

PPAR- α improves the recovery of lung function following acute respiratory distress syndrome by suppressing the level of TGF- β 1

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Abstract. Although peroxisome proliferator-activated receptor (PPAR)- α has been reported to be involved in preventing acute lung injury (ALI), the molecular regulation of post-ALI lung recovery remains to be fully elucidated. The aim of the present study was to characterize the mechanism by which PPAR- α prevents ALI and examine the role of PPAR- α in the recovery of lung function following acute respiratory distress syndrome (ARDS). Reverse transcription-quantitative-polymerase chain reaction and western blot analyses suggested that PPAR- α was effective in suppressing transforming growth factor (TGF)- β 1 in HLF cells and RAW 264.7 cells. In an ALI mouse model, PPAR- α treatment prior to stimulation with lipopolysaccharide (LPS) resulted in a decrease in the expression of TGF- β 1 in bronchoalveolar lavage fluid (BALF), peripheral blood and splenocytes. The injection of a virus expressing short hairpin PPAR- α into mice following LPS treatment resulted in a dose-dependent increase in lung resistance index and decrease in dynamic compliance, and a significant increase in BALF protein, which indicated PPAR- α was essential for the recovery of lung function following ALI. Of note, the serum expression of PPAR- α was inversely correlated with TGF- β 1 and negatively correlated with disease severity in patients with ARDS. These data suggested that PPAR- α was essential for the recovery of lung function following ALI by the suppression of TGF- β 1, which reveals a previously unappreciated mechanism controlling post-ALI lung recovery.

Introduction

Acute respiratory distress syndrome (ARDS), the most severe form of acute lung injury (ALI), is the leading cause of acute respiratory failure, and has a mortality rate of ~40% worldwide (1), with a variety of detrimental clinical disorders, including hypoxemia, respiratory distress and pulmonary edema (2,3). Although the morbidity and mortality rates associated with ARDS in patients has decreased due to advances in protective ventilation (4) and fluid conservative supportive treatments (5), those surviving suffer from significant physical impairments (6). Therefore, improved comprehension of the molecular mechanism of ARDS is urgently required.

In the majority of cases, ARDS is induced by inflammatory pulmonary diseases or bacterial sepsis, and Gram-negative bacteria are common culprits (7). The endotoxin of Gram-negative bacteria, lipopolysaccharide (LPS), has been reported to be important in eliciting lung inflammation by inducing proinflammatory cytokines, including tumor necrosis factor (TNF)- α , interleukin (IL)-6, IL-1 β and IL-8 (8), which increase the infiltration of inflammatory cells to the lungs in the development of ALI. Pro-inflammatory gene expression can be inhibited by the activation of peroxisome proliferator-activated receptor (PPAR)- α (9), which is a member of the ligand-activated transcription factors involved in the nuclear hormone receptor superfamily (10,11).

Transforming growth factor- β (TGF- β) signaling has been reported to be important in development and disease. TGF- β is known to be a major inducer of epithelial to mesenchymal transition via the small mothers against decapentaplegic (Smad)-dependent or Smad-independent pathways (12,13). Previous studies have reported that, through the stimulation of fibroblast proliferation, TGF- β 1 is involved in ARDS, which leads to the development of pulmonary fibrosis. Pretreatment with rosiglitazone, a ligand of PPAR- γ , can protect against ALI by repressing the activation of nuclear factor- κ B and inhibiting TGF- β signaling (14). However, whether PPAR- α is involved in TGF- β signaling, and whether PPAR- α is involved for the recovery of lung function following ALI, remain to be fully elucidated. The aim of the present study was to investigate the protective effects of PPAR- α in LPS-induced ALI *in vivo* and *in vitro*, and examine the underlying mechanisms involving the PPAR- α and TGF- β signaling pathway.

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Materials and methods

Patient selection. The present study was approved by the Ethics Committee of Zigong First People's Hospital. A total of 18 patients (including 8 females and 10 males, ages 55-75) with ARDS caused by sepsis were enrolled between June 2010 and October 2013. All peripheral blood samples were collected with written informed consent. The clinical characteristics of ARDS were summarized and disease severity was determined using the Acute Physiology and Chronic Health Evaluation (APACHE) II, the Murray Lung Injury Score (LIS) and the Simplified Acute Physiology Score (SAPS) II (15). The characteristics of patients with ARDS were as follows: i) PaO₂/FiO₂ \leq 300; ii) presence of bilateral pulmonary infiltrates on frontal chest radiograph; iii) no clinical evidence of left atrial hypertension; iv) requirement for positive pressure ventilation via an endotracheal tube; v) composite of oxygenation, compliance, positive end expiratory pressure and the appearance of chest radiograph (16). Blood was collected using an indwelling arterial catheter at the time of ICU admission (baseline) into sterilized, silicone-coated glass tubes, and at 3 and 7 days subsequently. Healthy blood donors were used as controls (also including 8 females and 10 males, ages from 55-75). Serum samples (4 ml) were obtained by centrifugation at 2,500 x g for 10 min at room temperature and frozen at -70°C until use. The buffy coat cell layer was carefully aspirated and buffy coat cells were suspended. Concentrations of TGF- β 1 and PPAR- α were determined using commercial, standardized enzyme-linked immunosorbent assay (ELISA) kits. The TGF- β 1 ELISA kit (cat. no. ADI-900-155) was from Enzo Life Sciences, Inc. (Farmingdale, NY, USA) and the PPAR- α ELISA kit (cat. no. 40,196) was from Active Motif, Inc. (Carlsbad, CA, USA). The lower detection limit for TGF- β 1 was 0.10 pg/ml and for PPAR- α was 0.30 pg/ml.

