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ATP2B1-AS1 exacerbates sepsis-induced cell apoptosis and inflammation by regulating miR-23a-3p/TLR4 axis

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Abstract
Background: Sepsis is a life-threatening disease with dominant mortality. Its early diagnosis and treatment can improve prognosis and reduce mortality. Long noncoding RNAs (lncRNAs) ATPase plasma membrane Ca2+ transporting 1 antisense RNA 1 (ATP2B1-AS1) is dysregulated and is involved in the progression of various diseases. Nevertheless, the role of ATP2B1-AS1 in sepsis remains unclear.
Methods: A human monocytic cell line, THP-1 cells, was stimulated to induce a model of sepsis *in vitro*. The levels of ATP2B1-AS1, miR-23a-3p, and TLR4 were assessed by real-time quantitative polymerase chain reaction. The role of ATP2B1-AS1 in cell apoptosis and inflammation was explored by flow cytometry, Western blot analysis and enzyme-linked immunosorbent serologic assay. The binding sites between ATP2B1-AS1 and miR-23a-3p, and between miR-23a-3p and TLR4 were predicted by BiBiServ and the Encyclopedia of RNA Interactomes (ENCORI) online sites, respectively, and confirmed by the luciferase assay.
Results: The level of ATP2B1-AS1 was increased in lipopolysaccharide (LPS)-treated THP-1 cells. LPS increased apoptosis ratio, relative protein expressions of pro-apoptotic factors, and relative messenger RNA (mRNA) level and concentrations of pro-inflammatory cytokines, but decreased the relative expression of anti-apoptosis protein and relative mRNA level and concentrations of anti-inflammatory factor. All these alterations were reversed with transfection of shATP2B1-AS1 into THP-1 cells. Moreover, ATP2B1-AS1 directly bound miR-23a-3p and negatively modulated the level of miR-23a-3p. Meanwhile, TLR4 was directly targeted by miR-23a-3p, and negatively and positively modulated by miR-23a-3p and ATP2B1-AS1, respectively.
Conclusion: ATP2B1-AS1 aggravated apoptosis and inflammation by modulating miR-23a-3p/TLR4 axis in LPS-treated THP-1 cells.

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Introduction

Sepsis is a life-threatening disease resulting from dysregulated inflammatory response to infection, which can rapidly progress to multiple organ injury and failure, septic shock, acidosis, and death.[1] Sepsis is associated with dominant mortality, which is estimated as 26.7%.[2] Moreover, it is shown that 41.9% sepsis patients treated in intensive care units (ICU) died before hospital discharge.[2] Despite great advancements in the treatment of sepsis, such as early identification according to clinical biomarkers and characteristics, noninvasive treatment approaches, and clinical administration in non-ICUs, its incidence and mortality remain high.[3,4] Fortunately, early diagnosis and treatment have been demonstrated to improve prognosis and diminish mortality of sepsis.[5] Therefore, it is of great importance to explore more biomarkers and therapeutic targets for the clinical treatment of sepsis.

Long noncoding ribonucleic acids (lncRNAs) are RNAs with more than 200 nucleotides that play important roles in regulating various biological processes.[6] Notably, it has been highlighted that lncRNAs are strongly involved in the diagnosis, treatment, and prognosis of sepsis.[7,8] More importantly, lncRNAs can mediate the messenger RNA (mRNA) stability control and the activation of translation through competitively sponging to microRNAs (miRNAs),[9] thereby participating in a wide range of diseases, including sepsis. For instance, lncRNA FGD5-AS1 attenuates inflammatory response via miR-133a-3p-dependent aquaporin-1 upregulation in lipopolysaccharide (LPS)-elicited sepsis.[10] LncRNA NEAT1 sponges miR-125 to downregulate mast cell expressed membrane protein 1 (MCEMP1) to inhibit immunity during sepsis.[11] LncRNA NKILA reduces cell viability and induces cell apoptosis, inflammation, and autophagy through miR-140-5p/CLDN2 axis in LPS-stimulated sepsis.[12] LncRNA ATPase plasma membrane Ca2+ transporting 1 antisense RNA 1 (ATP2B1-AS1), also known as long intergenic non-protein-coding RNA 936 (LINC00936), is harbored in chr12:89,708,954–89,713,726 with a length of 3626-bp.[13] ATP2B1-AS1 has been identified as one of the differentially expressed lncRNAs using microarray data analysis in colorectal cancer[14] and acute myocardial infarction.[15] Additionally, ATP2B1-AS1 modulates miR-330-5p/TLR4 axis to facilitate cerebral ischemia/reperfusion injury through MyD88/NF-κB signaling pathway.[16] ATP2B1-AS1 regulates endothelial permeability in diabetic retinopathy through miR-4729-dependent IQGAP2 downregulation.[17] ATP2B1-AS1/miR-221-3p/LAMA3 axis modulates the progression of ovarian cancer.[18] However, the role of ATP2B1-AS1 in sepsis is still unclear.

Thus, in order to address the role and the mechanism of ATP2B1-AS1 in sepsis, human monocytic cell line THP-1 cells were challenged with LPS to induce a model of sepsis *in vitro*. The role of ATP2B1-AS1 in apoptosis and inflammation was explored in LPS-induced THP-1 cells. Additionally, the downstream target of ATP2B1-AS1 was identified.

