CircRNA circFADS2 is Under-expressed in Sepsis and Protects Lung Cell From LPS-induced Apoptosis by Downregulating miR-133a

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Research

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Abstract

Background: It has been reported that circFADS2 and miR-133a play opposite roles in LPS-induced cell apoptosis, which contributes to the development of sepsis. This study was carried out to explore the interaction between circFADS2 and miR-133a in sepsis.

Methods: Expression of circFADS2 and miR-133a in plasma from both sepsis patients (n=62) and healthy controls (n=62) was studied by RT-qPCR. The effects of LPS on the expression of circFADS2 and miR-133a were also analyzed by RT-qPCR. The crosstalk between circFADS2 and miR-133a was analyzed by overexpression, followed by RT-qPCR. The role of circFADS2 and miR-133a in regulating the apoptosis of lung cells induced by LPS was analyzed by cell apoptosis assay.

Results: We found that circFADS2 was under-expressed in sepsis and miR-133a was overexpressed in sepsis. An inverse correlation between circFADS2 and miR-133a was observed across sepsis samples. In lung cells, LPS treatment decreased the expression of circFADS2 and increased the expression of miR-133a. In lung cells, circFADS2 overexpression decreased the expression of miR-133a. Moreover, circFADS2 overexpression reduced the enhancing effects of miR-133a overexpression on cell apoptosis induced by LPS.

Conclusion: Therefore, circFADS2 is under-expressed in sepsis and may protect lung cell from LPS-induced apoptosis by downregulating miR-133a.

Background

Sepsis is a major challenge in clinics that is caused by the body's responses to severe infections [1]. Sepsis is usually caused by infections of bacteria, viruses and fungus, with bacterial infection as the major cause [2]. With proper treatment, such as systemic antibodies within one hour of diagnosis, most patients with mild or moderate sepsis can get full recovery [3]. However, in severe cases, such as septic shock, organs failures may occur, leading to a mortality rate as high as 40% before hospitalization [4, 5]. Even worse, patients recovered from severe sepsis are prone to future infections [6], resulting in increased risk of death within 2 years after sepsis.

The failures of organs, such as the lungs, liver, kidney and heart, are the major causes of deaths among sepsis patients [7–9]. Therefore, the prevention and treatment of organ failures are the key for the survival of sepsis patients. With the increased elucidation of the molecular mechanism of sepsis, several molecular players, such as SIRT1 and MD2, have been proven to be potential targets for the treatment of organ failures, such as acute lung injuries, in sepsis patients [10–12]. However, more safe and effective targets are needed to be tested prior to clinical applications. Rather than coding proteins, circular RNAs (circRNAs) are covalently closed RNAs that can play critical roles in human diseases mainly by regulating gene expression [13, 14]. Therefore, circRNAs are potential targets to treat diseases. It has been reported that circRNA circFADS2 and miR-133a play opposite roles in LPS-induced cell apoptosis [15, 16], which

contributes to the development of sepsis [17]. We therefore analyzed the involvement of circFADS2 and miR-133a in sepsis and explored the interactions between them.

Methods

Sepsis and controls

At Minhang Hospital, Fudan University, this study enrolled a total of 62 sepsis patients (32 males and 30 females, 52.3+/-4.9 years) and 62 controls (32 males and 30 females, 52.4+/-4.8 years) between March 2018 and March 2020. All healthy controls were enrolled at physiological health center of this hospital. All healthy controls received systemic physiological exam and all parameters were within normal range. To exclude other factors that can also affect gene expression, this study did not include patients with initiated therapy, other clinical disorders and history of sepsis. All sepsis cases in this study were caused by bacterial infections. All sepsis patients were diagnosed by blood test to show the existent of bacteria. All patients and controls signed informed consent.

Blood and lung cells

All patients and healthy controls were subjected to blood (2 ml) extraction from the elbow vein of both sepsis patients and healthy controls prior to therapy. Blood samples were mixed with citric acids to a ratio of 10:1. The mixture was centrifuged for 15 min at 1200g to separate plasma.

