Tohoku J. Exp. Med., 2024, **263**, 69-79

MicroR-380-3p Reduces Sepsis-Induced Acute Kidney Injury via Regulating RAB1P to Restrain NF-κB Pathway

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Septic acute kidney injury (AKI) is a common complication in critically ill patients with high morbidity and mortality. This study intends to clarify the clinical value and molecular mechanism of microR-380-3p in septic AKI by recruiting patients with septic AKI and establishing septic AKI cell models. Patients with septic AKI were included and human kidney-2 (HK-2) cells were induced by lipopolysaccharide (LPS) to construct the AKI cell model of sepsis. The expression of microR-380-3p was detected by quantitative real-time RT-PCR (qRT-PCR). The expression of Bax, cleaved caspase 3, Bcl-2, p65, and p-p65 was detected by Western blot. The contents of inflammation and oxidation were determined by commercial kits. Bioinformatics predicted the binding target of microR-380-3p and a dual luciferase reporting system was used to verify the regulatory relationship between microR-380-3p and RAP1B. The concentration of microR-380-3p was elevated in patients with septic AKI and appeared to be a biomarker for these patients. Silenced microR-380-3p reversed the damage of LPS on HK-2 cells via promoting viability, inhibiting apoptosis, inflammation, and oxidation. RAP1B was a target of microR-380-3p and microR-380-3p exerted targeted inhibition of RAP1B expression level. Down-regulation of RAP1B reversed the influence of silenced microR-380-3p on HK-2 cells. MicroR-380-3p/RAP1B participated in activating the NF-κB pathway. MicroR-380-3p down-regulated RAP1B to exacerbate septic AKI, providing a potential therapeutic biomarker for septic AKI.

Keywords: diagnostic value; microR-380-3p; NF-κB pathway; RAP1B; septic AKI
Tohoku J. Exp. Med., 2024 May, **263** (1), 69-79.
doi: 10.1620/tjem.2023.J106

Introduction

Sepsis is a systemic inflammatory syndrome that is triggered by an infection and organ dysfunction (Zhang et al. 2022). It is usually caused by an uncontrolled immune response, cytokine storm, and oxidative stress leading to multiple organ failure, which is a major cause of death (Zhang et al. 2018). Acute kidney injury (AKI) is one of the common complications of sepsis (Heinzl et al. 2022). AKI is a type of common critical illness, caused by various causes and affecting many individuals, characterized by a rapid decline in renal function and acute tubular epithelial necrosis (Nature Reviews Disease Primers 2021). The incidence and mortality of AKI are increasing, and nearly one-third of the survivors will eventually develop end-stage renal disease, which severely affects the quality of life (Zeinali et al. 2022). The pathogenesis of AKI in sepsis is complex. Among them, inflammation and oxidative stress appear to be crucial causes of septic AKI (Wang et al. 2023). At the same time, the research on its pathophysiological mechanism has hindered the early prevention and treatment of AKI. Therefore, exploring the new pathogenesis of AKI and seeking new therapeutic targets for AKI is a hot issue.

Lipopolysaccharide (LPS) is a commonly occurring endotoxin and a known inducer of AKI (Guo et al. 2021b).

Received November 30, 2023; revised and accepted December 23, 2023; J-STAGE Advance online publication January 12, 2024
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LPS stimulates the release of various proinflammatory factors, and activates the concentration of reactive oxygen species, thus triggering the apoptosis of renal tubular cells. LPS-induced renal cell apoptosis is an important AKI injury model that is often used to study the complex etiology and pathophysiological mechanism of AKI (Nežić et al. 2020). MicroRNA is a class of non-coding single-stranded small RNA with a size of approximately 18 to 25 nucleic acids (Sun et al. 2020). Alteration in miRNA expression affects the expression of downstream genes that are involved in numerous diseases, while miRNAs are considered promising diagnostic markers (Ho et al. 2022; Xi et al. 2022). The roles of miRNA in predicting AKI are significant. Liu et al. (2020b) reveal that microR-452 could distinguish patients with septic AKI, predicting that microR-452 may be used as a biomarker. The primary function of microRNA is believed to be the post-transcriptional inhibition of gene expression (Jian et al. 2020). Research has shown that microRNA has the capability to control inflammatory response by targeting specific genes and plays an important role in septic AKI. MicroR-150-5p functions as a mediator in an inflammatory and oxidative situation of septic AKI through MEKK3/JNK pathway (Shi et al. 2021). The reduced microR-376b expression is evident in the patients with septic AKI, and in the inflammation of the kidney, microR-376b regulates the NF-κB pathway through targeting NFKBIZ and subsequently participating in inflammation and injury of kidney (Liu et al. 2020a). MicroR-380-3p is a highly researched miRNA in the research field. The expression of microR-380-3p is increased in patients with kidney injury caused by systemic lupus erythematosus, reflecting that microR-380-3p might be involved in renal injury (Navarro-Quiroz et al. 2016). Nevertheless, the pathogenesis of microR-380-3p is unclear in septic AKI.

This investigation evaluated the expression of serum levels of microR-380-3p in patients with septic AKI and examined its potential as a predictive biomarker. The mechanism of microR-380-3p was verified in a cell model induced by the LPS. Specifically, the cell viability, apoptosis, inflammation, and oxidation were used to reflect the injury of human kidney-2 (HK-2) cells and the regulatory function of microR-380-3p.

Materials and Methods

Ethics approval and consent to participate

The study protocol was approved by The Ethics Committee of Shanxi Bethune Hospital. All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki Declaration and later versions. In addition, informed consent has been obtained from the participants involved.

Screening abnormally expressed microRNAs in septic AKI

The GEO database was searched to screen differentially expressed microRNAs, leading to the discovery of the GES94717 dataset. This database measures the expression alternation of microRNAs in venous blood samples obtained from 6 patients with septic AKI and 3 healthy persons. The GEO2R program was employed to analyze the differentially expressed microRNAs, using $P < 0.05$ and $|\log FC| > 1$ as the criteria for identifying differently expressed microRNAs.

