Circular RNA circFADS2 is Overexpressed in Sepsis and Suppresses LPS-Induced Lung Cell Apoptosis by Inhibiting the Maturation of miR-15a-5p

Xiaoyang Hong  
Pediatric Intensive Care Unit, The Seventh Medical Center, PLA General Hospital, Beijing, 100700, PR

Shuanglei Li  
Department of Cardiovascular Surgery, PLA General Hospital, Beijing, 100853, PR.

Jie Wang  
Surgical Pediatric Intensive Care Unit, Children's Hospital Affiliated of Zhengzhou University, Zhengzhou City, Henan Province, 450018, PR

Zhe Zhao  
Pediatric Intensive Care Unit, The Seventh Medical Center, PLA General Hospital, Beijing, 100700, PR

Zhichun Feng (ZhichunFengBeijing@163.com)  
Pediatric Intensive Care Unit, The Seventh Medical Center, PLA General Hospital, Beijing

Research

Keywords: sepsis, circFADS2, miR-15a-5p, precursor, apoptosis

DOI: https://doi.org/10.21203/rs.3.rs-76069/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Circular RNA circFADS2 plays protective roles in LPS-induced inflammation, which promotes sepsis, suggesting its involvement of circFADS2 in sepsis. We then analysis the involvement of circFADS2 in LOS Expression of circFADS2, mature miR-15a-5p and miR-15a-5p precursor in plasma samples (collected at two time points) from sepsis patients (n=60) and healthy controls (n=60) was determined by RT-qPCR. The expression vector of circFADS2 was transfected in lung cells, followed by the measurement of the expression levels of mature miR-15a-5p and miR-15a-5p precursor to study the role of circFADS2 in the maturation of miR-15a-5p. Cell apoptosis was analyzed by cell apoptosis assay.

CircFADS2 was upregulated in sepsis and inversely correlated with mature miR-15a-5p, but not miR-15a-5p precursor. In lung cells, overexpression of circFADS2 decreased the levels of mature miR-15a-5p expression, but not miR-15a-5p precursor. LPS treatment decreased the expression levels of miR-15a-5p, and increased the expression levels of circFADS2. Cell apoptosis analysis showed that circFADS2 overexpression reduced the enhancing effects of miR-15a-5p overexpression on the apoptosis of lung cells induced by LPS.

Therefore, circFADS2 is upregulated in sepsis and suppresses LPS-induced lung cell apoptosis by inhibiting the maturation of miR-15a-5p.

Introduction

Sepsis refers to a severe clinical condition caused by the excessive body's responses to bacterial, viral and fungal infections [1]. Other than ICU treatment, there are no effective approaches for severe sepsis of septic shock [2]. As a consequence, sepsis is correlated with unacceptably high mortality rate [3]. It is estimated that 1 out 3 deaths in hospital is at least partially caused by sepsis [4]. Sepsis-caused inflammation and cell injuries bring damages to almost all important organs, such as lung [5, 6]. Acute lung injury or respiratory failure is a devastating complication of sepsis and causes either deaths or disabilities even after active treatment [6]. Therefore, the prevention and treatment of lung injury are critical for the recovery of sepsis patients.

Sepsis is essential a type of inflammatory disease, in which the involvement of multiple players is required [7, 8]. With the increased elucidation of the molecular mechanism of sepsis, some molecular factors, such as ALK, have been proven to be potential targets for the development of novel therapies against sepsis and sepsis-induced organ failures, such as targeted therapy to treat sepsis by regulating regulated gene expression [9, 10]. However, to date, effective targets for sepsis targeted therapy remain lacking. Circular RNAs, or circRNAs, are covalently closed single-strand RNA transcripts that participate in human diseases including sepsis may by regulating gene expression [11, 12], suggesting that they are potential targets for sepsis-targeted therapy. However, the function of most circRNAs in sepsis has not been elucidated. CircRNA circFADS2 in a recent study was proven to play protective role in LPS-induced cell apoptosis, which contributes to sepsis [14]. Our preliminary RNA-seq analysis revealed the altered the
expression of circFADS2 as well as inverse correlation with miR-15a-5p, which may promote pulmonary diseases [15]. This study was therefore performed to explore the role circFADS2 in sepsis and its interaction with miR-15a-5p, with a focus on sepsis-induced lung injury.

