Long non-coding RNA CASC7 Contributes to the Progression of Sepsis-Induced Liver Injury by Targeting miR-217/TLR4 Axis

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Abstract

Background: Sepsis is a system inflammation disease that can lead to liver injury. Long non-coding RNAs as crucial regulators participate in the regulation of sepsis-induced liver injury. However, the role of lncRNA CASC7 (CASC7) in the modulation of sepsis-induced liver injury remains elusive. Here, we aimed to explore the effect of CASC7 on the sepsis-induced liver injury.

Methods: The sepsis mouse model was established in BALB/c mice by the treatment of lipopolysaccharide (LPS). The effect of CASC7 on sepsis-induced liver injury was analyzed by Hematoxylin and Eosin (HE) staining, ELISA assays, TUNEL detection kit, CCK-8 assays, and Annexin V-FITC Apoptosis Detection Kit *in vivo* or *in vitro*. The mechanism investigation was performed using RNA pull-down, luciferase reporter gene assays, qPCR assays, and Western blot analysis.

Results: The expression of CASC7 was elevated in a time-dependent manner in the liver tissues of the sepsis mice and LPS-treated LO2 cells. The depletion of CASC7 decreased the LPS treatment-induced liver injury in the sepsis mice. The treatment of LPS enhanced the apoptosis in the sepsis mice, while the depletion of CASC7 blocked this enhancement in the system. The CASC7 knockdown inhibited the LPS-enhanced expression of TNF-α and IL-1β in the mice. CASC7 served as a sponge for the miR-217 in the liver cells. CASC7 promoted the progression of sepsis-induced liver injury by sponging miR-217. MiR-217 attenuated sepsis-induced liver injury by targeting TLR4.

Conclusions: Thus, we conclude that CASC7 contributes to the progression of sepsis-induced liver injury by targeting miR-217/TLR4 axis.

Background

Sepsis is an expensive, worldwide and deadly disease induced by the infection that causes systemic inflammatory response syndrome[1]. Sepsis occurs during the body replies to the infection, and next leads to injury to the organs or tissues, in which the incidence of sepsis death is nearly 25%-30%[2, 3]. During sepsis progression, the liver is a potential target of the abnormal inflammation processes [4]. It has been recognized that high rates and clinic death of sepsis-induced liver injury give it a significant public well-being problem[5]. Sepsis-induced liver injury is correlated with various molecular and cellular processes[6]. During the development of sepsis-induced liver injury, inflammation cytokine is able to damage various tissues or organs and dysregulate the immune response[7]. IL-1β and Tumor necrosis factor (TNF)-α control the immune response in the early stage, which are remarkably expressed and secreted by immune cells for transcriptional and post-translational modulation[8]. However, the mechanism of sepsis-induced liver injury is still poorly understood.

Long non-coding RNAs (LncRNAs) serve as the non-coding molecules with over 200 nucleotides, interacting with RNAs, DNAs and proteins to modulate transcription, chromatin remodeling and post-transcriptional modification[9]. LncRNAs is the emerging critical biological regulator and plays multiple roles in many physiological and pathological processes such as cell cycle, differentiation, apoptosis,

cardiovascular diseases, and liver disease[10, 11]. The aberrant regulation of lncRNAs have been observed in sepsis, and these lncRNAs may be linked with the development of sepsis-induced organ damage. It has been reported that LncRNA NEAT1 increases the inflammation response in sepsis-caused liver injury by the Let-7a/TLR4 signaling[12]. LncRNA MALAT1 modulates sepsis-produced cardiac dysfunction and inflammation by interacting with miR-125b and p38/MAPK/NF-κB[13]. LncRNA H19 serves as a competitive endogenous RNA of Aquaporin 1 to mediate the expression of miR-874in LPS-induced sepsis[14]. LncRNA HOTAIR elevates TNF-α production by the activation of the NF-κB pathway in the cardiomyocytes from the LPS-produced sepsis mouse model[15]. LncRNA CASC7 serves as a well-studied lncRNA in multiple disease models, such as cancer and spinal cord ischemia-reperfusion injury[16, 17]. However, the role of lncRNA CASC7 in the modulation of the progression of sepsis-induced liver injury remains unclear.

MicroRNAs (miRNAs) are identified as short non-coding RNAs with a length of approximately 20-25 nucleotides, which exert significant impacts on numerous biological processes[18]. MiRNAs are able to control gene expression in the post-transcriptional levels by pairing with target mRNAs at the 3' untranslated region (3' UTR)[19]. A substantial number of investigations have revealed that miRNAs are involved in the progression of sepsis-induced liver injury. For example, it has been reported that miR-155 aggravates the liver injury by regulating mitochondrial dysfunction and oxidative stress-mediated ER stress through targeting Nrf-2[20]. Increased serum levels of miR-122 serve as an independent biomarker of liver injury in inflammatory disorders[21]. Meanwhile, the role of miR-217 in the inflammation-related damage has been reported[22]. Moreover, Toll-like receptor 4 (TLR4), as an essential regulator in multiple physiological and pathological processes, including liver disease, has been identified to participate in the regulation of sepsis-related liver injury[23]. In addition, miR-217 is able to target TLR4 to modulate podocyte apoptosis[24]. However, the correlation of lncRNA CASC7 with miR-217 and TLR4 in the modulation of sepsis-induced liver injury is still elusive.

Lipopolysaccharide (LPS) serves as the endotoxin that is issued by bacterial membranes and interacts with receptors on the surface of endothelial cells, whereby working as a noxious factor that produces acute inflammation[25]. LPS has been identified to cause sepsis by modulating the oxidative stress or inflammatory factors, and the growth of endothelial cells[26]. In this study, we aimed to explore the function of lncRNA CASC7 in the development of sepsis-induced liver injury. The sepsis mouse model was established in BALB/c mice by the treatment of lipopolysaccharide (LPS). The effect of CASC7 and the underlying mechanism on the sepsis-induced liver injury were investigated in the sepsis mouse model and LPS-treated liver cells. We identified that CASC7 contributed to the progression of sepsis-induced liver injury by targeting miR-217/TLR4 axis.

Methods

Sepsis mouse model

The sepsis mouse model was established in BALB/c mice by the treatment of lipopolysaccharide (LPS, sigma, USA). The BALB/c mice (male, four-week-old) were purchased from the Academy of Military Medical Sciences (Beijing, China). Briefly, the BALB/c mice (male, four-week-old) (n=5) were saved in a humidity- and temperature-regulated place under the 12-hours usual dark/light circle with water and food. The mice were intraperitoneally injected with LPS (20 mg/kg) or the equal volume of saline. The mice were injected with the lentivirus carrying the CASC7 shRNA or corresponding control through tail vein. The lentivirus carrying the CASC7 shRNA corresponding control were obtained (GenePharma, China). The mice were euthanatized by cervical dislocation (CD) and harvest liver tissue and plasma samples for further analysis. The liver injury was analyzed by the Hematoxylin and Eosin (HE) staining in the mice. The levels of TNF-α and IL-1β were measured by the ELISA assays in the mice. Animal care and method procedure were authorized by the Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences Animal Ethics Committee.

Cell culture and treatment

The human liver LO2 cells were purchased in American Type Tissue Culture Collection. The cells were cultured in the medium of DMEM (Gibco, USA) containing 10% fetal bovine serum (Gibco, USA), 0.1 mg/mL streptomycin (Solarbio, China) and 100 units/mL penicillin (Solarbio, China) at a condition of 37 °C with 5% CO_2. The lentivirus carrying the CASC7 shRNA, miR-217 mimic, CASC7 overexpression vector, TLR4 overexpression vector and corresponding control were obtained (GenePharma, China). The transfection in the cells was performed by Liposome 3000 (Invitrogen, USA) according to the manufacturer's instructions. The levels of TNF-α and IL-1β were measured by the ELISA assays in the cells.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)

The apoptosis was analyzed by using the TUNEL detection kit (Roche, Germany) in the liver tissues of the mice according to the product’s guidance. After the staining of TUNEL, the ventricular samples were dyed by DAPI (Sigma, USA) to stain nuclear. Fluorescence was observed using a confocal microscope (Olympus Fluoview1000, Tokyo, Japan).

