Characterization of metabolic profile of intact non-tumor and tumor breast cells by high-resolution magic angle spinning nuclear magnetic resonance spectroscopy

Roberta M. Maria a, b, Wanessa F. Altei c, Adriano D. Andricopulo c, Amanda B. Beccheneri d, Márcia R. Cominetti d, Tiago Venâncio e, Luiz A. Colnago b, *

a Instituto de Química de São Carlos, Universidade de São Paulo, São Carlos, SP 13560-970, Brazil
b Embrapa Instrumentação, São Carlos, SP 13560-970, Brazil
c Instituto de Física de São Carlos, Universidade de São Paulo, São Carlos, SP 13560-970, Brazil
d Centro de Ciências Biológicas e da Saúde, Centro de Ciências Biológicas e da Saúde/Universidade Federal de São Carlos (CCBS/UFSCar), Departamento de Gerontologia, Laboratório de Biologia do Envelhecimento (LABEN), São Carlos, SP 13565-905, Brazil
e Departamento de Química, Centro de Ciências Exatas e de Tecnologia, Universidade Federal de São Carlos, São Carlos, SP 13565-905, Brazil

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1H high-resolution magic angle spinning nuclear magnetic resonance (1H HR–MAS NMR) spectroscopy was used to analyze the metabolic profile of an intact non-tumor breast cell line (MCF-10A) and intact breast tumor cell lines (MCF-7 and MDA-MB-231). In the spectra of MCF-10A cells, six metabolites were assigned, with glucose and ethanol in higher concentrations. Fifteen metabolites were assigned in MCF-7 and MDA-MB-231 1H HR–MAS NMR spectra. They did not show glucose and ethanol, and the major component in both tumor cells was phosphocholine (higher in MDA-MB-231 than in MCF-7), which can be considered as a tumor biomarker of breast cancer malignant transformation. These tumor cells also show acetone signal that was higher in MDA-MB-231 cells than in MCF-7 cells. The high acetone level may be an indication of high demand for energy in MDA-MB-231 to maintain cell proliferation. The higher acetone and phosphocholine levels in MDA-MB-231 cells indicate the higher malignance of the cell line. Therefore, HR–MAS is a rapid reproducible method to study the metabolic profile of intact breast cells, with minimal sample preparation and contamination, which are critical in the analyses of slow-growth cells.

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Nuclear magnetic resonance (NMR) spectroscopy has been used to study metabolic profiles of cancer cells and tissues from biopsies for more than two decades [1]. It has been used to differentiate malignant and benign tumors [2,3] as well as to evaluate breast cancer treatment [4]. The initial NMR analyses were performed on biopsies of solid tumors to assess responsiveness to therapy [5]; however, the resolutions of the spectra were extremely low with broad overlapping peaks, limiting the biochemical information [6]. To have more information about the metabolic profile, the NMR analyses were also performed in tissue extracts. Regardless, the extraction processes of both water- and fat-soluble analyses were laborious and time-consuming, and the direct comparison with histopathology was impossible [6].

The problem with resolution of NMR spectra of cells/tissues was solved by the introduction of the high-resolution magic angle spinning (1H HR–MAS) technique, which has been used since 1996 [7]. 1H HR–MAS reduces the line width of NMR spectra by spinning the sample at a magic angle (54.7°) to the static magnetic field [8,9]. With this technique, it is possible to obtain spectra with solution resolution even with intact cells/tissues and consequently the identification and quantification of a large number of metabolites [10]. Furthermore, the sample preparation protocol is simple and...
fast, with minimal sample preparation and contamination, which are critical in the analyses of slow-growth cells [10,11].

To enhance resolution and minimize the contribution of broad lines from large molecules or molecular assemblies, it is possible to use a T2 filter based on the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence [11] or to use the filter diagonalization method (FDM) [12] to process the time domain signal. Nowadays, 1H HR–MAS is a standard NMR protocol to obtain high-resolution spectra of heterogeneous materials such as tissues and cells.

The 1H HR–MAS method has been used to study breast cancer, which is estimated to be the cause of death of more than half a million women annually worldwide [13]. Cheng and coworkers [10] used 1H HR–MAS to demonstrate the potential and clinical relevance of this method as a valuable tool in the investigation of tumor pathology in a group of female breast ductal carcinoma patients, concluding that this method results in improved accuracy for breast cancer diagnosis. Sitter and coworkers [14] compared spectra of breast cancer tissue from 10 patients with conventional high-resolution spectra of perchloric acid extracts from the same tissue type. 1H HR–MAS metabolic profiles of breast cancer tissue and non-involved specimens have also been compared [15]. Bathen and coworkers [16] evaluated the use of metabolic phenotype as a tool for prediction of histological grade, hormone status, and axillary lymphatic spread in breast cancer patients by 1H HR–MAS. Biochemical alterations in tumor tissue are expected to be detectable before any visible morphological changes. The analysis and discovery of metabolic pathways in breast cancer may enhance not only diagnosis but also therapeutic response in breast cancer aggressiveness.

In this work, we used 1H HR–MAS NMR to investigate the difference between non-tumor cells (MCF-10A) and tumor cells (MDA-MB-231 and MCF-7) metabolism. MCF-10A is a non-tumor epithelial mammary gland/breast cell line isolated from a 36-year-old Caucasian woman [17]. MDA-MB-231 is a triple negative breast tumor cell (TNBC) line isolated from a 51-year-old Caucasian woman and is a heterogeneous subgroup of breast cancer characterized by the absence of expression of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor-2/neu [18]. This subtype of cancer represents approximately 15–20% of all breast cancer cases and is generally considered as the most severe subgroup of breast cancer, with a high relapse rate. The MCF-7 cell line is a triple positive breast tumor isolated in 1970 from a 69-year-old Caucasian woman [19]. It is characterized by the presence of estrogen receptors and human epidermal growth factor receptor-2/neu, capable of forming domes and with better prognosis when compared with MDA-MB-231 cells.

