ABSTRACT

The presence of fucosyl residues linked α1→3(4) to N-acetylglycosamine was demonstrated on the oligosaccharides from glycoproteins of 11 human neuroblastoma tumors from ten different patients. This finding is in complete agreement with the previous report that human neuroblastoma cell lines contained an unusually large proportion of metabolically incorporated l-[^3]Hfucose in this specific linkage (U. V. Santer and M. C. Glick, Cancer Res., 43: 4159-4166, 1983). Furthermore, the glycopeptides derived from the neuroblastoma tumors had a low percentage of fucose-containing biantennary oligosaccharides as determined by affinity to concanavalin A-Sepharose and in this characteristic were similar to glycopeptides from virus transformed and other tumor cells. To obtain these results, the tumor cells were labeled metabolically for 48 h with l-[^3]Hfucose. The cells were harvested and digested with Pronase, and the glycopeptides were isolated and treated with α1→3fucosidase from almond, specific for the release of fucose linked α1→3(4) to N-acetylglycosamine. A portion of the glycopeptides was characterized by serial affinity chromatography on immobilized concanavalin A and lentil lectin. The phenotypic similarity of the tumor cells to the cell lines, particularly CHP-134, included the paucity of biantennary oligosaccharides and the presence of fucosyl residues on the multiantennae of the glycopeptides.

INTRODUCTION

Glycoproteins from human neuroblastoma cell lines contain oligosaccharides with a high percentage of fucosyl residues in α1→3 linkage to an antennary GlcNAc(2→3). Although fucosyl residues have been found in this linkage (Fucα1→3GlcNAc) in isolated glycoproteins (4, 5), including α1→3-acid glycoprotein from liver metastases (6), normal human granulocytes (7) and promyelocytic leukemic cells (8), it is unusual to find the proportion which is present in most of the human neuroblastoma cell lines. Other cell types which were examined, including mouse neuroblastoma and human bladder carcinoma, contained less than 1% of the fucosyl residues on glycoproteins in this linkage (1, 2, 9). A number of antibodies specific for the epitope, Gal β1→4(Fucα1→3)GlcNAc, have been described (10) and tested against a variety of cells (11). Although some of these antibodies react with serum mucins (12) and carcinoembryonic antigen (13), most have been reported to react with glycolipids.

The high proportion of fucosyl residues as branch Fucα1→3GlcNAc on N-linked glycopeptides of human tumor cell lines which are of neural crest origin (2) has been investigated extensively in one of these cell lines, human neuroblastoma, CHP-134. The fucosyl residues were detected by almond fucosidase, specific for Fucα1→3GlcNAc, and it was shown that Fucα1→3GlcNAc was widely distributed among the glycopeptides. NMR analysis of a fraction in which 50% of metabolically incorporated radiofucose was released by almond fucosidase showed that 50% of the fucosyl residues was positioned as Fucα1→3GlcNAc on the antennae of a trimannosyl N-linked glycan, while the other half of the fucosyl residues was linked α1→6 to the asparagine-linked GlcNAc(3). The NMR analysis verified the specificity of the almond fucosidase and showed that the radioactive fucose had the same specific activity regardless of the position on the oligosaccharide.

Fucα1→3GlcNAc on a branch of an N-linked glycan radically alters the configuration of the oligosaccharide residues (14), and molecular recognition by antibodies is highly specific for fucosyl residues in a particular linkage (15). A high percentage of Fucα1→3GlcNAc could thus be expected to affect various phenotypic properties of neuroblastoma cells. These particular fucosyl residues are of importance, however, not only in the perspective of neuroblastoma tumors but also for glycoprotein synthesis. The gene product, N-acetylgalactosaminidase α1→3 fucosyltransferase, potentially responsible for the formation of Fucα1→3GlcNAc on glycoproteins, has been purified from human milk (16) and characterized in some lectin resistant cells (17, 18). An enzyme which catalyzes the formation of this linkage on glycolipids of a human lung cancer cell line has also been characterized (19). Of interest from the biosynthetic viewpoint, the purified milk enzyme also had the activity of α1→4 fucosyltransferase (16). Therefore, in addition to refuting the dogma that each glycosidic linkage is specified by one enzyme, this latter result suggested that the oligosaccharide portion of the glycoprotein may determine the exact fucosyl linkage (16).