CCK-8 assays

The cell viability was analyzed by the CCK-8 assays. About 5×10^3 cells were put into 96 wells and cultured for 12 hours. Then the cells were used for the transfection or treatment. After 0 hours, 24 hours, 48 hours, 72 hours, and 96 hours, the cells were added with a CCK-8 solution (KeyGEN Biotech, China) and culture for another 2 hours at 37°C. The ELISA browser was applied to analyze the absorbance at 450nm (Bio-Tek EL 800, USA).

Analysis of cell apoptosis

Approximately 2×10^5 cells were plated on 6-well dishes. Cell apoptosis was analyzed by using the Annexin V-FITC Apoptosis Detection Kit (CST, USA) according to the manufacture's instruction. Briefly, the cells were collected and washed by binding buffer (BD Biosciences, USA) and were dyed at 25 ℃, followed by the flow cytometry analysis.

Quantitative reverse transcription-PCR (qRT-PCR)

The total RNAs were extracted by TRIZOL (Invitrogen, USA) from the tissues of the mice and cells. The first-strand cDNA was synthesized using Stand cDNA Synthesis Kit (Thermo, USA) as the manufacturer's instruction. The qRT-PCR was carried out by applying SYBR Real-time PCR I kit (Takara, Japan). The standard control for mRNA/lncRNA and miRNA was GAPDH and U6, respectively. Quantitative determination of the RNA levels was conducted by SYBR GreenPremix Ex TaqTM II Kit (TaKaRa, Japan). The primer sequences are as follows:

CASC7 forward: 5'-ATCAACGTCAAGCTGGGAGG-3'

CASC7 reverse: 5'-CTTGTCCCCCGCTCGTTC-3'

TLR4 forward: 5'-TGGATACGTTTCCTTATAAG-3'

TLR4 reverse: 5'-GAAATGGAGGCACCCCTTC-3'

miR-217 forward:

5'-CATGCTCGAGCTTATCAAGGATAAAATACCATG-3'

miR-217 reverse:

5'-GTTACGGCCGCTTGAGATCTACTCTAATTTCTTTTTTAAC-3'

GAPDH forward: 5'-AAGAAGGTGGTGAAGCAGGC-3'.

GAPDH reverse: 5'-TCCACCACCCAGTTGCTGTA-3'

U6 forward: 5'-GCTTCGGCAGCACATATACTAA-3'

U6 reverse: 5'-AACGCTTCACGAATTTGCGT-3'

Western blot analysis

Total proteins were extracted from the cells or tumor tissues of the mice with RIPA buffer (CST, USA). The concentrations of protein were analyzed by using the BCA Protein Quantification Kit (Abbkine, USA). Same concentration of protein was divided by SDS-PAGE (12% polyacrylamide gels), transferred to PVDF membranes (Millipore, USA) in the subsequent step. The membranes were hindered with 5% milk and hatched overnight at 4°C with the primary antibodies for PARP TLR4 (1:1000) (Abcam, USA), cleaved

PARP (1:1000) (Abcam, USA), caspase3 (1:1000) (Abcam, USA), cleaved caspase3 (1:1000) (Abcam, USA) and GAPDH (1:1000) (Abcam, USA), in which GAPDH served as the control. Then, the corresponding second antibodies (1:1000) (Abcam, USA) were used for hatching the membranes 1 hour at room temperature, followed by the visualization by using an Odyssey CLx Infrared Imaging System. The results of Western blot analysis were quantified by ImageJ software.

Luciferase reporter gene assay

The luciferase reporter gene assays were conducted by applying the Dual-luciferase Reporter Assay System (Promega, USA). Briefly, the cells were treated with the miR-21 mimic or control mimic, the vector containing CASC7, CASC7 mutant, TLR4, and TLR4 mutant fragment were transfected in the cells by using Lipofectamine 3000 (Invitrogen, USA), followed by the analysis of luciferase activities, in which Renilla was applied as a normalized control.

RNA pull-down

Biotin-marked RNAs were transcribed by using biotin-UTP of MEGAscript T7 Kit (Thermo, USA) *in vitro* and purified by MEGAclear Kit (Thermo, USA) according to manufacturer's guidance, and then incubated with entire cell lysates. Biotin-labeled transcripts and interacted RNAs were isolated with streptavidin beads and then subjected to qPCR analysis

Statistical analysis

Data were presented as mean ± SD, and the statistical analysis was performed by GraphPad Prism 7 software. The unpaired Student's *t*-test was applied for comparing two groups, and the one-way ANOVA was applied for comparing among multiple groups. $P < 0.05$ were considered as statistically significant.

Results

The expression of lncRNA CASC7 is positively correlated with the sepsis-induced liver injury

To evaluate the correlation of lncRNA CASC7 with the sepsis-induced liver injury, the sepsis mouse model was established in BALB/c mice by the treatment of lipopolysaccharide (LPS). Hematoxylin and Eosin (HE) staining revealed the symptoms of liver injury in a time-dependent manner, such as disorder of liver structure, infiltration of neutrophils into the portal area and hepatic sinusoid, cytoplasm rarefaction, nodular necrosis, and karyopyknosis (Fig. 1A). Meanwhile, the levels of TNF-α and IL-1β were increased in a time-dependent manner in the infiltration of inflammatory cells ($P < 0.01$) (Fig. 1B). Moreover, the expression of lncRNA CASC7 was elevated by the treatment of LPS in a time-dependent manner in the liver tissues of the mice ($P < 0.01$) (Fig. 1C). Similarly, the treatment of LPS time-dependently up-regulated the expression of CASC7 in the LO2 cells ($P < 0.01$) (Fig. 1C). Together these data suggest that the expression of lncRNA CASC7 was positively correlated with the sepsis-induced liver injury.

The depletion of lncRNA CASC7 relieves sepsis-induced liver injury in vivo

Then, we investigated the effect of lncRNA CASC7 on the progression of sepsis-induced liver injury *in vivo*. To this end, the sepsis mice were injected with the lentivirus carrying the CASC7 shRNA or corresponding control shRNA. The efficiency of CASC7 shRNA was validated in the liver tissues of the mice ($P < 0.05$) (Fig. 2A). The depletion of CASC7 significantly decreased the LPS treatment-induced liver injury in the sepsis mice (Fig. 2B). The terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) analysis showed that the treatment of LPS enhanced the TUNEL-positive cells while the depletion of CASC7 could block this enhancement in the system, indicating that the CASC7 depletion attenuated LPS-induced apoptosis ($P < 0.05$) (Fig. 2C). In addition, the CASC7 knockdown inhibited the LPS-enhanced expression of TNF-α and IL-1β in the mice ($P < 0.05$) (Fig. 2D). Together these suggest that the depletion of lncRNA CASC7 relieves sepsis-induced liver injury *in vivo*.

LncRNA CASC7 serves as a sponge for the miR-217 in the liver cells

Next, we tried to explore the mechanism of lncRNA CASC7 in LPS-induced sepsis liver injury. The expression of miR-217 was reduced by the treatment of LPS in a time-dependent manner in the liver tissues of the mice ($P < 0.05$) (Fig. 3A). Similarly, the treatment of LPS time-dependently down-regulated the expression of miR-217 in the LO2 cells ($P < 0.05$) (Fig. 3B). Meanwhile, the depletion of lncRNA CASC7 significantly enhanced the expression of miR-217 in the LO2 cells ($P < 0.05$) (Fig. 3C). The bioinformatic analysis identified the potential interaction between lncRNA CASC7 and miR-217 by using Starbase 3.0v software (Fig. 3D). The efficiency of miR-217 mimic was validated in the LO2 cells ($P < 0.05$) (Fig. 3E). The miR-217 mimic attenuated the luciferase activities of CASC7 but failed to affect the CASC7 with the miR-217-binding site mutant in the LO2 cells ($P < 0.05$) (Fig. 3F). RNA pull-down assays showed that wild-type Bio-miR-217, but not mutant Bio- miR-217, could interact with CASC7 ($P < 0.01$) (Fig. 3G). Together these results suggest that lncRNA CASC7 serves as a sponge for the miR-217 in the liver cells.

