Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Expression Profile of IL-17 in lung tissues of patients with lung cancer and COPD and clinical significance

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ARTICLE INFO

Original paper

Article history:
Received: August 09, 2022
Accepted: September 22, 2022
Published: September 30, 2022

Keywords:

TALNEC2, miR-19a-3p, JNK, cerebral infarction, cell viability, inflammation, apoptosis

ABSTRACT

The purpose of this experiment was to analyze the expression and clinical significance of interleukin-17 (IL-17) in lung tissue of lung cancer patients with chronic obstructive pulmonary disease (COPD). For this aim, 68 patients with lung cancer and chronic obstructive pulmonary disease who were admitted to our hospital from February 2020 to February 2022 were selected as the research objects of the study group. The specimens were fresh lung tissue obtained after lobectomy; In addition, 54 healthy subjects were selected as the control group during the same period, and fresh lung tissue obtained by minimally invasive lung volume reduction was selected as the sample. The clinical baseline data of the two groups were observed and compared. The mean alveolar area, small airway inflammation score and Ma tube wall thickness were measured. The expression of IL-17 was detected by immunohistochemistry. Results showed that gender, average age and average body mass index of the two groups ($P > 0.05$); At present, the proportion of smokers, pulmonary artery systolic pressure, $PaCO_2$ level and CRP level in the study group are high, PaO_2 level, FEV_1% predicted value, FEV_1 / FVC and FIB level are low, and the number of acute exacerbations in the past year is high ($P > 0.05$). In the study group, the average alveolar area, the thickness of the Ma tube wall, the lymphocyte infiltration score of the tracheal wall and the total pathological score of the small airway were higher ($P > 0.05$). The expression of IL-17 in the airway wall and lung parenchyma was higher in the study group ($P > 0.05$). IL-17 expression in lung tissue of lung cancer patients with COPD was positively correlated with body mass index, and negatively correlated with CRP, FIB, FEV_1% predicted value and the number of acute exacerbations in the past year; CRP and the number of acute exacerbations in the past year were independent variables and independent influencing factors of IL-17 expression ($P < 0.05$). In conclusion, IL-17 is highly expressed in the lung tissues of patients with lung cancer and COPD, which may play an important role in the occurrence and development of the disease.

Doi: http://dx.doi.org/10.14715/cmb/2022.68.9.21

Introduction

Chronic obstructive pulmonary disease (COPD) refers to a kind of lung disease with airflow limitation-caused dyspnea. COPD patients on exertion will experience shortness of breath or dyspnea that can be relieved at rest, which is induced by smoking, dust in occupation, infection, chronic bronchiolitis or fog of fuels (1). COPD, as indicated by a previous study (2), is a kind of chronic inflammatory disease with the airway as the initial site of inflammation, which could be further advanced by the inflammation enhanced by the inhalation of harmful gas or substances and gradually diffuse to the whole lung and involve a variety of systems. As the research on the tumor etiology and pathogenesis deepens, cytokines have been found to play key roles in regulating the development and progression of tumors and the activity of the immune system of the host, where IL-17, as a recently found cytokine with the potent pro-inflammatory effect, has been confirmed to be highly expressed in some autoimmune diseases, including multiple sclerosis, inflammatory bowel diseases and rheumatoid arthritis; otherwise, some studies (3-5) have demonstrated that IL-17 is up-regulated in the bronchial epithelium and mucosal layer of COPD patients, suggesting the potential role of IL-17 in the chemotaxis and activation of inflammatory cells in the airway and airway injury and remodeling in COPD patients. Currently, IL-17 expression has been reported in some studies on COPD patients, yet there remains no evidence suggesting the expression profile of IL-17 in COPD patients complicated with lung cancer. As such, we, in this study, measured the expression profile of IL-17 in COPD patients with lung cancer and analyzed the potential clinical significance.

Materials and Methods

Subjects

In this study, 68 patients with COPD and lung cancer who were admitted to Cancer Hospital Affiliated with Chongqing University between February 2020 and February 2022 were assigned to the treatment group, and the specimens of fresh lung tissue were collected from the pulmonary lobectomy. Simultaneously, 54 subjects who took the physical examination at Cancer Hospital Affiliated with Chongqing University were assigned to the control group, and the specimens of fresh lung tissue were collected from minimally invasive lung volume reduction surgery. Subjects in the treatment group aged between 45 and 71 years

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old, while those in the control group were between 46 and 70 years old. Data of subjects were comparable between the two groups, and all subjects or their family had signed the written informed consent prior to the study. This study had been approved by the Ethical Committee of Cancer Hospital Affiliated with Chongqing University.

