

## Original Article

# Downregulation of miR-155 attenuates sepsis-induced acute lung injury by targeting SIRT1

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**Abstract:** Sepsis-induced acute lung injury (ALI) characterized by devastating hyperinflammatory response in the lungs is the ultimate cause of high mortality and morbidity in septic patients. miR-155 was reported to be significantly upregulated in sepsis-induced ALI cases and alleviated inflammation in septic lung injury in mouse and cell models. However, the detailed role of miR-155 and its underlying mechanism in sepsis-associated ALI remain to be further explored. *In vivo*, a cecal ligation and puncture (CLP)-induced ALI mouse model was successfully established. miR-155 expression was significantly higher in CLP mice compared with control mice. miR-155 inhibitor attenuated histopathological changes, lung apoptosis, lung inflammation, and increased the survival rate in CLP-induced ALI mice. *In vitro*, miR-155 expression increased in murine alveolar epithelial cells MLE-12 stimulated with lipopolysaccharide (LPS) and downregulation of miR-155 suppressed apoptosis and the release of inflammatory cytokines in LPS-stimulated MLE-12 cells. In addition, luciferase reporter assay and RNA immunoprecipitation (RIP) demonstrated that SIRT1 was a direct target of miR-155 in LPS-treated MLE-12 cells. Moreover, miR-155 partially reversed the inhibitory effects of SIRT1 on apoptosis and inflammatory response in LPS-stimulated MLE-12 cells. In summary, these results demonstrated that downregulation of miR-155 attenuated sepsis-induced ALI *in vivo* and *in vitro* by targeting SIRT1.

**Keywords:** miR-155, inflammation, SIRT1, sepsis, acute lung injury

## Introduction

Sepsis, a bacterial-infected complex systemic inflammatory response syndrome, leads to life-threatening multi-organ dysfunction syndrome (MODS) including cardiovascular system, liver, kidney, and lung [1]. Acute lung injury (ALI) and its severe manifestation acute respiratory distress syndrome (ARDS) characterized by devastating hyperinflammatory response in the lungs, are considered one of the most severe complications of sepsis, which cause high mortality and morbidity in septic patients [2]. Despite improvements in clinical therapy, approximately 50% of septic patients develop ALI and ARDS [3], which account for approximately 30% of the total mortality of patients with sepsis in recent years [4]. Thus, it is imperative to develop effective therapeutic approaches for the treatment of ALI.

Accumulating studies demonstrated that sepsis-induced ALI is generally caused by exces-

sive activation of the lung inflammatory response, excessive oxidative stress, and alveolar epithelial cell apoptosis [5]. Inflammatory cytokines and inflammatory mediators have been suggested to play a fundamental role in mediating, amplifying, and perpetuating sepsis-induced ALI [6]. Recent studies pointed out that the administration of cecal ligation and puncture (CLP) and lipopolysaccharide (LPS), a principal component of gram-negative bacterial endotoxin, were used for the establishment of sepsis-related lung injury models [7].

microRNAs (miRNAs) are a group of small, endogenous noncoding RNAs of 19-24 nucleotides that negatively regulate gene expression by incompletely binding to the 3'-untranslated region (UTR) of target mRNAs for translational repression or degradation [8]. It has been shown that miRNAs participate in the regulation of various basic biological processes, such as cell development, proliferation, survival, apoptosis, inflammation, and tumorigenesis [9].

## The role of miR-155 in ALI by targeting SIRT1

Emerging evidence has demonstrated that miRNAs are identified as important regulators in the pathogenesis and progression of ALI [10]. miR-155, a well-conserved miRNA across vertebrate species, is derived from exon 2 of the proto-oncogene B-cell integration cluster (*bic*) located on chromosome 21 [11]. Recently, miR-155 has aroused great attention due to its significance as a regulatory element of the immune system in various inflammatory transmitters and its association with hematological malignancies [12]. Notably, it was previously reported that miR-155 was significantly upregulated in sepsis-induced ALI cases and alleviated inflammation in septic lung injury in mouse and cell models by inducing autophagy [13, 14]. However, the detailed role of miR-155 and its underlying mechanism in sepsis-associated ALI remains to be further explored.

In the present study, we assessed the expression and function of miR-155 in a CLP mouse model of ALI and LPS-induced cell model of ALI, as well as the underlying mechanism.

