Research Square

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LncRNA CAIF Upregulates miR-16 Through Methylation to Suppress LPS-Induced Apoptosis of Cardiomyocytes

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Abstract

Background: Both lncRNA CAIF and miR-16 can inhibit LPS-induced inflammatory response. It is known that LPS plays crucial roles in sepsis. This study was therefore carried out to investigate the interactions between CAIF and miR-16 in sepsis.

Methods: Levels of CAIF (A) and miR-16 (B) in plasma samples from sepsis patients (n = 60) and healthy controls (n = 60) were measured by performing RT-qPCR. Correlations of levels of CAIF and miR-16 across plasma samples from sepsis patients (A) and healthy controls (B) were analyzed by linear regression.The effects of CAIF overexpression on miR-16 (B), and the effects of miR-16 overexpression on CAIF (C) were analyzed by RT-qPCR.

Results: We found that CAIF and miR-16 were downregulated in plasma of sepsis patients and they were positively correlated. In cardiomyocytes, LPS treatment led to downregulated CAIF and miR-16. Moreover, CAIF overexpression led to upregulated miR-16 and increased methylation of miR-16. However, miR-16 overexpression failed to significantly affect CAIF expression. Cell apoptosis analysis showed that CAIF and miR-16 overexpression suppressed LPS-induced apoptosis of cardiomyocytes. The combination of CAIF and miR-16 overexpression showed stronger effect.

Conclusion: CAIF may upregulate miR-16 through methylation to suppress LPS-induced apoptosis of cardiomyocytes.

1. Introduction

Upon infections, the human body will release chemicals into blood to fight ipe out invaders. However, body's responses to these chemical in some cases may be out of control, leading to the occurrence of sepsis [1, 2]. Sepsis causes the damages to multiple organs [3]. Without timely and proper treatment, organ damage caused by sepsis can be irreversible, leading to high mortality rate [4]. It has been estimated that more than 40% patients with severe sepsis will die of this disease [5]. Chronic heart failure (CHF) is usually complicated with sepsis [6]. In effect, sepsis is one of the major causes of death of patients with CHF [7]. At present, molecular mechanism of sepsis-induced CHF remains unclear, leading to difficulties in the development of novel treatment approaches.

It has been reported the development of sepsis and sepsis-induced organ failures requires the involvement of multiple molecular pathways [8]. In effect, understanding of the functionality of these molecular pathways provides novel insights to the development of targeted therapy, which aim to improve the conditions of sepsis by regulating sepsis-regulated gene expression [9, 10]. LPS-induced inflammatory responses play crucial role in sepsis [11]. It has been well established that many, non-coding RNAs (ncRNAs), such as miRNAs and long ncRNAs (> 200nt, lncRNAs) are involved in LPS-mediated inflammation [12], indicating their potential involvement in sepsis. Previous studies have showed that lncRNA CAIF and miR-16 could inhibit LPS-induced inflammatory response [13, 14]. This study was therefore carried out to investigate the interactions between CAIF and miR-16 in sepsis.

2. Materials And Methods

2.1 Study patients and plasma samples

A total of 60 sepsis patients (gender: 35 males and 25 females; age: 42 to 67 years, 53.6 ± 6.7 years) and 60 healthy controls (gender: 35 males and 25 females; age: 42 to 67 years, 53.6 ± 6.8 years) were enrolled at xx hospital between January 2017 and January 2019. Ethics Committee of this hospital approved this study. All sepsis patients were caused by bacterial infections. Sepsis patients complicated with other severe complications were excluded. Patients with initiated therapy were also excluded. All healthy controls received at health center of aforementioned hospital and all physiological functions of these controls were normal. All patients signed informed consent. Before therapy, all participants were fasted overnight and blood (5 ml) was extracted from the elbow vein of each participant. Blood was mixed with EDTA, followed by centrifugation for 10 min at 1200 g to separate plasma. Fresh plasma samples were stored in a liquid nitrogen tank.

2.2 Cell line and transient transfections

A human cardiomyocyte cell line AC16 (ATCC, USA) was used. Cell culture medium was composed of 12% FBS and 88% DMEM medium (1% penicillin and streptomycin). Cells were cultivated at 37 °C in a 5% CO_2 incubator with 95% humidity. Cells were harvested at 85% confluence to perform following experiments.

