, Transfection efficacies of miR-515-5p inhibitor and miR-515-5p mimics, respectively. **D**, Potential binding sequences between LINC00511 and miR-515-5p. **E**, Dual-luciferase reporter gene assay showed the luciferase activity in cardiomyocytes co-transfected with LINC00511-MT/LINC00511-WT and miR-515-5p mimics/NC. **F**, Relative level of LINC00511 in primary cardiomyocytes transfected with NC, miR-515-5p inhibitor or miR-515-5p mimics. **G**, Wild-type and mutant miR-515-5p were further constructed for luciferase reporter gene experiment. **H**, Luciferase reporter assay confirmed that LINC00511 targeted miR-515-5p. **I**, Overexpression of LINC00511 significantly decreased miR-515-5p in cardiomyocytes.

miRNAs are produced by the processing of primary transcripts through a series of nucleases. Based on the degree of complementary base pairing, miRNAs can guide the degradation of target mRNAs or inhibit their translation[28,29]. Current studies[30,31] have indicated that miRNAs participate in a variety of regulatory pathways, including development, viral defense, hematopoietic processes, organ formation, cell proliferation, and apoptosis, etc.

MiRNAs are closely related to tumor biology, serving as oncogenes or tumor suppressor genes[32-34]. Liu et al[35] have discovered the involvement of miRNAs in cardiomyocyte apoptosis. Of note, miRNA-15b disrupts hypoxia/reperfusion-induced cardiomyocyte apoptosis by down-regulating Bcl-2 and MAPK. The downregulation of miR-200c protects the cardiomyocytes from hypoxia-induced apoptosis by targeting GATA-4[36]. The inhibition of miR-363 protects cardiomyocytes from hypoxia-induced apoptosis through the Notch pathway[37]. Meanwhile, miRNA-122 regulates caspase-8

