



# Lipidomic differentiation between human kidney tumors and surrounding normal tissues using HILIC-HPLC/ESI-MS and multivariate data analysis



Eva Cífková<sup>a</sup>, Michal Holčapek<sup>a,\*</sup>, Miroslav Lísa<sup>a</sup>, David Vrána<sup>b</sup>, Bohuslav Melichar<sup>b</sup>, Vladimír Študent<sup>c</sup>

<sup>a</sup> University of Pardubice, Faculty of Chemical Technology, Department of Analytical Chemistry, Studentská 573, 532 10 Pardubice, Czech Republic

<sup>b</sup> Palacký University, Medical School and Teaching Hospital, Department of Oncology, I.P. Pavlova 6, 775 20 Olomouc, Czech Republic

<sup>c</sup> Palacký University, Faculty of Medicine and Dentistry, Department of Urology, I.P. Pavlova 6, 775 20 Olomouc, Czech Republic

## ARTICLE INFO

### Article history:

Received 27 January 2015

Received in revised form 24 March 2015

Accepted 5 July 2015

Available online 13 July 2015

### Keywords:

Lipidomics

Kidney cancer

Glycerophospholipids

HILIC-HPLC/ESI-MS

Multivariate data analysis

## ABSTRACT

The characterization of differences among polar lipid classes in tumors and surrounding normal tissues of 20 kidney cancer patients is performed by hydrophilic interaction liquid chromatography (HILIC) coupled to electrospray ionization mass spectrometry (ESI-MS). The detailed analysis of identified lipid classes using relative abundances of characteristic ions in negative- and positive-ion modes is used for the determination of more than 120 individual lipid species containing attached fatty acyls of different chain length and double bond number. Lipid species are described using relative abundances, providing a better visualization of lipidomic differences between tumor and normal tissues. The multivariate data analysis methods using unsupervised principal component analysis (PCA) and supervised orthogonal partial least square (OPLS) are used for the characterization of statistically significant differences in identified lipid species. Ten most significant up- and down-regulated lipids in OPLS score plots are also displayed by box plots. A notable increase of relative abundances of lipids containing four and more double bonds is detected in tumor compared to normal tissues.

© 2015 Elsevier B.V. All rights reserved.

## 1. Introduction

Kidney cancer ranks among 10 most common cancers for both men and women, representing approximately 3% of adult tumors [1,2]. Renal cell cancer (RCC) represents more than 90% of malignant kidney tumors. The principal histological RCC subtypes include clear cell RCC (70–80%), papillary RCC (10–15%) and chromophobe RCC (3–5%). RCC is characterized by the resistance to virtually all cytotoxic agents. Before the advent of targeted

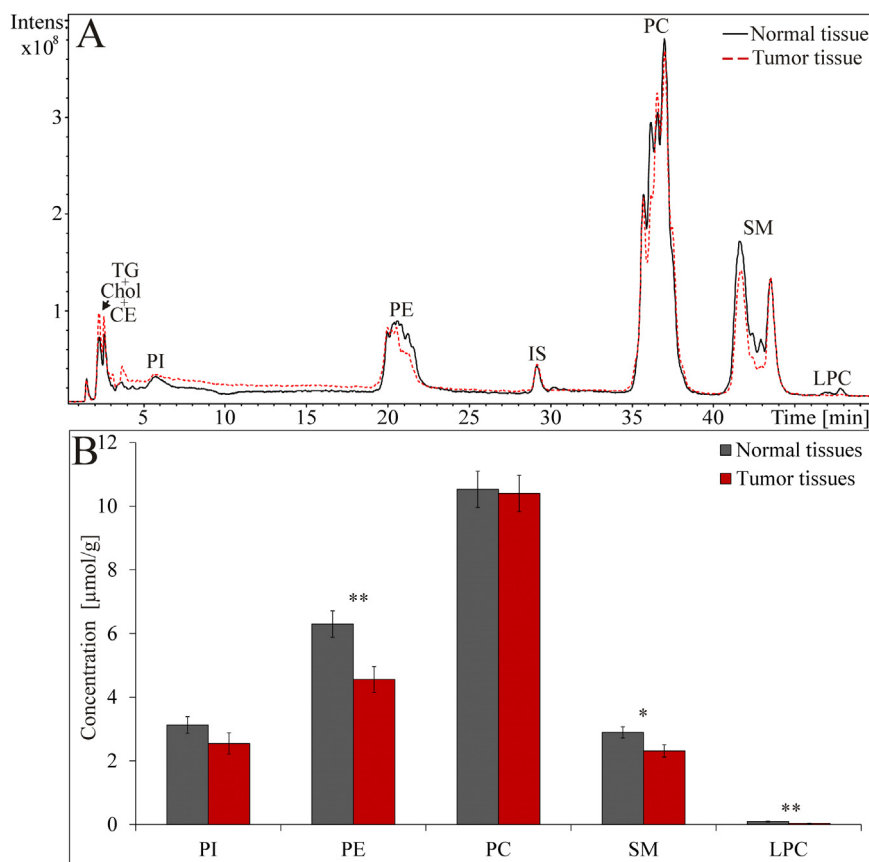
therapy, surgery was the only effective treatment of this tumor. However, only a small proportion of patients with metastatic disease could be cured with surgery. Cytokines, the only systemic agents with some reproducible activity, were effective only in a minority of patients. Targeted therapy has changed the natural course of metastatic RCC. In the last decade, a number of targeted agents have been introduced, including multiple tyrosine kinase inhibitors sunitinib, sorafenib, axitinib and pazopanib, the monoclonal antibody bevacizumab and mammalian target of rapamycin inhibitors everolimus and temsirolimus. Despite this progress, few, if any, patients are cured by currently available drugs. In the search of new effective therapies novel molecular targets associated with malignant transformation have to be identified.

