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ORIGINAL RESEARCH

Rs9839776 Genetic Variant of lncRNA SOX2OT Contributes to Susceptibility of Acute Kidney Injury in Sepsis Patients via Regulating SOX2OT/miR-9-5p Axis

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Purpose: Single nucleotide polymorphisms (SNPs) are commonly found in lncRNA, and can regulate its expression. The study examined the genotype and allele distributions of rs9839776 polymorphism in lncRNA SOX2OT in sepsis patients with acute kidney injury (AKI), as well as its expression changes. The function of SOX2OT in AKI cell model was also elucidated.

Patients and Methods: Serum SOX2OT levels were examined via qRT-PCR in 450 septic patients including 202 cases with AKI and 248 without. Genotyping of rs9839776 polymorphism was completed via Taqman real-time PCR. HK-2 cells were treated with LPS to mimic AKI, the cell viability, apoptosis and inflammatory response were evaluated after regulating SOX2OT levels. The function and pathways enriched by the downstream target genes were explored via GO and KEGG analysis.

Results: Rs9839776 CC genotype carriers were commonly observed in sepsis patients with AKI, and presented reduced levels of SOX2OT. Serum SOX2OT was lowly expressed in AKI patients, which can distinguish AKI patients from sepsis ones. In vitro, SOX2OT alleviated LPS-induced AKI via mediating cell proliferation, apoptosis and inflammatory response, which was reversed by miR-9-5p. GO and KEGG analysis uncovered significant links of miR-9-5p target genes with cytoskeleton in muscle cells, cell adhesion molecules and prolactin signaling pathway.

Conclusion: The CC genotype of rs9839776 polymorphism in SOX2OT could affect the susceptibility of AKI for sepsis patients, and its-mediated SOX2OT downregulation may serve as a biomarker for AKI. The underlying mechanism might be related to the mediation of the SOX2OT/miR-9-5p axis.

Keywords: sepsis, acute kidney injury, Rs9839776, SOX2OT/miR-9-5p axis

Introduction

Sepsis refers to a systemic inflammatory response syndrome that is triggered by the intrusion of pathogenic microbes like bacteria into the body.[1] The pathogenesis of sepsis is mainly related to pathogen infection, host factors and medical-related factors. Acute kidney injury (AKI) is a usual complication in patients with sepsis and is also one of the independent risk factors for the death of patients with sepsis.[2] Its pathogenesis is complex and involves multiple aspects such as inflammatory response, oxidative stress, abnormal energy consumption and cell metabolism, and autophagy imbalance.[3] AKI caused by sepsis can lead to prolonged hospital stays for patients, progression to chronic kidney disease and high mortality.[4] In the future, further in-depth research on the pathogenesis of acute kidney injury caused by sepsis is needed to explore more effective treatment methods to improve the survival rate and quality of life of patients.

Long noncoding RNA (lncRNA) is a class of non-coding RNA molecules whose transcript length is greater than 200 nucleotides. Unlike traditional protein-coding genes, lncRNAs do not have protein-coding capabilities. It is widely present in cells and plays an important regulatory role in a variety of biological processes.[5] In recent years, the role of

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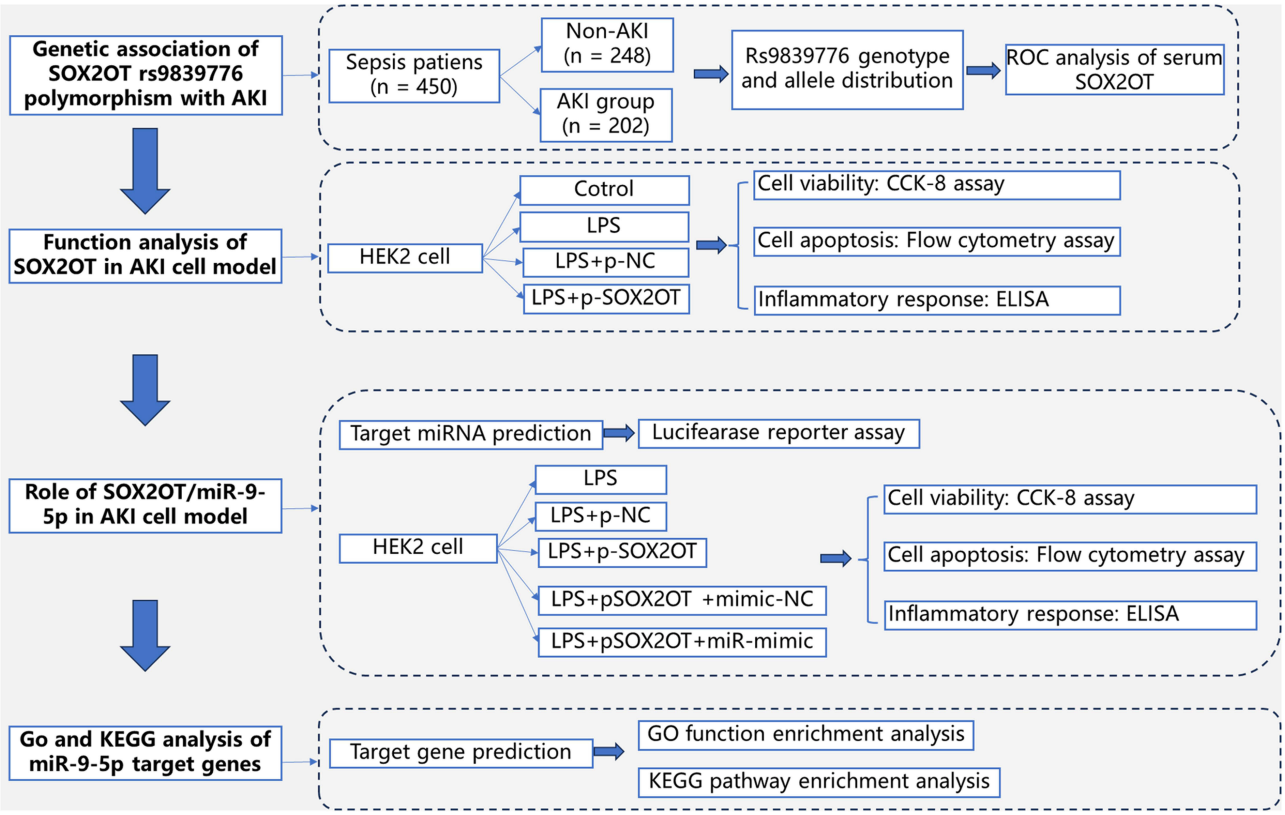
Graphical Abstract



lncRNA in sepsis complicated with AKI has attracted increasing attention.[6] Multiple studies have shown that lncRNA can participate in the regulation of inflammatory responses in sepsis complicated with AKI. For example, the expression of lncRNA small nucleolar RNA host gene 14 (SNHG14) is increased in lipopolysaccharide (LPS)-induced HK-2 cells, which stimulates oxidative stress, inflammation and apoptosis of HK-2 cells.[7] LncRNA MALAT1 also plays an important role in sepsis complicated with AKI. MALAT1 promotes the progression of AKI by sponging miR-205 and stimulating the release of inflammatory factors.[8]

LncRNA SOX2 overlapping transcript (SOX2OT) is a kind of non-coding RNA with important biological functions.[9] Previously, SOX2OT is reported to be down-regulated in sepsis rats, exacerbating LPS-induced inflammatory factors and sepsis-induced cardiac dysfunction.[10] Moreover, it plays an important role in many kinds of kidney diseases. In diabetic nephropathy, down-regulated SOX2OT has been detected and is involved in mesangial cell proliferation and fibrosis in diabetic nephropathy.[11] In addition, downregulated SOX2OT contributes to high-glucose-induced podocyte injury via mediating cell autophagy and miR-9/SIRT1 axis.[12] All evidence demonstrates the potential effect of lncRNA SOX2OT on sepsis-induced acute kidney injury.