### Materials and methods

#### *Animals and experimental protocol*

All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of the First Affiliated Hospital of Xinjiang Medical University. Healthy C57BL/6 male mice (6-8 weeks old, weight 20-24 g) were purchased from the Animals Experimentation Center of Fudan University (Fudan, China). Mice were maintained under specific pathogen-free conditions in a temperature-controlled room with a 12 h dark/light cycle for 1 week before the experiments, with free access to food and water. Mice were randomly divided into three groups ( $n = 10$  per group): sham group, CLP group, and CLP + miR-155 inhibitor group. A classic sepsis-induced ALI mouse model was established by the method of cecal ligation and puncture (CLP) as described previously [15]. Briefly, mice were anesthetized by intraperitoneal injection of 100 mg/kg body ketamine and 5 mg/kg xylazine after an overnight fast. After skin sterilization, a 2-cm ventral midline abdominal incision was made and the cecum was exposed. The cecum was ligated with a 3-0 silk suture between the terminal and ileocecal valve to avoid intestinal obstruction. The ligated cecum was then punctured

through-and-through twice using a 20-gauge needle. Finally, the cecum was placed back into the normal sterile peritoneal cavity and the abdomen was sutured layer by layer. Mice in the sham group underwent the same procedure but the cecum was neither ligated nor punctured. Mice in the CLP + miR-155 inhibitor group were intratracheally instilled with 50  $\mu$ l miR-155 inhibitor (2 mg/kg; GeneChem Co., Ltd., Shanghai, China) 24 h before CLP. Six days after the surgery, mice were euthanized and blood samples were collected. Lung tissues were excised for wet/dry ratio analysis, histological examination and biochemical analysis.

#### *Hematoxylin and eosin (HE) staining*

The part of left lung tissues from each mouse were fixed in 10% formalin and embedded in paraffin. After being sliced into 4  $\mu$ m-thick sections using a Leica RM2125RT microtome (Leica, Nussloch, Germany), the slides were then stained with hematoxylin and eosin, and then observed under a light microscopy (Olympus BX51, Olympus, Tokyo, Japan).

#### *Lung wet/dry (W/D) weight ratio*

The ratio of lung wet/dry wet was measured by dividing the wet by the dry weight to assess the severity of pulmonary edema. The fresh upper part of the right lung was cleansed of blood-stains and weighed to obtain the wet weight. The lung tissues were then dried in an oven at  $-80^{\circ}\text{C}$  for 72 h for the measurement of the dry weight.

#### *Myeloperoxidase (MPO) activity measurement*

After weighing, the lung tissues were homogenized in 0.5% hexadecyltrimethylammonium bromide lysis buffer (HTAB) and sonicated in an extraction buffer to obtain 10% of homogenate. After centrifugation at  $40,000 \times g$  for 10 min, MPO activity was measured using a commercially available assay kit (Jiancheng Bioengineering Institute, Nanjing, China) at 460 nm with a spectrophotometer (Shanghai Precision & Scientific Instrument Co. Ltd, China).

#### *Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) assay*

An In Situ Cell Death Detection Kit (Roche, Basel, Switzerland) was used to assess endo-

## The role of miR-155 in ALI by targeting SIRT1

thelial apoptosis. Paraffin-embedded sections were digested with proteinase K for 20 min and then incubated with the 50  $\mu$ l TUNEL reaction mixture at 37°C for 1 h. After three washes with PBS, the slides were labeled with 4',6-diamidino-2-phenylindole (DAPI) for 5 min at room temperature in the dark and washed three times for 5 min each. The sections were then observed with Eclipse 80i fluorescence microscope (Nikon, Tokyo, Japan). The number of TUNEL-positive cells was counted in twelve randomly selected fields under a magnification of 200 $\times$ .

### *Cell culture and transfection*

MLE-12 cells, a cell line derived from murine alveolar epithelial cells, were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and were maintained in DMEM (Hyclone, UT, USA) containing 10% FBS (Gibco, Grand Island, NY, USA), 100 U/ml penicillin, and 100  $\mu$ g/ml streptomycin at 37°C in a incubator with an atmosphere of 5% CO<sub>2</sub>. MLE-12 cells were seeded into 48-well plates at a density of 5  $\times$  10<sup>3</sup> cells/well and stimulated with 1  $\mu$ g/ml LPS (*Escherichia coli* LPS O55:B5; Sigma-Aldrich, St. Louis, MO, USA) for 24 h. Additionally, MLE-12 cells were transfected with miR-155 inhibitor, miR-155 mimic, miR-con, pcDNA, pcDNA-sirtuin 1 (SIRT1), miR-155 + SIRT1, or miR-con + SIRT1 (GeneChem Co., Ltd.) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) before LPS stimulation.

### *Flow cytometry analysis*

Cell apoptosis was measured by flow cytometry using the Annexin V-FITC Apoptosis Detection kit (BD Biosciences, Franklin Lakes, NJ, USA). MLE-12 cells treated as above were collected, washed twice with ice-cold PBS and resuspended with 400  $\mu$ l of 1  $\times$  binding buffer solution. Cells were then incubated with 10  $\mu$ l of Annexin-V fluorescein isothiocyanate (FITC) and 5  $\mu$ l propidium iodide (PI) at 4°C in the dark for 15 min. The apoptotic rate was analyzed with a FACScan flow cytometer (BD Biosciences).

