Original Article
Yonsei Med J 2021 May;62(5):461-469
https://doi.org/10.3349/ymj.2021.62.5.461

Yonsei Medical Journal
YMJ
pISSN: 0513-5796 · eISSN: 1976-2437

MIR210HG Aggravates Sepsis-Induced Inflammatory Response of Proximal Tubular Epithelial Cell via the NF-κB Signaling Pathway

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Purpose: Acute kidney injury (AKI) is a serious complication of sepsis and is characterized by inflammatory response. MicroRNA-210 host gene (MIR210HG) is upregulated in human proximal tubular epithelial cells under treatment of inflammatory cytokines. This study aimed to explore the role of MIR210HG in sepsis-induced AKI.
Materials and Methods: Cell viability was detected by a cell counting kit 8 assay. The levels of proinflammatory cytokines were detected by enzyme-linked immunosorbent assay kits. The protein levels of p65, IκBα, and p-IκBα were examined by western blot analysis. The nuclear translocation of nuclear factor kappa B (NF-κB) was detected by immunofluorescence assay. The histological changes of kidneys were analyzed by hematoxylin and eosin staining assay.
Results: Lipopolysaccharide (LPS) treatment significantly inhibited cell viability and increased productions of proinflammatory cytokines in proximal tubular epithelial cells (HKC-8). Additionally, MIR210HG levels in HKC-8 cells were increased by LPS treatment. MIR210HG silencing inhibited the LPS-induced cell inflammatory response. MIR210HG activated the NF-κB signaling pathway by promoting the phosphorylation of IκBα and nuclear translocation of p65. Rescue assays revealed that the MIR210HG-induced increase of cytokines levels and decline of cell viability were rescued by QNZ treatment. Knockdown of MIR210HG decreased blood urea nitrogen, serum creatinine, and proinflammatory cytokine levels in AKI rats. Moreover, the knockdown of MIR210HG protected against AKI-induced histological changes of kidneys in rats.
Conclusion: MIR210HG promotes sepsis-induced inflammatory response of HKC-8 cells by activating the NF-κB signaling pathway. This novel discovery may be helpful for the improvement of sepsis-induced AKI.

Key Words: MIR210HG, sepsis, AKI, NF-κB signaling

INTRODUCTION

Sepsis, a lethal systemic inflammatory disease, is caused by infectious factors, such as viruses, bacteria, or fungi, and contributes to multiple organ dysfunction syndromes, shock, and even death.[1,2] Acute kidney injury (AKI), also known as acute renal failure, is a serious complication of sepsis. Increasing studies have reported that acute functional injury, along with the inflammatory response, may occur in the kidney during sepsis.[3] Unfortunately, the physiologic or pathologic mechanisms of sepsis-induced AKI remain elusive. In addition, the impairment of renal function may contribute to increased fatality rate in septic patients.[4] The fatality rate of septic patients will be significantly reduced if the intervention treatment is performed in the AKI stage.[5] Therefore, studies to further explore the mechanisms of pathophysiological alterations at the early stage of kidney injury in septic patients, as well as to seek an effective therapeutic strategy for sepsis-induced AKI, are urgently needed.

Long non-coding RNAs (lncRNAs), which are transcripts with over 200 nucleotides in length and without an open reading frame, are unable to code proteins.[6,7] Although lncRNAs lack

Received: September 23, 2020 **Revised:** January 18, 2021
Accepted: February 23, 2021
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•The authors have no potential conflicts of interest to disclose.

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protein-encoding ability, they can modulate both posttranscriptional mRNA expression and chromatin levels via the genetic regulatory networks.[7] Recently, many studies have revealed that lncRNAs are dysregulated in AKI.[8-10] Additionally, these dysregulated lncRNAs exert effects on various biological processes, including cell viability, cell apoptosis, and inflammatory response. For instance, the upregulated lncRNA nuclear paraspeckle assembly transcript 1 predicts unfavorable prognosis of septic patients.[11] In addition, the lncRNA metastasis-associated lung adenocarcinoma transcript 1 promotes sepsis-induced cardiac dysfunction and inflammatory response by regulation of miR-125b.[12] Furthermore, homeobox A cluster antisense RNA 2 represses sepsis-induced AKI by binding with miR-106b-5p.[13] MicroRNA-210 host gene (MIR210HG) consists of 1965 nucleotides, and is transcribed by the gene ENSG00000247095.2 located on human chromosome 11p15.5.[14] Most of the studies on MIR210HG have focused on cancers, and revealed the oncogenic role of MIR210HG in lung cancer, hepatocellular carcinoma, osteosarcoma, and glioma.[14-17] Moreover, MIR210HG is upregulated by 5.69-fold changes in HKC-8 cells in hypoxic conditions at 0.1% O_2, and is upregulated by 2.35-fold changes in HKC-8 cells by the treatment of proinflammatory cytokines consisting of 50 ng/mL of interleukin (IL)-6, 50 ng/mL of tumor necrosis factor (TNF)-α, and 20 ng/mL of interferon-γ.[18] We hypothesized that MIR210HG plays a role in the inflammatory response of HKC-8 cells.

