Immunocchemical Detection of a Small Cell Lung Cancer-associated Ganglioside (FucGM₁) Antigen in Serum¹

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ABSTRACT

Recently, the ganglioside FucGM₁ (Fucα1-2Galβ1-3GalNAcβ1-4[NeuAcα2-3]-Galβ1-4Glcβ1-1Cer) was identified as a small cell lung cancer (SCLC) marker both in chemical and histochemical studies. In order to further determine whether the FucGM₁ ganglioside is shed from the tumor site and consequently is present in the serum of SCLC patients, we produced a series of new monoclonal antibodies raised against FucGM₁ and related glycolipids. Shedding of the FucGM₁ ganglioside was studied both in vitro and in vivo using SCLC cell lines and nude mice xenografts of SCLC cells as model systems, and finally immunocchemical analyses were performed on serum samples from patients with SCLC. High-performance thin-layer chromatography immunostaining demonstrated the presence of FucGM₁ in conditioned culture media obtained from FucGM₁-positive SCLC cell lines. Furthermore, tumor extracts of SCLC cell line xenografts in nude mice were positive for the FucGM₁ marker, and more importantly the marker was also present in serum samples from these mice. Twenty serum samples were obtained from patients with histologically verified SCLC. Eight patients had localized disease, and the remaining patients had disseminated cancer involving metastases to other organ sites. Sera from 4 of these patients were clearly positive, and 2 additional cases were found to be weakly positive. The positive patient sera were all from patients with extensive disease. Sera from 12 patients with non-SCLC and 20 healthy individuals were all found to be negative. These results clearly establish the FucGM₁ glycolipid as a potential serum marker of SCLC for which a sensitive immunocassay should be developed and tested using a larger series of serum samples.

INTRODUCTION

SCLC¹ comprises 20 to 25% of all lung cancer cases (1), and with a 5-year survival rate of 2 to 5% it is the histological subtype of lung cancer with the poorest prognosis (2). SCLC is surgically incurable due to the rapid proliferation and metastatic spread by the time of diagnosis (3), but SCLC tumors are generally sensitive to chemo- and radiation therapy, which emphasize the importance of accurate and early detection of SCLC for effective therapy.

SCLC has been characterized as a neuroendocrine tumor because of the presence of neuroendocrine differentiation markers and the presence of neurosecretory granules. Several serum markers have been suggested for SCLC, including neuron-specific enolase and the creatine kinase-BB isoenzyme. None of these markers has shown an absolute specificity for SCLC (4, 5).

Nilsson et al. (6) originally identified the glycolipid FucGM₁ (Fucα1-2Galβ1-3GalNAcβ1-4[NeuAcα2-3]-Galβ1-4Glcβ1-1Cer) as a selective tumor-associated marker of SCLC cells. FucGM₁ and related structures are illustrated in Fig. 1. Using specific monoclonal antibodies they analyzed glycolipid extracts of a variety of normal human tissues and tumor tissues by HPTLC immunostaining and found the FucGM₁ glycolipid to be strongly associated with SCLC although some normal tissues, excluding normal lung tissue, also contained the glycolipid (6–9). Recently, the SCLC-associated expression of FucGM₁ was further substantiated by immunohistology of frozen tissue sections, where the presence of FucGM₁ was demonstrated in 90% of the cases as compared to 12% positive cases observed in other lung cancers (10).

Gangliosides have been identified in serum samples from patients with malignant melanoma, presumably as a result of shedding from tumor cells, and the detection of these antigens in serum may provide an aid for diagnosis and follow-up (11–13). In this report, we present evidence that the FucGM₁ ganglioside is shed in vitro and in vivo from SCLC cells and that this antigen also may be detected in serum samples from SCLC patients.

MATERIALS AND METHODS

Cell Lines. NCI-H69, CALU-1, CALU-3, SK-MES, and SK-LU1 cells were obtained from the American Type Culture Collection (Rockville, MD). NCI-H345 and NCI-H510 cells were kindly provided by Dr. T. Schwartz (Laboratory of Molecular Endocrinology, Copenhagen, Denmark). GLC-14, GLC-16, and TOOS4A cells (14, 15) were kindly provided by Dr. K. Rygaard (State University Hospital, Copenhagen).

