

LncRNA GAS5 Participates in Pneumonia by Inhibiting Cells Apoptosis Through The Downregulation of MiR-155

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Research Article

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Abstract

Background: LncRNA GAS5 and miR-155 are reported to play opposite roles in lung inflammatory responses. Lung inflammation participates in pneumonia, indicating the involvement of GAS5 and miR-155 pneumonia. We then analyzed the potential interaction between GAS5 and miR-155 in pneumonia

Methods: GAS5 and miR-155 expression in plasma samples from pneumonia patients and controls was studied by RT-qPCR. The role of GAS5 in miR-155 RNA gene methylation in human Bronchial Epithelial Cells (HBEpCs) was analyzed by methylation analysis. Flow cytometry was applied to analyze cell apoptosis.

Results: GAS5 was downregulated in pneumonia and miR-155 was upregulated in pneumonia. GAS5 and miR-155 were inversely correlated. GAS5 overexpression decreased miR-155 expression in HBEpCs, while miR-155 overexpression showed no significant effects on GAS5 expression. In addition, GAS5 suppressed the apoptosis of HBEpCs induced by LPS and reduced the enhancing effect of miR-155 on cell apoptosis.

Conclusions: GAS5 may participate in pneumonia by inhibiting cells apoptosis through the downregulation of miR-155.

Introduction

Pneumonia is the infection of one or both lungs caused by viruses, bacteria and fungi [1,2]. Pneumonia is common in children [3]. Pneumonia affects about 0.28 episodes per child-year. That means that pneumonia annually affects more than 150 million children, 7-13% of which (11-20 million) are severe cases that require hospitalization [3]. Unfortunately, about 5 to 10 percent of hospitalized cases will die of pneumonia with 30 days after admission [4,5]. Pneumonia is usually treated with macrolide antibiotics, fluoroquinolones and tetracyclines [6,7]. Treatment outcomes are generally satisfactory, while side effects are not avoidable, leading to poor prognosis [6,7].

Besides infections, previous studies have shown the participation of molecular players in pneumonia [8,9]. In effect, functional analysis of these factors may improve the treatment of pneumonia [8,9]. ncRNAs not directly encode proteins but they regulate the expression of non-coding RNA genes and coding genes to play their roles, suggesting that targeting the expression of ncRNAs may assist the recovery of certain diseases [10,11]. However, the function of most ncRNAs in human diseases, such as pneumonia, remains unclear. Previous lncRNA GAS5 and miR-155 play opposite roles in lung inflammatory responses [12,13]. Lung inflammation participates in pneumonia [14], indicating the involvement of GAS5 and miR-155 in pneumonia. We then analyzed the potential interaction between GAS5 and miR-155 in pneumonia.

Materials And Methods

Research subjects

Research subjects of this study included both pneumonia patients (n=62, 37 males and 25 females, 1 to 3 years, 1.7 ± 0.5 years) and healthy controls (n=62, 37 males and 25 females, 1 to 3 years, 1.7 ± 1.4 years). All patients and controls signed informed consent. These participants were enrolled at Inner Mongolia University for Nationalities Hulunbuir People's Hospital between March 2018 and March 2020. This study obtained ethics approval from aforementioned hospital Ethics Committee. The pneumonia patients were caused by either viral (n=34) or bacterial (n=28) infections. The patients excluded other diseases and initiated therapy. All the 62 healthy controls received a systemic-level physiological examination at aforementioned hospital during the same time period. Please check Table 1 for clinical data of patients and controls. Informed consent was obtained from all individual participants included in the study. All patients provided written form informed consent. Procedures operated in this research were completed in keeping with the standards set out in the Announcement of Helsinki and laboratory guidelines of research in China.

Preparation of plasma samples

All patients (before the initiation of therapy) and healthy controls were fasted overnight, followed by extraction of blood (5ml) from each participant. Centrifuged in EDTA tubes (1200g for 10 min at room temperature) was performed to separate the supernatant (plasma). RNA extractions were performed within 6h after plasma preparations

Human Bronchial Epithelial Cells (HBEpC)

HBEpCs (PromoCell) were used. Cells were cultivated in Bronchial Epithelial Cell Medium (ScienCell). Cells were collected from passage 4-6 were used for subsequent experiments. Cells were cultivated in medium containing 1, 5, and 10ng LPS for 48h in cases the treatment with LPS.

Vectors, miRNAs and transfections

Backbone vector expressing pcDNA3.1-GAS5 was constructed. MiR-155 mimic and negative control (NC) miRNA were purchased from Sangon (Shanghai, China). Expression vector (1 μ g) or miRNA (50 nM) was transfected into HBEpCs using lipofectamine 2000 (Invitrogen). Empty vector or NC miRNA transfections were performed to serve as NC groups. Untransfected cells were used as control (C) cells. Cells were cultivated for further 48h after transfections before subsequent experiments.

RNA samples

Plasma samples and HBEpCs were subjected to total RNA extractions using Ribozol (Invitrogen). DNase I (Sangon) incubation for 2h at 37 °C was perform to remove DNA. RNA integrity was checked using 5% Urine-PAGE gel.