Patient selection and specimen obtain

A total of 90 patients with septic AKI and 87 healthy volunteers from Shanxi Bethune Hospital were chosen for this study from August 2020 to October 2022. The third international consensus for sepsis served as the basis for diagnosing patients with sepsis (Singer et al. 2016). All these septic patients were identified with AKI during the first 7 days as per the KDIGO guideline (Drüeke and Parfrey 2012). The patients with the following situations were excluded: immune diseases, malignant tumors, and chronic liver and kidney diseases. The average age and BMI of the septic AKI group (52 males and 38 females) were 52.07 ± 6.55 years (mean ± standard deviation, SD) and 25.82 ± 3.14 kg/m^2, respectively. The average age and body-mass index (BMI) of the control group (46 males and 41 females) were 53.45 ± 6.50 years and 25.09 ± 2.80 kg/m^2, respectively. No difference was indicated on these indicators. Patients had not received any treatment during the time of recruitment and sample collection. All participants willingly signed the informed consent form. The approval from the ethics review board of Shanxi Bethune Hospital was obtained prior to enrollment.

Fasting venous blood (5 ml) was gathered from the patients on the day of admission and from the control group during physical examination. The blood sample was centrifuged at 2,500 g for 2 minutes, and the serum was separated and stored in the refrigerator at −80°C.

Cell grouping and treatment

Human kidney-2 cells (HK-2) were purchased from Procell Life Science and Technology (Wuhan, China). HK-2 cells were cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin double antibody at 37°C and 5% CO_2. These cells were induced by 5 μg/ml LPS (Sigma, St Louis, MO, USA) for 24 hours to mimic the injury of sepsis, as described in previous work (Wang et al. 2022). The oligonucleotides, including microR-380-3p negative control (miR-NC), its inhibitor (miR-inhibitor), short hairpin of RAP1B (sh-RAP1B), and its negative control (sh-NC), were all sourced from GenePharm (Shanghai, China). HK-2 cells in the miR-NC group and miR-inhibitor group were respectively transfected with microR-380-3p control sequences (100 nM) or inhibitors (100 nM). The cells in the miR-inhibitor + sh-NC and sh-RAP1B group were transfected with miR-inhibitor companies with sh-NC (50 nM) or sh-RAP1B (50 nM), respectively. Lipofectamine 3000 used in the transfection experiment was from Thermo Fisher Scientific (Waltham, MA, USA)

and after 48 hours, all transfected cells were allowed for the next protocol.

Expression detection using qPCR

Total RNA was extracted using RNATripLS reagent (APPLYGEN, Beijing, China) and reverse transcribed with miR-Quant cDNA Synthesis Kit (Biobolai, Beijing, China) or PrimeScript RT-PCR kit (TaKaRa, Beijing, China). Acceptable purity requirements for RNA were A260/280 between 1.8 and 2.0. After the concentration and purity of RNA were analyzed by a spectrophotometer, 1 ng DNA was used for PCR amplification using qPCR detection premix (Thermo Fisher Scientific). Primers of miR-380-3p, RAP1B, and internal reference were purchased from Sangon (Shanghai, China). In a 20 μl reaction system, 10 μl premix, 0.4 μl each of 10 μM upstream and downstream primers, 2 μl of cDNA, and 7.2 μl of water were added. The reaction was carried out in the following manner. The reaction program consisted of 95°C for 20 s; 40 cycles of 95°C for 3 s and 60°C for 30 s. MicroR-380-3p upstream primer was 5′-CTCGCTTCGGCAGCACA-3′, downstream primer was 5′-CAGTGCGTGTCGTGGAGT-3′. RAP1B upstream primer was 5′-GATACTGCAGGAACGG AGCAGTTT-3′, and downstream primer was 5′-GTCTTG CCAGGTTCTGACCTTGTT-3′. U6 upstream primer was 5′-CGCAAGGATGACACGCAAAT-3′, and U6 downstream primer was 5′-GTGCAGGGTCCGAGGTATTC-3′. GAPDH upstream primer was 5′-TGTTCCTACCCCCA ATGTGTCCGTC-3′, and GAPDH downstream primer was 5′-CTGGTCCTCAGTGTAGCCCAAGATG-3′. After the PCR reaction, the relative expression of microR-380-3p and RAP1B was calculated by the $2^{-\Delta\Delta Ct}$ method with U6 and GAPDH as endogenous controls, respectively. Three biological replicates with three technical replicates each were used for this RT-qPCR experiment.

CCK-8 assay

The cell concentration was adjusted, and approximately 2×10^3 cells were seeded in a well of a 96-well plate. After 96 hours of culture, 10 ml of CCK-8 solution (Beyotime, Shanghai, China) was added to each well, and the culture was continued for another 4 hours. The absorbance at 450 nm was measured using a microplate reader.

Flow cytometry (FLC)

About 5×10^4 cells and 195 μl of Annexin V-FITC reagent (Beyotime) were added. An additional 5 μl of Annexin V-FITC reagent was added for mixing. Subsequently, 5 μl of propidium iodide dye (Beyotime) was added and incubated at room temperature in the dark for 20 minutes. Flow cytometry was used for detection.

Inflammation and oxidative stress of HK-2 cells

After 48 hours of culture, the supernatant of cells in each group was collected. The enzyme-linked immunosorbent assay (ELISA) kits were used for the expression detection of interleukin-6 (IL-6), interleukin-1beta (IL-1β), and tumor necrosis factor-alpha (TNF-α) (Beyotime). The levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malonic dialdehyde (MDA), and reactive oxygen species (ROS) were detected with commercial kits (Beyotime).

Predictive targets and verification

We used miRBDB (Version 6.0) (Chen and Wang 2020), TargetScan (Version 7.2) (Agarwal et al. 2015), miRWalk (Version 3) (Sticht et al. 2018), and miRTarbase (Version 8.0) (Huang et al. 2022) databases to predict and identify potential genes of microR-380-3p. The intersection of targets was documented in a Venn diagram, and the potential for dynamic correlations was analyzed through a protein-protein interaction (PPI) drawing.

