miR-186-5p regulates the inflammatory response of chronic obstructive pulmonary disorder by targeting HIF-1α

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Abstract. Chronic obstructive pulmonary disorder (COPD) is a chronic respiratory disease that is a major cause of morbidity and mortality worldwide. Previous studies have shown that miR-186-5p expression is significantly increased in COPD and is involved in multiple physiological and pathological processes. However, the role of miRNA-186-5p in the inflammatory response of COPD remains unclear. In this study, an *in vitro* model of COPD was established using lipopolysaccharide (LPS)-induced human bronchial epithelial cells (BEAS-2B). CCK-8 assays, flow cytometry, and a Muse cell analyzer were used to determine cell viability, cell cycle distribution, and apoptosis, respectively. The production of TNF-α and IL-6 were measured by ELISA. Reverse-transcription-quantitative PCR and western blotting were used to analyze mRNA and protein expression levels. The targeting relation between miR-186-5p and *HIF-1α* was discovered using dual-luciferase reporter assays. The results showed that transfection of miR-186-5p inhibitor inhibited cell proliferation and promoted cell apoptosis in the LPS-induced BEAS-2B cells. Inhibition of miR-186-5p markedly increased the levels of *TNF-α* and *IL-6*. miR-186-5p directly targeted and negatively regulated *HIF-1α* expression. In addition, inhibition of miR-186-5p increased the expression of the NF-κB pathway protein p-p65. In conclusion, it was found that inhibiting miR-186-5p may improve inflammation of COPD through *HIF-1α* in LPS-induced BEAS-2B cells, possibly by regulating NF-κB signaling. These findings provide a novel potential avenue for the clinical management of COPD. Future research is required to determine the mechanism of the interaction between miR-186-5p and *HIF-1α* in COPD.

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Introduction

Chronic obstructive pulmonary disorder (COPD) is a common, preventable, and treatable chronic disease characterized by persistent respiratory symptoms and airflow limitation. It is associated with an enhanced chronic inflammatory response of the airways and the lungs to a variety of noxious particles or gases (1,2) According to the 2019 Global Burden of Disease Study report, 3.3 million people died from COPD and there were 74.4 million disability-adjusted life years (DALYs) lost to COPD, and it is thus considered a global major public health problem (3). The etiology of COPD is well established to be influenced by environmental factors, especially smoking, and genetics (4-6). In China, given it has the world's largest number of smokers, combined with an ever-aging population, the COPD cases in China account for 25% of global COPD cases and it has thus become a significant economic burden (7). COPD is associated with chronic inflammation that predominantly affects the lung parenchyma and peripheral airways (8). However, the exact pathogenesis of COPD remains unclear. Therefore, an improved understanding of the inflammatory responses is essential for the development of COPD therapeutics and improved clinical treatment.

MicroRNAs (miRNAs/miRs) are endogenous non-coding RNA molecules that regulate gene expression by binding to the 3'-untranslated region (3'-UTR) of multiple target mRNAs for degradation or translational repression (9). miRNAs are involved in a wide variety of biological processes including inflammation, cell differentiation, cell proliferation, and cell apoptosis (10). Recent studies have demonstrated that several miRNAs play an important role in positive and negative regulation of the inflammatory response and participate in various regulatory network motifs in respiratory disease (11-13). Numerous studies have revealed that miRNAs have a role in the pathogenesis of COPD via critical molecular pathways and have become valuable biomarkers for the diagnosis and prognosis of COPD (14-17). In a previous study, a significant difference in the expression of miR-186-5p was found between healthy controls and COPD

patients (18). miR-186-5p has been shown to be involved in the regulation of the inflammatory responses of various diseases. For example, miR-186-5p knockdown repressed oxygen-glucose deprivation/reperfusion (OGD/R)-induced pyroptosis and suppressed lactate dehydrogenase and inflammatory cytokine release (19). iR-186-5p inhibition abolished the effects of SOX2-OT blocking on the inflammatory responses, proliferation, and apoptosis of OGD/R-challenged H2C9 cells (20). Moreover, miR-186-5p inhibitor reduced the inflammatory factors and oxidative stress in BV2 treated with lipopolysaccharide (LPS) and reduced apoptosis (21). Li *et al* (22) reported that miR-186-5p may regulate COPD dysfunction. However, the relevance of miR-186-5p in COPD and the underlying molecular mechanisms are unclear. Therefore, it is necessary to develop novel therapeutic methods and targets by understanding the mechanism of COPD pathogenesis mediated by miR-186-5p.