Moreover, the enzyme which was found in mouse melanoma (18) and Chinese hamster ovary cells (17), selected for lectin resistance, was not detected in the parent cell lines. In both cases a previously repressed enzyme of glycoprotein biosynthesis seems to be derepressed. Thus the derepression of this enzyme in human neuroblastoma may lead to the presence of Fucα1→3(4)GlcNAc on the tumor cells.

While the occurrence of a high proportion of glycoprotein Fucα1→3GlcNAc is limited to human tumors, a predominance of highly branched oligosaccharides on N-linked glycopeptides is characteristic of all tumor cell lines and is not selective for species, tumor type, or transforming virus. This was originally shown in virus transformed cells (20, 21), was then demonstrated in a wide variety of transformed and tumor cells (22), and most recently demonstrated by the lectin affinity of glycopeptides from mouse cells transformed with oncogenes, N-ras or H-ras (9).

Since such a high proportion of incorporated l-[3]Hfucose occurred as Fucα1→3(4)GlcNAc on the human neuroblastoma cell lines, we investigated (a) whether or not these fucosyl residues were found on the glycoproteins directly from the human neuroblastoma tumors, and (b) whether or not the glycoproteins contained a predominance of highly branched oligosaccharides. We report here the presence of Fucα1→3(4)GlcNAc on all the tumors examined and a more extensive characterization of glycopeptides of the tumor cells from the bone marrow and other sites.

MATERIALS AND METHODS

Materials. All medium and fetal calf serum were from Flow Laboratories, Rockville, MD. β-D-Glucosidase, the starting material for

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1 Supported by NIH Grants CA 14489 and CA 37853.

2 The abbreviations used are: GlcNAc, N-acetylglucosamine; Fuc, fucose (fu
cosyl); Gal, galactose (galactosyl); TLC, thin-layer chromatography; Con A, concanavalin A; NMR, nuclear magnetic resonance; αMMan, α-methyl manno-
pyranoside.

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purification of the almond α-L-fucosidase, was from Worthington Diagnostic Systems, Inc., Freehold, NJ; Pronase was from Calbiochem-Behring, La Jolla, CA; Con A- and lentil lectin-Sepharose and DEAE-Sephael were obtained from Pharmacia, Piscataway, NJ; soybean lectin was from Vector Laboratories, Burlingame, CA; Ficol-Hypaque (Lymphoprep) was from Litton Bionetics, Walkersville, MD; purified goat anti-mouse IgG was from Tago, Inc., Burlingame, CA. L-[5,6-3H]Fucose (60 Ci/mmol) and D-[6-3H]glucosamine (31.3 Ci/mmol) were from New England Nuclear, Boston, MA. L-368 culture supernatant was generously provided by Dr. Lois Lampson.

Radiolabeling and Harvesting of Primary Tumors. All of the solid tumors were obtained from surgery or autopsy within 1 h of removal. In all cases, the tumors were staged (23), and the medical records of the patients were available through Dr. Audrey Evans, Division of Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY. The tumors were minced and dispersed into Corning Primaria (25 cm²) and/or Falcon flasks (25 or 75 cm²). The number of flasks was estimated from the tumor size. The cells were allowed to settle overnight in RPMI 1640 medium containing 15% fetal calf serum and penicillin/streptomycin in an atmosphere of 5% CO₂ at 37°C. After 24 or 48 h the medium was replaced by centrifugation of the cells, and 2.5 or 5 μCi of L-[3H]fucose was added to a 25- or 75-cm² flask, respectively. In a few cases, 5 μCi of D-[6-3H]glucosamine were added to cells from the same tumor in one of the 75-cm² flasks. The cells were then incubated for 48 h and harvested by centrifugation. After one wash the cells were frozen at —40°C until further use. In some cases, the cell pellets were treated with trypsin (0.5 mg/25-cm² flask in 0.02 M Tris-HCl, pH 7.2, containing 0.15 M sodium chloride) for 4 min followed by the addition of an equalizing amount of soybean inhibitor. The material removed by trypsin was further processed as described (24) and designated membrane glycopeptides. The remaining cell pellet was washed once with 0.16 M sodium chloride and frozen at —40°C until further use.