### *Quantitative real-time PCR*

Total RNA was extracted from excised lung tissues or treated MLE-12 cells using TRIzol reagent (Invitrogen) and reversely transcribed into cDNA with the One Step PrimeScript mi-

RNA cDNA Synthesis Kit (Takara, Dalian, China). miR-155 expression was quantified with SYBR Green reagent (Takara) using Applied Biosystems 7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The thermocycling parameters were performed as follows: 10 min at 95°C, followed by 30 cycles of 5 s at 95°C, 30 s at 55°C, and 30 s at 72°C. The relative expression level was calculated using the 2<sup>- $\Delta\Delta$ Ct</sup> method and normalized to U6 small nuclear RNA (snRNA).

### *Inflammatory cytokines analysis*

The blood samples were centrifuged at 1500 rpm for 10 min at 4°C to collect the serum. The levels of tumor necrosis factor (TNF)- $\alpha$  and interleukin-1 $\beta$  (IL-1 $\beta$ ) in the serum or the treated MLE-12 cells were measured by commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits (Jiancheng Bioengineering Institute).

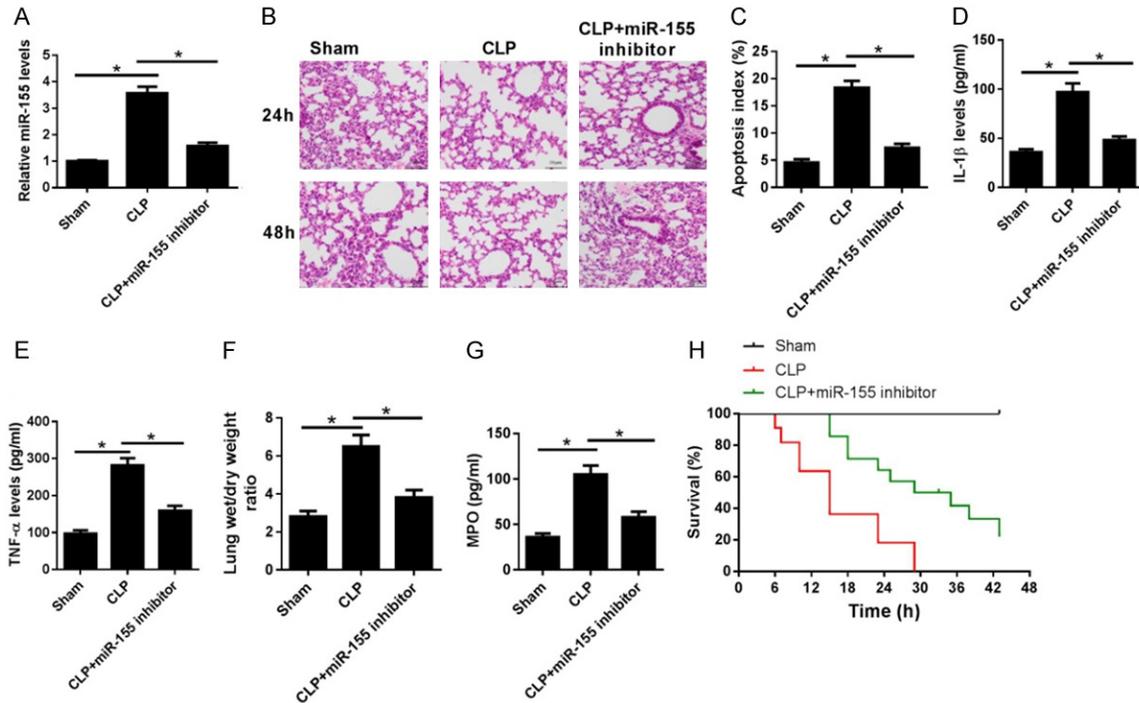
### *Western blot analysis*

Total proteins from treated MLE-12 cells were extracted using a modified Radioimmunoprecipitation Assay buffer (Sigma-Aldrich) and quantified by a BCA protein assay kit (Thermo Scientific, Rockford, IL, USA). Protein lysates (30  $\mu$ g/lane) were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by transfer onto polyvinylidene difluoride membranes (PVDF; Millipore, Billerica, MA, USA). After being blocked with 5% skimmed milk in 0.05% TBS-Tween-20 (v/v) for 2 h at room temperature, the membranes were gently incubated overnight at 4°C with the following primary antibody against SIRT1 (Cell Signaling Technology, Danvers, MA, USA) and  $\beta$ -actin (Cell Signaling Technology), followed by incubation with horseradish peroxidase (HRP)-conjugated secondary antibody IgG (Santa Cruz biotechnology, Santa Cruz, CA, USA) for 1 h at room temperature. Protein bands were detected with the enhanced chemiluminescence (ECL) system (Pierce Biotechnology, Rockford, USA) and analyzed with Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA).