HIF-1α is an important activator of inflammatory responses. Increased serum levels of *HIF-1α* are associated with the progression of COPD (23). The role of *HIF-1α* in the development and progression of COPD has also been demonstrated (24). Furthermore, NF-κB, the downstream target gene of HIF, is a central regulator of immunity and inflammation and is a key target in COPD therapy (25). In an osteosarcoma study, *HIF-1α* was identified as a downstream target of miR-186-5p, where it regulated osteosarcoma progression (26). However, the involvement of miR-186-5p in relation to *HIF-1α* in controlling inflammation of COPD remains unclear. Therefore, this investigation attempted to evaluate the association between miR-186-5p and *HIF-1α* and their roles in inflammation during COPD. It was found that interfering with miR-186-5p reduced LPS-induced BEAS-2B cell proliferation and promoted cell apoptosis, and miR-186-5p regulated the inflammatory response of COPD by targeting *HIF-1α*. These findings provide novel insights for further investigation of the pathogenesis of COPD and may eventually contribute to novel treatments for COPD.

Materials and methods

Cell culture and establishment of the COPD inflammation model. Human bronchial epithelial cells (BEAS-2B), purchased from Procell Life Science & Technology Co., Ltd., were incubated in DMEM (HyClone; Cytiva) with 10% FBS (HyClone; Cytiva), 100 IU/ml penicillin (Gibco; Thermo Fisher Scientific, Inc.), and 100 μg/ml streptomycin (Gibco; Thermo Fisher Scientific, Inc.). The cells were cultured in an incubator (Likang Biomedical Technology Co., Ltd.) at 37˚C, with 5% CO_2. BEAS-2B cells were induced with LPS (MilliporeSigma) (0, 1, 2, 5, 10, 20, or 40 mg/l) for 24 h to establish an *in vitro* inflammation model of COPD, and cells treated with 0.1% DMSO were used as a control. The cell inhibition rate and half maximal inhibitory concentration (IC_{50}) of LPS were detected and calculated using a Cell Counting Kit 8 (CCK-8) assay to evaluate the COPD inflammation model. Moreover, ELISA was used to detect the expression levels of inflammatory factors *IL-6* and *TNF-α* in the supernatant of cells.

Cell transfection. The miR-186-5p mimics, miR-186-5p inhibitor, and corresponding negative control (NCs) were synthesized by Suzhou Jima Gene Co. Ltd. Cells were plated into a 6-well plate and allowed to adhere. When the cell confluency reached 70-80%, Lipofectamine® 2000 (Procell Life Science & Technology Co., Ltd.) was used for transfection according to the manufacturer's protocol. The sequences of the miR-186-5p mimics, negative control of mimics (mimics-NC), miR-186-5p inhibitor and negative control of inhibitor (inhibitor-NC) are as follows: miR-186-5p mimics, 5'-CAAAGAAUUCUCCUUUUG GGCU-3'; mimics-NC, 5'-CGAUCGCAUCAGCAUCGA UUGC-3'; miR-186-5p inhibitor, 5'-AGCCCAAAAGGA GAAUUCUUUG-3'; and inhibitor-NC: 5'-CAGUACUUU UGUGUAGUACAA-3'.

Reverse transcription-quantitative PCR (RT-qPCR). RT-qPCR was used to measure the expression of miR-186-5p, *HIF-1α*, *IL-6*, and *TNF-α*. Total RNA was extracted from cells using TRIzol® according to the manufacturer's instructions. RNA was reverse transcribed into cDNA using a PrimeScript RT kit (Takara Bio, Inc.) according to the manufacturer's protocol. qPCR was performed using a SYBR Premix Ex Taq II kit (Takara Bio, Inc.). The expression levels of related genes were detected on the ABI 7500 (Applied Biosystems; Thermo Fisher Scientific, Inc.). Relative quantitative values were calculated using the $2^{-\Delta\Delta Cq}$ method (27). The relative expression was normalized to that of U6 or GAPDH. The primers used in this study were provided by Beijing Aogukang Biotechnology Co., Ltd. The sequences of the PCR primers were: *HIF-1α* forward, 5'-GCCTCTGTGATGAGGCTTACC-3' and reverse, 5'-CAG TGCAATACCTTCCATGTTGC-3'; *IL-6* forward, 5'-CTC CTTCTCCACAAGCGCC-3' and reverse, 5'-GATGCCGTC GAGGATGTACC-3'; *TNF-α* forward, 5'-TGTAGCCCATGT TGTAGCAAACC-3' and reverse, 5'-TGAGGTACAGGCCCT CTGAT; miR-186-5p forward, 5'-CGCCAAAGAATTCTC CTTTTGGGCT-3' and reverse, 5'-AGCCCAAAAGGAG AATTCTTTGGCG-3'; and U6 forward, 5'-TGGAACGCT TCACGAATTTGCG-3' and reverse, 5'-GGAACGATACAG AGAAGATTAGC-3'; GAPDH forward, 5'-ATCACTGCC ACCCAGAAGAC3' and reverse, 5'-TTTCTAGACGGCAGG TCAGG-3'.