CAIF expression vector was constructed using pcDNA3.1 vector (Invitrogen) as backbone. Negative control (NC) miRNA and miR-16 mimic were purchased from Sigma-Aldrich (USA). AC16 cells were transfected with expression vector (10 nM) or 40 miRNA (40 nM) using lipofectamine 2000 (Invitrogen) according to manufacturer's instruction. Control (C) cells were untransfected cells. NC cells were NC miRNA- or empty vector-transfected cells. Subsequent experiments were performed 48 h later. It is worth noting that, to mimic the conditions of sepsis, cells were incubated with 10 μg/ml LPS for 48 h before transfections.

2.3 RNA preparations

AC16 cells were used to extract total RNA using Ribozol (Invitrogen). In cases of LPS treatment, AC16 cells were incubated with LPS (Sigma-Aldrich) at dosage of 0, 2, 5 and 10 μg/ml for 48 h before use. All RNA samples were subjected to DNase I (Invitrogen) digestion to completely remove genomic DNA.

2.4 RT-qPCR assay

QuantiNova Reverse Transcription Kit (Qiangen) was used to reverse transcribe total RNA samples into cDNA samples. With cDNA samples as template, QuantiFast SYBR Green PCR Kit (Qiangen) was used to perform qPCR reactions with 18S rRNA as internal control to measure the levels of CAIF expression.

To measure the levels of mature miR-16 expression, addition of poly (A), miRNA reverse transcriptions and qPCRs were performed using All-in-One™ miRNA qRT-PCR reagent kit (GeneCopoeia). Three replicates

were performed for each experiment and $2^{-\Delta\Delta CT}$ method was used to analyze data.

2.5 Methylation-specific PCR (MSP)

Monarch® Genomic DNA Purification Kit (NEB) was used to extract genomic DNA from AC16 cells. Genomic DNA samples were converted using DNA Methylation-GoldTM kit (ZYMO RESEARCH). After that MSP was performed using Taq 2X master mix (NEB) to analyze the methylation of miR-16 gene.

2.6 Cell apoptosis assay

AC16 cells harvested at 48 h post-transfection were subjected to cell apoptosis assay using FITC Annexin V Apoptosis Detection Kit with PI (BioLegend). Briefly, AC16 cells were incubated with LPS at a dosage of 10 μg/ml for 48 h and cold PBS was used to wash AC16 cells. Following that, AC16 cells were incubated with FITC-annexin V and PI for 20 min in dark. Finally, apoptotic cells (early apoptosis) were separated by performing flow cytometry.

Statistical analysis

Three replicates were included in each experiment and data were expressed as mean ± SD values. Unpaired t test was used to compare two groups. Comparisons among multiple groups were performed using ANOVA Tukey test. Correlations were analyzed by linear regression. $p < 0.05$ was statistically significant.

3. Results

3.1 CAIF and miR-16 were downregulated in plasma of sepsis patients

Levels of CAIF and miR-16 in plasma samples from sepsis patients (n = 60) and healthy controls (n = 60) were measured by performing RT-qPCR. Compared with control group, plasma levels of CAIF were significantly lower in sepsis patients (Fig. 1A, $p < 0.05$). Similarly, plasma levels of miR-16 were also significantly lower in sepsis patients in comparison to control group (Fig. 1B, $p < 0.05$).

3.2 LPS induced downregulation of CAIF and miR-16 in AC16 cells

AC16 cells were incubated with LPS (Sigma-Aldrich) at dosage of 0, 2, 5 and 10 μg/ml for 48 h, followed by the measurement of expression levels of CAIF and miR-16. It was observed that LPS treatment led to downregulated CAIF (Fig. 2A) and miR-16 (Fig. 2B) in a dose-dependent manner ($p < 0.05$). Therefore, CAIF and miR-16 may participate in sepsis in a dose-dependent manner.

3.3 Levels of CAIF and miR-16 were positively correlated across plasma samples from sepsis patients

Correlations of levels of CAIF and miR-16 across plasma samples from sepsis patients (n = 60) and healthy controls (n = 60) were analyzed by linear regression. It was observed that plasma levels of CAIF and miR-16 were significantly and positively correlated across plasma samples from sepsis patients (Fig. 3A). However, no significant correlation between plasma levels of CAIF and miR-16 was observed across plasma samples from healthy controls (Fig. 3B).

