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The Overexpression of Sirtuin1 (SIRT1) Alleviated Lipopolysaccharide (LPS)-Induced Acute Kidney Injury (AKI) via Inhibiting the Activation of Nucleotide-Binding Oligomerization Domain-Like Receptors (NLR) Family Pyrin Domain Containing 3 (NLRP3) Inflammasome

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Sepsis-induced acute kidney injury (AKI) is threatening the patients with sepsis, and nucleotide-binding oligomerization domain-like receptors (NLR) family pyrin domain containing 3 (NLRP3) inflammasome is considered to play a critical role in this complication of sepsis and might be regulated by sirtuin1 (SIRT1). Thus, we explored the roles of NLRP3 and SIRT1 in the lipopolysaccharide (LPS)-induced AKI in the HK-2 cell line.

Material/Methods: Cell viability was assessed by Cell Counting Kit-8 (CCK-8). Apoptosis rate was measured by flow cytometry. Protein levels of interleukin (IL)-1β and IL-18 were tested by enzyme-linked immunosorbent assay (ELISA) and NLRP3, cleaved caspase-1, caspase-1 were tested by western blot. The mRNA levels of IL-1β, IL-18, and SIRT1 were quantified by qPCR.

Results: LPS could decrease cell viability and the expression of SIRT1 and elevate the expressions of IL-1β, IL-18, NLRP3, and cleaved caspase-1. However, the overexpression of SIRT1 could upregulate cell viability and expression of caspase-1 and downregulate apoptosis rate, expressions of NLRP3, IL-1β, IL-18, and cleaved caspase-1.

Conclusions: NLRP3 inflammasome could act as a critical regulator promoting the process of AKI induced by LPS, and the overexpression of SIRT1 might be able to suppress the activation of NLRP3 and therefore resist the kidney injury, showing promise to be used as a target in the treatment of sepsis-induced AKI.

MeSH Keywords: Acute Kidney Injury • Gene Expression Regulation, Archaeal • Inflammasomes • Lipopolysaccharides • Sirtuin 1

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Background

Sepsis is a complex clinical syndrome, the nature of which is a kind of uncontrolled systemic inflammatory response in the body, and can lead to excessive release of various inflammatory mediators, causing extensive damage of cells and tissues [1]. The kidney is the most vulnerable organ in the development of sepsis, and acute kidney injury (AKI) is one of the most common complications [2]. The incidence of renal damage is very high in cases of sepsis complications, and 50% of patients with sepsis have varying degrees of renal injury, which is closely related to the 2-year mortality rate for sepsis [3,4]. Moreover, there is evidence that the mortality rate of patients with AKI, especially those requiring dialysis treatments, can reach more than 70% [5]. Thus, the treatment for AKI induced by sepsis is urgently in need of being further studied.

Inflammasome is a large protein complex that promotes the production of inflammatory factors during infection and tissue damage [6]. The most studied inflammasome is nucleotide-binding oligomerization domain-like receptors (NLR) family pyrin domain containing 3 (NLRP3), which can recognize various pathogenic molecular patterns (PAMPs) and endogenous danger signals, and therefore regulate the processing and activation of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-18, and IL-33 by activating caspase-1 [7]. In the absence of external stimulation, the inflammasome is present in the cell in an inactive form. NLRP3 inflammasome is activated when cells are subjected to specific stimuli, such as hypoxia, bacterial toxins, uric acid crystals, or complement-mediated damage [6]. When NLRP3 is activated, the activated NLRP3 inflammasome activates caspase-1. Caspase-1 is hydrolyzed to cleaved caspase-1, which in turn activates IL-1 and IL-18, leading to pro-inflammatory responses [8,9]. Many studies have confirmed that NLRP3 inflammasome is a newly recognized as a mechanism that triggers various inflammatory reactions and plays an important role in the occurrence and development of various viruses, bacteria and fungi, as well as non-infectious inflammation such as gout, arthritis, and atherosclerosis [10]. Cao et al. pointed out that the activation of the NLRP3 inflammasome might contribute to the development of inflammatory responses of AKI, emphasizing its importance in the study of renal diseases [11]. Therefore, further investigation about NLRP3 is needed.

Sirtuin1 (SIRT1), one of the members of the Sirtuin family, is a nicotinamide adenine dinucleotide (NAD+) -dependent class III histone deacetylase [12]. In mammals,SIRT1 is mainly located in the nucleus and plays its role through ethylating [13]. Evidence has shown that SIRT1 has protective effects in a variety of renal diseases, such as AKI, and its main mechanism involves reducing oxygen free radicals, alleviating apoptosis, increasing autophagy, and stabilizing mitochondrial function [14]. Khajevand-Khazaei et al. reported that rutin could alleviate acute endotoxemic kidney injury in C57BL/6 mice via suppression of inflammation and upregulation of SIRT1 [15]. Gan et al. also reported that resveratrol could increase the survival rate of septic rats by inhibiting inflammatory factors to ease AKI and promotes nuclear factor kappa-light-chain-enhancer of activated B cells P65 (NF-κB-P65) deacetylation by upregulating SIRT1 [16]. Given these findings, we assumed that SIRT1 might play a critical role in the regulation of renal diseases.

Nowadays, lipopolysaccharide (LPS) is a widely used biological inducer to create the model of AKI [17]. Li et al. reported that LPS could remarkably damage HK-2 cells by reducing cell viability [18]. In view of the aforementioned facts, we hypothesized that the overexpression of SIRT1 might be able to suppress the activation of NLRP3 and therefore increase the survival of HK-2 cells injured by LPS-induced AKI. Thus, we explored whether LPS could lower the cell viability and the expression of SIRT1 and activate NLRP3 at gradient concentration, and whether the overexpression of SIRT1 could upregulate cell viability and downregulate apoptosis rate and activation of NLRP3 in the HK-2 cell line. Our study could thus provide a new understanding about the role of SIRT1 and NLRP3 in the LPS-induced AKI.

