

Overexpression of lncRNA MCM3AP-AS1 in Sepsis Suppresses the Maturation of miR-223 to Promote LPS-induced Lung Cells

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Research

Keywords: MCM3AP-AS1, miR-223, sepsis, miRNA maturation, apoptosis, lung

Posted Date: October 23rd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-95415/v1>

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Abstract

Background: Previous studies have showed that lncRNA MCM3AP-AS1 and miR-223 play opposite roles in LPS-induced inflammation, which contributes to the progression of sepsis. This study was therefore carried out to analyze the interactions between MCM3AP-AS1 and miR-223 in sepsis.

Methods: Plasma samples were obtained from 62 sepsis patients and 62 healthy controls. Expression of MCM3AP-AS1, miR-223 precursor and mature miR-223 in plasma samples was determined by RT-qPCR. In human bronchial epithelial cells (HBEpCs), the interaction between MCM3AP-AS1 and miR-223 was analyzed by overexpression experiments. Cell apoptosis assay was analyzed by cell apoptosis assay.

Results: We found that MCM3AP-AS1 was upregulated in sepsis, while miR-223 was downregulated in sepsis. MCM3AP-AS1 and mature miR-223 were inversely correlated, while MCM3AP-AS1 and miR-223 precursor were not. In HBEpCs, LPS treatment resulted in the upregulation of MCM3AP-AS1 and downregulation of miR-223. In HBEpCs, MCM3AP-AS1 overexpression downregulated mature miR-223 but failed to affect miR-223 precursor. In addition, MCM3AP-AS1 overexpression reduced the inhibitory effects of miR-223 on LPS-induced apoptosis of HBEpCs.

Conclusions: LncRNA MCM3AP-AS1 is upregulated in sepsis and may suppress the maturation of miR-223 to promote LPS-induced lung cells.

Background

As a serious clinical disorder, sepsis is caused by an overwhelming immune response in the body to bacteria, fungi, and viruses-caused infections [1]. Sepsis causes more deaths than many well-known causes of deaths, such as lung cancer, heart attacks and breast cancer [2]. It is estimated that, in the United States, sepsis affects about 1.7 million adults and causes about 250 000 deaths [3, 4]. Sepsis patients were mostly treated with antibiotics, in some cases intravenous fluids and oxygen supply are needed to maintain the normal function of critical organs [5, 6]. However, treatment outcomes are still unsatisfactory in some case and death is inevitable. Therefore, novel therapeutic approaches are needed.

Previous studies on the molecular mechanism of the pathogenesis of sepsis have characterized a considerable number of molecular alterations in the initiation, development and progression of sepsis [7, 8]. Increased understanding of the molecular mechanism of sepsis may provide novel insights to the development of therapeutic approaches, such as targeted therapy, which can be applied to regulate sepsis-related gene expression to suppress disease progression [9, 10]. Non-coding RNAs (ncRNAs) are RNA transcripts without protein-coding capacity but they regulate the expression of protein coding genes or other ncRNAs to participate in diverse human diseases, such as sepsis [11, 12]. Therefore, ncRNAs are potential targets for the treatment of sepsis. Previous studies have showed that lncRNA MCM3AP-AS1 and miR-223 play opposite roles in LPS-induced inflammation [13, 14], which contributes to the progression of sepsis. Therefore, they may have interactions in this disease. This study was therefore carried out to study the interaction between MCM3AP-AS1 and miR-223 in sepsis.

Results

MCM3AP-AS1, miR-223 precursor and mature miR-223 were altered in sepsis

Expression of MCM3AP-AS1, miR-223 precursor and mature miR-223 in plasma samples from both sepsis patients (n = 62) and healthy controls (n = 62) was determined by RT-qPCR. Compared to Control group, Sepsis group exhibited significantly upregulated MCM3AP-AS1 (Fig. 1A, $p < 0.01$), but significantly downregulated miR-223 precursor (Fig. 1B, $p < 0.01$) and mature miR-223 (Fig. 1C, $p < 0.01$). Therefore, altered expression of MCM3AP-AS1 and miR-223 may participate in sepsis.

MCM3AP-AS1 was inversely correlated with mature miR-223, but not miR-223 precursor across plasma samples from sepsis patients

Correlations between MCM3AP-AS1 and mature miR-223 (Fig. 2A) or miR-223 precursor (Fig. 2B) across plasma samples from sepsis patients (n = 62) were analyzed by linear regression. It was observed that MCM3AP-AS1 was inversely correlated with mature miR-223, but not miR-223 precursor across plasma samples. Therefore, MCM3AP-AS1 is likely involved in the maturation of miR-223 from precursor to mature miRNA.

MCM3AP-AS1 overexpression resulted in the downregulation of mature miR-223 in HBEpCs

To explore the interaction between MCM3AP-AS1 and miR-223, HBEpCs were transfected with either MCM3AP-AS1 expression vector or miR-223 mimic, followed by the confirmation of overexpression by RT-qPCR at 48 h post-transfection (Fig. 3A, $p < 0.05$). It was observed that cells with MCM3AP-AS1 expression vector transfection showed significantly downregulated mature miR-223, but not miR-223 precursor (Fig. 3B, $p < 0.05$). In contrast, miR-223 overexpression failed to significantly affect the expression of MCM3AP-AS1 (Fig. 3C).

