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Fatty Acid Composition of Ganglioside GM3 of Renal Glomerular epithelial SGE1 Cells during Spontaneous Dome Formation *in vitro*.

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ABSTRACT. **Ganglioside GM3, one of acidic components of membrane glycosphingolipids (GSL), has been known to change its content quantitatively during growth and differentiation of various cells in vitro.**

Detailed analysis of lipid portion of GM3 of rat renal glomerular SGE1 cells revealed that fatty acids with long carbon chains, especially that of C24:0 and C24:1 increased, while that of short C18:0 and C20:0 decreased after spontaneous dome formation.

Since not only fatty acid composition of neutral GSL, sulfatide and phospholipid but also composition of long-chain bases (LCB) did not change, it was suggested that only C24 fatty acid of GM3 specifically increased in relation to dome formation.

The spontaneous dome formation has been reported to be related with induction of cellular differentiation in many transporting epithelial cells. We thus assume that the change of fatty acid composition of GM3 is involved in cellular differentiation of SGE1 cells.

Ganglioside is one of the acidic membrane glycosphingolipids (GSL) and is composed of an oligosaccharide chain and a lipid portion. The lipid portion is called ceramide and composed of LCB combined with various fatty acids. There have been many reports on biological processes related with ganglioside changes. These processes include growth, development, differentiation, antigenicity, as well as recognition of bioeffectors such as hormones and bacterial toxins (1, 2, 3). Specificity and ability of gangliosides interacting with external ligands have generally been considered to be related to their oligosaccharide chain. However, recent reports suggest the biochemical and physicochemical importance of the lipidic hydrophobic portion, ceramide, of gangliosides and other GSL.

In biochemical studies of cultured cells, LCB modulates protein kinase C (4, 5) and inhibits tyrosine kinase activity associated with the epidermal growth factor receptor (6, 7, 8).

As to its physicochemical properties, LCB or ceramide is believed to play significant roles in the organization and function of the cell membrane. For instance, interaction with external ligands (9), lateral phase separation in artificial (10, 11) or microsomal membrane (12), and combination with glycosyltransferases involved in gangliosides biosynthesis (13) depend on the chemical features of LCB or ceramide moiety.

We previously reported the characterization of a rat renal glomerular epithelial cell line, SGE1, which we established (14, 15), and the behavior of GM3, a major membrane ganglioside (16). GM3 content specifically increased during growth and spontaneous dome formation of the cells on monolayer culture.

During the study, it was noted that the thin-layer chromatography (TLC) pattern of GM3 between SGE1 cells with or without domes were different. Because the GM3 band of SGE1 cells was doublet at the same position as the standard, GM3 molecules should have different polarities caused by fatty acids and LCB. The area of higher Rf peak (low polarity) of GM3 doublet was also greater than that of lower Rf peak (high polarity) in the cells with domes. However, the area of higher Rf peak of the cells without domes decreased while that of lower Rf peak increased. This showed that GM3 with low polarity was richer in the cells with domes than those without domes.

When Rf values were compared with the standard on reversed phase TLC, the variety of the polarity was suggested to be caused by the difference of fatty acid

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Abbreviations: GSL, glycosphingolipids; LCB, long-chain base; TLC, thin layer chromatography; GC, gas chromatography; GC-MS, GC-mass spectrometry.

length. Interestingly, almost all of GM3 in the cells with domes developed at the position of the C24 fatty acid, while GM3 in the cells without domes developed at the positions of both C24 and shorter fatty acids.

Spontaneous dome formation of cultured dog renal cells, MDCK, has been shown to be a marker for cellular differentiation (17). We supposed that GM3 of SGE1 cells changed its composition in relation to spontaneous dome formation along with cellular differentiation.

MATERIALS AND METHODS

Cells and culture conditions. Rat renal glomerular epithelial cells, SGE1, established and maintained in our laboratory, were used at less than the 50th passage (14, 15).

SGE1 cells were cultured under the condition described previously (16). At confluence, the medium was changed to serum-free DHFs medium (a 1 : 1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium) supplemented with 10 μl/ml ITS+ (Becton Dickinson Labware, Bedford, MA, USA) and 1 ng/ml EGF (Wako Pure Chemical Industries, Osaka, Japan). Dome formation was observed on the day after confluence. Cells were collected by rubber policeman before and after dome formation, washed with PBS and centrifuged at 1,000 rpm for 5 min. The pellet was resuspended with PBS and sonicated and lyzed by a cell sonifier (Ultrasonic Processor VP-5, Taitec, Koshigaya, Japan). A small portion of the pellet was used for determination of DNA content and the remainder was used for extraction of GSL.

