Promotion of Inflammation, Apoptosis, and Inhibition of Autophagy by Overexpression of lncRNA SNHG12 in Acute Kidney Injury

Jiaqing Zhang,[1#] Liang Li,[2#] Yanhui Yu,[3] Yan Fang,[1] Jian Li,[1] Jinji Li[4]

[1]Department of Nephrolgy, 901 Hospital of Joint Logistics Support Force of People Liberation Army, Hefei City, Anhui Province, 230031, China
[2]Department of Nephrology, Ezhou Central Hospital, Ezhou City, Hubei Province, 436000, China
[3]Department of Cadre 3 Ward 2, General Hospital of Western Theater of Chinese People's Liberation Army, Chengdu City, Sichuan Province, 610036, China
[4]Department of Nephrology, The Affiliated Hospital of Yanbian University, Yanji City, Jilin Province, 133000, China

[#]Jiaqing Zhang and Liang Li contributed equally to this work.

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Introduction. There is a dispute regarding the roles of newly discovered lncRNAs in acute kidney injury (AKI). Therefore, this study discussed long non-coding RNA (lncRNA) small nuclear host gene 12 (SNHG12) in AKI and its molecular mechanism.
Methods. Lipopolysaccharide (LPS) induction was treated into renal tubular epithelial cells (HK-2 cells) to induce septic AKI *in vitro*. In the cell model, SNHG12, miR-1270, and tubulin beta class I (TUBB) expression patterns, along with p-p65, cleaved caspase-3, Beclin-1, p62, and autophagy related 7 (ATG7) protein expressions, were determined by reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) and Western blot. Cell viability was evaluated by cell counting kit-8 (CCK-8) and lactate dehydrogenase (LDH) cytotoxicity assay, while apoptosis and inflammation were assessed by flow cytometry and enzyme-linked immunosorbent assay (ELISA), respectively. At last, the mechanistic interaction between SNHG12, miR-1270, and TUBB was identified.
Results. SNHG12 was highly expressed in LPS-induced HK-2 cells. Functionally, knocking down SNHG12 increased cell viability and autophagy, while inhibited LDH release, inflammation, and apoptosis. Mechanically, SNHG12 absorbed miR-1270 to upregulate TUBB expression, thereby aggravating inflammation, apoptosis, and inhibiting autophagy in AKI.
Conclusion. SNHG12 promotes inflammation, apoptosis, and autophagy by targeting the miR-1270/TUBB axis in AKI.

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Original Paper

INTRODUCTION

Acute kidney injury (AKI) is stimulated by several conditions, such as sepsis, ischemia/reperfusion, and various toxins, and is characterized by a rapid decrease in renal function in a short period of time and a high mortality rate.[1,2] Sepsis leads to severe systemic inflammation of the host and septic AKI accounts for about 50% of all AKI cases.[3] Recent epidemiological studies have revealed that even patients who experience mild or transient AKI are at greater risk of chronic kidney disease, cardiovascular disease, and death.[4,5] Considering the unfavorable outcomes of septic AKI, it is essential to elucidate the underlying regulatory mechanisms of AKI.

Lipopolysaccharide (LPS), a component of the

cell wall of gram-negative bacteria, is an endotoxin that is commonly applied to induce inflammation in experimental models. Currently, LPS-induced renal tubular epithelial cells (HK-2 cells) can secrete inflammatory factors such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α), which are mature cell models of septic AKI *in vitro*.[6-8] Non-coding RNAs (ncRNAs) are deeply involved in the development and repair of AKI[9] and long ncRNAs (lncRNAs) with 200 nucleotide extended transcripts that do not encode proteins or have limited coding capacity and are involved in kidney diseases, including AKI.[10] Indeed, lncRNAs play a regulatory role by acting as a sponge for microRNAs (miRNAs).[11] For example, lncRNA MALAT1 modulates inflammation in septic AKI through the absorption of miR-146a.[12] Small nuclear host gene 12 (SNHG12) has been considered a tumor-related lncRNA in various cancers, such as renal cell carcinoma,[13] oral squamous cell carcinoma[14] and colorectal cancer.[15] Besides, SNHG12 has been reported to be implicated in the progression of cardiovascular diseases.[16,17] Notably, SNHG12 has also been reported to affect endothelial cell function in LPS-induced acute lung injury.[18] However, the role of SNHG12 in AKI has not been reported.

MiRNAs are single-stranded RNA molecules of 17-24 nucleotides in length, and many miRNAs are considered to play crucial roles in AKI, such as miR-494[19] and miR-107.[20] It has been reported that miRNAs can regulate gene expression by binding to target messenger RNAs (mRNAs) to induce mRNA degradation or prevent mRNA protein translation.[21,22] The current study examined and screened the downstream miRNAs and mRNA of SNHG12, which were miR-1270, and tubulin beta class I (TUBB), respectively. It has been noted that increasing miR-1270 can inhibit inflammation and apoptosis in oxidized low-density lipoprotein (oxLDL)-injured THP-1 macrophages.[23] As with TUBB, it is encoded by a multi-gene family and forms microtubules in a heterodimer manner. Experimentally, TUBB has been studied and reported to be abnormally expressed in clear cell renal cell carcinoma.[24,25]

The current work established an AKI model in LPS-induced renal epithelial cells and tried to explore the function and molecular mechanism of SNHG12 through the miR-1270/TUBB axis, with the aim of developing new therapeutic targets and strategies for improving and treating AKI.

