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# LncRNA-MALAT1 influences myocardial infarction by regulating miR-30a/beclin-1 pathway

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**Abstract. – OBJECTIVE: Long non-coding ribonucleic acid-metastasis-associated lung adenocarcinoma transcript 1 (lncRNA-MALAT1) has been confirmed as a key factor involving in various physiological and pathological processes. The present study aims to investigate whether lncRNA-MALAT1 affects the process of myocardial infarction (MI) in rats by regulating the micro RNA (miR-30a)/Beclin-1 (BECN1) pathway.**

**MATERIALS AND METHODS: Twelve healthy male Sprague-Dawley rats were randomly selected and equally divided into sham-operation group and MI group. In MI group, a rat model of acute MI (AMI) was established by ligating the left anterior descending coronary artery. Rats in sham-operation group were set as the control. The messenger RNA (mRNA) expression levels of lncRNA-MALAT1, miR-30a, and BECN1 in the infarcted myocardial tissues were detected *via* real-time fluorescence quantitative polymerase chain reaction (qRT-PCR). The rat myocardial cell line H9c2 was cultured *in vitro* and then transfected with vectors of lncRNA-MALAT1, miR-30a, and BECN1. After that, qRT-PCR was performed to measure mRNA levels of lncRNA-MALAT1, miR-30a, and BECN1 in H9c2 cells. The protein level of BECN1 in cells was determined *via* Western blotting (WB) assay.**

**RESULTS: Expression levels of lncRNA-MALAT1 and BECN1 in the rat myocardium of MI group were up-regulated markedly ($p<0.01$), while miR-30a was down-regulated notably ($p<0.01$) compared with those in sham-operation group. After H9c2 cells were transfected with overexpression vectors of lncRNA-MALAT1 or miR-30a, expression levels of miR-30a ($p<0.01$) and BECN1 ($p<0.01$) were remarkably down-regulated, respectively.**

**CONCLUSIONS: LncRNA-MALAT1 up-regulates the expression of BECN1 by binding to miR-30a, thereby increasing the level of cell autophagy after MI.**

*Key Words:*
LncRNA-MALAT1, MiR-30a, Beclin-1, Rats, Myocardial infarction.

## Introduction

Myocardial infarction (MI) is one of the most serious coronary artery diseases, which is a fatal worldwide[1]. As one of the most severe cases among MI, acute MI (AMI) occurs in a short period and causes serious damages to myocardial tissues, leading to extremely high death and disability rates of patients. AMI is mainly induced by the rupture or erosion of atherosclerotic plaques, which causes thrombotic occlusion and interruption of the blood flow in the epicardial coronary artery, finally resulting in local ischemia[2]. For the patients with AMI attack, therefore, vascular recanalization of the infarcted blood vessels is of great significance. However, reperfusion may trigger secondary injury, expand the range of myocardial injury, and decrease the survival rate of the patients. Csonka et al[3] have demonstrated that reperfusion injury may induce myocardial necrosis with a final area of about 50%. Some studies have pointed out that myocardial cell apoptosis, necrosis, and autophagy jointly participate in the process of myocardial cell injury caused by ischemia/reperfusion.

Although necrosis is regarded as an uncontrolled modality of cell death, multiple modalities regulating cell death are stimulated in the infarcted myocardium. Their effects on the formation of the final area of MI have not been clarified[4]. Autophagy, a regulation model, is a degradation process of lysosomal proteins, while the roles of protein recycling in myocardial ischemia/reperfusion and cardiac protection during mitophagy remain unclear[5].

Long non-coding ribonucleic acids (lncRNAs) are a category of RNAs with a length of over 200 nt and they lack protein-encoding capacity[6]. LncRNAs are involved in the occurrence and development of many diseases in animals and exert vital effects on physiological and pathological ac-

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tivities of cells[7]. Researchers[8,9] have reported that lncRNAs can regulate heart development. According to a transcriptomic analysis, the dynamic regulation of lncRNAs occurs after heart failure and implantation of left ventricular assist device, suggesting that lncRNAs probably play crucial roles in the pathogenesis of heart diseases[10]. MicroRNAs (miRNAs), a kind of small non-coding, single-stranded RNAs encoded by endogenous genes, are involved in the post-transcriptional gene expression regulation. MiRNAs negatively regulate protein expressions in different physiological and pathological processes and perform critical functions in the myocardial injury[11,12].