Enrichment of Neuroblastoma from Bone Marrow. Metastatic cells were obtained on 2 occasions, 6 mo apart, from one patient with widespread, Stage IV neuroblastoma (23). The marrow was aspirated from the iliac crests into heparinized syringes. In the first sample, erythrocytes and polymorphs were removed by density centrifugation on Ficol-Hypaque at 500 × g for 45 min. In the second instance, the marrow was first subjected to soybean lectin agglutination by a modification (25) of the method of Reisner (26). The fraction of cells that were agglutinated by the lectin was recovered, washed in 0.2 M D-galactose followed by phosphate-buffered saline, and then subjected to density centrifugation as above. After density centrifugation, the interface cells were harvested and washed 2 times with phosphate-buffered saline. The neuroblastoma cells were agglutinated by soybean lectin (25, 26).

In order to further enrich for metastatic neuroblastoma, normal cells were preferentially removed by a panning procedure (27). The cells were exposed to the supernatant solution from the L368 murine hybridoma, which produces antibody against human β₂-microglobulin. The antibody binds to normal marrow cells but not to neuroblastoma (28). In prior experiments using artificial mixtures of the human neuroblastoma cell line, IMR-5, and normal blood mononucleocytes, the optimal concentration of antibody was determined for the panning procedure. The samples from the marrow were incubated for 60 min in RPMI-1640, supplemented with 10% pooled human serum and the optimal concentration of L368. Unbound L368 was then washed away by 3 cycles of centrifugation and resuspension in medium. The cells were resuspended at 5 × 10⁶/ml of medium and poured into plastic 10-cm dishes which had been precoated with purified anti-mouse IgG in 0.05 M Tris buffer, pH 9.5. After 1 h the nonadherent cells were removed from the dish with a pipet, and a sample was taken aside for microscopic examination. The bulk of the cells was firmly adherent to the plastic. It was difficult to count the neuroblastoma cells in the nonadherent fraction because of the high density of neuroblastoma cells to aggregate. However, as shown in Fig. 1, most of the cells had the morphological characteristics of neuroblastoma cells.

Radiolabeling and Harvest of Neuroblastoma Cell Lines and Fibroblast-like Cells Derived from the Primary Tumors. Human neuroblastoma cell lines, CHP-134 and CHP-382/JK, and clonal cell lines 1 and 4 were grown and harvested as described (1, 29). The cells were labeled with 5 μCi of L-[3H]fucose per 75-cm² flask for 48 h prior to harvest. CHP-382/JK and clonal cell lines 1 and 4 were harvested with trypsin (29), whereas CHP-134 cells were shaken from the flask in 1 ml of 0.16 M sodium chloride. After washing the cells 3 times in 0.16 M sodium chloride, the cell pellet was stored at —40°C until used. Fibroblast-like cells grew from the primary tumor tissue on some occasions. Three of these cell types were labeled metabolically with 5 μCi of L-[3H]fucose per 75-cm² flask for 48 h and harvested by trypsinization as described (30). The material removed by trypsin from these cells and CHP-382 cells was designated membrane glycopeptides and was lyophilized. The membrane glycopeptides and the remaining washed cell pellets were stored at —40°C until used.

Partial Purification of Membrane and Cell Glycopeptides. The metabolically labeled cells and membrane glycopeptides were digested exhaustively with Pronase as described (24) and separated from ions and free [3H]fucose by passage over a column (0.9 × 30 cm) of Biogel P-2 in water. Fractions of 1 ml were collected, and an aliquot was examined...
for radioactivity in a scintillation counter (2). The radioactive material which was eluted with the void volume was lyophilized and used for further characterization.