Material and Methods

Plasmids, cell culture, transfection, and grouping

The HK-2 cell line is obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and was cultured in RPMI-1640 Medium (Thermo Fisher, Waltham, USA). Each medium was supplemented with 10% fetal bovine serum (HyClone, USA) at 37°C with 5% CO_2. The cells were subcultured every 2 to 3 days. The overexpression plasmid of SIRT1 (pcDNA 3.1) and negative control (NC) plasmid were synthesized by Shanghai Genepharma Company (Genepharma, Shanghai, China). The LPS was purchased from Sigma (USA). For the exploration of the effect of LPS at gradient concentration, HK-2 cells were randomly divided into 5 groups with 5 different concentrations of LPS: 0 μg/mL, 1 μg/mL, 10 μg/mL, 20 μg/mL, and 50 μg/mL. For the investigation of the consequences of the overexpression of SIRT1, HK-2 cells were randomly divided into 5 groups after cell density reached over 90%: control group (blank control, cells without treatment), LPS group (cells with 10 μg/mL LPS), NC+ LPS group (cells with negative plasmid and 10 μg/mL LPS), SIRT1 + LPS group (cells with overexpressed SIRT1 plasmid and 10 μg/mL LPS), and SIRT1 group (cells with overexpressed SIRT1 plasmid). For the confirmation of transfection of SIRT1 into the HK-2 cell line and the measurement of the alteration of cell viability and apoptosis rate thereafter, cells were divided into 3 groups: control group (blank control, cells without

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treatment), NC group (cells with negative plasmid), and SIRT1 group (cells with overexpressed SIRT1 plasmid). Lipofectamine™ 2000 Transfection Reagent was used for transfection (Invitrogen, Waltham, USA). The cells were harvested 48 hours after transfection for subsequent studies.

Cell viability assay

Cell vitality was tested by Cell Counting Kit-8 (CCK-8) (Sigma-Aldrich, Shanghai, China). After 0 hours, 24 hours, and 48 hours of culturing, the cell viability was measured separately. Procedures were followed according to the manufacturer's instruction, and then the cells were incubated at 37°C in 96-well plate for 1 hour, and the optical density (OD) value for each well was measured at 450 nm wavelength on a microplate reader (Multiskan, Thermo, USA).

Measurement of apoptosis rate by flow cytometry

Annexin V-FITC Apoptosis Staining and Detection Kit (Abcam, Shanghai, China) was used to measure the apoptosis rate. We diluted 10× Annexin V Binding Buffer with dH_2O to 1× and kept it on ice. Cells of interest were collected by centrifugation at 800 rpm for 5 minutes and washed with ice-cold culture medium. Cells were resuspended at 10^5 to 10^6 cells/mL with 1× Annexin V Binding Buffer and gathered in an assay tube. We added 1 μL Annexin V-FITC Conjugate and 12.5 μL propidium iodide (PI) solution to the cell suspension. Cells were incubated for 10 minutes on ice in the dark. Cell suspension was diluted to a final volume of 250 μL per each assay with ice-cold 1× Annexin V Binding Buffer and then analyzed immediately by BD FACScanto II (Becton Dickinson, San Jose, CA, USA) flow cytometry.

Enzyme-linked immunosorbent assay (ELISA) for IL-1β, IL-18

Briefly, IL-1β was measured by IL-1 beta human ELISA Kit (Abcam, Shanghai, China), and IL-18 was measured by human IL-18 SimpleStep ELISA® Kit (Abcam, Shanghai, China). Briefly, 50 μL standard or cells were added to a 96-well plate. Then 50 μL of Antibody Cocktail was added to all the wells. Cells were then incubated at room temperature for 1 hour. Each well was aspirated and washed 3 times with 350 μL of 1× Wash Buffer PT. We added 100 μL TMB substrate to the wells and cells were incubated for 10 minutes. Then, 100 μL Stop Solution was added and the OD was read at 450 nm wavelength of UV.

Extraction and assessment of total proteins

Total proteins of the cells were extracted by RIPA buffer (Thermo Fisher, Waltham, USA). Samples were prepared in tubes. RIPA buffer was then added to cells in each tube, and kept on ice for 5 minutes. Supernatant was gathered after the tubes were centrifuged at 4°C, 14 000 g for 15 minutes. Five wells in the first vertical line of the plates were set as the standard according to the Pierce™ BCA Protein Assay Kit (Thermo Fisher, Waltham, USA) instructions, and standard protein liquid was diluted to 1, 0.5, 0.25, 0.125, 0.0625 g/mL respectively, and added to the 5 wells. Then 2 μL samples were added to each well. BCA reagent was then prepared following the manufacturer's instruction. To each well, 200 μL BCA reagent was added, and then the plate was placed in a 37°C incubator for 30 minutes. The OD value of the whole plate at 562 nm wavelength was then read on the microplate reader (Multiskan, Thermo, Waltham, USA). Standard curve (Y=A×X+B; Y stands for OD; X stands for protein concentration; A and B stand for parameters) was drawn in Microsoft EXCEL program. The concentration of the total proteins was calculated according to the standard curve.

