Fibroblast growth factor 18 alleviates hyperoxia-induced lung injury in mice by adjusting oxidative stress and inflammation


Department of Critical Care Medicine, People's Hospital of Rizhao, Rizhao, China

Abstract. – OBJECTIVE: Bronchopulmonary dysplasia (BPD) is one of the most common chronic lung diseases in infants, but the ways to prevent and treat BPD are still very limited. We tried to find an effective method for treating BPD by studying the effect of fibroblast growth factor 18 (FGF18) on hyperoxia-induced lung injury in mice.

MATERIALS AND METHODS: We placed newborn mice in high-oxygen environment (60-70%) and collected mouse lung tissue for histological examination at 3, 7, 14 and 21 days after birth. The correlation between FGF18 and BPD was studied by analyzing the expression of FGF18 in mouse lung tissue. In addition, we used exogenous FGF18 to stimulate primary mouse type II alveolar epithelial cells (AECs II), and detected changes in oxidative stress, inflammation and NF-κB signaling pathway activity of AECs II to analyze the effects of FGF18 on AECs II.

RESULTS: From the 7th day after the birth of the mouse, the lung tissue of the hyperoxia-induced mice suffered significant lung injury relative to the control group. The expression of FGF18 in lung tissue induced by hyperoxia was lower than that in the control group. Cell viability of AECs II stimulated by exogenous FGF18 increased, and FGF18 also reduced oxidative stress and inflammation levels of AECs II and inhibited the AECs II injury caused by hyperoxia. NF-κB signaling pathway activity in hyperoxia-induced lung increased, while exogenous FGF18 could reduce the expression and phosphorylation of NF-κB p65 in AECs II.

CONCLUSIONS: Hyperoxia-induced lung injury was accompanied by a decrease in FGF18. FGF18 can reduce oxidative stress and inflammation levels of AECs II by inhibiting the NF-κB signaling pathway, thereby reducing hyperoxia-induced cell injury.

Key Words: Fibroblast growth factor 18, Hyperoxia, Bronchopulmonary dysplasia, Oxidative stress, Inflammation.

Introduction

Bronchopulmonary dysplasia (BPD) is a common disease in preterm infants and the most common complication in very preterm infants. The Northway team first described BPD as a chronic lung disease in preterm infants in 1967. Their described preterm infants had a history of mechanical ventilation with high pressure and high oxygen concentration. The BPD histological phenotype is characterized by pulmonary parenchymal fibrosis, inflammation, and smooth muscle hypertrophy, which can lead to diffuse airway injury. However, the progression and aggravation of BPD can lead to alveolar dysplasia, alveolar sepal thickening, abnormal pulmonary microvascular network, mild airway and vascular smooth muscle hypertrophy, accumulation of interstitial fluid, abnormal deposition of extracellular matrix components, and pulmonary dysplasia. In recent years, with the development of medicine, the survival rate of very preterm infants has improved significantly, but it has not changed the higher incidence of BPD. Long-term complications of BPD include changes in lung function, increased susceptibility to infection, increased incidence of inflammatory lung disease, and airway hyperresponsiveness. In addition, approximately 40% of children with BPD develop pulmonary hypertension. This progressive increase in pulmonary arterial pressure can cause right heart hypertrophy and even death. In addition to respiratory sequelae, children with BPD may also develop neurological sequelae.

However, the ways to prevent and treat BPD are still very limited. The current treatments to reduce the symptoms of BPD are mechanical ventilation and drug therapy. Drug therapies include caffeine, diuretics, bronchodilators, hormones,
vitamin A, anti-oxidants, and inhaled NO\textsuperscript{8}. However, these drugs are limited to moderately active substances, mainly to relieve symptoms, so the treatment is not satisfactory.