The gene fragment of RAP1B, which contains the microR-380-3p binding site, was inserted into the pmirGOL luciferase reporter vector as a wild type of RAP1B (WT-RAP1B). Simultaneously, the RAP1B mutant recombinant plasmid (MUT-RAP1B) was constructed. They were co-transfected with microR-380-3p mimics or controls using Lipofectamine 3000. Double luciferase activities were detected with kits (Beyotime), determining the targeting binding relationship between microR-380-3p and RAP1B.

Western blot

Western blot was used in the detection of expression levels of apoptosis-related proteins. The protein supernatant was collected from cells by mixing it with RIPA protein lysate (Beyotime) and centrifuging for 20 minutes. The protein content was measured by the BCA method. The obtained protein sample was mixed with the BCA working solution (Beyotime), and allowed to stand for 30 minutes. The concentration was determined using a microplate reader. The protein standard was prepared and the concentration was measured to obtain a standard curve. The concentration of protein specimens was calculated by the standard curve. Protein samples were mixed with a 4 × SDS PAGE Loading Buffer in a 4:1 ratio and placed in boiling water for 5 minutes for protein denaturation. After SDS-PAGE electrophoresis and membrane transfer, the primary antibody reaction reagents of Bax, cleaved caspase 3, Bcl-2, GAPDH, p65, and p-p65 were added and incubated overnight at 4 degrees Celsius. After washing, an HRP-labeled secondary antibody was added and incubated for 1 hour at 37°C. Primary and secondary antibodies were obtained from Santa Cruz Biotech (Santa Cruz, CA, USA). ECL developing solution was added onto PVDF membrane and photographed using gel imaging followed by band gray value analysis using Image J. The protein concentration of RAP1B was also determined and the primer antibody of RAP1B was ordered from Abcam (Cambridge, MA, USA).

Statistical analysis

The results were obtained from three parallel repeated experiments. Statistical analysis and mapping were performed by GraphPad Prism 8.0. Shapiro-wilk test and Levin test are used to test the normal distribution and variance homogeneity of samples. An Independent sample t-test was used to compare data between the two groups that were normally distributed and had chi-squared variances. One-way and two-way ANOVA analysis methods were used for comparison among groups. The receiver operating characteristic (ROC) curve was conducted to predict the diagnosis of microR-380-3p. The sample size was estimated using PASS 11.0 software. A minimum of 70 subjects should be recruited in each group based on a 5% false-positive error rate (bilateral, alpha = 0.05), efficacy = 90%, beta = 0.1, and dropout rate = 20%. $P < 0.05$ is a significant difference.

Results

MicroR-380-3p is up-regulated in septic AKI patients

There were 49 down-regulated miRNAs and 601 up-regulated miRNAs exhibited in Fig. 1A. MicroR-380-3p is an up-regulated miRNA in the GSE94717 database (Fig. 1A).

To confirm expression changes, we recruited septic AKI patients (n = 90) and healthy persons (n = 87). Differently from the GSE94717, we selected the serum of patients with septic AKI. As depicted in Fig. 1B, the expression of microR-380-3p was increased in the serum of patients with septic AKI ($P < 0.001$, Fig. 1B), which was consistent with the finding of GSE94717 published by Huang et al. (2022). Furthermore, a ROC curve was generated to assess diagnostic accuracy, with an area under the curve of 0.910 (95% CI = 0.868-0.951), showing the diagnostic possibility of microR-380-3p (Fig. 1C). When the cut-off value was 1.27, the sensitivity was 0.822 and the specificity was 0.874 (Fig. 1C).

MicroR-380-3p contributes to the injury of HK-2

In the AKI model cells, the levels of microR-380-3p were elevated, indicating that the LPS might contribute to the increase of microR-380-3p expression ($P < 0.001$, Fig. 2A). The transfection of miR-inhibitor suppressed the expression of microR-380, indicating the high transfection efficiency of miR-inhibitor ($P < 0.001$, Fig. 2A).

The viability and the apoptosis of HK-2 cells were measured to reflect the influence of microR-380-3p on the bioactivities of HK-2 cells. As seen in Fig. 2B, the viability was inhibited by the LPS, while the decreased microR-380-3p expression countered this effect ($P < 0.001$). Results of FLC and the protein expression of Bax, cleaved caspase 3, and Bal-2 reflected the apoptotic levels. The

