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Metabolomic profiling of prostate cancer by matrix assisted laser desorption/ionization-Fourier transform ion cyclotron resonance mass spectrometry imaging using Matrix Coating Assisted by an Electric Field (MCAEF)☆

Xiaodong Wang [a,1], Jun Han [a], Darryl B. Hardie [a], Juncong Yang [a], Jingxi Pan [a], Christoph H. Borchers [a,b,*]

[a] University of Victoria-Genome British Columbia Proteomics Centre, Vancouver Island Technology Park, #3101-4464 Markham St., Victoria, BC V8Z 7X8, Canada
[b] Department of Biochemistry and Microbiology, University of Victoria, Petch Building Room 207, 3800 Finnerty Rd., Victoria, BC V8P 5C2, Canada

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ABSTRACT

In this work, we combined the use of two MALDI matrices (quercetin and 9-aminoacridine), a recently developed new matrix coating technique – matrix coating assisted by an electric field (MCAEF), and matrix-assisted laser desorption/ionization – Fourier transform ion cyclotron resonance mass spectrometry (MALDI-FTICRMS) to detect and image endogenous compounds in the cancerous and non-cancerous regions of three human prostate cancer (stage II) tissue specimens. After three rounds of imaging data acquisitions (i.e., quercetin for positive and negative ion detection and 9-aminoacridine for negative ion detection), and metabolite identification, a total of 1091 metabolites including 1032 lipids and 59 other metabolites were routinely detected and successfully localized. Of these compounds, 250 and 217 were only detected in either the cancerous or the non-cancerous regions respectively, although we cannot rule out the presence of these metabolites at concentrations below the detection limit. In addition, 152 of the other 624 metabolites showed differential distributions ($p < 0.05$, *t*-test) between the two regions of the tissues. Further studies on a larger number of clinical specimens will need to be carried out to confirm this large number of apparently cancer-related metabolites. The successful determination of the spatial locations and abundances of these endogenous biomolecules indicated significant metabolism abnormalities – e.g., increased energy charge and under-expression of neutral acyl glycerides, in the prostate cancer samples. To our knowledge, this work has resulted in MALDI-MS imaging of the largest group of metabolites in prostate cancer thus far and demonstrated the importance of using complementary matrices for comprehensive metabolomic imaging by MALDI-MS. This article is part of a Special Issue entitled: MALDI Imaging, edited by Dr. Corinna Henkel and Prof. Peter Hoffmann.

1. Introduction

Matrix-assisted laser desorption/ionization mass spectrometric imaging (MALDI-MSI) is a label-free molecular profiling technique that permits the direct determination of the spatial distribution and relative abundance of a wide range of molecules, including endogenous and exogenous compounds, peptides, and proteins in a biological sample such as tissue specimen [1–8]. Since its introduction by Caprioli and colleagues [9], MALDI-MSI has shown great potential for assisting pathologists in both diagnostic and prognostic decision making [1,2,4,6,7]. In

Abbreviations: 9-AA, 9-aminoacridine; ACN, acetonitrile; ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; CDP-DG, cytidine diphosphate-diacylglycerol; Cer, ceramide; CID, collision-induced dissociation; CL, cardiolipin; cPA, cyclic phosphatidic acid; DAG, diacylglycerol; ESI, electrospray ionization; FTICR, Fourier transform ion cyclotron resonance; GSL, glycosphingolipid; ITO, indium-tin oxide; MAG, monoacylglycerol; MALDI, matrix-assisted laser desorption/ionization; MCAEF, matrix coating assisted by an electric field; MSI, mass spectrometric imaging; PA, phosphatidic acid; PE, phosphatidylethanolamine; PE-Cer, ceramide phosphoethanolamine; PG, phosphoglycerol; PI, phosphatidylinositol; PI-Cer, ceramide phosphoinositol; PIN, prostatic intraepithelial neoplasia; PIP, phosphatidylinositol phosphate; PIP2, phosphatidylinositol bisphosphate; PS, phosphatidylserine; SM, sphingomyelin; TAG, triacylglycerol; TFA, trifluoroacetic acid; TOF, time-of-flight.

