THE RATIONALE, DESIGN AND METHODS OF NEW STUDIES

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Study of serum metabolic profiles of patients with non-small cell lung cancer with special emphasis on the smoking status of patients

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ABSTRACT

The project entitled "Study of serum metabolic profiles of patients with non-small cell lung cancer with special emphasis on the smoking status of patients" is a study based on metabolomics, which is the latest of the "omics" technologies and involves a comprehensive analysis of small molecule metabolites of a specific biological sample. High-throughput and sensitive analytical techniques used in metabolomic investigations are powerful tools in the field of oncology and aids understanding what is happening in cancer cells and searching for new cancer markers. The aim of the project is to determine whether lung cancer patients have a distinct serum metabolic profile and whether this profile is associated with patients' smoking status. The application of liquid chromatography-high-resolution mass spectrometry-based methodology along with advanced statistical methods will enable to select potential molecules that can be useful in early lung cancer detection..

Keywords: lung cancer, metabolomics, chronic-obstructive pulmonary disease, mass spectrometry.

General information

The project entitled "Study of serum metabolic profiles of patients with non-small cell lung cancer with special emphasis on the smoking status of patients" was founded by the National Science Centre, Poland within MINIATURA1 competition (grant number 2017/01/X/NZ7/02064). The duration of the grant is 12 months. The project is planned for 12 months, and it is run by the Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Poland in the cooperation with Department of Thoracic Surgery as well as Department of Pulmonology, Allergology and Respiratory Oncology, Poznan University of Medical Sciences, Poland. The principal investigator is Agnieszka Klupczyńska, Ph.D. and the total grant value is 49467 PLN. The project includes an internship of the principal investigator in Integrative Molecular Phenotyping Laboratory, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden. The study was approved by the ethics

committee at the Poznan University of Medical Sciences (Decision No. 746/17 and 314/18).

Research Project Objectives

The aim of the study is to perform serum untargeted metabolic profiling of lung cancer patients and patients with chronic obstructive pulmonary disease (COPD) as a control, non-cancer group. A novelty is the application of patient categorization based on the smoking status, which allows for distinguishing molecular markers associated with lung cancer from changes in the metabolic profile resulting from tobacco smoking. Lung cancer remains one of the major challenges in contemporary oncology. Although its incidence rate is similar to other malignant tumors, such as prostate cancer and breast cancer, lung cancer is characterized by 4–5 times higher death rate and it has been the main cause of malignant tumor-related deaths for years [1, 2]. Due to the high morbidity and mortality of lung cancer, there is a high demand for identification of cancer biomarkers that can contribute clinically relevant information. Tobacco smoking is the main factor in the development of lung cancer, and therefore the effect of this factor on the metabolic profiles of the patients should be taken into account in metabolomic experiments. So far the issue of the influence of smoking on the metabolic profiles of patients has been neglected in lung cancer biomarker studies and only a few of them present patient stratification by smoking status [3, 4]. Previous research of serum metabolome of lung cancer patients indicated alterations in many distinct groups of metabolites, such as amino acids, organic acids and acylcarnitines [5–7]. However, little is known about the differences between the levels of angiogenic markers between smoking and not smoking patients. Therefore, it should be investigated whether the observed differences in the metabolome result from the development of lung cancer or if they are the result of chronic smoking. The answer to the above research question can be obtained by profiling a broad spectrum of metabolites in serum samples taken from patients with lung cancer and a control group. Endogenous metabolites are at the end of a series of processes beginning with the genome followed by the transcriptome and proteome. Therefore, metabolome fills the gap between a genotype and phenotype. Components of metabolic profiles include such compound classes as amino acids, amines, organic acids, fatty acids, steroids, and sugars [8, 9].

Research Plan and Basic Concept

The project includes untargeted mass spectrometry-based metabolic profiling, which represents a promising and valuable tool in the analysis of complex cancer-associated metabolic changes [8, 10]. The study is conducted in serum samples derived from individuals with untreated non-small cell lung cancer and a matched non-cancer group (individuals with COPD). The resulting metabolite profiles will be subjected to univariate and multivariate statistical tests, and significant features will be identified using different databases (**Figure 1**).

The research plan includes the following steps:

1. Sample collection
   Collection of serum specimens from patients with newly diagnosed lung cancer and individuals with COPD (non-cancer group). Conducting a questionnaire survey among all individuals who donated blood samples. Selection of the samples of the study group (people with lung cancer) based on histopathological examination of tissue samples.
2. Untargeted metabolic profiling of serum samples using high-resolution mass spectrometry.
   Performing serum sample extraction and metabolite profiling using quadrupole-time-of-flight (Q-TOF) mass spectrometer coupled to a liquid chromatograph. Analysis of quality control samples.
3. Data analysis and identification of metabolite markers
   Univariate and multivariate statistical analyses performed on the obtained bioanalytic data together with clinical data. Significant feature identification. Characterization of correlations between the endogenous metabolites and the individual's health status (presence or absence of lung cancer). Estimation of the correlation of the obtained results (metabolite profiles) with the smoking status of patients. Estimation of the diagnostic value of the selected endogenous compounds.