### *Luciferase reporter assay*

The wild-type (WT) or mutated (MUT) 3'UTR of SIRT1 containing the predicted miR-155 bind-

## The role of miR-155 in ALI by targeting SIRT1



**Figure 1.** The effects of miR-155 inhibitor on CLP-induced ALI in mice. Mice were intratracheally instilled with miR-155 inhibitor 24 h prior to CLP. A. The expression of miR-155 in the sham group, CLP group, and CLP + miR-155 inhibitor group was examined by qRT-PCR. B. Histopathological analysis of lung tissues from the different groups. C. The apoptosis of lung epithelial cells from the different groups was determined by TUNEL staining (magnification 200  $\times$ ). D and E. The serum levels of TNF- $\alpha$  and IL-1 $\beta$  in the different groups were measured by ELISA. F. The ratio of lung wet/dry weight from the different groups. G. MPO activity of lung tissues from the different groups. H. The survival rates of the different groups were monitored at different times for 42 h. \* $P < 0.05$ .

ing sites were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) and cloned into a luciferase pMIR-REPORT vector (Promega Corporation, Madison, WI, USA). 293T cells were seeded into 24-well plates and cotransfected with the constructed luciferase plasmids and miR-155 mimic or miR-con using the Lipofectamine 2000 (Invitrogen). After 48 h transfection, firefly and Renilla luciferase activities were measured with a Dual-Luciferase Reporter Assay System (Promega Corporation).

### RNA immunoprecipitation (RIP)

Coimmunoprecipitation (Co-IP) of miRNA ribonucleoprotein complex (miRNP) with anti-argonaute1 (Ago1; Abcam, Cambridge, MA, USA) or IgG (Santa Cruz Biotechnology) was performed as previously reported [16]. RNA coimmunoprecipitated with anti-Ago1 or IgG antibodies was isolated using TRIzol (Invitrogen) and then subjected to qRT-PCR analysis for the demonstration of the binding sites between miR-155 and SIRT1.

