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Original Article

Molecular mechanism of miRNA-23a in sepsis-induced lung injury

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Abstract: Objective: MicroRNA-23a-3p (miR-23a) is a novel gene regulator involved in inflammation. This study aimed to explore the molecular mechanism of miR-23a in sepsis-induced lung injury both *in vitro* and *in vivo*. Methods: Lipopolysaccharide (LPS)- and ATP-stimulated human myeloid leukemia mononuclear cells (THP-1) and Human Bronchial Epithelial Cells (BEAS-2B) cell lines were used, while cecal ligation and puncture (CLP)-induced sepsis BABL/c mice were constructed. The mRNA expression levels of interleukin (IL)-18, IL-1β, and miR-23a were determined, and Western blotting was used to measure CXCR4/PTEN/PI3K/AKT signaling. The concentrations of cytokines and Nod-like receptor family pyrin domain-containing 3 (NLRP3) were determined using an enzyme-linked immunosorbent assay. Lung tissue of mice was subjected to hematoxylin-eosin staining for examining myocardial injury. Results: MiR-23a inhibited NLRP3 inflammasome activation in LPS- and ATP-stimulated THP-1 and BEAS-2B cells ($P<0.05$). Overexpression of miR-23a decreased the lactate dehydrogenase release rate in the cells ($P<0.05$). Meanwhile, miR-23a overexpression decreased the concentration and gene expression of IL-1β and IL-18 in CXCR4 positive cells ($P<0.05$). Conversely, miR-23a knockdown increased the concentration and gene expression of IL-1β and IL-18 ($P<0.05$). Additionally, PTEN and p53 proteins were up-regulated in miR-23a mimic group and down-regulated in miR-23a inhibitor group ($P<0.05$). Furthermore, miR-23a expression was decreased in sepsis-induced lung injury mice ($P<0.05$). MiR-23a overexpression reduced the sepsis-induced lung injury probably by inhibiting acetylcholinesterase activity and expression levels of IL-1β, IL-18, capase-1, and NLRP3 ($P<0.05$). Conclusion: miR-23a can significantly alleviate sepsis-induced lung injury in CLP-induced septic mice and LPS-stimulated cell lines by suppressing NLRP3 inflammasome activation and inflammatory response, while promoting the CXCR4/PTEN/PI3K/AKT pathway.

Keywords: Sepsis, miR-23a, NLRP3 inflammasome, inflammation, CXCR4, PTEN/PI3K/AKT

Introduction

Sepsis can be caused by excessive immune response to pathogen-induced infections [1]. Sepsis-induced acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are the leading causes of death in intensive care units [2]. In case of systemic inflammatory response syndrome, the recruitment and activation of pulmonary macrophages and pulmonary neutrophils promote the uncontrolled release of numerous pro-inflammatory factors and mediators, leading to ARDS [3]. Studies have found that when patients develop sepsis, several inflammatory factors and inflammatory mediators are either released in large quantities or are continuously activated. Therefore, the balance between pro-inflammatory factors and anti-inflammatory factors is disturbed, which causes impaired immune function and lung tissue damage [4]. The main cause of lung injury is inflammation caused by infection. Neutrophils, alveolar macrophages, alveolar epithelial cells, and lymphocytes contribute to the development of inflammation. In sepsis, proinflammatory cytokines and inflammatory mediators are absent, including tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, IL-8, and nitric oxide [5]. Anti-inflammatory cytokines such as IL-4, IL-10, IL-13, and IL-17 are involved in sepsis and sepsis-related lung injury [6]. Exploring the biological markers of sepsis-induced ALI is important for early diagnosis, as well as evaluation of treatment efficacy and prognosis.

miR-23 is highly conserved among species and can regulate various disease processes, such as cancer, inflammation, ischemia-reperfusion injury, and cognitive impairment [7-9]. Our previous study has confirmed that miRNA-23a-3p (miR-23a) can improve sepsis-induced lung injury through the PTEN/PI3K/AKT/p53 pathway. Meanwhile, other studies have shown that miR-23a can regulate the activation of Nod-like receptor family pyrin domain-containing 3 (NLRP3) inflammasomes in the inflammatory response of spinal glial cells through chemokine CXC receptor 4 (CXCR4) [10]. However, the role and molecular mechanisms of miR-23a in regulating NLRP3 inflammasome activation are not fully understood. It is also unclear whether the protective effect of miR-23a on sepsis-induced lung injury model mice is related to the activation of NLRP3 inflammatory bodies. In the present study, we aimed to explore the molecular mechanisms of miR-23a in sepsis-induced lung injury both *in vitro* and *in vivo*.

Materials and methods

Cell culture and treatment

THP-1 (Ber004-J3) and BEAS-2B (Ber004-D4) cell lines were purchased from BersinBio (Guangzhou, China) and cultured in modified RPMI-1640 (SH30909.01; HyClone, USA) containing 10% fetal bovine serum (FBS; FS101-02; TransGen Biotech) and penicillin-streptomycin (60162ES76; Yeasen, China) in an incubator at 37°C with 5% CO_2. When the cell confluence reached 70-80%, the cells were stimulated with 100 nM phorbol-12-myristate-13-acetate (PMA; P1585; Sigma-Aldrich, USA) for 24 h, and then cultured with 2 mL complete medium containing 1 μg/mL Lipopolysaccharide (LPS; L8880; Solarbio, Beijing, China) for 5.5 h. Subsequently, cells were treated with 5 mmol adenosine 5'-triphosphate (ATP) disodium salt hydrate (A1852; Sigma, St. Louis, MO, USA) for 0.5 h and cultured with 2 mL fresh complete medium for additional 24 h.

Cell transfection

Hsa-miR-23a-3p mimic (AUCACAUUGCCAGGGAUUUCC), mimic NC (UCACAACCUCCUAGAAAGAGUAGA), hsa-miR-23a-3p inhibitor (GGAAAUCCCUGGCAAUGUGAU), and inhibitor NC (UCUACUCUUUCUAGGAGGUUGUGA) were transfected into THP-1 and BEAS-2B cells using Hieff TransTM liposome transfection reagent (4080-2ES03; Yeasen, China). Briefly, 6 μL reagent was added to 60 μL serum-free medium and incubated at room temperature for 5 min. Next, 5 μL miRNA (20 μg) was added into the mixture and incubated for another 10 min. Finally, the mixture was added to the cells and cultured for 48 h.