MATERIALS AND METHODS

Cells and Treatments

HK-2 cells (Procell Life Science & Technology Co., Ltd., Wuhan, China) were kept in a humid environment at 37 °C and 5% CO_2 and cultured in Dulbecco's Modified Eagle's Medium (Procell Life Science & Technology Co., Ltd., Wuhan, China) containing 10% fetal bovine serum and 1% penicillin/streptomycin. When cells reached 80 to 90% confluence, they were passaged and transferred to a new medium the following day; thereafter, the medium was changed every 2 to 3 days. Cells were treated with LPS (1 mg/L, Sigma-Aldrich, USA) for 12 h.

Transfection of Cells

Small interfering RNA (siRNA) and pcDNA 3.1 overexpression vectors targeting SNHG12 and TUBB, as well as miR-1270 mimic/inhibitor and the negative controls were purchased from GenPharma (China). HK-2 cells were transiently transfected with Lipofectamine 3000 (Thermo, USA). After 48 hrs, the transfection efficiency was evaluated by western blot and RT-qPCR.

Lactate Dehydrogenase (LDH) Detection

HK-2 cells were seeded in a 96-well plate and the wells were assigned to control, treatment, maximum release, and spontaneous release. The maximum release group was added with 1 μL Triton X-100, while the spontaneous release group was with a cell-free medium. Then, the supernatant was centrifuged at 4 ℃ for 15 min, mixed with 25 μL matrix solution and 5 μL coenzyme I application solution, and reacted with 25 μL 2, 4-dinitrophenylhydrazine. After diluting with 250 μL sodium hydroxide or caustic soda (NaOH), LDH release rate was analyzed at 450 nm with a microplate reader. LDH release rate = (optical density (OD) value of target group - OD value of spontaneous release group) / (OD value of maximum release group - OD value of spontaneous well) × 100%.

Cell Counting Kit-8 (CCK-8) Test

In the 96-well plate inoculated with HK-2 cells, each well was cultured with 10 μL CCK-8 solution (Beyotime, China) for four hours and the

absorbance was read at 450 nm using a microplate reader. Cell viability = (OD experimental group - OD blank group) / (OD control group - OD blank group) × 100%.

Apoptosis

HK-2 cells were digested with trypsin (Yubo Biotech, Shanghai, China). After centrifugation, cell pellets were added with Annexin V-fluorescein isothiocyanate (FITC), propidium iodide (PI), and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffers at 1:2:50, as per the Annexin V-FITC Apoptosis Detection Kit (K201-100, Biovision, USA). HK-2 cells (1×10^6) were placed in 100 µL staining solution for 15 minutes and mixed with 1 mL HEPES buffer (PB180325, Procell, Wuhan, China) before reading the fluorescence of FITC and PI at 488 nm using 252 nm and 620 nm bandpass filter.

Enzyme-linked Immunosorbent Assay (ELISA)

Briefly, HK-2 cells were seeded into 24-well plates (2×10^5 cells/well) and transfected with the indicated plasmids. LPS (1 mg/L) was then added to each well for incubation. Afterwards, the cell culture supernatant was gathered, and interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) levels in the supernatant were examined by the help of commercial ELISA kits (GIBCO, USA).

Reverse Transcription Quantitative Real-time Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted from the cells using TRIzol reagents (Invitrogen). After determination of total RNA using NanoDrop 2000 (Thermo Scientific), complementary DNA (cDNA) was synthesized by using PrimeScript RT kit (Takara). qPCR was performed on the LightCycler 96 system (Roche) using SYBR Premix Ex Taq II (Takara, Beijing, China). The gene expression was quantified by $2^{-\Delta\Delta CT}$. The primer sequences of genes are shown in Table 1.

Table 1. Primers Sequences of Genes

	Sequences (5′-3′)
SNHG12	Forward: 5'- GCTGACAGGCGGATAAAACG-3'
	Reverse: 5'- AACCAGGTCCCCTGCATTTC-3'
miR-1270	Forward: 5'-GCGCTGGAGATATGGAAGAG-3'
	Reverse: 5'- TGGTGTCGTGGAGTCG-3'
TUBB	Forward: 5'- AACATGATGGCTGCCTGTGA -3'
	Reverse: 5'- ACGAGGTCGTTCATGTTGCT -3'
U6	Forward: 5'- CTCGCTTCGGCAGCACA-3'
	Reverse: 5'- AACGCTTCACGAATTTGCGT-3'
GAPDH	Forward: 5'- CACCCACTCCTCCACCTTTG-3'
	Reverse: 5'- CCACCACCCTGTTGCTGTAG-3'

Note: SNHG12, long non-coding RNA small nucleolar RNA host gene 12; miR-1270, microRNA-1270; TUBB, tubulin beta chain; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase

Western Blot

The total proteins of HK-2 cells were prepared with the protein extraction kit (Beyotime). After the protein concentration was determined by bicinchoninic acid (BCA) method, the total protein samples were transferred to the polyvinylidene fluoride (PVDF) membrane after isolating by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Then, cells were blocked with phosphate buffer saline (PBST) containing 5% bovine serum albumin (BSA) and incubated overnight with primary antibody solution at 4°C. Then, after rinsing by the use of Tris-buffered saline with Tween-20 (TBST), the membrane was treated with goat anti-rabbit secondary antibody (1/2000, ab6721, Abcam) for one hour. The protein images were developed by using a chemiluminescent reagent (abs920, Absin Bioscience, Shanghai, China), and analyzed by ImageJ software 1.48. Primary antibodies included cleaved caspase-3 (1/100, ab2302, Abcam), p-p65 (1/1000, 3033, Cell Signaling Technology), Beclin-1 (1/1000, 3495, Cell Signaling Technology), autophagy related 7 (ATG7; 1/1000, 2631, Cell Signaling Technology), p62 (5114, Cell Signaling Technology), and GAPDH (ab8245, Abcam).

