BMC Immunology

RESEARCH

Open Access

LncRNA CYP1B1-AS1 as a clinical biomarker exacerbates sepsis inflammatory response via targeting miR- 18a- 5p

Lixia Xu[1], Jingpo Li[2], Li Li[1], Qiushuang Zhang[3], Qiuju Feng[4] and Lijie Bai[5*]

Abstract

Background Sepsis, characterized by high morbidity and mortality, necessitates the identification of novel diagnostic and prognostic biomarkers to enhance patient outcomes. Prior research has highlighted the potential clinical utility of long non-coding RNAs (lncRNAs) in sepsis. This study aimed to investigate the clinical significance and underlying mechanisms of serum lncRNA Cytochrome P450 family 1 subfamily B member 1 antisense RNA 1 (CYP1B1-AS1) expression in sepsis.

Methods Differentially expressed lncRNAs in sepsis patients were explored via GEO database. Sepsis patients and Control subjects were included. An in vitro cellular model was established with LPS-stimulated THP- 1 cells. RT-qPCR assessed CYP1B1-AS1 and miR- 18a- 5p expression. ROC analysis evaluated diagnostic and predictive value. Kaplan-Meier curves and Cox regression analyzed the prognostic value of CYP1B1-AS1. Flow cytometry and ELISA assessed cell apoptosis and inflammatory factors levels. Dual luciferase reporter, RIP, and RNA pull down to validate target binding relationship.

Results The GSE217700 database shows that CYP1B1-AS1 was upregulated in sepsis. Serum levels of CYP1B1-AS1 were higher in sepsis patients than controls. CYP1B1-AS1 was positively correlated with SOFA and APACHE II scores and distinguished sepsis patients from controls. The 28-day mortality rate for sepsis patients was 29.31%. High CYP1B1-AS1 expression in sepsis patients predicts a worse prognosis and is a potential risk factor. CYP1B1-AS1 targets miR- 18a- 5p. Silencing CYP1B1-AS1 reduced LPS-inducted apoptosis and inflammatory factor promotion, which the miR- 18a- 5p inhibitor reversed.

Conclusion CYP1B1-AS1 serves as a biomarker for sepsis diagnosis and poor prognosis, potentially promoting inflammation and apoptosis by targeting miR- 18a- 5p.

Keywords Sepsis, CYP1B1-AS1, Inflammation, Diagnostic, Prognosis

*Correspondence:
Lijie Bai
Bailijie86@163.com
[1]Department of Oncology Nursing, Hebei General Hospital, Shijiazhuang City, Hebei Province 050000, China
[2]Department of Urology Nursing, Hebei General Hospital, Shijiazhuang City, Hebei Province 050000, China
[3]Department of Neurology Nursing, Hebei General Hospital, Shijiazhuang City, Hebei Province 050000, China
[4]Department of Acute and Critical Care, Hebei General Hospital, Shijiazhuang City, Hebei Province 050000, China
[5]Department of Coronary Intensive Care Unit CCU, Hebei General Hospital, No. 348, Heping West Road, Xinhua District, Shijiazhuang City, Hebei Province 050000, China

Introduction

Sepsis is a systemic inflammatory condition triggered by severe trauma, burns, infections, or surgery, often leading to organ failure and shock in severe cases [1]. Statistically, sepsis affects 31.5 million people globally annually, causing 5.3 million deaths [2]. The 28-day mortality rate ranges from 8.5 to 31.6% [3]. The WHO considers sepsis prevention and treatment to top global priority [3]. Sepsis is characterized by excessive inflammation triggered by overactivation of the innate immune system, leading to significantly elevated inflammatory mediators in peripheral blood and ultimately causing damage or even death to the host. Despite an in-depth understanding of the pathogenesis of sepsis, there has been no significant improvement in sepsis mortality in clinical treatment at home and abroad [4]. Low diagnostic specificity hampers research, detection, and prognosis. Comprehensive pathogenesis analysis and biomarker identification are crucial for early diagnosis, treatment, mortality reduction, and understanding of sepsis.

Long non-coding RNAs (lncRNAs) are a diverse subset of transcripts longer than 200 nt that do not encode proteins. Their abundance and dysfunction serve as biomarkers and affect sepsis by modulating immune function. Recent evidence indicates that lncRNA MCM3 AP-AS1 [5], SNHG8 [6], and LINC00265 [7] are implicated in sepsis and serve as potential biomarkers. Cytochrome P450 family 1 subfamily B member 1 antisense RNA 1 (CYP1B1-AS1), also known as C2orf58 or MGC34824, is a lncRNA located on human chromosome 2q22.2 with three exons. This novel lncRNA is abnormally regulated in lung adenocarcinoma [8] and acute myeloid leukemia [9], and has potential as a biomarker for glioblastoma [10]. Respiratory epithelial cells regulate local inflammation by interacting with immune cells. Notably, CYP1B1-AS1 is upregulated in COPD respiratory epithelial cells [11]. Recently, Aryashree et al. showed that *Coxiella burnetiid* infection regulates CYP1B1-AS1 expression, reducing host cell death and enhancing survival [12]. Using the GEO database, we identified differential expression of lncRNAs in sepsis patients compared to healthy individuals, noting a 7.27-fold increase in CYP1B1-AS1. Additionally, RNA sequencing by Zhao et al. (2021) also revealed a significant elevation of CYP1B1-AS1 in patients with sepsis [13]. Nevertheless, the clinical significance and molecular mechanisms of CYP1B1-AS1 in sepsis remain unknown.

Herein, we hypothesized that abnormally expressed CYP1B1-AS1 contributes to sepsis progression. To validate this, we analyzed its clinical significance in patients and investigated its molecular mechanisms. Our findings aim to offer new insights into sepsis treatment and management.

Materials and methods

Gene expression omnibus (GEO) public functional genomics data

The GEO (https://www.ncbi.nlm.nih.gov/geo/) serves as a publicly accessible repository for functional genomics data, encompassing high-throughput sequencing and microarray datasets. To obtain the human lncRNA expression database, specifically GSE217700, which comprises transcriptomics sequencing data from whole blood samples of four sepsis patients and four healthy controls. Using the GEO2R web tool, we identified differentially expressed lncRNAs between samples. Volcano plots were employed to visualize and analyze their differences based on absolute values. The study considered a statistically significant threshold of an absolute log2 fold change greater than 2.5 and a *P*-value less than 0.05.

Study approval

The study protocol was approved by the Ethics Committee of Hebei General Hospital and conducted following the Helsinki Declaration. All participants or their family members provided informed consent.

Participants

This study enrolled 116 patients with sepsis who were admitted to the Hebei General Hospital ICU over the period from May 2020 to February 2023. Inclusion criteria were as follows: (1) Meeting the diagnostic criteria for sepsis and septic shock as outlined in the "International Consensus on Sepsis and Septic Shock, third edition" published by the American College of Critical Care Medicine in 2016 [14]; (2) having complete clinical data; (3) Hospitalization for at least 24 h. Patients excluded from this study included those with various systemic malignancies, severe hematologic disorders, acute cardiovascular events, those who had received immunotherapy before admission, pregnant or lactating individuals, and those at the end of life. In addition, 92 subjects served as controls, selected based on the following criteria: (1) Undergoing physical examination at this hospital during the same period as the sepsis patients; (2) Matched sepsis patients in age (53.76 ± 8.48 years) and gender (58.62% male); (3) No history of sepsis or serious infections; (4) Free from malignant tumors and physical abnormalities.

Data collection

Demographic details and medical histories of all participants were gathered. Biochemical analysis measurements included serum creatinine (Scr) levels, albumin levels, white blood cell count (WBC), and C-reactive protein (CRP) concentration. Additionally, the levels of tumor necrosis factor (TNF-α), interleukin (IL)– 6, and IL- 1β were also recorded for the patients. CRP levels were measured by scattering turbidimetry using a Beckman

Coulter IMMAGE800 detector. Whole blood WBCs were measured using a Sysmex XN2000 automated hematology analyzer. Inflammatory factors such as TNF-α, IL- 6, and IL- 1β were measured by enzyme-linked immunosorbent assay (ELISA) using commercial kits.