Real-time quantitative PCR (RT-qPCR)

QuantiTect Reverse Transcription Kit (QIAGEN) was used to synthesize cDNA. After that, qPCRs were performed with cDNA samples as template using SYBR Green Master Mix (Bio-Rad) to measure the levels of GAS5 expression with GAPDH as internal control. To determine the level of mature miR-155 expression, poly (A) addition, reverse transcriptions and qPCRs were sequentially performed using GeneCopoeia All-in-One™ miRNA qRT-PCR Reagent Kit. The method of $2^{-\Delta\Delta Cq}$ was used for Ct value normalizations.

Methylation analysis

DNeasy Tissue Kit (Qiagen) was used to extract genomic DNAs following manufacturer's instructions. EZ DNA Methylation Lighting Kit was used to convert DNA. Routine PCR and MSP were performed and PCR products were separated using 2% agarose gel electrophoresis. Ultraviolet irradiation was used to visualize bands.

Apoptosis assay

Cells were cultivated in medium containing 10ng/LPS for 48h. After that, incubation with 70% ethanol was performed to achieve fixation. Following that, PI and Annexin-V FITC incubation was performed. Finally, apoptotic cells were analyzed by FACSCalibur instrument.

Statistical analysis

Three independent replicates were included in each experiment. Mean±SD values were used to express all data. Unpaired t test was used to compare patient and control groups. Multiple cell transfection groups were compared by ANOVA Tukey's test. P<0.05 was statistically significant.

Results

GAS5 and miR-155 expression was altered in pneumonia

GAS5 and miR-155 expression in plasma samples from both pneumonia (n=62) and healthy controls (n=62) was analyzed. RT-qPCR experiments illustrated that pneumonia patients were with downregulated GAS5 (Fig.1A, p<0.01). In contrast, significantly higher plasma level of miR-155 was observed in pneumonia group compared to control group (Fig.1B, p<0.01).

GAS5 and miR-155 were closely correlated with each other

Correlations between plasma levels of GAS5 and miR-155 across both pneumonia samples and control samples were analyzed by linear regression. GAS5 and miR-155 were inversely correlated across both pneumonia samples (Fig.2A) and control samples (Fig.2B).

GAS5 decreased miR-155 expression in HBEpCs through methylation

HBEpCs were overexpressed with GAS5 and miR-155 to analyze the crosstalk between them (Fig.3A, p<0.05). Interestingly, GAS5 decreased miR-155 expression (Fig.3B, p<0.05), while miR-155

overexpression showed no significant effects on GAS5 expression (Fig.3C). MSP experiments illustrated that cells transfected with GAS5 expression vector were with significantly increased methylation of miR-155 RNA gene (Fig.3D), indicating that GAS5 may downregulate miR-155 by increasing its methylation.

GAS5 suppressed the apoptosis of HBEpCs through miR-155

HBEpCs were treated with 1, 5, and 10ng LPS for 48h, followed by the determination of GAS5 and miR-155 expression. LPS treatment resulted in downregulation of GAS5 (Fig.4A, $p<0.05$) and upregulation of miR-155 (Fig.4B, $p<0.05$). Moreover, GAS5 overexpression inhibited LPS-induced apoptosis, while miR-155 overexpression promoted cells apoptosis. In addition, GAS5 reduced the enhancing effect of miR-155 on cell apoptosis (Fig.4C, $p<0.05$).

Discussion

This study analyzed the interactions between GAS5 and miR-155 in pneumonia. We found that GAS5 and miR-155 expression was altered in pneumonia and GAS5 may increase the methylation of miR-155 RNA gene to downregulate it, thereby inhibiting LPS-induced apoptosis of HBEpCs.

Li et al. reported that GAS5 could target miR-429/DUSP1 to suppress inflammation in alveolar epithelial cells, thereby inhibiting cell apoptosis [12]. It has been well established that lung inflammation promotes the aggregation of pneumonia [14], suggesting the potential involvement of GAS5 in pneumonia. This study is the first to report the downregulation of GAS5 in pneumonia. LPS-induced inflammatory responses and cell apoptosis promote the development of pneumonia. In this study we showed that LPS treatment downregulated GAS5 in HBEpCs, and overexpression of GAS5 resulted in decreased apoptotic rate of HBEpCs induced by LPS. Therefore, GAS5 may play protective role in pneumonia possibly by suppressing LPS-mediated cell apoptosis.

Tiwari et al. reported that increased expression of miR-155 in alveolar macrophages could serve as an inflammatory marker in obese patients [13]. In this study we showed that miR-155 was also upregulated in pneumonia patients, and LPS treatment resulted in the upregulation of miR-155 in a dose-dependent manner. Therefore, miR-155 may participate in inflammatory responses through a LPS-dependent manner. Our study also showed the enhancing effects of miR-155 on the apoptosis of HBEpCs induced by LPS. Therefore, we first reported that miR-155 could promote the development of pneumonia by increasing LPS-induced cell apoptosis.