Cell lines and cell culture. Human lung fibroblast (HLFs; IMR-90 cells) and *Mus musculus* monocyte/macrophage RAW 264.7 cells were purchased from American Type Culture Collection (Manassas, VA, USA) and cultured in Eagle's Minimum Essential Medium with 10% fetal bovine serum (FBS; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The RAW 264.7 macrophages were cultured in Dulbecco's modified Eagle's medium containing 10% FBS. The cells were cultured in a humidified incubator at 37°C in 5% CO₂.

Reagents. LPS (isolated from *Escherichia coli*) and TGF- β 1 (used at a dose of 10 μ g/ml) were purchased from Sigma-Aldrich; Merck Millipore (Darmstadt, Germany). Mouse monoclonal antibodies against TGF- β 1 were from Abcam (cat. no. PB190503; Cambridge, MA, USA) and rabbit monoclonal antibody against PPAR α was from Cell Signaling Technology, Inc. (cat. no. 2443S; Beverly, MA, USA). HRP-conjugated anti-mouse and anti-rabbit secondary antibodies were from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Specific small interfering short hairpin (sh)RNA targeting TGF- β 1 and PPAR- α and the negative control shRNA were all from Sigma-Aldrich; Merck Millipore. Following allowing cellular attachment to the plates, 2x10⁵ cells were treated with relative

shRNAs using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) RNA and protein were collected 72 h following treatment.

Induction of ARDS in mice. All experiments involving mice were performed in strict accordance with Animal Care and Use guidelines from Beijing Charles River Laboratory Animal Centre institutional committee (Beijing, China). A total of 30 (15 male and 15 female) mice (8-10 weeks old; 19-22 g) were purchased from Beijing Charles River Laboratory Animal Centre, and kept under standard conditions at room temperature (24°C) with a 12 h day/night cycle under specific pathogen-free conditions. At 3 h following intratracheal instillation of LPS (4 mg/kg) (17), the mice were administered with an intravenous injection in the tail vein of 10% chloral hydrate (3.5 ml/kg) for anesthetization at 4, 12 and 24 h. The mice were divided into the following groups: Vector group, injected with the same volume of pyrogen-free PBS; LPS group, stimulated with LPS; PPAR α group, pre-treated with PPAR α prior to stimulation with LPS. Following the successful induction of ALI, the mice were administered with a tail vein injection of adeno-associated viruses (AAV) carrying PPAR- α short hairpin RNA (shPPAR- α group) or with a scrambled (SCR) sequence as a control. Primary murine alveolar epithelial cells were isolated, as described previously (18).

RNA isolation and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. Total RNA was extracted from the cultured cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). For analysis of the expression of messenger RNA (mRNA), cDNA synthesis was performed by reverse transcription using the Transcriptor First Stand cDNA synthesis kit (Roche Diagnostics, Basel, Switzerland). The qPCR was performed in duplicate with a QuantiTect SYBR Green PCR kit (Qiagen, Inc., Valencia, CA, USA) on an Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.), according to manufacturer's protocol. The primers were as follows: TGF- β 1, forward 5'-CCACCTGCAAGACCATCG AC-3' and reverse: 5'-CTGGCGAGCCTTAGTTTGGAC-3'; PPAR γ , forward 5'-GAGATCATCTACACGATGCTGGC-3' and reverse 5'-CGCAGGCTTTTGAGGAAC-3'; and GAPDH, forward 5'-GAGAAGTATGACAACAGCCTC-3' and reverse 5'-ATGGACTGTGGTCATGAGTC-3'. All primers were purchased from Thermo Fisher Scientific, Inc. The PCR amplification was performed at 95°C for 1 min, followed by 35 cycles of 95°C for 15 sec, 60°C for 15 sec, and 72°C for 30 sec. The expression levels of genes were normalized against that of GAPDH and relative fold changes in mRNA expression were calculated using the formula 2^{- $\Delta\Delta$ C_q} (19).

Western blot analysis. Whole cell lysates were collected and protein from the cultured cells was extracted using RIPA lysis buffer on ice, following centrifugation at 4°C, 10,000 x g for 15 min, the supernatants were collected. The BCA protein assay kit (Thermo, USA) was used to determine the protein concentration, and 30 μ g from each sample was mixed with 4X SDS loading buffer and heated

