



# The downregulation of miR-129-5p relieves the inflammatory response in acute respiratory distress syndrome by regulating PPAR $\gamma$ -mediated autophagy

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**Background:** The peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) promotes autophagy and regulates the inflammatory response. However, the effects of the PPAR $\gamma$  on inflammation in acute respiratory distress syndrome (ARDS) are unclear. This study sought to explore the mechanism by which the downregulation of microRNA-129-5p (miR-129-5p) attenuates the inflammatory response in ARDS patients by regulating PPAR $\gamma$ -mediated autophagy.

**Methods:** Lipopolysaccharide (LPS) was used to establish an acute lung injury (ALI) mice model to simulate ARDS. GW9662 and pioglitazone were applied to inhibit and activate the PPAR $\gamma$ , respectively. Enzyme-linked immunoassays were used to detect inflammatory cytokines. The expressions of miR-129-5p, the PPAR $\gamma$ , and the autophagy-marker protein were detected by quantitative polymerase chain reaction (qPCR) or Western blot. Dual-luciferase reporter assays were used to verify the targeting relationship between miR-129-5p and PPAR $\gamma$  messenger RNA (mRNA). Human lung epithelial cells BEAS-2B transfected with the miR-129-5p inhibitor and/or the short interfering RNA PPAR $\gamma$  (si-PPAR $\gamma$ ) were applied to explore the mechanism.

**Results:** The results showed that pioglitazone promoted autophagy and relieved lung injury caused by LPS, while GW9662 exacerbated lung injury. MiR-129-5p directly targeted the PPAR $\gamma$ . Inhibiting the expression of miR-129-5p increased the level of the PPAR $\gamma$ , induced autophagy, and inhibited apoptosis and the inflammatory response. Conversely, silencing the PPAR $\gamma$  had the opposite effects, and blocked the protective effects of the miR-129-5p inhibitor.

**Conclusions:** The inhibition of miR-129-5p may induce autophagy and inhibit the inflammatory response by promoting the expression of the PPAR $\gamma$ , thereby relieving ARDS.

**Keywords:** Acute respiratory distress syndrome (ARDS); inflammatory response; autophagy; peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ); microRNA-129-5p (miR-129-5p)

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## Introduction

Acute respiratory distress syndrome (ARDS) is mainly manifested by the continuous activation of the inflammatory system. ARDS is a life-threatening pulmonary inflammatory reactive disease (1,2). Its clinical manifestations include respiratory distress, refractory hypoxemia, and decreased lung compliance. Epidemiological data indicate that the lives of 2.2 million patients are threatened by ARDS each year worldwide (3,4). As many as 40,070 patients die each year from ARDS; thus, ARDS remains an important public health issue (3,4). Acute lung injury (ALI) is a mild form of ARDS with a complex pathogenesis. Presently, an insufficient understanding of the pathophysiology of ALI affects the evaluation and treatment of ARDS.

The continuous activation of the inflammatory system has a close causal relationship with the progression and outcome of ARDS, and cell autophagy has been confirmed to be closely related to the inflammatory response (5). For example, when Toll-like receptor (TLR)-related pathways induce inflammatory responses, they can simultaneously trigger cellular autophagy, and cellular autophagy has a significant negative regulatory effect on TLR signals and inflammatory responses (6,7). Meng (8) showed that enhancing autophagy reduces inflammation and oxidative stress, and protects lung injury. Peroxisome proliferator-activated receptors (PPARs) are transcription factors. PPARs are mainly expressed in adipose tissue, but are also highly expressed in the kidneys and lungs. The PPAR $\gamma$  is one of the pathways that regulates autophagy and inflammation. In aged human dental pulp cells, the activation of PPARs maintains cell homeostasis by inducing antioxidant responses and autophagy, and inhibits the expression of inflammatory factors (9). Research has shown that the PPAR $\gamma$  activates autophagy (10-12). Further, the activation of the PPAR $\gamma$  has been shown to inhibit the inflammatory response against lung injury (13). In a lipopolysaccharide (LPS)-induced ALI model, PPAR $\gamma$  activation is related to the inhibition of the inflammatory response (14). However, it is still unclear whether the mechanism by which the PPAR $\gamma$  alleviates ALI is related to autophagy.

MicroRNA (miRNA) regulates gene expression at the post-transcriptional level. Studies have shown that the level of the PPAR $\gamma$  is regulated by miRNA, and participates in the process of inflammatory response and autophagy (15,16). Additionally, the intervention of miRNA may regulate autophagy flux by regulating the PPAR $\gamma$  expression (17,18).

However, in ALI, the upstream miRNA that regulates the PPAR $\gamma$  is still unclear. MiRNA-129-5p (miR-129-5p) is a newly discovered miRNA that regulates autophagy and inflammation, for example, it is showed that miR-129-5p can alleviate LPS-induced acute kidney injury (19). Further, it has been shown that miR-129-5p participates in adipogenesis by regulating autophagy (20). However, the role of miR-129-5p in ARDS remains unclear. This study sought to examine the role of the PPAR $\gamma$  in the regulation of autophagy in ARDS, and identify the key upstream regulatory miRNA, and its role in autophagy. We present the following article in accordance with the ARRIVE reporting checklist (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-979/rc>).