In the present work, the interaction between lncRNA-metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) with miR-30a and Beclin-1 (BECN1) in the myocardial cells were explored to better understand their roles in the pathogenesis of MI.

## Materials and Methods

### Main Materials

Sprague-Dawley rats, pentobarbital sodium (Shanghai Longsheng Chemical Co., Ltd., Shanghai, China), SYBR Green Real Time fluorescence quantitative Polymerase Chain Reaction (RT-PCR) Master Mix kit and RT Master Mix kit (TaKaRa, Otsu, Shiga, Japan), rat myocardial cell line (H9c2) (Shanghai Cell Bank of Chinese Academy of Sciences, Shanghai, China), Dulbecco's Modified Eagle's Medium (DMEM), Opti-MEM medium, fetal bovine serum (FBS) and phosphate-buffered saline (PBS; Gibco, Rockville, MD, USA), BECN1 antibody and β-actin antibody (Abcam, Cambridge, MA, USA), Lipofectamine 2000 and TRIzol (Invitrogen, Carlsbad, CA, USA), miRNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA), luciferase reporter gene assay system (Promega, Madison, WI, USA), bioluminescent plate reader (Modulus TM), and 0.22 μm pinhole filter (Millipore, Billerica, MA, USA). This study was approved by the Animal Ethics Committee of Nanjing Medical University Animal Center.

### Establishment of Rat Model of MI

The rat model of MI was established by ligating the left anterior descending coronary artery. All the rats were deprived of food and water for 8 h before the research. They were fixed on a heated operation table after anesthesia by intraperitoneal injection of 0.75% pentobarbital sodium. After tracheal intubation, the rats were connected to a rodent ventilator. The hair of the rats in the surgical area was shaved. Then, a continuous 3-lead electrocardiogram (ECG) monitoring system was connected to the rats. Next, the thoracic wall was cut open along the third and fourth intercostals of the left sternum to expose the heart. The pericardium was exposed. Then, the left anterior descending coronary artery was exposed and ligated at the proximal end 1 mm below the left atrium using a 5-0 suture. In MI group, the connection was removed at 30 min after ischemia and reperfusion was allowed for 60 min. The modeling of AMI was successful when the region of the anterior descending coronary artery turned white, with two or more places displaying the J-point elevation on the lead ECG higher than 0.2 mV. After that, the air in the thoracic cavity was gently squeezed out and the thoracic cavity was sutured layer by layer. The rats in sham-operation group underwent the same procedures except for the ligation.

### Culture of H9c2 Cell Lines

H9c2 cells were cultured in DMEM containing 10% FBS in an incubator at 37°C with 5% $CO_2$. Until 90% of confluence, they were washed with PBS twice, digested with 0.25% trypsin, and passaged at a density of 1:3.

### Cell Transfection

The cells in logarithmic growth phase were seeded into a 24-well plate at a density of $1\times10^6$ cells/mL and transfected with pcDNA-MALAT1, miR-30a mimics or pcDNA-BECN1 using Lipofectamine 2000. The plasmids were diluted to a final concentration of 50 nM by mixing with 250 μL of serum-free Opti-MEM medium, followed by incubation at room temperature for 5 min. Additionally, 5 μL of Lipofectamine 2000 was diluted using 250 μL of serum-free Opti-MEM medium and incubated at room temperature for 5 min after mixing evenly. Subsequently, the two solutions were mixed together for incubation at room temperature for 20 min. The mixture was applied to cells. Finally, the cells were cultured in the incubator with 5% $CO_2$ at 37°C. At 6-8 h, the complete medium was replaced and the cells were collected at 48 h.

