

LncRNA MALAT1 Forms a Negative Feedback Loop With lncRNA CRNDE in Sepsis to Regulate Lung Cell Apoptosis

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Research

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Abstract

Background: It has been reported that lncRNAs MALAT1 and CRNDE plays opposite roles in sepsis. Therefore, they may have crosstalk. We therefore explored the potential interaction between them in sepsis.

Methods: Plasma samples were collected from both sepsis patients (n=60) and healthy controls (n=60). Expression of MALAT1 and CRNDE in plasma samples before than after treatment was determined by RT-qPCR.

Results: Overexpression of MALAT1 and CRNDE in Human Bronchial Epithelial Cells (HBEpCs) was achieved to explore the relationship between them. The roles of MALAT1 and CRNDE in regulating the apoptosis of HBEpCs were analyzed by cell apoptosis assay. MALAT1 was upregulated in sepsis, while CRNDE was downregulated in sepsis. In lung cells, overexpression of MALAT1 and CRNDE mediated the downregulation of each other. With proper treatment, MALAT1 was downregulated in sepsis and CRNDE was upregulated in sepsis. In lung cells, LPS mediated the upregulation of MALAT1 and the downregulation of CRNDE. Cell apoptosis analysis showed that MALAT1 overexpression promoted the apoptosis of lung cells induced by LPS, and the overexpression of CRNDE played an opposite role. Moreover, CRNDE overexpression attenuated the effects of MALAT1 overexpression. **Conclusion:** MALAT1 may form a negative feedback loop with CRNDE in sepsis to regulate lung cell apoptosis.

Background

Sepsis is a severe clinical condition occurs when infections cause body's response to its own organs and tissues [1]. The most common cause of sepsis is bacterial infections [2]. The development of sepsis and septic shock results in failure of multiple organs, leading to unacceptable high mortality rate [3]. In the United States, sepsis affects about 1.7 million of new cases and causes about 270,000 deaths every year [4]. It is estimated that, even after effective treatment, such as the use of antibiotics, oxygen and intravenous fluids, more about 50% of patients with septic shock or severe sepsis will die of this disease [5]. Therefore, novel therapeutic approaches are needed to improve the survival of sepsis patients.

Infections are not sufficient for the development of sepsis [6]. In effect, more and more studies have elucidated that multiple molecular pathways are involved in the initiation and progression of all kinds of sepsis and the sepsis-induced organ failures [7, 8]. Increased understanding of the molecular mechanism of sepsis may provide novel targets for the treatment and prevention of sepsis-related organ dysfunctions by regulating the expression of critical genes [9]. Long non-coding RNAs (lncRNAs) have no protein-doing capacity, but with regulatory roles in gene expression, they have been proven to be critical players in human disease, such as sepsis [10, 11]. Therefore, lncRNAs may serve as potential targets for the treatment of sepsis. However, the function of most lncRNAs remains unclear. LPS-induced inflammatory response plays critical roles in sepsis [12]. It has been reported that lncRNAs MALAT1 and CRNDE have opposite roles in LPS-induced organ damage [13, 14], indicating the potential interaction between them.

This study was carried out to analyze the roles of MALAT1 and CRNDE in LPS and to explore the interactions between them.

Materials And Methods

Sepsis patients and healthy controls

Research subjects in this study were both sepsis patients (n=60, 34 males and 26 females; 38 to 62 years; 49.8 ± 5.6 year) and healthy controls (n=60, 34 males and 26 females; 38 to 62 years; 49.9 ± 5.7 year). All the participants were enrolled at No.1 Hospital Attached to Jiamusi University from May 2017 to May 2019. All sepsis patients were diagnosed for the first time. The main cause of sepsis was bacterial (n=32) or virus (n=28) infections. All patients survived for more than 3 months after admission. In view of the fact that other clinical disorders or treatments may also affect the expression of certain genes, this study excluded patients with initiated therapy or the ones complicated with other clinical disorders. Healthy controls were subjected to systemic physiological tests and all physiological functions were within normal range. All participants signed informed consent.