## Methods

### *Animals*

In this study, 20 C57BL/6 mice [specific-pathogen free (SPF), male, 18–20 g, 8–10 weeks old, Medical Animal Experimental Center of the Third Military Medical University] were randomly divided into the following four groups: (I) the sham group; (II) the ARDS group; (III) the ARDS + pioglitazone group; and (IV) the ARDS + GW9662 group (n=5). The animals were kept in the same environment (SPF Animal Laboratory) to eliminate interference.

LPS (10 mg) was dissolved in 5 mL of saline and stored at 4 °C. The mice were intraperitoneally injected with LPS (15 mg/kg) to establish an ARDS model. The mice in the ARDS + pioglitazone group also received pioglitazone (10 mg/kg, agonists of PPAR $\gamma$ , Selleck, China) for 4 consecutive days before LPS. At 6 h before treatment with LPS, the mice in the ARDS + GW9662 group were injected intraperitoneally with GW9662 [1 mg/kg, 10% (v/v), dissolved in DMSO, antagonists of PPAR $\gamma$ , Selleck, China]. The mice were sacrificed by cervical dislocation after 1% pentobarbital (50 mg/kg) anesthesia at 24 h after LPS injection, and blood and lung tissues were collected for the experiments. The animal research was approved by the Animal Experiment Committee of The First Affiliated Hospital of the Army Medical University (AMUWEC20182012). The experiment process complied with the institutional guidelines for the care and use of laboratory animals. Subsequent indicator testers did not know the specific groupings of the samples.

**Table 1** Primer sequences

Gene	Primer sequence
MiR-129-5p-F	5'-GGGGGCTTTTTGCGGTCTGG-3'
MiR-129-5p-R	5'-AGTGC GTGTCGTGGAGTC-3'
U6-F	5'-CTCGCTTCGGCAGCAGCATATACT-3'
U6-R	5'-ACGCTTCACGAATTTGCGTGTC-3'
PPAR $\gamma$ -R	5'-CGAAGAGCACCTGACGAACA-3'
PPAR $\gamma$ -F	5'-CTCGTCATTCCCAAAACGCC-3'
GAPDH-F	5'-GCATCTTCTGTGCAGTGCC-3'
GAPDH-R	5'-ACTGTGCCGTTGAATTTGCC-3'

### Wet-to-dry ratio of the lung

At 24 h after treatment with LPS, the right lungs were removed and the wet weight was determined. The lungs were incubated at 60 °C for 5 days to dry, and the weight was measured. The wet-to-dry weight ratio was then calculated.

### Hematoxylin and eosin (H&E)

The lung tissue was fixed with 4% (v/v) paraformaldehyde and embedded in paraffin, sectioned at 4  $\mu$ m thickness, and stained with H&E solution (Sigma-Aldrich, St. Louis, MO, USA).

### Enzyme-linked immunosorbent assay (ELISA)

Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-1 $\beta$ , and IL-6 in cell cultures were detected using an ELISA kit (Beyotime, Beijing, China). The antibody and reagent were added according to the manufacturer's instructions. The optical density (OD) at 450 nm was measured by a microplate reader.

### Cell cultures and transfections

Human lung epithelial cells BEAS-2B (ATCC, USA) were cultured in bronchial epithelial cell culture medium BEpiCM (#3221, ScenCell, USA). The culture environment was 37 °C and 5% carbon dioxide (CO<sub>2</sub>). Next, 0, 0.5, 1, or 2  $\mu$ g/mL of LPS (Sigma-Aldrich) was added to the culture medium, and the cells were cultured for 24 h. PBS (Sigma-Aldrich) was added to the 0  $\mu$ g/mL group as a control.

The miR-129-5p and PPAR $\gamma$  in the cells were downregulated by plasmid transfection. In brief, 2  $\mu$ L of Lipofectamine<sup>TM</sup> 3000 (Invitrogen, Waltham, USA), 40 pmol of miR-129-5p inhibitor, short interfering RNA PPAR $\gamma$  (si-PPAR $\gamma$ , F-5'-CTCCAATGTTCTCAA ACTTAC-3'; R-5'-GATGAGTCATGTAAGTTGACC-3') or negative control (NC) (GenePharma, Suzhou, China) were mixed in 50  $\mu$ L serum-free medium at room temperature for 15 min. The lipid compounds were diluted in 300  $\mu$ L of serum-free medium, and incubated with the cells at 37 °C with 5% CO<sub>2</sub> for subsequent experiments.

### Dual-luciferase reporter assay

The wild-type (WT)/mutated (MUT) PPAR $\gamma$  and miR-129-5p mimic/NC were both cloned into pMIR-REPORT Luciferase vectors (GenePharma, Shanghai, China). The BEAS-2B cells were transfected with both vectors using Lipofectamine 3000 for 24 h. The Dual-Luciferase Reporter 1000 Assay System (Promega, Madison, WI, USA) was used to evaluate luciferase activity.