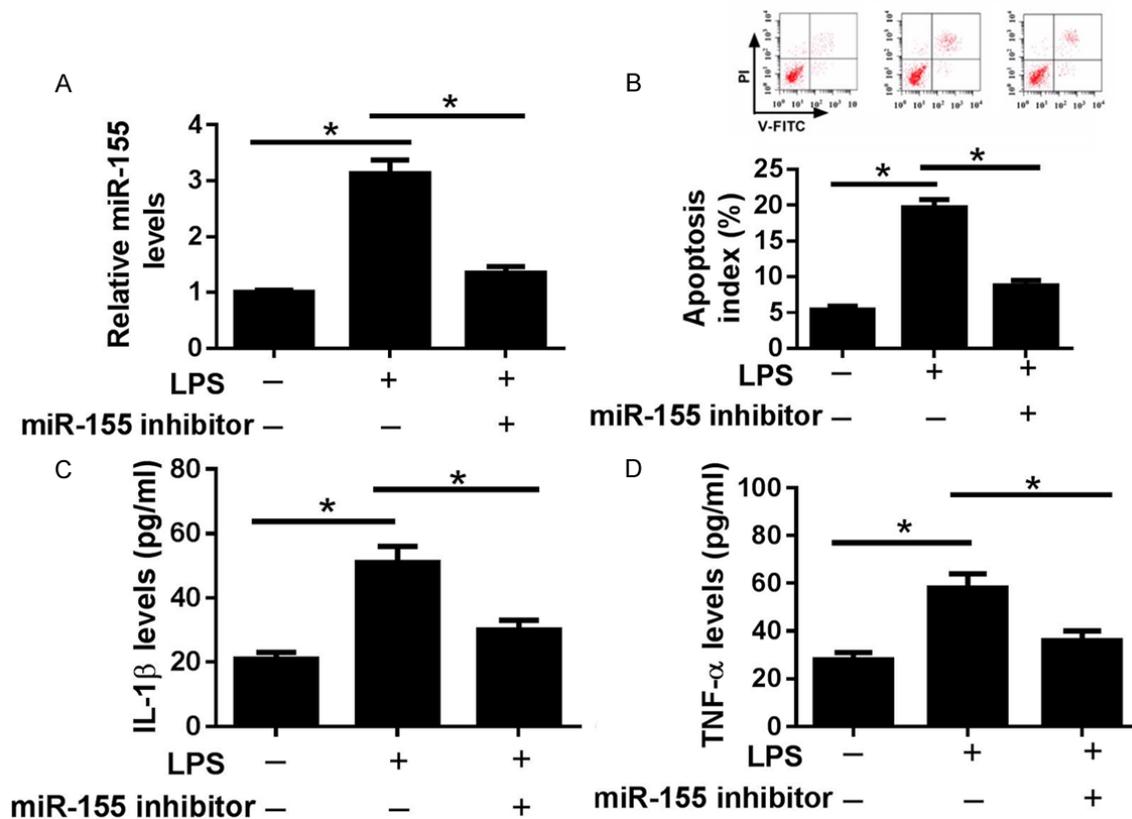
### Statistical analysis

All values are expressed as the mean  $\pm$  standard deviation (SD), and all statistical analyses were performed using GraphPad Prism (version 6.0; GraphPad Software, Inc., La Jolla, CA, USA). Comparison between two or more groups was carried out using independent sample *t*-test and one-way analysis of variance (ANOVA).  $P < 0.05$  was considered as statistically significant.

### Results

#### miR-155 inhibitor significantly attenuated CLP-induced ALI in mice

To characterize the roles of miR-155 in CLP-induced ALI in mice, mice were intratracheally instilled with miR-155 inhibitor 24 h prior to CLP. As shown in **Figure 1A**, miR-155 expression was significantly higher in CLP-challenged mice in comparison with sham group, which was remarkably undermined after suppressing

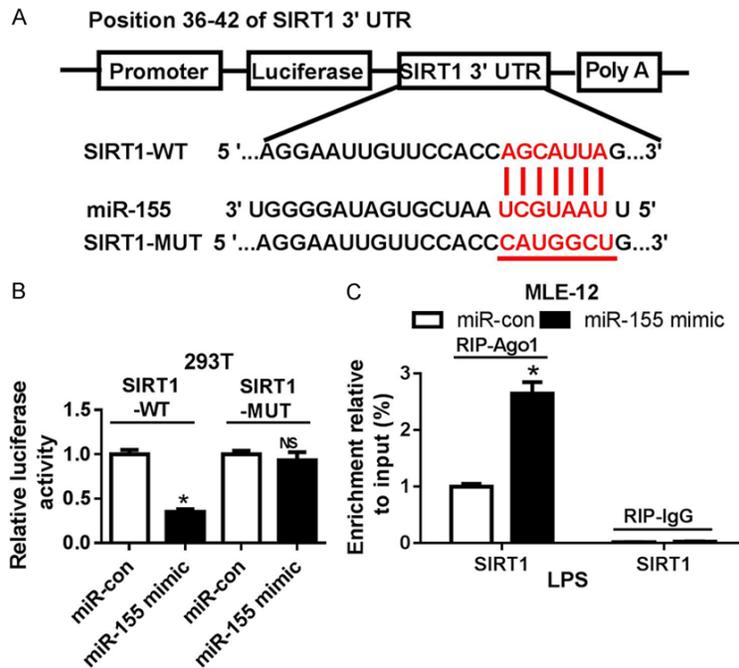


**Figure 2.** The effects of miR-155 inhibitor on LPS-induced apoptosis and inflammation in MLE-12 cells. MLE-12 cells were transfected with or without miR-155 inhibitor, followed by stimulation with 1  $\mu$ g/ml LPS for 24 h. A. qRT-PCR analysis was performed to detect the expression of miR-155 in the treated MLE-12 cells. B. Flow cytometry analysis was conducted to assess the apoptosis of the treated MLE-12 cells. C and D. ELISA was applied to measure the concentrations of TNF- $\alpha$  and IL-1 $\beta$  in the treated MLE-12 cells. \* $P$  < 0.05.

miR-155 expression. The histologic assessment of lung tissue was then carried out using HE staining and the results revealed that pathologic changes in the lung tissues from CLP mice were obvious, including hemorrhage in the alveolus, fibrin deposition, thickening of the alveolar wall, edema and polymorphonuclear cell infiltration (Figure 1B). However, miR-155 inhibition notably receded CLP-induced pathological changes in the lung tissues (Figure 1B). TUNEL assay showed that the apoptosis index of the lung epithelial cells in mice upon CLP challenge was distinctly increased compared with the sham group, but was greatly suppressed following the transfection of miR-155 inhibitor (Figure 1C). We further analyzed the effects of miR-155 inhibitor on inflammation in CLP-induced ALI model, the productions of inflammatory cytokines in the serum samples were measured by ELISA. The results demonstrated that the levels of TNF- $\alpha$  and IL-1 $\beta$  in the serum were drastically promoted in mice

after CLP challenge, and these increases were prominently attenuated by suppression of miR-155 (Figure 1D and 1E). Lung edema was evaluated using the ratio of lung wet/dry weight and the results suggested that the ratio of lung wet/dry weight in the CLP group was evidently higher than sham group, but dramatically decreased in the CLP + miR-155 inhibitor group (Figure 1F). MPO activity in lung tissues, a marker of the extent of neutrophil infiltration, was also evaluated and the results manifested that CLP challenge in mice induced a marked neutrophil infiltration in the lung tissues, as evidenced by the increased MPO activity, while this effect was apparently offset by miR-155 inhibitor (Figure 1G). Furthermore, we found that low expression of miR-155 by miR-155 inhibitor induced a substantial increase of survival rate of CLP mice (Figure 1H). Collectively, these results suggested that miR-155 inhibitor significantly ameliorated CLP-induced ALI in mice.