Single nucleotide polymorphisms (SNPs) are DNA sequence polymorphisms caused by variation of a single nucleotide at the genomic level. SNPs are widely found in the human genome and are one of the most common forms of human genetic variation. Previously, several SNPs have been identified to be related to AKI susceptibility for sepsis patients., such as rs361525 of TNF-α gene, rs721917 of SP-D gene and rs41275743 and rs4648143 of NFKB1 gene.[13,14] It has been reported that there are a large number of SNPs in lncRNA, which may affect the structure and function of lncRNA.[15] Rs9839776 is a common SNP of lncRNA SOX2OT, it can mediate the expression of SOX2OT.[16] The three genotypes CC, CT and TT of rs9839776 showed different associations with the disease in different studies. In Chinese breast cancer patients, rs9839776 CT/TT genotype carriers accounted for a relatively high proportion, and high

SOX2OT levels were detected.[16] Interestingly, rs9839776 shows a negative correlation with susceptibility to sepsis in children in southern China, and T allele may contribute to decreased sepsis risk.[15] However, its genetic association with sepsis-induced AKI has not been elucidated. Previously, downregulated SOX2OT has been reported to be related to kidney injury,[11] while CC genotype of rs9839776 is determined to contribute to the downregulation. Therefore, we speculated that rs9839776 CC genotype of SOX2OT might be positively related to AKI susceptibility for sepsis patients.

Therefore, sepsis patients with or without AKI were enrolled in the current study. The genotype and allele distributions of rs9839776 polymorphism in lncRNA SOX2OT were examined in the study groups. In addition, its expression changes and clinical value were also elucidated in sepsis patients. Moreover, the role of lncRNA SOX2OT in in vitro AKI cell models was explored, and the underlying mechanism was preliminary discussed.

Materials and Methods

Study Cohort

A total of 450 septic patients were recruited from Binzhou Medical University Hospital under the approval of the ethics committee of Binzhou Medical University Hospital, including 202 cases with AKI and 248 without AKI. The sample size of this case-control study was calculated using the G*Power analysis software. Power analysis is based on the formula used for sample size calculation: $N = p\ (1\text{-}p)\ (Z/E)^2$ with power value = 0.926.[17] The study primary endpoint was an incidence of AKI. AKI was defined as follows: serum creatinine (Scr) elevation that exceeds 0.3 mg/dL (26.5 μmol/L) within 48 hours, or serum creatinine elevation that is more than 1.5 times the baseline value, or urine output less than 0.5 mL/kg/h for over 6 hours.[18] According to the Kidney Disease Improving Global Outcomes (KDIGO) guidelines, Acute kidney injury (AKI) was categorized into stages 1–3.[19] Patients with the following conditions were excluded from the present study: (1) pregnant or lactating women; (2) those with chronic kidney disease; (3) cases with chronic respiratory disease; (4) those with missing information, such as age, gender, admission diagnoses, and discharge diagnoses. To avoid sampling bias, the two groups of patients were matched in terms of age and sex. In addition, the use of uniform questionnaires, detection methods and diagnostic criteria to ensure that the data collection process of the two groups is consistent, reducing the bias caused by different data collection methods. Conduct uniform training for the investigators involved in the research to ensure the consistency and accuracy of the investigation process.

Rs9839776 Polymorphism Genotyping

5 mL of anticoagulated blood was extracted from patients with sepsis during their admission. The anticoagulated blood samples were then utilized to extract genomic DNA with the help of the TIANamp Blood DNA Kit. Subsequently, the DNA was detected by means of an ultraviolet spectrophotometer, and the qualified DNA samples were preserved in a −80°C refrigerator for subsequent utilization. The rs11752942 polymorphism was genotyped by applying the Taqman real-time PCR method, which was described in previous studies. Moreover, 10% of the samples were randomly selected for repetition, and it was found that the results were completely consistent, with a concordance rate of 100%.

Cell Culture and Modeling

HK-2 cells were obtained from the Cell Bank of the Chinese Academy of Science (in Shanghai, China). These cells were maintained in DMEM (from Gibco, Carlsbad, CA, USA) supplemented with high-glucose medium (Gibco), 1% penicillin-streptomycin (Gibco), and 10% FBS (Gibco) under the conditions of 37°C and 5% CO_2. To mimic the sepsis-induced AKI cell model, HK-2 cells were exposed to various doses of LPS (2, 5 and 10 μg/mL) for 8 hours. Cells exposed to 0 μg/mL were applied as the control group.

Cell Transfection

HK-2 cells at the logarithmic growth stage were selected and inoculated into 96-well plates with 5×105 cells per well. When the cell fusion degree was 80%-90%, Lipofectamine 2000 was used for cell transfection. To regulate lncRNA SOX2OT, overexpression plasmids for SOX2OT were constructed in pcDNA 3.0 (p- SOX2OT) by Invitrogen (Grand Island, NY, USA), and the empty vector (pcDNA-NC) was set as the negative control. The sequences of miR-5p mimic

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and its corresponding negative control (mimic-NC), in addition to the miR-9-5p inhibitor and its inhibitor-NC, were fabricated to adjust the miR-9-5p levels within HK-2 cells. Once the transfection had proceeded for 6 hours, the existing medium was substituted with a new one, and the cells were incubated for another 48 hours. Thereafter, the transfected cells were harvested for the ensuing experiments.

RT-qPCR

Total RNA was extracted from serum and cells of each group by Trizol reagent. 1.0 μgRNA was used for cDNA Synthesis and reverse transcription was performed by PrimeSeript RT reagent Kit or Mir-X miRNA FirstStrand Synthesis Kit. Then TB Green Fast qPCR Mix and MirX miRNA qRT-PCR TB Green Kit were used for real-time fluorescence quantitative PCR. PCR condition was as follows: pre-denaturation at 95°C for 5 min; denaturation at 95°C for 30s, annealed at 56°C for 30s, extended at 72°C for 40s, a total of 30 cycles were completed. GAPDH was used as the internal reference for SOX2OT and U6 was for miR-9-5p. The relative expression levels of SOX2OT and miR-9-5p were calculated by $2^{-\Delta\Delta Ct}$ method.

CCK-8 Assay

HK - 2 cells at the logarithmic growth stage were selected and their density was adjusted to 2×10^4 cells/mL. 100 μL/well cells were inoculated into 96-well plates, which were cultured in an incubator for 24 h and then grouped and transfected according to the requirements. After continued culture for 24, 48, and 72 h, the cell culture plates were taken out at the corresponding time points, and 10 μL CCK-8 solution was added to each well, and cultured at 37 °C for 4 h. The optical density of each well at 450 nm was measured by enzyme labeling.

Flow Cytometry Assay

The transfected cells of each group were collected and re-suspended in 200 μL PBS. Subsequently, 10 μL Annexin V - FITC and 10 μL PI were added to the cells, followed by incubation at 4°C for 30 minutes under dark conditions. Subsequently, the cell apoptosis rate in each group was determined through flow cytometry. The apoptosis rate was equivalent to the aggregate of the early apoptosis rate and the late apoptosis rate.

