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The comparison of glycosphingolipids isolated from an epithelial ovarian cancer cell line and a nontumorigenic epithelial ovarian cell line using MALDI-MS and MALDI-MS/MS

Krishani K. Rajanayake [a], William R. Taylor [b], Dragan Isailovic [a, *]

[a] Department of Chemistry and Biochemistry, The University of Toledo, MS 602, 2801 W. Bancroft St., Toledo, OH, 43606, USA
[b] Department of Biological Sciences, The University of Toledo, MS 601, 2801 W. Bancroft St., Toledo, OH, 43606, USA

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ABSTRACT

Glycosphingolipids (GSLs) are important biomolecules, which are linked to many diseases such as GSL storage disorders and cancer. Consequently, the expression of GSLs may be altered in ovarian cancer cell lines in comparison to apparently healthy cell lines. Here, differential expressions of GSLs in an epithelial ovarian cancer cell line SKOV3 and a nontumorigenic epithelial ovarian cell line T29 were studied using matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) and MALDI-MS/MS. The isolation of GSLs from SKOV3 and T29 cell lines was carried out using Folch partition. GSLs were successfully detected by MALDI-MS, and structurally assigned by a comparison of their MALDI-MS/MS fragmentation patterns with MS/MS data found in SimLipid database. Additionally, LIPID MAPS was used to assign GSL ion masses in MALDI-MS spectra. Seventeen neutral GSLs were identified in Folch partition lower (chloroform/methanol) phases originating from both cell lines, while five globo series neutral GSLs were identified only in the Folch partition lower phase of SKOV3 cell line. Several different sialylated GSLs were detected in Folch partition upper (water/methanol) phases of SKOV3 and T29 cell lines. Overall, this study demonstrates the alteration and increased glycosylation of GSLs in an epithelial ovarian cancer cell line in comparison to a nontumorigenic epithelial ovarian cell line.

1. Introduction

Glycosphingolipids (GSLs) are complex biomolecules that are found in lipid rafts, which are small subdomains of the cell membrane [1]. They are involved in many biological functions such as ontogenesis (development of an organism), cell differentiation, cell adhesion and recognition, signal transduction and immunogenicity [2]. GSLs contain one or more carbohydrates attached to C-1 hydroxyl group of ceramide and are derived from a complex family of compounds called sphingolipids (SLs). Ceramide is the core structure of GSLs and consists of a sphingoid base with an amide-linked fatty acid. The major sphingoid base is sphingosine and the fatty acid of ceramide can be saturated or unsaturated with the number of carbon atoms ranging from 14 to more than 30 [1a]. But, most commonly the fatty acid chains of GSLs contain from 14 to 24 carbon atoms [3]. Glucosyl ceramide is the precursor for about 90% of mammalian GSLs and galactosyl ceramide is the precursor for about 10% of mammalian GSLs [2b]. More complex GSLs can be derived through the extension of the carbohydrate by the addition of different sugars in stepwise manner via glycosidic bonds. A large variety of GSLs is possible depending on the variations of the carbohydrate and the lipid structure. Mostly, the diversity of GSLs depends on the variation of the carbohydrate [1a].

GSLs are amphipathic compounds because of the hydrophilic head group and hydrophobic fatty acid and sphingosine alkyl chains. They are soluble in chloroform and chloroform/methanol mixtures, and less soluble in biologically compatible solvents such as water. Liquid-liquid extraction can be used to isolate lipids because the partition depends on polarity of lipids. A mixture of chloroform and methanol (2:1 in volume) can be used to extract pure lipids from tissues or cells as proposed by Folch [4]. The chloroform/methanol extract can be equilibrated with water or saline solution leading to separation of upper aqueous phase and lower organic phase (Folch partition). Folch partition allows total lipids to be partitioned into the lower phase leaving polar lipids and non-lipid contaminants in the upper phase [4c]. It has been applied

* Corresponding author.
E-mail address: Dragan.Isailovic@utoledo.edu (D. Isailovic).