Luciferase Reporter Gene Assay

Wild-type (WT) and mutant-type (MUT) sequences of SNHG12 and TUBB (WT/MUT)-SNHG12 and WT/MUT-TUBB) were constructed respectively according to miR-1270 putative binding sites. The synthesized sequences (Sangon, Shanghai, China) were cloned into the pmirGLO Dual-Luciferase miRNA target expression vector (Promega). These luciferase reporters and miR-1270 mimic or mimic NC were co-transfected into HK-2 cells using Lipofectamine 3000 (Thermo). After 48 hours, fluorescence activity was measured in the Dual-Luciferase® Reporter Assay System (Promega).

RNA Immunoprecipitation (RIP) Experiment

HK-2 cell lysate was incubated with RIP buffer

and added with magnetic beads coupled with human argonaute RISC catalytic component 2 (AGO2) or mouse immunoglobulin G (IgG). The complex was harvested with protease K, and the isolated immunoprecipitated RNA was quantitatively analyzed on a NanoDrop spectrophotometer (Thermo Scientific). At last, the purified RNA was tested by RT-qPCR.

Analysis of Data

Data were expressed as mean ± standard deviation (SD) and replicated at least three times. Shapiro-Wilk was practical for the normality test, after which Student t-test or one-way Analysis of Variance (ANOVA) was applied to compare data. Post hoc tests were done by using the Tukey's honest significant difference (HSD) method. The significance was set at $P < .05$.

RESULTS

SNHG12 Is High-expressed in LPS-treated HK-2 Cells

In HK-2 cells, LPS stimuli increased LDH release and decreased cell viability (Figures 1A, B). Flow cytometry showed that LPS enhanced apoptosis of HK-2 cells (Figure 1C), while ELISA results revealed an increase in TNF-α, IL-1β, and IL-6 levels in LPS-treated HK-2 cells (Figure 1D). Subsequently, the effects of LPS on proteins associated with inflammation, apoptosis, and autophagy were examined by Western blot. LPS treatment elevated cleaved caspase-3, p-p65, and p62, but inhibited ATG7 and Beclin-1 expression (Figure 1E). These data indicated that an AKI cell model was successfully established. Additionally, SNHG12 expression pattern presented an increase in LPS-treated HK-2 cells (Figure 1F). These findings showed that LPS reduced cell viability and autophagy while inducing cell apoptosis and inflammation as well as SNHG12 expression in HK-2 cells.

SNHG12 Knockdown Improves LPS-induced Inflammation, Apoptosis, and Promotes Autophagy

In LPS-treated HK-2 cells, SNHG12 expression was silenced due to transfection with si-SNHG12 (Figure 2A). Followed by that, the increased cell viability and autophagy, along with reduced LDH release, apoptosis rate, and production of inflammatory factors were observed in SNHG12-silenced HK-2 cells (Figures 2B-F). All above results suggested that SNHG12 knockdown increased cell viability and autophagy while inhibited inflammation and apoptosis in LPS-treated HK-2 cells.

SNHG12 Competitively Adsorbs miR-1270

Genetic information of SNHG12 was analyzed

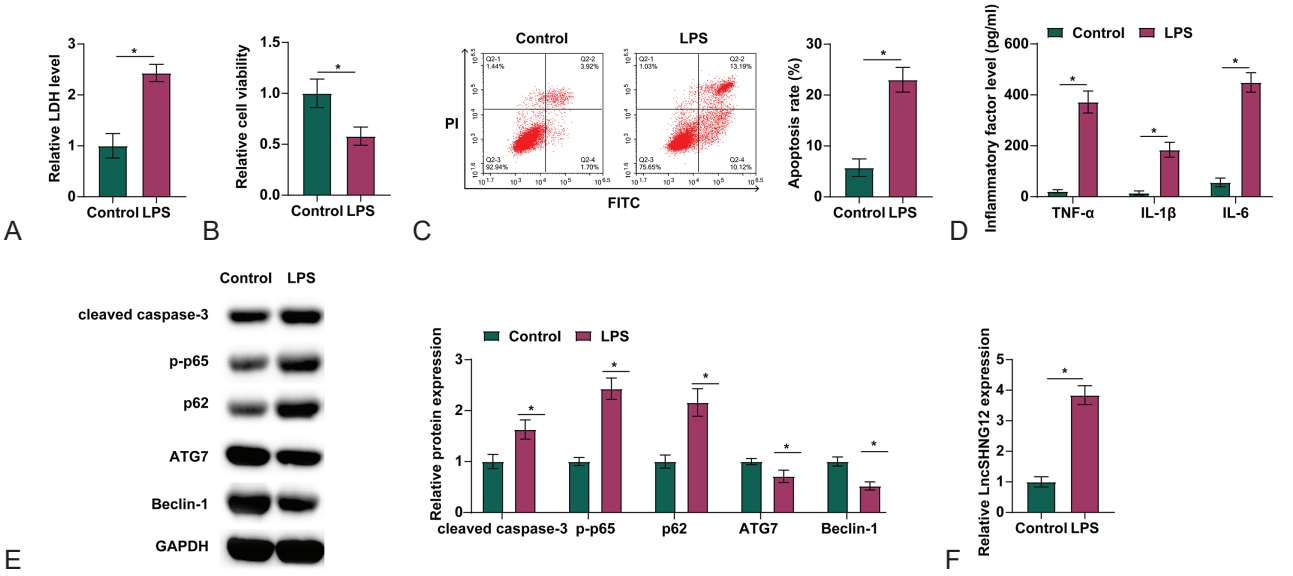

Figure 1. SNHG12 is highly expressed in LPS-treated HK-2 cells [A: LDH release; B: CCK-8 assay to detect cell viability; C: Flow cytometry to detect cell apoptosis rate; D: ELISA to analyze TNF-α, IL-1β, and IL-6 in cell supernatant; E: Western blot of cleaved caspase-3, p-p65, p62, ATG7, and Beclin-1; F: RT-qPCR to detect SNHG12 after LPS treatment (*P < .05)].