### Quantitative polymerase chain reaction (qPCR)

The total RNA was acquired, and the purity was detected. The RNA was reverse transcribed using a reverse transcription complementary DNA (cDNA) kit (TaKaRa, Dalian, China) to synthesize the cDNA. SYBR Green PCR Master Mix (TaKaRa, Dalian, China) and the PCR detection system (ABI 7500, Life Technology, Carlsbad, CA, USA) were applied to conduct the qPCR experiment. The PCR cycle was as follows: pretreatment at 95 °C for 10 min; followed by 40 cycles of 94 °C for 15 s, 60 °C for 1 min, and 4 °C for preservation. The comparative cycle threshold ( $\Delta\Delta$ Ct) was employed to analyze the expression of the RNAs. GAPDH and U6 expression was used for normalization. The primers are listed in *Table 1*.

### Western blot

The protein was extracted by protein lysate and detected using a BCA kit. Next, the protein was separated using SDS-PAGE (110 V, 100 min). The proteins were transferred to PVDF membranes (90 V, 90 min). The PVDF membranes were blocked in 5% non-fat milk for 1 h at room temperature. The anti-PPAR $\gamma$  (Abcam, San Francisco, CA, USA; ab45036, 57 kD), anti-Beclin1 (ab207612, 52 kD), LC3B (ab51520, 16/18 kD), and

GAPDH antibody (ab8245, 36 kD) diluted at 1:500 were added and incubated overnight at 4 °C. Next, the secondary antibodies (sc-516102/sc-2357; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) were diluted at 1:5,000, and added and incubated at room temperature for 2 h. Protein blot bands were detected by the Pierce<sup>TM</sup> ECL plus western blotting substrate (Thermo Fisher Scientific) in ChemiDoc MP (Bio-Rad, Hercules, CA, USA).

### Cell counting kit-8 (CCK-8) assays

In this study,  $2 \times 10^4$  cells (100  $\mu$ L) were seeded in 96-well plates, 10  $\mu$ L of CCK-8 (Beyotime Institute of Biotechnology, Beijing, China) medium was added, and the cells were cultured at 37 °C for 2 h. The OD at 450 nm was measured using a microplate reader (Tecan Infinite M200 Micro Plate Reader; LabX, Männedorf, Switzerland).

### Flow cytometry

The apoptosis rates were tested using flow cytometry (BD FACSCalibur, Becton Dickinson, USA) and the annexin V-FITC/PI kit (Millipore, USA). The reagents were added according to the manufacturers' instructions. Q2 + Q3 was the apoptotic rate.

### Statistical analysis

The experiments were repeated 3 times. All the statistical analyses were performed using GraphPad Prism 7. All the experimental data are presented as the mean  $\pm$  standard deviation. The *t*-test was used for comparisons between groups, and the Tukey test was used for comparisons among multiple groups. A *P* value <0.05 was considered statistically significant.

## Results

### PPAR $\gamma$ alleviates lung injury in LPS-induced ARDS mice by promoting autophagy and suppressing inflammatory

To analyze whether the mechanism of the PPAR $\gamma$  alleviating the inflammatory response of ALI is related to autophagy, we simulated the ARDS process *in vivo* by injecting LPS intraperitoneally into a mouse model of ALI. GW9662 and pioglitazone were used to inhibit and activate the PPAR $\gamma$ , respectively. By observing the pathological changes of lung tissue, we found that antagonizing the PPAR $\gamma$  with

GW9662 further aggravated lung tissue damage, while activating the PPAR $\gamma$  with pioglitazone alleviated LPS-induced lung injury to a certain extent (see *Figure 1A*). GW9662 further increased of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, and the wet-to-dry ratio caused by LPS. The activation of the PPAR $\gamma$  by pioglitazone reduced the inflammatory reaction and pulmonary edema caused by LPS (see *Figure 1B-1E*). Additionally, the GW9662 antagonism of the PPAR $\gamma$  caused the levels of autophagy-related proteins Beclin1 and LC3II/LC3I to reduce further. Pioglitazone not only upregulated the PPAR $\gamma$ , but also partially reversed the autophagy inhibition caused by LPS (see *Figure 1F*). This suggested that in the LPS-induced ARDS model, the level of the PPAR $\gamma$  and autophagy was reduced. Antagonizing the PPAR $\gamma$  with GW9662 further inhibited autophagy and exacerbated pulmonary edema and inflammatory damage. Conversely, the use of pioglitazone promoted autophagy levels and effectively relieved the lung injury caused by LPS. This suggested that the anti-inflammatory effects of the PPAR $\gamma$  might be related to autophagy.