Furthermore, the patient's Acute Physiology and Chronic Health Evaluation (APACHE II) scores, comprising three components: the Acute Physiology Score, Age Score, and Chronic Health Score, were assessed. The total score ranges from 0 to 71, with a higher score indicating a more severe condition and a poorer prognosis. Additionally, sequential organ failure assessment (SOFA) scores at 12 h of post-admission were also recorded. The SOFA score encompasses respiratory, coagulation, hepatic, cardiovascular, central nervous system, and renal system functions, and has a total range of 0 to 24. An elevated SOFA score signifies increased severity of illness. Additionally, 2 ml of peripheral blood was centrifuged at 12,000 rpm for 15 min to collect serum, which was then stored at – 80℃ for subsequent testing. Upon admission, patients underwent a 28-day short-term follow-up. Their survival status was documented, and the Kaplan-Meier method was employed to determine the cumulative survival rate.

Cell culture and treatment

The human monocytic leukemia THP- 1 cell line was acquired from the American Type Culture Collection (ATCC, Cat# TIB- 202, USA). Cells were maintained in RPMI- 1640 medium (Cat# 11875093, Gibco, USA) supplemented with 10% fetal bovine serum (Cat# F8318- 500ML, Sigma Aldrich, USA) and 1% penicillin/streptomycin (Cat# 15140155, Invitrogen, USA), and the experiments were carried out during the logarithmic growth period. A sepsis model was constructed in vitro by treating cells with 1 μg/ml lipopolysaccharide (LPS, Cat# L4391, Sigma Aldrich, USA) at 37℃ for 24 h, mimicking the sepsis environment as per established protocols [15].

Cell transfection

Small interfering RNA targeted CYP1B1-AS1 (si-CYP1B1-AS1, Cat# siB150423095235-1–5) and negative control (si-NC, Cat# siB06525141922-1–5), miR- 18a- 5p inhibitor (Cat# miR20000072-1–5), and inhibitor NC (Cat# miR2 N0000001-1–5) were obtained from RiboBio. Following a 24 h LPS stimulation of THP-1 cells, 50 mg/ml of si-CYP1B1-AS1, si-NC, or/and 50 nM miR- 18a- 5p inhibitor or inhibitor NC, were combined with the transfection reagent lipofectamine 3000 (Cat #L000015, Invitrogen, USA). The mixture was incubated at room temperature for 20 min and subsequently added dropwise to the cells. Transfection efficiency was assessed by RT-qPCR.

RNA extraction and real-time quantitative reverse transcription PCR (RT-qPCR)

Total RNA isolated from samples through GenElute Total RNA purification Kit (Cat# RNB100 - 50RXN, Sigma Aldrich, USA) or miRNeasy Serum/Plasma Kit (Cat# 217184, Qiagen, Germany). RNA was quantified using a NanoDrop 2000 (Thermo Fisher, USA) and the purity was assessed according to both absorption ratios A260/A280 and A260/A230 respectively in the range of 2 and 1.8–2.2 reflecting contaminant free samples. Subsequently, the total RNA of each sample was subjected to cDNA synthesis with PrimeScript RT Reagent kit (PerfectReal Time, Cat# RR047, Takara Biotechnology, China, for lncRNA) or TaqMan miRNA Reverse Transcription Kit (for miRNA, Cat# 4366597, Life Technologies, USA). 20 μl of fluorescent RT-qPCR system mixture (cDNA, primers, kit reagents, and ddH_2O) was prepared according to the SYBR Green qPCR Master Mix kit (Cat# K0221, ThermoFisher Scientific, USA) instructions and amplified using an ABI 7500 Fluorescence Quantitative PCR Instrument (Applied Biosystems) for amplification. CYP1B1-AS1 and miR- 18a- 5p mRNA expression were measured using the $2^{-\Delta\Delta Ct}$ methods, and normalized to the GPAHD and U6, respectively. The primer sequences used in this study were as follows: CYP1B1-AS1 forward 5'-AGACTCTGCTCTACCAGGGG- 3', reverse 5'-TGG TCACCTTGGAATGGCTC- 3'; miR- 18a- 5p forward 5'-TAAGGTGCATCTAGTGCA- 3', reverse 5'-CAGTG CGTGTCGTGGAGT- 3'; GAPDH forward 5'-GGTGG TCTCCTCTGACTTCAA- 3', reverse 5'-GTTGCTGTA GCCAAATTCGTTGT- 3'; U6 forward 5'-CGCTTCGG CAGCACATATAC- 3', reverse 5'AACGCTTCACGAAT TTGCGT- 3'. The PCR program settings are as follows: pre-denature cycle (1 cycle): 95℃ for 5 min, PCR cycle (40 cycles): 95℃ for 10 s, 60 ℃ for 30 s, and dissolution curve: 60–95℃ for 30 s, and increase the set point temperature after cycle two by 0.

Cell apoptosis assay

Apoptosis in transfected THP- 1 cells was evaluated using Annexin V-FITC/PI Apoptosis Detection Kit (Cat# E-CK- 211, Elabscience, USA) staining followed by flow cytometry. Cells were washed with chilled PBS, collected, and resuspended in a binding buffer. After adding 5 μl of PI and 5 μl of FITC reagent, the cells were stained at room temperature in the dark for 15 min. Subsequently, the stained cells were analyzed using a BD/FACS Aria II Flow cytometry (BD Biosciences, Piscataway, USA).

Enzyme-linked immunosorbent assay (ELISA)

The concentrations of cytokines, specifically tumor necrosis factor (TNF)-α (Cat# KAC1751), interleukin (IL)– 6 (Cat# KRC0061), IL- 8 (Cat# KHC0081), and IL-1β (Cat# KAC1211), in the culture supernatants, were

quantitatively determined using ELISA kits sourced from Invitrogen. ELISA was performed according to the manufacturer's instructions. Colorimetric changes were detected using a microplate reader set at 450 nm. Measurements were taken in triplicate.

Subcellular fractionation location

Following the rinsing of the THP- 1 cells with pre-chilled phosphate buffer, cellular lysis was achieved using a lysis buffer. Thereafter, the cytoplasmic and nuclear fractions were segregated employing the PARIS kit (Cat# AM1921, Thermo Fisher Scientific, USA) from Thermo Fisher Scientific, lnc. Subsequently, total RNA was extracted, and the expression of CYP1B1-AS1 was quantified via RT-qPCR analysis. The results were normalized to the endogenous reference gene GAPDH (serving as the cytoplasmic control) and U6 (serving as the nuclear control), respectively.

Luciferase reporter assay

The lncBook2 (https://ngdc.cncb.ac.cn/lncbook/home) and DIANA (https://rnasysu.com/encori/index.php) databases independently predicted the target miRNAs for CYP1B1-AS1. Subsequently, Venn analysis was employed to identify overlapping target miRNAs among the predictions. The pGL3 luciferase reporter plasmid, containing both wild-type (WT) and mutant (MUT) sequences of CYP1B1-AS1, was obtained from Suzhou GenePharma Co., Ltd. THP- 1 cells were transfected with either miR- 18a- 5p inhibitor or inhibitor NC using lipofectamine 3000 for 8 h at 37℃. Following 48 h, luciferase activity was measured using the Dual-luciferase reporter system (Cat# E1910, Promega, USA) and normalized to Renilla luciferase activity.

RNA immunoprecipitation (RIP) assay

The EZ-MAGNA RIP kit (Cat# 17–701, Millipore, USA) was prepared for RIP assay. Cells were treated with RIP lysis buffer and cell lysates were incubated with magnetic beads conjugated with anti-Ago2 or anti-IgG. Finally, immunoprecipitated was collected and RNA levels (CYP1B1-AS1 and miR- 18a- 5p) were determined.

RNA pull-down assay

Biotin-labeled miR- 18a- 5p probe (Bio-miR- 18a- 5p) and control probe (Bio-miR-NC) were obtained from RiboBio. The M-280 Streptavidin Dynabeads (Cat# 112.05D, Invitrogen, USA) were first incubated with the Bio-miR-18a- 5p and Bio-miR-NC probes, respectively at 37℃ for 2 h. Cells were then lysed and incubated with magnetic beads coated with the probes for 3 h at 4℃. Finally, RNA was isolated from the magnetic beads and then analyzed for MYP1B1-AS1 expression by RT-qPCR.

Statistical analysis

Data from at least three replicated experiments were analyzed using SPSS 3.0 and GraphPad Prism 9.0. Continuous variables were compared using unpaired t-tests (for normal distribution) or Mann-Whitney U tests (for non-normal distribution), and multiple groups were compared using ANOVA. Continuous data were reported as mean ± SD (for normal distribution) or median with interquartile range (for non-normal distribution). Quantitative data were analyzed using chi-square tests and reported as n (%). The diagnostic and predictive value of lncRNA was evaluated using ROC curves. Pearson Pearson coefficient was used for correlation analysis, Kaplan-Meier for survival analysis, and Cox regression for survival risk factor analysis. A P-value < 0.05 indicated significance.