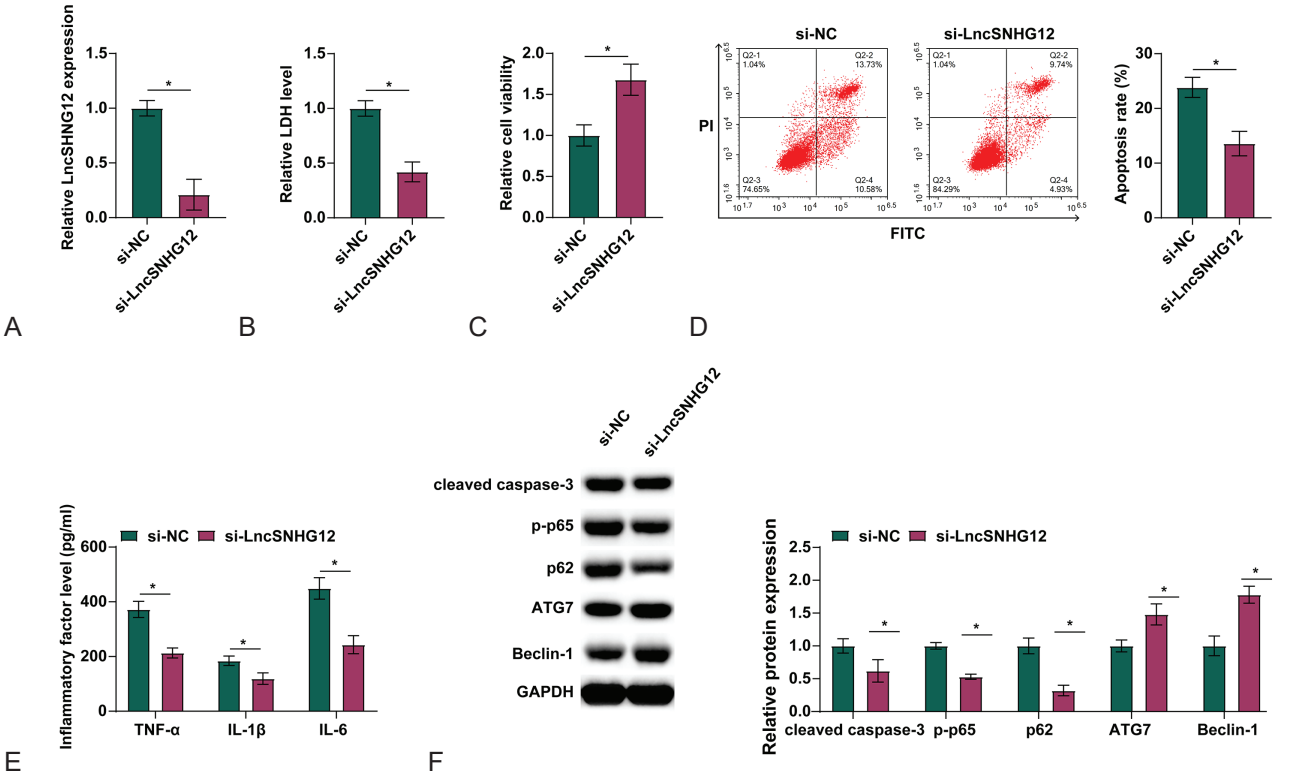

Figure 2. SNHG12 knockdown improves LPS-mediated inflammation, apoptosis, and autophagy [A: RT-qPCR to detect SNHG12; B: LDH release; C: CCK-8 assay to detect cell viability; D: Flow cytometry to detect cell apoptosis rate; E: ELISA to analyze TNF-α, IL-1β, and IL-6 in cell supernatant; F: Western blot to measure cleaved caspase-3, p-p65, p62, ATG7, and Beclin-1 (*P < .05)].

via the bioinformatics website (http://www.noncode.org). SNHG12 was located at the position of chromosome chr1: 28578537-28581872 [-], with a length of 757 bp (Figure 3A). Subsequently, the bioinformatics website, starBase, predicted the potential binding sites of SNHG12 and miR-1270 (Figure 3B). Based on that, luciferase reporter gene assay was conducted and ultimately it was recognized that miR-1270 mimic reduced the luciferase activity of WT-SNHG12 but had no effect on the luciferase activity of MUT-SNHG12 (Figure 3C). In addition, RIP experiments confirmed that SNHG12 and miR-1270 were enriched in Ago2 magnetic beads (Figure 3D). LPS reduced miR-1270 expression in HK-2 cells, but this change was restored after SNHG12 knockdown (Figure 3E). All these results revealed that SNHG12 could bind to miR-1270.