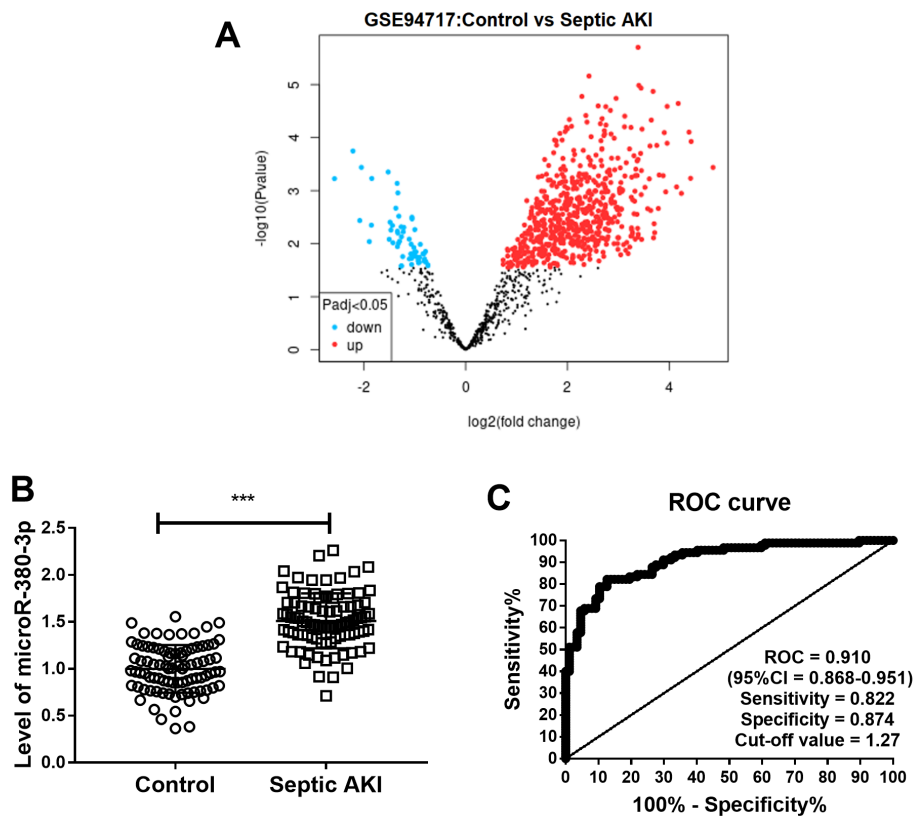

Fig. 1. The expression of microR-380-3p.
(A) The expression profile of GSE94717. (B) Overexpression of microR-380-3p in patients with septic acute kidney injury (AKI). (C) The receiver operating characteristic (ROC) curve of microR-380-3p. ***$P < 0.001$.

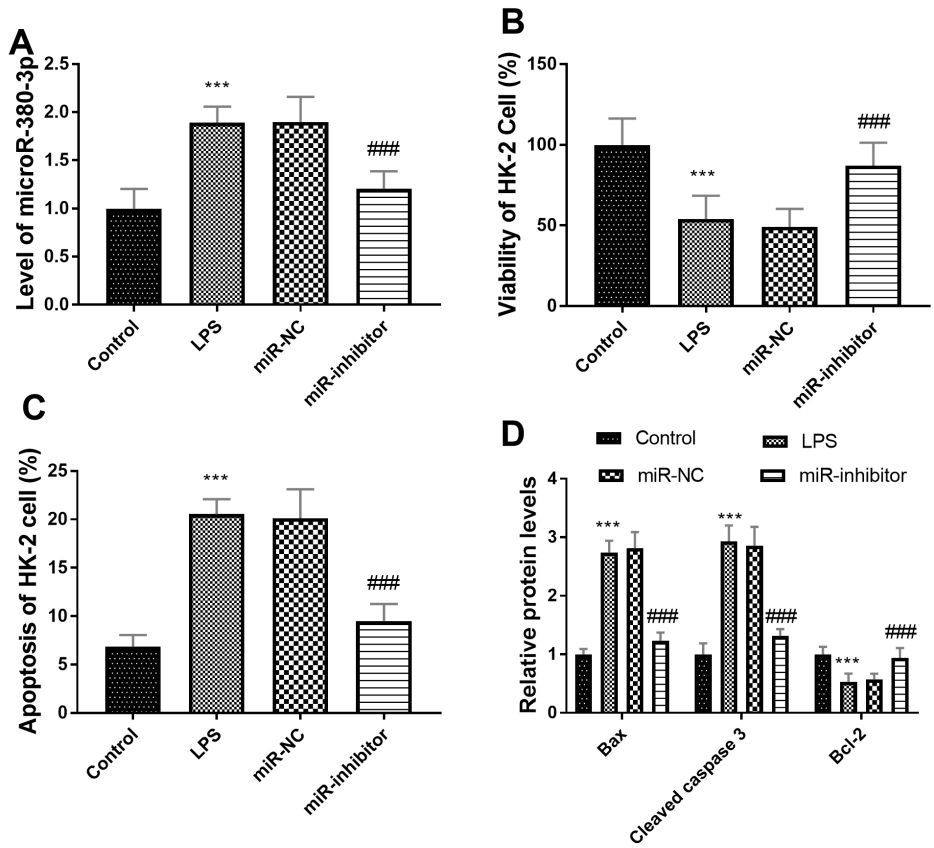

Fig. 2. Functions of microR-380-3p on viability and apoptosis of HK-2 cells.
(A) The microR-380-3p is up-regulated by lipopolysaccharide (LPS), while miR-inhibitor limited its expression. The relative expression of microR-380-3p in the control group was standardized to 1. (B) The function of microR-380-3p on viable cells. (C) The effect of microR-380-3p on cell apoptosis. (D) The levels of apoptotic-related protein were influenced by microR-380-3p. Error bars indicated SD (n = 5). ***$P < 0.001$, compared to control group; $^{###}P < 0.001$, compared to miR-NC group.

apoptotic cells in the LPS group were increased and decreased in the miR-inhibitor group (P < 0.001, Fig. 2C). The rise in Bax and cleaved caspase 3 and decreased Bcl-2 suggested the accelerated apoptotic percentage, which was opposite in the miR-inhibitor group ($P < 0.001$, Fig. 2D).

MicroR-380-3p expedites the inflammation and oxidative stress

To verify the correlation between the microR-380-3p effects on septic AKI and inflammatory response, serum IL-6, IL-1β, and TNF-α were assessed in different groups through ELISA. The LPS treatment increased cytokine expression associated with inflammation, but inflammatory disorders were suppressed when microR-380-3p was knocked down ($P < 0.001$, Fig. 3A). The oxidative situation in HK-2 cells was induced by LPS, while was suppressed by the reduction of microR-380-3p ($P < 0.001$, Fig. 3B).

RAP1B targets microR-380-3p

Five genes were identified through the use of four prediction websites and analyzed further through a Venn diagram (Fig. 4A). The investigation of PPI showed no interactions among PPP2R1B, RAP1B, REL, ERRFI1, and FSD1L (Fig. 4B). The related sequences between RAP1B and microR-380-3p were shown in Fig. 4C. The relative luciferase activities were increased in the WT-RAP1B and miR-inhibitor group ($P < 0.001$, Fig. 4D) while having no obvious difference in the MUT-RAP1B group ($P > 0.05$, Fig. 4D). Fig. 4E showed that the RAP1B mRNA expression was decreased in the LPS group and this expression was reversed by the inhibited microR-380-3p expression ($P < 0.001$). The protein expression of RAP1B declined in the LPS group and partly increased in the miR-inhibitor group ($P < 0.01$, Fig. 4F).