Plasma and treatment

Before therapy, all patients and healthy controls were subjected to blood (3cm) extraction under fasting conditions. Plasma samples were prepared by centrifuging the blood samples for 15min at 1200g. All patients were treated with antibiotics in combination with oxygen and intravenous fluids. At 3 months after treatment, fasting blood was also extract from each patient to prepare plasma samples. RNAs were extracted from plasma samples immediately after plasma preparation.

Human Bronchial Epithelial Cells (HBEpCs)

With lung injury in sepsis as a focus, HBEpCs (Sigma-Aldrich) were used as the cell model. Cell culture medium was Bronchial Epithelial Cell Medium (Cat. #3211; PromoCell). A 95% humidity and 5% CO₂ incubator (37°C) was used to cultivate cells. At passage 3 to 5, cells were harvested to perform following experiments. To explore the effects of LPS on gene expression, HBEpCs were cultivated in medium supplemented with LPS at doses of 0, 1, 2, 5, and 10 µg/ml for 48h before use.

Cell transfections

MALAT1 or CRNDE expression backbone vector was constructed with pcDNA3.1 vector (Invitrogen). MALAT1 or CRNDE expression vector (1µg) or empty vector (1µg, negative control (NC) group) was transfected into 10⁸ HBEpCs using lipofectamine 2000 (Invitrogen). After transfections, cells were cultivated for further 48h before use. To perform control (C) experiment, cells without transfections were cultivated until the end of cell culture.

RNA preparations

Isolation of total RNAs from both plasma samples and HBEpCs was performed using Ribozol reagent (Invitrogen). RNA integrity was tested using 5% Urine PAGE gel. Genomic DNA removal was performed with gDNA eraser for 2h at 37°C. OD values at 260 and 280 were measured and the ratio of 260 to 280 was calculated.

RT-qPCRs

Total RNA samples with a 260/280 ratio close to 2.0 (pure RNA) were used as template to synthesize cDNAs through reverse transcriptions, which were performed using SSRT IV system (Invitrogen). With cDNA samples as template, qPCRs were performed using SensiFAST™ Real-Time PCR Kit (Bioline) with 18S rRNA as internal control to normalize the expression levels of MALAT1 and CRNDE. Three technical replicates were included in each experiment and gene expression levels were normalized using the method of $2^{-\Delta\Delta CT}$.

Cell apoptosis assay

To induce cell apoptosis, HBEpCs with transfections were cultivated in medium supplemented with 10 µg/ml LPS for further 48h. Cell culture was performed in a 6-well cell culture plate with 8000 cells in 2 ml medium per well. Three wells were set for each experiment. After that, ice-cold PBS was used to wash cells and PI and FITC-annexin V (Sigma-Aldrich) were used to stain cells in dark for 20 min. After that, flow cytometry was used to separate apoptotic cells.

Statistical analysis

Mean±SD was used to express all data from 3 independent values. Gene expression levels in plasma samples were expressed as averaged values. Differences between two groups were analyzed by unpaired t test. Paired t test was used to compare two time points of the same group. Differences among multiple groups were analyzed by ANOVA Tukey's test. Correlation analyses were performed using linear regression. P<0.05 was set to be statistically significant.

Results

Altered expression of MALAT1 and CRNDE was observed in sepsis

Expression of MALAT1 and CRNDE in plasma samples from both sepsis patients (n=60) and healthy controls (n=60) was determined by RT-qPCR. Compared to Control group, Sepsis group exhibited significantly higher levels of MALAT1 (Fig.1A, p<0.01) and lower levels of CRNDE (Fig.1B, p<0.01). Therefore, altered expression of MALAT1 and CRNDE may participate in sepsis.