Materials and methods

Cell culture and treatment

THP-1 cells (CL-0233) were obtained from Procell (Wuhan, China) and cultured in Roswell Park Memorial Institute 1640 culture (RPMI-1640, PM150110; Procell) with 10% fetal bovine serum (FBS, 164210-50; Procell), 0.05-mM β-mercaptoethanol (PB180633; Procell), and penicillin-streptomycin (PB180120; Procell) with 5% CO_2 at 37°C. THP-1 cells with a density of 2000 cells per well were plated into 96-well plates and cultured at 37°C. Then, THP-1 cells were treated with LPS (L8880; Solarbio, Beijing, China) for 24 h to establish the sepsis model *in vitro*. In order to screen the optimal concentration of LPS, 0.5-, 1-, and 2-μg/mL LPS was used to incubate THP-1 cells for 24 h.

Cell transfection

Short hairpin RNA (shRNA) targeting ATP2B1-AS1 (shATP2B1-AS1), the corresponding negative control (shNC), miR-23a-3p mimic, NC mimic, miR-23a-3p inhibitor, and NC inhibitor were acquired from GenePharma (Shanghai, China). THP-1 cells were plated into 6-well plates with a density of 5×10^5 cells per well and incubated with 5% CO_2 at 37°C. When cell density reached 70–80%, the shATP2B1-AS1, shNC, miR-23a-3p mimic, NC mimic, miR-23a-3p inhibitor, and NC inhibitor were transfected into THP-1 cells with Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). After transfection for 48 h, cells were collected for the subsequent assays.

Flow cytometry

According to previous reports,[19,20] the apoptosis of THP-1 cells was evaluated by flow cytometry. THP-1 cells were seeded into 12-well plates with a density of 2.5×10^5 cells per well and hatched with 5% CO_2 at 37°C. After being yielded and rinsed with phosphate buffer saline (PBS, P1020; Solarbio), THP-1 cells were stained with Annexin V-FITC apoptosis detection kit (CA1020; Solarbio) and analyzed on a FACScan flow cytometry with the CellQuest software (BD Biosciences, NJ, USA) to determine cell apoptosis.

Real-time quantitative polymerase chain reaction (RT-qPCR)

Following a previous study,[21] THP-1 cells were treated with TRIzol reagent (15596026; Thermo Fisher Scientific, MA, USA) to extract total RNA. Then, total RNA (1 μg) was reverse transcribed into complementary DNA (cDNA) with the PrimeScript RT reagent kit (RR047A; Takara, Dalian, China). RT-qPCR was performed on the A PIKORed 96 (Thermo Fisher) by TB Green TM Premix Ex TaqTM Ⅱ (Tli RNaseH Plus) (RR820A; Takara). The PCR amplification conditions were 94°C for 10 min, 94°C for 10 s, and 55°C for 40 s in 40 cycles. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) and *U6* reference genes were applied as internal references of lncRNA, ATP2B1-AS1, and mRNA and miRNA, respectively. The expression levels of genes were presented with the comparative threshold cycle method ($2^{-\triangle\triangle CT}$ method). Primer sequences are exhibited in Table 1.

Western blot analysis

Western blot assays were carried out based on previous descriptions.[22,23] In brief, THP-1 cells were treated with

Table 1 Primer sequences used in the RT-qPCR.

Name	Forward (5′-3′)	Reverse (5′-3′)
ATP2B1-AS1	GGTAAATCGAGGCCCAGAGAT	ACGTGGATGACAGCGTGTGA
TNF-α	AGGACAGCAGAGGACCAGCTAAGAGG	TGCCACGATCAGGAAGGAGAAGAGG
IL-1β	TGGCGGACTCCAGCTACGAATCTC	TCTGTGGGCAGGGAACCAGCATCT
IL-6	TTCGGTACATCCTCGACGGCATCTCA	GCACAGCTCTGGCTTGTTCCTCACT
IL-10	GCCTAACATGCTTCGAGATC	TGATGTCTGGGTCTTGGTTC
TLR4	AGACCTGTCCCTGAACCCTAT	CGATGGACTTCTAAACCAGCCA
GAPDH	TGACTTCAACAGCGACACCCA	CACCCTGTTGCTGTAGCCAAA
miR-23a-3p	TCTCATATGCAGGAGCCACCA	GCAAGTTGCTGTAGCCTCCTTG
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT

radioimmunoprecipitation assay (RIPA) buffer (R0010; Solarbio), and the protein concentration was assessed by the BCA protein assay kit (PC0020; Solarbio). Protein samples (20 μg) were isolated and electrically transferred onto a polyvinylidene fluoride (PVDF) membrane (EMD Millipore; Billerica, MA, USA). The membranes were sealed with 5% skimmed milk (D8340; Solarbio) for 1 h at room temperature and incubated with the following antibodies overnight at 4°C targeting Bax (1:2000, ab182733; Abcam, Cambridge, UK), Bcl-2 (1:2000, ab196495; Abcam), cleaved Caspase 3 (1:500, ab2302; Abcam), Caspase 3 (1:500, ab13847; Abcam), TLR4 (1:1000, ab13556; Abcam), p-p65 (1:5000, ab86299; Abcam), p65 (1:500, ab16502; Abcam), and β-actin (1:5000, ab8227; Abcam). The membranes were visualized by the ECL Western blotting substrate (PE0010; Solarbio) after being probed with goat anti-rabbit immunoglobulin G (IgG) H&L (HRP, 1:20,000; Abcam) for 2 h at room temperature (PE0010; Solarbio). The gray value was examined by the QUANTITY ONE software (Bio-Rad, Hercules, CA, USA).

