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Research article

Running title: LncRNA CASC7 and LPS liver injury

Long non-coding RNA (lncRNA) cancer susceptibility candidate 7 (CASC7) contributes to the progression of lipopolysaccharide (LPS)-induced liver injury by targeting microRNA-217 (miR-217)/toll-like receptor 4 (TLR4) axis

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**Data availability:** The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.
ABSTRACT

It has been reported that long non-coding RNAs (lncRNAs) are involved in sepsis-induced liver injury, while the role of cancer susceptibility candidate 7 (CASC7) in liver injury induced by sepsis remains elusive. In our study, 62 patients and 55 healthy controls were enrolled from our hospital, from whom CASC7 and miR-217 in serum samples were detected by quantitative real-time PCR (qRT-PCR). Then the sepsis-induced liver injury mice model was established by lipopolysaccharide (LPS). The effect of CASC7 on liver injury induced by sepsis was confirmed by Hematoxylin and Eosin (HE) staining, ELISA assay, TUNEL assay, Annexin V-FITC Apoptosis assay and cell counting kit-8 (CCK-8) assay respectively. Besides, RNA pull-down, luciferase reporter gene assay, qRT-PCR, and western blot were used to evaluate the underlying mechanisms. In this study, LncRNA CASC7 was significantly increased while miR-217 was significantly decreased in patients with sepsis-induced liver injury compared with that in healthy controls. There was a negative association of CASC7 and miR-217 in serum samples from patients with sepsis-induced liver injury and healthy controls. And CASC7 was upregulated in a time-dependent manner in liver tissues of LPS-treated mice. It was found that knockdown of CASC7 reduced the liver injury induced by LPS in mice. Then In vitro, LPS treatment enhanced cell apoptosis, while knockdown of CASC7 inhibited the role of LPS in cell apoptosis. Moreover, knockdown of CASC7 suppressed the LPS-enhanced TNF-α and IL-1β expression. In addition, microRNA-217 (miR-217) was found to be a target of CASC7, and miR-217 mimic could reverse CASC7-promoted liver injury. Furthermore, toll-like receptor 4 (TLR4) was identified as the target of miR-217, and both CASC7 and miR-217 could downregulated the mRNA and protein level of TLR4. Additionally, TLR4 overexpression could reversed miR-217-inhibited or CASC7-promoted liver injury. Taken together, CASC7 contributes to the progression of LPS-induced liver injury via the miR-217/TLR4 axis.

KEYWORDS: Sepsis, liver injury, CASC7, miR-217, TLR4.
INTRODUCTION

Sepsis is a deadly infection that causes systemic inflammatory response syndrome [1-3], which accounts for nearly 25 to 30 percent of deaths [4, 5]. Sepsis, a critical condition arising from a severe infection, is characterized by the triggering of a systemic inflammatory response syndrome (SIRS) [1-3]. This inflammatory cascade can lead to widespread tissue damage and organ dysfunction, making it a significant contributor to mortality in healthcare settings. According to recent studies, sepsis is responsible for a substantial proportion of deaths in intensive care units, with estimates ranging from 25 to 30 percent [4, 5]. The high mortality rate underscores the urgency of early diagnosis and effective treatment strategies to mitigate the life-threatening consequences of this condition. During sepsis progression, the liver is a potential target of the abnormal inflammation processes [6]. Sepsis-induced liver injury is correlated with various molecular and cellular processes [7]. However, the mechanisms of liver injury induced by sepsis are were still poorly understood.

Long non-coding RNAs (LncRNAs), with over 200 nucleotides in length, have been shown to interact with RNAs, DNAs and proteins to modulate transcription, chromatin remodeling and post-transcriptional modification [8]. These LncRNAs also play important roles in various physiological processes such as cardiovascular diseases, cell cycle, apoptosis, differentiation, and liver disease. In fact, studies have shown that LncRNAs are crucial in sepsis or LPS-induced injury [9, 10]. The aberrant regulations of LncRNAs have been observed in sepsis and are linked with sepsis-induced organ damage. LncRNA NEAT1 increases the inflammation response in liver injury induced by sepsis by regulating the the Let-7a/TLR4 (Toll-like receptor 4) signaling pathway [11]. LncRNA MALAT1 modulates sepsis-produced cardiac dysfunction and inflammation by interacting with p38/MAPK/NF-κB and miR-125b [12]. LncRNA H19 serves as a competitive endogenous RNA of aquaporin 1 to mediate the expression of miR-874 in sepsis induced by LPS [13]. LncRNA
HOTAIR elevates the expression of TNF-α by activating the NF-κB pathway in cardiomyocytes of the LPS-produced sepsis mouse model [14]. In addition, IncRNA CASC7 (CASC7) is involved in multiple disease models, such as cancer and spinal cord ischemia-reperfusion injury [15, 16]. However, the function of CASC7 in sepsis is unknown.