Nuclear factor kappa B (NF-κB), a ubiquitous DNA-binding transcription factor, is a key member of the NF-κB signaling pathway, and is widely reported to be implicated with inflammatory response in sepsis-induced AKI.[19,20] Downregulation of tissue inhibitor of metalloproteinase 2 improves cell injury and inflammatory response by the NF-κB pathway in sepsis-induced AKI.[21] In addition, lidanpaidu prescription ameliorates lipopolysaccharide-induced AKI by suppressing the NF-κB signaling pathway.[22] However, the relationship between MIR210HG and the NF-κB signaling pathway remains unclear in AKI.

In the current study, we used lipopolysaccharide (LPS) to induce inflammatory response of immortalized human proximal tubular epithelial cells (HKC-8). Then, we evaluated the expression status and biological effects of MIR210HG in LPS-treated HKC-8 cells. Considering that the NF-κB signaling pathway is crucial for inflammatory response in AKI, we then investigated the relationship between MIR210HG and the NF-κB signaling pathway. Lastly, we performed cecal ligation and perforation on rats to imitate sepsis-induced AKI in vivo, and evaluated the role of MIR210HG in AKI rats.

MATERIALS AND METHODS

Cell lines

HKC-8 cells, obtained from the Chinese Academy of Science Cell Bank (Shanghai, China), were cultured in Dulbecco's modified Eagle medium (DMEM; Gibco, Grand Island, NY, USA)-nutrient mixture F-12 with 5% fetal bovine serum (FBS; GE Life Sciences, Beijing, China), 1% Primocin (Gibco), and 0.1% insulin-transferrin-selenium (Gibco). The HKC-8 cells were mycoplasma-negative, and were incubated at 37°C with 5% CO_2. HKC-8 cells were treated with 100 ng/mL of LPS for 24 h to induce inflammatory response. In the control group (Con), HKC-8 cells were treated with the same dose of cell culture medium.

Cell transfection

The short hairpin RNA targeting MIR210HG (sh-RNA#1/2) was used to knock down MIR210HG. The sh-NC served as the negative control. The full length of MIR210HG was subcloned into pcDNA3.1 vector to overexpress MIR210HG. Empty pcDNA3.1 vector served as a negative control for vector overexpressing MIR210HG. The vectors were synthesized by GenePharma (Shanghai, China) and transfected into HKC-8 cells by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

Cell viability measurement

The cell viability of HKC-8 cells was detected by a cell counting assay kit-8 (CCK-8; Beyotime, Shanghai, China) under the manufacturer's instructions. HKC-8 cells were seeded onto 96-well plates (5×10^3 cells per well). Forty-eight hours after transfection, the CCK-8 solution (10 μL) was added. Afterwards, HKC-8 cells were incubated in a humidified incubator with 5% CO_2 at 37°C for 2 h. Finally, the absorbance was detected by a microplate reader at a wavelength of 450 nm.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

The extraction of total RNA from HKC-8 cells or renal tissues of rats were performed by TRIzol Reagent (Invitrogen). The RevertAid First Strand cDNA Synthesis Kit (K1622, Thermo Fisher Scientific, Waltham, MA, USA) was applied for reverse transcription of MIR210HG. The qPCR was performed using the SYBR-Green PCR Master Mix kit (Applied Biosystems, Waltham, MA, USA). The relative expression level of MIR210HG was calculated using the $2^{-\Delta\Delta Ct}$ method. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as a control gene. The primer sequences are listed as follows: MIR210HG, forward, 5'-GCT TGG TAG AGT GTC ACG CC-3' and reverse, 5'-CAT CTG ACC GAG CCA GTT TG-3'; GAPDH, forward, 5'-TAT GAT GAT ATC AAG AGG GTA GT-3' and reverse, 5'-TGT ATC CAA ACT CAT TGT CAT AC-3'.

Preparation of nuclear extracts

The HKC-8 cell suspensions were prepared by trypsin-ethylene diamine tetra acetic acid treatment. The proteins were extracted from the cytoplasm and nucleus of HKC-8 cells using NE-PER™ Nuclear and Cytoplasmic Extraction Reagents

https://doi.org/10.3349/ymj.2021.62.5.461

(Thermo Fisher Scientific) according to the manufacturer's instructions.

Western blot

Western blot analysis was used to measure the protein levels. In brief, protein samples were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels and then transferred onto polyvinylidene fluoride membranes. After being coated with 5% non-fat milk, the membranes were incubated with primary antibodies against p65 (ab16502, Abcam, Cambridge, UK), β-tubulin (ab204947, Abcam), Lamin B (ab194109, Abcam), IκBα (ab32518, Abcam), p-IκBα (sc-52943, Sigma-Aldrich, Saint Louis, MO, USA), and GAPDH (ab9484, Abcam) at a dilution of 1:1000 overnight at 4°C. Next, the membranes were exposed to horseradish peroxidase-conjugated goat anti-rabbit (1:5000, ab205718, Abcam) secondary antibodies, and the signals were detected with an enhanced chemiluminescence detection system. The protein levels were quantified by the ImageJ software.

