European Review for Medical and Pharmacological Sciences 2016; 20: 44-53

Serum angiopoietin-like 4 is over-expressed in COPD patients: association with pulmonary function and inflammation

Y.-Q. WU[1,2], Y.-C. SHEN[1,2], H. WANG[1,2], J.-L. ZHANG[3], D.-D. LI[1,2], X. ZHANG[1,2], T. WANG[1,2], D. XU[1,2], B.-W. YING[3], L.-L. WANG[3], F.-Q. WEN[1,2]

[1]Department of Respiratory and Critical Care Medicine, West China Hospital of Sichuan University, Chengdu, Sichuan, People's Republic of China
[2]Division of Pulmonary Diseases, State Key Laboratory of Biotherapy of China, Chengdu, Sichuan, People's Republic of China
[3]Department of Laboratory Medicine, West China Hospital of Sichuan University, Chengdu, Sichuan, People's Republic of China

Yan-qiu Wu and Yong-chun Shen contributed equally to this work and share joint first authorship.

Abstract. – OBJECTIVE: Chronic obstructive pulmonary disease (COPD) is both a pulmonary and systematic disease, which will cause abnormal expression of some circulating factors. Angiopoietin-like protein 4 (ANGPTL4) has been reported to play important role in inflammatory responses and several diseases. However, whether it contributes to COPD is an open question. The aim of this study is to explore the potential relationship between ANGPTL4 and COPD.

PATIENTS AND METHODS: In this study, circulating levels of ANGPTL4, C-reactive protein (CRP), adiponectin, tumor necrosis factor (TNF)-α, matrix metalloproteinase (MMP)-9 and monocyte chemotactic protein (MCP)-1 in 73 COPD patients and 40 healthy volunteers were investigated using multiplex enzyme-linked immunosorbent assay Kits. Then, we analyzed the correlations between ANGPTL4 with other inflammatory mediators and pulmonary function.

RESULTS: Serum ANGPTL4 levels were significantly elevated in COPD patients compared with healthy controls (122.86 ± 38.59 ng/mL versus 99.03 ± 31.84 ng/mL, $p = 0.001$). Besides, serum ANGTPL4 levels were much higher in ever-smokers with COPD than in never-smokers with COPD (131.71 ± 32.92 ng/mL versus 113.25 ± 42.34 ng/mL, $p = 0.03$). More importantly, the concentrations of circulating ANGPLT4 correlated inversely with forced expiratory volume in 1 second (FEV_1) % predicted, an index of lung function in COPD ($r = -0.450$, $p < 0.001$) and in all participants ($r = -0.369$, $p < 0.001$), while correlated positively with CRP ($r = 0.312$, $p = 0.007$ for COPD; $r = 0.404$, $p < 0.001$ for total subjects), adiponectin ($r = 0.266$, $p = 0.004$ for total subjects), and MMP-9 ($r = 0.254$, $p = 0.03$ for COPD).

CONCLUSIONS: Our results suggest that circulating ANGPTL4 levels are up-regulated in COPD patients, and have correlations with pulmonary function and systematic inflammation in COPD, which provides a novel idea to further dig the pathogenic mechanisms of COPD, and justifies more studies to determine how ANGPTL4 contributes to COPD.

Key Words:
Chronic obstructive pulmonary disease, Angiopoietin-like protein 4, Cigarette smoke, Inflammation.

Introduction

Chronic obstructive pulmonary disease (COPD), characterized by irreversible airflow limitation; chronic airway inflammation, is the third leading cause of morbidity and mortality and bring about substantial health care burden in the world[1]. Except suffering from the inflammatory responses in respiratory system, COPD patients also have several extra-pulmonary manifestations, namely systemic inflammation, such as emaciation, skeletal muscle dysfunction, metabolic disorder and cardiovascular disorders[2], which will lead to abnormal expression of some circulating biomarkers, such as C-reactive protein (CRP), interleukin (IL), tumor necrosis factor (TNF), matrix metalloproteinase (MMP) and monocyte chemotactic protein (MCP)[3,4]. In recent years, growing studies have focused on seeking more biomarkers of COPD, offering

Corresponding Author: Fuqiang Wen, MD, Ph.D; e-mail: wenfuqiang.scu@gmail.com

more lines about the systemic inflammation of COPD. It has been reported that "spilling-over" of pulmonary inflammation, smoking, tissue hypoxia, lung hyperinflation, and skeletal muscle dysfunction may be the possible origins of systemic inflammation in COPD[5]. However, the exact mechanisms of systemic inflammation in COPD remain unclear[6].

Angiopoietin-like proteins (ANGPTLs) are a family of secreted proteins with a similar structure consisting of an N-terminal signal sequence, a unique region of variable length, a coiled-coil domain, and a large fibrinogen/angiopoietin like domain[7,8]. Angiopoietin-like protein 4 (ANGPTL4) is a member of this family, which is expressed in several tissues including liver, blood plasma, placenta, small intestine, heart and adipose tissue[9,10]. It has been demonstrated to regulate glucose homeostasis, insulin sensitivity and lipid metabolism, proliferation and apoptosis of vascular endothelial cells[11-13]. Several recent researches have shown that this multi-functional hormone also plays role in regulating inflammatory responses[14,15]. For example, ANGPTL4 expression in osteoblasts could be induced by IL-1β through c-Jun N-terminal kinase (JNK)-mitogen-activated protein kinase (MAPK) signaling pathway[16], and could also be stimulated by hypoxia through activation of hypoxia induced factor (HIF)-1α and peroxisome proliferator-activated receptors (PPARs)[17,18]. Moreover, ANGPTL4 was involved in LPS-induced lung injury[19] and tissue remodeling during lung injury[20]. Lu et al[21] previously reported that LPS, an activator of Toll-like receptor (TLR)-4, and Zymosan, a stimulator of TLR2 increased ANGPTL4 levels in liver, heart, muscle, adipose tissue, and serum.

