Lung Cancer 112 (2017) 69–74

Contents lists available at ScienceDirect

Lung Cancer

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

Serum lipid profile discriminates patients with early lung cancer from healthy controls

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ARTICLE INFO

Keywords:
Early detection
Lipidomics
Lung cancer screening
Mass spectrometry
Serum biomarkers

ABSTRACT

Objectives: The role of a low-dose computed tomography lung cancer screening remains a matter of controversy due to its low specificity and high costs. Screening complementation with blood-based biomarkers may allow a more efficient pre-selection of candidates for imaging tests or discrimination between benign and malignant chest abnormalities detected by low-dose computed tomography (LD-CT). We searched for a molecular signature based on a serum lipid profile distinguishing individuals with early lung cancer from healthy participants of the lung cancer screening program.

Materials and methods: Blood samples were collected from 100 patients with early stage lung cancer (including 31 screen-detected cases) and from a matched group of 300 healthy participants of the lung cancer screening program. MALDI-ToF mass spectrometry was used to analyze the molecular profile of lipid-containing organic extract of serum samples in the 320–1000 Da range.

Results: Several components of the serum lipidome were detected, with abundances discriminating patients with early lung cancer from high-risk smokers. An effective cancer classifier was built with an area under the curve of 0.88. Corresponding negative predictive value was 98% and a positive predictive value was 42% when the classifier was tuned for maximum negative predictive value. Furthermore, the downregulation of a few lysophosphatidylcholines (LPC18:2, LPC18:1 and LPC18:0) in samples from cancer patients was confirmed using a complementary LC–MS approach (a reasonable cancer discrimination was possible based on LPC18:2 alone with 25% total weighted error of classification).

Conclusions: Lipid-based serum signature showed potential usefulness in discriminating early lung cancer patients from healthy individuals.

1. Introduction

The majority of lung cancer cases are diagnosed at advanced stages, with consequent poor treatment outcomes. Apart from the reduction of exposure to tobacco smoke, screening for the detection of early stage disease has been considered the major strategy to reduce lung cancer mortality [1–3]. Results of the National Lung Screening Trial (NLST) showed that compared to chest x-ray examination, the low-dose computed tomography (LD-CT) screening is associated with a 20% reduction of lung cancer-specific mortality in a high-risk group of subjects defined by their smoking status [4]. However, due to low specificity of LD-CT (positive predictive value [PPV] of only 3.8% in the NLST), the vast majority of patients with screen-detected chest abnormalities are subjected to further expensive and potentially harmful diagnostic procedures, such as positron emission tomography, transthoracic or bronchoscopic biopsy and even to surgery. In our own experience, 75% of such patients underwent unnecessary further diagnostic work-up, including 25% subjected to invasive procedures [5]. To reduce overdiagnosis and decrease the costs, there is an urgent need for clinical tests supporting CT-based screening for the detection of lung cancer.

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http://dx.doi.org/10.1016/j.lungcan.2017.07.036
Received 17 November 2016; Received in revised form 11 July 2017; Accepted 31 July 2017

Such tests could either pre-select individuals for LD-CT examination or discriminate between benign and malignant chest abnormalities detected by LD-CT [6,7].

Several different components of blood, including circulating tumor cells, circulating DNA, micro RNA, autoantibodies and specific serum/plasma proteins, have been analyzed as potential early lung cancer biomarkers [8–10]. However, only a few blood-based assays were validated for diagnosis of screen-detected lung cancer, and none of them have found its wide application in clinical practice. Monitoring of cancer-related metabolites in blood is an emerging approach in the detection and diagnosis of different malignancies [11]. Among different types of metabolites, phospholipids and other classes of lipids seem to have particular relevance, as their levels in body fluids may differentiate cancer patients from healthy individuals [12]. Several studies demonstrated that profiling of serum or plasma samples using nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry (MS) can deliver metabolomic signatures discriminating patients with lung cancer from healthy individuals or patients with non-malignant lung diseases [13–24]. Examples of serum metabolites with a potential discriminating value are unsaturated and/or choline-containing lipids [14], various lysophosphatidylcholines (LPC) and sphingomyelins (SM), oleamid and acylcarnites [16], multicomponent panel comprised sphingosine, phosphorylcholine or other compounds involved in metabolism of sphingolipids [20], and certain free fatty acids (arachidonic acid, linoleic acids and their metabolites) [19]. However, lung cancer cohorts enrolled in all these studies included usually both early and advanced cancer cases, and none of these studies used material from lung cancer screening programs including high-risk controls. In the present study we used mass spectrometry-based profiling of a serum lipid fraction to identify molecular signature discriminating patients with early lung cancer from healthy participants of the LD-CT screening program.