MicroRNAs (miRNAs) are 20-25 nucleotides in length and play important roles in numerous biological processes [17]. One of their key functions is to regulate gene expression by binding to the 3′-untranslated region (3′-UTR) of mRNAs [18]. These miRNAs have also been implicated in the progression of liver injury caused by sepsis. For instance, miR-155 worsens liver injury by targeting Nrf-2 and inducing mitochondrial and ER stress through oxidative stress [19]. Increased serum expression levels of miR-122 served as an independent liver injury biomarker in inflammatory disorders [20]. Meanwhile, miR-217 was shown to be involved in inflammation-related damage [21]. Toll-like receptor 4 (TLR4) is an essential regulator in multiple physiological and pathological processes including liver disease, and it has been identified to be involved in sepsis-related liver injury [22]. MiR-217 can target TLR4 to modulate podocyte apoptosis [23]. However, the involvement of the miR-217/TLR4 axis in CASC7-regulated sepsis-induced liver injury remains uncertain.

As an endotoxin, lipopolysaccharide (LPS) interacts with receptors on the surface of endothelial cells, resulting in acute inflammation [24]. LPS is known to cause sepsis by modulating oxidative stress or inflammatory factors, and the growth of endothelial cells [25]. In this study, we aimed to investigate and validate the involvement of CASC7 in LPS-induced liver injury associated with sepsis. A sepsis mouse model was created by administering LPS to BALB/c mice. In various studies, both in vitro and in vivo models induced by LPS have been utilized to confirm its effects on liver injury [26, 27]. We hypothesized that CASC7 might regulate LPS-induced liver injury
progression via the miR-217/TLR4 axis. Our assay study aimed to evaluate confirm the functions roles and mechanisms of CASC7 in LPS-induced liver injury progression. This study can provide a potential therapeutic target for sepsis-induced liver injury.

MATERIALS AND METHODS

Patients and tissue specimens
In this study, 62 sepsis patients with liver injury and 55 healthy controls were enrolled from The First People’s Hospital of Gui Yang during January 2016 and March 2021 (Table 1). Serum samples were collected to detect the expression of CASC7, and miR-217 using q-PCR. The study protocol was approved by Ethical committee of The First People’s Hospital of Gui Yang (No.87645) according to the Helsinki declaration. Written informed consent was obtained from each patient.

Sepsis mouse model
BALB/c mice was used to establish the sepsis mouse model by lipopolysaccharide (LPS) treatment. LPS from Escherichia coli was procured from Sigma-Aldrich Co., LLC. (St. Louis, MO, USA). Briefly, BALB/c mice (male, four-week-old) (n = 25) were fed in a humidity- and temperature-regulated room with 12 h/12 h dark/light circle as well as free access to water and food. LPS (20 mg/kg) or the equal volume of saline was intraperitoneally injected into mice. Mice were treated with 1 μg/mL LPS to mimic sepsis in vitro, and 1 μg/mL LPS for different time points (6, 12, 24 and 48 h). Meanwhile, 1 μg/mL LPS treatment for 12 h was used for further functional assays. The mice were injected with lentivirus carrying the CASC7 shRNA (GenePharma, China) or corresponding control through tail vein. Hematoxylin and Eosin (HE) staining was performed to evaluate the liver injury in mice. ELISA assays were performed to measure the expression levels of TNF-α and IL-1β in mice. All procedures and animal care were authorized by the Animal Ethics
Committee of the First People’s Hospital of Guiyang (No. 67663).

