Glycoconjugate Journal (1995) **12**: 848–856

The expression of CMP-NeuAc: Galβ1,4GlcNAc α2,6 sialyltransferase [EC 2.4.99.1] and glycoproteins bearing α2,6-linked sialic acids in human brain tumours

HIROTAKA YAMAMOTO, YOICHI KANEKO, DAVID VANDERMULEN, DONNA KERSEY, EDWARD MKRDICHIAN, LEONARD CERULLO, JAN LEESTMA and JOSEPH R. MOSKAL*

The Chicago Institute of Neurosurgery and Neuroresearch, 2515 N. Clark St., Chicago, IL 60614, USA

Received 26 April 1995, revised 9 June 1995

The expression of CMP-NeuAc: Galβ1,4GlcNAc α2,6 sialyltransferase (α2,6-ST) [EC 2.4.99.1] and glycoproteins bearing α2,6-linked sialic acids were examined in primary human brain tumours and cell lines. 79% (19/24) of the meningiomas expressed α2,6-ST mRNA, 42% (10/24) of which showed very high expression. α2,6-ST mRNA expression was undetectable in normal brain tissue. In contrast, only 1/13 of the gliomas examined expressed detectable α2,6-ST mRNA. Metastases to the brain did not express measurable amounts of α2,6-ST mRNA. Less expression was found in malignant (i.e. anaplastic) compared to benign (i.e. meningothelial) meningiomas. Two-dimensional SDS-PAGE of glioma and meningioma proteins, followed by *Sambucus nigra* lectin staining, revealed the presence of a glycoprotein bearing α2,6-linked sialic acids, M_r = 53 kDa and a pI = 7.0 (MEN-1) that appeared in all seven of the meningiomas examined, but was expressed at barely detectable levels, if at all, in seven out of the seven glioblastomas examined. Thus, decreased α2,6-ST expression may play a role in the aggressive nature of anaplastic meningiomas, but appears to be virtually absent in all tumours of glial origin.

Keywords: glioblastoma, meningioma, sialyltransferase, *Sambucus nigra* lectin, α2,6-linked sialic acids

Introduction

CMP-NeuAc: Galβ1,4GlcNAc α2,6 sialyltransferase (α2,6-ST) [EC 2.4.99.1] transfers sialic acid to the non-reducing termini of sugar chains of N-linked glycoproteins [1, 2] and is a type II transmembrane protein which exists predominantly within the Golgi and trans-Golgi network [3, 4]. Although it was originally purified from bovine colostrum by Paulson *et al.* [5], the purified form isolated from rat liver [6] has been more extensively studied. The rat α2,6-ST gene has been cloned by Weinstein *et al.* [7] and shown to be quite complex, producing as many as five transcripts with distinct tissue specificity [8, 9, 33]. Liver expresses a 4.3 kb α2,6-ST mRNA and expresses the highest enzyme activity of any tissues tested. Tissues such as brain, ovary, and heart express only a 4.7 kb transcript and typically have >50-fold less enzyme activity than liver. In all tissues examined, mRNA levels correlated with enzyme activity [11].

It appears that α2,6-ST plays a key role in regulating the expression of cell-surface α2,6-linked sialoglycoconjugates which, like sialic acid-bearing glycoconjugates in general, may play a role in the regulation of cellular differentiation [12–16]. For example, α2,6-ST expression is a key step in the formation of multiple leukocyte cell-surface differentiation antigens [12], α2,6-ST activity can be induced in cultured human epithelial cells by the inflammation-related cytokines, tumour necrosis factor-α and interleukin-1 [13], and CD22-mediated intercellular adhesion can be regulated by the addition of α2,6-linked sialic acid [14].

α2,6-ST and glycoconjugates bearing α2,6-linked sialic acids also have been implicated in neoplasia and metastasis. α2,6-linked sialoglycoconjugate expression, as

*To whom correspondence should be addressed.

measured by *Sambucus nigra* agglutinin lectin binding, has been reported to be associated with the malignant transformation of human colonic mucosa [17]. Several-fold increases in α2,6-linked sialoglycoconjugate expression have been observed in metastatic colon carcinoma cell lines compared with their poorly metastatic parent. Upon neuraminidase treatment, the metastatic behaviour of these cells was reduced when injected into syngeneic mice [18]. Treatment of the human carcinoma cell line, Hep G2, with n-butyrate induced morphological differentiation and reduced α2,6-ST mRNA expression by greater than 90% [19, 20].

Studies on the role that cell-surface glycoconjugates play in brain tumorigenesis have focused primarily on ganglioside expression and regulation. Brain tumours have been reported, in general, to express elevations in GM3, GD3, and GD2, with concomitant reductions in the more complex polysialo-gangliosides [21–24]. Interestingly, 3′,6′-isoLD1, a ganglioside with a sialic acid linked α2,6 to an internal *N*-acetylgalactosamine, has been reported to be expressed in the human central nervous system during both gliagenesis and reactive astrocytosis, and in primary tumours [24].

