Expression of Connective Tissue Growth Factor in Pulmonary Vascular Remodeling with Chronic Obstructive Pulmonary Disease

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ABSTRACT Objective: To investigate the expression and significance of connective tissue growth factor (CTGF) in pulmonary vascular remodeling in patient with chronic obstructive pulmonary disease (COPD). Method: According to their lung function, 30 male lung carcinoma patients, who had smoking history and operation in thoracic surgery, were divided into 2 groups: control group (patients with normal lung function) and COPD stable group (patients with COPD), with 15 patients in each group. The lung specimens were obtained from adjacent noncancerous tissue. Morphmetric characteristic of the pulmonary vascular remodeling was observed by HE and Masson trichrome staining. The expression of CTGF and PCNA proteins in pulmonary muscular arteries were evaluated by immunohistochemistry methods. Results: (1) The expression of CTGF, PCNA, artery wall area / total area (WA%) and collagen thickness were increased significantly in pulmonary arteries of COPD group compared with those of the control group. (2) The expression of CTGF in smooth muscle cells was correlated with the thickness of the vessel wall, collagen and the expression of PCNA (r= 0.81, 0.68, 0.86, $P<0.05$). The smoking index was positively correlated with WA%, the expression of PCNA (r= 0.73, 0.99, $P<0.01$). Conclusion: Pulmonary vascular remodeling was evident in smokers without COPD, and the remodeling became more severe in patients with COPD. The expression of CTGF protein in pulmonary vessels in COPD patients tend to be higher than in that of the control group, and it may be involved in the process of pulmonary vascular remodeling.

Key words: Pulmonary disease; Obstructive; Pulmonary vascular remodeling; Connective tissue growth factor

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Introduction

Chronic obstructive pulmonary disease (COPD) is defined in terms of airflow obstruction that results from an inflammatory process affecting the airways and lung parenchyma. Despite major abnormalities affecting the airways and lung parenchyma, changes in pulmonary vessels represent an important component of the disease [1]. Pulmonary vascular remodeling leading to pulmonary hypertension and cor pulmonale is a characteristic feature of COPD. Hypoxia has been classically considered the major pathogenic mechanism of these changes. However, structural abnormalities of pulmonary arteries are not exclusive of advanced COPD, as they have been shown also in patient with mild COPD without arterial hypoxemia and in smokers with normal lung function [2]. Pulmonary vascular remodeling is characterized by thickening of all three layers of the blood vessel wall. The thickening is due to hypertrophy and/or hyperplasia of the predominant cell type within each of the layers, as well as increased deposition of extracellular matrix components and neomuscularisation of normally non-muscular arteries [3]. Connective tissue growth factor, an immediate early gene of the CCN family, is a new growth factor which is directly involved in the fibrotic response as a down-stream mediator of TGF-β [4]. CTGF is abundantly expressed in fibroblasts, smooth muscle cells and endothelial cells. It is an intercellular signaling molecule that regulates cell proliferation and extracellular matrix synthesis by autocrine and paracrine mechanisms [5]. However, its role in COPD pulmonary vascular remodeling is still unknown. This study investigated the changes of pulmonary vascular morphometric with COPD patients and the relationship between CTGF expression in the pulmonary vascular and pulmonary vascular remodeling.

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1 Materials and methods

1.1 Specimen collection

In this study 30 male lung carcinoma patients with smoking history were chosen from the Department of Chest Surgery of the Affiliated Hospital of Qingdao University Medical College. Preoperative lung functional measurements, partial pressure of oxygen (PaO_2) and smoking index were performed in all the patients. COPD was diagnosed according to the Global Initiative for Chronic Obstructive Pulmonary Disease guidelines. The 30 male patients were divided into two groups according to their lung functions: control group (patients with normal lung function) and COPD stable group (patients with COPD), with 15 cases in each group. The lung specimens were obtained from adjacent noncancerous tissue, and then fixed with 10% neutral formalin 12-24 hours. The clinical data of the patients in the two groups were showed in Table 1.