Western blot

Total proteins were prepared and mixed with loading buffer (Bio-Rad, Shanghai, China) and then loaded onto SDS-PAGE. The SDS-PAGE was put into electrophoresis apparatus with 100 V for 2 hours and then transferred to PVDF membrane. The membrane was blocked in 5% non-fat milk with PBST (Solarbio Life Sciences, Beijing, China) at room temperature for 2 hours. Anti-NLRP3 antibody (ab214185, Abcam, San Francisco, USA, 1: 1000), anti-caspase-1 antibody (ab62698, Abcam, San Francisco, USA, 1: 1000), cleaved caspase-1 (Asp297) (D57A2) (#4199, CST, MA, USA), anti-SIRT1 antibody (ab32441, Abcam, San Francisco, USA, 1: 1000), and anti-GAPDH antibody (ab181602, Abcam, San Francisco, USA, 1: 1000) were used separately to detect the target proteins at 4°C with shaking overnight. The membranes were then washed with PBST 3 times with shaking. The membrane was then probed with the secondary antibody IgG H&L (HRP) (ab6721, Abcam, San Francisco, USA, 1: 2000) for 1 hour at room temperature. Pierce™ ECL plus western blotting substrate (Thermo Fisher, Waltham, USA) was used to detect the proteins and images were taken.

Quantification of mRNA by qPCR

Total RNA was extracted by TRIzol reagent (Thermo Fisher, Waltham, USA) and the purity of extracted RNA was 1.8–2.2 (OD 260/OD 280). ABI High Capacity cDNA Reverse Transcription Kit (Thermo Fisher, Waltham, USA) was then used to obtain cDNA. Reaction system was designed based on templates and primers (Table 1) and prepared on ice. PCR progress was processed in qPCR machine (#6093, CFX96 Touch™, Bio-Rad, CA, USA). The reaction system was prepared as follows: 2× SYBER Green Master Mix (Roche, Shanghai, China) 12.5 μL, cDNA template 4 μL, upstream primer (10 μM) 0.5 μL, downstream primer (10 μM) 0.5μL, DPEC 7.5 μL. PCR procedure was set as follows: 95°C for 10 minutes, 95°C for 15 seconds, 60C for

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Table 1. Reaction system was designed based on templates and primers.

Genes	Primers
SIRT1	(F) 5'-CAGTGTCATGGTTCCTTTGC-3' (R) 5'-CACCGAGGAACTACCTGAT-3'
IL-1β	(F) 5'-CCACAGACCTTCCAGGAGAATG-3' (R) 5'-GTGCAGTTCAGTGATCGTACAGG-3'
IL-18	(F) 5'-GTGAACCCCAGACCAGACTG-3' (R) 5'-CCTGGAACACGTTTCTGAAAGA-3'
GAPDH	(F) 5'-ATGGTGAAGGTCGGTGTGAA-3' (R) 5'-TGGAAGATGGTGATGGGCTT-3'

1 minute, 72°C for 3 minute, 40 cycles. Data was analyzed by ABI SDS software (version 2.3).

Statistical analysis

Data was analyzed by GraphPad Prism 7.0 software. All data was shown as the mean ±SD. One-way analysis of variance was used to compare multiple groups. $P<0.05$ was considered to indicate statistical significance.

Results

LPS could decrease cell viability and elevate the expression of IL-1β and IL-18

In order to find out whether cell proliferation was influenced and NLRP3 might be activated, we measured the cell viability by CCK-8 and the expression of IL-1β and IL-18 by ELISA kit and qPCR. The results of our study showed that the cell viability was decreased as LPS concentration increased (Figure 1A, ** $P<0.01$), and the same was true for the expression of IL-1β and IL-18 mRNA and protein levels (Figure 1B–1D, ** $P<0.01$, * $P<0.05$). The results indicated that LPS could decrease cell proliferation and might activate NLRP3 in the HK-2 cell line.

LPS could decrease the expression of SIRT1 and raise NLRP3 and cleaved caspase-1 protein levels

To investigate whether there was alteration on the expression of SIRT1, and activation of NLRP3, we measured the protein levels of SIRT1, NLRP3, cleaved caspase-1, and caspase-1 by western blot and mRNA level of SIRT1 by qPCR. As shown in the study, the relative mRNA and protein levels of SIRT1 were