The studies reported here addressed three questions: (1) Is α2,6-ST expressed in brain tumours? (2) How does α2,6-ST expression in brain tumours relate to its expression in normal brain? (3) Are there any glycoconjugates bearing α2,6-linked sialic acids biosynthesized by brain tumours that might have clinical significance based on their pattern of expression or uniqueness to tumours?

Materials and methods

Cell culture

All established cell lines were maintained using Dulbecco's modified Eagle's medium (DMEM, containing 4.5 $g\,l^{-1}$ glucose) supplemented with 10% heat-inactivated fetal bovine serum (Whittaker BioProducts, Walkersville, MD): human glioblastoma, SNB-19 (generously provided by Dr Paul Kornblith, Department of Neurosurgery, University of Pittsburgh); human glioblastoma, U-87 MG (American Type Culture Collection [ATCC], Rockville, MD); human hepatocarcinoma, Hep G2 (ATCC); human epidermoid carcinoma, A431 (ATCC); human leukaemia, HL60 (ATCC); human neuroblastoma, IMR32 (ATCC). AlS and A5I are subclones of A431 cells that are growth stimulated by epidermal growth factor (EGF) and growth inhibited by EGF, respectively. Upon reaching 50% confluency, SNB-19 cells were treated with one of the following compounds for 24 h: 10 μM retinoic acid (Sigma Chemical Co., St Louis, MO), 1 mM dibutyryl cyclic-AMP (Boehringer Mannheim, Indianapolis, IN), or 3 mM sodium butyrate (Sigma Chemical Co., St Louis, MO). Controls were cells grown after addition of solvent alone (dimethyl sulfoxide or ethanol [Sigma Chemical Co., St Louis, MO]; final concentration in percent of total volume was 0.01%).

Northern analysis of α2,6-ST mRNA expression in human brain tumours

Human α2,6-sialyltransferase (α2,6-ST; EC 2.4.99.1) cDNA was cloned by using the reverse-transcriptase polymerase chain reaction (RT-PCR) and poly A + RNA from Hep G2 hepatocarcinoma cells. A sense primer, CTTCTGTTTGCAGTCATCTG (bp 489–508) and an antisense primer, AAATCTGTGACCTCTACCAT (bp 910–891) were used [26]. A 422 bp PCR product (bp 489–910) was cloned into pT7 Blue T vector (Novagen, Madison, WI) and the sequence of the insert was confirmed by the dideoxy termination method. The cDNA coding for human α2,6-ST cDNA was isolated from the gel after *Xba* I and *Bam* HI digestion of the vector and used as the template. Total RNA was isolated from clinical brain tumour specimens and cell cultures using guanidium isothiocyanate [27] followed by $CsCl_2$ centrifugation [28]. Thirty μg of total RNA per primary brain tumour and 30 μg of total RNA per tumour cell line per lane was electrophoresed in a formaldehyde containing agarose gel and transferred to Duralon nylon membranes (Stratagene, La Jolla, CA). After UV cross-linking, blots were hybridized with a ^{32}P-radiolabelled cDNA probe synthesized by using a random priming kit (Boehringer-Mannheim, Indianapolis, IN) and QuikHyb solution (Stratagene, La Jolla, CA). After washing at 60 °C, the blots were exposed to X-OMAT film (Kodak, Rochester, NY) and the films developed appropriately. The same filters were also hybridized with the 1.4 kb cDNA coding for α-tubulin to examine the integrity of RNA samples from clinical specimens.

Southern analysis of α2,6-ST in brain tumours

Twenty μg genomic DNA, isolated from each primary brain tumour specimen, was digested by *Eco* R1, electrophoresed in a 0.8% agarose gel and transferred to a Duralon nylon membrane (Stratagene, La Jolla, CA). After UV cross-linking, blots were hybridized with radiolabelled probe synthesized using a random priming kit (Boehringer Mannheim, Indianapolis, IN). The 0.4 kb insert of the human α2,ST cDNA was used as the template (as described above).

Immunohistochemistry and lectin histochemistry

Immunohistochemical analysis was performed with a polyclonal affinity purified antibody directed against rat α2,6-ST (supplied by Dr Karen Colley, Department of Biochemistry, University of Illinois School of Medicine) using the streptavidin-biotin complex (ABC) method. Streptavidin and biotin reagents were purchased from Amersham (UK) and used at 1:200 dilution. The speci-

mens were immediately fixed in buffered 10% formaldehyde, and sectioned at 6 μm. Deparaffinized sections were incubated in 0.3% H_2O_2 for 30 min in methanol to inhibit the endogenous peroxidase activity. The sections were subsequently incubated with primary antibody (1 μg ml^{-1}) diluted in 0.05 M Tris-HCl, pH 7.6, containing 10% normal goat serum at 4 °C overnight, biotin-labelled anti-rabbit Ig for 30 min at room temperature, and streptavidin-biotinylated peroxidase complex for 30 min. Each step was followed by three 10 min washes in 0.05 M Tris-HCl, pH 7.6. The peroxidase reaction was developed with 0.01% 3.3′-diaminobenzidine (Sigma, St Louis, MO) and 0.003% H_2O_2 in 0.05 M Tris-HCl, pH 7.6.

