

Effects and early diagnostic value of lncRNA H19 on sepsis-induced acute lung injury

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Abstract. Sepsis is an immune disease induced by microbial invasion. The molecular mechanism and value of long non-coding H19 (lncRNA H19) in sepsis remain largely unknown. The present study aimed to investigate the effects and early diagnostic value of lncRNA H19 on sepsis-induced acute lung injury (ALI). Serum samples from 85 septic patients and 76 healthy individuals were collected, and the expression of lncRNA H19 was assessed by the quantitative polymerase chain reaction (qPCR). Sprague-Dawley (SD) rats were subjected to cecal ligation and puncture (CLP) in order to construct models of sepsis-induced ALI. A total of 18 successfully modeled rats were randomly allocated into an lncRNA H19-ad group and a model group, and another 9 healthy SD rats from the same batch were selected as a control group. The samples of serum and lung tissue were collected. lncRNA H19 expression was quantified by qPCR, and levels of tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), IL-17, caspase-3, caspase-9, B-cell lymphoma-2 (Bcl-2), and BCL2-associated X (Bax) were measured by western blotting. A receiver operating characteristic (ROC) curve was employed to assess the diagnostic value of lncRNA H19 for septic patients. lncRNA H19 was downregulated in sepsis. Upregulation of lncRNA H19 inhibited TNF- α , IL-6, IL-17, caspase-3, caspase-9 and Bax and increased Bcl-2. The AUC of lncRNA H19 for early diagnosis of sepsis was 0.8197 (95% CI, 0.77 to 0.91). lncRNA H19 alleviated sepsis-induced ALI by inhibiting pulmonary apoptosis and inflammation, serving as a biochemical marker and therapeutic target for sepsis.

Introduction

Sepsis is an immune disease induced by microbial invasion (1) and has a higher incidence in the elderly (2). It not only exhibits

a high prevalence and fatality, but also may lead to subsequent kidney injury (3), cardiac autophagy (4), stroke (5), and lung injury (6-13). At present, due to the lack of a gold standard for early diagnosis (14), it is difficult to distinguish sepsis from other immune diseases, hindering its prevention and treatment. Understanding sepsis from the perspective of molecular biology is conducive to the discovery of reliable and accurate biomarkers for the early diagnosis and treatment.

Long non-coding H19 (lncRNA H19) which is located on human chromosome 11 and approximately 6,291-bp long, does not directly encode genes but regulates gene expression at the post-transcriptional level by pairing and binding to nucleic acid sequences, thereby participating in various diseases (15). According to Wan *et al*, lncRNA H19 induced inflammation and neuronal dysfunction in rats with ischemia-reperfusion injury via the microRNA (miR)-21/PDCD4 pathway (16). It also inhibited epithelial-mesenchymal transition of endothelial cells through tumor growth factor- β 1 (TGF- β 1) and Smad pathway in diabetic retinopathy (17). Bitarafan *et al* reported that upregulated lncRNA H19 may be involved in coronary artery diseases (18). In addition, lncRNA H19 was revealed to activate the signal transducer and activator of transcription/enhancer of zeste homolog 2 (STAT3/EZH2) pathway by downregulating let-7, thus accelerating malignant proliferation of esophageal cancer (19). Moreover, lncRNA H19 was capable of regulating pulmonary fibrosis through the TGF- β 1 pathway via miR-140 (20). It is worth mentioning that the downregulated lncRNA H19 may be associated with sepsis (21).

In the present study, lncRNA H19 was revealed to be downregulated in the serum of septic patients, and thus it was theorized that downregulated lncRNA H19 may be involved in sepsis development and is valuable for early diagnosis. Cecum ligation and puncture (CLP) was performed to establish rat models of sepsis-induced acute lung injury (ALI). Moreover, starBase2.0 demonstrated that lncRNA H19 carried sequence fragments that bound to miR-152-3p. Therefore, it was theorized that lncRNA H19 was likely to be involved in sepsis by regulating miR-152-3p. The lncRNA H19/miR-152-3p axis has been reported to play a role in myeloma (22), but there is no evidence that lncRNA H19 participates in sepsis through miR-152. Therefore, the present study investigated the association between the lncRNA H19/miR-152 axis and sepsis.

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Materials and methods

Septic patients. A total of 85 patients with sepsis (57 males and 28 females, aged 72.74 ± 6.68 years) admitted to the Second Affiliated Hospital of Nanjing Medical University (Nanjing, China) from January 2016 to March 2017 were enrolled. The inclusion criteria were as follows: patients diagnosed with sepsis. The exclusion criteria were as follows: Pregnant or lactating women; individuals ≥ 85 years old; patients with chronic lung-related diseases, advanced adenocarcinoma, mental illness, or those unwilling to cooperate with the treatment; patients with a history of sepsis or blood transfusion. Another 76 healthy individuals undergoing physical examinations were selected as a control group. The Ethics Committee of The Second Affiliated Hospital of Nanjing Medical University (Nanjing, China) approved the present study and patients who participated in this research, signed the informed consent and had complete clinical data.

Venous blood (5 ml) was sampled after 8 h fasting and stored in Eppendorf (EP) tubes without anticoagulant, and then centrifuged at $3 \times 10^3 \times g$ for 15 min at room temperature. Subsequently, the supernatant was placed in an EP tube without RNase, and centrifuged again at $1.2 \times 10^4 \times g$ for 5 min at 4°C . Supernatant was collected and stored at -40°C for testing.

Sepsis diagnostic criteria followed the guidelines for emergency treatment of sepsis/septic shock in China (2018) (23): Patients with infection or suspected infection were diagnosed with sepsis when sepsis-related sequential organ failure assessment (SOFA) score increased more than 2 points from the baseline (24).