1.2 Morphometric characteristics of pulmonary muscular arteries were observed with HE and MASSON Trichrome Staining

Serial sections were cut from formalin-fixed, paraffin-embedded tissue blocks. Every slide from each series was stained with Hematoxylin-Eosin (HE) and Masson Trichrome Staining (MASSON). The respiratory bronchioles and alveolar ducts accompanying small pulmonary artery whose the transverse section diameter was between100 and 200 μm were observed at × 20 and × 40 magnification. Vascular wall area / total area, collagen thickness were measured by computer-based image analysis system.

Table 1 The clinical data of patients in the two groups ($\bar{X}\pm S$)

Group	Gender	Number	Smoking index	PaO_2(mmHg)	FEV_1(%pred)	FEV_1/FVC (%)
control group	male	15	700.00± 261.86	90.28± 5.79	97.28± 5.28	84.93± 6.08
COPD group	male	15	1053.33± 354.29#	87.47± 5.89	69.87± 5.37☆	64.93± 3.99★
T value			-3.11	1.31	14.09	10.66
P value			＜0.01	>0.05	＜0.01	＜0.01

Note: # There was significant difference between the two groups, P <0.01

☆There was significant difference between the two groups, P <0.01

★There was significant difference between the two groups, P <0.01

1.3 Proliferating cell nuclear antigen (PCNA) and CTGF immunohistochemistry

Tissue sections of formalin-fixed and paraffin-embedded were immunotained with a mouse monoclonal antibody antihuman CTGF and PCNA. A mean of 10 arteries was analyzed in each subject. Positive immunoreactivity to CTGF and PCNA expression was shown by the appearance of the brown granular staining in nucleus or cytoplasm of vascular smooth muscle cells. The respiratory bronchioles and alveolar ducts accompanying small pulmonary artery whose the cross-sectional diameter was between 100 and 200μm were observed at × 20 and × 40 magnification. Meanwhile we detected the average absorbance of smooth muscle cells.

1.4 Statistical Analysis

All data are expressed as mean ± SD. Comparisons between the two groups were performed using t test. Correlations between variables were analyzed using the Pearson's coefficient, Probability values lower than 0.05 were considered significant.

2 Results

2.1 Morphometric Analysis

Morphometric measurements of pulmonary muscular arteries are summarized in Table 2. We examined a similar number of arteries per patient in each group. According to morphometric analysis, vessel wall area / total area and collagen thickness were enlarged in COPD group compared with those of the control group. The pulmonary artery wall of COPD group became thicker, and the vessel lumen became narrower. Significant difference existed between the two groups (P <0.05).

2.2 Immunohistochemical Analysis

In control group, positive immunoreaction to CTGF and PCNA antibody was observed in the smooth muscle cells of the wall of the pulmonary artery. Intense immunoreactivity to CTGF and PCNA antibody was observed in COPD groups. The expression of CTGF and PCNA in control group showed a trend to be lower than in COPD group. There was a significant difference between the two groups, as is shown in Table 2.

Table 2 WA%, collagen thickness and expression of PCNA and CTGF with two groups ($\bar{X}\pm S$)

Group	WA%	Collagen thickness(μm)	CTGF protein	PCNA protein
Control group	45.27± 5.36	13.91± 3.11	0.24± 0.09	0.19± 0.05
COPD group	54.63± 8.14#	16.45± 3.53※	0.33± 0.11☆	0.25± 0.06★
T value	-3.72	-2.09	-2.48	-3.24
P value	＜0.01	＜0.05	＜0.05	＜0.01

Note: # There was significant difference between the two groups, P <0.01

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★There was significant difference between the two groups, P <0.01

2.3 Correlation analysis

The expression of CTGF in smooth muscle cells correlated with the thickness of the vessel wall, collagen and the expression of PCNA (r= 0.81, 0.68, 0.86, P＜0.05). The smoking index was

positively correlated with WA%, the expression of PCNA (r= 0.73, 0.99, P＜0.01).