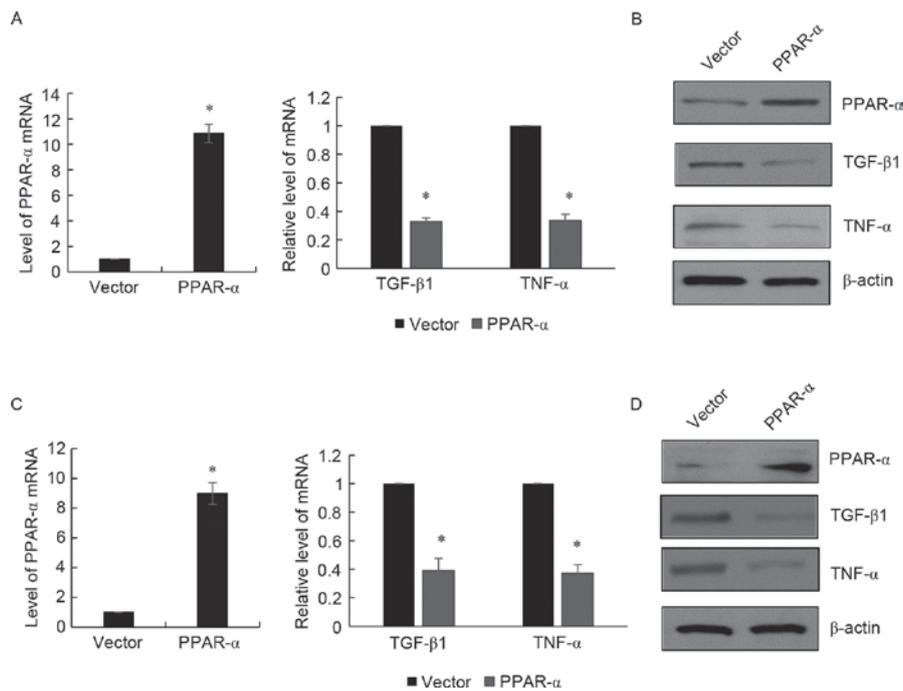


Figure 1. PPAR- α is effective in suppressing the activity of TGF- β in IMR-90 cells and RAW 264.7 cells. (A) RT-qPCR analysis was used to detect the mRNA levels of TGF- β 1, TNF- α and PPAR- α in IMR-90 cells treated with PPAR- α , compared with the vector group. Data are presented as the mean \pm standard deviation of three independent experiments. * P <0.05. GAPDH was used as an intrinsic control. (B) Western blot analysis was used to detect the protein levels of TGF- β 1, TNF- α and PPAR- α in IMR-90 cells treated with PPAR- α , compared with the vector group. β -actin was used as a control. (C) RT-qPCR analysis was used to detect levels of TGF- β 1, TNF- α and PPAR- α in RAW 264.7 cells treated with PPAR- α , compared with the vector group. (D) Western blot analysis was used to detect levels of TGF- β 1, TNF- α and PPAR- α in RAW 264.7 cells treated with PPAR- α , compared with the vector group. RT-qPCR, reverse transcription-quantitative polymerase chain reaction; TGF- β , transforming growth factor- β ; TNF- α , transforming growth factor- α ; PPAR- α , peroxisome proliferator-activated receptor- α .

at 100°C for 10 min. Protein samples were then separated by 10% SDS-polyacrylamide gel electrophoresis and transferred onto a PVDF membrane (EMD Millipore, Billerica, MA, USA). A mixture of 5% non-fat milk in Tris-buffered saline Tween-20 (TBST) was used to block the nonspecific proteins for 1 h at room temperature. The membrane blots were then probed with primary antibodies at 4°C overnight, as follows: PPAR- α (1:500), TGF- β 1 (1:1,000), TNF- α (1:1,000), and β -actin (1:2,000). The membranes were washed five times with TBST for 5 min, followed by incubation with horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. An enhanced chemiluminescent system (GE Healthcare Life Sciences, NJ, USA) was used to visualize the protein antigen. The signals were recorded using X-ray film (Kodak, Rochester, NY, USA). Images were captured, representative of three repeats.

ELISA. The levels of PPAR- α and TGF- β 1 in the bronchoalveolar lavage fluid (BALF), serum and cell culture supernatants were determined using sandwich ELISA, according to the manufacturer's protocol.

Statistical analysis. All statistical analyses were performed using SPSS 18.0 statistical software (SPSS, Inc., Chicago, IL, USA). Data were analyzed by comparing the mean \pm standard deviation from three experiments using Student's t-test P <0.05 was considered to indicate a statistically significant difference.

One-way analysis of variance with a Bonferoni correction was used for statistical analysis, followed by a Fisher's exact test, as necessary.

Results

PPAR- α is effective at suppressing TGF- β in HLF cells and RAW 264.7 cells. In order to investigate whether PPAR- α was involved in TGF- β signaling, the present study treated HLFs with PPAR- α and, 48 h following treatment, the cells were harvested. The mRNA and nuclear proteins were isolated, followed by the examination of TGF- β 1 using RT-qPCR and western blot analyses. It was found that PPAR- α inhibited the expression of TGF- β 1 in HLF cells at the mRNA level (Fig. 1A) and protein level (Fig. 1B); the suppression of TNF- α by PPAR- α was used as a positive control. To further confirm these findings, RAW 264.7 cells were used and a similar experiment was performed. As shown in Fig. 1C and D, the expression of TGF- β 1 was suppressed by the enforced expression of PPAR- α .

PPAR- α treatment downregulates the expression of TGF- β 1 in the mouse model of LPS-induced ALI. To further investigate the roles of PPAR- α and TGF- β 1 in ALI, LPS-induced ALI mice were used, and the expression levels of PPAR- α and TGF- β 1 were examined using RT-qPCR and western blot analyses. Following LPS treatment, in the murine lung, a decrease in the mRNA expression of PPAR- α and an increase in the mRNA