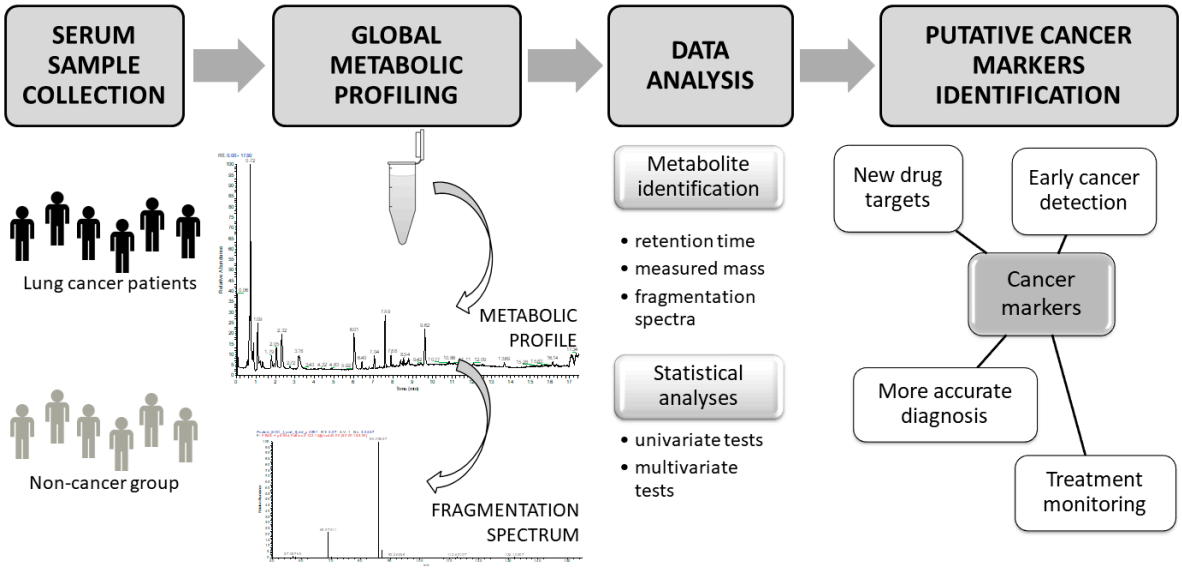

Figure 1. Basic concept of metabolomic studies in cancer research

Research Methodology

Patients and samples

Serum samples are collected from 2 groups of patients divided into subgroups based on smoking status: current smokers and ex-smokers (**Table 1**). Subgroups of smokers consist of patients smoking at least ten pack-years and a minimum of 10 cigarettes/day the past 6 months. In case of the ex-smokers criterion of minimum 2 years since smoking cessation is applied. Study participants are recruited in the Department of Thoracic Surgery, Poznan University of Medical Sciences and Department of Pulmonology, Allergology and Respiratory Oncology, Poznan University of Medical Sciences. Lung cancer diagnosis is performed by the histopathological examination of tissues. Blood samples are collected before the initiation of any anti-cancer treatment.

Table 1. Classification of patients enrolled to the study

Lung cancer patients (n = 40)		COPD patients (n = 40)	
Current smokers[1] (n = 20)	Ex-smokers[2] (n = 20)	Current smokers[1] (n = 20)	Ex-smokers[2] (n = 20)

Analytical methodology

The goal of the project will be reached with the use of untargeted metabolic profiling and by the application of high-resolution mass spectrometry. In untargeted metabolomic experiment all detectable metabolites in a specimen are analyzed, and as a result, a unique, global metabolic profile of samples is obtained. One of the most commonly used analytical platforms in global metabolomics is Q-TOF mass spectrometer coupled to liquid chromatograph and equipped with electrospray ionization source. Since Q-ToF instrument is not available at Poznan University of Medical Sciences, the assays will be performed in Integrative Molecular Phenotyping Laboratory, Division of Physiological Chemistry II, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden. The project includes an internship of the principal investigator in that foreign research centre. Integrative Molecular Phenotyping Laboratory is one of the leading metabolomic centres in Europe and has experience in metabolomic studies of respiratory diseases [11–13].

Data analysis

Due to its comprehensive nature, the untargeted metabolomics produces large dataset, which requires extensive data processing as well as advanced statistical methods for data analysis. Data processing consists of several steps, i.a. smoothing, chromatogram deconvolution, peak alignment, duplicate peak removing, peak filtering. For statistical analyses of data arising from high-throughput metabolomics specialized software such as MetaboAnalyst is used [14]. The performed tests will allow for select features that are significantly different between the two analyzed

groups (biomarker discovery). The next crucial and also challenging step in data analysis of global metabolic profiles is the reliable identification of detected significant signals. Metabolite identification is performed by matching accurate mass, retention time and tandem mass spectrometry fragmentation patterns to different chemical reference databases, i..a., the in-house library of standards acquired previously using the same instrument, Human Metabolome Database and others.

Measurable Effects and Expected Results

The proposed metabolomic research will broaden our pathophysiological understanding of cancer and will be used as a source of new potential cancer-associated biomarkers. The study will allow to better understand the effect of tobacco smoking on the complex and multidimensional pathogenesis of lung cancer. The obtained results will enable the design of further studies focused on a specific group of metabolites to better estimate their potential as lung cancer markers. The further plans involve the determination of a panel of identified metabolic markers using a targeted approach with the application of triple quadrupole mass spectrometry to obtain quantitative data and prove the clinical usefulness of the selected molecules.

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Conflict of interest statement
The authors declare no conflict of interest.

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