Criteria for inclusion of patients into the treatment group: Patients who were first diagnosed in accordance with the diagnostic criteria for lung cancer of *Chinese Medical Association guidelines for clinical diagnosis and treatment of lung cancer (Edition 2018)* (6) and the criteria for COPD of *Guidelines for primary care of chronic obstructive pulmonary disease (2018)* (7); patients with no history of chemotherapy or biological immunotherapy prior to the enrollment.

Criteria for inclusion of patients into the treatment group: Healthy subjects.

Criteria for exclusion: Patients with diseases in the respiratory system but COPD; patients complicated with diabetes mellitus or other endocrine or metabolic diseases; patients with a history of trauma, surgery, oral or intravenous administration of glucocorticoid within 3 months.

Determination of indicators

Clinical baseline data

Following the enrollment, we recorded the baseline data of all subjects, including the sex, age, smoking history, aggravation times within 1 year and body mass index. Then, 5 mL fasting venous blood was collected in the morning in a septic environment from the subjects of two groups and then prepared for centrifugation at 3000 r/min (r = 15 cm) for 10 min to obtain the serum. Serum samples were then stored at -80°C. An automatic Biochemical Analyzer (Shanghai Yuyan Instruments Co., Ltd., Shanghai, China) was used to determine the blood routine, arterial partial pressure of oxygen (PaO_2) and arterial partial pressure of carbon dioxide ($PaCO_2$). A Lung Function Calculator purchased from Shanghai Ouqi Electric Technology Co., Ltd (Shanghai, China) was utilized to determine the forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC), and accordingly, the ratio of FEV_1 to FVC was calculated. Enzyme-linked immunosorbent assay (ELISA) kit was used to determine the levels of C-reactive protein (CRP) and fibrinogen (FIB). Pulmonary arterial systolic pressure was also estimated by echocardiogram.

Pathological test

The average alveolar area, the score of small airway inflammation and the thickness of muscularized artery wall were evaluated by two pathologists blinded to the information of slides as per the methods of published literatures (8).

IL-17 expression

An immunohistochemistry kit purchased from Shanghai QiMing Biotechnology Co.,Ltd (Shanghai, China) was used to determine the expression of IL-17 in the following steps: Tissue samples were de-paraffinized, hydrated and sliced into sections in thickness of 3 mm and rinsed in the running water (3 min/time); sections were placed in 3% H_2O_2 for 15 min without light at room temperature, followed by washes in the running water (3 min/wash); sections were then incubated with the rabbit anti-human polyclonal anti-IL-17 antibody in a dilution of 1:50 at room temperature for 1 h, followed by washes in PBS (3 min/wash); then, PBS was removed and sections were stained in DAB, counterstained in hematoxylin for 1-2 min, differentiated in 0.1% hydrated chloride, blued in PBS and reaction was terminated according to the changes in color, followed by counterstaining, dehydrating, clearing and mounting the sections. For negative control, the primary antibodies were replaced by PBS.

Interpretation of results: Cells with brown and/or deep brown particles in the cytoplasm were taken as positive cells, where the strength of staining was scored within 0 and 3 points, respectively for no color, light yellow, brown and deep brown; positive rate of stained cells was also scored within 0 and 4 points, where 0 stood for the cells with no positive staining, 1 for cells with positive staining between 1% and 10%, 2 for those between 11% and 50%, 3 for 51% and 80% and 4 for those, not fewer than 80%. The expression of IL-17 was expressed by the multiply of the positive rate and the strength of stained cells.

Statistical methods

Data were processed by SPSS 26.0 software. Measurement data were first prepared for the Kolmogorov-Smirnov test to validate whether the data conformed to the normal distribution. Data in normal distribution were described as ($\bar{x} \pm s$). A pairwise comparison was performed by using the independent *t*-test. Enumeration data were described by ratio (%), and the comparison between two groups was performed by the *F* test. Pearson analysis was performed to detect the correlation of IL-17 expression with CRP, FIB, BMI, predicted value of FEV_1% and acute aggravation times within 1 year, and the underlying correlation was further validated in the Logistic Regression model. $P < 0.05$ suggested that the difference had statistical significance.

Results

Comparison of the clinical baseline data between two groups

Differences in the sex, average age and average BMI of patients between the two groups showed no statistical significance ($P > 0.05$). In the treatment group, the proportion of subjects with a smoking history, pulmonary arterial systolic pressure, $PaCO_2$ and CRP were all higher than those in the control group, while PaO_2, predicted value of FEV_1%, FEV_1/FVC and FIB were lower, and the times of acute aggravation within 1 year were more than those in the control group (all $P < 0.05$; Table 1).