Rat model of sepsis. A total of 27 healthy Sprague-Dawley (SD) male rats (aged 2-3 months, weighing 190-270 g; Hunan SJA Laboratory Animal Co., Ltd.) were randomly allocated into a control group, model group, and lncRNA H19 adenovirus (lncRNA H19-ad) group. The rats in the lncRNA H19-ad group were injected with lncH19 expression vectors (lentiviral packaging plasmids; cat. no. V79020; Shanghai Sangon Biotech Co., Ltd.) to upregulate lncRNA H19. The model group and lncRNA H19-ad group were anesthetized with 1% pentobarbital sodium (40 mg/kg body weight) to be modeled via CLP. CLP was performed at the middle of the abdomen, then the wound was sutured. The rats were raised in cages under constant temperature (23°C) and humidity conditions (60%), and were free to move and eat under an alternating 12-h light/dark cycle. The collection of serum samples was as follows: Venous blood (1 ml) was sampled from the tail of the rats, placed in an anticoagulant tube, then centrifuged at $3 \times 10^3 \times g$ at 4°C for 30 min to collect the supernatant. Subsequently, rats were sacrificed with cervical dislocation and received intraperitoneal injection of pentobarbital sodium (150 mg/kg body weight) until respiratory and cardiac arrest. Part of the pulmonary tissue and blood were obtained for pathological section examination and bacterial culture and identification, respectively. The modeling was determined by pathological sections and colony identification. In short, sepsis models were constructed in model and lncRNA H19-ad groups, and the lncRNA H19-ad group received an injection of pcDNA3-lncH19 expression vectors (lentiviral packaging plasmids), while the control group was not subjected to any treatment. Humane endpoints: Rats were

dying or suffering from severe organ failure that could not be treated effectively. No deaths were reported before the end point of the study. The cell line used to amplify lncRNA H19 was 293 cells (ATCC). For plasmid transfection, $2 \mu\text{g}$ plasmid was used for cell transfection, and the lentivirus was packaged with pMDL/pRSV-Rev/VSVG-mixed plasmid and lentivirus plasmid. The transfection kit used was Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). After transfection for 48 h, lentivirus supernatant was collected, and then centrifuged at $1.5 \times 10^3 \times g$ at 4°C for 5 min and stored at -80°C for subsequent experiments.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). After serum sampling, rats were sacrificed and part of the pulmonary tissue was ground into homogenate. A MiRNeasy Serum/Plasma Kit (cat. no. 217184; Qiagen GmbH) was applied to extract RNAs from serum, and TRIzol (Solarbio Life Sciences) was used to isolate total RNAs from pulmonary tissues. The optical density (OD) value of total RNAs at 260-280 nm was obtained by an ultraviolet spectrophotometer, and those with a value of $\text{OD}_{260}/\text{OD}_{280} > 1.8$ were used for subsequent qPCR. FastKing one-step reverse transcription-fluorescence quantitative kit (Beijing Tiangen Biotech Co., Ltd.) and ABI PRISM 7000 (Applied Biosystems; Thermo Fisher Scientific, Inc.) were employed for RNA quantification. The fluorescence quantification was performed using the TaqMan probe method (TaqMan; Applied Biosystems; Thermo Fisher Scientific, Inc.). lncRNA H19 and miR-152-3p primers were designed and synthesized by Sangon Bioengineering (Shanghai) Co., Ltd. The primers were as follows: lncRNA H19 forward, 5'-GTCCGGCCTTCCTGAACACCTT-3' and reverse, 5'-GCTTCACCTTCCAGAGCCGAT-3'; miR-152-3p forward, 5'-GCCTATAAA CATCCGACTG-3' and reverse, 5'-GATCGCTGTCGTGGAAGTCG-3'. The qPCR reaction system (50 μl) contained 1.25 μl forward primer, 1.25 μl reverse primer, 1.0 μl probe, 10 pg/g RNA template, 5 μl 50X ROX Reference Dye, and RNase-Free ddH₂O was added to a final volume of 50 μl . The reaction process was as follows: reverse transcription at 50°C for 30 min for 1 cycle; pre-denaturing at 95°C for 3 min for 1 cycle; denaturation at 95°C for 15 sec, annealing at 60°C for 30 sec, for a total of 40 cycles. ABI PRISM 7000 was used for analyses. U6 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as internal reference genes, and the data were normalized using the $2^{-\Delta\Delta\text{Ct}}$ method (25). The forward primer of GAPDH was 5'-CGGAATTCGTGAAGCTCGGAGTCAACGG-3'; and the reverse primer was 5'-CGGGATCCCAGGAGCGCAGGGTTAGTCA-3'. The upstream primer of U6 was 5'-ATTGGAACGATACAGAGAAGATT-3'; and the downstream primer was 5'-GGAACGCTTCACGAATTG-3'.

Western blotting. The protein extract consisted of the following reagents: protein inhibitor +20 mmol Tris-HCl solution (pH 7.5; both from Solarbio Life Science). After serum sampling, rats were sacrificed and part of the pulmonary tissue was ground into homogenate. The homogenate was mixed with protein extract (1 ml), and the mixture was pipetted repeatedly until the complete lysis of cells. The extract was centrifuged in a pre-cooled centrifuge ($1.6 \times 10^4 \times g$) for 20 min at 4°C . Protein concentration in the supernatant was determined by

bicinchoninic acid (BCA), and the proteins (20 μ g protein loaded per lane) were separated by sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (5% spacer gel and 10% separation gel). Then, the separated proteins were transferred to a nitrocellulose (NC) membrane and maintained for 1 h at room temperature [sealed with 5% skim milk/phosphate-buffered saline (PBS) solution]. Subsequently, the primary antibody against β -actin was added, and maintained overnight at 4°C. The NC membrane was washed three times with PBS solution, and then goat anti-rabbit secondary antibody [horseradish peroxidase (HRP) conjugate] was added and maintained for 1 h at room temperature. Finally, after washing with PBS solution, the NC membrane was visualized with enhanced chemiluminescence (ECL) solution. ImageJ software (version 1.60; National Institutes of Health) was used to analyze the relative protein expression levels. β -Actin was used as the internal reference protein, and the relative expression level of the protein was calculated as follows: Relative protein expression level=gray value of the test band/gray value of the β -actin band. Apoptosis-related proteins [caspase-3 (product code ab32351; 1:5,000), caspase-9 (product code ab32539; 1:1,000), B-cell lymphoma-2 (Bcl-2; product code ab196495; 1:2,000), and BCL2-associated X (Bax; product code ab32503; 1:10,000)], inflammatory factors [tumor necrosis factor- α (TNF- α ; product code ab205587; 1:1,000), interleukin (IL)-6 (product code ab208113; 1:1,000) and IL-17 (product ab218013; 1:1,000)], β -actin antibody, goat anti-rabbit secondary antibody H&L (DyLight® 488; product code ab96899; 1:20,000), cleaved caspase-3 (product code ab214430; 1:5,000) and cleaved caspase-9 (product code ab2324; 1:5,000) were all purchased from Abcam.