SNHG12 Affects Inflammation, Apoptosis, and Autophagy of LPS-induced HK-2 Cells by Regulating miR-1270

A functional rescue experiment was conducted by using si-SNHG12 and miR-1270 inhibitor in LPS-treated HK-2 cells. si-SNHG12 increased miR-1270 expression; however, this effect was reversed after co-transfection of miR-1270 inhibitor simultaneously (Figure 4A). Similarly, the elevated cell viability and autophagy as well as reduced LDH level, apoptosis and inflammation mediated by SNHG12 knockdown were reversed after miR-1270 silence together (Figure 4B-F). All these results suggested that SNHG12 promoted inflammation, apoptosis, and inhibited autophagy of LPS-induced HK-2 cells by regulating miR-1270.

MiR-1270 Targets TUBB

StarBase, Bioinformatics website, predicted the potential binding sites of miR-1270 and TUBB (Figure 5A). Subsequently, luciferase reporter assay and RIP assay further confirmed the interplay between miR-1270 and TUBB (Figure 5B, C). As analyzed, TUBB protein expression was elevated in HK-2 cells induced by LPS, but this phenomenon was offset upon SNHG12 knockdown or miR-1270 upregulation simultaneously (Figure 5D).

A

NONCODE TRANSCRIPT ID	NONHSAT001967.2
NONCODE Gene ID	NONHSAG000840.3
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Start Site	28578537
End Site	28581872
Strand	-
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Assembly	hg38
Other transcript Versions	NONHSAT001967.1(old version)

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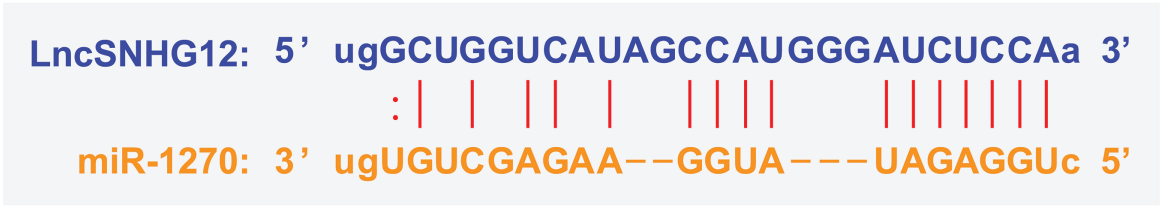

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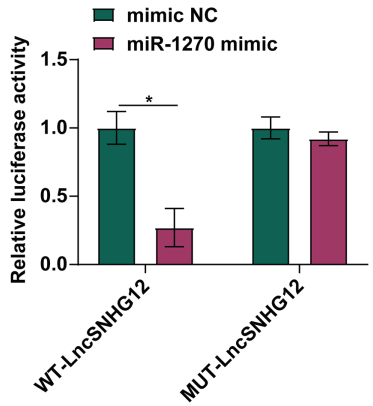

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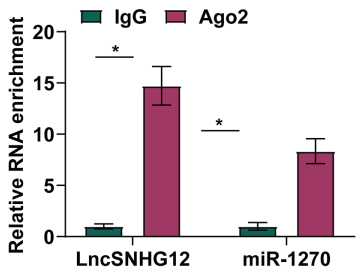

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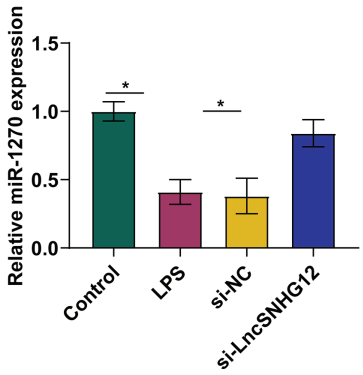

Figure 3. SNHG12 competitive adsorbs miR-1270 [A: Bioinformatics website http://www.noncode.org to query SNHG12 gene information; B: starBase bioinformatics website predicted the binding sites of SNHG12 and miR-1270; C: Dual luciferase reporter assay to detect the targeting relationship between SNHG12 and miR-1270; D: RIP experiment to detect the binding relationship between SNHG12 and miR-1270; E: RT-qPCR to detect miR-1270 (*P < .05)].

All these results indicated that miR-1270 directly targeted TUBB.

SNHG12 Affects Apoptosis, Inflammation, and Autophagy in AKI Via the miR-1270/TUBB Axis

Moreover, pcDNA 3.1-SNHG12 and si-TUBB were co-transfected into LPS-treated HK-2 cells, and it was discovered that pcDNA 3.1-SNHG12 elevated SNHG12 expression and TUBB protein expression as well as inhibiting miR-1270 expression; however, co-transfection of si-TUBB only reversed the effect of SNHG12 overexpression on TUBB expression (Figure 6A, B). Furthermore, overexpressed SNHG12 increased LDH level, apoptosis and inflammatory reaction, and reduced cell viability and autophagy, but these effects were suppressed after co-transfection of si-TUBB (Figure 6C-G). All these results suggest that SNHG12 promotes inflammation, apoptosis, and inhibits autophagy of LPS-induced HK-2 cells by elevating TUBB.