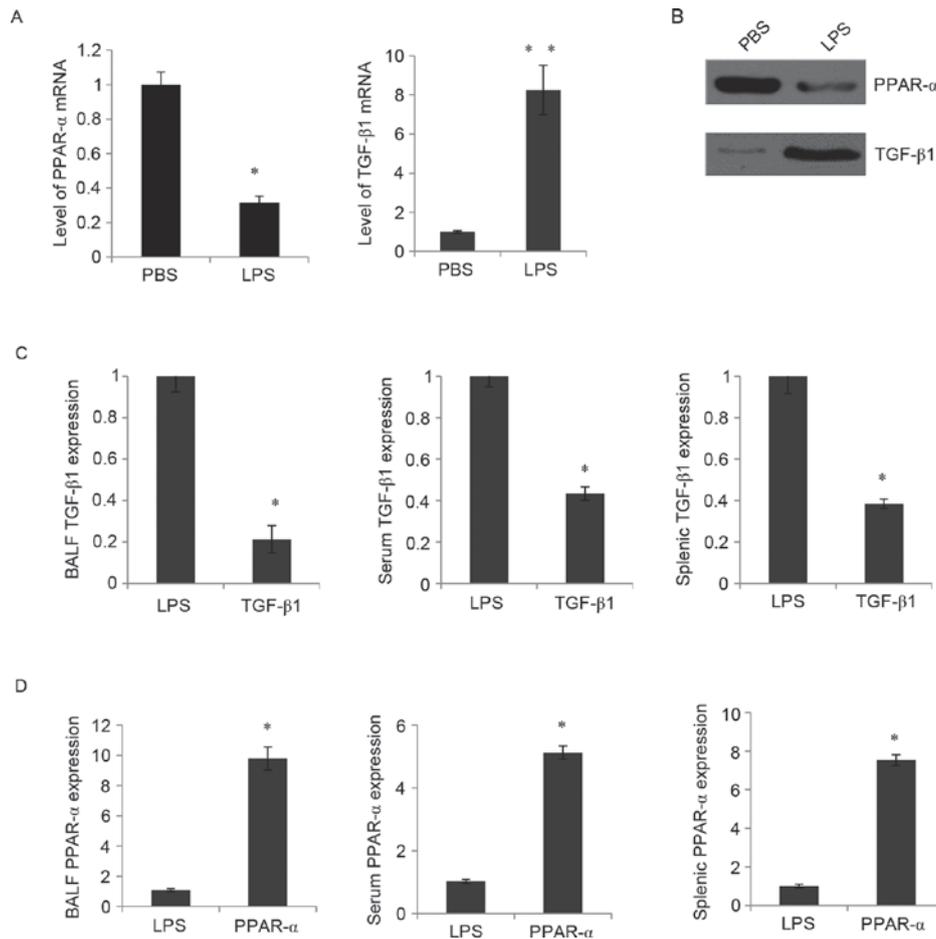


Figure 2. PPAR- α treatment downregulates the expression of TGF- β 1 in an LPS-induced acute lung injury mouse model. (A) Groups of mice were treated with LPS (or PBS as a control). The levels of TGF- β 1 and PPAR- α were determined using reverse transcription-quantitative polymerase chain reaction analysis. Data are presented as the mean \pm standard deviation of three replicates. GAPDH was used as intrinsic control. Experiments were performed in triplicate. * $P < 0.05$ and ** $P < 0.01$. (B) Groups of mice were treated with LPS or PBS. The levels of TGF- β 1 and PPAR- α were determined using western blot analysis. (C) Groups of mice were treated with LPS or with PPAR- α treatment prior to LPS. The levels of TGF- β 1 in BALF (left panel), peripheral blood (middle panel) and splenocytes (right panel) were determined. Data are presented as the mean \pm standard deviation. Experiments were performed in triplicate. * $P < 0.05$. (D) Groups of mice were treated with LPS, or PPAR- α prior to LPS. The levels of PPAR- α in BALF (left panel), peripheral blood (middle panel) and splenocytes (right panel) were determined. * $P < 0.05$. TGF- β , transforming growth factor- β ; PPAR- α , peroxisome proliferator-activated receptor- α ; BALF, bronchoalveolar lavage fluid; LPS, lipopolysaccharide.

expression of TGF- β 1 were observed using RT-qPCR analysis (Fig. 2A). Similarly, the western blot analysis showed that the protein expression PPAR- α was decreased and that of TGF- β 1 was significantly increased by treatment with LPS (Fig. 2B). PPAR- α treatment prior to stimulation with LPS resulted in a decrease in the mRNA expression levels of TGF- β 1 in the BALF, peripheral blood and splenocytes (Fig. 2C). Consistently, the present study found that the expression level of PPAR- α in the BALF was significantly elevated by treatment with the PPAR- α expression vector (Fig. 2D, left panel). The expression levels of PPAR- α in the peripheral blood and splenocytes were also upregulated (Fig. 2D, middle and right panels). These findings demonstrated that the enforced expression of PPAR- α downregulated the expression of TGF- β 1 in LPS-induced ALI mice.

Activation of PPAR- α is essential for recovery of lung function following ALI. The question of whether PPAR- α was involved in the recovery of lung function following ALI remained; therefore, to evaluate the role of PPAR- α in the

recovery of lung function, the present study developed a virus carrying the shPPAR- α or SCR sequence as a control. It was found that the RAW 264.7 cells, which were transduced with shPPAR- α , exhibited significantly decreased levels of PPAR- α (Fig. 3A). Following injecting of the virus expressing either shPPAR- α or SCR into the mouse following LPS treatment, the effects of PPAR- α on lung recovery were examined. Significantly decreased levels of PPAR- α in the mouse lung were detected using RT-qPCR analysis (Fig. 3B) and western blot analysis (Fig. 3C). Impaired lung function has been considered to cause a dose-dependent increase in lung resistance index (RI) and decrease in dynamic compliance (Cdyn) in response to a cholinergic stimulus (methacholine) and a significant increase in BALF protein (11). The analysis of pressure and flow waveforms indicated that the knockdown of PPAR- α resulted in a marked increase in RI (Fig. 3D), a notable decrease in Cdyn (Fig. 3E) and a significant increase in BALF protein (Fig. 3F). These data suggested that PPAR- α was essential for the recovery of lung function following ALI.