RAP1B mediates the effects of microR-380-3p

MicroR-380-3p inhibitors and RAP1B knockdown sequences were co-transfected into HK-2 cells to investigate the function of microR-380-3p/RAP1B axis in HK-2 cells, thus further revealing the mechanism of this signaling pathway in AKI. The mRNA levels and protein levels of RAP1B in the miR-inhibitor group were increased, while its level in the co-transfection group was decreased to a certain extent, indicating that microR-380-3p could inhibit the expression of RAP1B ($P < 0.001$, Fig. 5A, B).

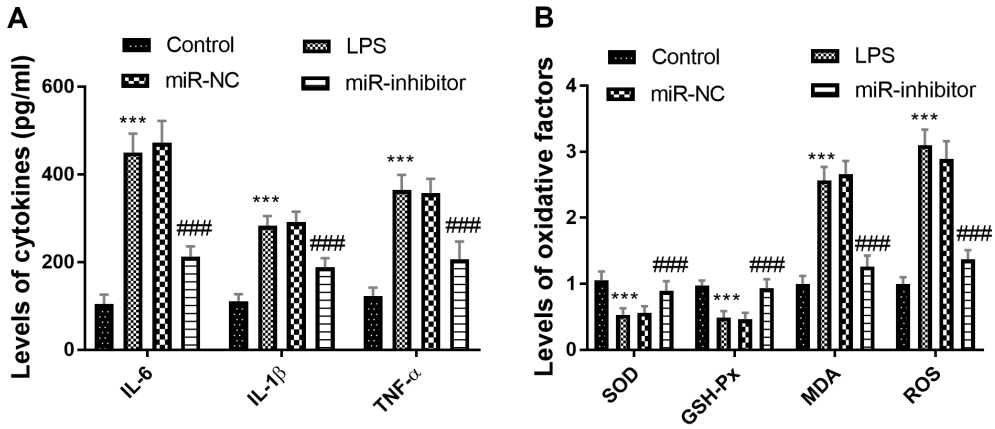

Fig. 3. Influence of microR-380-3p on inflammation and oxidation.
(A) The levels of inflammatory cytokines. (B) The levels of oxidative indicators. Error bars indicated SD (n = 5). ***$P < 0.001$, compared to control group; ###$P < 0.001$, compared to miR-NC group.

The cell viable ability of the miR-inhibitor group was enhanced, while this ability of the miR-inhibitor + sh-RAP1B group was reversed ($P < 0.001$, Fig. 5C). The FLC documented that the apoptosis ability of cells in the miR-inhibitor group declined, and the down-regulation of RAP1B offset the effect of microR-380-3p on the apoptosis ability ($P < 0.001$, Fig. 5D). The assay of apoptosis-related proteins showed that microR-380-3p suppressed the secretion of Bax and cleaved caspase 3 while promoting the content of Bcl-2, while the silenced RAP1B reversed these tendencies ($P < 0.001$, Fig. 5E). The inflammatory and oxidative state of HK-2 cells in the miR-inhibitor group was suppressed, while the down-regulation of RAP1B partly nullified the effect of microR-380-3p ($P < 0.001$, Fig. 5F, G).

Micro380-3p/RAP1B axis activities NF-κB pathway

For verification of whether the NF-κB pathway participates in the regulation of the microR-380-3p/RAP1B axis, we downloaded the genes related to the NF-κB pathway. The interconnections between RAP1B and these genes were analyzed. The results in Fig. 6A documented that RAP1B had five nodes in this PPI analysis, including PIK3CB, SYK, LYN, MAPK1, and MAPK3, which suggested that RAP1B might be implicated in the regulation of the NF-κB pathway. We assessed the expression of p-p65 and p65 to test this hypothesis. As shown in Fig. 6B, the relative p-p65/p65 levels were increased in the LPS group, however, the decreased microR-380-3p expression reversed the high relative levels of p-p65/p65 ($P < 0.001$, Fig. 6B). In the miR-inhibitor + sh-RAP1B group, the levels of p-p65/p65 were boosted compared to the miR-inhibitor + sh-NC group ($P < 0.001$, Fig. 6B).

Discussion

Sepsis is a syndrome of organ dysfunction that leads to organ dysfunction and circulatory disturbance, often affecting the kidney, and is an independent risk factor for AKI (Juan et al. 2021). Its pathogenesis is complex and may involve oxidative stress, microvascular dysfunction, immune inflammatory responses, and so on (Scapini et al. 2019). Predicting the occurrence and prognosis of septic AKI has important guiding significance for clinical treatment, but the current lack of sensitive and specific indicators leads to missing the best time for diagnosis and treatment (Guo et al. 2021a). Multiple studies have demonstrated the potential value of microRNA as a diagnostic tool for septic AKI. Some microRNAs become alternative diagnosis of AKI, such as microR-15a-5p, microR-22-3p, and microR-452 (Liu et al. 2020b; Zhang et al. 2021; Petejova et al. 2022). The expression of microR-574-5p can differentiate the AKI occurrence from septic patients, indicating the predictive potential of microRNAs in the clinic (Liu et al. 2021). MicroRNA has become a hot topic in the research of many diseases. Its basic function is to regulate the expression of target genes by inhibiting gene transcription. In kidney-related ailments, microRNAs are involved in the regulation of the occurrence and development mainly through manipulating cell proliferation, differentiation, and apoptosis. In the current study, we detected and researched the significance of the microR-380-3p in septic AKI.

