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TROP2 PROMOTES PROLIFERATION OF CHRONIC OBSTRUCTIVE PULMONARY AIRWAY BASAL CELLS VIA ERK/MAPK SIGNALING PATHWAY

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ABSTRACT

Objective: *To explore the mechanism of trophoblast cell surface antigen 2 (TROP2) promoting the proliferation of chronic obstructive pulmonary airway basal cells through the ERK/MAPK signalling pathway.*

Methods: *Fifty cases of COPD tissue and normal lung tissue were randomly selected between May 2016 to May 2017 in our hospital to detect the expression of trophoblast cell surface antigen 2 (TROP2) in COPD tissue and normal lung tissue. Airway basal cells were cultured, while basal cells were constructed. Four groups were set up to observe and compare the overexpression/silence ofTROP2 on cell proliferation in each group and the effect of TROP2 on airway basal cell proliferation in COPD via the ERK/MAPK signalling pathway: a blank control group (control), a pcDNA 3.1 group (constructing pcDNA 3.1 plasmids), a pcDNA.3-TROP2 group (over-expressingTROP2), and an siRNA-TROP2 group (silencing TROP).*

Results: *The expression rate of TROP2 in COPD airway epithelium was 52.00% (26/50), significantly higher than that in normal airway epithelium by 8.00% (4/50) ($P<0.05$). At 48 h and 72 h, the cell proliferation ability of pcDNA3.1-TROP2 group was significantly stronger than that of the pcDNA3.1 group ($P<0.05$), and there was no significant difference in cell proliferation ability between pcDNA3.1 group and blank control group ($P>0.05$). At 0 h and 24 h, there was no significant difference between the pcDNA3.1-TROP2 group, the blank control group and the pcDNA3.1 group ($P>0.05$). At 24 h, 48 h and 72 h, the cell proliferation ability of siRNA-TROP2 group was significantly weaker than that of the pcDNA.3 group ($P<0.05$), and the cell proliferation ability of pcDNA3.1 group was not significant compared with that of the blank control group ($P>0.05$). There was no significant difference between the siRNA-TROP2 group, the blank control group and the pcDNA3.1 group at 0 h ($P>0.05$). After overexpression of TROP2, the expression of p-ERK1/2 in the pcDNA3.1-TROP2 group was significantly higher than that in the blank control group and the pcDNA3.1 group ($P<0.05$), and the expression of p-ERK1/2 in the blank control group and pcDNA3.1 group was not significant ($P>0.05$). There was no significant difference in ERK1/2 expression in the three groups ($P>0.05$).*

Conclusion: *Overexpression of TROP2 can promote the proliferation of chronic obstructive lung airway basal cells through the ERK/MAPK signalling pathway.*

Keywords: *TROP2, ERK/MAPK signalling pathway, COPD, airway basal cells, proliferation.*

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Introduction

Chronic obstructive pulmonary disease (COPD) is a destructive heterogeneous disease. Although it is an airway disease, COPD can pose a threat to the entire body. It is a preventable and treatable disease[(1)]. According to a 2007 survey in China, the incidence of COPD in people over 40 years of age is 8%, making it an important cause of death in the rural population in China, and the disease causes the burden of individuals and families to rank first[(2)]. However, the exact pathogenesis of COPD has not been elucidated, and therefore, further study is needed to understand the optimum treatment in the clinic. Many factors lead to the onset of COPD: smoking, environmental pollution and other factors can induce COPD or lead to gradually increasing severity[(3)]. Therefore, we need to understand the pathogenesis

of COPD to explore an efficient therapeutic target for the treatment of COPD patients. Rapid proliferation of airway basal cells and changes in function can cause cell proliferation, thickening of the airway wall and other abnormalities. At present, it is recognized that the imbalance of basal cells is the key to the progression of COPD in smokers, and its role in the development of COPD is remarkable[4-5]. Therefore, exploring the mechanisms of airway basal cells plays an important role in early prevention and treatment of COPD. Trophoblast cell surface antigen 2 (TROP2) is a transmembrane glycoprotein that is expressed in normal tissues[6].

Clinical studies have shown that TROP2 is overexpressed in colon and pancreatic cancer and is closely related to the growth and migration of these tumours[7]. However, the expression level and mechanism of TROP2 in COPD airway basal cells are less studied, so this study aims to explore the mechanism of TROP2 promoting the proliferation of chronic obstructive lung airway basal cells through the ERK/MSPK signalling pathway.

