LPS-induced inflammatory response and apoptosis are mediated by Fra-1 upregulation and binding to YKL-40 in A549 cells

FEI WANG[1], WENXUAN LI[1], ZHEN LIU[1],
RONGHUA YU[2] and DALIAN WANG[1]

[1]Department of Pediatrics, Shanghai Fifth People's Hospital, Fudan University, Shanghai 200240;
[2]Department of Gastroenterology, Hepatology and Nutrition, Shanghai Children's Hospital, Shanghai Jiao Tong University, Shanghai 200062, P.R. China

Received April 14, 2021; Accepted July 16, 2021

DOI: 10.3892/etm.2021.10909

Abstract. Acute respiratory distress syndrome (ARDS) is a multifactorial syndrome that leads to increased morbidity and mortality in infants and children. The identification of novel biomarkers is critical for the treatment of ARDS. The present study aimed to investigate the effects of chitinase-3-like-1 protein (CHI3L1 or YKL-40) in an *in vitro* model of ARDS and to explore the potential underlying mechanisms. The *in vitro* model of ARDS was established in A549 alveolar epithelial type II cells, which were treated by lipopolysaccharide (LPS) to induce inflammation. Transfection was performed to alter YKL-40 expression. The mRNA and protein expression of YKL-40 was determined using reverse transcription-quantitative PCR and western blotting, respectively. Cell Counting Kit-8 and TUNEL assays were used to evaluate the cell viability and apoptosis, respectively. The production of cytokines was evaluated using specific ELISA kits. The relationship between YKL-40 and Fos-related antigen 1 (Fra-1) was verified using luciferase reporter and chromatin immunoprecipitation assays. The expression of the apoptotic proteins was detected using western blotting. The expression levels of YKL-40 and Fra-1 were increased in LPS-treated A549 cells. Higher levels of pro-inflammatory cytokines and induction of cell apoptosis were observed in LPS-treated A549 cells compared with the control. YKL-40 knockdown in LPS-treated A549 cells significantly decreased the production of pro-inflammatory cytokines and reduced cell apoptosis, whereas it concomitantly caused upregulation of Bax and downregulation of Bcl-2, cleaved caspase-3 and cleaved caspase-9. In addition, Fra-1 could directly bind to YKL-40 promoter and regulate its expression level. Overexpression of YKL-40 partly decreased the inhibitory effects of Fra-1 knockdown on the inflammatory response and induction of apoptosis. In summary, the findings from the present study indicated that Fra-1 could bind to YKL-40 and regulate its expression, whereas YKL-40 knockdown could further suppress LPS-induced inflammatory response and apoptosis in A549 cells. These data may provide novel evidence on the diagnosis and therapy of ARDS.

Correspondence to: Dr Dalian Wang, Department of Pediatrics, Shanghai Fifth People's Hospital, Fudan University, 128 Ruili Road, Shanghai 200240, P.R. China
E-mail: wangdalian205@163.com

Key words: chitinase-3-like-1 protein, Fos-related antigen 1, inflammatory response, apoptosis, acute respiratory distress syndrome

Introduction

Acute respiratory distress syndrome (ARDS) is a severe condition that may cause acute lung injury. ARDS is a multifactorial syndrome that leads to significant morbidity and mortality in infants and children, with a fatality rate ≤40% worldwide (1,2). ARDS usually results from trauma, hemorrhagic shock or toxic inhalation. However, the most common cause of this syndrome is bacterial sepsis (3). The development of severe inflammation involving pro-inflammatory cytokine production and neutrophil integration is the main feature of ARDS, which may account for its high mortality rate (4). Previous studies conducted on ARDS have not been successful in developing specific biomarkers and pharmacological targets for patients with ARDS. Therefore, the identification of novel targets for the treatment of ARDS is crucial.

At present, the pathogenesis of ARDS remains unclear. It has been recognized that ARDS is primarily induced by pathogenic inflammation. Lipopolysaccharide (LPS) is a main component of the cell wall of Gram-negative bacteria and has been reported to cause lung injury, which can subsequently progress into ARDS (5,6). Chitinase-3-like-1 protein (CHI3L1 or YKL-40) is also termed human cartilage glycoprotein-39. This protein is a member of the mammalian chitinase-like protein family and can be secreted by numerous cells, including macrophages, vascular smooth muscle cells, endothelial cells, chondrocytes and neutrophils (7,8). YKL-40 is considered as a pro-inflammatory cytokine and its circulating levels have been shown to be abnormally elevated in a wide range of inflammatory disorder-associated diseases, including purulent meningitis, rheumatoid arthritis and community-acquired pneumonia (9-12). In addition, accumulating evidence has shown that YKL-40 might play a crucial

pathogenic role in chronic obstructive pulmonary disease and hyperoxia-induced acute lung injury (13,14).

Interestingly, from the JASPAR database (http://jaspar.genereg.net/), a potential binding relationship was found between YKL-40 promoter and Fos-related antigen 1 (Fra-1). Fra-1 is a member of the Fos family of proteins and a component of the activator protein-1 transcription factor complex (15). Fra-1 is a transcription factor involved in various pathological processes, including cell proliferation and cell death, extracellular remodeling, inflammation and immune response (16).

To the best of our knowledge, whether YKL-40 or Fra-1 is involved in ARDS progression remains unknown. The present study investigated therefore the role of YKL-40 in LPS-induced ARDS and its potential underlying mechanisms.

Materials and methods

Cell culture and treatment. The human type II lung epithelial A549 cell line was obtained from the American Type Culture Collection and cultured in RPMI-1640 medium (Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Thermo Fisher Scientific, Inc.). The cells were maintained at 37˚C in a humidified incubator containing 5% CO_2. A549 cells were treated with increasing concentrations of LPS (100, 500, 1,000 and 1,500 ng/ml) for 12 h for stimulation (17).