**Cell culture and transfection**
LO2 liver cells (human) were purchased from American Type Tissue Culture Collection. Cells were cultured in DMEM (Gibco, USA) containing 100 units/mL penicillin (Solarbio, China), 0.1 mg/mL streptomycin (Solarbio, China) and 10% fetal bovine serum (Gibco, USA) at 37 °C with 5% CO2. LO2 cells were infected with the lentivirus (shCASC7 and shNC, MOI = 25) to stably knockdown CASC7. shNC, 5′- TGGACACTGTTGACCTCACTAATAA-3′, shCASC7′ 5'- TGGGAAACACATGGTCCAGCACTTTAA-3’. The lentivirus and infection regent HitransG A were obtained from Genechem (Shanghai, China). After incubation with 2 mg/ml puromycin for 48 h, shCASC7 and shNC LO2 cells were harvested by trypsin digestion and centrifugation. The lentivirus system (oe-CASC7 and oe-NC, MOI = 50) was used to infect LO2 cells to overexpress CASC7. Compared with the overexpression of full-length CASC7 lentiviral production (oe-CASC7), oe-NC is an empty lentiviral vector and used as a control. miR-217 mimic and miRNA NC were provided by Invitrogen (USA). Cell transfection was performed using lipofectamine 2000 (Invitrogen, USA) following the manufacturer’s instructions.

**Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)**
TUNEL detection kit (Roche, Germany) was used to detect cell apoptosis following the manufacturer’s instructions. After TUNEL staining, the liver samples were dyed by DAPI (Sigma, USA) to stain nuclear. Fluorescence was observed using a confocal microscope (Olympus Fluoview1000, Tokyo, Japan) [28].

**Histology and ELISA analyses**
Prior to paraffin embedding, livers were fixed in 4% paraformaldehyde at room temperature overnight and then transferred to 70% ethanol. Subsequently, organs were embedded and frozen using liquid nitrogen-cooled isopentane. Paraffin-embedded samples were then sectioned at 4-μm
thickness. For pathological analysis, paraffin sections were stained with HE (Nanjing Jiancheng Bioengineering Institute, China). The sections were observed under an optical microscope (CKX31SF; Olympus Corporation, Tokyo, Japan). Prior to ELISA analysis, liver samples were mechanically homogenized in protease-inhibitor (Sigma-Aldrich, USA) containing phosphate-buffered saline, and the homogenates were centrifuged at 11,330 g at 4 °C for 30 min. Protein concentrations of the supernatant fraction were measured using a bicinchoninic acid (BCA) protein measurement kit (R&D Systems, Minneapolis, MN, USA). TNF-α and IL-1β ELISA kits were purchased from R&D Systems. The sheep anti-mouse polyclonal antibody (1:200, MTA00B) was used to detect TNF-α, and mouse monoclonal antibody (1:1,000, MLB00C) was used to detect IL-1β [29].

**Cell counting kit-8 (CCK-8) assay**

CCK-8 assay was used to detect cell viability. Cells (5 × 103) were seeded into 96 wells and cultured for 12 h. Next, CCK-8 solution (KeyGEN Biotech, China) was added at 0, 24, 48, 72 and 96 h and cultured at 37 °C for another 2 h. ELISA browser was applied to analyze the results at the absorbance of 450 nm (Bio-Tek EL 800, USA) [30].

**Analysis of cell apoptosis**

To detect cell apoptosis, cells were seeded into 6-well dishes with 2 × 105 cells/well. Annexin V-FITC Apoptosis Detection Kit (CST, USA) was used to evaluate cell apoptosis following the manufacturer’s instructions. Briefly, binding buffer (BD Biosciences) was used to collect and wash the cells. Cells were dyed at 25 °C and analyzed by flow cytometry assay [31].