### Extraction of Total RNA and Quantitative RT-PCR (qRT-PCR)

The sequences of lncRNA-MALAT1, miR-30a, and BECN1 were obtained from the website

of NCBI database (http://www.ncbi.nlm.nih.gov/) and listed in Table I. The primers were designed using Premier 6.0 software and synthesized by Sangon Biotech. Co., Ltd. (Shanghai, China); TRIzol reagent was utilized to extract the total RNA and its concentration was measured *via* a NanoDrop spectrophotometer. 5 ng of total RNA extracted was synthesized into complementary deoxyribonucleic acid (cDNA) using the miRNA reverse transcription kit. For lncRNA-MALAT1 and BECN1, the total RNA was synthesized into cDNA using the random primers from the RT Master Mix kit. Next, qRT-PCR was performed using the SYBR Green RT-PCR Master Mix kit and ABI 7500 sequence detection system in accordance with the manufacturer's protocol. The transcription level was evaluated *via* the cycle threshold (Ct) value. The target amount of standardized internal reference was calculated through the $2^{-\Delta\Delta Ct}$ method.

### Western Blotting (WB)

Total proteins were extracted from tissues and cells, whose concentration was determined by the bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL, USA). Then, the proteins were separated *via* 8% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA), followed by sealing in 5% skim milk powder and 0.1% Tris-Buffered Saline and Tween 20 (TBST). Subsequently, membranes were incubated with primary antibody at 4°C overnight. After that, horseradish peroxidase (HRP)-labeled secondary antibody was added for incubation and the proteins to be detected were subjected to exposure using enhanced chemiluminescence (ECL) reagent. β-actin was used as an internal reference.

### Dual-luciferase Reporter Assay

H9c2 cells were co-transfected with wild-type/mutant-type luciferase vectors and overexpression plasmid/negative control for 48 h. Next, the luciferase activity in the transfected cells was determined using the luciferase reporter gene assay system combined with a bioluminescent plate reader. Three parallel controls were set in each assay and repeated independently three times.

### Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 software (IBM, Armonk, NY, USA) was used for data record and processing. The data from different treatment groups were presented as mean ± standard deviation ($\chi \pm s$). The independent-samples *t*-test was adopted for comparison between groups. $p<0.05$ suggested that the difference was statistically significant.

## Results

### Pathology and Messenger RNA (mRNA) Expressions in Myocardial Tissues of Rats

The results of HE staining showed no apparent pathological change in the myocardial tissues in sham-operation group. Myocardial fibers were arranged closely and regularly, and the overall morphology remained substantially the same (Figure 1A). However, there was a significant fracture of myocardial fibers, which were arranged loosely and irregularly in MI group (Figure 1B). QRT-PCR data showed that expression levels of lncRNA-MALAT1 and BECN1 in rat myocardium of the MI group were up-regulated markedly ($p<0.01$) (Figures 2A and 2C), while miR-30a was down-regulated notably ($p<0.01$) (Figure 2B) compared with those in sham-oper-

**Table I.** Primer sequences of lncRNA-MALAT1, miR-30a, BECN1 and GAPDH.

| Name | Sequences |
|---|---|
| LncRNA-MALAT1 F | 5'-GCAGTGTGCCAATGTTTCGT-3' |
| LncRNA-MALAT1 R | 5'-GCTGTTTCCTGCTCCGAGAT-3' |
| MiR-30a F | 5'-GACGGTACCTGGTGGAGAACAACTTCG-3' |
| MiR-30a R | 5'-CAGAAGCTTCATCAAACCTTCAATCCC-3' |
| BECN1 F | 5'-CCCAGCCAGGATGATGTCTAC-3' |
| BECN1 R | 5'-AGTCTCCGGCTGAGGTTCTC-3' |
| GAPDH F | 5'-AGTGCCAGCCTCGTCTCATA-3' |
| GAPDH R | 5'-GGTAACCAGGCGTCCGATAC-3' |