3.4 CAIF overexpression led to upregulated miR-16 through methylation

AC16 cells were transfected with CAIF expression vector or miR-16 mimic, and the overexpression of CAIF and miR-16 in AC16 cells was confirmed by performing RT-qPCR at 48 h post-transfection (Fig. 4A, $p < 0.05$). Compared with C and NC groups, CAIF overexpression led to upregulated miR-16 (Fig. 4B, $p < 0.05$), while miR-16 overexpression failed to significantly affect CAIF (Fig. 4C). MSP was performed to analyze the effects of CAIF overexpression on the methylation of miR-16 gene. Compared with cells transfected with empty pcDNA3.1 vector, cells transfected with CAIF expression vector showed significantly reduced methylation of miR-16 gene (Fig. 4D).

3.5 CAIF and miR-16 overexpression suppressed LPS-induced apoptosis of cardiomyocytes

After transfections, AC16 cells were incubated with LPS at a dosage of 10 μg/ml for 48 h, followed by the analysis of cell apoptosis. Compared with C and NC groups, CAIF and miR-16 overexpression suppressed LPS-induced apoptosis of cardiomyocytes. The combination of CAIF and miR-16 overexpression showed stronger effect (Fig. 5, $p < 0.05$).

Discussion

The interaction between CAIF and miR-16 was explored in this study. We found that CAIF and miR-16 were both downregulated in sepsis and CAIF may upregulate miR-16 through methylation to suppress LPS-induced apoptosis of cardiomyocyte.

The function of CAIF has been investigated in heart diseases [15, 16]. It was observed that CAIF was downregulated in end-stage cardiomyopathy and its expression levels were closely correlated with the poor survival of patients [15]. In addition, overexpression of CAIF can inhibit myocardial infarction and autophagy through the inactivation of p53-mediated myocardin transcription [16]. This study is the first to report the involvement of CAIF in sepsis. It is well known that heart failure is a common complication of sepsis [6]. In this study we showed that overexpression of CAIF suppressed the apoptosis of

cardiomyocytes induced by LPS. Therefore, CAIF may play protective role in sepsis by suppressing cell apoptosis.

It has been reported that miR-16 can induce the expression of CD40 to play anti-inflammatory role in LPS-induced myocarditis [14]. Consistently, our study showed that miR-16 played inhibitory effects on the apoptosis of cardiomyocyte-induced by LPS. LPS-induced inflammatory responses play crucial role in sepsis [11]. Therefore, miR-16 may also participate in sepsis. In this study we showed that miR-16 was downregulated in sepsis patients. Therefore, miR-16 may also play protective role in sepsis by suppressing cell apoptosis.

The key finding of the present study is that CAIF may upregulate miR-16 through methylation. However, the methylation-related factors that mediate the interaction between them remain to be further characterized. We observed that plasma levels of CAIF and miR-16 were positively correlated with each other only across plasma samples from sepsis patients but not the samples from healthy controls. Therefore, the interaction between CAIF and miR-16 may be mediated by certain pathological factors.

Conclusion

CAIF and miR-16 were both downregulated in sepsis. CAIF may upregulate miR-16 through methylation to suppress the apoptosis of cardiomyocyte induced by LPS.

Abbreviations

CHF: Chronic heart failure
ncRNAs: non-coding RNAs

Declarations

Acknowledgements

None

Funding statement

None

Ethics approval and consent to participate

Ethical approval was obtained from the Ethics Committee of the Shanxi Provincial People's Hospital (NO.X1520113). All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Availability of data and material

The datasets used and/or 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.

Authors' contributions

YW: manuscript writing and study design; YZ literature search and data analysis and statistical analysis. All authors read and approved the final manuscript.

References

[1] Singer M, Deutschman C S, Seymour C W, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA. 2016, 315(8): 801-810.

[2] Hotchkiss R S, Moldawer L L, Opal S M, et al. Sepsis and septic shock. Nat Rev Dis Primers. 2016, 2(1): 1-21.

[3] Churpek M M, Snyder A, Han X, et al. Quick sepsis-related organ failure assessment, systemic inflammatory response syndrome, and early warning scores for detecting clinical deterioration in infected patients outside the intensive care unit. Am J Respir Crit Care Med. 2017, 195(7): 906-911.