ELISA Assay

The transfected cells were collected and centrifuged at 1000 r/min for 10 min. The cell supernatant was collected, and the levels of TNF-α, IL-1β and IL-6 in the cell supernatant of each group were detected according to the operating instructions of the ELISA kit.

Target Gene Prediction

To examine the target miRNAs of lncRNA SOX2OT, the online dataset ENCORI was applied. The prediction of downstream target genes of miR-9-5p was carried out using multiple databases. Specifically, miRDB was employed with the criterion of a Target Score exceeding 80. TargetScan was used with the requirement of a Total context++ score being less than −0.25. microT was utilized with the condition of an interaction_score greater than 0.9. Additionally, the ENCORI database was involved, with the screening standard of CLIP-DATA being greater than or equal to 1. Subsequently, Venn diagrams were generated to visually represent the overlapping genes among these three databases. For the overlapping genes, the enriched terms were annotated by means of Gene Ontology (GO) analysis, while the enriched pathways were annotated through Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis respectively.

Luciferase Reporter Assay

The cells were subjected to cotransfection with either the miR-9-5p mimic or inhibitor, along with the wild-type (WT) or mutant seed region (MUT) of miR-9-5p in the 3'-UTR of SOX2OT. Lipofectamine 2000 (manufactured by Invitrogen, USA) was employed for the cell transfection operation. The relative luciferase activity was gauged using the Dual-Luciferase Reporter System (produced by Promega Corporation, USA) in accordance with the instructions provided by the manufacturer. The Renilla fluorescence activity was taken as the internal reference for this assessment.

GO and KEGG Analysis

Gene Ontology (GO) classification was performed to obtain the corresponding functions of miR-9-5p target genes. Then the KEGG pathway analysis was applied to annotate enriched pathways related to the overlapping target genes.

Statistical Analysis

Data analysis was conducted with the aid of SPSS 21.0 and GraphPad 9.0 software. The Chi square test was employed to examine the differences in categorical variables. For the comparison of differences in continuous variables among groups, the Student's *t* test and one-way ANOVA were utilized. The relationship between the rs9839776 polymorphism and AMI susceptibility was quantified by means of probability odds ratios (ORs) and 95% confidence intervals (CIs). The frequency of each genotype was analyzed using Hardy-Weinberg equilibrium (HWE). A *P*-value less than 0.05 signifies a significant difference.

Results

General Clinical Data Characteristics of the Study Subjects

Table 1 shows the general clinical data of the two study groups. The mean age was 44.94 years for the non-AKI group consisting of 119 males and 129 females. 97 males and 105 females constituted the AKI group, with the mean age of 45.87 years. There was no notable disparity in the distribution of age and gender between the two groups ($P > 0.05$). In contrast to the non-AKI group, cases in the AKI group exhibited high values of SOFA score ($P < 0.01$). Their comorbidities showed no notable disparity between the two groups ($P > 0.05$). The infection foci of all cases were recorded, respiratory system was the most common foci. In contrast to the non-AKI group, the infection foci of a high proportion of cases in AKI group were enriched in bloodstream, while less AKI cases occurred in abdominal cavity and urinary tract ($P < 0.01$).

Genotype and Allele Distributions of lncRNA SOX2OT rs9839776 Polymorphism

The genotype and allele distributions of SOX2OT rs9839776 polymorphism were displayed in Table 2, and HWE test indicated the representation of the enrolled study population ($P > 0.05$). CT genotype carriers were less observed in AKI group compared to non-AKI group, determining its genetic association with low AKI risk for sepsis patients (OR= 0.597, 95% CI = 0.399–0.892). There may be different genetic patterns in the association between gene polymorphism and

Table 1 Demographic and Clinical Characteristics of the Study Subjects

Items	No-AKI (n = 248)	AKI (n = 202)	*P* value
Age, year	44.94±7.87	45.87±8.25	0.220
Sex, male/female	119/129	97/105	0.994
SOFA score	10.18±4.13	12.10±2.95	< 0.001
Comorbidities, n (%)			
Hypertension	29 (11.69)	30 (14.85)	0.324
Diabetes mellitus	34 (13.71)	39 (19.31)	0.109
Infection foci, n (%)			< 0.001
Respiratory system	107 (43.15)	86 (42.57)	
Abdominal cavity	45 (18.15)	30 (14.85)	
Urinary tract	67 (27.02)	31 (15.35)	
Bloodstream	23 (9.27)	48 (23.76)	
Unknown	6 (2.41)	7 (3.47)	

Abbreviations: AKI, acute kidney injury; SOFA, Sequential Organ Failure Assessment.

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Table 2 Genotype and Allele Distributions of lncRNA SOX2OT rs9839776 Polymorphism in Sepsis Patients with or Without AKI

Genetic Models	Non-AKI group (n = 248)	AKI Group (n = 202)	χ 2	OR (95% CI)	*P*
Codominant					
CC	129 (52.02)	131 (64.85)	-	1	-
CT	99 (39.92)	60 (29.70)	6.364	0.597 (0.399–0.892)	0.012
TT	20 (8.06)	11 (5.45)	2.461	0.542 (0.250–1.175)	0.117
Dominant					
CC	129 (52.02)	131 (64.85)	-	1	-
CT/TT	119 (47.98)	71 (35.15)	7.518	0.588 (0.401–0.860)	0.006
Recessive					
CC/CT	228 (91.94)	191 (94.55)	-	1	-
TT	20 (8.06)	11 (5.45)	1.190	0.657 (0.307–1.404)	0.275
Alleles					
C	357 (71.98)	322 (79.70)	-	1	-
T	139 (28.02)	82 (20.30)	7.176	0.654 (0.479–0.893)	0.007
P^{HWE}	0.869	0.244			

Abbreviations: HWE, Hardy-Weinberg equilibrium; AKI, acute kidney injury; OR, odd ratio; CI, confidence interval.

disease. Dominant or recessive model analysis can reasonably group data according to possible genetic patterns, reduce the influence of confounding factors, more accurately evaluate the relationship between gene polymorphism and disease, and improve the reliability of research results. Therefore, the genetic association between SOX2OT rs9839776 polymorphism and AKI risk was further analyzed in dominant or recessive models. It was found that, SOX2OT rs9839776 polymorphism was significantly associated with a low risk of AKI for sepsis patients in the dominant model (OR = 0.588, 95% CI =0.401–0.860, $P < 0.01$). Under allelic model, the T allele of rs9839776 polymorphism was found to be related to a lower risk of AKI for sepsis patients compared with the C allele (OR = 0.654, 95% CI = 0.479–0.893, $P < 0.01$).

Correlation of lncRNA SOX2OT rs9839776 Polymorphism with AKI KDIGO Stages

All AKI patients were divided into three groups based on the AKI KDIGO stage, and the genotype and allele distributions of SOX2OT rs9839776 polymorphism were examined in cases with different stages of AKI. As demonstrated in Table 3, it was observed that the frequencies of the CC genotype and the C allele were notably elevated in patients at KDIGO stage 3. In contrast, the frequencies of the CT genotype, the TT genotype, as well as the T allele in patients at KDIGO stage 3 were significantly decreased when compared to those of the KDIGO stage 1 patients ($P < 0.05$). The findings indicated that SOX2OT rs9839776 CC genotype was associated with AKI severity of sepsis patients, and the AKI of cases carrying CT or TT genotypes were less severe.

