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ORIGINAL RESEARCH

Sigma-1 Receptor Rescues Autophagy Through AMPK/mTOR Signaling Pathway in Sepsis-Induced Acute Kidney Injury

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Purpose: This study aimed to investigate the effects of σ-1R on autophagy in sepsis-AKI and its potential involvement in the AMPK/mTOR signaling pathway.

Methods: The serum samples from patients were used to diagnose sepsis and sepsis-AKI using double-blind and randomized method and to quantify σ-1R and inflammatory cytokines using enzyme-linked immunosorbent assays. HK-2 cells induced by lipopolysaccharide (LPS) were employed as an in vitro model of sepsis-AKI. To evaluate the function of σ-1R in sepsis-AKI, siRNA and an overexpression plasmid targeting σ-1R were used. σ-1R and autophagy marker expressions were analyzed using quantitative real-time polymerase chain reaction and Western blot assays. Cell proliferation was evaluated using CCK-8 and EdU assays, and cell apoptosis was detected using flow cytometry and TUNEL staining assays. Phosphorylated proteins were detected in the AMPK/mTOR signaling pathway.

Results: In sepsis and sepsis-AKI, σ-1R levels were reduced, whereas the levels of interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α) increased. σ-1R promoted the proliferation of LPS-induced HK-2 cells while inhibiting apoptosis. Moreover, σ-1R enhanced autophagy, as evidenced by the upregulation of autophagy biomarkers LC3-II/LC3-I and Beclin 1. Furthermore, σ-1R promoted the phosphorylation of AMPK and ULK1 while inhibiting mTOR.

Conclusion: σ-1R utilizes the AMPK/mTOR signaling pathway to enhance autophagy in sepsis-AKI, indicating that σ-1R may serve as a promising target for sepsis-AKI diagnosis and therapy.

Keywords: sepsis-induced acute kidney injury, sigma-1 receptor, autophagy, AMPK/mTOR signaling pathway

Introduction

Acute kidney injury (AKI) is a prevalent medical condition that encompasses various fields of study. Its characteristic feature is a rapid decline in kidney function, primarily indicated by a sudden drop in glomerular filtration rate. This leads to the accumulation of nitrogen-containing compounds, resulting in swift imbalances in water, electrolytes, acid-base levels, and complications throughout the body.[1] AKI can be caused by various factors, including physiological harm, reduced blood flow to the kidneys (with or without cellular damage), toxicity to renal tubules, ischemia or blockage, inflammation and swelling of the tubulointerstitium, and the progression of underlying glomerular disease, all contributing to a decline in the glomerular filtration rate.[2] Sepsis-AKI, a frequent occurrence in severe sepsis and septic shock induced by sepsis,[3] is responsible for over half of AKI cases in the intensive care unit (ICU). However, only critically ill patients with a percentage of 5% experience AKI necessitating dialysis, resulting in a mortality rate ranging from 30% to 60%.[4,5] Sepsis, characterized by organ dysfunction, stems from an imbalanced response to infection[6] and is a frequently encountered severe illness in the emergency ICU, presenting a high occurrence and fatality rate in clinical settings.[7] Notably, sepsis-AKI differs from non-septic AKI, with a unique pathophysiological mechanism. Therefore, sepsis-AKI

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represents a significant medical challenge for healthcare professionals and warrants increased attention and recognition.[7] Recent studies have demonstrated that autophagy has a protective effect on AKI.[8] Autophagy is triggered in renal tubular epithelial cells during AKI. Inhibiting autophagy through pharmacological means or genetic manipulation may exacerbate AKI, whereas promoting autophagy can mitigate renal damage.[9] Nevertheless, the intricate regulatory process of autophagy in sepsis-AKI remains incompletely understood.

σ-1R, a non-opioid molecular chaperone protein,[10] is primarily located at the junction of the mitochondria and endoplasmic reticulum as well as in subcellular membrane components. It exerts its influence in various cells and tissues, including the heart, brain, liver, and lung.[11] In the context of neurodegenerative diseases, σ-1R's role in activating autophagy to eliminate damaged mitochondria or remove abnormal protein aggregates has been extensively studied to prevent cell death.[12] The involvement of σ-1R in autophagy regulation has been established, with elevated σ-1R levels causing damage to lysosomes and promoting autophagy.[13] σ-1R governs diverse functions in the nervous system, including neuropathy,[14] the interaction between the endoplasmic reticulum and mitochondria,[15] potassium channel activity,[16] G-protein–coupled receptor activity,[17] and microglial function.[18] Consequently, σ-1R is involved in the pathogenesis of some central and peripheral disorders, such as ischemic stroke[19] and Alzheimer's disease.[20] Its role in inflammation and structural remodeling is also significant.[21] Research on depression[22] has revealed that σ-1R overexpression in LPS-induced astrocytes suppresses inflammatory cytokines such as IL-1β, TNF-α, and inducible nitric oxide synthase (iNOS). This finding suggests that σ-1R may have the potential to alleviate depressive behavior caused by inflammation, making it a potential target for depression treatment.[23] However, there are currently no report on σ-1R role in sepsis-AKI, and it remains unclear whether the protective mechanism of σ-1R in sepsis-AKI.

Materials and Methods

Human Serum Samples

Twenty serum samples were collected from patients diagnosed with sepsis and/or AKI using double-blind and randomized method at the Affiliated Hospital of Nantong University (Nantong, China) between January and December 2022. All patients were diagnosed with sepsis-AKI according to the 2012 Kidney Disease Improving Global Outcomes (KDIGO).[24] Additionally, 10 serum samples were obtained from healthy volunteers to serve as normal controls.

Ethical Approval

The study was conducted in accordance with the guidelines outlined in the Helsinki Declaration and was approved by the Ethics Committee of the Affiliated Hospital of Nantong University (Approval No. 2022-K138-01). Written consent was obtained from all participants.

Enzyme-Linked Immunosorbent Assay (ELISA)

We used ELISA to measure the levels of σ-1R and inflammatory cytokines, including IL-1β, IL-6, and TNF-α, in the serum of the patients. Blood samples were collected from patients diagnosed with sepsis or sepsis-AKI and placed in serum separation tubes. These tubes were then refrigerated at 4°C overnight, and the supernatant was obtained by centrifugation for 20 min at 1000×g and subsequently stored at −80°C. Similarly, the supernatant from cell cultures was obtained by centrifugation for 20 min at 1000×g. Detection was carried out using ELISA kits following the manufacturer's protocol (MLbio, China) using approximately 50 μL of samples. The concentration of each sample was determined by constructing a standard linear regression curve using the standard samples provided in the kits. A microtiter plate reader (Bio-Tek, USA) was used to determine the optical density (OD) value of each well at 450 nm.

Cells, Culture and Treatments

Human kidney-2 (HK-2) cells obtained from ATCC were cultured in Dulbecco's modified Eagle's medium (DMEM) (Thermo Fisher Scientific, USA) containing 10% fetal bovine serum (FBS) (Thermo Fisher Scientific). Cells were grown at 37°C in an incubator with 95% air and 5% CO_2. HK-2 cells originate from normal kidneys and are immortalized by transduction of the E6/E7 gene of human papillomavirus 16 (HPV-16).