[4] Lelubre C, Vincent J L. Mechanisms and treatment of organ failure in sepsis. Nat Rev Nephrol. 2018, 14(7): 417-427.

[5] Seymour C W, Gesten F, Prescott H C, et al. Time to treatment and mortality during mandated emergency care for sepsis. N Engl J Med. 2017, 376(23): 2235-2244.

[6] Arfaras-Melainis A, Polyzogopoulou E, Triposkiadis F, et al. Heart failure and sepsis: practical recommendations for the optimal management. Heart Fail Rev. 2019: 1-12.

[7] Liu Y C, Yu M M, Shou S T, et al. Sepsis-induced cardiomyopathy: mechanisms and treatments. Front Immunol. 2017, 8: 1021.

[8] Pool R, Gomez H, Kellum J A. Mechanisms of organ dysfunction in sepsis. Crit Care Clin. 2018, 34(1): 63-80.

[9] Doyle J F, Forni L G. Update on sepsis-associated acute kidney injury: emerging targeted therapies. Biologics. 2016, 10: 149-156.

[10] Sun Y, Yao X, Zhang Q J, et al. Beclin-1-dependent autophagy protects the heart during sepsis. Circulation. 2018, 138(20): 2247-2262.

[11] Hung Y L, Fang S H, Wang S C, et al. Corylin protects LPS-induced sepsis and attenuates LPS-induced inflammatory response. Sci Rep. 2017, 7: 46299.

[12] Mandal S S, Obaid M. Long noncoding RNAs in immune response and inflammation. FASEB J. 2019, 33(1_supplement): 778.5-778.5.

[13] Qi K, Lin R, Xue C, et al. Long Non-Coding RNA (LncRNA) CAIF is Downregulated in Osteoarthritis and Inhibits LPS-Induced Interleukin 6 (IL-6) Upregulation by Downregulation of MiR-1246. Med Sci Monit. 2019, 25: 8019-8024.

[14] Li Q Q, Xi J, Li B Q, et al. MiR-16, as a potential NF-Kb-related miRNA, exerts anti-inflammatory effects on LPS-induced myocarditis via mediating CD40 expression: A preliminary study. J Biochem Mol Toxicol. 2019: e22426.

[15] Wu D, Zhou Y, Fan Y, et al. LncRNA CAIF was downregulated in end-stage cardiomyopathy and is a promising diagnostic and prognostic marker for this disease. Biomarkers. 2019, 24(8): 735-738.

[16] Liu C Y, Zhang Y H, Li R B, et al. LncRNA CAIF inhibits autophagy and attenuates myocardial infarction by blocking p53-mediated myocardin transcription. Nat Commun. 2018, 9(1): 1-12.