Enzyme-linked immunosorbent assay (ELISA). Venous blood (1 ml) sampled from the tail of laboratory rats was placed in an anticoagulant tube, then centrifuged ($3 \times 10^3 \times g$) at 4°C for 30 min to collect the supernatant. The ELISA kits (Abcam) detected the levels of IL-17 (product code ab214028), TNF- α (product code ab208348), and IL-6 (product code ab222503).

Wet to dry (W/D) weight ratio, myeloperoxidase (MPO), superoxide dismutase (SOD), malondialdehyde (MDA). After obtaining serum samples part of the pulmonary tissue was ground into homogenate. MPO was determined by colorimetry using an MPO colorimetric activity assay kit (cat. no. K744; BioVision, Inc.) according to the instructions of the kit. Trichloroacetic acid (10%) was added into ground tissue samples, and centrifuged at $4 \times 10^3 \times g$ at 4°C for 10 min. Subsequently, 0.6% thiobarbituric acid was added to the supernatant, followed by heating in a boiling water bath for 15 min. Subsequently the absorbance at 532, 600 and 450 nm was detected. Additionally, the SOD concentration was determined by a Super Oxide Disruption Activity Assay Kit (product no. ab65354; Abcam) according to the instructions of the kit.

The dry and wet weight of the right middle and lower lobes were measured to calculate the W/D weight ratio.

Dual-luciferase reporter gene assay. Lung epithelial cells (BEAS-2B; ATCC) were cultured at 37°C in a 5% CO₂ incubator with a culture medium system containing Dulbecco's modified Eagle's Medium (DMEM) (Hyclone; Cytiva), 10% fetal

bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), and 1% penicillin/streptomycin solution (100X; Solarbio Life Science). starBase2.0 (26) predicted the targeting binding loci between lncRNA H19 and miR-152-3p. GLO-H19-wild type (wt) and GLO-H19-mutant (mut) vectors were constructed and co-transfected with miR-152-3p mimics (5'-UCAGUGCAU GACAGAACUUGG-3') and NC-mimics (5'-ACUACUGAG UGACAGUAGA-3') (designed and synthesized by Sangon Biotech Co., Ltd.) respectively. The transfection kit used was Lipofectamine 2000. The transfected cells were cultured on 96-well plates for 48 h. Then, a Dual-luciferase reporter gene assay system (Promega Corporation) was employed to determine the luciferase activity. In this study, pGLO plasmid vectors (Bio-Rad Laboratories, Inc.) were used. The relative luciferase activity was measured as follows: Relative luciferase activity=luciferase activity of glowworm/internal reference luciferase activity of sea pansy.

Statistical analysis. The data were statistically analyzed with SPSS 20.0 (Asia Analytics formerly SPSS, China) and visualized with GraphPad Prism8.0 (GraphPad Software, Inc.). Measurement data were expressed by the mean \pm standard deviation ($\bar{X} \pm SD$). Between-group comparisons were performed by independent sample's t-test and multi-group comparisons by one way ANOVA, followed by pairwise post hoc comparisons (Dunnett's test). The counting data were expressed by cases/percentage [n(%)], and the inter-group comparisons adopted the χ^2 test. Pearson correlation analysis was used to determine the correlations between lncRNA H19 and patient data. Receiver operating characteristic (ROC) curve was employed to assess the diagnostic value of lncRNA H19 for septic patients. All data were analyzed with two-tailed tests. The confidence interval (CI) was set at 95%. $P < 0.05$ indicated a statistically significant difference.

Results

lncRNA H19 is expressed at a low level in septic patients and rats. Firstly, the serum samples of 85 septic patients and 76 healthy individuals were collected for lncRNA H19 quantification with qPCR. Serum lncRNA H19 in septic patients was significantly lower than that in healthy individuals (Fig. 1A). Subsequently, CLP was performed on rats to construct septic models. qPCR quantified the expression of lncRNA H19, and the results revealed that it was downregulated in the serum and pulmonary tissue of septic rats (model group) compared with the control group (Fig. 1B and C). The aforementioned results suggested a close association between lncRNA H19 and sepsis.

Clinical value of lncRNA H19. Correlations between lncRNA H19 and general data of septic patients were statistically analyzed. lncRNA H19 was not revealed to be correlated to sex and body mass index (BMI), but was correlated to age, history of alcoholism, smoking, white blood cell (WBC), procalcitonin (PCT), sequential organ failure assessment (SOFA) and acute physiology and chronic health evaluation II (APACHEII) scores, as well as sepsis-induced ALI (Table I). In addition, lncRNA H19 was negatively correlated to miR-152-3p. Therefore, lncRNA H19 may be a biomarker of sepsis.

Table I. Correlation between general data and H19 (n=85).

