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Archives of Medical Research 39 (2008) 388–396

Archives of Medical Research

ORIGINAL ARTICLE

Downregulation of Secretory Leukocyte Proteinase Inhibitor in Chronic Obstructive Lung Disease: The Role Of TGF-β/Smads Signaling Pathways

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Received for publication November 1, 2007; accepted January 22, 2008 (ARCMED-D-07-00519).

Background. Secretory leukocyte proteinase inhibitor (SLPI) is an important antileukoprotease in airway. The aim of the present study was to explore the expression of SLPI in the bronchi and lung tissues of chronic obstructive pulmonary disease (COPD) models and the regulative mechanism by transforming growth factor (TGF)β_1/Smads signal pathway in bronchial epithelial cell.

Methods. COPD rat model was established and was treated with or without TGFβ1 monoclonal antibody. Spirometry was conducted, and expressions of TGFβ_1, Smad4 and SLPI were examined by immunohistochemistry and reverse-transcription polymerase chain reaction (RT-PCR), respectively. The normal human bronchial epithelial cell (NHBE) was cultured, preincubated with or without siRNA (Smad4), and then stimulated with TGFβ_1. Expressions of Smad4 and SLPI were detected by immunocytochemistry, Western blot and RT-PCR, respectively.

Results. As compared with the model group, after treatment with TGFβ_1 monoclonal antibody, peak expiratory flow (PEF), forced expiratory volume in 0.3 sec ($FEV_{0.3}$) and $FEV_{0.3}$/forced vital capacity (FVC) in the TGFβ_1 monoclonal antibody intervention group were all significantly improved. Expression of SLPI was also improved, but expression of Smad4 was significantly decreased. Expression of SLPI in NHBE cells was inhibited by TGFβ_1 both at the mRNA level and the protein level. Furthermore, effect of TGFβ_1-inhibited expression of SLPI in NHBE cells was disengaged by siRNA (Smad4) both at the mRNA level and the protein level.

Conclusions. Decreased expression of SLPI in the COPD rat model may be mainly caused by the increased expression of TGFβ_1, and this process is probably related to the activation of Smads signal pathway.

Key Words: Chronic obstructive pulmonary disease, Transforming growth factor beta 1, Secretory leukocyte proteinase inhibitor, Smad4.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by airflow obstruction, which comprises emphysema and chronic bronchitis/bronchiolitis. Molecular mechanisms by which small airway obstruction occurs remain unknown. Recently, some studies indicated that an imbalance between neutrophil protease and surrounding antiprotease levels has been shown to be important in the pathogenesis of COPD (1,2). Secretory leukocyte proteinase inhibitor (SLPI), known as one of the most important antileukoproteases in airway, is a 12-kDa nonglycosylated, cationic protein produced by serous cells of the submucosal bronchial glands, nonciliated cells of the bronchial epithelium, and neutrophils. Its major physiological function is considered to be the inhibition of the destructive capacity of neutrophil elastase (NE) (3), and some data indicated that COPD is related to the reduction of SLPI (4,5). Little is known about the regulation of SLPI expression in the lung.

Other studies suggest some fibrogenic growth factors may be involved in the remodeling processes of the small airways (6,7). One of the most potent and extensively

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0188-4409/08 $–see front matter.
doi: 10.1016/j.arcmed.2008.02.002

studied growth factors is transforming growth factor (TGF)β_1, which induces fibroblast proliferation, increases production of collagen and other extracellular matrix proteins and decreases collagen degradation (8). TGFβ_1 is also chemotactic for neutrophils, macrophages and mast cells (9,10), and its major intracellular signaling effector is the Smad protein family. TGFβ1 is widely localized in the lung (11). Several studies recently demonstrated that there was a significant expression of TGFβ_1 in airway epithelial cells in subjects with COPD as compared with controls (12−14).

Low levels of SLPI and high levels of TGFβ_1 were observed in the bronchi and lung tissues of COPD. A recent study indicated that TGFβ_1 is a potent inhibitor of SLPI in a bronchial epithelial cell (15). However, whether decreased expression of SLPI in the bronchi and lung tissues of COPD is related to the increased expression of TGFβ_1 is unknown. If so, whether the role of SLPI is mediated through Smads signal pathway needs further investigation. To address the role of TGFβ_1/Smads on the regulation of SLPI in the bronchi and lung tissues of COPD, COPD model was established and the rats were treated with TGFβ_1 monoclonal antibody; the normal human bronchial epithelial cell (NHBE) line was cultured and then stimulated with TGFβ_1 and siRNA (Smad4). The relationship among the expression of SLPI, TGFβ_1 and Smad4 in the bronchi and lung tissues was then observed, and the role of TGFβ_1/Smads on the decreased expression of SLPI in NHBE cells was investigated.

