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Long non-coding RNA CASC2 ameliorates sepsis-induced acute kidney injury by regulating the miR-155 and NF-κB pathway

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Abstract. Sepsis is a systemic inflammatory response syndrome that can cause multiple-organ damage, including acute kidney injury (AKI). Studies have shown that the long non-coding RNA cancer susceptibility candidate 2 (CASC2) is involved in the occurrence and development of multiple human diseases, although its expression and role in AKI has not yet been reported. The present study demonstrated that the expression of CASC2 was significantly decreased in the serum of patients with sepsis compared with healthy subjects. In addition, the CASC2 level was negatively associated with the severity of AKI. Further experiments revealed that CASC2 promoted cell viability and inhibited inflammatory factor secretion, apoptosis and oxidative stress in lipopolysaccharide-stimulated human renal tubular epithelial HK-2 cells. Importantly, the current study observed that CASC2 was negatively associated with a pro-inflammatory microRNA (miR)-155. In addition, the upregulation of CASC2 significantly suppressed the nuclear factor κB (NF-κB) signaling pathway. In conclusion, the results of the present study suggested that CASC2 may serve as a potential target for treating sepsis-induced AKI by inhibiting the miR-155 and NF-κB pathway-mediated inflammation.

Introduction

Sepsis is a systemic inflammatory response syndrome caused by infection (1). The common clinical features of this disease are fever, tachycardia, shortness of breath and increased peripheral blood leukocytes (1). Sepsis involves various pathophysiological mechanisms, such as cell injury, apoptosis, oxidative stress and mitochondrial dysfunction, which are associated with the occurrence of renal injury (2). The probability of acute renal injury in patients with severe sepsis is 60% (3). The clinical diagnosis of acute kidney injury (AKI) can be determined by the increase of blood urea nitrogen and serum creatinine (4). However, the clinical outcome of this disease remains unsatisfactory as recent data has revealed that the mortality rate of patients with AKI is ≤22% (5). Therefore, it is necessary to develop an effective therapeutic strategy for acute kidney damage caused by sepsis.

Long non-coding RNA (lncRNA) is a class of non-protein coding RNAs that regulate the expression of genes at the transcriptional or post-transcriptional level (6). Recent studies have demonstrated that lncRNAs serve important roles in cell biology, including cell proliferation, differentiation, development, invasion, migration and apoptosis (7). An increasing number of studies have demonstrated that lncRNAs, such as H19 (8), MALAT1 (9), SNHG16 (10) and NEAT1 (9), are involved in sepsis-induced AKI.

The lncRNA cancer susceptibility candidate 2 (CASC2), located on chromosome 10q26, was originally reported to be downregulated in the endometrium (11). Subsequent studies have shown that CASC2 is involved in several human diseases (12). Notably, CASC2 can act as a tumor suppressor gene, which is able to inhibit cell proliferation, invasion and metastasis, and promote cell apoptosis in multiple human types of cancer, such as pancreatic carcinoma, papillary thyroid carcinoma and gastric cancer (13). Certain studies have recommended CASC2 as a good prognostic biomarker for tumors, such as hepatocellular carcinoma and pancreatic cancer (11,14-16). Another study has revealed that CASC2 expression is significantly reduced in renal cell carcinoma (17). However, to the best of our knowledge, whether CASC2 is involved in sepsis-induced AKI has not been studied. The aim of the present study was to investigate the effect of CASC2 on sepsis-induced AKI, as well as the underlying molecular mechanism.

Materials and methods

Sample collection. Blood samples were obtained from 20 healthy volunteers and 69 patients with sepsis and AKI after clinical diagnosis following the standards of the American College of Chest Physicians/Society of Critical Care Medicine consensus conference. All patients with

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sepsis-induced AKI and healthy volunteers from the Huai'an no. 1 People's Hospital of Nanjing Medical University were enrolled in this study between May 2015 and June 2018. The patient group included 30 males and 39 females, with a mean age of 43.4 (range, 23-72) years. Healthy volunteers included 8 males and 12 females, with a mean age of 45 (range, 25-58) years. All participants provided written informed consent, and ethical approval was granted by the Ethics Committee of Nanjing Medical University (Nanjing, China).

Cell culture. A human renal tubular epithelial HK-2 cell line was purchased from the American Type Culture Collection, and the 293 cell line was obtained from the Cell Bank of Chinese Academy of Sciences. HK-2 cells were cultured in keratinocyte serum immunization medium containing L-glutamine (Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and 100 mg/ml streptomycin (HyClone; GE Healthcare Life Sciences), and 293 cells were cultured in DMEM (high glucose; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (HyClone; GE Healthcare Life Sciences). To establish an *in vitro* lipopolysaccharide (LPS)-induced sepsis model, HK-2 cells were treated with 1 μg/ml LPS (Sigma-Aldrich; Merck KGaA) for 24 h.

Animals and grouping. A total of 20 male BALB/c mice (6-8 weeks old; 22-25 g) were purchased from Beijing HFK Biotechnology. Mice were maintained in a SPF-grade room at 18-22˚C and 40-60% humidity under a light/dark cycle of 12/12 h with free access to food and water. The mice were randomly divided into two groups after one week of feeding (sham surgery group, n=10; and model group, n=10). The sepsis mouse model was established as previously described (18). Briefly, mice were anesthetized with ketamine-xylazine (20-25 mg/kg body weight) and injected intraperitoneally with 15 mg/kg LPS. The control group was injected with the same amount of saline. All animal experiments were approved by the Animal Ethics Committee of the Nanjing Medical University and performed in accordance with the Guide for the Care and Use of Laboratory Animals.

