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MicroRNA-206 promotes lipopolysaccharide-induced inflammation injury via regulation of IRAK1 in MRC-5 cells

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ABSTRACT

Background: MicroRNAs (miRNAs) have been reported to play crucial role in the airway inflammatory diseases. However, the involvement of miR-206 in airway inflammatory diseases is still uninvestigated. The study aimed to explore the effect of miR-206 on lipopolysaccharide (LPS)-induced inflammation injury in MRC-5 cells, and point out a potential relevance for chronic obstructive pulmonary disease (COPD).

Methods: LPS was utilized to expose MRC-5 cells, then cell viability, cell migration, apoptosis, apoptosis-associated factors, as well as the concentrations and protein levels of IL-6 and IL-8 were explored. After transfected with miR-206 mimic and inhibitor, above parameters were reassessed in LPS-injured cells. Expression level of IRAK1 was examined in miR-206 mimic/inhibitor transfected cells by using RT-qPCR. The effect of IRAK1 on LPS-induced inflammation injury was investigated in MRC-5 cells after transfection with pc-IRAK1 and sh-IRAK1. The effects of miR-206 and IRAK1 on MEK/ERK and JNK pathways were determined by western blot assay.

Results: LPS significantly triggered inflammation injury in MRC-5 cells by inhibiting cell viability, suppressing the healing of scratches, inducing cell apoptosis, down-regulating Bcl-2 expression and up-regulating Bax, cleaved-Caspase-3 and cleaved-Caspase-9 expression, and concurrently increasing the concentrations and the protein levels of IL-6 and IL-8. MiR-206 overexpression aggravated LPS-induced inflammation injury in MRC-5 cells. Up-regulation of IRAK1 was observed in miR-206 mimic-transfected cells. Moreover, IRAK1 overexpression promoted LPS-induced inflammation injury in MRC-5 cells. MiR-206 activated MEK/ERK and JNK pathways by regulating IRAK1.

Conclusions: MiR-206 promotes LPS-induced inflammation injury through regulation of IRAK1 in MRC-5 cells.

1. Introduction

Chronic airway inflammatory diseases refer to chronic inflammatory diseases of the upper and lower airways, mainly encompassing bronchial asthma and chronic obstructive pulmonary disease (COPD) [1,2]. Smoking is known as a major cause of chronic airway inflammatory disease, several other factors such as air pollution, occupational exposure and genetics can also lead to chronic airway inflammatory disease [3,4]. The essential symptoms of chronic airway inflammatory disease include chronic cough, expectoration, shortness of breath or difficulty breathing [5]. Currently, there is still no cured treatment for chronic airway inflammatory disease, but its progression can be delayed. COPD is a common chronic airway inflammatory disease characterized by continuous airflow restriction [6]. The clinical diagnosis is ascertained by spirometry, Chest X-ray examination and Chest CT examination [7–9]. Pulmonary rehabilitation, bronchodilators, corticosteroids, and supplemental oxygen are main managements for treating COPD [10,11]. However, application of biological methods in the diagnosis and treatment of chronic airway inflammatory disease is still scarce, which arouses our strong research interest.

MicroRNAs (miRNAs) are small noncoding RNAs, which are well known to play crucial roles in post-transcriptional gene regulation, and to be linked to various biological processes [12,13]. Several miRNAs have been proven to be involved in affecting the onset or progression of chronic airway inflammatory diseases represented by COPD and asthma. For example, Wu et al. revealed that miR-126 and miR-21 were elevated in patients with asthma, which might be linked to the pathogenesis of asthma, and might be biomarkers of the therapy [14]. Soeda

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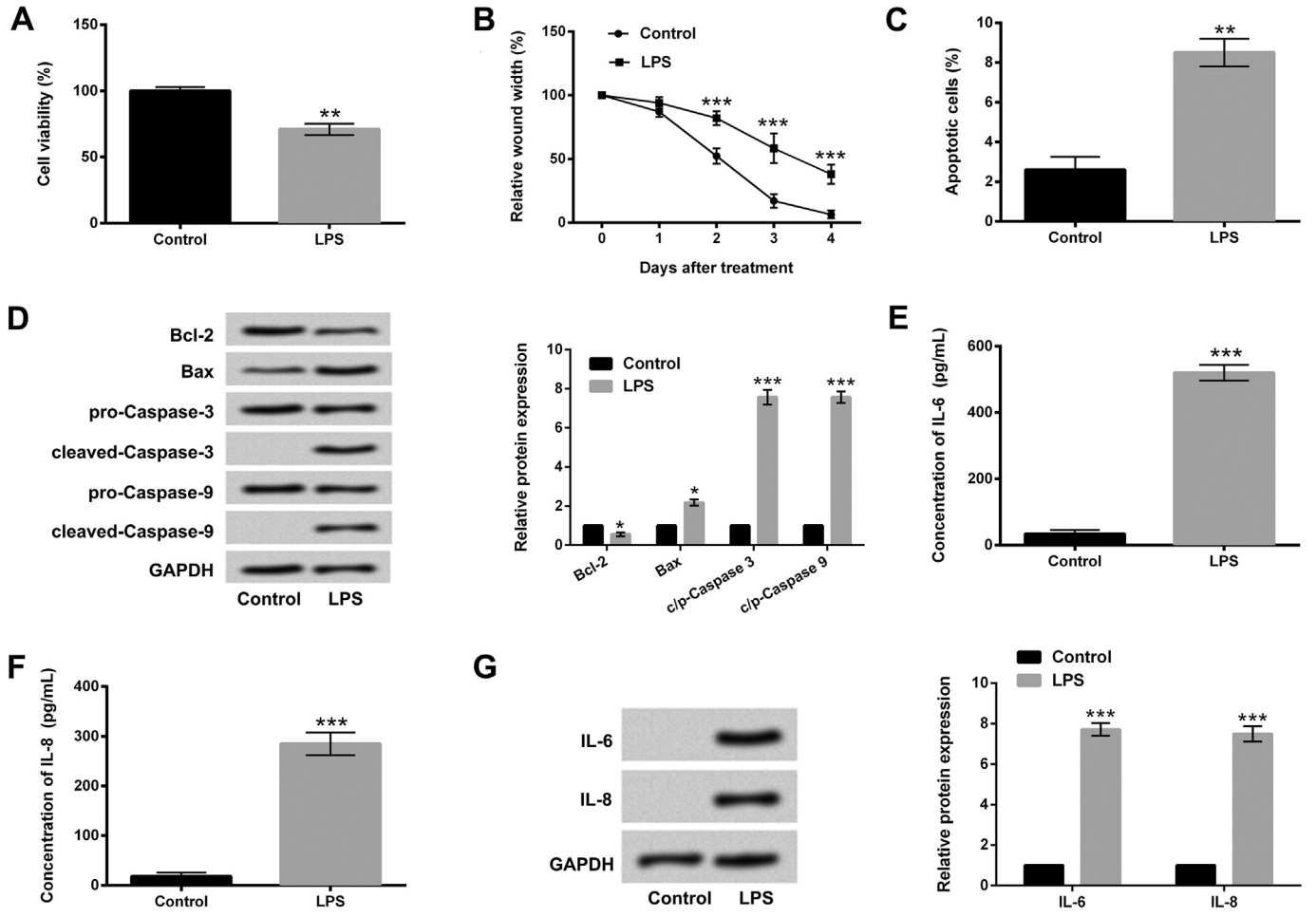