Lung cells were in this study were human bronchial epithelial cells (HBEpCs, Sigma-Aldrich). In a 5% CO_2 incubator with 95% humidity, HBEpCs were cultivated in bronchial Epithelial Cell Medium (Sigma-Aldrich) at 37°C to reach about 85% confluence prior to the subsequent assays. LPS treatment was performed by incubating HBEpCs in medium containing 0, 2, 4, 8 and 12μg/ml LPS under the aforementioned conditions for 48h.

Overexpression assays

Overexpression of circFADS2 and miR-133a was reached in HBEpCs by transfecting expression vector (1μg) or miRNA (50 nM) through transient transfections mediated by lipofectamine 2000 (Invitrogen). The expression vector of circFADS2 was constructed with pcDNA3.1 vector (Invitrogen) as backbone. Mimic of miR-133a and negative control (NC) miRNA were purchased from Sigma-Aldrich (USA). NC groups included cells transfected with empty vector or NC miRNA. C (control) group included cells without transfections. Cells were washed with fresh medium after incubation with transfection medium for 6h, followed by cell culture in fresh medium for further 48h prior to use.

RNA isolation and quality analysis

Total RNA in plasma and HBEpCs was extracted using Ribozol (VWR), followed by digestion with DNA eraser (Takara, Japan) until all samples reached and OD260/280 ratio close to 2.0, which indicated pure

RNA. Electrophoresis (5% urea-PAGE gel) was carried out to analyze the integrity of RNA samples. Only RNA samples with high purity and satisfactory integrity were subjected to the subsequent assays.

RT-qPCR assays

The expression of circFADS2 in both plasma and HBEpCs was analyzed using ReverTra Ace™ qPCR RT Kit (Toyobo, Japan). The internal control of circFADS2 was 18S rRNA. All-in-One™ miRNA qRT-PCR reagent kit (GeneCopoeia) was used to analyze the expression of mature miR-133a. The internal control of miR-133a was U6. All operations were performed followed by manufacturers' instructions. Ct values of circFADS2 and miR-133a were normalized to their endogenous controls following $2^{-\Delta\Delta CT}$ method.

Cell apoptosis analysis

HBEpCs collected at 48h post-transfection were subjected to cell apoptosis analysis. HBEpCs with transfections were transferred to a 6-well cell culture plate with 8000 cells in 1.5 ml medium per well. Three replicate wells were set for each transfection group. Each well was added with 10 μg/ml LPS, followed by cell culture under the aforementioned conditions for further 48h. After cell culture, ice-cold PBS was used to wash HBEpCs. PI and FITC-annexin V (Abcam) were used to stain cells for 20 min in dark. After that, flow cytometry was performed to analyze cell apoptosis.

Statistical analysis

Comparisons between sepsis and control groups were analyzed by unpaired t test. To compare multiple independent groups, ANOVA Tukey's test was performed. To analyze the correlations between circFADS2 and miR-133a, Pearson's correlation coefficient analysis was performed. $P<0.05$ was statistically significant.

Results

Sepsis patients showed altered expression of circFADS2 and miR-133a

RNA samples isolated from both sepsis patients (n = 62) and healthy controls (n = 62) were subjected to RT-qPCR to analyze differential gene expression in sepsis. Our data revealed that, compared to the 62 healthy controls, circFADS2 was under-expressed in sepsis (Fig. 1A, $p < 0.001$) and miR-133a was overexpressed (Fig. 1B, $p < 0.001$) in sepsis. Therefore, the downregulation of circFADS2 and the overexpression of miR-133a may participate in sepsis.

RNA samples isolated from both sepsis patients (n = 62) and healthy controls (n = 62) were subjected to RT-qPCR to analyze the differential expression of circFADS2 (A) and miR-133a (B) in sepsis. Ct values were normalized to corresponding internal controls following $2^{-\Delta\Delta CT}$ method. The sample with the

biggest ΔCT value was set to value “1”. Other samples were normalized to this sample to calculate relative gene expression. ***,$p < 0.001$.