To construct the sepsis-AKI model in-vitro according to a previous study,[25] HK-2 cells in the logarithmic phase of growth were plated in a 12-cm cell culture dish at a density of 2×10^5 cells per well. The cells were cultured for 24 h in an 37°C incubator. The medium was replaced with fresh DMEM containing 10% FBS and 1 μg/mL LPS (Sigma-Aldrich). The cells were cultured for 24 h at 37°C. Additionally, dihydrobromide (BD-1047) and hydrochloride (PRE-084) (both obtained from MedChemExpress) were used as the antagonist and agonist of σ-1R, respectively. The cells were treated with BD-1047 (10 μM) and PRE-084 (10 μM) for 48 h.[26,27]

Small Interfering RNAs, Plasmid, and Transfection

To achieve the downregulation or upregulation of endogenous σ-1R in cells, siRNAs and overexpression plasmids were designed and constructed. σ-1R-specific targeting siRNAs were designed based on the σ-1R sequence from the NCBI database (accession no. NM_005866). The siRNAs used were si-σ-1R-1, si-σ-1R-2, and si-σ-1R-3, respectively. A non-homologous siRNA sequence with human genes was used as a negative control (si-NC); the sequences are listed in Table 1. The σ-1R overexpression plasmid was constructed into the pcDNA3.1 vector (p-σ-1R) by OligoBio (Beijing, China), with the empty vector pcDNA3.1 serving as a negative control (p-NC). Lipofectamine® 2000 (Thermo Fisher Scientific) was used to transfect siRNAs and plasmids into cells following the manufacturer's instructions.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

QRT-PCR was used to assess the mRNA levels of the target genes. TRIzol® reagent (Thermo Fisher Scientific) was used to obtain total RNA from cells or tissues, following the manufacturer's protocols. The obtained RNA was diluted in 50 μL of RNase-free ddH_2O. A One-Step SYBR Green qRT-PCR kit (Thermo Fisher Scientific) was used for qRT-PCR following the manufacturer's instructions. The variation in the expression of target genes between the control group and each experimental group was analyzed using the $2^{-\Delta\Delta Ct}$ method,[28] and normalized to β-actin. The qPCR primer sequences were as follows: σ-1R, with the forward, 5′-ATCTCGGATACCATCATCTC-3′ and reverse, 5′-TGGGTAGAAGACCTCACT-3′. Additionally, β-actin primer sequences consisted of forward 5′-AGCGAGCATCCCCCAAAGTT-3′ and reverse 5′-GGGCACGAAGGCTCATCATT-3′.

Western Blot

Western blot was performed to assess the protein levels of these genes. Total proteins from the cells or tissues were extracted on ice for 30 min using RIPA extraction reagent (Promega). The mixture was centrifuged at 4°C for 20 min at 12,000 rpm. After quantification using the BCA method (Biosharp), 30 μg of protein was separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and then transferred onto a polyvinylidene difluoride (PVDF) membrane (Merck Millipore). Following a 1-h incubation at room temperature in 5% BSA, the membrane was exposed to the following primary antibodies at 4°C overnight: σ-1R (1:1,000 dilutions; #15168-1-AP; Proteintech), LC3 (1:2,000 dilutions; #14600-1-AP; Proteintech), Beclin 1 (1:1,000 dilutions; #11306-1-AP; Proteintech), AMPK (1:1,000 dilutions; #10929-2-AP; Proteintech), phospho-AMPK1 (S496; p-AMPK1; 1:1,000 dilutions; #BM4718; Boster), mTOR (1:5,000 dilutions; #66888-1-Ig; Proteintech), phospho-mTOR (Ser2448; p-mTOR; 1:5,000 dilutions, #67778-1-Ig; Proteintech),

Table 1 The Sequences of siRNAs Targeting σ-1R

Name		Sequence (5′-3′)
si-σ-1R-1	Sense	GGCUUGAGCUCACCACCUAdTdT
	Antisense	UAGGUGGUGAGCUCAAGCCdTdT
si-σ-1R-2	Sense	GCGAAGAGAUAGCGCAGUUdTdT
	Antisense	AACUGCGCUAUCUCUUCGCdTdT
si-σ-1R-3	Sense	CUUCCAGCGCGAAGAGAUAdTdT
	Antisense	UAUCUCUUCGCGCUGGAAGdTdT
si-NC	Sense	UUCUCCGAACGUGUCACGUdTdT
	Antisense	ACGUGACACGUUCGGAGAAdTdT

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ULK1 (1:1,000 dilutions, #20986-1-AP; Proteintech), phospho-ULK1 (Ser556; p-ULK1; 1:2,000 dilutions; #80218-1-RR; Proteintech), and β-actin (1:5,000 dilutions; #66009-1-lg; Proteintech). After washing in Tris-buffered saline with Tween 20 for 10 min three times, the membrane was exposed to horseradish peroxidase (HRP)-labeled Goat anti-rabbit IgG H&L (1:1,000 dilutions, #A0208; Beyotime) or HRP-labeled Goat anti-mouse IgG H&L (1:1,000 dilutions, #A0216; Beyotime) at 37°C for 1 h. Finally, BeyoECL Plus solution (Beyotime) was used to visualize the protein blots with a luminometer. Subsequently, the blots were scanned and analyzed using ImageJ software.

Cell Counting Kit (CCK)-8

CCK-8 assay was used to assess cell viability. Briefly, HK-2 cells in the logarithmic phase of growth were seeded into 96-well culture plates at a density of 1×10^4 cells/well based on the different experimental groups and then incubated for 24 h at 37°C. The cells in the experimental group were stimulated with LPS for 24 h. Subsequently, the cells were transfected with siRNAs or plasmids for 6 h. Post replacement with 100 μL of fresh medium, the cells were continue cultured for 24 h, 48 h, 72 h and 96 h. To analyze the effect on cell proliferation, 10 μL of CCK-8 solution (5 mg/mL) (Beyotime) was added to each well and incubated at 37°C for 1 h in the dark. OD values were measured at 450 nm at the same time point.

5-Ethynyl-2′-Deoxyuridine (EdU) Labeling Assay

Cell proliferation was evaluated using the EdU labeling assay. HK-2 cells in the logarithmic phase of growth were plated into 96-well plates at a concentration of 1×10^4 cells/well based on the different experimental groups and incubated for 24 h at 37°C. Cells in the experimental groups were stimulated with LPS for 24 h and then transfected with siRNAs or plasmids for 6 h. After replacement with 100 μL fresh DMEM, the cells were cultured for 48 h. The cells were stained using an Alexa Fluor 555-labeled EdU Cell Proliferation Kit (Beyotime) according to the manufacturer's protocol. After exposure to the click solution at room temperature for 30 min, protected from light, the cells were stained with 1× Hoechst 33342 solution at room temperature for 30 min protecting from light. Subsequently, EdU-positive (EdU^+) cells were observed using a fluorescence microscope (Olympus), and the proliferation rate was determined.

Flow Cytometry (FCM)

Flow cytometry (FCM) was employed to detect cell apoptosis following Annexin V-FITC and PI double staining using an Annexin V-FITC/PI Apoptosis Detection Kit (Beyotime). Briefly, HK-2 cells in the logarithmic phase of growth were plated into 6-well plates at a concentration of 2×10^5 cells/well based on the experimental groups and were cultured for 24 h in a 37°C incubator. Cells in the experimental group were stimulated with LPS for 24 h and then transfected with siRNAs or plasmids for 6 h. Next, 100 μL of fresh DMEM was added, and the cells were cultured for 72 h. Approximately 5×10^4 cells were obtained and suspended in 195 μL of Annexin V-FITC binding buffer, followed by the addition of 5 μL of Annexin V-FITC and 10 μL of PI to each well, and incubated for 10 min at 37°C to protect from light. Finally, the cells were identified and quantified using a flow cytometer (BD FACSVerse).

Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick-End-Labeling (TUNEL) Assay

The cell apoptosis index was determined using a One-Step TUNEL Apoptosis Assay Kit (Beyotime). Briefly, HK-2 cells in the logarithmic phase of growth were plated into 96-well plates at a concentration of 1×10^4 cells/well based on the different experimental groups and were incubated for 24 h at 37°C. Cells in the experimental group were stimulated with LPS for 24 h and then transfected with siRNAs or plasmids for 6 h. After replacement with 100 μL fresh DMEM, the cells were cultured for 72 h. After rinsing in phosphate-buffered saline (PBS), the cells were fixed in a 4% paraformaldehyde solution for 30 min, washed in PBS, and exposed to a 0.3% Triton X-100 solution for 5 min at room temperature. After two PBS washes, the cells were treated with 50 μL TUNEL detection solution, comprising 45 μL of fluorescent labeling solution and 5 μL of TDT enzyme, at 37°C for 60 min. After three washes with PBS, the cells were observed and imaged using a fluorescence microscope (Olympus).