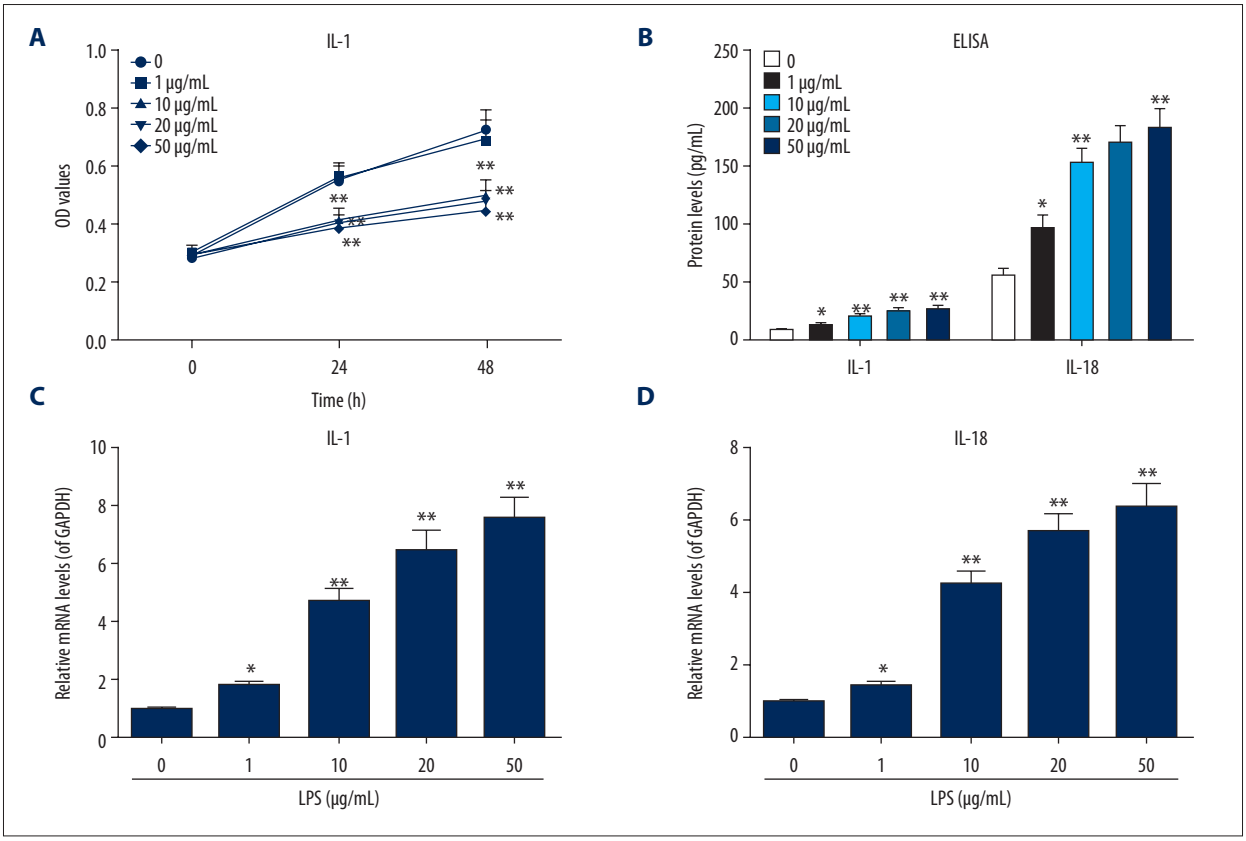

Figure 1. The cell viability and expression of IL-1β and IL-18 at each gradient concentration of LPS. (**A**) The cell viability over time. (**B**) The protein levels of IL-1β and IL-18. (**C**) Relative mRNA levels of IL-1β. (**D**) Relative mRNA levels of IL-18. Bars indicated means ±SD. ** $P<0.01$ and * $P<0.05$ versus control group. IL – interleukin.

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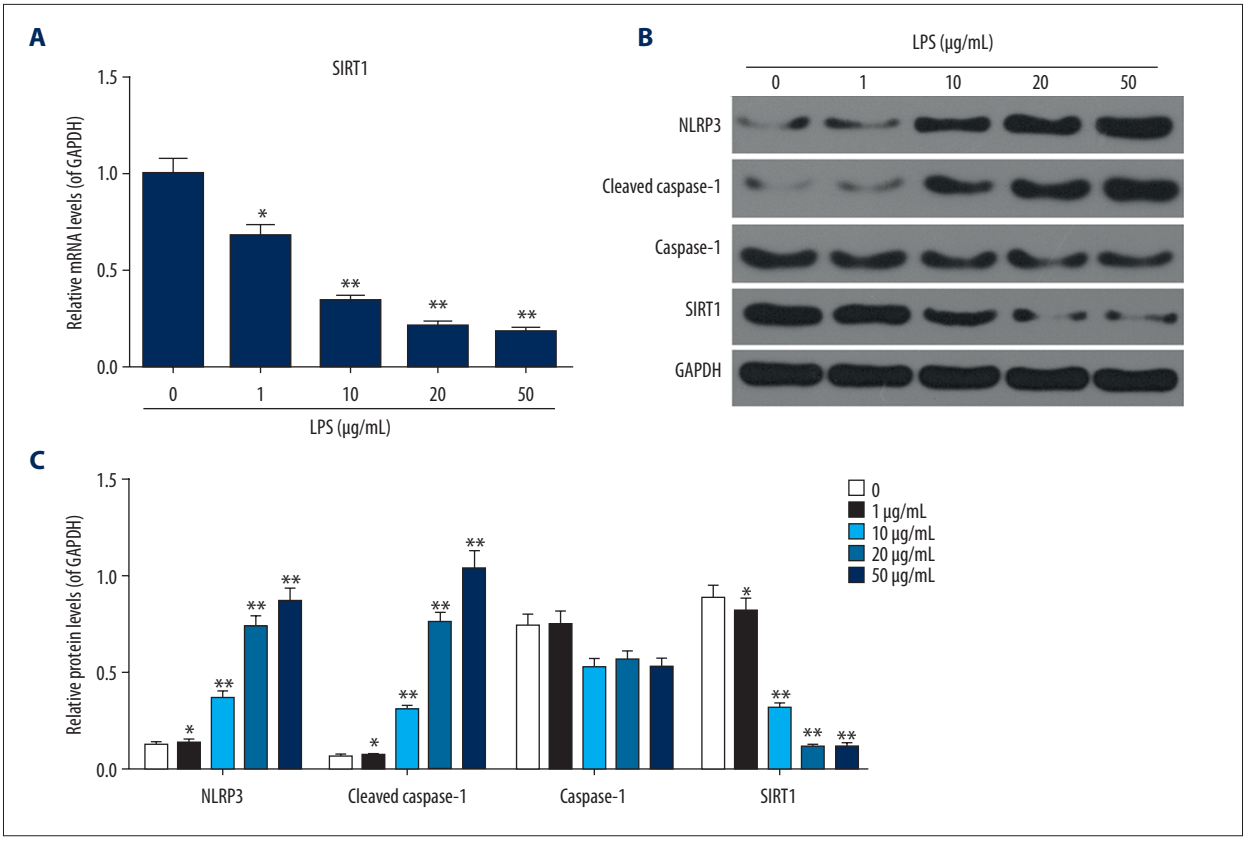