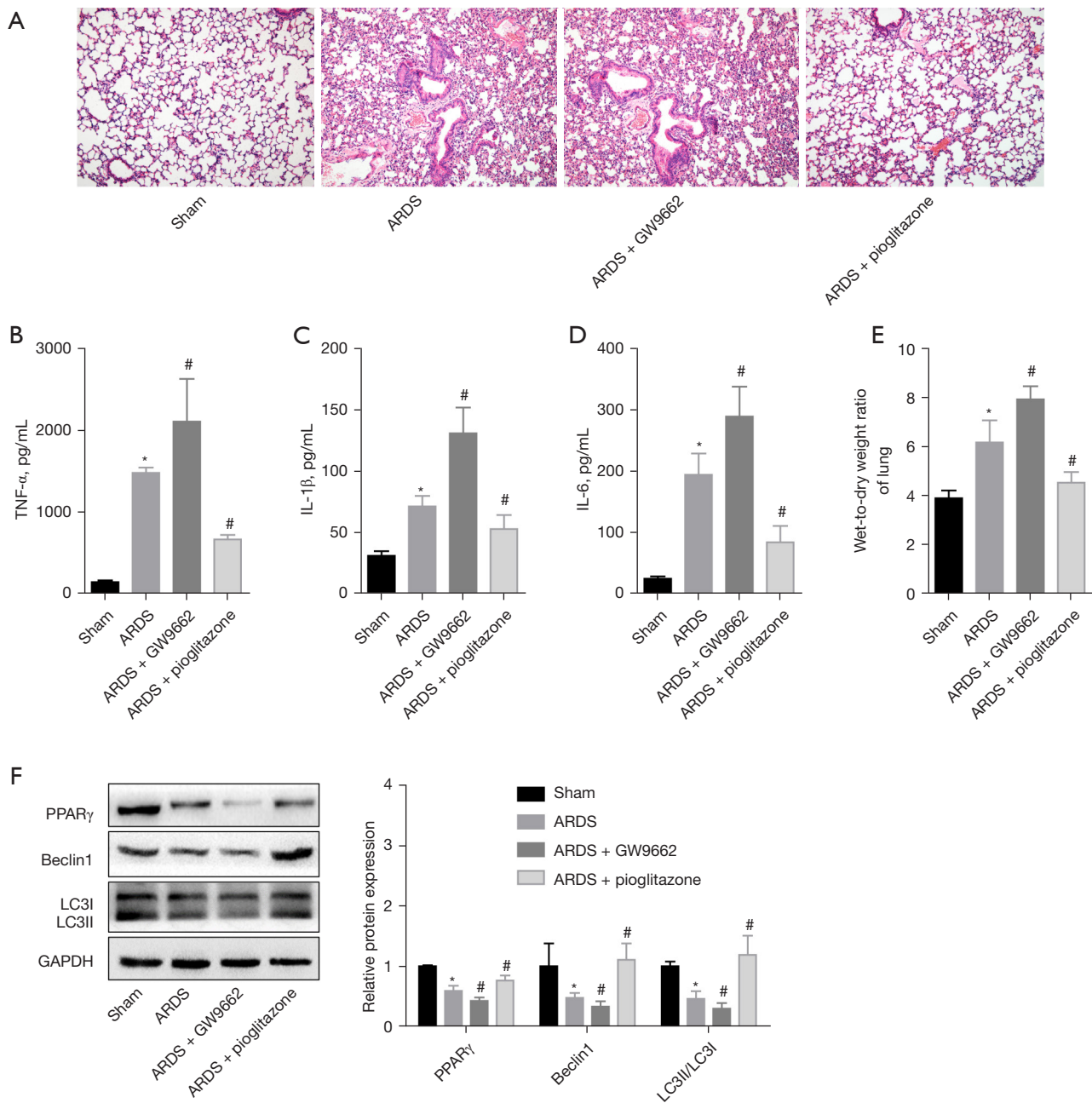
### MiR-129-5p directly targets PPAR $\gamma$

The prediction results showed that there were targeted binding sites between miR-129-5p and the 3'-untranslated region (3'-UTR) of the PPAR $\gamma$  messenger RNA (mRNA) (see *Figure 2A*). The dual-luciferase report verified that miR-129-5p directly targeted the PPAR $\gamma$  mRNA 3'-UTR (see *Figure 2B,2C*).

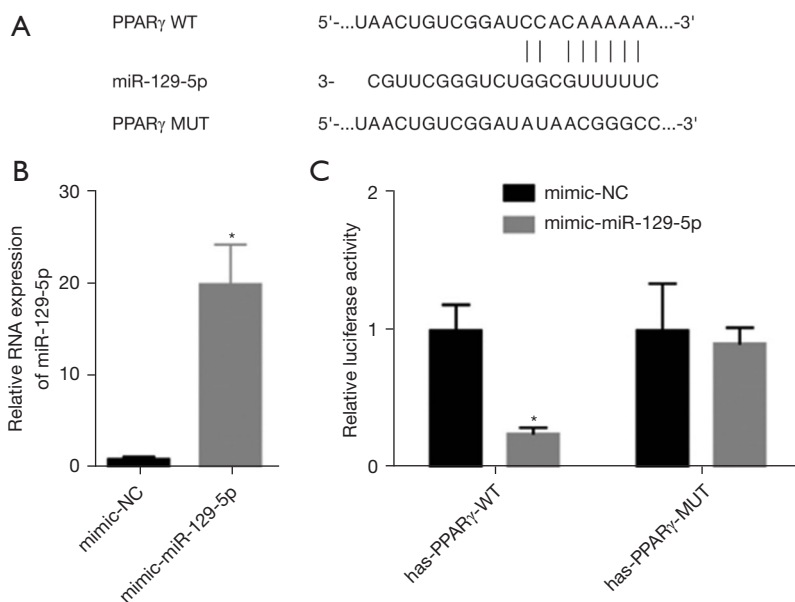
### LPS induces miR-129-5p and inhibits PPAR $\gamma$

The BEAS-2B cells and LPS were used to establish an *in vitro* ALI model to simulate ARDS. The results showed that LPS inhibited the growth and promoted the apoptosis of the BEAS-2B cells in a dose-dependent manner in the concentration range of 0–2  $\mu$ g/mL (see *Figure 3A,3B*). The results also showed that LPS promoted the levels of miR-129-5p, such that the levels of miR-129-5p increased 2-, 5-, and 6-fold at 0.5, 1 and 2  $\mu$ g/mL of LPS, respectively (see *Figure 3C*). Additionally, in a concentration range of 0–2  $\mu$ g/mL, LPS promoted the secretion of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 by BEAS-2B cells in a dose-dependent manner (see *Figure 3D-3F*).