Monoclonal Antibodies. MAbs directed to the FucGM₁ and FucGA₁ antigens were produced according to standard procedures (16). Purified glycolipids (2 µg), adsorbed on acid-treated Salmonella minnesota (50 µg), were used as antigens for i.v. immunization of BALB/c mice (3-month-old) 3–5 times with 2–3-week intervals between immunizations. Fusion with SP/2-0 or NS-1 hypoxanthine phosphoribosyltransferase-negative myeloma cells was performed 3 days after the last i.v. immunization. The screening and further selection of hybridomas with specificity for FucGM₁ (TKH5 and 1D7) was based on reactivity with purified glycolipids using enzyme-linked immunosorbent assay as well as HPTLC immunostaining techniques (17, 18). Both MAbs (TKH5 and 1D7) were identified as being of the IgG3 isotype. An additional MAb, TKH4, specifically bound FucGA₁ but did not react with FucGM₁ or other glycolipid antigens. The MAb MH2 was prepared similarly using a biosynthetically produced AG₃ ganglioside.⁴

Purification of Antibodies. One-tenth volume of 1.0 M Tris (pH 8.0) was added to the MAb-containing culture supernatant, adsorbed on a protein A-Sepharose column (Pharmacia, Uppsala, Sweden), washed with 100 µl Tris (pH 8.0), eluted with 100 µl glycine buffer (pH 3.0), and collected into tubes containing 0.1 volume of 1 M Tris, pH 8.2. The IgG3 anti-FucGM₁ MAbs (TKH5 and 1D7) were immediately

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The abbreviations used are: SCLC, small cell lung cancer; HPTLC, high-performance thin-layer chromatography; MAb, monoclonal antibody; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; FACS, fluorescence-activated cell sorter; PBS, phosphate-buffered saline; FucGM₁, Fucα1-2Galβ1-3GalNAcβ1-4[NeuAcα2-3]-Galβ1-4Glcβ1-1Cer; FucGA₁, fucosyl-asialo-GM₁.

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collected and dialyzed (molecular weight cutoff, 12,000–14,000; Spectra/por, Los Angeles, CA) against PBS (pH 7.4).

Culture of Cell Lines. NCI-H69, NCI-H345, NCI-H510, GLC-14, and GLC-16 cells were grown in RPMI 1640 containing 10% FCS. CALU-1 cells were grown in McCoy's 5a medium containing 10% FCS, whereas CALU-1, SK-MES, T0054A, and SK-LU1 cells were grown in modified Eagle's medium containing 10% FCS supplemented with 7 nonessential amino acids and sodium pyruvate. All lung cancer cell cultures were supplemented with L-glutamine, penicillin, and streptomycin (100 units/ml, 0.5 mg/ml). Conditioned media in a total volume of 50 ml from GLC-14, GLC-16, and NCI-H69 cells were obtained from cell cultures in the exponential growth phase. The NCI-H69 cells were also propagated in vivo as xenografts in athymic nude BALB/c mice. Hybridoma cells were cultured in RPMI 1640 containing 15% FCS supplemented with L-glutamine and sodium pyruvate. All cell cultures were routinely screened for possible Mycoplasma contamination at monthly intervals and were negative throughout these experiments.

Nude Mouse Xenografts. Cells (3–5 × 10⁶) of the cell lines GLC-14, GLC-16, NCI-H69, and T0054A were inoculated s.c. into the flanks of athymic nude BALB/c mice. When the xenografted mice showed symptoms of dehydration and loss of weight, the tumors and organs such as liver, lung, spleen, and mesenteric lymph nodes were collected. Blood samples were collected in heparinized test tubes containing aprotinin (Trasylol; Bayer, Leverkusen, Germany) at a final concentration of 2000 Kallikrein inhibitor units/ml. Tumor, organs, and plasma samples were immediately frozen at −80°C.

Serum Samples. Serum samples (700–800 µl) were obtained from 20 patients with histologically verified SCLC as classified according to WHO II criteria. The sera were obtained at the time of diagnosis and before the patients received chemotherapy, and all patients participated in a follow-up study. Eight patients had localized disease, and 12 patients had disseminated cancer involving metastases to other organ sites. Clinical information on these patients (age, sex, blood type, state of disease, and organ site involvement) and results from analyses of serum samples are summarized in Table 1. Twelve serum samples from patients with non-SCLC cancer as well as 20 serum samples from normal individuals were used as control sera.