Characteristics	n (%) or mean \pm SD	H19	t/r	P-value
Sex			1.851	0.0677
Male	57 (67.06)	0.75 \pm 0.13		
Female	28 (32.94)	0.69 \pm 0.16		
Age	72.74 \pm 6.68	0.73 \pm 0.21	0.784	0.0026
BMI	20.26 \pm 1.01	0.73 \pm 0.21	0.117	0.2871
History of alcoholism			2.660	0.0094
Yes	61 (71.76)	0.76 \pm 0.19		
No	24 (28.24)	0.65 \pm 0.11		
Smoking history			3.115	0.0025
Yes	54 (63.53)	0.77 \pm 0.17		
No	31 (36.47)	0.66 \pm 0.13		
WBC ($10^9/l$)	13.96 \pm 4.56	0.73 \pm 0.21	-0.739	<0.0001
MDA (nmol/ml)	16.95 \pm 4.66	0.73 \pm 0.21	-0.759	<0.0001
SOD (kU/l)	41.86 \pm 9.26	0.73 \pm 0.21	-0.794	<0.0001
PCT (ng/ml)	9.12 \pm 2.53	0.73 \pm 0.21	-0.647	<0.0001
SOFA	8.57 \pm 2.34	0.73 \pm 0.21	-0.713	0.0146
APACHEII	16.98 \pm 4.42	0.73 \pm 0.21	-0.687	0.0069
Sepsis type			2.510	0.0140
Sepsis without ALI	36	0.71 \pm 0.18		
Sepsis with ALI	49	0.69 \pm 0.15		
miR-152	1.36 \pm 0.16	0.73 \pm 0.21	-0.718	0.0125

BMI, body mass index; WBC, white blood cell; MDA, malondialdehyde; SOD, superoxide dismutase; PCT, procalcitonin; SOFA, sequential organ failure assessment; APACHEII, acute physiology and chronic health evaluation II; ALI, acute lung injury; miR, microRNA.

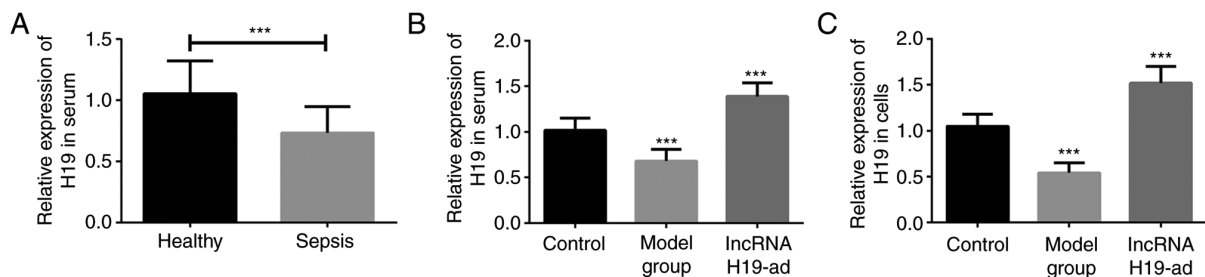


Figure 1. lncRNA H19 is expressed at a low level in sepsis. (A) lncRNA H19 was downregulated in the serum of septic patients; ***P<0.001. (B) lncRNA H19 was down-regulated in the serum of septic rats; ***P<0.001 vs. the control group. (C) lncRNA H19 was downregulated in the pulmonary tissue of septic rats; ***P<0.001 vs. the control group. lncRNA, long non-coding RNA.

ROC curve that was employed to assess the diagnostic value of lncRNA H19 demonstrated that the area under the curve (AUC) of lncRNA H19 for early diagnosis of sepsis was 0.8197 (95% CI, 0.77 to 0.91), while that for diagnosis of sepsis patients with ALI and without ALI was 0.9141 (95% CI, 0.87 to 0.96) and 0.8399 (95% CI, 0.77 to 0.91), respectively (Fig. 2; Table II). The outcomes indicated the significant value of lncRNA H19 for early diagnosis of sepsis.

Upregulation of lncRNA H19 reduces inflammation and pulmonary apoptosis in septic rats. Caspase-3, caspase-9, Bax, and Bcl-2 in pulmonary tissue were detected to investigate the effects of lncRNA H19 on sepsis. Lung injury is

characterized by pulmonary apoptosis, and the increase of pro-apoptotic proteins (caspase-3 and caspase-9) and the decrease of anti-apoptotic protein (Bcl-2) are capable of inducing apoptosis directly (27). Therefore, the aforementioned four proteins were indicators in the evaluation of pulmonary apoptosis. Caspase-3, caspase-9, and Bax were increased while Bcl-2 was decreased in septic rats compared to the control group. In addition, upregulation of lncRNA H19 suppressed caspase-3, caspase-9 and Bax and increased Bcl-2 expression compared to the model group (Fig. 3A and D). As a systemic inflammatory response syndrome, sepsis inevitably causes changes in inflammatory factors (TNF- α , IL-6 and IL-17) (28-30), thus the levels of these factors were determined

Table II. ROC curve of lncRNA H19.

Patients	AUC	SE	95% CI	P-value
Sepsis without ALI	0.8399	0.0377	0.77-0.91	<0.0001
Sepsis with ALI	0.9141	0.0247	0.87-0.96	<0.0001
Sepsis	0.8197	0.0322	0.76-0.88	<0.0001

ROC, receiver operating characteristic; lncRNA, long non-coding RNA; AUC, area under the curve; SE, standard error; CI, confidence interval; ALI, acute lung injury.

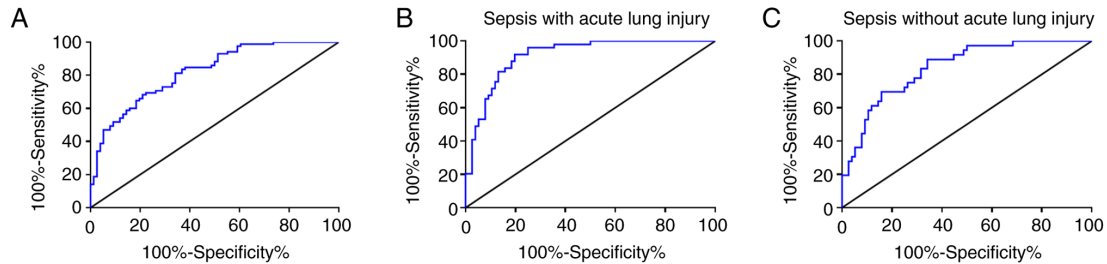


Figure 2. ROC analysis of lncRNA H19 for early diagnosis of sepsis. (A) lncRNA H19 for early diagnosis of sepsis, n=85; AUC=0.8197; 95% CI, 0.77 to 0.91. (B) lncRNA H19 for diagnosis of septic patients with ALI, n=49; AUC=0.9141; 95% CI, 0.87 to 0.96. (C) lncRNA H19 for diagnosis of septic patients without ALI, n=36; AUC=0.8399; 95% CI, 0.77 to 0.91. ROC, receiver operating characteristic; lncRNA, long non-coding RNA; ALI, acute lung injury; AUC, area under the curve; CI, confidence interval.