Figure 2. The expression of SIRT1, NLRP3, cleaved caspase-1 and caspase-1 at each gradient concentration of LPS. (**A**) Relative mRNA levels of SIRT1. (**B**) The original results of western blot of SIRT1, NLRP3, cleaved caspase-1 and caspase-1 proteins. (**C**) Relative protein levels of SIRT1, NLRP3, cleaved caspase-1 and caspase-1. Bars indicated means ±SD. ** *P*<0.01 and * *P*<0.05 versus control group. SIRT1 – sirtuin1; NLRP3 – nucleotide-binding oligomerization domain-like receptors (NLR) family pyrin domain containing 3; LPS – lipopolysaccharide.

decreased with the gradient increased concentration of LPS (Figure 2A–2C, ** *P*<0.01, * *P*<0.05). Relative protein levels of NLRP3 and cleaved caspase-1 were elevated as the concentration of LPS increased. However, there was no significance among the groups on the relative protein levels of caspase-1 with increased concentration of LPS (Figure 2A–2C, ** *P*<0.01, * *P*<0.05). Taken together, these results indicated that the expression of SIRT1 could be suppressed with the activation of NLRP3 due to the increased concentration of LPS.

The overexpression of SIRT1 could raise cell viability and lower apoptosis rate

To understand the role of SIRT1 in the model of AKI induced by LPS, we observed the cell viability and apoptosis rate by CCK-8 and flow cytometry separately. The results from our study showed that the relative mRNA and protein levels of SIRT1 were increased after the transfection (Figure 3A–3C, ** *P*<0.01, ^^ *P*<0.01). Notably, the cell viability in SIRT1 + LPS group was elevated when compared to the LPS or the NC + LPS group and there was no significance between the control group and the SIRT1 + LPS group (Figure 3D, ** *P*<0.01, ## *P*<0.01). The apoptosis rate was higher in the LPS group or NC + LPS group than the control group, and, moreover, the apoptosis rate in the SIRT1 + LPS group was less than the LPS group (Figure 3E, 3F, ** *P*<0.01, ## *P*<0.01, respectively). In addition, the cell viability and apoptosis rate in the NC + LPS group demonstrated no difference from the LPS group, and there was no difference between the control group and the SIRT1 group, indicating that the plasmid and SIRT1 overexpression could not affect the cell viability and apoptosis rate by themselves. Given these findings, we assume that the overexpression of SIRT1 could raise cell proliferation and lower the apoptosis rate induced by LPS.

The overexpression of SIRT1 could downregulate NLRP3 and cleaved caspase-1, and upregulate caspase-1 protein levels

To figure out the status of the activation of NLRP3 due to the overexpression of SIRT1, we measured the protein levels of SIRT1, NLRP3, cleaved caspase-1, and caspase-1 by western blot, and mRNA level of SIRT1 by qPCR. Our results revealed that

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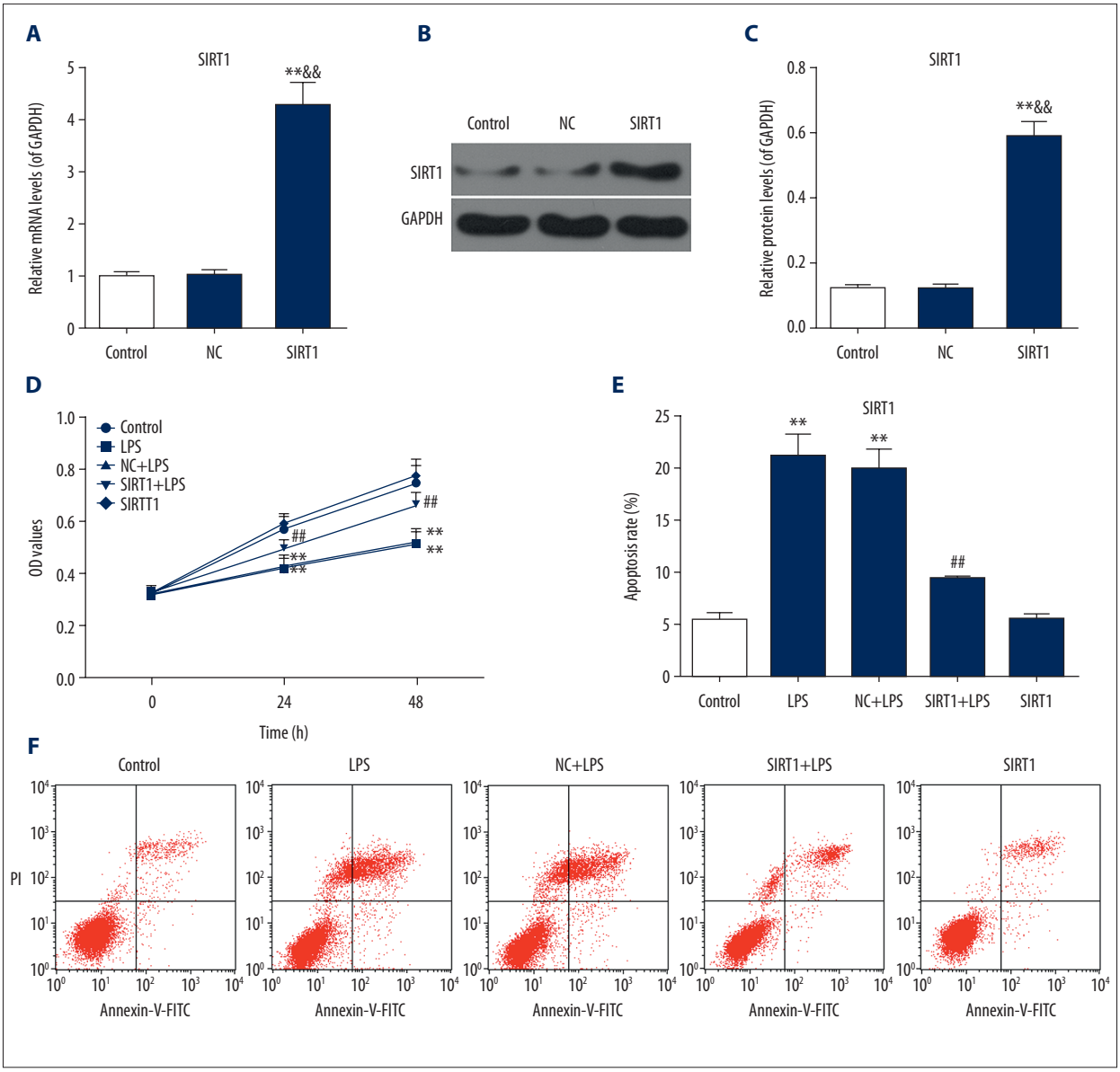