In order to estimate their relative amounts in the cells, DNA content was measured by the method using 4′, 6-diamidino-2-phenylindole (18). Calf thymus DNA (Worthington Biochemical, Freehold, NJ, USA) was used as a standard.

GSL and phospholipid of SGE1 cells. Extraction and purification of GSL from SGE1 cells were done by procedures described previously (16). Determination of content and detection of GSL were performed by two fluorescent labeling methods: amino-methylcoumarin and 5-hydroxytetralone, respectively (19, 20). Total phospholipid was determined by phosphate using Fiske-Sabbarow reagent (21).

Analysis of fatty acid, sugar and LCB composition. The fractions of GM3, total neutral GSL, sulfatide, and total phospholipid were methanolyzed in 3% hydrogen chloride methanol solution at 85°C for 3 h (22). Fatty acid methyl ester was extracted with n-hexane and directly analyzed by capillary-gas chromatography (GC) and GC-mass spectrometry (GC-MS). Sugar and LCB were identified by GC as trimethylsilyl derivatives.

GC analyses of fatty acid methyl ester, sugar and LCB were performed on a Hitachi G3000 GC apparatus equipped with a flame ionization detector and a Chromapack glass capillary column (CP-Wax52CB; 25 × 0.53 mm I.D.) for fatty acid methyl ester, and an OV-1-coated silica capillary column (25 × 0.53 mm I.D.) for sugar and LCB. The operative conditions were as follows. For fatty acid methyl ester the column temperature was 150°C at start and 230°C at end with 2°C /min rate of increase; for sugar and LCB it was 190°C at start and 260°C at end with 0.5°C/min rate of increase. The carrier gas was helium at flow rate of 0.4 kg/cm^2. Peak identification was based on identical retention time with known standard run under the same condition.

To confirm composition of fatty acid methyl ester, GC-MS was done by a Shimadzu QP-5000 apparatus connected with a DB-5 fused-silica glass capillary column (30 m × 0.32 mm I.D.).

RESULTS

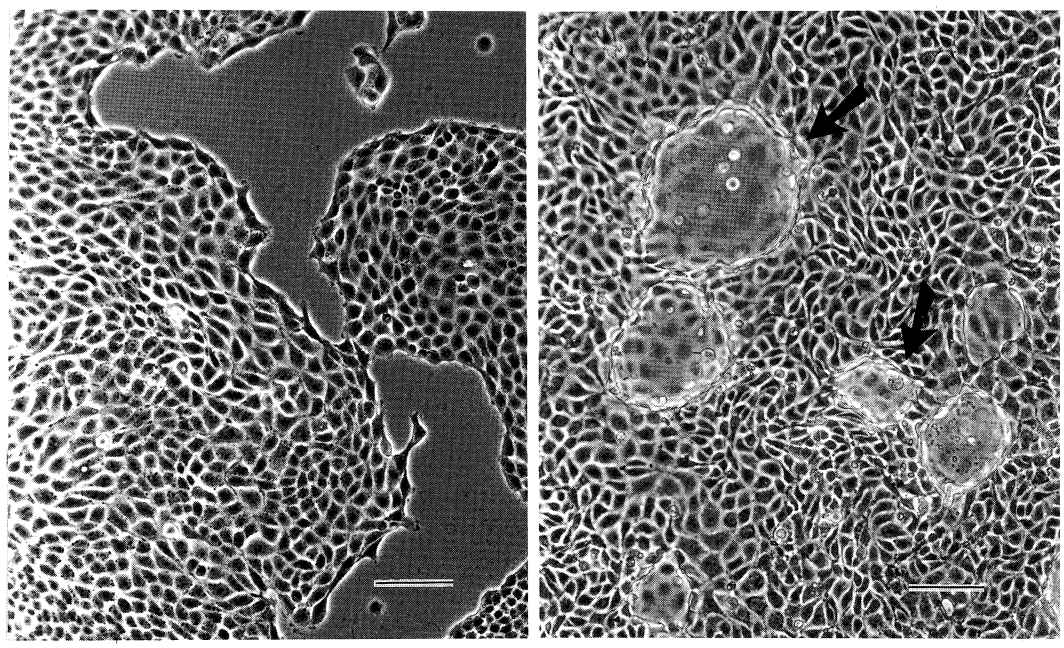
Spontaneous dome formation of SGE1 cells in monolayer culture. SGE1 cells in culture exhibit a distinctive polygonal epithelial shape in cobblestone-like sheet and normally grow as a typical monolayer until confluency. The cells, however, begin to show architectural changes soon after the confluency, and begin to form hemispherical multicellular domes here and there. The number of cells forming the domes are approximately 10–30 (Fig. 1). We designated these cultures as SGE1 cells with domes or without domes. The area which the domes occupied in monolayer was vertically calculated as 13.4±2.7% (n=4).