Based on these studies above all, we learned that ANGPTL4 was an acute phase protein and had important functions in pulmonary inflammatory diseases, which rationalized a speculation that ANGPTL4 also played role in the COPD. However, studies on the correlation between COPD and ANGPTL4 are unavailable. Therefore, we designed this preliminary clinical study to explore whether ANGPTL4 is associated with COPD.

Patients and Methods

Subjects

We recruited 73 COPD patients from the Outpatient Department of West China Hospital and 40 smoking status, sex, Body Mass Index (BMI) and age-matched healthy volunteers from the hospital's physical examination center from November 2013 to October 2014. All subjects experienced a chest X-ray and a standard lung function test using a Spirotel® spiro-meter purchased from Mir Medical International Research Srl (Rome, Italy). Patients with COPD were diagnosed according to Global Initiative for Chronic Obstructive Lung Disease criteria, that is a lower-than-70% ratio of forced expiratory volume in 1 second to forced vital capacity (FEV_1/FVC) and (b) a less-than-12% increase in FEV_1 after inspiring bronchodilator[1]. None of them had ever been given inhaled glucocorticoid or antibiotics, and no one suffered an acute exacerbation within the last 12 weeks before our study. We gathered all the participants' basic healthy information by performing questionnaires and physical examinations. Patients with coronary heart disease, metabolic syndrome and other pulmonary diseases were excluded. This study was approved by the Institutional Review Board for Human Studies of West China Hospital of Sichuan University, China. Informed consents were obtained from all volunteers.

Blood Sampling and Analysis of Circulating ANGPTL4 and Blood Parameters

The fasting blood samples of all subjects were taken from cubital vein, and serum was isolated immediately for detection. Circulating ANGPTL4, CRP, adiponectin, TNF-α, MMP-9 and MCP-1 levels were determined using Human Magnetic Luminex Screening Assay (LXSAHM; R&D Systems, Inc. Minneapolis, MN, USA) on bio-plex200 platform (Bio-Rad, Hercules, CA, USA). The lower limits of detection were 86 pg/mL for ANGPTL4, 116 pg/mL for CRP, 148 pg/mL for adiponectin, 1.2 pg/mL for TNF-α, 13.6 pg/mL for MMP-9 and 9.9 pg/mL for MCP-1. All procedures were carried out strictly according to the manufacturer's instructions. Manipulators performing tests were blinded to the clinical characteristics of the subjects. The serum lipid was measured in the Department of Laboratory Medicine of West China Hospital.

Statistical Analysis

Continuous data were expressed as mean ± standard deviation (SD), whereas categorical data were presented as frequency and percent. After the normal distributions of these data were test-

ed, variables with skewed distributions such as CRP, adiponectin, TNF-α, MMP-9 and MCP-1 were log-transformed before further analyses. After the homogeneity of variance test, comparisons of parameters were analyzed by Student unpaired t test, one-way analysis of variance or Chi-square tests when appropriate. Pearson's correlation coefficient test was used for correlation analyses. Data analyses were carried out with SPSS 19.0 for Windows (IBM, Chicago, IL, USA). $p < 0.05$ was considered to be the threshold of statistical significance. Pictures were drawn using Graphpad Prism5.0 (GraphPad Software, San Diego, CA, USA).

Results

Characteristics of Subjects

Clinical characteristics of COPD and healthy control subjects were summarized in Table I and Table II. There were no differences between control subjects and COPD patients with respect to the age, sex, BMI, smoking pack-years and serum lipid. However, FEV_1, FEV_1 % predicted, and FEV_1/FVC between the two groups were significantly different ($p < 0.01$). In multi-groups analyses, the age, BMI, and blood oxygen saturation of all the groups were similar. There were no differences between smoking-COPD group and healthy smokers in gender composition and smoking pack years.

Increased serum ANGPTL4 Levels in Patients with COPD

As shown in Figure 1A, serum ANGPTL4 levels of COPD patients (122.86 ± 38.59 ng/mL) were significantly higher than that of healthy controls (99.03 ± 31.84 ng/mL) ($p = 0.001$). In addition, the circulating ANGPTL4 levels had relationship with smoking history, because we found that it was higher expressed in smoking-COPD than in never-smoking COPD (131.71 ± 32.92 ng/mL versus 113.25 ± 42.34 ng/mL, $p = 0.03$), smoking healthy (103.70 ± 36.51 ng/mL, $p = 0.01$) and never-smoking healthy (95.91 ±

Table I. Characteristics of patients with COPD and healthy subjects.

Characteristic	Healthy controls	COPD	*p*-value
Sex (m/f)	22/18	51/22	0.150*
Age	59.50±11.43	61.29 ± 10.83	0.412
Smoking status			0.329*
non-smoker (%)	24 (60)	34 (46.6)	
former smoker (%)	5 (12.5)	9 (12.3)	
current smoker (%)	11 (27.5)	30 (41.1)	
Pack years (ever-smokers)	24.03 ± 12.45	28.09 ± 18.12	0.418
SO_2	96.80 ± 1.64	96.48 ± 1.55	0.304
FEV_1 (L)	2.56 ± 0.73	1.95 ± 0.49	<0.001
FVC (L)	3.38 ± 0.89	3.33 ± 0.69	<0.001
FVE_1 % predicted	108.15 ± 16.35	81.21 ± 16.18	<0.001
FEV_1/FVC	79.39 ± 5.58	58.98 ± 9.02	<0.001
BMI	23.72 ± 3.60	22.83 ± 2.70	0.138
TG (mmol/L)	1.64 ± 0.69	1.34 ± 0.62	0.062
TC (mmol/L)	4.72 ± 0.80	4.54 ± 0.83	0.368
HDL (mmol/L)	1.35 ± 0.34	1.51 ± 0.38	0.076
LDL (mmol/L)	2.67 ± 0.74	2.35 ± 0.81	0.105
ANGPTL4 (ng/mL)	99.03 ± 31.84	122.86 ± 38.59	0.001
CRP (ug/mL)#	1.08 ± 1.21	2.36 ± 2.88	0.001
Adiponectin (ug/mL)#	5.27 ± 2.37	7.76 ± 4.73	0.001
TNF-α (pg/mL)#	15.97 ± 22.60	14.58 ± 22.92	0.962
MMP-9 (ng/mL)#	337.49 ± 192.46	301.62 ± 268.51	0.077
MCP-1 (pg/mL)#	290.73 ± 86.00	332.26 ± 311.87	0.590