Statistical Analyses

All quantitative data are shown as the mean values ± standard deviation (SD) and were statistically analyzed using GraphPad Prism 8.0 (GraphPad Software, Inc). Differences between two groups were compared using Student's *t*-test (parametric), while 1-way analysis of variance (ANOVA) was performed to compare differences among three or more groups, following Tukey's multiple comparison tests. Differences were considered statistically significant when the p-value was < 0.05.

Results

σ-1R Decrease and Inflammatory Cytokines Increase in Sepsis and Sepsis-AKI

The primary indication for sepsis is inflammation, primarily driven by elevated levels of inflammatory cytokines.[29] We employed ELISA to measure σ-1R and the levels of inflammatory cytokines including IL-1β, IL-6 and TNF-α, in sepsis and sepsis-AKI. A total of 30 serum samples were collected, including 10 cases of sepsis, 10 cases of sepsis-AKI, and 10 samples from healthy volunteers who served as normal controls. The results revealed that in both sepsis and sepsis-AKI, σ-1R levels were significantly reduced compared to those in the control group (Figure 1A), while IL-1β, IL-6, and TNF-α levels were elevated (Figure 1B–D). Notably, σ-1R levels were lower in sepsis-AKI than in sepsis (Figure 1A), whereas

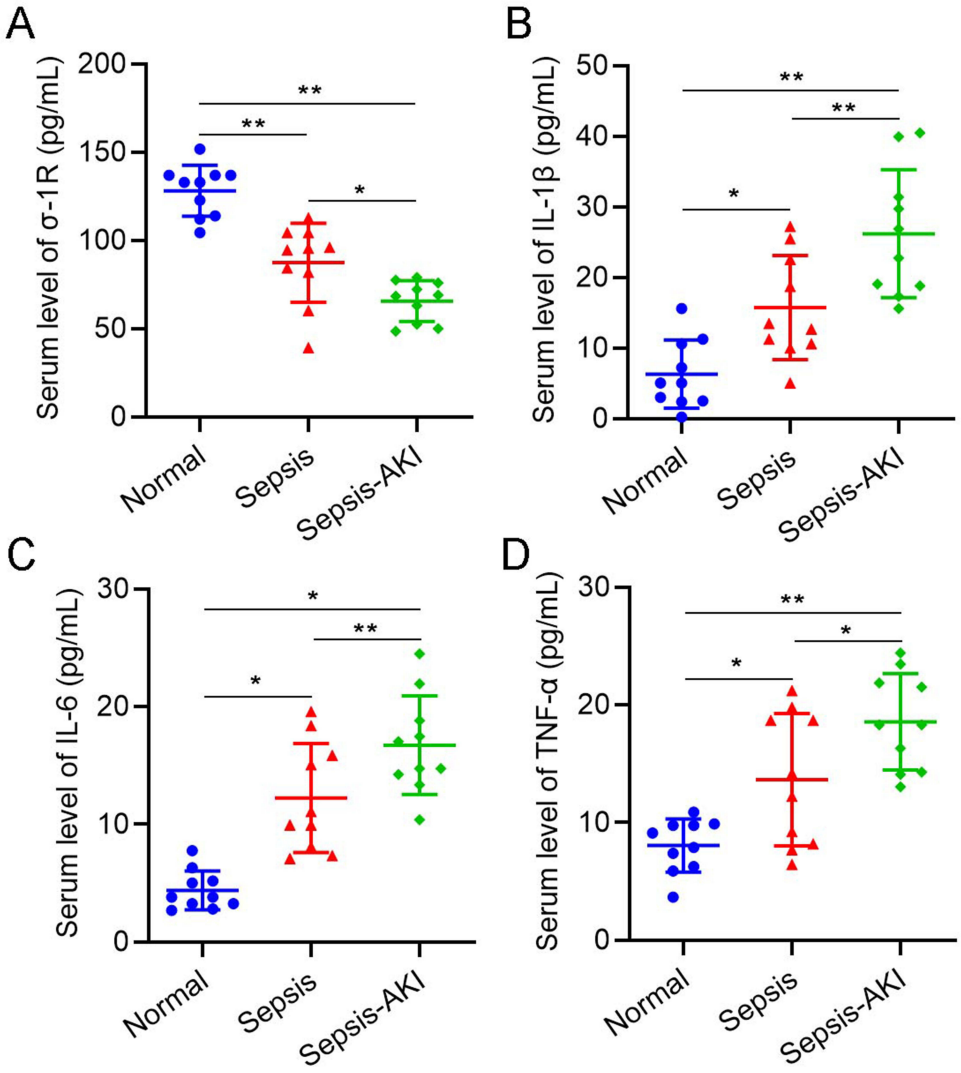

Figure 1 Levels of σ-1R and inflammatory cytokines in sepsis and sepsis-AKI patients, as determined by ELISA. (**A**) σ-1R serum levels. (**B**) IL-1β serum levels. (**C**) IL-6 serum levels. (**D**) TNF-α serum levels. *P<0.05, **P<0.01, as indicated in the figures.

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IL-1β, IL-6, and TNF-α levels were higher in sepsis-AKI than in sepsis (Figure 1B–D). These findings suggest that both sepsis and sepsis-AKI correlate with decrease of σ-1R levels and increase of inflammatory cytokine levels, indicating a more pronounced inflammatory response in patients with sepsis than in those with sepsis alone.

σ-1R Decrease in LPS-Induced HK-2 Cells While Inflammatory Cytokines Increase

To elucidate the role of σ-1R in sepsis-AKI, we used LPS-induced HK-2 cells as an in-vitro model. QRT-PCR and Western blotting were used to detect σ-1R mRNA and protein levels, respectively, and ELISA was used to measure inflammatory cytokine levels. We observed a significant reduction in σ-1R expression at both the mRNA and protein levels in LPS-induced HK-2 cells compared to that in non-LPS-induced HK-2 cells (Ctrl; Figure 2A and B). ELISA results indicated elevated concentrations of inflammatory cytokines IL-1β, IL-6, and TNF-α compared to those in the control group in LPS-induced HK-2 cells (Figure 2C–E). These findings suggest that LPS induces an increase in the levels of inflammatory cytokines in HK-2 cells.

