Human Uterine Endometrial Adenocarcinoma: Characteristic Acquirement of Synthetic Potentials for II\(^3\)SO\(_3\)-LacCer and Ganglio Series Sulfoglycosphingolipids after Transfer of the Cancer Cells to Culture\(^1\)

Kaneyuki Kubushiro, Katsumi Tsukazaki, Jun Tanaka, Kiyoshi Takamatsu, Kazushige Kiguchi, Mikio Mikami, Shirou Nozawa, Yoshitaka Nagai, and Masao Iwamori\(^2\)

Department of Obstetrics and Gynecology, Keio University School of Medicine, 35 Shinano-machi, Shinjuku-ku [K. T., T. K., K. K., M. M., S. N.]; Tokyo Metropolitan Institute of Medical Science, 3-18-22 Honkomomoge, Bunkyo-ku [Y.N.]; and Department of Biochemistry, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, [M. I.], Tokyo

ABSTRACT

The acidic glycosphingolipid composition of human uterine endometrial adenocarcinoma was compared with those of normal uterine endometrium at the proliferative and the secretory phases. Upon chemical composition analysis, no significant transformation-associated change of these glycolipids was observed. However, when cancer cells from the patients with human uterine endometrial adenocarcinoma were transferred to culture, the composition of glycosphingolipids, particularly sulfoglycosphingolipids, was significantly altered after the 70th doubling time. II\(^3\)SO\(_3\)-GalCer, which was contained in the original tissues of uterine endometrial adenocarcinomas, disappeared completely from the cultured cells at the 70th doubling time, whereas II\(^3\)SO\(_3\)-LacCer and ganglio series sulfoglycosphingolipids, which were originally contained in a trace amount or not present at all in the cancer tissues, became the major components in the total acidic glycosphingolipids in the cultured cells. Also, among cell lines established from several gynecological cancers, which include uterine cervical squamous carcinoma, uterine endometrial adenocarcinoma, ovarian clear cell carcinoma, choriocarcinoma, uterine sarcoma, ovarian sarcoma, and vulvar melanoma, only those cells derived from uterine endometrial adenocarcinoma expressed II\(^3\)SO\(_3\)-LacCer and ganglio series sulfoglycosphingolipids and the synthetic activities of these sulfoglycolipids, indicating that uterine endometrial adenocarcinoma cells characteristically lose the sulfotransferase to GalCer and acquire the sulfotransferase to LacCer after being transferred to culture in vitro. Thus, the unique sulfoglycosphingolipids and sulfotransferase are useful markers for the characterization of uterine endometrial adenocarcinoma among human gynecological cancers.

INTRODUCTION

The expression of glycosphingolipids on the cell surface is strictly controlled, either at the translational level or at the enzymatic level, and is closely associated with cellular differentiation, proliferation, and morphogenesis (1, 2). As shown in our previous paper (3), sulfatide, II\(^3\)SO\(_3\)-GalCer, has been characterized as a molecule expressed specifically in the secretory phase of human uterine endometrium, in which the cellular differentiation in association with the menstrual cycle is regulated with sex steroid hormones, estrogen and progesterone, and the sulfotransferase responsible for the synthesis of sulfatide has been thought to be activated along with the periodic action of sex steroid hormones during the period from menstruation to ovulation. Steroid hormones are also known to have a significant effect upon the triggering, promotion, or progression of several human gynecological cancers (4), but the expression of glycosphingolipids including sulfoglycosphingolipids in the gynecological cancers has not been clearly characterized yet. Abnormal expression of glycosphingolipids by neo-synthesis or retarded synthesis is frequently observed in several transformed cells and is a useful marker not only in discriminating the cancer cells from the normal cells but also in resolving cellular functions, such as infiltration, metastasis, and protection from the host immune defense system of the cancerous cells. Information on the glycosphingolipids of the gynecological cancers is therefore quite important in the detection of the markers mentioned above (5-7) and also provides a clue to the hormonal regulation of glycosphingolipid metabolism. Accordingly, we attempted to analyze the glycosphingolipid composition of human uterine endometrial adenocarcinoma in comparison with those of the normal uterine endometrium at the proliferative and the secretory phases, as well as that of cultured cells at a different doubling time. Our analysis show that, as a result of culture, uterine endometrial adenocarcinoma-derived cells characteristically lost the synthetic potential of sulfatide and acquired the synthetic ability of II\(^3\)SO\(_3\)-LacCer. These findings indicate that the metabolism of sulfoglycosphingolipids is significantly regulated in uterine endometrial adenocarcinoma as well as in the normal uterine endometrium.