To analyze the changes of the PPAR $\gamma$ -related pathways and autophagy levels in ARDS simulated by LPS, the PPAR $\gamma$  and the autophagy-related proteins Beclin1 and



**Figure 1** PPAR $\gamma$  alleviates lung injury in LPS-induced ARDS mice by promoting autophagy and suppressing inflammatory. (A) H&E staining was applied to detect effects of the PPAR $\gamma$  antagonized/activated by GW9662/pioglitazone on LPS-induced lung tissue damage in ARDS mouse models ( $\times 100$ ). (B-D) Effects of GW9662 and pioglitazone on TNF- $\alpha$ , IL-1 $\beta$ , and IL-6. (E) Effects of GW9662 and pioglitazone on pulmonary edema. (F) Effects of GW9662 and pioglitazone on the PPAR $\gamma$ , Beclin1 and LC3 proteins. \* $P < 0.05$  vs. sham group; # $P < 0.05$  vs. ARDS group. ARDS, acute respiratory distress syndrome; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; IL, interleukin; PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ ; LPS, lipopolysaccharide; H&E, hematoxylin and eosin.



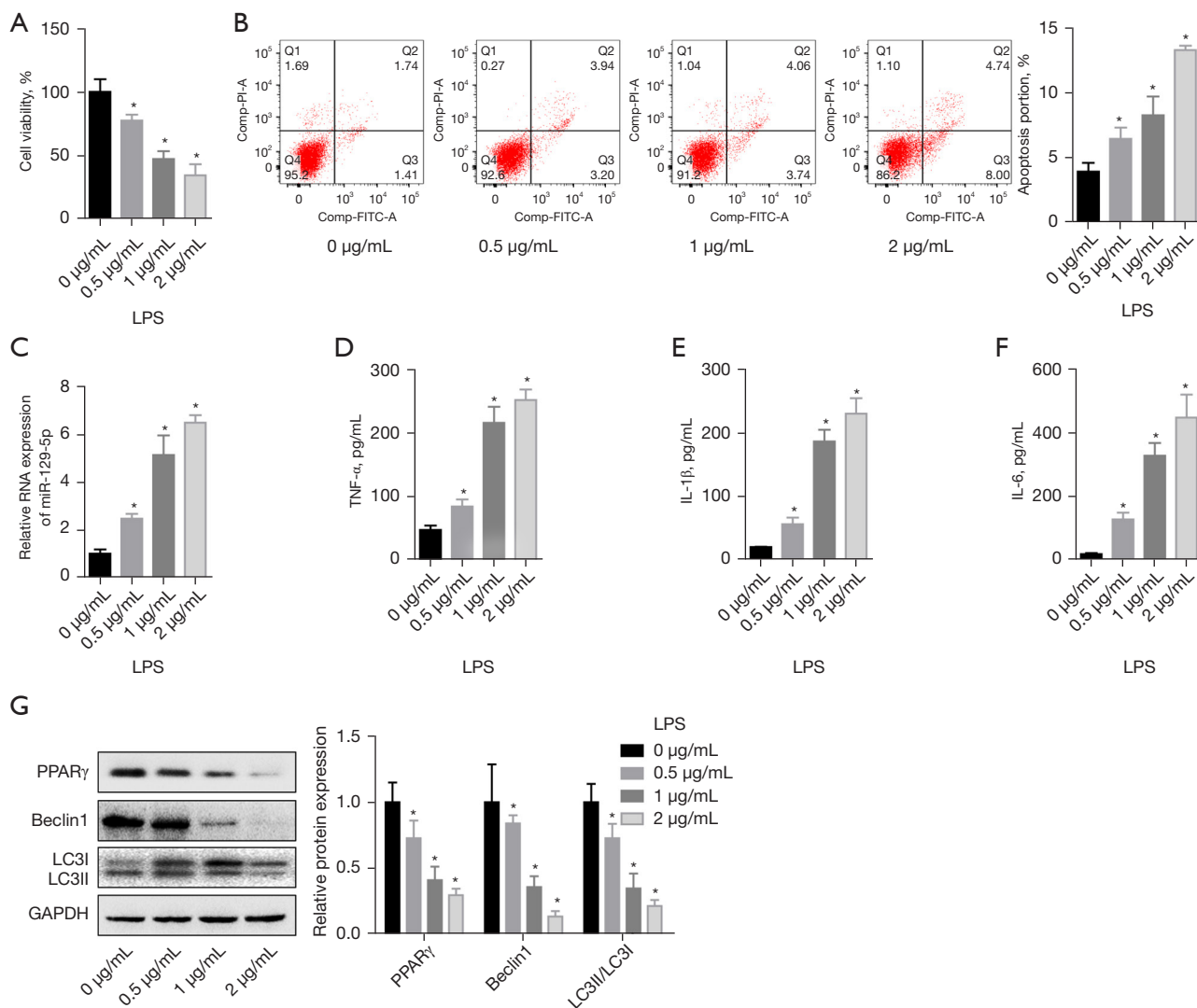
**Figure 2** MiR-129-5p directly targets PPAR $\gamma$ . (A) The binding site of miR-129-5p to the 3'-UTR region of the PPAR $\gamma$  mRNA. (B,C) The dual-luciferase report verified that miR-129-5p directly targeted the 3'-UTR region of the PPAR $\gamma$  mRNA. \* $P < 0.05$  vs. mimic-NC group. PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ ; WT, wild-type; MUT, mutated; miR-129-5p, microRNA-129-5p; NC, negative control; 3'-UTR, 3'-untranslated region; mRNA, messenger RNA.