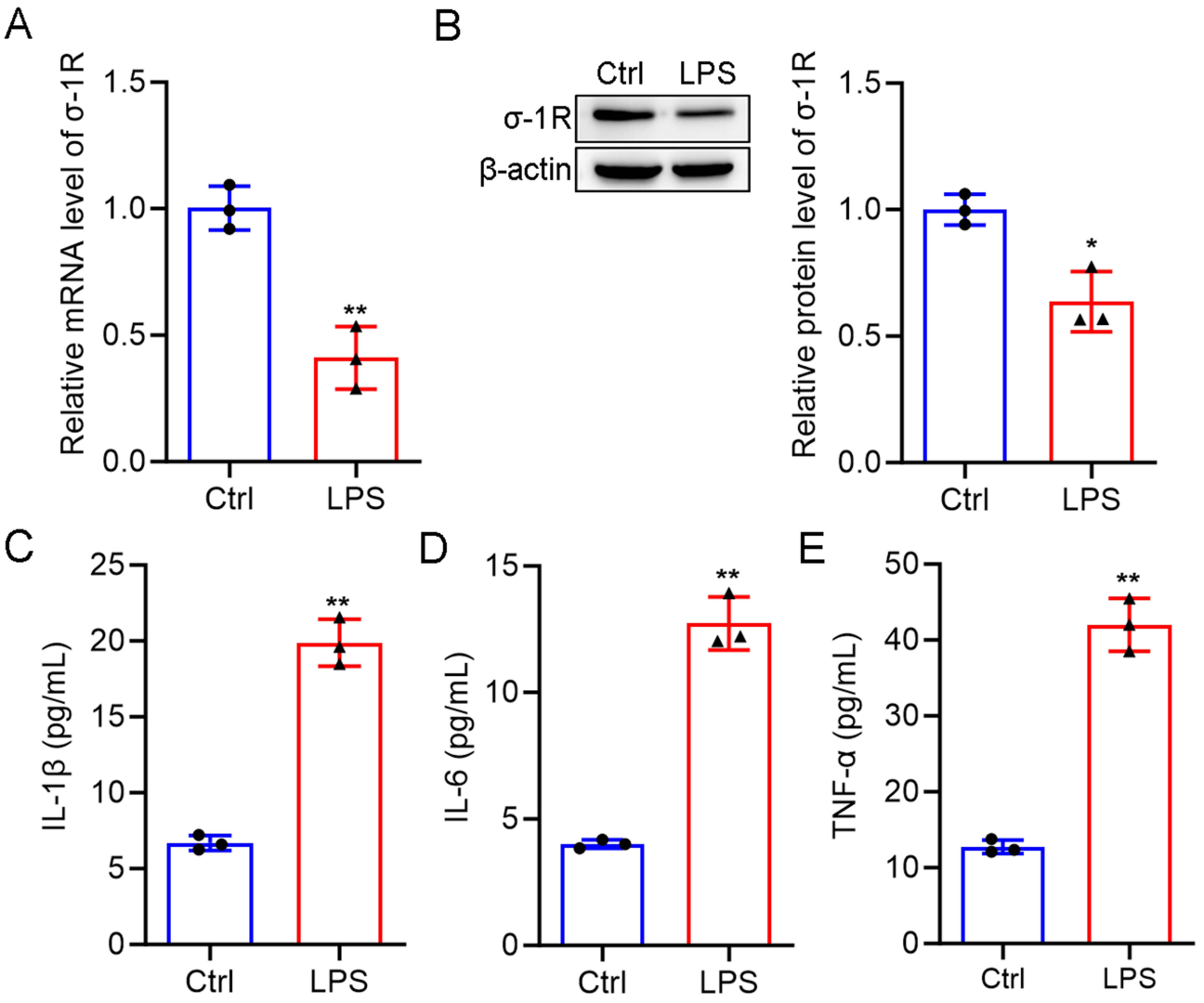

Figure 2 Expression of σ-1R and inflammatory cytokines in LPS-induced HK-2 cells. (**A**) The mRNA levels of σ-1R were detected by qRT-PCR. (**B**) The protein levels of σ-1R were detected by Western blot. (**C**) IL-1β levels were detected by ELISA. (**D**) IL-6 levels were detected by ELISA. (**E**) TNF-α levels were detected by ELISA. *P<0.05, **P<0.01 vs Ctrl (non-LPS-treated HK-2 cells).

Expression of σ-1R Regulated by siRNAs or Overexpression Plasmid in LPS-Induced HK-2 Cells

To monitor the changes in the levels of σ-1R in LPS-induced HK-2 cells, we designed specific siRNAs targeting σ-1R (si-σ-1R-1, si-σ-1R-2, and si-σ-1R-3). QRT-PCR was used to screen for siRNAs in HK-2 cells treated with LPS. The results showed that all three siRNAs effectively reduced σ-1R mRNA levels compared with those in LPS-induced HK-2 cells transfected with si-NC (Figure 3A). Among these, si-σ-1R-1 (si-σ-1R) exhibited the best silencing ability and was chosen for subsequent investigations. Additionally, Western blot revealed a significant reduction in σ-1R protein levels when cells were transfected with si-σ-1R compared to si-NC-transfected cells (Figure 3B). Furthermore, we constructed a σ-1R overexpression plasmid (p-σ-1R) and transfected it into the LPS-induced HK-2 cells. We observed that p-σ-1R significantly increased σ-1R mRNA and protein levels compared to the empty plasmid negative control (p-NC)-treated cells (Figure 3C and D). These findings suggest that si-σ-1R and p-σ-1R can modulate σ-1R expression in LPS-induced HK-2 cells.

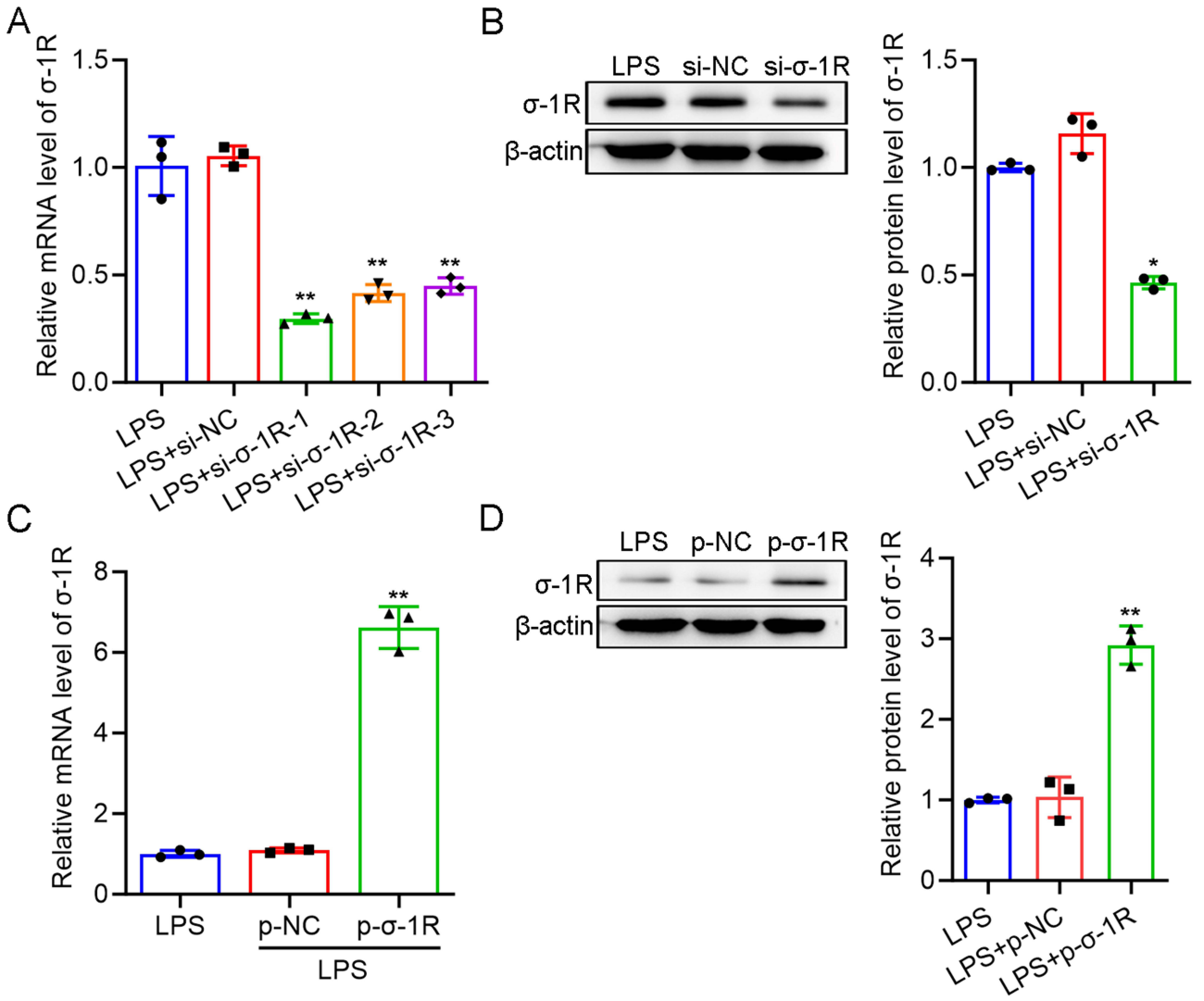

Figure 3 Expression levels of σ-1R regulated by siRNA or overexpression plasmid in HK-2 cells induced by LPS. (**A**) The mRNA levels of σ-1R inhibited by siRNAs (si-σ-1R-1, si-σ-1R-2, and si-σ-1R-3) detected by qRT-PCR. **P<0.01 vs si-NC transfected cells. (**B**) The protein levels of σ-1R inhibited by si-σ-1R detected by Western blot. *P<0.05 vs si-NC transfected cells. (**C**) The mRNA levels of σ-1R upregulated by overexpression plasmid p-σ-1R detected by qRT-PCR. **P<0.01 vs p-NC transfected HK-2 cells. (**D**) The protein levels of σ-1R upregulated by p-σ-1R detected by Western blot. **P<0.01 vs p-NC transfected cells.