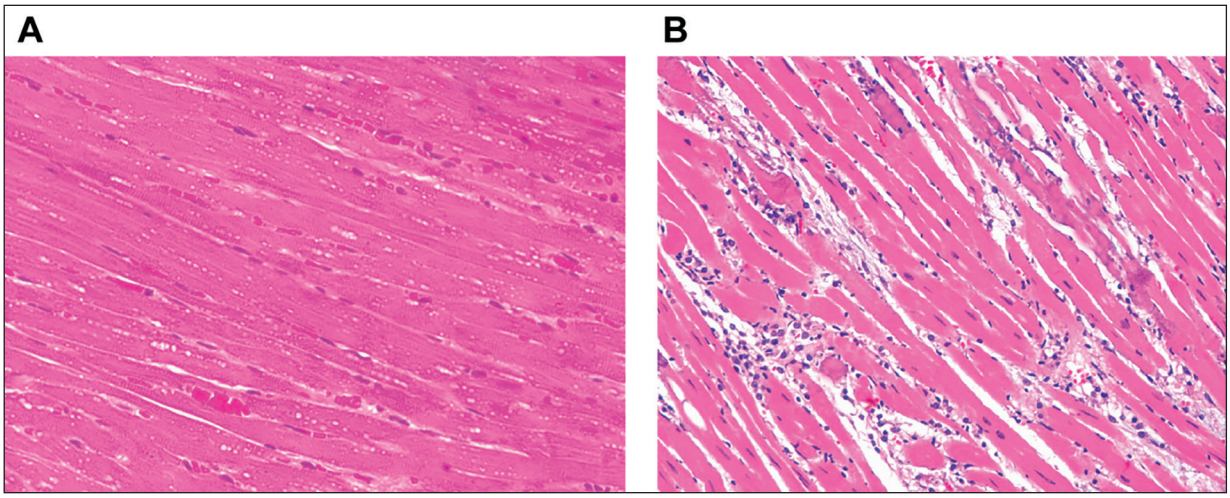


**Figure 1.** HE staining results for pathology of myocardial tissues in rats (magnification ×40). **A,** sham-operation group, **B,** MI group.

ation group. WB assay results indicated that the protein level of BECN1 in MI group was remarkably higher than that in sham-operation group ($p$<0.01) (Figure 2D).

### *Impact of LncRNA MALAT1 on Myocardial Cells of Rats*

To detect the potential interaction between lncRNA-MALAT1 with miR-30a and BECN1,