2. Materials and methods

2.1. Characteristics of the study groups

Study material was collected during the Pomerania Lung Cancer Screening Program performed at the Medical University of Gdańsk, Poland between 2008 and 2010. This project used LD-CT in individuals 50–75 years of age who were current or former tobacco smokers (at least 20 pack-year). Blood samples were collected before the diagnosis from about 3600 cases subjected to screening, including 31 with screen-detected lung cancer. The latter group was supplemented with 69 cases of asymptomatic, incidentally detected stage IA-IIB lung cancer patients with similar characteristics considering smoking habits, sex and age collected with this same protocol from clinical cases (Table 1). From all lung cancer patients blood samples were collected before any therapeutic intervention. Each cancer case was accompanied by 3 pair-matched controls regarding sex, age and smoking exposure, selected from lung cancer-free participants of the screening program (Table 1). The appropriate Ethics Committee of the Medical University of Gdańsk approved the study (NKEBN/42/2009) and all subjects provided written informed consent indicating their conscious and voluntary participation.

2.2. Sample preparation

Peripheral blood was collected into a 5 mL BD Vacutainer Tube, incubated for 30 min at room temperature to allow clotting, and then centrifuged at 1000*g* for 10 min to remove the clot. The serum was aliquoted and stored at −70 °C. Extraction of a lipid fraction was performed according to a modified Folch method [25]. In brief, 25 μL of serum was mixed with 350 μL of 1:1 methanol/chloroform mixture (v/v) containing antioxidants: 0.01% (w/v) 2,6-di-*tert*-butyl-4-methylphenol and 0.005% (w/v) retinol. The mixture was vortexed and incubated for 25 min at 4 °C. Then, 100 μL of water was added to the mixture, vortexed for another 10 s and centrifuged (5 min, 15,000*g*). Chloroform phase (the bottom one) was kept and stored at −70 °C, until performing mass spectrometry analysis (within one week).

Table 1
Characteristics of the study groups.

Group	Controls n = 300	Cancer cases n = 100
Clinical stage	NA	
IA		42
IB		21
IIA		21
IIB		10
IIIA		6
Pathology type	NA	
adenocarcinoma		62
squamous cell carcinoma		36
NSCLC, not otherwise specified		2
Sex		
female	138	46
male	162	54
Age (years) [median]	49–76 [62]	49–77 [62]
Smoking (pack-year) [median]	10–90 [30]	10–80 [30]

NA, not applicable.

2.3. Registration of mass spectra

Samples were analyzed using ultrafleXtreme MALDI-ToF mass spectrometer (Bruker Daltonics, Bremen, Germany). A sample of 0.6 μL of the extract was mixed directly on stainless steel target plate (Ground steel target, Bruker) with 0.6 μL of matrix solution − 30 μg/μL of 6-aza-2-thiothymine (ATT) dissolved in 30% ethanol containing 0.1% trifluoroacetic acid. Positive ions were recorded in the 320–1000 Da range using a reflectron mode of the analyzer. For each sample, four technical replicas were registered. Samples were spotted in a random sequence to avoid "batch effect".

2.4. Spectral data processing

The pre-processing of spectra included baseline identification and removal, spectral alignment and averaging of technical repeats (with outlier detection done using the Dixon procedure), and normalization of the total ion current (TIC). In the second step the spectral components, which reflected lipid ions recorded at defined *m/z* values, were identified using decomposition of mass spectra into their Gaussian components as described in details elsewhere [26]. The initial set of Gaussian components was further processed to merge overlapping components (components with mean value closer than 3 standard deviations) and to remove components presumably representing the residual baseline (components with extremely high variance and low height), which resulted in further dimension reduction. These final 2401 components were used to compute features (i.e. relative abundance) of registered spectra (termed spectral components afterward) for all samples by the operations of convolutions with Gaussian masks. Hypothetical identification of selected lipid species was performed based on annotation of masses of modelled spectral components at the Human Plasma Standard Reference Material (SRM1950) lipid database [27], available at http://www.lipidmaps.org/data/results/nist/index.html; potential candidates were filtered based on their biological relevance (i.e., previous detection in human blood) and assuming mass tolerance below 0.5 Da.

2.5. LC–MS analysis of selected lipids

The LPC17:1 standard (3.82 ng; Avanti Polar Lipids, Inc.) was added