# TGF - B RECEPTOR INHIBITORS AFFECT THE PROLIFERATION OF CHRONIC OBSTRUCTIVE PULMONARY FIBROBLASTS BY REGULATING THE EXPRESSION OF GROWTH FACTORS

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***ABSTRACT***

***Objective****: To analyze the effect of TGF - β receptor inhibitors on the proliferation of COPD fibroblasts by regulating the expression of growth factors.*

***Methods:*** *Twenty-four healthy male SD rats were randomly divided into a normal group, a model group, and a TGF - β receptor inhibitor group, with 8 rats in each group. Every group except the normal one used the cigarette smoke inhalation method to establish the rat COPD model. After the model was established successfully, the TGF - β receptor inhibitor was dissolved in 5ml of normal saline and given to the rats in the TGF - β receptor inhibitor group for atomization inhalation treatment. The normal group rats in the model group and the model group were given the same volume of saline atomization inhalation. Changes in lung tissue morphology, TGF - β 1, and bFGF were observed, and the proliferation and apoptosis of fibroblasts were compared.*

***Results:*** *Compared with the model group, the bronchociliary structure of the TGF - β receptor inhibitor group was complete, and the infiltration of inflammatory cells in the alveoli was slightly relieved. The level of TGF - β 1 and bFGF in the model group's lung tissue was significantly higher than that of the normal group (P<0.05), and the level of TGF - β 1 and bFGF in the TGF - β receptor inhibitor group's lung tissue was lower than that of the model group (P<0.05). The proliferation activity of fibroblasts in the model group was significantly higher than that of the normal group (P<0.05). The proliferation activity of fibroblasts in the TGF - β receptor inhibitor group was significantly lower than that of the model group (P<0.05). The apoptosis index of fibroblasts in the model group was significantly higher than that of the normal group (P<0.05). The apoptosis index of fibroblasts in the TGF - β receptor inhibitor group was significantly higher than that of the model group (P<0.05).*

***Conclusion:*** *TGF - β receptor inhibitors can significantly inhibit the proliferation and promote the apoptosis of fibroblasts in COPD rats. The mechanism may be related to the regulation of TGF - β 1, bFGF, and other growth factors.*

***Keywords:*** *TGF - β receptor inhibitor, growth factor, COPD, fibroblast, proliferation.*

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## Introduction

Chronic obstructive pulmonary disease (COPD) is a form of chronic bronchitis and emphysema with the characteristics of airflow obstruction. The disease often develops progressively, which is related to the abnormal inflammatory reaction of harmful gases and particles. The patients usually show symptoms such as chronic cough, expectoration, shortness of breath, or dyspnea, which can develop into cor pulmonale and respiratory failure(1). The morbidity and mortality rates of chronic obstructive pulmonary disease are high. The incidence rate of the disease is 9%~10%. The epidemiological study shows that

in the coming years, chronic obstructive pulmonary disease will rank as the third deadliest disease globally[2]. The main pathological basis of COPD is chronic inflammation and structural destruction of airway wall and lung parenchyma, which leads to lumen stenosis and airflow obstruction. Airflow obstruction is often the reason for excessive deposition of the extracellular matrix in a patient's airway wall. Therefore, understanding the pathological characteristics of airflow obstruction and its occurrence matrix is a prerequisite for effective prevention and treatment[3]. Transforming Growth Factor β 1 (TGF - β 1) is a kind of fibrogenic cytokine that exists in all tissues, especially bone, lung, kidney, and placenta. In recent years, TGF - β has played an increasingly important role in the reconstruction of COPD, and it is often expressed in acute or chronic lung diseases and promotes the proliferation of fibrocytes and the synthesis of fibrin[4].

Some scholars have found that TGF - β 1 can promote the proliferation of stromal cells by inhibiting epithelial cells and endothelial cells[5], but the effect on COPD fibroblasts is still unclear. Thus, this study aims to analyze the effect of TGF - β receptor inhibitors on COPD fibroblast proliferation by regulating the expression of growth factors.