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Effects of σ-1R Regulation on the Expression of Inflammatory Cytokines in LPS-Induced HK-2 Cells

To investigate the effect of σ-1R regulation on inflammatory cytokine levels, we used si-σ-1R and p-σ-1R to manipulate the LPS-induced σ-1R expression in HK-2 cells. ELISA was used to assess cytokine levels of IL-1β, IL-6, and TNF-α. Compared to cells transfected with si-NC, si-σ-1R-transfected cells exhibited upregulation of IL-1β (Figure 4A), IL-6 (Figure 4B), and TNF-α levels (Figure 4C). Conversely, p-σ-1R-transfected cells showed decreased levels of these cytokines compared with p-NC-transfected cells (Figure 4A–C). These findings suggest that σ-1R regulates inflammatory cytokine levels during sepsis or sepsis-AKI.

Effects of σ-1R Regulation on the Cell Proliferation and Apoptosis in LPS-Induced HK-2 Cells

Cell proliferation was evaluated using CCK-8 and EdU labeling assays, whereas cell apoptosis was detected using FCM and TUNEL staining assays. CCK-8 results indicated that cell growth was significantly inhibited by si-σ-1R compared to that of si-NC-transfected cells. Conversely, p-σ-1R-transfected cells exhibited enhanced cell growth compared to p-NC-transfected cells (Figure 5A). The EdU labeling assay produced similar cell proliferation results (Figure 5B). FCM analysis demonstrated that si-σ-1R-transfected cells exhibited increased apoptosis compared to si-NC-transfected cells, whereas p-σ-1R-transfected cells showed significant inhibition of apoptosis compared to p-NC-transfected cells (Figure 5C). TUNEL staining confirmed the presence of apoptosis (Figure 5D).

Effects of σ-1R Regulation on Autophagy Through the AMPK/mTOR Signaling Pathway

Recent findings showed that autophagy played a protective role in AKI,[30,31] and σ-1R has been implicated in autophagy regulation.[20] The signaling pathway of AMPK/mTOR is a critical regulator of cellular autophagy and has been implicated in sepsis.[32] To assess the autophagic process, we examined the expression levels of Light chain 3 (LC3) and Beclin 1, which were autophagy-related proteins.[33] si-σ-1R-transfected cells exhibited decreased the protein expressions of σ-1R (Figure 6A and B), LC3-II/LC3-I (Figure 6A and C) and Beclin 1 (Figure 6A and D), compared with si-NC-transfected cells. BD-1047, a specific functional σ-1R antagonist,[34] served as a reference for σ-1R inhibition and effectively suppressed the protein levels of σ-1R (Figure 6A and B), LC3-II/LC3-I (Figure 6A and C) and Beclin 1 (Figure 6A and D) in LPS-induced HK-2 cells. We also examined the levels of phosphorylated ULK1, mTOR, and

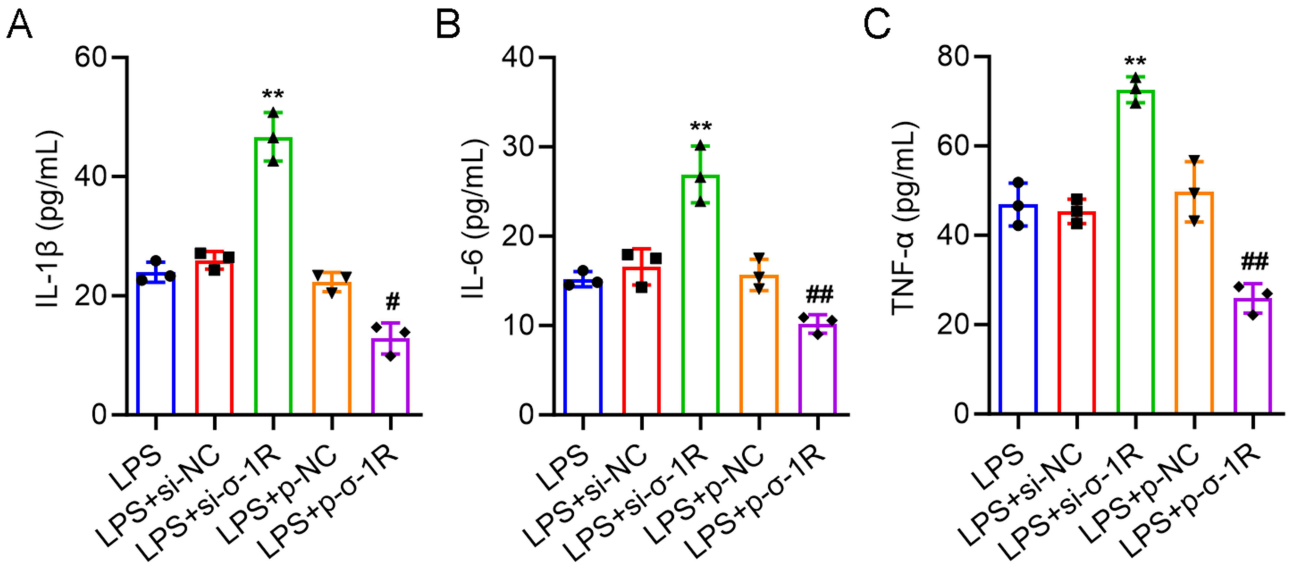

Figure 4 Levels of inflammatory cytokines regulated by σ-1R in LPS-induced HK-2 cells, as detected by ELISA. (**A**) Levels of IL-1β. (**B**) Levels of IL-6. (**C**) Levels of TNF-α. **P<0.01 vs si-NC transfected cells; #P<0.05, ##P<0.01 vs p-NC transfected cells.