Fig. 1. LPS induced inflammation injury in MRC-5 cells
MRC-5 cells were treated with LPS, then LPS (A) inhibited cell viability, (B) increased the percentage of wound width, (C) induced apoptosis, (D) down-regulated anti-apoptotic factor (Bcl-2) and up-regulated pro-apoptotic factors (Bax, cleaved-Caspase-3 and cleaved-Caspase-9), (E and F) increased the concentrations of IL-6 and IL-8, and (G) up-regulated the protein levels of IL-6 and IL-8. $N = 3$, data were emerged as the mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

et al. found that miR-106b expression was down-regulated in the plasma from COPD patients, and there was a negative correlation between miR-106b level and the duration of disease [15]. MiR-206 is an important miRNA, which acts as a key regulator in muscle differentiation and maintenance and repair of neuromuscular junctions [16,17]. Previous literature reported that miR-206 was significantly increased in patients with COPD, and was associated with circulating inflammatory cytokines in severe COPD patients [18]. Outside of this, miR-206 has been reported to regulate lipopolysaccharide (LPS)-triggered inflammatory response in human astrocytes and microglia [19,20]. Moreover, LPS as an inducer has been applied to build research model to mimic airway inflammatory diseases especially COPD in vivo experiments [21,22]. Zhang et al. discovered that LPS could repress lung fibroblasts proliferation via regulating IL-6 and IL-8 release [23]. According to these researches, our study investigated the influences of miR-206 in airway inflammatory diseases, and disclosed the potential relevance between miR-206 and COPD. LPS was utilized as an inducer in the current research to build cell injury model to mimic airway inflammatory disease.

Interleukin-1 receptor associated kinase 1 (IRAK1), a serine/threonine protein kinase related to Interleukin 1R (IL-1R) and toll-like receptors (TLRs) signal transduction, plays an important role in innate immunity and inflammation diseases [24,25]. Recent studies demonstrated that miRNAs exhibited the regulatory effect on several inflammation diseases by regulating IRAK1 expression [26,27]. However, whether IRAK1 can affect the effect of miR-206 on airway inflammatory diseases remains unclear. Fibroblasts are the main source of extracellular matrix, and the alteration of fibroblast function leads to the failure of complete repair after lung parenchymal destruction, which may be one of the important reasons for the pathological process of emphysema. Togo et al. demonstrated that the pulmonary fibroblasts of COPD patients are defective in repair function [28]. Therefore, human lung fibroblast MRC-5 cells (25–30 generations) was utilized in this study. The study aimed to explore the effect of miR-206 on LPS-induced inflammation injury in human lung fibroblast MRC-5 cells, as well as uncovered the regulatory mechanism of IRAK1 in inflammation response.

2. Materials and methods

2.1. Cell culture and treatment

Human lung fibroblast MRC-5 cells (ATCC® CCL-171™, Manassas, VA, USA) were cultured in Dulbecco's modied Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin (all from Gibco, Carlsbad, CA, USA). MRC-5 cells were maintained in an incubator with 5% CO_2 at 37 °C. After incubation, cells were stimulated with 10 μg/mL LPS (Sigma-Aldrich, St, Louis, MO, USA) for 12 h in the subsequent experiments.