Immunofluorescence (IF)

The cells in a 6-well plate were fixed in 4% paraformaldehyde (PFA) at room temperature for 30 min and treated with 1 mL 0.5% TritonX-100 for 20 min. After washing the cells thrice with PBS, 5% bovine serum albumin (BSA) blocking solution (AR0004; Boster Bioengineering Co., Ltd., Wuhan, China) was added, followed by incubation at room temperature for 30 min. Then, cells were incubated with primary antibodies anti-CXCR4 (Proteintech, IL, USA) at 4°C overnight, followed by 30 min of incubation with Alexa Fluor-labeled Goat anti-rabbit IgG (H + L) secondary antibody (A0468; Beyotime, Guangzhou, China) at room temperature. The cells were then stained with 4,6-Diamidino-2-phenylindole dihydrochloride (DAPI; AR1176; Boster Bioengineering Co., Ltd., Wuhan, China) in the dark for 10 min. Finally, the anti-fluorescence attenuation sealing agent (AR1109; Boster Bioengineering Co., Ltd., Wuhan, China) was added, and the cells were observed under a laser confocal microscope (LSM800; Carl Zeiss, Oberkochen, Germany).

Lactate dehydrogenase (LDH) release

Lactate dehydrogenase (LDH) release was measured using LDH cytotoxicity test kits (C0017; Beyotime, Guangzhou, China). Briefly, 24 h after the cells were transfected with miR-23a mimic, mimic negative control (NC), miR-23a inhibitor, and inhibitor NC, the supernatant was collected and centrifugated at 1000 rpm for 5 min. Next, 50 μL supernatant was added in 96-well plates, and 50 μL LDH detection working fluid was added to the plates, which were then incubated at room temperature in the dark for 30 min. Subsequently, 50 μL LDH stop solution was added, and the absorbance was measured under a microscope at 490 nm.

Real-time fluorescent quantitative PCR (qRT-PCR)

The mRNA expression of IL-18, IL-1β, and miR-23a was determined using qRT-PCR. Briefly,

total cellular RNA was extracted from cells using RNeasy kit (Qiagen, Valencia, CA, USA) and reverse-transcribed into cDNA. Thereafter, qRT-PCR was performed using the ABI-Prism 7300 system (Applied Biosystem, Waltham, MA, USA) according to the manufacturer's instructions. The PCR thermocycling conditions included pre-denaturation at 95°C for 5 min, 40 amplification cycles consisting of denaturation at 95°C for 15 s, primer annealing at 60°C for 20 s, and primer extension at 72°C for 20 s. U6 was used as the internal control for miR-23a, and GAPDH was used as the internal control for IL-18 and IL-1β. Expression of miR-23a was analyzed using the $2^{-\Delta\Delta Ct}$ method [11]. The primers used in this study were as follows: IL-18: TGGCTGCTGAACCAGTAGAAG (Forward), GAGGCCGATTTCCTTGGTCA (Reverse); IL-1β: CCACCTCCAGGGACAGGATA (Forward), TCAACACGCAGGACAGGTAC (Reverse); GAPDH: AACGGATTTGGTCGTATTGGG (Forward), CCTGGAAGATGGTGATGGGAT (Reverse); miR-23a: ATCACATTGCCAGGGATTTCC; and U6: CTCGCTTCGGCAGCACA (Forward), AACGCTTCACGAATTTGCGT (Reverse).

Magnetic bead separation

BEAS-2B and THP-1 cell lines were collected and resuspended with sorting buffer. After cells were incubated with primary anti-F4/80 at 4°C for 30 min and washed with sorting buffer, and the cell concentration was adjusted to 1×10^8/mL. Next, 80 μL cell suspension and 20 μL anti-rabbit IgG microbeads were added and incubated at 4°C for 15 min. After incubation, cells were centrifuged at 300 g for 10 min and resuspended with 500 μL sorting buffer. The resulting homogenate was subjected to magnetic bead separation using an autoMACS Pro Separator. Briefly, 15 μL magnetic beads (Invitrogen, CA, USA) were added into the cell homogenate and incubated for 30 min at 37°C with mild shaking using a ThermoMixer (MultiTherm, Benchmark, USA). Then, the magnetic beads were magnetically separated from the mixture and re-dissolved in 60 μL PBS. Consequently, the magnetically labeled cells were obtained.

Enzyme linked immunosorbent assay (ELISA)

Protein expression of capase-1 (Bes11052M; BersinBio, China), NLRP3 (Bes11072M; BersinBio, China), IL-6 (Bes11002M; BersinBio, China), IL-1β (Bes11001H; BersinBio, China), IL-18 (Bes11009H; BersinBio, China), and TNF-α (Bes11026M; BersinBio, China) were measured using ELISA detection kits by following the manufacturer's instructions.

Western blot

Protein expression of ASC, pro-caspase-1 + p10 + p12, caspase-1 + p10, PTEN, PI3K, AKT, and p53 in cells were measured using Western blotting analysis. Briefly, cells were lysed with radioimmunoprecipitation (RIPA) assay lysis buffer (Thermo Fisher Scientific, Inc., Waltham, MA, USA), and the protein concentration was measured using a bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Then, 20 μg of protein was separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% gel, and transferred to a polyvinylidene fluoride (PVDF) membrane (ISEQ0001; EMD Millipore, Billerica, MA, USA). Following blocking with 5% BSA for 12 h at 4°C, the membranes were incubated with primary antibodies anti-ASC (10500-1-AP; Proteintech, IL, USA), pro-caspase-1 + p10 + p12 (ab179515; Abcam, MA, USA), caspase-1 + p10 (PA5-105049; Thermo Fisher, Waltham, MA, USA), PTEN (22034-1-AP; Proteintech, IL, USA), PI3K (60225-1-Ig; Proteintech, IL, USA), AKT (10176-2-AP; Proteintech, IL, USA), and p53 (60283-2-Ig; Proteintech, IL, USA). Further, they were incubated with HRP conjugate goat anti-rabbit IgG (H + L) (SA00001, Proteintech, IL, USA) for 2 h at room temperature. The membranes were then visualized using enhanced chemiluminescence (ECL) reagent (Amersham; GE Healthcare, Chicago, IL, USA). Meanwhile, the band intensities were analyzed using Quantity One software (Bio-Rad, CA, USA).

