Differential expression of fucosyl GM1 and a disialoganglioside with a NeuAcα2–6GalNAc linkage (GD1e) in various rat ascites hepatoma cells

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A distinct difference in ganglioside composition among various rat ascites hepatomas and Yoshida sarcoma was observed on TLC-immunostaining with anti-fucosyl GM1 antibody, and chemical and enzymatic analyses. Yoshida sarcoma and ascites hepatomas, AH13, AH66F and AH66, but not the other 9 tumor cell lines investigated, specifically contained a disialoganglioside, NeuAcα2–3Galβ1–3(NeuAcα2–6)GalNAcβ1–4Galβ1–4Glcβ1–1ceramide (GD1e), whereas the 9 ascites hepatoma cells without GD1e contained fucosyl GM1. The differential expression of fucosyl GM1 and GD1e in various tumor cell lines indicates that different cell lineages express distinct metabolic pathways for gangliosides, and that the gangliosides are useful markers for distinguishing tumor cell lines.

Ganglio-series ganglioside *(Rat ascites hepatoma)* *TLC-immunostaining*

1. INTRODUCTION

In our previous study [1], we found that gangliosides containing ganglio-*N*-tetraose were widely distributed in various rat extraneural tissues, being present in erythrocytes, white blood cells, bone marrow and testis at particularly high concentrations. In rat erythrocytes, GM1, fucosyl GM1 and GD1a were the major gangliosides, and the high concentration of GM1 and fucosyl GM1 was a tissue characteristic of erythrocytes among the various rat tissues. Although fucosyl GM1 was only present in rat liver in a trace amount, it became detectable in the liver after malignant transformation with chemical carcinogens [2]. Also, accumulation of various gangliosides containing ganglio-*N*-tetraose was reported in rat hepatomas 27 [3] and H35 [2]. Probably, all rat tissues have the potential to synthesize various ganglioside molecules belonging to the ganglio-series and the tissues after transformation may be modulated to activate the different biosynthetic pathways which are generally suppressed in normal tissues. To elucidate the mode of modification of ganglioside metabolism during transformation, we analyzed gangliosides from various rat ascites hepatomas and Yoshida sarcoma, and found a distinct difference in the expression of polar gangliosides among the tumor cell lines.

2. MATERIALS AND METHODS

2.1. *Materials*

GM3, GM1, GD1a, GD1b and GT1b, and fucosyl GM1 were isolated from human brain and bovine thyroid, respectively, according to [4,5]. Anti-asialo GM1 and anti-fucosyl GM1 antisera were prepared by immunizing rabbits with the purified antigens and their antigen specificities confirmed as reported in [6]. Rat ascites hepatoma cells, AH130, AH109, AH44, AH272, AH41C, AH60C, AH414, AH7974, AH66, AH66αF, AH66F and AH13, and Yoshida sarcoma cells (approx. 1×10^8) at a stationary growing state were kindly donated by Dr H. Sato, Sasaki Institute, Tokyo [7].

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2.2. *Preparation and analysis of gangliosides from hepatoma and sarcoma cells*

Extraction of total lipids followed by fractionation into neutral and acidic lipids by DEAE-Sephadex A-25 (acetate form) column chromatography was carried out according to [5,6].

2.3. *TLC-immunostaining of fucosyl GM1 on thin-layer plates with rabbit anti-fucosyl GM1 antiserum [8]*

A thin-layer plate (Polygram; Macherey-Nagel, Düren, FRG), after development of gangliosides from the cells, was incubated with the blocking buffer [1% polyvinylpyrrolidone (PVP), 1% ovalbumin and 0.02% NaN_3 in phosphate-buffered saline (PBS)] at 37°C for 15 min, followed by incubation with anti-fucosyl GM1 antiserum diluted 1:200 with dilution buffer (3% PVP in PBS) at 37°C for 2 h. After washing the plate 5 times with washing buffer (0.1% Tween 20 in PBS), the plate was reacted with blocking buffer at 37°C for 15 min, followed by incubation with peroxidase-conjugated anti-rabbit IgG antiserum diluted 1:1000 for 1 h at 37°C. The enzyme activity remaining on the plate was visualized by incubation with 0.003% H_2O_2 and 4-chloro-1-naphthol in Tris-HCl buffer (50 mM, pH 7.4) containing 200 mM NaCl at 37°C for 15 min.