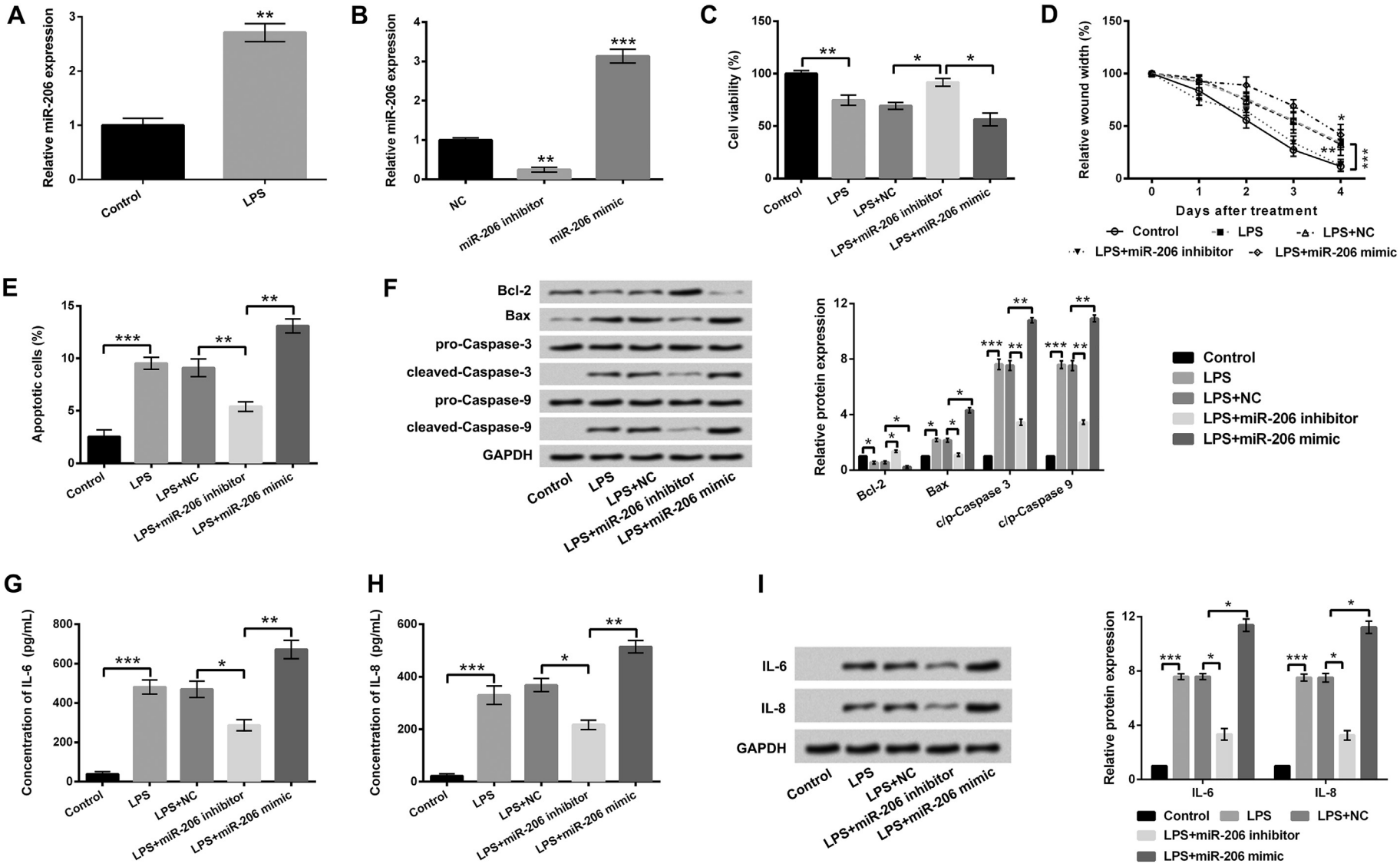

Fig. 2. MiR-206 aggravated LPS-induced inflammation injury in MRC-5 cells

(A) MRC-5 cells were administrated with LPS, and the expression level of miR-206 was enhanced by LPS exposure. (B) MRC-5 cells were transfected with miR-206 mimic and inhibitor, the expression level of miR-206 was up-regulated by miR-206 overexpression, but down-regulated by miR-206 inhibition. These transfected cells were treated with LPS, then results showed that miR-206 overexpression (C) suppressed cell viability, (D) increased wound width, (E) induced apoptosis, (F) down-regulated Bcl-2 and up-regulated Bax, cleaved-Caspase-3 and cleaved-Caspase-9, (G and H) improved the concentrations of IL-6 and IL-8, and (I) up-regulated the protein levels of IL-6 and IL-8. $N = 3$, data were emerged as the mean ± SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

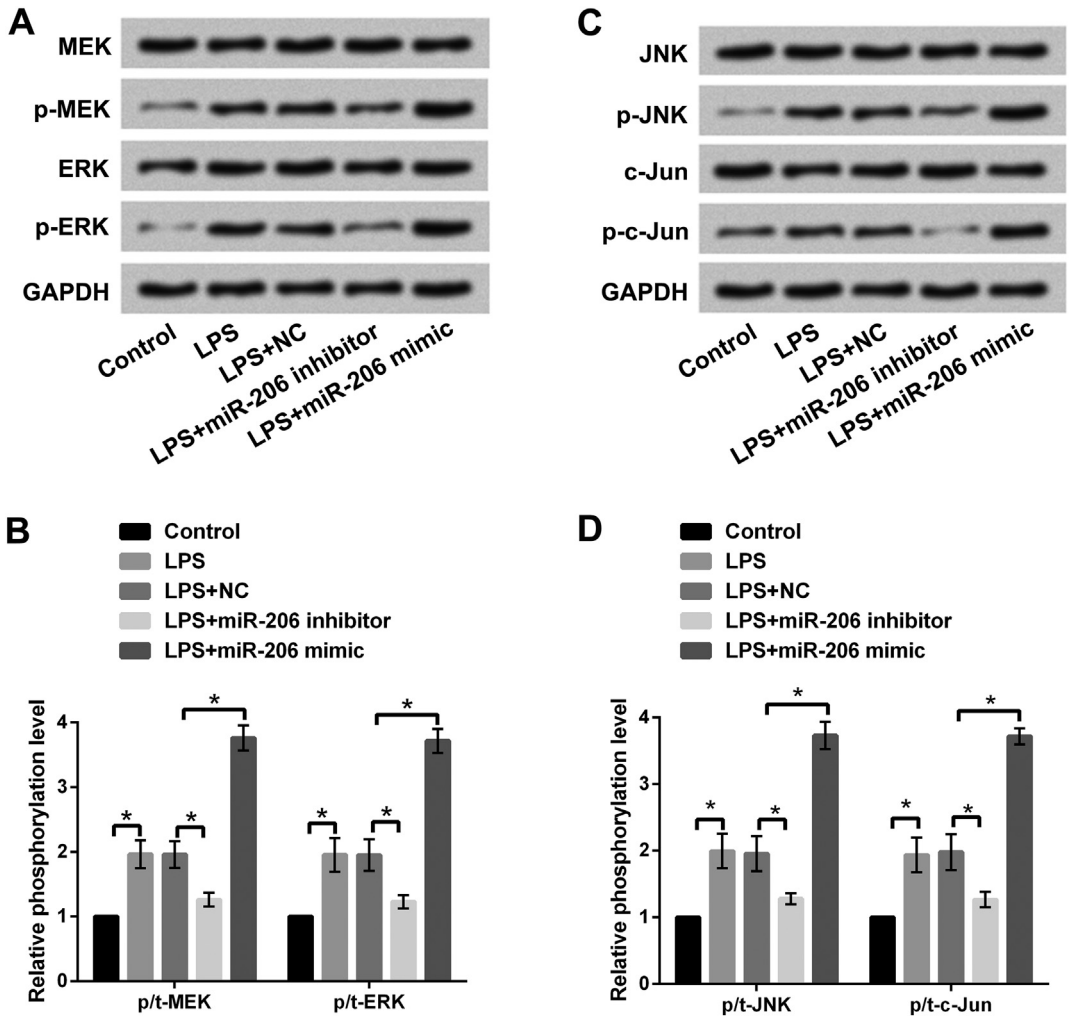

Fig. 3. MiR-206 activated MEK/ERK and JNK pathways in LPS-treated MRC-5 cells
After transfection with miR-206 mimic and inhibitor and simultaneously treated with LPS, the protein levels of (A and B) phosphorylated (p)-MEK and p-ERK, as well as (C and D) p-JNK and p-c-Jun were up-regulated by miR-206 overexpression, but down-regulated by miR-206 suppression in LPS-treated MRC-5 cells. $N = 3$, data were emerged as the mean ± SD. * $P < 0.05$.