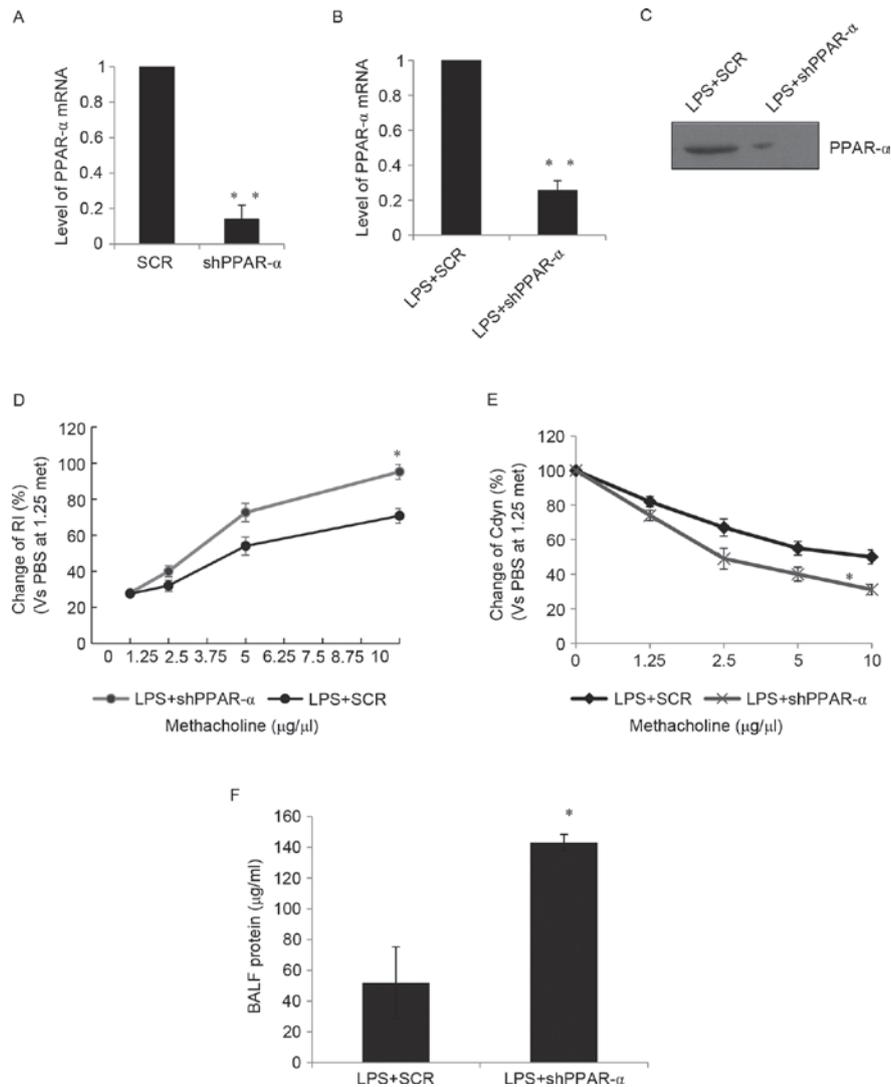


Figure 3. PPAR- α activation is essential for recovery of lung function following acute lung injury. (A) Effect of PPAR- α knockdown by shPPAR- α was confirmed in RAW 264.7 cells. Cells were transfected with AAV-shPPAR- α or SCR, reverse transcription-quantitative polymerase chain reaction analysis was used to detect levels of PPAR- α . (B) mRNA levels of PPAR- α in the mouse lung were detected by injecting AAV expressing either shRNA for PPAR- α or SCR following LPS treatment. (C) Protein levels of PPAR- α in the mouse lung were detected by injecting AAV expressing either shRNA for PPAR- α or SCR following LPS treatment. The effects of PPAR- α on lung recovery were determined by PPAR- α knockdown using (D) RI and (E) Cdyn in response to a high dose methacholine. (F) Total protein levels in BALF were measured using the bovine serum albumin standard curve to record the absorbance at 595 nm. * $P < 0.05$; ** $P < 0.01$. PPAR- α , peroxisome proliferator-activated receptor- α ; LPS, lipopolysaccharide; AAV, adeno-associated viruses; sh, short hairpin RNA; BALF, bronchoalveolar lavage fluid; SCR, scrambled sequence; RI, resistance index; Cdyn, dynamic compliance.

Suppression of TGF- β by PPAR- α improves recovery of lung function following ALI. In order to further evaluate the association between TGF- β with PPAR- α in the recovery of lung function following ALI, TGF- β 1 was inhibited in the mouse lung following LPS treatment by injecting AAV expressing shTGF- β 1 (or SCR as a control). TGF- β 1-knockdown efficiency was first confirmed in the mouse lung. It was found that the application of these viruses significantly decreased the levels of TGF- β 1 (Fig. 4A), resulting in a significant decrease in RI (Fig. 4B), a significant increase in Cdyn (Fig. 4C) and a significant decrease in BALF protein (Fig. 4D). These data suggested that shTGF- β 1 was essential for the recovery of lung function following ALI. The results showed that the knockdown of PPAR- α and knockdown of TGF- β 1 in the mouse lung partially reduced the shPPAR- α -impaired recovery of lung function, as detected by RI (Fig. 4E), Cdyn (Fig. 4F) and BALF protein (Fig. 4G). Together, these experiments suggested that PPAR- α

was essential for the recovery of lung function following ALI, possibly through the suppression of TGF- β .