LncRNA CASC7 promotes the progression of sepsis-induced liver injury by targeting miR-217

We then evaluated whether CASC7 promoted the progression of sepsis-induced liver injury by targeting miR-217 in the liver cells. The overexpression of CASC7 inhibited the cell viability, while miR-217 mimic could rescue the cell viability in the LPS-treated LO2 cells ($P < 0.05$) (Fig. 4A). The treatment of miR-217 mimic was able to reverse the CASC7 overexpression-enhanced levels of TNF-α and IL-1β in the LPS-treated LO2 cells ($P < 0.05$) (Fig. 4B). The CASC7 overexpression-induced apoptosis was attenuated by the miR-217 mimic in the LPS-treated LO2 cells ($P < 0.05$) (Fig. 4C). The expression of cleaved PARP (c-PARP) and cleaved caspase3 (c- caspase3) elevated by CASC7 overexpression was reduced by the treatment of miR-217 mimic in the LPS-treated LO2 cells ($P < 0.05$) (Fig. 4D). Together these data indicate that lncRNA CASC7 promotes the progression of sepsis-induced liver injury by sponging miR-217 *in vitro*.

Mir-217 Attenuates Sepsis-induced Liver Injury By Targeting Tlr4

Next, we explored the downstream target of miR-217 in the liver cells. The bioinformatic analysis identified the miR-217-targeted site in TLR4 3' UTR by using miRDB and miRmap software (Fig. 5A). The miR-217 mimic attenuated the luciferase activities of TLR4 but failed to affect the TLR4 with the miR-217-binding site mutant in the LO2 cells ($P < 0.01$) (Fig. 5B). The protein expression of TLR4 was reduced by the treatment of miR-217 mimic in the cells ($P < 0.01$) (Fig. 5C). The depletion of CASC7 down-regulated while the overexpression of CASC7 up-regulated the protein levels of TLR4 in the cells ($P < 0.01$) (Fig. 5D). The depletion of CASC7 reduced the LPS-elevated expression of TLR4 in the cells ($P < 0.01$) (Fig. 5E). The efficiency of TLR4 overexpression was validated in the cells ($P < 0.01$) (Fig. 5F). The overexpression of TLR4 was able to block the miR-217 mimic-increased cell viability of the LO2 cells ($P < 0.05$) (Fig. 5G). The miR-217 mimic-attenuated cell apoptosis was enhanced by the TLR4 overexpression in the cells ($P < 0.05$) (Fig. 5H). Together these data indicate that miR-217 attenuates sepsis-induced liver injury by targeting TLR4 *in vitro.*

Discussion

Sepsis is a systemic inflammatory disease caused by severe trauma, burns and postoperative infections, leading to multiple organ failure, including liver injury [27]. The incidence of occurrence and death of sepsis-induced liver injury is high [4]. Nevertheless, the mechanism of sepsis-induced liver injury is still elusive. In the present study, we identified that lncRNA CASC7 contributed to the progression of sepsis-induced liver injury by modulating miR-217/TLR4 axis.

The pathogenesis of sepsis is complex, and lncRNAs have been well-recognized to participate in the modulation of sepsis development. It has been well-identified that multiple lncRNAs are involved in the development of sepsis-induced liver injury. For example, lncRNA colorectal neoplasia, differentially displayed, relieves sepsis-caused liver damage by targeting miR-126-5p [28]. Circulating lncRNA NEAT1 is correlated with an unfavorable prognosis, high severity, and increased risk in sepsis patients [29]. LncRNA SNHG16 changes the consequences of miR-15a/16 on the LPS-produced inflammation pathway [30]. LPS increases sepsis development by stimulating the lncRNA HULC/miR-204-5p/TRPM7 axis in the HUVECs [31]. LncRNA TapSAKI increases inflammatory injury and urine-derived sepsis injury [32]. The impact of lncRNA HOTAIR on serious organ injury in the sepsis rat model by mediating miR-34a/Bcl-2 axis has been reported [33]. LncRNA NEAT1 plays an essential role in the sepsis-produced severe injury by mediating miR-204 and changing the NF-κB signaling [34]. Enhanced expression of lncRNA HULC and UCA1 is needed for the pro-inflammatory response of the LPS-induced sepsis in the endothelial cells [35]. In the present study, we identified that the expression of lncRNA CASC7 was elevated in the sepsis mice. It presents a novel function of lncRNA CASC7 in the modulation of the progression of sepsis-induced liver injury, providing valuable evidence for the fundamental role of lncRNAs in the development of sepsis-induced liver injury.

As a primary component of non-coding RNA and the significant interplay factors with lncRNAs in the physiological and pathological processes, miRNAs are also involved in the modulation of sepsis-induced liver injury. It has been reported that the repression of miRNA 155 inhibits sepsis-caused liver damage by

the inactivation of the JAK/STAT signaling [36]. Paclitaxel alleviates liver damage of the septic mouse by relieving inflammation response through microRNA-27a/TAB3/NF-κB axis[37]. MCPIP1 alleviates lipopolysaccharide-produced liver injury by controlling the expression of SIRT1 by modulation of miR-9[38]. MiR-103a-3p is able to inhibit sepsis-produced liver injury by mediating HMGB1[39]. MiR-21 is needed to remote and local ischemic preconditioning in the protection of various organs against sepsis[40]. The restraint of miRNA 195 inhibits multiple organ injury and apoptosis in the sepsis mouse models[41]. MicroRNA-30e represses apoptosis and increases hepatocyte proliferation in puncture and cecal ligation-induced sepsis by the modulation of JAK/STAT signaling through interacting with FOSL2[42]. Puncture and cecal ligation-induced sepsis is correlated with the inhibited expression of adenylyl cyclase nine and enhanced expression of miR142-3p[43]. Our data demonstrated that lncRNA CASC7 served as a sponge for miR-217 in the liver cells and lncRNA CASC7 promoted the progression of sepsis-induced liver injury by sponging miR-217. It presents the valuable information that miR-217 is involved in the CASC7-mediated sepsis-induced liver injury progression, providing another evidence that miR-217 participate in the modulation of sepsis-induced liver injury development.

TLR4 has been involved in the development of sepsis-induced liver injury. TLR4 antagonist eritoran tetrasodium inhibits liver ischemia and reperfusion injury by the repression of high-mobility group box protein B1 (HMGB1) signaling[44]. Dexmedetomidine-regulated the attenuation against sepsis liver damage

rely on the downregulation of TLR4 and MyD88/NF-κB signaling, partly through anti-inflammatory cholinergic mechanisms[45]. Trichostatin preserves the liver against sepsis damage by the inhibition of the TLR4 signaling[46]. The treatment of Green Tea extract in the obese mice with nonalcoholic steatohepatitis reclaims the liver metabolome in the correlation with inhibiting endotoxemia/TLR4/NFκB-regulated inflammation[47]. Experimental sepsis-produced mitochondria biogenesis is reliant on TLR9, TLR4, and signaling in the liver[48]. The inhibition of TLR4 reduces bacterial clearance in murine abdominal sepsis and in a corrective setting[49]. Leukadherin-1-regulated stimulation of CD11b represses LPS-produced pro-inflammation response in the macrophages and preserves mice upon endotoxic shock by pressing the interaction of LPS-TLR4[50]. Extracellular histones serve as the mediators of destruction by TLR4 and TLR2 in the fatal liver injury mouse model[51]. The protecting impacts of apilarnil against lipopolysaccharide related liver damage in rats by TLR4/ HMGB-1/NF-κB signaling have been reported[52]. TLR4 controls platelet capacity and leads to organ injury and coagulation abnormality in hemorrhagic resuscitation and shock[53]. In the present study, we revealed that miR-217 attenuated sepsis-induced liver injury by targeting TLR4. It indicates that TLR4 plays a crucial role in the modulation of sepsis-induced liver injury.

