EFFECT OF TRANSFORMING GROWTH FACTOR-B 1 ON ECM EXPRESSION AND CELL BEHAVIOR OF AIRWAY SMOOTH MUSCLE CELLS IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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ABSTRACT

Objective: *To analyze the effect of transforming growth factor-β1 (TGF-β1) on the expression of extracellular matrix (ECM) and cell behavior of airway smooth muscle cells in patients with chronic obstructive pulmonary disease (COPD).*

Methods: *Airway smooth muscle cells isolated from COPD patients were cultured in vitro and treated with TGF-β1(10ng/ml). The expression levels of chemokine-1/8 (CXCL-1/8), interleukin-6 (IL-6), matrix metalloproteinase-10, 12 (MMP-10, 12), fibronectin and collagen-8 α 1 (col8 α 1) were detected by ELISA after TGF-β1 stimulated airway smooth muscle cells; the effects of TGF-β1 on the adhesion and repair function of airway smooth muscle cells were observed.*

Results: *There was no significant difference in the expression of cxcl-1 between the two groups (P>0.05). The expression of CXCL-8 and IL-6 in the TGF-β1 group was significantly higher than that in the control group (P<0.05). The expression of MMP-3 and MMP-10 in TGF-β1 group was significantly higher than that in control group (P<0.05). The expression of fibronectin and col8 α 1 protein in airway smooth muscle cells in TGF-β1 group was significantly higher than that in control group (P<0.05). Compared with the control group, TGF-β1 group significantly enhanced the adhesion and repair ability of airway smooth muscle cells (P<0.05).*

Conclusion: *TGF-β1 can significantly increase the expression of extracellular matrix protein in COPD patients, which has a tendency to improve the adhesion of airway smooth muscle cells and wound repair ability of COPD patients, which provides reference for clinical treatment of COPD.*

Keywords: *Transforming growth factor -β1, chronic obstructive pulmonary disease, airway smooth muscle cells, extracellular matrix, cellular behavior.*

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Introduction

Chronic obstructive pulmonary disease (COPD) has a high incidence and great harm. The incidence of Chronic obstructive pulmonary disease (COPD) is about 8.2% in people over 40 years old in China, ranking the fourth cause of death in the world. Seriously harm people's health and increase social and economic burden[(1)]. COPD is a preventable and treatable disease characterized by airflow restriction, which develops progressively and is associated with abnormal inflammatory responses to harmful gases and particles. The clinical manifestations of COPD patients are chronic cough, expectoration, shortness of breath and dyspnea, which can be further developed into pulmonary heart disease and respiratory failure[(2)]. Currently, the pathogenesis of COPD is not clear, and it is generally believed to be related to chronic inflammation of the airway and lung, protease-related genes, oxidative stress-related genes, etc.[(3)]. Airway inflammation and airway remodeling are the pathological basis of airflow restriction in COPD patients. In recent years, it has been found that Transforming growth

factor-β1 (TGF-β1) is a group of oligodimer polypeptide growth factors, which are derived from airway epithelial cells and platelets in the lung. It is increased in COPD, asthma, pulmonary fibrosis and other acute or chronic pulmonary diseases[4].

Some scholars have found that TGF-β1 can promote the proliferation and migration of airway smooth muscle cells and the synthesis of Extracellular matrix (ECM)[5]. This study aimed to analyze the effects of TGF-β1 on THE EXPRESSION of EMC and cell behavior in airway smooth muscle of COPD patients.

Materials and methods

Experimental reagents and instruments

DMEM culture medium and phosphate buffer (Wuhan Yipu Biotechnology Co., LTD.); Fetal bovine serum (Wuhan Punosai Life Technology Co., LTD.); Hepes buffer (Shanghai Zhenyu Biotechnology Co., LTD.); Pancreatic protein Digestive Enzyme (Emmetall Technology Co., LTD.); PCR detection box (Guangzhou Jianlun Biotechnology Co., LTD.); TGF-β1 (PeproTech China); Enzyme conjugate agent (Shenyang Wanshi Biotechnology Co., LTD.); TMB color solution (Huzhou Yingchuang Biotechnology Co., LTD.); Elisa Kit (Guangzhou Jianlun Biotechnology Co., LTD.); DAB Chromogenic agent (Shanghai Rongweida Industrial Co., LTD.).