Results

Serum CYP1B1-AS1 is significantly elevated in patients with sepsis

The GSE217700 database identified multiple differentially expressed lncRNAs, with CYP1B1-AS1 significantly upregulated by 7.27-fold (Fig. 1A). To validate CYP1B1-AS1's role in sepsis, we regressively conducted clinical trials and included sepsis patients. Demographic and medical histories were similar between groups (P> 0.05, Table 1). However, sepsis patients had higher Scr, WBC, CRP, and inflammatory factors (TNF-α, IL- 1β, IL- 6, and IL- 8) levels, and lower Albumin levels compared to the controls (P< 0.05, Table 1). Crucially, sepsis patients had significantly higher serum CYP1B1-AS1 levels compared to controls (P< 0.001, Fig. 1B).

Elevated serum CYP1B1-AS1 correlates with sepsis severity and may serve as a marker for it

Subsequently, we analyzed the correlation of serum CYP1B1-AS1 with biochemical indices and inflammatory markers. In sepsis patients, CYP1B1-AS1 levels positively correlated with CRP (r= 0.499), TNF-α (r= 0.636), IL- 6 (r= 0.719), IL- 1β (r= 0.625), and IL- 8 (r= 0.590, Table 2). It also correlated positively with the SOFA (r= 0.774) and APACHE II (r= 0.611) scores, which gauge disease severity (Fig. 1C-D). Furthermore, CYP1B1-AS1 had a sensitivity of 78.45% and specificity of 82.61% for identified sepsis patients from controls (AUC = 0.857, Fig. 1E).

CYP1B1-AS1 levels may predict unfavorable outcomes in sepsis patients

We analyzed the prognostic significance of CYP1B1-AS1 in sepsis patients, finding that the 28-day mortality rate was 29.31%. Serum CYP1B1-AS1 expression was noticeably higher in non-survivors than in survivors (P< 0.001, Fig. 2A). The ROC curve demonstrated that CYP1B1-AS1 expression predicted a high risk of 28-day mortality with

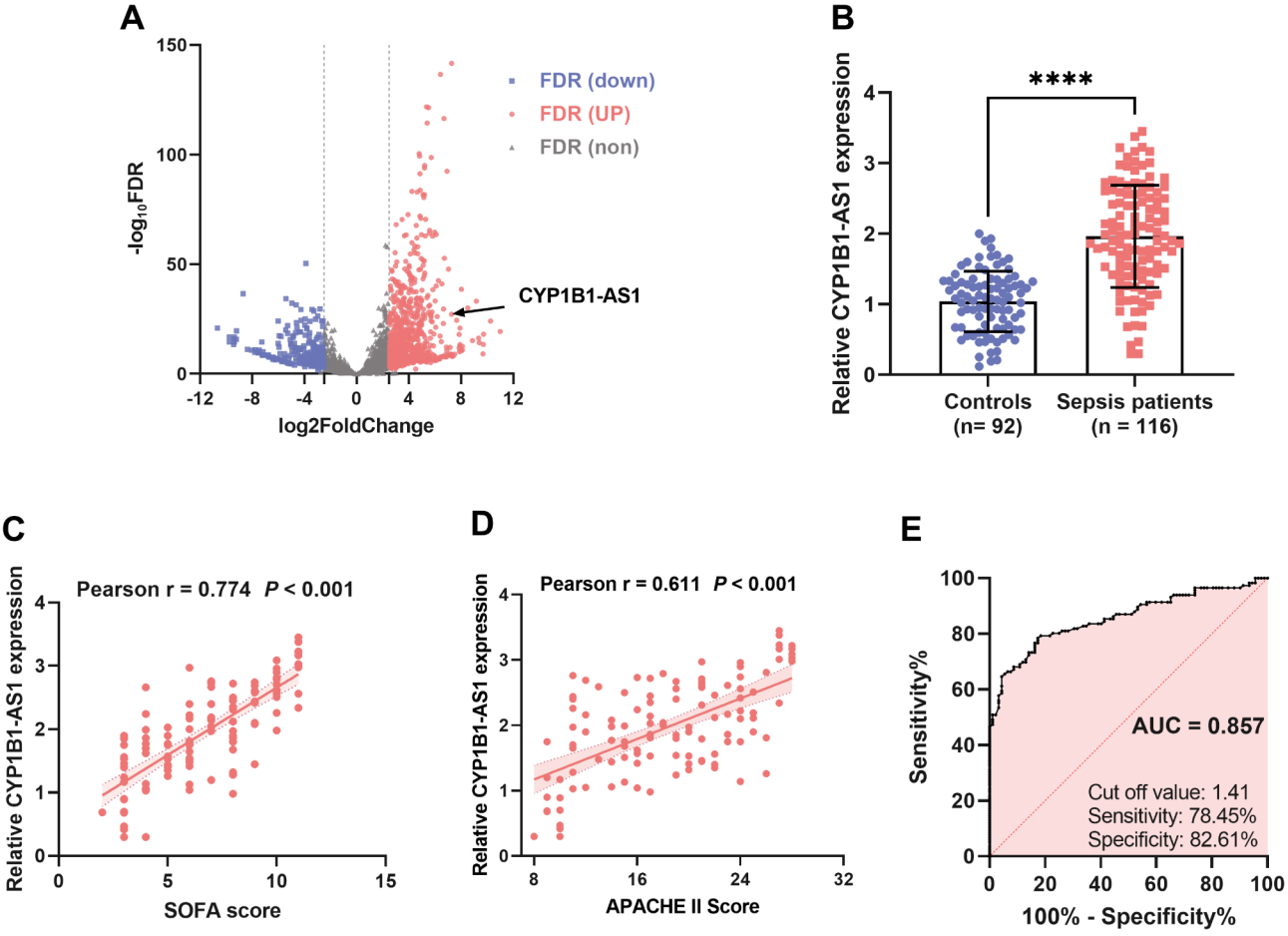

Fig. 1 Elevated serum CYP1B1-AS1 has potential as a diagnostic biomarker of sepsis. **A** Differential expression volcano plots of lncRNA in sepsis from the GSE217700 database. **B** Serum CYP1B1-AS1 expression in the subjects. **C**-**D** Correlation analysis between serum CYP1B1-AS1 and severity scores (SOFA and APACHE II score). **E** ROC curves were employed to evaluate the diagnostic utility of CYP1B1-AS1 in sepsis patients

73.53% sensitivity and 92.68% specificity (AUC = 0.891, Fig. 2B). Based on the median CYP1B1-AS1 expression in sepsis patients, they were divided into high and low-expression groups. Patients in the high expression group had a poorer prognosis than those in the low group (P = 0.0074, Fig. 2C). Cox regression analysis revealed that similar to the APACHE II and SOFA score, CYP1B1-AS1 (HR: 5.592, 95%CI: 1.249–25.036, P = 0.024) was a potential risk factor for 28-day mortality in sepsis (Table 3).

CYP1B1-AS1 inhibition reverses LPS- reduces apoptosis and inflammation

LPS-stimulated THP- 1 cells modeled sepsis. Figure 3A shows CYP1B1-AS1 mRNA increased dose-dependently after 48 h of LPS (P < 0.05). It also rose time-dependently with 1 μg/mL LPS over 0–48 h (P < 0.005, Fig. 3B). Subsequent studies focused on 1 μg/mL LPS for 24 h. siRNA targeting CYP1B1-AS1 significantly reduced its expression compared to si-NC (P < 0.05, Fig. 3C), and si-CYP1B1-AS1 #1 was chosen for further studies. LPS increased CYP1B1-AS1 mRNA levels compared to controls, but this was reversed by si-CYP1B1-AS1 (P < 0.05, Fig. 3D). Additionally, LPS-induced apoptosis was significantly reduced by si-CYP1B1-AS1 (P < 0.05, Fig. 3E). Notably, LPS increased pro-inflammatory factor release (TNF-α, IL- 6, IL- 8, and IL- 1β) in THP- 1 cells, which was partially reduced by low CYP1B1-AS1 expression (P < 0.05, Fig. 3F).