Enzyme-linked-immunosorbent serologic assay (ELISA)

Concentrations of inflammatory cytokines in cell culture supernatant were measured by using commercial ELISA kits, including Human tumor necrosis factor-α (TNF-α) ELISA kIT (SEKH-0047; Solarbio), Human Interleukin (IL)-1β ELISA kIT (SEKH-0002; Solarbio), Human IL-6 ELISA kIT (SEKH-0013; Solarbio), and Human IL-10 ELISA kIT (SEKH-0018; Solarbio) following the instructions for use. The absorbance was detected with a microplate reader (Thermo Fisher Scientific) at 450 nm.

Luciferase assay

The binding sites between ATP2B1-AS1 and miR-23a-3p were predicted with BiBiServ online sites (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid?id=rnahybrid_view_submission), while those between miR-23a-3p and TLR4 were predicted with the Encyclopedia of RNA Interactomes (ENCORI) online sites (https://starbase.sysu.edu.cn/). The wild-type (WT) and mutant (MUT) ATP2B1-AS1 or TLR4 were cloned into pGL3-basic luciferase vector (Promega, Madison, WI, USA), and transfected into THP-1 cells. Both cells were then co-transfected with miR-23a-3p mimics or NC mimics (GenePharma) with Lipofectamine 3000 (Invitrogen). The luciferase activity was examined by Promega kit (Promega), after 48 h of transfection in line with the instruction manual.

Statistical analysis

Data were displayed as mean ± standard deviation (SD). The difference was determined by the Student's *t*-test between two groups, or by the one-way analysis of variance (ANOVA) for three or more groups, followed by *post hoc* Bonferroni test using the SPSS 26.0 software (IBM, Armonk, NY, USA). $P < 0.05$ was considered as statistically significant.

Results

ATP2B1-AS1 was highly expressed in LPS-induced THP-1 cells

An *in vitro* cell model of sepsis was first established by treating cells with LPS. In order to screen the optimal concentration of LPS, 0.5-, 1-, and 2-μg/mL LPS was used to incubate THP-1 cells for 24 h. Both 1- and 2-μg/mL LPS treatment significantly increased the early and late stages of apoptosis of THP-1 cells accompanied with a decrease in live cells, while 0.5-μg/mL LPS treatment did not exhibit the same effect on THP-1 cells (Figures 1A and B). Additionally, all three concentrations of LPS had no influence on necrosis cells. Moreover, all three concentrations of LPS markedly enhanced the relative expression of ATP2B1-AS1 in THP-1 cells (Figure 1C). Combining the collected data (Figures 1A–C), it was shown that the level of ATP2B1-AS was higher when the concentration of LPS was 1 μg/mL. When the concentration of LPS was increased, the expression of ATP2B1-AS did not elevate and the apoptosis ratio enhanced, indicating more cell deaths. Therefore, concentration of 1 μg/mL was chosen for the subsequent assays. Altogether, ATP2B1-AS1 was upregulated in LPS-challenged THP-1 cells.

Downregulation of ATP2B1-AS1 inhibited LPS-induced apoptosis of THP-1 cells

In order to investigate the role of ATP2B1-AS1 in sepsis, shRNA targeting ATP2B1-AS1 was prepared. Transfection