Establishment of sepsis-induced lung injury mice model

BABL/c male mice were randomly divided into five groups with three mice in each group: sham operation control group (Ctrl), cecal ligation and puncture (CLP)-induced lung injury group (CLP), transfected miR-23a agomir group (CLP + miR-23a), transfected NC group (CLP + NC), and simple transfection reagent group (CLP + PEI). Briefly, mice were fed in cages at room temperature (25 ± 2)°C, with relative humidity of (50 ± 5)%, and a light and dark circle of 12 h/12 h each day. During the 7 days prior to the experiment, the mice had free access to pel-

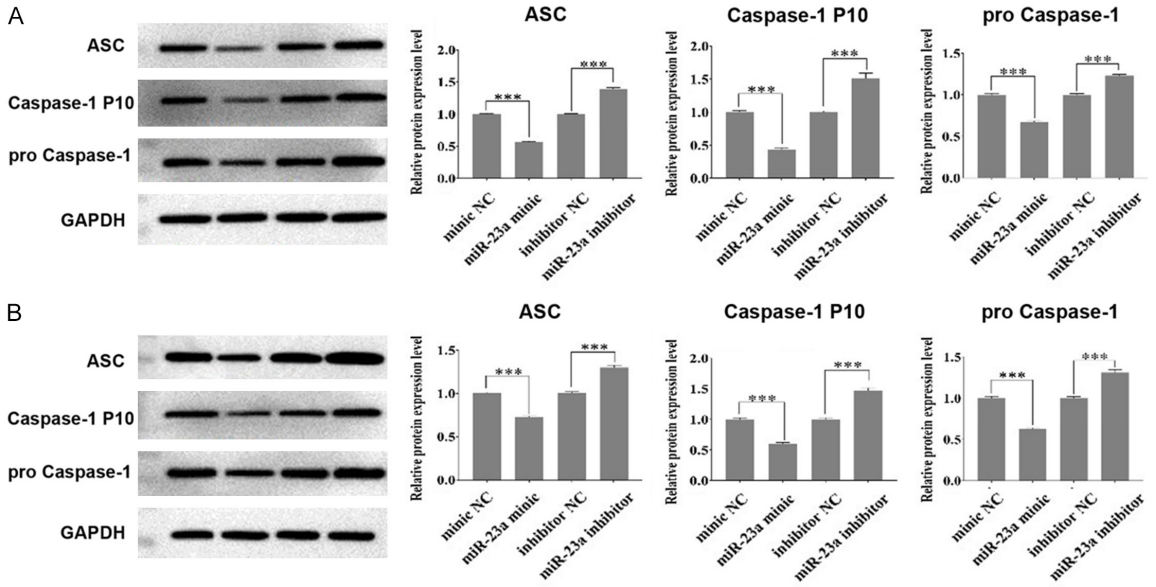

Figure 1. Effects of miR-23a on NLRP3 inflammasome activation. Western blot of LPS- and ATP-stimulated THP-1 (A) and BEAS-2B (B) cells. The experiment was performed in triplicates. Compared with matched group, *P<0.05, ***P<0.001. NLRP3: nod-like receptor family pyrin domain-containing 3; ASC: caspase-1 recruitment domain; LPS: lipopolysaccharide.

leted feed and water. One hour before the CLP operation, mice were treated with miR-23 mimic, mimic NC, and transfection reagent via tail vein injection. The 4 μL plasmids (5 μg/μL in RNA free H_2O) were mixed with 3.2 μL *in vivo*-jet PEITM (PT-201-10G, Polyplus-transfection, Strasbourg, France) and 5% glucose solution to obtain a total volume of 50 μL, which was intravenously injected into mice via tail veins. In the CLP model group and CLP + miR-23a group, the cecum of mice was punctured, and an appropriate amount of intestinal content was extruded. The cecum was then placed back in the abdominal cavity, and the incision was sutured. In the Crtl group, the cecum was pulled out and retracted. Lung samples were collected 24 h after the CLP. The left lung tissue solution of each group of mice with bronchoalveolar lavage fluid (BALF) was collected and stored at -80°C. The right lung of the mice in each group without BALF was directly removed, fixed with formaldehyde, and stored at 4°C.

Hematoxylin-eosin (H&E) staining

The formaldehyde-fixed lung tissues of mice in Ctrl, CLP, CLP + miRNA-23a, CLP + NC, and CLP + PEI groups were washed slowly with running water for 4-8 h. The embedding pieces were then placed in 70% ethanol overnight. In the morning, these pieces were first soaked in 80%, 90%, 100% A, and 100% B ethanol for 1 h and in xylene A and xylene B for 30 min until the tissue mass became brown, yellow, or dark red and transparent. Subsequently, the pieces were placed in paraffin tanks A, B, and C for 1.5 h (60°C) and embedded in the embedding instrument B for at least 30 min. The 4 μm thick slices obtained using a Leica slicer were stained using H&E staining.

Acetylcholinesterase (AchE) activity

AchE activity in lung tissue was measured using AchE activity assay kits (D799813-0050, Sangon Biotech (Shanghai) Co., Ltd., Shanghai, USA). Briefly, 25 g lung tissue of mice was homogenized in extraction buffer and centrifuged at 4°C at a speed of 8000 g for 10 min. Subsequently, the supernatants were collected. Then, 1-5 μL of supernatant was added into a 96-well plate, and the volume was adjusted to 50 μL using the AChE Assay Buffer. Absorbance was measured under a microplate reader at 412 nm.

Statistical analysis

All experiments were performed in triplicates, and all data were processed using the Statistical Product and Service Solutions (SPSS)

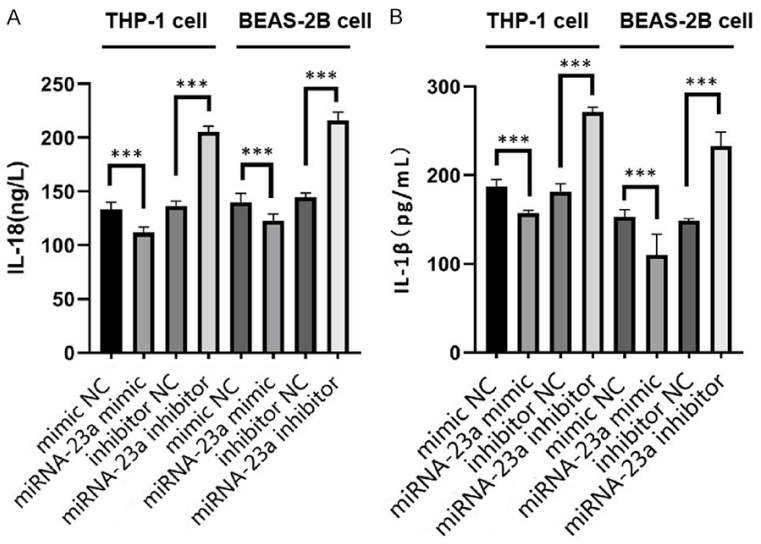

Figure 2. Effects of miR-23a on inflammatory factor release. IL-18 (A) and IL-1β (B) expression in the LPS- and ATP-stimulated THP-1 and BEAS-2B cells. The experiment was conducted in triplicates. Compared with matched group, *P<0.05, ***P<0.001. NC: negative control; IL: Interleukin; LPS: lipopolysaccharide; ATP: Adenosine triphosphate.