CCK-8 assay. Cell proliferation was evaluated using a CCK-8 assay (Biyuntian Co., Ltd.) according to the manufacturer's protocol. A total of $5x10^3$ cells per well were resuspended and seeded in a 96-well plate and incubated for 24 h. After culture for 24, 48, 72, and 96 h, respectively, 10 μl CCK-8 solution was added to each well and incubated for 4 h in the humidified incubator. An Epoch microplate reader was used to detect the absorbance at 450 nm (BioTek Instruments, Inc.).

Cell apoptosis assay. Cell apoptosis was detected using a Muse Annexin V & Dead Cell Kit (MilliporeSigma). Stably transfected cells and empty vector-transfected control cells were collected. Cells were washed in PBS and resuspended in media supplemented with 1% FBS. Next, 100 μl Muse Annexin V & Dead Cell Reagent was added to the cell suspension, gently mixed, and incubated in the dark for 20 min at room temperature. The percentage of apoptotic cells was determined using the Muse Cell Analyzer (Luminex Corp), according to the manufacturer's instructions.

Cell cycle assay. Stably transfected cells and empty vector-transfected control cells were collected. The adherent cells were digested with pancreatin, and the cells were re-suspended in DMEM. Next, cells were centrifuged at 2,000 x g for 5 min, and the supernatant was discarded. The cells were washed twice with PBS for 5 min and fixed with pre-cooled 70% ethanol at 4˚C overnight. The following day, cells were washed with PBS twice, and then incubated with propidium iodide (PI) in the dark for 30 min. PI staining was detected using a flow cytometer (Gallios, Beckman Coulter, Inc.) and analyzed using FlowJo (version 10.7.1; FlowJo LLC).

Western blotting. Total proteins from the cells were extracted using RIPA lysis buffer (CWBIO), and the protein concentrations were measured using a BCA Protein Assay Kit (Biyuntian Co., Ltd.). Protein samples were separated using a 10% SDS gel by SDS-PAGE and transferred to PVDF membranes. Subsequently, the membranes were blocked using 5% non-fat milk and incubated with primary antibodies against HIF-1α (1:500; Wanleibio Co., Ltd.; cat. no. WL01607), p-p65 (1:1,000; Affinity Bioscience; cat. no. AF2006), p65 (1:1,000; ProteinTech Group, Inc.; cat. no. 66535-1-1g), and β-actin (1:5,000; ProteinTech Group, Inc.; cat. no. 66009-1-lg) overnight at 4˚C. Subsequently, membranes were incubated with a goat anti-rabbit horseradish peroxidase-conjugated IgG secondary antibody (1:10,000; Zen Bio; cat. no. 511203) for 1 h at room temperature. Signals were visualized using enhanced chemiluminescence.

Cytokine quantification. ELISA kits (cat nos. JL10208 and JL14113; Shanghai Jianglai Biotechnology Co., Ltd.) were used to measure the levels of TNF-α and IL-6 in the supernatant of LPS-induced BEAS-2B cell culture medium, according to the manufacturer's protocol.

Dual-luciferase reporter gene assay. The potential target of miR-186-5p and *HIF-1α* was predicted using TargetScan (http://www.targetscan.org). A dual-luciferase reporter assay was performed to confirm the predicted interactions. The region that contained the miR-186-5p binding site on *HIF-1α* was inserted into the luciferase pGL3 reporter vector. This was followed by co-transfection with luciferase plasmids and with miR-186b-5p mimics, inhibitor, mimics-NC, or inhibitor-NC using Lipofectamine® 2000 (Thermo Fisher Scientific, Inc.), to confirm binding between miR-186b-5p and *HIF-1α*. After 48 h, the luciferase activity was measured using a Dual-Luciferase Reporter Assay System (Promega Corporation).