Interestingly, GAS5 and miR-155 play opposite roles in LPS-induced cell apoptosis. We also observed the inverse correlation between GAS5 and miR-155 across plasma samples from both pneumonia patients and healthy controls. In addition, GAS5 overexpression mediated the downregulation of miR-155. It is known that lncRNAs may regulate the expression of miRNAs through methylation [16]. In this study we showed that GAS5 could downregulate miR-155 through methylation. However, other mechanisms may exist. Future studies are needed.

In conclusion, GAS5 is downregulated in pneumonia and miR-155 was upregulated in pneumonia. GAS5 may downregulate miR-155 through methylation to suppress LPS-induced apoptosis of HBEpCs.

Declarations

Ethical Approval and Consent to participate

Informed consent was obtained from all individual participants included in the study. Ethics Committee of Inner Mongolia University for Nationalities Hulunbuir People's Hospital approved this study. All patients provided written form informed consent. Procedures operated in this research were completed in keeping with the standards set out in the Announcement of Helsinki and laboratory guidelines of research in China.

Consent to publish

Not Applicable

Availability of supporting data

The data that support the findings of this study are available on request from the corresponding author: * Yue Ma, Department of Respiratory and Critical Medicine, Clinical Medical College of Hulunbeier, Inner Mongolia University for Nationalities Hulunbuir People's Hospital, No.20 Shengli Avenue, Hulunbuir City. Email address: YueMa678@163.com

The data are not publicly available due to their containing information that could compromise the privacy of research participants.

Competing interests

All other authors have no conflicts of interest.

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Funding

Not Applicable.

Authors' contributions

Xiaoping Wang, Ping Guo, Yue Ma: study concepts, literature research, clinical studies, data analysis, experimental studies, manuscript writing and review; Jiahui Tian: study design, literature research, experimental studies and manuscript editing; Jie Li: definition of intellectual content, clinical studies, data acquisition and statistical analysis; Na Yan: data acquisition, manuscript preparation and data analysis; Xin Zhao: data acquisition and statistical analysis.

Acknowledgements

Not Applicable.

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Table

Table 1 Clinical data of patients and controls

	Pneumonia (n=62)	Control (n=62)
RBC ($\times 10^{12}/L$)	3.43 \pm 0.67	4.99 \pm 0.43
WBC ($\times 10^9/L$)	11.23 \pm 2.78	6.92 \pm 1.72
PLT ($\times 10^9/L$)	203.43 \pm 61.98	267.81 \pm 70.12
HB (g/L)	109.52 \pm 20.53	134.62 \pm 18.59
HCT	0.38 \pm 0.033	0.43 \pm 0.043
DD (mg/L)	5.98 \pm 1.01	3.11 \pm 0.87
PaO ₂ (mmHg)	57.45 \pm 18.23	86.37 \pm 14.12
PaCO ₂ (mmHg)	34.89 \pm 7.48	40.39 \pm 9.77

Figures

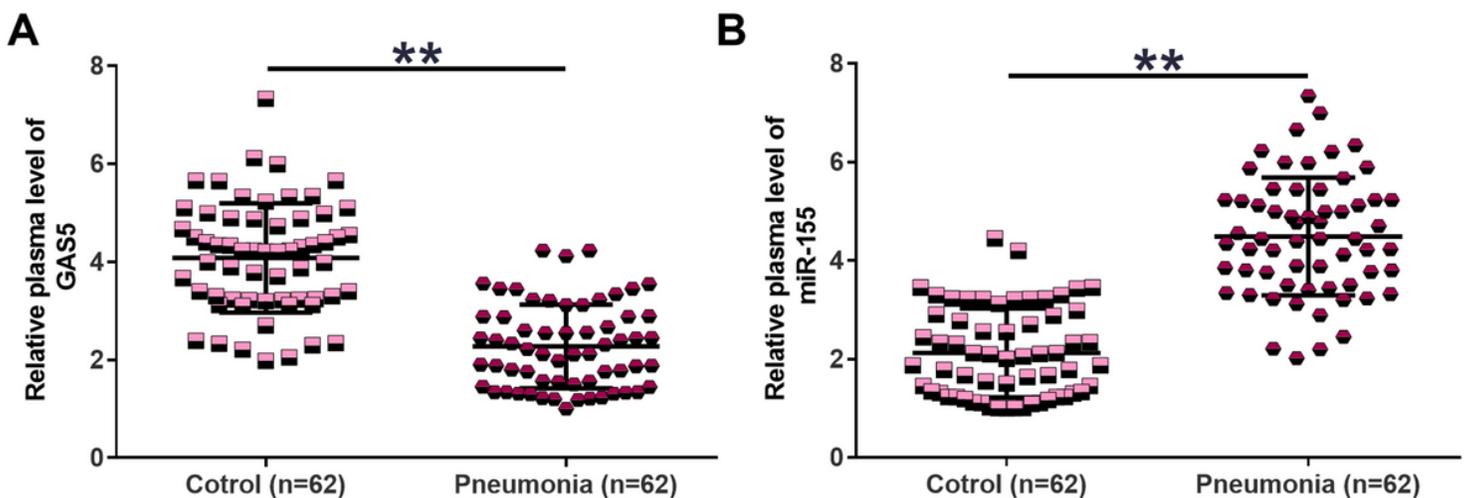


Figure 1

GAS5 and miR-155 expression was altered in pneumonia GAS5 (A) and miR-155 (B) expression in plasma samples from both pneumonia (n=62) and healthy controls (n=62) was determined by RT-qPCR.