CYP1B1-AS1 is a target of miR- 18a- 5p

Sublocalization analysis showed CYP1B1-AS1 was mainly in the cytoplasm (Fig. 4A), hinting at its potential as a ceRNA for miRNAs. DIANA and lncBook2 predicted CYP1B1-AS1 target miRNAs, including miR- 18a- 5p (Fig. 4B). The putative binding sequences between CYP1B1-AS1 and miR- 18a- 5p are presented in Fig. 4C. Furthermore, a dual luciferase reporter assay showed that the miR- 18a- 5p inhibitor reduced CYP1B1-AS1-WT luciferase activity but not CYP1B1-AS1-MUT (Fig. 4D). Ago2, a score RISC component, enriched both CYP1B1-AS1 and miR- 18a- 5p compared to IgG (P < 0.05, Fig. 4E). RNA pull-down experiments revealed the

Table 1 Clinical baseline characteristics of healthy controls and patients with sepsis

Parameters	Controls ($n = 92$)	Sepsis patients ($n = 116$)	*P* value
Demographics			
Age (years), mean ± SD	55.77 ± 9.02	53.76 ± 8.48	0.101
BMI (kg/m^2), mean ± SD	23.73 ± 4.25	24.26 ± 4.17	0.365
Male, n (%)	45 (48.91)	68 (58.62)	0.207
Smoke, n (%)	40 (43.48)	54 (46.55)	0.676
Drink, n (%)	41 (44.57)	57 (49.14)	0.576
Medical history, n (%)			
Hypertension	31 (33.70)	46 (39.67)	0.390
Hyperlipidemia	37 (40.22)	39 (33.61)	0.385
Diabetes	32 (34.78)	47 (40.52)	0.472
Biochemical indicators, median (IQR)			
Scr (mg/dL)	0.85 (0.72, 1.01)	1.60 (1.16, 2.05)	< 0.001
Albumin (g/L)	44.76 (39.42, 51.63)	32.42 (25.64, 38.31)	< 0.001
WBC (× 10^9/L)	5.92 (5.07, 6.85)	18.37 (15.25, 22.67)	< 0.001
CRP (mg/L)	4.95 (3.96, 6.93)	106.47 (79.91, 136.31)	< 0.001
Disease severity, mean ± SD			
SOFA score		7.00 (4.00, 9.00)	-
APACHE II score		18.50 (14.00, 23.00)	-
Inflammatory cytokine, median (IQR)			
TNF-α (pg/mL)	29.70 (23.76, 36.63)	199.00 (150.98, 250.47)	< 0.001
IL- 6 (pg/mL)	13.12 (9.90, 18.12)	51.48 (37.62, 72.27)	< 0.001
IL- 8 (pg/mL)	15.35 (12.87, 20.79)	112.34 (81.51, 142.51)	< 0.001
IL- 1β (pg/mL)	25.25 (19.31, 32.18)	182.16 (137.12, 216.32)	< 0.001

Abbreviations: *BMI* Body mass index, *Scr* Serum creatinine, *WBC* White blood cell, *CRP* C-reactive protein, *SOFA* Sequential organ failure assessment, *APACHE II* Acute physiology and chronic health evaluation II, *IL* Interleukin, *TNF* Tumor necrosis factor, *SD* Standard deviation, *IQR* Interquartile range

Table 2 Correlation of serum LncRNA CYP1B1-AS1 levels with biochemical indices and inflammatory factors in the subjects

Parameters	Controls		Sepsis patients	
	P value	*r*	*P* value	*r*
Scr	0.195	0.136	0.031	0.200
Albumin	0.066	− 0.192	0.053	−0.180
WBC	0.169	0.145	0.011	0.236
CRP	0.075	0.188	0.000	0.499
TNF-α	0.636	0.050	0.000	0.636
IL- 6	0.533	0.066	0.000	0.719
IL- 8	0.588	0.057	0.000	0.625
IL- 1β	0.333	0.102	0.000	0.590

enrichment of CYP1B1-AS1 by bio-miR- 18a- 5p ($P < 0.05$, Fig. 4F). Furthermore, sepsis patients had significantly lower serum miR- 18a- 5p levels compared to controls ($P < 0.001$, Fig. 4G) and a positive correlation with serum CYP1B1-AS1 ($P < 0.001$, $r = -0.603$, Fig. 4H). LPS suppressed miR- 18a- 5p expression in THP- 1 cells, but this was restored by si-CYP1B1-AS1 ($P < 0.001$, Fig. 4I).

miR- 18a- 5p downregulation partly reversed the effects of miR- 18a- 5p silencing on LPS-induced cell damage in THP- 1 cellls

In THP- 1 cells, miR- 18a- 5p levels significantly decreased with increasing doses (0, 0.5, 1, 2 μg/ml) after 48 h and with time (0, 12, 24, 48 h) at 1 μg/ml LPS ($P < 0.05$, Fig. 5A-B). Moreover, the miR- 18a- 5p inhibitor markedly decreased its expression ($P < 0.05$, Fig. 5C). In LPS-induced THP- 1 cells, low CYP1B1-AS1 expression boosted miR- 18a- 5p levels, which the inhibitor then suppressed ($P < 0.005$, Fig. 5D). Additionally, miR- 18a- 5p suppression reversed the reduction in apoptosis due to CYP1B1-AS1 downregulation in these cells ($P < 0.05$, Fig. 5E). Crucially, the miR- 18a- 5p inhibitor restored the suppression of silenced CYP1B1-AS1 on inflammatory factors in THP- 1 cells ($P < 0.05$, Fig. 5F).

Discussion

Sepsis, triggered by severe infection and mediated by innate immune cells (neutrophils, monocytes, macrophages), poses a significant threat due to its rapid progression, severity, and high mortality rates. Despite common interventions such as fluid resuscitation, antibiotic therapy, and vasopressor use, sepsis prognosis remains poor. Moreover, differentiating sepsis from other immune disorders is challenging, delaying early treatment and often leading to multi-organ dysfunction or high morbidity and mortality. CRP, an inflammation indicator, and procalcitonin, a bacteremia marker, were widely used as sepsis biomarkers, yet they have limited value in early diagnosis and prognosis

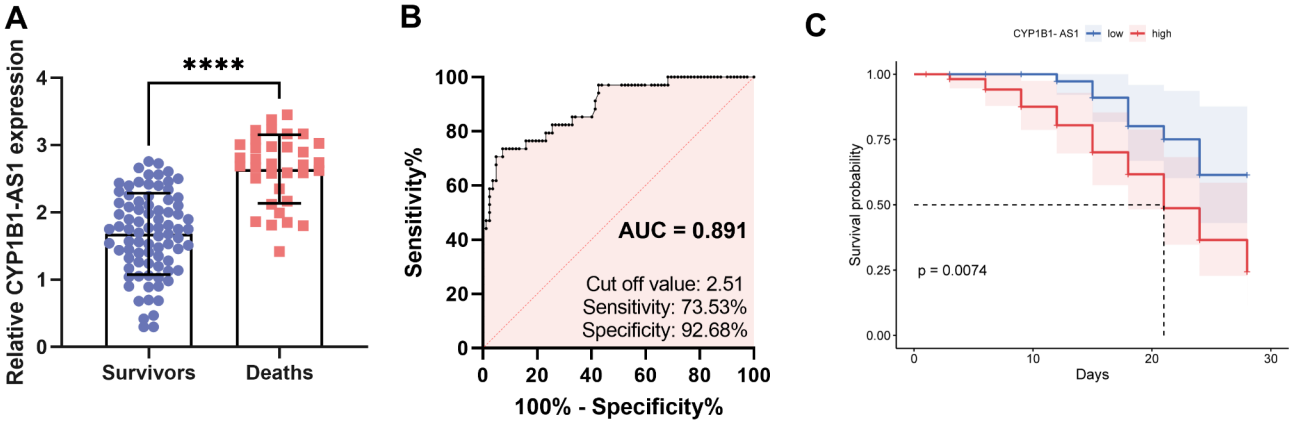

Fig. 2 CYP1B1-AS1 levels may predict unfavorable outcomes in sepsis patients. **A** Serum CYP1B1-AS1 expression in death and survival patients with sepsis at 28 days of follow-up. **B** The ROC curve was employed to examine the predictive significance of serum CYP1B1-AS1 for mortality in sepsis patients. **C** Kaplan-Meier curves depicted cumulative survival rates, with group differences assessed by the log-rank test. **** $P < 0.0001$

Table 3 Cox regression analysis of independent risk factors for 28-day mortality in patients with sepsis