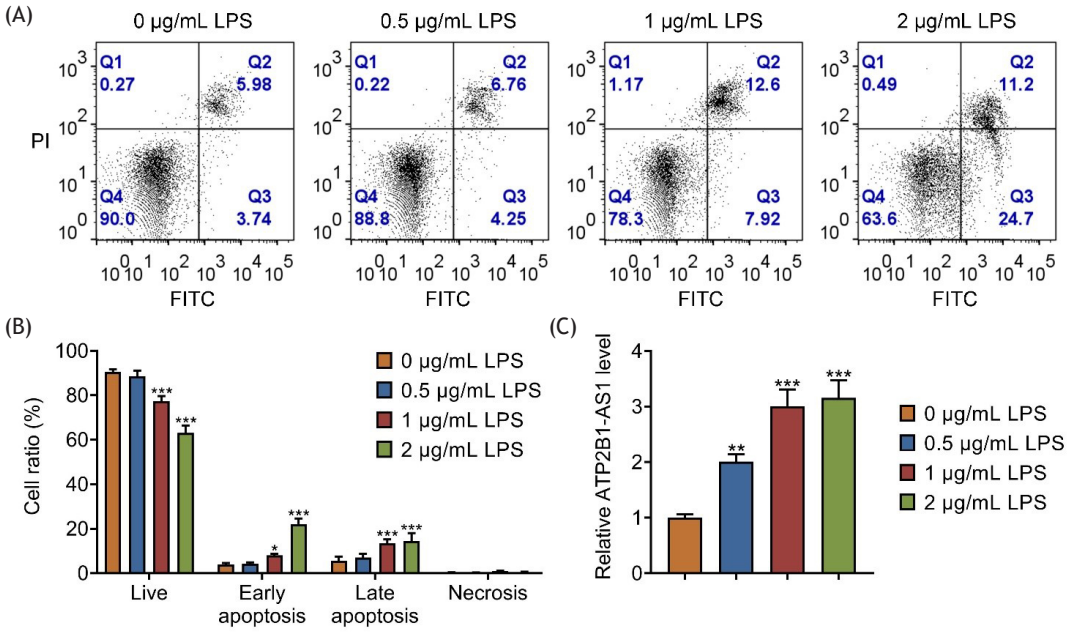

Figure 1 ATP2B1-AS1 was upregulated in LPS-stimulated THP-1 cells. THP-1 cells were treated with 0-, 0.5-, 1-, and 2-μg/mL LPS for 24 h. (A and B) The apoptosis rate was detected by flow cytometry. Among them, cells in the bottom left quadrant Q4 (AnnexinV-FITC)-/PI- were living cells, cells in the bottom right quadrant Q3 (AnnexinV-FITC)+/PI- were early apoptotic cells, cells in the upper right quadrant Q2 (AnnexinV-FITC)+/PI+ were advanced apoptotic cells, and cells in the upper left quadrant Q1 (AnnexinV-FITC)-/PI+ were necrotic cells. (C) The relative expression of ATP2B1-AS1 was examined by RT-qPCR. The data were expressed after normalized with *GAPDH*. **P < 0.01 and ***P < 0.001 vs. 0 μg/mL LPS.

of shATP2B1-AS1 significantly attenuated the LPS-induced relative level of ATP2B1-AS1 (Figure 2A) and the apoptosis ratio in THP-1 cells (Figures 2B and C). Meanwhile, LPS administration signally elevated the relative protein levels of Bax and cleaved Caspase 3/Caspase 3 and decreased the relative protein expression of Bcl-2, which were all notably reversed with the ATP2B1-AS1 silencing (Figure 2D). Therefore, knockdown of ATP2B1-AS1 suppressed apoptosis in LPS-exposed THP-1 cells.

Knockdown of ATP2B1-AS1 reduced LPS-evoked release of inflammatory cytokines in THP-1 cells

Next, the role of ATP2B1-AS1 in inflammation was also explored in LPS-challenged THP-1 cells. The mRNA expressions in LPS-induced THP-1 cells and the concentrations of TNF-α, IL-6, and IL-1β in the cell culture supernatant were prominently upregulated, which were observably counteracted with transfection of shATP2B1-AS1 (Figures 3A and B). In addition, interference of ATP2B1-AS1 significantly rescued the LPS-induced levels of IL-10 in both THP-1 cells and cell culture supernatant (Figures 3A and B). Collectively, silencing of ATP2B1-AS1 attenuated the release of inflammatory cytokines in LPS-induced THP-1 cells.

ATP2B1-AS1 could bind miR-23a-3p

Since lncRNA generally sponge to miRNA to regulate the expression of downstream genes, the potential targets of ATP2B1-AS1 were predicted by BiBiServ online site. Results demonstrated (Figure 4A) that ATP2B1-AS1 could bind miR-23a-3p because of the complementary base pairing. Moreover, direct binding between ATP2B1-AS1 and miR-23a-3p was verified by luciferase assay results, as indicated by a decrease in the relative luciferase activity upon co-transfection of ATP2B1-AS1-WT and miR-23a-3p mimics, but not ATP2B1-AS1-MUT and miR-23a-3p mimics (Figure 4B). Transfection of shATP2B1-AS1 observably reduced the relative ATP2B1-AS1 levels in both THP-1 cells and LPS-challenged THP-1 cells, indicating a viable interfering efficiency of shATP2B1-AS1 (Figure 4C, upper part). Meanwhile, transfection of shATP2B1-AS1 into THP-1 cells markedly increased the relative miR-23a-3p levels, LPS treatment notably deceased the relative miR-23a-3p levels, and interference of ATP2B1-AS1 prominently restored the LPS-induced relative miR-23a-3p levels in THP-1 cells (Figure 4C, lower part). Taken together, ATP2B1-AS1 directly bound miR-23a-3p and negatively modulated the expression of miR-23a-3p.

MiR-23a-3p targeted TLR4

More importantly, the downstream targets of miR-23a-3p were analyzed by ENCORI online sites. TLR4 was predicted to be one of the targets of miR-23a-3p because of complementary base pairing (Figure 5A), which was confirmed by luciferase assay results (Figure 5B). Transfection of miR-23a-3p inhibitors into THP-1 cells effectively decreased the relative miR-23a-3p level but significantly enhanced the relative TLR4 mRNA level (Figure 5C). In addition, suppression of miR-23a-3p prominently enhanced the relative protein