Materials And Methods

Blood extraction

This study enrolled 50 sepsis patients (28 males and 22 females) and 50 healthy controls (28 males and 22 female) at The Seventh Medical Center, PLA General Hospital between May 2018 and May 2020. Age range of sepsis patients and healthy controls were both 40–68 years, with a median age of 54 years. Therefore, these two groups of participates have similar age and gender distributions. Sepsis patients were excluded from other severe clinical disorders and any therapy initiated therapy within 3 months prior to admission. All sepsis patients were caused by bacterial infections. Healthy controls showed normal physiological functions in systemic physiological exams. The aforementioned hospital Ethics Committee approved this study. Informed consent was signed by all participants. Patients were treated with systemic antibiotics. Blood (3 ml) was extracted under fasting conditions from patients and controls on day 1 and day 8 (after treatment for 1 week) after admission.

Plasma preparation and human bronchial epithelial cells (HBEpCs)

Blood samples were mixed with citric acid to a ratio of 10:1. The mixture was transferred to centrifuge tubes and plasma samples were separated by centrifugation at 1300 g for 15 min at room temperature.

HBEpCs from Sigma-Aldrich (USA) were treated with LPS to serve as the cell model of sepsis. Bronchial Epithelial Cell Medium (Sigma-Aldrich) was used to cultivate cells at 37 °C, 95% humidity and 5% CO₂. To perform LPS treatment, HBEpCs were cultivated in medium supplemented with 0, 1, 2, 4, 8 and 10 µg/ml LPS (Sigma-Aldrich) for further 48 h prior to the subsequent assays.

Vector, miRNA and transfections

Expression vector of circFADS2 was constructed with pcDNA3.1(+) CircRNA Mini Vector (Addgene) as backbone. Mimic of miR-15a-5p and miRNA negative control (NC) were the products of Sigma-Aldrich. HBEpCs were subjected to transfections with either 1 µg circFADS2 expression vector or 45 nM miR-15a-5p mimic using lipofectamine 2000 (Invitrogen). NC experiments were performed by transfecting empty vector or miRNA NC into HBEpCs, and untransfected cells were included as control (C) cells. Fresh medium was used to cultivate HBEpCs for further 48 h prior to the subsequent assays.
**RNA samples**

Plasma samples and HBEpCs were subjected to RNA isolation using Trizol (Invitrogen). At 37 °C, RNA samples were digested for 2 h with DNase I (Invitrogen) to remove genomic DNA. RNA integrity was analyzed by electrophoresis (5% urea-PAGE gel). OD 260/280 ratios were determined and RNA samples with an OD 260/280 ratio close to 2.0 (pure RNA) were used in the subsequent experiments.

**RT-qPCR analysis**

The preparation of cDNA samples was performed using SS-RT-III system (Invitrogen). All-in-One qPCR Mix (BioCat GmbH) was used to perform all qPCRs with GAPDH as internal control to analyze the expression of circFADS2.

The expression of miR-15a-5p precursor was analyzed by All-in-One™ miRNA qRT-PCR reagent kit (GeneCopoeia) and sequence-specific primers were used in both reverse transcriptions (RTs) and qPCRs. The same kit was used to analyze the expression of mature miR-15a-5p through following steps: 1) addition of poly (A); 2) RTs with poly (T) as reverse primer; 3) qPCRs with poly (T) as reverse primer. The internal control was U6.

Each PCR was performed in three technical replicates, and $2^{-\Delta\Delta CT}$ method was used for the normalization of Ct values to corresponding controls.

**Cell apoptosis analysis**

The apoptosis of HBEpCs was analyzed at 48 h after cell transfection. In brief, 2 ml medium containing 20,000 cells were transferred to each well of 6-well plate. After the addition of 10 µg/ml LPS, cells were cultivated at 37 °C for further 48 h, followed by washing with pre-cold PBS. Cells were resuspended in binding protein, following by staining with PI and FITC-annexin V (Sigma-Aldrich) for 15 min. Cell apoptosis was then analyzed by flow cytometry.