Immunofluorescent staining

HKC-8 cells treated with LPS were placed on a cover slide in 12-well plates. Then, HKC-8 cells were washed three times with phosphate buffer saline (PBS; Invitrogen), followed by fixation for 10 minutes with 4% paraformaldehyde at room temperature, and washed again. Afterwards, HKC-8 cells were blocked with 1% bovine serum albumin and incubated with primary antibody anti-NF-kB (ab16502, Abcam). Subsequently, the secondary antibody fluorescein isothiocyanate-conjugated immunoglobulin G (IgG) was added. Finally, the slides were rinsed, mounted, and viewed on a confocal microscope (FV1000, Olympus, Japan) at 488 nm.

Enzyme-linked immunosorbent assay

The levels of TNF-α, IL-6, and IL-1β in cell supernatants or kidneys of rats in each group were measured with ELISA kits according to the manufacturer's instructions.

Animal model establishment

A total of 40 Sprague Dawley rats (male, 200–250 g, 8–10 weeks) were purchased from Vital River company (Beijing, China), and were randomly divided into four groups (n=10 in each group): sham group, sham+sh-RNA#1 group, AKI group, and AKI+sh-RNA#1 group. To induce AKI, we anesthetized the rats by intraperitoneal injection of 2% sodium pentobarbital (80 mg/kg). Subsequently, the rats were placed on the laboratory bench, and their abdomens were shaved and disinfected with 70% isopropanol. Then, an incision (1.5 cm) was made in the midline of rat abdomen to fully expose the cecum, and the cecum (1 cm away from the cecum tail) was ligated with a sterile sewing silk (no. 4). Afterwards, the blind end was perforated by a 20-gauge needle. After restoring the cecum to the abdominal cavity, the abdominal incision was sutured. Finally, the rats were moved to a thermostatic blanket for rewarming. The sham-operated rats underwent similar operation without cecal ligation and perforation. Three days later, all rats were killed, and the kidneys and blood of rats were collected. All experimental procedures were approved by the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (IRB No. 2018-077). The ethics committee of Jiangsu Taizhou People's Hospital approved the current study.

Adeno-associated virus injection

Adeno-associated virus (AAV) (serotype 9, Vigene Biosciences, Shanghai, China) was packaged, rinsed, and titrated for injection. AAV9 (1×10^{12} copies) containing the shRNA#1 targeting MIR210HG was injected into rats through the tail vein 2 weeks before model establishment.

Renal function measurement

The blood urea nitrogen (BUN) and serum creatinine (SCr) levels in the serum of rats were detected by a Hitachi 7060 automatic biochemistry analyzer (Hitachi, Japan) and a creatinine assay kit (BioAssay Systems, Hayward, CA, USA) according to the manufacturer's instructions.

Hematoxylin & eosin staining assay

H&E staining assay was conducted for histopathological analysis of the rats' kidneys, as described previously.[10] First, sections (4 μm) of rat renal tissues were immobilized in 10% buffer formalin, dehydrated in ethanol, clarified in xylene, and embedded in paraffin resin. Then, hematoxylin and eosin were separately utilized to immerse the sections for 10 min and 1 min. Afterwards, a microscope was used to visualize the morphological changes.

Statistical analysis

Statistical analysis was conducted with SPSS software. Data are presented as the mean±standard deviation. The unpaired Student's t-tests or one-way analysis of variance were used to compare the differences between the groups. Pearson correlation analysis was used to analyze the correlation of expressions between MIR210HG and inflammatory cytokines. $p<0.05$ was considered statistically significant.

RESULTS

MIR210HG level is increased by LPS treatment in renal epithelial cells

LPS treatment significantly inhibited cell viability (Fig. 1A). Moreover, the levels of inflammatory cytokines (IL-1β, IL-6, and TNF-α) in HKC-8 cells were significantly increased by LPS treatment (Fig. 1B). Next, we evaluated the expression status of MIR210HG in HKC-8 cells. The result of RT-qPCR demonstrated that MIR210HG level was increased by LPS treatment