Materials and methods

Subjects

• Randomly selected 50 cases of COPD tissues and normal lung tissues preserved in our hospital from May 2016 to May 2017 and signed the informed consent with the patients.

• Airway basal cells were cultured and constructed simultaneously.

Main instruments and reagents

Instruments

CO_2 cell incubator was purchased from Juchuang Environmental Equipment Co., Ltd. High-pressure automatic steam sterilizer was purchased from Shanghai Huotong Experimental Instrument Co., Ltd. Optical microscope was purchased from Shanghai Bingyu Optical Instrument Co., Ltd. The microscope with a photographic device was purchased from Shanghai Vanke Instrument Co., Ltd. The protein electrophoresis apparatus was purchased from Bunsen Health Technology Co., Ltd. Protein transmissometer was purchased from Shanghai Aiyan Biotechnology Co., Ltd. The enzyme marker was purchased from Beijing Yiobai Science & Trade Co., Ltd. High-speed and low-temperature centrifuge was purchased from Huawei Kechuang Technology Co., Ltd. Low-speed centrifuge was purchased from Beckman, USA; –80 °C ultra-low temperature refrigerator was purchased from Beijing Ailis Biotechnology Co., Ltd. PCR reverse transcription apparatus was purchased from ThermoFisher; RNA and protein denaturation apparatus were purchased from Thermo Company; the automatic PCR amplification instrument was purchased from Tianchang Technology Co., Ltd.

Reagents

The medium was purchased from Thermo Fly Company. Trypsin cell digestive juice was purchased from Shanghai Yaxin Biotechnology Co., Ltd. Dimethyl sulfoxide was purchased from Shandong Xichen Chemical Technology Co., Ltd. Cell lysis fluid was purchased from Wuxi Jerian Instrument Equipment Co., Ltd. BCA protein quantitative kit was purchased from Enzyme-Linked Organisms in Shanghai. 5×loading buffer was purchased from Hangzhou Ford Biotechnology Co., Ltd. Tween-20 was purchased from Jiangsu Hai'an Petrochemical Plant. Pre-dyed protein was purchased from Shanghai Liji Biotechnology Co., Ltd. Bovine serum albumin was purchased from Yancheng Saibao Biotechnology Co., Ltd. Super-sensitive ECL chemiluminescence kit was purchased from ABCAM Company.

Horseradish peroxidase-labelled secondary antibody was purchased from Western Chemical Technology Co., Ltd. Primary antibody and secondary antibody removal fluid was purchased from Biyuntian Company. Protein extraction kit was purchased from Solabal. Perthiamide was purchased from Shanghai Lianshuo Baowei Biotechnology Co., Ltd. SDS-PAGE protein loading buffer was purchased from Shenyang Wanban Biotechnology Co., Ltd. Trizol was purchased from Chongqing Plico Biotechnology Co., Ltd. A reverse transcription kit was purchased from Hangzhou Bev Medical Technology Co., Ltd. CCK-8 kit was purchased from Qingdao Dafei Biotechnology Co., Ltd. TROP primer was purchased from Nanjing Sahongrui Biotechnology Co., Ltd. GAPDH was purchased from Wuhan Vickers Technology Co., Ltd.

Methods

• Airway basal cells were isolated and cultured for 12 h, and fluid was changed once every other day. The cell growth integration degree reached 80%, and the basal cells of the airway were stained with immunofluorescence. Then their purity was detected.

• Blank control group (Control), pcDNA3.1 group (negative control, pcDNA3.1 plasmid construction), pcDNA.3-TROP2 group (overexpression-

TROP2), siRNA-TROP2 group (silent TROP) were established.

- TROP2 expression in lung tissues and normal adjacent tissues was detected by immunohistochemistry.
- Airway basal cells were inoculated on a culture plate and transfected with TROP2 when the cell fusion degree reached 90%. The transfection effect was verified after 48 h of continuous culture in an incubator at 37 °C.
- siRNA sequence was designed, and the airway basal cells were inoculated on the culture plate and transfected when the fusion degree reached 50%.
- CCK-8 was used to detect cell viability. When the cells were grown to 80% of the base area, CCK-8 was added to the cells and cultured in a medium and incubated at 5% CO_2 at 37 °C.
- Western blot: the proteins were extracted and determined, and ECL chemiluminescence was performed after gel filling, sample loading, electrophoresis, film transfer and immunohybridisation.

Statistical methods

SPSS24.0 was used to analyze the data of each group. The OD value of cells in each group was expressed as ($\bar{x}\pm s$), the Mann-Whitney U test was used for comparison between groups, and P<0.05 was considered significant.