Serum levels of PPAR- α are negatively correlated with TGF- β 1 in patients with ARDS. To further investigate the clinical significance of the above-mentioned findings, the present study detected the serological expression levels of PPAR- α and TGF- β 1, which revealed that PPAR- α was inversely correlated with TGF- β 1 in patients with ARDS (Fig. 5A; $P < 0.05$). In addition, the correlation with disease activity in patients with ARDS was analyzed. It was found that serum PPAR- α was positively associated with the ratio of PaO₂/FiO₂, whereas the serum level TGF- β 1 was negatively associated with PaO₂/FiO₂ (Fig. 5B; $P < 0.05$).

Using the LIS (Fig. 5C; $P < 0.05$) and SAPS II (Fig. 5D; $P < 0.05$) scoring methods to determine disease activity, it was revealed that the serum expression level of PPAR- α

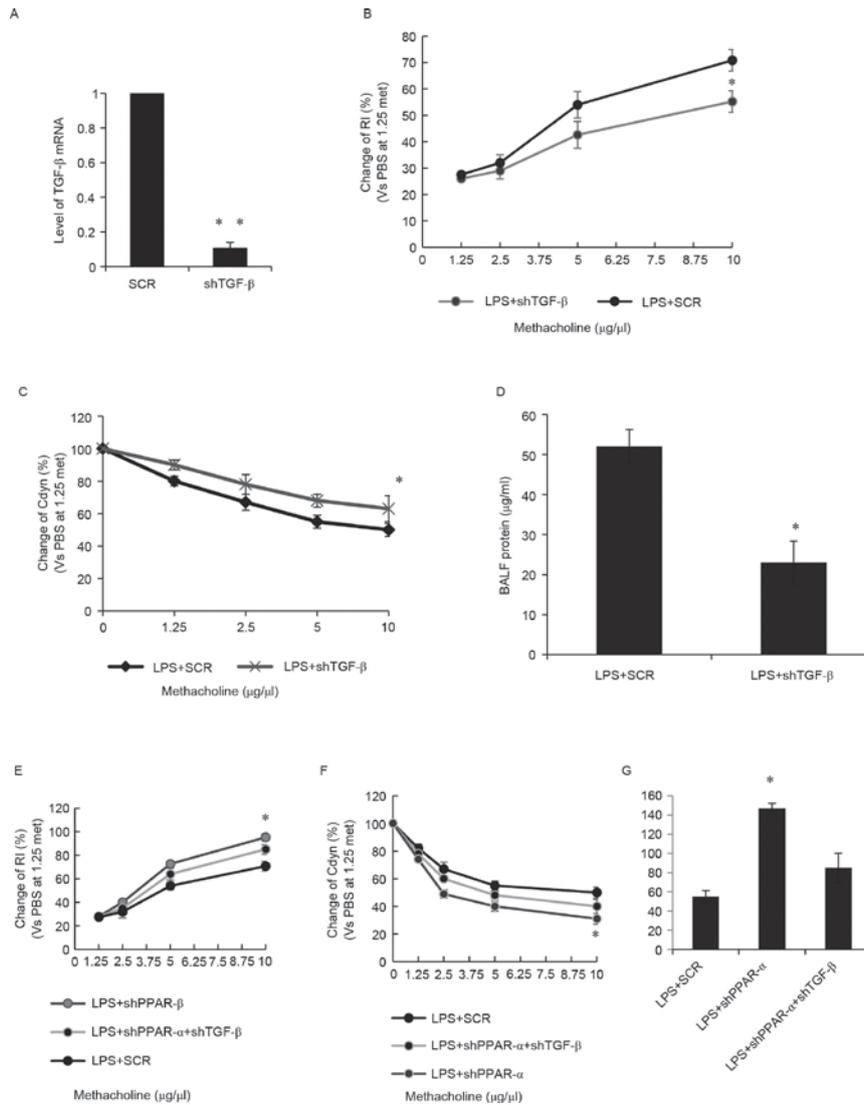


Figure 4. Suppression of TGF- β 1 by PPAR- α improves recovery of lung function following ALI. (A) Effect of TGF- β 1 knockdown was confirmed in the mouse lung by injecting AAV expressing either shRNA for TGF- β 1 or SCR following LPS treatment. Reverse transcription-quantitative polymerase chain reaction analysis was performed. (B) Effects of TGF- β 1 knockdown on lung recovery were determined according to RI in response to a high dose methacholine. (C) Effects of TGF- β 1 knockdown on lung recovery were determined using Cdyn in response to high dose methacholine. (D) Total protein in BALF was measured using the bovine serum albumin standard curve to record the absorbance at 595 nm. (E) Mice were treated with SCR, shPPAR- α or shPPAR- α plus shTGF- β 1 following LPS treatment. RI in response to high dose methacholine was determined. (F) Cdyn in response to high dose methacholine were determined. (G) Total protein in BALF was determined to measure recovery of lung function following ALI. * P <0.05; ** P <0.01. TGF- β , transforming growth factor- β ; LPS, lipopolysaccharide; ALI, acute lung injury; AAV, adeno-associated viruses; sh, short hairpin RNA; BALF, bronchoalveolar lavage fluid; SCR, scrambled sequence; RI, resistance index; Cdyn, dynamic compliance.

was inversely correlated with these indices, whereas the serum expression level of TGF- β 1 was consistent with these indices. No significant correlation was observed between the APACHE II scoring method and PPAR- α or TGF- β 1 serum levels (Fig. 5E; P >0.05). On the whole, these results suggested that PPAR- α was negatively correlated with TGF- β 1 and involved in the pathogenesis of ARDS.