Fibroblast growth factor 18 (FGF18) was first isolated from rat embryos by Ohbayashi et al\textsuperscript{9}. They found that FGF18 is a unique secreted signaling molecule in the adult lung and developing tissues. FGF18 exists in many cell types, and its function is not limited to bone development. FGF18 is a pleiotropic growth factor that stimulates the proliferation of numerous mesenchymal and epithelial cells, including lungs, kidneys, heart, testes, spleen, skeletal muscle and brain\textsuperscript{10}. FGF18 was significantly expressed in embryos and neonatal lungs. McGowan and McCoy\textsuperscript{11} have suggested that FGF18 plays an important role in the development of pulmonary alveoli in the late stages of embryonic lung development. In early lung development, FGF18 signaling is mainly mediated by FGFR2b and FGFR2c. The abnormal expression of FGF18 results in functional and structural abnormalities in multiple organs\textsuperscript{12}. Ohbayashi et al\textsuperscript{13} has found that mice with FGF18 gene knockout (-/-) developed rib deformities, leading to a decrease in chest volume and respiratory failure. Usui et al\textsuperscript{14} studied the lungs of FGF18 -/- mice and found that the lung size of FGF18 -/- mice was slightly smaller than that of normal mice. FGF18 -/- mice showed a decrease in the alveolar space, the occurrence of interstitial compartments, the insertion of capillaries and a decrease in cell proliferation, but the expression of marker genes in lung epithelium was not affected. Therefore, we hypothesized that FGF18 plays an important role in the occurrence and development of neonatal lung disease. We made the mouse BPD model and detected the expression of FGF18 in mouse lung tissue, then we verified effect of FGF18 on alveolar epithelial cells in vitro. Through this study, we hoped to provide new targets and theoretical foundations for the clinical treatment of BPD in infants.

**Materials and Methods**

**Animals and BPD Model**

A total of 80 C57/BL6 newborn mice were used in this study. All mice were housed in People’s Hospital of Rizhao Animal Center. We divided newborn mice equally into a hyperoxia group and a control group. The mice in the hyperoxia group were placed in a special closed breeding box and the oxygen concentration in the box was maintained at 60-70%. Mice in the control group were routinely reared. We took 6 mice from each group at 3, 7, 14 and 21 days after birth and collected lung tissue for subsequent experiments. This investigation was approved by the Animal Ethics Committee of People’s Hospital of Rizhao Animal Center.

**Histology and Hematoxylin-Eosin (HE) Staining**

We soaked fresh mouse lung tissue in 4% paraformaldehyde for more than 24 hours. Then, we put the lung tissue into different concentrations of gradient alcohol for dehydration and then put them into xylene alcohol and xylene solution in order. Then, we put the lung tissue in the xylene paraffin solution and paraffin solution to make paraffin blocks. We used a microtome (LEICA RM2235, Köln, Germany) to make paraffin blocks into 5 μm sections. Paraffin sections were then baked in a 37°C incubator for 3 days.

Prior to HE staining, we dried the sections by baking them in a 55°C incubator for 1 hour. Then, we put the sections in xylene and gradient alcohol for dewaxing and hydration. After washing the sections in running water for 3 minutes, we stained the cell nucleus in the hematoxylin staining solution (Beyotime, Shanghai, China) for 1 minute. We, then, rinsed the sections with running water for 3 minutes and used 1% hydrochloric acid alcohol for differentiation for 3 seconds. If the blue is too dark, we will continue to use 1% hydrochloric acid alcohol for differentiation; if the blue is too light, we will restain the nucleus. We, then, stained the cytoplasm with eosin staining solution (Beyotime, Shanghai, China) for 1 minute. Finally, we put the sections in gradient alcohol and xylene in turn and mounted the slides with neutral gum. A high power optical microscope (LEICA, Köln, Germany) was used to observe the staining results.