## The role of miR-155 in ALI by targeting SIRT1



**Figure 3.** The interaction between miR-155 and SIRT1 in MLE-12 cells. **A.** Alignment between the predicted miR-155 binding site and mutant site of the SIRT1 3'-UTR and miR-155. **B.** Luciferase reporter assay was performed to detect luciferase activity in 293T cells after cotransfection with miR-155 mimic or miR-con and the constructed luciferase reporter plasmids. **C.** RIP analysis with Ago1 antibody was used to confirm the binding status between miR-155 and SIRT1 in MLE-12 cells. \* $P < 0.05$ .

*miR-155 inhibitor remarkably alleviated LPS-induced apoptosis and inflammatory response in MLE-12 cells*

To further address the role of miR-155 on LPS-induced ALI *in vitro*, murine alveolar epithelial cells MLE-12 cells were transfected with miR-155 inhibitor, followed by stimulation with 1  $\mu\text{g}/\text{ml}$  LPS for 24 h. miR-155 expression was abnormally upregulated in MLE-12 cells stimulated with LPS, while the increase of miR-155 expression in LPS-treated MLE-12 cells was greatly counteracted by miR-155 inhibitor (**Figure 2A**). In addition, flow cytometry analysis hinted that LPS stimulation triggered a substantial elevation of apoptosis in MLE-12 cells, which was obviously reversed by miR-155 inhibitor (**Figure 2B**). Moreover, ELSA results revealed that LPS treatment strikingly promoted the release of inflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  in MLE-12 cells (**Figure 2C** and **2D**). However, miR-155 suppression apparently undermined the LPS-induced increase in TNF- $\alpha$  and IL-1 $\beta$  levels (**Figure 2C** and **2D**). Together, these results indi-

cated that miR-155 inhibitor remarkably alleviated LPS-induced apoptosis and inflammatory response in MLE-12 cells.

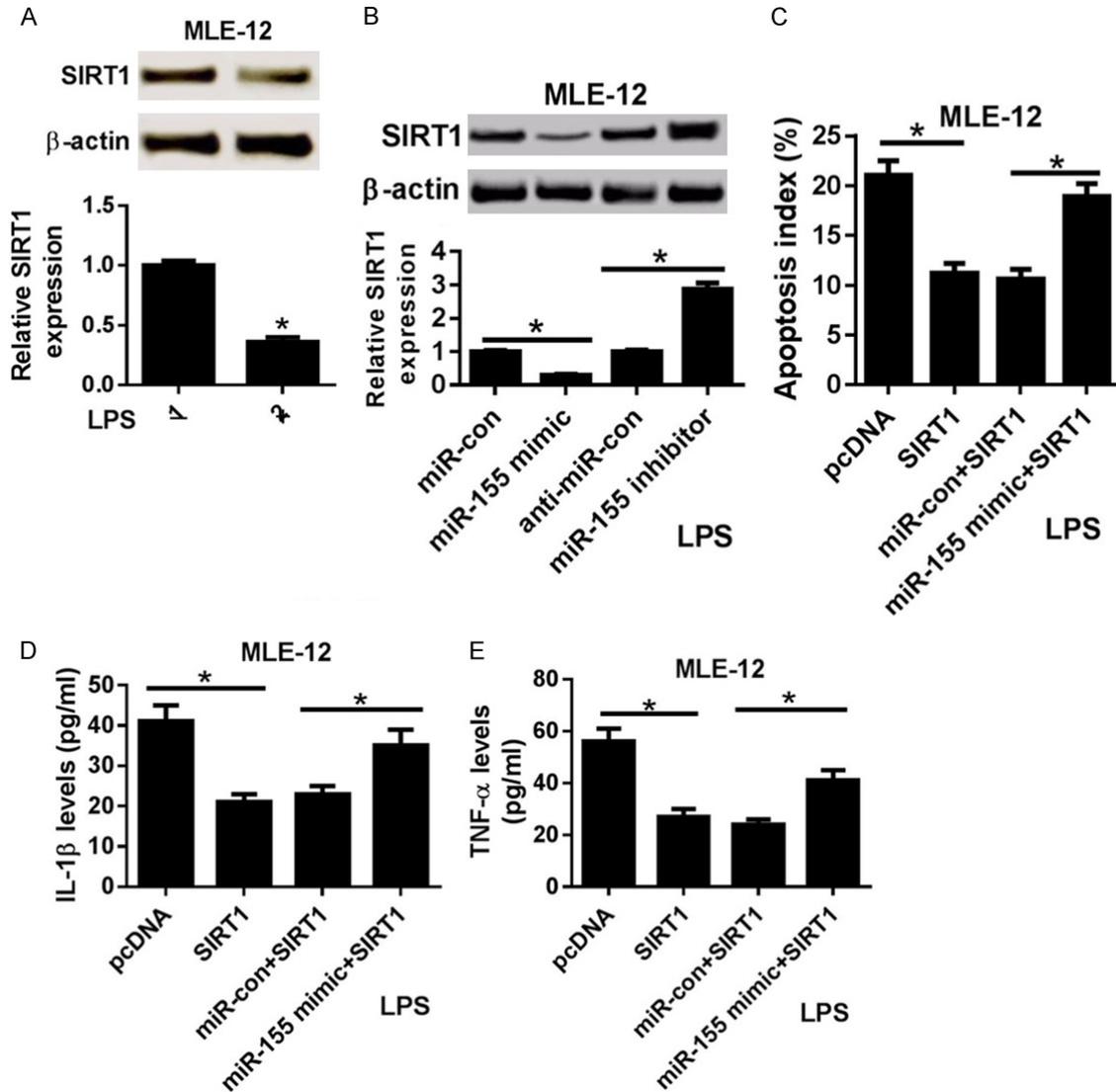
*SIRT1 was identified as a target of miR-155 in LPS-stimulated MLE-12 cells*