MATERIALS AND METHODS

Cells. A portion of the cancerous tissues from patients with uterine endometrial adenocarcinoma, which were obtained from the university hospital of the University of Tokyo, were cut into small pieces, washed once, and incubated with 0.25% trypsin in calcium- and magnesium-free PBS. The cells liberated were cultured in Ham’s F-12 medium with 10% fetal calf serum in an atmosphere of 5% CO\(_2\). The following cancer cells, all of which were established from pathologically well-defined cancerous tissues (8, 9), were also used in this experiment: SNG-II and SNG-M from uterine endometrial adenocarcinoma: SKG-I, SKG-II, and SKG-IIa from uterine cervical squamous carcinoma: RMG-I and RMG-II from ovarian clear cell carcinoma: NJG from choriocarcinoma: SKN from uterine sarcoma: RKN from ovarian sarcoma: and GAK from vulvar melanoma. In addition, HeLa cells originally established from uterine cervical squamous carcinoma were used for comparison. All cells were cultured in the medium described above.

Tissues. Human uterine endometria from patients suffering from myoma uteri and uterine endometrial adenocarcinoma were obtained from the university hospitals of Keio University and the University of Tokyo. The phase of normal uterine endometrium was determined histochemically, and the tissues were stored at \(-20^\circ\text{C}\) until glycosphingolipid analysis.

Glycosphingolipids. Standard glycosphingolipids used in this experiment were purified in our laboratory from the following sources: II\(^3\)NeuAc-LacCer from human erythrocytes; II\(^3\)SO\(_3\)-GalCer and II\(^3\)NeuAc\(_2\)-LacCer from human brain, and II\(^3\)SO\(_3\)-LacCer, II\(^3\)SO\(_3\)-GalCer, and II\(^3\)SO\(_3\)-NeuAc-LacCer from human tumors. The acidic glycosphingolipid composition of human uterine endometrial adenocarcinoma as well as in the normal uterine endometrium at the proliferative and the secretory phases. Upon chemical composition analysis, no significant transformation-associated change of these glycolipids was observed. However, when cancer cells from the patients with human uterine endometrial adenocarcinoma were transferred to culture, the composition of glycosphingolipids, particularly sulfoglycosphingolipids, was significantly altered after the 70th doubling time. II\(^3\)SO\(_3\)-GalCer, which was contained in the original tissues of uterine endometrial adenocarcinomas, disappeared completely from the cultured cells at the 70th doubling time, whereas II\(^3\)SO\(_3\)-LacCer and ganglio series sulfoglycosphingolipids, which were originally contained in a trace amount or not present at all in the cancer tissues, became the major components in the total acidic glycosphingolipids in the cultured cells. Also, among cell lines established from several gynecological cancers, which include uterine cervical squamous carcinoma, uterine endometrial adenocarcinoma, ovarian clear cell carcinoma, choriocarcinoma, uterine sarcoma, ovarian sarcoma, and vulvar melanoma, only those cells derived from uterine endometrial adenocarcinoma expressed II\(^3\)SO\(_3\)-LacCer and ganglio series sulfoglycosphingolipids and the synthetic activities of these sulfoglycolipids, indicating that uterine endometrial adenocarcinoma cells characteristically lose the sulfotransferase to GalCer and acquire the sulfotransferase to LacCer after being transferred to culture in vitro. Thus, the unique sulfoglycosphingolipids and sulfotransferase are useful markers for the characterization of uterine endometrial adenocarcinoma among human gynecological cancers.

INTRODUCTION

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GgCer, and II3SO2-GgCer from human kidney.