**p<0.01.

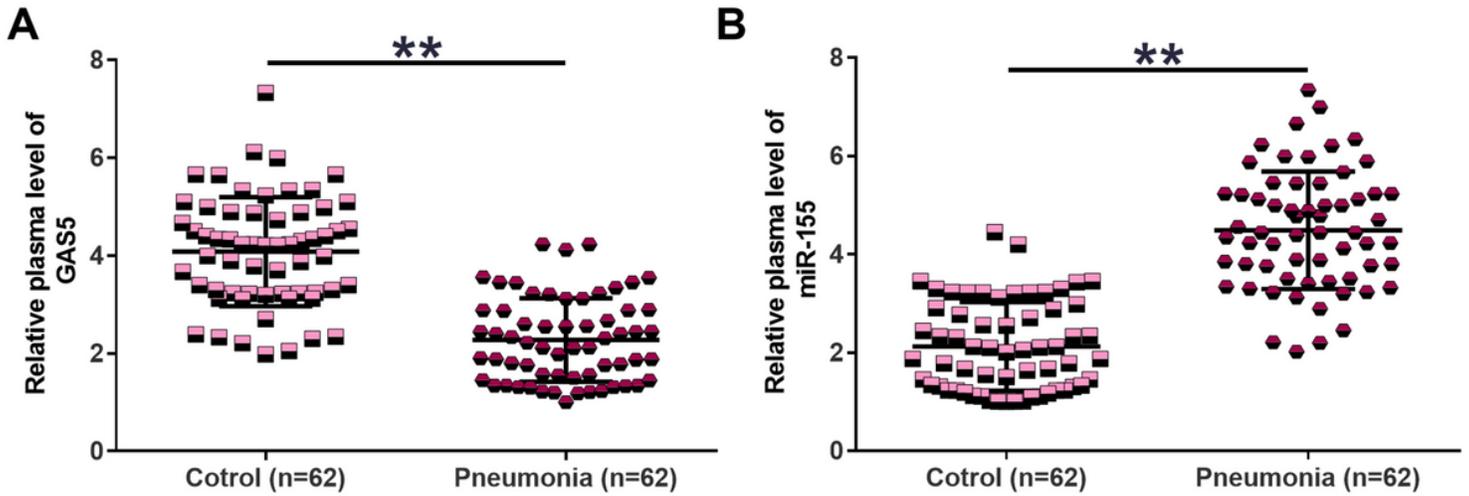


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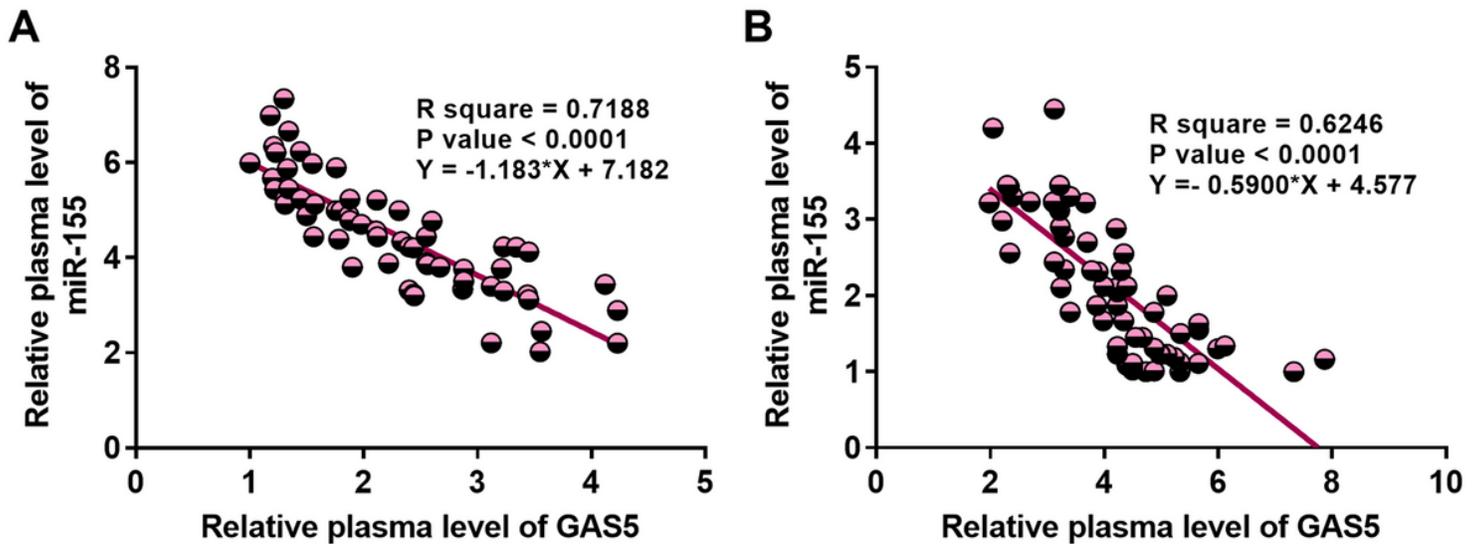