Immunofluorescence Staining Procedure. One million to 5 million cells were incubated with 100 µl protein A-purified TKH5 or 1D7 MAb (dilution: 1:100) in PBS for 1 h and washed 3 times, followed by incubation with 100 µl FITC-conjugated goat anti-mouse immunoglobulin (code F-261; Dako A/S, Glostrup, Denmark) at a final dilution of 1:80. All procedures were done at 4°C. An IgG3 monoclonal antibody of nonrelated specificity was used for control staining. Immunofluorescence staining was analyzed by fluorescence microscopy as well as by FACS analysis.

FACS Analysis. One hundred µl of cell suspension adjusted to 10⁶ cells/ml were incubated with 100 µl of MAbs TKH5 or 1D7 Mabs (dilution: 1:100 in PBS) for 1 h at 4°C and washed three times, followed by incubation at the same temperature for 1 h with FITC-conjugated anti-mouse antibodies. After three washings in PBS, the cells were resuspended in 200 µl sheath fluid (Becton Dickinson, Mountain View, CA) and analyzed in a FACSscan flow cytometer (Becton Dickinson). Control samples were incubated with nonrelated IgG3 MAb and FITC-conjugated anti-mouse immunoglobulin only. Five thousand cells were analyzed for each sample and gated to include only intact viable cells.

Extraction of FucGmi from Conditioned Media and Sera. Serum samples (700–800 µl) and conditioned media (50 ml) were extracted using isopropanol alcohol:hexane:serum (55:20:20, v/v/v). Control samples consisted of complete media and 10% FCS only. Total lipid extracts were evaporated to dryness under N₂, resuspended in PBS containing 0.1 M KCl, and bound on a Bond-Elute C18 disposable cartridge column (Analytichem International, Harbor City, CA). Columns were washed with PBS and distilled H₂O and eluted with chloroform:methanol (2:1, v/v). The eluate was dried under N₂, dissolved in 30 µl chloroform:methanol (2:1, v/v), and analyzed by HPTLC in a volume of 5 µl.

Extraction from Tumors, Organs, and Cell Lines. Tissues and cell lines were homogenized in and extracted twice with isopropanol alcohol:hexane:water (55:25:20, v/v/v). The extraction and purification were performed as described above.

Table 1 Summary of clinical data and presence or absence of FucGmi in serum samples for patients with histologically verified SCLC

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Blood group (ABO, rhesus)</th>
<th>State of disease</th>
<th>Organ site involvement</th>
<th>FucGmi in serum HPTLC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66</td>
<td>M</td>
<td>O; Rh+</td>
<td>Disseminated</td>
<td>Liver, bone marrow</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>F</td>
<td>O; Rh+</td>
<td>Localized</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>65</td>
<td>M</td>
<td>A; Rh+</td>
<td>Localized</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>57</td>
<td>M</td>
<td>A; Rh−</td>
<td>Disseminated</td>
<td>Liver, bone marrow</td>
<td>(+)</td>
</tr>
<tr>
<td>5</td>
<td>65</td>
<td>M</td>
<td>B; Rh+</td>
<td>Disseminated</td>
<td>Liver, bone marrow</td>
<td>(+)</td>
</tr>
<tr>
<td>6</td>
<td>64</td>
<td>M</td>
<td>A; Rh+</td>
<td>Localized</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>44</td>
<td>M</td>
<td>O; Rh+</td>
<td>Disseminated</td>
<td>Liver, bone marrow</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>65</td>
<td>M</td>
<td>A; Rh−</td>
<td>Disseminated</td>
<td>Liver</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>54</td>
<td>F</td>
<td>A; Rh+</td>
<td>Localized</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>57</td>
<td>F</td>
<td>O; Rh+</td>
<td>Disseminated</td>
<td>Liver, bone marrow</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>44</td>
<td>M</td>
<td>A; Rh+</td>
<td>Localized</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>57</td>
<td>F</td>
<td>O; Rh−</td>
<td>Disseminated</td>
<td>Liver, bone marrow</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>63</td>
<td>M</td>
<td>A; Rh−</td>
<td>Disseminated</td>
<td>Liver, skin-metastases</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>33</td>
<td>F</td>
<td>A; Rh+</td>
<td>Localized</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>15</td>
<td>62</td>
<td>F</td>
<td>B; Rh+</td>
<td>Disseminated</td>
<td>Liver</td>
<td>–</td>
</tr>
<tr>
<td>16</td>
<td>65</td>
<td>M</td>
<td>B; Rh−</td>
<td>Localized</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>17</td>
<td>52</td>
<td>M</td>
<td>O; Rh+</td>
<td>Disseminated</td>
<td>Bone marrow</td>
<td>–</td>
</tr>
<tr>
<td>18</td>
<td>67</td>
<td>M</td>
<td>A; Rh+</td>
<td>Localized</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>19</td>
<td>67</td>
<td>M</td>
<td>B; Rh+</td>
<td>Localized</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>20</td>
<td>57</td>
<td>M</td>
<td>A; Rh+</td>
<td>Localized</td>
<td></td>
<td>–</td>
</tr>
</tbody>
</table>