Figures

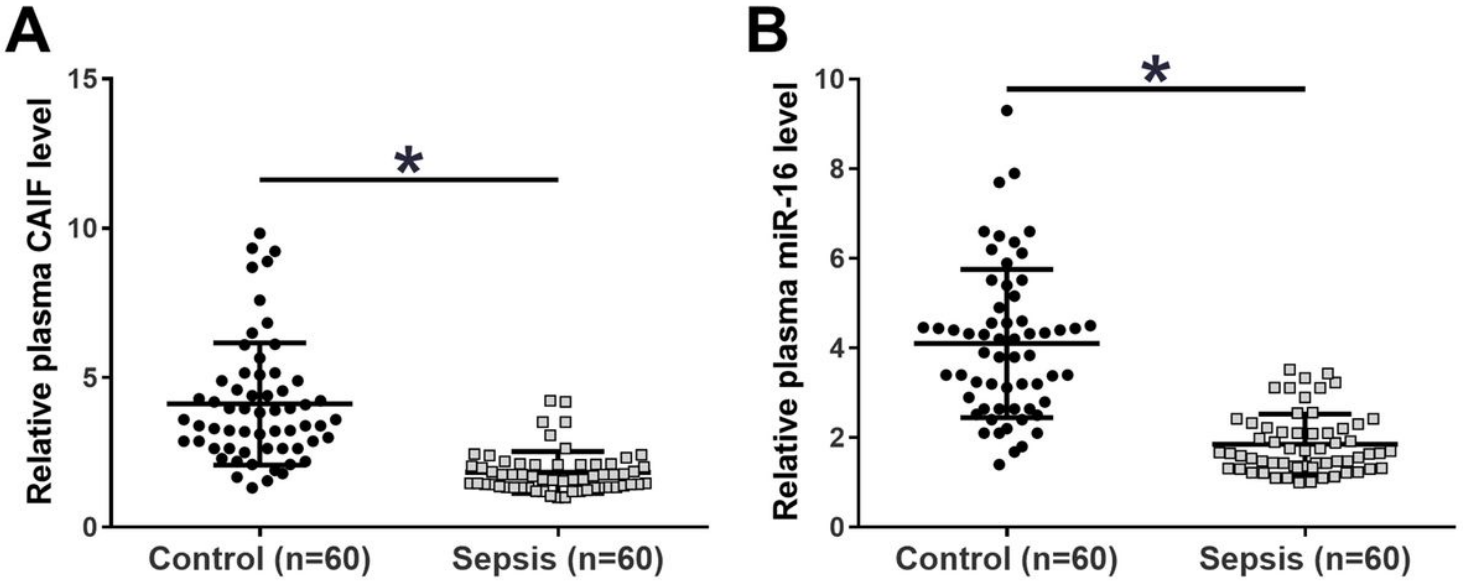

Figure 1

CAIF and miR-16 were downregulated in plasma of sepsis patients Levels of CAIF (A) and miR-16 (B) in plasma samples from sepsis patients (n=60) and healthy controls (n=60) were measured by performing RT-qPCR. PCR reactions were repeated 3 times and mean values were presented and compared.*,p<0.05.