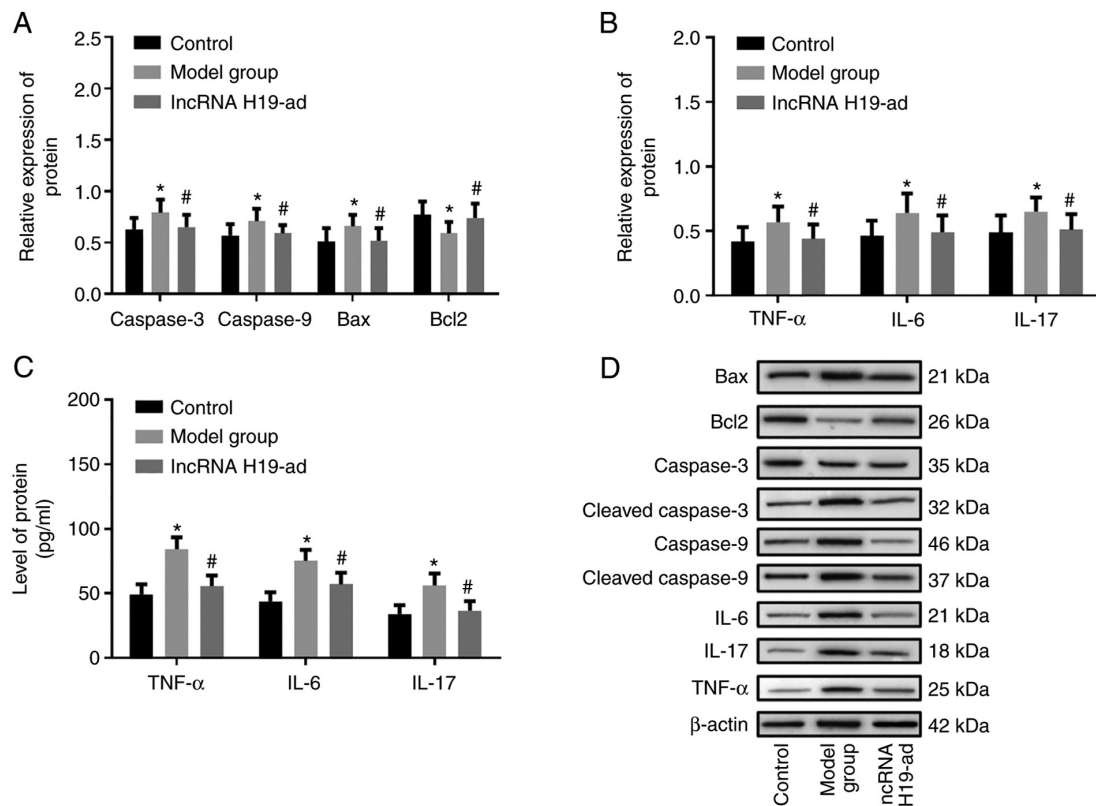


Figure 3. Upregulation of lncRNA H19 reduces pulmonary inflammation and apoptosis in septic rats. (A) lncRNA H19 downregulated caspase-3, caspase-9, Bax and upregulated Bcl-2. (B) Upregulation of lncRNA H19 reduced TNF- α , IL-6 and IL-17 in pulmonary tissue. (C) Upregulation of lncRNA H19 reduced TNF- α , IL-6 and IL-17 in serum. (D) Western blotting of caspase-3, caspase-9, Bax, Bcl-2, TNF- α , IL-6 and IL-17. *P<0.05 vs. the control group; #P<0.05 vs. the model group. lncRNA, long non-coding RNA; Bax, BCL2-associated X; Bcl-2, B-cell lymphoma-2; TNF- α , tumor necrosis factor- α ; IL, interleukin.

as well to evaluate the inflammatory response. TNF- α , IL-6, and IL-17 were increased in the pulmonary tissue and serum of

septic rats compared to the control group, while upregulation of lncRNA H19 reduced their levels compared with the model