Blood biochemical index. The blood samples of the mice were collected and centrifuged at 3,000 x g for 10 min at 4˚C to obtain the serum. Then, the blood biochemical indicators were detected using a diagnostic kit and a biochemical analyzer.

Cell transfection and nuclear factor κ-B (NF-κB) inhibitor treatment. The CASC2 overexpression plasmid was constructed using the pcNDA3 expression vector (pc-CASC2) (Guangzhou Ribobio Co. Ltd). The mircRNA-155 (miR-155) mimic and inhibitor, as well as the corresponding negative control (NC), were purchased from Guangzhou Ribobio Co., Ltd., and the sequences were as follows: miR-155 forward, 5'-ACGCTCAGTTAATGCTAATCGTGATA-3' and reverse, 5'-ATTCCATGTTGTCCACTGTCTCTG-3'. The cells were transfected with miR-155 (1 μg/well) or negative control (1 μg/well) using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.). Following 48-h transfection, cells were subjected to subsequent experiments. The NF-κB inhibitor QNZ (EVP4593) was purchased from MedChem Express, and the final concentration was 10 μM.

MTT assay. HK-2 cells ($1x10^4$) were seeded in 96-well plates. After 12 h of culture, cells were treated with or without LPS for 24 h and then washed three times in PBS. Subsequently, 500 μg/ml MTT (Merck KGaA) was added to each well. A total of three h later, 200 μl dimethyl sulfoxide solution (Merck KGaA) was added. Finally, the samples were measured at 570 nm using a microplate reader (Bio-Rad Laboratories, Inc.).

Cell apoptosis analysis. Cell apoptosis was measured using the Annexin V-FITC/7-AAD kit (BD Biosciences; Becton, Dickinson and Company) according to the manufacturer's protocol. Samples were analyzed using a FACSCanto flow cytometer (BD Bioscience) Data analysis was performed using the FlowJo10.0 software (TreeStar, Inc.).

ELISA. Mouse peripheral blood (1 ml) was collected by enucleating eyeballs prior to euthanasia and centrifuged at 10,000 x g for 10 min at 4˚C. Subsequently, the serum was used to detect the concentration of tumor necrosis factor (TNF)-α (cat. no. BMS607-3), interleukin (IL)-6 (cat. no. BMS603-2), IL-8 (cat. no. BMS6001) and IL-1β (cat. no. BMS6002) using ELISA kits (Thermo Fisher Scientific, Inc.).

Superoxide measurement. HK-2 cells treated with or without LPS were incubated for 1 h and then were collected. Following centrifuged at 300 x g for 5 min at 4˚C, the pellet was resuspended in 900 μl Krebs buffer containing 5 mmol KCl, 130 mmol NaCl, 1 mmol $MgCl_2$, 1 mmol K_2HPO_4, 5 mmol $CaCl_2$ and 20 mmol HEPES (pH 7.4), supplemented with 1 mg/ml bovine serum albumin (Sigma-Aldrich; Merck KGaA). Subsequently, the samples were transferred into a measuring chamber. Finally, the suspension was mixed with 100 μl lucigenin (final concentration $4x10^4$ mmol/l) and evaluated using a chemiluminescence analyzer.

Detection of nitrite. The nitrite content in HK-2 medium was measured using the Measure-iTTM High-Sensitivity Nitrite Assay kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol.

RT-qPCR. Total RNA was isolated from the cells using the TRIzol reagent (Thermo Fisher Scientific, Inc.). For the detection of CASC2, cDNA was synthesized from 1 μg RNA using the PrimeScript RT reagent kit (Takara Bio, Inc.) under the following conditions: 42˚C for 2 min, 37˚C for 15 min and 85˚C for 5 sec. QPCR was performed using a SYBR Premix Ex Taq kit (Takara Bio, Inc.) with a three-step PCR protocol. The thermocycling conditions were as follows: 95˚C for 2 min, followed by 38 cycles of 95˚C for 5 sec, 60˚C for 30 sec and 72˚C for 30 sec. GAPDH served as an internal reference gene. The primers used were as follows: CASC2 forward, 5'-TAC AGGACAGTCAGTGGTGGT-3' and reverse, 5'-ACATCT AGCTTAGGAATGTGGC-3'; GAPDH forward, 5'-AGG TCGGTGTGAACGGATTTG-3' and reverse, 5'-TGTAGA CCATGTAGTTGAGGTCA-3'. For the detection of miR-155, cDNA was generated using the TaqMan MicroRNA Reverse Transcription kit (Thermo Fisher Scientific, Inc.) at 42˚C for 60 min and 95˚C for 3 min. Subsequently, qPCR was performed using a TaqMan Universal Master Mix II kit (Thermo Fisher

Scientific, Inc.). The thermocycling conditions were as follows: 94˚C for 3 min, followed by 30 cycles of 95˚C for 30 sec, 55˚C for 30 sec and 72˚C for 1 min and a final extension at 72˚C for 3 min. U6 snRNA served as an internal reference gene. The following primers were used: miR-155 forward, 5'-TTAATG CTAATCGTGATAGGGG-3' and reverse, 5'-TCATGCCGT TAGGTAGCGTA-3'; U6 snRNA forward, 5'-ATTGGAACG ATACAGAGAAGATT-3' and reverse, 5'-GGAACGCTTCAC GAATTTG-3'. The relative expression of lncRNA or miRNA were normalized to GAPDH or U6 using a $2^{-\Delta\Delta Cq}$ method as previously described (19).

