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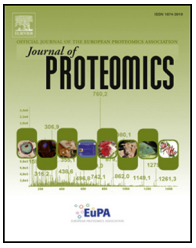

Identification of proteomic signatures associated with lung cancer and COPD

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ABSTRACT

Lung cancer (LC) and chronic obstructive pulmonary disease (COPD) commonly coexist in smokers, and the presence of COPD increases the risk of developing LC. The aim of this study was to identify distinct proteomic profiles able to discriminate these two pathological entities. Protein content was assessed in the bronchoalveolar lavage (BAL) of 60 patients classified in four groups: COPD, COPD and LC, LC without COPD, and control with neither COPD nor LC. Proteins were separated into spots by bidimensional polyacrylamide gel electrophoresis (2D-PAGE) and examined by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF/TOF). A total of 40 proteins were differentially expressed in the LC and/or COPD groups as compared with the control group. Distinct protein profiles were identified and validated for each pathological entity (LC and COPD). The main networks involved were related to inflammatory signalling, free radical scavenging and oxidative stress response, and glycolysis and gluconeogenesis pathways. The most relevant signalling link between LC and COPD was through the NF-κB pathway.

In conclusion, the protein profiles identified contribute to elucidate the underlying pathogenic pathways of both diseases, and provide new tools of potential use as biomarkers for the early diagnosis of LC.

Abbreviations: 2D-PAGE, Two dimensional electrophoresis; AKR1B10, Aldo-keto reductase family 1, member B10; AKR1C3, Aldo-keto reductase family 1, member C3; ALDH3A1, Aldehyde dehydrogenase 3 family, member A1; ALDOA, Aldolase A; AMY1A, Alpha 1 amylase; AMY2A, Alpha 2 amylase; ANXA1, Annexin A1; ANXA2, Annexin A2; ANXA5, Annexin A5; ARHGDIB, Rho GDP dissociation inhibitor beta; BAL, Bronchoalveolar lavage fluid; C3A, Complement C3; CA1, Carbonic anhydrase 1; CAPS, Calcyphosine; CAT, Catalase; CFL1, Cofilin 1; COPD, Chronic obstructive pulmonary disease; CRP, C-reactive protein; CTSD, Cathepsin D; ENO1, Alpha enolase; EZR, Ezrin; FBP1, Fructose-1,6-bisphosphatase 1; GSR, Glutathione reductase; GSTA1, Glutathione S-transferase alpha 1; GSTA2, Glutathione S-transferase alpha 2; GSTP, Glutathione S-transferase pi 1; HSP70, Heat shock protein 70; IDH1, Isocitrate dehydrogenase 1; IPA, Ingenuity Pathways Analysis; IκB, Inhibitor of κB; LC, Lung cancer; LCN2, Lipocalin 2; MALDI-TOF/TOF, Matrix-assisted laser desorption/ionization time of flight-time of flight mass spectrometry; MMP, Matrix metalloproteinase; NF-κB, Nuclear factor κB; NSCLC, Non-small cell lung cancer; PEBP4, Phosphatidylethanolamine-binding protein 4; PKM2, Pyruvate kinase 2; PPIA, Peptidylprolyl isomerase A (cyclophilin A); PRDX1, Peroxiredoxin 1; PRDX2, Peroxiredoxin 2; PRDX5, Peroxiredoxin 5; PYGM, Glycogen phosphorylase; SCC, Squamous cell carcinoma; SELENBP1, Selenium binding protein 1; SERPINB1, Serpin peptidase inhibitor, clade B, member 1 (leukocyte elastase inhibitor); TKT, Transketolase; TPPP3, Tubulin polymerization-promoting protein family member 3 (CGI-38); TXN, Thioredoxin

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Biological significance
Sequence coverage. The protein sequence coverage (95%) was estimated for specific proteins by the percentage of matching amino acids from the identified peptides having confidence greater than or equal to 95% divided by the total number of amino acids in the sequence.
Ingenuity Pathways Analysis. Mapping of our proteins onto biological pathways and disease networks demonstrated that 22 proteins were linked to inflammatory signalling (p-value: $1.35 * 10^{-08}$–$1.42 * 10^{-02}$), 15 proteins were associated with free radical scavenging and oxidative stress response (p-value: $4.93 * 10^{-11}$–$1.27 * 10^{-02}$), and 9 proteins were related with glycolysis and gluconeogenesis pathways (p-value: $7.39 * 10^{-09}$–$1.58 * 10^{-02}$).