* Data shown in Fig. 4.
Preparation of Glycolipids. FucGM₁ was purified from bovine thyroids as previously described (19, 20), and fucosyl-asialo-GM₁ (FucGM₃) was prepared from purified FucGM₁ by acid hydrolysis at 100°C. AG₃₄ and AG₄₉ were prepared biosynthetically from FucGM₁ and FucAGA₁ using purified blood group A transferase. G₃₄ ganglioside was isolated from bovine brain, and asialo-GM₁ was prepared by acid hydrolysis of GM₁ ganglioside. Other α₁-2-fucosyl-containing glycolipids were prepared as previously described (21).

HPTLC Immunostaining of Glycolipids. The total ganglioside fraction isolated from tissues and sera (5 μl/30 μl ganglioside extract obtained from 700-800 serum samples) were separated on HPTLC plates (Merck, Darmstadt, Germany) using a chloroform:methanol: H₂O (50:40:10, v/v/v) solvent system. HPTLC plates were briefly air dried and immersed in 0.5% 5-poly(isobutylmethacrylate) beads (Polysciences, Inc., Warrington, PA) in ether for 2 min and blocked at room temperature with 5% bovine serum albumin in PBS for 30 min followed by overnight incubation at 4°C with the MAb TKH5. Rabbit anti-mouse IgG:IgM (Code Z-109; Dako A/S) were exposed at room temperature for 1.5 h at a final concentration of 1:1000, and finally the plates were labeled with ¹²⁵I-protein A (specific activity, 30 mCi/mg total protein A) (Amersham International, Amersham, United Kingdom). X-OMAT AR films (Kodak H 165-1496) were exposed overnight with dried plates.

RESULTS

Characterization of Monoclonal Antibodies. Two hybridomas (TKH5 and 1D7) producing monoclonal antibodies that bound FucGM₁ but not FucG₃₄, or any of a panel of additional standard glycolipids were isolated from two independent fusions. Both of these MAbs were of the IgG3 isotype and were found to be specific for the FucGM₁ relevant structures as shown in Fig. 2. Further attempts to produce MAb of other isotypes (IgG or IgM) against the FucGM₁ antigen were unsuccessful. One additional hybridoma (TKH4) that produced an IgM isotype MAb that bound FucG₃₄ but not FucGM₁ or other standard glycolipids was also generated. The MAb TKH5 specifically labeled 7 of 10 different SCLC cell lines tested but none of 38 other carcinomas of various origins.

Immunofluorescence Staining of Cell Lines. Six different SCLC cell lines were selected for further study. Immunofluorescence staining of these cell lines showed that 3 of the 6 selected SCLC cell lines were positive when stained with either of the 2 different MAb (TKH5 or 1D7). No difference in staining intensity was detected when unfixed cells were compared to cells fixed in 4% formaldehyde or 96% ethanol. Fixation in acetone, however, partially abolished the staining. Neuraminidase pretreatment of the cells did not affect binding. Neuraminidase treatment of purified FucGM₁ glycolipid, even in the presence of detergent (sodium taurocholate), did not result in desialylation to any significant degree, presumably because the sialic acid residue in FucGM₁ is positioned at the internal galactose. Indirect immunofluorescence staining resulted in a homogeneous ring-shaped membrane-associated fluorescence staining pattern. The percentage of immunofluorescence-positive cells varied among different SCLC cell lines as demonstrated by immunofluorescence and the FACS analysis shown in Fig. 3. Ten % of the cells from the GLC-16 and NCI-H69 cell lines were positive for the FucGM₁ antigen. This percentage was much higher in GLC-14 cells, where as many as 80% of the cells were positive. The cell lines NCI-H345, NCI-H510, and T0054A were all negative in immunofluorescence, as were all four non-SCLC cell lines included in this study.

