Cytokine 111 (2018) 342–349

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Serum cytokine profiles in patients with chronic obstructive pulmonary disease associated pulmonary hypertension identified using protein array

Yi Zhang[a,1], Peiyi Lin[a,1], Cheng Hong[b], Qian Jiang[b], Yue Xing[a], Xiaoyan Tang[a], Huilin Jiang[a], Shuhong Luo[c,d], Xiaohui Chen[a,*]

[a] The Second Affiliated Hospital of Guangzhou Medical University, 250 Changangdong Rd., Guangzhou, Guangdong 510260, China
[b] State Key Laboratory of Respiratory Diseases, Guangzhou Institute of Respiratory Disease, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China
[c] Guangzhou Raybiotech, Inc., 79 Ruihe Road, Guangzhou, 515000, China
[d] RayBiotech, Inc., 3607 Parkway Lane Suite 200, Norcross, GA 30092, USA

ARTICLE INFO

Keywords:
Chronic obstructive pulmonary disease
Pulmonary hypertension
Antibody array
Biomarkers

ABSTRACT

Pulmonary hypertension (PH) is a common complication of chronic obstructive pulmonary disease (COPD) and is a significant risk factor for hospitalization and shortened life expectancy. Therefore, developing new serum biomarkers for early diagnosis and prognosis of COPD associated PH is crucial. In the present study, a solid-phase antibody array simultaneously detecting multiple proteins was used to search specific COPD associated PH biomarkers, with COPD patients and healthy subjects as control groups. As a result, compared to the COPD and healthy groups, the levels of MCP-4, SDF-1 alpha, CCL28, Adipsin, IL-28A, CD40 and AgRP were uniquely altered in COPD patient serum with pulmonary hypertension. Among these proteins, CCL28, MCP-4, CD40, AgRP and IL-28A were identified to be differentially expressed in COPD patients with hypertension, indicating that these cytokines may serve as novel biomarkers for the diagnosis and prognosis of COPD associated pulmonary hypertension.

1. Introduction

Pulmonary hypertension (PH) is primarily caused by chronic obstructive pulmonary disease (COPD), which is a global lung disease with high mortality and morbidity, and expected to be the fourth leading cause of death in the world in 2030 [1]. PH is an important factor for acute exacerbation of COPD and increases the hospitalization rate and the mortality of the patients with COPD [2].

PH is characterized by pulmonary vascular remodeling, including medial hypertrophy, adventitial thickening, intimal proliferative changes, and fibrosis. Although right heart catheterization (RHC) is the gold standard for diagnosing and staging PH [3], it is invasive and can be complicated with bleeding, pneumothorax, and other risks. RHC may not be widely available in patients with COPD. Clinically, Ultrasonic cardiogram (UCG) can be an effective measure to assess pulmonary artery systolic pressure. However, UCG has low sensitivity, specificity, and predictive values in patients with COPD mainly because the technical drawbacks in finding good windows [4,5]. Thus far, PH remains a clinical dilemma with regard to accurate risk assessment and efficient management due to a lack of sufficient clinical or paraclinical variables for clinical decisions.

Biomarkers, involving normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention, are considered to be a useful, noninvasive tool for better diagnoses, accurate risk assessment, and appropriate management. Biomarkers are being increasingly recognized to have great clinical value. Recently, more and more studies have investigated specific PH medications in COPD [6–13] with various inclusion criteria. However, the identification of specific PH biomarkers in COPD is rare. Therefore, identifying specific biomarkers for early diagnosis of COPD associated PH is urgently needed.

New, promising strategies for biomarker discovery include microarray assay and mass-spectrometry for the protein level detection [14]. However, mass-spectrometry suffers from high false positive rates and poor sensitivity. Antibody microarrays are a novel technology simultaneously detecting multiple proteins with the advantage of being high-throughput amenable [15]. The aim of the present study was to identify specific PH biomarkers to improve PH diagnosis and clinical

* Corresponding author.
E-mail address: cxhgz168@126.com (X. Chen).
[1] These authors have contributed equally to this work.

https://doi.org/10.1016/j.cyto.2018.09.005
Received 20 March 2018; Received in revised form 9 September 2018; Accepted 11 September 2018

Table 1
Participant characteristics for antibody array.

Variables	COPD + PH group	COPD group	Control group	p Value (ANOVA)
Number	6	6	6	NA
Age (yr)	65.83 ± 4.75	62.50 ± 7.61	58.33 ± 8.96	0.983
Sex (M/F)	6/0	6/0	6/0	NA
Severity of PH (mmHg)	Mild, (6.7%) Moderate, (50.0%) Severe, (33.3%)	NA	NA	

Severity of PH (mmHg): mild (41–50), moderate (51–60), severe > 60. There is no significant difference in age among the three groups.

Table 2
Participant characteristics for ELISA.

Variables	COPD + PH group	COPD group	Control group	p Value (ANOVA)
Number	20	20	19	NA
Age (yr)	62.35 ± 6.24	62.20 ± 8.28	62.37 ± 5.95	0.872
Sex (M/F)	18/2	18/2	17/2	NA
Severity of PH (mmHg)	Mild, (40%) Moderate, (30%) Severe, (30%)	NA	NA	

Severity of PH (mmHg): mild (41–50), moderate (51–60), severe > 60. There is no significant difference in age among the three groups.