DISCUSSION

Septic AKI involves various pathophysiological processes such as inflammation and apoptosis, but

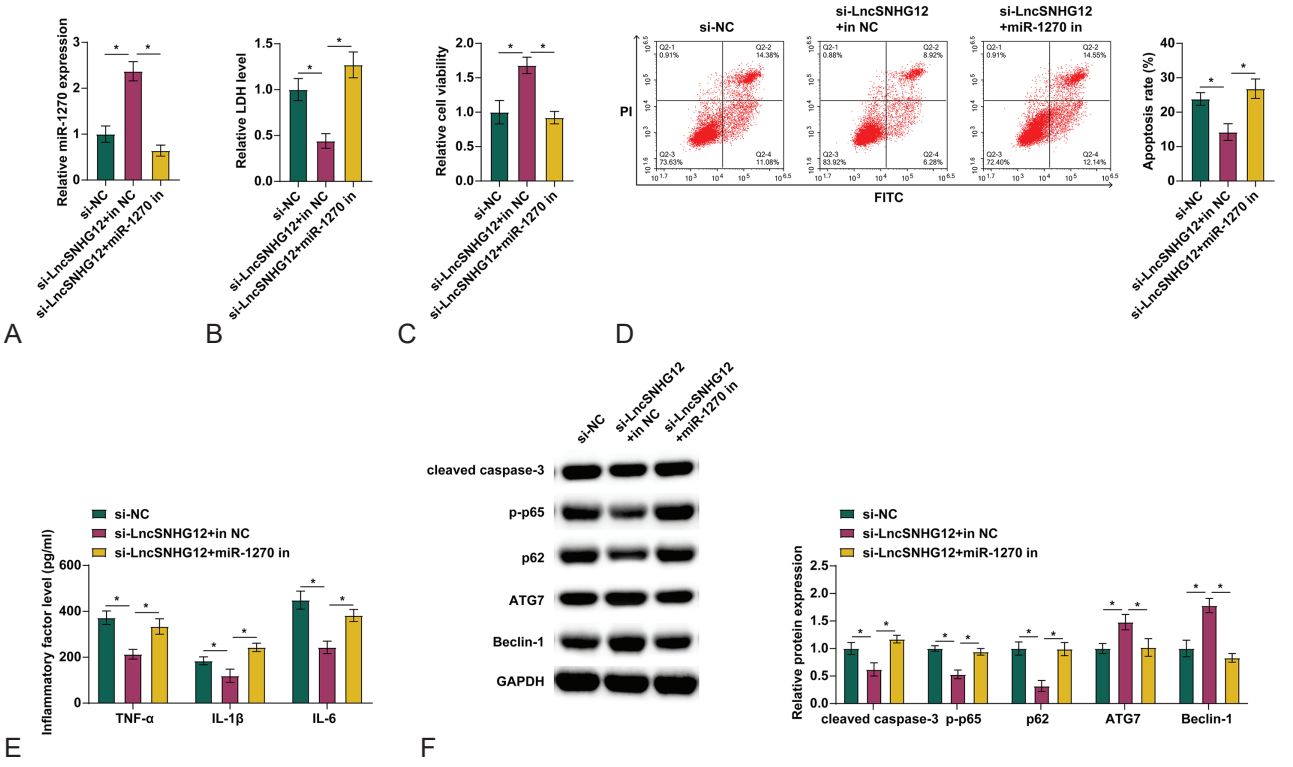

Figure 4. SNHG12 affects LPS-induced inflammation, apoptosis, and autophagy by miR-1270 [A: RT-qPCR to detect miR-1270; B: LDH release; C: CCK-8 assay to detect cell viability; D: Flow cytometry to detect cell apoptosis rate; E: ELISA to analyze TNF-α, IL-1β, and IL-6 in cell supernatant; F: Western blot to measure cleaved caspase-3, p-p65, p62, ATG7, and Beclin-1. (*P < .05)].