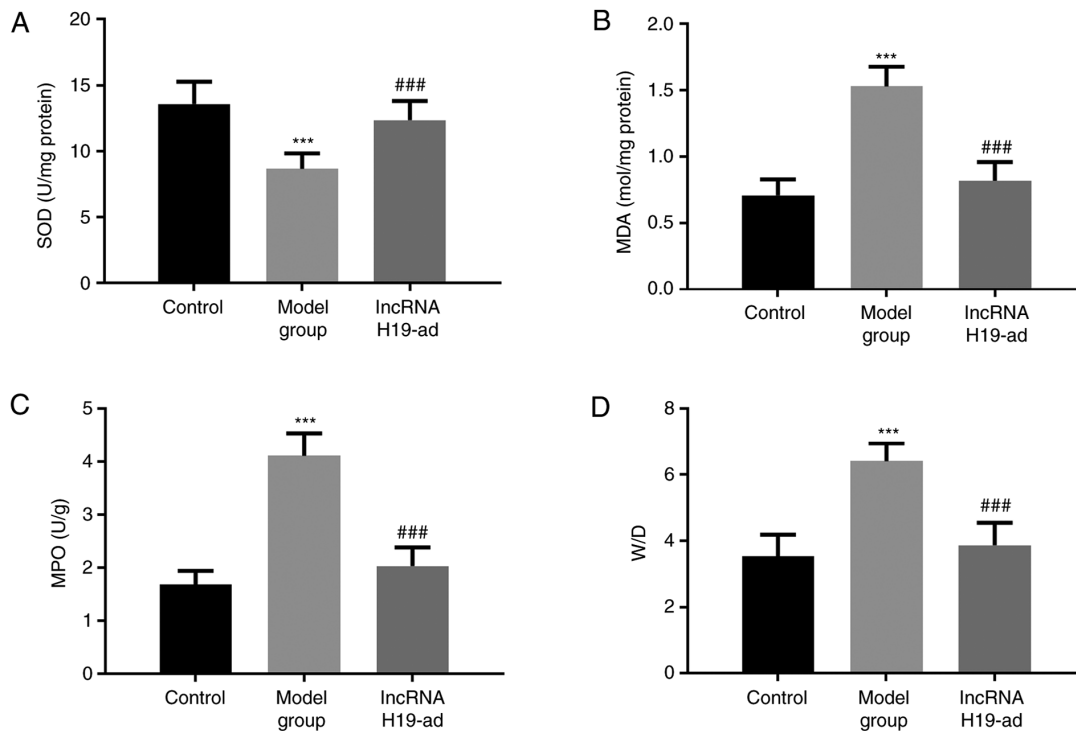


Figure 4. Upregulation of lncRNA H19 improves pulmonary function in septic rats. (A) Effects of lncRNA H19 on SOD. (B) Effects of lncRNA H19 on MDA. (C) Effects of lncRNA H19 on MPO. (D) Effects of lncRNA H19 on W/D. ***P<0.001 vs. the control group; ###P<0.001 vs. the model group. lncRNA, long non-coding RNA; SOD, superoxide dismutase; MDA, malondialdehyde; MPO, myeloperoxidase; W/D, wet to dry.

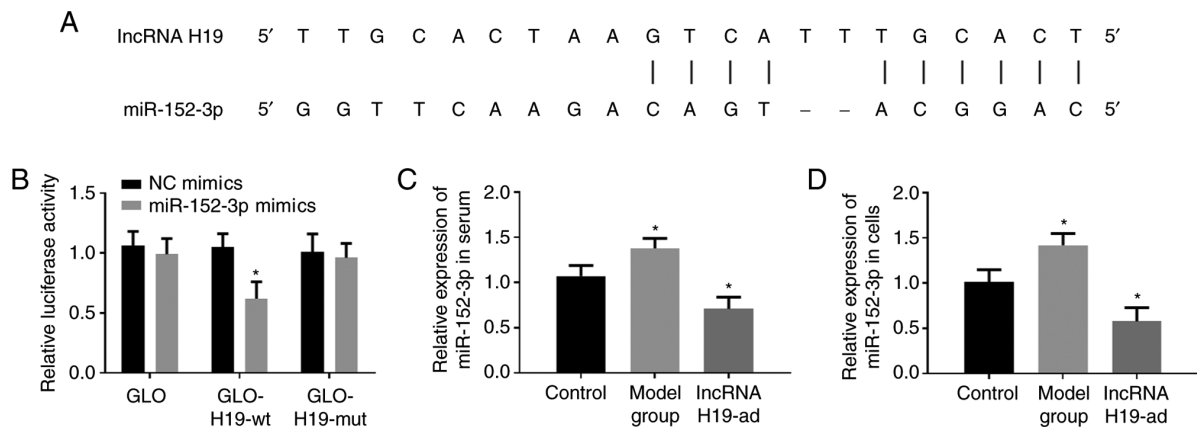


Figure 5. miR-152-3p is the target gene of lncRNA H19. (A) lncRNA H19 was capable of binding to miR-152-3p. (B) Dual-luciferase reporter gene assay. *P<0.05 vs. the NC-mimics group. (C) Upregulation of lncRNA H19 suppressed the expression of miR-152-3p in serum; *P<0.05 vs. the control group. (D) Upregulation of lncRNA H19 suppressed miR-152-3p in cells; *P<0.05 vs. the control group. lncRNA, long non-coding RNA; miR, microRNA.

group (Fig. 3B and C). These results indicated that upregulation of lncRNA H19 reduced pulmonary inflammation and apoptosis in septic rats.

Upregulation of lncRNA H19 improves pulmonary function in septic rats. The effects of lncRNA H19 on pulmonary function were evaluated by W/D, MPO, SOD, and MDA (Fig. 4). Compared with the control group, SOD was decreased while MDA, MPO and W/D were increased in septic rats. Compared with model group, upregulation of lncRNA H19 increased SOD and decreased MDA, MPO and W/D. Therefore, upregulation of lncRNA H19 improved pulmonary function in septic rats.

miR-152-3p is the target gene of lncRNA H19. starBase2.0 predicted that lncRNA H19 was capable of binding to miR-152-3p (Fig. 5A). Moreover, upregulation of lncRNA H19 suppressed the expression of miR-152-3p in serum and tissue (Fig. 5C and D). Therefore, it was theorized that miR-152-3p could bind to and negatively regulate lncRNA H19. Dual-luciferase reporter gene assay was adopted to verify the authenticity of the binding sites. The results revealed that the co-transfection of lncRNA H19-wt and miR-152-3p-mimics decreased the luciferase activity, while no significant changes were observed by other co-transfection combinations (Fig. 5B). The aforementioned results indicated that lncRNA H19 downregulated miR-152-3p by binding to it.

Discussion

How to perform accurate early diagnosis of sepsis, a thorny public health issue, is the focus of present sepsis research. Due to their highly active participation in the progression of sepsis, non-coding RNAs have become a research hotspot. Thus, seeking biomarkers for early diagnosis of sepsis from non-coding RNAs has also been considered a new approach to challenge sepsis. Fang *et al* observed that lncRNA H19 was downregulated in the peripheral blood of septic patients (21), and the aberrant downregulation was also reported in our study. Therefore, it is theorized that the downregulated lncRNA H19 may have potential value for early diagnosis of sepsis and participate in its process.