MCM3AP-AS1 overexpression reduced the inhibitory effects of miR-223 on LPS-induced apoptosis of HBEpCs

HBEpCs were cultivated in medium containing 0, 2, 4, 6, 8 or 10 $\mu\text{g/ml}$ LPS for 48 h, followed by the determination of the expression of MCM3AP-AS1 and miR-223 by RT-qPCR. It was observed that LPS treatment resulted in the upregulation of MCM3AP-AS1 (Fig. 4A) and downregulation of both miR-223 mature miRNA and precursor (Fig. 4B) in a dose-dependent manner ($p < 0.05$). The roles of MCM3AP-AS1 and miR-223 in regulating the apoptosis of HBEpCs induced by LPS (10 mg/ml , 48 h) were analyzed by cell apoptosis assay. It was observed that MCM3AP-AS1 overexpression increased cell apoptosis, while miR-223 overexpression decreased cell apoptosis. In addition, MCM3AP-AS1 overexpression reduced the inhibitory effects of miR-223 on LPS-induced apoptosis of HBEpCs (Fig. 4C, $p < 0.05$).

Discussion

This study mainly analyzed the interaction between MCM3AP-AS1 and miR-223 in sepsis. We found that MCM3AP-AS1 was upregulated in sepsis and miR-223 was downregulated in sepsis. In addition, MCM3AP-AS1 may suppress the maturation of miR-223 to promote LPS-induced apoptosis of HBEpCs.

The function of MCM3AP-AS1 has been mostly investigated in cancers [15]. It has been reported that MCM3AP-AS1 is usually upregulated in cancers and promotes cancer development and progression mainly by regulating cancer cell behaviors, such as suppressing cell apoptosis [15]. In a recent study, Gao et al. reported that MCM3AP-AS1 promoted the apoptosis of chondrocyte induced by LPS by regulating the axis of miR-142-3p/HMGB1 [13]. Therefore, MCM3AP-AS1 may play opposite roles in regulating the apoptosis of different types of cells. LPS-induced inflammation plays a critical role in sepsis [16], indicating the potential involvement of MCM3AP-AS1 in sepsis. This study is the first to report the upregulation of MCM3AP-AS1 in sepsis. Lung injury is commonly observed in patients with sepsis [17]. In this study we observed that MCM3AP-AS1 overexpression resulted in the increased apoptosis of HBEpCs induced by LPS. In HBEpCs, LPS treatment resulted in increased expression of MCM3AP-AS1. Therefore, MCM3AP-AS1 may promote cell apoptosis in a LPS-dependent manner to aggravate sepsis.

Different from the role of MCM3AP-AS1, miR-223 plays an opposite role in LPS-induced inflammation by suppressing lung cell apoptosis by interacting with RHOB to regulate TLR4/NF- κ B signaling [14]. In this study we observed the downregulation of both mature miR-223 and miR-223 precursor in sepsis. In addition, LPS treatment also downregulate both mature miR-223 and miR-223 precursor in HBEpCs. The maturation of miRNA includes three steps: 1) transcription to form pri-miRNA; 2) pri-miRNA to precursor miRNA; 3) precursor miRNA to mature miRNA [18]. In this study we showed that MCM3AP-AS1 overexpression suppress the generation of mature miR-223 but not miR-223 precursor. Therefore, MCM3AP-AS1 overexpression may inhibit the maturation of miR-223 at step 3. However, the mechanism remains to be further explored.

Conclusion

In conclusion, MCM3AP-AS1 is upregulated in sepsis. MCM3AP-AS1 may inhibit the maturation of miR-223 to promote the apoptosis of lung cells induced by LPS.

Materials And Methods

Sepsis and healthy controls

From June 2017 to June 2019, 62 sepsis patients (40 males and 22 females; 41 to 64 years; 52.1 ± 5.9 year) and 62 healthy controls (40 males and 22 females; 41 to 64 years; 52.3 ± 5.7 year) were enrolled at Affiliated Hospital of Binzhou Medical University. This study was approved by this hospital Ethics Committee. All healthy controls were enrolled at healthy center of this hospital after they received systemic physiological examination. All physiological parameters of healthy controls were normal. All sepsis patients were diagnosed for the first time. Bacterial ($n = 30$) or virus ($n = 32$) infections were found

to be the major causes of sepsis. In view of the fact that other clinical disorders and therapies may also affect gene expression, we excluded patients complicated with other clinical disorders or patients who received therapy within 100 days before admission. All participants signed informed consent.

Plasma Samples And Human Bronchial Epithelial Cells (hbepcs)

Fasting blood (3 ml) was extracted from each participant before breakfast on the next day of admission. All blood samples were transferred to EDTA tubes, followed by centrifugation for 15 min at 1200 g to prepare plasma samples. Plasma samples were subjected to RNA extraction immediately after preparation.

Cell model in this study is HBEpCs (Sigma-Aldrich, USA). Cells were cultivated in Bronchial Epithelial Cell Medium (Sigma-Aldrich). Cell culture was performed in a 95% humidity and 5% CO₂ incubator at 37 °C. To mimic sepsis, cells were cultivated in medium containing 0, 2, 4, 6, 8 or 10 µg/ml LPS for 48 h before the subsequent experiments. Cells were collected from passage 3 to passage 5 were used.

Transient Cell Transfections

With backbone vector pcDNA3.1 (Invitrogen), expression vector of MCM3AP-AS1 or miR-223 was constructed. Negative control (NC) miRNA and mimic of miR-223 were obtained from Sigma-Aldrich. HBEpCs collected at about 85% humidity were transfected with 1 µg expression vector or 40 nM miRNA through lipofectamine 2000 (Invitrogen)-mediated transfections. To perform NC experiments, HBEpCs were transfected with empty vector or NC miRNA. To perform control (C) experiments, cells without transfections were cultivated until the end of experiments. Cells were cultivated with transfection mixture for 6 h, followed by washing with fresh medium. After that, cells were cultivated in fresh medium for 48 h before use.