Analysis of Glycosphingolipids. The extraction and analysis of glycosphingolipids from tissues and cells were carried out according to the methods described previously (10). Briefly, the cells (1 x 10^9) and tissues were hypophhilized and the lipids were extracted with chloroform:methanol:water (20:10:1, 10:20:1, and 10:10:1 by volume) at 40°C. After determination of the cholesterol and lipid-bound phosphorus in the combined lipid extracts by GLC (11) and colorimetry (12), respectively, the lipids were fractionated into neutral and acidic lipids by DEAE-Sephadex column chromatography. Then, neutral and acidic glycosphingolipids were prepared from the neutral and acidic lipid fractions by acetylation, Florisil column chromatography, and deacytlation (13) and by mild alkaline hydrolysis and dialysis (10), respectively. A quantitative determination of the individual glycosphingolipids was performed by TLC densitometry, as described previously (14). In short, neutral and acidic glycosphingolipids were developed on TLC with chloroform:methanol:water (0.5:3:8 by volume) and chloroform:methanol:0.5% calcium chloride in water (55:45:10 by volume), respectively. The spots were located with orcinol-sulfuric acid reagent for neutral and sulfoglycosphingolipids and with resorcinol-hydrochloric acid for gangliosides. The density of the spots was measured with a TLC densitometer (CS-910; Shimadzu Co., Kyoto, Japan) at 430 nm for the orcinol-positive spots and at 570 nm for the resorcinol-positive spots, respectively. The control wave length was set at 710 nm.

35S Incorporation into Sulfolipids of the Cells. Carrier-free H235SO4 (100 µCi; specific activity, 30 Ci/mol) was added to the cells at the confluent stage in a 25-cm² dish and cultured for an additional 72 h. After the dish was washed with the culture medium, the cells were harvested by scraping with a rubber policeman and washed twice with medium and once with calcium- and magnesium-free Hanks' solution. The lipids were extracted from the cell pellet with 3 ml of chloroform:methanol:water (70:30:2 and 10:90:4 by volume) and chloroform:methanol:0.5% calcium chloride in water (55:45:10 by volume), respectively. The spots were located with orcinol-sulfuric acid reagent for neutral and sulfoglycosphingolipids and with resorcinol-hydrochloric acid for gangliosides. The density of the spots was measured with a TLC densitometer (CS-910; Shimadzu Co., Kyoto, Japan) at 430 nm for the orcinol-positive spots and at 570 nm for the resorcinol-positive spots, respectively. The control wave length was set at 710 nm.

RESULTS

Glycosphingolipids of Normal Human Uterine Endometrium and Uterine Endometrial Adenocarcinoma. TLCs of acidic glycosphingolipids from tissues of normal uterine endometrium at the secretory phase and uterine endometrial adenocarcinoma are shown in Figs. 1 and 2. As already reported in our previous paper (3), II3SO2-GalCer (sulfatide), II3NeuAc^ GalCer (GM3), and II3NeuAc^2-GalCer (GD3) were the major acidic glycosphingolipids and the concentration of II3SO2-GalCer was characteristically increased in the secretory phase of normal uterine endometrium. In uterine endometrial adenocarcinoma tissues, the concentration of II3NeuAc-LacCer was not significantly
Fig. 2. TLC of the acidic glycosphingolipids from the tissues of human uterine endometrium (I-1 and II-1), the cells from tissue I-1 at the 10th (I-2) and the 70th (I-3) doubling times, and the cells from tissue II-1 at the 70th doubling time (II-2).

<table>
<thead>
<tr>
<th>Table 1 Concentrations of cholesterol, phospholipids, sulfoglycosphingolipids, and II3NeuAc-LacCer in normal human endometrium and cancerous tissues from patients with uterine endometrial adenocarcinoma</th>
<th>µmol/g dry wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissues*</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>Normal uterine at the secretory phase</td>
<td>36.96</td>
</tr>
<tr>
<td>2</td>
<td>26.11</td>
</tr>
<tr>
<td>3</td>
<td>18.79</td>
</tr>
<tr>
<td>Uterine endometrial adenocarcinoma</td>
<td>23.98</td>
</tr>
<tr>
<td>5</td>
<td>20.35</td>
</tr>
<tr>
<td>6</td>
<td>28.89</td>
</tr>
<tr>
<td>7</td>
<td>24.52</td>
</tr>
<tr>
<td>8</td>
<td>20.90</td>
</tr>
<tr>
<td>9</td>
<td>37.80</td>
</tr>
<tr>
<td>10</td>
<td>27.24</td>
</tr>
<tr>
<td>11</td>
<td>23.55</td>
</tr>
<tr>
<td>12</td>
<td>20.47</td>
</tr>
<tr>
<td>I-1</td>
<td>30.32</td>
</tr>
<tr>
<td>II-1</td>
<td>26.67</td>
</tr>
</tbody>
</table>

*Numbers of tissues correspond to those in Figs. 1 and 2.