In GSE94717, the concentration of microR-380-3p is up-regulated in patients with septic AKI through analysis by GEO2R program. As an autoimmune disease, systemic lupus erythematosus is a reason for kidney injury (Bonnemaison et al. 2019). In lupus nephritis patients with systemic lupus erythematosus, the concentration of microR-380-3p is increased and its increased levels may serve as a risk predictor in diagnosing patients with systemic lupus erythematosus companied with renal injury (Navarro-Quiroz et al. 2016). As we all know, septic AKI is a type of kidney damage caused by a systemic inflammatory response, which has a strong correlation to inflammation

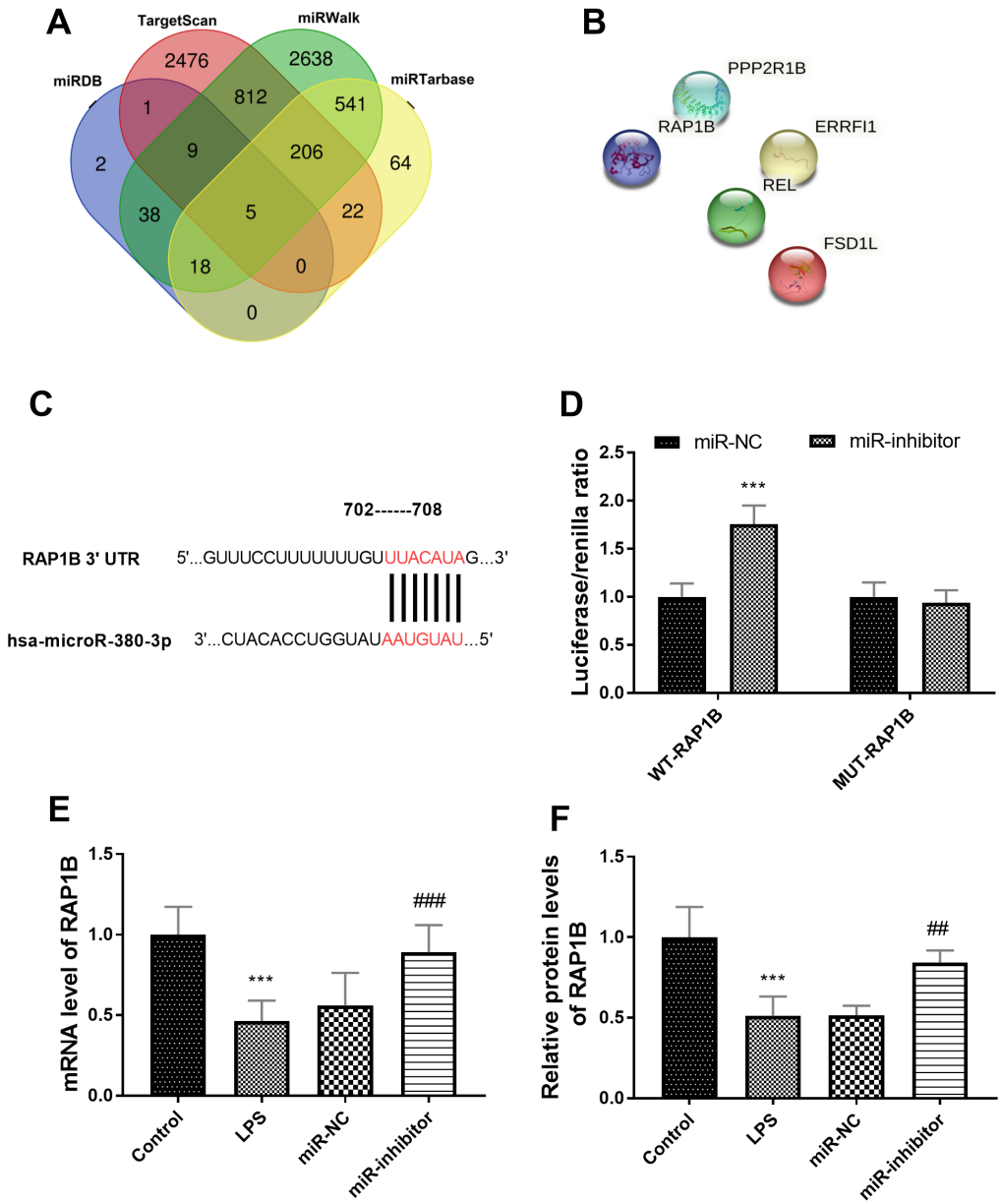

Fig. 4. Target of microR-380-3p.
(A) Venn diagram was drawn to analyze the intersection of genes. (B) The protein-protein interaction (PPI) of five targets. (C) The putative bases between *RAP1B* and microR-380-3p. (D) Luciferase reporter assay certified the targeted relationship. (E) The mRNA levels of *RAP1B*. The mRNA level of *RAP1B* in the control group was standardized to 1. (F) The relative protein levels of RAP1B. The protein level of RAP1B in the control group was standardized to 1. Error bars indicated SD (n = 5). ***$P < 0.001$, compared to miR-NC or control group; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, compared to miR-NC group.

(Ni et al. 2021). The up-regulation of microR-380-3p promotes the levels of IL-6, IL-1β, and TNF-α in rat models of spinal cord injury, implying that microR-380-3p has an expansionary effect on inflammation (Li et al. 2020). In the current observation, the expression of microR-380-3p was increased in patients with septic AKI, suggesting that the alternation of microR-380-3p levels may be involved in septic AKI. In addition, the ROC curve demonstrated its predictive capability, suggesting that the detection of microR-380-3p was likely to be a diagnostic biomarker for patients with septic AKI due to its high sensitivity and specificity.