GSL and phospholipid of SGE1 cells. GSL composition of SGE1 cells with or without domes has been analyzed and reported before (16). It was shown that sulfatide developed over GM3 on a TLC-plate, showing the same position as the standard. Ganglioside fraction was mainly composed of GM3, GM1, GD1a and GM2. GM3 was the major component among them, occupying over 80%.

Contents of GSL and phospholipid per mg DNA of the cells are shown in Table I. In cells with domes, all of the lipid content except that of GM3 increased to 1.2 times that of the cells without domes. While GM3 content was less among all other lipids in the cells without domes, it increased to 3.3 times that of the cells with domes. It was therefore suggested that GM3 content of SGE1 cells specifically changed in relation to dome formation.

Fatty acid, sugar and LCB composition. The major fatty acids of GM3 in the cells were C16:0, C18:0, C18:1, C22:0, C24:0 and C24:1, regardless of dome formation (Fig. 2a). In the case of cells without domes, C16 and C18 fatty acids comprised 50% of the total. By contrast, in the cells with domes, C16 and C18 fatty acids decreased to 32.9% of the total, with C18:0 fatty acid decreased from 17.5% to 6.6%. On the other hand, long C24:0 and C24:1 fatty acids increased 2.1-folds (19.0% to 39.5%) and 2.5-fold (4.1% to 10.2%), respectively, compared to cells without domes.

Total neutral GSL was composed of C16:0, C18:0,



(a) (b)

Fig. 1. Phase contrast photomicrographs of SGE1 cells in monolayer culture. a, typical monolayer of cobblestone-like sheet at confluent stage, without domes. b, formation of domes (arrow) at post-confluent stage. Both bars indicate 0.1 mm.

C18:1, C22:0, C24:0 and C24:1 fatty acids (Fig. 2b). The ratios of these fatty acids were almost constant among the cells with or without domes. The exceptions were C18:1 and C22:0 fatty acids, which increased from 7.1% to 13.7% and decreased from 10.1% to 4.2%, respectively. C24:0 and C24:1 fatty acids, which occupied 38.0% of total fatty acid, did not change regardless of dome formation.

Sulfatide was composed mainly of C14:0, C16:0, C18:0, C18:1, C18:2 and C24:1 fatty acids (Fig. 2c). C18 fatty acid was a half of the total and it increased slightly except for a loss of C18:3, while C16:0 decreased from 43.3% to 31.7% and C24:1 increased from 1.8% to 8.2% after dome formation.

Phospholipid was composed of C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C20:0, C20:1, C20:2 and C24:0 fatty acids (Fig. 3). While the amount of C24 fatty acid was very small, C18 fatty acid including C18:0, C18:1 and C18:2 was a major component and occupied 56.8% and 59.6% in the cells with or without domes, respectively. Every fatty acid included in phospholipid did not differ significantly except C20:2 fatty acid which increased from 3.2% to 11.5% after dome formation.

As shown in Table II, only LCB with C18 existed in GM3 of the cells regardless of domes. As for a sugar ratio of GM3, Gal : Glc : NeuAc was 1.00 : 0.99 : 0.89 for the cells with domes, and 1.00 : 1.14 : 0.83 for the cells without domes.

Table I. CONTENT OF GM3, TOTAL NEUTRAL GSL, SULFATIDE AND PHOSPHOLIPID IN SGE1 CELLS

	Content in SGE1 cells (nmol/mg DNA)	
	Without domes	With domes
GM3	2.81±1.84*	9.34±1.66
Total neut. GSL**	7.48±1.01	8.70±1.55
Sulfatide	7.96±4.28	9.64±1.24
Phospholipid	590±270**	810±160

* Values represent means±S.D. of at least 3 samples.