Figure 3. The expression of SIRT1 in control, NC, and SIRT1 groups, and the cell viability and apoptosis rate in control, LPS, NC + LPS, SIRT1 + LPS, and SIRT1 groups. (**A**) Relative mRNA levels of SIRT1. (**B**) The original results of western blot of SIRT1 protein. (**C**) Relative protein levels of SIRT1. (**D**) The cell viability over time. (**E**) The apoptosis rates. (**F**) The original results of apoptosis rate by flow cytometry. Bars indicated means ±SD. ** $P<0.01$ and * $P<0.05$ versus control group; ^^ $P<0.01$ versus NC group; ## $P<0.01$ versus LPS group. SIRT1 – sirtuin1; LPS – lipopolysaccharide.

the expression of SIRT1 was elevated in the SIRT1 group when compared to the control group, and the expression of SIRT1 was higher in the SIRT1 + LPS group than the LPS group or the NC + LPS group which had the lowest level of the expression of SIRT1 (Figure 4A–4C ** $P<0.01$, ## $P<0.01$).The level of NLRP3 and cleaved caspase-1 protein in the SIRT1 + LPS group were less than in the LPS or the NC + LPS group, and without doubt, the level of caspase-1 in the SIRT1 + LPS group was higher than in the LPS group or the NC + LPS group (Figure 4B, 4C, ** $P<0.01$, ## $P<0.01$, * $P<0.05$, # $P<0.05$). There was no difference between the LPS group and the NC+LPS group on each of molecules tested, meaning that the plasmid itself could not affect the results. These results indicated that the overexpression of SIRT1 could suppress the activation of NLRP3 to some extent.

The overexpression of SIRT1 could decrease the expression of IL-1β and IL-18

To further investigate the status of the activation of NLRP3, we measure the levels of IL-1β and IL-18 by ELISA kit.

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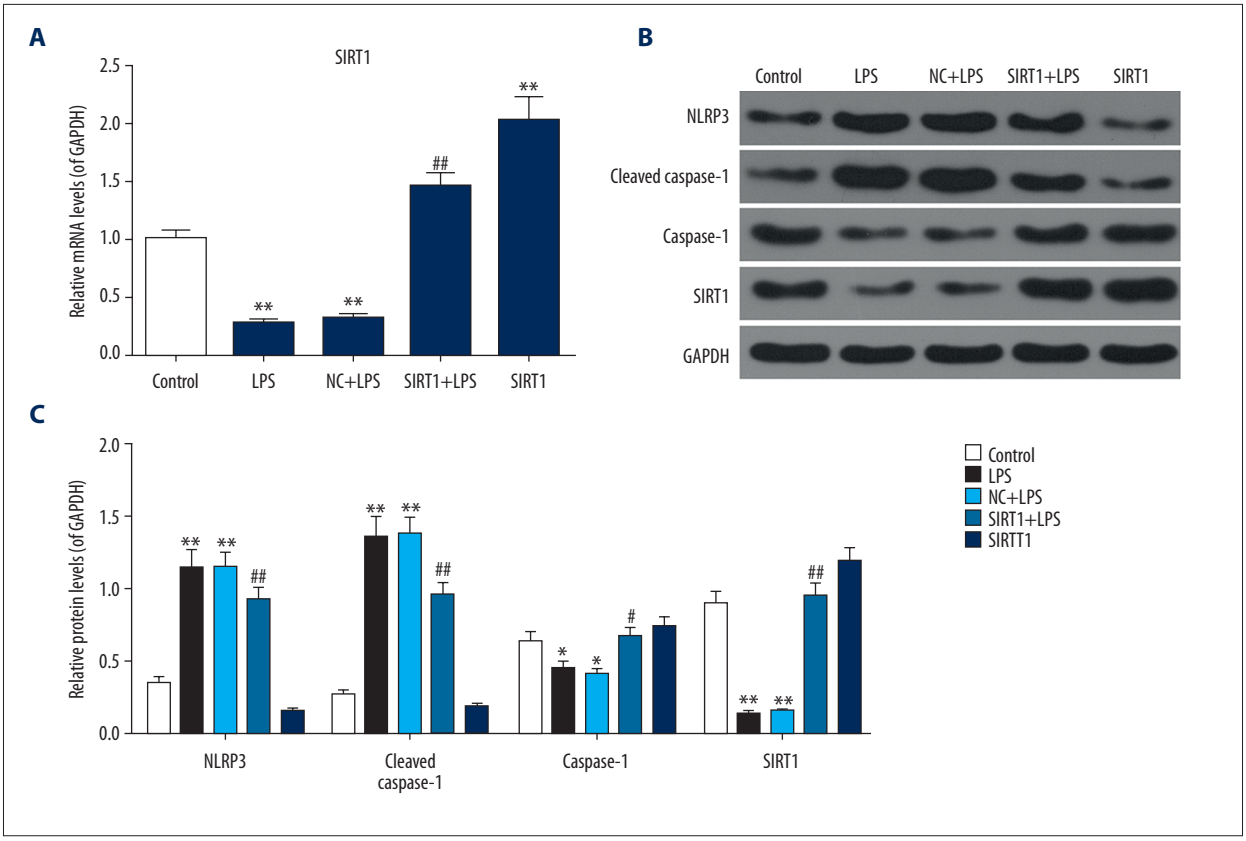