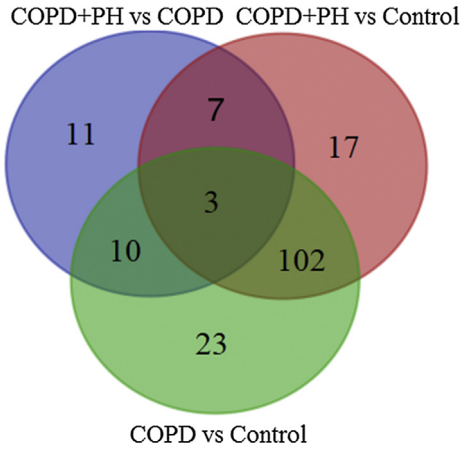

Fig. 1. Venn diagram analysis. The differentially expressed proteins between the COPD + PH, COPD and healthy control groups were analyzed by Venn diagram to identify specific COPD associated PH biomarkers.

Table 3
The information of seven specific COPD associated PH biomarkers.

		COPD vs Control		COPD + PH vs Control		COPD + PH vs COPD	
Targets	Gene ID	p value	Fold change	p value	Fold change	p value	Fold change
MCP-4	6357	1.000	0.997	0.008	3.422	0.007	3.433
CD40	958	1.000	0.927	0.002	0.605	0.009	0.653
SDF-1a	6387	1.000	0.769	0.033	1.858	0.007	2.415
CCL28	56,477	1.000	0.932	0.001	2.550	0.001	2.736
Adipsin	1675	1.000	1.023	0.008	1.463	0.011	1.429
AgRP	181	1.000	0.882	0.009	0.450	0.041	0.510
IL-28A	282,616	1.000	1.176	0.003	3.882	0.005	3.301

management in patients with COPD.

2. Materials and methods

2.1. Patients

6 COPD patients were recruited based on the Global Initiative for Chronic Obstructive Lung Disease criteria (Called COPD group). Another 6 COPD patients suffering from PH who were diagnosed by a right heart catheterization with a mean pulmonary artery pressure of 51–60 mmHg were also recruited (Named COPD + PH group). Patients who had causes of PH other than COPD were not enrolled in the study. 6 healthy volunteers with similar demographic characteristics were included as a control group. Subjects were from the Respiratory Department at the 2nd Affiliated Hospital of Guangzhou Medical University. Clinical data of the participants is listed in Table 1. This study was approved by the Ethics Committee of the 2nd Affiliated Hospital of Guangzhou Medical University (2017-hs-09-01) and all participants signed written informed consent forms prior to their inclusion in the study.

2.2. Antibody array assay

Serum obtained from the participants was used to research specific PH biomarkers by Human Cytokine Antibody Array (GSH-CAA-440, RayBiotech Company, Norcross, GA, USA), according to the manufacturer's instructions. This antibody array simultaneously detects 440 cytokines in a single experiment by utilizing a sandwich technique with 440 antibody dots arranged in four duplicates printed onto the glass. Briefly, serum samples were diluted (1:2) and added into the array pools to incubate with capture antibodies overnight. After washing, the arrays were incubated with a biotin-conjugated anti-cytokine antibody mix for 2 h at room temperature. Cy3-conjugated streptavidin was added to bind with biotin from the detection antibodies and the fluorescent signal was detected using an InnoScan 300 Microarray Scanner (Innopsys, France). Signal values were captured with Mapix software. The data was normalized using positive control values from the array with the RayBiotech analysis tool, specifically designed to analyze the data of Human Cytokine Antibody Array G series 440 with Microsoft Excel technology.

2.3. ELISA

ELISA kits (RayBiotech, Norcross GA, USA) were utilized to validate the result of the antibody array, according to the manufacturer's instructions. Briefly, serum samples were diluted at different dilution factors based on individual serum biomarkers. Samples were coated on the plates overnight at 4 °C. The plates were washed with wash buffer and biotin-conjugated antibody was added (1:80) into the ELISA plate for incubation (2 h). HRP-conjugated streptavidin was added (1:1000) to catalyze the TMB reagent. Finally, the catalytic reaction was stopped with the addition of sulfuric acid. For each step, each well was incubated at 100 µl. Finally, the OD_{450} was determined using a microplate reader (ELx800NB, Biotek, Winooski, CT, USA).

2.4. RT-qPCR validation

Peripheral blood mononuclear cell (PBMC) were isolated from whole blood collected from COPD + PH, COPD and control using Ficoll-Hypaque (Lonza, Walkersville, MD). Total RNA was extracted from PBMC using the Trizol method (Life Technologies, Carlsbad, California), then was reverse-transcribed using SuperScriptIII reverse transcriptase (first Strand Kit; Invitrogen, Carlsbad, CA), a mixture of random hexamers and specific primers (MCP-4, Forward primer (5′ to 3′): ATGTGATCACCACCAG CAGG. Reverse primer (5′ to 3′): ATTCTG GACCCACTT CTCCT; CCL28, Forward primer (5′ to 3′): 5′-CAGAGAG