**Immunohistochemical (IHC) Staining**

After the sections were baked in a 55°C incubator for 1 hour and dewaxed, we washed the sections in running water for 3 minutes. We then heated the sections to 95°C in citrate buffer for 15 minutes. After the citrate buffer naturally cooled to room temperature, we took the sections and washed the sections with phosphate-buffered saline (PBS). 3% H\textsubscript{2}O\textsubscript{2} is used to repair antigens on lung tissue. We then blocked the non-specific antigen using 10% goat serum.
After 1 hour, we used primary antibody dilutions (FGF18. ab71794; p65, ab16502, Abcam, Cambridge, MA, USA) to incubate the lung tissue at 4°C overnight. After washing the sections with PBS, we incubated the lung tissue with secondary antibody dilutions (GeneTech, Shanghai, China) for 2 hours at room temperature. We then washed the sections with PBS and developed color using diaminobenzidine (DAB). Finally, we stained the nuclei with hematoxylin and observed the staining results with a high power optical microscope (LEICA, Köln, Germany).

**Isolation and Culture of Primary Mouse Type II Alveolar Epithelial Cells (AECs II)**

After collecting mouse lung tissue, we cut the lung tissue into about 1 mm³ size using sterile scissors. We then rinsed the lung tissue fragments using D-Hank solution. Then, we put the lung tissue fragments into 0.25% dispase (containing 0.01 g/L DNase and 5% fetal bovine serum (FBS)). After the lung tissue fragments were digested in a refrigerator at 4°C overnight, we put the lung tissue fragments in a 37°C incubator and continued to incubate for 2-4 hours. After the lung tissue fragments are digested into cell suspension, we blow cells to make them fall off. We then filtered the cell suspension using a sieve and centrifuged it. We purified AECs II by density gradient centrifugation and cultured AECs II using Dulbecco's Modified Eagle Medium/F12 medium (Gibco, Rockville, MD, USA) containing 15% FBS (Gibco, Rockville, MD, USA). AECs II were cultured in a 37°C incubator containing 5% CO₂, and we changed the cell culture medium every 3 days. Recombinant mouse FGF18 (100 ng/mL) (Abcam, Cambridge, MA, USA) was used to treat AECs II 10.

**Cell Counting Kit 8 (CCK8) Assay**

We seeded AECs II in 96-well plates and treated the cells differently according to experimental needs. Each well contains approximately 5000 cells and 100 μL of culture medium. After 3, 7 and 10 days, we removed 96-well plates and added 10 μL of CCK8 reagent (Dojindo Molecular Technologies, Kumamoto, Japan) to each well. We then placed the 96-well plate in the 37°C incubator for another 2 hours. Finally, we used a microplate reader (Molecular Devices, Santa Clara Valley, CA, USA) to detect the absorbance (OD) of each well of a 96-well plate at a wavelength of 450 nm. We set up a blank group and a control group. The blank group had only medium and no cells. The control group had media and cells but was not treated. Cell viability = (OD sample - OD blank) / (OD control - OD blank).

**Protein Isolation and Western Blot**

We discarded the culture medium of the six-well plate and washed the cells with pre-cooling PBS. We then added radioimmunoprecipitation assay (RIPA) lysate (Beyotime, Shanghai, China) containing a protease inhibitor to each well. After full lysis, we transferred the protein lysate to an Eppendorf (EP) tube for centrifugation (12000 rpm, 15 minutes, 4°C). We, then, collected the supernatant and used the bicinchoninic acid (BCA) kit (Beyotime, Shanghai, China) to detect the protein concentration. We added an appropriate amount of 5 × loading buffer to the EP tube and heated it with a dry thermostat for 20 minutes. The extracted protein was stored in a refrigerator at -80°C.

We configured 10% of the gel and placed it in the electrophoresis tank. After adding the electrophoresis solution, we gently pull out the electrophoresis comb and add an equal amount of protein. After constant voltage electrophoresis (80 V) and constant current (0.28 A) transfer, we transferred the protein to polyvinylidene difluoride (PVDF) membrane (Roche, Basel, Switzerland). We then blocked non-specific antigens using 5% skim milk. After incubating the PVDF membrane at 4°C overnight with primary antibody dilution (p65, ab16502; p-p65, ab97726, Abcam, Cambridge, MA, USA), we washed the PVDF membrane with PBS-tween (PBST). We, then, incubated the PVDF membrane with secondary antibody dilution (Abcam, Cambridge, MA, USA) for 2 hours at room temperature. After washing the PVDF membrane with PBST, we used developer for color development.