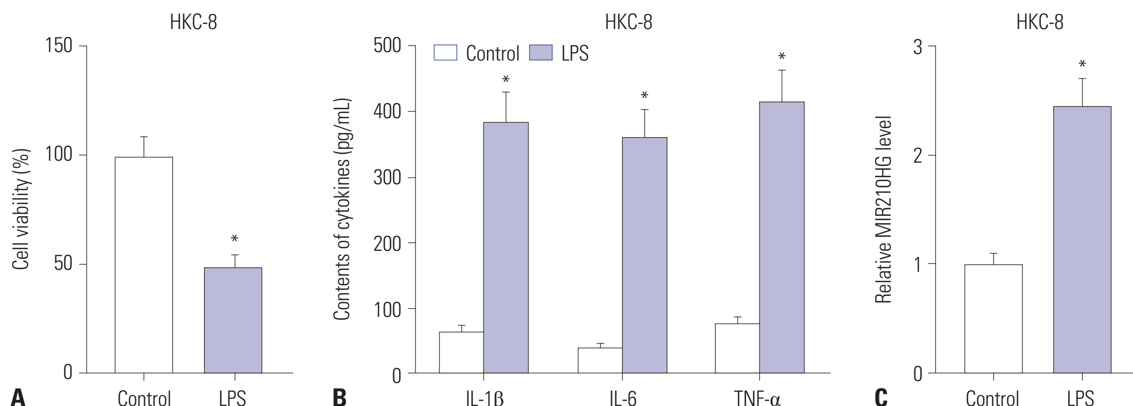

Fig. 1. MIR210HG levels were increased by LPS treatment in proximal tubular epithelial cells. (A) The CCK-8 assay was used to detect cell viability of HKC-8 cells under LPS stimulation. (B) The levels of inflammatory cytokines (IL-1β, IL-6, and TNF-α) in HKC-8 cells under LPS stimulation were detected by ELISA. (C) RT-qPCR analysis was used to measure MIR210HG levels in HKC-8 cells under LPS stimulation. **p*<0.05. MIR210HG, MicroRNA-210 host gene; LPS, lipopolysaccharide; IL, interleukin; TNF, tumor necrosis factor.

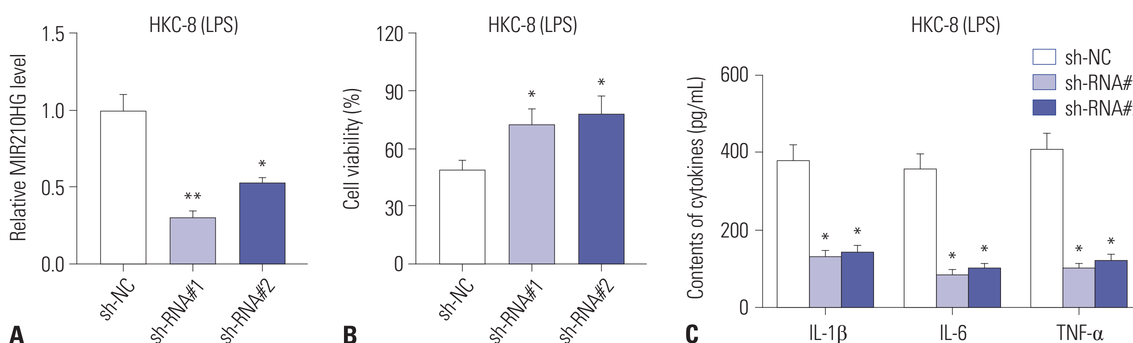

Fig. 2. MIR210HG silencing alleviated inflammatory response and cell injury. (A) The knockdown efficacy of MIR210HG sh-RNA#1/2 was evaluated by RT-qPCR. (B) The effect of MIR210HG sh-RNA#1/2 on HKC-8 cell viability. (C) The effects of MIR210HG sh-RNA#1/2 on levels of inflammatory cytokines (IL-1β, IL-6, and TNF-α). **p*<0.05, ***p*<0.01. MIR210HG, MicroRNA-210 host gene; IL, interleukin; TNF, tumor necrosis factor.

in HKC-8 cells (Fig. 1C).

MIR210HG downregulation alleviates inflammatory response and cell injury

We knocked down MIR210HG levels by transfecting shRNA#1/2 targeting MIR210HG into HKC-8 cells. As shown in Fig. 2A, MIR210HG levels were reduced by transfection of shRNA#1/2. In addition, CCK-8 assay showed that MIR210HG silencing significantly improved cell viability of HKC-8 cells (Fig. 2B). Results of ELISA revealed that MIR210HG silencing significantly decreased the levels of IL-1β, IL-6, and TNF-α in LPS-treated HKC-8 cells (Fig. 2C).

MIR210HG silencing inactivates the NF-κB signaling

The NF-κB signaling has been widely reported to regulate sepsis-induced inflammatory response.[23,24] We hypothesized that MIR210HG may modulate inflammatory response by targeting the NF-κB signaling pathway. To validate our hypothesis, western blot analysis was conducted. According to Fig. 3A, the increase of nucleus p65 expression and the decline of cytoplasm p65 expression induced by LPS were restored by MIR210HG silencing. Moreover, the increase in p-IκBα protein levels resulting from LPS treatment was rescued by MIR210HG silencing (Fig. 3B). In addition, results of immunofluorescence staining assay demonstrated that the promotive effect of LPS treatment on NF-κB nuclear translocation was rescued by MIR210HG silencing (Fig. 3C).