**Detection of Fucα1→3(4)GlcNAc** from almonds specific for Fucα1→3(4)GlcNAc (31) was as described (2) and was used previously to detect Fucα1→3GlcNAc (2, 3). Two aliquots (approximately 200 cpm each) of the Pronase-digested [3H]-fucose-labeled membrane and/or cell glycopeptides were combined with [3H]fucose-containing glycopeptides from the human neuroblastoma cell line CHP-134 (50 cpm) in 25 ml of 0.1 M citric acid-0.2 M dibasic sodium phosphate buffer, pH 5.5. The fraction of [14C]-glycopeptides was determined by liquid scintillation counting. The release of [3H]-age varied from 5-30% with the different tumors. Among the glycopeptides were obtained from the tumor fragments in sufficient, the cells from cell line CHP-134 and Tumor 7 both period after an initial adjustment period of 24-48 h. For ex-viable, and the cells incorporated L-[3H]fucose over a 48-h rate the membrane material from the rest of the cell, or to do more extensive study of the glycopeptides (Tumors 7 and 8). In the TLC system, the glycopeptides remained at the origin, whereas Fuc migrated 9 cm. The position of Fuc which served as a standard was determined with orcinol. Radioactivity in a scintillation counter (2). The radioactive material which was eluted with the void volume was lyophilized and used for further characterization.

**RESULTS**

**Presence of Fucα1→3(4)GlcNAc.** Glycopeptides from 11 human neuroblastoma tumors obtained from a variety of anatomical sites of 10 patients were examined for the presence of fucosyl residues which were released by almond α-L-fucosidase, specific for Fucα1→3(4)GlcNAc. All of the tumors contained Fucα1→3(4)GlcNAc (Table 1).

The tumor tissue, obtained immediately after excision, was viable, and the cells incorporated L-[3H]fucose over a 48-h period after an initial adjustment period of 24–48 h. For example, the cells from cell line CHP-134 and Tumor 7 both incorporated 2 × 10^6 cpm per 10^6 cells. In all cases radiolabeled glycopeptides were obtained from the tumor fragments in sufficient amount to partially characterize them. In some cases (Tumors 6, 6A, and 11), sufficient cells were available to separate the membrane material from the rest of the cell, or to do more extensive study of the glycopeptides (Tumors 7 and 8). In one other case (Tumor 6) it was possible to passage the cells while they maintained their tumor phenotype.

Approximately 70% of the tumors contained glycopeptides with greater than 10% of the total radioactive Fuc susceptible to release by the almond α-L-fucosidase (Table 1), and only one tumor contained glycopeptides with less than 7%. The percentage varied from 5–30% with the different tumors. Among the tumors examined, there appeared to be no correlation to the percentage of Fucα1→3(4)GlcNAc present and the stage or position of the tumor (Table 1). For example, Tumors 5 and 10, derived from the mediatium, both contained a high proportion of fucosyl residues as Fucα1→3(4)GlcNAc (17 and 27%, respectively), even though Tumor 5 was Stage II and Tumor 10 was Stage IV. Nor was there any correlation to the prognosis of the patients.3 The variation in the percentage of total fucosyl residues susceptible to almond α-L-fucosidase among the individual tumors may partially reflect the mixed populations of cells derived from the tissue. Erythrocytes were the major contaminant, however, and no difference was observed using the same tumor more or less contaminated with erythrocytes (Tumors 6 and 6A; Table 1).

Neuroblastoma cells which were enriched from the bone marrow also had a high percentage of fucosyl residues as Fucα1→3(4)GlcNAc. On 2 occasions with a 6-mo interval between bone marrow aspirates from the same individual, the glycopeptides of the neuroblastoma cells (Tumors 7 and 8; Table 1) had 28 and 19% of fucosyl residues as Fucα1→3(4)GlcNAc. The cell preparations contained about 90% tumor cells (Fig. 1).

In 3 cases (Tumors 1, 2, and 8; Table 1), examination was made of fibroblast-like cells which grew out from the tumor preparations after more than 1 wk. The cells contained 4.4, 2.3, and 4.7% Fucα1→3(4)GlcNAc residues detectable with the almond a-L-fucosidase. The fibroblast-like cells were not cloned. Therefore, at least in the case of fibroblasts from Tumors 1 and 8 (Table 1), the residual tumor cells which were present probably accounted for the Fucα1→3(4)GlcNAc observed.

A cell line, CHP-382/JK (29), was established from Tumor 9 (Table 1). This cell line and 2 clones derived from it contained 7–10% of fucosyl residues as Fucα1→3(4)GlcNAc. Thus, the activity of the α1→3(4) fucosyltransferase was maintained in the cell line, conserving the phenotypic expression of the tumor.