Statistical analysis. All statistical tests were performed using GraphPad Prism version 8.0 (GraphPad Software Inc.). Data are presented as the mean ± SD of three repeats. A Student's t-test (unpaired) was used to compare differences between two groups. A one-way ANOVA was used to evaluate the statistical significance between multiple groups, and a Tukey's Honey Significant Difference post hoc test was used to identify which specific groups exhibited significant differences. A two-sided P<0.05 was considered to indicate a statistically significant difference.

Table I. Proliferation inhibition rate of BEAS-2B cells following treatment with different concentrations of LPS for 24 h

Group	Inhibitory rate of proliferation (%, Mean ± SD)
0.1% DMSO	1.55±0.76
LPS, mg/l	
1	0.84±0.08
2	2.58±1.25
5	5.87±2.14[a]
10	19.03±2.36[b]
20	44.06±6.43[b]
40	73.29±4.77[b]

[a]P<0.05, [b]P<0.01. SD, standard deviation; LPS, lipopolysaccharide.

Results

COPD inflammation model. To establish an *in vitro* model of COPD, BEAS-2B cells were exposed to increasing concentrations of LPS to determine the optimum concentration. The results showed that exposure to varying concentrations of LPS, namely 5 mg/l (5.87%), 10 mg/l (19.03%), 20 mg/l (44.06%), and 40 mg/l (73.29%), resulted in significant inhibition of cell proliferation when compared to the control group treated with DMSO (P<0.05, Table I). The IC_{50} value for LPS was 22.65±3.03 mg/l (Fig. 1A, P<0.05). LPS treatment resulted in a significant concentration-dependent decrease in the number of viable cells starting at a concentration of 10 mg/l with an inhibition rate of 19% (P<0.01). To verify whether 10 mg/l LPS induced cellular inflammation, the expression levels of inflammatory factors IL-6 and TNF-α in the supernatant of cells were determined using ELISA. The results indicated that the expression of IL-6 (Fig. 1B) and TNF-α (Fig. 1B) in the LPS-induced BEAS-2B cells were significantly higher than that in the control group (P<0.05). Therefore, in the subsequent experiments, cells were pretreated with 10 mg/l LPS for 24 h.

Transfection efficacy. To evaluate the impact of miRNA-186-5p on COPD progression, miRNA-186-5p mimic or miRNA-186-5p inhibitor were transfected into the LPS-induced COPD cells. The transfection efficacies of miRNA-186-5p mimic and miRNA-186-5p inhibitor were examined in LPS-induced BEAS-2B cells (Fig. 2). The results showed that transfection of miRNA-186-5p mimic significantly increased the expression of miRNA-186-5p in LPS-induced BEAS-2B cells, while transfection of miRNA-186-5p inhibitor significantly inhibited the levels of miRNA-186-5p in LPS-induced BEAS-2B cells (P<0.001).

Inhibition of miR-186-5p reduces proliferation and induces apoptosis in LPS-induced BEAS-2B cells. To investigate the effect of miR-186-5p on the proliferative activity of LPS-induced BEAS-2B cells, CCK-8 assays were performed (Fig. 3). The results showed that the miRNA-186-5p inhibitor significantly decreased the viability of LPS-induced BEAS-2B