**Statistical analysis**

Heml 1.0 software was used to plot heatmaps to express the expression of circFADS2 and miR-15a-5p in plasma samples from patients to analyze their expression during treatment. Unpaired test was used to compare the expression of circFADS2 and miR-15a-5p between patients and controls. Mean ± SD values were used to express the data of in vitro cell experiments and ANOVA Tukey’s test was used for data comparisons. Correlations were analyzed by Pearson’s correlation coefficient. P < 0.05 was deemed statistically significant.

**Results**
Altered expression of circFADS2 and miR-15a-5p in sepsis is likely induced by LPS

Expression of circFADS2, mature miR-15a-5p and miR-15a-5p precursor in plasma samples from sepsis patients (n = 50) and healthy controls (n = 50) collected prior to treatment was analyzed by RT-qPCR. Compared to controls, circFADS2 was significantly upregulated in sepsis patients, while mature miR-15a-5p and miR-15a-5p precursor were significantly downregulated in sepsis patients (Fig. 1A, p < 0.01). To test whether the altered expression was cases by LPS, HBEpCs were cultivated in medium supplemented with 0, 1, 2, 4, 8 and 10 µg/ml LPS (Sigma-Aldrich) for further 48 h, followed by RT-qPCR to determine gene expression. It was observed that LPS treatment increased the expression of circFADS2, and increased the expression of mature miR-15a-5p and miR-15a-5p precursor in a dose-dependent manner (Fig. 2B, p < 0.05).

Systemic antibiotics treatment regulated the expression of circFADS2 and miR-15a-5p

Expression of circFADS2, mature miR-15a-5p and miR-15a-5p precursor in plasma samples of sepsis patients was also determined after the treatment of systemic antibiotics for 1 week (day 8). Heml 1.0 software was used to plot heatmaps to express the expression of circFADS2 and miR-15a-5p in plasma samples from patients to analyze their expression during treatment. It was observed that treatment downregulated the expression of circFADS2 (Fig. 2A), and increased the expression of mature miR-15a-5p (Fig. 2B) and miR-15a-5p precursor (Fig. 2B) in plasma samples of sepsis patients.

CircFADS2 overexpression suppressed the maturation of miR-15a-5p in HBEpCs

Pearson's correlation coefficient analysis was performed to analyze the correlation between expression levels of CircFADS2 and miR-15a-5p across plasma samples from sepsis patients (day 1). It was observed that circFADS2 was inversely correlated with mature miR-15a-5p (Fig. 3A), but not miR-15a-5p precursor (Fig. 3B). The expression vector of circFADS2 was transfected in lung cells. Overexpression of circFADS2 was determined every 24 h until 96 h. It was observed that overexpression of circFADS2 was achieved from 48 h to 96 h (Fig. 3C, p < 0.05). The measurement of the expression levels of mature miR-15a-5p and miR-15a-5p precursor was then performed to study the role of circFADS2 in the maturation of miR-15a-5p. It was observed that circFADS2 overexpression decreased the expression of mature miR-15a-5p, but not miR-15a-5p precursor in HBEpCs (Fig. 3C, p < 0.05).

Overexpression of circFADS2 suppressed LPS-induced apoptosis of HBEpCs through miR-15a-5p

The role of circFADS2 and miR-15a-5p in regulating the apoptosis of HBEpCs after the treatment of 10 µg/ml LPS for 48 h was analyzed by cell apoptosis assay. CircFADS2 overexpression decreased cell apoptosis, while miR-15a-5p increased cell apoptosis. In addition, circFADS2 overexpression reduced the enhancing effects of miR-15a-5p overexpression on cell apoptosis (Fig. 4, p < 0.05).

Discussion
This study explored the involvement of circFADS2 in sepsis and studied its potential interactions with miR-15a-5p. We found that circFADS2 was overexpressed in sepsis and it may suppress the maturation of miR-15a-5p to suppress the apoptosis of HBEpCs induced by LPS.