Figure 2

Correlation analysis between GAS5 and miR-155 Correlations between plasma levels of GAS5 and miR-155 across both pneumonia samples (A) and control samples (B) were analyzed by linear regression

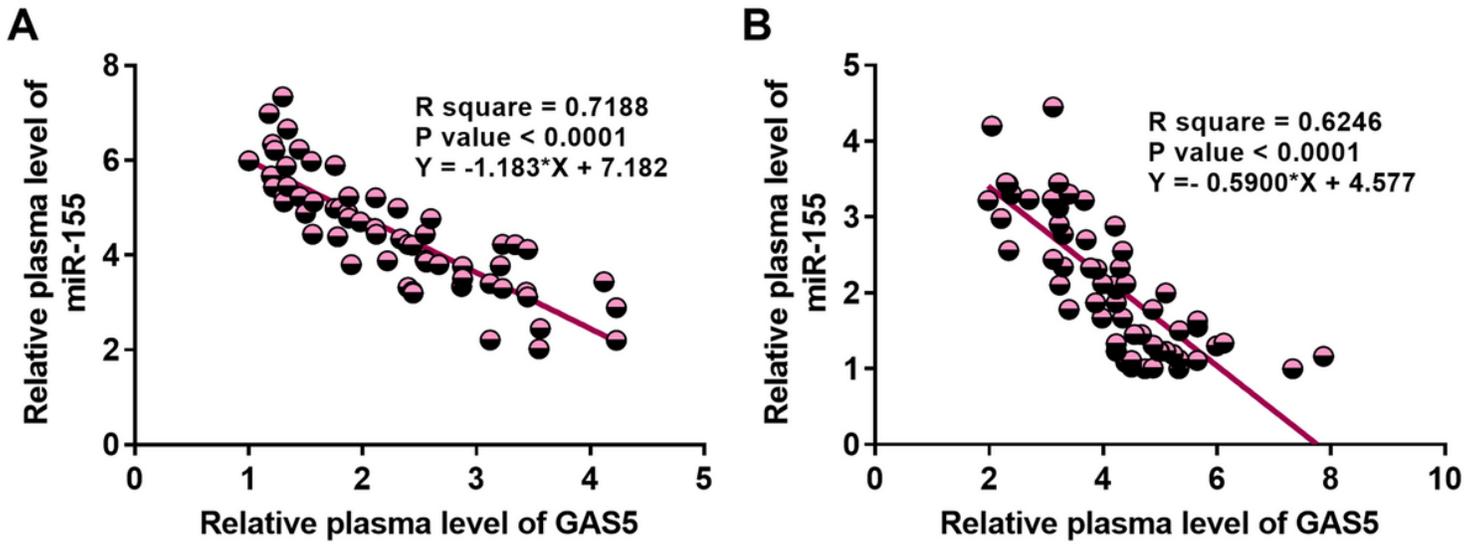


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Correlation analysis between GAS5 and miR-155. Correlations between plasma levels of GAS5 and miR-155 across both pneumonia samples (A) and control samples (B) were analyzed by linear regression