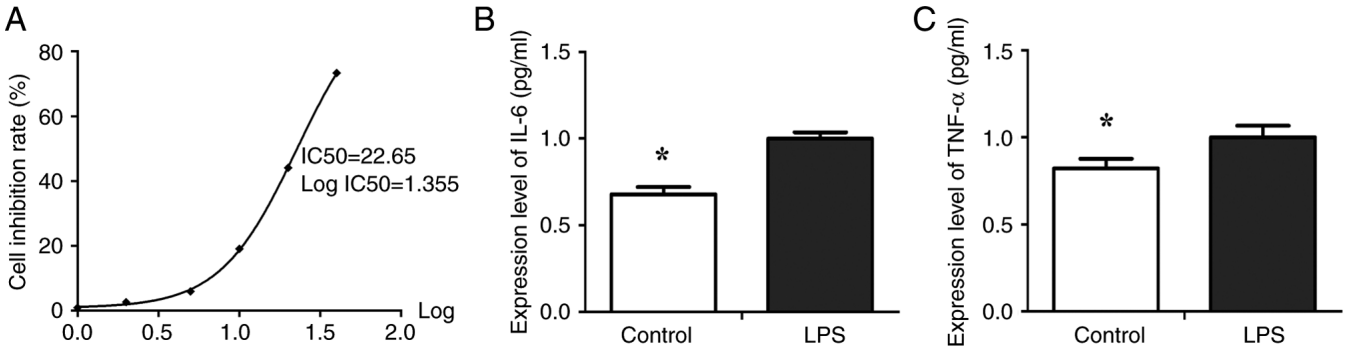

Figure 1. LPS-induced BEAS-2B cells were used to establish a COPD inflammatory model *in vitro*. (A) The inhibition rate curve of proliferation of BEAS-2B cells treated with different concentrations of LPS for 24 h were analyzed using CCK-8 assays. (B and C) The expression of IL-6 and TNF-α in the supernatants of BEAS-2B cells treated with 10 mg/l LPS were determined using ELISA kits. Data are presented as the mean ± SD of three repeats. *P<0.05. LPS, lipopolysaccharide; Control, no LPS treatment.

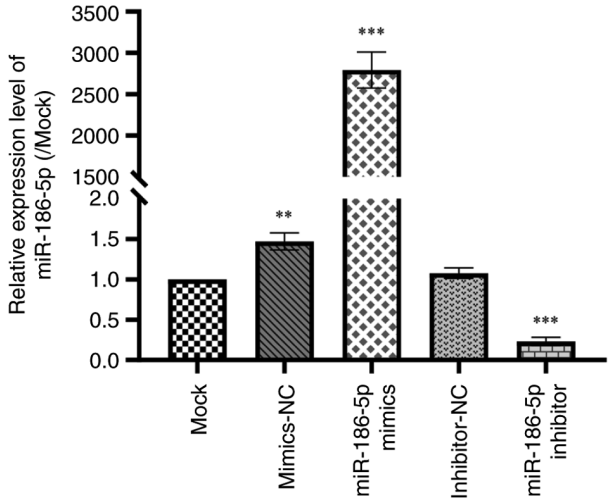

Figure 2. Expression of miR-186-5p in BEAS-2B cells treated with 10 mg/l LPS were detected. Data are presented as the mean ± SD of three repeats. **P<0.01, ***P<0.001. LPS, lipopolysaccharide; Mock, LPS + Lipofectaime® 2000; NC, negative control.

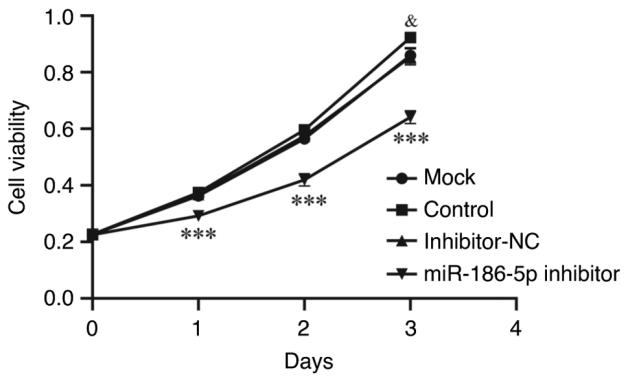

Figure 3. Cell viability analysis following transfection with miR-186-5p in BEAS-2B cells treatted with 10 mg/l. Data are presented as the mean ± SD of three repeats. ***P<0.001 miR-186-5p inhibitor vs. mock, $^{\&}$P<0.05 Inhibitor-NC vs. control. LPS, lipopolysaccharide; NC, negative control; Mock: LPS+Lip2000; miR, microRNA; NC, negative control.

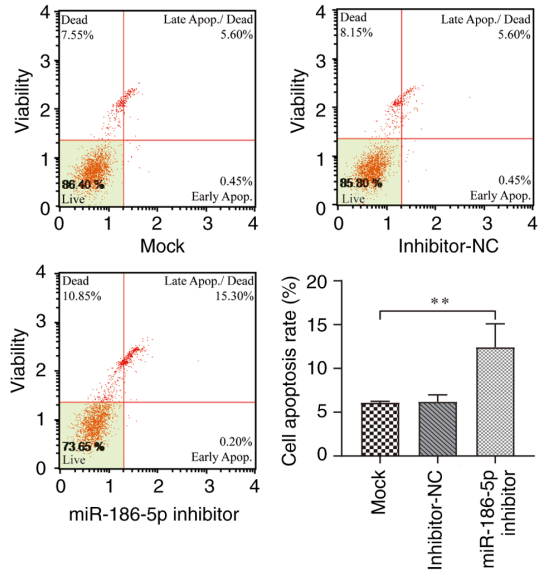

Figure 4. Effect of miR-186-5p on the apoptosis of BEAS-2B cells treated with 10 mg/l LPS was detected using a Muse flow cytometry assay. Data are presented as the mean ± SD of three repeats. **P<0.01. LPS, lipopolysaccharide; Mock, LPS + Lipofectaime® 2000; miR, microRNA; NC, negative control; Apop, apoptosis.