Parameters	HR	95%CI		*P* value
		Lower	Higher	
Age	2.01	0.798	5.061	0.139
Male	2.016	0.741	5.485	0.17
BMI	1.193	0.486	2.927	0.7
Smoke	1.922	0.855	4.324	0.114
Drink	1.014	0.39	2.638	0.976
Hypertension	1.474	0.582	3.735	0.413
Hyperlipidemia	1.806	0.662	4.93	0.248
Diabetes	1.785	0.752	4.237	0.189
Scr	2.63	0.856	8.083	0.091
Albumin	0.59	0.239	1.452	0.25
WBC	2.331	0.91	5.972	0.078
CRP	2.073	0.822	5.226	0.123
TNF-α	2.374	0.679	8.306	0.176
IL- 6	1.823	0.518	6.414	0.349
IL- 8	1.366	0.531	3.515	0.518
IL- 1β	2.365	0.757	7.382	0.138
APAHCE II score	2.923	1.029	8.303	0.044
SOFA score	3.868	1.066	14.033	0.040
CYP1B1-AS1	5.592	1.249	25.036	0.024

BMI Body mass index, *APACHE* Acute physiology and chronic health evaluation, *SOFA* Sequential organ failure assessment, *Scr* Serum creatinine, *WBC* White blood cells, *CRP* C-reactive protein, *PCT* Procalcitonin, *TNF-α* Tumor necrosis factor-α, *IL* Interleukin

prediction. CRP, while useful for infection diagnosis, struggles to differentiate sepsis. PCT, less specific, also increases significantly in non-infectious conditions like severe trauma and surgery [16]. Recently, the qSOFA score, based on three clinical criteria, was introduced as a bedside tool. However, studies have shown that the qSOFA score had high specificity but low sensitivity, insufficient for early patients' detection during disease progression [17]. Therefore, identifying biomarkers with high sensitivity and specificity for early sepsis identification is crucial for risk stratification, prognosis assessment, and molecularly targeted therapy. Yet, current biomarkers lack specificity, research progresses slowly, and treatment options are limited by uncontrolled inflammation and persistent immune suppression [18]. Discovering new biomarkers and blocking trauma-induced inflammation may inhibit sepsis progression. This study reveals that CYP1B1-AS1 is significantly upregulated in sepsis patients, correlating positively with inflammation levels and predicting poor prognosis. Silencing CYP1B1-AS1 inhibits the inflammation response by targeting miR- 18a- 5p, offering new insights for sepsis management and treatment.

LncRNAs exert an impact on the inflammatory response by modulating the expression profiles of inflammatory mediators, as demonstrated in previous studies. Specifically, select lncRNAs have been shown to regulate key inflammatory cytokines such as IL- 6, IL- 1β, and TNF-α, therefore influencing the progression of sepsis [19]. Prior investigations have demonstrated that lncRNA PRKCQ-AS1 partakes in the sepsis inflammatory process and holds promise as a prognostic biomarker [20]. In addition, LINC00265 was associated with systemic inflammation and disease severity in sepsis [7]. CYP1B1-AS1, being a newly discovered lncRNA, exhibits aberrant expression patterns in lung adenocarcinoma [8] and acute myeloid leukemia [9]. Moreover, it shows potential to serve as a novel biomarker in the contest of glioblastoma [10]. Respiratory epithelial cells modulate local inflammation by engaging with macrophages and dendritic cells with CYP1B1-AS1 notably elevated in COPD epithelial cells [11]. We screened the GEO databases to detect differentially expressed lncRNAs between sepsis patients and controls, revealing a 7.27-fold upregulation of CYP1B1-AS1. Zhao et al. (2020) through

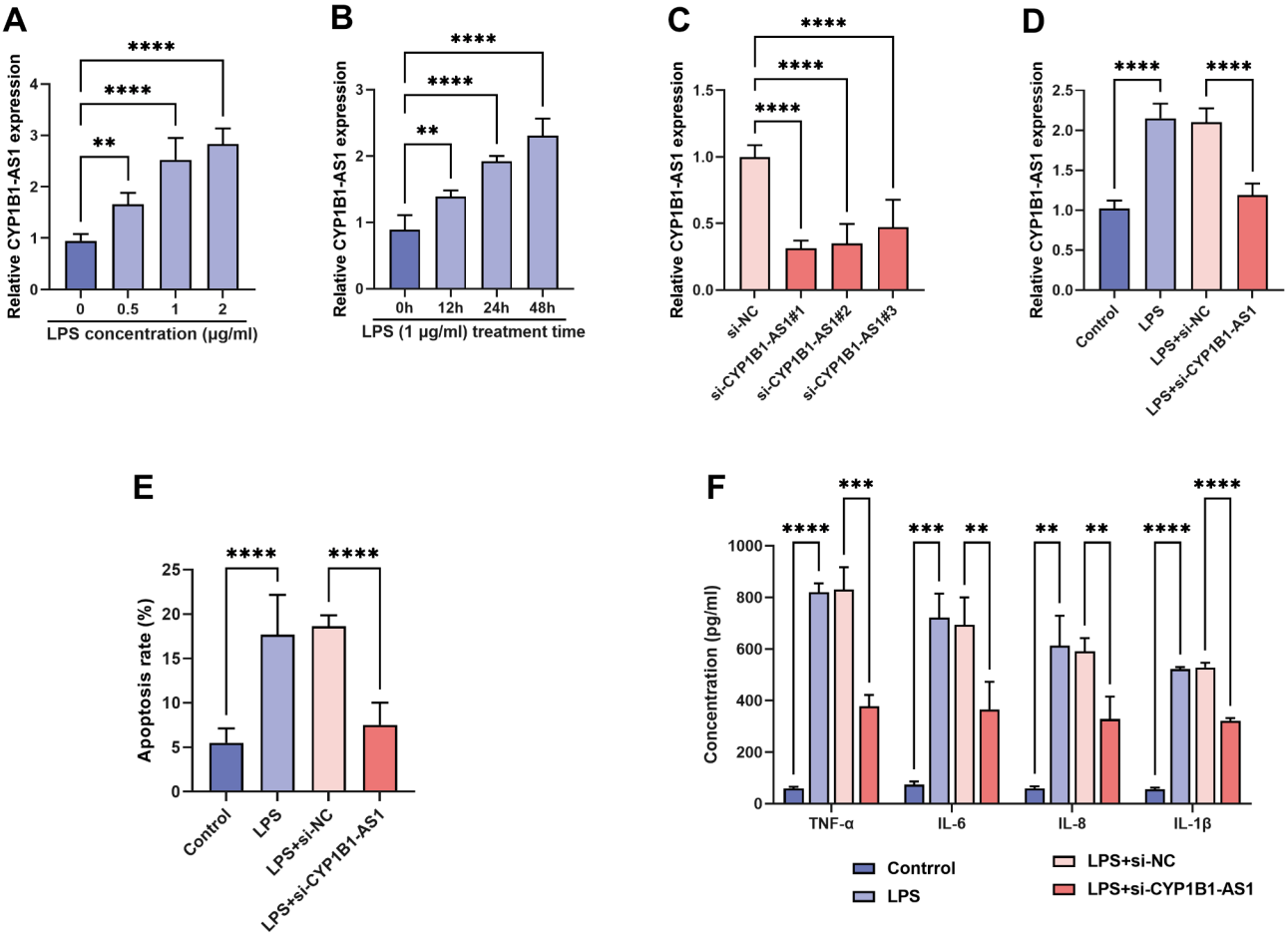

Fig. 3 Silencing CYP1B1-AS1 mitigates LPS-induced effects on THP- 1 cell proliferation, apoptosis, and inflammation. **A** Impact of LPS concentrations on CYP1B1-AS1 levels in THP- 1 cells after 48 h. **B** Impact of LPS induction (1 μg/ml) on CYP1B1-AS1 levels in THP-1 cells. **C** RT-qPCR assessment of CYP1B1-AS1 siRNA transfection efficiency in THP- 1 cells. **D** RT-qPCR analysis of CYP1B1-AS1 expression in THP- 1 cells treated with LPS and si-CYP1B1-AS1. E-F. Flow cytometry and ELISA assay were used to assess cell apoptosis and inflammation factors in LPS and si-CYP1B1-AS1-treated cells. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

RNA sequencing also identified a significant 3.37-fold elevation of CYP1B1-AS1 in sepsis patients [13]. Furthermore, CYP1B1-AS1 was notably increased during the inflammatory response induced by Clostridium burnetiid, the pathogen responsible for Q fever [12], although the precise mechanism remains elusive. In accordance with prior research, our study also revealed a notable elevation in serum CYP1B1-AS1 levels.