** Total neut. GSL was estimated as the total amout of each neutral GSL.

DISCUSSION

We previously reported that the change of GM3 content of SGE1 cells was related with spontaneous dome formation which had been attributed to cellular differentiation (16). In the course of study, we noticed a significant difference in GM3 quality of the cells with or without domes, which suggested that dome formation was caused by the change in fatty acid.

In the present study, GC analysis revealed that C24:0 and C24:1 fatty acids of GM3 became 2.1 and 2.5 times higher respectively in the cells with domes. On the other hand, fatty acids of total neutral GSL did not change even after dome formation in spite of having high content of C24. Among the fatty acids of the other acidic GSL, sulfatide, only C16:0 decreased whereas C18:0,

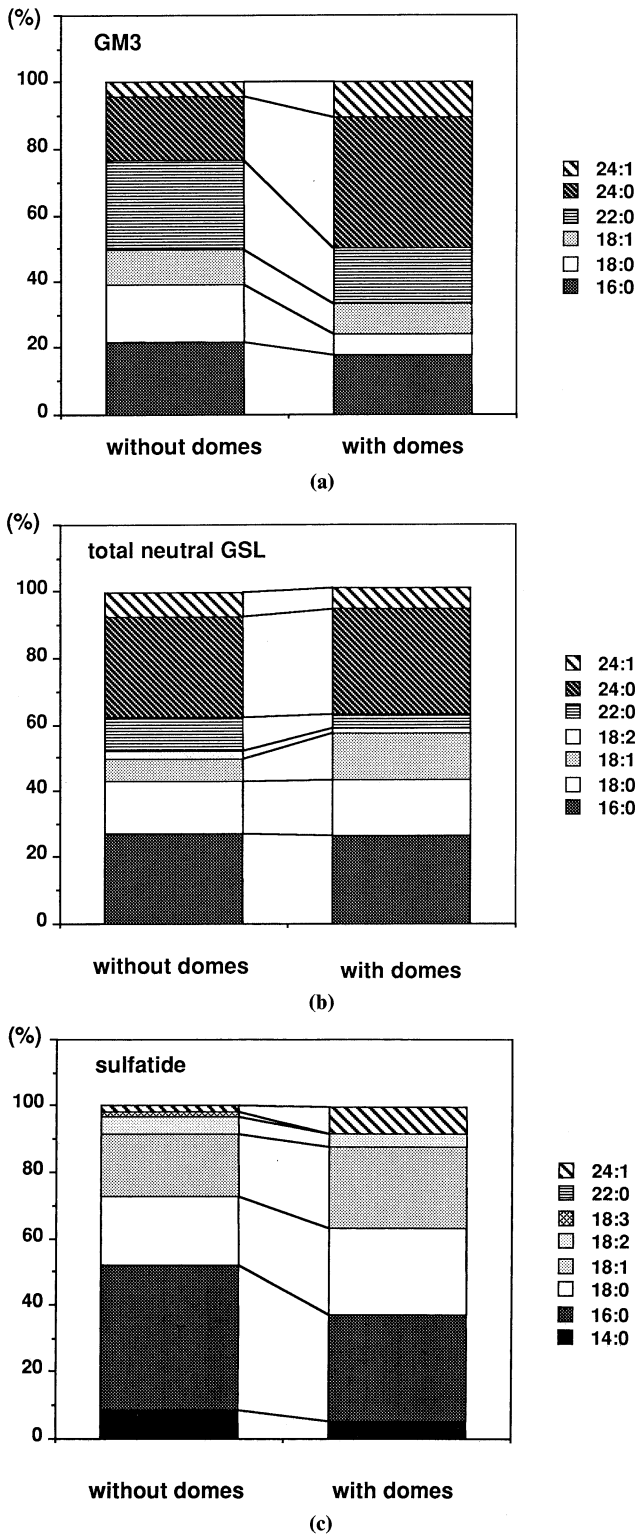


Fig. 2. Differences of fatty acid composition in SGE1 cells with or without domes. a, GM3, b, total neutral GSL; c, sulfatide. All of the columns represent relative percentages compared to total amount of fatty acid as 100%. Each result is expressed as the mean of 3 experiments.