LC3 were detected. We found that the levels of the PPAR $\gamma$  protein decreased to about 0.7, 0.4, and 0.3 for 0, 0.5, 1, and 2  $\mu\text{g/mL}$  of LPS, respectively. The changing trend of autophagy-related proteins were also reflected in the range of 0–2  $\mu\text{g/mL}$ , such that LPS inhibited autophagy in a quantitative manner (see *Figure 3G*). Thus, in the LPS-induced *in vitro* ARDS model, the miR-129-5p and inflammatory factor levels were elevated, while the PPAR $\gamma$  and autophagy fluxes were decreased.

#### ***PPAR $\gamma$ -induced autophagy alleviates the inflammatory response and apoptosis of lung epithelial cells caused by LPS***

The BEAS-2B cells were cultured at 1  $\mu\text{g/mL}$  of LPS to induce acute injury, and simulate the ARDS environment *in vitro*. Before adding LPS, the cells were transfected with the miR-129-5p inhibitor and/or the si-PPAR $\gamma$  plasmid. We found that LPS induced the upregulation of miR-129-5p and the downregulation of the PPAR $\gamma$  mRNA and PPAR $\gamma$  protein. The addition of the miR-129-5p inhibitor promoted the expression of the PPAR $\gamma$  mRNA and PPAR $\gamma$ . Additionally, si-PPAR $\gamma$  blocked the promotion of the miR-129-5p inhibitor on the PPAR and PPAR $\gamma$ . The PPAR $\gamma$

mRNA and PPAR $\gamma$  had no significant effects on the level of miR-129-5p (see *Figure 4A-4C*). In the presence of 1  $\mu\text{g/mL}$  of LPS, downregulating the expression of miR-129-5p promoted cell survival and reduced the apoptosis rate. Additionally, the downregulation of the PPAR $\gamma$  further increased the damage of LPS to cells, and the co-transfection of the si-PPAR $\gamma$  blocked the protective effect of the miR-129-5p inhibitor on the cells (see *Figure 4D,4E*). In the presence of 1  $\mu\text{g/mL}$  of LPS, the transfection of the miR-129-5p inhibitor restored the levels of Beclin1 protein and LC3II/LC3I to promote autophagy, and inhibited the cells from secreting TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 inflammatory factors into the culture medium. Inhibiting the expression of the PPAR $\gamma$  protein further reduced the levels of Beclin1 and LC3II/LC3I to inhibit autophagy, and blocked the promotion effect of the miR-129-5p inhibitor on autophagy and inhibition of inflammatory factors (see *Figure 4F-4I*). This suggested that the transfection of the miR-129-5p inhibitor increased the level of the PPAR $\gamma$  protein, promoted autophagy, inhibited the secretion of inflammatory factors, and alleviated the apoptosis induced by LPS. Additionally, this effect was blocked by the si-PPAR $\gamma$  plasmid.