Western blotting. This assay was performed as previously described (20). Primary antibodies against human p-IκBα^{Ser32} (14D4; 1:1,000; cat. no. 2859S; Cell Signaling Technology, Inc.), IκBα (ab32518, Abcam), p50 (E381; 1:1,000; cat. no. ab32360), p65 (E379; 1:1,000; cat. no. ab32536) and GAPDH (6C5; 1:1,000; cat. no. ab8245; Abcam) were used.

Histological examination. The kidney was fixed in 4% paraformaldehyde (4˚C, 24 h) and embedded in paraffin. Sections (4-μm thick) were cut and processed, and then stained with hematoxylin for 5 min at room temperature, differentiated by 0.6% hydrochloric alcohol for 30 sec, stained with eosin (1%) at room temperature for 1 min, sterilized with 80% ethanol for 2 min, 95% ethanol for 2 min, 100% ethanol for 2 min, 100% ethanol for 2 min. Finally, samples were visualization by light microscopy (magnification, x400; Olympus Corporation).

Electrophoretic mobility shift assay (EMSA). The NF-κB activity was measured by EMSA as previously described (21). Briefly, HK-2 cells were pretreated with AP (1 mg/ml) and then treated with LPS (10 mg/ml) for 30 min. Subsequently, nuclear extracts isolated from these cells were mixed with binding buffer [20 mM HEPES-NaOH (pH 7.9), 100 mM NaCl, 10% glycerol, 2 mM EDTA and 0.2% NP-40], Poly(dI-dC) and 32P-labelled NF-κB oligonucleotide probes (Promega Corporation) for 30 min at room temperature. Finally, the DNA-nuclear protein complexes were separated using 10% PAGE, followed by DNA binding detection by autoradiography.

Luciferase reporter assay. Luciferase reporter assay was performed on 293 cells using dual-luciferase reporter assay kit (Promega Corporation). Cells were transfected with Luc reporter plasmid containing wild-type or mutant CASC2 (synthesized by Shanghai Sangon Biotech Co., Ltd.) using Lipofectamine® 3000 (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Luciferase activities were measured at 48 h post-transfection. *Renilla* luciferase was used as an internal reference.

Bioinformatics. The prediction of the interaction between CASC2 and miR-155 was performed using Starbase 3.0 (http://starbase.sysu.edu.cn).

Statistical analysis. All data analyses were carried out using SPSS19.0 software (IBM Corp.) and all graphs were made using Graph Prism 6.0 software (GraphPad Software, Inc.). Student's t-test was used to compare the differences between two groups and one-way analysis of variance followed by Tukey-Kramer post hoc test was used to compare the differences among multiple groups. All experiments were performed three times and the obtained data are expressed as the mean ± standard error of mean. $^{*}P<0.05$ was considered to indicate a statistically significant difference.

Results

CASC2 expression is significantly decreased in sepsis-induced AKI. To investigate the role of the lncRNA CASC2 in sepsis, the expression of CASC2 mRNA was first measured in serum obtained from sepsis patients and control groups by RT-qPCR analysis. It was found that, compared with the control group, the expression level of CASC2 was significantly decreased in sepsis patients (Fig. 1A). Interestingly, the present study observed that the expression level of CASC2 decreased with the severity of AKI (Fig. 1B) and was inversely associated with the level of creatinine clearance rate (Fig. 1C). Next, a sepsis-induced AKI mouse model was established and confirmed by the pathological changes observed in the kidneys and certain biochemical indicators of blood (Fig. S1). Consistently, the expression of CASC2 is significantly downregulated in mice with AKI caused by sepsis (Fig. 1D). The above data suggest that CASC2 may play a protective role in sepsis-induced AKI.

CASC2 promotes cell viability and inhibits inflammatory factor secretion, apoptosis and oxidative stress in LPS-stimulated HK-2 cells. To assess the extract function of CASC2 in sepsis-induced AKI, LPS was used to induce sepsis in a cell model. Then, the CASC2 overexpression plasmid was transfected into HK-2 cells (Fig. 2A). The effect of CASC2 upregulation on cell viability, apoptosis, inflammatory factor expression and oxidative stress in LPS-stimulated HK-2 was examined. It was found that the overexpression of CASC2 increased cell viability (Fig. 2B) and reduced the percentage of apoptotic cells in LPS-treated HK-2 cells (Fig. 2C). The results of the ELISA showed that the concentration of TNF-α, IL-6, IL-8 and IL-1β in the cell suspension was significantly increased following LPS stimulation, while the overexpression of CASC2 significantly decreased the secretion of these inflammatory factors (Fig. 2D). In addition, the present study observed that CASC2 overexpression significantly suppressed the production of superoxide and nitrite in HK-2 cells after LPS stimulation (Fig. 2E and F). However, CASC2 overexpression did not have a significant effect on normal HK-2 cells (Fig. 2). Altogether, these results demonstrate that the upregulation of CASC2 may contribute to the inhibition of the inflammatory response, apoptosis and oxidative stress in sepsis-induced AKI.