To explore the underlying mechanism underlying the protective effects of miR-155 on sepsis-induced ALI, bioinformatics analysis was performed to predict the potential targets of miR-155. As shown in **Figure 1A**, bases 36-42 of the 3'UTR of SIRT1 mRNA was predicted to be a binding site for miR-155. To confirm the direct interaction between miR-155 and SIRT1, SIRT1 3'UTR luciferase reporter plasmids containing the WT or MUT binding sites for miR-155 were constructed and luciferase reporter assay was performed. As shown in **Figure 3B**, miR-155 overexpression significantly reduced the luciferase activity of the plasmids containing the WT 3'UTR of SIRT1 in 293T cells, but showed no significant effect on the luciferase activity of the plasmids containing the MUT 3'UTR of SIRT1. Furthermore, RIP analysis showed that SIRT1 mRNA could be specifically recruited to the miRNP complex isolated using anti-Ago1 antibody following miR-155 mimic transfection (**Figure 3C**). These findings suggested that miR-155 can directly target SIRT1 in LPS-stimulated MLE-12 cells.

*miR-155 partially reversed the inhibitory effects of SIRT1 on apoptosis and inflammatory response in LPS-stimulated MLE-12 cells*

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The expression pattern of SIRT1 in LPS-induced ALI *in vitro* was also determined by western blot. As shown in **Figure 4A**, SIRT1 protein level was dramatically decreased in LPS-treated MLE-12 cells with respect to control cells. To assess the effect of miR-155 on the protein level of SIRT1, MLE-12 cells were transfected with miR-155 mimic, miR-155 inhibitor, or



**Figure 4.** miR-155 partially reversed the inhibitory effects of SIRT1 on apoptosis and inflammatory response in LPS-stimulated MLE-12 cells. A. Western blot analysis of SIRT1 level in MLE-12 cells stimulated with or without LPS. B. Western blot analysis of SIRT1 level in MLE-12 cells transfected with miR-155 mimic, miR-155 inhibitor, or matched controls, followed by LPS stimulation. C. Flow cytometry analysis was performed to evaluate apoptosis in MLE-12 cells after transfection with SIRT1, pcDNA or combined with miR-155 mimic or miR-con, followed by LPS stimulation. D and E. The levels of TNF- $\alpha$  and IL-1 $\beta$  were measured by ELISA in MLE-12 cells after transfection with SIRT1, pcDNA or combined with miR-155 mimic or miR-con, followed by LPS stimulation. \* $P < 0.05$ .

respective controls, followed by stimulation with LPS. Western blot analysis showed that SIRT1 level was significantly decreased in miR-155 mimic-transfected MLE-12 cells, but drastically increased in miR-155 inhibitor-introduced MLE-12 cells (Figure 4B). Considering the aforementioned results, we further investigated the effects of miR-155 on the functions of SIRT1 in LPS-induced MLE-12 cells. MLE-12 cells were transfected with SIRT1, pcDNA, or combined with miR-155 mimic or miR-con, fol-

lowed by treatment with LPS. The subsequent flow cytometry analysis revealed that SIRT1 overexpression led to a marked inhibition of apoptosis in MLE-12 cells followed by LPS stimulation, while miR-155 mimic greatly abolished SIRT1-mediated inhibition of apoptosis (Figure 4C). Furthermore, ELISA results demonstrated that the levels of TNF- $\alpha$  and IL-1 $\beta$  were notably declined by regained expression of SIRT1, which were distinctly restored following cotransfection with SIRT1 and miR-155 mimic (Figure

**4D and 4E).** These results demonstrated that miR-155 partially reversed the inhibitory effects of SIRT1 on apoptosis and inflammatory response in LPS-stimulated MLE-12 cells.