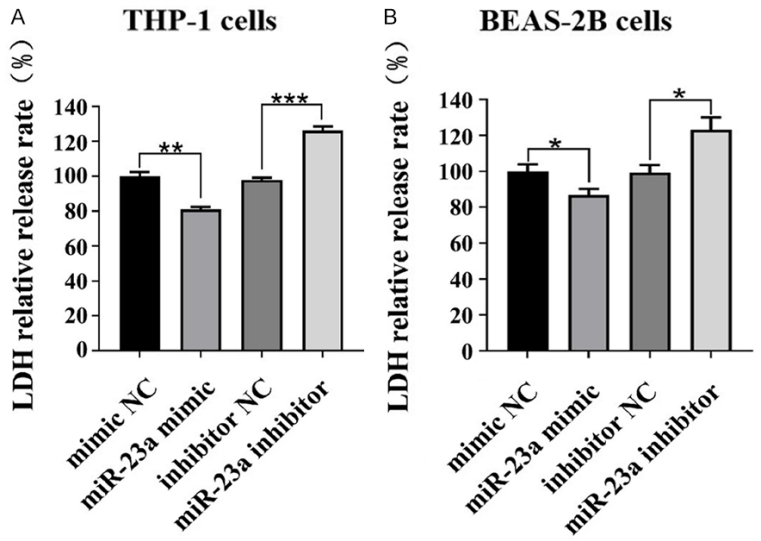

Figure 3. Effects of miR-23a on LDH release rate. LDH release rate in the LPS- and ATP-stimulated THP-1 (A) and BEAS-2B (B) cell lines. The experiment was conducted in triplicates. Compared with matched group, *P<0.05, **P<0.01, ***P<0.001. LDH: lactate dehydrogenase; NC: negative control.

software version 21.0 (SPSS Inc., Chicago, IL, USA). Data were represented as mean ± standard deviation (SD). The Student's t-test for two groups or one-way analysis of variance (ANOVA) combined with a post hoc Bonferroni test for multiple groups was used for comparison (*P*-value <0.05 was considered statistically significant).

Results

MiR-23a suppresses NLRP3 inflammasome activation

To investigate the effects of miR-23a on NLRP3 inflammasome activation in LPS- and ATP-stimulated cells, protein expression of caspase-1 recruitment domain (ASC), caspase-1 P10, and pro-caspase-1 were detected using Western blotting. Compared with the control group, protein expression of ASC, caspase-1 P10, and pro-caspase-1 were significantly decreased in the miR-23a mimic group and increased in the miR-23a inhibitor group in THP-1 (**Figure 1A**) and BEAS-2B (**Figure 1B**) cells ($P<0.01$), indicating that miR-23a suppressed NLRP3 inflammasome activation.

MiR-23a inhibits inflammatory factor release

The effects of miR-23a on inflammatory factor release were determined using ELISA (**Figure 2**). The results showed that the concentrations of IL-18 (**Figure 2A**) and IL-1β (**Figure 2B**) in the miR-23a mimic group significantly decreased in LPS- and ATP-stimulated THP-1 and BEAS-2B cells compared to those in the mimic NC group ($P<0.01$). In contrast, the concentration of IL-18 in the miR-23a inhibitor group significantly increased as compared with that in the inhibitor NC group ($P<0.01$).

MiR-23a inhibits LDH release rate

The effect of miR-23a on inflammatory factor release was measured in LPS- and ATP-stimulated THP-1 (**Figure 3A**) and BEAS-2B (**Figure 3B**) cells. Compared with the control group, the LDH release rate was significantly decreased following overexpression of miR-23a

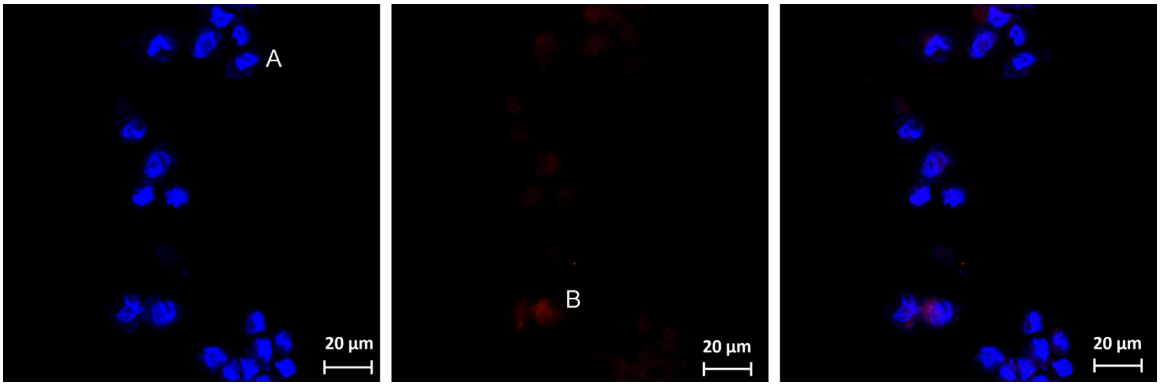

Figure 4. Representative immunofluorescence images of THP-1 and BEAS-2B cells showing CXCR4-positive and CXCR4-negative after magnetic bead separation (× 400). The location of F40/80 (A) and CXCR4 (B) proteins in LPS and ATP-stimulated CXCR4-positive and CXCR4-negative THP-1 and BEAS-2B cells. LPS: lipopolysaccharide; ATP: Adenosine triphosphate; CXCR4: CXC chemokine receptor 4.

but increased after inhibition of miR-23a (P<0.05).