Plasma samples from sepsis patients showed an inverse correlation between circFADS2 and miR-133a

The differential expression pattern of circFADS2 and miR-133a in sepsis may indicate the potential crosstalk between them. To explore the interaction between them, the first step is to analyze the correlations. Pearson’s correlation coefficient analysis revealed that circFADS2 and miR-133a were inversely and significantly correlated across sepsis samples (Fig. 2A). However, correlation analysis revealed no close correlation between circFADS2 and miR-133a across control samples (Fig. 2B). The close correlation between them indicates the potential interaction between them.

The differential expression pattern of circFADS2 and miR-133a in sepsis may indicate the potential crosstalk between them. To explore the interaction between them, Pearson’s correlation coefficient analysis was performed to analyze the correlations between circFADS2 and miR-133a across sepsis samples (A) and control samples (B).

Overexpression of circFADS2 decreased the expression of miR-133a in HBEpCs

Expression vector of circFADS2 and the mimic of miR-133a were transfected into HBEpCs (pre-treated with 12 μg/ml LPS for 48 h) to further explore the crosstalk between circFADS2 and miR-133a. Overexpression of circFADS2 and miR-133a was analyzed by RT-qPCR every 24 h until 96 h. It was observed that circFADS2 and miR-133a were overexpressed as each time point (Fig. 3A, $p < 0.05$). It was observed that circFADS2 overexpression decreased the expression of miR-133a (Fig. 3B, $p < 0.05$). However, overexpression of miR-133a failed to significantly alter the expression of circFADS2 at each time point (Fig. 3C). Therefore, circFADS2 may downregulate miR-133a in HBEpCs.

Expression vector of circFADS2 and the mimic of miR-133a were transfected into HBEpCs (pre-treated with 12 μg/ml LPS for 48 h) to further explore the crosstalk between circFADS2 and miR-133a (A). The effects of circFADS2 overexpression on the expression of miR-133a (B) and the effects of miR-133a overexpression on the expression of circFADS2 (C) were also analyzed by RT-qPCR. *,$p < 0.05$.

LPS treatment altered the expression of circFADS2 and miR-133a in HBEpCs

LPS treatment was performed by incubating HBEpCs in medium containing 0, 2, 4, 8 and 12 μg/ml LPS under the aforementioned conditions for 48 h, followed by detecting the expression of circFADS2 and miR-133a through RT-qPCR. It was observed that LPS treatment decreased the expression of circFADS2 (Fig. 4A, $p < 0.05$) and increased the expression of miR-133a (Fig. 4B, $p < 0.05$). Therefore, the altered expression of circFADS2 and miR-133a in sepsis is likely induced by LPS.

LPS treatment was performed by incubating HBEpCs in medium containing 0, 2, 4, 8 and 12 μg/ml LPS under the aforementioned conditions for 48 h, followed by detecting the expression of circFADS2 (A) and miR-133a (B) through RT-qPCR. *,$p < 0.05$.

Overexpression of circFADS2 suppressed the apoptosis of HBEpCs induced by LPS through miR-133a

The effects of the overexpression of circFADS2 and miR-133a on the apoptosis of HBEpCs induced by LPS were analyzed by cell apoptosis assay. Our data showed that circFADS2 overexpression decreased cell apoptosis and miR-133a overexpression increased cell apoptosis. In addition, circFADS2 overexpression reduced the enhancing effects of miR-133a overexpression on cell apoptosis induced by LPS (Fig. 5, $p < 0.05$).

The effects of the overexpression of circFADS2 and miR-133a on the apoptosis of HBEpCs induced by LPS were analyzed by cell apoptosis assay.*,$p < 0.05$.

Discussion

In this study the involvement of circFADS2 and miR-133a in sepsis and the potential crosstalk between them in sepsis were explored. We found that circFADS2 and miR-133a were altered in sepsis. Interestingly, circFADS2 and miR-133a play opposites roles in apoptosis of HBEpCs induced by LPS, and circFADS2 may suppress cell apoptosis by downregulating miR-133a.