Results

TROP2 expression in COPD and normal lung tissues

TROP2's expression rate in COPD airway epithelium was 52.00% (26/50), significantly higher than that in normal airway epithelium, 8.00% (4/50) (P<0.05), as shown in Figure 1.

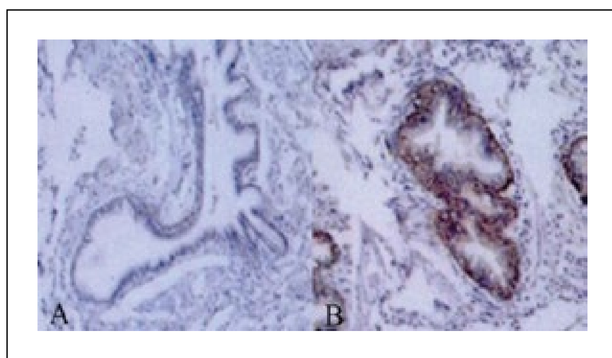

Figure 1: TROP2 positive expression in normal lung tissues and COPD tissues.
A: TROP2 is positively expressed in normal lung tissues; B: TROP2 is positively expressed to a much greater extent in COPD tissues.

Effect of overexpressionTROP2 on cell proliferation

At 48 h and 72 h, the proliferation capacity of the pcDNA3.1-TROP2 group was significantly higher than that of pcDNA3.1 group (P<0.05).

The proliferation ability of pcDNA3.1 group was not significantly different from that of the blank control group (P>0.05). At 0 h and 24 h, there was no significant difference between the pcDNA3.1-TROP2 group, the blank control group and the pcDNA3.1 group (P>0.05), as shown in Figure 2.

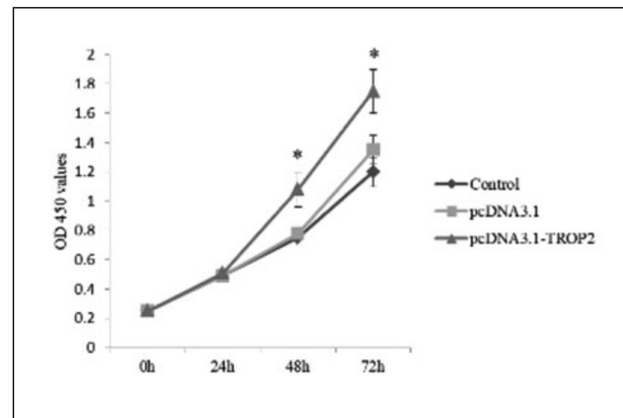

Figure 2: Effect of overexpressionTROP2 on cell proliferation.
*Note: compared with pcDNA3.1 group, *P<0.05.*

Effect ofTROP2 silencing on cell proliferation

At 24 h, 48 h and 72 h, the proliferation capacity of siRNA-TROP2 cells was significantly lower than that of pcDNA-3 (P<0.05).

The proliferation ability of the pcDNA3.1 group was not significantly different from that of the blank control group (P>0.05).

There was no significant difference between the siRNA-TROP2 group and blank control group and the pcDNA3.1 group at 0 h (P>0.05), as shown in Figure 3.

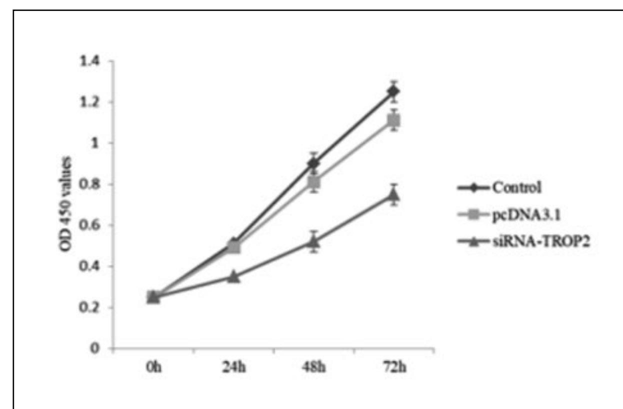

Figure 3: Effect of TROP2 silencing on cell proliferation.
*Note: compared with pcDNA3.1 group, *P<0.05.*

Effects ofTROP2 on the proliferation of basal airway cells in COPD via the ERK/MAPK signalling pathway

After the over-expression of TROP2, the expression of p-ERK1/2 in the pcDNA3.1-TROP2 group was significantly higher than that in the blank control group and the pcDNA3.1 group (P<0.05), and there was no significant difference in the expression of p-ERK1/2 in the blank control group and the pcDNA3.1 group (P>0.05). There was no significant difference in ERK1/2 expression among the three groups (P>0.05), as shown in Figure 4.