## Materials and methods

### *Experimental animal*

All animal experiments should strictly abide by the ethical regulations related to experimental animals. Twenty-four healthy male SD rats, aged 3 months and weighing 180 to 220g, were selected and purchased from the experimental animal center of the Institute of Radiology, Chinese Academy of Sciences (license No.: SCXK Jin 2017-0002). The rats were fed in an air-conditioned room with a temperature of 21oC to 25oC and humidity of 50% to 60%. The rats were kept in a 12-hour circadian rhythm and given water and food for one week.

### *Experimental reagents and instruments*

TGF - β receptor inhibitors were purchased from the Selleck company in the United States. Fetal bovine serum was purchased from Sijiqing biological company in Hangzhou. Trypsin was purchased from the GIBCO company in the United States. Chloral hydrate was purchased from Sinopharm Group Chemical Co., Ltd. TGF - β 1, bFGF ELISA kits were purchased from Nanjing Xinfan Biotechnology Co., Ltd. Tetrazolium salt (MTT) was purchased from sigma Co., Ltd. Hematoxylin and Yihong were purchased from Beijing zhongshanjinqiao Biotechnology Co., Ltd.

The inverted fiber microscope was purchased from Olympus, Japan. The centrifuge was purchased from Shanghai Anting Scientific Instrument Factory. Pipette and enzyme labeling instruments were purchased from Eppendorf Ebender China Co., Ltd. The –80oC ultra-low temperature refrigerator and 4oC refrigerator were purchased from Qingdao Haier Co., Ltd. The flow cytometer was purchased from Shanghai Jingke Industrial Co., Ltd. The ultraclean workbench was purchased from Nuair in the United States. Different types of surgical knife handles, medical needles and threads, eye scissors, and other hand tools were purchased from Shanghai Jiading Medical Equipment Factory.

### *Establishment and grouping of the COPD model in rats*

The COPD rat model was established using cigarette smoke inhalation as a means of testing. The change of airflow obstruction and bronchitis was caused by this inhalation of cigarette smoke. Twenty-four rats were randomly divided into a normal group, a model group, and a TGF - β receptor inhibitor group, with 8 rats in each group. With the exception of the normal group, the rat groups were passive smoking in the self-made smoke box. Eight black Camellia cigarettes were ignited each time, making the concentration in the smoke box around 6%. This procedure was carried out for 30 min. at a time, twice a day, 4 hrs. each time, for a total of 45 days. The rats in the model group and TGF - β receptor inhibitor group were given lipopolysaccharide (3mg/kg) on the first and 15th day after smoking, and the rats in the normal group were given normal saline of the same volume. On the 45th day, the lung function indexes were examined, and the lung compliance and airway resistance were calculated. Modeling criteria was as follows.

Lung compliance was less than 3.5ml/cm$H_2O$, airway resistance was more than 6.0cm$H_2O$/ml/s, and rats had symptoms of cough and expectoration. After the successful establishment of the model, the TGF - β receptor inhibitor was dissolved in 5ml of normal saline and given aerosol inhalation treatment to rats in the TGF - β receptor inhibitor group, 20 min. at a time, twice a day. Each treatment kept the oxygen flow at 5-9ml /min, and rats in the normal group and the model group were given aerosol inhalation equal to the volume of normal saline.

***Observation index***

• The HE staining method was used to observe the morphological changes of lung tissue in each group. After two weeks of successful modeling and drug treatment, the rats were killed by the femoral artery bleeding method.

Then the right lung tissue was taken, fixed with 10% formaldehyde, and then HE staining was performed by a pathological section machine after being embedded with paraffin.

The staining results were analyzed by two pathologists with a double-blind method, inverted microscope, and pathological image analyzer.

• After the rats were killed, 200g of the lower left lung tissue was taken and added to the centrifuge tube containing PBS solution. The homogenizer was used for 8000r/min homogenization for 5 min. The homogenizer was used for 2000r/min centrifugation for 10 min. The supernatant was mixed with the cell lysate and centrifuged for 5 min at 8000r/min. The supernatant was used for enzyme-linked immunosorbent in the levels of TGF - β 1, and basic fibroblast growth factor (bFGF) in lung tissue of the rats in each group was detected by assay and ELISA.