**RNA Isolation and Quantitative Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR)**

We used liquid nitrogen to grind mouse lung tissue into powder, and then added it to EP tubes containing 1 mL of TRizol reagent (Invitrogen, Carlsbad, CA, USA). After mixing, we add 200 μL of chloroform to the EP tube and shake it for 15 seconds. After centrifugation (12000 rpm, 15 minutes, 4°C), we collected 400 μL of the upper layer of colorless liquid and transferred it to a new EP tube. Then, we added 400 μL of isopropanol to the EP tube and mixed them. After centrifuga-
tion (12000 rpm, 10 minutes, 4°C), we collected the RNA sediment and washed the RNA with 75% ethanol and centrifuged again (8000 rpm, 5 minutes, 4°C) to collect the RNA sediment. After the sediment has dried, we add 50 μL of diethyl pyrocarbonate (DEPC) water (Beyotime, Shanghai, China) to each EP tube to dissolve the RNA. After measuring the RNA concentration using a spectrophotometer, we stored the RNA solution in a -80°C refrigerator.

The HiScript II Q RT SuperMix for qPCR kit (Vazyme, Nanjing, China) was used for reverse transcription. We added 1 μg of template RNA, 4 μL of 5 × HiScript II Q RT SuperMix and RNase-free ddH₂O to 20 μL to each well of a 96-well plate. The obtained complementary deoxyribose nucleic acid (cDNA) was stored in a refrigerator at -20°C. We, then, used the SYBR Green Master Mix kit (Vazyme, Nanjing, China) to perform qPCR according to the manufacturer’s instructions. The primer sequences were as follow: FGF18 sense sequences, GTGCTTCCAGGTTCAGGT; FGF18 anti-sense sequences, GCTGCTTCCGACTCACA; SP-C sense sequences, TGTAGGGGCTCTGCTCAT; SP-C anti-sense sequences, GTCTGCTCGCTCACTCG; p65 sense sequences, CCAGGGTGTGTCCATGTCT; p65 anti-sense sequences, GTGTGGGAGCTGGTTCA; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) sense sequences, GGATGCTGCCCTTACCC; GAPDH anti-sense sequences, GTTCACACCGACCTTCACC. The expression of endogenous GAPDH was used as a control. 2^−ΔΔCT is used to represent the relative expression of mRNA.

Flow Cytometry
Flow cytometry was used to detect the reactive oxygen species (ROS) level in AECs II. After using trypsin to digest the cells, we collected the cells and prepared single-cell suspension. We added 5 μL of 2,7-dichlorofluorescent yellow diacetate (DCFH-DA) (Beyotime, Shanghai, China) to 1 ml of single-cell suspension and incubated at 37°C in the dark for 30 minutes. Then, we removed the supernatant by centrifugation (1500 rpm, 5 minutes, 4°C) and added 10% FBS for incubation at 37°C for 20 minutes. After centrifugation again (1500 rpm, 5 minutes, 4°C) to remove the supernatant, we added pre-cooling PBS and detected the fluorescence intensity by flow cytometry within 1 hour.

Enzyme-Linked Immunosorbent Assay (ELISA)
We collected the cell culture fluid and collected the supernatant by centrifugation (3000 rpm, 20 minutes, 4°C). We then diluted the standards in the ELISA kits (R&D Systems, Emeryville, CA, USA) to different concentrations and added them to the plate. We then added the diluted samples to the microtiter plate for incubation at 37°C for 30 minutes. Then, we removed the liquid in the microplate and washed it 5 times with the washing solution. We added 50 μL of enzyme-labeled reagent to each well except for the blank wells. After incubation and washing, we add developer to each well and incubate it for 15 minutes in the dark. After adding the stop solution, we used a microplate reader (Molecular Devices, Santa Clara Valley, MD, USA) to detect the OD of each well at a wavelength of 450 nm. The concentration of the sample was calculated according to the OD and standard curve.