Conclusion

In conclusion, we discovered that lncRNA CASC7 contributed to the progression of sepsis-induced liver injury by targeting miR-217/TLR4 axis. Our finding provides new insights into the mechanism by which

CASC7 modulates sepsis-induced liver injury development. LncRNA CASC7, miR-217, and TLR4 may serve as potential targets for the treatment of sepsis-induced liver injury.

Abbreviations

lncRNAs, long non-coding RNAs; miRNAs, microRNAs; 3' UTR, 3' untranslated region; LPS, lipopolysaccharide; HE, Hematoxylin and Eosin; TNF-α, Tumor necrosis factor-α; TLR4, Toll-like receptor 4; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

Declarations

Ethics approval and consent to participate

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.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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Availability of data and materials

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

Authors' contributions

XQZ were dedicated to the study concepts, study design, definition of intellectual content, and statistical analysis; TZ carried out the literature research; YHT, WYJ, and WWY were involved in the experimental studies and data acquisition; HW carried out the data analysis; XQZ and GL were dedicated to the manuscript preparation, manuscript editing, and manuscript review. All authors have read and approved this article.

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References

1. Cecconi M, Evans L, Levy M, Rhodes A. Sepsis and septic shock. Lancet. 2018;392(10141):75–87.
2. Czura CJ: "Merinoff symposium 2010: sepsis"-speaking with one voice. Mol Med. 2011;17(1–2): 2–3.
3. Walkey AJ, Lagu T, Lindenauer PK. Trends in sepsis and infection sources in the United States. A population-based study. Ann Am Thorac Soc. 2015;12(2):216–20.
4. Woznica EA, Inglot M, Woznica RK, Lysenko L. Liver dysfunction in sepsis. Adv Clin Exp Med. 2018;27(4):547–51.
5. Vincent JL, Opal SM, Marshall JC, Tracey KJ. Sepsis definitions: time for change. Lancet. 2013;381(9868):774–5.
6. Cohen J, Vincent JL, Adhikari NK, Machado FR, Angus DC, Calandra T, Jaton K, Giulieri S, Delaloye J, Opal S, et al. Sepsis: a roadmap for future research. Lancet Infect Dis. 2015;15(5):581–614.
7. Schulte W, Bernhagen J, Bucala R. Cytokines in sepsis: potent immunoregulators and potential therapeutic targets–an updated view. Mediators Inflamm. 2013;2013165974.
8. Tsay TB, Yang MC, Chen PH, Lai KH, Huang HT, Hsu CM, Chen LW. Blocking TNF-alpha enhances Pseudomonas aeruginosa-induced mortality in burn mice through induction of IL-1beta. Cytokine. 2013;63(1):58–66.
9. Bhan A, Soleimani M, Mandal SS. Long Noncoding RNA and Cancer: A New Paradigm. Cancer Res. 2017;77(15):3965–81.
10. Kopp F, Mendell JT. Functional Classification and Experimental Dissection of Long Noncoding RNAs. Cell. 2018;172(3):393–407.
11. Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. Nature. 2018;553(7689):446–54.
12. Zhang CC, Niu F. LncRNA NEAT1 promotes inflammatory response in sepsis-induced liver injury via the Let-7a/TLR4 axis. Int Immunopharmacol. 2019;75105731.
13. Chen H, Wang X, Yan X, Cheng X, He X, Zheng W. LncRNA MALAT1 regulates sepsis-induced cardiac inflammation and dysfunction via interaction with miR-125b and p38 MAPK/NFkappaB. Int Immunopharmacol. 2018;5569–76.
14. Fang Y, Hu J, Wang Z, Zong H, Zhang L, Zhang R, Sun L. LncRNA H19 functions as an Aquaporin 1 competitive endogenous RNA to regulate microRNA-874 expression in LPS sepsis. Biomed Pharmacother. 2018;1051183–91.

15. Wu H, Liu J, Li W, Liu G, Li Z. LncRNA-HOTAIR promotes TNF-alpha production in cardiomyocytes of LPS-induced sepsis mice by activating NF-kappaB pathway. Biochem Biophys Res Commun. 2016;471(1):240–6.
16. Gong X, Liao X, Huang M. LncRNA CASC7 inhibits the progression of glioma via regulating Wnt/beta-catenin signaling pathway. Pathol Res Pract. 2019;215(3):564–70.
17. Liu Y, Pan L, Jiang A, Yin M. Hydrogen sulfide upregulated lncRNA CasC7 to reduce neuronal cell apoptosis in spinal cord ischemia-reperfusion injury rat. Biomed Pharmacother. 2018;98856–62.
18. Lu TX, Rothenberg. ME: MicroRNA. J Allergy Clin Immunol. 2018;141(4):1202–07.
19. Zhou Q, Huang SX, Zhang F, Li SJ, Liu C, Xi YY, Wang L, Wang X, He QQ, Sun CC, et al.: MicroRNAs: A novel potential biomarker for diagnosis and therapy in patients with non-small cell lung cancer. Cell Prolif. 2017;50(6).
20. Yang ZB, Chen WW, Chen HP, Cai SX, Lin JD, Qiu LZ. MiR-155 aggravated septic liver injury by oxidative stress-mediated ER stress and mitochondrial dysfunction via targeting Nrf-2. Exp Mol Pathol. 2018;105(3):387–94.
21. Roderburg C, Benz F, Vargas Cardenas D, Koch A, Janssen J, Vucur M, Gautheron J, Schneider AT, Koppe C, Kreggenwinkel K, et al. Elevated miR-122 serum levels are an independent marker of liver injury in inflammatory diseases. Liver Int. 2015;35(4):1172–84.
22. Yang X, Li D, Qi YZ, Chen W, Yang CH, Jiang YH. MicroRNA-217 ameliorates inflammatory damage of endothelial cells induced by oxidized LDL by targeting EGR1. Mol Cell Biochem. 2020.
23. Park JS, Choi HS, Yim SY, Lee SM. Heme Oxygenase-1 Protects the Liver from Septic Injury by Modulating TLR4-Mediated Mitochondrial Quality Control in Mice. Shock. 2018;50(2):209–18.
24. Jin LW, Pan M, Ye HY, Zheng Y, Chen Y, Huang WW, Xu XY, Zheng SB. Down-regulation of the long non-coding RNA XIST ameliorates podocyte apoptosis in membranous nephropathy via the miR-217-TLR4 pathway. Exp Physiol. 2019;104(2):220–30.
25. Fang H, Liu A, Chen X, Cheng W, Dirsch O, Dahmen U. The severity of LPS induced inflammatory injury is negatively associated with the functional liver mass after LPS injection in rat model. J Inflamm (Lond). 2018;1521.
26. Baradaran Rahim V, Khammar MT, Rakhshandeh H, Samzadeh-Kermani A, Hosseini A, Askari VR. Crocin protects cardiomyocytes against LPS-Induced inflammation. Pharmacol Rep. 2019;71(6):1228–34.
27. Sagy M, Al-Qaqaa Y, Kim P. Definitions and pathophysiology of sepsis. Curr Probl Pediatr Adolesc Health Care. 2013;43(10):260–3.
28. Li Y, Song J, Xie Z, Liu M, Sun K. Long noncoding RNA colorectal neoplasia differentially expressed alleviates sepsis-induced liver injury via regulating miR-126-5p. IUBMB Life. 2020;72(3):440–51.
29. Huang Q, Huang C, Luo Y, He F, Zhang R. Circulating lncRNA NEAT1 correlates with increased risk, elevated severity and unfavorable prognosis in sepsis patients. Am J Emerg Med. 2018;36(9):1659–63.