Rna Preparation

RNAzol reagent (Sigma-Aldrich) was used to isolate RNAs from both plasma samples and HBEpCs. All RNA samples were subjected to genomic DNA removal using DNase I (Invitrogen) at 37 °C for 100 min. RNA integrity was check using a 5% Urea-PAGE gel. OD 260/280 ratios of RNA samples were determined to check RNA purity.

Rt-qpcrs

RNA samples with an OD 260/280 ratio about 2.0 were reverse transcribed into cDNA samples using SSRT IV system (Invitrogen). SensiFAST™ Real-Time PCR Kit (Bioline) was used to perform qPCRs to

determine the expression of MCM3AP-AS1 and miR-223 precursor. The internal control MCM3AP-AS1 was 18S rRNA, and the internal control of miR-223 was U6. Sequence-specific primers were used. Expression of mature miR-223 was determined using GeneCopoeia All-in-One™ miRNA qRT-PCR reagent kit. Mature miRNAs were first added with poly (A), followed by miRNA RTs and miRNA qPCRs. Three technical replicates were included in each experiment and Ct values were normalized based on $2^{-\Delta\Delta CT}$ method.

Cell Apoptosis Assay

At 48 h post-transfection, HBEPcs were cultivated in medium supplemented with 10 mg/ml LPS for another 48 h. After that, ice-cold PBS was used to wash the cells and staining with PI and FITC-annexin V (Sigma-Aldrich) was performed in dark for 20 min. After that, flow cytometry was performed to separate apoptotic cells.

Statistical analysis

Data of gene expression in plasma samples of both patients and controls were expressed as average values of 3 technical replicates and were compared paired t test. Data of multiple cell transfection groups or LPS treatment groups were expressed as mean \pm SD values and compared by ANOVA Tukey's test. Correlations were analyzed by linear regression. $P < 0.05$ was deemed statistically significant.

Declarations

Ethics approval and consent to participate

This study was approved by Ethics Committee of Affiliated Hospital of Binzhou Medical University.

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by no funder.

Authors' contributions

Guarantor of integrity of the entire study—Zhang Quanyi. Study concepts—Gong Chunzhi. Study design—Gong Chunzhi. Definition of intellectual content—Gong Chunzhi. Literature research—Wang Jianying. Clinical studies—Wang Jianying. Experimental studies—Liu Shupeng. Data acquisition—Li Zunfeng. Data analysis—Wang Jianying. Statistical analysis—Zhang Quanyi. Manuscript preparation—Liu Shupeng. Manuscript editing—Sun Chao. Manuscript review—Liu Zhaoguo.

Acknowledgements

The authors would like to express our gratitude for those who have critically reviewed this manuscript and those who give us help during this experiment.