Materials and Methods

COPD Animals Model

Thirty six grade SPF healthy male Wistar rats, 10−12 weeks of age, initially weighing 200 ± 20 g, were provided by the experimental animal center of Central South University. Animals were divided into three random groups: COPD model group (COPD group), TGFβ_1 monoclonal antibody intervention group (anti-TGFβ_1 group) and control group, 12 rats in each group. COPD model group rats were anesthetized intraperitoneally with 10% chloral hydrate. After their tracheas were cannulated, they were established by intratracheal instillation of lipopolysaccharide (LPS 200 μg/200 μL) twice on the 1st and 30th day, respectively, and were exposed to the smoke of three commercial unfiltered cigarettes for 30 min each day, for a total of 2 months. Moreover, they received normal saline (0.5 mL) via the tail vein twice on the 6th and 19th day, respectively. Drug intervention group rats received TGFβ_1 monoclonal antibody (TB21, Abcam, London, England) 0.5 mg (1 mg/mL) via the tail vein twice on the 6th and 19th day, respectively, and other treatments were taken as the COPD model group. Control group rats were treated with intratracheal instillation of normal saline 0.1 mL and received air only in the smoke exposure Plexiglas box. Other treatments were the same as the COPD model group (16,17). The study protocol was in accordance with the guidelines for animal research and was approved by the Ethical and Research Committee of the hospital.

Lung Function Test

Rats were anesthetized intraperitoneally with 10% chloral hydrate and then placed supine. After the tracheas and esophagus were both cannulated, they were connected to an animal lung function instrument (BUXCO, Wilmington, NC), respectively. Peak expiratory flow (PEF), forced expiratory volume (0.3 sec) ($FEV_{0.3}$) and $FEV_{0.3}$/forced vital capacity (FVC) were then performed and calculated by computer (16).

Tissue Preparation

After the lung function tests, rats were exsanguinated. The right posterior and anterior pulmonary lobes were stored in liquid nitrogen for use later in the reverse-transcription polymerase chain reaction (RT- PCR) assay. Bronchoalveolar lavage was performed by instilling 0.1 mol/L PBS into the left lung three times and 3 mL for three times. Each bronchoalveolar lavage fluid (BALF) sample was centrifuged, and the supernatant was stored for use later in enzyme-linked immunosorbent assay (ELISA). The right middle lobe was inflated via the trachea using 4% paraformaldehyde at 25 cm H_2O for 30 min and fixed in 4% paraformaldehyde for $>$48 h. A lung block from the lobe was embedded in paraffin and finally sliced into 5-μm-thick sections for further study. The study protocol was in accordance with the guidelines for animal research and was approved by the Ethical and Research Committee of the hospital.

Cell Culture

Normal human bronchial epithelial (NHBE) cells were purchased from the cell culture collection center of Central South University. They were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% newborn calf serum and were grown in tissue culture flasks in a humidified gas environment with 95% air and 5% carbon dioxide at 37°C. For subculture, cells were detached with 1% trypsin. After reaching 80% confluence, cells were seeded into six-well plates at a density of 1×10^5 cells per well to grow again to 80% confluence (18). After 24 h in serum-free medium: 1) cells were stimulated with TGFβ_1 (Santa Cruz Biotechnology, Santa Cruz, CA) (10 ng.mL^{-1}, 48) (TGFβ_1 group), 2) cells were preincubated with the small interfering RNA (siRNA) against Smad4 [Smad4 siRNA (human), Santa Cruz Biotechnology, sc-29484] (2 μg/mL, 5 h) then stimulated with TGFβ_1 (10 ng.mL^{-1}, 48 h), (Smad4 siRNA Group 3), cells were preincubated with the negative control siRNA (Santa Cruz Biotechnology, sc-36869) (2 μg/mL, 5 h) then stimulated with TGFβ_1