30. Wang W, Lou C, Gao J, Zhang X, Du Y. LncRNA SNHG16 reverses the effects of miR-15a/16 on LPS-induced inflammatory pathway. Biomed Pharmacother. 2018;1061661–67.
31. Chen X, Song D. LPS promotes the progression of sepsis by activation of lncRNA HULC/miR-204-5p/TRPM7 network in HUVECs. Biosci Rep. 2020;40(6).
32. Shen J, Liu L, Zhang F, Gu J, Pan G. LncRNA TapSAKI promotes inflammation injury in HK-2 cells and urine derived sepsis-induced kidney injury. J Pharm Pharmacol. 2019;71(5):839–48.
33. Jiang ZJ, Zhang MY, Fan ZW, Sun WL, Tang Y. Influence of lncRNA HOTAIR on acute kidney injury in sepsis rats through regulating miR-34a/Bcl-2 pathway. Eur Rev Med Pharmacol Sci. 2019;23(8):3512–19.
34. Chen Y, Qiu J, Chen B, Lin Y, Chen Y, Xie G, Qiu J, Tong H, Jiang D. Long non-coding RNA NEAT1 plays an important role in sepsis-induced acute kidney injury by targeting miR-204 and modulating the NF-kappaB pathway. Int Immunopharmacol. 2018;59252–60.
35. Chen Y, Fu Y, Song YF, Li N. Increased Expression of lncRNA UCA1 and HULC Is Required for Pro-inflammatory Response During LPS Induced Sepsis in Endothelial Cells. Front Physiol. 2019;10608.
36. Lv X, Zhang Y, Cui Y, Ren Y, Li R, Rong Q. Inhibition of microRNA155 relieves sepsisinduced liver injury through inactivating the JAK/STAT pathway. Mol Med Rep. 2015;12(4):6013–8.
37. Yang Q, Zhang D, Li Y, Li Y, Li Y. Paclitaxel alleviated liver injury of septic mice by alleviating inflammatory response via microRNA-27a/TAB3/NF-kappaB signaling pathway. Biomed Pharmacother. 2018;971424–33.
38. Han S, Li Z, Ji P, Jia Y, Bai X, Cai W, Li X, Yang C, Yang Y, Yang K, et al. MCPIP1 alleviated lipopolysaccharide-induced liver injury by regulating SIRT1 via modulation of microRNA-9. J Cell Physiol. 2019;234(12):22450–62.
39. Chen L, Lu Q, Deng F, Peng S, Yuan J, Liu C, Du X. miR-103a-3p Could Attenuate Sepsis-Induced Liver Injury by Targeting HMGB1. Inflammation. 2020.
40. Jia P, Wu X, Dai Y, Teng J, Fang Y, Hu J, Zou J, Liang M, Ding X. MicroRNA-21 Is Required for Local and Remote Ischemic Preconditioning in Multiple Organ Protection Against Sepsis. Crit Care Med. 2017;45(7):e703-e10.
41. Zheng D, Yu Y, Li M, Wang G, Chen R, Fan GC, Martin C, Xiong S, Peng T. Inhibition of MicroRNA 195 Prevents Apoptosis and Multiple-Organ Injury in Mouse Models of Sepsis. J Infect Dis. 2016;213(10):1661–70.
42. Ling L, Zhang SH, Zhi LD, Li H, Wen QK, Li G, Zhang WJ. MicroRNA-30e promotes hepatocyte proliferation and inhibits apoptosis in cecal ligation and puncture-induced sepsis through the JAK/STAT signaling pathway by binding to FOSL2. Biomed Pharmacother. 2018;104411–19.
43. Risoe PK, Ryg U, Wang YY, Rutkovskiy A, Smedsrod B, Valen G, Dahle MK. Cecal ligation and puncture sepsis is associated with attenuated expression of adenylyl cyclase 9 and increased miR142-3p. Shock. 2011;36(4):390–5.
44. McDonald KA, Huang H, Tohme S, Loughran P, Ferrero K, Billiar T, Tsung A. Toll-like receptor 4 (TLR4) antagonist eritoran tetrasodium attenuates liver ischemia and reperfusion injury through inhibition of

high-mobility group box protein B1 (HMGB1) signaling. Mol Med. 2015;20639–48.
45. Zi SF, Li JH, Liu L, Deng C, Ao X, Chen DD, Wu SZ. Dexmedetomidine-mediated protection against septic liver injury depends on TLR4/MyD88/NF-kappaB signaling downregulation partly via cholinergic anti-inflammatory mechanisms. Int Immunopharmacol. 2019;76105898.
46. Kim SJ, Park JS, Lee DW, Lee SM. Trichostatin A Protects Liver against Septic Injury through Inhibiting Toll-Like Receptor Signaling. Biomol Ther (Seoul). 2016;24(4):387–94.
47. Sasaki GY, Li J, Cichon MJ, Riedl KM, Kopec RE, Bruno RS. Green Tea Extract Treatment in Obese Mice with Nonalcoholic Steatohepatitis Restores the Hepatic Metabolome in Association with Limiting Endotoxemia-TLR4-NFkappaB-Mediated Inflammation. Mol Nutr Food Res. 2019;63(24):e1900811.
48. Carchman EH, Whelan S, Loughran P, Mollen K, Stratamirovic S, Shiva S, Rosengart MR, Zuckerbraun BS. Experimental sepsis-induced mitochondrial biogenesis is dependent on autophagy, TLR4, and TLR9 signaling in liver. FASEB J. 2013;27(12):4703–11.
49. van Lieshout MH, van der Poll T, van't Veer C. TLR4 inhibition impairs bacterial clearance in a therapeutic setting in murine abdominal sepsis. Inflamm Res. 2014;63(11):927–33.
50. Yao X, Dong G, Zhu Y, Yan F, Zhang H, Ma Q, Fu X, Li X, Zhang Q, Zhang J, et al.: Leukadherin-1-Mediated Activation of CD11b Inhibits LPS-Induced Pro-inflammatory Response in Macrophages and Protects Mice Against Endotoxic Shock by Blocking LPS-TLR4 Interaction. Front Immunol. 2019;10215.
51. Xu J, Zhang X, Monestier M, Esmon NL, Esmon CT. Extracellular histones are mediators of death through TLR2 and TLR4 in mouse fatal liver injury. J Immunol. 2011;187(5):2626–31.
52. Doganyigit Z, Okan A, Kaymak E, Pandir D, Silici S. Investigation of protective effects of apilarnil against lipopolysaccharide induced liver injury in rats via TLR 4/ HMGB-1/ NF-kappaB pathway. Biomed Pharmacother. 2020;125109967.
53. Ding N, Chen G, Hoffman R, Loughran PA, Sodhi CP, Hackam DJ, Billiar TR, Neal MD. Toll-like receptor 4 regulates platelet function and contributes to coagulation abnormality and organ injury in hemorrhagic shock and resuscitation. Circ Cardiovasc Genet. 2014;7(5):615–24.