In this article, we report the 1H HR–MAS NMR spectra that show significant differences between the metabolic profile of non-tumor breast cells (MCF-10A) and tumor breast cells (MCF-7 and MDA-MB-231) as well as between the two tumor lines. These comparisons between HR–MAS spectra of breast cell lines have not been previously reported in the literature. The non-tumor cells showed high content of glucose and ethanol, and the tumor cells showed high levels of phosphocholine. Acetone was absent in non-tumor cells but present in both types of tumor cells. The acetone content was higher in MDA-MB-231 than in MCF-7 cells. Therefore, the 1H HR–MAS NMR spectra can be used in early detection, with monitoring of the disease being a valuable tool in cancer research.

Materials and methods

Non-tumor and tumor cell samples

MCF-10A (ATCC CRL-10371) cells were obtained from Peter MacCallum cancer centre (Australia), MCF-7 (ATCC HTB 22) and MDA-MB-231 (ATCC HTB 26) cells were obtained from the Rio de Janeiro cell bank (BCRJ, Rio de Janeiro, RJ, Brazil). The MCF-10A cell line was derived from non-tumor epithelial cell from human mammary gland and was maintained in Dulbecco’s modified Eagle’s medium/nutrient mixture F-12 (DMEM/F-12, Invitrogen, Carlsbad, CA, USA) supplemented with 5% fetal horse serum (Thermo Fisher Scientific, Waltham, MA, USA), 1% penicillin-streptomycin (Thermo Fisher Scientific), 10 μg/ml bovine insulin (Sigma–Aldrich), 20 ng/ml epidermal growth factor (EGF; Sigma–Aldrich), and 0.5 mg/ml hydrocortisone (Sigma–Aldrich). All cells were maintained at 37 °C in a humidified atmosphere (95% air and 5% CO2).

MCF-7 and MDA-MB-231 tumor cell lines were chosen because of the high impact of cancer on the human mortality. MCF-7 cells were maintained in DMEM (Thermo Fisher Scientific) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Thermo Fisher Scientific), and MDA-MB-231 cells were maintained in Leibovitz’s L-15 medium (Cultilab, Campinas, SP, Brazil), both supplemented with l-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 mg/ml). Cells were seeded at a density of 1.4 × 106 cells/well in 75-cm2 cell culture flasks and incubated (37 °C with 5% CO2) for 72 h to allow the cells to attach to the flasks. The medium was removed, and subcultures were obtained by treating the cell with trypsin in phosphate-buffered saline (PBS; Sigma–Aldrich) for 2 min. Subsequently, 4 ml of culture medium was added, and cells were centrifuged at 1500 rpm for 5 min to completely separate the pellets. Cell viability was tested by Trypan Blue exclusion prior to the experiments. After centrifugation, the pellet was resuspended in D2O and centrifuged for NMR analysis. Each cell line was prepared in triplicate for the NMR analysis.

1H HR–MAS NMR analyses

Each cell compartment was packed into a zirconium HR–MAS rotor containing 20 μl of deuterium oxide and sodium trimethylsilyl-[2,2,3,3-2H4]-1-propionate (TMSP) as an internal standard, and it was spun at the magic angle (54.7° relative to the magnetic field z-direction). 1H HR–MAS spectroscopy was performed from 9.4 T (T) (400.21 MHz for 1H) and 14.1 T (600 MHz for 1H) using a DRX 400 Bruker NMR spectrometer and an Avance 600 Bruker NMR spectrometer, respectively, and a 5-KHz spinning rate. The spectra were acquired with a 1.5-s presaturation pulse, an acquisition time of 4.63 s (32 K points), a 4-s recycle delay, and an accumulation of 256 transients. In addition, a CPMG spin–echo train was used before acquisition by applying 120 cycles separated by 1.2 ms of echo time. The free induction decay (FID) signal was multiplied by a 1.0-Hz (0.0025 ppm) line broadening and zero filled 2-fold for Fourier transformation. For automatic phasing and baseline correction, Advanced Chemistry Development (ACD) Labs software was used. The samples were also analyzed using two-dimensional (2D) NMR spectroscopy such as COSY (correlation spectroscopy), 1H–13C-HSQC (proton-carbon heteronuclear single-quantum correlation spectroscopy), and 1H–13C-HMBC (proton-carbon heteronuclear multiple bond correlation spectroscopy). The assignments of 1H HR–MAS spectra of non-tumor and tumor cells were performed using published data [4,15] and correlated spectroscopy (2D homonuclear and heteronuclear NMR experiments such as COSY, 1H–13C-HSQC, and 1H–13C-HMBC). Online databases, such as the human metabolome database (HMDB) and Chenomx software, were also used for assignment.

Origin 9.0 software (OriginLab, Northampton, MA, USA) was used for the normalization of signal areas of non-tumor and tumor cells. Each spectrum baseline was corrected, if necessary, to eliminate the amplitude offset. Then, each spectrum was normalized by dividing the amplitude of each data point from 1.0 to 4.5 ppm by the sum of the amplitude of all data points in the same region. The