**Quantitative reverse transcription-PCR (qRT-PCR)**

TRIZOL (Invitrogen, USA) was used to extract the total RNAs from mice and cell tissues. The cDNA Synthesis Kit (Thermo, USA) was used to synthesize the first-strand cDNA following the manufacturer’s instructions. SYBR Real-time PCR I kit (Takara, Japan) was used to perform qRT-
PCR. GAPDH and U6 were used as the internal control for mRNA and lncRNA, respectively. SYBR Green Premix Ex TaqTM II Kit (TaKaRa, Japan) was used to detect the expression levels of genes [32]. The primer sequences are as follows:

CASC7 forward: 5′-ATCAACGTAAGCTGGGAGG-3′;
CASC7 reverse: 5′-CTTGTCCCCCGCTCGTTC-3′;
TLR4 forward: 5′-TGGATACGTTTCTTATAAG-3′;
TLR4 reverse: 5′-GAAATGGAGGCACCCCTTC-3′;
MiR-217 forward: 5′-CATGCTCGAGCTTATCAAGGATAAAATACCATG-3′;
MiR-217 reverse: 5′-GTTACGGCCGCTTGAGATCTACTCTATTCTTTTTTAAC-3′;
GAPDH forward: 5′-AAGAAGGTGGTGAAGGCG-3′;
GAPDH reverse: 5′-TCCACCACCCAGCTTGCTGTA-3′;
U6 forward: 5′-GCTTCGGCAGCACATATACTAA-3′;
U6 reverse: 5′-AACGCTTCACGAATTTCGCT-3′.

Western blot analysis
RIPA buffer (CST, USA) was used to extract total proteins from cells or liver of mice. BCA Protein Quantification Kit (Abbkine, USA) was used to measure protein concentrations. Next, SDS-PAGE (12% polyacrylamide gels) was used to separate the protein samples, which were then transferred to PVDF membranes (Millipore, USA). After that, 5% milk was used to hinder the membranes, followed by incubation with primary antibodies for PARP TLR4 (1:1,000) (Abcam, USA), cleaved PARP (1:1,000) (Abcam, USA), caspase3 (1:1,000) (Abcam, USA), cleaved caspase3 (1:1,000) (Abcam, USA) and GAPDH (1:1,000) (Abcam, USA) were added at 4 °C overnight, GAPDH was
used as the control. Then, for hatching the membranes, corresponding second antibodies (1:1,000) (Abcam, USA) were used for incubation at room temperature for 1 h. Images were visualized using an Odyssey CLx Infrared Imaging System. The results of Western blot were quantified by ImageJ software [33].

**Luciferase reporter gene assay**  
The wild-type (WT) sequence and mutant-type (MUT) sequence (binding site mutation with miR-21) of CASC7 and TLR4 were amplified and cloned into PGL4 reporter plasmid, respectively. LO2 cells were cotransfected with the fusion plasmid and miR-21 or miR-NC. At 48 h post-transfection, the luciferase activity in these transfected cells was examined using the Dual Luciferase Assay System (Promega, Madison, WI, USA) following the manufacturer’s instructions [34].

**RNA pull-down assay**  
Using biotin-UTP of MEGAscript T7 Kit (Thermo, USA) and MEGAclear Kit (Thermo, USA), biotin-marked RNAs were transcribed in vitro and purified following the manufacturer’s instructions, and then incubated with the cell lysates [35]. Streptavidin beads were used to isolate the biotin-labeled transcripts and interacted RNAs, which were then subjected to qPCR analysis.

**Ethical statement**  
This study was carried out in accordance with the ethical guidelines of the Declaration of Helsinki and approved by the ethics and research committees of The First People’s Hospital of Gui Yang (No. 67663). Consent to participate Not applicable. Consent for publication Not applicable.

**Statistical analysis**  
All experiments were repeated at least for three times independently. Data were presented as mean ± standard deviation (SD). GraphPad Prism 7 software was used to conduct statistical analysis.
Unpaired Student’s t-test was applied to compare two groups. One-way ANOVA was applied to compare multiple groups. P < 0.05 was considered as statistically significant.

RESULTS
Expression of IncRNA CASC7 and miR-217 in patients with sepsis-induced liver injury
Compared with that in healthy controls, the expression of IncRNA CASC7 in sepsis patients with liver injury was significantly increased (Fig. 1A), while miR-217 expression was significantly decreased (Fig. 1B). Then, the correlation analysis showed that there was a negative association of CASC7 expression and miR-217 expression both in healthy controls (Fig. 1C) and sepsis patients with liver injury (Fig. 1D).