Notes: Data are presented as mean ± SD. *: The chi-squared test was used to test the significance of the difference in gender and smoker proportions. #: As distributions of these parameters were skewed, log-transformed values were used in the statistical analyses. *Abbreviations:* COPD: chronic obstructive pulmonary disease; SO_2: oxygen saturation; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; BMI: Body Mass Index; TG: triglyceride; TC: total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; ANGPTL4: angiopoietin-like protein 4; CRP: C-reactive protein; TNF-α: tumor necrosis factor-α; MMP-9: matrix metalloprotein-9; MCP-1: monocyte chemotactic protein[1].

Table II. Characteristics of multi-groups.

Characteristic	NS-H	S-H	NS-COPD	S-COPD	*p*-value
Sex (m/f)	9/17	13/1	9/22	42/0	<0.001**
Age	61.00±10.01	57.25±13.29	60.57±10.89	61.95±10.89	0.563
BMI	23.64±3.73	23.85±3.52	23.43±2.60	22.27±2.70	0.181
Pack years	0	24.03±12.45	0	28.09±18.12	<0.001**
FEV1 (L)	2.23±0.66	3.06±0.53	1.82±0.42	2.06±0.53	<0.001
FVC (L)	2.81±0.78	3.97±0.51	2.98±0.55	3.65±0.65	<0.001
FVE_1 % predicted	107.29±18.16	109.44±13.65	84.54±16.54	78.13±15.41	<0.001
FEV_1/FVC	81.12±5.55	76.80±4.68	61.37±8.37	56.79±9.15	<0.001
SO_2	97.13±1.30	96.31±1.99	96.49±1.29	96.47±1.77	0.310
TG	1.69±0.71	1.60±0.70	1.38±0.75	1.31±0.50	0.291
TC	4.68±0.70	4.59±0.59	4.38±0.81	4.68±0.83	0.368
HDL	1.29±0.41	1.41±0.23	1.51±0.40	1.52±0.37	0.285
LDL	2.78±0.89	2.56±0.58	2.12±0.70	2.54±0.87	0.081
ANGPTL4	95.91±28.71	103.70±36.51	113.25±42.34	131.71±32.92	0.001
CRP (ug/mL)#	1.07±1.18	1.09±1.29	1.41±1.59	3.38±3.45	0.001
Adiponectin (ug/mL)#	5.35±2.33	5.16±2.51	8.78±5.29	6.83±2.40	0.002
TNF-α (pg/mL)#	16.92±24.97	14.54±19.19	11.74±16.42	17.20±27.56	0.709
MMP-9 (pg/mL)#	360.45±202.21	303.06±177.48	243.31±188.90	355.33±318.33	0.115
MCP-1 (pg/mL)#	304.39±104.43	270.25±42.03	352.98±437.64	313.18±111.78	0.869

Notes: Data are presented as mean ± SD. #: The data were log-transformed in the statistical analyses. **: There's no difference between healthy smokers and smokers with COPD. *Abbreviations:* NS-H: never smoking-healthy; S-H: smoking-healthy (including former smokers); NS-COPD: never smokers with chronic obstructive pulmonary disease; S-COPD: smokers with chronic obstructive pulmonary disease (including former smokers); SO_2: oxygen saturation; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; BMI: Body Mass Index; TG: triglyceride; TC: total cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein; ANGPTL4: angiopoietin-like protein 4; CRP: C-reactive protein; TNF-α: tumor necrosis factor-α; MMP-9: matrix metalloproteinase-9; MCP-1: monocyte chemotactic protein[1].