3 Discussion

The previous studies have showed all layers of the vessel wall appear to be involved in the progress of pulmonary vascular remodeling, and the smooth muscle cells proliferation was the most prominent [6]. Those pathological changes could be explained with the combined effects of hypoxia, inflammatory reaction and the toxic effects of cigarette smoke and so on [7]. A recent study of the patients with lung resection showed that structural changes of the pulmonary vessels were recognized in patients with mild COPD without hypoxemia and in smokers with normal lung function [2]. Since these changes were more prominent in smokers than in non-smokers, which might be related to tobacco smoking. This suggested that tobacco smoke played an important role in the causation of pulmonary vascular remodeling pathology in COPD [8]. There was a positive correlation between the smoking index and WA%, the expression of PCNA in this study, (r= 0.73, 0.99, P＜0.01). This study also showed that pulmonary vascular remodeling is evident in smokers without COPD, and the remodeling became more severe in patients with COPD, which suggested that the cigarette smoking could induce selective endothelial dysfunction in pulmonary arteries, smooth muscle cell proliferation in small pulmonary vessels. The pulmonary vascular remodeling in the COPD patients with cigarette smoking history was more serious than in other smokers, which might be caused by the pulmonary vascular damage as a result of the emphysema. Therefore, smoking may lead directly to pulmonary vascular remodeling with COPD patients, which is consistent with the previous animal experimental results [9].

The results of this study showed an increased expression of CTGF in pulmonary arteries of patients with COPD, and the expression of CTGF was associated with the proliferation of the smooth muscle. By contrast, in the patients in the control group, the immunohistochemical expression of CTGF in pulmonary arteries tends to be low. This study was mainly addressed to investigate the potential role of CTGF in the pathogenesis of pulmonary vascular remodeling that takes place in the initial stage of COPD. At this stage, remodeling is characterized by smooth muscle cells proliferation and extracellular matrix deposition in the pulmonary arteries. An important discovery of this investigation is the more intense expression of CTGF in the wall of pulmonary arteries in patients with COPD than in smokers with normal lung function. In pulmonary vessels, CTGF can be expressed by the smooth muscle cells. Interestingly, the expression of CTGF in smooth muscle cells correlated with the thickness of the arterial wall, collagen deposition and the expression of PCNA. The CTGF played a potential role in the pathogenesis of pulmonary vascular remodeling, and the CTGF may induce and modulate vascular smooth muscle cells proliferation and migration of matrix metalloproteinase [10-11]. Experimental studies in animal models showed that CTGF could promote smooth muscle cells proliferation [12-13]. The potential mechanisms that implicate CTGF in the early vascular remodeling of COPD do not seem to be related to hypoxia, as the majority of the patients in our series had normal arterial partial pressure of oxygen. Indeed, we found a weak correlation between PaO_2 values and protein of CTGF in lung tissue. Therefore, it is necessary to consider other potential triggers for the increased CTGF activity at early stages of COPD. Consequently, CTGF might contribute to the structural remodeling of pulmonary arteries in the early stage of COPD, presumably by enhancing the proliferation of smooth muscle cells.

In summary, the study showed that pulmonary vascular remodeling was evident in smokers without COPD, and the remodeling became more severe in patients with COPD. The CTGF may induce and modulate vascular smooth muscle cells proliferation and deposition of collagen [11], which are two important aspects in the process of pulmonary vascular remodeling. The previous studies have found that antifibrotic substances can inhibit collagen synthesis and then prevent pulmonary vascular remodeling, so they are applied in the treatment of pulmonary hypertension [14]. Current treatment strategies for pulmonary arterial hypertension have shifted from vasodilator to anti-proliferative drugs [15]. We have confirmed that CTGF involves in vascular remodeling process as its effect on the proliferation of pulmonary artery smooth muscle cells. It is believed that CTGF will probably be a new therapy target to vascular remodeling of COPD.