Lipids play an essential role in many biological processes, including the formation of cellular or intracellular membranes and compartments, the energy storage, the synthesis of hormones and the signal transduction [2–4]. The disruption of lipid metabolism and associated signaling pathways alters cellular function resulting in a spectrum of disorders, including the cancer. For example, palmitic acid (16:0) is a substrate for the formation of lipids required for the cellular proliferation and tumorigenic

**Abbreviations:** aCN, average carbon number; aDB, average double bond; CE, cholesteryl esters; CN, carbon number; DB, double bond; ESI, electrospray ionization; HILIC, hydrophilic interaction liquid chromatography; HPLC, high-performance liquid chromatography; Chol, cholesterol; IS, internal standard; LPA, lysophosphatidic acids; LPC, lysophosphatidylcholines; MDA, multivariate data analysis; MS, mass spectrometry; NP, normal phase; OPLS, orthogonal partial least square; PC, phosphatidylcholines; PCA, principal component analysis; PE, phosphatidylethanolamines; PI, phosphatidylinositols; RCC, renal cell cancer; RF, response factor; RP, reversed phase; SM, sphingomyelins; TG, triacylglycerol.

\* Corresponding author. Fax: +420 466037068.

E-mail address: [Michal.Holcapek@upce.cz](mailto:Michal.Holcapek@upce.cz) (M. Holčapek).



**Fig. 1.** (A) Positive-ion HILIC-HPLC/ESI-MS of total lipid extracts of normal (black line) and tumor (red line) tissues of a patient with clear cell type of kidney cancer. HPLC conditions: column Spherisorb Si (250 × 4.6 mm, 5 μm), flow rate 1 mL/min, separation temperature 40 °C, gradient 0 min – 94% A + 6% B, 60 min – 77% A + 23% B, where A is acetonitrile and B is 5 mM aqueous ammonium acetate. (B) Comparison of average concentrations [μmol/g] of individual lipid classes in normal and tumor tissues for 20 patients with their standard errors. Peak annotation: TG – triacylglycerols, Chol – cholesterol, CE – cholesteryl esters, PI – phosphatidylinositols, PE – phosphatidylethanolamines, IS – internal standard, PC – phosphatidylcholines, SM – sphingomyelins, LPC – lysophosphatidylcholines. Statistically significant differences according to T-test are indicated by an asterisk, where \* refers to the significance  $p \leq 0.05$  and \*\*  $p \leq 0.01$ . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

lipid signaling, while lysophosphatidic acids (LPA) and some specific eicosanoids serve as proliferative receptors. On the other hand, ceramides and sphingosines have antiproliferative and proapoptotic activity and are involved in the programmed cell death [3–7]. Furthermore, arachidonic acid (20:4) belongs to major *n*-6 polyunsaturated fatty acids that affect increased production of inflammatory mediators [4].

Many studies addressing the lipidomic analysis of various biological samples benefit from high sensitivity and selectivity of the coupling of high-performance liquid chromatography and mass spectrometry (HPLC/MS). The separation in normal phase (NP) [8,9] or hydrophilic interaction liquid chromatography (HILIC) systems [10] enables the lipid class separation according to the polarity. Reversed phase (RP) HPLC on nonpolar stationary phases is frequently used for the separation of individual lipid species according to the fatty acyl chain length [11]. The quantitative analysis of lipids can be performed using the MS with the direct infusion (shotgun lipidomics) or HPLC/MS approaches. Shotgun is the most frequently used technique due to the rapid nontargeted analysis using precursor ion and neutral loss scans, which are well characterized for many lipid classes [12–14]. HPLC/MS approach was developed for the quantitation of separated lipid classes using the internal standard (IS) per each lipid class [15] or the combination of the single IS and response factors (RF) of individual lipid classes related with this IS [16,17].

Lipidomic analysis of RCC tissues and normal kidney tissues was performed using HPLC/MS [18], where significantly increased lev-

els of cholesteryl esters (CE) and triacylglycerols (TG) and decreased levels of phosphatidylethanolamines (PE) and sphingomyelins (SM) in tumor tissues were reported. Mass spectrometry imaging using matrix-assisted laser desorption/ionization or desorption electrospray ionization was used for the relative comparison of tumor and surrounding normal tissues [19–22]. Increased absolute intensities for PI 18:0/20:4, PS 18:0/18:1 and PI 22:4/18:0 were reported [19,21] in kidney tumor tissues using the desorption electrospray ionization imaging. Analyses of lipids in plasma from RCC patients using <sup>31</sup>P nuclear magnetic resonance showed decreased concentrations of lysophosphatidylcholines (LPC) in comparison with healthy volunteers [23].

Multivariate data analysis (MDA) is a valuable approach for the evaluation of extensive data sets, providing significantly more information compared to the univariate data analysis [24–27]. The principal component analysis (PCA) is the most widespread nonsupervised method for the visualization of data sets using converted uncorrelated variables called principal components. Score plots serve for the projection of clustering of observed data, while loading plots describe clustering patterns. Orthogonal partial least square (OPLS) is a supervised method, where the group identification is set up in model parameters. OPLS method may use S-plots for a better visualization of clustering patterns including the influence of magnitude and reliability of variables.

The aim of the present study is to characterize lipidomic differences between kidney tumors and surrounding normal tissues in a cohort of 20 kidney cancer patients using our validated HILIC-

Download English Version:

<https://daneshyari.com/en/article/7616904>

Download Persian Version:

<https://daneshyari.com/article/7616904>

[Daneshyari.com](https://daneshyari.com)