Serum SOX2OT Levels of Cases with Difference Genotypes of rs9839776 Polymorphism and its Diagnostic Value Analysis in AKI

According to qRT-PCR results, CC genotype carriers exhibited significantly low values of serum SOX2OT compared to CT/TT genotype carriers in either sepsis patients with or without AKI (Figure 1A, $P < 0.001$). Moreover, in comparison with non-AKI group, serum SOX2OT levels were significantly downregulated in AKI patients (Figure 1B, $P < 0.001$). In

Table 3 Genotype and Allele Distributions of lncRNA SOX2OT rs9839776 Polymorphism in Sepsis Patients with Different AKI KDIGO Stage

Genotype/Allele	AKI KDIGO Stage			*P* value
	1 (n = 59)	2 (n = 102)	3 (n = 41)	
Genotype				
CC	27 (45.76)	71 (69.61)	33 (80.49)	0.001
CT	25 (42.37)	28 (27.45)	7 (17.07)	0.019
TT	7 (11.86)	3 (2.94)	1 (2.44)	0.035
Alleles				
C	79 (66.95)	170 (83.33)	73 (89.02)	< 0.001
T	39 (33.05)	34 (16.67)	9 (10.98)	

Abbreviations: AKI, acute kidney injury; KDIGO, Kidney Disease Improving Global Outcomes.

light of the dysregulated SOX2OT in AKI patients, its diagnostic value was further examined. As seen in Figure 1C, the outstanding diagnostic performance of serum SOX2OT in differentiating AKI for sepsis patients was presented by ROC curve, with the AUC of 0.938.

Protective Role of SOX2OT in LPS-Treated HK-2 Cell

HK-2 cells were treated with different doses of LPS to mimic sepsis-induced AKI in vitro. As seen in Figure 2A, in LPS-stimulated HK-2 cells, the expression of SOX2OT was reduced in a dose-dependent manner, and difference reached an extremely significant level when LPS dose was 5 μg/mL. Consequently, 5 μg/mL LPS was chosen for further investigation. To uncover the precise functions of SOX2OT in septic AKI, p-SOX2OT or p-NC was introduced into LPS-induced HK-2 cells. The level of SOX2OT was significantly upregulated following p-SOX2OT transfection when compared to the p-NC group, which demonstrated the successful transfection of p-SOX2OT (Figure 2B). As CCK-8 assay indicated, an obvious inhibition of cell viability was observed in LPS-triggered HK-2 cells, but after p-SOX2OT transfection, the cell viability was promoted again (Figure 2C). In addition, the enhanced cell apoptosis of cell models was suppressed by SOX2OT overexpression (Figure 2D). Similarly, in LPS-stimulated HK-2 cells, the pro-inflammatory cytokines of TNF-α, IL-1β and IL-6 were released in large quantities, but p-SOX2OT transfection significantly inhibited this trend

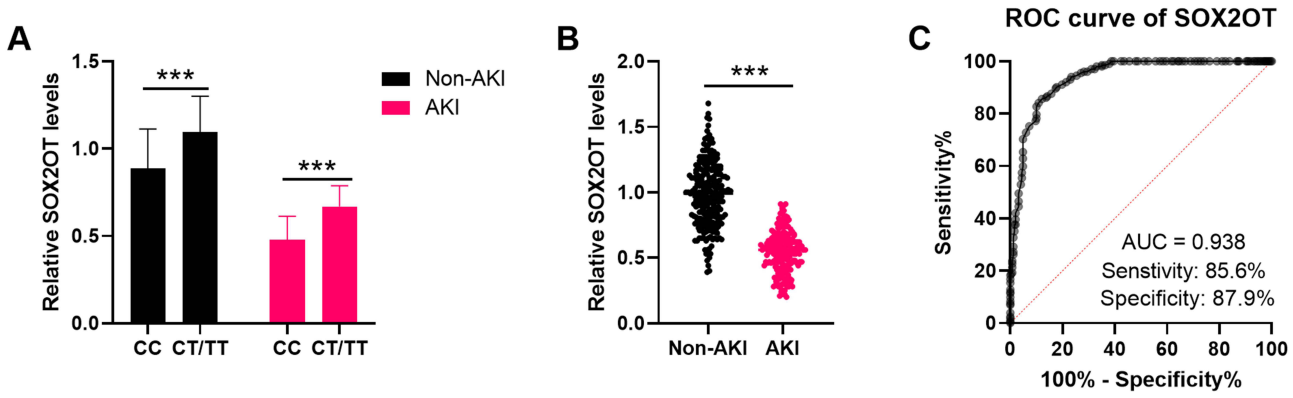

Figure 1 Serum SOX2OT levels of cases with difference genotypes of rs9839776 polymorphism and its diagnostic value analysis. (**A**) Serum SOX2OT levels of cases with difference genotypes of rs9839776 polymorphism. (**B**) Serum SOX2OT levels in sepsis patients with or without AKI. (**C**) ROC curve of serum SOX2OT in differentiating AKI from septic cases. *** $P < 0.001$.

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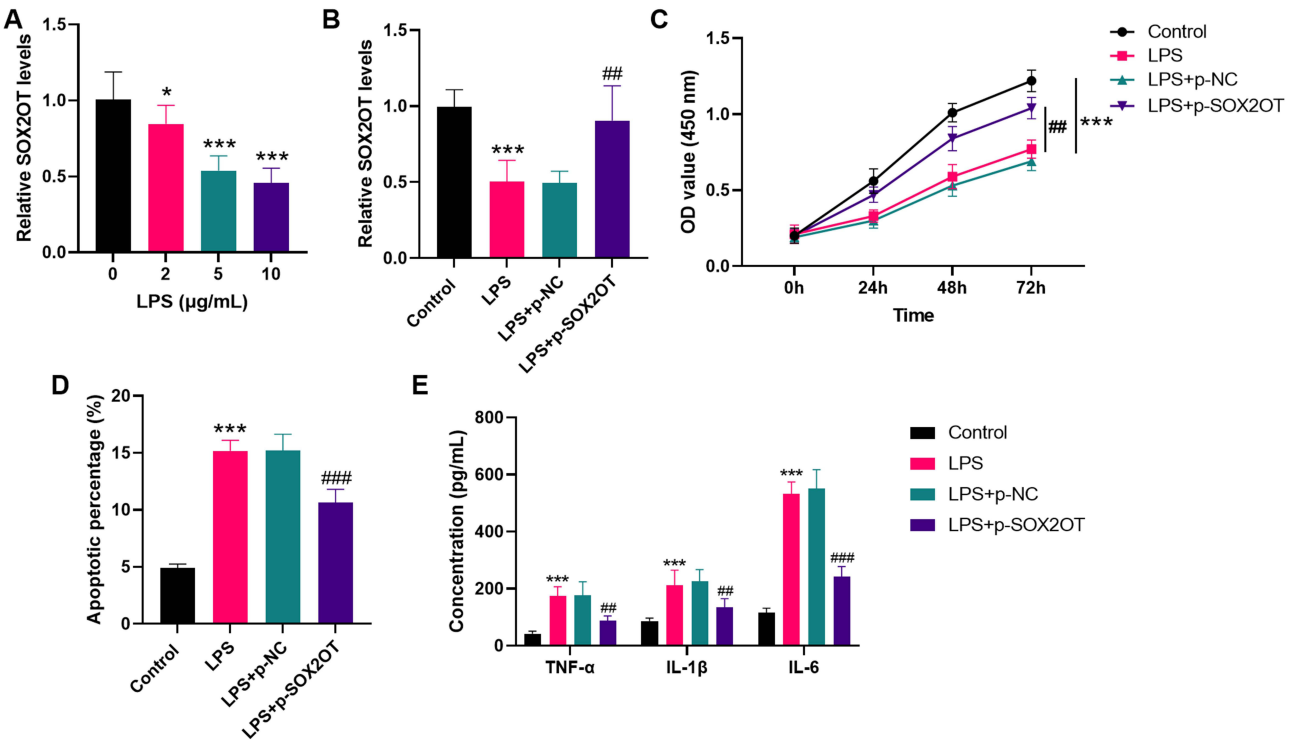