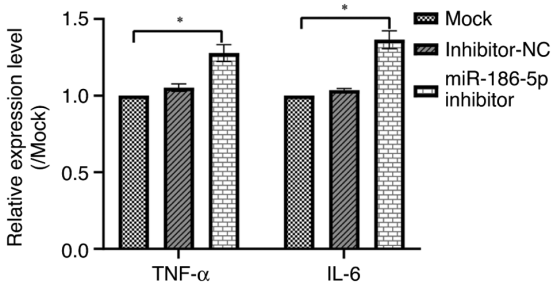

Figure 5. mRNA expression levels of TNF-α and IL-6 in BEAS-2B cells treated with 10 mg/l LPS. Data are presented as the mean ± SD of three repeats. *P<0.05. LPS, lipopolysaccharide; Mock, LPS + Lipofectaime® 2000. miR, microRNA; NC, negative control.

cells between days 0 and 3 compared with the mock group (P<0.001). Apoptosis of LPS-induced BEAS-2B cells was detected by flow cytometry after transfection for 48 h (Fig. 4). The findings revealed that the apoptotic rate of LPS-induced

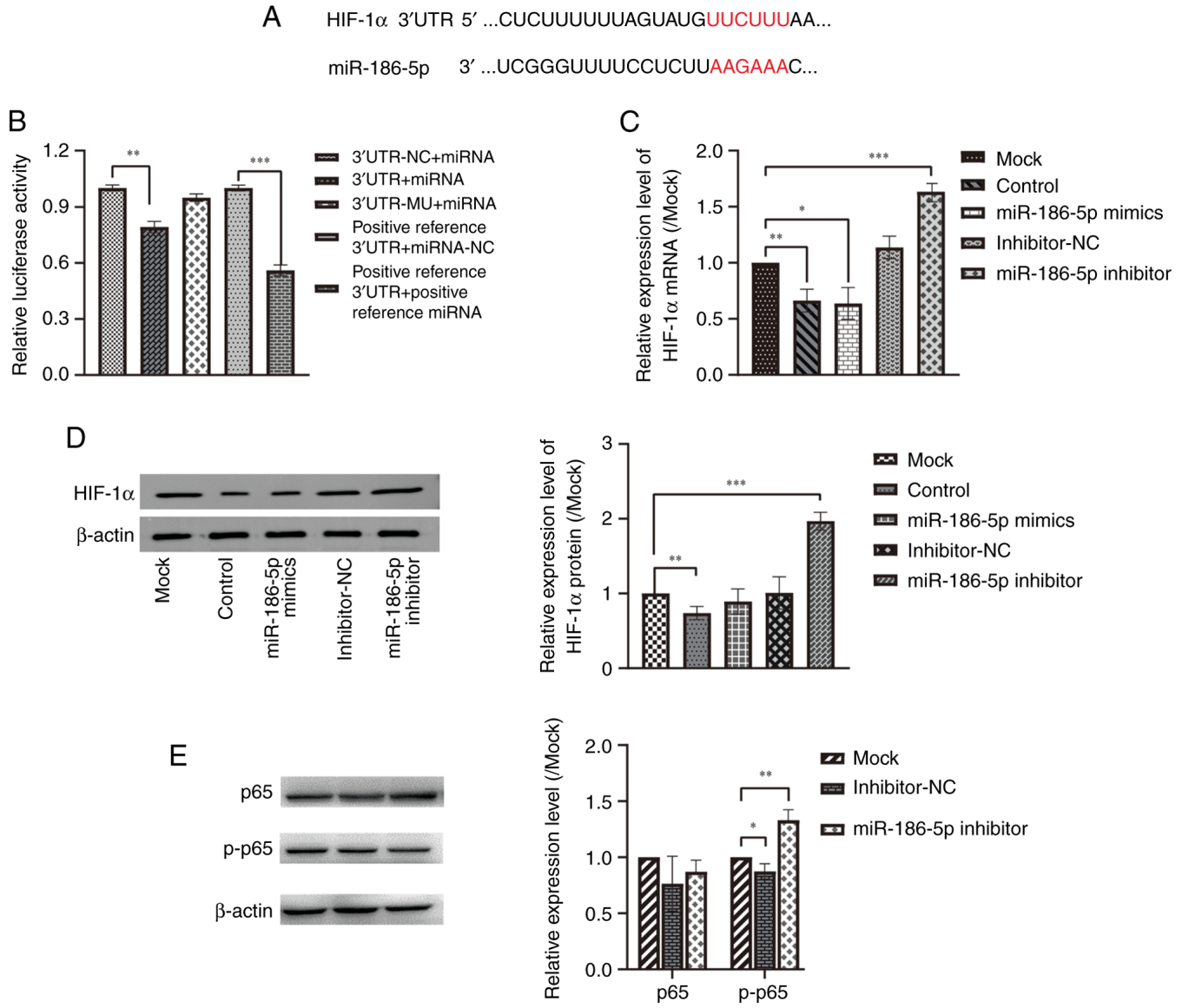

Figure 6. Effect of miR-186-5p on the expression of HIF-1α, inflammatory cytokines, and p-p65. (A) Predicted miR-186-5p binding sites on HIF-1α 3'UTR. The binding sites are labeled in red letters. (B) Dual-luciferase reporter assays demonstrated that miR-186-5p bound to HIF-1α. (C) mRNA and (D) protein expression levels of HIF-1α in BEAS-2B cells treated with 10 mg/l LPS. (E) p65 and p-p65 protein expression in BEAS-2B cells treated with 10 mg/l LPS after transfection with miR-486-5p inhibitor. *P<0.05, **P<0.01, ***P<0.001. Data are presented as the mean ± SD. Mock, LPS + Lip2000; Control, without LPS; LPS, lipopolysaccharide; miR, microRNA; UTR, untranslated region; NC, negative control.

BEAS-2B cells in the miR-186-5p inhibitor group was significantly higher than that in the mock group (P<0.01). Additionally, cell cycle distribution was also analyzed. The results of cell cycle distribution indicated no significant difference between the mock and miR-186-5p inhibitor groups in the G0/G1, S, and G2/M phase, respectively (Fig. S1, P>0.05). These findings suggest that inhibition of miR-186-5p decreased the proliferation of and induced apoptosis in LPS-induced BEAS-2B cells but had no effect on the cell cycle.

Role of miR-186-5p in inflammation. To further evaluate the inflammatory effects of miR-186-5p, the levels of inflammatory cytokines (*TNF-α* and *IL-6*) in LPS-induced BEAS-2B cells. The results indicated that miR-186-5p inhibitor significantly increased the levels of *TNF-α* and *IL-6* compared with that in the control group (Fig. 5, P<0.05).