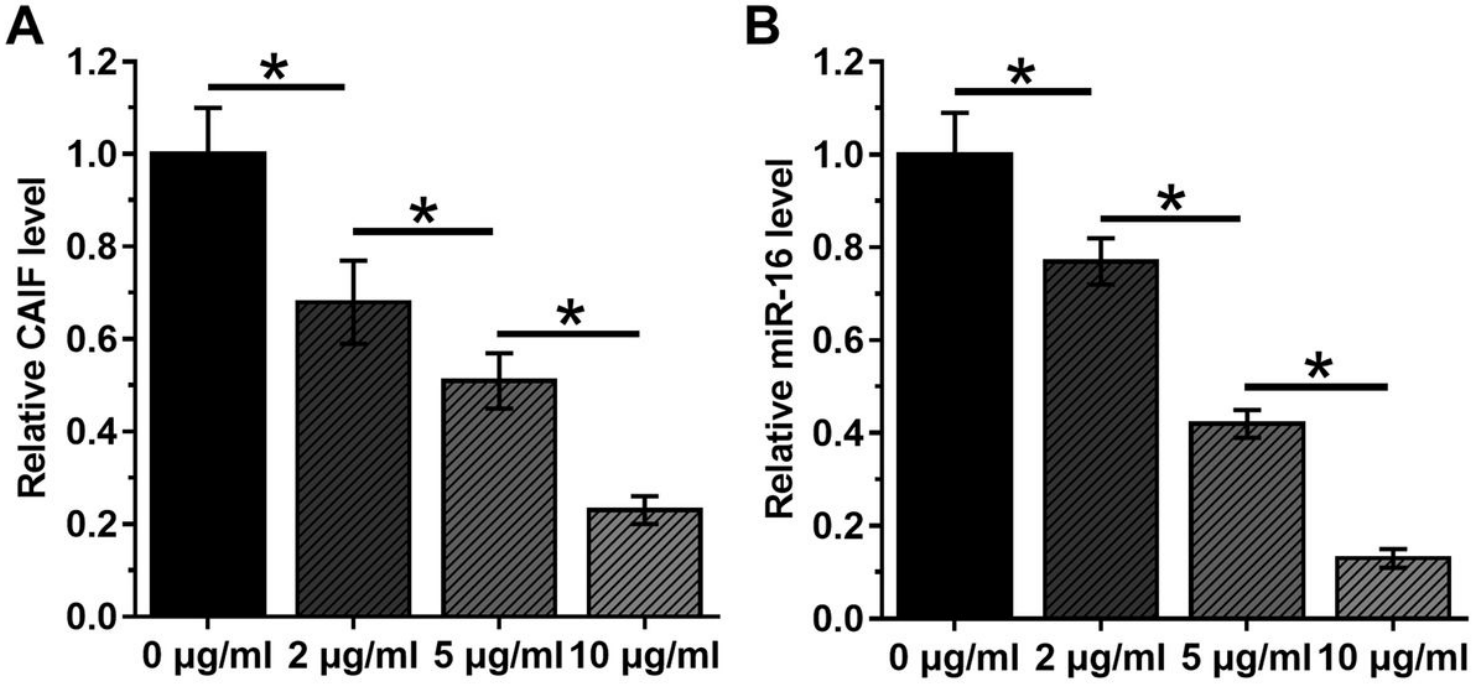

Figure 2

LPS induced downregulation of CAIF and miR-16 in AC16 cells AC16 cells were incubated with LPS (Sigma-Aldrich) at dosage of 0, 2, 5 and 10μg/ml for 48h, followed by the measurement of expression levels of CAIF (A) and miR-16 (B). Experiments were repeated 3 times and mean±SD values were compared.*,p<0.05.

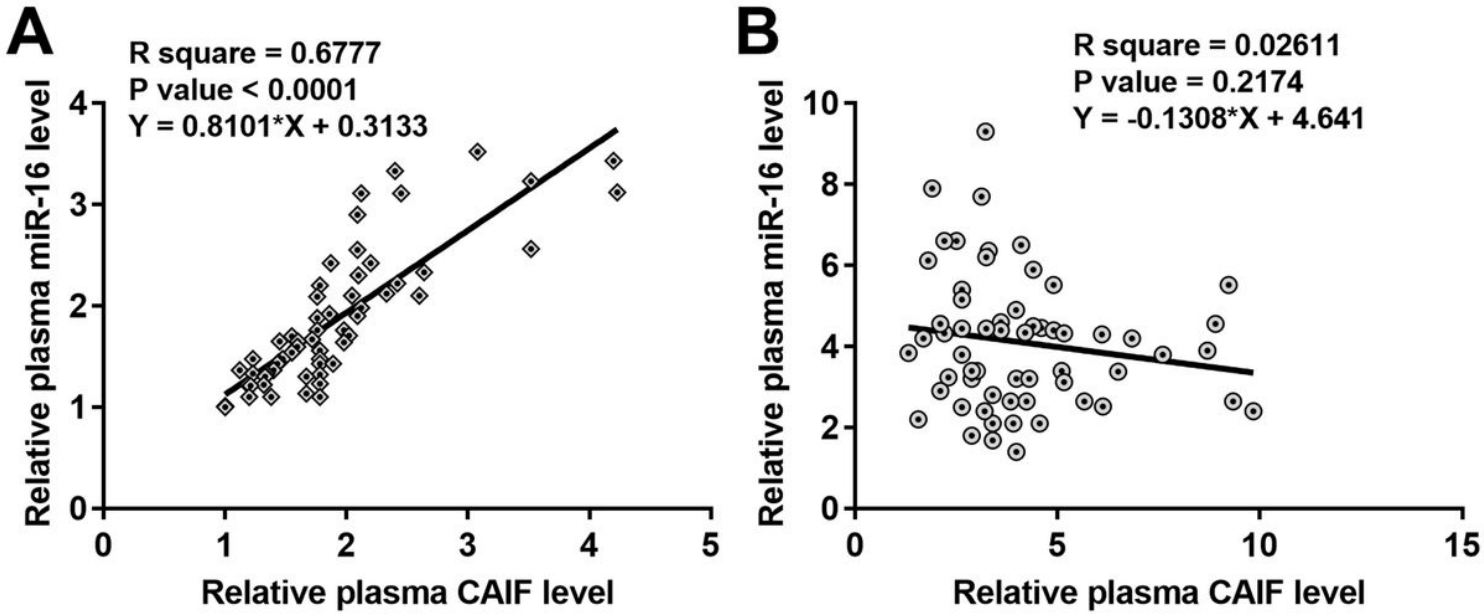

Figure 3

Levels of CAIF and miR-16 were positively correlated across plasma samples from sepsis patients Correlations of levels of CAIF and miR-16 across plasma samples from sepsis patients (A) and healthy controls (B) were analyzed by linear regression.