The functionality of circFADS2 has been explored in cancer biology [18, 19]. In lung cancer and colorectal cancer, circFADS2 is overexpressed and promotes cancer progression by regulating cancer cell behaviors, such as increasing cell proliferation and invasion[18, 19]. Besides that, a recent study reported that circFADS2 could interact with miR-498/mTOR axis to protect chondrocyte from LPS-induced apoptosis [15]. It is well known that LPS-induced cell apoptosis and inflammation are critical contributors to sepsis [17], indicating the involvement of circFADS2 in sepsis. In this study we showed that circFADS2 was downregulated in sepsis and overexpression of circFADS2 decreased the apoptosis of HBEpCs induced by LPS. Therefore, circFADS2 may play protective roles in sepsis-induced lung injury. However, in vivo animal model experiments are needed to further confirm the function of circFADS2 in sepsis. In addition, our study showed that LPS treatment resulted in the downregulation of circFADS2 in HBEpCs. Therefore, the downregulation of circFADS2 is likely induced by LPS.

It has been reported that miR-133a in sepsis can target SIRT1 to aggregate inflammation [16]. Consistently, our study showed that miR-133a was overexpressed in sepsis and overexpression of miR-133a increased the apoptosis of HBEpCs under LPS treatment. Moreover, in this study we also showed that the downregulation of miR-133a in sepsis is also likely induced by LPS. However, the upstream regulator of miR-133a in sepsis is unknown. In this study we showed that circFADS2 could downregulate the expression of miR-133a in LPS-treated HBEpCs. However, we observed the close correlation between circFADS2 and miR-133a across sepsis samples, but not control samples. Therefore, the interaction between circFADS2 and miR-133a is likely mediated by certain pathological factors.

Conclusion

In conclusion, circFADS2 is downregulated in sepsis, and the overexpression of circFADS2 may protect lung injuries in sepsis by reducing LPS-induced apoptosis of HBEpCs by downregulating miR-133a.

Abbreviations

not applicable.

Declarations

Acknowledgements
not applicable.

Authors' contributions

GS and XianL designed the study. AN and XiaoL carried out experiments and wrote the manuscipt, GS and XianL revised the paper, FN,XiaoL, JN,ZX, LJ,HW and HL collected patient specimens and related information. JN,ZX, LJ,HW and HL contributed to analysing the data. All authors reviewed the results and approved the final version of the manuscript.

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Availability of data and materials
The analyzed data sets generated during the present study are available from the corresponding author on reasonable request

Ethics approval and consent to participate
This study was approved by Minhang Hospital, Fudan University Ethics Committee and all the patients or parents/ guardians of patients provided written informed consent.

Consent for publication
not applicable.

