



THE RATIONALE, DESIGN AND METHODS OF NEW STUDIES

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Proteomic and metabolomic strategy of searching for biomarkers of genital cancer diseases using mass spectrometry methods

Jan Matysiak¹, Paweł Dereziński¹, Agnieszka Klupczyńska¹, Joanna Hajduk¹, Agata Światły¹, Szymon Plewa¹, Agnieszka Lemańska², Patryk Jaźwiński¹, Paulina Banach², Ewa Nowak-Markwitz², Zenon J. Kokot¹

¹ Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Poland

² Gynecologic Oncology Department, Poznan University of Medical Sciences, Poland

ABSTRACT

The project entitled "Proteomic and metabolomic strategy of searching for biomarkers of genital cancer diseases using mass spectrometry methods" is a study based on novel omics techniques. The main assumption of this research is the development of innovative model of searching of biomarkers in ovarian and prostate cancers using proteomics and metabolomics methodologies supported by bioinformatics analysis. The innovatory strategy based on the latest achievements in the field of mass spectrometry will allow for the implementation of the unique studies for discovery new biomarkers, which are useful in prediction, diagnosis and treatment of the genital cancers. To date, there is no comprehensive data including set of proteins and other endogenous compounds involved in the development and differentiation of these diseases. Therefore, the proposed approach may contribute to the discovery of biomarkers with high sensitivity and specificity, which will provide new information about genital cancers characterization.

Keywords: ovarian cancer, prostate cancer, proteomics, metabolomics.

General information

The project entitled "Proteomic and metabolomic strategy of searching for biomarkers of genital cancer diseases using mass spectrometry methods" was awarded a grant in the OPUS8 Competition organized by the Polish National Science Center (grant number: 2014/15/B/NZ7/00964). The project is planned for 36 months and it is run by the Department of Inorganic and Analytical Chemistry and the Gynecologic Oncology Department, at Poznan University of Medical Sciences, Poland. The principal investigator is Professor Zenon J. Kokot, Ph.D. The total grant value is 798,590.00 Polish Zloty.

This project will provide new prospective characterization of reproductive system cancers including ovarian and prostate cancers. Due to low specificity and sensitivity of diagnostics methods, this malignancies often reach advanced stages, when the sufficient treatment become problematic. Today, diagnosis of prostate cancer is mainly based on per rectum examination and on measurement of Prostate Specific Antigen (PSA) in blood [1]. While ovarian cancer diagnosis is conducted with the use of transvaginal ultrasound examination and estimation of two markers: cancer antigen 125 (CA 125) and human epididymis protein 4 (HE 4)[2]. However, none of clinical tools provide early detection of these malignancies. If the proper

early diagnosis is made, cancers can be curable with standard therapies including surgery and chemotherapy. Bearing in mind, that malignancies, which affect reproductive system, are serious worldwide problem [3, 4], this project will focus on finding new potential indicators of these pathologies. Better characterization of diseases may contribute to understanding of tumorigenesis and to develop novel diagnostic and treatment approaches. Close cooperation between scientists and clinicians will allow for a thorough and complete insight into the subject.

Ethics

The project was approved by Bioethical Committee of Poznan University of Medical Sciences, Poland (Decision No. 165/16) on 4th February 2016.

Research Project Objectives

The goal of this project is to develop innovative model of discriminatory indicators of selected reproductive system cancers using modern methods including mass spectrometry techniques: nanoLC-MALDI-TOFMS (nano liquid chromatography – matrix assisted laser desorption ionization – time of flight mass spectrometry), LC-ESI-QqQ-MS/MS (liquid chromatography – electrospray ionization-triple quadrupole – tandem mass spectrometry) and advanced bioinformatics. Recently, proteomics and metabolomics studies are widely used to characterize many diseases and pathologies like cancers [5–7]. Mass spectrometry coupled with liquid chromatography enables detection and identification of thousands endogenous molecules (e.g. amino acids, proteins, peptides) in body fluids. Therefore, this project is focused on complementary metabolomics and proteomics characterization of serum and urine in order to identify compounds which may improve diagnostic and treatment of ovarian and prostate cancers. The term biomarker has been present in medical terminology for many years to define variable, which may be marked or measured in order to evaluate important physiological and pathophysiological processes. Biomarkers can play essential role in modern diagnostic methods [8] and also in undertaking therapeutic decisions [9]. Moreover, discovery of new potential disease indicators will provide knowledge about pathomechanisms. Currently, improvement of analytical methods led to increase of selectivity, resolution, precision, accuracy and specificity. It result in more detailed analysis and enable deeper description of tumorigenesis. Therefore, in this proj-

ect, compilation of data derived from proteomics and metabolomics study based on advanced bioinformatics analysis will provide model of the most discriminative features describing reproductive system cancers. There are two main hypothesis in this project:

- Determination of the qualitative and quantitative correlation within a multiprotein/multi-peptide panels of markers of ovarian and prostate malignancies will allow for development of rapid and non-invasive diagnostic methods.
- Analysis of amino acid and other low molecular components profiles can contribute to determination of the metabolomic differences between clinical and histological types of ovarian and prostate cancers.

