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Peroxiredoxins and tropomyosins as plasma biomarkers for lung cancer and asbestos exposure

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ABSTRACT

The prognosis of lung cancer is poor due to late diagnosis, the lack of established screening programs, and the paucity of early biomarkers for high-risk populations. Plasma proteome analysis was used to identify novel biomarkers for diagnosing lung cancer, and to unravel the mechanisms of underlying pathogenesis. Plasma proteins obtained from asbestos-exposed lung cancer cases detected by CT screening, asbestos-exposed subjects, clinical lung cancer patients, and healthy tobacco smokers, 5–6 cases in each group, were separated by two-dimensional gel electrophoresis, and identified with tandem mass spectrometry (LC–MS/MS). Nine proteins were selected for immunological confirmation in a test or validation set of plasma samples from an additional 49 clinical lung cancer cases, 66 asbestos-exposed patients, and 107 healthy tobacco smokers. Twenty-eight unique proteins were differentially expressed between the four study groups ($p < 0.05$). Peroxiredoxin 1 (PRX1) was detected as a novel plasma marker for lung cancer ($p = 0.001$). We also confirmed the previously found association of serum amyloid A with lung cancer ($p < 0.001$). High plasma levels of tropomyosin 4 (TPM4: $p < 0.001$) and peroxiredoxins 1 and 2 (PRX2: $p < 0.001$) correlated with asbestos exposure or a diagnosis of asbestosis. PRX1 and PRX2 exhibited an inverse correlation with tobacco smoking ($p < 0.001$). Plasma peroxiredoxins 1 and 2, and tropomyosin 4 were shown to associate with asbestos-exposure, and peroxiredoxin 1 with lung cancer. High plasma levels of peroxiredoxin 1 may result from genetic damage caused by reactive oxygen species. This study has identified several biomarkers worthy of further investigation in lung cancer and asbestos-related diseases.

1. Introduction

Each year lung cancer is responsible for over one million deaths worldwide [1]. In the developed countries, approximately 15% of patients live 5 years after the diagnosis, but this percentage has not improved substantially during the last decades. Despite the well-known risk factors, and the straightforward recognition of high-risk individuals namely tobacco smokers and those exposed to carcinogens especially to asbestos, lung cancer is usually diagnosed at a late stage. A recent study shows, however, that screening with low-dose computed tomography (CT) can reduce mortality from lung cancer [2]. A small fraction, between five to seven percent, of lung cancer cases is considered as being attributable to asbestos [3]. Furthermore, carcinogens in tobacco smoke and asbestos fibers act synergistically adding to the lung cancer risk [4].

Exposure to asbestos accounts for over half of all occupational cancers [5], although the use of asbestos has been banned in most developed countries for decades. This is due to the long latency period between the exposure to asbestos fibers and the development of cancer [6]. Excessive generation of reactive oxygen species (ROS) in cells is believed to play a substantial role in tobacco smoke- and asbestos-induced genetic damage. ROS affect cellular homeostasis, and have a role in inflammation, cell proliferation, and apoptosis [7] (reviewed in). Several previous studies [8–14] have shown that certain chromosomal aberrations are more common in asbestos-exposed than in non-asbestos-exposed individuals' lung cancer. One such aberration is a deletion of chromosomal region 19p, this being also associated with smoking in lung adenocarcinoma patients [8,14]. The 19p region harbors a tumor suppressor Kelch-like ECH-associated protein 1 gene (KEAP1) that controls the degradation of a transcription factor NRF2. NRF2 regulates a number of cytoprotective genes, including peroxiredoxin 1 [15,16].

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Genetic aberrations associated with lung cancer have triggered clear interest, but surprisingly there are very few studies reporting changes in the proteome. Despite remarkable methodological advances, biomarker research is hampered by lack of marker specificity, method sensitivity, and sample matrix complexity. For instance, plasma is among the most accessible biological materials available, but due to its complexity, attempts to determine the plasma proteome are challenging [17]. Statistical guidelines that take into account sample size and collection, study layout, and address the need of validation are, therefore, crucial in the search for markers in cancer prediction [18].

In this study, two-dimensional gel-electrophoresis (2-DE) coupled to a mass spectrometer was used to compare the plasma proteomes between four matched groups with differing asbestos-exposure or lung cancer statuses. The results were confirmed by immunological validation in samples from over 200 additional individuals with lung cancer or at high risk of contracting lung cancer. Among other findings, we have identified a novel plasma marker for lung cancer, peroxiredoxin 1, which is an antioxidant protein known to posses multiple functions relevant in lung carcinogenesis.

