A New Biomarker Panel in Bronchoalveolar Lavage for an Improved Lung Cancer Diagnosis

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Introduction: The enormous biological complexity and high mortality rate of lung cancer highlights the need for new global approaches for the discovery of reliable early diagnostic biomarkers. The study of bronchoalveolar lavage samples by proteomic techniques could identify new lung cancer biomarkers and may provide promising noninvasive diagnostic tools able to enhance the sensitivity of current methods.

Methods: First, an observational prospective study was designed to assess protein expression differences in bronchoalveolar lavages from patients with (n = 139) and without (n = 49) lung cancer, using two-dimensional gel electrophoresis and subsequent protein identification by mass spectrometry. Second, validation of candidate biomarkers was performed by bead-based immunoassays with a different patient cohort (204 patients, 48 controls).

Results: Thirty-two differentially expressed proteins were identified in bronchoalveolar lavages, 10 of which were confirmed by immunoassays. The expression levels of APOA1, CO4A, CRP, GSTP1, and SAMP led to a lung cancer diagnostic panel that reached 95% sensitivity and 81% specificity, and the quantification of STMN1 and GSTP1 proteins allowed the two main lung cancer subtypes to be discriminated with 90% sensitivity and 57% specificity.

Conclusions: Bronchoalveolar lavage represents a promising noninvasive source of lung cancer specific protein biomarkers with high diagnostic accuracy. Measurement of APOA1, CO4A, CRP, GSTP1, SAMP, and STMN1 in this fluid may be a useful tool for lung cancer diagnosis, although a further validation in a larger clinical set is required for early stages.

Key Words: Bronchoalveolar lavage, Proteomics, Biomarker, Lung cancer, Immunoassay.

(*J Thorac Oncol.* 2014;9: 1504–1512)

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Disclosure: Drs. Ruiz-Argüello, Nagore, Martinez, and Hormaeche are employees at Proteomika S.L.U., the company that developed the diagnostic tool. Dr. Uribarri is a PhD student supported by the Basque Government (Programa Ikertu). The other authors declare no conflict of interest.

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DOI: 10.1097/JTO.0000000000000282

ISSN: 1556-0864/14/0910-1504

INTRODUCTION

Lung cancer remains the most common cause of cancer-related deaths worldwide.[1] It is divided into two major clinicopathological classes: small-cell lung cancer (SCLC), representing approximately 15% of cases, and non–small-cell lung cancer (NSCLC), which accounts for 85% of cases.[2] NSCLC is commonly treated with surgery, while SCLC usually responds better to chemotherapy and radiotherapy.[3] However, the clinical outcome of conventional therapies remains very poor (15% 5-year survival) mainly due to difficulties with early diagnosis.[4]

Bronchoscopy can be considered as the primary diagnostic method in patients with suspected pulmonary carcinoma and is also necessary to select the appropriate therapeutic strategy.[5] It is less invasive than other tissue procurement methods, carries a small risk of complications, and has high specificity, although its sensitivity is relatively poor.[5,6] It is expected that approaches that do not require the collection of tumor cells, such as the detection of molecular markers in bronchial fluids, will enhance the sensitivity of cytological examination (30–80% depending on tumor accessibility)[6,7] and improve the diagnostic accuracy. Traditionally, blood has been the biological fluid used for noninvasive biomarker analysis, but detection of low-abundance tumor proteins in this complex mixture can be very challenging. Bronchoalveolar lavage (BAL) represents an alternative source of more specific lung cancer biomarkers due to its vicinity to tumor cells, its less complex protein composition, and the fact that it can be obtained through minimally invasive methods, compared with biopsies.[8] A number of potential biomarkers have already been found in bronchial fluids from lung cancer patients,[5] but few have proved to be useful in the clinic, because of their low sensitivity, specificity, and reproducibility.[9,10] Thus, identification and validation of diagnostic biomarkers for early detection and subtype classification of lung cancer patients is urgently needed.