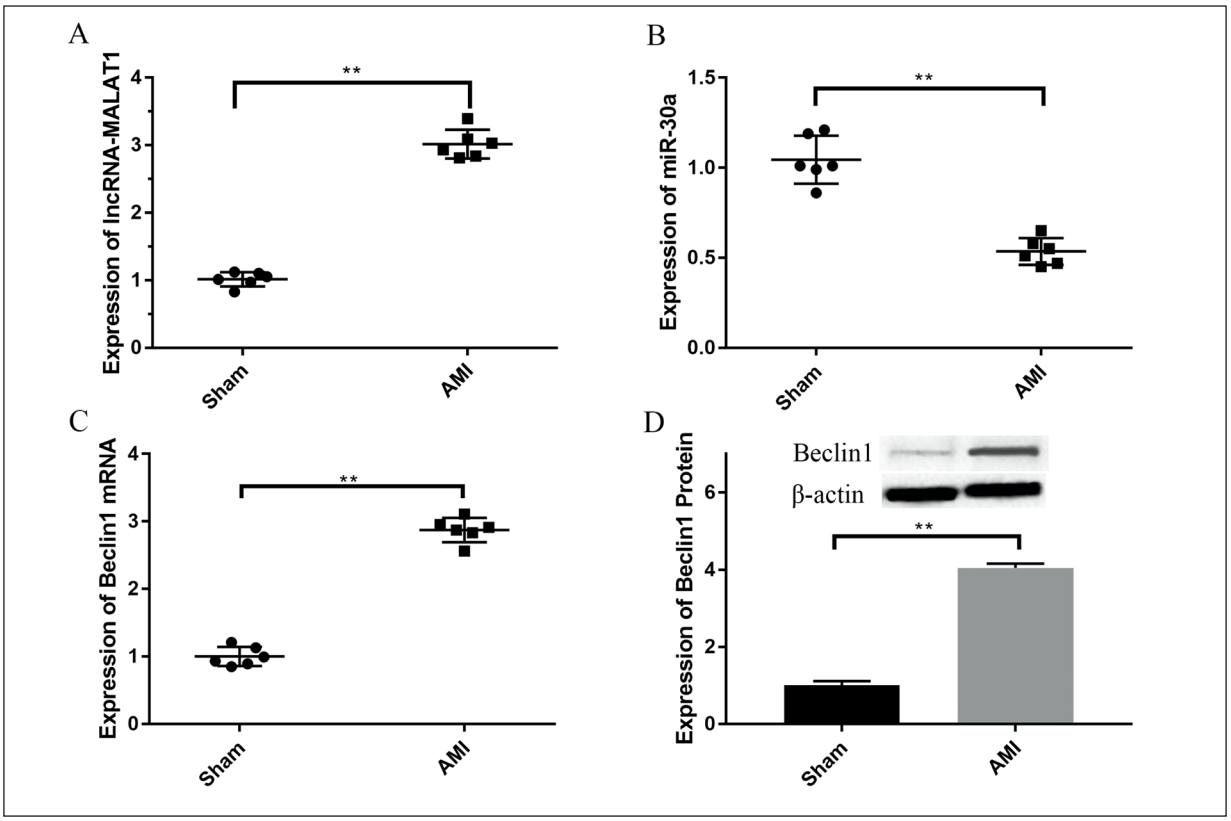


**Figure 2.** MRNA expressions of lncRNA-MALAT1, miR-30a, BECN1, and expression of BECN1 protein in myocardial tissues. **A,** MRNA expression level of lncRNA-MALAT1 in sham-operation group and MI group (**$p$<0.01), **B,** MRNA expression level of miR-30a in sham-operation group and MI group (**$p$<0.01), **C,** MRNA expression level of BECN1 in sham-operation group and MI group (**$p$<0.01), **D,** Expression of BECN1 protein in sham-operation group and MI group (**$p$<0.01).

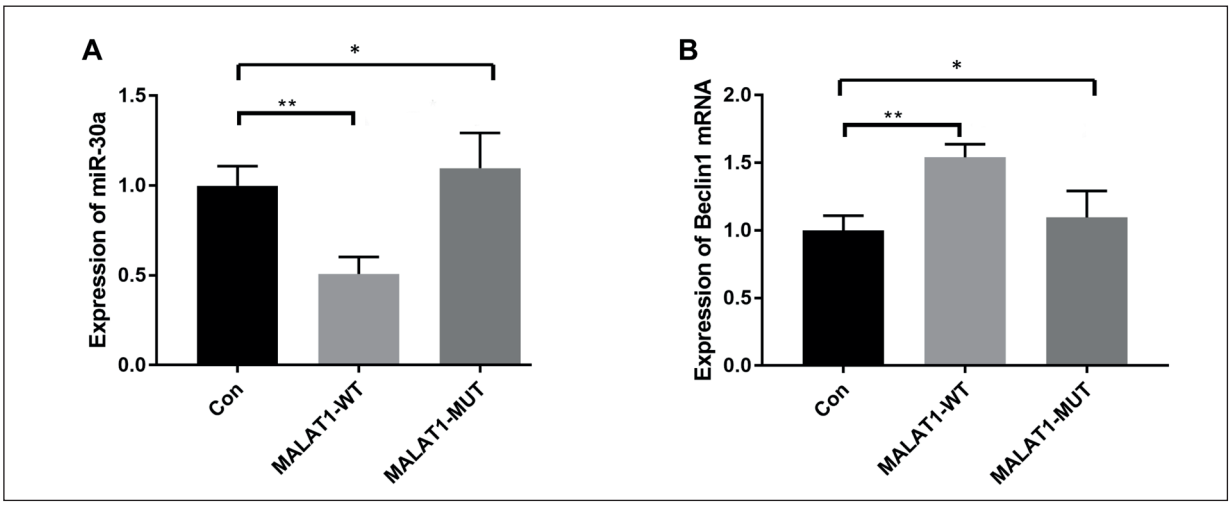


**Figure 3.** Impacts of transfecting H9c2 cell lines with wild-type lncRNA-MALAT1 plasmid and mutant-type lncRNA-MALAT1 plasmid on mRNA expression levels of miR-30a and BECN1, $^{**}p<0.01$, $^{*}p>0.05$.

