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# Long non-coding RNA H19 protects acute myocardial infarction through activating autophagy in mice

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**Abstract. – OBJECTIVE: We investigate the effect of long non-coding RNA H19 in acute myocardial infarction (AMI) and the underlying mechanism.**

**MATERIALS AND METHODS: C57BL/6 mice were subjected to AMI and injected with lentivirus pcDNA-H19. After AMI procedures for 3 weeks, cardiac function was detected by echocardiography. The infarct size was stained by triphenyltetrazolium chloride. H19 expression in mice was measured by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). Protein expressions of LC3, Beclin-1, and ATG-7 in mice were measured by Western blot.**

**RESULTS: Our results indicated that H19 expression was significantly downregulated in the infarcted myocardium. Overexpression of H19 after injection with pcDNA-H19 in mice could reduce infarct size and improve cardiac function through upregulating the ratio of LC3-II/I and expressions of Beclin-1 and ATG-7.**

**CONCLUSIONS: Overexpression of H19 could protect AMI in mice via activating autophagy.**

*Key Words:*
Acute myocardial infarction, Long non-coding RNA, H19, Autophagy.

## Introduction

Acute myocardial infarction (AMI) is one of the most serious ischemic heart diseases. It has been the leading cause of cardiovascular morbidity and mortality nowadays[1]. Cardiac remodeling after AMI is characterized by ventricle dilation and cardiac dysfunction. Although cardiomyocyte death, hypertrophy and interstitial fibrosis within the infarcted myocardium are all associated with cardiac remodeling[2,3], the underlying molecular mechanisms of post-AMI cardiac remodeling are still not been fully elucidated. Autophagy is a highly conserved cell survival mechanism maintaining cellular homeostasis and cell adaptation under external stimuli[4]. Autophagy plays an important role in the regulation of cardiac structure and function. Activation of autophagy responses to acute ischemic injury, cardiac hypertrophy, fibrosis and heart failure as an adaptive mechanism[5,6]. Therefore, autophagy regulation may be an effective strategy to attenuate cardiac remodeling after AMI. Long non-coding RNAs (lncRNAs) are a class of transcribe molecules with over 200 nucleotides, which participate in various biological processes[7]. LncRNA H19 is an imprinted gene that participates in the embryonic development and growth control[8]. Recent investigations[9-11] have demonstrated that H19 is closely associated with the regulation of cardiac hypertrophy, fibrosis and diabetic cardiomyopathy. However, the specific role of H19 in AMI has not been completely elucidated. In the present study, we first detected the expression of H19 in AMI mice. The regulatory effect of H19 on autophagy was further explored.

## Materials and Methods

### Ethics Statements

Animal experiments were implemented in accordance with the Guide for the Care and Use of Laboratory Animals published by the NIH (National Institutes of Health). Ten-week-old male C57BL/6 mice weighing 20-30 g were housed in a room at 22°C with 12:12 hour light/dark cycles and fed with standard food and water. This study was approved by the Animal Ethics Committee of The Second People's Hospital of Liaocheng Animal Center.

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### Mice Model and Treatment

The mice were anaesthetized by pentobarbital (50 mg/kg, intraperitoneal injection), intubated and ventilated. A left horizontal incision was made at the third intercostal space. The left anterior descending (LAD) was ligated using 8-0 silk suture. Myocardial ischemia was measured by the electrocardiograph performance (ST segment elevation). The sham-operated mice were underwent an identical surgical procedure without ligature. The mice were randomly divided into 4 groups: sham group, AMI group, AMI + pcDNA-H19 group (infarcted myocardium injected with lentivirus pcDNA-H19), and AMI + pcDNA vector group (infarcted myocardium injected with empty vector). Each group had 15 mice. After 3 weeks, animals were sacrificed and the heart samples were harvested for analysis.

### Echocardiography

After 3 weeks of operation, the mice were anesthetized by isoflurane and the cardiac function was detected using a rodent animal ultrasonic instrument (Vevo 2100, Visual Sonics, Toronto, Canada). The left ventricular (LV) end diastolic diameter (LVEDD), LV end systolic diameter (LVESD), LV ejection fraction (LVEF) and fractional shortening (FS) were calculated.

### Assessment of Myocardial Infarct Size

Evans blue dye was perfused into the aorta to demarcate the ischemic area-at-risk. Hearts were excised and sliced. The heart sections were stained with 1% triphenyltetrazolium chloride at 37°C for 20 min and then fixed with 4% paraformaldehyde at room temperature for 8 h. Infracted myocardium was carefully separated from the non-infarcted myocardium and weighed. The infarct size was presented as a percentage of the ischemic area-at-risk.

### Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total RNA in infarcted tissue was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Complementary Deoxyribose Nucleic Acid (cDNA) synthesis was using the reverse transcription (RT) reagent according to instructions (TaKaRa Otsu, Shiga, Japan). Real-time PCR reaction system was performed by the Eppendorf (EP) Mastercycler ep realplex. The primer sequences (5'-3') were as follows: H19, (forward) CACAACGTGCTCTGCGTTGA, (reverse) GACTCAAGCCCTACTTGGGTA; Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), (forward) CTGGAGAAACCTGCCAAGTA, (reverse) TGTTGCTGTAGCCGTATTCA. The relative expression levels of H19 mRNA were normalized to GAPDH.

### Western Blot

Western blot analysis was used to determinate the protein expression of relative genes that extracted from the infarcted myocardium. Briefly, gel electrophoresis was performed to separate the different molecular weight proteins and then transferred onto polyvinylidene difluoride membranes. These membranes were incubated with anti-LC3, anti-Beclin-1 and anti-ATG-7 (Cell Signaling Technology, Danvers, MA, USA) for overnight at 4°C. Membranes were washed in Tris-buffered saline and Tween (TBST) (Beyotime, Shanghai, China) and then incubated with the horseradish peroxidase (HRP)-conjugated secondary antibody at room temperature for another 2 h. Western Blot Detection kit and Image J software (Rawak Software, Inc., Hamburg, Germany) were used to measure the blot signal and density.

### Statistical Analysis

All results were presented as means ± standard deviations (SD). Differences among different groups were analyzed by using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). A value of $p < 0.05$ was indicated to be statistically significant.

## Results

### The Expression of lncRNA H19 in Infarcted Myocardium

As shown in Figure 1A, the expression of lncRNA H19 was decreased in the infarcted myocardium of AMI mice compared with that of sham mice ($p < 0.05$). After injection with lentivirus pcDNA-H19, H19 expression was significantly increased in the infarcted myocardium ($p < 0.05$).

### Overexpression of H19 Reduced I nfarct Size and Improved Cardiac Function in AMI Mice

As shown in Figure 1B, the myocardial infarct size was significantly larger in AMI mice compared with that of the sham mice ($p < 0.05$). Overexpression of H19 reduced the myocardial

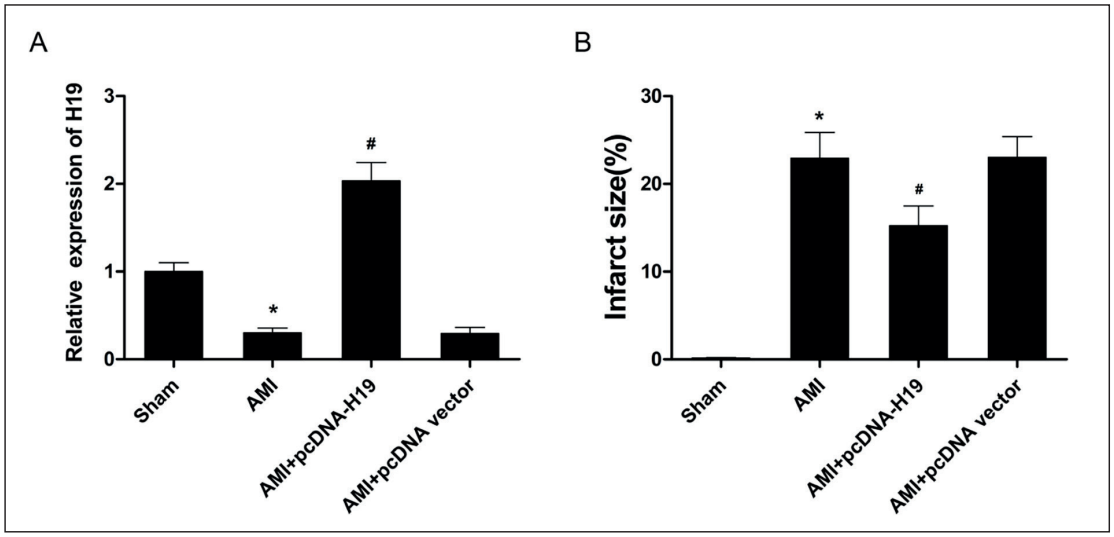


**Figure 1.** The expression of H19 and infarct size. ***A,*** The expression of H19 in each group. ***B,*** The infarct size in each group. $^{*}p < 0.05$ *vs.* sham group, $^{\#}p < 0.05$ *vs.* AMI group.

infarct size in contrast to the AMI group ($p < 0.05$). Compared with the sham group, LVEDD was significantly increased, whereas LVFS and LVEF were significantly decreased in the AMI group ($p < 0.05$). Nevertheless, overexpression of H19 in infarcted myocardium could markedly decrease LVEDD, but increase LVFS and LVEF ($p < 0.05$) (Figure 2).