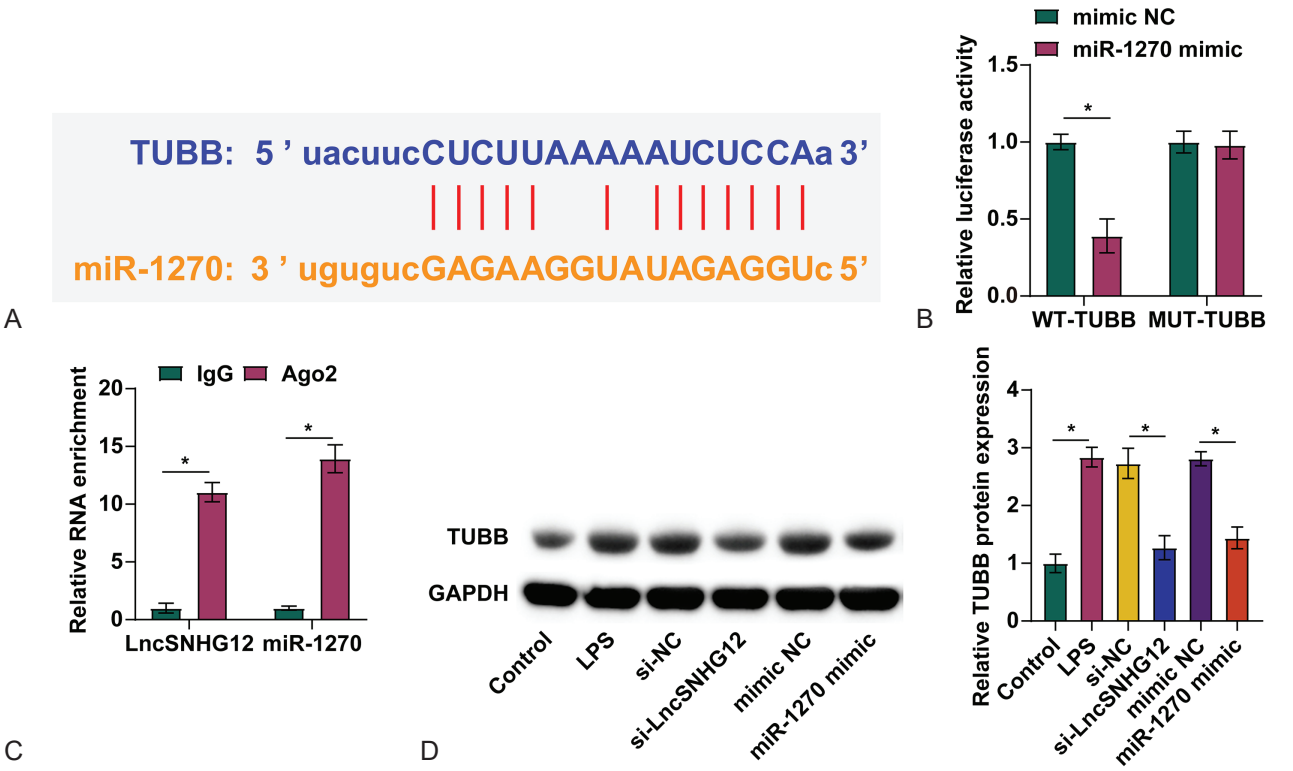

Figure 5. miR-1270 targets TUBB expression [A: starbase bioinformatics website predicted the binding sites of miR-1270 and TUBB; B: Dual luciferase reporting assay to detect the targeting relationship between miR-1270 and TUBB; C: RIP experiment to detect the binding relationship between miR-1270 and TUBB; D: Western blot to measure TUBB (*P < .05)].

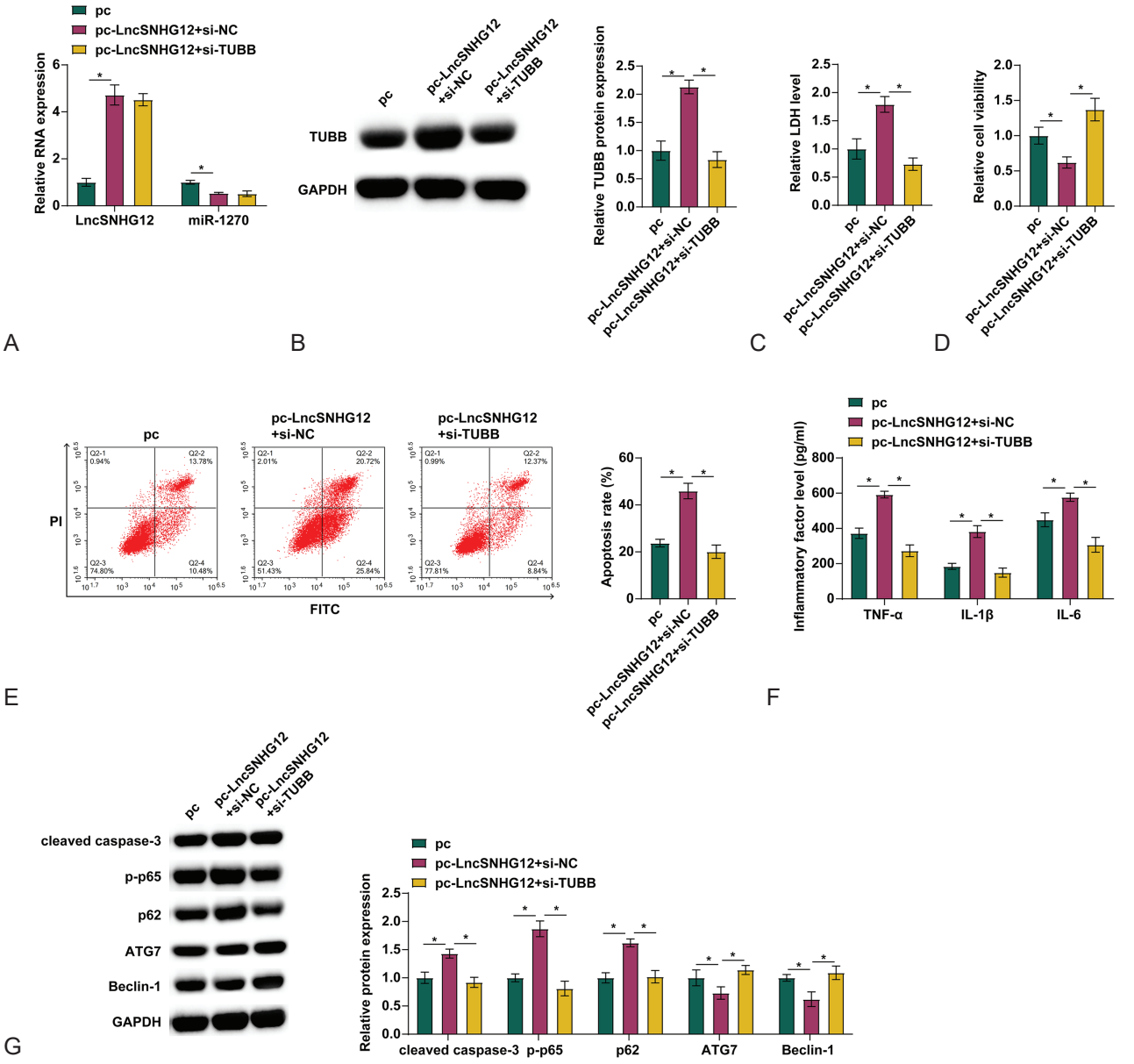

Figure 6. SNHG12 affects apoptosis, inflammation, and autophagy of renal cells through miR-1270/TUBB [A: RT-qPCR to detect SNHG12 and miR-1270; B: Western blot to measure TUBB; C: LDH release; D: CCK-8 assay to detect cell viability; E: Flow cytometry to detect cell apoptosis; F: ELISA to analyze TNF-α, IL-1β, and IL-6; G: Western blot of autophagy-related proteins (*P < .05)].