The expression of CASC7 is positively correlated with liver injury induced by LPS
To explore whether CASC7 was involved in sepsis-related liver injury, the sepsis mouse model was established by LPS treatment. The results showed that LPS could induce liver injury including disorder of liver structure, infiltration of neutrophils into the portal area and hepatic sinusoid, cytoplasm rarefaction, nodular necrosis, and karyopyknosis in a time-dependent manner (Fig. 2A). Meanwhile, the expression levels of ALT, TNF-α and IL-1β were increased in a time-dependent manner (Fig. 2B and C, P < 0.01). Furthermore, the expression levels of CASC7 were elevated by LPS treatment in the mouse liver tissues (Fig. 2D, P < 0.01) and LO2 cells in a time-dependent manner (Fig. 2E, P < 0.01). Together, these data suggested that the expression of CASC7 was positively correlated with LPS-induced liver injury.

Knockdown of CASC7 relieves liver injury induced by LPS in vivo
Next, the effect of CASC7 on the progression of LPS-induced liver injury was investigated in vivo. Firstly, specific short hairpin RNAs (shRNAs) against CASC7 was used to knockdown the expression of CASC7. By transfecting three CASC7 shRNAs, we found that sh-CASC7#1 could significantly downregulate the expression of CASC7 (Fig. 3A, P < 0.05). Knockdown of CASC7
significantly decreased the liver injury induced by LPS in mice (Fig. 3B). TUNEL assay showed that LPS treatment increased TUNEL-positive cell number, while knockdown of CASC7 blocked this increase, indicating that knockdown of CASC7 attenuated liver injury induced by LPS (Fig. 3C, P < 0.05). In addition, it was found that knockdown of CASC7 inhibited LPS-enhanced expression of ALT (Fig. 3D), TNF-α (Fig. 3E) and IL-1β (Fig. 3F) in mice (P < 0.05) (Fig. 3D and E). Altogether, these results suggested that knockdown of CASC7 relieves LPS-induced liver injury in vivo.

**MiR-217 is a target of CASC7**

Next, we explored the mechanism of CASC7 in liver injury induced by LPS, and we found that the expression levels of miR-217 were reduced by LPS treatment in a time-dependent manner in the mouse liver tissues (Fig. 4A, P < 0.05). Similarly, LPS treatment down-regulated the expression of miR-217 time-dependently in the LO2 cells (Fig. 4B, P < 0.05). Meanwhile, knockdown of CASC7 enhanced (P < 0.05), and overexpression of CASC7 inhibited (P < 0.001) the expression of miR-217 in LO2 cells (Fig. 4C and D). Then, the potential interaction between CASC7 and MiR-217 was predicted by Starbase 3.0v software (Fig. 4E). The efficiency of miR-217 mimic was detected in LO2 cells (Fig. 4F, P < 0.05). The miR-217 mimic attenuated the luciferase activities of CASC7, but not CASC7 with miR-217-binding site mutant in LO2 cells (Fig. 4G, P < 0.05). In addition, RNA pull-down assay showed that wild-type Bio-miR-217, but not mutant Bio-miR-217, could interact with CASC7 (Fig. 4H, P < 0.01). Altogether, these results suggested that miR-217 is a target of CASC7. CASC7 promoted LPS-induced liver injury progression by targeting miR-217. We then evaluated whether CASC7 promoted the progression of liver injury induced by LPS by targeting miR-217 in the liver cells. The results showed that Overexpression of CASC7 inhibited the cell viability, while miR-217 mimic could rescue the cell viability inhibited by CASC7 overexpression in LPS-treated LO2 cells.
The treatment of miR-217 mimic was able to reverse the CASC7 overexpression-enhanced expression levels of IL-1β and TNF-α in LO2 cells treated by LPS (Fig. 5B, P < 0.05). The CASC7 overexpression-induced apoptosis was reduced by miR-217 mimic in LPS-treated LO2 cells (P < 0.05) (Fig. 5C). The elevated expression of cleaved caspase3 (c-caspase3) and cleaved PARP (c-PARP) by overexpression of CASC7 was reduced by miR-217 mimic treatment in LPS-treated LO2 cells (Fig. 5D, P < 0.05). Altogether, these results indicated that CASC7 promotes the progression of liver injury induced by LPS by targeting miR-217 in vitro.