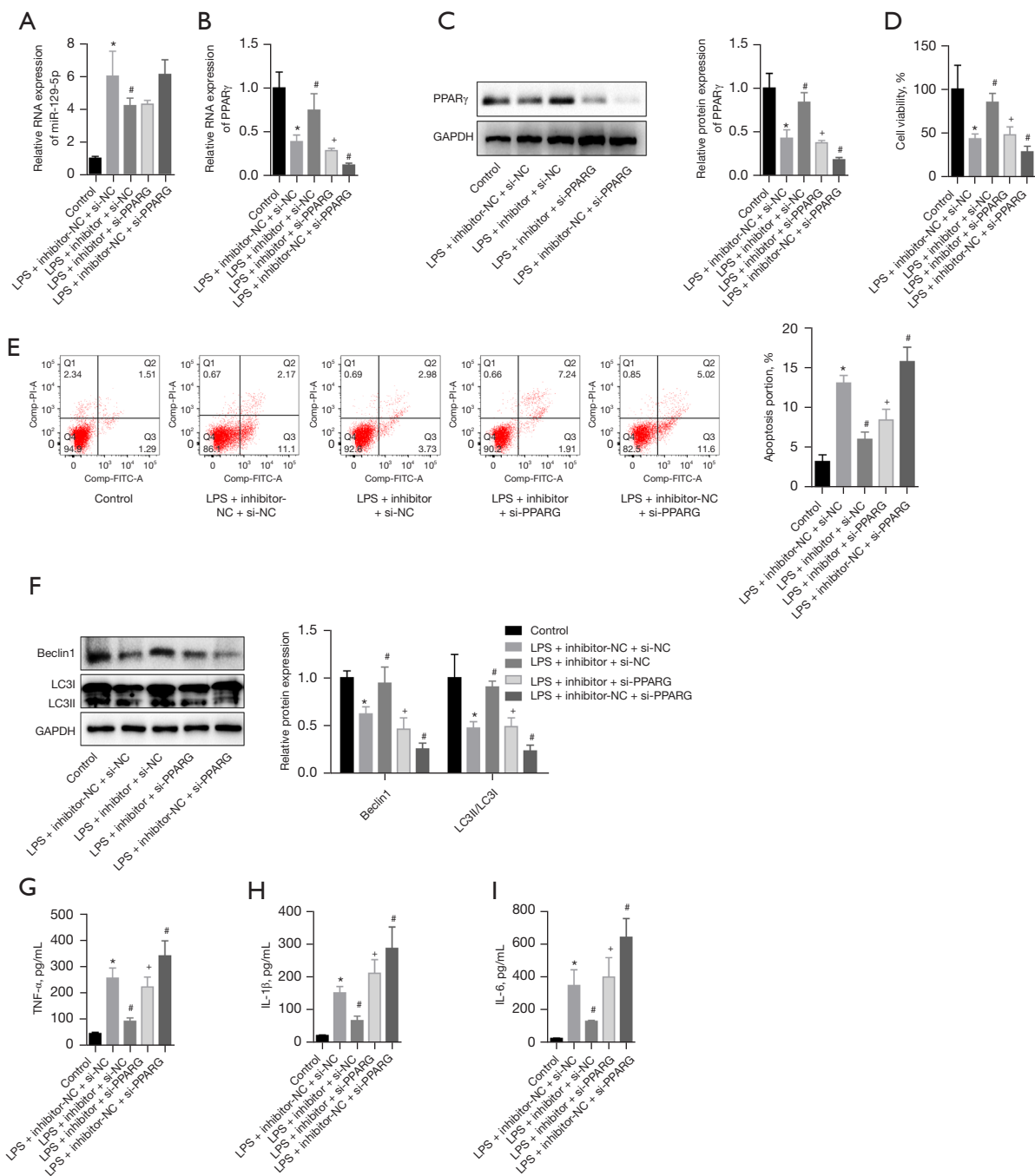
**Figure 3** LPS-induced *in vitro* ARDS model promotes the inflammatory response, apoptosis and inhibits autophagy in a dose-dependent manner. (A) Cell viabilities of human lung epithelial cells BEAS-2B at different LPS concentrations. (B) Apoptotic rates of BEAS-2B at different LPS concentrations. (C) MiR-129-5p levels of BEAS-2B at different LPS concentrations. (D-F) The concentrations of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 in cell culture medium under different concentrations of LPS. (G) The expression levels of the PPAR $\gamma$ , Beclin1 and LC3 proteins under different concentrations of LPS. \* $P < 0.05$  vs. 0  $\mu\text{g/mL}$  group. LPS, lipopolysaccharide; miR-129-5p, microRNA-129-5p; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; IL, interleukin; PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ ; ARDS, acute respiratory distress syndrome.

## Discussion

In this study, we found that in ARDS, miR-129-5p is upregulated and autophagy is suppressed. In *in vitro* and *in vivo* ARDS models, the downregulation of miR-129-5p may promote PPAR $\gamma$  expression and play a role in the promotion of autophagy. This may cause the promotion

of autophagy and the inhibition of inflammatory response, thus preventing lung injury.

Autophagy is the degradation of proteins and organelles mediated by lysosomes in eukaryotes. It is a physiological process in which the material is recycled and maintained in the internal environment homeostasis (21). Autophagy not only occurs during the normal physiological metabolism



**Figure 4** PPAR $\gamma$ -induced autophagy alleviates the inflammatory response and apoptosis of lung epithelial cells caused by LPS. (A) Effects of transfection of the miR-129-5p inhibitor and/or the si-PPAR $\gamma$  plasmid on miR-129-5p levels in BEAS-2B cells. (B,C) Effects of transfection of the miR-129-5p inhibitor and/or the si-PPAR $\gamma$  plasmid on the PPAR $\gamma$  mRNA and PPAR $\gamma$  protein levels in BEAS-2B cells. (D,E) Effects of transfection of the miR-129-5p inhibitor and/or the si-PPAR $\gamma$  plasmid on cells viability and apoptosis. (F) Effects of transfection of the miR-129-5p inhibitor and/or the si-PPAR $\gamma$  plasmid on Beclin1 and LC3 proteins. (G-I) Effects of transfection of the miR-129-5p inhibitor and/or the si-PPAR $\gamma$  plasmid on TNF- $\alpha$ , IL-1 $\beta$ , and IL-6. \* $P < 0.05$  vs. control group; # $P < 0.05$  vs. LPS + inhibitor-NC + si-NC group; † $P < 0.05$  vs. LPS + inhibitor + si-NC group. LPS, lipopolysaccharide; NC, negative control; si-PPAR $\gamma$ , short interfering RNA PPAR $\gamma$ ; PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ ; miR-129-5p, microRNA-129-5p; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; IL, interleukin.



of cells, but is also a defense mechanism that enables cells to cope with adverse external environmental factors (22). The disturbance of cell autophagy or autophagy-related genes is a key factor causing the inflammatory response. Research has shown that in a mouse model of ALI induced by LPS, autophagy inhibits the inflammatory response and relieves lung injury by regulating the NF- $\kappa$ B pathway (23). In an LPS-induced ARDS mice model, Parkin-dependent mitophagy autophagy was shown to inhibit apoptosis and relieve lung injury (24). However, the mechanism by which ARDS-related autophagy is regulated is unknown.