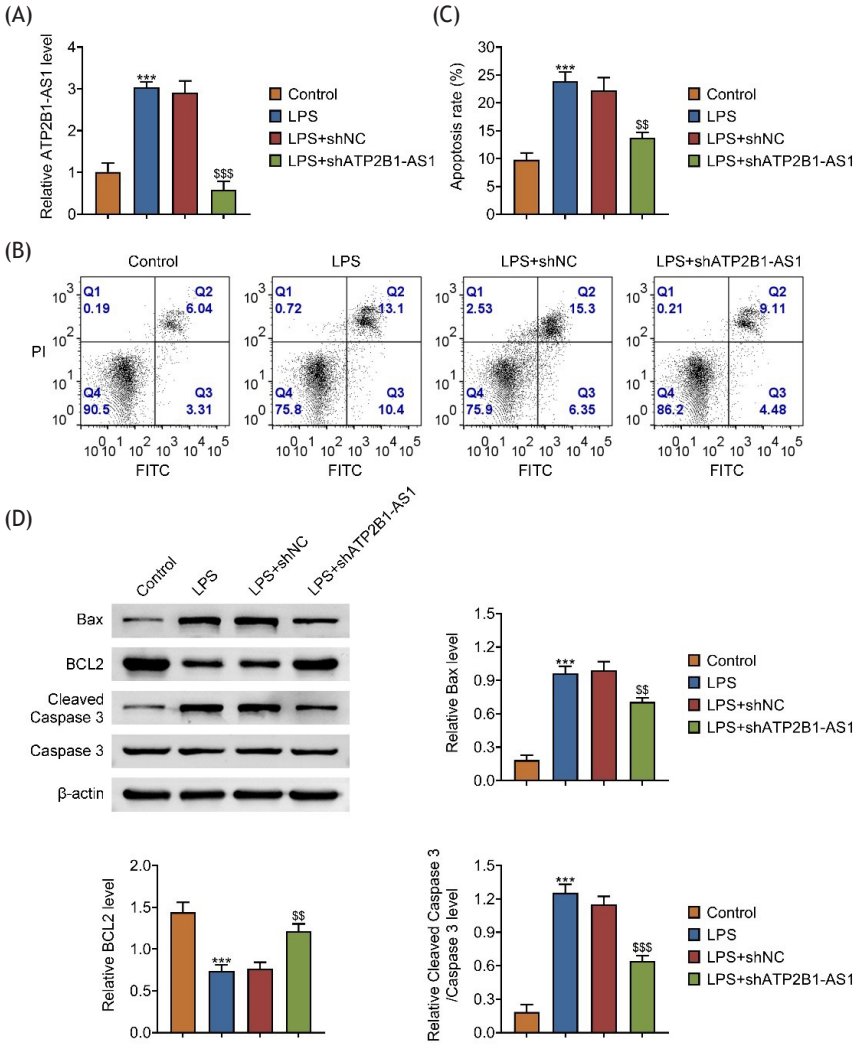

Figure 2 Interference of ATP2B1-AS1 restrained LPS-induced apoptosis of THP-1 cells. THP-1 cells were transfected with shATP2B1-AS1 and stimulated with 1-μg/mL LPS for 24 h. (A) The relative expression of ATP2B1-AS1 was determined by RT-qPCR. The data were expressed after normalization with *GAPDH*. (B and C) The apoptosis ratio was analyzed by flow cytometry. (D) The relative protein expressions of Bax, Bcl-2, cleaved Caspase 3, and Caspase 3 were examined by Western blot analysis. The data were expressed after normalized with β-actin. **P < 0.01 vs. control; $^{\$\$}$P < 0.01 and $^{\$\$\$}$P < 0.001 vs. LPS.

level of TLR4 and its downstream protein p65 (Figure 5D). Moreover, silencing of ATP2B1-AS1 observably reduced the relative TLR4 mRNA level, and LPS induced the relative TLR4 mRNA level in THP-1 cells (Figure 5E). Similar results were also observed in the relative protein expression of TLR4 and its downstream protein p65 (Figure 5F). Briefly, these data demonstrated that TLR4 was targeted by miR-23a-3p and positively regulated by ATP2B1-AS1.

Discussion

In the current study, THP-1 cells were stimulated with LPS to induce an *in vitro* sepsis model. The level of ATP2B1-AS1 was elevated in LPS-treated THP-1 cells. Silencing of ATP2B1-AS1 attenuated the LPS-elicited apoptosis and inflammation in THP-1 cells. Moreover, ATP2B1-AS1 directly bound miR-23a-3p and negatively modulated the level of miR-23a-3p. Meanwhile, TLR4 was directly targeted by miR-23a-3p, and was modulated negatively and positively by miR-23a-3p and ATP2B1-AS1, respectively. Taken together, ATP2B1-AS1 exacerbated apoptosis and inflammation by modulating miR-23a-3p/TLR4 axis in LPS-evoked THP-1 cells.

A large number of lncRNAs have been discovered to be dysregulated in sepsis, which is closely associated with its diagnosis, treatment, and prognosis.[7,8] Upregulation of lncRNA NEAT 1 is interrelated in biochemical indicators, acute respiratory distress syndrome risk, disease severity, and 28-day mortality in sepsis.[24] Circulating lncRNA MALAT1 is upregulated and can indicate disease severity, risk, and survival in patients with sepsis.[25] Circulating lncRNA ZFAS1 is downregulated and is negatively correlated with inflammatory markers, disease severity, and disease risk, and predicts poor prognosis of patients with sepsis.[26] ATP2B1-AS1, a novel lncRNA, is highly expressed in cerebral ischemia/reperfusion injury[16] and myocardial infarction.[13,27] In line with these findings, our results consistently revealed