Cell transfection. Short hairpin (sh) RNA targeting Fra-1 (shRNA-Fra-1-1/2), sh-RNA targeting YKL-40 (sh-YKL-40-1/2) and negative control shRNA (sh-NC) were obtained from Shanghai GenePharma Co., Ltd. A YKL-40 overexpression plasmid (pcDNA3.1-YKL-40) and a Fra-1 overexpression plasmid (pcDNA3.1-Fra-1) were commercially constructed by Shanghai GenePharma Co., Ltd. and the empty pcDNA 3.1 vector (pcDNA 3.1) was used as the negative control. A549 cells were seeded in 6-well plates ($2x10^5$ cells/well) in a humidified incubator containing 5% CO_2 at 37˚C. When cells reached 70-80% confluence, they were transfected with sh-NC (500 ng/μl), sh-YKL-40-1/2 (500 ng/μl), sh-Fra-1-1/2 (500 ng/μl), pcDNA3.1-Fra-1 (15 nM), pcDNA3.1-YKL-40 (15 nM) or pcDNA 3.1 (15 nM) using Lipofetamine® 2000 reagent (Thermo Fisher Scientific, Inc.) for 48 h according to the manufacturer's instructions. Following transfection, cells were cultured at 37˚C in a humidified incubator containing 5% CO_2 for 48 h and the transfection efficiency was determined using reverse transcription-quantitative (RT-q) PCR and western blotting. After 48 h transfection, cells were harvested for subsequent experiments.

Cell viability assay. Cell viability was determined using the Cell Counting Kit-8 (CCK-8) assay. Cells were seeded in 96-well plates at the density of $1x10^3$ cells/well and treated with LPS (0, 100, 500, 1,000 and 1,500 ng/ml) for 12 h. CCK-8 solution (10 μl; Dojindo Molecular Technologies, Inc.) was added to the cells that were incubated for an additional 3 h. The absorbance was read at 450 nm on a microplate reader (Bio-Rad Laboratories, Inc.).

RT-qPCR. Total RNA was extracted from cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and reverse-transcribed into cDNA using PrimeScript™ RT Master Mix kit (Takara Bio, Inc.) according to the manufacturer's instructions. The mRNA levels of the genes were assessed by RT-qPCR according to the SYBR Premix Ex-Taq Kit (Takara Bio, Inc.). The primers used were as follows: YKL-40, forward, 5'-CCTGCTCAGCGCAGCACTGT-3' and reverse, 5'-GCTTTTGACGCTTTCCTGGTC-3'; Fra-1, forward, 5'-AGGAACTGACCGACTTCCTG-3' and reverse, 5'-CAGCTCTAGGCGCTCCTTC-3', and GAPDH, forward, 5'-AGCCACATCGCTCAGACA-3' and reverse, 5'-GCCCAATACGACCAAATCC-3'. The qPCR thermocycling conditions were as follows: 5 min at 95˚C, 40 cycles of 10 sec at 95˚C, 20 sec at 59˚C and 30 sec at 72˚C. The relative expression levels were normalized to endogenous control and were expressed as $2^{-\Delta\Delta Cq}$ (18).

Western blotting. Cells were lysed in RIPA buffer (Wuhan Servicebio Technology Co., Ltd.) on ice for 30 min. Following centrifugation at 4˚C, 12,000 x g for 15 min, the supernatant was collected and the protein concentration was determined using the BCA method. Proteins (30 μg/lane) were separated by 10% SDS-PAGE and subsequently transferred onto PVDF membranes. Membranes were blocked with 5% skimmed milk for 1 h at room temperature and incubated with primary antibodies against YKL-40 (1:1,000; cat. no. ab180569; Abcam), Fra-1 (1:1,000; cat. no. ab124722; Abcam), Bcl-2 (1:2,000; cat. no. ab182858; Abcam), Bax (1:1,000; cat. no. ab32503; Abcam), cleaved caspase 3 (1:500; cat. no. ab2302; Abcam), caspase 3 (1:500; cat. no. ab13847; Abcam), cleaved caspase 9 (1:500; cat. no. ab2324; Abcam), caspase 9 (1:1,000; cat. no. ab32539; Abcam) and GAPDH (1:2,500; cat. no. ab9485; Abcam) at 4˚C overnight. Membranes were that incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (1:2,000; cat. no. ab6721; Abcam) at room temperature for 2 h. Enhanced chemiluminescence reagent (Cytiva) was used to detect the signal on the membrane. The data were analyzed via densitometry using ImageJ software version 1.46 (National Institutes of Health) and normalized to expression of the internal control GAPDH.

ELISA. Transfected cells were treated with 500 ng/ml LPS for 12 h. Subsequently, the cell supernatant was collected. The levels of tumor necrosis factor-α (TNF-α), interleukin (IL)-6 and IL-1β in the cell supernatant were measured using ELISA kits (cat. no. 555220 for IL-6; cat. no. 557953 for IL-1β; and cat. no. 555212 for TNF-α; BD Biosciences) according to the manufacturer's instructions.

TUNEL assay. Apoptosis was detected *in vitro* using TUNEL staining. Following the cell treatment, cells were fixed with 4% formaldehyde for 10 min at room temperature and permeabilized with 0.1% Triton X-100 for 2 min at room temperature. TUNEL assay Kit (Roche Diagnostics) was used to detect the percentage of apoptotic cells according to the instructions provided by the manufacturer. The fluorescent images were captured using an inverted fluorescence microscope (magnification, x200).

Chromatin immunoprecipitation (ChIP) assay. ChIP assay was performed using the EpiQuik Chromatin Immunoprecipitation Assay Kit (EpiGentek) according to the

manufacturer's instructions. An antibody against Fra-1 (1:30; cat. no. ab252421; Abcam) or IgG (1:100; cat. no. ab172730; Abcam) was used for immunoprecipitation. Subsequently, gel electrophoresis and RT-qPCR were performed to detect the DNA fragments at the predicted YKL-40 promoter binding sites.

Luciferase reporter assay. According to the JASPAR database (http://jaspar.genereg.net/), a potential binding relationship between YKL-40 promoter and Fra-1 was predicted. Then, this binding relationship was verified by Luciferase reporter assay. In brief, the pcDNA 3.1-Fra-1 or pcDNA 3.1 vector was co-transfected into A549 cells together with a luciferase reporter plasmid driven by the full length (FL) of YKL-40 promoter, or mutated YKL-40 promoter (targeting 2 E-Box motifs, named E1 Del and E2 Del, respectively) using Lipofetamine® 2000 reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. After 48 h of transfection, the Dual Reporter Assay System (Promega Corporation) was used to measure luciferase activity. The relative luciferase activity was normalized to that of *Renilla* luciferase.