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Figures

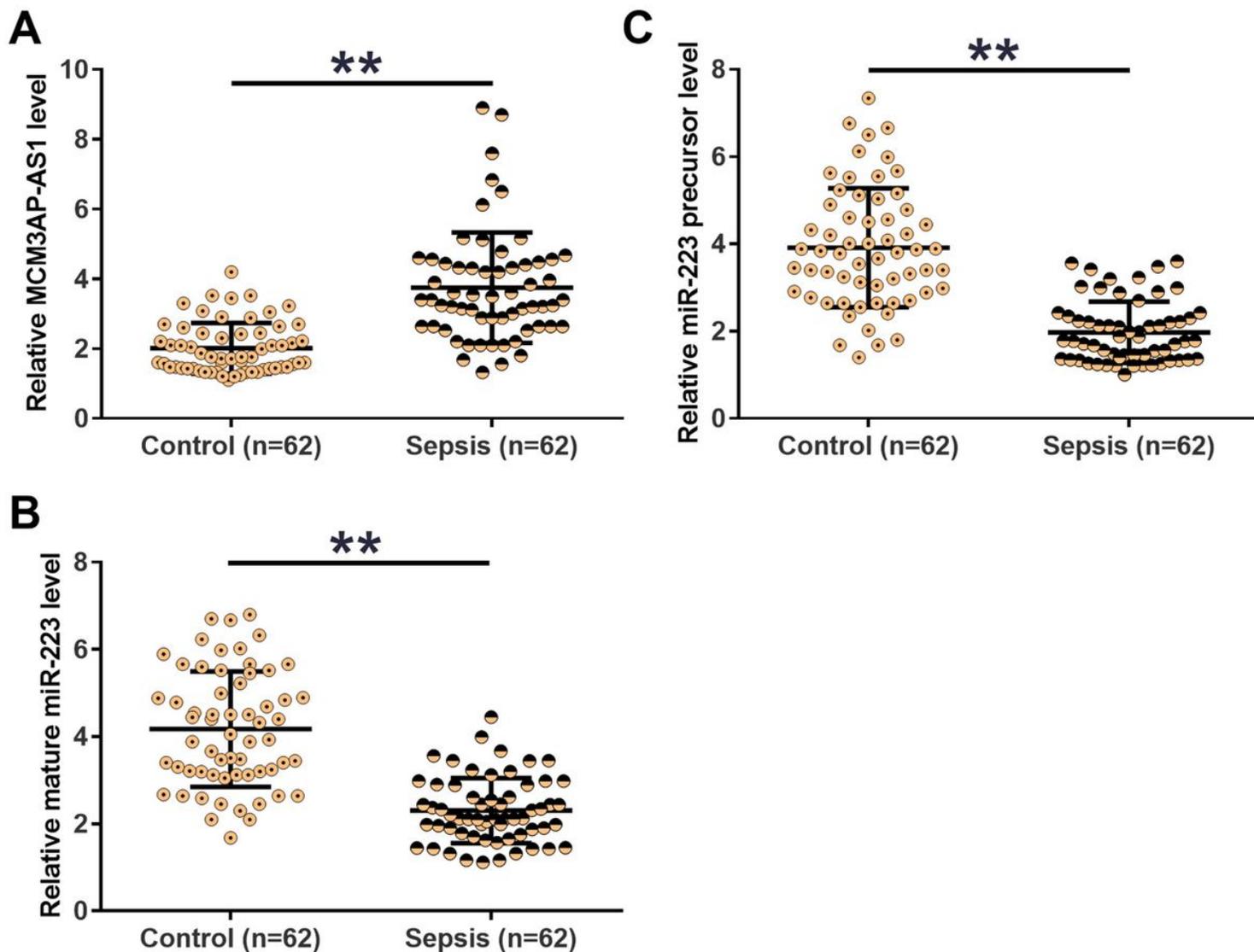


Figure 1

MCM3AP-AS1, miR-223 precursor and mature miR-223 were altered in sepsis. Expression of MCM3AP-AS1 (A), miR-223 precursor (B) and mature miR-223 (C) in plasma samples from both sepsis patients (n=62) and healthy controls (n=62) was determined by RT-qPCR. Data of gene expression in plasma samples of both patients and controls were expressed as average values of 3 technical replicates and were compared paired t test. **, $p < 0.05$.