Comparison of the average area of alveoli, inflammation score of the small airway and MA wall thickness between two groups

In the treatment group, the average area of alveoli and MA wall thickness of patients were all larger than those of subjects in the control group, while the score of lymphocyte infiltration into the airway wall and the pathological score of small airway were also higher (all $P < 0.05$; Table 2).

Comparison of IL-17 expression in lung tissues between two groups

Expression of IL-17 in the airway wall and pulmonary

Table 1. Comparison of the clinical baseline data between two groups.

Clinical baseline data		Control group (n=54)	Treatment group (n=68)	x^2/t	*P*
Sex [n (%)]	Male	29 (53.70)	36 (52.94)	0.007	0.933
	Female	25 (46.30)	32 (47.06)		
Average of age ($\bar{x}\pm s$, years)		56.49±13.27	54.18±14.52	0.906	0.367
Average of BMI ($\bar{x}\pm s$, kg/m^2)		24.58±4.29	24.13±4.38	0.569	0.571
Smoking [n (%)]		15 (27.78)	42 (61.76)	13.967	0.001
Pulmonary arterial systolic pressure ($\bar{x}\pm s$, mmHg)		29.87±2.99	37.15±3.74	11.650	0.001
PaO_2 ($\bar{x}\pm s$, mmHg)		86.13±8.64	67.25±6.74	13.560	0.001
$PaCO_2$ ($\bar{x}\pm s$, mmHg)		37.26±3.75	45.28±4.56	10.420	0.001
Predicted value of FEV_1% ($\bar{x}\pm s$, %)		79.26±6.43	51.08±5.16	26.860	0.001
FEV_1/FVC ($\bar{x}\pm s$, %)		82.47±9.35	57.24±5.68	18.390	0.001
CRP ($\bar{x}\pm s$, mg/L)		0.39±0.02	7.82±0.84	64.930	0.001
FIB ($\bar{x}\pm s$, g/L)		2.97±0.34	2.58±0.29	6.834	0.001
Times of acute aggravation within 1 year ($\bar{x}\pm s$, n)		-	2.37±0.26	66.930	0.001

Note: PaO_2, arterial partial pressure of oxygen; FEV_1%, predicted value of forced expiratory volume in 1 s; FVC, forced vital capacity; CRP, C-reactive protein; FIB, fibrinogen.

Table 2. Comparison of the average area of alveoli, inflammation score of the small airway and MA wall thickness between two groups ($\bar{x}\pm s$)

Group	Case (n)	Average area of alveoli (μm^2)	Score of lymphocyte infiltration into the airway wall (point)	Pathological score of small airway (point)	MA wall thickness (μm)
Control group	54	51.36±5.28	27.69±2.78	48.23±4.86	118.67±11.89
Treatment group	68	118.47±11.89	58.23±6.19	108.59±11.07	152.49±15.26
t		38.540	33.640	37.290	13.370
P		0.001	0.001	0.001	0.001

Note: MA, muscularized artery in pulmonary acinus.

Table 4. Univariate correlation analysis and multivariate Logistic regression analysis for the expression of IL-17 in the lung tissue of COPD patients with lung cancer.

Independent variable	Univariate correlation analysis		Multivariate Logistic regression analysis	
	r	*P*	*β*	*P*
CRP	**-0.357**	**0.001**	**-0.209**	**0.011**
FIB	**-0.182**	**0.029**	-	-
BMI	**0.189**	**0.025**	-	-
Predicted value of FEV_1%	-0.206	**0.015**	-	-
Times of acute aggravation within 1 year	-0.237	**0.008**	**-0.187**	**0.023**

parenchyma of patients in the treatment group was higher than that in the control group ($P < 0.05$; Table 3 and Figure 1).

Univariate correlation analysis and multivariate Logistic regression analysis for the expression of IL-17 in the lung tissue of COPD patients with lung cancer

Results of Pearson correlation analysis showed that the expression of IL-17 in the lung tissue of COPD patients with lung cancer was in positive correlation with BMI, but in negative correlation with CRP, FIB predicted value of FEV_1% and times of acute aggravation within 1 year. Then, with IL-17 expression as the dependent variable, CRP, FIB, BMI, predicted value of FEV_1% and times of acute aggravation within 1 year as independent variables, these variables would be set as 1 for any increase or 0 for normal and further integrated into the binary classification multivariate Logistic Regression models for analysis, and we found that CRP and times of acute aggravation within 1 year were the independent factors affecting the expression of IL-17 ($P < 0.05$; Table 4).