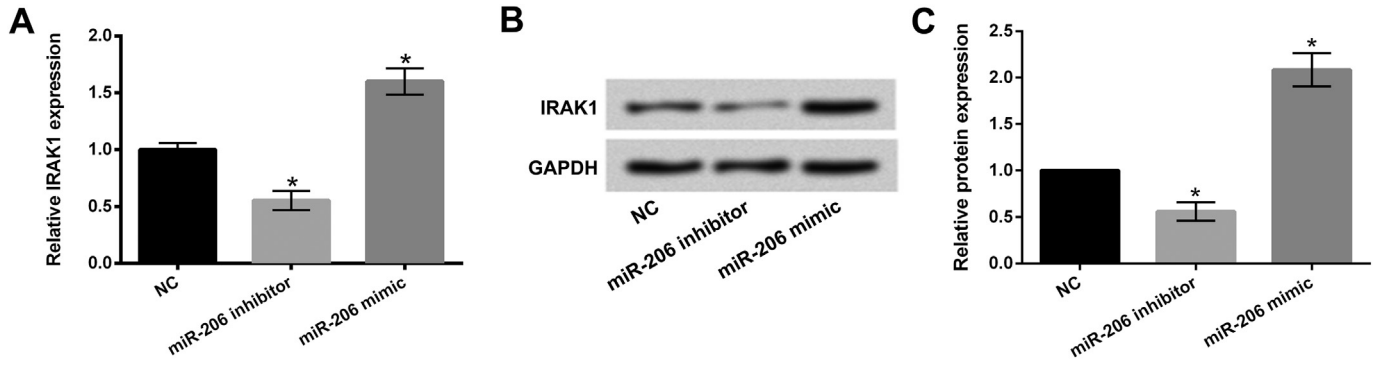

Fig. 4. MiR-206 up-regulated IRAK1 expression in MRC-5 cells
MRC-5 cells were transfected with miR-206 mimic and inhibitor, then the (A) mRNA expression and (B and C) protein levels of IRAK1 were enhanced by miR-206 overexpression, but declined by miR-206 suppression in MRC-5 cells. $N = 3$, data were emerged as the mean ± SD. * $P < 0.05$.

2.2. Transfection

MiR-206 mimic, miR-206 inhibitor, IRAK1 targeted siRNA (si-IRAK1) and the corresponding controls were synthesized by GenePharma Co. (Shanghai, China). The full-length IRAK1 sequences were constructed in pcDNA3.1 plasmid (pc-IRAK1, GenePharma). The empty pcDNA3.1 served as a control group. Lipofectamine 3000 reagent (Life Technologies Corporation, Carlsbad, CA, USA) was used for cell transfection. After 48 h transfection, cells were harvested for the following experiments.

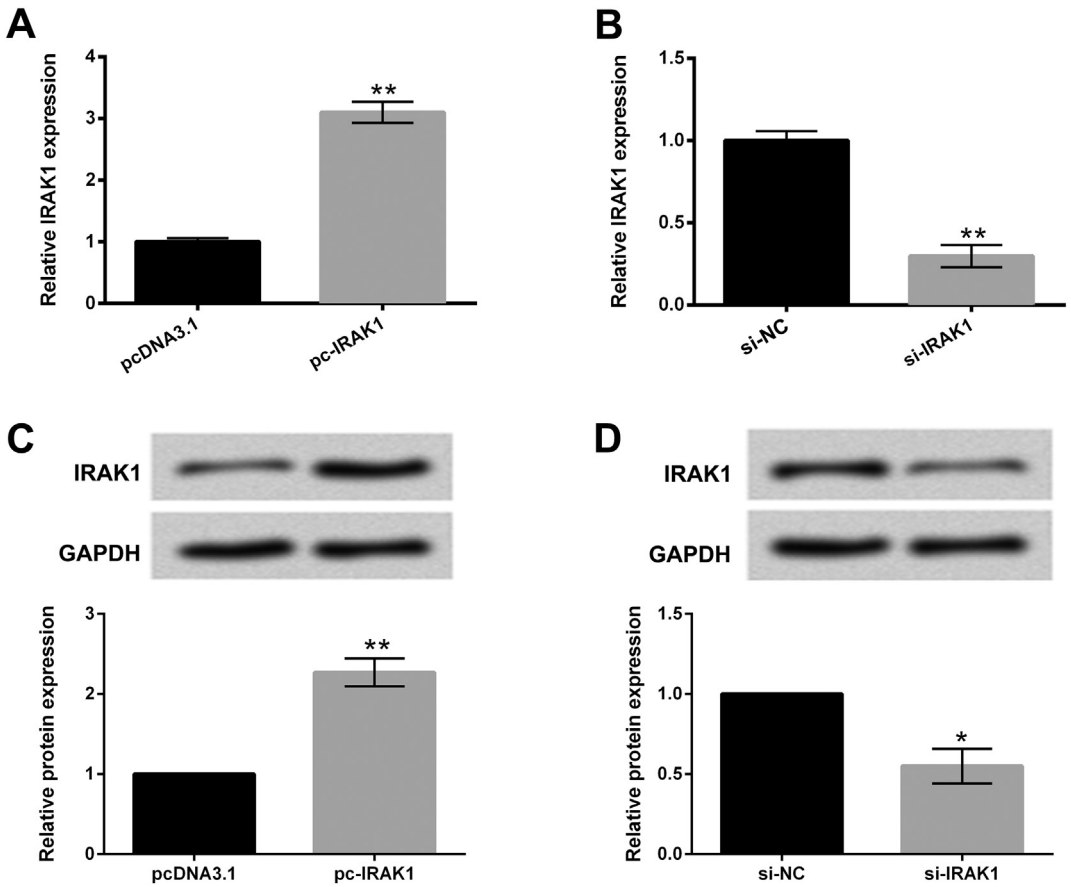

Fig. 5. The transfection efficiency of IRAK1
MRC-5 cells were transfected with pc-IRAK1, si-IRAK1 and the corresponding controls (pcDNA3.1 and si-NC). (A and B) The mRNA expression level of IRAK1 was up-regulated by IRAK1 overexpression, but down-regulated by IRAK1 silence in MRC-5 cells. (C and D) The protein level of IRAK1 was down-regulated by IRAK1 silence in MRC-5 cells. $N = 3$, data were emerged as the mean ± SD. * $P < 0.05$, ** $P < 0.01$.