Nevertheless, it is not realistic to explain any cancer as a disorder of one single protein. Therefore, high-throughput molecular techniques could represent an alternative strategy for the selection of a panel comprising a combination of different biomarkers. Proteomics, a powerful tool for global evaluation of protein expression, has been widely applied in cancer research, allowing the efficient identification of accurate and reproducible differentially expressed proteins in complex biological samples.[9]

Several proteomic studies have used BAL samples to assess differential protein profiles in a number of lung-related

diseases such as asthma, sarcoidosis, and cystic fibrosis,[11] but the suitability of BAL as a source of NSCLC biomarkers, was not reported until 2011 by Oumeraci et al.,[8] based on a preliminary proof of concept using six lung cancer samples. Very recently, Pastor et al.[12] have published the first proteomic study of NSCLC in BAL using two-dimensional polyacrilamide gel electrophoresis (2D-PAGE). However, to date no study has evaluated the protein profile of lung cancer BAL by 2D-PAGE, including both SCLC and NSCLC, and performing a comprehensive validation of the results with a different patient cohort and an alternative technique.

In the present study, BAL samples from lung cancer patients (n = 139) were compared with those from subjects with nonmalignant pulmonary diseases (n = 49) by 2D-PAGE with the aim of finding candidate diagnostic biomarkers. Thirty-two proteins significantly up- or down-regulated were identified by mass spectrometry (MS). Ten of them were individually validated using a different patient cohort and technique (bead-based immunoassays). Furthermore, a panel comprising five of those biomarkers led to a useful tool for an accurate diagnosis of lung cancer. Moreover, the quantification of two biomarkers was found to differentiate NSCLC and SCLC patients.

MATERIALS AND METHODS

Study Design

The main objective was to identify protein biomarkers that could be useful in the clinics to improve lung cancer-specific diagnostic. For that purpose, two prospective and observational studies were carried out in two phases: (1) a biomarker discovery phase, where 188 BAL samples from lung cancer patients (n = 139; 43 SCLC and 96 NSCLC) and control subjects (n = 49) were subjected to 2D-PAGE analysis, and differentially expressed proteins identified by MS; and (2) a biomarker validation and diagnostic model generation phase, where identified candidate proteins were quantified in 252 BAL samples (49 control subjects and 204 cancer patients, 63 SCLC and 141 NSCLC) by in-solution bead-based immunoassays. A multivariate model was generated by logistic regression analysis.

Study Subjects and Samples

All patients were recruited at Cruces Hospital (Barakaldo, Spain). Study protocols were approved by the corresponding ethical committee and all patients signed an informed consent form. Subjects with suspected lung cancer, who were finally diagnosed with another respiratory-related disease (including pneumonia, bronchitis, sarcoidosis, and chronic bronchopathy) were classified as controls. Patients with either SCLC or NSCLC at different clinical stages were included. The diagnosis was confirmed by anatomical pathology. Characteristics of the study population are summarized in Table 1.

BAL specimens were collected during bronchoscopy for routine diagnostic purposes, by flushing the airways with saline fluid to harvest surrounding cells. After centrifugation

TABLE 1. Patient Demographics and Clinical Profiles

	Discovery Set		Validation sestet	
Demographic Characteristics	**Control Patients (*n* = 49)**	**Cancer Patients (*n* = 139)**	**Control Patients (*n* = 48)**	**Cancer Patients (*n* = 204)**
Age (mean ± SEM)	56.4 ± 14.5	63.6 ± 10.3	54.9 ± 14.0	63.0 ± 10.7
Sex				
Male	38	123	38	177
Female	11	16	10	27
Smoking habit				
Nonsmokers	13	5	14	8
Ever smokers	36	134	34	196
Histology				
SCLC		43		63
NSCLC		96		141
Adenocarcinoma		29		59
Squamous cell carcinoma		64		80
Others		3		2
Clinical stage Total (SCLC/NSCLC)				
I		14 (2/12)		14 (2/12)
II		4 (1/3)		4 (0/4)
III		47 (13/34)		60 (17/43)
IV		65 (25/40)		109 (40/69)
Undetermined		9 (2/7)		17 (4/13)

SEM, standard error of the mean; SCLC, small-cell lung cancer; NSCLC, non–small-cell lung cancer.