MALAT1 was downregulated and CRNDE was upregulated after treatment

MALAT1 and CRNDE expression in plasma samples from sepsis patients at 3 months after treatment was also determined by RT-qPCR. Compared to pretreatment levels, significantly lower levels of MALAT1

(Fig.2A, $p<0.05$) and higher levels of CRNDE (Fig.2B, $p<0.05$) were observed after treatment.

MALAT1 and CRNDE negatively regulated each other in HBEpCs

Expression vector of MALAT1 or CRNDE was transfected into HBEpCs, and the transfections were confirmed by RT-qPCR at 48h post-transfection (Fig.3A, $p<0.05$). It was observed that MALAT1 overexpression downregulated CRNDE (Fig.3B, $p<0.05$). In addition, CRNDE overexpression also resulted in the downregulation of MALAT1 (Fig.3C, $p<0.05$). Therefore, MALAT1 and CRNDE may form a negative regulation loop in HBEpCs. Correlations between MALAT1 and CRNDE across plasma samples from both sepsis patients (Fig.3D) and healthy controls (Fig.3E) were analyzed by linear regression. It was observed that MALAT1 and CRNDE were inversely and significantly correlated with each other across both types of plasma samples. Therefore, MALAT1 and CRNDE may also interact with each other in the human body.

MALAT1 and CRNDE interact with each other to regulate the apoptosis of HBEpCs induced by LPS

HBEpCs were cultivated in medium supplemented with LPS at doses of 0, 1, 2, 5, and 10 $\mu\text{g/ml}$ for 48h, followed by RT-qPCR to determine the expression of MALAT1 and CRNDE. It was observed that LPS treatment resulted in the upregulation of MALAT1 (Fig.4A, $p<0.05$) and downregulation of CRNDE (Fig.4B, $p<0.05$) in a dose-dependent manner. Cell apoptosis analysis showed that MALAT1 overexpression promoted the apoptosis of HBEpCs induced by LPS, and the overexpression of CRNDE played an opposite role. Moreover, CRNDE overexpression attenuated the effects of MALAT1 overexpression (Fig.4C, $p<0.05$).

Discussion

In this study we explored the interactions between MALAT1 and CRNDE in sepsis. We found that MALAT1 was upregulated and CRNDE was downregulated in sepsis. In addition, MALAT1 and CRNDE may form a negative regulation loop to participate in the apoptosis of HBEpCs induced by LPS.

MALAT1 is a well-characterized lncRNA in cancer biology [15]. In many types of cancers, MALAT1 was upregulated and promotes cancer development mainly by regulating cancer-related gene expression [15]. In a recent study, Yong et al. reported that MALAT1 is upregulated in mice model of sepsis and promotes the apoptosis of skeletal muscle cells by downregulating BRCA1 to promote disease progression [13]. Consistently, we observed the upregulation of MALAT1 in patients with sepsis. Interestingly, MALAT1 overexpression promoted the apoptosis of HBEpCs induced by LPS. Therefore, MALAT1 may regulate the apoptosis of multiple kinds of cells induced by LPS to participate in the injuries of multiple organs. In addition, in cancer biology MALAT1 can promote cancer development by suppressing cell apoptosis. Therefore, MALAT1 may play opposite roles in cell apoptosis in different types of cancers.

lncRNA CRNDE is also a characterized oncogenic lncRNA with multiple functions in cancer biology [16]. Wang et al. reported that CRNDE was downregulated in mice model of sepsis and upregulated miR-181a-5p to suppress cell apoptosis [14]. In our study we also observed the downregulation of CRNDE in

patients with sepsis and the inhibitory effects of CRNDE on the apoptosis of HBEpCs induced by LPS. Interestingly, LPS treatment regulated both MALAT1 and CRNDE in dose-dependent manner. Therefore, MALAT1 and CRNDE may participate in sepsis in a LPS-dependent manner.