2.4. *Structural determination of gangliosides*

The structures of the gangliosides purified by DEAE-Sephadex and Iatrobead column chromatography were determined after permethylation [9], sialidase (*Vibrio cholerae*; Calbiochem, La Jolla, CA) and α-fucosidase (Sigma, St. Louis, MO) treatment [5], and immunostaining of the hydrolytic products with anti-asialo GM1 antibody on thin-layer plates.

3. RESULTS AND DISCUSSION

3.1. *Gangliosides from various rat ascites hepatoma and sarcoma cells*

A thin-layer chromatogram (TLC) of gangliosides from various rat ascites hepatoma and Yoshida sarcoma cells is shown in fig.1. Different cells varied in ganglioside composition from each other. Although the relative concentration of GM3 appeared to be characteristic of each cell type, a distinct difference in the ganglioside composition was observed in the occurrence of ganglioside X, which was present in AH66F and AH13 ascites hepatoma cells and Yoshida sarcoma cells, but not in the other hepatoma cells, even in a trace amount (table 1). Ganglioside X was the major ganglioside in AH66F (lane 11 in fig.1).

3.2. *Structure of ganglioside X*

Ganglioside X had a lower mobility than that of GD1a and showed an elution position corresponding to that of a disialoganglioside on the ganglioside map [4]. *V. cholerae* neuraminidase was able to remove the sialic acid residue completely, and the product was proved to be asialo GM1 by comparing its mobility and reactivity with those of anti-asialo GM1 antibody on a thin-layer plate (fig.2). Permethylation analysis of the partially

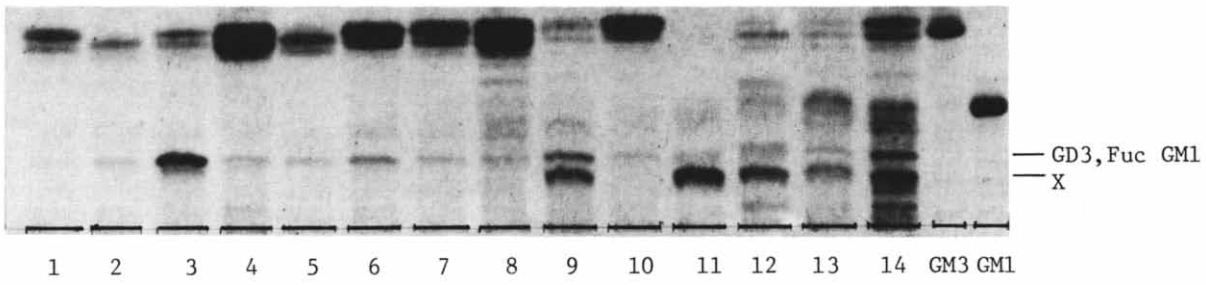

Fig.1. TLC of gangliosides from various rat ascites hepatoma and sarcoma cells. Gangliosides (5–15 μg lipid-bound sialic acid) were developed with chloroform-methanol-0.5% $CaCl_2$ (55:45:10, by vol.) and the spots located by the resorcinol method. 1, AH130; 2, AH109; 3, AH44; 4, AH272; 5, AH41C; 6, AH60C; 7, AH414; 8, AH7974; 9, AH66; 10, AH66αF; 11, AH66F; 12, AH13; 13, 14, Yoshida sarcoma.