Competing interests
The authors declare that they have no competing interests

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Figures

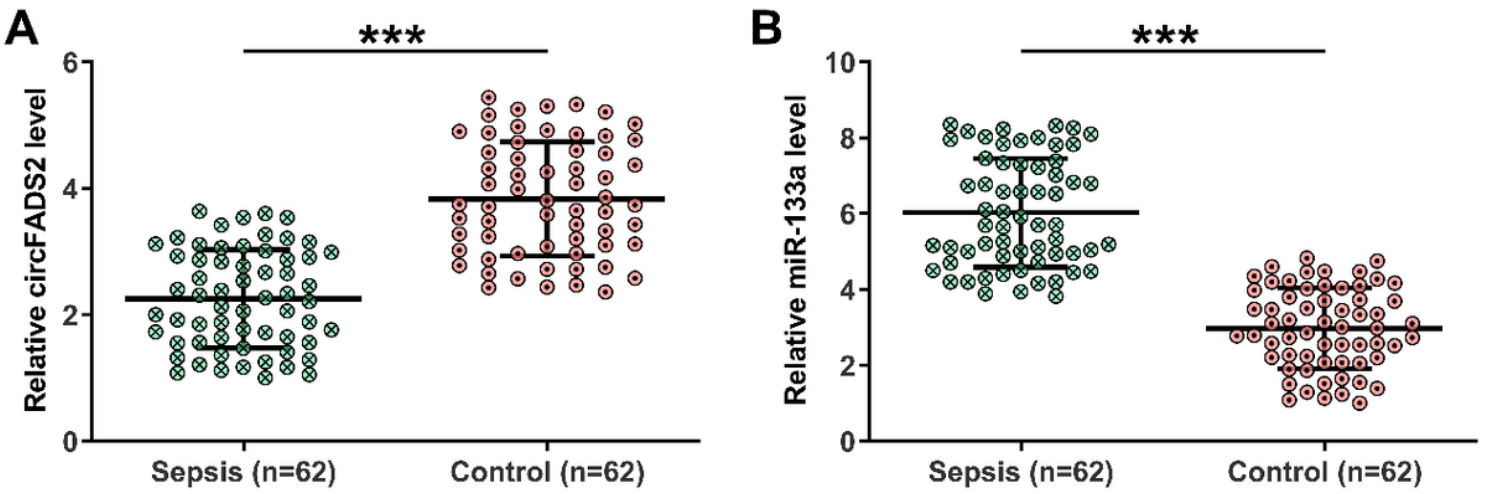

Figure 1

Sepsis patients showed altered expression of circFADS2 and miR-133a RNA samples isolated from both sepsis patients (n=62) and healthy controls (n=62) were subjected to RT-qPCR to analyze the differential expression of circFADS2 (A) and miR-133a (B) in sepsis. Ct values were normalized to corresponding internal controls following 2-ΔΔCT method. The sample with the biggest ΔCT value was set to value “1”. Other samples were normalized to this sample to calculate relative gene expression. ***,p<0.001.