Immunohistochemical Detection of FucGM₁ from SCLC Cell Lines, Tumors, and Serum Samples from SCLC Xenografted Nude Mice. The results from HPTLC immunostaining of cells, supernatants, nude mouse xenograft tumors, and mouse sera are summarized in Table 2. The cell extracts obtained from the immunofluorescence-positive cells cultured in vitro and in vivo were found to be positive for the ganglioside FucGM₁. The ganglioside FucGM₁ was also detected in the conditioned media from 2 of 3 positive cell lines. In addition, in athymic nude mice xenografted with these cell lines, mouse sera were positive in 2 of the 3 cases. The cell line GLC-14 showed similar results in both model systems. This was in contrast to the cell lines GLC-16 and NCI-H69 which were only positive for the antigen in either one of the two model systems. In the case of GLC-16 FucGM₁ was identified in conditioned media but not in xenograft serum. NCI-H69 cells showed the opposite pattern. The reason for this controversy is unknown, but the two cell lines,
IMMUNOCHEMICAL DETECTION OF FucGM1 ANTIGEN IN SERUM

Fig. 3. FACS evaluation of SCLC cells stained for FucGM1. A, the 3 different FucGM1-positive SCLC cell lines (GLC-14, GLC-16, and NCI-H69). First row, increase in fluorescence intensity due to staining with the MAb TKH5; second row, background staining with the secondary antibody (FITC-conjugated goat anti-mouse immunoglobulin) of cells. An 80% increase after specific staining was noted in GLC-14 cells as compared to a 10% increase observed in GLC-16 as well as with NCI-H69 cells. B, corresponding immunofluorescence staining profiles of the 3 different FucGM1-negative SCLC cell lines (NCI-H345, NCI-H510, and TOO54A).

Table 2 Summary of immunochromatographic detection of FucGM1

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Immuno-fluorescence positive cells (%)</th>
<th>HPTLC immunostaining of cell extracts</th>
<th>HPTLC immunostaining of conditioned media</th>
<th>HPTLC immunostaining of xenograft tumor extracts</th>
<th>HPTLC immunostaining of sera xenografted nude mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLC-14</td>
<td>80</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GLC-16</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NCI-H69</td>
<td>10</td>
<td>+</td>
<td>Negative</td>
<td>ND*</td>
<td>ND, Negative</td>
</tr>
<tr>
<td>NCI-H345</td>
<td>0</td>
<td>Negative</td>
<td>ND</td>
<td>ND</td>
<td>ND, Negative</td>
</tr>
<tr>
<td>NCI-H51</td>
<td>0</td>
<td>Negative</td>
<td>ND</td>
<td>ND</td>
<td>ND, Negative</td>
</tr>
<tr>
<td>TOO54A</td>
<td>0</td>
<td>Negative</td>
<td>ND</td>
<td>ND</td>
<td>ND, Negative</td>
</tr>
</tbody>
</table>

* ND, not determined.

Table 2 continued...

GLC-16 and NCI-H69, showed only a small fraction (10%) of positive cells in culture, whereas GLC-14 was more homogeneously positive (80%) (Table 2). The immunoreactive FucGM1 antigen from different sources defined by the MAb TKH5 migrated slightly differently in HPTLC. The mobility of immunoreactive FucGM1 from the established cell lines (in cell extracts or from nude mouse xenograft tumors) migrated as a double band in contrast to a slower-migrating single FucGM1 immunoreactive band, when media or sera were analyzed, presumably due to differences in the ceramide composition (22). The FucGM1 antigen was also detectable by HPTLC immunostaining in total extracts from livers of mice xenografted with the 80% FucGM1-positive GLC-14 cell line suggesting the presence of SCLC metastases in the liver. In contrast to this, tissues from lung, spleen, and mesenteric lymph nodes were found to be consistently negative, similar to the organs from nude mice used as controls.

Detection of FucGM1 in Serum Samples from SCLC Patients. The results of HPTLC immunostaining of 18 of a total of 20 patient serum samples are illustrated in Fig. 4. Fig. 4A illustrates the staining of the FucGM1 ganglioside by the MAb TKH5. Sera from patients 1, 10, 13, and 18 stained positively, and only trace amounts were detected in serum samples from patients 4 and 6. The FucGM1-positive samples were found only for patients with extended disease. Sera from patients with localized disease or other histological types of lung cancer or from 20 healthy individuals were negative for FucGM1. Fig. 4A (Lanes 2 and 3) illustrates the HPTLC immunostaining pattern of the MAbsth TKH4 and MH2 raised against FucGA1 and AGM1, respectively (for structures see Fig. 1). None of the FucGM1-negative sera were positive for these alternative glycosylation variants of the ganglioside core structure. At least one patient serum from an individual with blood group A (patient 18) was strongly positive for the FucGM1 ganglioside but not for the AGM1 glycolipid (MH2) (Fig. 4).