### Overexpression of H19 Activated Autophagy in Infarcted Myocardium

As shown in Figure 3, the ratio of LC3-II/I and expressions of Beclin-1 and ATG-7 were significantly decreased in AMI mice compared with those of the sham mice ($p < 0.05$). Overexpression of H19 in infarcted myocardium could markedly increase the ratio of LC3-II/I and expressions of Beclin-1 and ATG-7 ($p < 0.05$).

## Discussion

In this study, we first established AMI mouse model to explore the potential effect of lncRNA H19 on AMI development. Our results indicated that H19 expression was significantly downregulated in the infarcted myocardium, which might be associated with cardiac dysfunction and impaired autophagy. Furthermore, we found that overexpression of H19 could reduce infarct

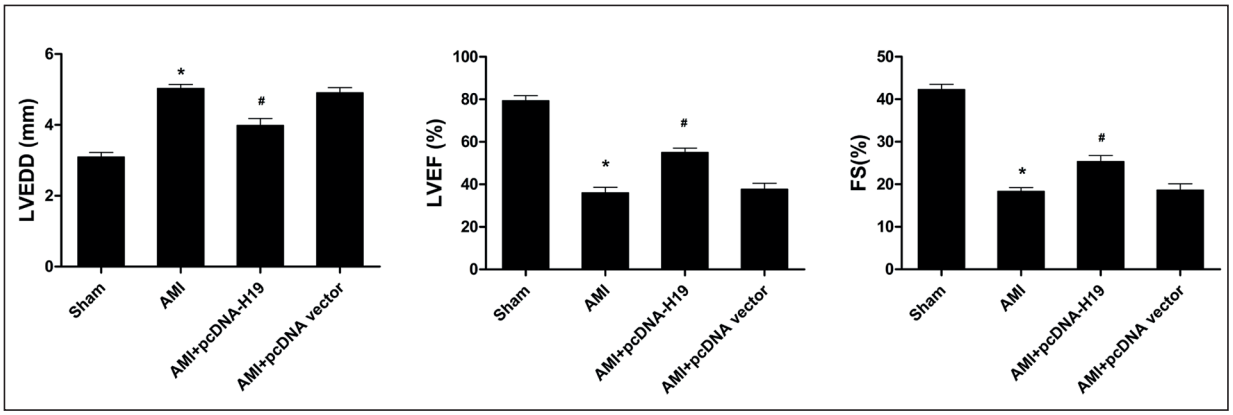


**Figure 2.** Cardiac function in each group. LVEDD: left ventricular (LV) end diastolic diameter; LVEF: LV ejection fraction; LVFS: LV fractional shortening. $^{*}p < 0.05$ *vs.* sham group, $^{\#}p < 0.05$ *vs.* AMI group.