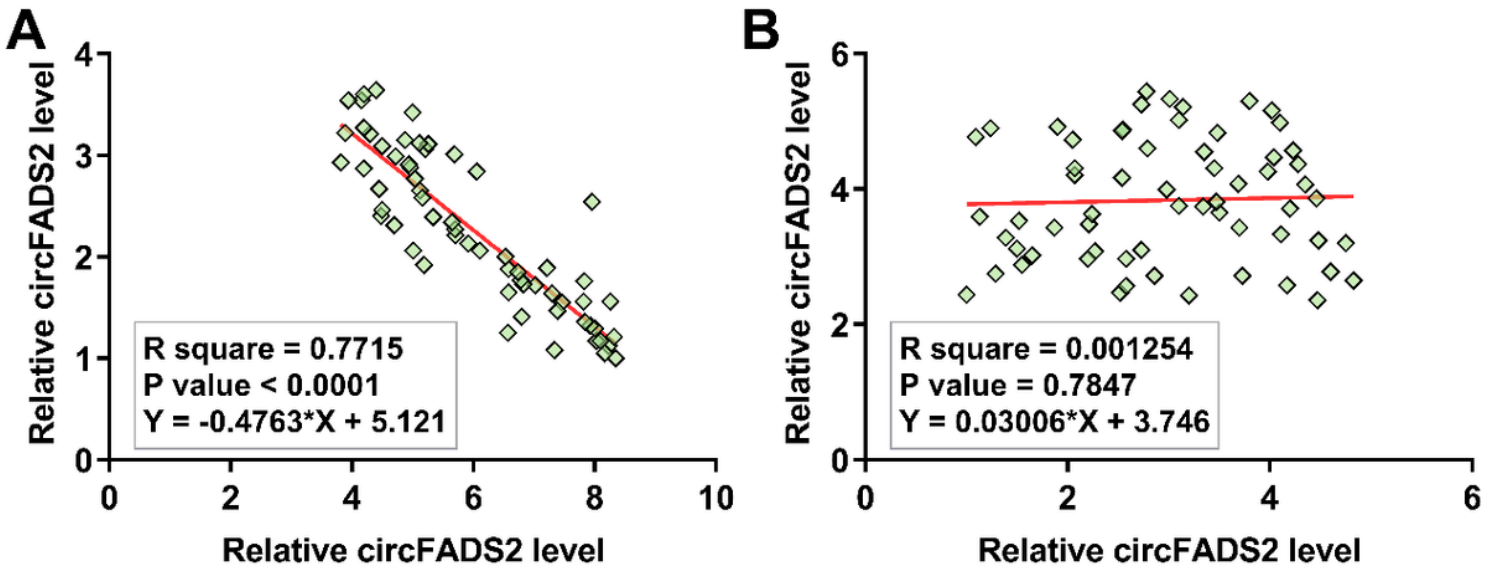

Figure 2

Plasma samples from sepsis patients showed an inverse correlation between circFADS2 and miR-133a The differential expression pattern of circFADS2 and miR-133a in sepsis may indicate the potential crosstalk between them. To explore the interaction between them, Pearson's correlation coefficient analysis was performed to analyze the correlations between circFADS2 and miR-133a across sepsis samples (A) and control samples (B).