Figure 4. The expression of SIRT1, NLRP3, cleaved caspase-1 and caspase-1 in control, LPS, NC + LPS, SIRT1 + LPS, and SIRT1 groups. (**A**) Relative mRNA levels of SIRT1. (**B**) The original results of western blot of SIRT1, NLRP3, cleaved caspase-1 and caspase-1 proteins. (**C**) Relative protein levels of SIRT1, NLRP3, cleaved caspase-1 and caspase-1. Bars indicated means ±SD. ** $P<0.01$ and * $P<0.05$ versus control group; ## $P<0.01$ and # $P<0.05$ versus LPS group. SIRT1 – sirtuin1; NLRP3 – nucleotide-binding oligomerization domain-like receptors (NLR) family pyrin domain containing 3; LPS – lipopolysaccharide.

The results showed that the expression of IL-1β and IL-18 in the LPS group and the NC + LPS group were elevated when compared to the control group (Figure 5A–5C, ** $P<0.01$, ## $P<0.01$). Significantly, the expressions of IL-1β and IL-18 in the SIRT1 + LPS group were lower than in the LPS group or the NC + LPS group, and showed no difference from the control group (Figure 5A–5C, ** $P<0.01$, ## $P<0.01$). There was no difference between the LPS group and the NC+LPS group on IL-1β and IL-18 levels, suggesting that the plasmid itself could not affect the results. We assumed that the activation of NLRP3 might be depressed due to the overexpression of SIRT1.

Discussion

In this study, we showed that LPS could lower cell viability and the expression of SIRT1 and elevate the expression of IL-1β, IL-18, NLRP3, and cleaved caspase-1 at gradient concentration, and found that the overexpression of SIRT1 could upregulate cell viability and caspase-1 and downregulate apoptosis rate, NLRP3, IL-1β, IL-18, and cleaved caspase-1 in the HK-2 cell line. Our results provide a potential path to reduce the damage of human renal proximal tubular cells.

IL-1β, which has a wide range of biological effects, can play a role in various progressions of condition, such as fever or other inflammatory responses [19]. IL-1β can bond with IL-1β receptor I to activate the NF-κB, MAPK pathway through a series of kinase phosphorylation steps, so as to promote the expression of genes related with inflammation [20]. IL-1β and IL-18 can also increase the expression of other cytokines such as IL-6 and TNF-α, upregulate the expression of adhesion molecules, and promote the exudation of inflammatory cells [21]. IL-18 is a multipotent cytokine produced mainly by activated mononuclear macrophages that mediates ischemic AKI and can be excreted from urine [22]. The caspase family is a protease system and operates as the ultimate pathway of apoptosis, standing in the center of the apoptotic mechanism of cells [23,24]. Our results showed that the expression of IL-1β, IL-18, cleaved caspase-1, caspase-1, and NLRP3 were elevated due to the treatment of LPS, and it seemed that they were related to the concentration of LPS positively. Besides, the results revealed that

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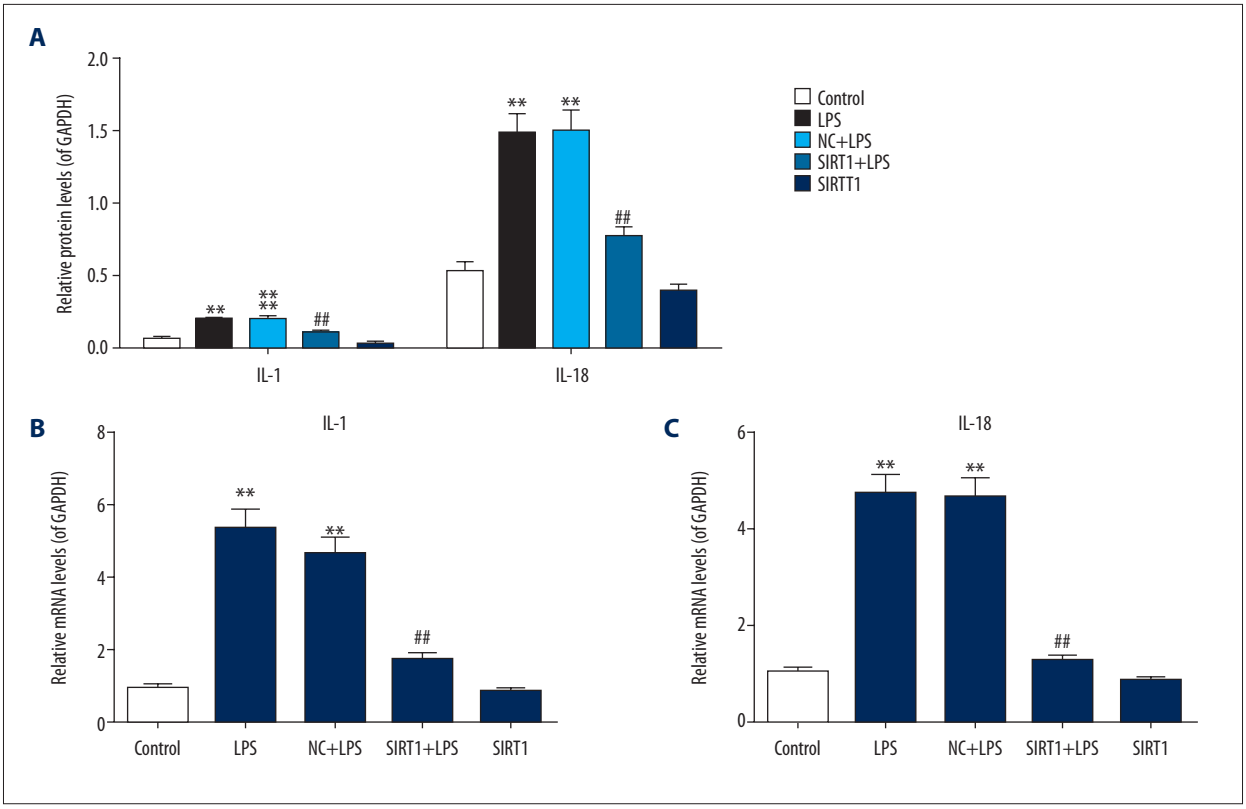