Ultra-clean workbench (Beijing Jiayuan Xingye Technology Co., LTD.); Inverted microscope (Beijing Jinda Sunshine Technology Co., LTD.); Carbon dioxide cell incubator (Shanghai Xinyu Biotechnology Co., LTD.); Ultra-pure water system (Beijing Zeping Technology Co., LTD.); PH meter (Shanghai Fuze Trading Co., LTD.); Centrifuge (Nanjing Beiden Medical Co., LTD.); Low temperature refrigerator (Haier Group of China); Electronic balance (Beijing Jiayuan Xingye Technology Co., LTD.); Enzyme-linked immunoassay (Wuhan Famous Instrument Co., LTD.); Microscope imaging system (Shanghai Fuze Trading Co., LTD.).

Culture of airway smooth muscle cells

Primary airway smooth muscle cells were anatomically isolated from COPD patients with grade 2-6 airway smooth muscle tissue. The experiment used well-grown airway smooth muscle cells. All the smooth muscle cells used in the experiment were between the 2nd and 7th generations, and mycoplasma was detected. The airway smooth muscle cells were inoculated on 96-well culture plates with 1×10^4 cells per square centimeter and cultured in cell growth medium. The cells were digested by trypsin when the flask was 70~80% full, and then cultured at 37°C and $5CO_2$ for 72h.

Airway smooth muscle cells were stimulated with TGF-β1 10ng/ml and cultured at 37°C and $5CO_2$ for 72h. Extracellular matrix sediments in 96-well plates were collected, and the treated 96-well plates were labeled and stored in a refrigerator at -20°C for future use.

Detection method

• Expressions of chemokines-1/8 (CXCL-1/8) and interleukin-6 (IL-6) in airway smooth muscle cells stimulated by TGF-β1 were detected by ELISA method: Cxcl-1/8 and IL-6 primary antibodies were fixed on a 96-well plate, 100 μL of primary antibody was added to each well, and the antibody was incubated overnight at 4°C, and then the excess antibodies were washed with 0.05% Tween-20/PSB buffer. Add 300 μL 1% bovine serum protein to each well and shake it on a shaker at low speed for 1h. Standard curves of CXCL-1/8 and IL-6 were configured in the resting culture medium. 100 μL of standard was added into each well and the standard was shaken at low speed for 2h on the shaking table. Excl-1/8 and IL-6 secondary antibody 100 μL were added to each well and shaken at low speed for 1h on the shaking table. Add 100 μL of enzyme-conjugate working solution to each well, shake it on the shaking table at low speed for 30min, and then add TMB chromogenic agent for chromogenic reaction.

The chromogenic reaction can be terminated when the chromogenic curve of the standard product shows gradient changes. The absorbance wavelength of 450nm and 570nm is set by enzyme-linked immunoassay to read the absorbance value of the sample.

• The expression of Matrix metalloproteinases (MMP-10, 12) after TGF-β1 stimulated airway smooth muscle cells was detected by ELISA, using the same method as (1).

• The extracellular matrix components fibronectin and Collagen protein (COL8α1) of airway smooth muscle cells stimulated by TGF-β1 were determined by ELISA.

• Cell adhesion test: Add 100 μL cell growth medium to the 96-well plate and incubate at 37°C and $5CO_2$ for 72h. Discard the culture medium and add 100 μL resting medium to the 96-well plate and

incubate at 37°C and 5CO_2 for 24h. Add TGF-β1 10ng/mL resting medium to the 96-well plate. Culture at 37°C and 5CO_2 for 72h.