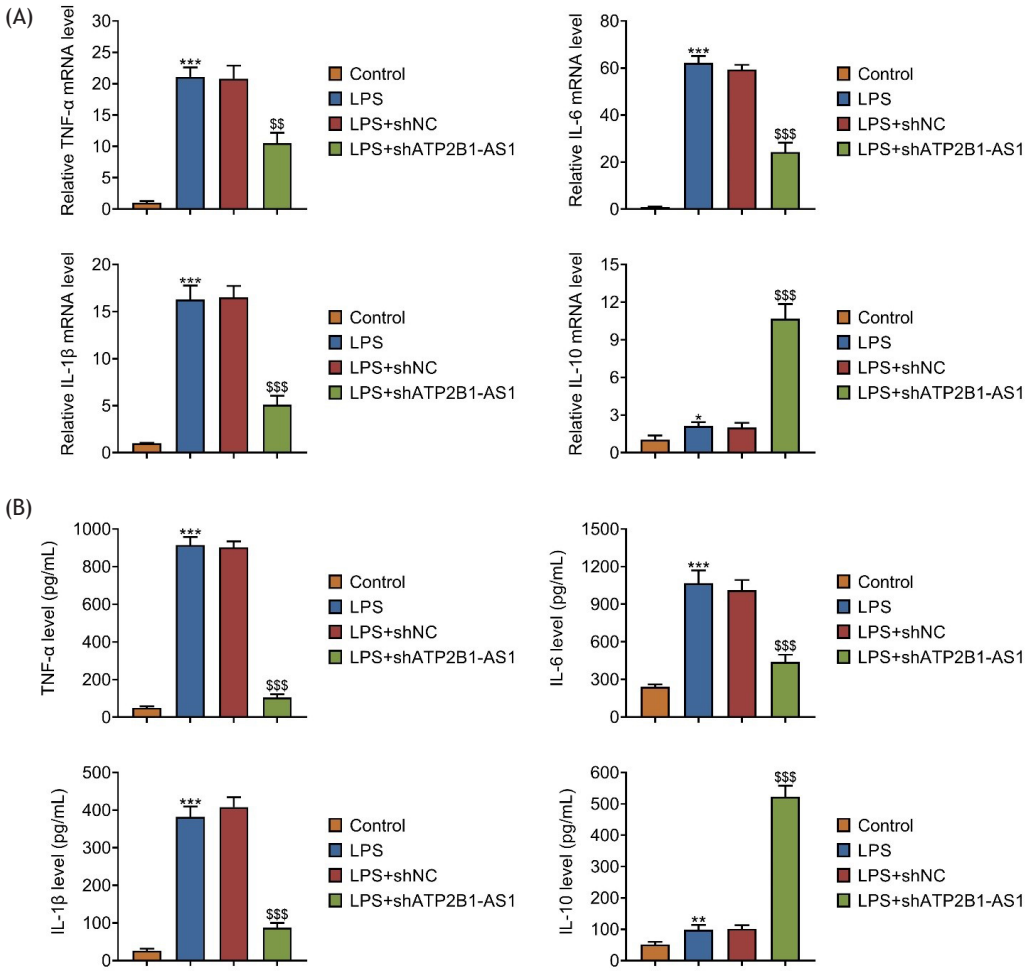

Figure 3 Knockdown of ATP2B1-AS1 diminished LPS-induced release of inflammatory cytokines in THP-1 cells. THP-1 cells were transfected with shATP2B1-AS1 and stimulated with 1-μg/mL LPS for 24 h. (A) The mRNA expression levels of TNF-α, IL-6, IL-1β, and IL-10 were measured by RT-qPCR in THP-1 cells. The data were expressed after normalized with *GAPDH*. (B) The concentrations of TNF-α, IL-6, and IL-1β in cell culture supernatant were detected by ELISA. $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. control; $^{\$\$}P < 0.01$ and $^{\$\$\$}P < 0.001$ vs. LPS.

that the level of ATP2B1-AS1 was enhanced in LPS-induced THP-1 cells. Thus, ATP2B1-AS1 might be a marker for the diagnosis of sepsis, and a potential target for the treatment of sepsis.

Several important pathogenic mechanisms are tightly involved in the development of sepsis, among which inflammation and apoptosis are nonnegligible.[28] Numerous studies have shown that attenuation of inflammation and apoptosis can contribute to the improvement of sepsis. Inhibition of apoptosis and inflammation by knockdown of miR-181a represses sepsis through regulating Nrf2- and NF-κB-signaling pathways.[29] MiR-128-3p can alleviate sepsis by suppressing apoptosis and inflammation by targeting TGFBR2.[30] In addition, lncRNAs also participate in the regulation of apoptosis and inflammation in sepsis. Downregulation of lncRNA GAS5 suppressed apoptosis and inflammation to relieve sepsis through miR-23a-3p/TLR4 axis.[31] Silencing of lncRNA NKILA restrains apoptosis and inflammation by miR-140-5p/CLDN2 axis in LPS-evoked sepsis.[12] Here, LPS evoked an increase in the apoptosis ratio and the levels of pro-apoptotic factors and pro-inflammatory cytokines with the decreased levels of anti-apoptosis protein and anti-inflammatory factor, which were reversed by the interference of ATP2B1-AS1 in THP-1 cells. Similar results, that downregulation of ATP2B1-AS1 dampens apoptosis and the inflammation cytokines level, have been verified in myocardial infarction[13] and cerebral ischemia/reperfusion injury.[16] Collectively, these outcomes elucidated that ATP2B1-AS1 restrained apoptosis and inflammation in LPS-stimulated THP-1 cells.