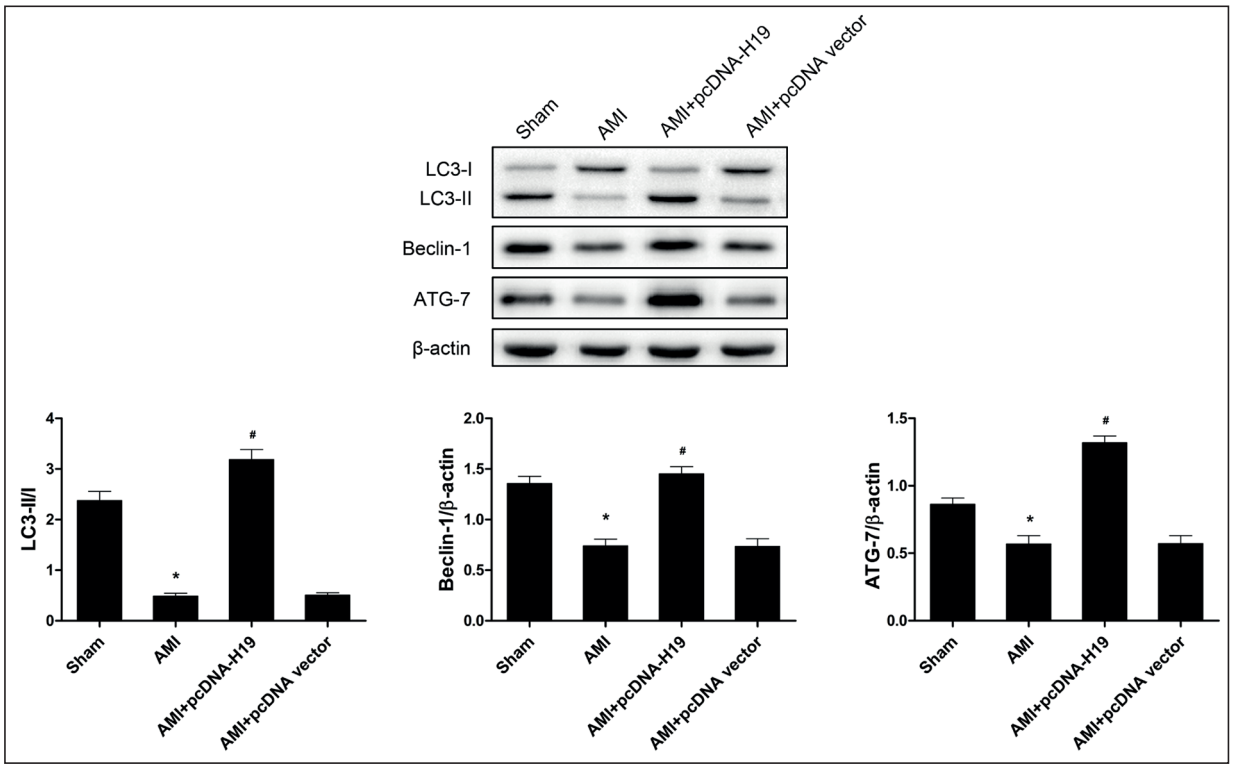


**Figure 3.** Overexpression of H19 activates autophagy in infarcted myocardium. The ratio of LC3-II/I and expressions of Beclin-1 and ATG-7 in each group. *$p < 0.05$ *vs.* sham group, $^{\#}p < 0.05$ *vs.* AMI group.

size and improve cardiac function through activation of autophagy. H19 is a highly conserved imprinted lncRNA and plays a distinct role in cell proliferation, differentiation, apoptosis and invasion[8]. H19 serves as a competing endogenous RNA by sponging miRNAs. For instance, Gong et al[12] reported that upregulation of H19 could attenuate hypoxia-induced cardiomyocyte apoptosis by sponging miRNA-139. However, Huang et al[10] demonstrated that upregulation of H19 could induce cardiac fibrosis by sponging miR-455. In addition, H19 can also act as a precursor of miR-675 to negatively regulate cardiac hypertrophy[9]. Zhang et al[13] reported that H19/miR-675 axis promoted cardiomyocyte apoptosis in rats with dilated cardiomyopathy. Nevertheless, Li et al[14] found that H19/miR-675 axis inhibited cardiomyocyte apoptosis in rats with diabetic cardiomyopathy. Through literature review, the specific function of H19 remains controversial. It is speculated that H19 plays different roles as a promoter or suppressor mainly depending on the pathogenesis or developmental stage of different diseases. In this study, our results indicated that H19 was downregulated in the infarcted myocardium. Upregulation of H19 after transfecting pcDNA-H19 could reduce infarct size and improve cardiac function. Autophagy is a highly regulated energy-dependent process, in which organelles and long-lived proteins are recycled and degraded[15]. Autophagy is regulated by autophagy-related genes (Atgs), and beclin-1 is required for the vesicle nucleation. Autophagosome formation is involved in the conversion of the soluble form of LC3-I to the autophagic vesicle-associated form LC3-II, which is considered as a marker of autophagy[16]. Activation of autophagy has been reported to protect acute ischemic death and cardiac hypertrophy[6,17]. However, excessive autophagy may result in cardiomyocyte death and heart failure[18, 19]. Wu et al[20] found that autophagy was activated in the acute phase of AMI. However, sustained myocardial ischemia impaired autophagy and was associated with post-AMI cardiac remodeling. The present study revealed that the ratio of LC3-II/I and expressions of Beclin-1 and ATG-7 were significantly decreased, indicating that autophagy was inhibited after 3 weeks of AMI. The inhibited autophagy was associated with

the dilated ventricle and impaired cardiac function. Zhuo et al[11] recently reported that overexpression of H19 could inhibit autophagy in diabetic cardiomyopathy. Whereas, in this study, we found that overexpression of H19 could activate autophagy through upregulating the ratio of LC3-II/I and expressions of Beclin-1 and ATG-7. However, the specific relationship between H19 and autophagy in AMI still needs to be further explored *in vitro*.

## Conclusions

Firstly we identified that overexpression of H19 could protect heart against AMI. The molecular mechanism is associated with the activation of autophagy. Our results provide new insights into understanding the molecular mechanisms of AMI. Further research is needed to investigate the role of H19 in AMI *in vitro*.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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