Toluidine blue, paraformaldehyde, dodecyl sulphate working solution were configured, and paraformaldehyde, toluidine blue, and ammonium dodecyl sulphate working solution were successively added to the 96-well plate. The 96-well plate was placed in the enzyme linked immunodetection, and the wavelength was set at 595nm to read the absorbance value of the sample.

• Wound repair test: Add cell growth medium to the black well plate provided in the Oris cell migration kit, and culture at 37°C and 5CO_2 for 72h. Discard the medium and add 100 μL cell resting medium, continue to culture for 24h. Add TGF-β1 10ng/ mL resting medium to the 96-well plate for 72h. Install the mask provided with the Oris Cell Migration Kit on the bottom of the black 96-well plate and place the silicone plug into each hole of the 96-well plate, which is immediately adjacent to the bottom of the hole and aligned with the baffle light leakage. The airway smooth muscle cells were mixed with the green fluorescent probe, and incubated at 37°C and 5CO_2 for 30min without light.

The 96-well plate was placed in the ELISA, and the wavelength was set at 485nm to read the absorbance value of the sample.

Statistical methods

All data in this study were analyzed by SPSS21.0 software package, and all measurement data were expressed by ($\bar{x}\pm s$). T was used to test the comparison between TGF-β1 group and the control group, $P<0.05$ was considered to be statistically significant.

Results

Effects of TGF-β1 on cxCL-1/8 and IL-6 expression in airway smooth muscle cells

There was no significant difference in CXCL-1 expression between the two groups ($P>0.05$), the expressions of CXCL-8 and IL-6 in airway smooth muscle cells in TGF-β1 group were significantly higher than those in the control group, and the differences were statistically significant ($P<0.05$), see Table 1.

Effects of TGF-β1 on the expression of MMP-3 and MMP-10 in airway smooth muscle cells

The expression of MMP-3 and MMP-10 in airway smooth muscle cells in TGF-β1 group was significantly higher than that in the control group, and the difference was statistically significant ($P<0.05$). See Table 2.

Group	n	CXCL-1 (pg/ml)	CXCL-8 (pg/ml)	IL-6 (ng/L)
TGF-β1group	8	42.36±5.47	35.62±7.40	1.57±0.41
Control group	8	43.41±4.78	18.02±5.69	0.05±0.02
t		0.409	5.333	10.473

Table 1: Effects of TGF-β1 on CXCL-1/8 and IL-6 expression in airway smooth muscle cells ($\bar{x}\pm s$).

Group	n	MMP-3 (pg/ml)	MMP-10 (pg/ml)
TGF-β1group	8	1235.12±127.45	521.67±152.34
Control group	8	945.27±114.03	211.30±45.78
t		4.794	5.519
P		0.001	0.001

Table 2: Effects of TGF-β1 on the expression of MMP-3 and MMP-10 in airway smooth muscle cells ($\bar{x}\pm s$).

Effects of TGF-β1 on ECM protein deposition in airway smooth muscle cells

The expression levels of extracellular fibronectin and COL8α1 protein in TGF-β1 group were significantly higher than those in the control group, and the differences were statistically significant ($P<0.05$). See Table 3

Group	n	Fibronectin	COL8α1
TGF-β1group	8	0.52±0.16	0.13±0.06
Control group	8	0.35±0.09	0.08±0.02
t		2.619	2.236
P		0.020	0.042

Table 3: Effects of TGF-β1 on ECM protein deposition in airway smooth muscle cells ($\bar{x}\pm s$).

Effects of TGF-β1 on adhesion and repair function of airway smooth muscle cells

Compared with the control group, TGF-β1 group significantly enhanced the adhesion ability and repair ability of airway smooth muscle cells ($P<0.05$). Shown in Table 4.

Group	n	Adhesion function (nm)	Repair function (nm)
TGF-β1group	8	0.32±0.11	1520.36±547.28
Control group	8	0.21±0.08	1301.24±565.23
t		3.288	4.059
P		0.038	0.014

Table 4: Effects of TGF-β1 on the adhesion and repair function of airway smooth muscle cells ($\bar{x}\pm s$).