Figure 5. The expression of IL-1β and IL-18 in control, LPS, NC + LPS, SIRT1 + LPS, and SIRT1 groups. (**A**) The protein levels of IL-1β and IL-18. (**B**) Relative mRNA levels of IL-1β. (**C**) Relative mRNA levels of IL-18. Bars indicated means ±SD. ** $P<0.01$ versus control group; ## $P<0.01$ versus LPS group. IL – interleukin; SIRT1 – sirtuin1; LPS – lipopolysaccharide.

the effective concentration of LPS on the HK-2 cell line would be above 10 μg/mL. It is of note that Chunzhi et al. found that NLRP3 was increased in the LPS-induced AKI animal model of BABL/c mice [25]. Furthermore, Shen et al. reported that NLRP3 was increased in the model of contrast-induced AKI in the HK-2 cell line, and proved that silencing the expression of NLRP3 could attenuate contrast-induced apoptosis [26]. It is widely accepted that the activation of NLRP3 requires 2 signals, on one hand, the activation of NF-κB through toll-like receptors, tumor necrosis factor receptors, or IL-1β receptor signals promote the expression of inflammatory cytokine precursors such as pro-IL-1β and pro-IL-18 [27,28]. On the other hand, by interacting with its adapter apoptosis-associated speck-like protein (ASC), NLRP3 recruits and transforms pro-caspase-1 into its active form, cleaved caspase-1, shearing pro-IL-1β and pro-IL-18 to their mature forms, namely, IL-1β and IL-18 [27,29]. These evidences suggested that NLRP3 might be activated in the LPS-induced cell model of AKI and therefore result in the decreasing of cell viability, which seems to have positive connection with the concentration of LPS, too [26,29]. The expression of SIRT1 was found in our results to be suppressed, and it seemed to be worse as the concentration of LPS increased. Zhang et al. revealed that the expression of SIRT1 was decreased in the LPS-induced cell model of AKI as well [30]. Taken together, these findings indicate that the expression of SIRT1 could surely be suppressed in this cell model.

It has been shown that inhibition of NLRP3 activation has a significant effect of reducing tissue inflammatory damage and inhibiting apoptosis. Jiang et al. showed that CORM-2 inhibited the TXNIP/NLRP3 inflammasome pathway and prevented LPS-induced lung injury [31]. Hyperin is thought to attenuate mouse AKI caused by LPS by inhibiting TLR4 and NLRP3 signaling pathways [25]. The results of the Wang et al. study showed that inhibition of NLRP3 inflammasome protein expression may be a potential method for the treatment of sepsis-induced AKI [32]. Recently, studies have also shown that SIRT1 also has the effect of inhibiting NLRP3 inflammasome, but little has been studied on its role in AKI [15,16]. As shown in our study, SIRT1 could be successfully overexpressed in the HK-2 cell line and therefore elevate the proliferation and decrease the apoptosis rate of these cells. Fan et al. found that SIRT1 deficiency led to the raised apoptosis rate of cells in ischemia/reperfusion-induced AKI [14]. In addition, Qu et al. proved that SIRT1 could promote proliferation and inhibit apoptosis of human malignant glioma cell lines [33]. This indicates that the overexpression of SIRT1 might be able to raise the proliferation and decrease the apoptosis rate of HK-2 cells. Evidently, our study also showed

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that the expression of NLRP3 cleaved caspase-1, IL-1β, and IL-18 were decreased and the expression of caspase-1 was elevated after the overexpression of SIRT1 in the HK-2 cell line. Li et al. reported that SIRT1 could inhibit inflammatory response partly through regulation of NLRP3 inflammasome in vascular endothelial cells [34]. Zhang et al. revealed that arctigenin could attenuate ischemic stroke via SIRT1-dependent inhibition of NLRP3 inflammasome as well [35]. Given these evidences, we suggest that the overexpression of SIRT1 might be able to promote the proliferation and resist the apoptosis of HK-2 cells through downregulating the activation of NLRP3 inflammasome, protecting the cells from the damage caused by LPS-induced AKI.

Conclusions

In conclusion, our study revealed that NLRP3 inflammasome could act as a critical regulator promoting the process of AKI induced by LPS, and the overexpression of SIRT1 might be able to suppress the activation of NLRP3 and therefore resist kidney injury, showing the promising to be used as a target in the treatment of sepsis-induced AKI.

Conflict of interest

None.

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