Statistical Analysis
We used Statistical Product and Service Solutions (SPSS) 21.0 statistical software (IBM, Armonk, NY, USA) for analysis and GraphPad Prism 7.0 software (La Jolla, CA, USA) to draw the bar chart. All measurement data were represented as mean ± SD (standard deviation). Differences between two groups were analyzed by using the Student’s t-test. Comparison between multiple groups was done using One-way ANOVA test followed by Post-Hoc Test (Least
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Significant Difference). \( p < 0.05 \) indicated the significant difference. All experiments were repeated 3 times.

**Results**

*FGF18 Expression Increased in Lung Tissue of Hyperoxia-Induced Mice*

We first determined whether the mouse BPD model was successfully made by HE staining. We collected mouse lung tissue after 3, 7, 14 and 21 days of modeling, and our HE staining results showed that the lungs of the mice did not change significantly after 3 days. After 7, 14 and 21 days, typical BPD-like changes (thickening of alveolar septum, inflammatory cell infiltration, and bleeding) were seen in the lung tissue of hyperoxia-induced mice (Figure 1A). IHC staining was then used to detect differences in FGF18 expression. The expression of FGF18 in the lung tissue of mice in the control group did not change significantly with time, while the expression of FGF18 in the lung tissue of mice in the hyperoxia group decreased with time (Figure 1B). RT-PCR also detected FGF18 mRNA expression in mouse lung tissue and FGF mRNA expression was also reduced in hyperoxia-induced mice (Figure 1C).

![Figure 1](image-url) **Figure 1.** FGF18 expression increased in lung tissue of hyperoxia-induced mice. A, HE staining of mice in control group and hyperoxia group (magnification: 200×); B, FGF18 expression in mice lung tissue of control group and hyperoxia group was determined by IHC staining (magnification: 200×); C, mRNA expression of FGF18 in mice lung tissue. (** means the difference is statistically significant, \( p < 0.05 \).)
Exogenous FGF18 Reduces
Hyperoxia-Induced Injury of
Mouse AECs II

Mice were divided into control group and hyperoxia group. After a week of modeling, we extracted AECs II from mice in control group and hyperoxia group. Then, we culture AECs II for 10 days with recombinant mouse FGF18. AECs II were divided into a control group, a FGF18 group, a hyperoxia group and a hyperoxia + FGF18 group. We detected the proliferation ability of AECs II by CCK8 assay after 3, 7 and 10 days of culturing AECs II. CCK8 assay found that at different time points, the proliferation ability of AECs II in the hyperoxia group was reduced, while FGF18 could significantly increase cell viability (Figure 2A). In addition, we observed changes in ROS levels in AECs II by flow cytometry. Compared with the control group, the ROS level of the AECs II in the hyperoxia group increased, and FGF18 could inhibit the pro-oxidation induced by hyperoxia (Figure 2B). SP-C is an important marker of alveolar epithelial cell injury. We detected the expression of SP-C in AECs II after 7 days by IF staining. The expression of SP-C in AECs II in the hyperoxia group was significantly increased, while the SP-C in AECs II in the FGF18 group was lower than that in the control group. At both the base level and the hyperoxia level, FGF18 can reduce the expression of SP-C in AECs II (Figure 2C). RT-PCR detected SP-C mRNA expression and the results were similar to those obtained by IF staining (Figure 2D).