By analyzing the relationship between the general data and lncRNA H19, the close association between downregulated lncRNA H19 and sepsis was revealed. In addition, ROC curve demonstrated that the AUC of lncRNA H19 for the diagnosis of sepsis was 0.8197 (95% CI, 0.76 to 0.88, $P < 0.0001$). Therefore, lncRNA H19 was revealed as a biomarker of sepsis and achieved high early diagnostic value.

In addition, the association between lncRNA H19 and sepsis was also explored by upregulating lncRNA H19. Pulmonary apoptosis and inflammatory responses are pathological manifestations of sepsis-induced ALI (27), therefore, studying the effect of lncRNA H19 on both could assess its effects on this disease. It was determined that upregulated lncRNA H19 not only alleviated inflammatory responses in septic rats by downregulating TNF- α , IL-6 and IL-17, but also inhibited pulmonary apoptosis by downregulating caspase-3, caspase-9, and Bax. In addition, upregulated lncRNA H19 also improved pulmonary function (W/D, MPO, SOD, and MDA). It is worth mentioning that upregulation of lncRNA H19 triggered downregulation of miR-152-3p in pulmonary tissue. starBase predicted that lncRNA H19 was capable of binding to miR-152-3p. Dual-luciferase reporter gene assay demonstrated that co-transfection of lncRNA H19-wt and miR-152-3p significantly decreased luciferase activity, indicating that lncRNA H19 alleviated sepsis-induced ALI by downregulating miR152.

miR-152-3p is located on human chromosome 11 with a length of approximately 87bp. The most common biological function of miR-152-3p is to reduce mRNA stability at a post-transcriptional level (22). Numerous studies have revealed that it participates in the pathological pathways of a number of diseases. In liver-related diseases, miR-152-3p has been revealed to affect liver cell viability and liver fibrosis through cyclin-dependent kinase 8 (CDK8) and GLI family zinc finger 3 (GLI3) (31,32). Downregulated miR-152-3p promoted the growth of liver cancer cells (33). In addition, aberrantly expressed miR-152-3p has also been revealed to be associated with prostate cancer, keloids, and sepsis (34-36). According to our findings, as a downstream target gene of lncRNA H19, miR-152-3p may mediate the regulation of lncRNA H19 on pulmonary apoptosis and inflammation. Thus, the lncRNA H19/miR-152-3p axis exhibits potential therapeutic value for sepsis, and upregulating lncRNA H19 may alleviate sepsis-induced ALI.

Our study explored the relationship between lncRNA H19 and sepsis in terms of diagnostic value and molecular biology, and reported that lncRNA H19 was valuable for early diagnosis

of sepsis and was capable of inhibiting its progression via miR-152-3p. In the future, the target genes and signaling pathways located downstream of miR-152-3p will be investigated and the molecular network of lncRNA H19 in sepsis will be improved, in order to provide more systematic and reliable references for sepsis treatment. There are several limitations in the present study, such as failure to further detect more indicators and lack of survival analyses due to limited time and equipment support. We will address these limitations in our future work.

In conclusion, lncRNA H19 was revealed as a potential early diagnostic biomarker for sepsis. Moreover, it alleviated sepsis-induced ALI and reduced inflammation in rats by downregulating miR-152-3p, which revealed that upregulation of lncRNA H19 may hinder the progression of sepsis.

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Availability of data and materials

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

Authors' contributions

YZ designed the study and drafted the manuscript. LS, MZ and LH were responsible for the collection and analysis of the experimental data. HC revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The Second Affiliated Hospital of Nanjing Medical University (Nanjing, China). Patients who participated in this research, signed the informed consent and had complete clinical data.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Lever A and Mackenzie I: Sepsis: Definition, epidemiology, and diagnosis. *BMJ* 335: 879-883, 2007.
2. Martin-Loeches I, Guia MC, Valleccoccia MS, Suarez D, Ibarz M, Irazabal M, Ferrer R and Artigas A: Risk factors for mortality in elderly and very elderly critically ill patients with sepsis: A prospective, observational, multicenter cohort study. *Ann Intensive Care* 9: 26, 2019.