H9c2 cells were transfected with overexpression plasmid of lncRNA-MALAT1. It is shown that overexpression of MALAT1 down-regulated miR-30a ($p<0.01$) and up-regulated BECN1 ($p<0.01$) (Figure 3).

### Influence Relation Between LncRNA MALAT1 and MiR-30a in H9c2 Cell Lines

To further verify the interaction between miR-30a and lncRNA-MALAT1, H9c2 cells were co-transfected with wild-type lncRNA-MALAT1 plasmid/mutant-type lncRNA-MALAT1 plasmid and miR-30a mimic/negative control, respectively. According to the results, the luciferase activity of wild-type lncRNA-MALAT1 could be inhibited by miR-30a overexpression ($p<0.01$), but that of mutant-type lncRNA-MALAT1 was not repressed remarkably ($p>0.05$) (Figure 4A, 4B). Dual-luciferase reporter assay indicated the binding between lncRNA MALAT1 and miR-30a.

### Influence Relation Between MiR-30a and BECN1 in H9c2 Cell Lines

Similarly, H9c2 cells were co-transfected with wild-type BECN1/mutant-type BECN1

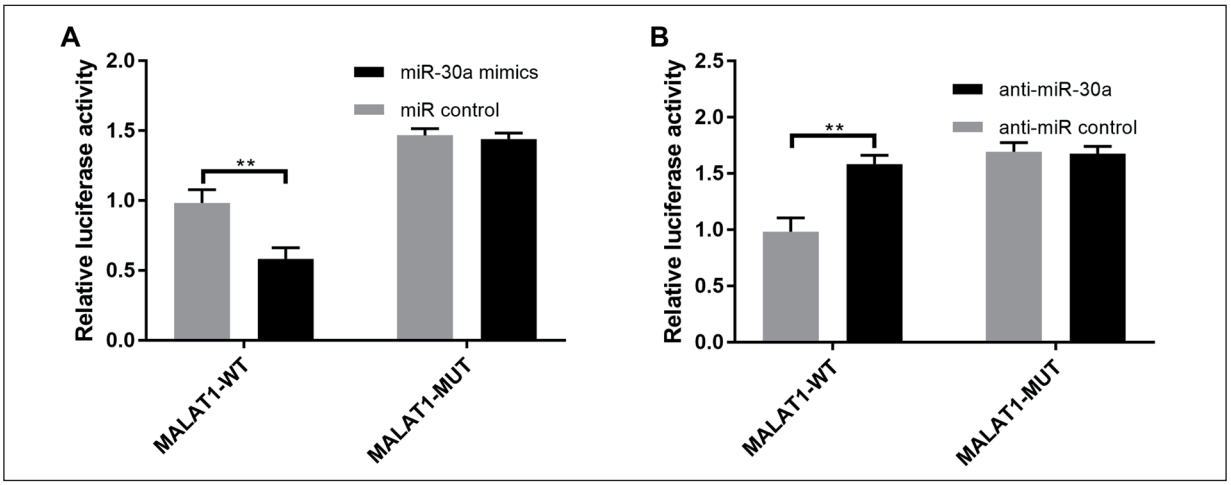


**Figure 4.** Interrelations under the actions of wild-type lncRNA-MALAT1 plasmid and mutant-type lncRNA-MALAT1 plasmid as well as miR-30a mimics and anti-miR-30a verified *via* luciferase reporter gene assay system. **A,** Relative luciferase activity of lncRNA-MALAT1 under the action of miR-30a mimics ($^{**}p<0.01$), **B,** Relative luciferase activity of lncRNA-MALAT1 under the action of anti-miR-30a ($^{**}p<0.01$).