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* Corresponding author at: University of Victoria-Genome British Columbia Proteomics Centre, #3101-4464 Markham St., Vancouver Island Technology Park, Victoria, BC V8Z 7X8, Canada.
E-mail address: christoph@proteincentre.com (C.H. Borchers).
[1] Present address: Centre for Imaging & Systems Biology, College of Life and Environmental Sciences, Minzu University of China, 27 Zhongguancun South St., Beijing, 100081, China.

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the past decade, MALDI-MSI has received an increasing amount of attention and is increasingly being used for the discovery of potential disease biomarkers directly from surgery-removed tissue sections, including those from chronic kidney disease [10], brain tumor [11], renal cell adenocarcinoma [12], breast cancer [13–15], colon cancer's liver metastasis [16], thyroid papillary cancer [17], gastric cancer [18,19], and ovarian cancer [20]. Despite the enormous progress has been made in MALDI tissue imaging [1,21], the percentage of biomolecules that can currently be imaged by MALDI-MS is still very limited, compared to the tens of thousands of biomolecules that exist in a cell or tissue [22–24]. For this reason, it is clear that the MALDI-MSI has not yet reached its full potential. In order to improve this situation, we recently described a technique called matrix coating assisted by an electric field (MCAEF), for overall enhancement of MALDI-MSI [25]. In MCAEF a static electric field is applied to sliced tissue sections during MALDI matrix coating so as to increase the concentration of the analytes in the deposited matrix layer [25]. Compared with the MCAEF-free matrix coating, MCAEF typically results in a general increase in the sensitivity of lipid and protein detection across the board in subsequent MALDI-MS analyses, as well as resulting in the successful imaging of a larger number of analytes [25].

Prostate cancer, a glandular cancer in the male reproductive system, is one of the most common malignancies in men [26]. Prostate cancer displays a broad range of clinical outcomes, from relatively indolent and harmless, to lethal metastatic disease [27]. It can be slow-growing and some men who develop prostate cancer may live many years without ever having the cancer detected. However, prostate cancer has become the second most frequently diagnosed cancer (after skin cancer) and the sixth leading cause of cancer death in men worldwide [26,28]. Prostate cancer is classified as the most commonly diagnosed cancer and a leading cause of cancer death among men in North America [29]. In Europe, prostate cancer became the third most diagnosed cancer after colorectal and breast cancers, with 417,000 cases in 2012 [30]. The current diagnostic tools, such as blood prostate-specific antigen test [31,32], histological Gleason grading of biopsy specimens (Gleason score) [33,34], and clinical tumor-node-metastasis staging [35] seem unable to accurately stratify individual prostate cancer patients at early stages of the disease [36–39]. For example, the standard needle biopsy-based procedure, a commonly-used method for prostate cancer diagnosis can have a high false-negative rate if none of the cancer cells pass through the biopsy needles, which delays cancer diagnosis and treatment, as well as increasing the anxiety and costs for supposedly cancer-free men [40,41]. Given the heterogeneity of the disease and the wide range of clinical outcomes, the main challenge facing physicians is to distinguish patients with non-malignant tumors from malignant prostate cancer, and to monitor early-stage prostate cancer. There is therefore a clear need for novel prognostic biomarkers with improved sensitivity and specificity.

MALDI-MSI with positive-ion detection has already been used to explore potential protein biomarkers for prostate cancer [42,43]. Potential biomarker proteins include a fragment of mitogen-activated kinase kinase kinase 2 (*m/z* 4355) [42,44], a fragment of cAMP-regulated phosphoprotein 19 (*m/z* 4965) [44], apolipoprotein C-I (*m/z* 6633) [44], apolipoprotein A-I (*m/z* 28,079) [44], apolipoprotein A-II (*m/z* 7805) [44], protein S100-A6 (*m/z* 10,179) [44], protein S100-A8 (*m/z* 10,851) [44], β-microseminoprotein (*m/z* 10,762) [44], tumor protein D52 (*m/z* 12,389) [44], α-1-acid glycoprotein 1 (*m/z* 21,560) [44], and heat shock protein β-1 (*m/z* 22,782) [44], and several large peptides identified only by their molecular masses (*e.g.*, *m/z* 2753 and 6704, high expression in non-cancerous glands *vs.* *m/z* 5002, overexpression in cancerous glands) [43,44]. For metabolomic imaging, Goto et al., reported higher expression of phosphatidylinositols [45] and decreased expression of lysophosphatidylcholine (16:0/OH) in prostate cancer [46]. In these studies, however, only a very limited numbers of metabolites were imaged [45,46].