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to extract GSLs from cells or tissues successfully [5]. Moreover, extraction of GSLs can be optimized by using different solvents, such as isopropanol and hexane, along with chloroform and methanol [5b].

Purification, identification, structural characterization and quantitation are important steps which should be achieved to understand the diversity and functions of GSLs in biological systems. Identification, structural elucidation and quantitation of GSLs in cells or tissues are possible with combination of conventional and high-throughput analytical techniques. These include thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), immunochemical methodologies, mass spectrometric techniques, and HPLC coupled to mass spectrometry (HPLC-MS) [3,6]. TLC has been used for a long time to separate and quantify GSLs because of its easiness, cost effectiveness and ability to separate GSLs [7]. However, HPLC [8] and HPLC-MS are widely used for high-throughput separation and quantitation of GSLs [7a,8e,9]. MS is a very sensitive technique, which can be used to detect GSLs that are present in very low amounts in biological samples. GSL structures have been studied using different MS instruments involving ion sources such as MALDI [8e,10], electrospray ionization (ESI) [5b,9d,10e,11] and atmospheric pressure chemical ionization (APCI) [7a]. Tandem mass spectrometry (MS/MS) and multistage MS/MS (MS^n) provide more structural information and can be used for structural elucidation of GSLs [9d,10a,11], while mass spectrometric imaging (MSI) enables the detection of GSLs in thin tissue sections [10b,12]. Additionally, nuclear magnetic resonance (NMR) spectroscopy and methylation studies can be used to determine the anomeric configuration and linkages of sugar units of GSLs [1a].

GSLs are important bioactive molecules, which are accumulated in the human body due to some genetic defects causing disorders including Niemann-Pick type C disease [13] and lysosomal storage diseases (LSDs), such as Gaucher disease, Fabry disease, Tay-Sachs disease and Sandhoff disease [14]. GSLs have also been studied because of their roles in some kidney diseases [15]. It has been shown that expression and glycosylation of GSLs change due to cancer, and numerous tumor-associated GSL antigens have been reported [2a,16]. Namely, GSL changes in ovarian [10b,17], breast [5b], gastric, colorectal, lung, and pancreatic cancer [16b] have been studied. Generally, GSLs are expressed more in tumors than in normal cells or tissues. The aberrantly glycosylated GSLs are accumulated in tumors and can promote the tumor progression [16b,18].

Ovarian cancer is a leading health issue and shows the greatest mortality rate among women's reproductive cancers [19]. Previous studies have reported that acidic GSLs, such as sulfatides (sulfated GSLs) [10b,17a] and gangliosides (sialic acid containing GSLs) [17b], are elevated in ovarian cancer. Makhlouf et al. have reported the increase in total sulfatides in ovarian cancer tissues while Santin et al. have reported the increase in total gangliosides in plasma and ascites fluid from ovarian cancer patients [17]. However, the characterization of individual sulfatides or gangliosides, which are most responsible for the elevation of total amount of GSLs, has not been done. Not only sulfatides and/or gangliosides are elevated in ovarian tumors but also some other GSLs, such as neutral GSLs, can be altered in ovarian tumors. For example, Jacob et al. recently reported that neutral GSL P_1 is expressed in ovarian cancer tissues and cultured cancer cells based on flow cytometry and LC-ESI-MS/MS analysis [20]. Consequently, they have reported GSL P_1 as a novel tumor-associated carbohydrate antigen. Furthermore, GSL expressions in different ovarian cancer cell lines, including SKOV3, were studied using flow cytometry, and a few types of GSLs, such as globo series, neolacto series and sialic acid-containing GSLs (gangliosides), were detected using GSL-specific antibodies [21]. The results of this study indicated that different ovarian, colon, and breast cancer cell lines express distinct GSLs.