**MiR-217 attenuates liver injury induced by CASC7 by targeting TLR4 in vitro**

Next, we explored downstream target of miR-217 in vitro and found targeted site of miR-217 in 3’-UTR of TLR4 (Fig. 6A). The miR-217 mimic attenuated the luciferase activities of TLR4 but not the TLR4 with miR-217-binding site mutant in LO2 cells (Fig. 6B, P < 0.01). The expression levels of TLR4 were significantly reduced by overexpression of miR-217 at both mRNA (P < 0.01) and protein (P < 0.05) levels (Fig. 6C and D). In addition, knockdown of CASC7 down-regulated, while overexpression of CASC7 up-regulated the expression of TLR4 at mRNA (P < 0.01) and protein (P < 0.05) levels in vitro (Fig. 6E and F). Knockdown of CASC7 reduced the LPS-elevated expression levels of TLR4 in vitro (Fig. 6G and I, P < 0.01). The efficiency of TLR4 overexpression was validated in vitro (Fig. 6H, P < 0.01). The overexpression of TLR4 could block the miR-217 mimic-increased or CASC7 decreased LO2 cell viability (Fig. 7A, P < 0.05). The miR-217 mimic-attenuated cell apoptosis or CASC7 increased cell apoptosis was enhanced by the overexpression of TLR4 in the cells (Fig. 7B, P < 0.05). Altogether, these results suggested that miR-217 attenuates liver injury induced by CASC7 by targeting TLR4 in vitro.
DISCUSSION
Sepsis is a systemic inflammatory disease caused by postoperative infections, severe trauma, and or burns, leading to multiple organ failure including liver injury [36]. The incidence of occurrence and death of liver injury induced by sepsis is high [6]. Nevertheless, the mechanism of sepsis-induced liver injury is still elusive. In this study, CASC7 was found to contribute to LPS-induced liver injury progression by modulating the miR-217/TLR4 axis.

It has been well-identified that multiple lncRNAs are involved in sepsis-induced liver injury development. For example, lncRNA colorectal neoplasia relieves sepsis-caused liver damage by targeting miR-126-5p [37]. Circulating lncRNA NEAT1 is correlated with an unfavorable prognosis, high severity, and increased risk in sepsis patients [38]. LncRNA SNHG16 changes miR-15a/16 consequences on the inflammation pathway induced by LPS [39]. LPS increases sepsis development by stimulating the HULC/miR-204-5p/TRPM7 axis in HUVECs [40]. LncRNA TapSAKI increases inflammatory injury and urine-derived sepsis injury [41]. The impact of lncRNA HOTAIR on serious organ injury in the sepsis rat has been reported to be mediated by the miR-34a/Bcl-2 axis [42]. LncRNA NEAT1 plays an essential role in severe injury produced by sepsis by mediating miR-204 and the NF-κB signaling [43]. The enhanced expression of lncRNA HULC and UCA1 is a necessary condition for the pro-inflammatory response of endothelial cell sepsis induced by LPS [44]. Previous studies have shown that hydrogen sulfide upregulated lncRNA CasC7 to reduce neuronal cell apoptosis in spinal cord ischemia-reperfusion injury rat [16]. In the present study, we identified that the expression of CASC7 was elevated in LPS-treated mice. It suggests that CASC7 is involved in LPS-induced liver injury.

As a primary component of ncRNA in the pathological and physiological processes, miRNAs involve in the modulation of liver injury induced by sepsis. It has been reported that miR-155
regulates the JAK/STAT signaling pathway and further regulates sepsis-caused liver damage [45]. Paclitaxel alleviates liver damage of the septic mouse by regulating the miR-27a/TAB3/NF-κB axis [46]. MCPIP1 alleviates liver injury induced by LPS by regulating the expression of SIRT1 mediated by miR-9 [47]. MiR-103a-3p can inhibit liver injury induced by sepsis by mediating HMGB1 [48]. MiR-21 was also shown to be involved in sepsis [49]. MiR-195 inhibits multiple organ injury and apoptosis in the sepsis mouse models [50]. MiR-30e represses apoptosis and increases hepatocyte proliferation in puncture and cecal ligation-induced sepsis by regulating the JAK/STAT signaling mediated by FOSL2 [51]. Puncture and cecal ligation-induced sepsis is correlated with the inhibited expression of adenylyl cyclase nine and enhanced expression of miR-142-3p [52]. Our data demonstrated that CASC7 targeted on miR-217 in liver cells and promoted LPS-induced liver injury progression by sponging miR-217. Our findings present valuable information that miR-217 is involved in CASC7-mediated liver injury progression induced by LPS.