Statistical analysis. GraphPad Prism 5 software (GraphPad Software, Inc.) was used for statistical analysis. The experimental data were presented as the means ± standard deviation of three independent experiments. Data were compared using one-way ANOVA followed by Tukey's post hoc test when appropriate. $P<0.05$ was considered to indicate a statistically significant difference.

Results

YKL-40 expression is upregulated in LPS-treated A549 cells. LPS was used to mimic the development of ARDS in A549 cells. Cell viability was decreased following treatment with increasing concentrations of LPS (0, 100, 500, 1,000 and 1,500 ng/ml; Fig. 1A). Furthermore, the mRNA and protein expression of YKL-40 was significantly upregulated (Fig. 1B and C) following LPS treatment. To retain a relative cell viability >50% after LPS stimulation, a concentration of 500 ng/ml LPS was used for induction of ARDS in subsequent experiments.

YKL-40 knockdown inhibits the inflammatory response and apoptosis in LPS-treated A549 cells. To explore the role of YKL-40 in LPS-treated A549 cells, cells were transfected with sh-YKL-40-1/2. The results indicated that the mRNA and protein expression of YKL-40 was significantly downregulated following transfection, notably with sh-YKL-40-2, which was used in subsequent experiments (Fig. 2A and B). A549 cells were treated with LPS and transfected or not with sh-YKL-40. The concentration of certain inflammatory cytokines was detected to assess the effect of YKL-40 on the induction of inflammation in LPS-treated A549 cells. The results demonstrated that LPS treatment induced a severe inflammatory response, with significantly increased levels of TNF-α, IL-6 and IL-1β (Fig. 2C). However, the increase in TNF-α, IL-6 and IL-1β release following treatment with LPS was significantly inhibited in sh-YKL-40-transfected cells, indicating that YKL-40 silencing caused a significant inhibition in

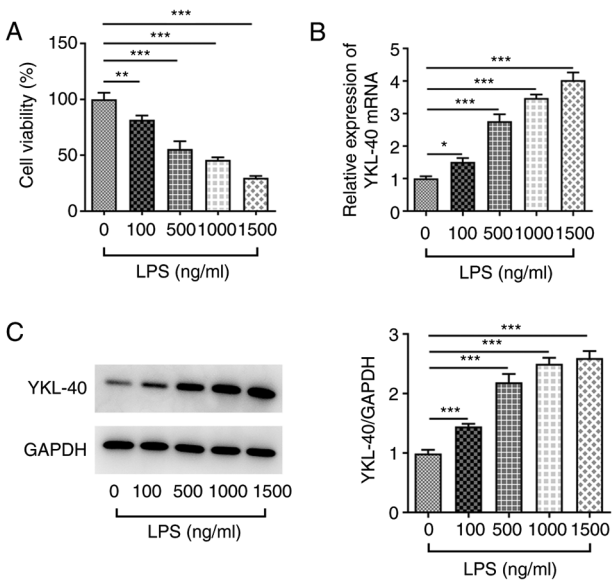

Figure 1. YKL-40 expression is upregulated in LPS-treated A549 cells. (A) A549 cells were treated with increasing concentration of LPS (0, 100, 500, 1,000 and 1,500 ng/ml) and cell viability was detected using Cell Counting Kit-8 assay. (B) mRNA and (C) protein expression of YKL-40 was detected by reverse transcription quantitative PCR and western blotting, respectively. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$. YKL-40, chitinase-3-like-1 protein; LPS, lipopolysaccharide.

LPS-induced inflammation in A549 cells. Furthermore, cell apoptosis was also evaluated. The results demonstrated that the number of apoptotic cells was elevated following LPS treatment. This effect was partly abolished following transfection with sh-YKL-40 (Fig. 2D and E). In addition, LPS caused downregulation of the Bcl-2 protein and upregulation of Bax, cleaved caspase-3 and cleaved caspase-9 proteins (Fig. 2F). These effects were partly reversed following cell transfection with sh-YKL-40.

Fra-1 directly binds to YKL-40 promoter and regulates YKL-40 expression. The potential mechanism underlying the role of YKL-40 in LPS-treated A549 cells was investigated. Firstly, according to the JASPAR database (http://jaspar.genereg.net/), a potential binding relationship between YKL-40 promoter and Fra-1 was predicted, and the potential binding sites (named E1 and E2) between Fra-1 and the YKL-40 promoter are displayed in Fig. 3A. The expression level of Fra-1 was significantly increased following cell transfection with pcDNA3.1-Fra-1. The results from luciferase reporter assay demonstrated that the transcriptional activity of YKL-40 was increased following Fra-1 overexpression (Fig. 3B and C). To clarify which binding site was mainly responsible for the increase in transcriptional activity, E1 and E2 were deleted. The results indicated that the change in the transcriptional activity was more apparent when E1 was deleted, indicating that this region was mainly responsible for binding to Fra-1 (Fig. 3D). Furthermore, the results from ChIP assay revealed that Fra-1 was enriched at the YKL-40 promoter within the E1 region, demonstrating the binding association between the YKL-40 promoter and Fra-1 (Fig. 3E).