The functionality of circFADS2 has been characterized in cancer biology [16, 17]. It was observed that circFADS2 was overexpressed in lung cancer and could sponge miR-498 to promote the proliferation and invasion of cancer cells [16]. In another study, circFADS2 was reported to be upregulated in colorectal cancer and predicted the poor survival of patients [17]. Besides that, circFADS2 plays protective roles in LPS-treated chondrocytes by interacting with miR-498/mTOR axis [13]. In this study we first showed the upregulation of circFADS2 in sepsis patients. In addition, LPS treatment increased the expression of circFADS2 in HBEpCs. Therefore, the upregulation of HBEpCs in sepsis patients is likely induced by LPS. Interestingly, we showed that circFADS2 suppressed the apoptosis of HBEpCs induced by LPS. Therefore, LPS-inducible circFADS2 attenuated the enhancing effects of LPS on cell apoptosis, suggesting the feedback regulation between circFADS2 and LPS. Totally, our data showed the protective role of circFADS2 in sepsis.

MiR-15a-5p induces the apoptosis of pulmonary artery smooth muscle cells by interacting with VEGF/p38/MMP-2 signaling pathway [15]. In this study, we showed that miR-15a-5p was downregulated in sepsis and the downregulation is likely induced by LPS. Moreover, overexpression of miR-15a-5p increased the apoptosis of HBEpCs induced by LPS, suggesting that miR-15a-5p also participate in sepsis-induce lung injury.

It has been well established that the function of circRNA in human diseases is to regulate gene expression [11, 12]. Besides that, IncRNAs may also sponge miRNAs to suppress their functions. Consistently, circFADS2 can sponge miR-498 [13]. Interestingly, our data showed that circFADS2 could suppress the maturation of miR-15a-5p. However, the mechanism is unclear. Our future studies will focus on the role of circFADS2 in the transportation of miR-15a-5p precursor from nucleus to cytoplasm, which is required for the maturation of miR-15a-5p.

In conclusion, circFADS2 is overexpressed in sepsis and it may suppress the maturation of miR-15a-5p to suppress the apoptosis of HBEpCs induced by LPS.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Availability of data and materials**
The data that support the findings of this study are available on request from the corresponding author: Zhichun Feng*, Pediatric Intensive Care Unit, The Seventh Medical Center, PLA General Hospital, No. 5 Nanmencang, Dongshitiao, Dongcheng District, Beijing, 100700, PR. China. Email address: ZhichunFengBeijing@163.com. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

Competing interests

The authors declare that they have no competing interests.

Funding

No funding.

Authors' contributions

Xiaoyang Hong and Shuanglei Li designed and carried out the study. Xiaoyang Hong, Shuanglei Li, Jie Wang, Zhe Zhao and Zhichun Feng participated in experiments and statistical analysis. Xiaoyang Hong and Shuanglei Li wrote the manuscript. Zhichun Feng revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No. 81400309) and Capital's Funds for Health Improvement and Research (Grant No. 2020-2-5093).

References

1. Hotchkiss R S, Moldawer L L, Opal S M, et al. Sepsis and septic shock. Nat Rev Dis Primers. 2016, 30;2:16045.
2. Prescott H C, Angus D C. Enhancing recovery from sepsis: a review. JAMA. 2018, 319(1): 62-75.
3. Ames S G, Davis B S, Angus D C, et al. Hospital variation in risk-adjusted pediatric sepsis mortality. Pediatr Crit Care Med. 2018, 19(5): 390-396.
4. 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.
5. Bauer M, Coldewey S M, Leitner M, et al. Deterioration of organ function as a hallmark in sepsis: the cellular perspective. Front Immunol. 2018, 9: 1460.
6. Petronilho F, Florentino D, Danielski L G, et al. Alpha-lipoic acid attenuates oxidative damage in organs after sepsis. Inflammation. 2016, 39(1): 357-365.

