Changes in Glycosphingolipids Accompanying the Differentiation of Human Squamous SQCC/Y1 Cells

Toshiki Tatsumura, Toshio Ariga, Robert K. Yu, and Alan C. Sartorelli

ABSTRACT

SQC/C/Y1 cells grow as a monolayer in culture and differentiate when maintained in the plateau phase; in absence of serum these cells differentiate more rapidly. The differentiation is characterized by the stratification of the culture to form a structure consisting of several cellular layers, synthesis of specific keratins, and the attainment of the capacity to form a cornified cell membrane. The stratification process is indicative of the importance of cell-cell interactions during maturation. To study the relationship between membrane glycosphingolipids (GSLs) and the state of differentiation of SqCC/Y1 cells, GSLs were measured in cultures grown in the presence or absence of fetal calf serum. Glycolipids were isolated from the sonicated cells prepared from 9 x 10^6 to 1.0 x 10^7 cells according to a previously described procedure (23) using bovine serum albumin as the standard. Total cellular protein was determined by the method of Lowry et al.

INTRODUCTION

GSLs are important constituents of the plasma cell membrane of virtually all vertebrate tissues (1, 2). They constitute part of the glycoconjugate network extending from the membrane surface and are important in governing the properties and functions of the cells (1-4). In recent years, considerable evidence has accumulated which demonstrates that the composition of GSLs can undergo marked changes during cellular growth, differentiation, and oncogenic transformation (5-17).

SQC/C/Y1, a human squamous cell carcinoma, can be rapidly induced to undergo terminal differentiation to form cornified cells by culturing the cells in serum-free medium (18-20). Thus, SQCC/Y1 cells represent a useful system for evaluating the role of GSLs in the maturation of these malignant keratinocytes. In this paper, the GSL composition of differentiated and undifferentiated SQCC/Y1 cells was measured. Changes in gangliosides and neutral GSLs occurred with the attainment of the mature state, suggesting that these lipids were of importance to the induction and/or maintenance of the differentiated phenotype.

MATERIALS AND METHODS

Cell Culture. The human buccal squamous carcinoma cell line, SQC/C/Y1, was grown under two conditions (19). A complete medium was used that consisted of a 1:1 (v/v) mixture of DMEM and Ham's F-12 medium (Grand Island Biological Co., Grand Island, NY), supplemented with 10% heat-inactivated fetal calf serum (Grand Island Biological Co.) and 50 µg/ml of gentamycin sulfate. A chemically defined medium was used to enhance the cellular differentiation process. It consisted of a 1:1 mixture of DMEM and Ham's F-12 medium supplemented with 5 µg/ml of insulin, 5 µg/ml of transferrin, and 5 ng/ml of selenious acid (ITS Premix; Collaborative Research, Inc. Lexington, MA). The cells were incubated at 37°C in a humidified atmosphere of 95% air/5% CO2. They were seeded initially at concentrations of 1.5 to 7.0 x 10^5 cells/175-mm flask (Becton Dickinson Labware, Oxnard, CA) and allowed 24 to 48 h to attach. After this period, the medium was replaced by one of six different media: complete medium containing EGF, RA, or neither; a chemically defined medium containing EGF, RA, or neither; a chemically defined medium containing EGF, RA, or neither; a chemically defined medium containing EGF, RA, or neither; a chemically defined medium containing EGF, RA, or neither; a chemically defined medium containing EGF, RA, or neither.

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1This research was supported in part by USPHS Grants CA-02817 from the National Cancer Institute and NS-11853 from the NIH.

2The abbreviations used are: GSL, glycosphingolipid; Cer, ceramide (N-acetyl-D-glucosamine)-N-alkyl-2-hexosylamine; Glc, glucose; Gal, galactose; NeuAc, N-acetylneuraminic acid; GM1, GM2, GM3, GM4; GalNAc, N-acetylgalactosamine; Lac, lacosamine; Asn, asparagine; Asp, aspartate; GlcNAc, N-acetylglucosamine; N-acetylneuraminic acid; A, acetate; B, butyrate; Ac, acetate; GPH, glycoprotein IIb/IIIa; EGF, epidermal growth factor; RA, retinoic acid; TLC, thin-layer chromatography; PAGE, polyacrylamide gel electrophoresis; LDH, lactic dehydrogenase; TCA, trichloroacetic acid; C, carbon; Cu, copper; D, dopamine; O2, oxygen; CO2, carbon dioxide; x, x-section; and Y, Y-section.