MIR210HG promotes inflammatory response by activating the NF-κB signaling pathway

To confirm whether MIR210HG modulates inflammatory response via the NF-κB signaling, rescue assays were designed. First, RT-qPCR analysis indicated that the level of MIR210HG was overexpressed by the transfection of pcDNA3.1/MIR210HG into HKC-8 cells (Fig. 4A). Additionally, western blot analysis revealed that the increase of NF-κB expression in the nucleus induced by MIR210HG was offset by the treatment of QNZ {4-N-[2-(4-phenoxyphenyl) ethyl] quinazoline-4, 6-diamine, an inhibitor of the NF-κB signaling pathway} (Fig. 4B). Furthermore, the increase of concentrations of inflammatory cytokines induced by MIR210HG overexpression was neutralized by QNZ treatment (Fig. 4C). Finally, CCK-8 assay showed that the suppressive effect of MIR210HG overexpression on cell viability was rescued by QNZ treatment (Fig. 4D).

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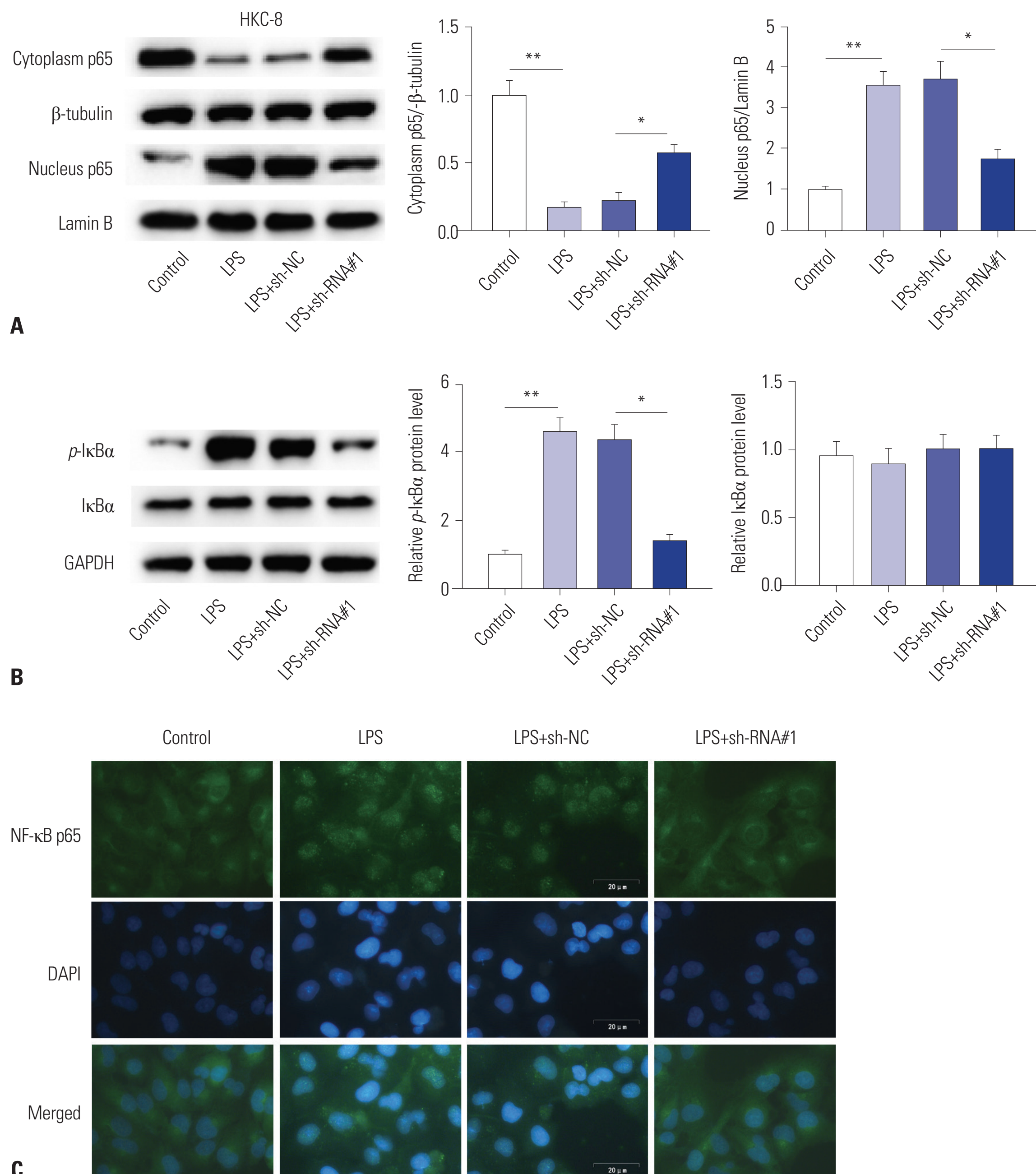

Fig. 3. MIR210HG silencing inactivated the NF-κB signaling pathway. (A and B) The western blot analysis was conducted to examine the protein levels of p65, phosphorylated IκBα, and IκBα in HKC-8 cells. (C) The immunofluorescent staining assay was used to analyze the nuclear translocation of NF-κB in HKC-8 cells. *p<0.05, **p<0.01. MIR210HG, MicroRNA-210 host gene; LPS, lipopolysaccharide; IL, interleukin; TNF, tumor necrosis factor; NF-κB, nuclear factor kappa B.