Tr, trace amount.

different from those in normal endometria at the proliferative and secretory phases (Table 1) (3) and no difference was observed between the ganglioside compositions of normal and cancerous tissues. In addition, the concentrations of I3SO3-GalCer and I3SO3-LacCer were varied among individual cancerous tissues examined and were not characteristic for endometrial adenocarcinoma, being within the levels of those in the proliferative and secretory phases of normal tissues.

Alteration of the Acidic Glycosphingolipid Composition in Uterine Endometrial Adenocarcinoma after Transfer of the Cells to Culture. The tissue of human uterine endometrial adenocarcinoma was composed of morphologically homogeneous cells. In order to further characterize uterine endometrial adenocarcinoma-derived cells, tissues I-1 and II-1 shown in Fig. 2 were successfully transferred to culture. These cells obtained were morphologically homogeneous at the 10th doubling time. The glycosphingolipids of the cells at the 10th doubling time (I-2) were changed from those of the original tissues (I-1). In particular, bands corresponding to I3SO3-LacCer and I3SO3-Gg3Cer, both of which were not detected in the original tissues, newly appeared in I-2 cells (Fig. 2). A more distinct change in the glycosphingolipid composition was observed in I-3 and II-2 cells at the 70th doubling time. As shown in Fig. 2, the acidic glycosphingolipid migrating close to I3SO3-LacCer became a major component in I-3 and II-2 cells, which were derived from tissues I-1 and II-1, respectively. I3SO3-GalCer, which was contained in the original tissues completely disappeared from the cells by the 70th doubling time. In contrast, the concentration of I3SO3-LacCer in cells I-3 and II-2 at the 70th doubling time was significantly increased compared to that in the original tissues. Then we compared the glycosphingolipid composition of several human gynecological cancer-derived cell lines (Table 2). A glycosphingolipid composition with a high concentration of I3SO3-LacCer similar to those in I-3 and II-2 was observed only in cell lines SNG-II and SNG-M, which were established from uterine endometrial adenocarcinoma in 1981 and 1975, respectively. I3SO3-LacCer was not detected in any of the cells from uterine cervical squamous carcinoma, ovarian clear cell carcinoma, choriocarcinoma, uterine sarcoma, ovarian sarcoma, or vulvar melanoma (Table 3), indicating that active synthesis of I3SO3-LacCer is characteristic of uterine endometrial adenocarcinoma-derived cells, and loss of the synthetic activity of I3SO3-GalCer and new acquirement of the synthetic potential for I3SO3-LacCer occur after transfer of the endometrial adenocarcinoma cells to culture. The highest concentrations of sulfoglycosphingolipids, corresponding to the mobilities of I3SO3-LacCer and I3SO3-Gg3Cer, were observed in SNG-II, where they comprised 8.43 and 3.20% of the total glycosphingolipids, and they were the primary negatively charged groups of membrane-associated lipids in SNG-II, as well as those in the other endometrial adenocarcinoma-derived cells (Table 3).

Structural Determination of Sulfoglycosphingolipids in the Uterine Endometrial Adenocarcinoma-derived Cells. Two acidic glycosphingolipids, showing mobilities on TLC identical to those of I3SO3-LacCer and I3SO3-Gg3Cer, respectively, were the major sulfoglycosphingolipids in endometrial adenocarcinoma-derived cells SNG-II. These two components were con-
Table 2 Several human gynecological cancer cells