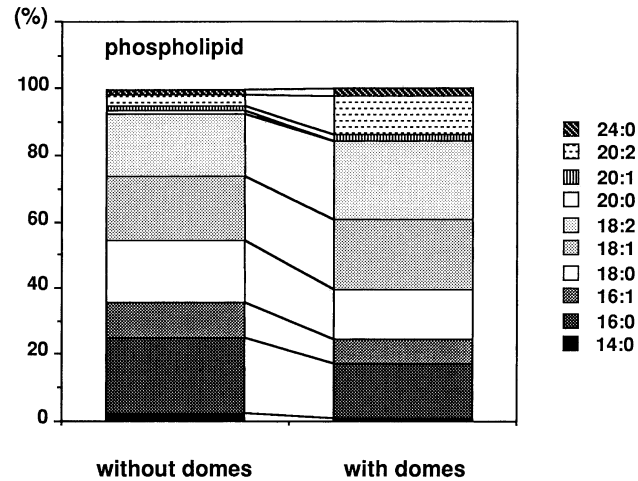


Fig. 3. Differences of fatty acid composition of phospholipid in SGE1 cells with or without domes. All of the columns represent relative percentages compared to total amount of fatty acid as 100%. Each result is expressed as the mean of 3 experiments.

C18:1 and C24:1 increased. Sulfatide is believed to have a role in transmembrane transport (23). In the present case of dome formation of monolayer cells, transmembranous secretion seemed to accelerate, hence increase of sulfatide concentration may be associated with dome formation. Elongation of fatty acid chains, which was documented in GM3, may be related to this phenomenon as well.

Total phospholipid mainly had C16, C18 and C20 fatty acids, the amounts of which did not show any change in cells, regardless of dome formation, except for C20:2. The amount of C24 fatty acid was very small in the cells regardless of dome formation. We thus conjectured that only C24 fatty acid of GM3 specifically increased in relation to dome formation.

This fact has been verified by the immuno-histochemical staining of SGE1 cells with an anti-GM3 monoclonal antibody (M2590). The cells without domes were not stained by M2590, but the antibody positively recog-

Table II. Composition of sugar and long chain bases of GM3 in SGE1 cells

	Composition in SGE1 cells	
	Without domes	With domes
Sugar		
Glc	1.00*	1.00
Gal	1.14	0.99
NeuAc	0.83	0.89
Long chain bases		
C18	0.53	0.61

* Values represent ratios of each sugar and LCB compared to Glc as 1.00.

nized the dome forming cells (16). Besides, ELISA and TLC-immunostaining showed that M2590 recognized GM3 with long fatty acids, but not that with short fatty acids (24). Therefore, it is possible that only dome forming cells were stained by M2590, because those cells had GM3 with long fatty acids.

In our present study, we investigated why fatty acid but not LCB changed along with dome formation. This can be explained as follows. Firstly, we would conjecture that it is caused by the physicochemical properties of fatty acids of the cell membrane. One report recently showed that the change of fatty acid length affected the physicochemical properties of the membrane more than LCB, because the LCB length is often rather homogeneous, whereas fatty acid length commonly ranges from C16 to C24 (25). A ^2H NMR analysis confirmed the induction of phase separation of GSL having longer fatty acids, particularly in orderly phospholipid bilayer (25).

Phospholipids also have fatty acid molecules in them, but they are mainly composed of C16, C18 and C20. In our study, C16, C18 and C20 fatty acids comprised 97% of total phospholipid in SGE1 cells regardless of dome formation (Fig. 3). Change of fatty acids in phospholipid related with dome formation has never been reported previously.

Secondly, we suppose that the fatty acids play a more important role in cellular differentiation than LCB does. In cultured granular cells related with aging, it was reported that not fatty acids but C20:1 LCB increased while C18:1 LCB was common (26, 27), suggesting the preferential use of C20:1 LCB in the process of ganglioside biosynthesis. When cellular differentiation occurs, C24 fatty acid could be preferentially used in the process of ganglioside biosynthesis. Whether for cellular aging or differentiation, fatty acids and LCB might each have their own respective roles.

One report has indicated the relationship between the ceramide portion of ganglioside and the cellular differentiation of renal tubular epithelial cells, MDCK (28). Exogenous gangliosides of GM3 and GD3 derived from different sources stimulated dome formation in a dose-dependent manner. It is suggested that the difference in the ceramide portion of GM3 and GD3 might give an effect to promote dome formation, because these gangliosides might vary in their fatty acid compositions of ceramide and include long fatty acids.

In conclusion, the present study positively showed the relationship between fatty acid composition and spontaneous dome formation, cellular differentiation in vitro, of cultured renal glomerular epithelial cells. We conclude that an elongation of fatty acid in GM3 are closely related to cell differentiation.

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