Table 1

Contents of fucosyl GM1 and GD1e in various rat ascites hepatoma cells

Cell	Fucosyl GM1	GD1e
AH130	3.41	n.d.
AH109A	9.95	n.d.
AH44	914.54	n.d.
AH272	30.11	n.d.
AH41C	18.24	n.d.
AH60C	74.73	n.d.
AH414	11.87	n.d.
AH7974	12.49	n.d.
AH66	n.d.	458.90
AH66αF	45.81	n.d.
AH66F	n.d.	867.94
AH13	n.d.	67.95
Yoshida sarcoma	n.d.	365.85

Fucosyl GM1 and GD1e were determined densitometrically after locating the spots with anti-fucosyl GM1 antiserum and resorcinol reagent, respectively. Detection limits of TLC-immunostaining (fucosyl GM1) and resorcinol reaction (GD1e) were 1 and 100 ng, respectively. n.d., not detected

methylated aldohexitol acetates showed the presence of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl glucitol, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl galactitol, 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl galactitol and 1,3,5,6-tetra-*O*-acetyl-2-deoxy-2-*N*-methylacetamido-4-mono-*O*-methyl galactitol in equimolar amounts (fig.3). *N*-Acetylneuraminic acid was the sole sialic acid. Thus, the structure of ganglioside X was concluded to be NeuAcα2 – 3Galβ1 – 3(NeuAcα2 – 6)GalNAcβ1–4Galβ1–4Glcβ1–1ceramide (GD1e), which is identical with that of the compound reported for AH7974F by Taki et al. [10].

3.3. *TLC-immunostaining of gangliosides from various rat ascites hepatoma and sarcoma cells with anti-fucosyl GM1 antiserum*

The antibody titer of anti-fucosyl GM1 antiserum on ELISA was 1:20480, and the antibody reacted with fucosyl GM1 to a great extent but only slightly with fucosyl asialo GM1, and not at all with GM3, GD3, GM1, GD1a, GD1b, GT1b or asialo GM1 on TLC-immunostaining. More than 5 ng fucosyl GM1 was detectable by this pro-

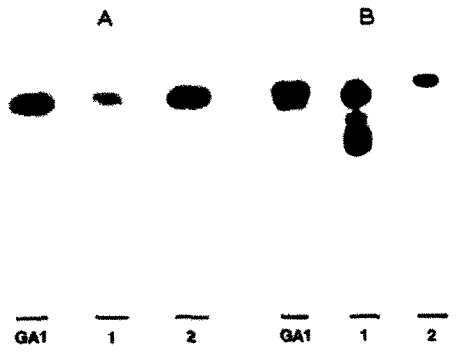

Fig.2. TLC-immunostaining with anti-asialo GM1 antibody (A) and orcinol staining (B) of the hydrolytic products of fucosyl GM1 (1) and ganglioside X (2) from rat ascites hepatoma cells. For TLC-immunostaining, about 10 ng fucosyl GM1 and ganglioside X were hydrolyzed with formic acid and neuraminidase, respectively. GA1, asialo GM1.

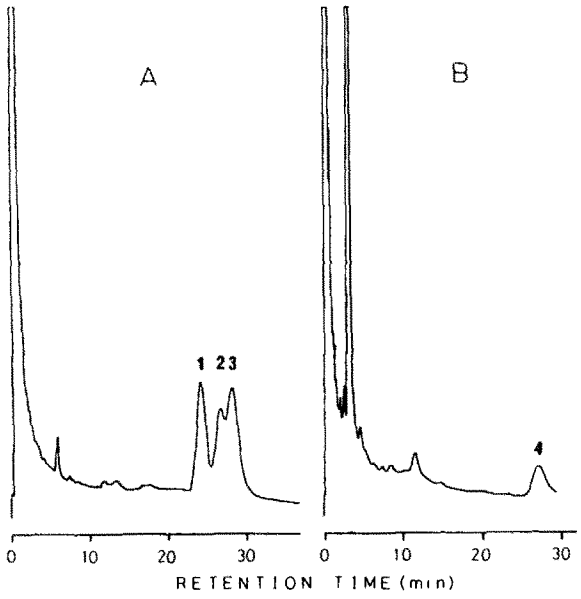

Fig.3. GLC of the partially methylated aldohexitol acetates from ganglioside X. A, 2% OV-275; B, 1% OV-17. Peaks 1–4: 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methyl galactitol; 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl galactitol; 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl glucitol; 1,3,5,6-tetra-*O*-acetyl-2-deoxy-2-*N*-methylacetamido-4-mono-*O*-methyl galactitol, respectively. Each peak was identified by comparing the retention times and mass spectra.