CASC2 negatively regulates the expression of miR-155 in sepsis-induced AKI. Next, the current study set out to explore the underlying molecular mechanism of CASC2 downregulation in the pathogenesis of sepsis-induced AKI. Considering that lncRNA can act as an endogenous RNA to competitively bind to miRNA, the expression of several miRNAs associated with sepsis was measured in HK-2 cells after CASC2 overexpression (8,10,20,22-25). Intriguingly, the present

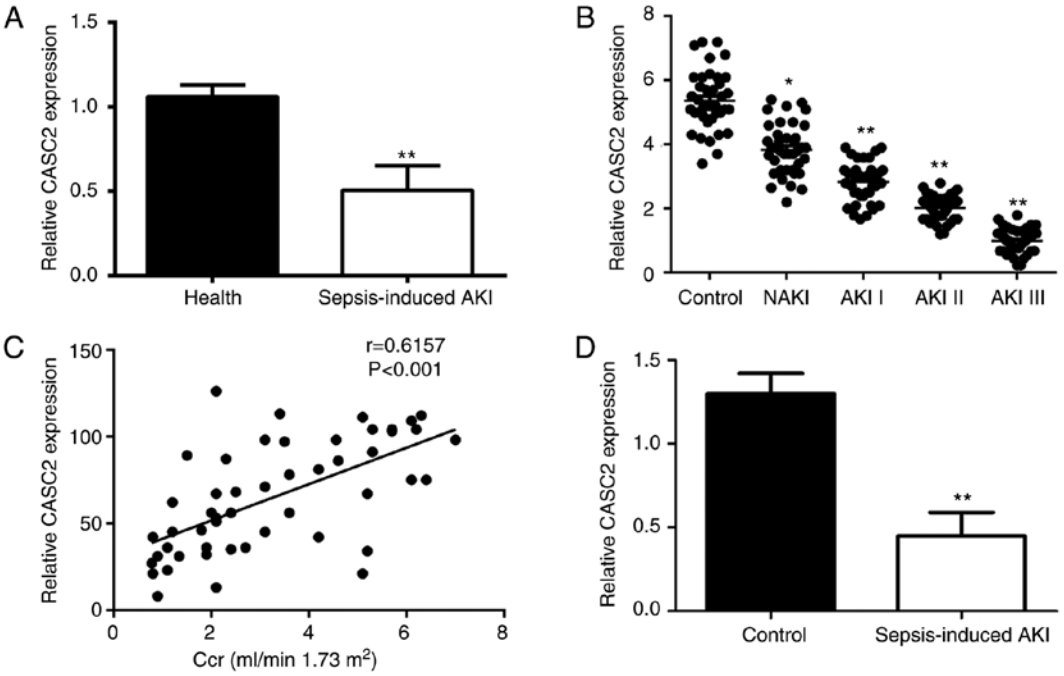

Figure 1. CASC2 expression is significantly decreased in sepsis-induced AKI. (A) RT-qPCR analysis of the expression level of CASC2 in 69 sepsis patients and 20 healthy controls. (B) The relative expression level of CASC2 in different degrees of AKI. AKI I, AKI II, AKI III represent the degree of acute kidney injury. Results were compared with the control group. (C) Pearson correlation analysis showing the correlation between CASC2 expression and Ccr levels. Ccr=(140-age) x body weight (kg)/72 x Scr (mg/dl) or Ccr=[(140-age) x body weight (kg)]/[0.818 x Scr (μmol/l)]. Females calculated the result x0.85. (D) RT-qPCR analysis of the expression of CASC2 in the kidney from control and sepsis-induced AKI mice. *P<0.05 and **P<0.01. AKI, acute kidney injury; CASC2, cancer susceptibility candidate 2; Ccr, Creatinine clearance rate; NAKI, no acute kidney injury; Scr, serum creatinine; RT-q, Reverse transcription-quantitative.

study found that only miR-155 was significantly increased in LPS-stimulated HK-2 cells when CASC2 was overexpressed (Fig. 3A). In addition, bioinformatics predictions showed that CASC2 can directly bind to miR-155, as demonstrated by the luciferase reporter assay (Fig. 3B). In accordance with these findings, it was noticed that there was a negative regulation of CASC2 and miR-155 in the serum of patients with sepsis (Fig. 3C). To further verify whether the increased expression of miR-155 is responsible for sepsis-induced AKI, the miR-155 inhibitor was then transfected into HK-2 cells after LPS stimulation. As expected, the inhibition of miR-155 partially reversed cell viability, apoptosis, cytokine secretion and oxidative stress (Fig. 3D-H). On the contrary, the transfection of the miR-155 mimic into LPS-stimulated HK-2 cells markedly weakened the protective role of CASC2 (Fig. 3D-H). Altogether, these data indicate that CASC2 overexpression can prevent HK-2 cells from LPS-induced injury at least partly by inhibiting miR-155 expression.