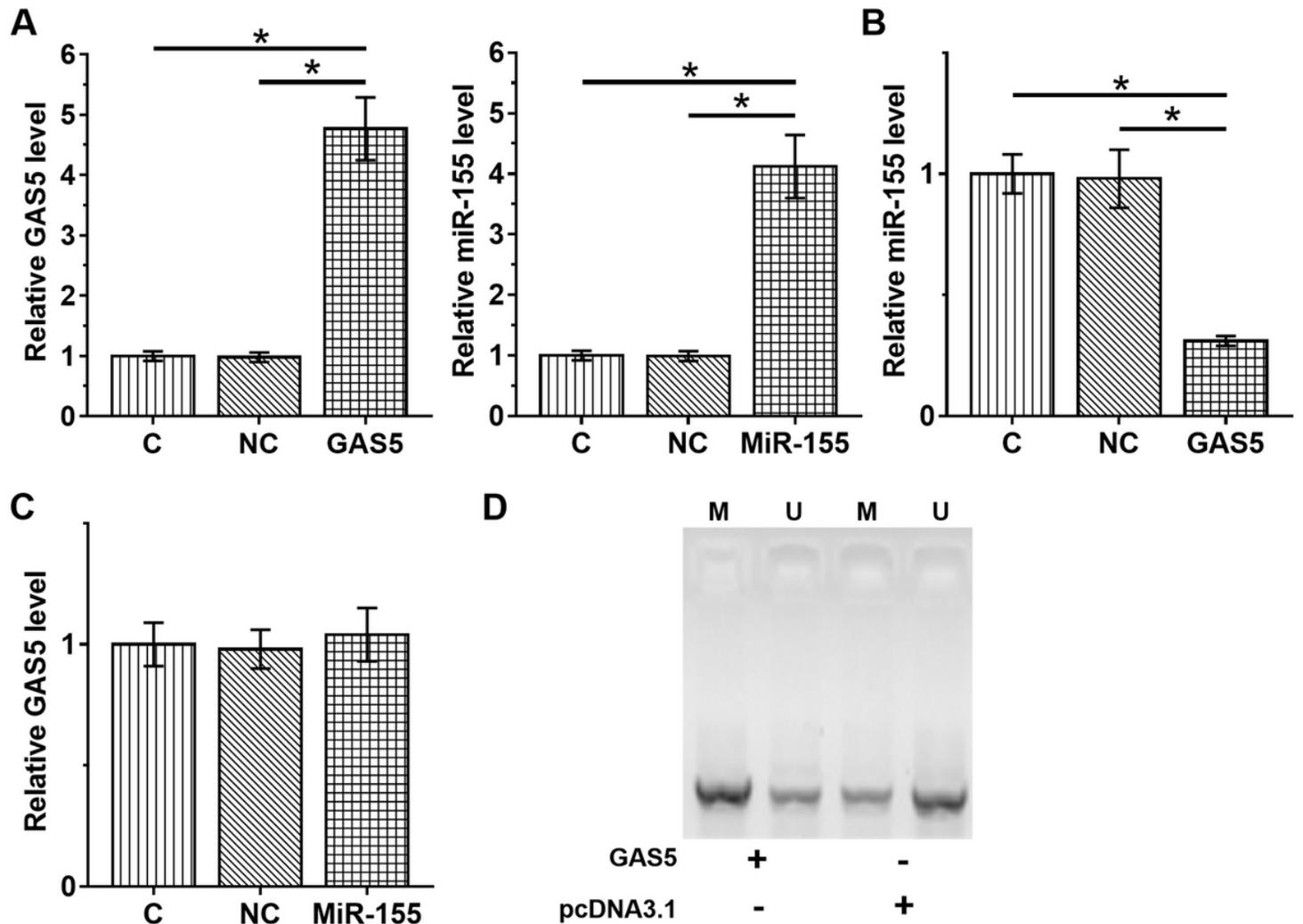


Figure 3

The interaction between GAS5 and miR-155 in HBEpCs GAS5 and miR-155 were overexpressed in HBEpCs through overexpression (A). The role of GAS5 in regulating miR-155 expression (B), and the role of miR-155 in regulating GAS5 expression (C) were studied by RT-qPCR. MSP was performed to analyze the effects of GAS5 overexpression on the methylation of miR-155. * $p < 0.05$.