Knockdown of MIR210HG mitigates renal injury by targeting the NF-κB signaling pathway

We then further investigated the role of MIR210HG in animals. RT-qPCR analysis showed that MIR210HG was significantly upregulated in renal tissues of AKI rats. MIR210HG was effectively knocked down by the injection of AAV-sh-MIR210HG in renal tissues of control rats and AKI rats (Fig. 5A). As shown in Fig. 5B and C, BUN and SCr levels were higher in AKI rats than in control rats. Knockdown of MIR210HG triggered a significant decrease of BUN and SCr levels in the serum of AKI rats. Levels of inflammatory cytokines were higher in kidneys of AKI rats than that of control rats, and MIR210HG silencing reduced the concentrations of inflammatory cytokines (Fig. 5D). In addition, H&E staining assay revealed that the distorted renal tubules as well as apoptotic or necrotic phenotypes of renal tubular epithelial cells were observed in the AKI group, and MIR210HG silencing mitigated these histological changes (Fig. 5E). The kidney index (kidney/body weight) was increased in AKI rats, and MIR210HG silencing reduced the kidney index in AKI rats (Fig. 5F). Subsequently, we found that the

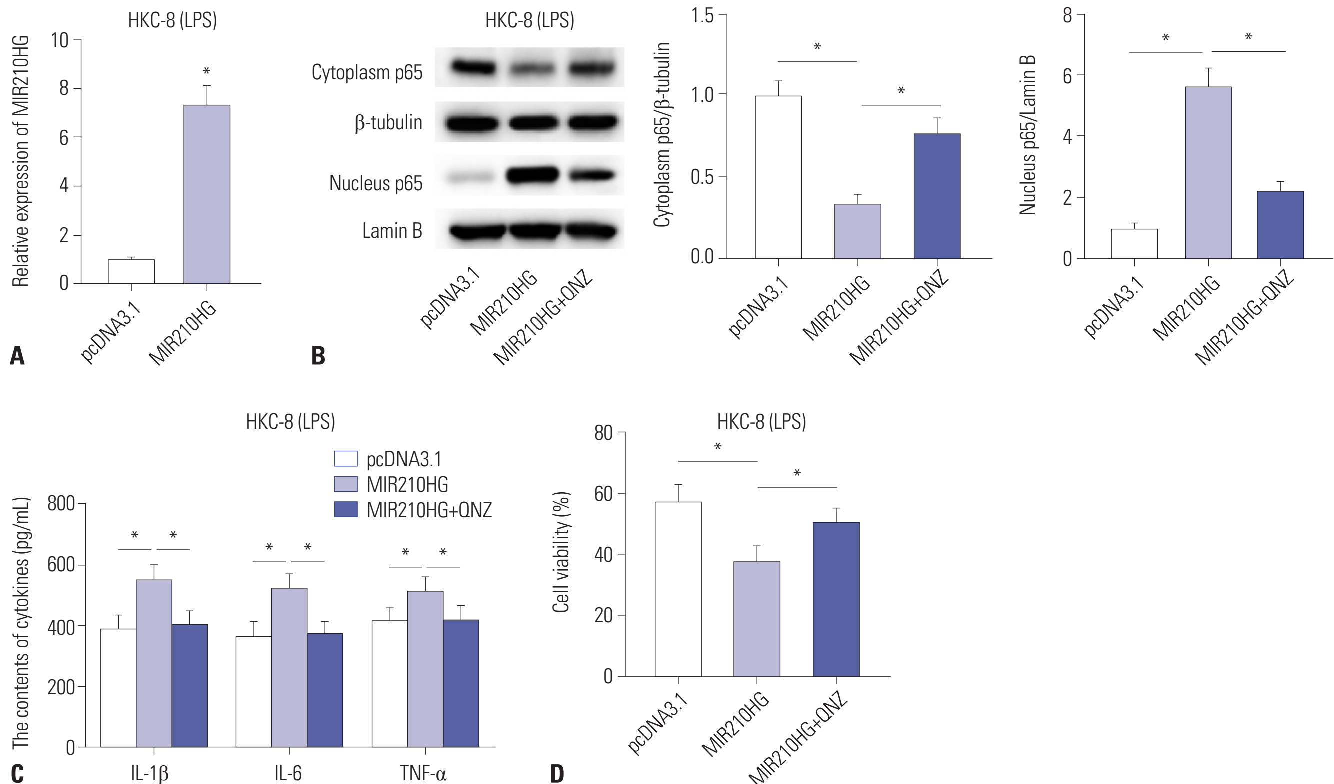

Fig. 4. MIR210HG overexpression promotes inflammatory response by activating the NF-κB signaling pathway. (A) The overexpression efficacy of pcDNA3.1/MIR210HG was evaluated by RT-qPCR. (B) The effects of MIR210HG and QNZ on NF-κB nuclear translocation were evaluated by immunofluorescent staining assay. (C) The effects of MIR210HG and QNZ on levels of inflammatory cytokines (IL-1β, IL-6, and TNF-α). (D) The effects of MIR210HG and QNZ on HKC-8 cell viability. *$p<0.05$. MIR210HG, MicroRNA-210 host gene; IL, interleukin; TNF, tumor necrosis factor; NF-κB, nuclear factor kappa B.

nuclear translocation of p65 and phosphorylation of IκBα were promoted in kidneys of AKI rats. MIR210HG silencing inhibited the nuclear translocation of p65 and the phosphorylation of IκBα (Fig. 5G and H).