Effects of miR-23a/CXCR4 on inflammatory factor release

To evaluate the molecular mechanism of miR-23a on inflammatory factor release, CXCR4-positive and CXCR4-negative cells were separated using the magnetic beads. As shown in **Figure 4**, F40/80 (blue) and CXCR4 proteins (red) were mainly localized in the cytoplasm. In CXCR4 positive cells, the intracellular concentrations of IL-1β and IL-18 precursor (**Figure 5A**) and cell supernatant production of mature IL-1β and IL-18 (**Figure 5B**) were significantly decreased in the miR-23a mimic group while increased in the miR-23a inhibitor group as compared with the control group (P<0.01). Similarly, the gene expression of IL-1β and IL-18 were significantly down-regulated in the miR-23a mimic group but up-regulated in the miR-23a inhibitor group when comparing with the control group (P<0.01; **Figure 5C**). There were no significant differences in the expression of IL-1β and IL-18 among all groups in CXCR4 negative cells (P>0.05; **Figure 5A**, **5B** and **5D**).

Effects of miR-23a/CXCR4 on the activation of the PTEN/PI3K/AKT signaling pathway

To investigate the effects of miR-23a/CXCR4 on sepsis-related PTEN, PI3K, and AKT expression levels, CXCR4 positive THP-1 and BEAS-2B cell were transfected with miR-23a after stimulation with LPS. As shown in **Figure 6**, protein expression of PTEN and p53 were significantly up-regulated in the miR-23a mimic group and down-regulated in the miR-23a inhibitor group when comparing with the control group (P< 0.01). It is indicated that miR-23a overexpression inhibited PI3K protein levels, and miR-23a knockdown increased PI3K expression levels (P<0.01). In contrast, there was no significant difference in the expression of AKT protein among each group of CXCR4-positive cells (P>0.05; **Figure 6A**). Furthermore, no significant difference was found in the expression of PTEN, PI3K, AKT, and p53 proteins in CXCR4-negative cells (P>0.05; **Figure 6B**).

Decreased expression of miR-23a in sepsis-induced lung injury mice

Expression of miR-23a-3p in lung tissue of sepsis-induced lung injury BABL/c mice was measured using qRT-PCR. As shown in **Figure 7**, miR-23a-3p expression was significantly decreased in CLP, CLP + NC, and CLP + PEI groups when comparing with the Ctrl group (P<0.01), indicating the downregulation of miR-23a in sepsis-induced lung injury mice. After miR-23a was intravenously injected into mice, the expression of miR-23a was obviously increased in the CLP group as compared with that in the CLP + NC and CLP + PEI groups (P<0.01).

MiR-23a reduces sepsis-induced lung injury in mice

H&E staining results suggested that the integrity of lung tissue was destroyed, and the alveolar wall and tracheal wall were significantly thickened in the CLP group as compared with

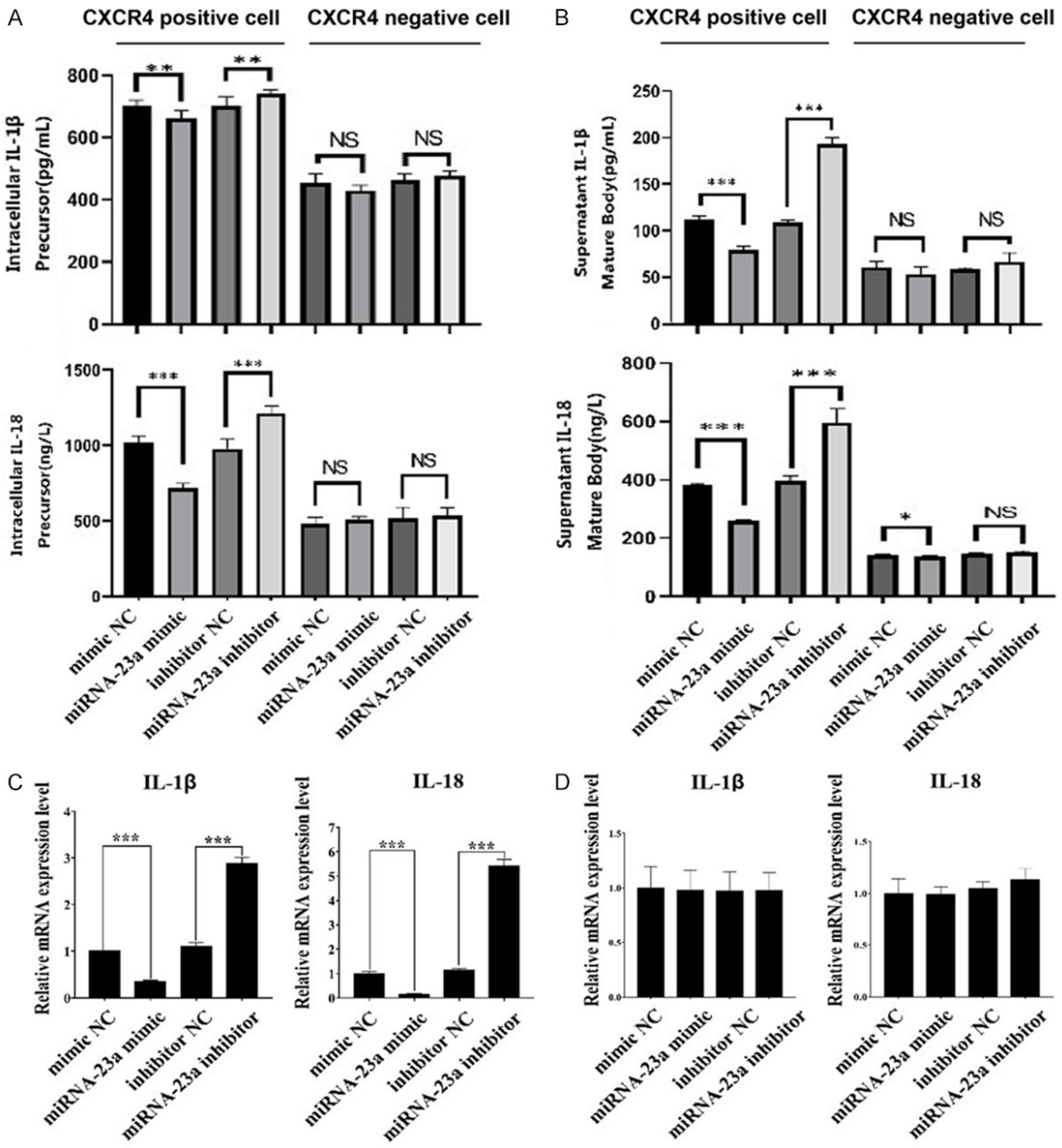

Figure 5. Effects of miR-23a/CXCR4 on inflammatory factor release. The intracellular concentrations of IL-1β and IL-18 precursor (A), and cell supernatant production of mature IL-1β and IL-18 (B) were measured in cells stimulated with LPS and ATP. The relative gene expression of IL-1β and IL-18 were measured using qRT-PCR in CXCR4-positive (C) and CXCR4-negative cells (D). The experiment was conducted in triplicates. Compared with matched group, *P<0.05, **P<0.01, ***P<0.001. NC: negative control; IL: Interleukin; LPS: lipopolysaccharide; ATP: Adenosine triphosphate; CXCR4: CXC chemokine receptor 4.

those in the Ctrl group. The alveolar wall and tracheal wall of the CLP + miR-23a group were thinner than that of the CLP group. However, there was no significant difference in the thickness of the alveolar wall and tracheal wall between the CLP + NC and CLP + PEI groups and the CLP group (**Figure 8**).