Figure 2 Protective role of SOX2OT in LPS-treated HK-2 cell. (**A**) The expression of SOX2OT in HK-2 cells treated with different doses of LPS. (**B**) SOX2OT levels in HK-2 cells after cell transfection. (**C**) Cell viability of HK-2 cells under different treatments. (**D**) Cell apoptosis of HK-2 cells under different treatments. (**E**) Concentrations of pro-inflammatory cytokines of TNF-α, IL-1β and IL-6 in HK-2 cells under different treatments. *** $P < 0.001$ relative to control group; ### $P < 0.001$ relative to LPS group.

(Figure 2E). These data indicated that SOX2OT could alleviate sepsis-induced AKI and modulate LPS-triggered inflammatory response in HK-2 cells.

SOX2OT Binds with miR-9-5p

Figure 3A shows the potential binding seeds between SOX2OT and miR-9-5p, which was predicted by ENCORI. Then their binding capacity was verified via dual luciferase reporter assay. Luciferase activity of WT-SOX2OT group was significantly suppressed when co-transfected with miR-9-5p mimic, while it was enhanced when co-transfected with miR-9-5p inhibitor (Figure 3B). But no significant difference was observed in MUT- SOX2OT group (Figure 3B). Clinically, remarkably increased miR-9-5p levels were detected in the serum of AKI patients compared to non-AKIs, and they were negatively correlated with serum SOX2OT levels (Figure 3C and D). In vitro, miR-9-5p levels upregulated gradually along with increasing LPS concentration in HK-2 cells (Figure 3E). All findings proved that SOX2OT could negatively regulate the expression of miR-9-5p.

Overexpression of miR-9-5p Reverses the Effect of SOX2OT on HK-2 Cells

To further explore the regulatory network involving SOX2OT and miR-9-5p, both miR-9-5p and lncRNA SOX2OT were co-transfected into HK-2 cells. Intriguingly, as depicted in Figure 4A, the expression level of miR-9-5p was notably reduced in the p-SOX2OT group when compared to that in the p-NC group. However, the co-transfection of p-SOX2OT and miR-9-5p mimic led to an increase in the expression of miR-9-5p. Moreover, the overexpression of SOX2OT was found to promote cell proliferation and suppress cell apoptosis. This effect was weakened by the up-regulation of miR-9-5p, as shown in Figure 4B and C. More than that, miR-9-5p overexpression also reversed the effects of SOX2OT on the release of TNF-α, IL-1β and IL-6 in LPS-treated HK-2 cells (Figure 4D). The results revealed that overexpression of miR-9-5p reversed the effect of SOX2OT on HK-2 cells's proliferation, apoptosis and inflammatory response.

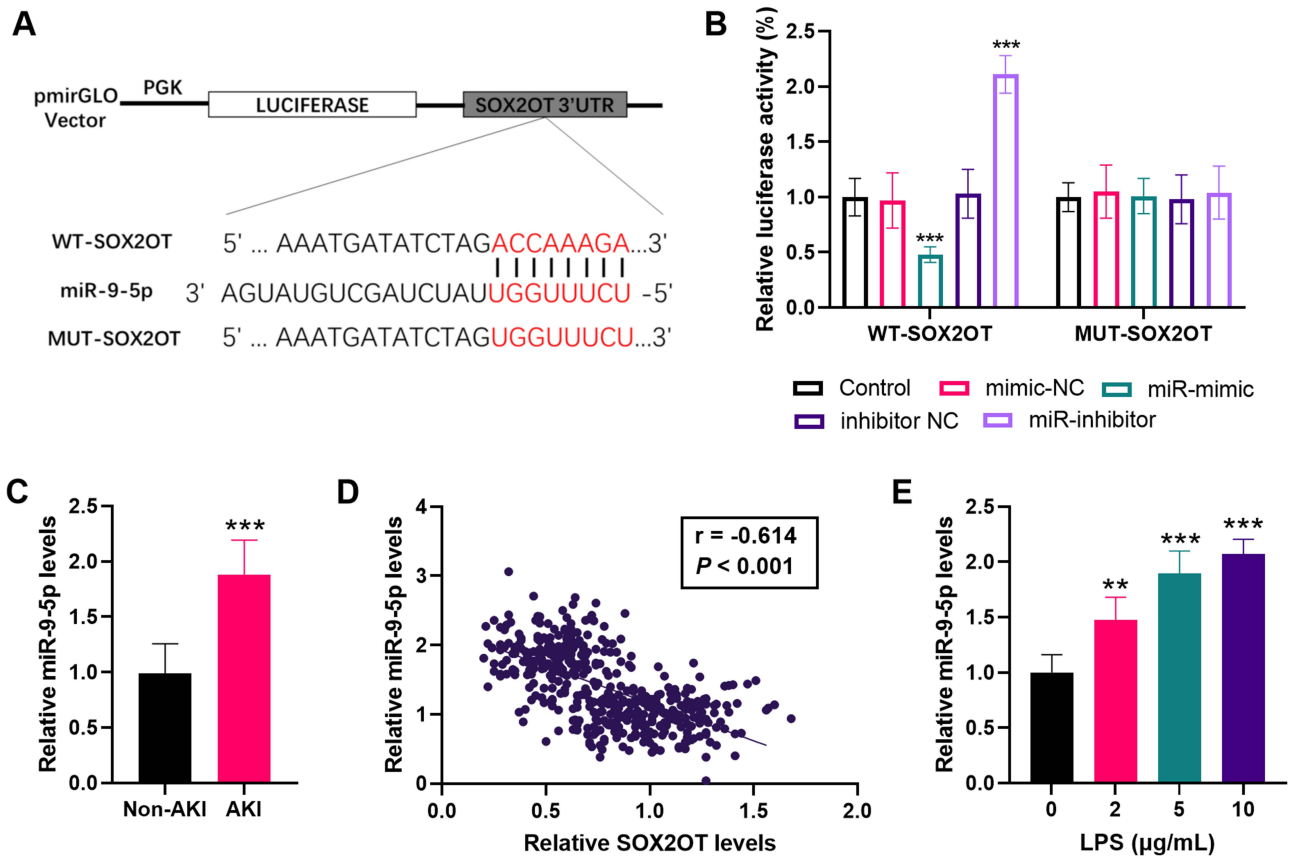

Figure 3 SOX2OT binds with miR-9-5p. (**A**) Binding sites of SOX2OT containing miR-9-5p and dual-luciferase reporter vector. (**B**) Luciferase activity of HK-2 cells transfected with different luciferase reporter vectors after mediating miR-9-5p levels. (**C**) Serum miR-9-5p levels in sepsis patients with or without AKI. (**D**) The expression of miR-9-5p in HK-2 cells treated with different doses of LPS. (**E**) The expression of miR-9-5p in HK-2 cells treated with different doses of LPS. *** $P < 0.001$.