The current research established a cell model of septic AKI by exposing HK-2 cells to LPS. In this cell model, the expression of microR-380-3p was increased, the viability was decreased, and the apoptosis was improved. Through transfecting microR-380-3p inhibitors into HK-2 cell models, the expression of microR-380-3p was suppressed. With the decrease of microR-380-3p, the decreased cell viability and accelerated apoptosis were reversed, indicating that

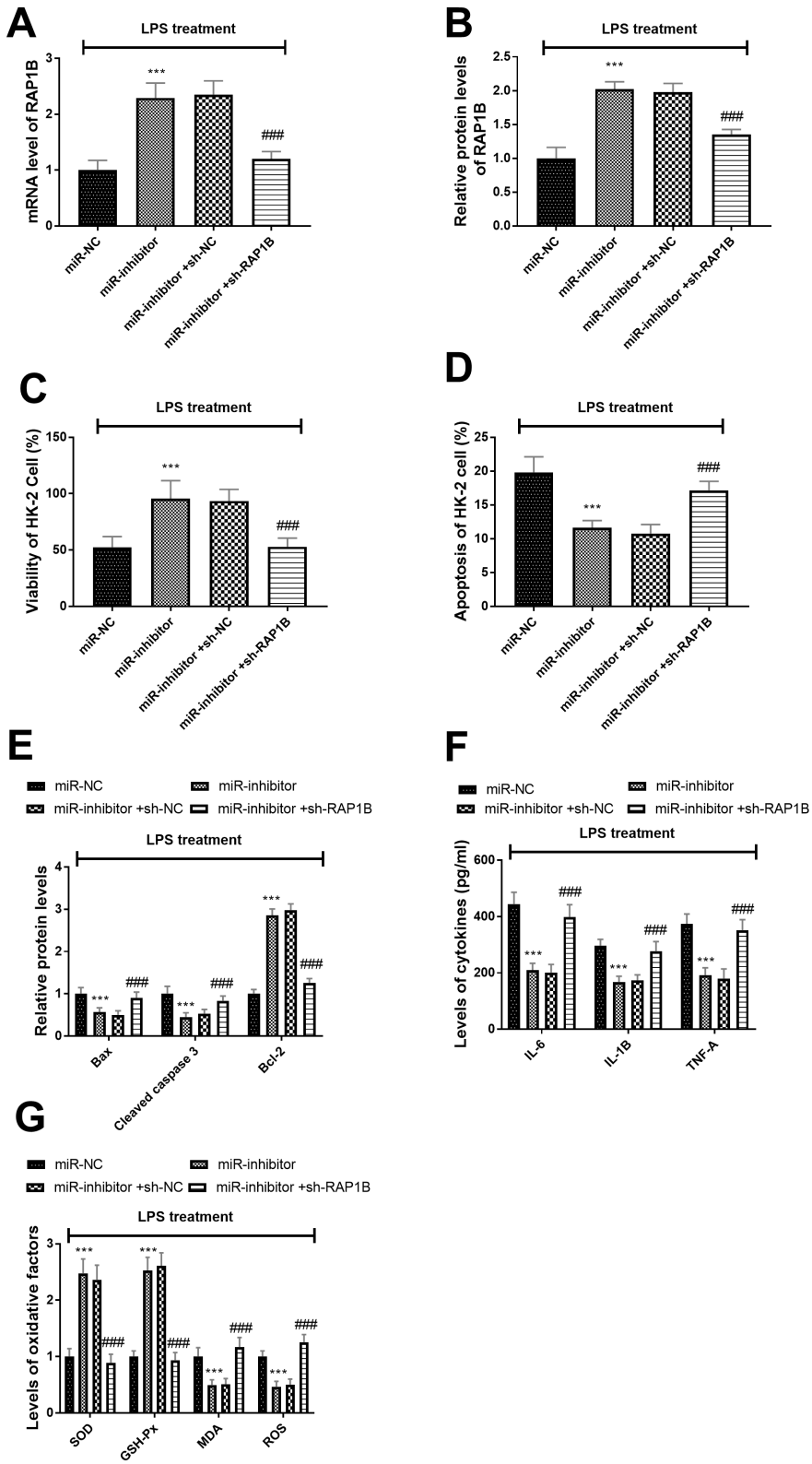

Fig. 5. Function of RAP1B on HK-2 cells.

(A) The mRNA levels of RAP1B were regulated by microR-380-3p and sh-RAP1B. The mRNA level of RAP1B in the control group was standardized to 1. (B) The relative protein levels of RAP1B. The relative protein level of RAP1B in the control group was standardized to 1. (C) The mediated effect of RAP1B on cell viability. (D) The apoptotic percentage was reduced, while knockdown of RAP1B reversed this trend. (E) RAP1B regulated the apoptotic-relative protein. (F) The silenced RAP1B reversed the function of microR-380-3p on inflammation. (G) Oxidative stress of HK-2 cells was controlled by RAP1B. Error bars indicated SD (n = 5). ***$P < 0.001$, compared to miR-NC group; $^{###}P < 0.001$, compared to miR-inhibitor + sh-NC group.

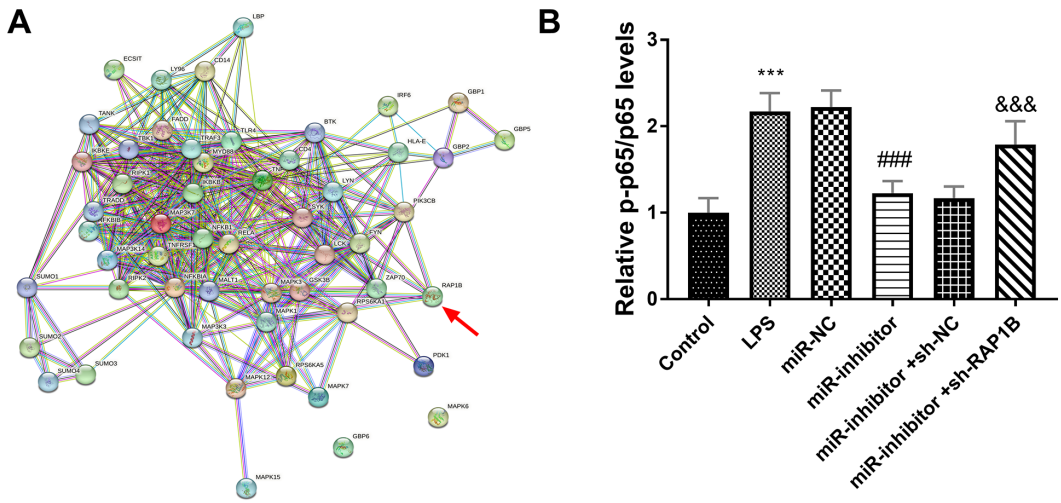

Fig. 6. The involvement of NF-κB pathway.
(A) The protein-protein interaction (PPI) of RAP1B with NF-κB signaling. The relative level of p-p65/p65 in the control group was standardized to 1. (B) The relative levels of p-p65/p65. Error bars indicated SD (n = 5). $^{***}P < 0.001$, compared to control group; $^{\#\#\#}P < 0.001$, compared to miR-NC group; $^{\&\&\&}P < 0.001$, compared to miR-inhibitor + sh-NC group.