2.3. Cell viability assay

After transfection with the plasmids of miR-206 mimic, miR-206 inhibitor, pc-IRAK1, si-IRAK1 and their controls, cells were collected and incubated in 96-well plate and treated with 10 μg/mL LPS for 12 h. Then, 10 μL Cell Counting Kit-8 solution (CCK-8, Dojindo Molecular Technologies, Gaithersburg, MD) were added to each well of 96-well plate, and incubated at 37 °C for 1 h in humidified 95% air and 5% CO_2. Absorbance of each well was measured at 450 nm by using a Microplate Reader (Bio-Rad, Hercules, CA, USA).

2.4. Wound healing assay

Cells were cultured in 60 mM dishes until confluence, and a wound was created in monolayer with a 200 μL sterile pipette tip after cells were pre-treated with 50 μM mytomicin C for 3 h. Subsequently, cells were rinsed twice with phosphate buffer saline (PBS), and incubated in new medium. The pictures of a specific position on the scratched areas were taken at 1, 2, 3 and 4 days by an inverted microscope (Leica, Wetzlar, Hessen, Germany). The wound widths were measured and the relative wound widths were calculated by using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

2.5. Apoptosis assay

After treatment or transfection, MRC-5 cells were rinsed twice with PBS. Then, cells were suspended in 300 μL binding buffer as well as 5 μL Annexin V-FITC solution (Sigma-Aldrich) was supplemented in the suspension. After incubation with Annexin V-FITC at room temperature in the dark for 15 min, 5 μL PI solution (Sigma-Aldrich) was added into the cell suspension. After staining for 10 min, the cells were analyzed by using FACS can (Beckman Coulter, Fullerton, CA). The percentage of apoptotic cells was analyzed by using FlowJo software (Tree Star Software, San Carlos, CA, USA).

2.6. Enzyme-linked immunosorbent assay (ELISA) for IL-6 and IL-8

Culture supernatant from MRC-5 cells after treatment with LPS or transfection with different plasmids was collected. The corresponding ELISA kits of IL-6 and IL-8 (R&D Systems, Abingdon, UK) were performed to analyze the concentrations of IL-6 and IL-8 in different treated cells. The experimental procedures were carried out according to the instructions of the ELISA kits.

2.7. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from cells transfected with miR-206 mimic, miR-206 inhibitor, pc-IRAK1, si-IRAK1 and their corresponding control by using Trizol reagent (Life Technologies Corporation). The cDNA was synthesized by using Taqman MicroRNA Reverse Transcription Kit and PrimeScript® RT reagent kit (Takara, Tokyo, Japan). RT-qRCR assay was performed by using the TaqMan® Universal Master Mix II (Applied Biosystems, Foster City, CA). U6 and GAPDH were used as internal control for the expression of miR-206 or IRAK1. Data in this study were analyzed by using the $2^{-\Delta\Delta Ct}$ method [29].