CASC2 attenuates sepsis-induced AKI via the NF-κB signaling pathway. It has been reported that the NF-κB signaling pathway plays an important role in sepsis-induced AKI (20). Therefore, IκBα, p50 and p65 were used as surrogate markers of NF-κB activation. As shown in Fig. 4A and B, LPS significantly activated the NF-κB signaling pathway in LPS-treated HK-2 cells. Importantly, the overexpression of CASC2 distinctly decreased the expression levels of p50 and p65 in the cytoplasm and nucleus. Consistent with these data, EMSA results confirmed that the upregulation of CASC2 significantly inhibited the activation of the NF-κB pathway (Fig. 4C). However, the inhibition of NF-κB activity did not alter miR-155 expression (Fig. 4D) and the suppression of miR-155 did not affect the activity of NF-κB in LPS-stimulated HK-2 cells (Fig. S2A-C). Collectively, these findings demonstrate that CASC2 can also inhibit the NF-κB signaling pathway to protect sepsis-induced AKI, which is independent of miR-155.

Discussion

Sepsis can cause multiple-organ dysfunction and even death, thus posing a major threat to human life (26). The most common complication of sepsis is AKI, which is closely associated with its high mortality (27). Therefore, it is important to determine its pathogenesis and find an effective method for treating sepsis-induced kidney injury. In this study, an LPS-induced sepsis cell model was established to investigate the role and underlying mechanism of the lncRNA CASC2 in sepsis-induced AKI.

In recent years, an increasing number of studies have focused on the biological function of lncRNAs; it has been reported that lncRNAs have a different expression pattern in the renal innate cells under disease conditions (28,29). Indeed, numerous lncRNAs serve important roles in several kidney diseases, including diabetic nephropathy, renal inflammation and fibrosis, renal transplant rejection, renal cell carcinoma and kidney injury (30). However, the role of the majority of lncRNAs in sepsis-induced AKI has not been explored. The present study showed for the first time to the best of our knowledge that the lncRNA CASC2 can ameliorate sepsis-induced AKI by targeting the miR-155 and NF-κB pathway.