The SOFA and APACHE II scores are widely utilized clinical metrics for evaluating sepsis severity, reflecting a range of physiological parameters, chronic health, and overall patient status [21]. Our research revealed a strong positive correlation between CYP1B1-AS1 levels and SOFA and APACHE II scores, indicating its utility in gauging disease severity. Elevated CYP1B1-AS1 levels may correspond to more severe illness necessitating more intensive treatment. ROC analysis demonstrated that CYP1B1-AS1 exhibits high sensitivity and specificity, effectively distinguishing between controls and patients, and predicting unfavorable outcomes. High serum CYP1B1-AS1 levels indicate a poor prognosis, necessitating vigilant monitoring and intensive treatment. In conclusion, our study suggests that CYP1B1-AS1 levels can serve as a sensitive and specific guide for the early diagnosis of sepsis and indicate a poor prognosis for patients. Our findings offer novel insights into the search for clinical biomarkers for sepsis. LPS, a key component of Gram-negative bacteria's membrane, acts as an endotoxin to activate cells like epithelia, endothelia, and monocyte macrophages via signal transduction. This activation triggers the release of pro-inflammatory cytokines (IL- 1β, TNF-α, IL- 6, and IL- 8), which in turn can induce a systemic inflammatory response and toxic effects on internal organs. Therefore, LPS-induced cellular models have become a common tool for sepsis simulation in vitro [22]. Our study revealed a concentration- and time-dependent upregulation of CYP1B1-AS1 in response to LPS.

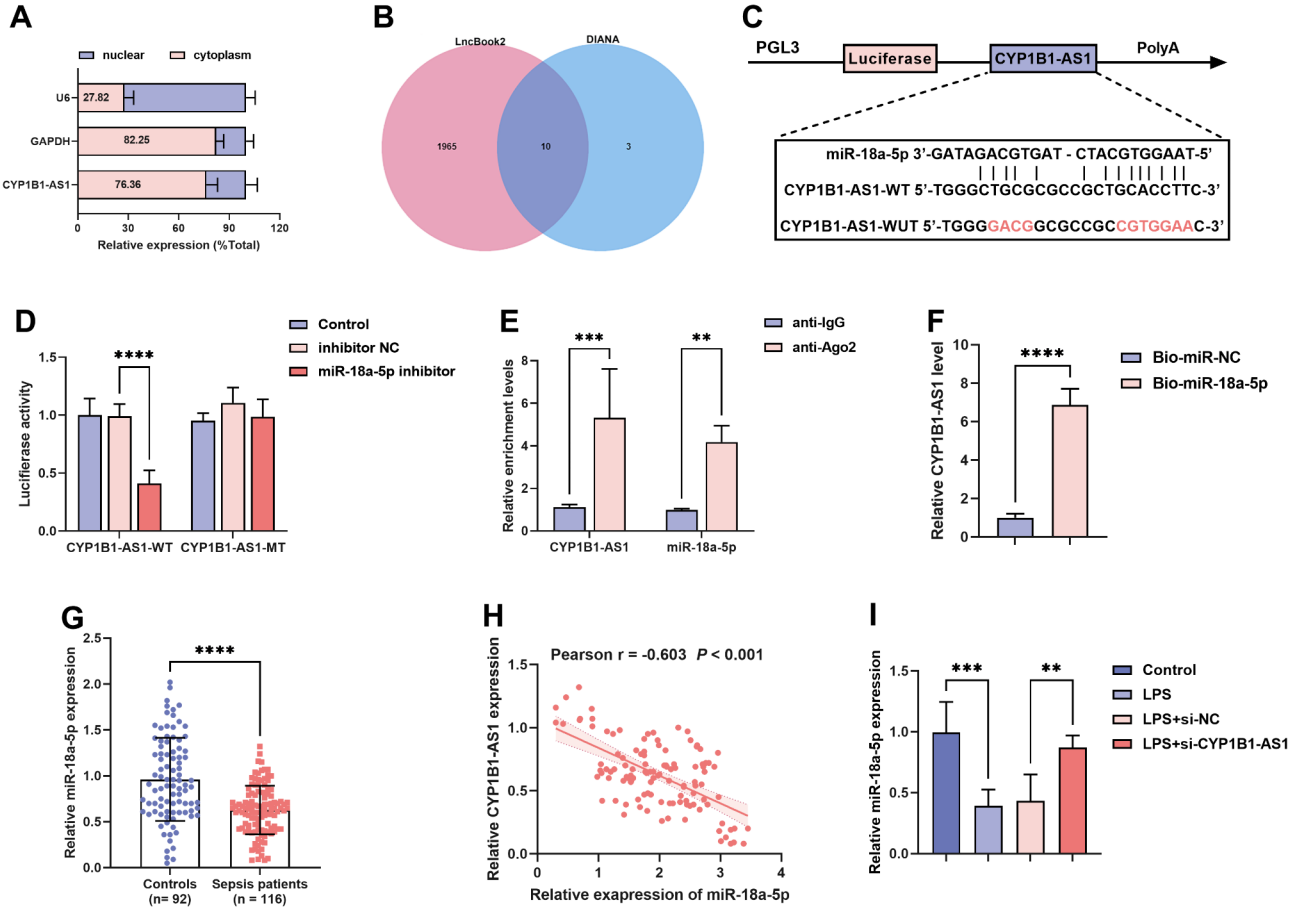

Fig. 4 CYP1B1-AS1 directly interacts with miR- 18a- 5p. **A** Investigation of CYP1B1-AS1 subcellular localization in THP- 1 cells. **B** Venn diagram presented the overlapping target miRNAs of CYP1B1-AS1 predicted by LncBooK2 and DIANA databases. **C** The putative binding sequences were presented. The targeting relationship between CYP1B1-AS1 and miR- 18a- 5p was validated using Dual-luciferase report assay (**D**), RIP assay (**E**), and RNA pull-down assay (**F**). **G** The serum miR- 18a- 5p in the subjects was detected. **H** Pearson coefficient was conducted to examine the correlation between serum miR- 18a- 5p and CYP1B1-AS1 levels in patients with sepsis. **I** The mRNA levels of miR- 18a- 5p were altered in THP- 1 cells that were induced with LPS and transfected with si-CYP1B1-AS1. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

Concurrently, silencing of CYP1B1-AS1 significantly reduced LPS-induced excessive release of IL-β, TNF-α, IL- 6, and IL- 8 inflammatory factors. In conclusion, the findings suggest that inhibition of CYP1B1-AS1 levels alleviates the inflammatory response in sepsis.

miRNAs are a class of short, single-stranded non-coding RNA molecules [23, 24]. Emerging evidence shows that miRNA exerts an essential role in the biological process of sepsis. Emerging evidence shows that miR- 182 -5p [25], miR-21 [26], and miR- 16 -5p [27] regulate inflammation, pyroptosis, and acute kidney injury, influencing sepsis. Cytoplasmic lncRNAs regulate gene expression by competitively binding to miRNAs, reducing their inhibitory effect on target mRNAs. Our study found that CYP1B1-AS1 predominantly enriched in the cytoplasm, suggesting it may regulate miRNAs. Bioinformatics prediction and further validation revealed that CYP1B1-AS1 has a targeted binding site with miR- 18a- 5p. miR- 18a-5p is involved in lung ischemia-reperfusion injury [28] and melatonin alleviates hyperoxia-induced lung injury by modulating it [29]. Moreover, miR- 18a- 5p has been recognized as an inflammation-related miRNA. Specifically, exosome miR- 18a- 5p has been linked to the advancement of small intestinal colitis [30]. Furthermore, isoproterenol was shown to suppress cardiomyocyte inflammation and mitigate hypoxia-reoxygenation-triggered myocardial injury using miR-18a- 5p [31]. miR- 18a- 5p affects inflammation and apoptosis in COPD bronchial cells [32]. Low serum miR- 18a- 5p predicts severe COVID-19 outcomes [33]. Jeon et al. (2023) identified 25 differentially expressed miRNAs, including miR- 18a- 5p, in plasma exosomes from 135 sepsis patients compared to 11 controls using microarray analysis [34]. Consistent with Jeon et al., we also found that miR- 18a- 5p was downregulated in sepsis patients. Novelly, we showed miR- 18a- 5p targets CYP1B1-AS1, and inhibiting