LncRNAs participate in both physiological processes and diseases mainly by regulating the expression of protein-coding genes or other non-coding RNAs, such as miRNAs [17]. However, the interaction between different lncRNAs remains unclear. In this study we showed that MALAT1 and CRNDE could form a negative feedback regulation loop to participate in the LPS-induced apoptosis of HBEpCs. Therefore, our study enriched our understanding of the functions of lncRNAs. Our future studies will explore the mechanism.

Conclusions

In conclusion, MALAT1 is upregulated in sepsis and CRNDE is downregulated in sepsis. In addition, MALAT1 and CRNDE may negatively regulate each other to regulate the apoptosis of HBEpCs induced by LPS.

Abbreviations

lncRNAs: long ncRNAs; HBEpCs : Human Bronchial Epithelial Cells

Declarations

Acknowledgements

Not applicable.

Authors' contributions

CY: study concepts, study design, literature research, experimental studies, manuscript preparation and editing; MHH: definition of intellectual content, literature research experimental studies, manuscript preparation and editing; YPT and XLB: literature research, experiments work and manuscript writing. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Ethical approval was obtained from the Ethics Committee of Inner Mongolia Baogang Hospital. Written informed consent was obtained from all individual patients included in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

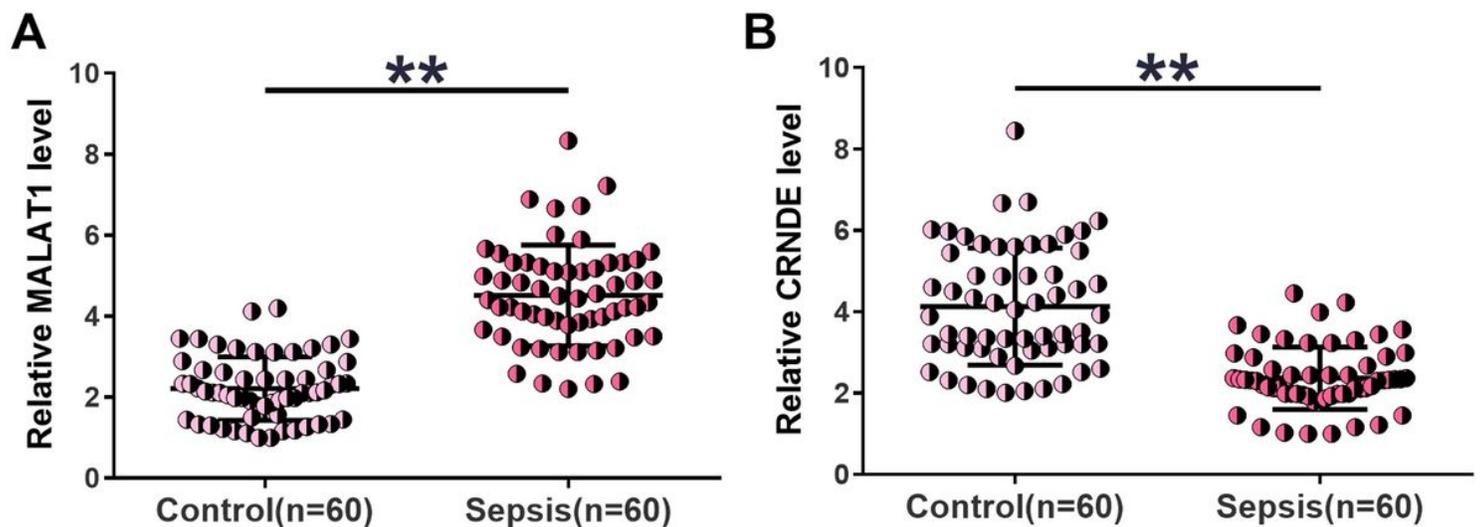


Figure 1

Altered expression of MALAT1 and CRNDE was observed in sepsis. Expression of MALAT1 (A) and CRNDE (B) in plasma samples from both sepsis patients (n=60) and healthy controls (n=60) was determined by RT-qPCR. Average values of three technical replicates were presented. **, $p < 0.01$.

