Journal of the American Society of Cytopathology (2016) 5, 3–8

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jascyto.org/

ORIGINAL ARTICLE

Morphologically compatible mass spectrometric analysis of lipids in cytological specimens

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Received 17 August 2015; accepted 14 October 2015

KEYWORDS
Mass spectrometry;
Ambient ionization;
Cytopathology;
Ancillary techniques;
Cytospin;
Non-gynecologic cytopathology

Introduction Modern lipid analysis requires mass spectrometric techniques, though to date these have been developed and applied primarily to histological serial sections. As such, there has been little emphasis on using mass spectrometry in such a way that the same specimen can yield both chemical and morphological information. Here, we present a mass spectrometric method that enables measurement of lipids from cells on cytospin slides in a way that preserves the cells for subsequent cytomorphologic evaluation.

Materials and methods Standardized cultures of MDA-MB-231, a breast cancer cell line, were prepared as cytospins and subjected to analysis using a Prosolia Flowprobe sampling and ionization source attached to a Thermo Scientific Quadrupole-Orbitrap mass spectrometer. Chemical compositions were deduced with accurate mass measurements and fragmentation of high intensity peaks to further determine chemical structure. After mass spectrometry, the slides were stained and cover-slipped, and the cells were reviewed for cytomorphologic features of breast cancer. These were compared to control slides of the same cellular concentration that had not been subjected to this analysis.

Results Spectra from samples of all cellular concentrations demonstrated characteristic qualitative features that were discovered to represent phosphatidylcholines, phosphatidylglycerols, and phosphatidylserines with fragmentation and accurate mass matching. Cytomorphologic analysis demonstrated excellent preservation of the cells subjected to the Flowprobe analysis.

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http://dx.doi.org/10.1016/j.jasc.2015.10.001

Conclusion Direct extraction, ionization, and identification of lipids is possible from cytologic preparations in such a way that the analyzed material is preserved and useful for subsequent microscopic analysis.

Introduction

The boundary between the interior and exterior of eukaryotic cells contains numerous molecules embedded in a lipid bilayer. The lipids that constitute the bilayer are key to structural integrity, signal transduction, association within tissues, post-translational modification of proteins, and protein trafficking.[1-5] Despite the central role of lipids in many biological processes, the majority of tissue diagnoses necessarily ignore lipids because lipids dissolve in the hydrophobic solvents used for fixation and paraffin embedding. Additionally, antibodies for lipids are often insufficiently specific.[6]

Cancer cells alter their metabolic pathways to accommodate for various survival needs including rapid proliferation, which requires de novo lipogenesis, a process that is usually reserved for hepatocytes and adipocytes.[7] Other investigators have noted a relationship between malignant transformation and changes in the distribution of specific lipid classes.[8] Moreover, unique cellular phospholipid profiles have been found to be associated with specific tumors.[9-11] Thus, cellular lipid compositions may be useful as biomarkers to identify and classify cancer types.

The most common modern methods for analysis of lipids use mass spectrometry (MS). In the mass spectrometer, ionized lipids are separated and detected on the basis of their mass (m) to charge (z) ratio (m/z). In order for this separation to occur, lipids must be converted from solids or liquids into gas phase ions. To do this, they must absorb large quantities of energy without degrading. Currently, the most convenient methods for supplying that energy are matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI). MALDI methods require the coating of the sample with matrix-crystalline molecules that absorb the wavelength of light emitted by the laser in the MALDI instrument. As the matrix absorbs laser energy, it undergoes rapid heating and expansion that results in an explosion of the matrix and the analyte into gas phase for ion transfer toward the mass analyzer. ESI methods involve the passage of a sample through a highly charged capillary. The effluent is aerosolized at the end of the capillary, and the strong electric field charges the droplets. As the droplets fly towards the mass analyzer, they are desolvated by the surrounding ambient gas.

Classical ESI and MALDI methods require the specimen to be compromised (by matrix coating and repeated laser pulses wherein the absorbed energy can induce degrading) or sacrificed entirely (by homogenization prior to capillary injection and spraying). Thus, in order to compare those methods with microscopic evaluation, the specimen must be sufficiently large for some to be spared for mass spectrometry and homogenous enough that the portion allocated for mass spectrometry is identical to the portion allocated for morphology based evaluation. Neither is typical in the practice of cytopathology, so experience with mass spectrometric methods in cytopathology is limited.

Ambient ionization techniques[12,13] perform the ionization process outside the mass spectrometer under conditions that are potentially compatible with microscopy. These have been used with tissue sections,[14-16] and the histomorphology appears to be intact even after mass spectrometric analysis.[9,17] The use of ambient ionization techniques on cytologic specimens has not been performed, however. In this paper, we demonstrate a cytomorphology-compatible ambient ionization mass spectrometric method for lipid research.

Materials and methods

Reagents

RPMI-1640, fetal bovine serum (FBS; Hyclone), TrypLE Express enzyme, HPLC grade methanol, and chloroform were obtained from Fisher Scientific (Pittsburgh, Penn.). Penicillin-streptomycin, buffered saline, and 95% ethanol were obtained from Sigma Aldrich (St. Louis, Mo.). Quik-dip stain was obtained from Mercedes Medical (Sarasota, Fla.) and used according to the manufacturer's instructions.

Cell culture

The breast cancer cell line MDA-MB-231 was maintained in RPMI1640 medium with 10% FBS 1% penicillin-streptomycin at 37°C and 5% CO_2-humidified incubator to near confluence.

Cytologic preparation

Cells were harvested by dissociation with TrypLE Express near confluence, re-suspended in Normosol, quantified using a hemocytometer and diluted to concentrations of 0.4×10^5, 0.6×10^5, 0.8×10^5, 1.0×10^5, and 2.0×10^5 cells/mL in Normosol. Cytologic preparations were performed in duplicate for each cellular concentration. For each slide, a 400 μL aliquot was transferred to a Shandon EZ Single cytofunnel with white filter card (Thermo Scientific, Grand Island, N.Y.) and centrifuged at 1000 rpm for 5 minutes. After centrifugation and removal from the funnel device, one of the duplicate slides was allowed to air dry without any manipulation; the other slide was subjected to Flowprobe-MS analysis, described in detail subsequently. Afterwards, both slides were stained in Quik-dip and coverslipped.