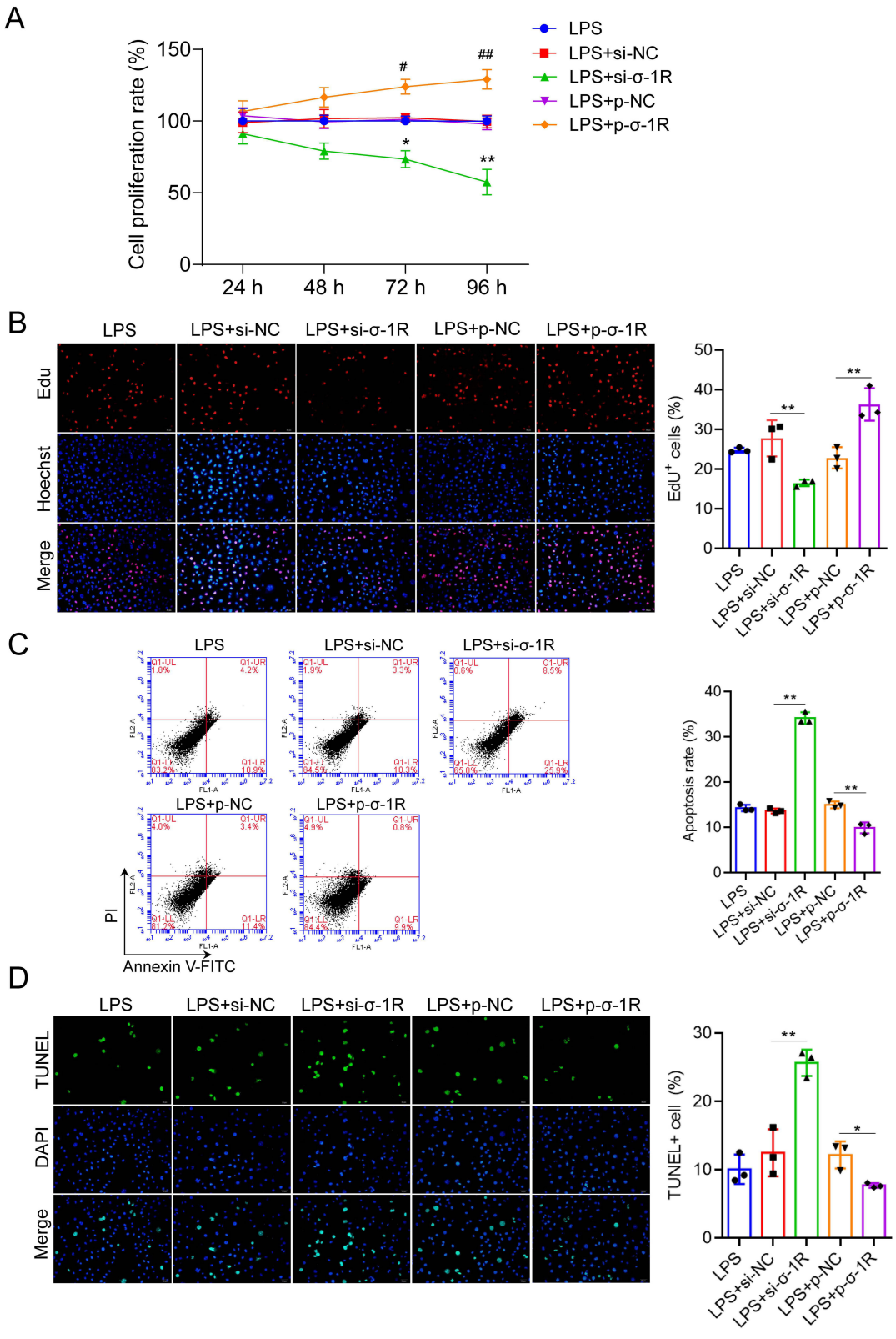

Figure 5 Effects of σ-1R expression on the proliferation and apoptosis of LPS-induced HK-2 cells. (**A**) Cell viabilities regulated by si-σ-1R or p-σ-1R detected by CCK8 assay. *P<0.05, **P<0.01 vs si-NC transfected cells; #P<0.05, ##P<0.01 vs p-NC transfected cells. (**B**) Cell proliferation abilities regulated by si-σ-1R or p-σ-1R detected by EdU labeling assay; scale bar=20 μm. **P<0.01 as indicated in the figures. (**C**) Cell apoptosis regulated by si-σ-1R or p-σ-1R, detected by FCM. **P<0.01 as indicated in the figures. (**D**) Cell apoptosis regulated by si-σ-1R or p-σ-1R detected by TUNEL staining; scale bar=20 μm. *P<0.05, **P<0.01 as indicated in the figures.

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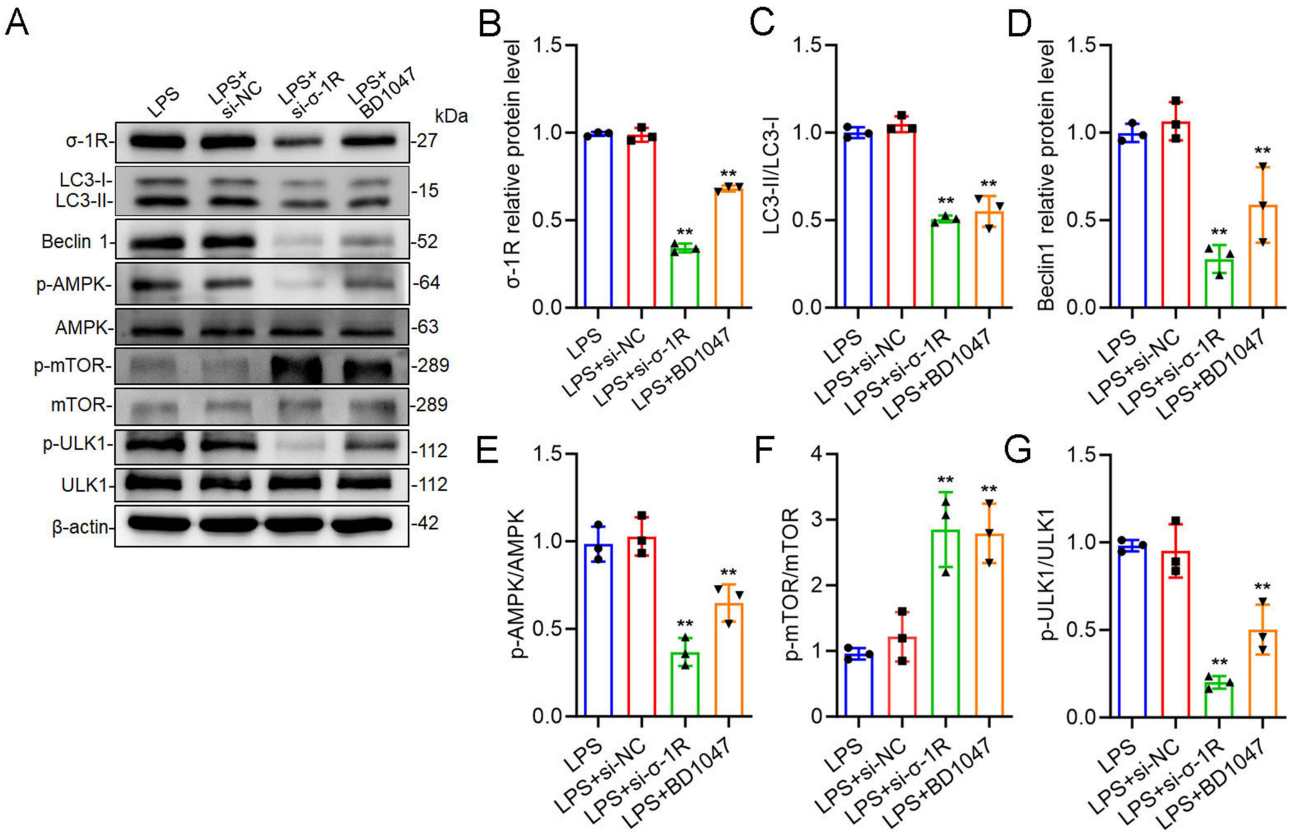

Figure 6 Regulation of autophagy levels and the AMPK/mTOR signaling pathway by σ-1R downregulation in LPS-induced HK-2 cells. (**A**) Representative blots of σ-1R, LC3-II/LC3-I, Beclin 1, AMPK, mTOR, ULK1, and phosphorylated AMPK, mTOR and ULK1, also the internal control β-actin, with BD1047 used as the σ-1R inhibition positive control. (**B-D**) Protein levels of σ-1R, LC3-II/LC3-I, and Beclin 1 regulated by si-σ-1R. (**E-G**) Phosphorylation levels of AMPK, mTOR, and ULK1 regulated by si-σ-1R. **P<0.01 vs si-NC transfected cells.