**Binding of the Tumors and Cell Lines to Immobilized Con A and Lentil Lectin.** The binding of glycopeptides to immobilized lectins can be used to give tentative structural information concerning branching and composition of the oligosaccharides (32). Using [3H]fucose-labeled glycopeptides, Con A-Sepharose binding has been used to indicate that the bound oligosaccharides are biantennary (33, 34), whereas those bound to lentil lectin-Sepharose after Con A are triantennary or biantennary. In addition, binding to

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3 A. Evans, personal communication.
both lectins requires N-linked oligosaccharides of a specific core composition (summarized in Ref. 32).

Table 2 presents the data derived after binding of the [3H]-fucose-containing glycopeptides from 11 neuroblastoma tumors to Con A- and lentil lectin-Sepharose in series. The striking pattern which was noted was the small percentage of Con A binding glycopeptides. A similar pattern was also seen with the neuroblastoma cell lines (Table 2). Thus the cell lines maintained another phenotypic expression of the tumor cells.

The paucity of [3H]fucose-labeled biantennary glycopeptides derived from the tumors was similar to those reported for virus transformed and other tumor cells (22). Moreover, the fibroblast-like cells derived from the tumors had a higher proportion of glycopeptides binding to Con A (Table 2), a result similar to that reported for other fibroblasts (1, 2, 30). Less than 30% of the [3H]glycopeptides derived from all but one of the tumors was biantennary, the numbers varying from 12–29%. All of the tumors had proportionally less fucose-containing glycopeptides as biantennary than those of the fibroblast-like cells which contained 43–68% biantennary glycopeptides (Table 2).

Characterization of Glycopeptides from Bone Marrow-derived Neuroblastoma. The [3H]fucose-labeled preparations from the bone marrow which were enriched (Fig. 1) in neuroblastoma (Tumors 7 and 8; Table 1) were digested exhaustively with Pronase, and the small components were removed by passage over Biogel P-2.

The charge distribution of the glycopeptides from both bone marrow preparations as shown by the profile after chromatography on DEAE-Sephalac was similar to that of cell line CHP-134 (Table 3). More than 30–40% of the fucose-labeled glycopeptides contained no or weak charge, whereas approximately 30% was highly charged, since these glycopeptides were eluted from DEAE-Sephalac with 0.2–0.3 M ammonium acetate. The initially charged groups of glycopeptides from the bone marrow neuroblastoma also had a distribution similar to the cell line CHP-134.

The glycopeptides from the bone marrow derived neuroblastoma (Tumor 7) and CHP-134 were collected by charge groups, and each fraction was treated with almond α-L-fucosidase. Consistent with the finding that Fuc-linked α1→3GlcNAc and sialic acid are usually not found on the same branch of a glycopeptide (37), there was a correlation between the decreased percentage of enzyme-releasable Fuc and increased charge of the glycopeptides. This is shown in Table 3, which gives comparative data for the cell line, CHP-134, prepared in the same way as the bone marrow cells. The latter data were consistent with those previously reported for membrane and cellular glycopeptides of CHP-134 treated separately (2). The amount of Fuc released by almond fucosidase in all the charge classes was added and found equivalent to that from the total tumor or CHP-134 cells, 28.7% and 29.9%, respectively.

The binding affinity to immobilized Con A and lentil lectin in series of the fucose-labeled glycopeptides from the bone marrow neuroblastoma (Tumors 7 and 8; Table 2) was also similar to cell line CHP-134 (Table 2). Only 19% and 23% of the glycopeptides from Tumors 7 and 8, respectively, bound to Con A-Sepharose and were eluted with 0.2 M ammonium acetate. Of the [3H]glycopeptides which did not bind to immobilized Con A, 20% bound to lentil-Sepharose and was eluted with 0.2 M ammonium acetate. Similar to glycopeptides from CHP-134, approximately 60% did not bind to these affinity columns. Previous studies with CHP-134 glycopeptides demonstrated that 10% of these unbound glycopeptides bound to lentil-Sepharose after the removal of branch Fuc by the almond α-L-fucosidase (2), and another 20% bound to erythro-agglutinating phytohemagglutinin (3), suggesting the addition of an intersecting GlcNAc.