GO Function and KEGG Pathway Enrichment of miR-9-5p Target Genes

To examine the potential function and signaling pathway affected by miR-9-5p, miRDB, ENCORI, and TargetScan were used to predict the target genes of miR-9-5p, and 76 overlapping targets were obtained via VENN plot (Figure 5A). The function and pathways enriched by the overlapping target genes were explored via GO and KEGG analysis. As shown in Figure 5B, the enrichment of biological processes related to basement membrane organization, cellular response to insulin stimulus and cell-matrix adhesion. Commonly enrichment in cellular components included basement membrane, collagen-containing extracellular matrix and presynaptic membrane (Figure 5C). In molecular functions, the target genes were mainly enriched in extracellular matrix structural constituent and cadherin binding (Figure 5D). KEGG analysis identified significant associations with cytoskeleton in muscle cells, cell adhesion molecules and prolactin signaling pathway (Figure 5E).

Discussion

In light of the close association of SOX2OT rs9839776 polymorphism with sepsis in China,[15] its genetic association with AKI development in sepsis patients was first explored in the current study. The results determined that a large proportion of rs9839776 CC genotype carriers were observed in sepsis patients with AKI, and the C allele was identified to be a risk genetic factor for AKI development in sepsis patients. Moreover, the CC genotype carriers presented reduced levels of SOX2OT. It was speculated that the genetic association between rs9839776 polymorphism and AKI development in sepsis patients might be related to its mediation in SOX2OT levels. Previously, several studies have determined the essential role of SOX2OT in kidney injury. For example, downregulated SOX2OT has been detected in diabetic nephropathy patients, which contributes to mesangial cell proliferation and fibrosis.[11] In addition, SOX2OT can alleviate high glucose-induced podocyte injury, and miR-9/SIRT1 axis is involved in the progress.[12] Based on the present clinical

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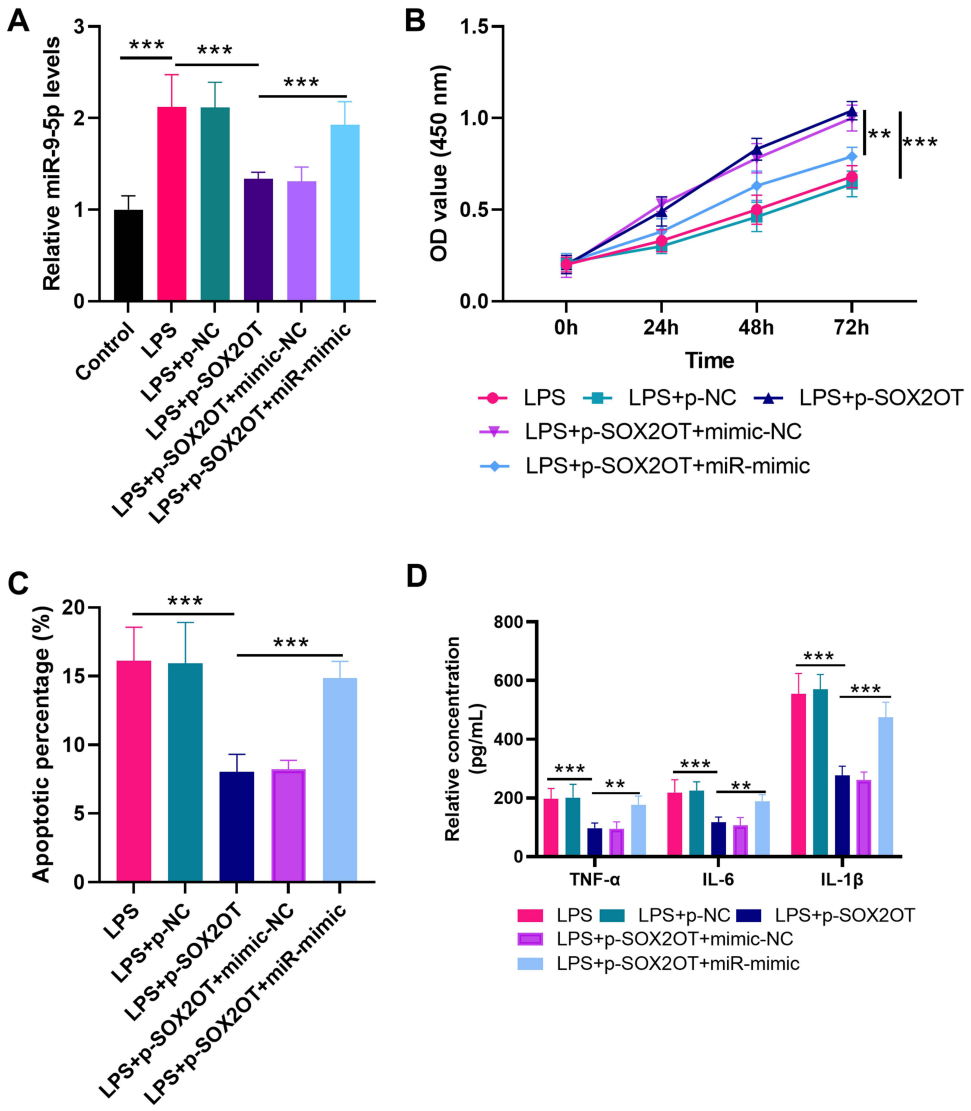

Figure 4 Overexpression of miR-9-5p reverses the effect of SOX2OT on HK-2 cells. (**A**) miR-9-5p levels in HK-2 cells after cell transfection. (**B**) Cell viability of HK-2 cells under different treatments. (**C**) Cell apoptosis of HK-2 cells under different treatments. (**D**) Concentrations of pro-inflammatory cytokines of TNF-α, IL-1β and IL-6 in HK-2 cells under different treatments. *** $P < 0.001$.

data, SOX2OT was at low expression in the serum of AKI patients, indicating that it might be involved in the development of AKI in sepsis patients. Moreover, the ROC curve determined that serum SOX2OT can distinguish AKI patients from sepsis ones, suggesting its diagnostic value for AKI in sepsis patients.