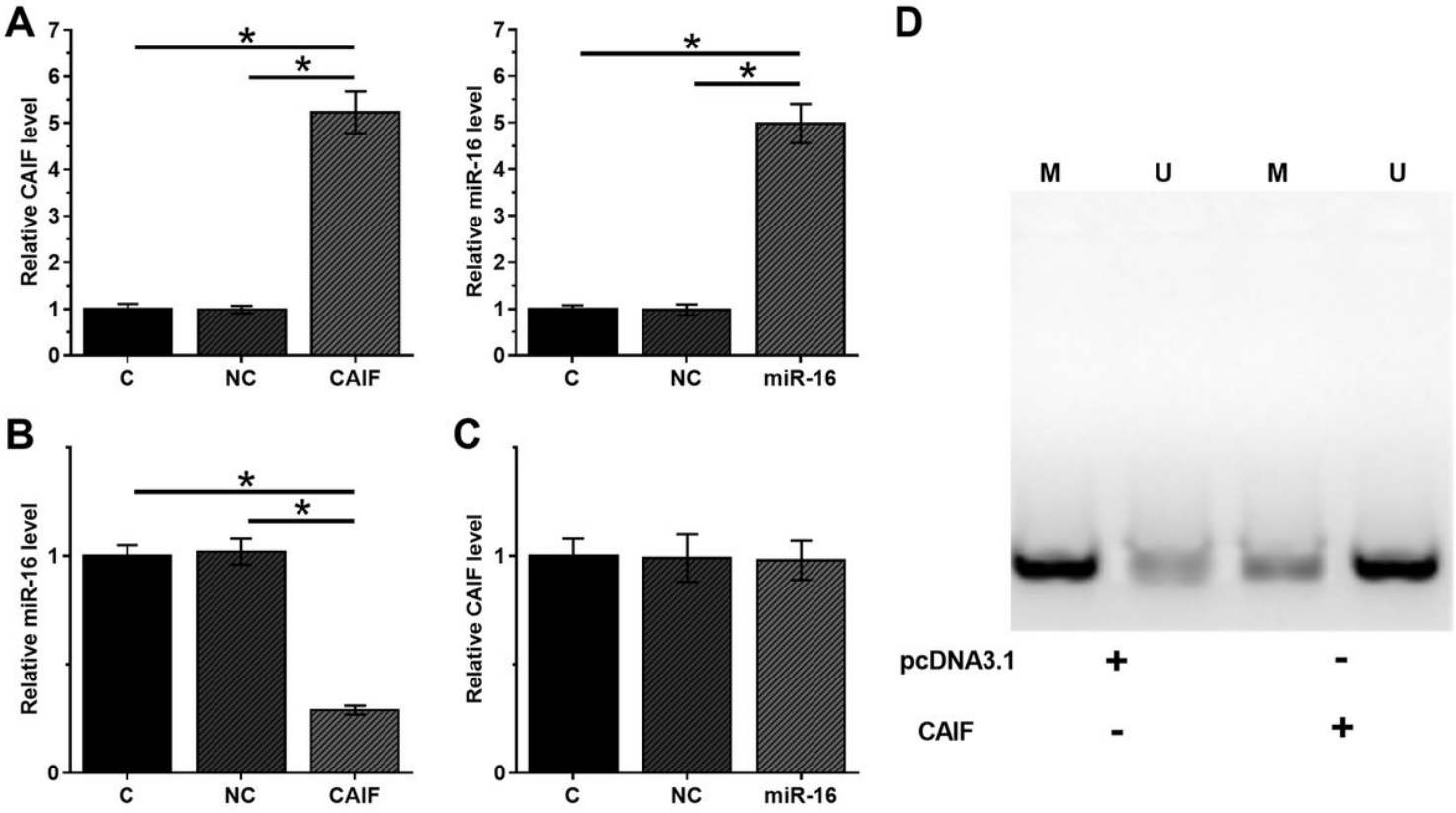

Figure 4

CAIF overexpression led to upregulated miR-16 through methylation AC16 cells were transfected with CAIF expression vector or miR-16 mimic, and the overexpression of CAIF and miR-16 in AC16 cells was confirmed by performing RT-qPCR at 48h post-transfection (A). The effects of CAIF overexpression on miR-16 (B), and the effects of miR-16 overexpression on CAIF (C) were analyzed by RT-qPCR. MSP was performed to analyze the effects of CAIF overexpression on the methylation of miR-16 gene (D). Experiments were repeated 3 times and mean±SD values were compared.*,$p<0.05$.