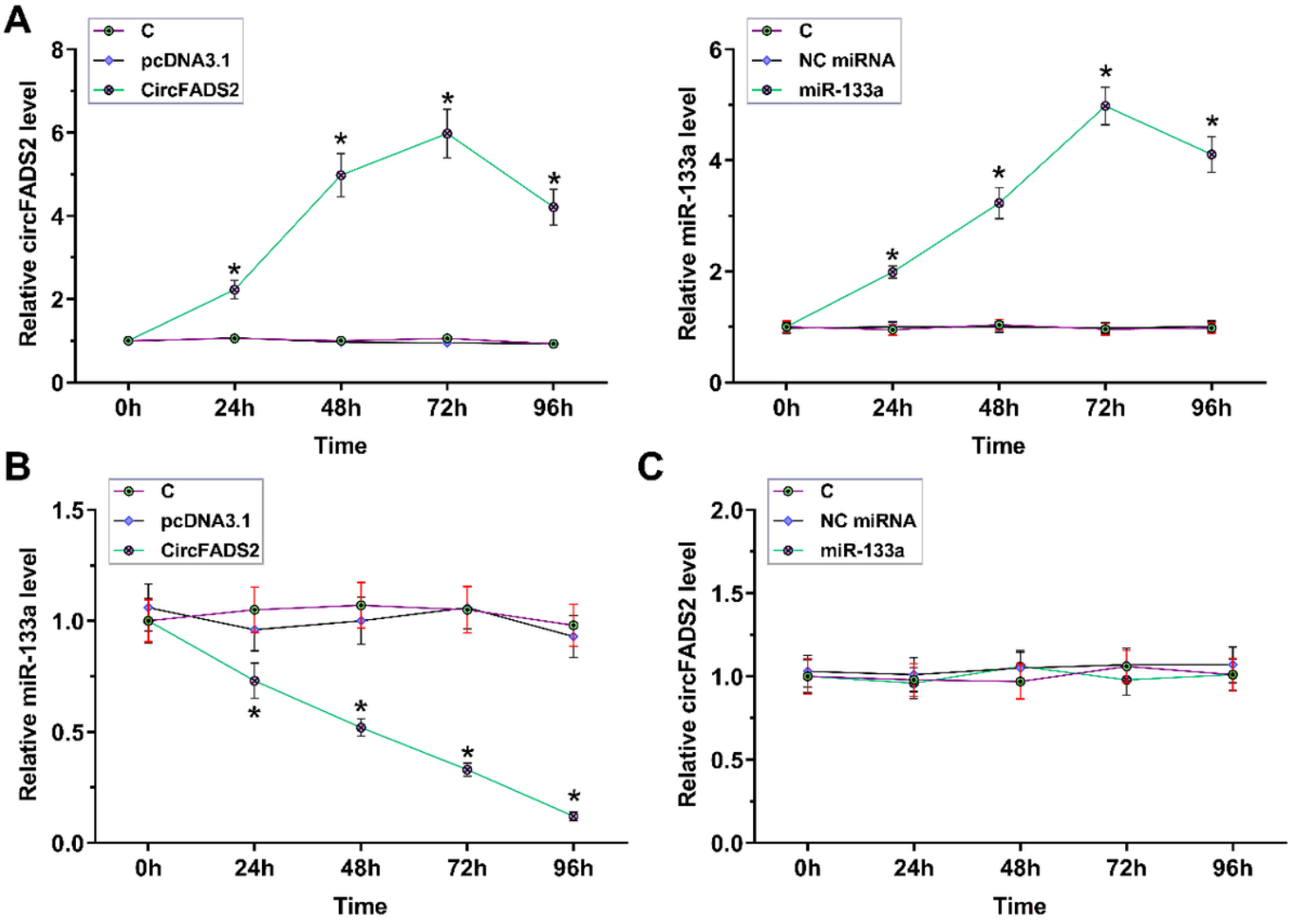

Figure 3

Overexpression of circFADS2 decreased the expression of miR-133a in HBEpCs Expression vector of circFADS2 and the mimic of miR-133a were transfected into HBEpCs (pre-treated with 12μg/ml LPS for 48h) to further explore the crosstalk between circFADS2 and miR-133a (A). The effects of circFADS2 overexpression on the expression of miR-133a (B) and the effects of miR-133a overexpression on the expression of circFADS2 (C) were also analyzed by RT-qPCR. *,p<0.05.

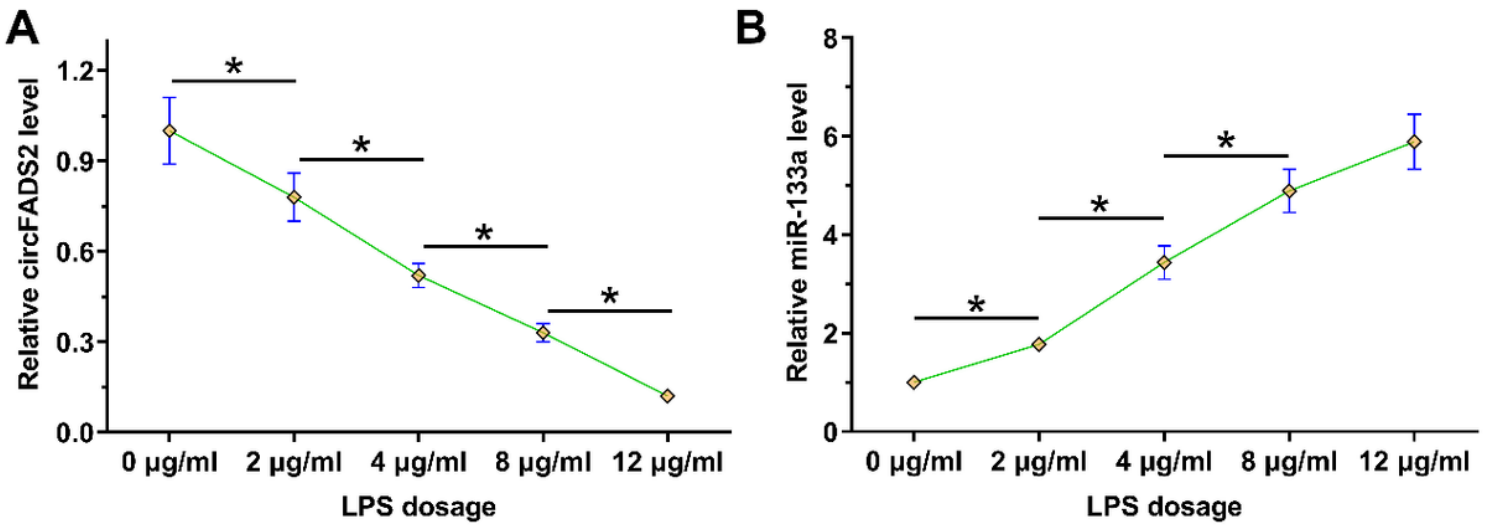

Figure 4

LPS treatment altered the expression of circFADS2 and miR-133a in HBEpCs LPS treatment was performed by incubating HBEpCs in medium containing 0, 2, 4, 8 and 12μg/ml LPS under the aforementioned conditions for 48h, followed by detecting the expression of circFADS2 (A) and miR-133a (B) through RT-qPCR. *,$p<0.05$.