TLR4 also involves in the development of sepsis-induced liver injury. For example, by inhibiting the high-mobility group box protein B1 (HMGB1) signaling pathway, TLR4 antagonist eritoran tetrasodium to inhibit hepatic ischemia-reperfusion injury [53]. Dexmedetomidine-regulated the attenuation against sepsis liver damage through downregulation of the TLR4 and MyD88/NF-κB signaling [54]. Trichostatin protects the liver against sepsis damage by inhibiting the TLR4 signaling [55]. Green tea extract can restore liver metabolism, and its effect is related to the inhibition of inflammation regulated by endotoxemia/TLR4/NF-κB [56]. In addition, by inhibiting LPS-TLR4 interactions, leukuadhesin-1 protects against endotoxic shock in mice [57]. We revealed that miR-217 attenuated liver injury induced by sepsis by targeting TLR4. It indicates that TLR4 involves in the modulation of LPS-induced liver injury. Then both CASC7 and miR-
217 could regulate the expression of TLR4 in vitro. Additionally, TLR4 overexpression could reversed CASC7/miR-217 function in vitro.

CONCLUSION
In conclusion, we found that CASC7 contributed to sepsis-induced liver injury progression by targeting the miR-217/TLR4 axis. CASC7, miR-217 and TLR4 may serve as potential targets for LPS-induced liver injury treatment. Our results help to expand the understanding of molecular mechanisms involved in sepsis.

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### TABLES AND FIGURES WITH LEGENDS

**Table 1.** Clinical characteristics of sepsis patients (n=62) and healthy controls (n=55)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Healthy controls</th>
<th>Patients</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55±12</td>
<td>56±15</td>
<td>0.12</td>
</tr>
<tr>
<td>Gender (male, %)</td>
<td>36</td>
<td>43</td>
<td>0.32</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.33±3.22</td>
<td>26.72±3.13</td>
<td>0.72</td>
</tr>
<tr>
<td>WBC count (10⁹/L)</td>
<td>12.45±6.23</td>
<td>17.22±7.23</td>
<td>0.032</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>45.26±18.34</td>
<td>68.56±20.56</td>
<td>0.015</td>
</tr>
<tr>
<td>Procalcitonin (ng/mL)</td>
<td>7.82±4.11</td>
<td>15.36±6.58</td>
<td>0.008</td>
</tr>
<tr>
<td>BUN (mmol/l)</td>
<td>9.32±3.23</td>
<td>19.33±5.32</td>
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<tr>
<td>Serum creatinine (µM)</td>
<td>78.56±32.54</td>
<td>286.22±89.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ccr (ml/min 1.73 m²)</td>
<td>71.23±13.35</td>
<td>38.22±11.56</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BMI: Body mass index; WBC: White blood cell; CRP: C-reactive protein; BUN: Blood urea nitrogen; Ccr: Creatinine clearance rate.
Figure 1. CASC7 expression was negatively associated with miR-217 in clinical. The expression of CASC7 (A) and miR-217 (B) was detected in patients with sepsis with liver injury. Then analysis of these CASC7 expression and miR-217 expression in healthy controls (C) and patients with liver injury (D) was identified. * P < 0.05. All experiments were performed in triplicate.
Figure 2. CASC7 expression is positively correlated with the liver injury induced by sepsis.

(A-C) BALB/c mice were used to establish the sepsis mouse model by the treatment of lipopolysaccharide (LPS concentration = 1 μg/ml) (n = 5). (A) Hematoxylin and Eosin (HE) staining was used to analyze the liver injury at the indicated time in the mice. (B and C) ALT, TNF-α and IL-1β levels were measured by ELISA assays at the indicated time in mice. (D) CASC7 expression was tested by qPCR assays at the indicated time in the liver tissues of the mice. (E) The LO2 cells were treated with LPS (1 μg/ml). qPCR assays were used to determine the expression of CASC7 at the indicated time in the cells. Statistic significant differences were indicated: * P < 0.05, ** P < 0.01. Data are presented as mean ± SD. All experiments were performed in triplicate.
Figure 3. CASC7 depletion relieves sepsis-induced liver injury in vivo. (A-D) LPS or co-treated with LPS and control shRNA or CASC7 shRNA were used to treat BALB/c mice. (A) CASC7 expression was tested by qPCR assays at the indicated time in the liver tissues of the mice. (B) HE staining was used to analyze the liver injury in mice. (C) TUNEL analysis was used to
measure the apoptosis in liver tissues of mice. (D and E) ALT, (E) TNF-α and (F) IL-1β levels were measured by the ELISA assays in the mice. Statistic significant differences were indicated: * P < 0.05, ** P < 0.01. Data are presented as mean ± SD. All experiments were performed in triplicate.