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cultures, however, decreased markedly due to the increased competency was observed in complete medium; this degree of differentiation of SqCC/Yl cells were measured at confluency.

Differentiation in the cultures was not significantly decreased by EGF or RA. The number of adherent cells in the RA-treated medium, the number of differentiated cells increased about 4-fold; under these conditions, EGF and RA caused 66.5% and 58% inhibition of the maturation process, respectively.

Neutral glycolipids were measured, and the results are shown in Table 1 and Fig. 1. The major glycolipids had the mobilities of globotriaosyl ceramide (Gb3), and almost equal amounts of GlcCer, LacCer, and Gb4 were detected. In addition, three minor unknown glycolipids were observed, none of which migrated with the Forssman antigen, asialo-GM1, or ceramide pentasaccharide. Cells that underwent differentiation in defined medium expressed distinct changes in GSL patterns as compared to their undifferentiated counterparts. Thus, SqCC/Y1 cells grown in defined medium were characterized by a lower content of neutral glycolipids than those in complete medium.

This lower content appeared to be mainly attributable to a decrease in Gb4 and Gb3, since a distinct increase in simple components of the GSLs, such as LacCer and GlcCer, occurred. The GlcCer levels were especially elevated, being 1.6-fold higher than cells maintained in complete medium. The addition of EGF to the complete medium produced no effects on the content and the distribution of neutral glycolipids of Gb4, Gb3, and LacCer, but an increase in the level of GlcCer was observed. In contrast, the EGF treatment of the cells grown in defined medium produced a partial increase in the glycolipid content and a shift in the distribution profile of the glycolipids of one that resembled that of SqCC/Y1 cells grown in serum-containing medium. In cells grown in the complete medium, RA induced a slight decrease in the neutral glycolipid content, but no major alteration in the distribution profile of the glycolipids. However, an increase in the glycolipid content was prominent in RA-treated cells grown in defined medium. This increase was in the Gb3 and Gb4 fractions, with a marked decrease in GlcCer content (Table 1).

The major ganglioside component in SqCC/Y1 cells was Gb3. In addition, three other minor gangliosides, which comigrated on TLC consistently with authentic Gb2, Gb3, and Gb4 were detected (Figs. 2 and 3). When the TLC plates were developed in the basic solvent system, the Gb3 standard was found to migrate faster than Gb4. A similar finding was seen in the test sample (Fig. 3), which confirmed that the increased material present in the TLC plate of the gangliosides was Gb3. The identities of Gb4 and Gb3 were further confirmed by a thin-
layer chromatographic immunostaining method (26) (data not shown). No fucose and glucosamine were detected by gas chromatographic analysis of sugar components in both differentiated and nondifferentiated cells.

All of the gangliosides found in cells cultured in the complete medium were also seen in SqCC/Yl cells treated with EGF or RA in complete medium and in the chemically defined medium.

However, the proportion of various individual gangliosides varied markedly under the different culture conditions. Thus, exposure of SqCC/Yl cells to EGF or RA in complete medium did not produce significant alterations in the content and distribution profile of gangliosides, whereas cells grown in defined medium were characterized by a lower content of gangliosides, with the most striking changes noted in the differentiated cells being a prominent increase in the levels of GD, which were about 3.1-fold greater than that present in undifferentiated SqCC/Yl cells (Table 2). No GMI component was detected in the differentiated cells. The addition of EGF or RA to the defined medium produced a partial increase in ganglioside content and a decrease in GD, a finding in keeping with the prevention of the attainment of the differentiated state by both of these agents.

The fatty acid composition of the total neutral glycolipid fraction from SqCC/Yl cells was measured by gas-liquid chromatography; a wide range of chain lengths (C18:0, C18:1, C20:0, C22:0, C23:0, C24:1, and C24:0), characteristic of extraneuronal GSLs, was found. The dichotomy noted in fatty acid chain lengths accounted for the appearance of doublet bands of GSLs noted in this cell line.