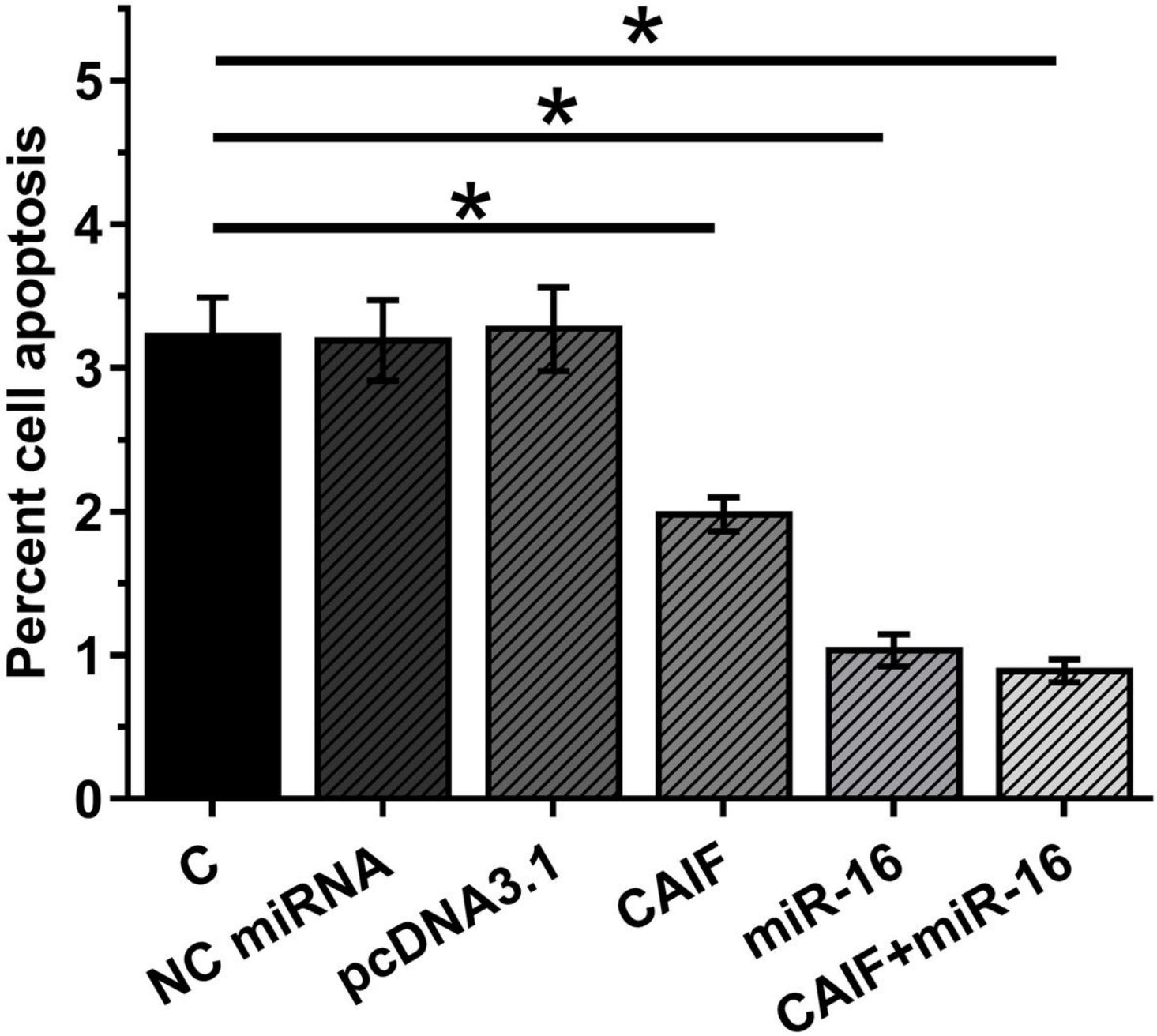

Figure 5

CAIF and miR-16 overexpression suppressed LPS-induced apoptosis of cardiomyocytes After transfections, AC16 cells were incubated with LPS at a dosage of 10 μg/ml for 48h, followed by the analysis of cell apoptosis. Experiments were repeated 3 times and mean±SD values were compared.*,$p<0.05$.