**Figure 4.** CASC7 targets miR-217 in liver cells. (A) BALB/c mice were used to establish the sepsis mouse model by the treatment of lipopolysaccharide (LPS, 20 mg/kg) (n = 5). MiR-217 expression was tested by qPCR assays at indicated time in liver tissues of mice. (B) LPS (1 μg/ml) was used to treat the LO2 cells. MiR-217 expression was determined by qPCR assays at the indicated time in the cells. (C) Control shRNA or CASC7 shRNA were transfected into LO2 cells. MiR-217 expression was assessed by qPCR assays. (D) MiR-217 expression was detected in LO2 cells transfected with pcCASC7. (E) Using Starbase 3.0v software, the potential interaction
between CASC7 and miR-217 was identified. (F) MiR-217 expression was determined by qPCR assays. (G) The luciferase activities of wild type CASC7 (CASC7 WT), and CASC7 with the miR-217-binding site mutant (CASC7 MUT). (H) RNA pull-down assay was used to analyze the interaction of CASC7 and miR-217. Statistic significant differences were indicated: * P < 0.05, ** P < 0.01. Data are presented as mean ± SD. All experiments were performed in triplicate.

Figure 5. CASC7 promotes LPS-induced liver injury progression by targeting miR-217. (A-D) The LO2 cells were co-treated with LPS (1 μg/ml) and CASC7 overexpression vector, or LPS (1 μg/ml), or co-treated with LPS (1 μg/ml), CASC7 overexpression vector, and miR-217 mimic. (A) CCK-8 assay was used to measure the cell viability. (B) TNF-α and IL-1β levels were measured by the ELISA assays in the cells. (C) Flow cytometry was used to measure cell apoptosis. (D) PARP, cleaved PARP (c-PARP), caspase3, cleaved caspase3 (c-caspase3), and GAPDH
expression was evaluated by Western blot. Statistic significant differences were indicated: * P < 0.05, ** P < 0.01. Data are presented as mean ± SD. All experiments were performed in triplicate.

Figure 6. MiR-217 targets TLR4 in liver cells, and TLR4 expression could be regulated by CASC7 or MiR-217. (A) Using miRDB and miRmap software, the interaction of miR-217 and TLR4 3’ UTR was identified. (B and C) The LO2 cells were treated with control mimic or miR-
217 mimic. (B) The luciferase activities of TLR4 with the miR-217-binding site mutant (TLR4 MUT) and wild type TLR4 (TLR4 WT) were determined in cells. (C and D) TLR4 expression was measured by qRT-PCR and western blot assay in cells. (E and F) LO2 cells were treated CASC7 shRNA, CASC7 overexpression vector, or the corresponding control. TLR4 expression was tested by western blot and qRT-PCR. (G) LPS (1 μg/ml), co-treated with LPS (1 μg/ml) and control shRNA or CASC7 shRNA were transfected into LO2 cells. TLR4 expression was assessed by western blot and qRT-PCR. (H and I) LO2 cells were transfected with TLR4 overexpression vector or control vector. western blot and qRT-PCR assay were used to measure TLR4 expression. Statistic significant differences were indicated: * P < 0.05, ** P < 0.01. Data are presented as mean ± SD. All experiments were performed in triplicate.

Figure 7. TLR4 could reversed the miR-217 mimic-increased or CASC7 decreased function in liver cells. (a) CCK-8 assay was used to measure cell viability. (b) Flow cytometry was used
to measure cell apoptosis. Statistic significant differences were indicated: * P < 0.05, ** P < 0.01.

Data are presented as mean ± SD. All experiments were performed in triplicate.