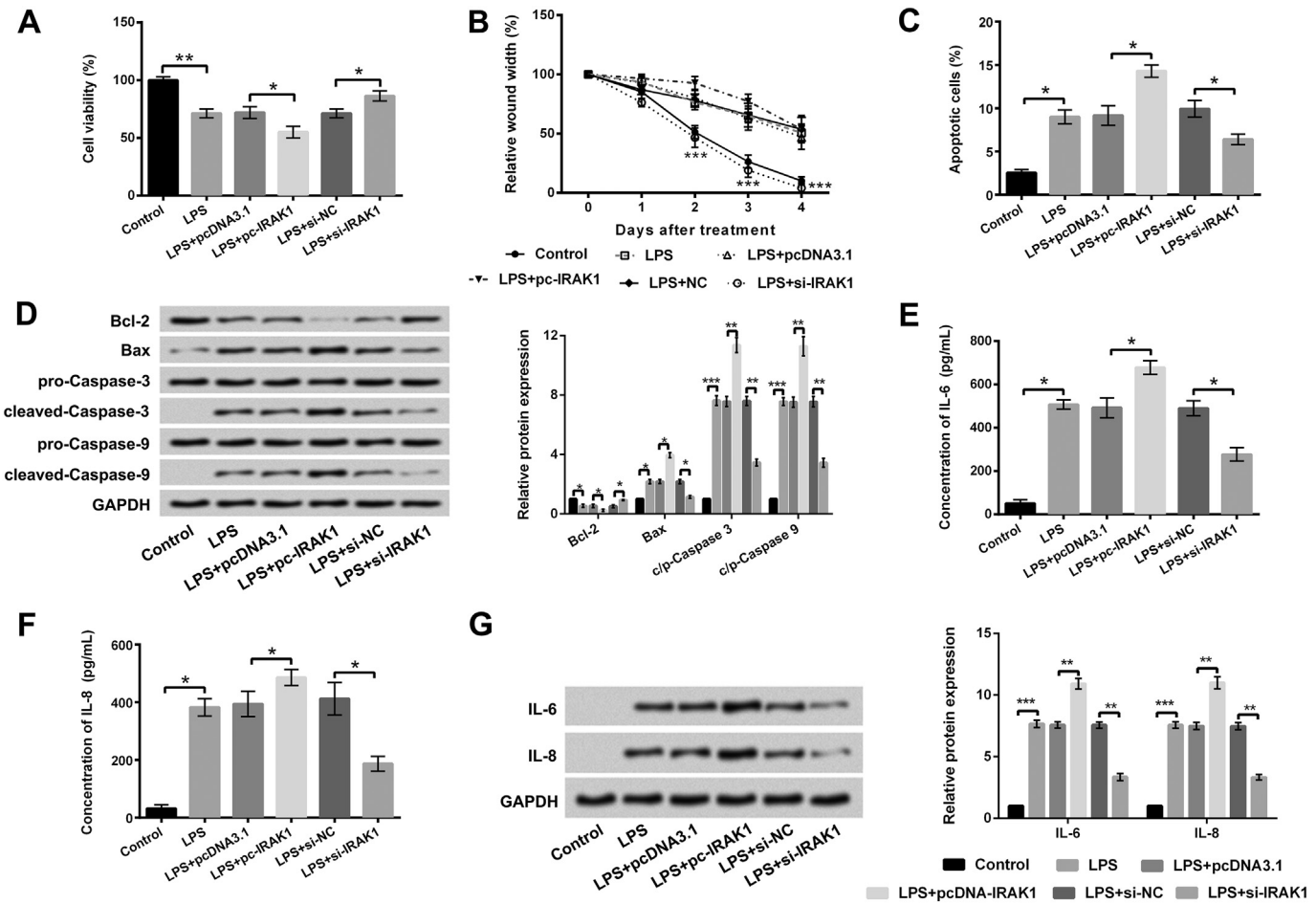

Fig. 6. IRAK1 promoted LPS-induced inflammation injury in MRC-5 cells
MRC-5 cells were transfected with pc-IRAK1, si-IRAK1 and the corresponding controls (pcDNA3.1 and si-NC), as well as treated with LPS. IRAK1 overexpression (A) inhibited cell viability, (B) restrained the healing of scratches, (C) induced apoptosis, (D) down-regulated Bcl-2 and up-regulated Bax, cleaved-Caspase-3 and cleaved-Caspase-9, (E and F) raised the concentrations of IL-6 and IL-8, and (G) up-regulated the protein levels of IL-6 and IL-8. $N = 3$, data were emerged as the mean ± SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

2.8. Western blot assay

Treated or transfected cells were washed with PBS, and the protein samples was extracted from these cells by using RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) containing protease inhibitor cocktail (Sigma-Aldrich). The proteins were quantified using the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out to separate the protein samples and then these proteins were transferred to nitrocellulose membranes. The membranes were blocked in 5% bovine serum albumin (BSA), and were incubated with the primary antibodies overnight at 4 °C. The primary antibodies included Bcl-2 (ab32124), Bax (ab32503), pro-Caspase-3 (ab32150), cleaved-Caspase-3 (ab2302), pro-Caspase-9 (ab135544), cleaved-Caspase-9 (ab2324), IL-6 (ab6672), IL-8 (ab7747), IRAK1 (ab238), mitogen-activated protein/extracellular signal-regulated kinase (MEK, ab32091), phosphorylated (p)-MEK (ab96379), extracellular signal-regulated kinase (ERK, ab32537), p-ERK (ab131438), c-Jun N-terminal kinase (JNK, ab199380), p-JNK (ab47337), c-Jun (ab32137), p-c-Jun (ab30620) and GAPDH (ab181602, Abcam, Cambridge, MA). The second antibody of horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (ab205718, 1:2000, Abcam) was subsequently incubated with the membranes for 1 h at room temperature. Lumi-Light Western Blotting Substrate (Sigma-Aldrich), an enhanced chemiluminescence reagent, was used to exhibit the signals.

2.9. Statistical analysis

All data were shown as the mean ± standard deviation (SD). Graphpad 6.0 statistical software (GraphPad Software, Inc., San Diego, CA, USA) was applied for statistical analyses in this study. The Student *t*-test was used to analyze the statistical significance between two groups. A one-way analysis of variance (ANOVA) followed by Holm-Sidak pos-hoc test was used to analyzed the statistical significance in three or more groups. *P*-value of < 0.05 was considered to be a statistically significant difference.

3. Results

3.1. LPS induced inflammation injury in MRC-5 cells

MRC-5 cells were treated with LPS for 12 h, and cell viability, migration, apoptosis and pro-inflammatory factors (IL-6 and IL-8) were detected. We observed that cell viability was significantly inhibited by LPS treatment ($P < 0.01$, Fig. 1A). The healing of scratches was inhibited by LPS treatment ($P < 0.001$, Fig. 1B). The apoptosis analytical results showed that LPS treatment evidently increased the percentage of apoptotic cells ($P < 0.01$), and concurrently down-regulated Bcl-2 expression and up-regulated Bax, cleaved-Caspase-3 and cleaved-Caspase-9 expression in MRC-5 cells ($P < 0.05$ or $P < 0.001$, Fig. 1C and D). Besides, the concentrations and the protein levels of IL-6 and IL-8