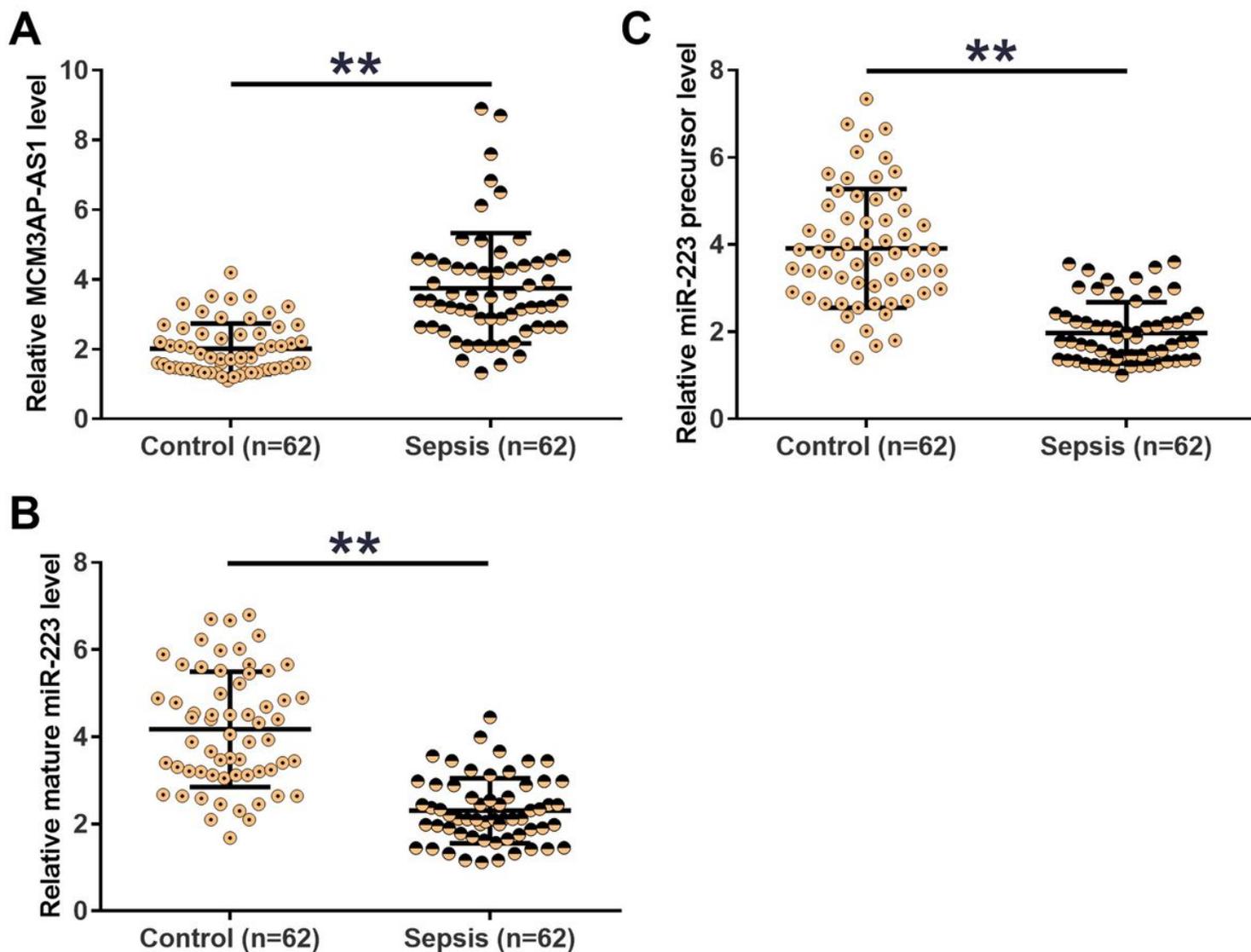


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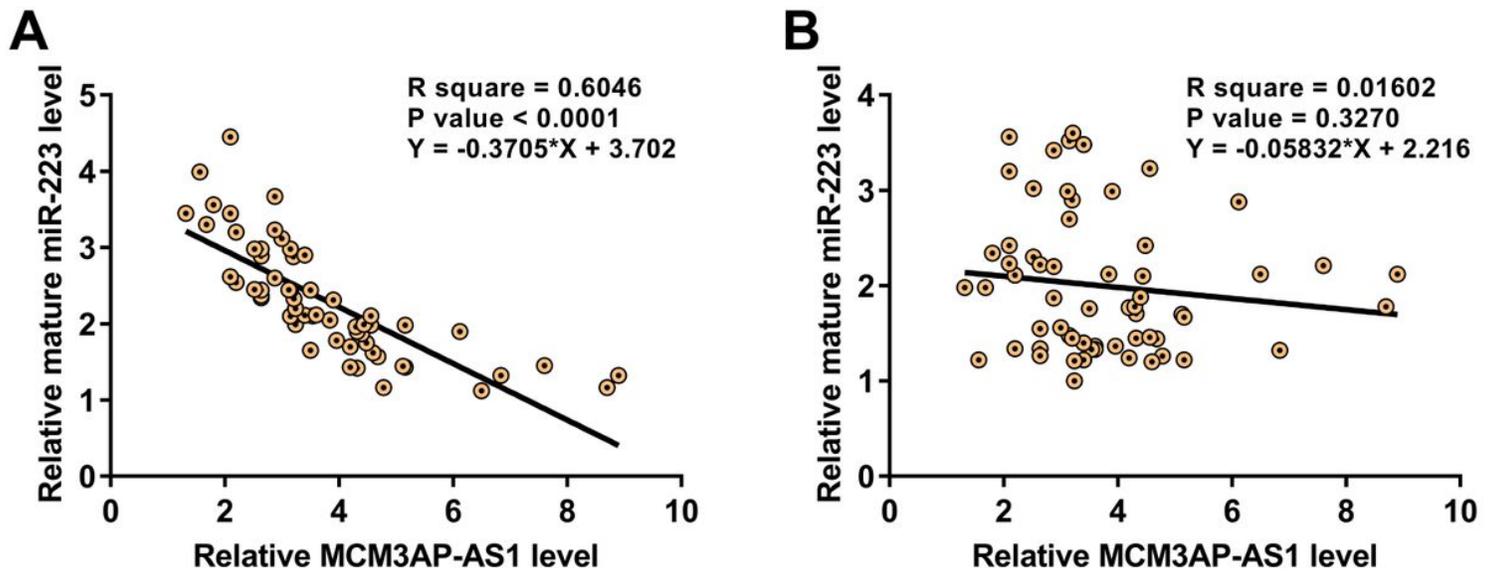


Figure 2

MCM3AP-AS1 was inversely correlated with mature miR-223, but not miR-223 precursor across plasma samples from sepsis patients. Correlations between MCM3AP-AS1 and mature miR-223 (A) or miR-223 precursor (B) across plasma samples from sepsis patients (n=62) were analyzed by linear regression.

