Immunochemical Detection of a Small Cell Lung Cancer-associated Ganglioside (FucGM₁) Antigen in Serum

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ABSTRACT

Recently, the ganglioside FucGM₁ (Fucα1-2Galβ1-3GaINAcβ1-4[NeuAcα2-3]Galβ1-4GlcF1-1Cer) 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 immunochemical 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 serum 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 ganglioside as a potential serum marker of SCLC for which a sensitive immunoassay 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 chemotherapeutic agents. 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 (Fucα1-2Galβ1-3GaINAcβ1-4[NeuAcα2-3]Galβ1-4GlcF1-1Cer) 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 TOOS54A 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 mg), adsorbed on acid-treated Salmonella minnesota (50 mg), 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 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 AG₄₃ ganglioside.*

Purification of Antibodies. Ten-thousand 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 ml Tris (pH 8.0), eluted with 100 ml glycine buffer (pH 3.0), and collected into tubes containing 0.1 ml of 1 M Tris, pH 8.2. The IgG₃ anti-FucGM₁ MAbs (TKH5 and 1D7) were immediately

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2 To whom requests for reprints should be addressed, at Department of Tumor Cell Biology, The Fibiger Institute, Danish Cancer Society, Ndr. Frihavnsgade 70, DK-2100 Copenhagen, Denmark.

3 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, Fucα1-2Galβ1-3GaINAcβ1-4[NeuAcα2-3]Galβ1-4GlcF1-1Cer; FucGA₁, fucosyl-asialo-GM₁.

ceramide: NeuAc, neuraminic acid.

Toms of dehydration and loss of weight, the tumors and organs such as athymic nude BALB/c mice. When the xenografted mice showed symp

GLC-16, NCI-H69, and T0054A were inoculated s.c. into the flanks of experiments.

cell cultures were routinely screened for possible Mycoplasma contam

Transylol; Bayer, Leverkusen, Germany) at a final concentration of

and AGMI. The MAbs TKH5, TKH4, and MH2 with specificity for FucGMi, Fuc-

tra/por, Los Angeles, CA) against PBS (pH 7.4).

collected and dialyzed (molecular weight cutoff, 12,000-14,000; Spec-

cel cultures were supplemented with L-glutamine, penicillin, and strep
grown in modified Eagle's medium containing 10% PCS supplemented

and GLC-16 cells were grown in RPMI 1640 containing 10% FCS.

Ulf, glucose; Gal, galactose. and AGMI. The MAbs TKH5, TKH4, and MH2 with specificity for FucGMi, Fuc-

Fig. 1. Schematic representation of the gangliosides FucGM1, Fuc-asialo-GM1, and AGMI. The MAb TKH5, TKH4, and MH2 with specificity for FucGM1, Fuc-asialo-GM1, and AGMI, respectively, are indicated. Glc, glucose; Gal, galactose, GlcNAc, N-acetylgalactosamine; GalNAc, N-acetylgalactosamine; Fuc, fucose; Cer, ceramide; NeuAc, neuraminic acid.

Table 1 Summary of clinical data and presence or absence of FucGM1 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>FucGM1 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>Disseminated</td>
<td>Liver, bone marrow</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, bone marrow</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>54</td>
<td>M</td>
<td>O; Rh+</td>
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<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>Disseminated</td>
<td>Bone marrow</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>Localized</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>65</td>
<td>M</td>
<td>B; Rh−</td>
<td>Disseminated</td>
<td>Bone marrow</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>52</td>
<td>M</td>
<td>O; Rh+</td>
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<td>-</td>
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<tr>
<td>18</td>
<td>67</td>
<td>M</td>
<td>A; Rh+</td>
<td>Disseminated</td>
<td>Liver, bone marrow</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 (FucAGA1) was prepared from purified FucGM1 by acid hydrolysis at 100°C. AGM1 and AGA1 were prepared biosynthetically from FucGM1 and FucAGA1 using purified blood group A transferase.3 GM1 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 biosynthetically from FucGM1 and FucAGA1 using purified blood group A transferase.4 GMI ganglioside was isolated from bovine brain, and fucosyl-asialo-GM1 (FucGMI) was prepared from purified FucGM1 by acid hydrolysis at 100°C. AGM1 and AGA1 were prepared biosynthetically from FucGM1 and FucAGA1 using purified blood group A transferase.4 GM1 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 μl 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 FucGA1, 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 M Abs of other isotypes (IgG or IgM) against the FucGM1 antigen were unsuccessful. One additional hybridoma (TKH4) that produced an IgM isotype MAb that bound FucGA1 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 M Abs (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.3 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 immunohistochemistry 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.