Based on these findings, the role of Fra-1 was investigated in LPS-treated A549 cells. Both the protein and mRNA

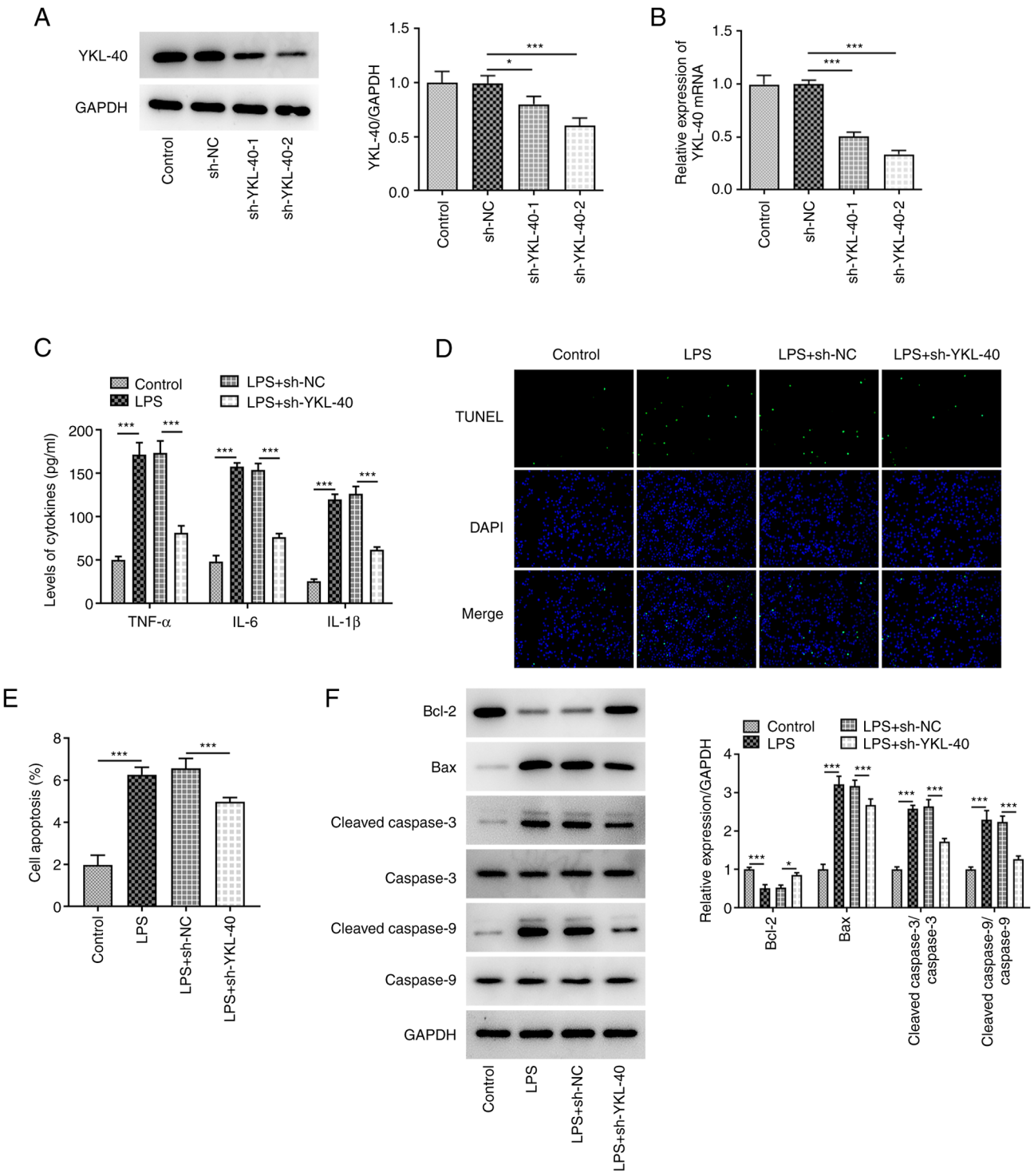

Figure 2. YKL-40 knockdown inhibits the inflammatory response and cell apoptosis in LPS-treated A549 cells. A549 cells were transfected with sh-NC or sh-YKL-40-1/2 and the (A) protein and (B) mRNA expression of YKL-40 was detected by western blotting and reverse transcription quantitative PCR, respectively. (C) sh-YKL-40-2 was used for subsequently experiment, and A549 cells were treated with LPS with or without sh-YKL-40 transfection. Inflammatory cytokines TNF-α, IL-6 and IL-1β production was measured using ELISA kits. (D) TUNEL assay was used to detect apoptotic cells. (E) The apoptotic cells were quantified. (F) Western blotting was used to evaluate the expression of apoptosis-related proteins. *P<0.05 and ***P<0.001. YKL-40, chitinase-3-like-1 protein; LPS, lipopolysaccharide; sh, short hairpin RNA; NC, negative control; TNF-α, tumor necrosis factor-α; IL, interleukin.

expression of Fra-1 was significantly increased following treatment of A549 cells with LPS (Fig. 4A and B). Cell transfection with sh-Fra-1-1/2 was successful (Fig. 4C), and sh-Fra-1-1 was used for subsequent experiments due to its higher transfection efficacy. In addition, Fra-1 knockdown decreased LPS-induced increase in YKL-40 expression level (Fig. 4D). Fra-1 expression was upregulated in LPS-treated A549 cells, and the data indicated that Fra-1 could directly bind to the YKL-40 promoter and positively regulate its expression.

YKL-40 overexpression partly abolishes the inhibitory effects of Fra-1 knockdown on LPS-induced inflammation and