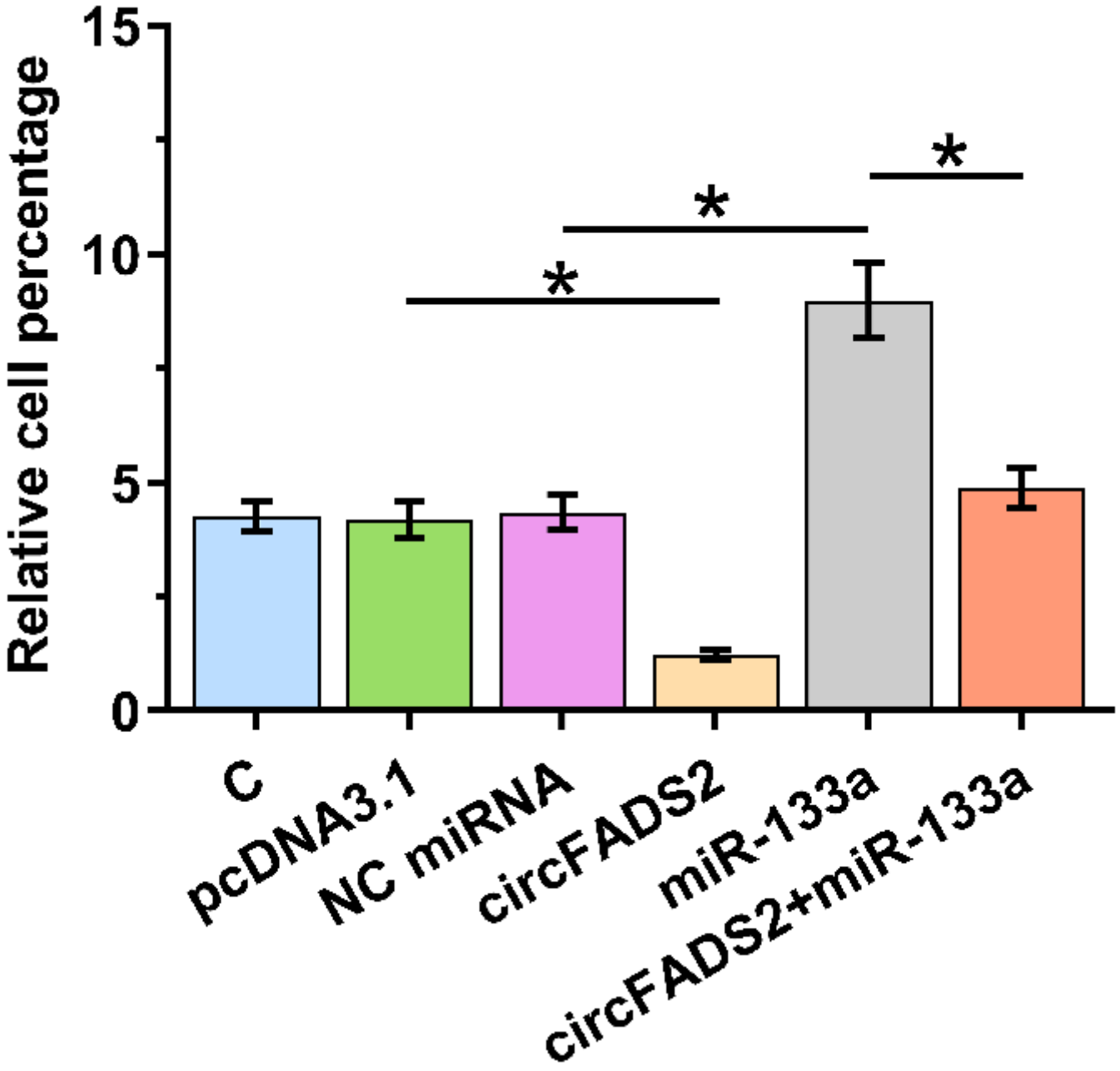

Figure 5

Overexpression of circFADS2 suppressed the apoptosis of HBEpCs induced by LPS through miR-133a The effects of the overexpression of circFADS2 and miR-133a on the apoptosis of HBEpCs induced by LPS were analyzed by cell apoptosis assay.*,$p<0.05$.