Talanta 150 (2016) 88–96

Contents lists available at ScienceDirect

Talanta

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

High resolution mass spectrometry coupled with multivariate data analysis revealing plasma lipidomic alteration in ovarian cancer in Asian women

Yangyang Zhang [a], Yingying Liu [b], Lin Li [a,c], Jinchao Wei [a], Shaoxiang Xiong [a], Zhenwen Zhao [a,c,*]

[a] Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry Chinese Academy of Sciences, Beijing Mass Spectrum Center, Beijing 100190, China
[b] Department of Obstetrics and Gynecology, Peking University Shougang Hospital, Beijing 100144, China
[c] Graduate School, University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Article history:
Received 23 October 2015
Received in revised form
25 November 2015
Accepted 10 December 2015
Available online 11 December 2015

Keywords:
Mass spectrometry
Lipidomics
Ovarian cancer
Multivariate data analysis
Glycerophospholipid metabolism

ABSTRACT

Ovarian cancer (OC) is the most common cause of death from gynecologic malignancies in women. The identification of reliable diagnostic biomarkers for the early detection of this deadly disease is critical for reducing the mortality rate of OC. Plasma lysophosphatidic acid (LPA) levels were increased from OC patients vs. healthy controls. Therefore, lipidomics may represent an excellent developing prospect for the discovery of diagnostic biomarkers of OC. In this study, a nontargeted lipidomics approach based on ultra performance liquid chromatography-electrospray ionization-QTOF-mass spectrometry (UPLC-ESI-QTOF-MS) combined with multivariate data analysis, including principal component analysis (PCA) and (orthogonal) partial least squared discriminant analysis [(O)PLS-DA] was applied for the investigation of potential diagnostic biomarkers in plasma of OC patients. Patients with OC could be distinguished from healthy individuals and patients with benign gynecological tumor disease by this method, which shows a significant lipid perturbation in this disease. With the assistance of high resolution and high accuracy of MS and MS/MS data, the potential markers including lysophosphatidylcholines (LPCs), phosphatidylcholines (PCs) and triacylglycerols (TGs) with specific fatty acid chains, were identified. Interestingly, LPCs were up-regulated and PCs and TGs were down-regulated, compared OC group with benign tumor and normal control groups, and the glycerophospholipid metabolism emerged as a key pathway, in particular, the phospholipase A2 (PLA2) enzyme activity, that was disregulated in the disease. This study may provide new insight into underlying mechanisms for OC and proves that MS-based lipidomics is a powerful method in discovering new potential clinical biomarkers for diseases.

1. Introduction

Ovarian cancer (OC) is the most common cause of death from gynecologic malignancies in women of all ages in the Western world and caused an estimated 14,270 deaths in 2014 in the United States [1]. Early stage (I/II) of OC has an excellent prognosis with a survival rate of over 90%, but approximate 80% of all reported cases are caught in the advanced stages (III/IV) with the 5-year survival rate at only 11% [2,3]. Given the success of treatment for early stage disease, a reliable diagnostic marker for OC at an early stage is intuitively attractive. The highly glycosylated CA-125 protein is a FDA approved blood test for the diagnosis of OC; however, this marker lacks the sensitivity and specificity to be used either as an early warning marker or for population screening [4–7]. HE4, a new biomarker for OC patients, shows similar sensitivity and specificity with CA125 in the diagnosis of ovarian cancer patients, having a slightly better efficacy in premenopausal patients [8–10]. Thus, the identification of reliable diagnostic biomarkers for the early detection of this deadly disease is critical for reducing the mortality rate of OC.

Lipidomics is a growing research field that studies cellular lipidomes on a large scale and at the intact-molecule level. Recent accumulating evidence suggests that cancer and metabolic diseases are connected with the dysregulation of lipid metabolism [11]. It was reported that lysophosphatidic acid (LPA) and lysophosphotidylinositol (LPI) are significantly increased in OC ascites compared with nonmalignant disease ascites (e.g., liver failure)

* Corresponding author at: Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry Chinese Academy of Sciences, Beijing Mass Spectrum Center, Beijing 100190, China.
E-mail address: zhenwenzhao@iccas.ac.cn (Z. Zhao).

http://dx.doi.org/10.1016/j.talanta.2015.12.021
0039-9140/

[12]. In addition, ample evidences support plasma LPA levels were increased from OC patients vs. healthy controls [13,14]. Therefore, lipidomics represents an excellent developing prospect for the discovery of diagnostic biomarkers of OC [15–18].

Multiple recent advances in mass spectrometric approaches have greatly extended the analytical capabilities to facilitate the accurate analysis of global lipids to provide new insights into lipidomic pathways and functions [19–22]. A lipidomic LC/MS platform based on chromatographic retention, high mass resolution and accuracy, MS/MS analysis, and quantitation software enables analysis of complex samples by LTQ-FT MS for lipid droplets, finding enhanced sensitivity for detection of PE and DG species [23]; Plasma metabolomic investigation of hepatocellular carcinoma patients was reported by ultra performance liquid chromatography-electrospray ionization-quadrupole time-of-flight mass spectrometry, downregulated molecules of interest included 11 LPCs in the plasma of HCC patients provide new insights into the pathobiology of the disease [16]; a uniform DHB layer-assisted MALDI-FTICR MS-based approach was developed for the rapid analysis of lipid extracts from cell pellets, which showed higher throughput and is more convenient, presenting an alternative approach for lipidomics study [21]. Thus, MS-based lipidomic analyses have significantly expanded our knowledge related to human physiology and pathology [24].