Fig. 2. Titers of gangliosides tested in enzyme-linked immunosorbent assay with three different antibodies. Binding specificity of the monoclonal antibodies TKH5, 1D7, and TKH4 to purified glycolipids. Abscissa, concentrations of gangliosides FucGM1, FucGA1, and asialo-GM1 starting at 100 ng followed by 2-fold dilutions. Antibodies were used as undiluted culture supernatants from hybridoma cells.

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 FucGM\ gradient antigen in SCLC cells stained for FucGM. A, the 3 different FucGM\-positive SCLC cell lines (GLC-14, GLC-16, and NCI-H69). First row, increase in fluorescence intensity due to staining with MAb TKH5; second row, background staining with 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 NCI-H69 cells. B, corresponding immunofluorescence staining profiles of the 3 different FucGM\-negative SCLC cell lines (NCI-H345, NCI-H510, and TOO54A).

**Table 2: Summary of immunochemical detection of FucGM.**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Immunofluorescent positive cells (%)</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>100</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GLC-16</td>
<td>100</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NCI-H69</td>
<td>10%</td>
<td>++ Negative</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NCI-H345</td>
<td>0</td>
<td>Negative ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>NCI-H51</td>
<td>0</td>
<td>Negative ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>TOO54A</td>
<td>0</td>
<td>Negative ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

**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 FucGM\ is present in the conditioned media from FucGM\-positive SCLC cell cultures, as well as in tumors and serum samples from nude mice xenografted with the same FucGM\-positive SCLC cells. The ganglioside FucGM\ was also detectable by HPTLC immunostaining in total extracts from livers of mice xenografted with the 80% FucGM\-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 FucGM\ 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 FucGM\ 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 FucGM\-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 FucGM\.

Fig. 4A illustrates the HPTLC immunostaining pattern of the MAb TKH4 and MH2 specific for FucGM\, respectively (for structures see Fig. 1). None of the FucGM\-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 FucGM\ ganglioside but not for the AGM\ glycolipid (MH2) (Fig. 4).

**Fig. 4.** HPTLC immunostaining of 18 SCLC patient sera of a total of 20 analyzed, by the use of MAbs TKH5, TKH4, and MH2. Sera (700–800 /ul) were extracted as described in “Materials and Methods,” and the extract was dissolved in 30 /ul chloroform:methanol (2:1, v/v). A 5-ml sample was applied per lane. The solvent system for HPTLC was chloroform:methanol:H2O (50:40:10, v/v/v). A, MAb TKH5 specific for FucGM\; B, MAb TKH4 specific for Fuc-asialo-GM\; C, MH2 specific for AGM\, HGAM, Fuc-GM\, Fuc-asialo-GM\ (standards).
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 vector 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 A\textsubscript{A2} histo-blood group subgrouping could influence these results (28). Interestingly, the asialo derivative of FucGM1, Fuc\textsubscript{a}, 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 chemotherapeutic 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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5. Jaques, G., Bepler, G., Holle, R., Wolf, M., Hannich, T., Crepp, C, and Hannich, T. Characterization of three small cell lung cancer cell lines (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 A\textsubscript{A2} histo-blood group subgrouping could influence these results (28). Interestingly, the asialo derivative of FucGM1, Fuc\textsubscript{a}, 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.

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


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