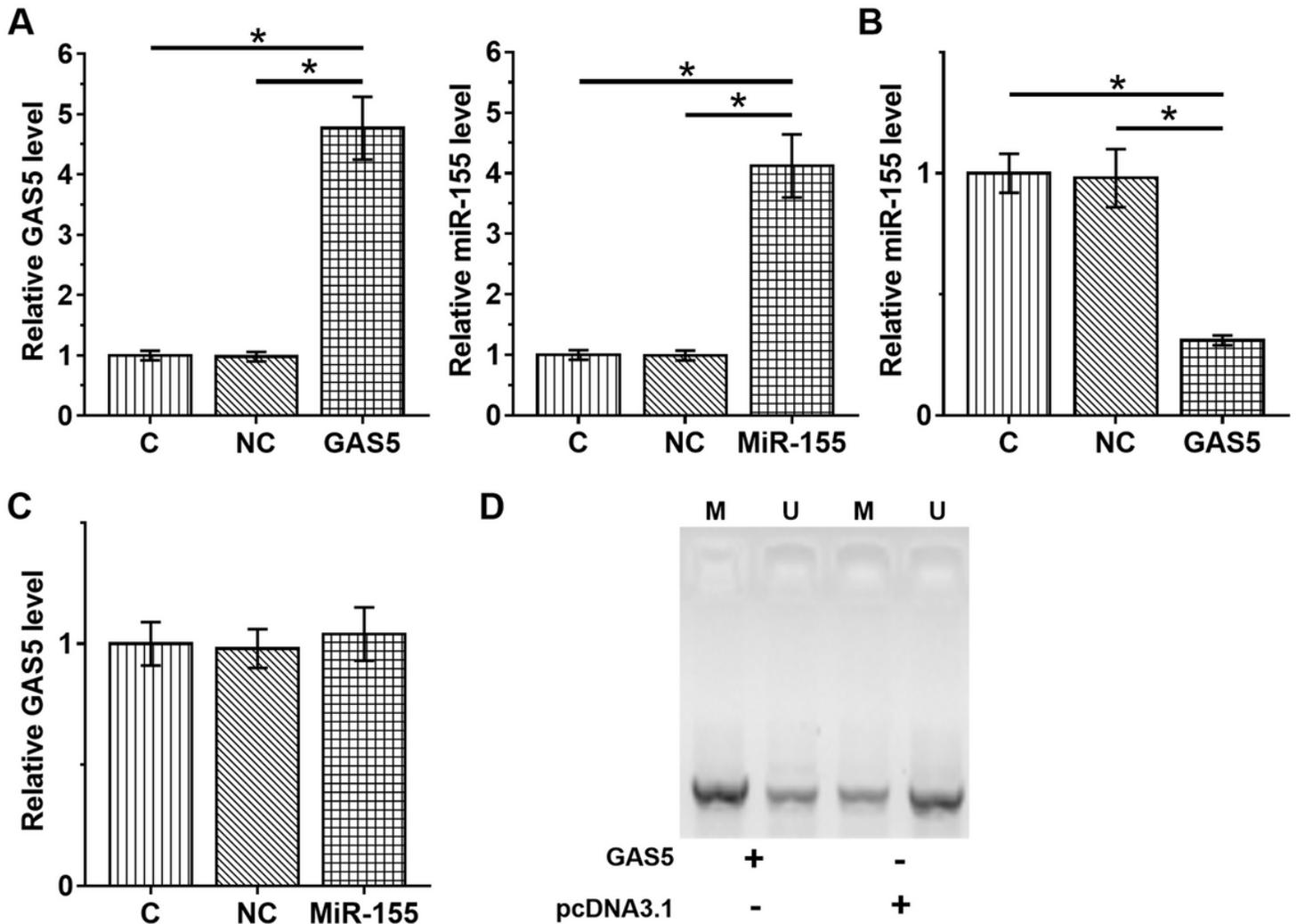


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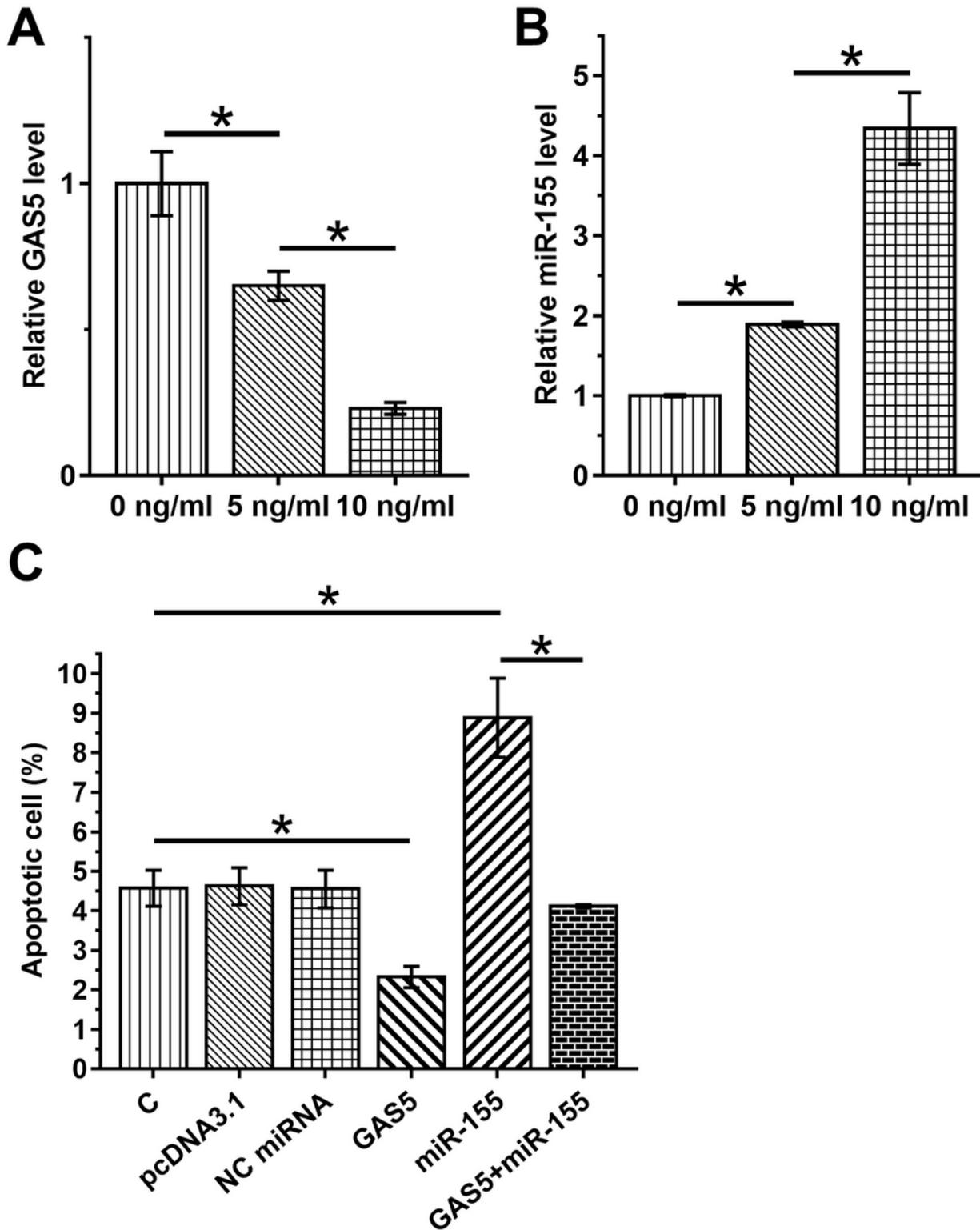