microR-380-3p had beneficial effects in the bio-functional recovery of HK-2 cells. LPS treatment resulted in increased protein levels of Bax and cleaved caspase 3 and a decreased level of Bcl-2, while the inhibition of microR-380-3p reversed these tendencies, indicating that microR-380-3p ameliorated the cell apoptosis. Cytokine levels are strongly associated with the development of sepsis-induced AKI, suggesting that systemic inflammatory mediators play an important role (Dewitte et al. 2020). In this study, we detected the cytokines' levels, such as IL-6, IL-1β, and TNF-α to reflect the inflammatory response of HK-2 cells. Notably, the inflammation was induced by the LPS, while was suppressed by the knockdown of microR-380-3p. In diabetic retinopathy, microR-380-3p increases and contributes to inflammatory response (Luo et al. 2022). In chronic obstructive pulmonary disease, the overexpression of microR-380-3p shows a promotion effect of inflammation (Wu 2020). Oxidative stress plays a key role in the pathogenesis of AKI (Rui et al. 2022). The inhibited SOD and GSH-Px, together with the improved MDA and ROS of the LPS group indicated that LPS caused the oxidative stress. The inhibition of microR-380-3p reversed the oxidative stress induced by LPS, suggesting that microR-380-3p improved oxidative activity and contributed to septic AKI.

RAP1B belongs to the RAS oncogene family and is associated with several diseases (Mitra et al. 2003). RAP1B protects against the dysfunction of tubular cells evoked by high glucose, indicating that RAP1B plays a protective role in diabetic nephropathy (Xiao et al. 2014). In current study, we found that there are five target genes in microR-380-3p, which are *RAP1B*, *PPP2R1B*, *ERRFI1*, *REL* and *FSD1L*. We reviewed the previous studies of these target genes, and found that *RAP1B* has been widely researched in renal disease, so this paper focuses on the target gene of *RAP1B*. *RAP1B* was certified as a direct target of microR-380-3p by the results of luciferase reporter assay, which reported that the inhibitors of microR-380-3p enhanced the relative luciferase activity of WT-RAP1B group. The mRNA and protein levels of RAP1B were decreased in the HK-2 cells under LPS circumstance but declined microR-380-3p reversed the diminished RAP1B expression, indicating that RAP1B levels were controlled by LPS and microR-380-3p. Additionally, the sh-RAP1B was cloned for the purpose of investigating the function of RAP1B. The cell viability, apoptosis, inflammation, and oxidative stress in the miR-inhibitor group were meliorated by the knockdown of microR-380-3p, indicating that RAP1B plays a role in the effect of microR-380-3p in HK-2 cells. The overexpression of RAP1B prevents acute inflammation through regulating the bio-function of neutrophils (Chowdhury et al. 2022). In retinal endothelial cells, RAP1B is a crucial regulator in the migration of lung endothelial cells, suggesting the influence of RAP1B on normal cell functions (Chrzanowska-Wodnicka et al. 2008). Taken together, RAP1B is a downstream target in the regulation of microR-380-3p of HK-2 cells.

NF-κB pathway is a non-canonical pathway that regulates immune disorder components, inflammatory responses, cell viability, and apoptosis of cells (Sun 2017; Shokri et al. 2019). Zhou et al. (2020) report that RAP1B regulates NF-κB, and thus participates in the development of papillary thyroid carcinoma, indicating that RAP1B is a regulator of NF-κB pathway. In the current study, we downloaded the genes of NF-κB pathway and identified a correlation between RAP1B and the pathway, suggesting its potential significance in the NF-κB signaling pathway. In addition, p-p65 and p65 were proteins related to NF-κB pathway, and their levels were detected to reflect the activa-

tion of NF-κB pathways. The findings indicated that the relative p-p65/p65 levels were increased irritated by LPS, whereas, the suppression of microR-380-3p reversed this influence. The RAP1B acted as a regulator of NF-κB pathway, indicating that microR-380-3p/RAP1B played a role in septic AKI by modulating NF-κB pathway. In the mouse septic AKI models, the p-p65 of NF-κB pathway is induced by LPS, and the renal inflammation is ameliorated by Fisetin through suppressing NF-κB signaling (Ren et al. 2020). Similarly, Tacrolimus assuages LPS-stimulated AKI via regulating NF-κB pathway (Hu et al. 2022). Overall, NF-κB may participate in septic AKI under the control of microR-380-3p/RAP1B axis. We validated the diagnostic value of microR-380-3p, a result that shows its potential as a diagnostic marker. In addition, we found that microR-380-3p is associated with the mechanism of septic kidney injury, and it can regulate the progression of this disease through the RAP1B/NF-κB pathway. This mechanistic study suggests that microR-380-3p is intrinsically linked to sepsis kidney injury, which is more valuable to study than other microRNAs. However, this investigation includes some weaknesses, including a small sample size and a single institution due to its retrospective nature. It is necessary to conduct further prospective research with a large sample size and multiple institutions was imperative to validate these findings of the present findings.

In conclusion, the expression of microR-380-3p was enhanced in patients with septic AKI and had a high possibility as a diagnostic biomarker. MicroR-380-3p contributed to HK-2 cell damage caused by LPS through inhibiting cell viability, facilitating cell inflammation, oxidative state, and apoptosis. RAP1B was a target of microR-380-3p and mediated the function of microR-380-3p. NF-κB pathway was involved in the microR-380-3p/RAP1B axis.

Acknowledgments

This study was supported by TLR4/NF-κB signaling pathway regulates mitochondrial function in sepsis mechanism of acute kidney injury in sepsis (202203021222350).

Conflict of Interest

The authors declare no conflict of interest.

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