DISCUSSION

A number of risk factors, such as endothelial dysfunction, intrarenal hemodynamic changes, and inflammatory cell infiltration of renal parenchyma, are involved in the progression of sepsis-mediated renal injury.[25,26] In the advanced stage of sepsis, tissue perfusion and microcirculatory blood flow are inhibited. The failure of microcirculation and mitochondrial damage of renal tubular epithelial cell may exacerbate renal tubular epithelial cell injury.[3,27] Numerous studies have shown that inflammatory response and anti-inflammatory response are strongly associated with the development of sepsis-induced renal injury.[3,28,29] Additionally, the levels of cytokines are related to the severity of septic AKI. Inflammatory cytokines can directly inhibit cell viability and further exacerbate sepsis-induced AKI.[30] Therefore, further exploration of mechanisms underlying the sepsis-induced AKI is urgently needed. In the present study, HKC-8 cells were treated with LPS to mimic the cell injury in AKI. We found that LPS treatment significantly inhibited cell viability and levels of proinflammatory factors such as TNF-α, IL-6, and IL-1β. These results revealed that LPS can induce cell injury, which was significantly associated with inflammatory response. A previous study showed that MIR210HG is upregulated in hypoxia or cytokine treated HKC-8 cells.[18]

Several lncRNAs were reported to modulate inflammatory response in sepsis-induced AKI. Downregulation of lncRNA taurine-upregulated gene 1 contributes to inflammatory response and promotes cytokine secretion during the development of sepsis-induced AKI.[24] Moreover, lncRNA plasmacytoma variant translocation 1 aggravates LPS-induced septic AKI by regulating TNF-α and cell viability.[23] In the present study, the knockdown of MIR210HG promoted cell viability and inhibited inflammatory response.

The NF-κB signaling pathway is a critical regulator of inflammatory response in sepsis-induced AKI.[22,31] Treatment of LPS contributes to the activation of IκBα phosphorylation by binding with related receptors. The phosphorylated IκBα can be ubiquitinated and degraded. As a result, NF-κB is released from the cytoplasmic NF-κB/IκBα complex. Then, the cytoplasmic NF-κB is translocated into the nucleus rapidly, ultimately initiating the transcription of downstream target genes.[32,33] Given that various lncRNAs regulate inflammatory response by tar-

https://doi.org/10.3349/ymj.2021.62.5.461

geting the NF-κB signaling pathway, we hypothesized that MIR210HG regulates inflammatory response in this way. As expected, the knockdown of MIR210HG reduced the p-IκBα/IκBα ratio and nucleus p65/cytoplasm p65 ratio, which indicated that the silencing of MIR210HG inhibited the phosphorylation of IκBα and inactivated the nuclear translocation of NF-κB.