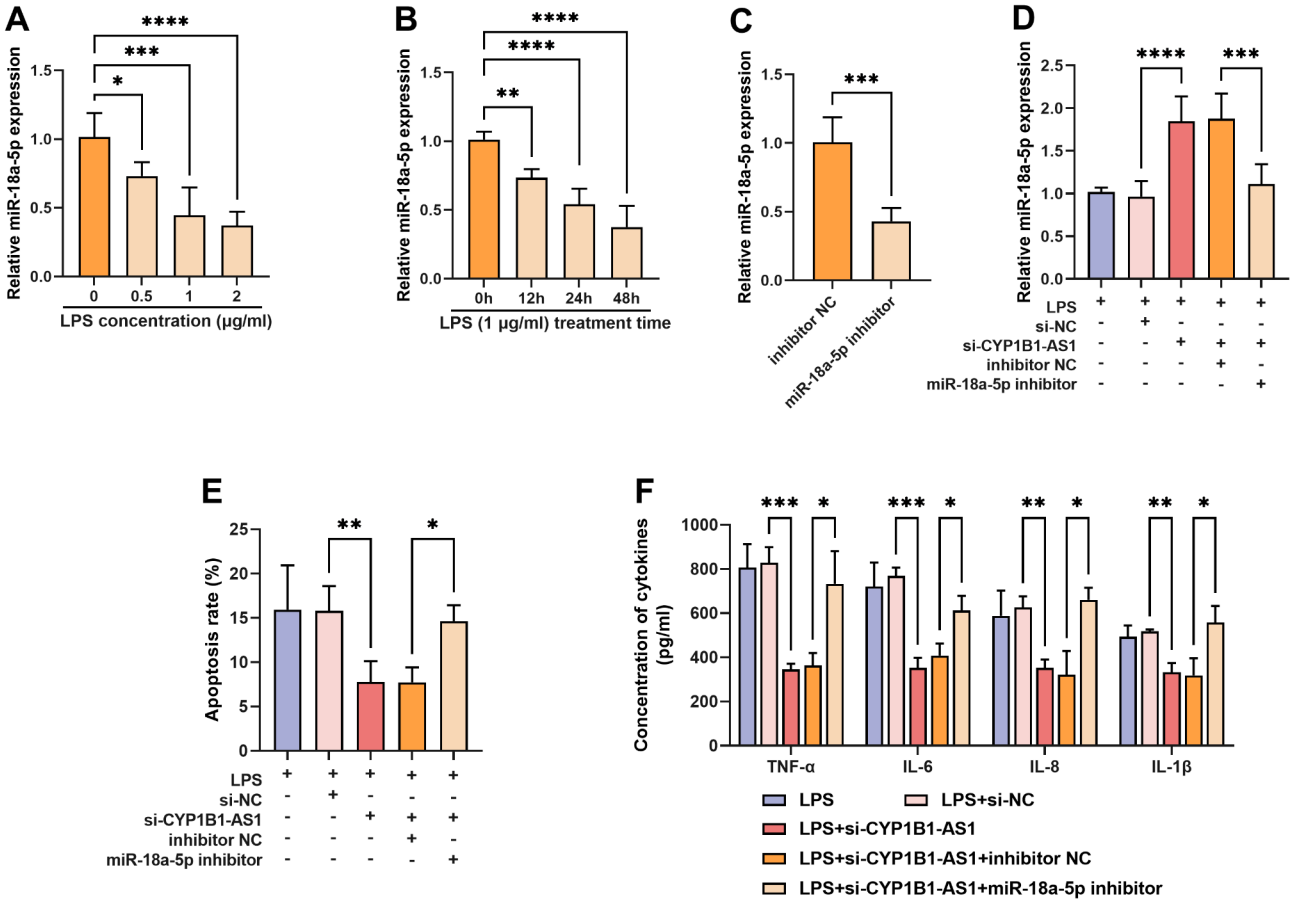

Fig. 5 miR- 18a- 5p downregulation partly reversed the effects of miR- 18a- 5p silencing on LPS-induced cell damage in THP- 1 cells. **A** Changes in miR-18a- 5p levels in THP- 1 cells after 48 h of induction with various LPS concentrations. **B** miR- 18a- 5p levels vary in THP- 1 cells induced by 1 μg/ml LPS for different durations. Transfection with miR- 18a- 5p inhibitor alone (**C**) or LPS-induced silencing of CYP1B1-AS1 alters THP- 1 cells (**D**). **E**-**F** Flow cytometry and ELISA assay were used to assess cell apoptosis, and inflammation factors in LPS, si-CYP1B1-AS1, and miR- 18a- 5p inhibitor CO-treated cells. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

miR- 18a- 5p reduced CYP1B1-AS1's effects on LPS-induced inflammation and apoptosis. Previous studies found that miR- 18a- 5p exacerbated the progression of small intestinal myositis by regulating RORA [30]. RORA is closely associated with sevoflurane to alleviate sepsis inflammation and endothelial permeability injury [35]. What's more, ginsenoside GRg1 has been identified as target IGF1 to ameliorate sepsis-induced acute lung injury [36]. Prior research has shown that miR- 18a- 5p plays a role in COPD progression by regulating IGF1 [32]. Therefore, we hypothesize that miR-18a- 5p may contribute to sepsis progression by target RORA, IGF1 or other mRNAs; however, the exact mechanism requires further investigation.

In conclusion, CYP1B1-AS1 may promote sepsis by targeting miR- 18a- 5p. Inhibiting CYP1B1-AS1 boosts miR- 18a- 5p, reducing macrophage inflammation and apoptosis thus alleviating sepsis. We acknowledge that this study has some further limitations. Firstly, the relatively small sample size may affect the generalizability of the results and statistical significance. Secondly, the relatively short follow-up period of this study focused on the early stages of patient outcomes, and longer follow-up studies are needed to gain insight into the prognostic significance of CYP1B1-AS1 in sepsis. Furthermore, a comprehensive analysis of the target genes of CYP1B1-AS1/miR- 18a- 5p and the associated signaling pathways in sepsis progression is required. Our future studies will focus on addressing these limitations.

In conclusion, our experiments uniquely identify CYP1B1-AS1 as a promising biomarker for diagnosing and predicting sepsis outcomes. Reducing CYP1B1-AS1 expression, which upregulates miR- 18a- 5p, effectively mitigates inflammation and sepsis severity. This finding advances sepsis treatment strategies.

Acknowledgements
Not applicable.

Authors' contributions
All authors contributed to the study conception and design. Study concept and design: L.X. X., and L.J. B.; analysis and interpretation of data: J.P. L., L. L., Q.S. Z., and Q.J. F.; drafting of the manuscript: L.X. X.; critical revision of the manuscript for important intellectual content: L.J. B.; statistical analysis: Q.S. Z, and Q.J. F.

Funding
This study was funded by Medical Science Research Project of Hebei (20171232).

Data availability
The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study protocol was approved by the Ethics Committee of Hebei General Hospital and conducted following the Helsinki Declaration. All participants or their family members provided informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 13 January 2025 / Accepted: 4 April 2025
Published online: 16 April 2025