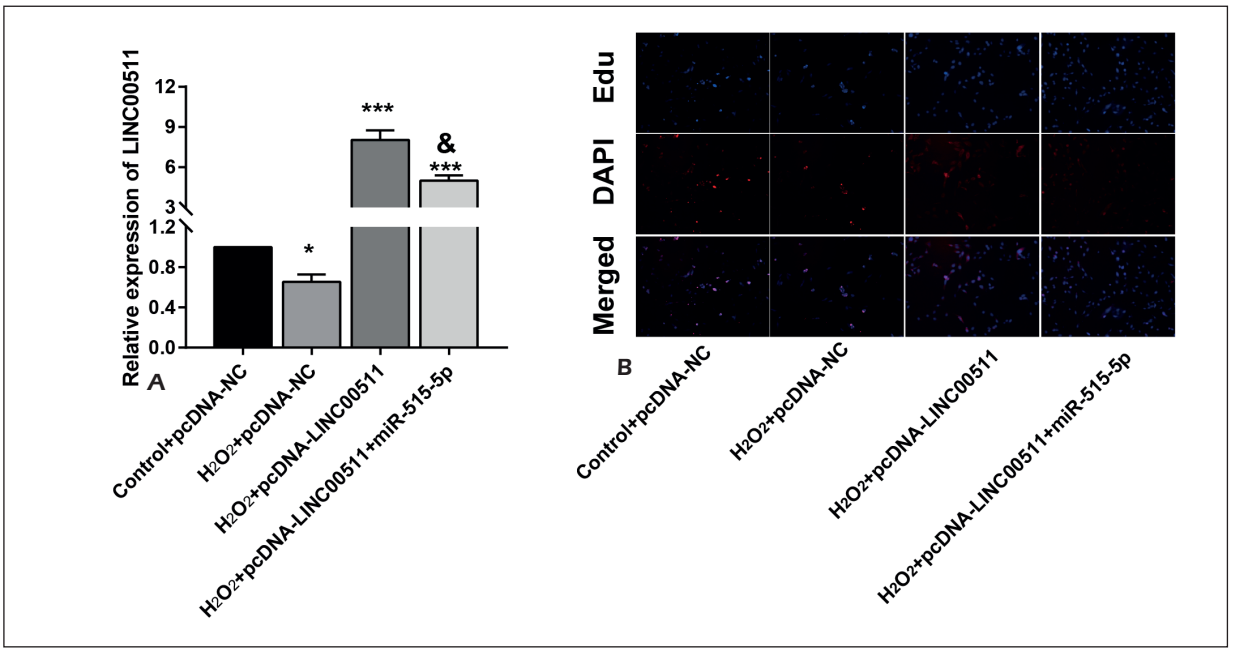

Figure 4. LINC00511 accelerated cardiomyocyte proliferation by targeting miR-515-5p. **A**, Relative level of LINC00511 in primary cardiomyocytes transfected with control + pcDNA-NC, H_2O_2 + pcDNA-NC, H_2O_2 + pcDNA-LINC00511 or H_2O_2 + pcDNA-LINC00511 + miR-515-5p mimics. **B**, DAPI-labeled, EdU-labeled and merged images of primary cardiomyocytes transfected with control + pcDNA-NC, H_2O_2 + pcDNA-NC, H_2O_2 + pcDNA-LINC00511 or H_2O_2 + pcDNA-LINC00511 + miR-515-5p mimics (magnification × 100).

and promotes the apoptosis of cardiomyocytes in mouse[38]. Cardiomyocyte apoptosis has also been observed associated with the development of HF. During the pathological progression of HF, miRNAs are important regulators of cardiomyocyte apoptosis[39,40].

In this paper, miRNA-515-5p was found significantly up-regulated following I/R injury. MiRNA-515-5p acted as the direct target of LINC00511 and was negatively correlated with LINC00511. Therefore, it was speculated that LINC00511 might absorb miRNA-515-5p to exert its promoting effect on cardiomyocyte proliferation. Our rescue experiments demonstrated that the overexpression of miRNA-515-5p reversed the promoting effect of LINC00511 on the proliferative rate of H_2O_2-treated cardiomyocytes.

Conclusions

This study demonstrated that LINC00511 regulated the proliferative ability of cardiomyocytes following I/R by targeting miRNA-515-5p.

Conflict of Interests

The authors declared that they have no conflict of interests.

References

1) Long B, Li N, Xu XX, Li XX, Xu XJ, Guo D, Zhang D, Wu ZH, Zhang SY. Long noncoding RNA FTX regulates cardiomyocyte apoptosis by targeting miR-29b-1-5p and Bcl2l2. Biochem Biophys Res Commun 2018; 495: 312-318.

2) Zhang Q, Lu L, Liang T, Liu M, Wang ZL, Zhang PY. MAPK pathway regulated the cardiomyocyte apoptosis in mice with post-infarction heart failure. Bratisl Lek Listy 2017; 118: 339-346.

3) Takemura G, Kanamori H, Okada H, Miyazaki N, Watanabe T, Tsujimoto A, Goto K, Maruyama R, Fujiwara T, Fujiwara H. Anti-apoptosis in nonmyocytes and pro-autophagy in cardiomyocytes: two strategies against postinfarction heart failure through regulation of cell death/degeneration. Heart Fail Rev 2018; 23: 759-772.

4) Toischer K, Zhu W, Hunlich M, Mohamed BA, Khadjeh S, Reuter SP, Schafer K, Ramanujam D, Engelhardt S, Field LJ, Hasenfuss G. Cardiomyocyte proliferation prevents failure in pressure overload but not volume overload. J Clin Invest 2017; 127: 4285-4296.

5) Xu XY, Zhang J, Qi YH, Kong M, Liu SA, Hu JJ. Linc-ROR promotes endometrial cell proliferation by activating the PI3K-Akt pathway. Eur Rev Med Pharmacol Sci 2018; 22: 2218-2225.

6) Yu SY, Dong B, Zhou SH, Tang L. LncRNA UCA1 modulates cardiomyocyte apoptosis by targeting miR-143 in myocardial ischemia-reperfusion injury. Int J Cardiol 2017; 247: 31.

7) Li X, Wang H, Yao B, Xu W, Chen J, Zhou X. LncRNA H19/miR-675 axis regulates cardiomyocyte apoptosis by targeting VDAC1 in diabetic cardiomyopathy. Sci Rep 2016; 6: 36340.