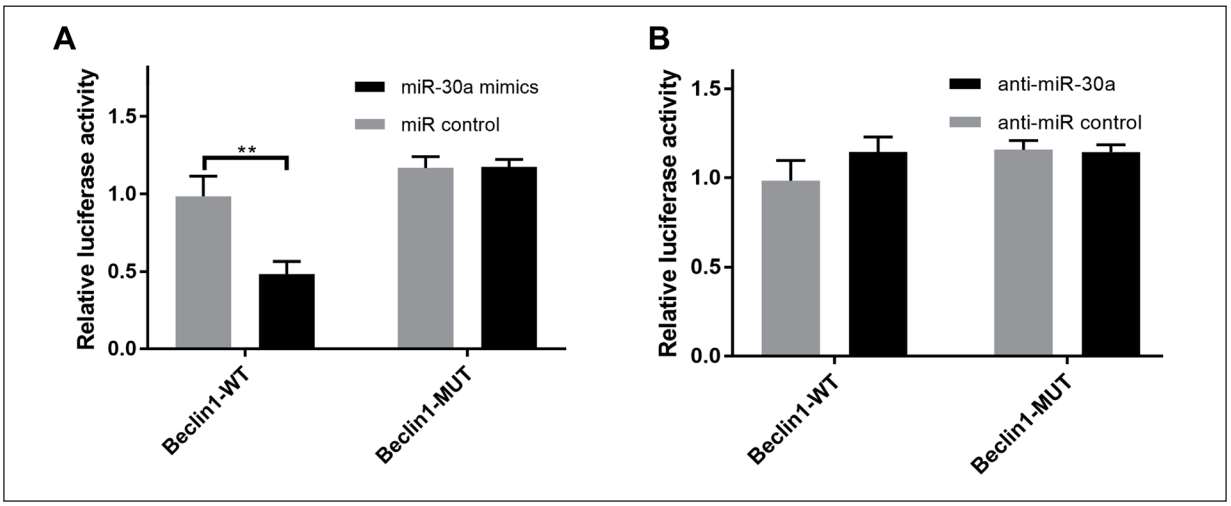


**Figure 5.** Interrelations under the actions of wild-type BECN1 and mutant-type BECN1, as well as miR-30a mimics and anti-miR-30a testified *via* luciferase reporter gene assay system. **A,** Relative luciferase activity of BECN1 under the action of miR-30a mimics (**$p$<0.01), **B,** Relative luciferase activity of BECN1 under the action of anti-miR-30a (**$p$<0.01).

and miR-30a mimics/negative control, respectively. Luciferase activity markedly decreased in wild-type group ($p$<0.01), while it remained unchangeable in mutant-type group ($p$>0.05) (Figure 5A, 5B). A regulatory loop MALAT1/miR-30a/BECN1 was identified.

### *Impact of LncRNA-MALAT1 on Protein Expression of BECN1*

The protein expression of BECN1 was up-regulated in H9c2 cells overexpressing lncRNA-MALAT1 ($p$<0.01) (Figure 6A). In contrast, it was down-regulated after the silence of lncRNA-MALAT1 ($p$<0.01) (Figure 6B).

## Discussion

AMI is a fatal disease and reperfusion is the most effective therapeutic method for MI induced by acute artery occlusion. However, reperfusion would lead to secondary myocardial injury. Reperfusion-mediated cell injuries include cell apoptosis and necrosis, which further