MiR-23a suppresses NLRP3 expression and inflammatory factor release in sepsis-induced lung injury mice

The effects of miR-23a on inflammatory factor release in sepsis-induced lung injury were evaluated using ELISA (**Figure 9**). The concentra-

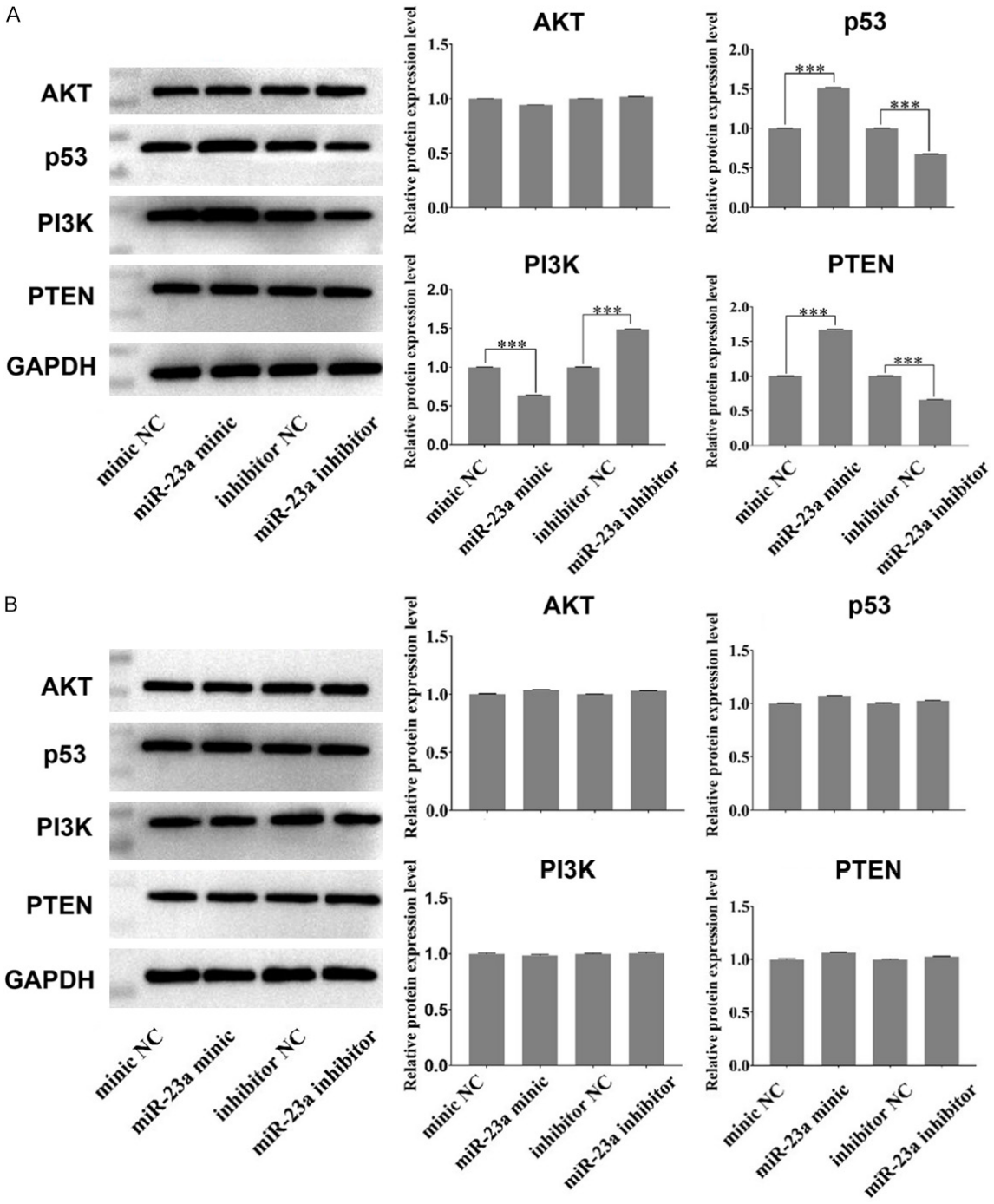

Figure 6. Effects of miR-23a/CXCR4 on the activation of the PTEN/PI3K/AKT signaling pathway. CXCR4-positive (A) and CXCR4-negative (B) THP-1 and BEAS-2B cell lines were treated with miR-23a after stimulation with LPS and ATP, and the protein expression of PTEN, p53, PI3K, and AKT were measured using Western blot. The experiment was conducted in triplicates. NC: negative control; LPS: lipopolysaccharide; ATP: Adenosine triphosphate; CXCR4: CXC chemokine receptor 4. Compared with matched group, ***P<0.001.

tions of IL-1β, IL-18, capase-1, and NLRP3 in the lungs of mice in the CLP group were significantly higher than those in the Ctrl group (*P*<0.01). However, the overexpression of miR-23a significantly decreased the concentrations of IL-18, capase-1, and NLRP3 (*P*<0.01). There was no significant difference in the concentrations of IL-18, capase-1, and NLRP3 between

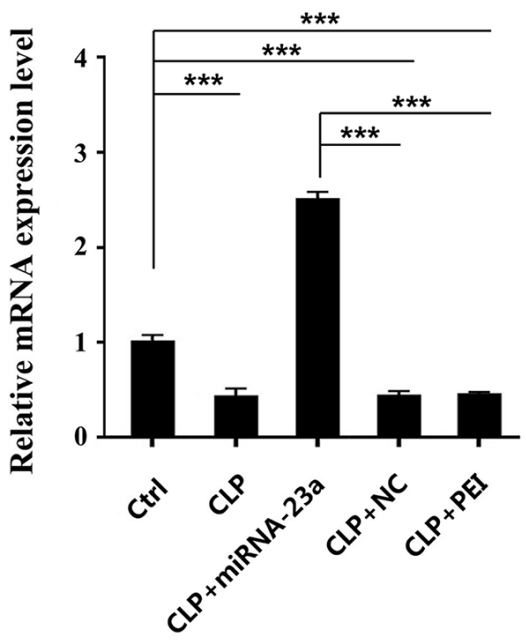

Figure 7. miR-23a expression in each group of mice (n=3 for each group). Ctrl: control; CLP: cecal ligation and puncture; NC: negative control; PEI: in vivo-jet PEITM transfection reagent. Compared with matched group, ***P<0.001.

the CLP + NC group and the CLP + PEI group (P>0.05).