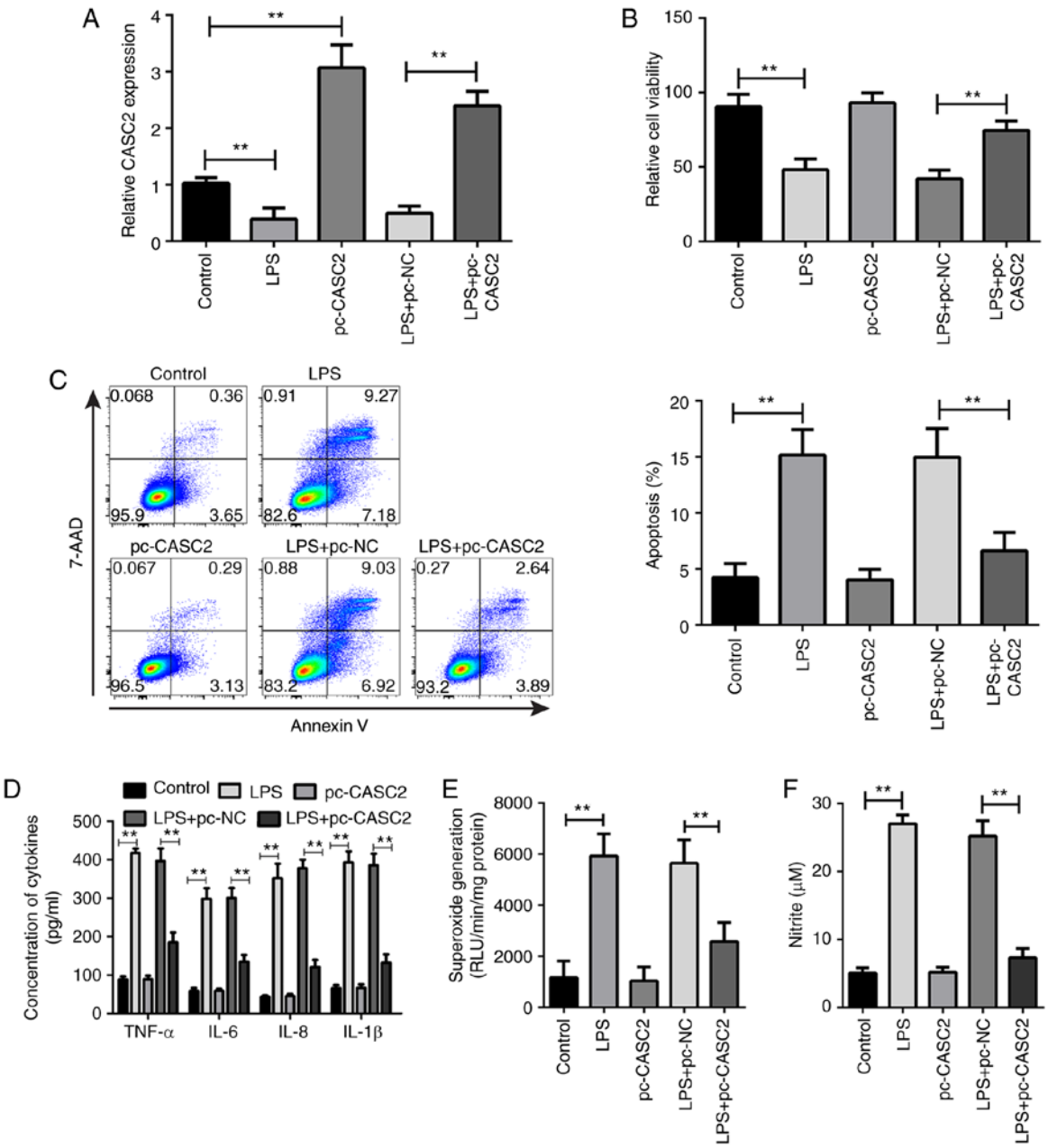

Figure 2. CASC2 promotes cell viability and inhibits inflammatory factors secretion, apoptosis and oxidative stress in LPS-stimulated HK-2 cells. (A) The expression of CASC2 in HK-2 cells from the control, LPS, pc-CASC2, LPS+pc-NC and LPS+pc-CASC2 groups. (B) The relative viability of HK-2 cells in control, LPS, pc-CASC2, LPS+pc-NC and LPS+pc-CASC2 groups. (C) Flow cytometric analysis of the apoptosis of HK-2 cells in control, LPS, pc-CASC2, LPS+pc-NC and LPS+pc-CASC2 groups. (D) ELISA analysis of the expression level of TNF-α, IL-6, IL-8 and IL-1β in control, LPS, pc-CASC2, LPS+pc-NC and LPS+pc-CASC2 groups. The production of (E) superoxide and (F) nitrite in HK-2 in the control, LPS, pc-CASC2, LPS+pc-NC and LPS+pc-CASC2 groups. **P<0.01. CASC2, cancer susceptibility candidate 2; LPS, lipopolysaccharide; IL, interleukin; NC, negative control; TNF, tumor necrosis factor.

Previous studies have shown that CASC2 plays an important role in multiple physiological and pathological processes, such as cell differentiation, osteoarthritis, pulmonary hypertension and tumor progression (15,31,32), while its expression and function in kidney disease, particularly sepsis-induced AKI, remains unclear. In the present study, it was found that the CASC2 expression was evidently reduced in patients with sepsis-induced AKI, compared with healthy controls. In addition, the CASC2 level was negatively associated with the severity of AKI. Furthermore, the decrease in CASC2 expression was verified in mice and a cell model of sepsis. These data indicate that the downregulation of CASC2 may be harmful to sepsis-induced AKI. Thus, CASC2 may also function as a diagnostic marker for sepsis-induced AKI.

Apoptosis is cell suicide or programmed cell death, which is also involved in sepsis-induced AKI (33). The present study found that CASC2 overexpression downregulates LPS-induced apoptosis in HK-2 cells. This led the current study to hypothesize that CASC2 can prevent sepsis-induced AKI by inhibiting the apoptotic pathway. The inflammatory response is recognized as a major cause of sepsis-induced AKI, due to the release of a large number of pro-inflammatory factors in the organism during sepsis (34). An increase in the levels of the 4 most common pro-inflammatory cytokines