Here, the differences between GSL expressions in ovarian cancer SKOV3 cells and nontumorigenic ovarian T29 cells were analyzed by MALDI-MS. GSLs in these epithelial cell lines were investigated in this study because epithelial ovarian cancer is the most common type of ovarian cancer [22]. SKOV3 cell line was derived from a human ovarian adenocarcinoma [23], and has been used widely to study ovarian cancer [21]. T29 cells are immortalized ovarian surface epithelial cells that originate from the outer epithelial layer of normal human ovaries. They were cultured and transfected with SV40 sequences to express the large T and small t antigens to inactivate p53 and Rb proteins [24]. Next, these cells were immortalized by introduction of a full-length hTERT cDNA [24].

The main goals of this study were to identify and characterize GSLs present in cancerous SKOV3 and noncancerous T29 ovarian cells, and to compare GSL expressions in both cell lines. These goals were achieved by the analysis of GSLs extracted from ovarian cells using MALDI-MS and MALDI-MS/MS and by searching through LIPID MAPS and SimLipid databases. The results indicate that several different neutral GSLs are expressed in SKOV3 cells in comparison to T29 cells, with differences being mostly due to aberrant glycosylation of neutral GSLs. A few different sialylated GSLs isolated from SKOV3 and T29 cells were also detected.

2. Results and discussion

Fig. 1 shows MALDI-MS spectra of GSLs in Folch partition lower phases of SKOV3 (Fig. 1a) and T29 (Fig. 1b) cells acquired in positive ion mode using 2,5-dihydroxybenzoic acid (DHB) as the matrix. The singly-charged sodiated GSL ions with *m/z* 806.6, 832.6, 834.6, 884.5, 968.6, 994.6, 996.6, 1046.6, 1130.6, 1156.7, 1158.7, 1197.7, 1199.7, 1249.6, 1277.7, 1333.7, 1359.7, 1361.8, 1411.7, 1495.8, 1523.8 and 1549.8, which are observed in the mass spectrum of Folch partition lower phase (LP)-SKOV3 sample (Fig. 1a), were identified as neutral GSLs using MS/MS (Figs. 2, 3, and S1, and Table 1) and SimLipid and LIPID MAPS databases. Singly-charged sodiated ions with *m/z* 806.6, 832.6, 834.6, 884.5, 968.6, 994.6, 996.6, 1046.6, 1130.6, 1156.7, 1158.7, 1197.7, 1199.7, 1249.6, 1333.7, 1359.7 and 1361.8 were also identified as neutral GSLs in LP-T29 sample (Fig. 1b). When both mass spectra in Fig. 1 are considered, GSL ions with *m/z* 806.6, 832.6, 834.6, 884.5, 968.6, 994.6, 996.6, 1046.6, 1130.6, 1156.7, 1158.7, 1197.7, 1199.7, 1249.6, 1333.7, 1359.7 and 1361.8 are found in both cell lines (Table 1). The GSL ions with *m/z* values of 1277.7, 1411.7, 1495.8, 1523.8 and 1549.8 were found only in the Folch partition lower phase of SKOV3 cells (Table 1) as discussed below.

MALDI-MS/MS analyses in positive ion mode were performed for each of the aforementioned parent ions, and the MS/MS peak lists were fed into the SimLipid and searched specifically for the GSLs. All the matched GSLs were scored using SimLipid, and the score was assigned based on the number of matched fragments in GSL MS/MS spectra. Most of GSLs in SKOV3 and T29 cell lines were identified based on the highest score assigned by SimLipid. In few cases (GSL ions with *m/z* values of 1277.7, 1359.7 and 1361.8), corresponding GSLs were identified depending on matched fragment *m/z* values with higher peak intensities rather than the higher number of matched low-intensity fragments. GSL fragments were also assigned using a fragmentation tool of ChemDraw (PerkinElmer) software and a standard nomenclature for carbohydrate and ceramide backbone fragmentations [25]. Most of the B and Y ions, which are formed due to the cleavages of glycosidic bonds, were matched with *m/z* values of GSL fragments. In addition to B and Y ions, C and Z ions were also observed and these ions were used to get information about the head groups of GSLs. A