<table>
<thead>
<tr>
<th>Original cancer</th>
<th>Age of patient (yr)</th>
<th>Date of primary culture</th>
<th>Cells</th>
<th>Chromosome</th>
<th>Doubling time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine endometrial adenocarcinoma</td>
<td>43</td>
<td>Nov. 17, 1981</td>
<td>SNG-II</td>
<td>46 diploid</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>Nov. 28, 1975</td>
<td>SNG-M</td>
<td>46 aneuploid</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>Mar. 15, 1989</td>
<td>I</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>May 10, 1989</td>
<td>II</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Uterine cervical squamous carcinoma</td>
<td>40</td>
<td>Jul. 30, 1974</td>
<td>SKG-I</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>Jul. 9, 1975</td>
<td>SKG-II</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>Aug. 24, 1978</td>
<td>SKG-IIIa</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Ovarian clear cell adenocarcinoma</td>
<td>34</td>
<td>Jan. 4, 1983</td>
<td>RMG-I</td>
<td>47 aneuploid</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>53</td>
<td>Oct. 1982</td>
<td>RMG-II</td>
<td>101 aneuploid</td>
<td>72</td>
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<tr>
<td>Choriocarcinoma</td>
<td>29</td>
<td>May 8, 1978</td>
<td>NJG</td>
<td>78 aneuploid</td>
<td>80</td>
</tr>
<tr>
<td>Uterine sarcoma</td>
<td>52</td>
<td>Jul. 3, 1975</td>
<td>SKN</td>
<td>112 aneuploid</td>
<td>36</td>
</tr>
<tr>
<td>Ovarian sarcoma</td>
<td>45</td>
<td>Jul. 18, 1978</td>
<td>RKN</td>
<td>76 aneuploid</td>
<td>31</td>
</tr>
<tr>
<td>Vulvar melanoma</td>
<td>86</td>
<td>Dec. 26, 1978</td>
<td>GAK</td>
<td>67 aneuploid</td>
<td>72</td>
</tr>
</tbody>
</table>

Table 3 Concentrations of acidic glycosphingolipids in several human gynecological cancer-derived cells

<table>
<thead>
<tr>
<th>Original cancer</th>
<th>Cell line</th>
<th>II \textsuperscript{3}NeuAc-LacCer</th>
<th>I \textsuperscript{3}sulfo-GalCer</th>
<th>I \textsuperscript{3}sulfo-LacCer</th>
<th>I \textsuperscript{3}sulfo-GgJ3Cer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine endometrial adenocarcinoma</td>
<td>SNG-II</td>
<td>0.06</td>
<td>0.79</td>
<td>0.30</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>SNG-M</td>
<td>0.19</td>
<td>0.51</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>I-2</td>
<td>0.76</td>
<td>0.20</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>I-3</td>
<td>0.31</td>
<td>0.30</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II-2</td>
<td>0.24</td>
<td>0.32</td>
<td>0.02</td>
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</tr>
<tr>
<td>Uterine cervical squamous carcinoma</td>
<td>SKG-I</td>
<td>1.01</td>
<td>Tr</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>SKG-II</td>
<td>0.14</td>
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<td></td>
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<tr>
<td></td>
<td>SKG-IIIa</td>
<td>0.04</td>
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<td></td>
<td>HeLa</td>
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<td>Ovarian clear cell carcinoma</td>
<td>RMG-I</td>
<td>0.05</td>
<td>Tr*</td>
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<tr>
<td></td>
<td>RMG-II</td>
<td>0.19</td>
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<tr>
<td>Choriocarcinoma</td>
<td>NJG</td>
<td>0.02</td>
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<tr>
<td>Uterine sarcoma</td>
<td>SKN</td>
<td>0.48</td>
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<tr>
<td>Ovarian sarcoma</td>
<td>RKN</td>
<td>0.46</td>
<td>Tr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vulvar melanoma</td>
<td>GAK</td>
<td>1.24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Tr, trace amount.

Fig. 3. Negative ion FABMS spectrum of the upper sulfoglycosphingolipid from the SNG-II cell. About 5 \mu g of the isolated upper sulfoglycosphingolipid were dissolved in triethanolamine and analyzed by negative ion FABMS, as described in the text.