miR-186-5p targets and regulates HIF-1α. Predictions from the TargetScan database (http://www.targetscan.org) showed that miR-186-5p bound to the 3'-UTR of *HIF-1α* (Fig. 6A). Dual-luciferase reporter gene assays showed that the relative luciferase activity of the 3' UTR + miRNA group was significantly reduced compared with the 3' UTR-NC + miRNA group (Fig. 6B, P<0.01), indicating that *HIF-1α* was the downstream target of miRNA-186-5p. Moreover, RT-qPCR and western blotting further confirmed that miR-186-5p could regulate the expression of *HIF-1α*. The results revealed that the relative expression levels of *HIF-1α* mRNA were significantly lower in the control (LPS-induced COPD model) group (P<0.01) and miR-186-5p mimics group (P<0.05), while the relative expression levels of *HIF-1α* mRNA were significantly increased in the miR-186-5p inhibitor group (P<0.001) compared with the

mock group (Fig. 6C). Western blotting results showed that the relative expression levels of HIF-1α were significantly decreased in the control group (P<0.01), while the relative expression levels of HIF-1α were significantly increased in the miR-186-5p inhibitor group (P<0.001) compared with the mock group (Fig. 6D). These findings demonstrate that miR-186-5p could target and regulate the expression of *HIF-1α*.

To investigate whether the downregulation of miR-186-5p affected the NF-κB inflammatory pathway, the protein expression levels of p-65, a crucial factor in the NF-κB pathway, and its activated form, p-p65, were determined. The results indicated that the miR-186-5p inhibitor group exhibited a significant increase in the relative expression levels of p-p65 protein (P<0.05) compared to the mock group, while the relative expression levels of p65 protein remained unchanged (Fig. 6E). These results suggest that the downregulation of miR-186-5p may enhance the expression of p-p65.

