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Lipid mapping by desorption electrospray ionization mass spectrometry in a murine breast DMBA carcinogenesis model

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ABSTRACT

Lipids are molecules with a large structural diversity inserted in various cytological and pathological processes, such as in breast cancer. The knowledge of the metabolic and lipid profiles in breast cancer helps to identify and classify neoplastic and non-neoplastic areas and aids therefore in precise histological diagnosis and in the identification of new promising therapeutic targets. The present study aimed to compare non-neoplastic samples of Sprague-Dawley rat breast lesions to a DMBA chemical carcinogenesis model via DESI-MS imaging. The model Sprague-Dawley rats were treated with the carcinogen compound DMBA, and frozen mammary samples with or without tumors were imaged. The developed ductal carcinomas showed indeed contrasting lipid relative abundances as compared to non-neoplastic breast tissue. Most lipids belong to the fatty acyl family, and were detected in greater abundance in the non-neoplastic cancer samples. The lipid ion of m/z 336 was observed only in the neoplastic areas. This is the first study that demonstrates changes in lipid profile of mammary tumors in rats treated with DMBA, and the promising results seems to encourage and serve as a guide for further lipidomic studies regarding breast cancer.

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1. Introduction

Recently, mass spectrometry imaging (MSI) has been established as a powerful technique for obtaining spatial distribution maps, identifying and (relatively or absolutely) quantifying various biological molecules, including drugs, lipids, hormones, peptides and proteins, directly on the surface of biological tissues from histological slides [1,2]. Several ionization techniques for MSI are available, including matrix-assisted laser desorption/ionization, MALDI, particularly suited for proteomic studies [3], and desorption electrospray ionization, DESI, the technique of choice for lipidomic investigations directly from tissue samples [1,2]. DESI has been widely applied in MSI with the advantage of having no interference from added matrix, as occurs in MALDI, since the ionization takes place externally to the mass spectrometer and samples can be directly analyzed, with minimal or no preparation [4]. The formation of ions by DESI is based on charged micro droplets of solvents that firstly collide with the sample surface, then analytes are extracted into the liquid film and release secondary droplets. The secondary droplets are pick up following by electrospray solvent evaporation processes, producing dry ions of the analytes that finally reach the mass spectrometer [1,4]. The solvents employed in micro droplets formation determine the quality of DESI and a mixture methanol: water is widely used [2].

Methods such as high performance liquid chromatography (HPLC) and gas chromatography (CG) coupled to mass spectrometry are commonly applied for lipid separation and identification, but these methods are not able to provide information about spatial distribution as MSI. Analysis of spatial distribution improves the information obtained by histochemical techniques and supports pathological diagnosis [2]. DESI-MSI provides two-dimensional images that uses the relative abundance of selected biomolecules

Abbreviations: DESI, desorption electrospray ionization; DMBA, dimetilbenz(α)anthracene; H&E, hematoxylin-eosin; HexSph, hexosylsphingosine; MALDI, matrix-assisted laser desorption/ionization; MSI, mass spectrometry imaging.

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such as lipids as "pixels" [2,5,6]. Lipids are dissolved in micro droplets that enter in the mass spectrometer, providing chemical details, *m*/*z* values and hence the distribution in the tissue [2,4]. An imaging experiment is carried out by scanning the sample in the *x* and *y* directions through spraying of charged micro droplets [2,4].

DESI-MSI has been demonstrated to be quite effective for a variety of lipid classes such as fatty acids, glycerophospholipids, glycerolipids, steroids and sphingolipids [2,5]. Lipids are exquisitely designed molecules with a great variety of structural features and fine-tuned physicochemical properties playing therefore key roles in various cytological processes and cell signaling [1]. The analysis and characterization of lipids in biological tissues are therefore an essential step in life sciences that assists in the investigation of lipid function in healthy biological tissues, whereas unique changes in the profile, relative abundances and/or function of the various groups of lipids have been associated to many pathological processes [2]. Tissue lipidomics analysis can hence support morphological investigations, diagnosis and characterization of pathophysiological processes [2,7], such as neurodegenerative and cardiovascular diseases and neoplastic processes [1,2]. Tumor tissues have also been found to display drastic changes in lipid relative abundances when compared to normal tissues [7]. It is also known that oncogenes such as AKT and PTEN can be activated from lipid signaling [7]. Lipids have therefore been found to function as important biomarkers for gliomas, gastric, liver and prostate tumors, lymphoma and breast cancer [1,2,7,8].