Exogenous FGF18 Reduces
Hyperoxia-Induced Inflammation
of Mouse AECs II

We detected the expression of inflammatory factors in the culture medium of AECs II by ELISA. The results showed that at different time points, hyperoxia induction increased the expression of interleukin (IL)-1β (Figure 3A), IL-6 (Figure 3B), IL-8 (Figure 3C), tumor necrosis factor (TNF)-α (Figure 3D), Macrophage inflammatory protein-2 (MIP-2) (Figure 3E) and transforming growth fac-

Figure 2. Exogenous FGF18 reduces hyperoxia-induced injury of mouse AECs II. A, AECs II viability was determined by CCK8 assay; B, ROS level in AECs II was determined by flow cytometry; C, protein expression of SP-C in AECs II after 7 days were determined by IF staining (magnification: 200×); D, mRNA expression of SP-C in AECs II were determined by RT-PCR. (**” means the difference is statistically significant, p<0.05).
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Exogenous FGF18 reduces hyperoxia-induced inflammation of mouse AECs II. IL-1β (A), IL-6 (B), IL-8 (C), TNF-α (D), MIP-2 (E) and TGF-β (F) in culture medium of AECs II were detected by ELISA. (*“*” means the difference is statistically significant, $p<0.05$).

**Figure 3.** Exogenous FGF18 reduces hyperoxia-induced inflammation of mouse AECs II. IL-1β (A), IL-6 (B), IL-8 (C), TNF-α (D), MIP-2 (E) and TGF-β (F) in culture medium of AECs II were detected by ELISA. (*“*” means the difference is statistically significant, $p<0.05$).

Exogenous FGF18 Inhibits NF-κB Signaling Pathway Activity in Mouse AECs II

We first detected the expression of p65 in lung tissue of normal and hyperoxia-induced mice by IHC staining. With the extension of modeling time, the expression of p65 in the hyperoxia group increased gradually and was higher than that in the control group (Figure 4A). We also indicated the protein and mRNA expression of p65 in AECs II after 7 days by IF staining (Figure 4B) and RT-PCR (Figure 4C). The expression of p65 in AECs II stimulated by FGF18 decreased significantly. The results of Western blot showed that FGF18 reduced p65 phosphorylation level (Figure 4D, 4E).

**Discussion**

BPD is a kind of chronic lung disease, which is related to premature birth and is a clinically high incidence of lung disease. BPD is the most common complication of preterm birth, and the prevalence of BPD in preterm infants less than
32 weeks is 12.3% to 30.0%\(^5\). Respiratory and neurological sequelae often occur in infants with BPD. BPD models are mainly made by hyperoxia induction method, mechanical ventilation induction method, bleomycin intrapulmonary injection method, hyperoxia/hypoxia induction method, hypoxia induction method, endotoxin induction method and transgenic method\(^16\). High oxygen exposure is an important factor in the occurrence and development of classic BPD and new BPD. Therefore, the hyperoxia induction method is still the most common method for making BPD models\(^7\). We kept newborn mice under high oxygen environment and detected the morphological changes of mouse lung tissue by HE staining. The pathological changes of the lung tissue of

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**Figure 4.** Exogenous FGF18 inhibits NF-KB signaling pathway activity in mouse ACEs II. A, p65 expression in mice lung tissue of control group and hyperoxia group was determined by IHC staining (magnification: 200×); B, protein expression of p65 in AECs II after 7 days were determined by IF staining (magnification: 200×); C, mRNA expression of p65 in AECs II were determined by RT-PCR; D, p65 and p-p65 protein expression in AECs II were determined by Western blot, E, phosphorylation level of p65. ("*" means the difference is statistically significant, \(p<0.05\)).
the hyperoxia group mice proved that the BPD model was successfully made in mice. In addition, with the aggravation of lung injury in mice, the expression of FGF18 protein and mRNA in lung tissue gradually decreased, suggesting that lung injury caused by BPD may be related to the decrease of FGF18. Therefore, we extracted mouse primary AECs II and used recombinant mouse FGF18 to stimulate cells to detect the effect of FGF18 on proliferation, oxidative stress and damage marker SP-C of AECs II. Our study found that FGF18 reduced the oxidative stress level and SP-C of AECs II and increased the activity of AECs II. This indicated that FGF18 improved the injury of AECs II well. In addition, under the action of FGF18, the expression of inflammatory factors in the culture fluid of AECs II was also greatly reduced. With the extension of hyperoxia-induced time, the activity of NF-kB signaling pathway in the lung tissue of mice also gradually increased, indicating that the NF-kB signaling pathway is involved in the pathogenesis of BPD. However, the expression and phosphorylation level of p65 in FGF18-treated AECs II were significantly reduced, indicating that FGF18 inhibited the activity of NF-kB signaling pathway in AECs II induced by hyperoxia. Therefore, FGF18 has a good application prospect for the treatment of BPD.