In this study, a nontargeted lipidomics approach based on ultra performance liquid chromatography-electrospray ionization-quadrupole time-of-flight-mass spectrometry (UPLC-ESI-QTOF-MS) in conjunction with multivariate data analysis, including principal component analysis (PCA) and (orthogonal) partial least squared discriminant analysis [(O)PLS-DA] was applied for the rapid investigation of potential diagnostic biomarkers in plasma of OC patients. The high resolution mass spectrometry and its corresponding MS/MS data were combined to identify the structure of these potential biomarkers. Additionally, these data were as well uploaded to MetaboAnalyst website (www.metaboanalyst.ca) for pathways analysis using MetPA software to identify the disregulated pathways.

2. Experimental

2.1. Materials

Lipid standards, 1-lauroyl-2-hydroxy-sn-glycero-3-phosphocholine (12:0 LPC) and 1-myristoyl-2-hydroxy-sn-glycero-3-phosphate (14:0 LPA) were purchased from Avanti Polar Lipids (Birmingham, AL). HPLC-grade methanol (MeOH), acetonitrile (CH_3CN), isopropanol (IPA), chloroform($CHCl_3$), formic acid as well as ammonium formate were purchased from Sigma or Fisher Scientific. Ultrapure water from Milli-Q purification system (Millipore Corporation, USA) was used. All of the above materials were used as-received condition without further purification.

2.2. Sample collection and lipids extraction

2.2.1. Participants

Plasma samples of Asian women were a gift from Dr. Yingying Liu, including 27 patients with OC (C group, age range: 37–74, 55.2 ± 10.7 years old), 27 patients with benign gynecological tumor (Artful bursa benign tumor, B group, age range: 8–76, 47.2 ± 17.6 years old), and 11 unaffected controls (N group, age range: 30–71, 45.9 ± 13.1 years old) enrolled at the Shougang Hospital of Peking University. Blood samples were collected in EDTA-containing tubes and centrifuged at $1750 \times g$ for 15 min at room temperature. Plasma samples were aliquoted into siliconized eppendorf tubes (SafeSeal microcentrifuge tubes; PGC Scientifics, Frederick, MD) and frozen at −80 °C until use. The project was approved by the Institutional Review Board, and written informed consent forms were signed by participants.

2.2.2. Lipids extraction

Lipids were extracted by methanol method [25]. In brief, 20 μl of plasma was transferred into 1000 μL of methanol containing 12:0 LPC (1 nmol, used for quality control in positive-ion detection mode) and 14:0 LPA (20 pmol, used for quality control in negative-ion detection mode). After vortexing and centrifugation (10,000 g, 5 min, room temperature), 2 μL of the supernatant or 2 μL of 10X dilution were loaded into mass spectrometer for negative-ion or positive-ion lipids analysis, respectively.

2.3. UPLC-ESI-QTOF mass spectrometry

Lipids profile analysis was performed using UPLC-ESI-QTOF-MS (Xevo G2 Q-TOF MS, Waters). Samples were loaded through a LC system (I-class Acquity ultra performance liquid chromatography, Waters) with an auto sampler. The mobile phase A was isopropanol/acetonitrile/formic acid (90:10:0.1, v/v/v) containing 10 mM ammonium formate; The mobile phase B was acetonitrile/water/formic acid (70:30:0.1, v/v/v) containing 10 mM ammonium formate. A CSH C18 column (1.7 μm, 2.1 mm ID × 100 mm, Waters) was used for separation of lipids. The column was maintained at 55 °C. The UPLC separations were 20 min/sample using the following scheme: (1) 0 min, 70% B; (2) 2 min, 57% B; (3) 2.1 min, 50% B; (4) 12 min, 46% B; (5) 12.1 min, 30% B; (6) 18 min, 1% B; (7) 18.1 min, 70% B; (8) 20 min, 70% B. All the changes are linear, and the flow rate was set to 400 μL/min.

The MS system was operated using both the positive-ion (ESI+) and negative-ion (ESI-) modes. The m/z range was set at 100–1500. The ionization source temperature was set at 110 °C. Nitrogen was used as the dry gas and collision gas. The cone gas flow was set at 15 L/h, and the desolvation gas flow was set at 600 L/h, and the desolvation gas temperature was set at 320 °C. In positive ion mode, the capillary voltage was 3.5 kV, the sampling cone voltage was 35 V, and the extraction cone voltage was 3.0 V. In negative ion mode, the capillary voltage was 2.8 kV, the sampling cone voltage was 35 V, and the extraction cone voltage was 3.0 V, Multiplexed data acquisition (MS^E), a kind of data independent acquisition modes was performed for simultaneously acquiring MS and MS/MS data with high resolution and high accuracy. MS/MS data were obtained for all the ions observed in the preceding MS scan. The lock spray was used to ensure reproducibility and accuracy. Leucine enkephalin (200 pg/ml) was used as lock mass in ESI- (m/z 554.2615) and ESI+ (m/z 556.2615). The lock spray frequency was set to 5 s, and the lock mass data were averaged over a total of 10 scans.

2.4. Data preprocessing

Data obtained by QTOF were processed by Markerlynx XS and EZ info software (Waters). Firstly, the intensity of each ion was normalized to generate a data matrix that consisted of the m/z value, the retention time, and the normalized peak area. Next, unsupervised segregation was assessed with principal components analysis (PCA) using pareto-scaled data. PCA data were visualized by plotting the PCA scores; each point represents one retention time/mass-to-charge (m/z) pair. Thus, the loadings plot gives an indication of the lipids that most strongly influence the patterns in the score plot. To maximize class discrimination and biomarkers identification, the data were further analyzed using the (O)PLS-DA method, whereas S-plots or loadings plot were calculated to visualize the relationship between covariance and correlation within the (O)PLS-DA data. Discriminating variables