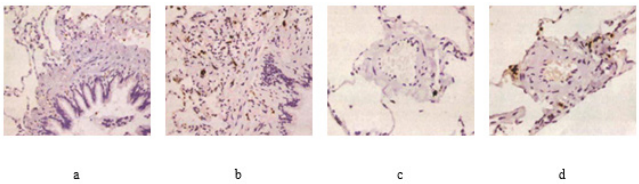

Figure 1. Immunohistochemistry figures of IL-17 in lung (×400). Note: a, Expression of IL-17 in the alveolar wall of subjects in the control group; b, Expression of IL-17 in the alveolar wall of subjects in the treatment group; c, Expression of IL-17 in the MA wall of a pulmonary acinus of subjects in the control group; d, Expression of IL-17 in the MA wall of a pulmonary acinus of subjects in the treatment group.

Discussion

COPD, as a kind of chronic inflammation frequently

seen in the airway, pulmonary parenchyma and vessels, could be exacerbated by the massively released inflammatory mediators and cytokines after the activation of inflammatory cells, yet there remains insufficient evidence to clarify the pathogens of COPD (9). Lung cancer, as a kind of malignant tumor originating from the bronchial mucosa or gland of the lung, is believed to be possibly correlated with smoking, diet, heredity and history of pulmonary diseases, which, however, have yet to be fully clarified. Besides, lung cancer is always neglected at the early stage due to the symptoms that are unapparent to attract the attention of patients, but with the progression and metastasis of lung cancer and the development of complications, patients may further encounter wheezing, trachyphonia, fever, blood-stained sputum and hemoptysis, but some patients may also experience the skin manifestations, including scleroderma, dermatomyositis and hyperkeratosis of the skin (10-11). In COPD patients with lung cancer, the immune system in the airway, lung and body becomes imbalanced, and under such circumstances, the inflammatory cascade will be further amplified and persist for a long time; the following interaction between them will eventually trigger the development, progression and deterioration of the disease.

IL-17, as a key member of the family of inflammatory mediators, promotes the release of a variety of inflammatory mediators, increases specifically in response to the inflammation and is able to mediate the inflammation and the acquired immune responses (12-13). Previous literatures (14-15) have reported that IL-17, a proinflammatory cytokine of T lymphocyte that is not only secreted by the Th17 cells, a subgroup of $CD4^+$ cells but also $CD8^+$T cells, could induce the massive secretion of perforin and granzyme that may injure the lung tissue. Results of this study demonstrated that in COPD patients with lung cancer, IL-17 was highly expressed in the alveolar wall, small airway wall and MA wall, suggesting the pivotal roles of IL-17 in the injury to the alveolar wall, development of emphysema, airway inflammation and remodeling of the pulmonary artery in COPD. CRP, as an acute phase protein, is found to be critical to the development of COPD, and, as such, it is used as an early inflammatory indicator for COPD (16). Our work also showed that CRP increased in COPD patients with lung cancer, and the exacerbation in inflammation could further aggravate the injury to the lung tissue and airway and make the disease hard to control. Therefore, in clinical treatment, it is necessary to decrease the level of CRP and alleviate inflammation and lung injury. Moreover, COPD is a chronic disease of the lung and shares the same pathogen – smoking with lung cancer (17-22). Data analysis in this study also indicated that smoking was the independent factor that affected the expression of IL-17 in COPD patients with lung cancer. This may attribute to the fact that smoking is one of the major environmental factors leading to the development of COPD, and the exposure of patients to smoking, dust, smog and conventional biological fuels, as the potential risk factors for COPD, could somehow result in the inflammation in airway and lung. Hence, smoking should be immediately withdrawn during clinical treatment to minimize the possibility of disease deterioration.

Overall, our data showed that IL-17 is upregulated in the airway wall and pulmonary parenchyma of COPD patients with lung cancer, and the expression of IL-17 is in positive correlation with BMI, but in negative correlation with CRP, FIB, predicted value of FEV_1% and times of acute aggravation within 1 year, while CRP and times of acute aggravation within 1 year are the independent risk factors for the expression of IL-17. Thus, IL-17 acts as a bridge connecting the innate immune and acquired immune to facilitate inflammation. However, this study is limited by the following factors: small sample size, single center and the territory restriction to the case origin, so in future work, we will carry out a multi-center study and expand the sample size and origin, so as to solidify the accuracy of this study.

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