Functionally, an in vitro sepsis-induced AKI cell model was established in HK-2 cells.[20,21] Consistent with the findings in clinical samples, downregulated SOX2OT was detected in LPS-treated HK-2 cells. Then the rescue experiments determined that SOX2OT could alleviate sepsis-induced AKI and modulate LPS-triggered inflammatory response in HK-2 cells. The findings were consistent with previous evidence about the role of SOX2OT in different types of nephropathy. For example, based on the microarray analysis results, the downregulation of SOX2OT is determined in diabetic nephropathy mice, indicating its regulatory role in the pathogenesis of diabetic nephropathy.[22] Moreover, SOX2OT is determined to alleviate mesangial cell proliferation and fibrosis induced by diabetic nephropathy through mediating Akt/mTOR-regulated autophagy.[11] In addition, a recent study also reports the involvement of SOX2OT in oxidative stress and apoptosis of renal tubular epithelial cells in the progress of diabetic nephropathy, and SIRT1 is identified to be a downstream target gene.[23] Besides, in high glucose-induced podocyte injury, SOX2OT also exerts a protective role through mediating cell autophagy via

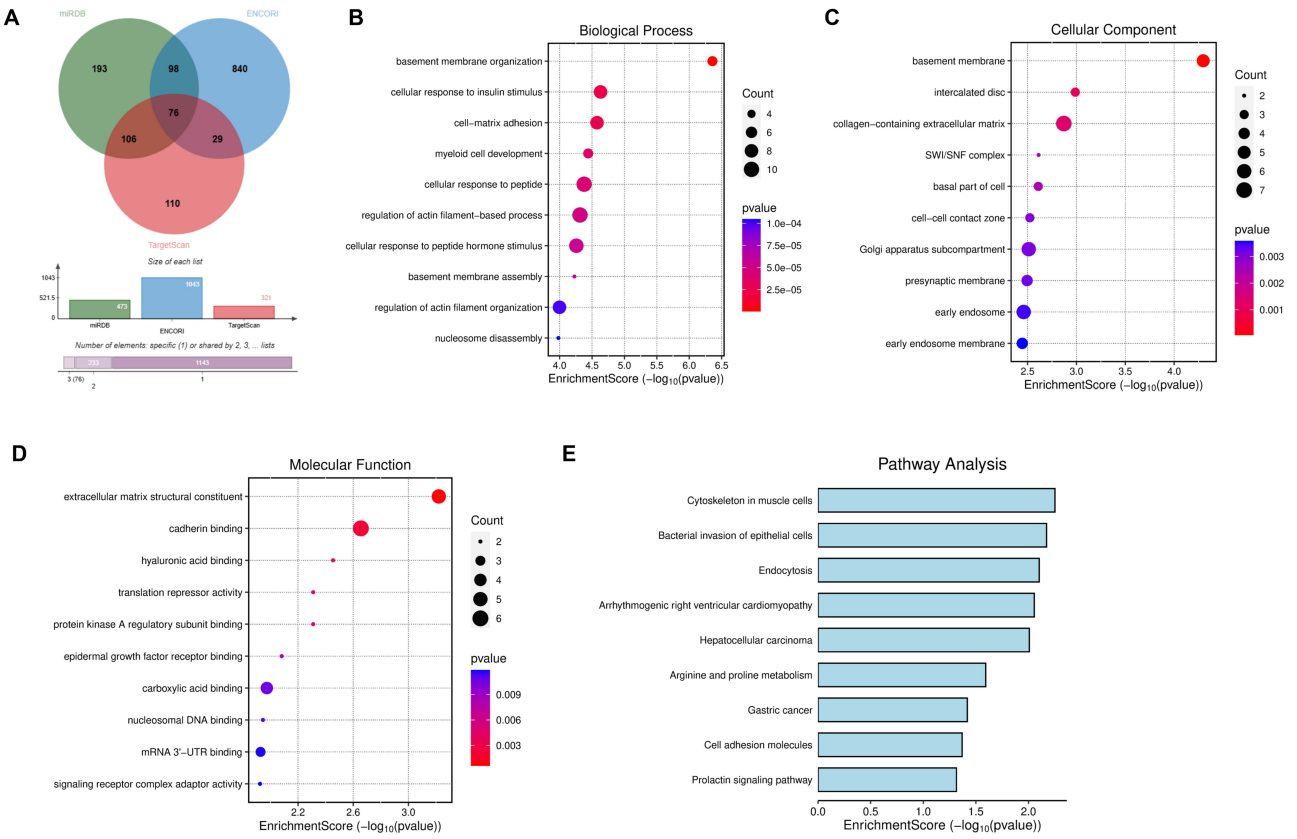

Figure 5 GO function and KEGG pathway analysis of miR-9-5p downstream target genes. (**A**) Overlapping target genes of miR-9-5p that were predicted by miRDB, ENCORI, and TargetScan online datasets. (**B**) The top ten enriched biological processes of overlapping target genes according to GO function analysis. (**C**) The top ten enriched cellular components of overlapping target genes according to GO function analysis. (**D**) The top ten enriched molecular functions of overlapping target genes according to GO function analysis. (**E**) The top ten enriched pathways related to overlapping target genes.

the miR-9/SIRT1 axis.[12] All evidence supported our conclusion that lncRNA SOX2OT can alleviate LPS-induced AKI in HK-2 cells via mediating cell proliferation, apoptosis and inflammatory response.

Competing endogenous RNA (ceRNA) is an important regulatory mechanism of lncRNAs.[24] LncRNAs can compete with messenger RNA (mRNA) to bind microRNAs (miRNAs) through the ceRNA mechanism, thereby regulating gene expression. Based on the current results, miR-9-5p was identified to be a candidate gene of lncRNA SOX2OT. Consistently, the binding relationship between SOX2OT and miR-9-5p has been reported in previous research.[12,25] In recent years, the expression of miR-9-5p in sepsis has attracted much attention, miR-9-5p may be involved in the pathophysiological process of sepsis through various pathways.[26] A previous study has shown that miR-9-5p is highly expressed in septic mice, and downregulating its expression can inhibit the secretion of LPS-induced inflammatory cytokine, and the mechanism of action is related to inhibiting the activation of TGF-β/Smad2 signaling.[26] Moreover, miR-9-5p is also involved in LPS-induced podocyte injury, miR-9-5p downregulation can alleviate podocyte injury through inducing cell autophagy.[12]

To gain a better understanding of the potential mechanisms and pathways that interactions may occur between SOX2OT/miR-9-5p axis and AKI, the enrichment analysis was conducted on overlapping target genes of miR-9-5p. GO enrichment analysis revealed that miR-9-3p target genes were predominantly linked to basement membrane organization, cell-matrix adhesion and structural constituent, and cadherin binding. KEGG analysis uncovered significant links with cytoskeleton in muscle cells, cell adhesion molecules and prolactin signaling pathway. It is known that during AKI, the disruption of the actin cytoskeleton along with the impairment of cell-extracellular matrix adhesion results in the loss of epithelial cell polarity and the detachment of cells.[27] That might be the potential mechanism of the role of SOX2OT/miR-9-5p axis in sepsis-induced AKI.

However, several limitations should be noted here. First of all, the limited size of our clinical samples may lead to certain biases in the results. Therefore, the genetic susceptibility between SOX2OT rs9839776 polymorphism and AKI

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needs to be further verified in large-scale samples. In addition, the mechanism study of SOX2OT in septic patients with concurrent AKI is limited to in vitro experiments. In the future, in vivo studies are needed to further explore its participation mechanism. The downstream signaling pathways also await further investigation.

Conclusion

In conclusion, rs9839776 polymorphism in SOX2OT could affect the susceptibility of AKI for sepsis patients, and rs9839776 CT genotype was related to low AKI risk and less AKI severity. Moreover, the in vitro experiments indicated that the underlying mechanism might be related to the mediation of SOX2OT/miR-9-5p axis. However, the genetic association between SOX2OT rs9839776 polymorphism and AKI risk should be verified in a larger cohort of septic patients, and the underlying mechanism should be explored in vivo. According to the findings, the detection of SOX2OT levels and rs9839776 genotypes might be helpful for the early diagnosis of AKI in septic patients clinically. Strengthening the monitoring of patients at high risk and achieving early intervention may improve the prognosis of patients. In addition, during the treatment and rehabilitation stage of sepsis patients, the detection of SOX2OT can also be used as one of the indicators to evaluate the recovery of renal function, providing a reference for the subsequent rehabilitation and follow-up of patients.