The PPARs are activated after binding to their ligands, and they then interact with PPAR response elements upstream of the target gene promoter, ultimately regulating the transcription of the target gene and the metabolic process in the cell. In a LPS-induced acute inflammatory injury model of RAW264.7 cells *in vitro*, the expression levels of TNF- $\alpha$  and other inflammatory factors were upregulated, and the level of the PPAR $\gamma$  protein was inhibited. The activation of the PPAR $\gamma$  inhibits the inflammatory response by inhibiting the activation of NF- $\kappa$ Bp65 (25). Decreasing the methylation level of the PPAR $\gamma$  gene promoter inhibits the expression level of inflammatory genes (26). The PPAR $\gamma$  is also an important protein that regulates the autophagy pathway (27,28). Based on this, we speculated that the PPAR $\gamma$  may inhibit the inflammatory response by promoting autophagy, and alleviate the lung injury caused by ARDS. LPS-induced ALI was used to simulate an ARDS mice model, and pioglitazone and GW9662 were applied to activate and antagonize the PPAR $\gamma$ , respectively. The results showed that the activation of the PPAR $\gamma$  significantly relieved lung injury and pulmonary edema, inhibited the expression of inflammatory factors, and promoted autophagy. GW9662 aggravated inflammatory damage and inhibited autophagy. Research has shown that pioglitazone relieves ARDS (29). Defects in autophagy activate inflammasomes, and promote the release of cytokines, such as IL-1 and IL-18 (30). Thus, the anti-inflammatory effects of the PPAR $\gamma$  may be related to autophagy.

MiRNA-related targeted regulatory mechanisms have recently been found to be related to ARDS (31,32). MiRNAs regulate the inflammatory response by regulating the target gene expression, and participate in ARDS and lung injury (31,32). MiR-129-5p promotes the inflammatory response and regulates autophagy. During the inflammatory response of cardiomyocytes, the miR-129-5p level is significantly upregulated and involved in promoting the apoptosis of cardiomyocytes (33). In

adipose-derived stem cells, inhibiting the level of miR-129-5p has also been shown to regulate the immunosuppressive response and reduce the release of inflammatory factors (34). However, miR-129-5p has been shown to have anti-inflammatory effects (35,36). Recent research has shown that miR-129-5p has anti-autophagy effects (37-39). During hydrogen peroxide, infection, and tumor-related autophagy, the increased expression of miR-129-5p is related to the inhibition of autophagy (37-39). Through *in vitro* experiments, this study showed that the targeting of miR-129-5p inhibits the expression of the PPAR $\gamma$ . Further, downregulating the level of miR-129-5p promotes the PPAR $\gamma$  expression, promotes autophagy, and inhibits inflammatory response and apoptosis. Additionally, silencing the PPAR $\gamma$  blocks the protective effects of miR-129-5p. This indicates that in the LPS-induced ARDS model, the decrease of the PPAR $\gamma$  caused by miR-129-5p may promote the inflammatory response, and inhibit autophagy, thereby leading to the inflammatory response.

In conclusion, activating the PPAR $\gamma$  might promote autophagy, inhibit the inflammatory response, and alleviate lung injury induced by LPS. The PPAR $\gamma$  is under the targeted regulation of miR-129-5p. MiR-129-5p may induce autophagy and inhibit the inflammatory response by promoting the expression of the PPAR $\gamma$ . The mechanism by which miR-129-5p regulates autophagy, and its role in ARDS still need to be confirmed. Additionally, the clinical significance of miR-129-5p in ARDS and its relationship with prognosis require further exploration. The mechanism by which miR-129-5p regulates autophagy and its role in ARDS still needs to be confirmed by *in vivo* studies.

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## Footnote

**Reporting Checklist:** The authors have completed the ARRIVE reporting checklist. Available at <https://atm.amegroups.com/article/view/10.21037/atm-22-979/rc>

**Data Sharing Statement:** Available at <https://atm.amegroups.com/article/view/10.21037/atm-22-979/dss>

**Conflicts of Interest:** All authors have completed the ICMJE uniform disclosure form (available at <https://atm.amegroups.com/article/view/10.21037/atm-22-979/coif>). All authors report that this study received funding from the General project of National Natural Science Foundation of China (project No. 81670070), and the Chongqing Research Program of Basic Research and Frontier Technology (project No. cstc2017jcyjAX0338). The authors have no other conflicts of interest to declare.

**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The animal research was approved by the Animal Experiment Committee of The First Affiliated Hospital of the Army Medical University (AMUWEC20182012). The experiment process complied with the institutional guidelines for the care and use of laboratory animals.

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