its pathogenesis has not been fully elucidated.[26-28] The release of inflammatory factors and apoptosis of renal cells are the direct causes of sepsis-induced AKI. In addition, alterations of autophagy have been demonstrated in both acute and chronic kidney diseases.[29,30] Autophagy is a lysosomal degradation pathway that breaks down cytoplasmic components by forming autophagosomes and autolysosomes.[31] During the acute phase of injury in AKI, autophagy is induced in proximal tubular cells, and works as an intrinsic protective mechanism.[32] During recovery of AKI, autophagy requires to be inactivated for tubular cell proliferation and tubular repair due to defects in tubular cell proliferation that persist in autophagy.[33] Tightly-regulated autophagy also helps phagocytosis clearing up dead cells, promoting restrictive antigen presentation, inhibiting inflammation, and maintaining self-tolerance of proximal tubules, all of which would contribute to normal kidney repair.[34] Studies have confirmed that ncRNAs play a key role in processing septic AKI. For example,

lncRNA TapSAKI regulates HK-2 cell apoptosis and inflammatory response in septic AKI.[35,36] In search of new therapeutic modalities, the current work was aimed at demonstrating the role and potential mechanism of SNHG12 in regulating inflammation, apoptosis, and autophagy in AKI.

LncRNAs can regulate protein expression at the post-transcriptional level, change cell signaling pathways in affected organs, and participate in the development of AKI. For example, lncRNA KCNQ1OT1 aggravates septic AKI by activating the p38/NF-κB pathway.[37] LncRNA NEAT1 promotes LPS-induced HK-2 cell injury through the NF-κB signaling pathway.[38] Similarly, the results of the present study showed that SNHG12 expression was abnormally upregulated in LPS-induced HK-2 cells. SNHG12 has been reported to affect inflammation and autophagy processes in atherosclerosis and ischemia/reperfusion.[39-41] In septic AKI, SNHG12 downregulation increased cell viability and autophagy while inhibited inflammation and apoptosis in LPS-treated HK-2 cells. Consistent with our findings, it has been reported that SNHG14 hinders cell proliferation and autophagy while enhances cell apoptosis and inflammatory cytokine production in LPS-stimulated HK-2 cells.[42]

Accumulating evidence has supported that lncRNAs affect the progression of diseases by regulating downstream mRNA through interaction with miRNAs.[43] SNHG12 has been reported to interact with multiple miRNAs to exert functions in disease. SNHG12 ameliorates brain microvascular endothelial cell injury by targeting miR-199a.[40] SNHG12 promotes proliferation and epithelial mesenchymal transition in hepatocellular carcinoma through targeting HEG1 via miR-516a-5p.[44] Likewise, our study also found that SNHG12 interacted with miR-1270 in septic AKI. For all we know, miRNAs are considered crucial participators in septic AKI. For example, increased miR-128 prevents LPS-induced glomerular podocyte injury.[45] In septic AKI, miR-107 affects TNF-α secretion and apoptosis in renal cells.[20] In addition, miR-1270 has been documented to act as a tumor suppressor in cancers[46], and also inhibits the inflammatory response in atherosclerosis.[23] Herein, our study performed rescue assays to validate that miR-1270 silencing reversed the effect of SNHG12 knockdown on inhibiting inflammation, apoptosis, and promoting autophagy in LPS-induced HK-2 cells, which suggested that miR-1270 may also play a protective role in septic AKI, which is consistent with the findings of previous studies.[47]

Another important finding of our study was that SNHG12 functioned as a ceRNA that competed with miR-1270 to regulate the expression of TUBB. TUBB encodes β tubulin protein that belongs to the β-tubulin family. TUBB forms a dimer with α-tubulin and serves as a structural component of microtubules, which are necessary for cell division and intracellular signaling and transport.[48] TUBB has been shown to have a variety of pathological effects, among which elevated TUBB level has been observed in breast cancer tumors.[49] Besides, high expression of TUBB is correlated with the worse survival of lung adenocarcinoma.[50] More importantly, TUBB has been reported to be upregulated in clear cell renal cell carcinoma patients.[25] Our study further supported that SNHG12 promoted AKI progression via elevating TUBB.

Our study was not conducted in vivo, which is a limitation of this study. At the same time, relevant studies in patients with AKI samples are needed in the years to come.

CONCLUSION

SNHG12 is upregulated in LPS-induced HK-2 cells and enhances apoptosis and inflammation while inhibiting autophagy in renal epithelial cells by targeting the miR-1270/TUBB axis. Our study might offer a promising therapeutic target for the intervention of AKI.

ACKNOWLEDGMENTS

Not applicable.

FUNDING

Not applicable.

CMPETING INTERESTS

The authors have no conflicts of interest to declare.

ETHICAL STATEMENT

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

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

AUTHORS' CONTRIBUTIONS

Conceptualization, J.Q. Zhang and L. Li; methodology, J.Q. Zhang, L. Li and J.J. Li; formal analysis, Y.H. Yu and J.J. Li; investigation, Y.H. Yu, Y. Fang and J. Li; data curation, Y. Fang and J. Li; writing—original draft preparation, J.Q. Zhang and L. Li; writing—review and editing, J.J. Li; project administration, J.J. Li. All authors have read and agreed to the published version of the manuscript.

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Correspondence to:
Jinji Li
Department of Nephrology, The Affiliated Hospital of Yanbian University, No. 1327 Bozi Street, Yanji City, Jilin Province, 133000, China
E-mail: lijinji938@outlook.com

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