MiR-23a decreases AChE activity in sepsis-induced lung injury mice

The effects of miR-23a on AChE activity in sepsis-induced lung injury mice were investigated (**Figure 10**). The activity of AChE in the CLP group increased significantly as compared with that in the Ctrl group (P<0.01). However, the overexpression of miR-23a significantly decreased the AChE activity (P<0.01). There was no significant difference in the AChE activity between the CLP + NC and CLP + PEI groups (P>0.05).

Discussion

The excessive inflammation, immunosuppression, and tissue damage caused by sepsis can increase the susceptibility to secondary infection, considerably aggravating the patients' illness and ultimately leading to death [12]. miR-23a participates in the host response against sepsis and acts as a potential biomarker for sepsis [13]. In this study, we found that miR-23a significantly improved sepsis-induced lung injury in CLP-induced septic mice and LPS-stimulated cell lines by suppressing NLRP3 inflammasome activation and activating the CXCR4/PTEN/PI3K/AKT pathway.

MiRNAs are abundantly expressed in eukaryotes and have extensive regulatory effects in information exchange, cell proliferation and apoptosis, tumor growth, and disease infection [14, 15]. miR-23a is highly conserved among species and regulates various disease processes, such as cancers, inflammations, Harada Miuji syndrome, and cognitive impairment [16]. The expression of miR-23a is down-regulated in septic cells [17]. Recent studies demonstrated that the miR-23a serum level in patients with sepsis differed significantly from control patients [13]. Ge et al. found a significant reduction in the expression of miR-23a in patients with acute kidney injury caused by sepsis [18]. In the present study, we successfully established a CLP mouse model of sepsis-induced ALI and found that the miR-23a expression was inhibited in lung tissue of BABL/c mice with sepsis-induced lung injury, and miR-23a overexpression reduced lung injury induced by sepsis.

The pathophysiology of sepsis is complex. Recently, it has been shown that the activation of NLRP3 inflammasome is closely related to the occurrence and development of sepsis [19, 20]. Studies have demonstrated that a variety of important pathogen molecular patterns (PAMPs), which can induce sepsis, activate NLRP3 inflammasomes *in vivo* and mediate the maturation and release of inflammatory factors in IL-1 family, leading to immune cell death [21]. The activation process of NLRP3 inflammasome includes protein component transcription and protein composite assembly. PAMPs activate intracellular signal transduction pathways downstream of pattern recognition receptors to start the transcriptional expression of components (also known as the first signal) such as LPS. Following recognition by TLR4, LPS activates cell NF-κB and MAPK signaling pathways to initiate the transcriptional expression of inflammatory factor precursors and NLRP3. Other activators such as ATP activate the protein complex assembly, known as signal 2. The specific molecular mechanism of regulation of the second signal in the NLRP3 inflammasome assembly is not clear. However,

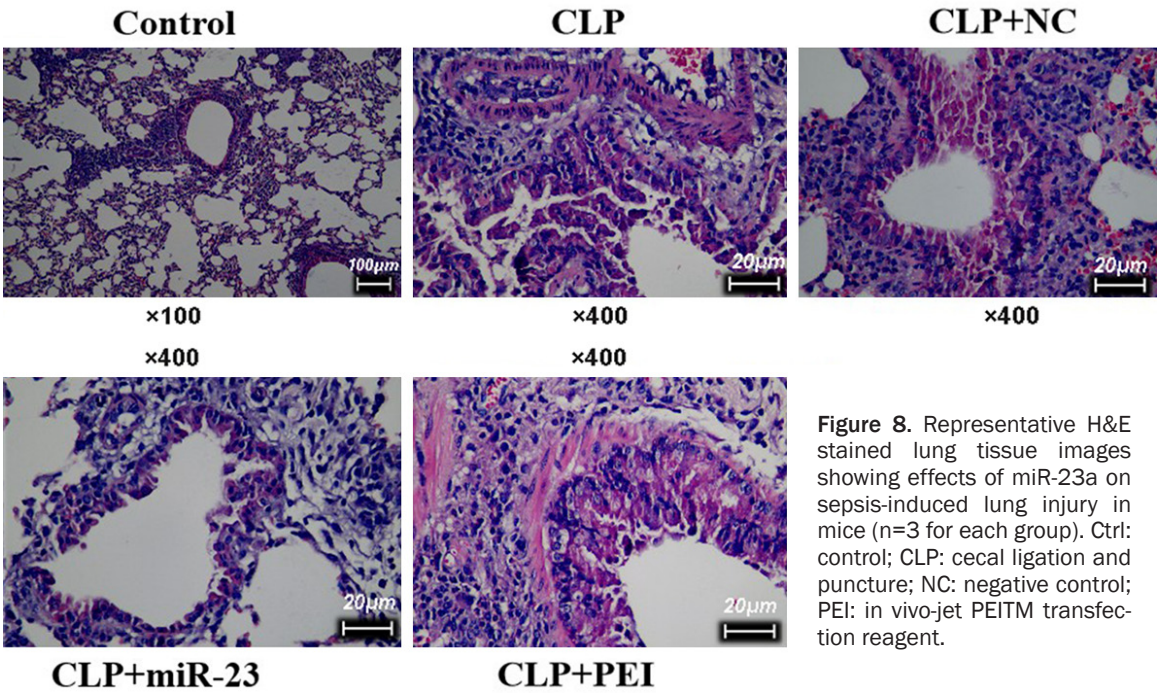

Figure 8. Representative H&E stained lung tissue images showing effects of miR-23a on sepsis-induced lung injury in mice (n=3 for each group). Ctrl: control; CLP: cecal ligation and puncture; NC: negative control; PEI: in vivo-jet PEITM transfection reagent.