### Table 2 Binding affinity of glycopeptides from tumors and cell lines to immobilized Con A and lentil lectin

<table>
<thead>
<tr>
<th>Neuroblastoma tumors or cell lines</th>
<th>Con A bound</th>
<th>Lentil bound</th>
<th>Lentil unbound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor 1</td>
<td>29</td>
<td>10</td>
<td>57</td>
</tr>
<tr>
<td>Tumor 2</td>
<td>29</td>
<td>5</td>
<td>62</td>
</tr>
<tr>
<td>Tumor 3</td>
<td>20</td>
<td>22</td>
<td>61</td>
</tr>
<tr>
<td>Tumor 4</td>
<td>17</td>
<td>22</td>
<td>61</td>
</tr>
<tr>
<td>Tumor 5</td>
<td>22</td>
<td>15</td>
<td>58</td>
</tr>
<tr>
<td>Tumor 6</td>
<td>15 and 24</td>
<td>33 and 20</td>
<td>51 and 56*</td>
</tr>
<tr>
<td>Tumor 7</td>
<td>19</td>
<td>20</td>
<td>59</td>
</tr>
<tr>
<td>Tumor 8</td>
<td>23</td>
<td>23</td>
<td>65</td>
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<tr>
<td>Tumor 9</td>
<td>18</td>
<td>19</td>
<td>63</td>
</tr>
<tr>
<td>Tumor 10</td>
<td>12</td>
<td>12</td>
<td>65</td>
</tr>
<tr>
<td>Tumor 11</td>
<td>35 and 27</td>
<td>14 and 7*</td>
<td>50 and 66*</td>
</tr>
</tbody>
</table>

### Table 3 Comparison of glycopeptides from bone marrow neuroblastoma and cell line CHP-134

<table>
<thead>
<tr>
<th>Fraction from DEAE-Sephalac eluting buffer (mm)</th>
<th>% of total in fraction</th>
<th>% released by almond α-L-fucosidase</th>
<th>% of total in fraction</th>
<th>% released by almond α-L-fucosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow radioactivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CHP-134 radioactivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0.5</td>
<td>15</td>
<td>43</td>
<td>26</td>
<td>51</td>
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<tr>
<td>60</td>
<td>17</td>
<td>35</td>
<td>10</td>
<td>24</td>
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<tr>
<td>200</td>
<td>18</td>
<td>37</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>300</td>
<td>19</td>
<td>38</td>
<td>20</td>
<td>32</td>
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</tbody>
</table>

* Membrane and remaining cell fraction, respectively.  
* Membrane fraction.
to the glycopeptides. It remains to be determined if similar binding characteristics will be obtained with bone marrow neuroblastoma.

Radioactive glycopeptides were derived from only 3 x 10⁶ bone marrow neuroblastoma cells, and thus there was not sufficient material to characterize by unequivocal methods. Interpretation of the lectin binding characteristics (32), however, shows that, of the fucose-containing oligosaccharides from the bone marrow neuroblastoma, 20% was biantennary, and another 20% was triantennary or biantennary with an intersecting GlcNAC, since the latter 2 types cannot be distinguished by lentil-Sepharose.

[1H]Fucose and [1H]Glucosamine Incorporation into Glycolipids. Incorporation of L-[1H]fucose into the glycolipids was examined in Tumors 1 and 10. After Pronase digestion, the tumor samples were extracted with chlorormethanol (3:1). Only 1-3% of the incorporated radioactive fucose was recovered in the lipid fraction of either tumor. A duplicate fraction of Tumor 1 gave the same result. In addition, when Tumor 10 was labeled with D-[1H]glucosamine, only 3% of the radioactivity was in the lipid fraction.