Figures

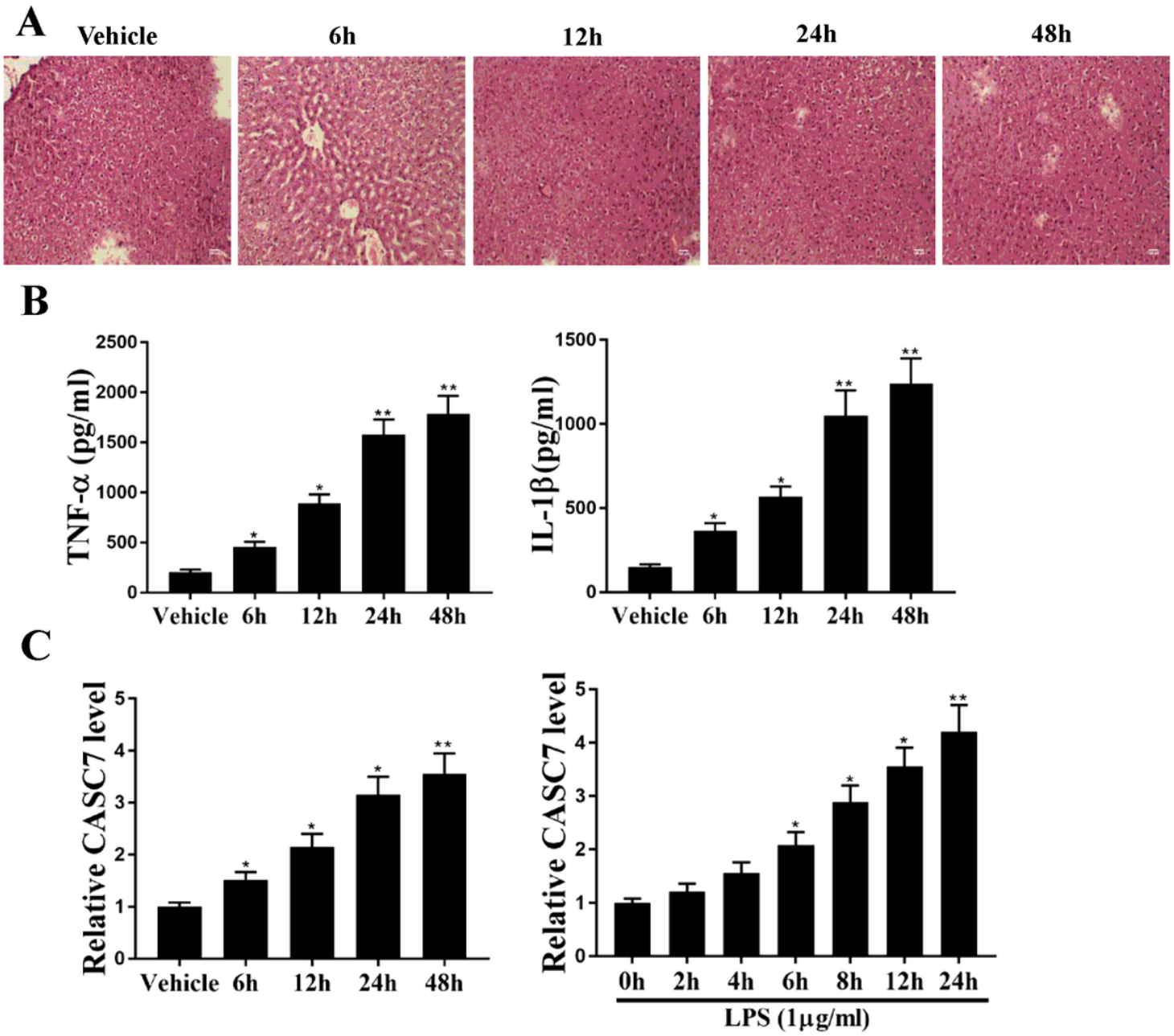

Figure 1

The expression of lncRNA CASC7 is positively correlated with the sepsis-induced liver injury. (A-C) The sepsis mouse model was established in BALB/c mice by the treatment of lipopolysaccharide (LPS, 20 mg/kg) (n = 5). (A) The liver injury was analyzed by the Hematoxylin and Eosin (HE) staining at the indicated time in the mice. (B) The levels of TNF-α and IL-1β were measured by the ELISA assays at the indicated time in the mice. (C) The expression of lncRNA CASC7 was tested by qPCR assays at the indicated time in the liver tissues of the mice. (D) The LO2 cells were treated with LPS (1 μg/ml). The expression of lncRNA CASC7 was determined by qPCR assays at the indicated time in the cells. Data are presented as mean ± SD. Statistic significant differences were indicated: * $P < 0.05$, ** $P < 0.01$.

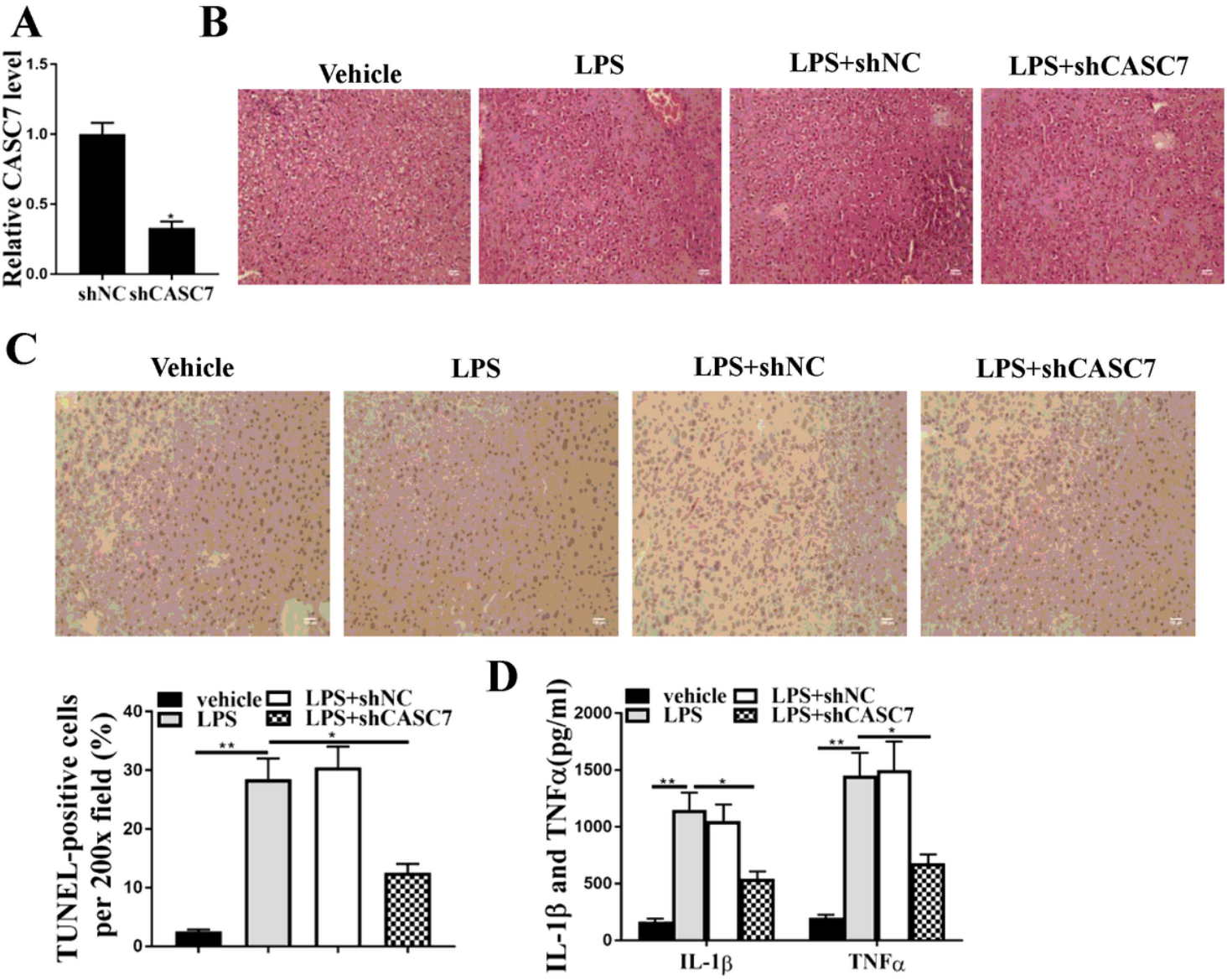

Figure 2

The depletion of lncRNA CASC7 relieves sepsis-induced liver injury in vivo. (A-D) The BALB/c mice were treated with LPS, or co-treated with LPS and control shRNA or CASC7 shRNA. (A) The expression of lncRNA CASC7 was tested by qPCR assays at the indicated time in the liver tissues of the mice. (B) The liver injury was analyzed by the HE staining in the mice. (C) The apoptosis was measured by the TUNEL analysis in the liver tissues of the mice. (D) The levels of TNF-α and IL-1β were measured by the ELISA assays in the mice. Data are presented as mean ± SD. Statistic significant differences were indicated: * $P < 0.05$, ** $P < 0.01$.