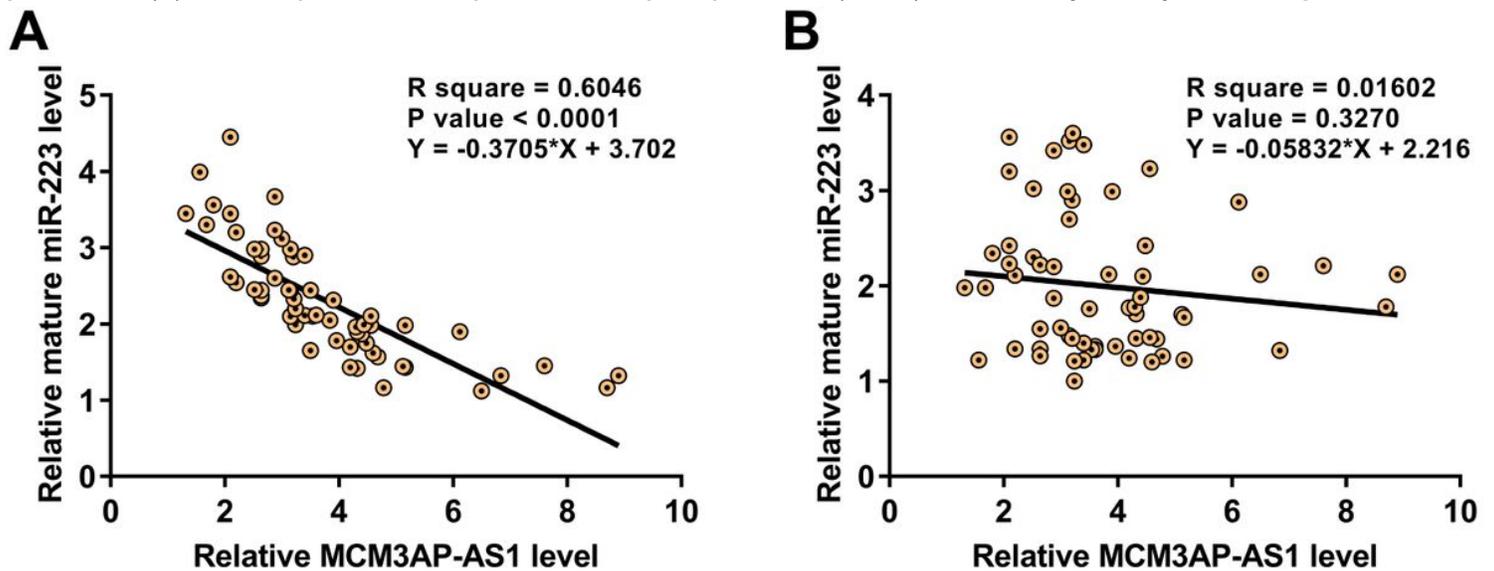


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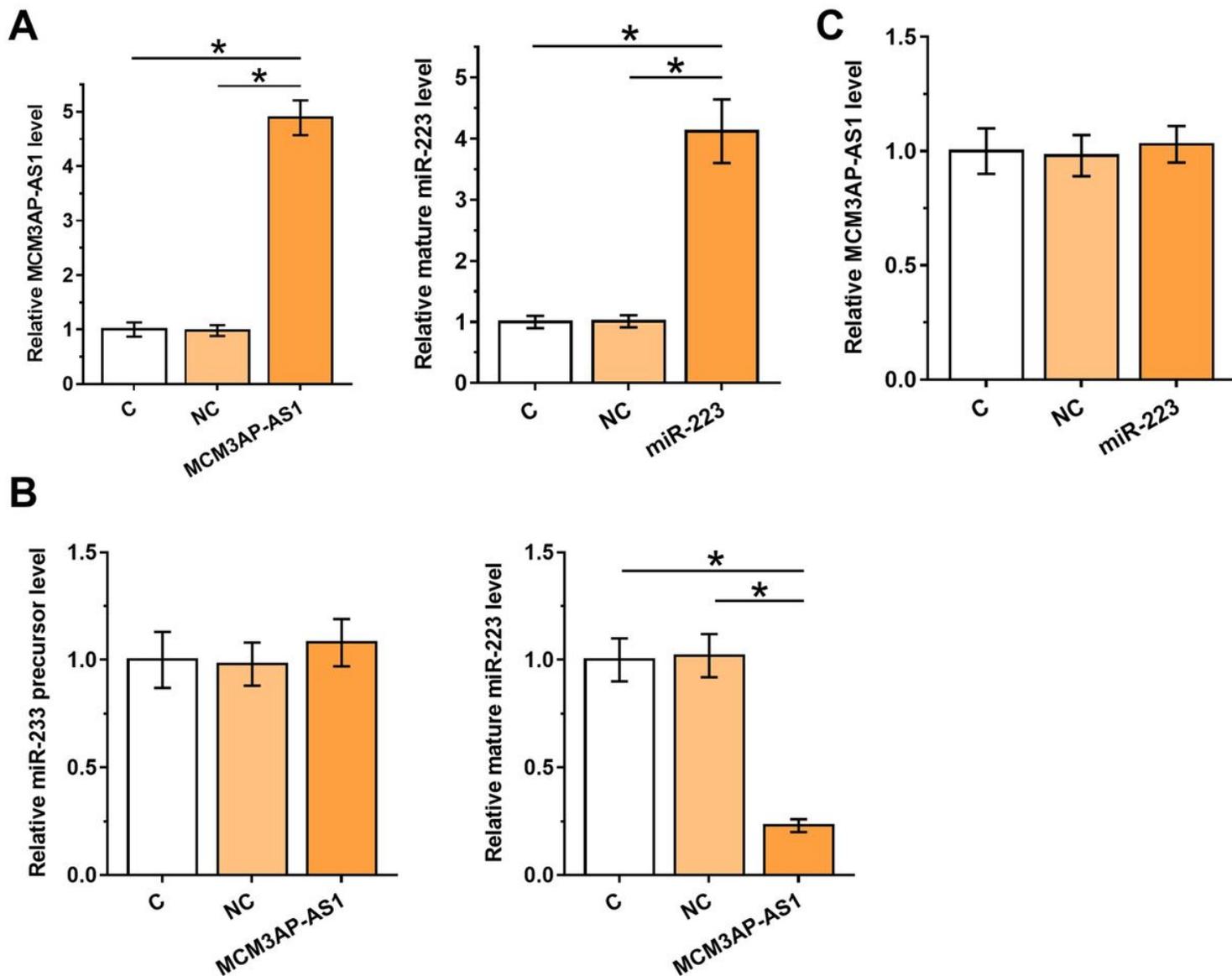


Figure 3

MCM3AP-AS1 overexpression resulted in the downregulation of mature miR-223 in HBEpCs. To explore the interaction between MCM3AP-AS1 and miR-223, HBEpCs were transfected with either MCM3AP-AS1 expression vector or miR-223 mimic, followed by the confirmation of overexpression by RT-qPCR at 48h post-transfection (A). The effects of MCM3AP-AS1 expression vector transfection on mature miR-223 and miR-223 precursor expression (B), and the effects of miR-223 overexpression on the expression of MCM3AP-AS1 (C) were also analyzed by RT-qPCR. Mean±SD values of three independent replicates were presented. *, $p < 0.05$.