AMPK in the AMPK/mTOR signaling pathway. The results indicated that si-σ-1R inhibited the levels of phosphorylated AMPK (p-AMPK) and ULK1 (p-ULK1) while increasing p-mTOR compared to cells transfected with si-NC (Figure 6A, E–G). BD-1047 showed similar results (Figure 6A,E–G).

Furthermore, increased σ-1R expression led to a significant increase in σ-1R, LC3 and Beclin 1 expression in LPS-induced HK-2 cells transfected with p-σ-1R (Figure 7A–C). The highly selective σ-1R agonist, PRE-084[35] was used as a positive control for σ-1R promotion. The results demonstrated that, in LPS-induced HK-2 cells, PRE-084 also enhanced the expression of LC3-II/LC3-I and Beclin 1 (Figure 7A, C and D). Additionally, p-σ-1R increased the phosphorylation levels of AMPK and ULK1, while inhibiting the phosphorylation of mTOR, compared with p-NC-transfected cells (Figure 7A, E–G). PRE-084 exhibited similar effects (Figure 7A, E–G).

These results suggest that σ-1R plays a crucial role in the autophagy process of sepsis-AKI, potentially promoting autophagy, at least in part, through the AMPK/mTOR signaling pathway (Figure 8).

Discussion

An imbalance in the body's response to infection can lead to sepsis, a condition characterized by organ dysfunction.[6] This is a frequently occurring, severe illness commonly encountered in the emergency ICU, with a significant incidence rate and high fatality rate in clinical settings.[7] Severe sepsis and septic shock often result in sepsis-AKI complications.[3] Despite advances in medical technologies, which have improved capacity management, internal environment monitoring, and organ support, the mortality rate associated with sepsis-AKI remains high. This necessitates a re-evaluation of our conventional understanding of sepsis-AKI.[36] Although significant research has been conducted on the pathogenesis of sepsis and sepsis-AKI, there is still a pressing need for new, effective targeted treatments. Therefore, gaining

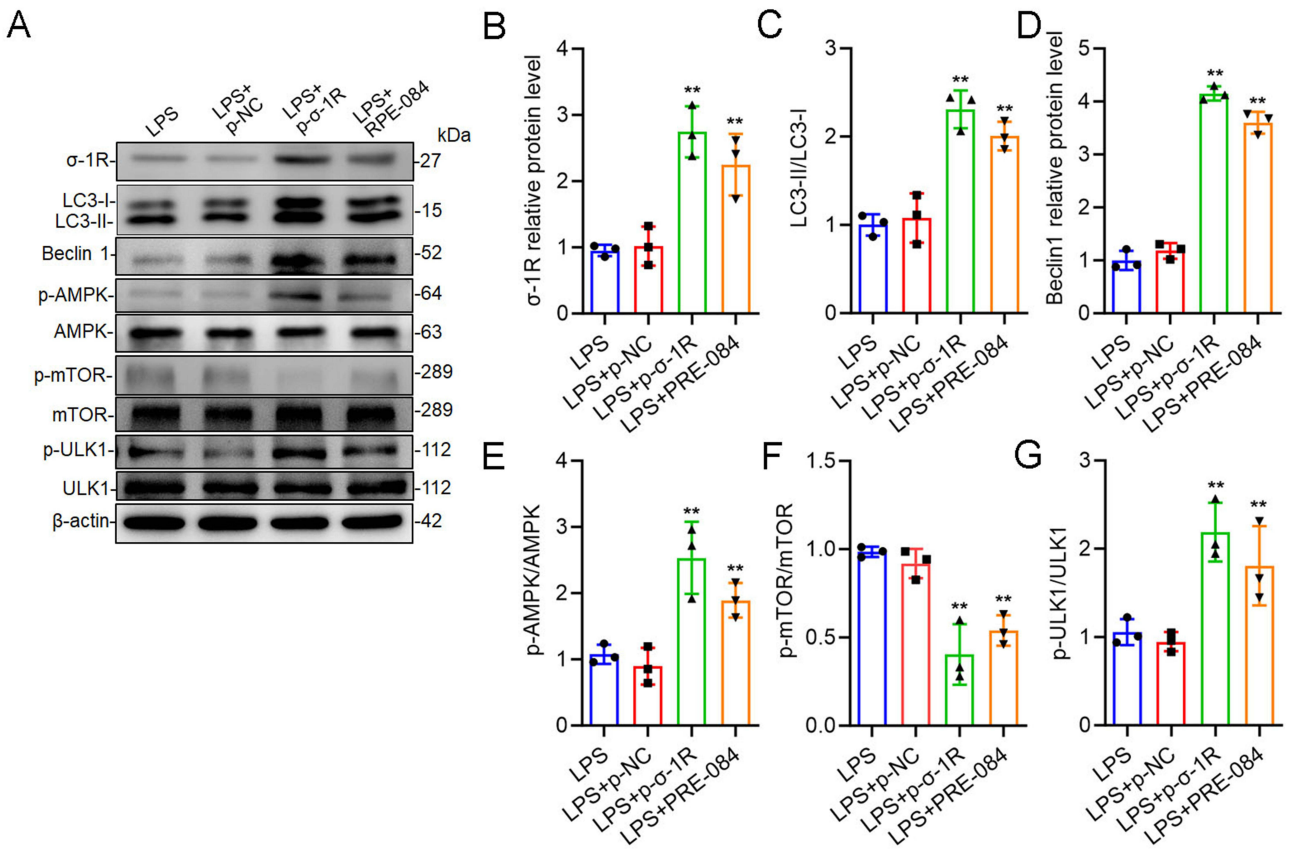

Figure 7 Regulation of autophagy levels and the AMPK/mTOR signaling pathway by σ-1R upregulation in LPS-induced HK-2 cells. (**A**) Representative blots of σ-1R, LC3-II /LC3-I, Beclin 1, AMPK, mTOR, ULK1, and phosphorylated AMPK, mTOR and ULK1, also the internal control β-actin, with PRE-084 used as the σ-1R upregulation positive control. (**B-D**) Protein levels of σ-1R, LC3-II/LC3-I and Beclin 1 regulated by p-σ-1R. (**E-G**) Phosphorylation levels of AMPK, mTOR, and ULK1 regulated by p-σ-1R. **P<0.01 vs p-NC transfected cells.

a comprehensive understanding of the immune mechanisms underlying sepsis-AKI is of paramount importance in its identification and management. This study introduced a novel and promising molecule that can be used to diagnose and treat sepsis-AKI.

Early detection and timely intervention in AKI can greatly improve prognosis given its high prevalence and fatality rate. However, the current approach to AKI diagnosis and treatment follows the KDIGO guidelines, which rely on elevated serum creatinine levels or reduced urine output for identification.[37] Unfortunately, this method may miss opportunities for early intervention. To address this challenge, several molecules, including neutrophil gelatinase-associated lipid carrier protein (NGAL),[38] kidney injury molecule 1 (KIM-1),[39] cysteine protease inhibitor C (Cys-C),[40] tissue inhibitor of metalloproteinase 2 (TIMP-2),[41] insulin-like growth factor binding protein 7 (IGFBP7)[42] and Wnt5a,[43] are anticipated to serve as diagnostic indicators for AKI. However, none of these markers have been validated in clinical settings. Supportive measures, such as blood transfusion, dialysis, or addressing the underlying causative conditions, are typically recommended for the routine management of AKI. Nonetheless, specific medications for AKI have not been widely utilized.[44] Given the ongoing lack of understanding of the causes of sepsis-AKI, it is imperative to research and develop diagnostic biomarkers and therapeutic medications for AKI.