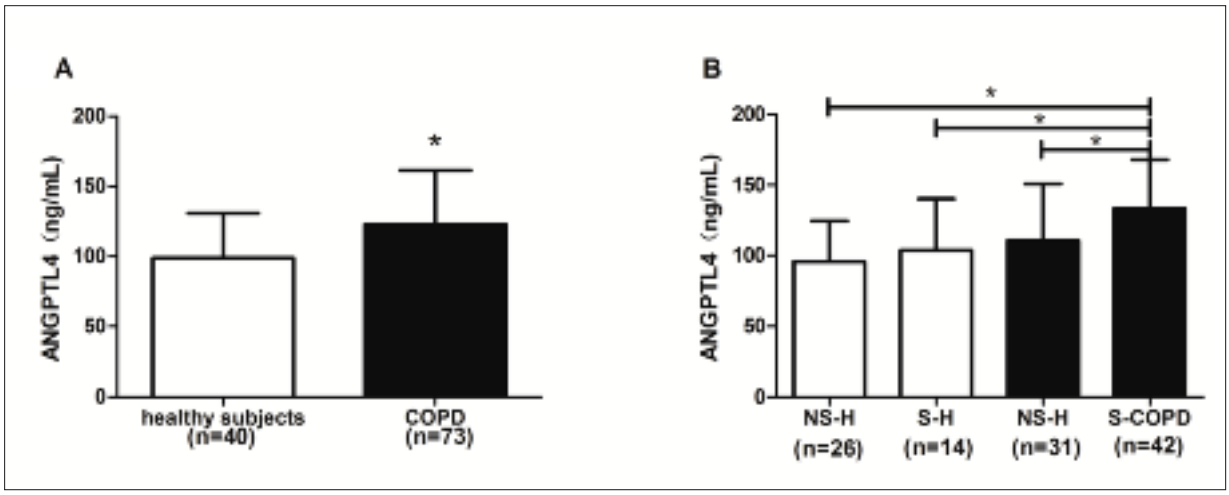

Figure 1. Levels of ANGPTL4 in the plasma from healthy group and chronic obstructive pulmonary disease (COPD) group. ***A,*** COPD patients had a significantly elevated ANGPTL4 levels when compared with healthy controls ($p = 0.001$). ***B,*** ANGPTL4 was higher expressed in smoking-COPD than in never-smoking COPD ($p = 0.03$), smoking healthy ($p = 0.01$) and never-smoking healthy ($p < 0.001$). Circulating ANGPTL4 levels were measured using multiplex enzyme-linked immunosorbent assay. The expression of ANGPTL4 was presented as the mean (error bar) and compared by unpaired Student's t-test. (*: $p < 0.05$). *Abbreviations:* COPD: chronic obstructive pulmonary disease; ANGPTL4: angiopoietin-like protein 4; NS-H: never smoking-healthy; S-H: smoking-healthy (including former smokers); NS-COPD: never smokers with chronic obstructive pulmonary disease; S-COPD: smokers with chronic obstructive pulmonary disease (including former smokers).

28.71 ng/mL, $p < 0.001$) (Figure 1B). Similar results were found in CRP, adiponectin (Tables I and II).

ANGPTL4 was Correlated with Lung Function and Other Serum Biomarkers

Serum levels of ANGPTL4 correlated inversely with FEV_1 % predicted in COPD and in all subjects (r = -0.450, $p < 0.001$; r = -0.369, $p < 0.001$, respectively, Figure 2A). Similar results were found between ANGPTL4 and FEV_1/FVC (r = -0.383, $p = 0.001$ for COPD; r = -0.461, $p < 0.001$ for all participants, Figure 2B). Conversely, ANGPTL4 levels correlated positively with CRP in patients and in all enrolled subjects (r = 0.312, $p = 0.007$; r = 0.404, $p < 0.001$, respectively, Figure 3A). Moreover, ANGPTL4 was also associated with adiponectin in total subjects (r = 0.266, $p = 0.004$, Figure 3B) and MMP-9 in COPD (r = 0.254, $p = 0.03$, Figure 3C), whereas ANGPTL4 did not correlate with TNF-α or MCP-1. All the correlation coefficients were summarized in the Table III.

Discussion

In this study, we investigated the circulating ANGPTL4 levels in 73 COPD cases and 40 controls using multiplex enzyme-linked immunosorbent assay. We observed that the serum levels of ANGPTL4 were remarkably elevated in COPD patients, and that circulating ANGPTL4 had relationship with smoking history. Further analyses demonstrated that ANGPTL4 levels had significant associations with indicators of lung function decline (FEV_1 % predicted and FEV_1/FVC) in COPD patients and systemic inflammation (CRP, adiponectin, and MMP-9). To the best of our knowledge, this is the first study to explore the associations between ANGPTL4 with lung function and systematic inflammation in COPD.

The angiopoietin-like proteins were a family of secreted proteins that played important roles in energy metabolism[9,22]. Furthermore, It has been proved that ANGPTL4 might also play role in other conditions, such as acute phase response[21], rheumatoid arthritis[23], carcinoma[24], hypoxic (low oxygen) conditions[25], oxidative stress[26], pneumonia[27] and lipopolysaccharide-induced acute lung injury[19]. Previous studies have measured the circulating ANGPTL4 levels in several diseases[23, 24,28, 29]. However, whether ANGPTL4 is related with COPD was not covered in previous studies.

In our study, we observed a marked elevation of serum ANGPTL4 levels in COPD patients, indicating ANGPTL4 might contribute to the pathogenesis of COPD, being accordant with numerous indirect evidences linking ANGPTL4 with this disease. Firstly, LPS and several cytokines (Tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and interferon (IFN)-γ) could increase the expression of ANGPTL4 in heart,

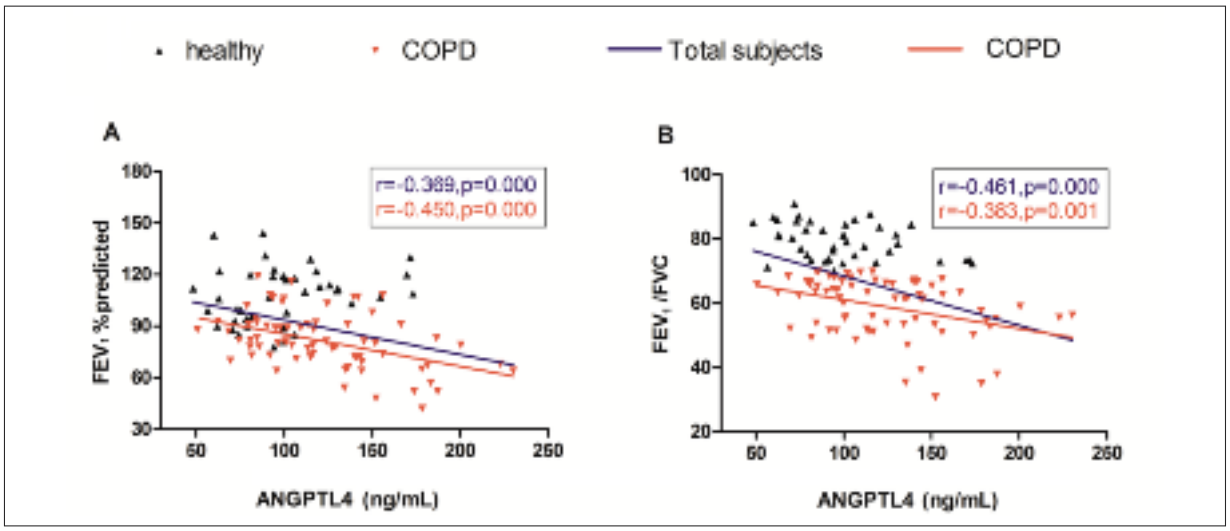

Figure 2. Correlations between serum ANGPTL4 levels and lung function (FEV_1 % predicted, FEV_1/FVC). The orange solid line denotes the line of best fit in COPD, the blue one represents the line of best fit in all subjects, and Pearson's correlation coefficient is presented as an r-value. *Abbreviations:* COPD: chronic obstructive pulmonary disease; ANGPTL4: angiopoietin-like protein 4; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity.