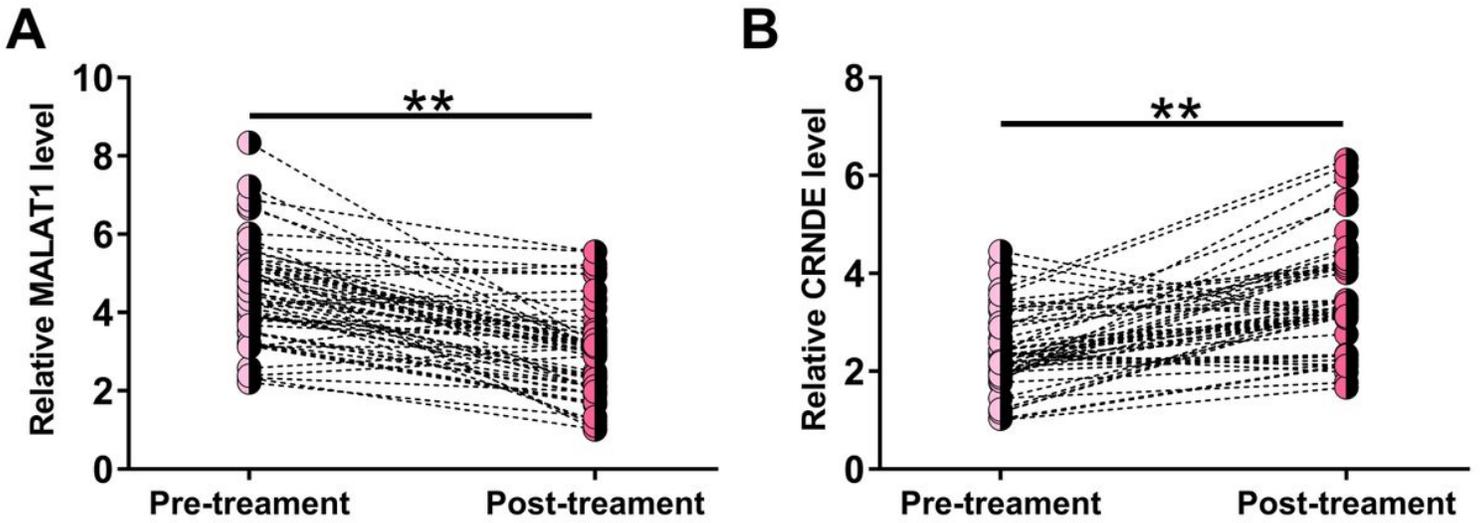


Figure 2

MALAT1 was downregulated and CRNDE was upregulated after treatment MALAT1 and CRNDE expression in plasma samples from sepsis patients at 3 months after treatment was also determined by RT-qPCR. Pre- and post-treatment levels of MALAT1 (A) and CRNDE (B). **, $p < 0.01$.