8) Uchida S, Dimmeler S. Long noncoding RNAs in cardiovascular diseases. Circ Res 2015; 116: 737-750.

9) Yu BY, Dong B. LncRNA H19 regulates cardiomyocyte apoptosis and acute myocardial infarction by targeting miR-29b. Int J Cardiol 2018; 271: 25.

10) Wu T, Wu D, Wu Q, Zou B, Huang X, Cheng X, Wu Y, Hong K, Li P, Yang R, Li Y, Cheng Y. Knockdown of long non-coding RNA-ZFAS1 protects cardiomyocytes against acute myocardial infarction via anti-apoptosis by regulating miR-150/CRP. J Cell Biochem 2017; 118: 3281-3289.

11) Li J, Li L, Li X, Wu S. Long noncoding RNA LINC00339 aggravates doxorubicin-induced cardiomyocyte apoptosis by targeting miR-484. Biochem Biophys Res Commun 2018; 503: 3038-3043.

12) Li J, Li Y, Meng F, Fu L, Kong C. Knockdown of long non-coding RNA linc00511 suppresses proliferation and promotes apoptosis of bladder cancer cells via suppressing Wnt/β-catenin signaling pathway. Biosci Rep 2018; 38. pii: BSR20171701.

13) Li C, Liu H, Yang J, Yang J, Yang L, Wang Y, Yan Z, Sun Y, Sun X, Jiao B. Long noncoding RNA LINC00511 induced by SP1 accelerates the glioma progression through targeting miR-124-3p/CCND2 axis. J Cell Mol Med 2019; 23: 4386-4394.

14) Sun CC, Li SJ, Li G, Hua RX, Zhou XH, Li DJ. Long intergenic noncoding RNA 00511 acts as an oncogene in non-small-cell lung cancer by binding to EZH2 and suppressing p57. Mol Ther Nucleic Acids 2016; 5: e385.

15) Zhao X, Liu Y, Li Z, Zheng S, Wang Z, Li W, Bi Z, Li L, Jiang Y, Luo Y, Lin Q, Fu Z, Rufu C. Linc00511 acts as a competing endogenous RNA to regulate VEGFA expression through sponging hsa-miR-29b-3p in pancreatic ductal adenocarcinoma. J Cell Mol Med 2018; 22: 655-667.

16) Ding J, Yang C, Yang S. LINC00511 interacts with miR-765 and modulates tongue squamous cell carcinoma progression by targeting LAMC2. J Oral Pathol Med 2018; 47: 468-476.

17) Lu G, Li Y, Ma Y, Lu J, Chen Y, Jiang Q, Qin Q, Zhao L, Huang Q, Luo Z, Huang S, Wei Z. Long noncoding RNA LINC00511 contributes to breast cancer tumourigenesis and stemness by inducing the miR-185-3p/E2F1/Nanog axis. J Exp Clin Cancer Res 2018; 37: 289.

18) Yan L, Wu X, Liu Y, Xian W. LncRNA Linc00511 promotes osteosarcoma cell proliferation and migration through sponging miR-765. J Cell Biochem 2018. doi: 10.1002/jcb.27999. [Epub ahead of print].

19) Peana D, Domeier TL. Cardiomyocyte Ca (2+) homeostasis as a therapeutic target in heart failure with reduced and preserved ejection fraction. Curr Opin Pharmacol 2017; 33: 17-26.

20) Sharifi-Sanjani M, Oyster NM, Tichy ED, Bedi KJ, Harel O, Margulies KB, Mourkioti F. Cardiomyocyte-specific telomere shortening is a distinct signature of heart failure in humans. J Am Heart Assoc 2017; 6. pii: e005086.

21) Braunwald E. The war against heart failure: the Lancet lecture. Lancet 2015; 385: 812-824.

22) Lu YY, Cheng CC, Tsai CF, Lin YK, Lee TI, Chen YC, Chen SA, Chen YJ. Discrepant effects of heart failure on electrophysiological property in right ventricular outflow tract and left ventricular outflow tract cardiomyocytes. Clin Sci (Lond) 2017; 131: 1317-1327.