The pathogenesis of BPD-induced lung injury involves many factors and is very complicated. Among them, oxidative stress and inflammatory response are the two main pathogenic factors of BPD. When oxidative damage occurs, the anti-oxidation-reduction system in tissue cells begins to function. The system develops its anti-oxidant capacity through the synthesis of anti-oxidants such as reduced glutathione (GSH) and superoxide dismutase (SOD) in the body and the intake of corresponding anti-oxidants to protect tissues from injury caused by oxidative stress. When tissue cells are damaged by oxidative stress, a large amount of ROS will be produced, including $O_2^-$ and $H_2O_2$, etc., and $H_2O_2$ is the main substance in the oxidative stress process. When stimulated by ROS, the body regulates cells to produce transcription factors that resist oxidative stress damage and activates a variety of downstream protective genes including major anti-oxidant protein genes γ-glutamylcysteine synthetase (γ-GCS) and SOD genes. γ-GCS is the rate-limiting enzyme for reduced GSH synthesis in vivo. Therefore, an increase in γ-GCS content and biological activity can lead to increased GSH synthesis, which enhances the ability of the body's tissue and cells to resist oxidative stress. GSH and SOD can prevent the formation of ROS, and they play an important role in improving tissue oxidation and avoiding toxic damage. Endesfelder et al. showed that caffeine has anti-oxidant effects and relieves hyperoxia-induced lung injury in rats. Mai et al. also found that asiaticoside as an anti-oxidant can reduce hyperoxia-induced lung injury in rat lung tissues, thereby alleviating lung injury in BPD rats. Therefore, the anti-oxidative and anti-apoptotic effects of FGF18 on AECs II can serve as a strong theoretical basis for its treatment of BPD.

The inflammatory response plays an important role in hyperoxia-induced lung injury. Under the conditions of hyperoxia exposure, the expression of inflammatory and anti-inflammatory factors in the lung tissue changes and loses its balance. NF-kB is a key early transcription factor that mediates the inflammatory response in the inflammatory pathway. NF-kB is widely present in various cytoplasms and is a type of homodimer or heterodimer composed of 5 functional subunits of its family proteins. The most common combination in the human body is a heterodimer composed of its functional subunits p65 (RelA) and p50 (NF-κB1), which combined with NF-kB inhibitory protein (1-κB) and formed trimers. After the cells are extracellularly stimulated by infection, edema, oxidation, and antigens, the inhibitory protein 1-κB can be phosphorylated and degraded, leading to the activation of NF-Kb. Our study found that FGF18 can effectively inhibit the expression and phosphorylation of p65, leading to a decrease in the activity of the NF-kB signaling pathway. This may be a key mechanism for FGF18 to protect AECs II. FGF-18 is particularly important for the stability of lung tissue structure and function. However, it is not clear whether FGF-18 is involved in the occurrence and regulation of BPD. This is the first study to investigate the effect of FGF-18 on BPD, and we hope our work would provide new targets for the clinical treatment of BPD.

**Conclusions**

In summary, this is the first study to discover the therapeutic effect of FGF18 on hyperoxia-induced BPD. We hope that the results of our research can provide new targets and theoretical basis for the clinical treatment of BPD.
Conflict of Interest
The Authors declare that they have no conflict of interests.

References