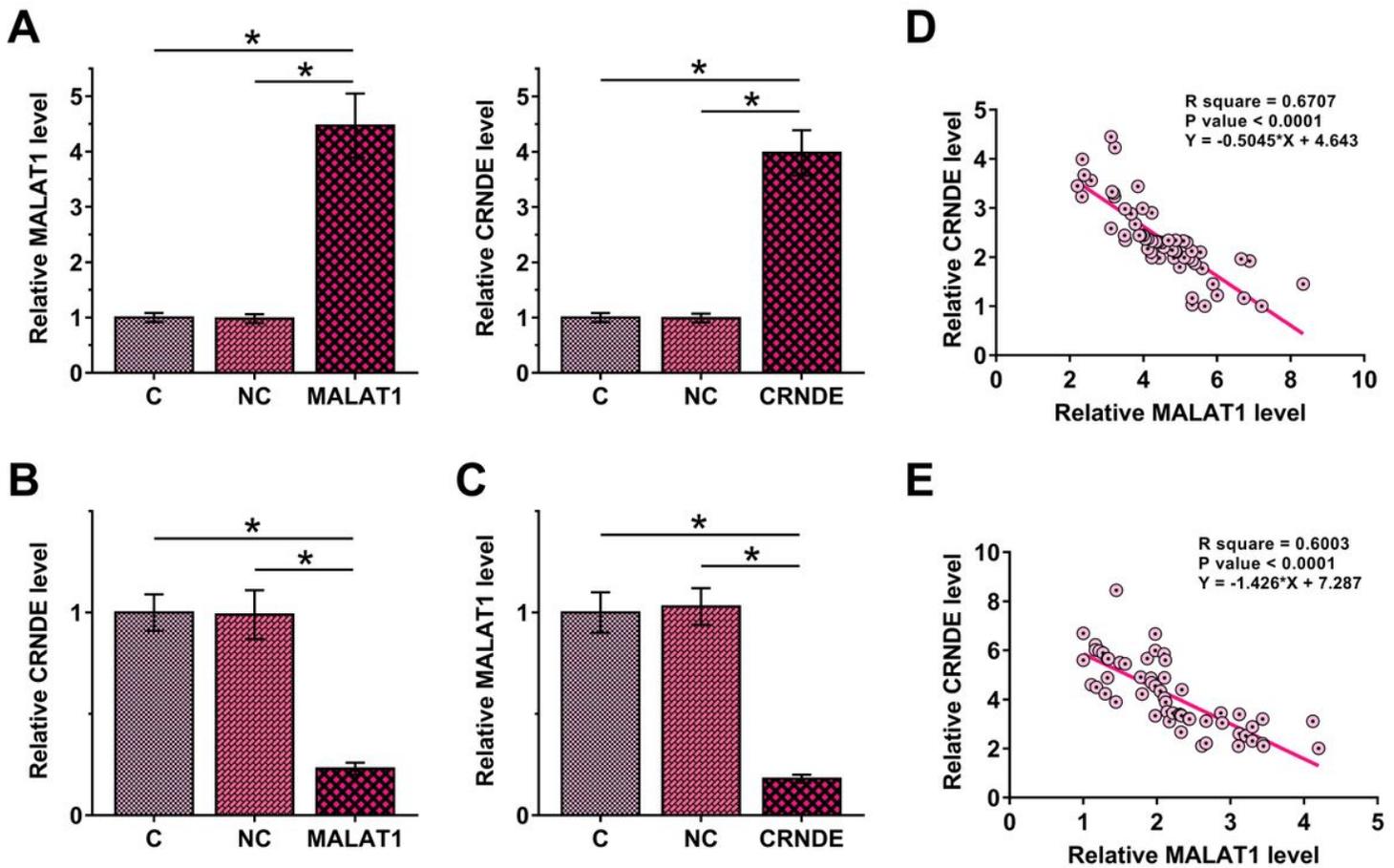


Figure 3

MALAT1 and CRNDE negatively regulated each other in HBEpCs. Expression vector of MALAT1 or CRNDE was transfected into HBEpCs, and the transfections were confirmed by RT-qPCR at 48h post-transfection (A). The effects of MALAT1 overexpression on CRNDE (B), and the effects of CRNDE overexpression on MALAT1 (C) were also explored by RT-qPCR. Correlations between MALAT1 and CRNDE across plasma samples from both sepsis patients (D) and healthy controls (E) were analyzed by linear regression. Mean±SD values of three independent replicates were presented and compared. *, $p < 0.05$.