The relative intensity of each molecular ion was identical to those of sulfolactosyl ceramide (Fig. 3). Also, lactosylceramide was obtained by solvolysis, and 1,4,5-tri-O-acetyl-2,3,6-tri-O-methylglucitol and 1,3,5-tri-O-acetyl-2,4,6-tri-O-methylgalactitol were identified by permethylation, indicating that the upper sulfoglycosphingolipid is determined to be the 3-O-sulfolactosylceramide, \text{II}^3\text{SO}_3\text{LacCer}. Conversely, molecular ions of the slowly migrating sulfoglycosphingolipid indicated by negative ion FABMS, appeared in the region of \textit{m/z} 1143 to 1255, and the fragmented ions caused by the cleavage at the glycosidic linkage of N-acetylgalactosamine from the nonreducing end of the carbohydrate chain were recognized at \textit{m/z} 940 to 1052 (Fig. 4). Also, GgJ3Cer was found to be produced after solvolysis by TLC immunostaining with anti-GgJ3Cer antiserum, followed by peroxidase-conjugated anti-rabbit IgG antibody. In addition, 1,4,5-tri-O-acetyl-2,3,6-tri-O-methylglucitol, 1,3,4,5-tetra-O-acetyl-2,6-di-O-methylgalactitol, and 1,5-di-O-acetyl-2-deoxy-2-N-methylacetamido-3,4,6-tri-O-methylgalactitol were detected at a ratio of 1:1:1. Thus, the structure of the slowly migrating sulfoglycosphingolipid was confirmed to be that of \text{II}^3\text{SO}_3\text{GgJ3Cer}. In accordance with the results obtained by...
negative ion FABMS analyses, of the fatty acid and long-chain base compositions by GLC revealed that II3SO3-LacCer and II3SO3-Gg4Cer contained palmitic to lignoceric acids, and the primary long-chain base was composed of 4-sphingenine. Neither hydroxy fatty acids nor phytosphingosine were detected in either of the sulfoglycosphingolipids from SNG-II. In addition, although we were unable to determine the complete structure due to its low concentration, the occurrence of II3SO3-Gg3Cer in the SNG-II, SNG-M, I-3, and II-2 cells was strongly suggested by TLC immunostaining of the acidic glycosphingolipids after solvolysis with anti-Gg,Cer antiserum and the peroxidase-conjugated anti-rabbit IgG antibody.

Biosynthetic Activities of Various Sulfoglycosphingolipids in Tissues of Human Uterine Endometrial Adenocarcinoma and Cells Established from Various Human Gynecological Cancers. Fig. 5 shows the radioautogram of the total lipid extracts of 12 human gynecological cancer cells after the cells were main- tained in the confluent stage in a medium containing 100 µCi of H235SO4 for 72 h. 35S-labeled glycolipids were observed only in the 1-1, 1-3, and II-2 cells. Thus, the pathway, LacCer—II3SO3-LacCer—II3SO3-Gg3Cer—II3SO3-Gg4Cer, is characteristically activated in cells established from human uterine endometrial adenocarcinoma.

DISCUSSION

As is clearly shown in this communication, the acidic glycosphingo- lipid composition of human uterine endometrial adenocarcinoma was found to be greatly altered when the cells were transferred to culture and was characteristic as an acquired phenotype among the cells from the other gynecological cancers, uterine cervical squamous carcinoma, ovarian clear cell adenocarcinoma, choriocarcinoma, uterine sarcoma, ovarian sarcoma, and vulvar melanoma. The major sulfoglycosphingolipids in the endometrial adenocarcinoma cells after transfer to culture were II3SO3-lactosyl and II3SO3-ganglio-N-triaosylceramides. Also, II3SO3-ganglio-N-tetraosylceramide was found in the endometrial adenocarcinoma-derived cells. II3SO3-galactosylceramide, sulfatide, which was contained in the original cancer tissues of uterine endometrial adenocarcinoma, was not detected in the cells after culturing for the 70th doubling time by either TLC analysis with orcinol reagent or labeling experi-
ments with $[^{35}S]$-sulfate. However, I$^{35}SO_4$-GalCer and its biosynthetic activity were still detected in the cells at the 10th doubling time, suggesting that the pathway for the synthesis of I$^{35}SO_4$-GalCer is slowly inactivated during the course of culture. By contrast, the pathway LacCer$\rightarrow$I$^{35}SO_4$-LacCer$\rightarrow$I$^{35}SO_4$-Gg$_3$Cer$\rightarrow$I$^{35}SO_4$-Gg$_2$Cer was characteristically expressed in all of the endometrial adenocarcinoma-derived cells examined, eliciting a useful marker for discriminating the uterine endometrial adenocarcinoma-derived cells from the other gynecological cancer-derived cells. Possible factors causing the metabolic shift of glycosphingolipids are entirely obscure at present, but several circumstances, including nutritional supplementation and cell-to-cell or cell-to-extracellular matrix interaction, are thought to affect the metabolism. In the case of culture of cancerous cells, we observed a change in the content of gangliosides due to replacement of glucose with galactose plus pyruvic acid in the synthetic medium (22), but modification of basic metabolism to activate a new pathway could not be achieved by exchange of the components in the culture medium. Therefore, change in the glycosphingolipid composition of the cultured cancerous cells did not seem to occur easily without induction of cellular differentiation. However, it is certain that supplementation of several compounds, such as growth factors, hormones, ions, and vitamins, which are supposed to affect cellular proliferation as well as the metabolism, differs greatly between in vivo and in vitro conditions, and it is probable that an enzyme, the production and activity of which are suppressed in vivo, is activated by culture in vitro. In this connection, the pathway for the synthesis of I$^{35}SO_4$-LacCer is thought to be inactive in vivo but to be carried in a latent form, because I$^{35}SO_4$-LacCer was detectable in some of the cancerous tissues. On the other hand, the pathway for the synthesis of I$^{35}SO_4$-GalCer in normal uterine endometrium was strictly regulated by estrogen and progesterone, which were also closely related to transformation of endometrial cells, as was indicated in our previous paper (3).