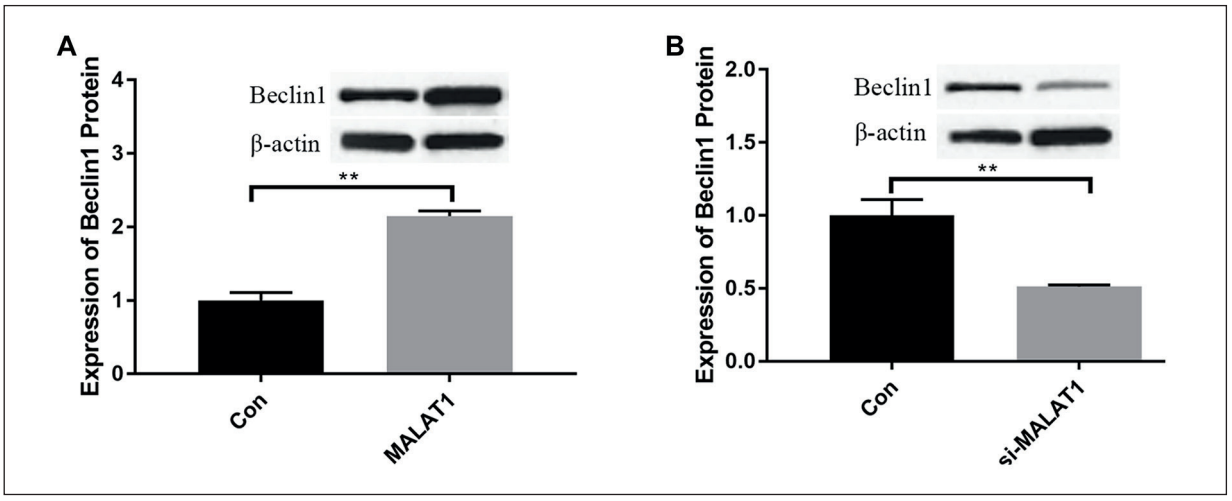


**Figure 6.** Impact of lncRNA-MALAT1 on expression of BECN1 protein. **A,** Expression of BECN1 protein after overexpression of lncRNA-MALAT1 (**$p$<0.01), **B,** Expression of BECN1 protein after inhibition on expression of lncRNA-MALAT1 (**$p$<0.01).

aggravates the disease. In the present work, the rat model of AMI was established by myocardial ischemia/reperfusion. It was found that the expressions of lncRNA-MALAT1 and BECN1 were up-regulated, while that of miR-30a was down-regulated in the course of MI.

As an important member of the lncRNA family, lncRNA-MALAT1 was discovered in non-small cell lung cancer[13]. LncRNA-MALAT1 is expressed in a variety of tissues and tumors. It is highly conserved in mammals, which are capable of participating in epigenetic regulation and cell cycle regulation through the specific recruitment of SR protein family members. Besides, it performs vital functions in angiogenesis[14]. Since lncRNA-MALAT1 is highly expressed in endothelial cells and activated by hypoxia-induction, it plays a vital role in the endothelial cell proliferation[15]. Our findings showed that lncRNA-MALAT1 was up-regulated after MI, which may be stimulated by the hypoxic environment during MI.

Furthermore, lncRNA-MALAT1 was found to directly bound to miR-30a and negatively regulated its level. Zhu et al[16] suggested that miR-30a is involved in regulating the post-transcriptional process of autophagy gene BECN1, which is capable of attenuating rapamycin-induced autophagy. Moreover, BECN1 protein serves as a marker of autophagosome in the process of MI induced by angiotensin II[17].

The autophagy is maintained at a relatively low level in the cardiac tissues in the case of normal physiological activities[18] and it is involved in the pathological process of myocardial ischemia/reperfusion. Myocardial cells can activate the autophagy under hypoxia/reoxygenation environemnt[19]. In this report, BECN1 was activated following MI. Moreover, the expression of lncRNA-MALAT1 was up-regulated as well. It is speculated that the hypoxic environment activated the expression level of lncRNA-MALAT1, which sponged miR-30a to up-regulate BECN1 level. The heart is featured by high energy consumption. It needs a great amount of energy to maintain its normal physiological activities. The interrupted blood flow triggered by MI directly results in the loss of energy source and oxygen molecules. Increased cell autophagy level can sustain the normal physiological activities of cells and reduce cell apoptosis to some extent by recycling amino acids, fatty acids, and other components in the organelles and cytoplasmic components. However, the excessive utilization of organelles and cytoplasmic components in cells by the autophagy may remarkably increase the content of lysosomal enzyme, further inducing autophagic cell death, and ultimately leading to irreversible cell death[20]. Nevertheless, in-depth researches are needed to explore the related physiological mechanisms.

## Conclusions

The above results demonstrate that lncRNA-MALAT1 is up-regulated in the process of MI, which promotes the expression of autophagosome marker BECN1 by binding to miR-30a, providing an effective therapeutic approach and relevant theoretical basis for clinical protection and treatment of MI.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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