References

[1] Barbera JA, Peinado VI, Santos S. Pulmonary hypertension in chronic obstructive pulmonary disease [J]. Eur Respir, 2003, 21: 892-905

[2] Nilsson I, Shibuya M, Wennstrom S. Differential activation of vascular genes by hypoxia in primary endothelial cells [J]. Exp Cell Res, 2002, 299: 476-485

[3] Santos S, Peinado VI, Ramirez J, et al. Characterization of pulmonary vascular remodeling smokers and patients with mild COPD [J]. Eur Respir, 2002, 19: 632-638

[4] Grotendorst GR. Connective tissue growth factor: a mediator of TGF-β action on fibroblasts [J]. Cytokine Growth Factor Rev, 1997, 8: 171-179

[5] Grotendorst GR, Okochi H, Hayashi N. A novel transforming growth factor-B response element controls the expression of the connective tissue growth factor gene [J]. Cell Growth Differ, 1996, 7: 469-480

[6] Yu TZ, Ma CT. Effect of hypoxia on angiogenesis converting enzyme activity and gene expression of pulmonary artery smooth muscle cells [J]. Chinese Pathophysiology, 1998, 50: 625-628

[7] Curina A, Sapienza P, Corvino V, et a1. Nicotine induces Platelet-derived growth factor release and cytoskeletal alteration in aortic smooth muscle cells [J]. Surgery, 2000, 127: 72-78

[8] Peinado VI, Barbera JA, Abate P, et al. Inflammatory reaction in pulmonary muscular arteries of patients with mild chronic obstructive

pulmonary disease [J]. Respir Crit Care Med, 1999, 59: 1605-1611

[9] Wright JL, Levy RD, Churg A. Pulmonary hypertension in chronic obstructive pulmonary disease: current theories of pathogenesis and their implications for treatment [J]. Thorax, 2005, 60: 605-609

[10] Xie S, Sukkar MB, Issa R, et al. Regulation of TGF-beta1 induced connective tissue growth factor expression in airway smooth muscle cells [J]. Physiol Lung Cell Mol Physiol, 2005, 288: 68-76

[11] Lee YS, Byun J, Kim JA, et al. Monocrotaline induced pulmonary hypertension correlates with upregulation of connective tissue growth factor expression in the lung [J]. Exp Mol Med, 2005, 37: 27-35

[12] Tian F, Xu YJ, Zhang ZX, et al. CTGF in the smoke exposure in rat pulmonary vascular expression of the relationship between pulmonary vascular remodelin [J]. Chinese Pathophysiology, 2008, 24: 7-11

[13] Wang R, Xu YJ, Liu XS, et al. Expression of connective tissue growth factor and cycling D1 in pulmonary vascular remodeling in rats exposed to smoke [J]. Chinese Journal of Tuberculosis and Respiratory Diseases, 2010, 33: 679-683

[14] Tozzi CA, Poiani GJ, McHugh NA, et al, Recombinant human relaxing reduces hypoxic pulmonary hypertension in the rat [J]. Pulm Pharmacol Ther, 2005, 18: 346-353

[15] Rubin LJ. Therapy of pulmonary hypertension: the evolution from vasodilators to antiproliferative agents [J]. Respir Crit Care Med, 2002, 166: 1308-1309

结缔组织生长因子在慢性阻塞性肺疾病血管重建中表达研究

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摘要 目的：探讨结缔组织生长因子(CTGF)在慢性阻塞性肺疾病(COPD)血管重建中的表达及意义。方法：将30例有吸烟史的男性鳞癌需要手术的患者按其肺功能结果分成二组，对照组：(肺功能正常组)；COPD稳定期组：(肺功能异常组)，每组15例，标本来自于癌旁的肺组织，肺血管重塑的形态学观察行HE和MASSON三色染色，行免疫组化来观察CTGF蛋白、PCNA蛋白在肺血管平滑肌中的表达。结果：(1) COPD组肺动脉管壁面积/管总面积(WA%)、管壁的胶原厚度、肺动脉平滑肌中CTGF蛋白及PCNA蛋白的表达与对照组相比差异有统计学意义。(2) CTGF与管壁面积/管总面积(WA%)、管壁的胶原厚度及血管平滑肌中PCNA表达呈正相关，(r值分别为0.81、0.68、0.86, P＜0.05)。吸烟指数与管壁面积/管总面积及PCNA的表达呈正相关(r= 0.73, 0.99, P＜0.01)。结论：单纯吸烟者即有血管重建，吸烟伴COPD者血管重建更加严重，CTGF在COPD患者肺血管中的表达较对照组高，可能参与了COPD血管重建过程。

关键词：肺疾病；阻塞性；肺血管重建；结缔组织生长因子

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