3. Gómez H and Kellum JA: Sepsis-induced acute kidney injury. *Curr Opin Crit Care* 22: 546-553, 2016.
4. Sun Y, Cai Y and Zang QS: Cardiac autophagy in sepsis. *Cells* 8: 141, 2019.
5. Shao IY, Elkind MSV and Boehme AK: Risk factors for stroke in patients with sepsis and bloodstream infections. *Stroke* 50: 1046-1051, 2019.
6. Chen X, Wang T, Song L and Liu X: Activation of multiple toll-like receptors serves different roles in sepsis-induced acute lung injury. *Exp Ther Med* 18: 443-450, 2019.
7. Wu J, Yan X and Jin G: Ulinastatin protects rats from sepsis-induced acute lung injury by suppressing the JAK-STAT3 pathway. *J Cell Biochem* 120: 2554-2559, 2019.
8. Zheng H, Liang W, He W, Huang C, Chen Q, Yi H, Long L, Deng Y and Zeng M: Ghrelin attenuates sepsis-induced acute lung injury by inhibiting the NF- κ B, iNOS, and Akt signaling in alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol* 317: L381-L391, 2019.
9. Park I, Kim M, Choe K, Song E, Seo H, Hwang Y, Ahn J, Lee SH, Lee JH, Jo YH, *et al*: Neutrophils disturb pulmonary microcirculation in sepsis-induced acute lung injury. *Eur Respir J* 53: 1800786, 2019.
10. Zou Y, Bao S, Wang F, Guo L, Zhu J, Wang J, Deng X and Li J: FN14 blockade on pulmonary microvascular endothelial cells improves the outcome of sepsis-induced acute lung injury. *Shock* 49: 213-220, 2018.
11. Aziz M, Ode Y, Zhou M, Ochani M, Holodick NE, Rothstein TL and Wang P: B-1a cells protect mice from sepsis-induced acute lung injury. *Mol Med* 24: 26, 2018.
12. Wang J, Gong S, Wang F, Niu M, Wei G, He Z, Gu T, Jiang Y, Liu A and Chen P: Granisetron protects polymicrobial sepsis-induced acute lung injury in mice. *Biochem Biophys Res Commun* 508: 1004-1010, 2019.
13. Englert JA, Bobba C and Baron RM: Integrating molecular pathogenesis and clinical translation in sepsis-induced acute respiratory distress syndrome. *JCI Insight* 4: e124061, 2019 (Online ahead of print).
14. Delahanty RJ, Alvarez J, Flynn LM, Sherwin RL and Jones SS: Development and evaluation of a machine learning model for the early identification of patients at risk for sepsis. *Ann Emerg Med* 73: 334-344, 2019.
15. Rezaei M, Mokhtari MJ, Bayat M, Safari A, Dianatpuor M, Tabrizi R, Asadabadi T and Borhani-Haghighi A: Long non-coding RNA H19 expression and functional polymorphism rs217727 are linked to increased ischemic stroke risk. *BMC Neurol* 21: 54, 2021.
16. Wan P, Su W, Zhang Y, Li Z, Deng C, Li J, Jiang N, Huang S, Long E and Zhuo Y: lncRNA H19 initiates microglial pyroptosis and neuronal death in retinal ischemia/reperfusion injury. *Cell Death Differ* 27: 176-191, 2020.
17. Thomas AA, Biswas S, Feng B, Chen S, Gonder J and Chakrabarti S: lncRNA H19 prevents endothelial-mesenchymal transition in diabetic retinopathy. *Diabetologia* 62: 517-530, 2019.
18. Bitarafan S, Yari M, Broumand MA, Ghaderian SM, Rahimi M, Mirfakhraie R, Azizi F and Omrani MD: Association of increased levels of lncRNA H19 in PBMCs with risk of coronary artery disease. *Cell J* 20: 564-568, 2019.
19. Chen MJ, Deng J, Chen C, Hu W, Yuan YC and Xia ZK: lncRNA H19 promotes epithelial mesenchymal transition and metastasis of esophageal cancer via STAT3/EZH2 axis. *Int J Biochem Cell Biol* 113: 27-36, 2019.
20. Wang X, Cheng Z, Dai L, Jiang T, Jia L, Jing X, An L, Wang H and Liu M: Knockdown of long noncoding RNA H19 represses the progress of pulmonary fibrosis through the transforming growth factor β /Smad3 pathway by regulating miR-140. *Mol Cell Biol* 39: e00143-19, 2019.
21. Fang Y, Hu J, Wang Z, Zong H, Zhang L, Zhang R and Sun L: lncRNA H19 functions as an Aquaporin 1 competitive endogenous RNA to regulate microRNA-874 expression in LPS sepsis. *Biomed Pharmacother* 105: 1183-1191, 2018.
22. Zheng JF, Guo NH, Zi FM and Cheng J: Long non-coding RNA H19 promotes tumorigenesis of multiple myeloma by activating BRD4 signaling by targeting miR-152-3p. *Mol Cell Biol* 40: e00382-19, 2020.
23. Liu XW, Ma T, Cai Q, Wang L, Song HW and Liu Z: Elevation of serum PARK7 and IL-8 levels is associated with acute lung injury in patients with severe sepsis/septic shock. *J Intensive Care Med* 34: 662-668, 2019.
24. Ferreira FL, Bota DP, Bross A, Mélot C and Vincent JL: Serial evaluation of the SOFA score to predict outcome in critically ill patients. *JAMA* 286: 1754-1758, 2001.
25. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
26. Li JH, Liu S, Zhou H, Qu LH and Yang JH: starBase v2.0: Decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. *Nucleic Acids Res* 42: D92-D97, 2014.
27. Xie W, Lu Q, Wang K, Lu J, Gu X, Zhu D, Liu F and Guo Z: miR-34b-5p inhibition attenuates lung inflammation and apoptosis in an LPS-induced acute lung injury mouse model by targeting progranulin. *J Cell Physiol* 233: 6615-6631, 2018.
28. Murray A, Gow AJ, Venosa A, Andres J, Malaviya R, Adler D, Yurkow E, Laskin JD and Laskin DL: Assessment of mustard vesicant lung injury and anti-TNF- α efficacy in rodents using live-animal imaging. *Ann N Y Acad Sci* 1480: 246-256, 2020.
29. Chepurnova DA, Samoilova EV, Anisimov AA, Verin AD and Korotaeva AA: Compounds of IL-6 receptor complex during acute lung injury. *Bull Exp Biol Med* 164: 609-611, 2018.
30. Gouda MM and Bhandary YP: Acute lung injury: IL-17A-mediated inflammatory pathway and its regulation by curcumin. *Inflammation* 42: 1160-1169, 2019.
31. Yin T, Liu MM, Jin RT, Kong J, Wang SH and Sun WB: miR-152-3p modulates hepatic carcinogenesis by targeting cyclin-dependent kinase 8. *Pathol Res Pract* 215: 152406, 2019.
32. Li L, Zhang L, Zhao X, Cao J, Li J and Chu G: Downregulation of miR-152 contributes to the progression of liver fibrosis via targeting Gli3 in vivo and in vitro. *Exp Ther Med* 18: 425-434, 2019.
33. Wang WL, Yu DJ and Zhong M: lncRNA HAGLROS accelerates the progression of lung carcinoma via sponging microRNA-152. *Eur Rev Med Pharmacol Sci* 23: 6531-6538, 2019.
34. Moya L, Meijer J, Schubert S, Matin F and Batra J: Assessment of miR-98-5p, miR-152-3p, miR-326 and miR-4289 expression as biomarker for prostate cancer diagnosis. *Int J Mol Sci* 20: 1154, 2019.
35. Wang R, Bai Z, Wen X, Du H, Zhou L, Tang Z, Yang Z and Ma W: miR-152-3p regulates cell proliferation, invasion and extracellular matrix expression through by targeting FOXF1 in keloid fibroblasts. *Life Sci* 234: 116779, 2019.
36. Dong L, Li H, Zhang S and Yang G: miR-148 family members are putative biomarkers for sepsis. *Mol Med Rep* 19: 5133-5141, 2019.



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