LncRNA can sponge to miRNAs to competitively impede their function, thereby modulating the expression level of miRNA target genes.[32] Here, we found that ATP2B1-AS1 directly bound miR-23a-3p and negatively modulated the level of miR-23a-3p. MiR-23a-3p has been revealed to be downregulated in LPS-treated RAW264.7 cells and patients with sepsis.[33] More importantly, miR-23a-3p has been highlighted to be sponged by different lncRNAs to take part in a variety of diseases. For instance, lncRNA PAHRF sponges miR-23a-3p to mediate proliferation and apoptosis in pulmonary arterial hypertension.[34] LncRNA GAS5 sponging miR-23a-3p induces apoptosis and inflammation in sepsis.[31] Also,

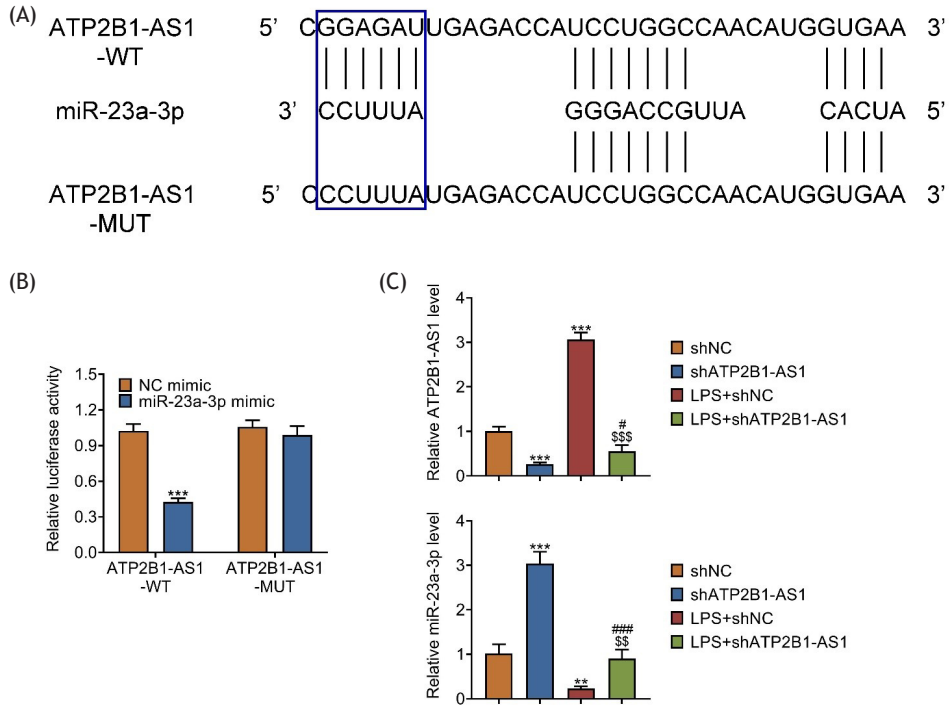

Figure 4 ATP2B1-AS1 directly bound miR-23a-3p but negatively modulated the level of miR-23a-3p. (A) The binding sites between ATP2B1-AS1 and miR-23a-3p were predicted by BiBiServ online sites. (B) The direct binding between ATP2B1-AS1 and miR-23a-3p was examined by luciferase assay. ***P < 0.001 vs. NC mimic. (C) The relative levels of ATP2B1-AS1 and miR-23a-3p were detected by RT-qPCR in THP-1 cells. The data were expressed after normalized with *GAPDH* or *U6*. **P < 0.01 and ***P < 0.001 vs. shNC; $^{\$\$}$P < 0.01 and $^{\$\$\$}$P < 0.001 vs. shATP2B1-AS1; $^{\#}$P < 0.05 and $^{\#\#\#}$P < 0.001 vs. LPS+shNC.

our results showed that miR-23a-3p could further directly target TLR4, and negatively regulate the TLR4 expression. The direct targeting relationship between miR-23a-3p and TLR4 has been verified in LPS-treated THP-1 cells, in which upregulation of TLR4 dampens the suppressive effects of miR-23a-3p upregulation on LPS-evoked apoptosis and inflammation.[31] TLR4 is identified as a prominent risk of posttraumatic sepsis.[35] TLR4 expression combined with C-reactive protein is a reliable biomarker for the diagnosis of late-onset neonatal sepsis.[36] In addition, TLR4, as one of the classical pattern recognition receptors, can be recognized by LPS to induce the activation of downstream signaling pathway, such as nuclear factor-κB (NF-κB) signaling pathway. TLR4/NF-κB pathway is closely related to inflammation and apoptosis.[37,38] Activated TLR4 can induce the release of inflammatory cytokines, including IL-1β and TNF-α, which are mediated by NF-κB.[39] TLR4/NF-κB pathway also plays a significant role in different diseases, including sepsis.[40,41] In the current study, LPS enhanced the relative protein level of p-p65/p-65 in THP-1 cells, which indicated the activation of NF-κB pathway in sepsis. Furthermore, suppression of ATP2B1-AS1 decreased the LPS-induced relative protein level of p-p65/p-65 in THP-1 cells. Thus, in combination with the results that suppression of miR-23a-3p elevated the relative protein level of p-p65/p-65, the results indicated that the role of ATP2B1-AS1/miR-23a-3p/TLR4 axis in LPS-challenged THP-1 cells was closely related to the NF-κB pathway. Taken together, the role of ATP2B1-AS1 in LPS-treated THP-1 cells was related to the miR-23a-3p/TLR4 axis.

Conclusion

Our results suggested that the level of ATP2B1-AS1 was enhanced in LPS-induced THP-1 cells. ATP2B1-AS1 contributed to apoptosis and inflammation of LPS-challenged THP-1 cells by regulating miR-23a-3p/TLR4 axis. However, several limitations remain to be discussed in the future research. An *in vivo* model and *in vivo* experiments need to be conducted in the future study. In addition, the role of NF-κB pathway can be explored in the regulation of ATP2B1-AS1/miR-23a-3p/TLR4 axis in LPS-stimulated THP-1 cells. Briefly, our study identified that ATP2B1-AS1 might be a marker for the diagnosis of sepsis as well as a potential target for its treatment.