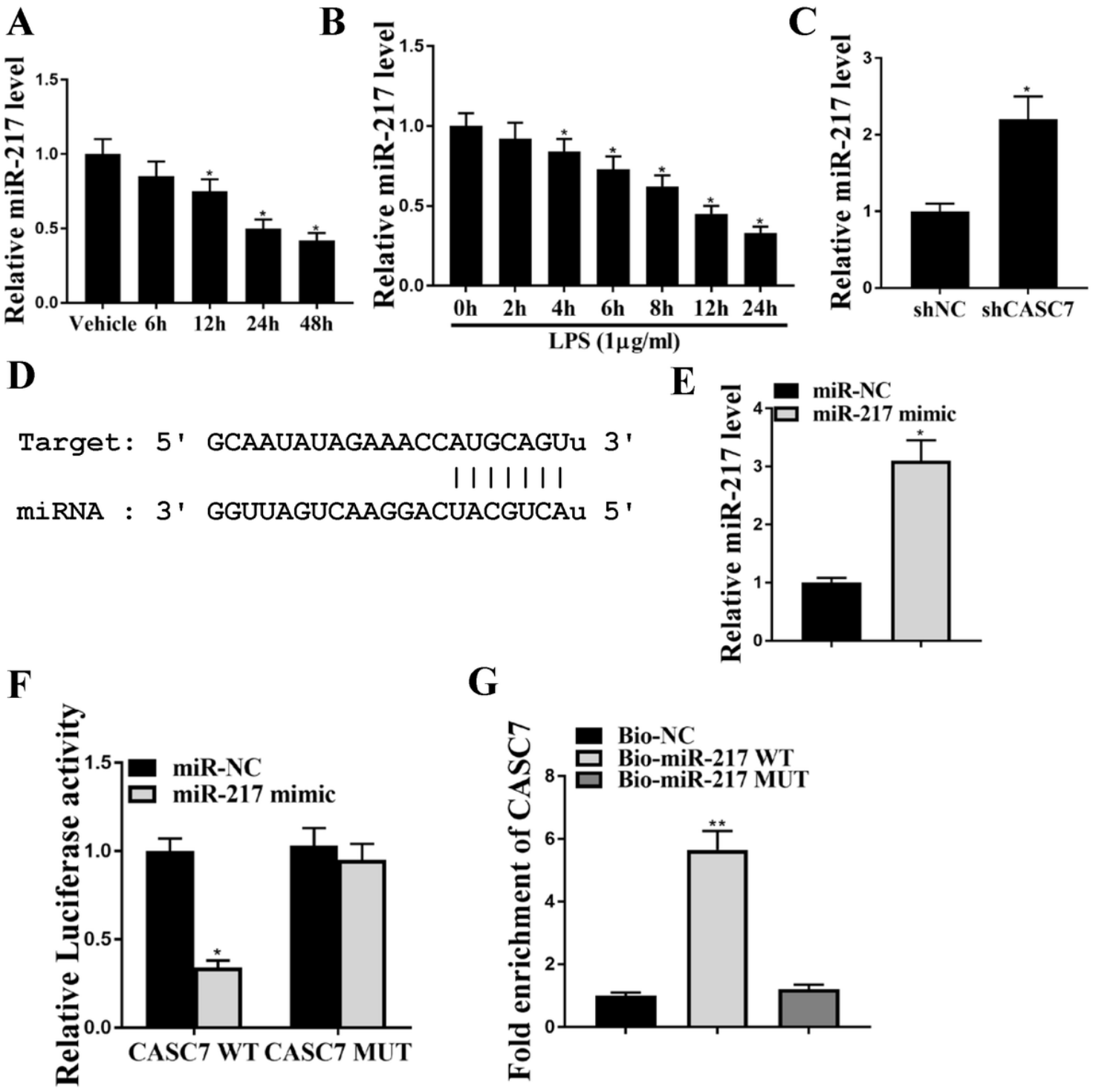

Figure 3

LncRNA CASC7 serves as a sponge for the miR-217 in the liver cells. (A) The sepsis mouse model was established in BALB/c mice by the treatment of LPS (20 mg/kg) (n = 5). The expression of miR-217 was tested by qPCR assays at the indicated time in the liver tissues of the mice. (B) The LO2 cells were treated with LPS (1 μg/ml). The expression of miR-217 was determined by qPCR assays at the indicated time in the cells. (C) The LO2 cells were treated with control shRNA or CASC7 shRNA. The expression of miR-217 was assessed by qPCR assays in the cells. (D) The potential interaction between lncRNA CASC7 and miR-

217 was identified by the bioinformatic analysis using Starbase 3.0v software. (E and F) The LO2 cells were treated with miR-217 mimic or control mimic. (E) The expression of miR-217 was examined by the qPCR assays in the cells. (F) The luciferase activities of wild type CASC7 (CASC7 WT), and CASC7 with the miR-217-binding site mutant (CASC7 MUT) were determined by luciferase reporter gene assays in the cells. (G) The interaction of CASC7 and miR-217 was analyzed by the RNA pull-down assays. Data are presented as mean ± SD. Statistic significant differences were indicated: * P < 0.05, ** P < 0.01.