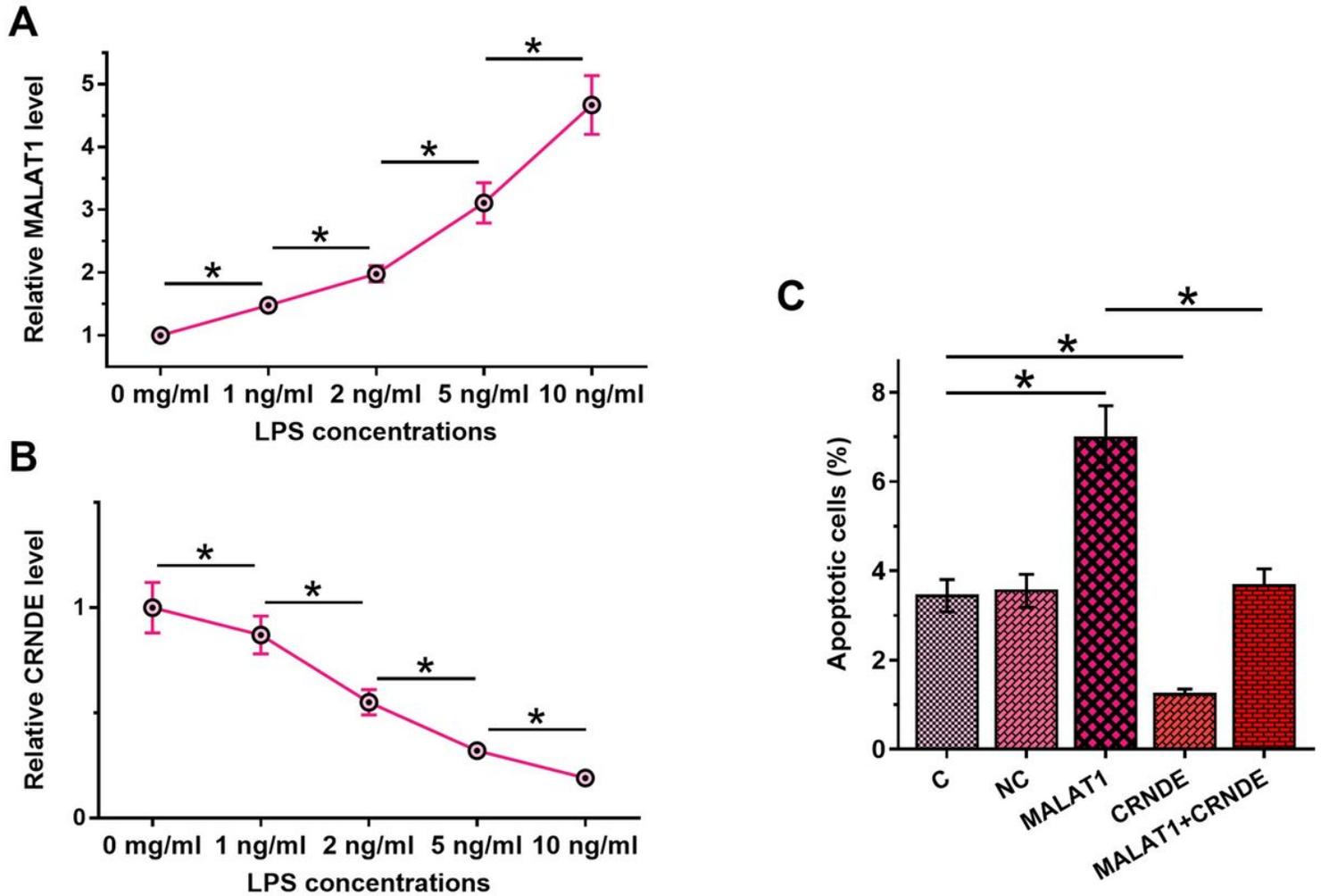


Figure 4

MALAT1 and CRNDE interact with each other to regulate the apoptosis of HBEpCs induced by LPS. HBEpCs were cultivated in medium supplemented with LPS at doses of 0, 1, 2, 5, and 10 $\mu\text{g/ml}$ for 48h, followed by RT-qPCR to determine the expression of MALAT1 (A) and CRNDE (B). Cell apoptosis assay was performed to analyze the roles of MALAT1 and CRNDE in regulating the apoptosis of HBEpCs induced by LPS (C). Mean±SD values of three independent replicates were presented and compared. *, $p < 0.05$.