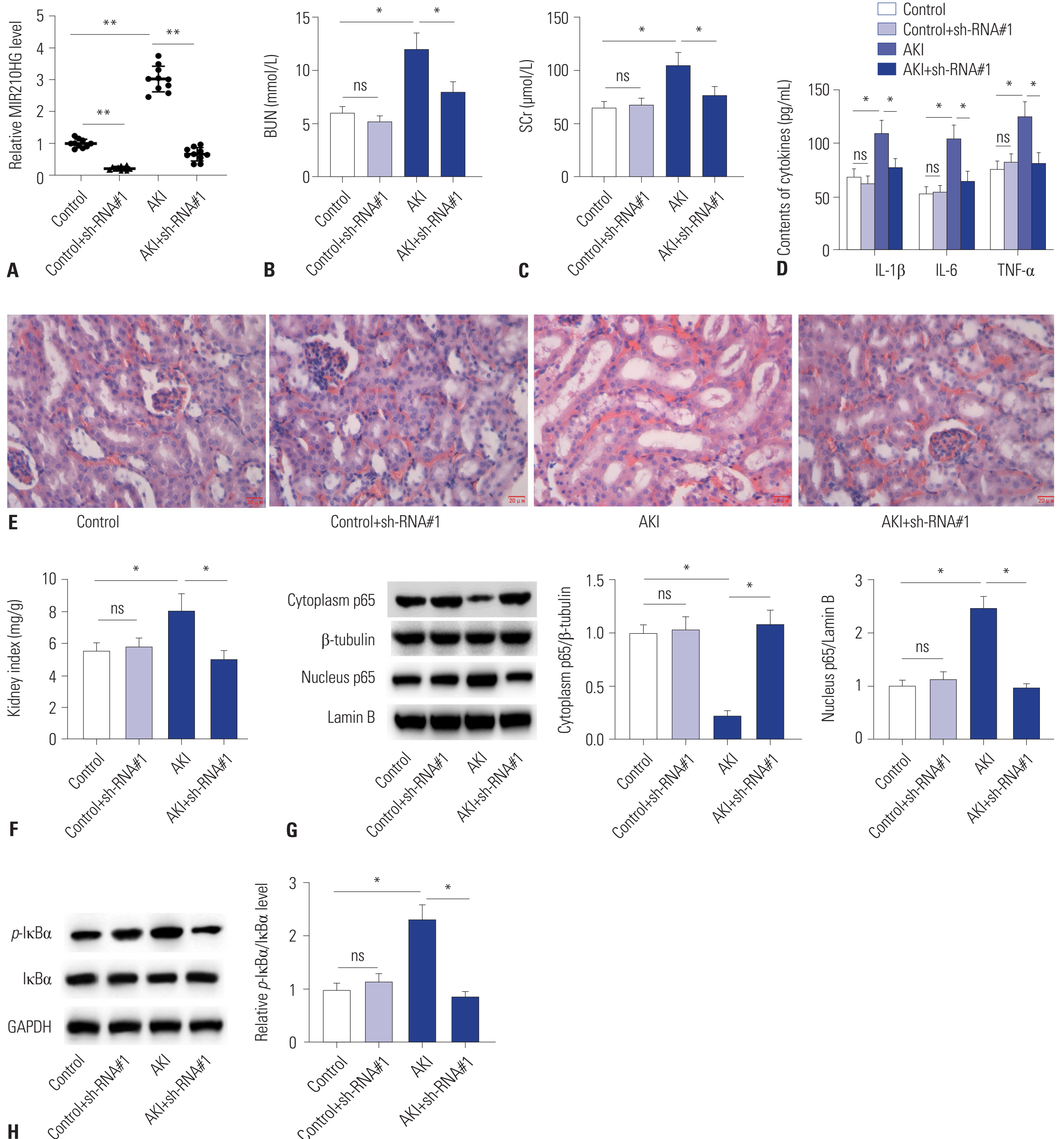

Fig. 5. Knockdown of MIR210HG mitigates renal injury by the NF-κB signaling pathway. (A) The levels of MIR210HG in renal tissues of control and AKI rats injected with AAV-shRNA#1 (n=10). (B and C) The measurement of BUN and SCr levels in serum of control and AKI rats (n=10). (D) The detection of inflammatory cytokine (IL-1β, IL-6, and TNF-α) levels in kidneys of control and AKI rats (n=10). (E) The examination of histological changes in renal tissues of rats (n=10). (F) Changes of kidney index in control rats, MIR210HG-silenced control rats, AKI rats, and MIR210HG-silenced AKI rats (n=10). (G and H) Western blot analysis was conducted to examine the protein levels of p65, phosphorylated IκBα, and IκBα in renal tissues of rats (n=10). *$p<0.05$, **$p<0.01$. ns, no significance; MIR210HG, MicroRNA-210 host gene; AKI, acute kidney injury; BUN, blood urea nitrogen; SCr, serum creatinine; IL, interleukin; TNF, tumor necrosis factor; NF-κB, nuclear factor kappa B; AAV, adeno-associated virus.

In contrast, MIR210HG overexpression activated the nuclear translocation of NF-κB. However, this result was rescued by the treatment of QNZ. The rescue assays suggested that the effects of MIR210HG on cell viability and inflammatory response were counteracted by QNZ treatment, which further confirmed that MIR210HG regulated sepsis-induced AKI by the NF-κB signaling pathway. Lastly, we established an AKI rat model by CLP operation. MIR210HG was upregulated in the renal tissues of CLP rats. Importantly, MIR210HG promoted the levels of proinflammatory cytokines in vivo.

MIR210HG can upregulate the expression of STAT3 and downregulate the expression of miR-874.[34] STAT3 was widely reported to induce kidney injury.[35-37] MiR-874 was reported to alleviate renal injury[38] and reduce high glucose induced inflammatory response of human renal tubular epithelial cells.[39]

In conclusion, for the first time, this study demonstrated that MIR210HG promotes LPS-induced inflammatory response of proximal tubular epithelial cells by targeting the NF-κB signaling pathway. This novel discovery may be helpful for the improvement of sepsis-induced AKI. However, our study had some limitations. First, the regulatory mechanisms of MIR210HG on the NF-κB signaling were not investigated. Second, the effects of MIR210HG on other cells, such as HK-2 and endothelial cells, remain to be explored.

AUTHOR CONTRIBUTIONS

Conceptualization: Shuai Deng. Data curation: all authors. Formal analysis: Shuai Deng. Investigation: Shuai Deng, Bin Gu, and Zheng Yu. Methodology: Shuai Deng and Bin Gu. Project administration: Shuai Deng. Resources: Shuai Deng and Zheng Yu. Software: Shuai Deng and Zhen Shen. Supervision: Shuai Deng. Validation: Shuai Deng. Visualization: Shuai Deng. Writing—original draft: all authors. Writing—review & editing: all authors. Approval of final manuscript: all authors.

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