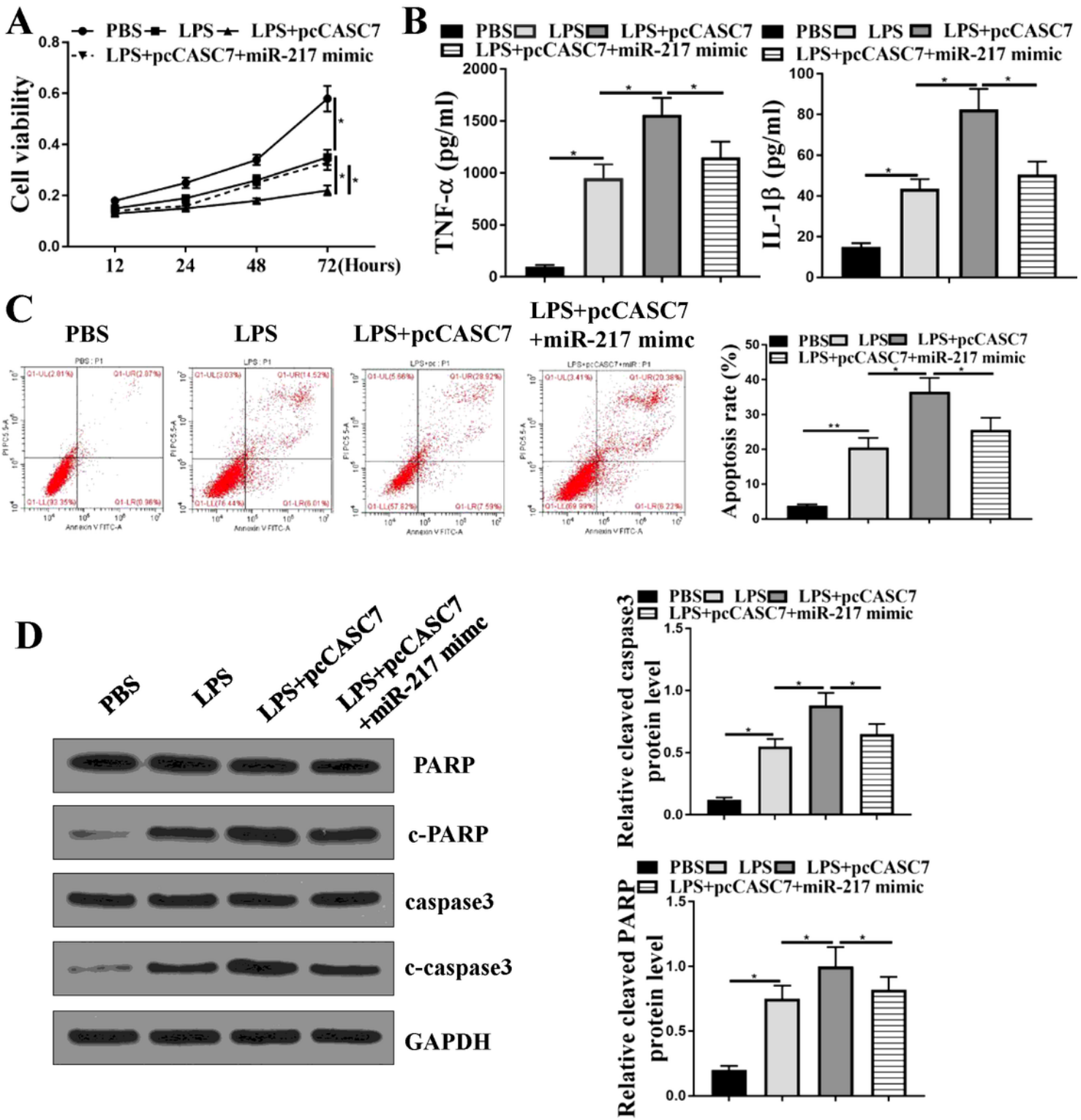

Figure 4

LncRNA CASC7 promotes the progression of sepsis-induced liver injury by targeting miR-217. (A-D) The LO2 cells were treated with LPS (1 μg/ml), co-treated with LPS (1 μg/ml) and CASC7 overexpression vector, or co-treated with LPS (1 μg/ml), CASC7 overexpression vector, and miR-217 mimic. (A) The cell viability was measured by CCK-8 assays in the cells. (B) The levels of TNF-α and IL-1β were measured by the ELISA assays in the cells. (C) The cell apoptosis was measured by flow cytometry analysis in the cells. (D) The expression of PARP, cleaved PARP (c-PARP), caspase3, cleaved caspase3 (c-caspase3), and GAPDH was analyzed by Western blot analysis in the cells. The results of Western blot analysis were quantified by ImageJ software. The original western blot of figure 4D was shown in sfigure 1. Data are presented as mean ± SD. Statistic significant differences were indicated: * $P < 0.05$, ** $P < 0.01$.

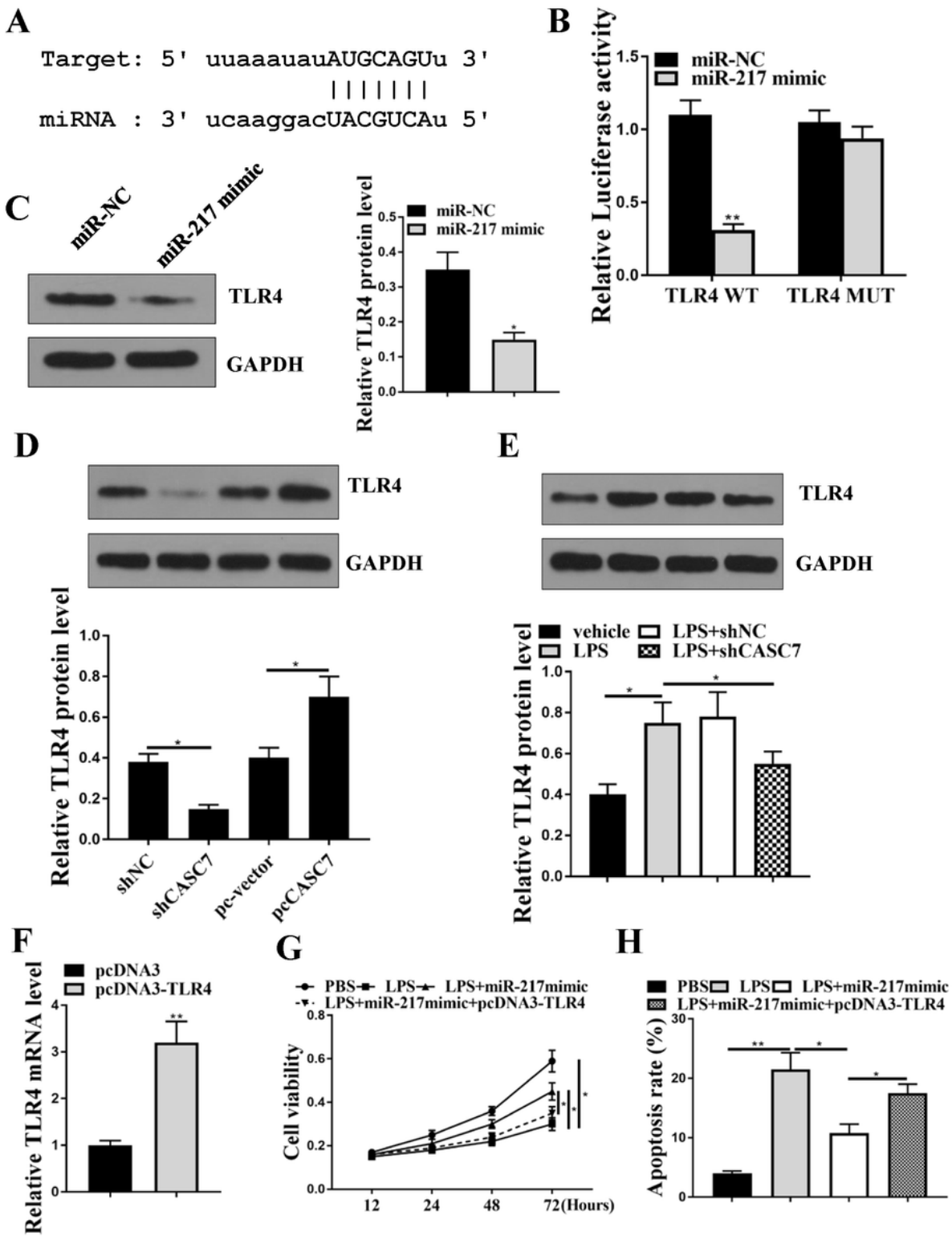

Figure 5

MiR-217 attenuates sepsis-induced liver injury by targeting TLR4. (A) The interaction of miR-217 and TLR4 3′ UTR was identified by bioinformatic analysis using miRDB and miRmap software. (B and C) The LO2 cells were treated with miR-217 mimic or control mimic. (B) The luciferase activities of wild type TLR4 (TLR4 WT), and TLR4 with the miR-217-binding site mutant (TLR4 MUT) were determined by luciferase reporter gene assays in the cells. (C) The expression of TLR4 and GAPDH was measured by

Western blot analysis in the cells. The results of Western blot analysis were quantified by ImageJ software. The original western blot of figure 5C was shown in sfigure 2. (D) The LO2 cells were treated CASC7 shRNA, CASC7 overexpression vector, or the corresponding control. The expression of TLR4 and GAPDH was tested by Western blot analysis in the cells. The results of Western blot analysis were quantified by ImageJ software. The original western blot of figure 5D was shown in sfigure 2. (E) The LO2 cells were treated with LPS (1 μg/ml), co-treated with LPS (1 μg/ml) and control shRNA or CASC7 shRNA. The expression of TLR4 and GAPDH was assessed by Western blot analysis in the cells. The results of Western blot analysis were quantified by ImageJ software. The original western blot of figure 5E was shown in sfigure 2. (F) The LO2 cells were treated with TLR4 overexpression vector or control vector. The expression of TLR4 was measured by qPCR assays in the cells. (G and H) The LO2 cells were treated with LPS (1 μg/ml), co-treated with LPS (1 μg/ml) and miR-217 mimic, or co-treated with LPS (1 μg/ml), miR-217 mimic and TLR4 overexpression vector. (G) The cell viability was measured by CCK-8 assays in the cells. (H) The cell apoptosis was measured by flow cytometry analysis in the cells. Data are presented as mean ± SD. Statistic significant differences were indicated: * $P < 0.05$, ** $P < 0.01$.

Supplementary Files

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