To provide a more comprehensive profile of metabolite changes in prostate cancer, we combined the use of two complementary MALDI matrices (i.e., quercetin for positive and negative ion detection and 9-aminoacridine (9-AA) for negative ion detection) with MCAEF [25] and MALDI-Fourier transform ion cyclotron resonance (FTICR)-MS for the detection and imaging of the endogenous compounds including lipids, nucleotides, and other metabolites in three human prostate cancer (stage II) clinical tissue specimens.

2. Materials and methods

2.1. Chemicals and reagents

Quercetin and 9-AA were obtained from Sigma-Aldrich (St. Louis, MO, USA). LC/MS grade methanol, isopropanol, acetonitrile (ACN), water, 25% ammonium hydroxide (NH_4OH), formic acid (FA), and trifluoroacetic acid (TFA), together with HPLC grade ethanol and chloroform, were also obtained from Sigma-Aldrich. The "ESI tuning mix" solution was purchased from Agilent Technologies (Santa Clara, CA, USA).

2.2. Materials

The use of the human tissue samples involved in this study was approved by the Ethics Committee of the University of Victoria. Three human prostate cancer specimens (I, II, and III) were obtained from BioServe Biotechnologies (Beltsville, MD, USA). The tissue specimens were acquired from three 62 ± 2-year-old male patients during prostate cancer surgical removal, with the patient's informed consent. According to the accompanying pathological classification information, all the three patients were diagnosed with prostate cancer at stage II. Tissue specimen I was diagnosed as high-grade prostatic intraepithelial neoplasia (PIN)-2/3 (PRSTAT05), specimens II and III were diagnosed as adenocarcinoma (PRSTAT03). The tissue samples were stored at −80 °C until used. These three tissue specimens were also used in our previous study on protein biomarker screening based on MALDI-TOF/TOF MS analysis [44].

2.3. MALDI imaging

2.3.1. Tissue sectioning

The prostate specimens were sectioned into 12-μm thick tissue slices at −20 °C inside a Microm HM500 cryostat (Waldorf, Germany). Serial tissue sections were immediately thaw-mounted onto 25 mm × 75 mm indium-tin oxide (ITO)-coated electrically conductive microscopic glass slides obtained from Bruker Daltonics (Bremen, Germany). Before matrix application, the tissue mounted slides were placed under a vacuum of 0.1 psi for 15 min in a Savant SPD1010 SpeedVac Concentrator (Thermo Electron Corporation, Waltham, MA, USA).

2.3.2. Histological staining

To obtain histological images of prostate tissue sections, hematoxylin and eosin (H&E) staining was performed according to a previously reported procedure [47]. H&E staining has been used for at least a century and is still used for recognizing various tissue types and the morphologic changes that form the basis of contemporary cancer diagnosis. Based on H&E staining, non-cancerous regions and cancerous region can be easily distinguished by their different staining colors, *i.e.*, blue in the cancerous region compared to pink in the non-cancerous region.

2.3.3. Matrix coating

Quercetin was dissolved in a mixed methanol:H_2O:25% NH_4OH (80:20:0.4, v/v) solution at a matrix concentration of 2.6 mg/mL, as has been described previously [48,49]. 9-AA was prepared at a concentration of 20 mg/mL in a mixed ACN:H_2O:TFA (70:30:0.2, v/v) solution, and this matrix was mainly used for the detection of nucleotides by