Funding

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Competing interests

The authors stated that there was no conflict of interest to disclose.

Ethics approval

This research had no human or animal participants to perform experiments by any of its authors.

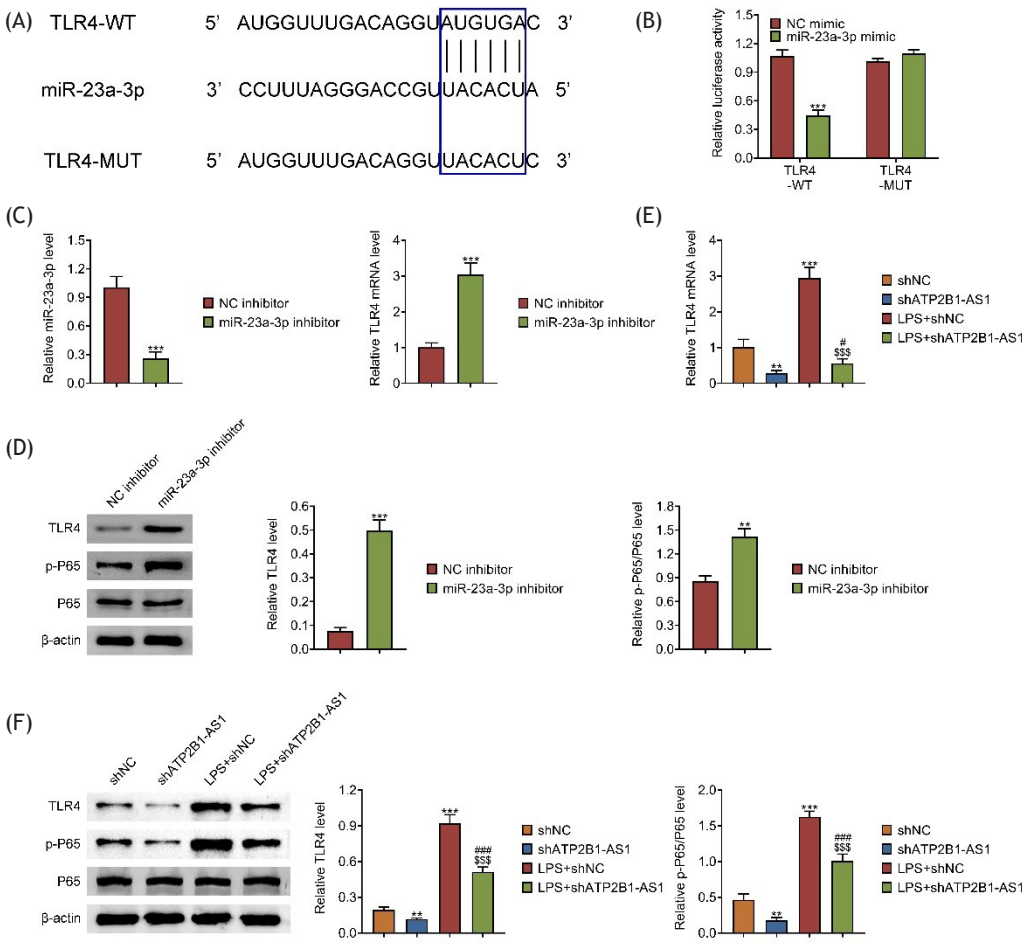

Figure 5 MiR-23a-3p targeted TLR4. (A) The binding sites between miR-23a-3p and TLR4 were predicted by ENCORI online sites. (B) The direct binding between miR-23a-3p and TLR4 was determined by luciferase assay. ***P < 0.001 vs. NC mimic. (C) The relative levels of miR-23a-3p and TLR4 were examined by RT-qPCR in THP-1 cells. The data were expressed after normalized with *U6* or *GAPDH*. ***P < 0.001 vs. NC inhibitor. (D) The relative protein expressions of TLR4, p-p65, and p65 were detected by Western blot analysis. The data were expressed after normalized with β-actin. **P < 0.01 and ***P < 0.001 vs. NC inhibitor. (E) The relative levels of TLR4 were measured by RT-qPCR in THP-1 cells. The data were expressed after normalized with *GAPDH*. **P < 0.01 and ***P < 0.001 vs. shNC; $^{\$\$\$}$P < 0.001 vs. shATP2B1-AS1; $^{\#}$P < 0.05 vs. LPS+shNC. (F) The relative protein expressions of TLR4, p-p65, and p65 were detected by Western blot analysis. The data were expressed after normalized with β-actin. **P < 0.01 and ***P < 0.001 vs. shNC; $^{\$\$\$}$P < 0.001 vs. shATP2B1-AS1; $^{\#\#\#}$P < 0.001 vs. LPS+shNC.

Consent to participate statement

Written informed consent was obtained from a legally authorized representative(s) of an anonymized patient for information published in this study.

Data availability

The authors declare that all data supporting the findings of this study are available within the paper, and any raw data can be obtained from the corresponding author upon request.

Author Contributions

Xin Yang and Liqun Lu designed and carried the study. Xin Yang, Liqun Lu, Chan Wu, and Feng Zhang supervised data collection, and analyzed and interpreted the collected data. Xin Yang prepared the manuscript and reviewed its draft for publication. All authors had read and approved the final manuscript.

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