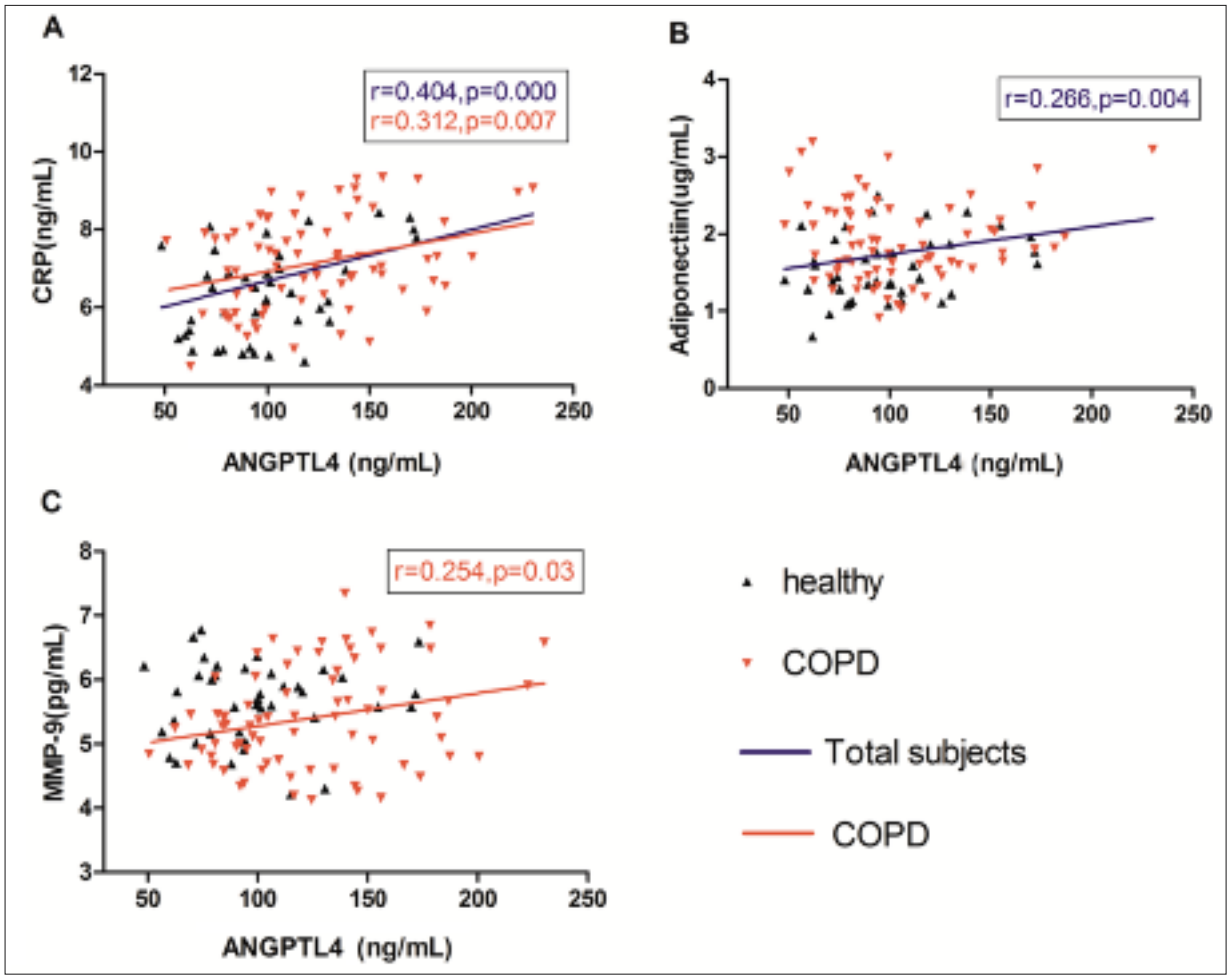

Figure 3. Correlations between serum levels of ANGPTL4 with CRP, MMP-9, and adiponectin. CRP, MMP-9 and adiponectin were log-transformed in the statistical analyses. The orange solid line denotes the line of best fit in COPD, the blue one represents the line of best fit in all subjects, and Pearson's correlation coefficient is presented as an r-value. *Abbreviations:* COPD: chronic obstructive pulmonary disease; ANGPTL4: angiopoietin-like protein 4; CRP: C-reactive protein; MMP-9: matrix metalloprotein-9.

Table III. Correlations between ANGPTL4 and other parameters.

	ANGPTL4 (in COPD)	ANGPTL4 (in total subjects)
FEV_1 % predicted	r = -0.450, $p<0.001$	r = -0.369, $p<0.001$
FEV_1/FVC	r = -0.383, $p = 0.001$	r = -0.461, $p<0.001$
CRP#	r = 0.312, $p = 0.007$	r = 0.404, $p<0.001$
Adiponectin#	r = 0.153, $p = 0.197$	r = 0.266, $p = 0.004$
MMP-9#	r = 0.254, $p = 0.030$	r = 0.140, $p = 0.138$
TNF-α#	r = 0.009, $p = 0.940$	r = 0.038, $p = 0.688$
MCP-1#	r = -0.700, $p = 0.557$	r = 0.023. $p = 0.812$

Notes: The correlations between variables were analyzed using Pearson's correlation analysis. Pearson's correlation coefficient is presented as an r-value. #: As distributions of these parameters were skewed, log-transformed values were used in the statistical analyses. *Abbreviations:* COPD: chronic obstructive pulmonary disease; FEV^1: forced expiratory volume in one second; FVC: forced vital capacity; ANGPTL4: angiopoietin-like protein 4; CRP: C-reactive protein; TNF-α: tumor necrosis factor-α; MMP-9: matrix metalloprotein-9; MCP-1: monocyte chemotactic protein[1].