DISCUSSION

Shedding of tumor-associated antigens resulting from aberrant glycosylation may have important diagnostic applications (23, 24). In cultures of melanoma cells, monosialo- and disialo-gangliosides were found to be shed in conditioned media (25–27), and disialogangliosides have been identified in serum samples from patients with malignant melanoma (11–13).

In this study, we have demonstrated that the ganglioside antigen FucGM1 is present in the conditioned media from FucGM1-positive SCLC cell cultures, as well as in tumors and serum samples from nude mice xenografted with the same FucGM1-positive SCLC cells. The ganglioside FucGM1 was also...
detected in total extracts of liver tissues from tumor-bearing mice but not from other organs analyzed. Since no histologically verified metastases could be detected in liver sections from tumor-bearing mice, it is, however, uncertain whether this positive reaction was due to the presence of micrometastases. Interestingly, only the serum samples from mice xenografted with the SCLC cell line expressing high levels of FucGM₁ (GLC-14 cells) or the in vivo propagated cell line (NCI-H69) shed detectable levels of the ganglioside antigen. Although this could be interpreted as if only a few SCLC cells actually shed antigen to serum, the combined findings of antigen shedding in vitro and in vivo suggest that the results obtained more likely are due to limited sensitivity of the HPTLC immunostaining method used for the detection of the FucGM₁ antigen. Using similar analytical techniques, but including extraction of the glycolipid antigen from small amounts of patient serum, 4 serum samples of a total of 20 analyzed were found to be positive for FucGM₁. All FucGM₁-positive serum samples were from patients with large tumor load and extensive disease, and the frequency of positive samples corresponds to a detection rate of 25% in all SCLC patients analyzed with disseminated disease.

Total lipid extracts from SCLC patient sera were also analyzed for alternative glycosylation products which theoretically could be present as a result of different ABO blood group status (28). The blood group A- variant of gangliosides was not detected, although at least one of the patients belonging to blood group A strongly expressed FucGM₁ (patient 18). The MAb MH2 with specificity for the AGM₁ glycolipid, however, had less affinity than the anti-FucGM₁ MAbs. Additional factors such as A₁/A₂ histo-blood group subgrouping could influence these results (28). Interestingly, the asialo derivative of FucGM₁, FucG₁₄, was not detected in any serum samples either. This result could be due to the relative sialidase resistance of the internal sialic acid in the FucGM₁ ganglioside.

The currently most accepted serum markers for SCLC are neuron-specific enolase and the creatine kinase-BB isoenzyme. At the time of diagnosis, elevated concentrations of neuron-specific enolase and the creatine kinase-BB isoenzyme have been detected in 70% of plasma samples from SCLC patients with disseminated disease followed by subsequent changes in plasma levels as a result of chemotherapy responses (29–32). However, both of these serum markers have also been detected in patients with non-SCLC (4, 5). No false-positive reactions for the ganglioside FucGM₁ have been found in our present limited analysis of patients with non-SCLC cancers or in the normal controls using HPTLC immunostaining. In the present study, the lipids were extracted from small volumes and prepared for analysis by HPTLC immunostaining, which implies that a high concentration of antigen was required for detection. This method was chosen here because we explicitly wanted to combine immunological detection with a more direct chemical method to visualize and confirm the nature of the serum glycolipid antigen. The FucGM₁ carbohydrate structure is generally considered not to occur in glycoproteins (28). It is possible that the development of more sensitive immunoassays for FucGM₁, which will require smaller amounts of antigen, will further increase the frequency of FucGM₁-positive sera from patients with SCLC. Attempts to achieve this have thus far been hampered by the poor stability of the present FucGM₁-specific MAbS after further purification.

In this paper we have demonstrated that the SCLC-associated antigen glycolipid FucGM₁ may be detected in sera of patients with SCLC. This finding could have further implications for the classification of lung cancer and the diagnosis of SCLC.

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REFERENCES

IMMUNOCHEMICAL DETECTION OF FucGM_{2} ANTIGEN IN SERUM


Immunochemical Detection of a Small Cell Lung Cancer-associated Ganglioside (FucG\textsubscript{M1}) Antigen in Serum


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