References

1. Gao Z, Huang D. lncRNA GAS5–mediated miR–23a–3p promotes inflammation and cell apoptosis by targeting TLR4 in a cell model of sepsis. Mol Med Rep. 2021;24(1):510.
2. Zhang Q, Wang C, Li S, Li Y, Chen M, Hu Y. Screening of core genes prognostic for sepsis and construction of a ceRNA regulatory network. BMC Med Genomics. 2023;16(1):37.
3. Zhang W, Chen B, Chen W. LncRNA GAS5 relates to Th17 cells and serves as a potential biomarker for sepsis inflammation, organ dysfunctions and mortality risk. J Clin Lab Anal. 2022;36(5):e24309.
4. ShilinLi, Hu Y. Identification of four mitochondria-related genes in sepsis based on RNA sequencing technology. BMC Immunol. 2024;25(1):32.
5. Wei Y, Bai C, Xu S, Cui M, Wang R, Wu M. Diagnostic and Predictive Value of LncRNA MCM3AP-AS1 in Sepsis and Its Regulatory Role in Sepsis-Induced Myocardial Dysfunction. Cardiovasc Toxicol. 2024;24(10):1125–38.
6. Liu Y, Sun F, Wang X, Guo G. Long non-coding RNA small nucleolar RNA host gene 8 (SNHG8) sponges miR-34b-5p to prevent sepsis-induced cardiac dysfunction and inflammation and serves as a diagnostic biomarker. Arch Med Sci. 2024;20(4):1268–80.
7. Cui Y, Jiang N, Liu X, Huang J, Chen W. LINC00265 can Serve as a Potential Biomarker for Predicting Increased Disease Risk, Systemic Inflammation, Disease Severity and Poor Prognosis in Sepsis. Immunol Invest. 2024;53(4):640–51.
8. Wang J, Shu J. Construction of RNA Methylation Modification-immune-related lncRNA Molecular Subtypes and Prognostic Scoring System in Lung Adenocarcinoma. Curr Med Chem. 2024;31(12):1539–60.
9. Cheng Y, Wang X, Qi P, Liu C, Wang S, Wan Q, Liu Y, Su Y, Jin L, Liu Y, et al. Tumor Microenvironmental Competitive Endogenous RNA Network and Immune Cells Act as Robust Prognostic Predictor of Acute Myeloid Leukemia. Front Oncol. 2021;11:584884.
10. Ye T, Li LL, Peng XM, Li Q. CYP1B1-AS1 Is a Novel Biomarker in Glioblastoma by Comprehensive Analysis. Dis Markers 2021;2021:8565943.
11. Misiukiewicz-Stepien P, Mierzejewski M, Zajusz-Zubek E, Goryca K, Adamska D, Szelag M, Krenke R, Paplinska-Goryca M. RNA-Seq Analysis of UPM-Exposed Epithelium Co-Cultivated with Macrophages and Dendritic Cells in Obstructive Lung Diseases. Int J Mol Sci. 2022;23(16):9125.
12. Arunima A, Niyakan S, Butler SM, Clark SD, Pinson A, Kwak D, Qian X, de Figueiredo P, van Schaik EJ, Samuel JE. CYP1B1-AS1 regulates CYP1B1 to promote Coxiella burnetii pathogenesis by inhibiting ROS and host cell death. Res Sq. 2024. https://doi.org/10.21203/rs.3.rs-5390645/v1
13. Guo X, Qin Y, Wang L, Dong S, Yan Y, Bian X, Zhao C. A competing endogenous RNA network reveals key lncRNAs associated with sepsis. Mol Genet Genomic Med. 2021;9(1):e1557.
14. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8):801–10.
15. Jiang L, Li J. lncRNA GMDS–AS1 upregulates IL–6, TNF–alpha and IL–1beta, and induces apoptosis in human monocytic THP–1 cells via miR–96–5p/caspase 2 signaling. Mol Med Rep. 2022;25(2):67.
16. Luo X, Chen X, Gu Y, Jia H, Lin X, Wang L, Feng J. LncRNA FENDRR/ miR-424-5p serves as a diagnostic biomarker for sepsis and its predictive value for clinical outcomes. Immunobiology. 2025;230(2):152870.
17. Chimwaza Y, Hunt A, Oliveira-Ciabati L, Bonnett L, Abalos E, Cuesta C, Souza JP, Bonet M, Brizuela V, Lissauer D, et al. Early warning systems for identifying severe maternal outcomes: findings from the WHO global maternal sepsis study. EClinicalMedicine. 2025;79:102981.
18. Cheng Y, Cao X, Zhang J, Chen D, Zhu J, Xu L, Qin L. Dysregulated lncRNAs are Involved in the Progress of Sepsis by Constructing Regulatory Networks in Whole Blood Cells. Front Pharmacol. 2021;12:678256.
19. Bai Y, Gao J, Yan Y, Zhao X. The significance of long chain non-coding RNA signature genes in the diagnosis and management of sepsis patients, and the development of a prediction model. Front Immunol. 2024;15:1450014.
20. Ding X, Liang W, Xia H, Liu Y, Liu S, Xia X, Zhu X, Pei Y, Zhang D. Analysis of Immune and Prognostic-Related lncRNA PRKCQ-AS1 for Predicting Prognosis and Regulating Effect in Sepsis. J Inflamm Res. 2024;17:279–99.
21. Diao Y, Zhao Y, Li X, Li B, Huo R, Han X. A simplified machine learning model utilizing platelet-related genes for predicting poor prognosis in sepsis. Front Immunol. 2023;14:1286203.
22. Zhang TN, Wen R, Yang YH, Yang N, Liu CF. Integration of transcriptomic, proteomic, and metabolomic data to identify lncRNA rPvt1 associations in lipopolysaccharide-treated H9C2 cardiomyocytes. Front Genet. 2023;14:1278830.
23. Ma L, Liu H, Shao P, Lv Q. Upregulated miR-146b-3p predicted rheumatoid arthritis development and regulated TNF-alpha-induced excessive proliferation, motility, and inflammation in MH7A cells. BMC Immunol. 2024;25(1):36.
24. Jin Q, Liu C, Cao Y, Wang F. miR-486-5p predicted adverse outcomes of SCAP and regulated K. pneumonia infection via FOXO1. BMC Immunol. 2024;25(1):33.
25. Ma C, Liu K, Wang F, Fei X, Niu C, Li T, Liu L. Neutrophil membrane-engineered Panax ginseng root-derived exosomes loaded miRNA 182-5p targets NOX4/Drp-1/NLRP3 signal pathway to alleviate acute lung injury in sepsis: experimental studies. Int J Surg. 2024;110(1):72–86.
26. Zhao X, Xie J, Duan C, Wang L, Si Y, Liu S, Wang Q, Wu D, Wang Y, Yin W, et al. ADAR1 protects pulmonary macrophages from sepsis-induced pyroptosis and lung injury through miR-21/A20 signaling. Int J Biol Sci. 2024;20(2):464–85.
27. Li H, Duan J, Zhang T, Fu Y, Xu Y, Miao H, Ge X. miR-16-5p aggravates sepsis-associated acute kidney injury by inducing apoptosis. Ren Fail. 2024;46(1):2322688.
28. Xiao K, Song L, Hu Y, He W, Hou F, Yan P, Xu J, Wang K, Tao Y, Li D et al. Novel Role of miR-18a-5p and Galanin in Rat Lung Ischemia Reperfusion-Mediated Response. Oxid Med Cell Longev 2021;2021:6621921.
29. Zou D, Liao J, Xiao M, Liu L, Xu M. Melatonin alleviates hyperoxia-induced lung injury through elevating MSC exosomal miR-18a-5p expression to repress PUM2 signaling. FASEB J. 2024;38(16):e70012.
30. Chen Y, Yuan X, Li Y, Chen J, Wu S, Jiang A, Miao X, Shu Q. Circulating exosomal microRNA-18a-5p accentuates intestinal inflammation in Hirschsprung-associated enterocolitis by targeting RORA. Am J Transl Res. 2021;13(5):4182–96.
31. Jin C, Yuan S, Piao L, Ren M, Liu Q. Propofol synergizes with circAPBB2 to protect against hypoxia/reoxygenation-induced oxidative stress, inflammation, and apoptosis of human cardiomyocytes. Immun Inflamm Dis. 2023;11(8):e952.
32. Liu P, Zhang H, Zeng H, Meng Y, Gao H, Zhang M, Zhao L. LncRNA CASC2 is involved in the development of chronic obstructive pulmonary disease via targeting miR-18a-5p/IGF1 axis. Ther Adv Respir Dis. 2021;15:17534666211028072.
33. Haroun RA, Osman WH, Eessa AM. Prognostic significance of serum miR-18a-5p in severe COVID-19 Egyptian patients. J Genet Eng Biotechnol. 2023;21(1):114.

34. Shin B, Lee JY, Im Y, Yoo H, Park J, Lee JS, Lee KY, Jeon K. Prognostic implication of downregulated exosomal miRNAs in patients with sepsis: a cross-sectional study with bioinformatics analysis. J Intensive Care. 2023;11(1):35.
35. Ni W, Zou Z, Jiang P, Wang S. Sevoflurane alleviates inflammation, apoptosis and permeability damage of human umbilical vein endothelial cells induced by lipopolysaccharide by inhibiting endoplasmic reticulum stress via upregulating RORalpha. Prostaglandins Other Lipid Mediat. 2024;172:106821.
36. Zhong K, Huang Y, Chen R, Pan Q, Li J, Xi X. The protective effect of ginsenoside Rg1 against sepsis-induced lung injury through PI3K-Akt pathway: insights from molecular dynamics simulation and experimental validation. Sci Rep. 2024;14(1):16071.

Publisher's Note