Accordingly, the potentials of sulfotransferase should be retained even after transformation.

In cell lines derived from renal tubules, a single sulfotransferase was proven to be responsible for sulfation of GalCer and LacCer, but the activities to the different substrates were regulated positively or negatively by different cations (23). In the endometrial adenocarcinoma-derived cells, in which a precursor, GalCer, was contained in a significantly high concentration, the sulfotransferase with substrate specificity similar to that in the renal tubule-derived cells might be directed to choose LacCer as the substrate in the cultured condition. In contrast to the rather heterogeneous condition insufficient to select a single substrate in the uterus, the cells in culture might be exposed to a relatively homogeneous condition to sulfate only LacCer. Thus, sulfotransferase for the synthesis of I$^{35}SO_4$-LacCer must be one of the uterine endometrial adenocarcinoma-associated phenotypes. On the other hand, as a result of active production of I$^{35}SO_4$-LacCer, II$^{35}$NeuAc-LacCer was found to be reduced in concentration, probably due to the fact that LacCer was a common substrate for the synthesis of I$^{35}SO_4$-LacCer and II$^{35}$NeuAc-LacCer. Therefore, alteration of negative charge in the cell surface might occur after transfer of the uterine endometrial adenocarcinoma to culture.

As shown in Tables 1 and 3, it was clear that LacCer sialyltransferase and LacCer sulfotransferase were compensatedly activated. However, by examining the degree of differentiation of the cells after intracutaneous transplantation of the cells to nude mice, we further found that the concentrations of II$^{35}$NeuAc-LacCer and I$^{35}SO_4$-LacCer were closely related to the degree of differentiation of endometrial adenocarcinomaderived cells. Thus, the composition and metabolism of sulfoglycosphingolipids acquired after transfer of the cells to culture were phenomena reflecting the type of original uterine endometrial adenocarcinoma. Although the cell biological properties of the cells expressing the ganglio series sulfoglycosphingolipids as the major acidic glycosphingolipids are unclear, several sulfated glycolipids are known to show tissue-specific localization and associate closely with the functions of the tissues; i.e., the I$^{35}SO_4$-GalCer with α-hydroxy fatty acids in the mammary myelin sheath is associated with myelin formation (24), and the I$^{35}SO_4$-GalGD in the mammary testis is associated with spermatogenesis (25, 26), the sulfated glucuronic acid-containing glycolipids are present in the human brain and T-lymphocytes (27), and the I$^{35}SO_4$-Gg$_3$Cer was found in mouse intestine (28). I$^{35}SO_4$-LacCer, I$^{35}SO_4$-Gg$_2$Cer, and I$^{35}SO_4$-Gg$_4$Cer in this report were also identified in the rat kidney (29) and are assumed to be related to kidney functions such as ion transport.

Since an egg passing through the endometrium can be fertilized only by sperm and sulfolipids are involved in the adhesion molecule, the sulfolipids in the endometrium may play an essential role in the processes of fertilization or in the adhesion process of the fertilized egg. The uterine endometrial adenocarcinoma-derived cells, having unique sulfoglycosphingolipids, will provide a clue to the elucidation of the possible function of sulfoglycolipids in the endometrium and of the hormonal regulation of cellular proliferation and transformation in the endometrium.

REFERENCES


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Human Uterine Endometrial Adenocarcinoma: Characteristic Acquisition of Synthetic Potentials for II 3SO3-LacCer and Ganglio Series Sulfoglycosphingolipids after Transfer of the Cancer Cells to Culture

Kaneyuki Kubushiro, Katsumi Tsukazaki, Jun Tanaka, et al.


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