Figure 4

GAS5 suppressed the apoptosis of HBEpCs through miR-155 HBEpCs were cultivated in medium containing 1, 5, and 10ng LPS for 48h, followed by the determination of GAS5 (A) and miR-155 (B) expression by RT-qPCR. Cell apoptosis assay was performed to analyze the role of GAS5 and miR-155 in regulating the apoptosis of HBEpCs (C). Mean±SD values were presented and compared. *p<0.05.

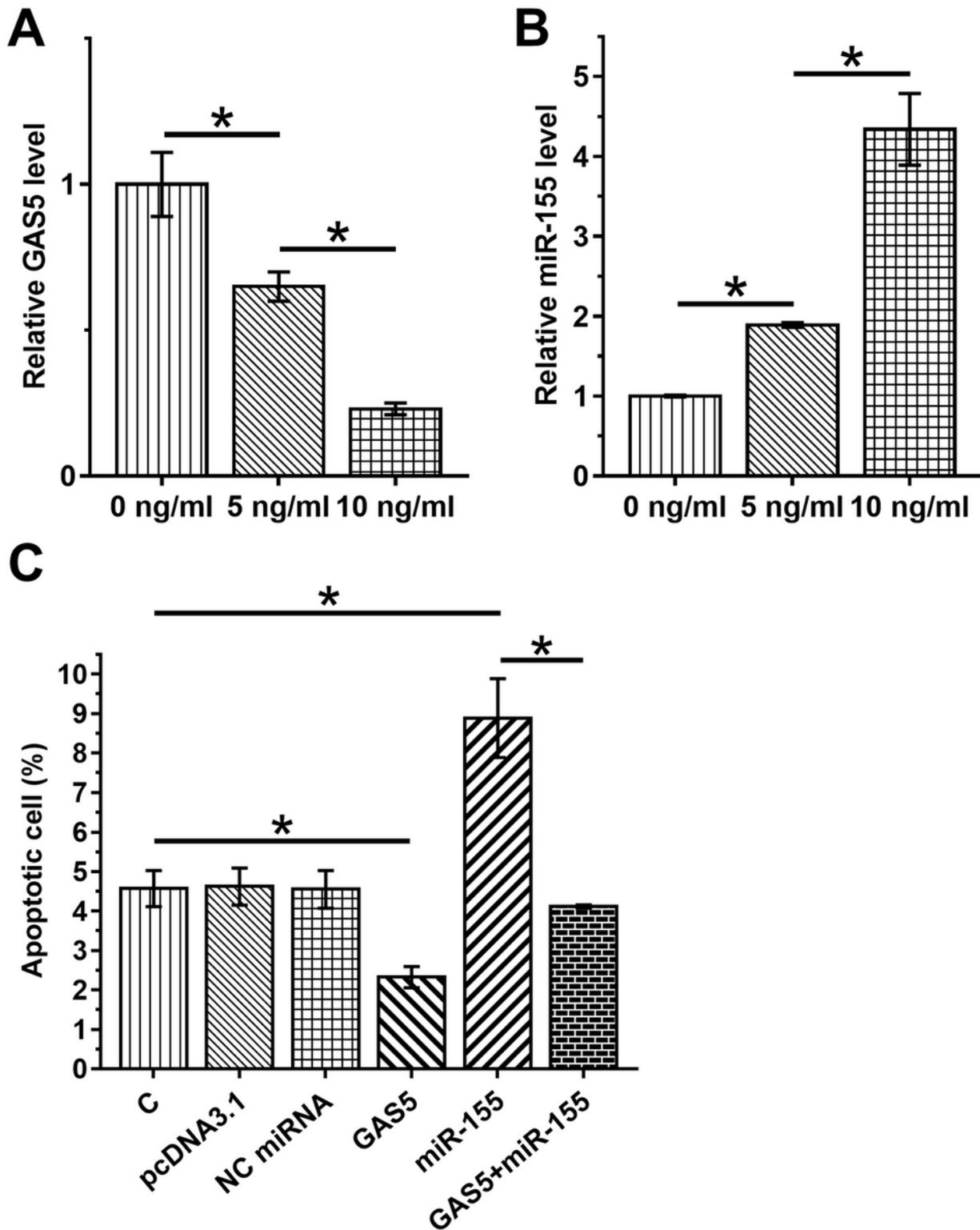


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