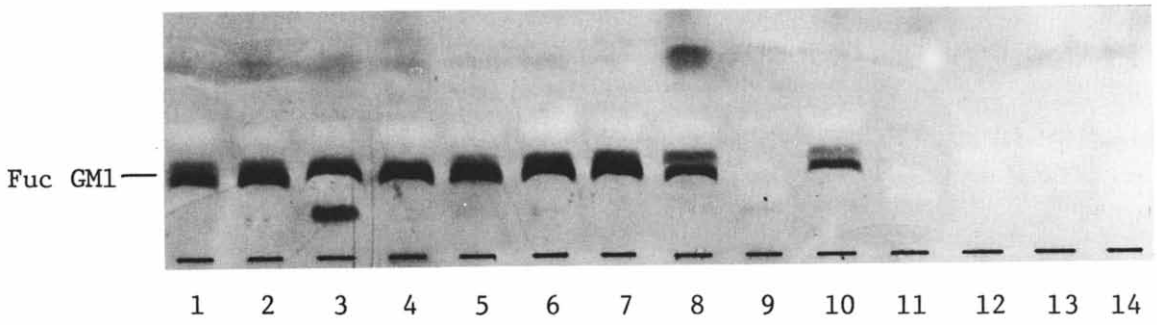

Fig.4. TLC-immunostaining of gangliosides from various rat ascites hepatoma and sarcoma cells with anti-fucosyl GM1 antibody. Numbers as in fig.1.

cedure. As shown in fig.4, antigen detectable with anti-fucosyl GM1 antibody was present in rat ascites hepatoma cells, AH130, AH109, AH44, AH272, AH41C, AH60C, AH414, AH7974 and AH66αF, but not in rat ascites hepatoma cells, AH66, AH66F and AH13, or Yoshida sarcoma cells, even in a trace amount (table 1). After isolation by DEAE-Sephadex and Iatrobead column chromatography, the structure of the antigen was determined by the same procedure as described above, and it was concluded to be fucosyl GM1 from the following evidence: (i) permethylation analysis of the partially methylated aldohexitol acetates showed 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl glucitol, 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methyl galactitol, 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methyl galactitol, 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl fucitol and 1,3,5-tri-*O*-acetyl-2-deoxy-2-*N*-methylacetamido-4,6-di-*O*-methyl galactitol in equimolar amounts; (ii) the product obtained on formic acid treatment was identical to asialo GM1 (fig.1) on TLC-immunostaining; and (iii) α-fucosidase treatment yielded GM1. Thus, fucosyl GM1 and GD1e were found to be differentially expressed among different cell lines of rat ascites hepatoma cells. This indicates strongly that these cells may be classified into two groups by utilizing the above two gangliosides as markers. Cells having fucosyl GM1 should express the synthetic pathway for the ganglioside via the fucosylation of GM1, and the other cells without fucosyl GM1 might preferentially express the pathway through sialylation at the 6-position of GalNAc in GM1b since GM1b has been detected as the only monosialoganglioside precursor in AH66F (Sunada et al., unpublished). Recently the attention of many investigators has been drawn to the metabolic modulation or regulation of glycolipids through induction or activation of glycosyl transferases during cellular differentiation, transformation [11–13] and genomic DNA segment transfection [14]; accordingly, the two cell lineages mentioned here should be useful for understanding the mechanism of the metabolic change of the ganglio-series gangliosides. The detailed ganglioside compositions of the above cells will be reported elsewhere.

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