Discussion

COPD is a globally recognized and prevalent disease, and although advances in clinical treatment have seen notable progress, the pathogenesis of COPD remains poorly understood. In this study, the potential role of miR-186-5p in COPD inflammation was investigated by inducing BEAS-2B cells with LPS to establish an *in vitro* COPD model. The results suggested that miR-186-5p regulated the inflammatory response of lung epithelial cells through targeted interactions with *HIF-1α* in COPD. It was also shown that interfering with miR-186-5p was associated with reduced LPS-induced BEAS-2B proliferation and enhanced LPS-induced BEAS-2B apoptosis.

miR-186-5p has been shown to play an important role in various diseases. Recent studies have suggested that miR-186 can regulate cancer cell growth, proliferation, migration, apoptosis, and other processes, and is associated with a variety of physiological and pathological processes (28,29). In our previous study, it was found that the expression of miR-186-5p was upregulated in COPD compared to the control group (19). Li *et al* (22) found, using bioinformatics analysis, that miR-186-5p may be involved in regulating COPD dysfunction blocks. The results of the present study found that downregulation of miR-186-5p inhibited the proliferation of LPS-induced BEAS-2B cells, promoted apoptosis in these cells, and significantly increased the levels of inflammatory factors (*TNF-α* and *IL-6*). However, further research is needed to confirm the impact of miR-186-5p on the inflammatory response in COPD. For example, in future studies, western blotting will be used to detect the expression of proliferation-related genes and determine whether the use of apoptosis inhibitors and necrosis inhibitors will rescue cell death promoted by miR-186-5p knockdown.

Furthermore, the findings of the present study suggested that miR-186-5p could target and regulate the expression of *HIF-1α* in COPD. Our previous study showed that miRNA-186 was associated with the expression of *HIF-1α* in COPD (30). *HIF-1α* serves as a key regulator of cellular oxygen homeostasis during the development of inflammation and various disorders. It activates a wide range of genes involved in multiple processes, including glycolysis, angiogenesis, proliferation, migration, autophagy, and apoptosis, amongst other processes (31). Several studies have investigated the relationship between *HIF-1α* and COPD and found that *HIF-1α* expression is increased in COPD patients, resulting in upregulated expression of inflammatory factors, which is associated with disease severity (23,24,32). Furthermore, the *HIF-1α* signaling pathway has been shown to be an important signaling pathway that drives COPD progression to lung cancer (33). The present study investigated the role of miR-186-5p in regulating *HIF-1α* expression and its impact on COPD inflammation. However, the direct effect of *HIF-1α* on COPD inflammation was not confirmed. In addition, HIF inhibitors were not used to ascertain whether the inflammatory response induced by miR-186-5p could be rescued. Therefore, in future studies, it is necessary to investigate the direct impact of *HIF-1α* on COPD inflammation and observe whether the inflammatory response induced by miR-186-5p can be suppressed using HIF inhibitors.

IL-6 can trigger a number of pro-inflammatory cytokines and chemokines, and has been reported to exhibit extensive crosstalk with NF-κB at multiple mechanistic levels to regulate immune processes, as well as promote the development of COPD by activating the NF-κB signaling pathway (34). The NF-κB signaling pathway is a vital pro-inflammatory pathway that regulates the levels of inflammatory factors in COPD patients' bronchial epithelial cells (25,35). In the present study, the results showed that miR-186-5p interference increased *HIF-1α* expression whilst also upregulating the production of pro-inflammatory cytokines *TNF-α* and *IL-6* and considerably increasing the phosphorylation of p65, a key regulator of the NF-κB signaling pathway. Thus, the findings indicate that miR-186-5p inhibits *HIF-1α*, which in turn contributes to the inflammatory response in COPD. The underlying mechanism may be associated with the NF-κB signaling pathway in epithelial cells. However, this study also has the limitation of using a single cell line to construct the COPD model. Therefore, further studies using multiple cell lines are needed to validate the findings and assess the generalizability of the results.

In conclusion, the results of the present study suggested that miR-186-5p may regulate the inflammatory response of COPD by targeting *HIF-1α* through regulating NF-κB signaling, which could potentially impact the development and progression of COPD. This discovery provides novel insights for the treatment of COPD, and future research should further investigate the underlying mechanism of the interaction between miR-186-5p and *HIF-1α*, as well as the specific role of this interaction in COPD inflammation.

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Availability of data and materials

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

Authors' contributions

YF, JZ and JC wrote the original draft of the manuscript, and created figures, tables and visual representations of the data. YZ, RM, BZ and LZ analyzed and interpreted the data. YF, JZ, JC, YD and TX conceived and designed the study. QL, CH, SL and LL performed the experiments. YF, YD and TX confirm the authenticity of all 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 have declared that they have no conflict of interest.

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