Ethics Statement

This study was approved by the Binzhou Medical University Hospital Medical Board and conducted with the informed consent of the subjects. The experiments were carried out in accordance with the guidelines of the Helsinki Declaration.

Disclosure

The author(s) report no conflicts of interest in this work.

References

1. Rello J, Valenzuela-Sanchez F, Ruiz-Rodriguez M, Moyano S. Sepsis: a review of advances in management. *Adv Ther*. 2017;34(11):2393–2411. doi:10.1007/s12325-017-0622-8
2. Manrique-Caballero CL, Del Rio-Pertuz G, Gomez H. Sepsis-associated acute kidney injury. *Crit Care Clin*. 2021;37(2):279–301. doi:10.1016/j.ccc.2020.11.010
3. Zarbock A, Koyner JL, Gomez H, Pickkers P, Forni L. Acute disease quality initiativeg. Sepsis-associated acute kidney injury-treatment standard. *Nephrol Dial Transplant*. 2023;39(1):26–35. doi:10.1093/ndt/gfad142
4. Kuwabara S, Goggins E, Okusa MD. The pathophysiology of sepsis-associated AKI. *Clin J Am Soc Nephrol*. 2022;17(7):1050–1069. doi:10.2215/CJN.00850122
5. Wang P, Chen W, Zhao S, Cheng F. The role of LncRNA-regulated autophagy in AKI. *Biofactors*. 2023;49(5):1010–1021. doi:10.1002/biof.1980
6. Alsaab HO, Alaqile AF, Alsaeedi RN, Alzahrani MS, Almutairy B. Long journey on the role of long non-coding RNA (lncRNA) in acute kidney injury (AKI). *Pathol Res Pract*. 2024;263:155591. doi:10.1016/j.prp.2024.155591
7. Shi C, Zhao Y, Li Q, Li J. lncRNA SNHG14 plays a role in sepsis-induced acute kidney injury by regulating miR-93. *Mediators Inflamm*. 2021;2021:5318369. doi:10.1155/2021/5318369
8. Wang B, Wang Y, Xu K, et al. Resveratrol alleviates sepsis-induced acute kidney injury by deactivating the lncRNA MALAT1/MiR-205 axis. *Cent Eur J Immunol*. 2021;46(3):295–304. doi:10.5114/ceji.2021.109195
9. Wang Y, Wu N, Luo X, Zhang X, Liao Q, Wang J. SOX2OT, a novel tumor-related long non-coding RNA. *Biomed Pharmacother*. 2020;123:109725. doi:10.1016/j.biopha.2019.109725
10. Bai X, Yang L, Liu R, et al. Long noncoding RNA SOX2OT ameliorates sepsis-induced myocardial injury by inhibiting cellular pyroptosis through mediating the EZH2/Nrf-2/NLRP3 signaling pathway. *J Inflamm Res*. 2024;17:3115–3127. doi:10.2147/JIR.S451643
11. Chen K, Yu B, Liao J. LncRNA SOX2OT alleviates mesangial cell proliferation and fibrosis in diabetic nephropathy via Akt/mTOR-mediated autophagy. *Mol Med*. 2021;27(1):71. doi:10.1186/s10020-021-00310-6
12. Zhang Y, Chang B, Zhang J, Wu X. LncRNA SOX2OT alleviates the high glucose-induced podocytes injury through autophagy induction by the miR-9/SIRT1 axis. *Exp Mol Pathol*. 2019;110:104283. doi:10.1016/j.yexmp.2019.104283
13. Fatani SH, ALrefai AA, Al-Amodi HS, Kamel HF, Al-Khatieb K, Bader H. Assessment of tumor necrosis factor alpha polymorphism TNF-alpha (−238) (rs 361525) as a risk factor for development of acute kidney injury in critically ill patients. *Mol Biol Rep*. 2018;45(5):839–847.
14. Liu J, Yao J, Zhang L, et al. Surfactant protein D (SP-D) gene polymorphism rs721917 is an independent predictor of acute kidney injury development in sepsis patients: a prospective cohort study. *Ann Intensive Care*. 2020;10(1):5. doi:10.1186/s13613-019-0617-5
15. Wu Z, Yu Y, Fu L, et al. LncRNA SOX2OT rs9839776 polymorphism reduces sepsis susceptibility in Southern Chinese children. *J Inflamm Res*. 2020;13:1095–1101. doi:10.2147/JIR.S281760
16. Tang X, Gao Y, Yu L, et al. Correlations between lncRNA-SOX2OT polymorphism and susceptibility to breast cancer in a Chinese population. *Biomark Med*. 2017;11(3):277–284. doi:10.2217/bmm-2016-0238
17. James E, Bartlett JWKI. Organizational research: determining appropriate sample size in survey research. *Inf Technol Learn Perform J*. 2001;19(1):43.

18. Force ADT, Ranieri VM, Rubenfeld GD, et al. Acute respiratory distress syndrome: the Berlin definition. *JAMA*. 2012;307(23):2526–2533. doi:10.1001/jama.2012.5669
19. Khwaja A. KDIGO clinical practice guidelines for acute kidney injury. *Nephron Clin Pract*. 2012;120(4):c179–184. doi:10.1159/000339789
20. Wang Y, Xi W, Zhang X, et al. CTSB promotes sepsis-induced acute kidney injury through activating mitochondrial apoptosis pathway. *Front Immunol*. 2022;13:1053754. doi:10.3389/fimmu.2022.1053754
21. Huang G, Bao J, Shao X, et al. Inhibiting pannexin-1 alleviates sepsis-induced acute kidney injury via decreasing NLRP3 inflammasome activation and cell apoptosis. *Life Sci*. 2020;254:117791. doi:10.1016/j.lfs.2020.117791
22. Zhang X, Shang J, Wang X, et al. Microarray analysis reveals long non-coding RNA SOX2OT as a novel candidate regulator in diabetic nephropathy. *Mol Med Rep*. 2018;18(6):5058–5068. doi:10.3892/mmr.2018.9534
23. Ye G, Hu ML, Xiao L. Forkhead box A2-mediated lncRNA SOX2OT up-regulation alleviates oxidative stress and apoptosis of renal tubular epithelial cells by promoting SIRT1 expression in diabetic nephropathy. *Nephrology*. 2023;28(3):196–207. doi:10.1111/nep.14139
24. Hu C, Li J. Identification of lncRNA-miRNA-mRNA ceRNA network as biomarkers for acute kidney injury. *Am J Transl Res*. 2023;15(9):5730–5746.
25. Li G, Li X, Li Z, et al. Sox2ot /miR-9 /Cthrc1 promote proliferation and migration of Schwann cells following nerve injury. *Neuroscience*. 2023;519:47–59. doi:10.1016/j.neuroscience.2023.03.009
26. Zhen J, Chen W, Zhao L, Zang X, Liu Y. A negative Smad2/miR-9/ANO1 regulatory loop is responsible for LPS-induced sepsis. *Biomed Pharmacother*. 2019;116:109016. doi:10.1016/j.biopha.2019.109016
27. Chen A, Zhang J, Yan Z, et al. Acidic preconditioning induced intracellular acid adaptation to protect renal injury via dynamic phosphorylation of focal adhesion kinase-dependent activation of sodium hydrogen exchanger 1. *Cell Commun Signal* 2024;22(1):393. doi:10.1186/s12964-024-01773-w