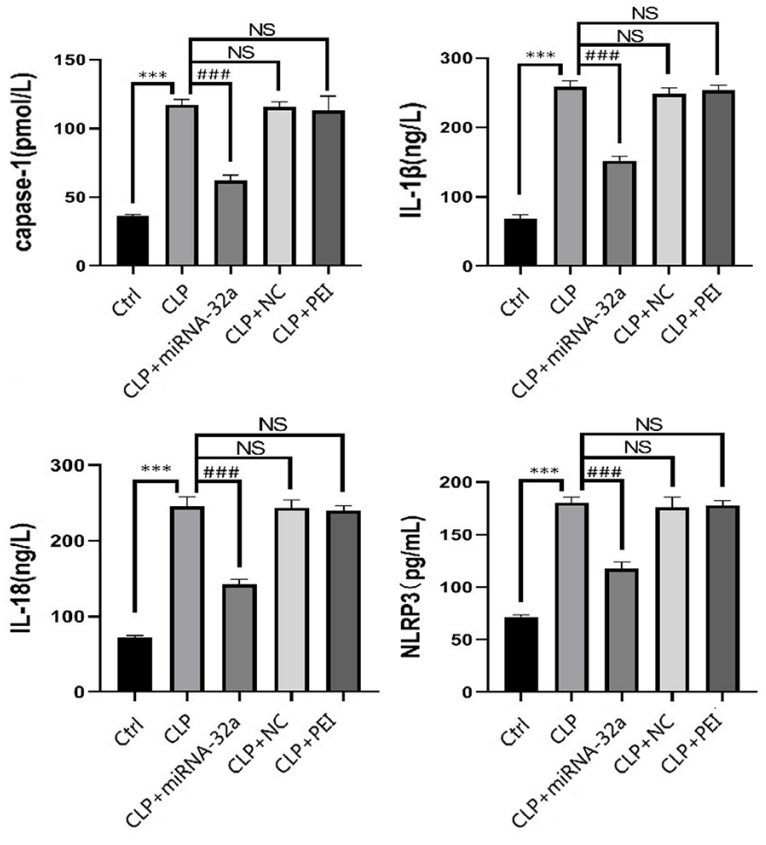

Figure 9. Effects of miR-23a on NLRP3 expression and inflammatory factor release in sepsis-induced lung injury mice. IL-1β, IL-18, capase-1, and NLRP3 expression in the lung tissue of sepsis-induced BABL/c mice (n=3 for each group). Ctrl: control; CLP: cecal ligation and puncture; NC: negative control; PEI: in vivo-jet PEITM transfection reagent. Compared with matched group, ###P<0.001.

ATP promotes the outflow of intracellular potassium ions and mediates the assembly of NLRP3, ASC, and caspase-1 into protein chimeras to activate the inflammatory response [22]. It functions by cleaving pro-caspase-1 to active caspase-1, resulting in the maturation and release of IL-1β and IL-18 [23]. LDH catalyzes the reversible conversion of pyruvate into lactate, and its level is positively associated with the severity of the sepsis-induced injury [24, 25]. miR-23a is involved in regulating the inflammatory response during septic insult via autophagy signaling [26]. AChE serves as an important diagnostic and therapeutic target in sepsis. In this study, we found that miR-23a suppressed NLRP3 inflammasome activation to inhibit IL-1β and IL-18 expression, LDH release, and AChE activity.

CXCR4 is part of an LPS sensing co-clustering complex that modulates TLR4 activation

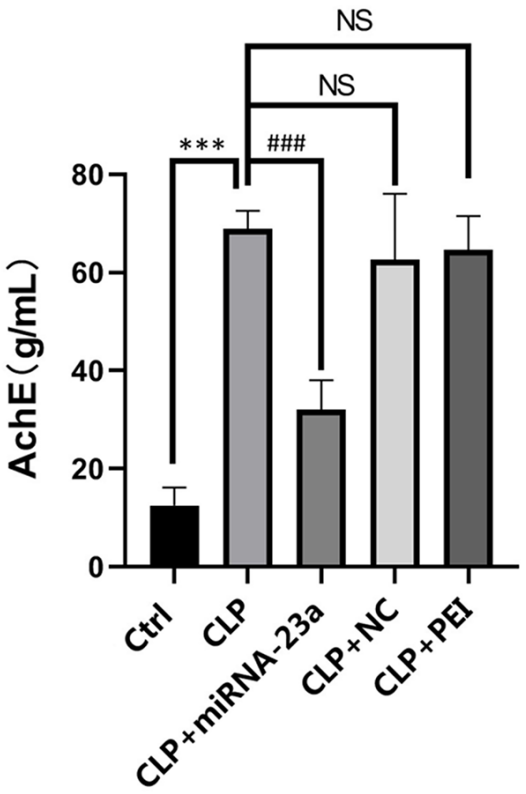

Figure 10. Effects of miR-23a on AChE activity in sepsis-induced lung injury mice (n=3 for each group). AChE: Acetylcholinesterase; Ctrl: control; CLP: cecal ligation and puncture; NC: negative control; PEI: in vivo-jet PEITM transfection reagent. Compared with the Ctrl group, ***P<0.001; Compared with the CLP group, ###P<0.001.

[27, 28]. Following TLR4 activation, the two subunits of PI3K, P85, and P110 in the PI3K/AKT pathway get activated and undergo a conformational change, resulting in phosphorylation of AKT and the subsequent signaling activation. However, PTEN is a negative regulator of the PI3K/AKT signaling pathway, which can dephosphorylate PIP3 and inhibit the phosphorylation of AKT [29]. In sepsis, the PI3K/AKT pathway negatively regulates inflammatory processes, and the inhibition of this signaling increases the susceptibility to polymicrobial sepsis [30]. In the course of ALI, inflammation is promoted, which causes increased concentrations of inflammatory factors such as IL-6, IL-1β, and TNF-α secreted by alveolar macrophages [31]. In this study, we provided evidence that miR-23a overexpression suppressed inflammation through activation of the CXCR4/PTEN/PI3K/AKT pathway.

There were some limitations in this study. Human miR-23a and mouse miR-23a share different sequences. Therefore, the functions of miR-23a may be differ in different species. Further study using human samples is still needed. There are also other possible mechanisms involved in sepsis induced lung injury. Further study on the mechanisms is also needed.

Conclusion

We found that miR-23a was down-regulated in mice with sepsis-induced lung injury. miR-23a can significantly improve sepsis-induced lung injury in CLP-induced septic mice and LPS-stimulated cell lines by suppressing NLRP3 inflammasome activation and inflammatory response via activation of the CXCR4/PTEN/PI3K/AKT pathway. This study indicated that miR-23a can serve as a potential therapeutic target for the treatment of sepsis-induced lung injury.

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Disclosure of conflict of interest

None.

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