muscle, and adipose tissue[21]. It was well recognized that IL-1β and TNF-α could be induced in COPD[30,31], and that IFN-γ could cause pulmonary emphysema in the lung of adult murine[32]. This might be one of the explanations why the serum ANGPTL4 levels were increased in COPD. Secondly, LPS could induce the expression of ANGPTL4 in human alveolar epithelial A549 cells and in the lung tissues of mice by promoting nuclear factor kappa B (NF-kB) p65 expression and suppressing sirtuin (SIRT1) expression[19]. It has been reported that NF-KBp65 was over-expressed in the lung tissue from COPD patients[33], and SIRT1 was decreased in the lung tissue of smokers and COPD patients[34]. We could deduce that the up-regulation of ANGPTL4 in COPD patients might have correlations with the activation of NF-KB and the reduction of SIRT1. Besides, ANGPTL4 could be induced by the transforming growth factor-β (TGF-β)[35]. TGF-β has been proved to be elevated in COPD, and participate in the inflammatory and remodeling process of airway disease (including COPD)[36]. Moreover, protein kinase C (PKC) activators, including phorbol-12-myristate-13-acetate (PMA) and platelet-derived growth factor α could induce ANGPTL4 in normal lung cell types and carcinoma cell lines. In human airway smooth muscle (HASM) cells, PMA induced ANGPTL4 through activating the PKC, extracellular signal-regulated kinase (ERK), and JNK signal pathways[20]. To our knowledge, the activations of these signal pathways were also involved in the physiopathology of COPD[37-40]. Although the exact mechanism of the increase of ANGPTL4 could not be elucidated, all the evidences above predicted the potential role of ANGPTL4 in COPD and our study extended the story by providing direct evidence for the first time that ANGPTL4 may be involved in COPD.

In addition, we also found ANGPTL4 levels were higher in smokers with COPD than in never-smokers with COPD, healthy smokers and healthy never-smokers, indicating that circulating ANGPTL4 levels had relations with smoking history and perhaps different mechanisms exist between smokers-COPD and never-smokers COPD. Due to the limited subjects within this study, we can't make a final conclusion whether the differences between COPD smokers with COPD non-smokers and healthy non-smokers attributed to the difference of smoking status and gender. However, the result that the expression of ANGPTL4 in smokers with COPD differed from smokers without COPD suggested that ANGPTL4 might be involved in the development of COPD induced by smoking.

More importantly, we also observed that there was an inverse correlation between ANGPTL4 and lung function (FEV_1 % predicted and FEV_1/FVC) in patients and in all participants, indicating that ANGPTL4 had relationship with the severity of airway obstruction. Previous studies[41] have shown that increase in the thickness of small airways, loss of parenchymal caused by emphysema, and mucus hypersecretion contributed to the airway obstruction in COPD. Despite the evidences linking ANGPTL4 with this COPD above, the exact role of ANGPTL4 in airflow obstruction could not be elucidated and needed further researches. Meanwhile, our present study identified that ANGPTL4 correlated positively with CRP, adiponectin and MMP-9. CRP was an acute phase protein that is synthesized mainly by the hepatocytes in response to inflammation or tissue damage[42]. Matrix metalloprotease (MMP)-9, an elastolytic endopeptidase released by inflammatory cells such as activated macrophages, has been thought to be involved in the pathogenesis of COPD[43]. Circulating CRP and MMP-9 levels were higher in COPD, and were associated with FEV_1 decline, thus they have been regarded as valid biomarker of systemic inflammation in COPD[3,44]. Therefore, the positive correlations between ANGPTL4 with CRP and MMP-9 in COPD patients suggested that ANGPTL4 might function as a biomarker of the severity of systemic inflammation in COPD. Adiponectin, a well-known adipokine, was predominantly secreted by adipocytes and had anti-inflammatory, anti-atherosclerotic, and anti-diabetic properties[45]. Serum adiponectin levels were elevated in COPD and contributed to metabolic derangements in the pathophysiology of emphysema[46]. The result that ANGPTL4 correlated positively with adiponectin indicated that ANGPTL4 might contribute to the metabolic disturbance in COPD. Of cause, larger studies should be conducted to figure out how ANGPTL4 was induced in COPD and which signaling pathways were involved in during the pathogenesis of COPD.

This study aimed to investigate whether ANGPTL4 has correlation with COPD. The results confirmed our hypothesis by showing serum ANGPTL4 levels were significantly higher in COPD and have relations with pul-

monary function and systemic inflammation. However, the relatively small number of subjects meant that we could not assess the relations between ANGPTL4 with lung function and inflammation in different stages of COPD and might not draw an absolutely right conclusion. Because the majority of our COPD patients were characterized by relatively mild airway obstruction, additional studies recruiting patients with more severe COPD are needed to verify our findings. Moreover, since we chose only 4 indicators of systemic inflammation (CRP, TNF-α, MMP-9 and MCP-1) as the indicators of inflammation, we also couldn't explain how ANGPTL4 contributes COPD and further studies are needed to elucidate the potential mechanisms under the findings presented in our study. In addition, without computed tomography to detect the presence of emphysema, we could not illuminate how ANGPTL4 correlated with lung function and inflammation in different phenotypes of COPD.