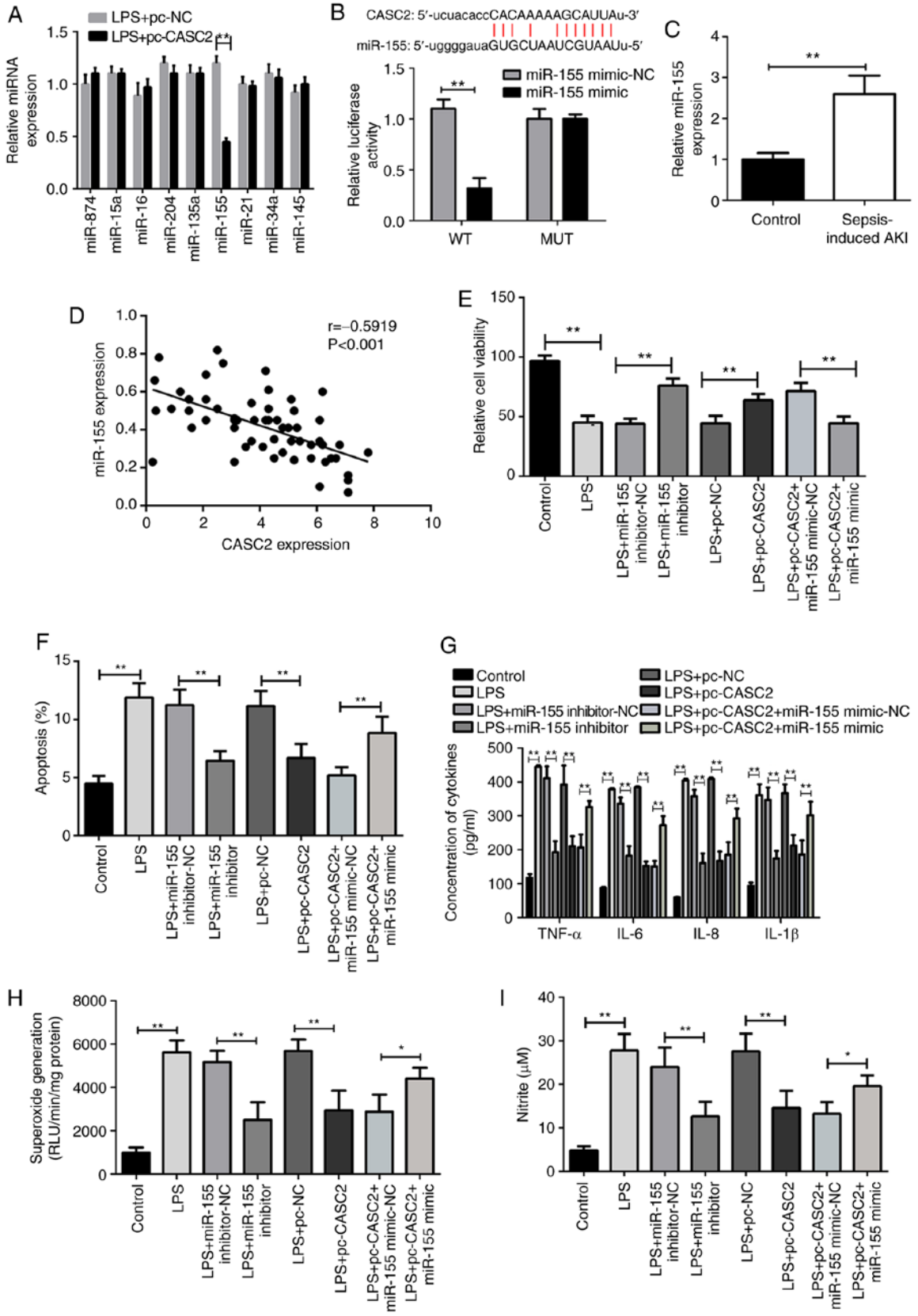

Figure 3. CASC2 negatively regulates the expression of miR-155 in sepsis-induced AKI. (A) RT-qPCR analysis of the expressions of miR-874, miR-15a, miR-16, miR-204, miR-135a, miR-155, miR-21, miR-34a and miR-145 in HK-2 cells from LPS+pc-NC and LPS+pc-CASC2 groups. (B) Relative luciferase activity in 293 cells after co-transfected with wt or mut CASC2 and miR-155 mimic or control miRNA (miR-155 mimic-NC), determined by the luciferase reporter assay. (C) Reverse transcription-quantitative PCR analysis of the expression of miR-155 in the kidney from the control and sepsis-induced AKI mice. (D) Pearson correlation analysis showing the negative relationship between the expression of CASC2 and miR-155 in the serum of 69 sepsis patients and 20 healthy controls. The (E) cell viability, (F) cytokines concentrations, (G) apoptosis, (H) superoxide generation and (I) nitrite production in HK-2 cells from control, LPS, LPS+miR-155 inhibitor-NC, LPS+miR-155 inhibitor, LPS+pc-NC, LPS+pc-CASC2, LPS+pc-NC+miR-155 mimic-NC, LPS+pc-NC+miR-155 mimic groups. *P<0.05 and **P<0.01. CASC2, cancer susceptibility candidate 2; LPS, lipopolysaccharide; miR, microRNA; NC, negative control; AKI, acute kidney injury; wt, wild-type; mut, mutant; IL, interleukin; TNF, tumor necrosis factor.