2. Methods

2.1. Subjects

Study subjects were recruited from two institutions in Finland. Asbestos-exposed lung cancer patients were participants in a cross-sectional CT-screening conducted in a health survey study of asbestos-exposed workers at the Finnish Institute of Occupational Health (FIOH) in 2003–2004. Asbestos-exposed patients without lung cancer were subjects coming to the clinical control in 2006–2008 to the Occupational Medicine Outpatient Clinic of FIOH due to occupational asbestos exposure. Specialists in occupational medicine and occupational hygiene at FIOH evaluated the probability and intensity of asbestos exposure on the basis of their work history [19]. Those subjects having definite or probable asbestos exposure were considered as being exposed. Clinical lung cancer cases were consecutive patients diagnosed and treated for lung cancer at the Lapland Central Hospital, Rovaniemi, Finland, between 2003 and 2009. The stage of lung cancer was classified according to the latest WHO principles. Healthy, asymptomatic tobacco smokers with more than 20 pack years were recruited in Lapland Central Hospital as a part of the health promotion campaign against tobacco smoking conducted between 2003 and 2005. Participants gave informed consent for the use of their interview data, and blood specimen in this study. The ethics committees of the Finnish Occupational Health Institute, the Hospital Districts of Helsinki and Uusimaa, and the Hospital District of Lapland reviewed and approved the study protocols (7/93, Dnro 75/E2/01, 3/9/03).

In the initial 2-DE screening for differentially expressed proteins, groups of five to six individuals were selected as follows: subjects with lung cancer and significant occupational asbestos exposure (A), subjects with significant occupational asbestos exposure (B), subjects with clinical lung cancer (C), and healthy tobacco smokers (D). All subjects were males, and were matched for smoking and age (Table 1). For all subjects, the exclusion criteria were age less than 40 years, smoking duration less than ten years, and, for healthy smokers (D) and smokers with asbestos exposure (B), any diagnosed cancer (allowing basal cell carcinoma of the skin). Lung cancer patients (C) with any other cancer were omitted from this study. Healthy smokers (D) and clinical lung cancer patients (C) were evaluated to ensure they had not been significantly exposed to asbestos. Since the focus of this study was to detect protein expression changes associated with lung cancer or asbestos exposure, and smoking is known to affect protein expression in the lungs, all patients were chosen to be current or former smokers. Patient characteristics are given in Table 1.

2.2. Protein identification

The training set (Table 1) plasma samples for 2-DE analyses were depleted of albumin and immunoglobulin G. The remaining proteins were separated first with IPG strips in three pH intervals, and second on SDS-PAGE gels (GE Healthcare Uppsala, Sweden). Differentially expressed protein spots were excised from the gels, in-gel digested, and identified in a tandem mass spectrometer. A detailed description of the methods used is given in the online supplement.

2.3. Immunoassay

Immunological analyses of the test set (includes the training set), and the validation set (Table 1) plasma samples were performed with commercially available primary antibodies for retinol-binding protein 4 (RBP4), transthyretin (TTHY), serum amyloid A component (SAA), serum amyloid P component (SAMP), apolipoprotein A-I (APOA1), complement component 5 (CO5), tropomyosin 3 (TPM3), tropomyosin 4 (TPM4), peroxiredoxin 1 (PRX1), and peroxiredoxin 2 (PRX2). A detailed description of the methods used is given in the online supplement.

2.4. Statistics

Statistical analysis were performed with GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA), and PASW Statistics 18.0 software (IBM Corp., Somers, NY, USA). A detailed description of the methods used is given in the online supplement.

3. Results

3.1. Identified proteins and protein selection

Screening of potential biomarkers was performed in 23 plasma samples from the study subjects, who had been subdivided into four groups (Table 1). Table 2 summarizes the identification results of nine differentially expressed proteins chosen for verification after comparing the 2-DE protein patterns across all groups. Other identifications are presented in Table S1. A total of 36 protein spots and 28 distinctive proteins were found to be differentially expressed and out of these 36 spots, 20, 9, and 7 were identified from pH intervals 3–6, 5–8, and 7–10 (Fig. 1). Twelve differentially expressed protein spots remained unidentified on the 2-DE gels.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.lungcan.2012.03.024.

Out of the 28 distinctive proteins, 9 were chosen for validation in the training or the test set (Table 2). TPM3, TPM4, CO5, SAMP, and PRX2 were selected for validation on the basis of previously obtained chromosome aberration results [12]. APOA1 and SAA were selected for their previously reported linkage to lung cancer [20,21]. Apolipoprotein B48 (B100) was not chosen for validation, since its plasma levels correlated with differences in body mass indexes (BMI) of the groups (data not shown). BMI information was available only on subjects of the training set, and did not play a part in any further analyses. The role of RBP4 in cancer is less obvious, but expression changes in lung cancer have been reported [22]. TTHY is a plasma carrier protein for RBP4, and was therefore chosen for validation together with RBP4.

The immunoblots of TPM3, TPM4, PRX1, PRX2, and SAA from the training set are presented in Fig. 2A. The results from the immunological validation of TPM3, TPM4, PRX1, and PRX2 in the test set are presented in Fig. 2B. The training set results are presented in