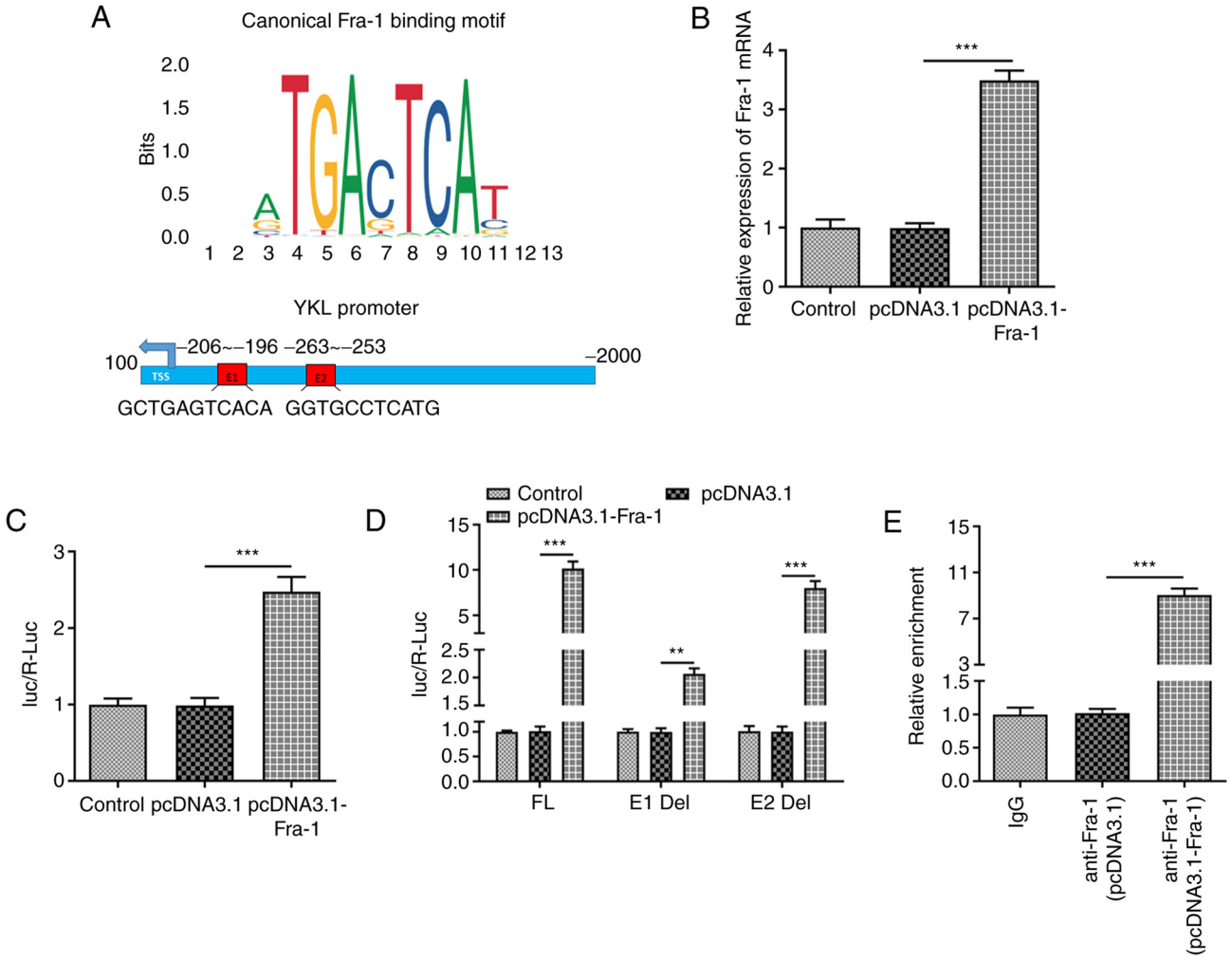

Figure 3. Fra-1 directly binds to YKL-40 promoter. (A) Binding sites between Fra-1 and YKL-40 promoter was predicted using the JASPAR database. (B) A549 cells were transfected with pcDNA3.1 or pcDNA3.1-Fra-1 and the mRNA expression of Fra-1 was detected by RT-qPCR. (C) Luciferase activity of YKL-40 promoter transcriptional activity following Fra-1 overexpression. (D) Luciferase activity of YKL-40 promoter deletion mutants (E1 Del and E2 Del) following Fra-1 overexpression. (E) Immunoprecipitated chromatin fragments obtained by the ChIP assay were analyzed by RT-qPCR. **P<0.01 and ***P<0.001. Fra-1, Fos-related antigen 1; YKL-40, chitinase-3-like-1 protein; ChIP, chromatin immunoprecipitation; RT-qPCR, reverse transcription-quantitative PCR; Del, deletion; IgG, immunoglobulin G.

apoptosis in A549 cells. YKL-40 overexpression was established following cell transfection with pcDNA3.1-YKL-40 (Fig. 5A). Fra-1 knockdown decreased LPS-induced elevated production of TNF-α, IL-6 and IL-1β and reduced apoptotic cell numbers in LPS-induced A549 cells (Fig. 5B and C, respectively). This reduction in apoptosis was accompanied by increased expression of Bcl-2 and decreased expression of Bax, cleaved caspase 3 and cleaved caspase 9 (Fig. 5D). These alterations were partly abolished by YKL-40 overexpression (Fig. 5B-D).

Discussion

ARDS is a life-threatening pulmonary inflammatory disease characterized by pulmonary edema, refractory hypoxemia and multiple organ failure (19). Although YKL-40 is regarded as a pro-inflammatory cytokine and is involved in various inflammatory diseases, its role in the pathophysiology of ARDS remains unknown. In the present study, LPS-treated A549 cells were used to establish an *in vitro* model of ARDS. Upregulation of YKL-40 expression was observed in LPS-treated A549 cells. Furthermore, YKL-40 knockdown inhibited the inflammatory response and induction of cell apoptosis in LPS-treated A549 cells. These phenomena were partly regulated by the transcription factor Fra-1. These data suggested a potential role of YKL-40 in the development of ARDS and may provide a potential target for ARDS treatment.

Accumulating evidence has confirmed that excessive production of pro-inflammatory cytokines and uncontrolled systemic inflammatory response account for impaired lung function in ARDS (20,21). Therefore, the reduction in the production of pro-inflammatory cytokines and the maintenance of the balance between the anti-inflammatory and the pro-inflammatory response may be a promising therapeutic strategy for ARDS. It has been previously reported that certain anti-inflammatory genes can be used as drug targets for ARDS treatment. For example, Pooladanda *et al* (6) reported that nimbolide could ameliorate LPS-induced ARDS partly by inhibiting inflammation. Zhou *et al* (22) demonstrated that the