Breast cancer is the most common and the leading cause of death among female malignancies worldwide, which affects more than a million women each year [9,10]. The heterogeneity and molecular complexity of breast cancer makes it difficult to create therapeutic strategies for prevention, diagnosis and treatment of this major disease [11]. DESI-MSI can therefore be a useful tool in the identification and classification of neoplastic and non-neoplastic areas of breast cancer, assisting in the diagnosis of histological subtypes and providing information regarding intraoperative procedures for tumor removal [1,5]. Conventional techniques, such as radiography and magnetic resonance imaging, are limited in providing information regarding surgical applications for the removal of the tumor [5]. It is known that neoplastic areas show a different metabolic profile than non-neoplastic breast tissue, pointing to the suitability of MS imaging for lipidomic investigations of breast cancer [3,5,12].

Sprague-Dawley and Wistar-Furth rats, as well as mice, are widely used as chemically induced experimental animal models for the study of mammary carcinogenesis [13,14]. Rats have great chance of developing breast neoplasms and the developed lesions greatly resemble human lesions [13,15]. Among the current chemical animal models, the dimethylbenz(α)anthracene (DMBA) model using young and virgin Sprague-Dawley rats is noteworthy [13,14,16].

The analysis of non-neoplastic and neoplastic mammary samples by MSI techniques is still in its infancy. Nevertheless, certainly DESI-MSI should function as a suitable tool for helping histological and pathophysiological studies, as well as in clinical field, contributing to the diagnosis, characterization and monitoring of disease progression. We herein present the first lipidomic comparison of normal rat breast tissues and neoplastic tissues from animals belonging to the DMBA chemical carcinogenesis rat model.

2. Material and methods

2.1. Animals and tumor induction

Virgin female Sprague-Dawley rats from the Multidisciplinary Center for Biological Investigation at the Laboratory for Animal Science of the University of Campinas (CEMIB-UNICAMP), aged 35–40 days (200–250 g), were used. The rats were housed in a temperature and humidity-controlled facility in the with a 12 h light/dark cycle. The rats were kept in plastic cages (5 animals per cage), and fed rodent food and water *ad libitum*. Malignant breast neoplasms were induced by a single dose of 7,12-dimethylbenz(α)anthracene (DMBA) at a concentration of 100 mg/kg diluted in 1 mL of soybean oil and given intragastrically by gavage (DMBA Group, n = 5). At same time, the control group was treated with 1 mL of soybean oil given intrasgastrically (Control Group, n = 5). All procedures were approved by the local Ethics Committee (CEUA-UNICAMP; protocol number 2335-1), in accordance to National guidelines (established by the Brazilian Society of Laboratory Animal Science [SBCAL], former College for Animal Experimentation [COBEA]), and NIH standards.

2.2. Tumor specimens

At the end of each experimental protocol, the rats were euthanized by deep anesthesia with isoflurane and cervical dislocation. Mammary glands were excised and macroscopically examined. The volume of each individual neoplasm was calculated following the equation $V = [(abc)\pi]/6$ (where a, b and c represent the greater dimensions of the tumors). The tissues were stored at −80 °C or proceeded to classic paraffin histological staining with five-μm sections with hematoxylin-eosin (H&E).

Ten to fifteen-μm thick sections were obtained from frozen tissue embedded in optimal cutting temperature (OCT) compound containing a representative slice of the largest tumor or normal breast using a cryostat (Leica, Wetzlar, Germany) at −21 °C. The slides were stained with hematoxylin-eosin (according to standard protocol) or proceeded for DESI-MSI analysis. For the slides stained with H&E, images were acquired using a CCD camera 212 (LEICA CTR 5000) and the software package LEICA Q Win Plus V. 3.2.0. for Windows.

2.3. DESI-MSI analysis

The experiments were conducted using a Q Exactive mass spectrometer (Thermo Fisher, Bremen, Germany) and a coupled DESI source with a 2D moving stage (Prosolia, Indianapolis, USA). The operating parameters were as follow: spray voltage 5 kV; the automatic gain control (AGC) was turned on and 1 microscans were summed to create each spectrum. The DESI source conditions were as follow: nitrogen sheath gas pressure of 160 psi; incident angle of 55°; tip-to-surface distance of 2 mm; tip-to-inlet distance of 4–6 mm. Methanol was used as spray solvent and delivered by the instrument syringe pump at a volumetric flow rate of 1.5 μL/min. The data were obtained from *m*/*z* 100 to 1200 in the negative ion mode by continuously scanning the DESI spray across the surface at spatial resolution of ca. 200 × 200 μm. The data was processed by BioMap software (www.maldi-msi.org) to create the selective ion images of the scanned sample surface.

2.4. Statistical analyses and lipid identification

Differences between groups were analyzed using an unpaired *t*-test. A *p* value of less than 0.05 was considered statistically significant. All tests were performed using the GraphPad Prism 5.0 software package for Windows.

Lipid identification was performed using the LIPID MAPS database (www.lipidmaps.org). The matches with the lower delta value compared to the inputted *m*/*z* on the database were selected for putative identification.

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