Discussion

One of the primary findings of the present study was that PPAR- α was effective in suppressing TGF- β 1 in HLF cells and RAW 264.7 cells. The present study further analyzed the levels of TGF- β 1 and PPAR- α in the mouse lung following LPS treatment. In the murine lung, there was a decrease in the

mRNA expression of PPAR- α and an increase in the mRNA expression of TGF- β 1. Treatment with PPAR- α prior to stimulation with LPS resulted in a dose-dependent decrease in the expression levels of TGF- β 1 in BALF, peripheral blood and splenocytes. The above data indicated that PPAR- α treatment downregulated the expression of TGF- β 1 in the LPS-induced ALI model *in vitro* and *in vivo*. Taken together, the present study demonstrated that the enforced expression of PPAR- α ameliorated the development of ALI using the LPS-induced model. Following the report that TNF- α is one of the primary cytokines involved in the response to LPS, the present study revealed that, in addition to TNF- α , TGF- β 1 was another key regulator in LPS-induced ALI.

In order to further elucidate the molecular mechanism underlying post-ALI lung recovery, the present study further

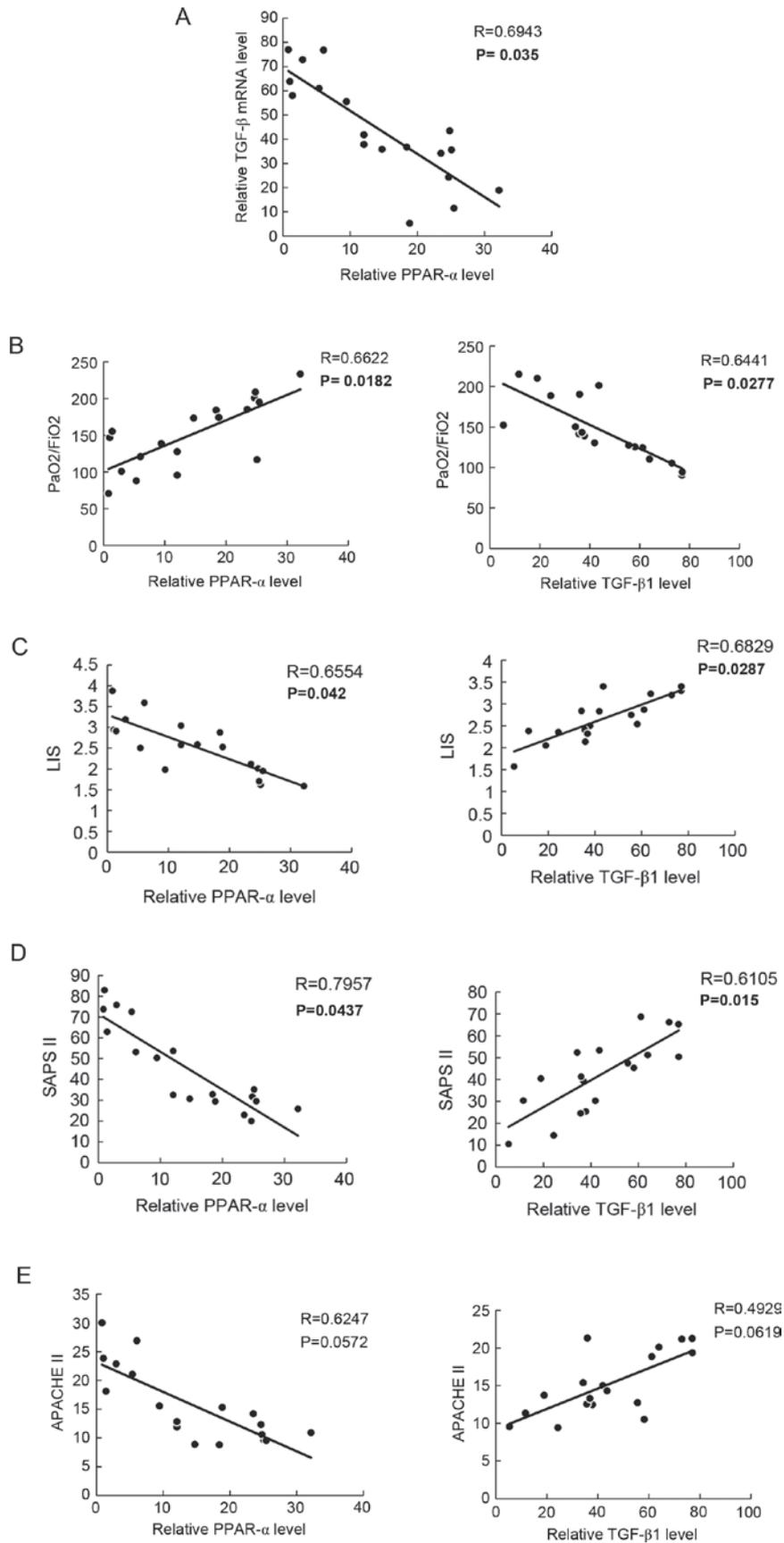


Figure 5. Levels of serum PPAR- α are negatively correlated with TGF- β 1 in patients with ARDS. (A) Analysis of the correlation between serological PPAR- α and serological TGF- β 1 by logistic regression analysis. (B) Analysis of the correlation between serum expression of PPAR- α or TGF- β 1 and the ratio of PaO₂/FiO₂. Each point represents the result from one patient. (C) Analysis of the correlation between serum expression of PPAR- α or TGF- β 1 and the LIS scoring method. (D) Analysis of the correlation between serum expression of PPAR- α or TGF- β 1 and the SAPS II scoring method. (E) Analysis of the correlation between serum expression of PPAR- α or TGF- β 1 and the APACHE II scoring method. ARDS, acute respiratory distress syndrome; TGF- β , transforming growth factor- β ; PPAR- α , peroxisome proliferator-activated receptor- α ; APACHE, Acute Physiology and Chronic Health Evaluation; LIS, Murray Lung Injury Score; SAPS, Simplified Acute Physiology Score.

focused on whether the suppression of TGF- β 1 by PPAR- α was involved in the recovery of lung function following ALI. The knockdown of PPAR- α and knockdown of TGF- β 1 in the mouse lung partially reduced the shPPAR- α impaired recovery of lung function, and it was concluded that the activation of PPAR- α and suppression of TGF- β 1 were essential for the recovery of lung function. The clinical significance of PPAR- α being inversely correlated with TGF- β 1 in patients with ARDS was consistent with this mechanism. Taken together, the results of the present study provided evidence supporting critical role of PPAR- α in the suppression of TGF- β 1 in lung recovery, and revealed a novel mechanism controlling post-ALI lung recovery.

References

1. Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, Stern EJ and Hudson LD: Incidence and outcomes of acute lung injury. *N Engl J Med* 353: 1685-1693, 2005.
2. Ware LB and Matthay MA: The acute respiratory distress syndrome. *N Engl J Med* 342: 1334-1349, 2000.