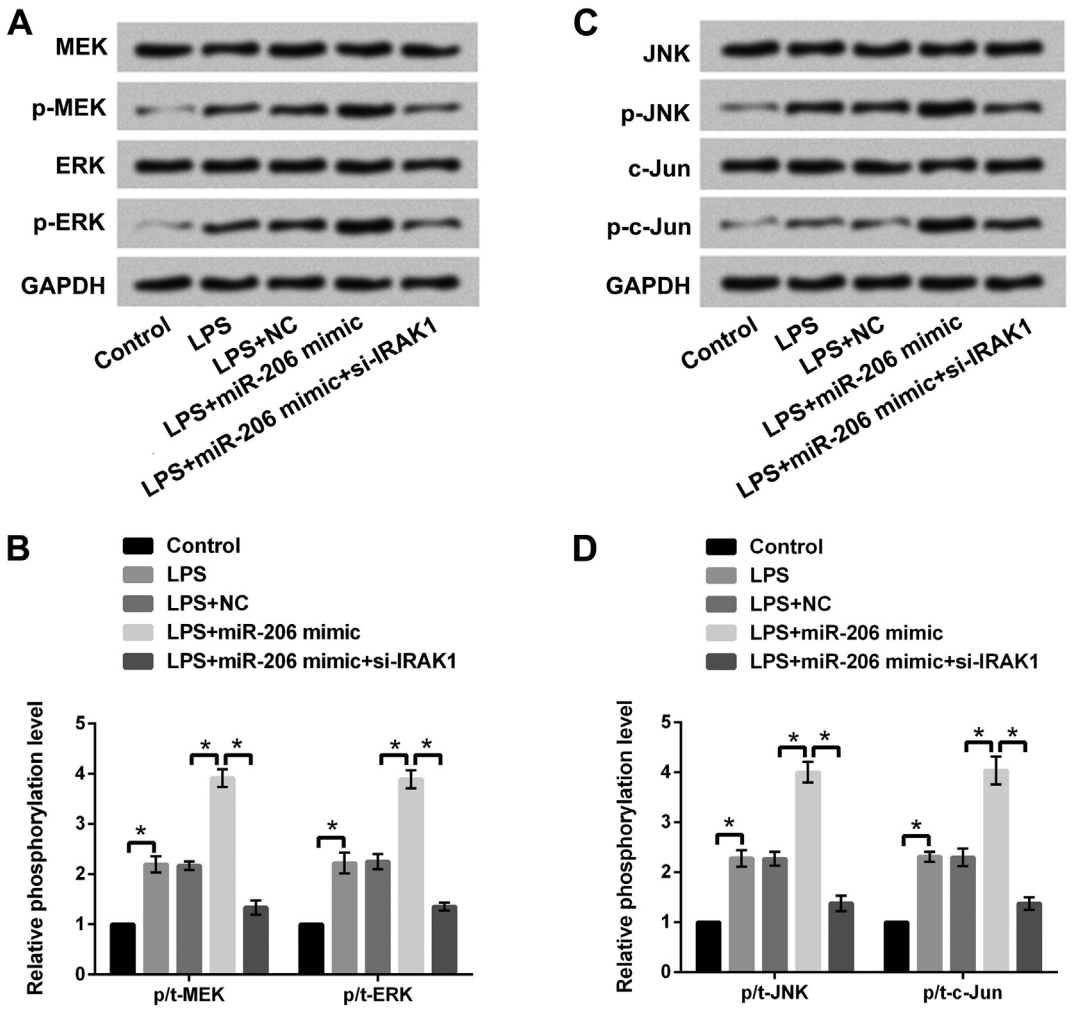

Fig. 7. MiR-206 activated MEK/ERK and JNK pathways via regulating IRAK1 in LPS-treated MRC-5 cells
MRC-5 cells were transfected with miR-206 mimic alone or co-transfected with si-IRAK1. After treatment with LPS, the protein levels of (A and B) p-MEK and p-ERK, as well as (C and D) p-JNK and p-c-Jun were up-regulated by miR-206 overexpression, but the promoting effect was reversed by IRAK1 silence in LPS-treated MRC-5 cells. $N = 3$, data were emerged as the mean ± SD. * $P < 0.05$.

were observably increased in LPS-treated cells as relative to that in control group ($P < 0.001$, Fig. 1E–G). Based on these results, we concluded that LPS induced inflammation injury in MRC-5 cells.

3.2. MiR-206 aggravated LPS-induced inflammation injury in MRC-5 cells

After exposure with LPS, we observed that miR-206 expression was significantly elevated in MRC-5 cells ($P < 0.01$, Fig. 2A). Before investigating the involvement of miR-206 in LPS-induced inflammation injury, miR-206 inhibitor and miR-206 mimic were transfected into MRC-5 cells to alter miR-206 expression. Results presented in Fig. 2B showed that relative miR-206 expression was decreased in miR-206 inhibitor-transfected cells, and increased in miR-206 mimic transfected cells ($P < 0.01$ or $P < 0.001$). The next experiments results revealed that miR-206 inhibition restored cell viability ($P < 0.05$), accelerated the healing of scratches ($P < 0.01$), reduced apoptosis ($P < 0.01$), as well as augmented anti-apoptotic factor (Bcl-2) expression, but abated pro-apoptotic factors (Bax, cleaved-Caspase-3 and cleaved-Caspase-9) expression in LPS-treated cells ($P < 0.05$ or $P < 0.01$, Fig. 2C–F). Further, suppressed miR-206 prominently inhibited the productions and the protein levels of IL-6 and IL-8 in LPS-treated cells ($P < 0.05$ or $P < 0.01$, Fig. 2G–I). However, miR-206 overexpression showed the opposite effects on cell viability, wound width, apoptosis and the productions of pro-inflammatory cytokines ($P < 0.05$, $P < 0.01$ or $P < 0.001$, Fig. 2B–I). These results implied that miR-206 inhibition alleviated LPS-induced inflammation injury, but miR-206 overexpression aggravated this process in MRC-5 cells.

3.3. MiR-206 activated MEK/ERK and JNK pathways in LPS-treated MRC-5 cells

The effect of miR-206 on MEK/ERK and JNK signaling pathways was explored in MRC-5 cells after treatment with LPS. Results in Fig. 3A and B showed that LPS treatment up-regulated the expression levels of phosphorylated MEK and ERK (p-MEK and p-ERK) in MRC-5 cells ($P < 0.05$). After transfection with miR-206 inhibitor and miR-206 mimic, we observed that the protein level of p-MEK and p-ERK were declined by miR-206 inhibition, but raised by miR-206 overexpression in LPS-treated cells ($P < 0.05$). Similarly, the phosphorylated JNK and c-Jun (p-JNK and p-c-Jun) were also notably increased by LPS treatment ($P < 0.05$). However, suppression of miR-206 reversed the promoting effect of LPS on JNK pathway. MiR-206 overexpression showed opposite regulatory effect of miR-206 inhibition on JNK pathway ($P < 0.05$, Fig. 3C and D). These data indicated that miR-206 activated MEK/ERK and JNK signaling pathways in LPS-treated MRC-5 cells.

3.4. MiR-206 up-regulated IRAK1 expression in MRC-5 cells

After transfection with miR-206 inhibitor and miR-206 mimic, the mRNA and protein levels of IRAK1 were determined in these cells by RT-qPCR and western blot assays. Results demonstrated that mRNA and protein levels of IRAK1 were remarkably decreased in miR-206 inhibitor-transfected cells ($P < 0.05$). However, up-regulation of IRAK1 was found in miR-206 mimic-transfected cells ($P < 0.05$, Fig. 4A–C). These data uncovered that miR-206 could positively regulate the expression level of IRAK1. Therefore, we speculated that IRAK1 might be a crucial regulator in LPS-induced inflammation injury in MRC-5 cells.

3.5. IRAK1 promoted LPS-induced inflammation injury in MRC-5 cells

To confirm whether IRAK1 was involved in regulating the process of LPS-induced inflammation injury in MRC-5 cells, the expression plasmids of pc-IRAK1, si-IRAK1 and their controls (pcDNA3.1 and si-NC) were transfected into MRC-5 cells to change IRAK1 expression. Results in Fig. 5A and B revealed that the mRNA expression of IRAK1 was observably up-regulated in pc-IRAK1 transfected cells as relative to that in pcDNA3.1 transfected cells, but was down-regulated in si-IRAK1 transfected cells as relative to that in si-NC transfected cells ($P < 0.01$). Similarly, the protein level of IRAK1 was also elevated in pc-IRAK1-transfected cells ($P < 0.01$), meanwhile was declined in si-IRAK1 transfected cells ($P < 0.05$, Fig. 5C and D). These data indicated that the expression vectors of pc-IRAK1 and si-IRAK1 were successfully transfected into MRC-5 cells.