Conclusions

Despite the limitations of our present study, this is the first evidence that ANGPTL4 may play role in COPD. Combined analysis of the correlation of serum ANGPTL4 concentration with lung function and inflammation indicators of COPD gives us a clue that ANGPTL is involved in airway obstruction and systemic inflammatory reaction of COPD, and it has a latent ability of serving as a systemic biomarker for COPD severity. This study provides a new idea to further understand the mechanism responsible for the pathogenesis and development of COPD.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (81230001, 81300032, 81470236, 31171103), National Key Technology Research and Development Program of the Ministry of Science and Technology of China during the "12th Five-Year Plan" (2012BAI05B02, 2014BAI08B04) and Projects in the Science and Technology Pillar Program from the Department of science and technology of Sichuan province (2015SZ0151). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Vestbo J, Hurd Ss, Agusti Ag, Jones Pw, Vogelmeier C, Anzueto A, Barnes Pj, Fabbri Lm, Martinez Fj, Nishimura M, Stockley Ra, Sin Dd, Rodriguez-Roisin R. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2013; 187: 347-365.
2) Macnee W. Systemic inflammatory biomarkers and co-morbidities of chronic obstructive pulmonary disease. Ann Med 2013; 45: 291-300.
3) Higashimoto Y, Iwata T, Okada M, Satoh H, Fukuda K, Tohda Y. Serum biomarkers as predictors of lung function decline in chronic obstructive pulmonary disease. Respir Med 2009; 103: 1231-1238.
4) Wang Y, Zheng Y, Zhai Yl, Liu Fq, Ding N. Comparative analysis of MCP-1 and TF in elderly patients with acute exacerbations of COPD and its clinical significance. Eur Rev Med Pharmacol Sci 2015; 19: 215-219.
5) Agusti A. Systemic effects of chronic obstructive pulmonary disease: what we know and what we don't know (but should). Proc Am Thorac Soc 2007; 4: 522-525.
6) Barnes Pj, Celli Br. Systemic manifestations and comorbidities of COPD. Eur Respir J 2009; 33: 1165-1185.
7) Hato T, Tabata M, Oike Y. The role of angiopoietin-like proteins in angiogenesis and metabolism. Trends Cardiovasc Med 2008; 18: 6-14.
8) Kersten S. Regulation of lipid metabolism via angiopoietin-like proteins. Biochem Soc Trans 2005; 33: 1059-1062.
9) Kersten S, Mandard S, Tan Ns, Escher P, Metzger D, Chambon P, Gonzalez Fj, Desvergne B, Wahli W. Characterization of the fasting-induced adipose factor FIAF, a novel peroxisome proliferator-activated receptor target gene. J Biol Chem 2000; 275: 28488-28493.
10) Kersten S, Lichtenstein L, Steenbergen E, Mudde K, Hendriks Hfj, Hesselink Mk, Schrauwen P, Muller M. Caloric Restriction and Exercise Increase Plasma ANGPTL4 Levels in Humans via Elevated Free Fatty Acids. Arterioscler Thromb Vasc Biol 2009; 29: 969-974.
11) Xu A, Lam Mc, Chan Kw, Wang Y, Zhang J, Hoo Rl, Xu Jy, Chen B, Chow Ws, Tso Aw, Lam Ks. Angiopoietin-like protein 4 decreases blood glucose and improves glucose tolerance but induces hyperlipidemia and hepatic steatosis in mice. Proc Natl Acad Sci U S A 2005; 102: 6086-6091.
12) Sukonina V, Lookene A, Olivecrona T, Olivecrona G. Angiopoietin-like protein 4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue. Proc Natl Acad Sci U S A 2006; 103: 17450-17455.
13) Mandard S, Zandbergen F, Van Straten E, Wahli W, Kuipers F, Muller M, Kersten S. The fasting-induced adipose factor/angiopoietin-like protein 4 is physi-

cally associated with lipoproteins and governs plasma lipid levels and adiposity. J Biol Chem 2006; 281: 934-944.

14) Guo L, Li Sy, Ji Fy, Zhao Yf, Zhong Y, Lv Xj, Wu Xl, Qian Gs. Role of Angptl4 in vascular permeability and inflammation. Inflamm Res 2014; 63: 13-22.

15) Tjeerdema N, Georgiadi A, Jonker Jt, Van Glabbeek M, Alizadeh Dehnavi R, Tamsma Jt, Smit Jw, Kersten S, Rensen Pc. Inflammation increases plasma angiopoietin-like protein 4 in patients with the metabolic syndrome and type 2 diabetes. BMJ Open Diabetes Res Care 2014; 2: e000034.

16) Noh Jm, Shen C, Kim Sj, Kim Mr, Kim Sh, Kim Jh, Park Bh, Park Jh. Interleukin-1beta Increases Angptl4 (FIAF) Expression via the JNK Signaling Pathway in Osteoblastic MC3T3-E1 Cells. Exp Clin Endocrinol Diabetes 2015; 123: 445-60.

17) Ge H, Cha Jy, Gopal H, Harp C, Yu X, Repa Jj, Li C. Differential regulation and properties of angiopoietin-like proteins 3 and 4. J Lipid Res 2005; 46: 1484-1490.

18) Gealekman O, Burkart A, Chouinard M, Nicoloro Sm, Straubhaar J, Corvera S. Enhanced angiogenesis in obesity and in response to PPARgamma activators through adipocyte VEGF and ANGPTL4 production. Am J Physiol Endocrinol Metab 2008; 295: E1056-1064.

19) Guo L, Li S, Zhao Y, Qian P, Ji F, Qian L, Wu X, Qian G. Silencing angiopoietin-like protein 4 (ANGPTL4) protects against lipopolysaccharide-induced acute lung injury via regulating SIRT1 /NF-kB pathway. J Cell Physiol 2015; 230: 2390-402.

20) Stapleton Cm, Joo Jh, Kim Ys, Liao G, Panettieri Ra, Jr., Jetten Am. Induction of ANGPTL4 expression in human airway smooth muscle cells by PMA through activation of PKC and MAPK pathways. Exp Cell Res 2010; 316: 507-516.

21) Lu B, Moser A, Shigenaga Jk, Grunfeld C, Feingold Kr. The acute phase response stimulates the expression of angiopoietin like protein 4. Biochem Biophys Res Commun 2010; 391: 1737-1741.

22) Yoon Jc, Chickering Tw, Rosen Ed, Dussault B, Qin Y, Soukas A, Friedman Jm, Holmes We, Spiegelman Bm. Peroxisome proliferator-activated receptor gamma target gene encoding a novel angiopoietin-related protein associated with adipose differentiation. Mol Cell Biol 2000; 20: 5343-5349.