• MTT method was used to detect the proliferation of fibroblasts in each group.

• The apoptosis of fibroblasts was detected by flow cytometry.

***Statistical methods***

All the measurement data of this study are expressed by (x±s), the mean comparison between the two groups is tested by independent sample t, the mean comparison between multiple groups is analyzed by ANOVA, and P<0.05 is regarded as statistically significant. The data in this study are analyzed by the SPSS 20.0 software package.

## Results

***Comparison of lung histomorphology in each group***

The results of HE staining in the normal group showed no obvious abnormality in the airway wall, alveolar structure, or alveolar wall. The morphological structure of bronchial cilia was complete and arranged in an orderly fashion, and there was no infiltration of inflammatory cells in evidence.

In the model group, the ciliary structure of the bronchus was disordered, and a large portion of the bronchus fell off. Compared with the model group, the bronchociliary structure of the TGF - β receptor inhibitor group was complete, and the infiltration degree of inflammatory cells in the alveoli was slightly relieved. See Figure 1.

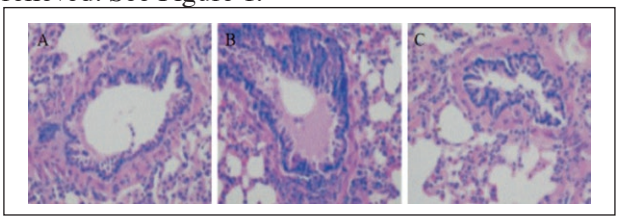


**Figure 1:** Comparison of HE staining results of lung histomorphology in each group: (A) normal group (B) model group (C) TGF - β receptor inhibitor group.

***Comparison of the levels of TGF - β 1 and bFGF in the lung tissue of rats in each group***

The level of TGF - β 1 and bFGF in the lung tissue of the model group was significantly higher than that of the normal group (P<0.05). The level of TGF - β 1 and bFGF in the lung tissue of the TGF - β receptor inhibitor group was lower than that of the model group (P<0.05). See Table 1.

| Group | Cases | TGF-β1 (μg/L) | bFGF (pg/ml) |
|---|---|---|---|
| Normal group | 8 | 2.32±0.12 | 5.18±0.80 |
| Model group | 8 | 7.31±0.23* | 8.35±0.82* |
| TGF-β receptor inhibitor group | 8 | 4.56±1.28# | 6.39±0.54# |

**Table 1:** Comparison of TGF - β 1 and bFGF levels in lung tissue of rats in each group ($\bar{x}$±s).
*Note: Compared with the normal group *P<0.05; Compared with the model group #P<0.05.*

***Comparison of proliferation activity of fibroblasts in each group***

The proliferation of fibroblasts in the model group was significantly higher than that in the normal group (P<0.05). The proliferation activity of fibroblasts in TGF - β receptor inhibitor group was significantly lower than that of the model group (P<0.05). See Table 2.

| Group | Cases | 24h | 48h | 72h |
|---|---|---|---|---|
| Normal group | 8 | 0.26±0.04 | 0.25±0.06 | 0.12±0.01 |
| Model group | 8 | 0.31±0.04* | 0.35±0.02* | 0.19±0.02* |
| TGF-β receptor inhibitor group | 8 | 0.23±0.03# | 0.28±0.02# | 0.14±0.01# |

**Table 2:** Comparison of fibroblast proliferation in each group ($\bar{x}$±s).
*Note: Compared with the normal group *P<0.05; Compared with the model group #P<0.05.*

***Comparison of fibroblast apoptosis in each group***

Flow cytometry showed that the apoptosis index of fibroblasts in the model group was significantly higher than that of the normal group (P<0.05).