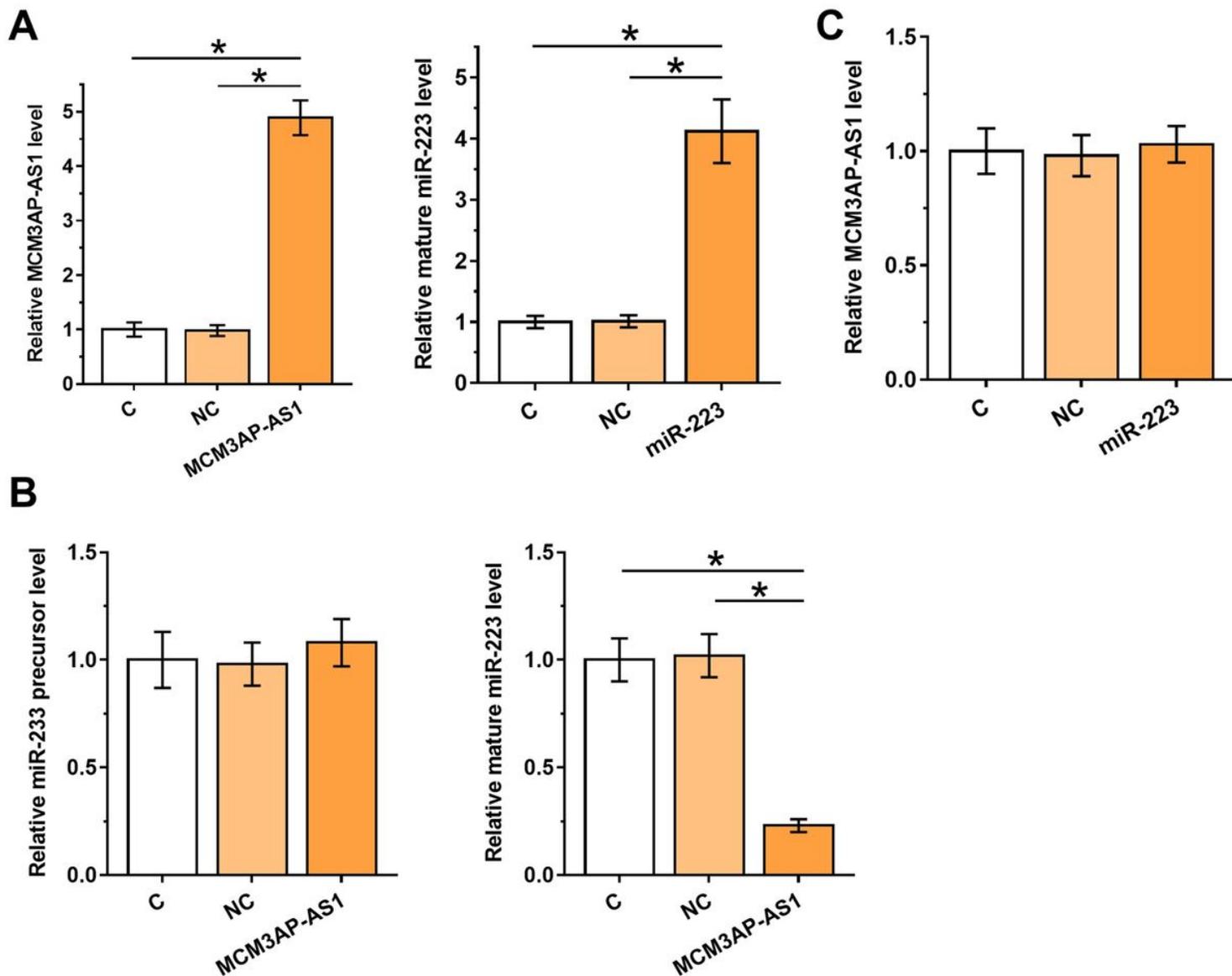


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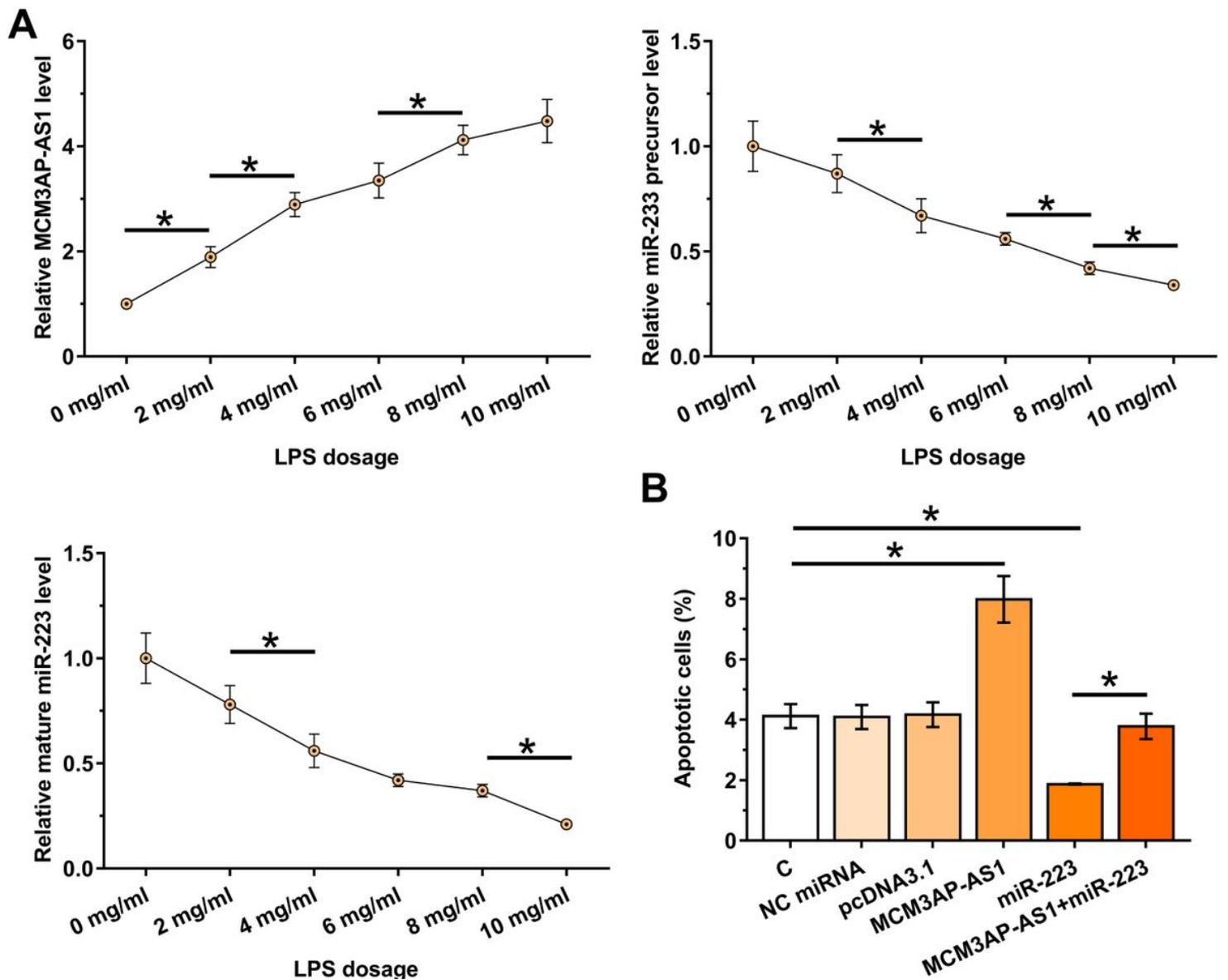