23) Swales C, Athanasou Na, Knowles Hj. Angiopoietin-like 4 is over-expressed in rheumatoid arthritis patients: association with pathological bone resorption. PLoS One 2014; 9: e109524.

24) Yi J, Pan Bz, Xiong L, Song Hz. Clinical significance of angiopoietin-like protein 4 expression in tissue and serum of esophageal squamous cell carcinoma patients. Med Oncol 2013; 30: 680.

25) Zhou L, Wang Lm, Song Hm, Shen Yq, Xu Wj, Xu Jh, Liu Y, Yan Ww, Jiang Jf. Expression profiling analysis of hypoxic pulmonary disease. Genet Mol Res 2013; 12: 4162-4170.

26) Georgiadi A, Lichtenstein L, Degenhardt T, Boekschoten Mv, Van Bilsen M, Desvergne B, Muller M, Kersten S. Induction of cardiac Angptl4 by dietary fatty acids is mediated by peroxisome proliferator-activated receptor beta/delta and protects against fatty acid-induced oxidative stress. Circ Res 2010; 106: 1712-1721.

27) Li L, Chong Hc, Ng Sy, Kwok Kw, Teo Z, Tan Eh, Choo Cc, Seet Je, Choi Hw, Buist Ml, Chow Vt, Tan Ns. Angiopoietin-like 4 increases pulmonary tissue leakiness and damage during influenza pneumonia. Cell Rep 2015; 10: 654–663.

28) Robciuc Mr, Naukkarinen J, Ortega-Alonso A, Tyynismaa H, Raivio T, Rissanen A, Kaprio J, Ehnholm C, Jauhiainen M, Pietilainen Kh. Serum angiopoietin-like 4 protein levels and expression in adipose tissue are inversely correlated with obesity in monozygotic twins. J Lipid Res 2011; 52: 1575-1582.

29) Clement Lc, Mace C, Avila-Casado C, Joles Ja, Kersten S, Chugh Ss. Circulating angiopoietin-like 4 links proteinuria with hypertriglyceridemia in nephrotic syndrome. Nat Med 2014; 20: 37-46.

30) Joppa P, Petrasova D, Stancak B, Tkacova R. Systemic inflammation in patients with COPD and pulmonary hypertension. Chest 2006; 130: 326-333.

31) Chung Kf. Cytokines in chronic obstructive pulmonary disease. Eur Respir J Suppl 2001; 34: 50s-59s.

32) Wang Z, Zheng T, Zhu Z, Homer Rj, Riese Rj, Chapman Ha, Jr., Shapiro Sd, Elias Ja. Interferon gamma induction of pulmonary emphysema in the adult murine lung. J Exp Med 2000; 192: 1587-1600.

33) Szulakowski P, Crowther Aj, Jimenez La, Donaldson K, Mayer R, Leonard Tb, Macnee W, Drost Em. The effect of smoking on the transcriptional regulation of lung inflammation in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2006; 174: 41-50.

34) Rajendrasozhan S, Yang Sr, Kinnula Vl, Rahman I. SIRT1, an antiinflammatory and antiaging protein, is decreased in lungs of patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2008; 177: 861-870.

35) Padua D, Zhang Xh, Wang Q, Nadal C, Gerald Wl, Gomis Rr, Massague J. TGFbeta primes breast tumors for lung metastasis seeding through angiopoietin-like 4. Cell 2008; 133: 66-77.

36) Yang Yc, Zhang N, Van Crombruggen K, Hu Gh, Hong Sl, Bachert C. Transforming growth factor-beta1 in inflammatory airway disease: a key for understanding inflammation and remodeling. Allergy 2012; 67: 1193-1202.

37) Kim S, Nadel Ja. Fibrinogen binding to ICAM-1 promotes EGFR-dependent mucin production in human airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 2009; 297: L174-183.

38) Dempsey Ec, Cool Cd, Littler Cm. Lung disease and PKCs. Pharmacol Res 2007; 55: 545-559.

39) Chen Y, Thai P, Zhao Yh, Ho Ys, Desouza Mm, Wu R. Stimulation of airway mucin gene expression by

interleukin (IL)-17 through IL-6 paracrine/autocrine loop. J Biol Chem 2003; 278: 17036-17043.

40) Hoshino S, Yoshida M, Inoue K, Yano Y, Yanagita M, Mawatari H, Yamane H, Kijima T, Kumagai T, Osaki T, Tachiba I, Kawase I. Cigarette smoke extract induces endothelial cell injury via JNK pathway. Biochem Biophys Res Commun 2005; 329: 58-63.

41) Barnes Pj, Shapiro Sd, Pauwels Ra. Chronic obstructive pulmonary disease: molecular and cellular mechanisms. Eur Respir J 2003; 22: 672-688.

42) Pepys Mb, Hirschfield Gm. C-reactive protein: a critical update. J Clin Invest 2003;111:1805-1812.

43) Lim S, Roche N, Oliver Bg, Mattos W, Barnes Pj, Chung Kf. Balance of matrix metalloprotease-9 and tissue inhibitor of metalloprotease-1 from alveolar macrophages in cigarette smokers. Regulation by interleukin-10. Am J Respir Crit Care Med 2000; 162: 1355-1360.

44) Montano M, Sansores Rh, Becerril C, Cisneros J, Gonzalez-Avila G, Sommer B, Ochoa L, Herrera I, Ramirez-Venegas A, Ramos C. FEV_1 inversely correlates with metalloproteinases 1, 7, 9 and CRP in COPD by biomass smoke exposure. Respir Res 2014; 15: 74.

45) Shehzad A, Iqbal W, Shehzad O, Lee Ys. Adiponectin: regulation of its production and its role in human diseases. Hormones (Athens) 2012; 11: 8-20.

46) Carolan Bj, Kim Yi, Williams Aa, Kechris K, Lutz S, Reisdorph N, Bowler Rp. The association of adiponectin with computed tomography phenotypes in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2013; 188: 561-566.