The apoptosis index of fibroblasts in the TGF - β receptor inhibitor group was significantly higher than that of the model group (P<0.05). See Table 3.

| Group | Cases | 24h | 48h | 72h |
|---|---|---|---|---|
| Normal group | 8 | 1.02±0.4 | 1.02±0.4 | 1.02±0.4 |
| Model group | 8 | 4.32±2.75* | 5.41±2.25* | 7.36±2.31* |
| TGF-β receptor inhibitor group | 8 | 17.25±4.12# | 19.63±4.52# | 21.36±4.02# |

**Table 3:** Comparison of fibroblast apoptosis in each group ($\bar{x}\pm s$).
*Note: Compared with the normal group *P<0.05; Compared with the model group #P<0.05.*

## Discussion

Chronic obstructive pulmonary disease (COPD) is characterized by incomplete reversible airflow limitation, which is related to the abnormal inflammatory reaction of the lung to harmful gases and particles found in, for example, cigarette smoke and smog. The pathogenesis is still not fully understood. Its high incidence and mortality rate contribute to huge economic and social problems worldwide[6]. Relevant data show that the cellular and humoral immune functions of COPD patients are significantly lower than that of normal people, so immunotherapy may be an effective way to impede the progress of COPD and improve the lives of those who suffer from it[7]. TGF - β 1 is an important fibrogenic cytokine and most closely related to pulmonary fibrosis. TGF - β 1 is mainly derived from airway epithelial cells and platelets in the lung and plays an important role in regulating cell growth, differentiation, and a variety of physiological and pathological processes, mainly in promoting the proliferation of smooth muscle cells and extracellular matrix deposition[8-9].

In patients with COPD, inhalation of irritant gas and other factors may cause airway inflammation, leading to airway epithelial damage, which activates a multitude of inflammatory cells that accumulate in the airway wall and lung tissue.

Macrophages synthesize TGF - β 1 through autocrine and paracrine, which can react on macrophages, expressing inflammatory mediators and amplifying inflammatory response. Therefore, TGF - β 1 in airways and alveoli during inflammation, the expression in epithelial cells, vascular smooth muscle cells, and lung tissue increased significantly[10-11]. In recent years, scholarly research on TGF - β has shifted in focus to cell growth, differentiation, and immune regulation. Some scholars have found that activation of TGF - β to promote the proliferation of fibroblasts is an important factor in the lead up to pulmonary fibrosis[12]. In addition, the TGF - β 1 signal also plays an important role in lung development and in the process of injury repair and remodeling. At present, TGF - β 1 is considered to be a strong chemokine of fibroblasts, which can promote the expression of related growth factors[13].

Based on the role of TGF - β 1 in pulmonary fibroblasts, this study used TGF - β receptor inhibitors to treat rats with COPD. The results showed that the structure of bronchi cilia in the model group was disordered, a large portion of cilia fell off, and numerous inflammatory cells infiltrated the alveoli and developed into pulmonary emphysema. The rats in the TGF - β receptor inhibitor group were atomized with TGF - β receptor inhibitors for two weeks. Compared with the model group, the hair structure was complete, and the infiltration degree of inflammatory cells in the alveoli was slightly alleviated, which suggests that the TGF - β receptor inhibitor could effectively improve the lung structure and inflammatory response of COPD rats. Further analysis of the TGF - β receptor inhibitor's effects on the proliferation and apoptosis of rat fibroblasts showed that the proliferation activity of fibroblasts in the TGF - β receptor inhibitor group was significantly lower than that of the model group, and the apoptosis index was significantly higher than that of the model group (P<0.05). Further analysis of the mechanism of action showed that the levels of TGF - β 1 and bFGF in the lung tissue of the TGF - β receptor inhibitor group were lower than those of the model group (P<0.05). BFGF is a multifunctional cell growth factor, and its primary role is to promote cell division, so it has attracted attention for its function of promoting the repair of damaged tissue and vascular production[14]. Relevant data show that bFGF is involved in airway remodeling of asthma and shows the effects of chemotaxis, cell migration, and apoptosis inhibition[15].

In conclusion, TGF - β receptor inhibitors can significantly inhibit the proliferation of fibroblasts while promoting apoptosis in COPD rats. The mechanism may be related to the regulation of TGF - β 1, bFGF and other growth factors.

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