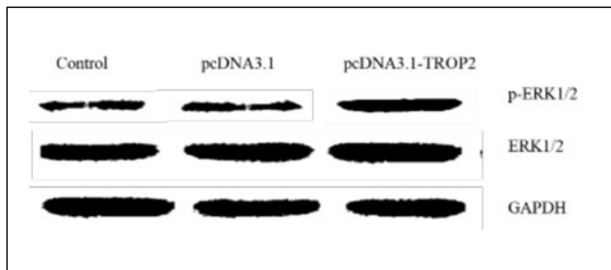

Figure 4: TROP2's effect on the proliferation of COPD airway basal cells via the ERK/MAPK signalling pathway.

Discussion

Airway basal cells have the function of maintaining normal airway epithelial structure, and their proliferation is the earliest pathological change of COPD. Still, the mechanism of their proliferation has not been clarified[8]. Under normal circumstances, airway basal cell hyperplasia has the function of accelerating airway epithelial regeneration and repair, which can be used as a repair mechanism to deal with bodily injury.

However, abnormal basal cell hyperplasia can lead to pathological changes in the body, which is a phenomenon of airway epithelial remodelling in early COPD[9]. Abnormal hyperplasia of basal cells not only causes restricted airflow but also leads to squamous metaplasia and mucous cell proliferation[10]. Literature has shown that COPD can promote pulmonary senescence, and if basal cells senescence, epithelial regeneration and repair function will be lost[11]. Studies on airway basal cell hyperplasia in COPD are relatively few, so further studies are needed on its regulatory mechanism.

TROP2 was first discovered in epigenetic tumours. Many reports have confirmed that TROP2 plays an important role in regulating the growth of stem cells and has the ability of self-renewal[12]. Some scholars have shown that TROP2 is abnormally expressed in basal prostatic cells, and only cells with high expression have some stem cell characteristics[13]. Clinical studies have shown that TROP2 is not expressed in normal liver tissues of mice, but it can be expressed in undifferentiated oval cells when the liver is damaged[14].

TROP2 has the function of self-renewal and tissue repair. The ERK/MAPK signalling pathway can participate in cell growth and development and promote cell transformation into malignant tumour cells, playing a significant role in tumour initiation and progression. Studies have shown that the ERK/MAPK signalling pathway is activated during the development of breast cancer. In addition, the ERK/MAPK signalling pathway is a key pathway for regulating cell proliferation, and its abnormal activation will lead to the transformation of many tumour phenotypes into malignant tumours. Studies have confirmed that inhibiting the activation of the ERK/MAPK signalling pathway can inhibit the proliferation of liver cancer and breast cancer tumour cells[15].

In this study, the expression of TROP2 in COPD and normal lung tissues was measured by immunohistochemistry. It was found that the expression rate of TROP2 in the airway epithelium of COPD patients was 52.00% (26/50), significantly higher than that in the normal airway epithelium of 8.00% (4/50) (P<0.05). These results suggest that TROP2 may be involved in the pathogenesis of COPD. At 48 h and 72 h, the proliferation capacity of the pcDNA3.1-TROP2 group was significantly higher than that of the pcDNA3.1 group (P<0.05).

The proliferation ability of the pcDNA3.1 group was not significantly different from that of the blank control group (P>0.05). These results suggested that TROP2 overexpression could enhance the proliferation of airway basal cells. At 24 h, 48 h and 72 h, the proliferation capacity of siRNA-TROP2 cells was significantly lower than that of pcDNA-3 cells (P<0.05). The proliferation ability of the pcDNA3.1 group was not significantly different from that of the blank control group (P>0.05). These results suggest that TROP2 silencing could inhibit the proliferation of airway basal cells.

After the over-expression of TROP2, the expression of p-ERK1/2 in the pcDNA3.1-TROP2 group was significantly higher than that in the blank control group and the pcDNA3.1 group (P<0.05), and there was no significant difference in the expression of p-erk1/2 in the blank control group and the pcDNA3.1 group (P>0.05). There was no significant difference in ERK1/2 expression among the three groups (P>0.05). These results suggest that p-ERK2

may be involved in the regulation of basal cell proliferation. To sum, TROP2 overexpression promotes basal cell proliferation, and overexpression of TROP2 can promote basal cell proliferation of the COPD airway through the ERK/MAPK signalling pathway.

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