Research Plan and Basic Concept

The main purpose of this project is to extend knowledge about indicators in selected reproductive system malignancies. This goal will be reached with the use of low molecular compounds (including amino acids), peptides and protein analysis. Comparison of healthy control group samples and group of patients with cancer will be possible. Proteomic and metabolomic studies will enable detection of novel peptides, proteins and low molecular compounds as potential biomarkers, which can differentiate healthy individuals and oncology patients. Deep analysis of those molecules is essential from the point of view of the project's goal.

In particular, the planned studies should allow for:

- Development and implementation of new, selective methods which will provide characterization of serum and urine biomarkers of ovarian and prostate cancer using mass spectrometry techniques
- Compilation data derived from proteomic and metabolomic analysis. Determination of correlation between them and health condition in patients, results of the routinely conducted diagnostic tests (CA125, PSA) and histopathological analysis.
- Creation of modern biomarker panel which may evaluate the risk of reproductive system cancer development and contribute to early diagnosis, which will minimize invasive methods currently used in clinical trials.
- Extension knowledge about tumorigenesis and neoplasia at metabolomic as well as proteomic levels. Deep analysis of the studied diseases will provide tools not only for diagnostics but also for monitoring results of undertaken treatment and to evaluate its efficacy.

Research Methodology

The project will be performed with the use of serum and urine samples collected from patients with ovarian and prostate malignancies. Moreover, samples from healthy individuals will be examined. The main analysis will be conducted with the use of 2 tandem mass spectrometers coupled with liquid chromatography systems: LC-ESI-QqQ-MS (4000 QTRAP, AB Sciex) and nanoLC-MALDI-TOF/TOF-MS (UltrafleXtreme, Bruker). The project is planned for 3 years and contains the following stages:

Stage I Optimization of the method for determination of levels of 42 amino acids in both serum and urine. The novel analytical method will be performed using a set of LC-ESI-QqQ-MS/MS.

- Selection and optimization of depletion and enrichment strategies for proteomics studies. Sample pretreatment which enables analysis of low abundance proteins and peptides is crucial step in analysis. Different methods including combinatorial ligand library, immunodepletion, magnetic beads and solid phase extraction will be tested.
- Optimization of approach for protein-peptide profiling of serum samples. Optimization of detection and identification of the most discriminative peaks using MALDI-TOF/TOF-MS.

Stage II

- Collection of serum and urine samples derived from patients with ovarian (around 100 samples) and prostate (around 100 samples) malignancies. Recruitment of healthy individuals to control group (around 200 samples). All participants of the study signed consent to publish their (anonymized) data for scientific purposes. Questionnaire was performed among all participants.
- Measurement of 42 amino acids levels in urine and serum samples using a LC-ESI-QqQ-MS system.
- Protein-peptide profiling of collected serum samples using MALDI-TOF-MS. Analysis will be performed in the mass range 1–10 kDa. Identification of the most discriminative peaks using nanoLC-MALDI-TOF/TOF-MS. Semi-quantitative analysis of identified proteins and peptides based on isobaric labeling iTRAQ.

Stage III

- Statistical and chemometric analysis of the obtained data using special software: Statistica, MetaboAnalyst, ClinPro Tools, MATLAB. Comparison and compilation of the results obtained from metabolomics, proteomic and clinical studies.

Investigation of correlation between the results and patient's health condition.

Measurable Effects and Expected Results

The project combined proteomics and metabolomics studies to deep characterization of reproductive system cancers. According to close collaboration between scientists and clinicians, we expect that the use novel strategy will extend knowledge about these malignancies. Compilation of the obtained data will allow for understanding pathomechanisms accompanying development of the diseases. Moreover, this project will provide new data on potential biomarkers, which can be further used as diagnostic tools. Broadening the knowledge about tumorigenesis might also contribute to development of new targeted therapies. The improvement of unique advanced analytical strategies in the field of mass spectrometry will also allow for investigation other diseases in the future.

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Conflict of interest statement

The authors declare no conflict of interest.

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Correspondence address:

Prof. Zenon J. Kokot, PhD
Department of Inorganic and Analytical Chemistry
Poznan University of Medical Sciences, Poland
6 Grunwaldzka Street, 60-780 Poznan, Poland
phone: +48 618546610
fax: +48 618546609
email: zkokot@ump.edu.pl