7. Bosmann M, Ward P A. The inflammatory response in sepsis. Trends Immunol. 2013, 34(3): 129-136.

8. Kaukonen K M, Bailey M, Pilcher D, et al. Systemic inflammatory response syndrome criteria in defining severe sepsis. N Engl J Med. 2015, 372(17): 1629-1638.

9. Li R, Guo C, Li Y, et al. Therapeutic targets and signaling mechanisms of vitamin C activity against sepsis: a bioinformatics study. Brief Bioinform. 2020, 11;bbaa079.

10. Zeng L, Kang R, Zhu S, et al. ALK is a therapeutic target for lethal sepsis. Sci Transl Med. 2017, 18;9(412):eaan5689.

11. Beltrán-García J, Osca-Verdegal R, Nacher-Sendra E, et al. Circular RNAs in Sepsis: Biogenesis, Function, and Clinical Significance. Cells. 2020, 9(6): 1544.

12. Haque S, Harries L W. Circular RNAs (circRNAs) in health and disease. Genes (Basel). 2017, 8(12): 353.

13. Li G, Tan W, Fang Y, et al. circFADS2 protects LPS-treated chondrocytes from apoptosis acting as an interceptor of miR-498/mTOR cross-talking. Aging (Albany NY), 2019, 11(10): 3348-3361.

14. 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.

15. Zhang W, Li Y, Xi X, et al. MicroRNA-15a-5p induces pulmonary artery smooth muscle cell apoptosis in a pulmonary arterial hypertension model via the VEGF/p38/MMP-2 signaling pathway. Int J Mol Med. 2020, 45(2): 461-474.

16. Zhao F, Han Y, Liu Z, et al. circFADS2 regulates lung cancer cells proliferation and invasion via acting as a sponge of miR-498. Biosci Rep. 2018, 31;38(4):BSR20180570.

17. Xiao Y S, Tong H Z, Yuan X H, et al. CircFADS2: A potential prognostic biomarker of colorectal cancer. Exp Biol Med (Maywood). 2020: 1535370220929965.

**Figures**
Altered expression of circFADS2 and miR-15a-5p in sepsis is likely induced by LPS. Expression of circFADS2, mature miR-15a-5p and miR-15a-5p precursor in plasma samples from sepsis patients (n=50) and healthy controls (n=50) collected prior to treatment was analyzed by RT-qPCR (A), \( **p<0.01 \). To test whether the altered expression was caused by LPS, HBEpCs were cultivated in medium supplemented with 0, 1, 2, 4, 8 and 10 μg/ml LPS (Sigma-Aldrich) for further 48h, followed by RT-qPCR to determine gene expression. (C), *, \( p<0.05 \).

Figure 1
Systemic antibiotics treatment regulated the expression of circFADS2 and miR-15a-5p. Expression of circFADS2, mature miR-15a-5p and miR-15a-5p precursor in plasma samples of sepsis patients was also determined after the treatment of systemic antibiotics for 1 week (day 8). Heml 1.0 software was used to plot heatmaps to express the expression of circFADS2 (A), mature miR-15a-5p (B) and miR-15a-5p precursor (C) in plasma samples from patients to analyze their expression during treatment.
Figure 3

CircFADS2 overexpression suppressed the maturation of miR-15a-5p in HBEpCs Pearson’s correlation coefficient analysis was performed to analyze the correlation between expression levels of CircFADS2 and mature miR-15a-5p (A) or miR-15a-5p precursor (B) across plasma samples from sepsis patients (day 1). The expression vector of circFADS2 was transfected in lung cells. Overexpression of circFADS2 was determined every 24h until 96h (C). The measurement of the expression levels of mature miR-15a-5p and miR-15a-5p precursor was then performed to study the role of circFADS2 in the maturation of miR-15a-5p (D). *, compared to C or NC group, p<0.05.

Figure 4

Overexpression of circFADS2 suppressed LPS-induced apoptosis of HBEpCs through miR-15a-5p The role of circFADS2 and miR-15a-5p in regulating the apoptosis of HBEpCs after the treatment of 10 µg/ml LPS for 48h was analyzed by cell apoptosis assay. *, p<0.05.