**DISCUSSION**

Cellular GSL composition undergoes changes which have been shown to be associated with development and oncogenic transformation (13–17, 29, 30). Although changes in GSLs have been reported in cultured cells treated with differentiating agents such as sodium butyrate, dimethyl sulfoxide, and RA (31), effects on GSL composition are different for cells of diverse lineages (13, 16). In the present study, the major ganglioside component of SqCC/Yl cells was shown to be GMI. Three other minor gangliosides were tentatively identified as GMI, GMI, and GD. SqCC/Yl cells contained GalCer, LacCer, Galb, and Galb as their major neutral glycolipids. In addition, three unknown minor neutral glycolipids were noted when the cells were treated with EGF or RA. A decrease in neutral GSLs was associated with the differentiation of this cell line that was almost exclusively due to a decrease in Gal and Galb. This finding suggests that a block in the galactosylation of LacCer may occur with the differentiation of these cells. This is consistent with the observations that, in certain cell lines, the globo-series of glycolipids are more closely associated with the undifferentiated state and that their synthesis decreases with differentiation (31, 32). Concomitantly, a significant increase in the proportions of simple GSLs, such as LacCer and GalCer, occurred in differentiated SqCC/Yl cells.

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**Table 1** The ganglioside composition ofSqCC/Yl cells grown in complete and defined medium.

Each determination was the average of 3 experiments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Compostion (% of the total area)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glc, Gb, LacCer, GlcCer</td>
</tr>
<tr>
<td>Complete medium</td>
<td></td>
</tr>
<tr>
<td>+EGF</td>
<td></td>
</tr>
<tr>
<td>+RA</td>
<td></td>
</tr>
<tr>
<td>Defined medium</td>
<td></td>
</tr>
<tr>
<td>+EGF</td>
<td></td>
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<td>+RA</td>
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<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sialic acid (µg/mg protein)</th>
<th>Compostion (% of the total area)</th>
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<tbody>
<tr>
<td>Complete medium</td>
<td></td>
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<tr>
<td>+EGF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+RA</td>
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<tr>
<td>Defined medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+EGF</td>
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<tr>
<td>+RA</td>
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* Estimated by densitometric scanning.

<table>
<thead>
<tr>
<th>Composition</th>
<th>GMI</th>
<th>GMI</th>
<th>GMI</th>
<th>GMI</th>
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<tbody>
<tr>
<td>Complete</td>
<td>1.8</td>
<td>0.1</td>
<td>5.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Defined</td>
<td>1.5</td>
<td>0.1</td>
<td>17.7</td>
<td>1.8</td>
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* Mean ± SD.
EGF or RA, which inhibited differentiation in the defined medium, the GSL composition was comparable to that of the nondifferentiated cells. This observation suggests that the pathways leading to Gb and Ga synthesis are suppressed during the maturation of this cell line, leading to an accumulation of precursor glycolipids such as LacCer. A decrease in more complex GSLs and a corresponding increase in simpler GSLs have been observed in a number of cell lines after oncogenic transformation (13, 16, 17).

SqCC/Y1 cells possess four identifiable gangliosides, GM3, GMI, and GD3. Although fucose-containing gangliosides have been reported to be present in a number of carcinoma cell lines (33–36), fucolipids were not detected in these malignant keratinocytes. The predominant ganglioside in SqCC/Y1 cells is GMI, which is known to be the major ganglioside in a variety of cells of extraneural origin (37, 38). The GD3 level increased markedly in maturing cultures. This change may be related to the degree of differentiation of SqCC/Y1 cells in that the increase was more prominent at 9 days of culturing than in cells cultured only for 6 days in the chemically defined medium. Ganglioside GD3 has been frequently associated with cells committed to differentiation (e.g., neuroectodermal cells, reactive glioma) (39, 40) and those undergoing neoplastic transformation (7, 39). In agreement with the concept that an increase in GD3 is associated with the differentiated state, cells grown in the chemically defined medium with EGF or RA, both of which interfered with the maturation process, had levels of GD3 comparable to undifferentiated cells. Since GD3 is derived from GMI, it is tempting to speculate that the activity of the sialyltransferase responsible for the formation of GD3 from GMI might be significantly enhanced in the differentiated state (41). Further studies are needed to establish this postulate.

GMI was not detected in cells undergoing differentiation in the defined medium. Furthermore, the disappearance of GMI could be prevented by the addition of EGF or RA. Thus, it would be interesting to determine whether cells committed to the differentiation pathway have lost their ability to synthesize the more complex gangliosides such as GMI.

REFERENCES

7. Rosenfelder, G., Young, W. J., and Hakomori, S. Structures and organization of cell surface glycolipids. De
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