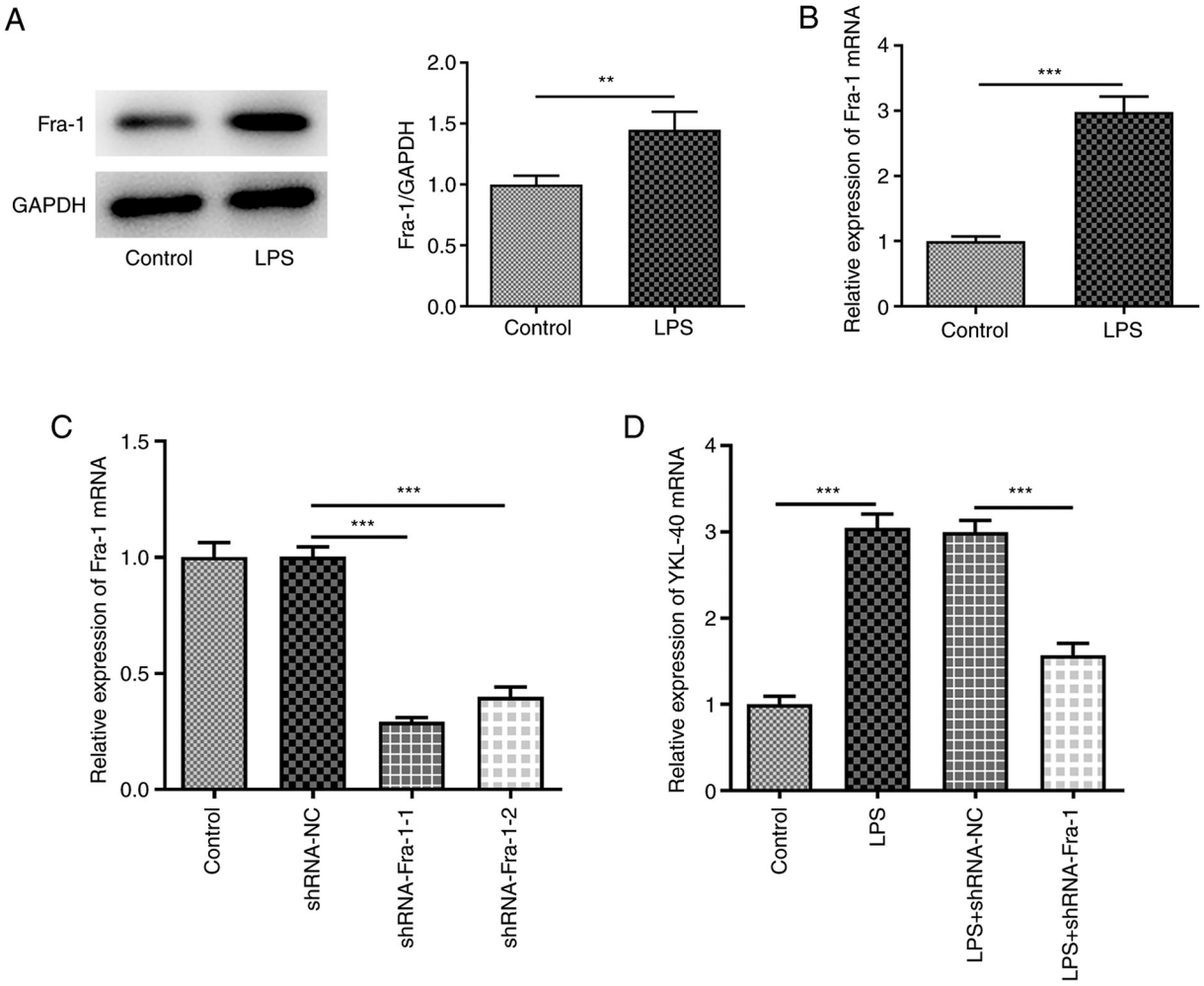

Figure 4. Fra-1 expression is upregulated in LPS-treated A549 cells and regulates YKL-40 expression. A549 cells were stimulated with LPS (500 ng/ml) and the (A) protein and (B) mRNA expression of Fra-1 was evaluated by western blotting and reverse transcription quantitative PCE. (C) A549 cells were transfected with sh-NC or sh-Fra-1-1/2, and the mRNA expression of Fra-1 was measured. (D) sh-Fra-1-1 was used for subsequent experiments, A549 cells were treated with LPS with or without sh-Fra-1 transfection, then the mRNA expression of YKL-40 was measured. **P<0.01 and ***P<0.001. Fra-1, Fos-related antigen 1; LPS, lipopolysaccharide; YKL-40, chitinase-3-like-1 protein; sh, short hairpin RNA; NC, negative control.

long non-coding RNA NEAT1 is highly expressed following LPS-induced lung injury in mice or A549 cells. In addition, suppression of NEAT1 expression restrains LPS-induced production of the inflammatory cytokines TNF-α, IL-6 and IL-1β via high mobility group box 1/receptor for advanced glycation end products signaling. In addition, microRNA (miR)-297, miR-150 and miR-216a are expressed at low levels in ARDS. These miRs exert protective effects against LPS-induced injury in A549 cells by reducing inflammatory cytokine secretion (17,23,24). The present study reported an excessive production of TNF-α, IL-6 and IL-1β in LPS-treated A549 cells, which was consistent with the findings reported previously. Furthermore, YKL-40 expression was upregulated following A549 cell exposure to LPS. YKL-40 knockdown markedly decreased the production of TNF-α, IL-6 and IL-1β, which partially alleviated the inflammatory response in LPS-treated A549 cells.

A previous study reported that Fra-1 could impair inflammatory response and suppress inflammation-induced chondrogenesis in fracture healing (25). In addition, Fra-1 can mediate some anti-fibrotic effects in the lung via the regulation of pro-inflammatory, pro-fibrotic and anti-fibrotic gene expression (26). Fra-1 is notably activated during inflammatory lung injury *in vivo*. Fra-1-null mice are less susceptible to LPS-induced lung injury and mortality than wild-type mice (27), indicating that Fra-1 serves a key role in the lung inflammatory response. In line with previous studies, the present study demonstrated that Fra-1 expression was upregulated in LPS-treated A549 cells. It is interesting to note that Fra-1 was predicted to bind to YKL-40 promoter from the JASPAR database. This prediction was subsequently verified by ChIP and luciferase reporter assays, and Fra-1 could positively regulate the expression of YKL-40. In addition, the inhibitory effects of Fra-1 knockdown on LPS-induced inflammatory response and cell apoptosis in A549 cells were partly abolished by YKL-40 overexpression, indicating that the functional role of YKL-40 in LPS-treated A549 cells may be partly mediated by Fra-1.