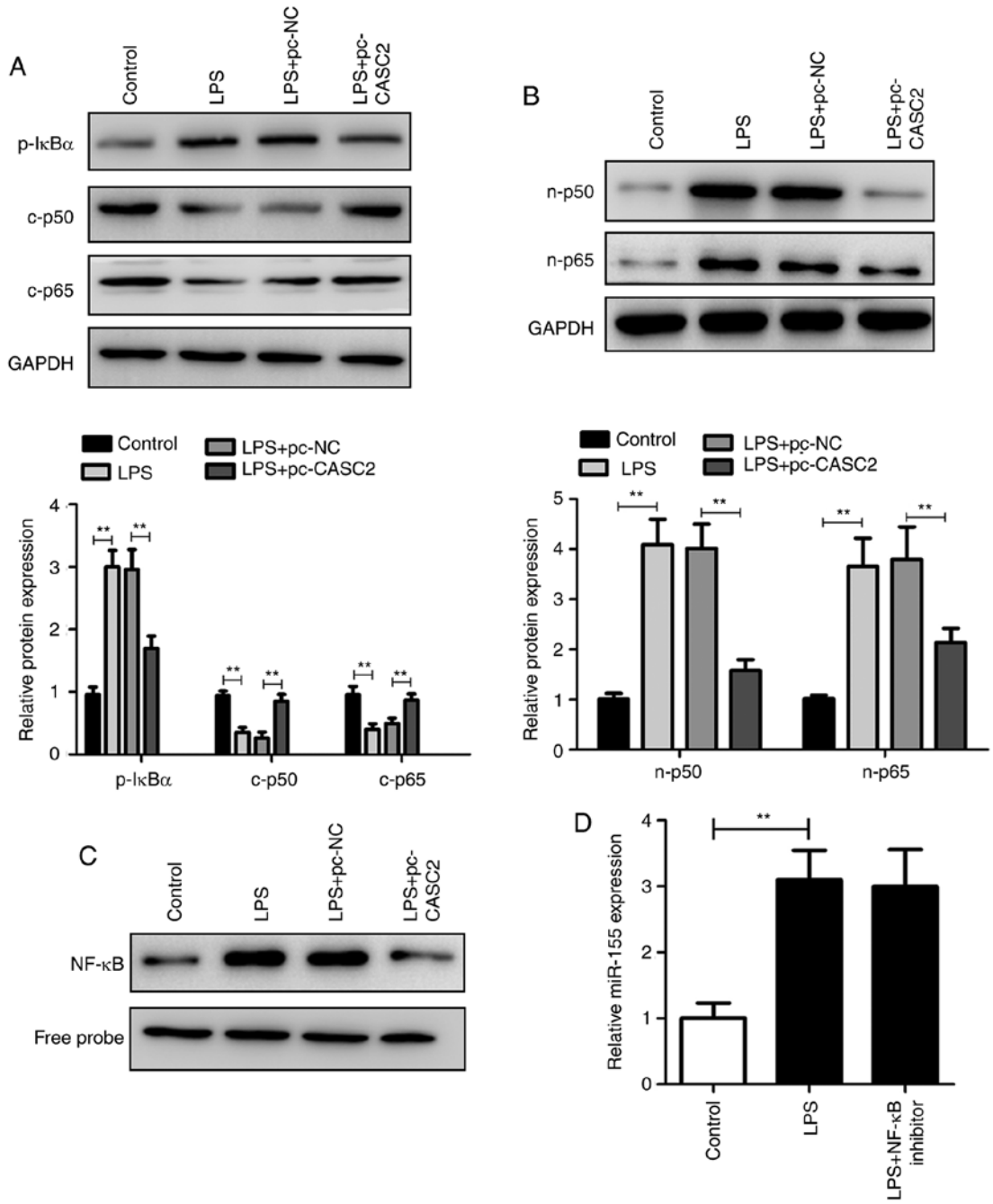

Figure 4. CASC2 attenuates sepsis-induced AKI via the NF-κB signaling pathway. (A) Western blot analysis of the expression of p-IκBα, t-IκBα, c-p50 and c-p65 in the HK-2 cells from the control, LPS, LPS+pc-NC and LPS+pc-CASC2 groups. The expression of p-IκBα was normalized to t-IκBα, and the expression of c-p50 and c-p65 was normalized to GAPDH. (B) Western blot analysis of the expression of n-p50 and n-p65 in the HK-2 cells from the control, LPS, LPS+pc-NC and LPS+pc-CASC2 groups. (C) EMSA analysis of the activity of the NF-κB pathway in HK-2 cells from control, LPS, LPS+pc-NC and LPS+pc-CASC2 groups. (D) Reverse transcription-quantitative PCR analysis of miR-155 expression in HK-2 cells from control, LPS and LPS+NF-κB inhibitor groups. **P<0.01. CASC2, cancer susceptibility candidate 2; LPS, lipopolysaccharide; miR, microRNA; NC, negative control; AKI, acute kidney injury; NF, nuclear factor; p-, phosphorylated; t-, total; c-p50, cytosolic p50; n-p50, nuclear p50.

(TNF-α, IL-6, IL-8 and IL-1β) was observed in a septic cell model. Specifically, it was found that the overexpression of CASC2 inhibits the LPS-induced expression of inflammatory factors in HK-2 cells. On the other hand, the increase of free radicals has been observed in multiple organs during sepsis. Therefore, reducing oxidative stress is one of the ways to interfere with sepsis (35,36). The present results showed that the production of superoxide and NO in HK-2 cells was increased under LPS stimulation, which was reversed following CASC2 overexpression. However, the present data showed that the overexpression of CASC2 has no significant effect on normal HK-2 cells. These data suggest that CASC2 may function mainly in disease conditions, such as AKI.

A large number of studies have identified the interaction between lncRNA and miRNA (37). In the current study, it was confirmed that CASC2 can directly bind to miR-155 to inhibit its expression. Further investigation revealed that CASC2 overexpression can ameliorate LPS-induced injury in HK-2

cells, while inhibiting miR-155 partially reversed these effects, suggesting that CASC2 can interact with miR-155 to affect the progression of sepsis-induced AKI. Also, these findings hint that there may be other mechanisms. NF-κB plays a key role in inflammatory diseases (38) and studies have confirmed that the activation of NF-κB can increase the secretion of cytokines, resulting in multiple-organ injury in sepsis (39). In particular, the present study noticed that CASC2 can inhibit the activation of the NF-κB signaling pathway in sepsis-induced AKI, which also contributes to alleviating disease progression. Although studies have reported that the NF-κB pathway can regulate the expression of miR-155 (40-42), current data found that the inhibition of NF-κB activity did not alter miR-155 expression and the suppression miR-155 did not affect the activity of NF-κB in the present research model. Therefore, the mechanism through which CASC2 inhibits the NF-κB pathway requires further research.

In summary, these results demonstrated that CASC2 expression was significantly decreased in sepsis-induced AKI. In addition, overexpression of CASC2 was confirmed to attenuate LPS-induced damage in human renal tubular epithelial HK-2 cells by targeting the miR-155 and NF-κB pathway. Therefore, CASC2 may be a potential therapeutic target for sepsis induced AKI.

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Availability of data and materials

All data generated or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

MW designed the study, performed experiments and wrote the manuscript. JW and FS performed the clinical study. KZ and TJ contributed to the animal experiments and data analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was performed in accordance with the Declaration of Helsinki. This study was approved by the Ethics Committee of the Huai'an no. 1 People's Hospital of Nanjing Medical University. According to the approval that was received, informed consent was not required. All animal experiments were approved by the Animal Ethics Committee of the Nanjing Medical University and performed in accordance with the Guide for the Care and Use of Laboratory Animals.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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