DISCUSSION

Glycopeptides from 11 human neuroblastoma tumors obtained from 10 different patients were examined, and all contained fucosyl residues linked α1→3(4)GlcNAC as determined by a specific α-L-fucosidase from almonds (Table 1). The percentage of fucosyl residues recovered as Fucα1→3(4)GlcNAC (5-30%) indicated that the neuroblastomas may vary in the expression of this phenotype. A natural variation among neuroblastoma tumors in the expression of the products of N-acetylglucosaminide α1→3 fucosyltransferase is possible. Indeed, previous examination of a number of human neuroblastoma cell lines established the presence of branch Fucα1→3(4)GlcNAC in all the cell lines, with a 5-fold range in the level of expression (2). Thus a 5- to 6-fold variation in the presence of Fucα1→3(4)GlcNAC residues among the tumors examined (Table 1) is not surprising.

Another natural variation was observed in neuroblastomas in the amplification of N-myc, the oncogene considered characteristic of this tumor (29). The level of N-myc amplification may vary as much as 30-fold between different cell lines (38) and 100-fold in primary neuroblastoma (39). Interestingly, human neuroblastoma CHP-382/JK and clonal cell lines which were shown to contain only one homogeneously staining region with amplified N-myc per cell (29) had only 7-9% of the fucosyl residues as Fucα1→3(4)GlcNAC, whereas CHP-134 cells which contained 3 copies of amplified N-myc (29) contained 30% of these fucosyl residues. The other cell type found to contain these fucosyl residues in unusually large amounts was retinoblastoma (2). Retinoblastoma cell lines and tumors have been reported to contain amplified copies of N-myc (40). It is thus a working hypothesis that the activity of N-acetylglucosaminide α1→3 fucosyltransferase may be related to the amplification of this oncogene in these human tumors. An exception to this hypothesis is the fact that the cell line SK-N-SH contained these fucosyl residues (2) even though it is one of the few human neuroblastomas which does not contain an amplified N-myc (38). SK-N-SH contains a ras-related oncogene, N-ras (41). Moreover, amplified copies of N-myc have not been observed thus far in Stage IVS tumors (42).

The presence of an active α1→3 fucosyltransferase in neuroblastoma may be responsible for the formation of fucosyl residues as Fucα1→3GlcNAC*. This enzyme may be derepressed in the tumor cells, since in the surrounding cellular population, represented by the fibroblast-like cells, the resultant products of the enzyme were not as pronounced as in the tumor cells. Derepression of this enzyme has been reported previously in certain lectin resistant mutants (17, 18) and, in the latter case, correlated with mutants which were less metastatic (18, 43).

As reported previously, cells transfected with human tumor DNA (9), virus transformed cells (21), and tumor cells (22) contained less biantennary or Con A-Sepharose binding glycopeptides than their normal counterparts. The binding to Con A-Sepharose of the [1H]fucose-labeled glycopeptides from the neuroblastoma tumors was similar to those from cell lines CHP-134 and CHP-382 (Table 2). Thus, the tumor cells showed a paucity of glycopeptides bound to Con A-Sepharose. One striking example, Tumor 9, contained 18% glycopeptides which bound to Con A (Table 2), and the subsequently isolated cell line CHP-382/JK and clones thereof contained similar percentages of Con A-Sepharose bound glycopeptides (Table 2). Again, a phenotypic expression of the tumor glycopeptides was conserved by the cell lines.

The similarity between the glycopeptides from bone marrow derived human neuroblastoma cells (Tumors 7 and 8; Table 1) and those of the human neuroblastoma cell line CHP-134 was remarkable. Both contained a high percentage of the fucosyl residues susceptible to almond α-L-fucosidase. The binding characteristics to immobilized Con A and lentil (Table 2) indicated a complement of bi- and triantennary glycopeptides which were similar. The charge characteristics were also similar (Table 3). Furthermore, the percentage in each charge class of fucosyl residues susceptible to release by almond α-L-fucosidase was similar.

We conclude that a number of properties of glycoproteins expressed by human neuroblastoma tumor cells are maintained in the cultured tumor cell lines. These include the presence of Fucα1→3(4)GlcNAC on the glycopeptides and a paucity of biantennary oligosaccharides on the glycoproteins. These characteristics of the glycopeptides can be directly exploited to produce monoclonal antibodies to neuroblastoma and perhaps other human tumors. The phenotypic similarity to the human neuroblastoma cell line, CHP-134, makes this a highly feasible project.

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REFERENCES

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