This study presented some limitations. The present study only performed *in vitro* experiments and lacked results from *in vivo* and clinical research. *In vivo* experiments and clinical research are of great significance to verify the role of YKL-40 in ARDS and should be performed in our future work. In addition,

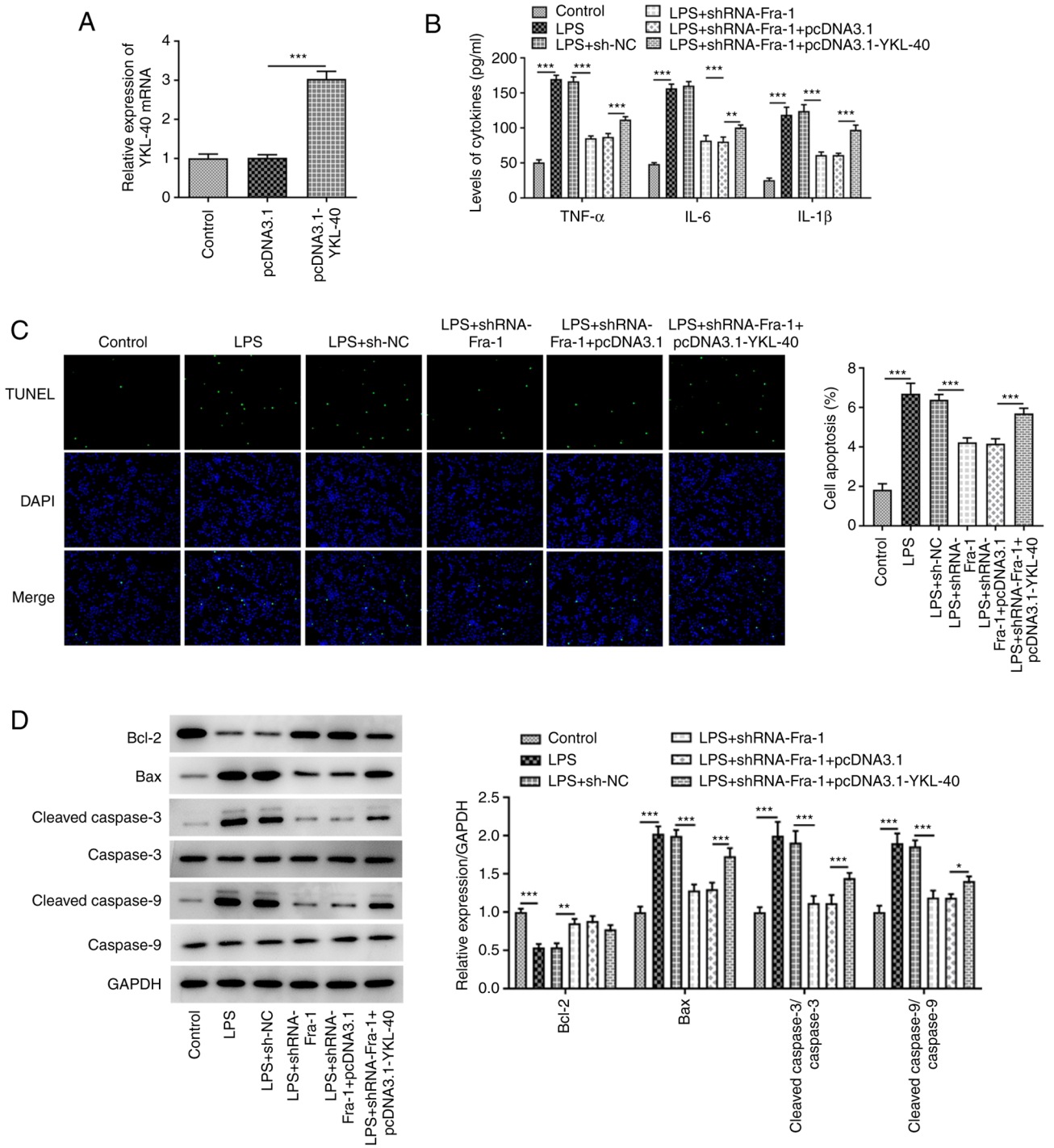

Figure 5. YKL-40 overexpression partly abolishes the inhibitory effects of Fra-1 knockdown on LPS-induced inflammation and apoptosis in A549 cells. A549 cells were transfected with pcDNA3.1 or pcDNA3.1-YKL-40 and the mRNA expression of YKL-40 was evaluated. (A) LPS-treated A549 cells were transfected with sh-NC or sh-Fra-1 or co-transfected with sh-Fra-1 and pcDNA3.1 or pcDNA3.1-YKL-40. (B) Production of inflammatory cytokines, including TNF-α, IL-6 and IL-1β, was measured using ELISA kits. (C) TUNEL assay was used to detect apoptotic cells, and the apoptotic cells were quantified. (D) Western blotting was used to evaluate the protein expression of apoptosis-related proteins. *P<0.05, **P<0.01 and ***P<0.001. YKL-40, chitinase-3-like-1 protein; Fra-1, Fos-related antigen 1; LPS, lipopolysaccharide; sh, short hairpin RNA; NC, negative control; TNF-α, tumor necrosis factor-α; IL, interleukin.

the transcription factors predicted to bind to YKL-40 promoter are multiful. Whether the other transcription factors can also directly bind to YKL-40 promoter and regulate YKL-40 requires some clarification, and the potential mechanism underlying the role of the other unknown potential transcription factors and YKL-40 during ARDS progression should be further investigated.