23) Foley JF. Heart failure inhibitor. Sci Signal 2017; 10: 487.

24) Tham YK, Bernardo BC, Ooi JY, Weeks KL, McMullen JR. Pathophysiology of cardiac hypertrophy and heart failure: signaling pathways and novel therapeutic targets. Arch Toxicol 2015; 89: 1401-1438.

25) Katanasaka Y, Suzuki H, Sunagawa Y, Hasegawa K, Morimoto T. Regulation of cardiac transcription factor GATA4 by post-translational modification in cardiomyocyte hypertrophy and heart failure. Int Heart J 2016; 57: 672-675.

26) Ashur C, Frishman WH. Cardiosphere-derived cells and ischemic heart failure. Cardiol Rev 2018; 26: 8-21.

27) Yan H, Ma F, Zhang Y, Wang C, Qiu D, Zhou K, Hua Y, Li Y. MiRNAs as biomarkers for diagnosis of heart failure: a systematic review and meta-analysis. Medicine (Baltimore) 2017; 96: e6825.

28) Zhang J, Xing Q, Zhou X, Li J, Li Y, Zhang L, Zhou Q, Tang B. Circulating miRNA21 is a promising biomarker for heart failure. Mol Med Rep 2017; 16: 7766-7774.

29) Shah P, Bristow MR, Port JD. MicroRNAs in heart failure, cardiac transplantation, and myocardial recovery: biomarkers with therapeutic potential. Curr Heart Fail Rep 2017; 14: 454-464.

30) Dangwal S, Schimmel K, Foinquinos A, Xiao K, Thum T. Noncoding RNAs in heart failure. Handb Exp Pharmacol 2017; 243: 423-445.

31) Vegter EL, van der Meer P, de Windt LJ, Pinto YM, Voors AA. MicroRNAs in heart failure: from biomarker to target for therapy. Eur J Heart Fail 2016; 18: 457-468.

32) Tutar L, Ozgur A, Tutar Y. Involvement of miRNAs and pseudogenes in cancer. Methods Mol Biol 2018; 1699: 45-66.

33) Masood N, Basharat Z, Khan T, Yasmin A. Entangling relation of micro RNA-let7, miRNA-200 and miRNA-125 with various cancers. Pathol Oncol Res 2017; 23: 707-715.

34) Garofalo M, Croce CM. MicroRNAs: master regulators as potential therapeutics in cancer. Annu Rev Pharmacol Toxicol 2011; 51: 25-43.

35) Liu Y, Yang L, Yin J, Su D, Pan Z, Li P, Wang X. MicroRNA-15b deteriorates hypoxia/reoxygenation-induced cardiomyocyte apoptosis by down-regulating Bcl-2 and MAPK3. J Investig Med 2018; 66: 39-45.

36) Chen Z, Zhang S, Guo C, Li J, Sang W. Downregulation of miR-200c protects cardiomyocytes from hypoxia-induced apoptosis by targeting GATA-4. Int J Mol Med 2017; 39: 1589-1596.

37) Meng X, Ji Y, Wan Z, Zhao B, Feng C, Zhao J, Li H, Song Y. Inhibition of miR-363 protects cardiomyocytes against hypoxia-induced apoptosis through regulation of Notch signaling. Biomed Pharmacother 2017; 90: 509-516.

38) Zhang ZW, Li H, Chen SS, Li Y, Cui ZY, Ma J. MicroRNA-122 regulates caspase-8 and promotes the apoptosis of mouse cardiomyocytes. Braz J Med Biol Res 2017; 50: e5760.

39) Wang H, Cai J. The role of microRNAs in heart failure. Biochim Biophys Acta Mol Basis Dis 2017; 1863: 2019-2030.

40) Ali SM, Salma U, Zhang B, Chen J, Zhuang J, Ping Z. Diagnostic, prognostic, and therapeutic value of circulating miRNAs in heart failure patients associated with oxidative stress. Oxid Med Cell Longev 2016; 2016: 5893064.