Discussion

COPD due to their disability fatality rate is high, the burden of social and economic, number of cases is more, has become a serious public health problem that nots allow to ignore, COPD in China is more obvious impact on people's work and productivity, the same is endangering people's physical health, influence people's life quality is an important chronic respiratory system disease[6].

Now about COPD pathogenesis is not fully clear, its pathogenesis is associated with a variety of factors, major risk factors including smoking, pollution of the environment, occupational dust, genetic factors, such as COPD airway and pulmonary vascular pathological changes including pulmonary parenchyma, the chronic inflammation, can further leading to loss of airway wall, pulmonary vascular structure, with the further development of the illness, Eventually, pulmonary hypertension, pulmonary heart disease and right heart failure may occur[7-8]. Some scholars have found through studies that the cellular immune function and humoral immune function of COPD patients are decreased, and regulatory immunotherapy may be an effective method to delay the progression of COPD, improve the prognosis of patients and improve the quality of life[9]. TGF-β1 is a multifunctional growth factor that regulates cell proliferation and differentiation. It is a 25KD polypeptide with homologous double chain, composed of 112 amino acids, and has the effect of promoting fiber cell proliferation and fibrin synthesis. TGF-β1 is mainly expressed in bronchi, alveolar epithelial cells and alveolar macrophages, and is widely involved in airway inflammation and airway remodeling, and plays an important role in bronchial morphological change, extracellular matrix generation and tissue fibrosis[10-11].

TGF-β1 transmits signals from the cell membrane to the nucleus mainly through membrane receptors and the Smads protein family, which plays an important role in regulating the synthesis, degradation and cell differentiation of ECM. In recent years, TGF-β1 has been gradually found to play an important role in renal interstitial fibrosis, gastrointestinal diseases, cardiovascular and cerebrovascular diseases, pulmonary interstitial fibrosis, bronchial asthma, COPD and other diseases, and plays a role in promoting the secretion of fibronectin, glycoprotein and collagen by regulating the immune response[12]. In recent years, many scholars have found that the increase of TGF-β1 is associated with many pulmonary inflammatory diseases related to oxidation/antioxidant imbalance[13]. Some foreign scholars have found that the expression of TGF-β1 mRNA in the mucous membrane of patients with chronic bronchitis and asthma is significantly higher than that of normal people, and is positively correlated with the thickness of basement membrane and the number of fibroblasts, indicating that excessive synthesis of TGF-β1 is closely related to the occurrence and development of pulmonary diseases[14]. Other scholars found through animal experiments that the expression of TGF-β1 in bronchopulmonary tissue, airway and alveolar epithelium of experimental COPD rats was significantly increased, and its concentration in pulmonary epithelial cells and fibroblasts of COPD patients was also significantly higher[15].

This study examined the inflammatory response of airway smooth muscle cells from COPD patients to TGF-β1 stimulation, and found that the expression of CXCL-8 and IL-6 in airway smooth muscle cells in TGF-β1 group was significantly higher than that in the control group ($P<0.05$), chemokine and inflammatory factors showed that the experimental cell model was stable and reliable. The expression of MMP-3 and MMP-10 in airway smooth muscle cells in TGF-β1 group was significantly higher than that in control group ($P<0.05$). The expression levels of extracellular fibronectin and COL8α1 protein in TGF-β1 group were significantly higher than those in the control group ($P<0.05$), suggesting that TGF-β1 can increase the deposition of extracellular matrix fibronectin in COPD airway smooth muscle cells. Compared with the control group, TGF-β1 group significantly enhanced the adhesion and repair ability of airway smooth muscle cells ($P<0.05$).

In conclusion, TGF-β1 can significantly increase extracellular matrix protein gene expression in COPD patients, and has a trend of improving cell adhesion and wound repair ability of COPD airway smooth muscle cells, providing reference for clinical treatment of COPD.

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