Taken together, the results from the present study demonstrated a high expression of YKL-40 in LPS-induced ARDS. In addition, YKL-40 knockdown exerted some protective effects against LPS-induced inflammatory response and apoptosis in A549 cells. The transcription factor Fra-1 could directly bind to the YKL-40 promoter and was partly responsible for mediating the function of YKL-40. These findings indicated that

YKL-40 may represent a promising target for the development of therapeutic strategies for ARDS.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DW made substantial contributions to the conception and design of the study. FW and WL made substantial contributions to data acquisition. FW, ZL and RY were responsible for the development of the study methodology, analysis and interpretation of the data. DW, FW and WL were involved in drafting the manuscript or revising it critically for important intellectual content. DW and FW confirm the authenticity of the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Amigoni A, Pettenazzo A, Stritoni V and Circelli M: Surfactants in acute respiratory distress syndrome in infants and children: Past, present and future. Clin Drug Investig 37: 729-736, 2017.
2. Matthay MA and Zemans RL: The acute respiratory distress syndrome: Pathogenesis and treatment. Annu Rev Pathol 6: 147-163, 2011.
3. Ware LB and Matthay MA: The acute respiratory distress syndrome. N Engl J Med 342: 1334-1349, 2000.
4. Jung YJ, Park YY, Huh JW and Hong SB: The effect of human adipose-derived stem cells on lipopolysaccharide-induced acute respiratory distress syndrome in mice. Ann Transl Med 7: 674, 2019.
5. Li D, Sun T, Chi L, Zhao D and Li W: Acupoint catgut embedding improves the lipopolysaccharide-induced acute respiratory distress syndrome in rats. Biomed Res Int 2020: 2394734, 2020.
6. Pooladanda V, Thatikonda S, Bale S, Pattnaik B, Sigalapalli DK, Bathini NB, Singh SB and Godugu C: Nimbolide protects against endotoxin-induced acute respiratory distress syndrome by inhibiting TNF-α mediated NF-κB and HDAC-3 nuclear translocation. Cell Death Dis 10: 81, 2019.
7. Lee CG, Hartl D, Lee GR, Koller B, Matsuura H, Da Silva CA, Sohn MH, Cohn L, Homer RJ, Kozhich AA, *et al*: Role of breast regression protein 39 (BRP-39)/chitinase 3-like-1 in Th2 and IL-13-induced tissue responses and apoptosis. J Exp Med 206: 1149-1166, 2009.
8. Johansen JS: Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodelling, fibroses and cancer. Dan Med Bull 53: 172-209, 2006.
9. Kastrup J: Can YKL-40 be a new inflammatory biomarker in cardiovascular disease? Immunobiology 217: 483-491, 2012.
10. Jafari-Nakhjavani MR, Ghorbanihaghjo A, Bagherzadeh-Nobari B, Malek-Mahdavi A and Rashtchizadeh N: Serum YKL-40 levels and disease characteristics in patients with rheumatoid arthritis. Caspian J Intern Med 10: 92-97, 2019.
11. Ostergaard C, Johansen JS, Benfield T, Price PA and Lundgren JD: YKL-40 is elevated in cerebrospinal fluid from patients with purulent meningitis. Clin Diagn Lab Immunol 9: 598-604, 2002.
12. Nordenbaek C, Johansen JS, Junker P, Borregaard N, Sorensen O and Price PA: YKL-40, a matrix protein of specific granules in neutrophils, is elevated in serum of patients with community-acquired pneumonia requiring hospitalization. J Infect Dis 180: 1722-1726, 1999.
13. Tong X, Wang D, Liu S, Ma Y, Li Z, Tian P and Fan H: The YKL-40 protein is a potential biomarker for COPD: A meta-analysis and systematic review. Int J Chron Obstruct Pulmon Dis 13: 409-418, 2018.
14. Sohn MH, Kang MJ, Matsuura H, Bhandari V, Chen NY, Lee CG and Elias JA: The chitinase-like proteins breast regression protein-39 and YKL-40 regulate hyperoxia-induced acute lung injury. Am J Respir Crit Care Med 182: 918-928, 2010.
15. Wagner EF and Eferl R: Fos/AP-1 proteins in bone and the immune system. Immunol Rev 208: 126-140, 2005.
16. Burch PM, Yuan Z, Loonen A and Heintz NH: An extracellular signal-regulated kinase 1- and 2-dependent program of chromatin trafficking of c-Fos and Fra-1 is required for cyclin D1 expression during cell cycle reentry. Mol Cell Biol 24: 4696-4709, 2004.
17. Xi X, Yao Y, Liu N and Li P: MiR-297 alleviates LPS-induced A549 cell and mice lung injury via targeting cyclin dependent kinase 8. Int Immunopharmacol 80: 106197, 2020.
18. 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.
19. Bein T, Briegel J and Annane D: Steroids are part of rescue therapy in ARDS patients with refractory hypoxemia: Yes. Intensive Care Med 42: 918-920, 2016.
20. Zhao D, Ding R, Mao Y, Wang L, Zhang Z and Ma X: Heparin rescues sepsis-associated acute lung injury and lethality through the suppression of inflammatory responses. Inflammation 35: 1825-1832, 2012.
21. Hu H, Shi D, Hu C, Yuan X, Zhang J and Sun H: Dexmedetomidine mitigates CLP-stimulated acute lung injury via restraining the RAGE pathway. Am J Transl Res 9: 5245-5258, 2017.
22. Zhou H, Wang X and Zhang B: Depression of lncRNA NEAT1 antagonizes LPS-evoked acute injury and inflammatory response in alveolar epithelial cells via HMGB1-RAGE signaling. Mediators Inflamm 2020: 8019467, 2020.
23. Li P, Yao Y, Ma Y and Chen Y: MiR-150 attenuates LPS-induced acute lung injury via targeting AKT3. Int Immunopharmacol 75: 105794, 2019.
24. Li P, Yao Y, Ma Y and Chen Y: MiR-30a-5p ameliorates LPS-induced inflammatory injury in human A549 cells and mice via targeting RUNX2. Innate Immun 27: 41-49, 2021.
25. Yamaguchi T, Takada Y, Maruyama K, Shimoda K, Arai Y, Nango N, Kosaki N, Takaishi H, Toyama Y and Matsuo K: Fra-1/AP-1 impairs inflammatory responses and chondrogenesis in fracture healing. J Bone Miner Res 24: 2056-2065, 2009.
26. Rajasekaran S, Vaz M and Reddy SP: Fra-1/AP-1 transcription factor negatively regulates pulmonary fibrosis in vivo. PLoS One 7: e41611, 2012.
27. Rajasekaran S, Tamatam CR, Potteti HR, Raman V, Lee JW, Matthay MA, Mehta D, Reddy NM and Reddy SP: Visualization of Fra-1/AP-1 activation during LPS-induced inflammatory lung injury using fluorescence optical imaging. Am J Physiol Lung Cell Mol Physiol 309: L414-L424, 2015.