1. Introduction

Lung cancer (LC) is the leading cause of cancer death worldwide, with a 5-year survival rate of 15%, which is among the lowest of all cancers [1]. The most important risk factor for the development of lung cancer is the smoking of tobacco, with smokers presenting a 10-fold higher risk to develop LC, on average, than non-smokers. Cigarette smoking is also the most important risk factor for chronic obstructive pulmonary disease (COPD). Interestingly, COPD, which is characterized by a chronic inflammation of the lower respiratory tract, is also a major independent risk factor for the development of lung carcinoma among long-term smokers. In fact, the presence of COPD increases the risk of LC up to 4–5 fold [2]. Furthermore, 50–70% of patients diagnosed with LC have spirometric evidence of COPD [3]. Lung cancer and COPD share risk factors and may therefore also likely share similar pathogenic pathways. Numerous mechanisms have been proposed for the common pathogenesis of LC and COPD, which involve chronic inflammation, epithelial-to-mesenchymal transition, oxidative stress, altered DNA repair and cellular proliferation [3–5]. Understanding the common mechanisms of these lung diseases is critical for the development of new methods of prevention, diagnosis and treatment.

Bronchoalveolar lavage (BAL) is a unique biological fluid that allows for appropriate sampling of the soluble protein content of the airway lumen. Comparative analyses have documented that certain proteins are present at higher levels in BAL than in plasma, suggesting that they are specifically produced in the airway tract [6]. However the plasma could be used as a source of biomarkers for early lung cancer diagnosis, blood has the great advantage of being readily accessible and that its collection is minimally invasive [7].

These proteins are therefore good candidates for becoming lung-specific biomarkers [8]. Proteomic technologies such as 2D-PAGE, 2D-DIGE, SELDI-TOF-MS technology, ICAT and iTRAQ, are valuable tools to expand the understanding of these processes. These technologies have been used to analyse different biological fluids including cell lysates, cell secretome, serum, plasma, tumour tissue, BAL, sputum and saliva [9,10]. Several studies have assessed differential BAL protein profiles obtained by 2D-PAGE analysis in smokers versus never smokers [11,12] and have also tried to identify profiles associated with a higher risk of developing COPD [13–15]. Regarding LC patients, a number of 2D-PAGE studies have been performed to explore the protein content of plasma or tumour tissue, focusing on understanding the molecular basis of cancer pathogenesis [16–18], as well as on the identification of new diagnostic, prognostic or predictive markers in lung cancer [19,20]. However, to the best of our knowledge, no study has evaluated to date the protein profile by 2D-PAGE in BAL of lung cancer patients. We believe that analysing the protein composition of BAL by a high-throughput technology, given its vicinity to tumour cells and potential enrichment in tumour-derived proteins may provide more reliable information and may therefore prove to be a more accurate tool for clinical use.

In the present study, the soluble protein content of the airway tract was assessed by 2D-PAGE and MALDI-TOF/TOF in the BAL of 60 patients classified in four groups: COPD, COPD and LC, LC without COPD, and control (neither COPD nor LC). As COPD and LC may share some common pathogenic pathways, the aim of this study was to identify distinct proteomic profiles that were able to discriminate these two pathological entities. Analysis of the main protein networks involved could also contribute to improve our understanding of the underlying mechanisms implicated in the development of each disease, and lead to the discovery of new biomarkers of potential diagnostic or therapeutic utility.

2. Materials and methods

2.1. *Patients and samples*

From 2009 to 2011, a total of 60 patients from the Hospital Universitario Virgen del Rocío were included in the study. Samples were obtained from four groups of patients: patients with COPD (n = 15), patients with LC (n = 15), patients with LC and COPD (n = 15), and patients with neither LC nor COPD (control group; n = 15). Characteristics of the study population are summarized in Table 1. Patients were required to meet all the following eligibility criteria: 1) patients were under pneumologist consultation due to haemoptysis and/or a pulmonary nodule; 2) patients were requested by their treating physician a flexible bronchoscopy for diagnostic purposes; 3) patients were smokers or ex-smokers of >20 pack-year; and 4) patients had to be older than 40 years of age. Exclusion criteria included: 1) diagnosis of a neoplastic disease other than non-small cell lung cancer (NSCLC); 2) active pulmonary tuberculosis; 3) previous lung resection; 4) history of drug abuse; and 5) the presence of other acute or chronic inflammatory disease. The study protocol was approved by the Hospital's Ethical Committee and a written informed consent was obtained from all patients prior to study entry.

Subjects were prepared with a combination of topical anaesthesia (20% benzocaine spray to the pharynx plus 2%