Figure 4

MCM3AP-AS1 overexpression reduced the inhibitory effects of miR-223 on LPS-induced apoptosis of HBEpCs. HBEpCs were cultivated in medium containing 0, 2, 4, 6, 8 or 10 $\mu\text{g/ml}$ LPS for 48h, followed by the determination of the expression of MCM3AP-AS1 (A) and miR-223 (B) by RT-qPCR. The roles of MCM3AP-AS1 and miR-223 in regulating the apoptosis of HBEpCs induced by LPS (10mg/ml, 48h) were analyzed by cell apoptosis assay (C). Mean \pm SD values of three independent replicates were presented. *, $p < 0.05$.

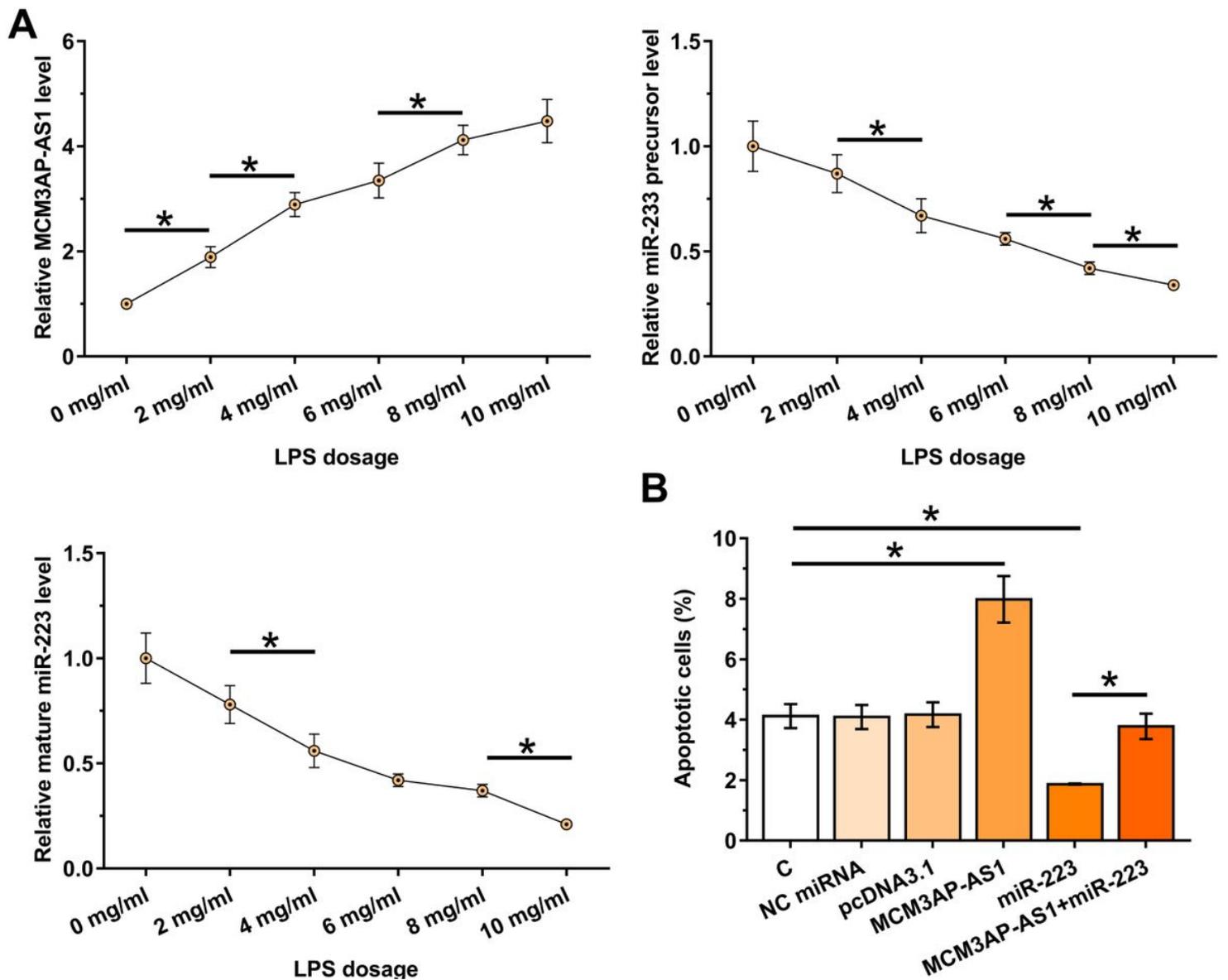


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