In recent years, research has shown that autophagy plays a defensive role against AKI.[8] Autophagy activation is observed in renal tubular epithelial cells during AKI. Inhibiting autophagy through pharmacological or genetic methods can lead to increased severity of AKI, while promoting autophagy can help mitigate renal injury.[9] However, the intricate regulatory mechanisms governing autophagy in AKI are not fully understood. Autophagy is considered crucial for maintaining intracellular stability and managing stress,[9] and serves as a conservative degradation system involving multiple steps. To maintain cellular balance, autophagy captures intracellular pathogens, damaged proteins, and

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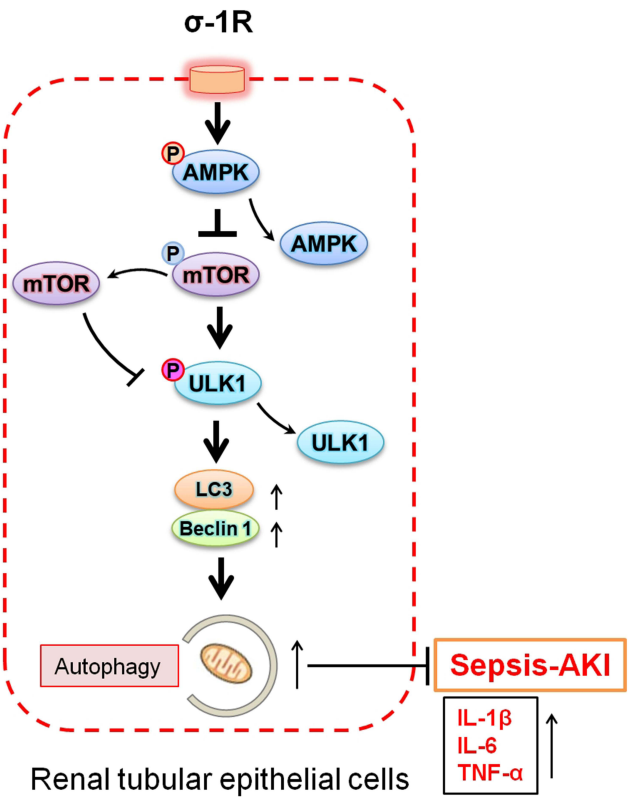

Figure 8 Schematic diagram proposing the σ-1R mechanism of autophagy activation in sepsis-AKI. σ-1R activates AMPK/mTOR, leading to autophagy activation. Additionally, σ-1R regulates the proliferation and apoptosis of HK-2 cells induced by LPS and inhibits the levels of inflammatory cytokines.

dysfunctional organelles, which are then degraded within lysosomes.[45] The pathogenesis of AKI involves upregulation of the autophagy pathway in response to various stress conditions such as hypoxia, cellular starvation, endoplasmic reticulum stress, nutrient and growth factor deprivation, and oxidative damage. Some studies have indicated that autophagy inhibits the activation of NLRP3 inflammasomes, consequently suppressing the secretion of inflammatory cytokines such as IL-1β and IL-18.[46] Furthermore, σ-1R has been demonstrated to be involved in the regulation of autophagy.[13] There is also evidence suggesting that σ-1R aggregation leads to the formation of autophagosomes in the mitochondria-associated endoplasmic reticulum membrane. However, the knockout of σ-1R hinders the proper functioning of autophagy clearance.[47] Additionally, σ-1R agonists have demonstrated the ability to stimulate autophagy, enhancing the removal of impaired mitochondria.[48] During this investigation, the concentrations of σ-1R were found to be reduced in individuals diagnosed with sepsis or sepsis-AKI, with a particularly significant decrease observed in sepsis-AKI. Conversely, there was an elevation in the levels of inflammatory cytokines, IL-1β, IL-6 and TNF-α.

The upregulation of lactic acid production and gene expression related to glycolysis can be induced by sepsis or LPS in the kidney tissue and HK-2 cells, suggesting an enhancement in aerobic glycolysis. Renal injury caused by sepsis in septic mouse models and HK-2 cells induced by LPS can be ameliorated by inhibiting aerobic glycolysis.[49] Recent research has demonstrated the pivotal role of fundamental autophagy in maintaining proper proximal tubule function in the kidney. Moreover, impairment of essential autophagy proteins not only impairs renal function, but also elevates the levels of p62 and oxidative stress. Sepsis can induce autophagy in various organs, with the kidneys being particularly affected.[50] This process involves the removal of impaired mitochondria and mitigation of oxidative stress.[51] Furthermore, autophagy is important in sepsis-induced AKI, and inhibiting autophagy leads to AKI development in sepsis.[32,52] These results suggest that autophagy and related pathways hold potential as targets for AKI treatment.

A study revealed high levels of σ-1R molecular chaperone protein expression in the renal cortex of the kidney.[53] Alterations in σ-1R expression in the kidneys and its activation have been strongly associated with the development of

various kidney diseases, including renal injury and gender-specific renoprotection.[54] Research on diabetic nephropathy has shown a notable increase in σ-1R levels in distal renal tubules, contributing to renal fibrosis mediated by TGF-β.[55] In hyperoxaluria-induced nephrolithiasis, a decrease in σ-1R expression in mitochondria-associated endoplasmic reticulum membranes has been linked to apoptosis, renal damage, and calcium oxalate crystal deposition.[55] Moreover, evidence suggests that dehydroepiandrosterone protects against kidney injury induced by hypertension by increasing σ-1R levels in ovariectomized rats.[56] Some studies have indicated that σ-1R activation can confer renoprotection by enhancing the heat shock response.[57] Additionally, activation of σ-1R in proximal renal tubular cells through σ-1R agonist ligands has been shown to improve renal function, post-ischemic survival and structural damage.[53] Nevertheless, the precise role of σ-1R in sepsis-induced AKI remains uncertain.

The study demonstrated that the levels of the inflammatory cytokines, IL-1β, IL-6 and TNF-α increased, while σ-1R decreased in an in vitro sepsis-AKI model using LPS-induced HK-2 cells. Moreover, there was a notable increase in the expression of autophagy indicators LC3-II/LC3-I and Beclin 1. The modulation of σ-1R expression resulted in corresponding changes in IL-1β, IL-6, and TNF-α levels. Decreased σ-1R levels suppressed the growth of LPS-induced HK-2 cells, whereas increased σ-1R levels promoted cell growth. Conversely, downregulation of σ-1R increased apoptosis in LPS-induced HK-2 cells, whereas σ-1R increase suppressed apoptosis.

Rapamycin is a well-known inhibitor of the autophagic mTOR pathway in many diseases. AMPK, acting as a negative regulator of mTOR, is essential in the anti-inflammatory process and has also been documented in sepsis-AKI.[58] Compounds such as PRE-084 and Anavex 2-73, which activate the σ-1R receptor, can stimulate autophagy by enhancing the phosphorylation of ULK1.[13] In this study, we further examined the impact of σ-1R on the AMPK/mTOR autophagy signaling pathway in sepsis-AKI. Our findings indicate that decrease of σ-1R leads to inhibition of phosphorylated AMPK and ULK1 levels, whereas phosphorylated mTOR levels increase. Conversely, upregulation of σ-1R enhances phosphorylated AMPK and ULK1 levels, while inhibiting phosphorylated mTOR. This suggests that σ-1R may partially promote autophagy via the AMPK/mTOR signaling pathway.

Conclusions

In conclusion, we have provided initial insights into the expression patterns of the autophagy-associated gene σ-1R in individuals with sepsis and sepsis-AKI. Our findings indicated that σ-1R suppressed in sepsis, particularly sepsis-AKI, and elevated σ-1R levels show an inverse relationship with inflammatory cytokine concentrations in the serum, including IL-1β, IL-6, and TNF-α levels. σ-1R appears to regulate cell growth, cell death, and inflammation in LPS-induced HK-2 cells while enhancing autophagy via the AMPK/mTOR signaling pathway. These findings suggest that σ-1R is a potential diagnostic marker and a therapeutic target for sepsis-AKI.

Data Sharing Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Disclosure

The authors state no conflicts of interest in this work.

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