### Discussion

In the present study, we observed the upregulation of miR-155 in CLP mice and MLC-12 cells stimulated with LPS. Moreover, we demonstrated that miR-155 inhibitor diminished CLP-induced ALI in mice and LPS-induced apoptosis and inflammatory response in MLE-12 cells. Mechanistically, we found that SIRT1 was identified as a target of miR-155 and miR-155 partially reversed the inhibitory effects of SIRT1 on apoptosis and inflammatory response in LPS-stimulated MLE-12 cells. These results suggested that miR-155 protected mice from sepsis-induced ALI by targeting SIRT1.

Severe sepsis, a systemic inflammatory syndrome in response to severe infection and surgery [17], is the most common risk factor for ALI development with high morbidity and mortality in critical ill patients [18]. ALI is characterized by pulmonary interstitial edema, alveolar and bronchial epithelial cell apoptosis, and alveolar damage. The CLP-induced ALI model is a widely accepted murine polymicrobial model, which is currently considered as the gold standard for the research of sepsis-associated mechanism [19]. In the present study, we successfully established the CLP-induced ALI mouse model, as evidenced by hemorrhage in the alveolus, fibrin deposition, thickening of the alveolar wall, edema and polymorphonuclear cell infiltration, lung apoptosis, overproduction of the inflammatory cytokines IL-1 $\beta$  and TNF- $\alpha$ , neutrophil infiltration, and an increased mortality rate. However, miR-155 inhibitor remarkably ameliorated CLP-induced ALI in mice. In addition, murine alveolar epithelial cells MLE-12 stimulated with LPS has been widely used as an ALI model [20]. Herein, we found that LPS could trigger apoptosis and release of inflammatory cytokine levels in MLE-12 cells, and suppression of miR-155 significantly attenuated LPS-induced apoptosis and inflammatory response in MLE-12 cells. Collectively, these data suggested that miR-155 inhibitor ameliorated sepsis-induced ALI *in vivo* and *in vitro*, consistent with previous studies [13, 14].

SIRT1, a conserved NAD<sup>+</sup>-dependent class III histone deacetylase, was found to be mostly

localized to the nucleus, where it plays a crucial role in transcriptional repression via histone deacetylation [21]. SIRT1 is constitutively expressed in most cells and has been well-established as a master regulator of senescence, cell lifespan extension, apoptosis, and oxidative stress responses [22]. In addition, increasing evidence has demonstrated that SIRT1 plays an important role in repressing inflammatory response by controlling the production of inflammatory cytokines [23]. Therefore, SIRT1 has been considered as a potential target of negative regulation of inflammation and is involved in various inflammatory disorders [24]. Previous studies demonstrated that SIRT1 suppressed acute lung inflammation during sepsis by controlling inflammasome activation pathway in a murine sepsis model induced by CLP [25]. In the present study, we showed that SIRT1 was downregulated in LPS-treated MLE-12 cells, which was in accordance with the previous studies [25, 26]. Moreover, we provided the first evidence that SIRT1 was a direct target of miR-155, as demonstrated by luciferase reporter assay and RIP. Moreover, rescue experiments demonstrated that miR-155 partially reversed the inhibitory effects of SIRT1 on apoptosis and inflammatory response in LPS-stimulated MLE-12 cells, suggesting that miR-155 promoted apoptosis and inflammatory response in LPS-stimulated MLE-12 cells by targeting SIRT1. Similarly, it was previously reported that downregulation of miR-199a attenuated sepsis-induced ALI through the suppression of excessive inflammatory response and the inhibition of cellular apoptosis by targeting SIRT1 [27].

In summary, we demonstrated that miR-155 was upregulated in CLP-induced ALI mouse model and the LPS-induced ALI cell model. Moreover, downregulation of miR-155 remarkably attenuated sepsis-induced ALI *in vivo* and *in vitro* by targeting SIRT1, contributing to a better understanding of the detailed role of miR-155 and its molecular mechanism in sepsis-induced ALI. Therefore, our study may provide a potential therapeutic target for the treatment of sepsis-induced ALI.

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## Disclosure of conflict of interest

None.

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## The role of miR-155 in ALI by targeting SIRT1

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