3. Tausch HW: Treatment of acute (Adult) respiratory distress syndrome. The holy grail of surfactant therapy. *Biol Neonate* 77 (Suppl 1): S2-S8, 2000.
4. Acute Respiratory Distress Syndrome Network, Brower RG, Matthay MA, Morris A, Schoenfeld D, Thompson BT and Wheeler A: Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med* 342: 1301-1308, 2000.
5. Reisinger MW, Moss M, Clark BJ; National Heart, Lung, and Blood Institute Acute Respiratory Distress Syndrome Network Investigators: Brief versus full alcohol use disorders identification test in national heart, lung, and blood institute acute respiratory distress syndrome network clinical trials. *Crit Care Med* 43: e382-e385, 2015.
6. Thompson BT and Matthay MA: The Berlin definition of ARDS versus pathological evidence of diffuse alveolar damage. *Am J Respir Crit Care Med* 187: 675-677, 2013.
7. Martin MA and Silverman HJ: Gram-negative sepsis and the adult respiratory distress syndrome. *Clin Infect Dis* 14: 1213-1228, 1992.
8. Bhatia M and Mochhala S: Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *J Pathol* 202: 145-156, 2004.
9. Chen C, Xu S, Wang WX, Ding YM, Yu KH, Wang B and Chen XY: Rosiglitazone attenuates the severity of sodium taurocholate-induced acute pancreatitis and pancreatitis-associated lung injury. *Arch Med Res* 40: 79-88, 2009.
10. Schaefer MB, Pose A, Ott J, Hecker M, Behnk A, Schulz R, Weissmann N, Günther A, Seeger W and Mayer K: Peroxisome proliferator-activated receptor-alpha reduces inflammation and vascular leakage in a murine model of acute lung injury. *Eur Respir J* 32: 1344-1353, 2008.
11. Delerive P, De Bosscher K, Besnard S, Vanden Berghe W, Peters JM, Gonzalez FJ, Fruchart JC, Tedgui A, Haegeman G and Staels B: Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappaB and AP-1. *J Biol Chem* 274: 32048-32054, 1999.
12. Derynck R and Zhang YE: Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* 425: 577-584, 2003.
13. Rastaldi MP: Epithelial-mesenchymal transition and its implications for the development of renal tubulointerstitial fibrosis. *J Nephrol* 19: 407-412, 2006.
14. Wu M, Melichian DS, Chang E, Warner-Blankenship M, Ghosh AK and Varga J: Rosiglitazone abrogates bleomycin-induced scleroderma and blocks profibrotic responses through peroxisome proliferator-activated receptor-gamma. *Am J Pathol* 174: 519-533, 2009.
15. Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, Legall JR, Morris A and Spragg R: The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med* 149: 818-824, 1994.
16. Murray JF, Matthay MA, Luce JM and Flick MR: An expanded definition of the adult respiratory distress syndrome. *Am Rev Respir Dis* 138: 720-723, 1988.
17. Wang H, Xu L, Zhao J, Wang D, Guo R, Wang J, Gong W, Liu T, Zhang Y and Dong L: Regulatory mechanism of pyrrolidine dithiocarbamate is mediated by nuclear factor- κ B and inhibits neutrophil accumulation in ARDS mice. *Exp Ther Med* 8: 614-622, 2014.
18. Rastaldi MP: Epithelial-mesenchymal transition and its implications for the development of renal tubulointerstitial fibrosis. *J Nephrol* 19: 407-412, 2006.
19. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.