Subsequently, we found that IRAK1 overexpression significantly inhibited cell viability ($P < 0.05$), slowed the healing of scratches ($P < 0.001$), induced apoptosis ($P < 0.05$) and down-regulated Bcl-2 expression, but up-regulated Bax, cleaved-Caspase-3 and cleaved-Caspase-9 expression ($P < 0.05$ or $P < 0.01$) in LPS-treated cells (Fig. 6A–D). Apart from these, the concentrations and protein levels of IL-6 and IL-8 were all increased by IRAK1 overexpression in LPS-treated cells ($P < 0.05$ or $P < 0.01$, Fig. 6E and G). Whereas, IRAK1 silence manifested the contrary results as those mentioned above ($P < 0.05$ or $P < 0.01$, Fig. 6A and G). There data implied that IRAK1 promoted LPS-induced inflammation injury in MRC-5 cells, which was similar with the effect of miR-206 on LPS-induced inflammation injury.

3.6. MiR-206 activated MEK/ERK and JNK pathways via regulating IRAK1 in LPS-treated MRC-5 cells

Finally, the regulatory mechanism of miR-206 in MEK/ERK and JNK signaling pathways was investigated. Above results have clarified that miR-206 overexpression activated MEK/ERK and JNK signaling pathways in LPS-treated cells. After co-transfection with miR-206 mimic and si-IRAK1 and treatment with LPS, we observed that the protein levels of p-MEK, p-ERK, p-JNK and p-c-Jun were all down-regulated in these cells ($P < 0.05$, Fig. 7A–D). There was no obvious difference of MEK, ERK, JNK and c-Jun protein levels in different treated groups (Fig. 7A–D). These data indicated that miR-206 activated MEK/ERK and JNK pathways might through regulating IRAK1 expression in LPS-treated MRC-5 cells.

4. Discussion

With the serious environmental pollution, lung-related diseases, especially chronic airway inflammatory diseases, have become the main causes of morbidity and mortality in developed and developing countries [30,31]. To find new biomarkers and early diagnostic methods for chronic airway inflammatory diseases has aroused our research interest. Recently, increasing evidence has found that miRNAs play an important role in the occurrence and development of chronic airway inflammatory diseases through various mechanisms [32]. In our study, we constructed an inflammation injury model induced by LPS in MRC-5 cells to mimic the pathogenesis of airway inflammatory disease, as well as investigated the effect of miR-206 on LPS-induced inflammation injury in MRC-5 cells.

MiRNAs consist of 19 to 25 nucleotides, which can control cell growth, differentiation, apoptosis, adhesion and cell death [33]. It has been demonstrated that miRNAs are involved in the physiological and pathological mechanisms of various airway inflammatory diseases [34]. As a typical chronic airway inflammatory disease, numerous studies have focused on COPD. A prominent research has discussed the importance of miRNAs in COPD [35]. Moreover, repressed miR-146a-5p in COPD fibroblasts has been corroborated to evoke a more pro-inflammatory phenotype, contributing to chronic inflammation [36]. In a mouse animal study, increased or decreased miRNAs expression has been proven to disturb the growth of normal respiratory epithelial cells, indicating that lung tissue is already dependent on the expression of miRNAs during embryonic development [34]. Li et al. showed that up-regulation of miR-196 could promote the onset and development of COPD [37]. These are enough to prove the importance of miRNAs in the occurrence and development of COPD. However, the effects of miR-206 on COPD remain unclear. The preliminary exploration in our study displayed that miR-206 overexpression aggravated LPS-induced inflammation injury in MRC-5 cells by mediating cell viability, migration, apoptosis, and the productions of IL-6 and IL-8. Our study preliminary testified the impacts of miR-206 on airway inflammatory disease *in vitro*, as well as disclosed the possible correlation for COPD. Further researches are still needed to explore the functions of miR-206 *in vivo* and other cell lines related to COPD.

IRAK1 is one of the most important connectors and pivots of TLRs/IL-1 signaling pathway which is involved in mediating various signaling pathways, such as ERK1/2, p38MAPK, and JNK [38,39]. It is also well known that IRAK is involved in the TLR-mediated innate immune response to LPS [40]. Recent study from Lee et al. displayed that inhibition of miR-146 could alleviate inflammation via inhibiting IRAK1, thereby relieving the symptoms of COPD patients [41]. In our study, we investigated the effect of IRAK1 on LPS-induced inflammation injury in MRC-5 cells. The results showed that the expression level of IRAK1 was up-regulated in miR-206-mimic transfected MRC-5 cells, indicating a positive correlation between miR-206 and IRAK1. More importantly, we observed that IRAK1 overexpression similarly promoted LPS-induced inflammation injury in MRC-5 cells. These data indicated that IRAK1 might be a key regulator in the development of airway inflammatory disease, and might also be involved in mediating the pathogenesis of COPD.

Cumulative evidences suggest that MEK/ERK and JNK signaling pathways play crucial role in various inflammation diseases, including COPD [42,43]. Inhibition of ERK has confirmed to protect LPS-induced lung injury [44]. One study from Li et al. demonstrated that AS-703026, a novel MEK/ERK inhibitor could inhibit LPS-induced TNFα production in monocytes of COPD patients [45]. Gu et al. revealed that mesenchymal stem cells (MSCs) alleviated airway inflammation and emphysema in COPD by down-regulating cyclooxygenase-2 (COX-2) through MAPK and ERK signaling pathways [46]. JNK signaling is activated by cytokines implicated in inflammation and apoptosis, which acted plays a prominent role in COPD [47]. In the present study, we found that miR-206 promoted the activations of MEK/ERK and JNK signaling pathways in LPS-treated MRC-5 cells. But, the promoting effect of miR-206 on MEK/ERK and JNK signaling pathways was obviously reversed by IRAK1 silence. These data hinted that miR-206 activated MEK/ERK and JNK signaling pathways by regulating IRAK1 expression in LPS-treated cells.

In summary, the study demonstrated that miR-206 promoted LPS-induced inflammation injury by activation of MEK/ERK and JNK signaling pathways in MRC-5 cells. IRAK1 has been proven to be involved in mediating this process. The research preliminary testified the regulatory impacts of miR-206 in airway inflammation, and possibly have a connection to diseases like COPD. Further studies are still necessary to

confirm the precise roles of miR-206 in COPD.

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Conflict of interest

The authors declare no conflict of interest.

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