Preparation of tissue extracts for 2-dimensional SDS-polyacrylamide gel electrophoresis

Primary brain tumour tissues were placed immediately into liquid nitrogen upon resection. Cells from tissue culture experiments were harvested by washing three times with phosphate-buffered saline (PBS), followed by scraping with a rubber policeman and pelleting by centrifugation (800 × **g**, 5 min, 4 °C), and were frozen at − 80 °C until use. Upon thawing, the pellets (or tumour specimens) were washed in PBS, and homogenized in solubilization buffer using a 1 cm diameter Teflon-glass homogenizer on ice. Solubilization buffer contained 9.5 M urea, 2% NP-40, 2% pH 9-11 ampholytes (Sigma Chemical Co., St Louis, MO), 5% 2-mercaptoethanol (Sigma Chemical Co., St Louis, MO), 0.2 mM phenylmethylsulfonyl fluoride (Boehringer Mannheim, Indianapolis, IN) and 2 μg ml^{-1} pepstatin A (Sigma Chemical Co., St Louis, MO).

Two-dimensional SDS-PAGE of proteins

The total protein homogenates were centrifuged at 10500 × **g** at 4 °C and the protein concentrations of the supernatants were determined using a modification of the Bradford method [29]. Sixty-five μg of total cellular protein from each tissue was applied to each gel. Electrophoresis of the protein extracts was performed in two-dimensions according to standard procedures [30], with minor modifications, using an SE250 mini-gel system (Hoefer Scientific Instruments, San Francisco, CA) in conjunction with a Brinkman/Lauda RM6 circulating water bath and EPS 500/400 power supply (Pharmacia, Piscataway, NJ). The pH gradient in the first dimension, from approximately pH 5 to 8, was established in 1.5 mm diameter tube gels using a combination of ampholytes. The actual gradient was verified directly by cutting prefocused IEF tube gels (6 cm length) into 2.5 mm segments (in triplicate), incubating in distilled, deionized water and measuring the pH with a small bore combination electrode. The second dimension was run on 8% acrylamide slab gels (7 × 9 cm) with a 5% acrylamide stacking gel. By running biotinylated SDS-PAGE molecular weight (MW) standards (BioRad Laboratories, Richmond, CA) in a separate lane, essentially linear separation was demonstrated in the 30–200 kDa range.

Lectin blot analysis of glycoproteins

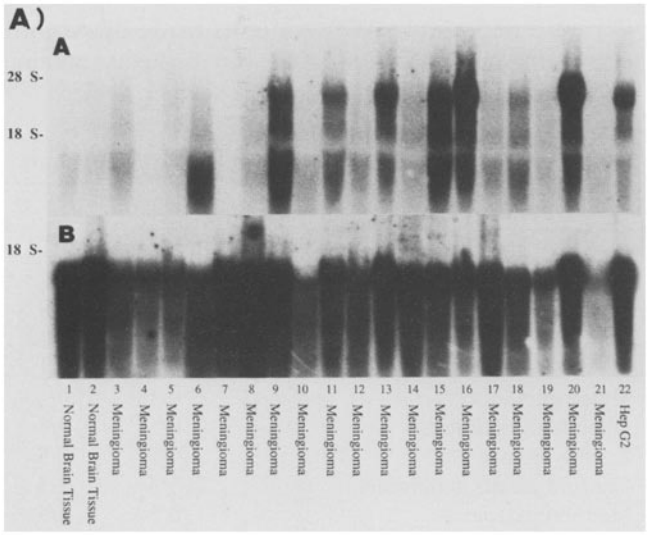
After separation, proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore Corp., Bedford, MA; [31]) by semi-dry electroblotting at ca. 1 mA cm^{-2} for 40 min with a Pharmacia Model 2117-250 Novablot unit. "Protein Blotting Protocols for the Immobilon-P Transfer Membrane" (Millipore Corp. publication) were followed as adapted from published procedures [32]. After washing with distilled, deionized water, some proteins could be visualized with the reversible, general protein stain, Ponceau S (Sigma Chemical Co., St Louis, MO), which was washed out with the blocking buffer used in subsequent lectin staining procedures. Specific oligosaccharide moieties on the isolated, separated, and immobilized glycoproteins were identified with biotinylated lectins. PVDF membranes were washed with distilled water and incubated in 50 mM Tris buffered saline (TBS), pH 7.5, containing 0.1% Tween-20 for 1 h to block non-specific binding. The membranes were then incubated with 7 ml of TBS containing 10 μg ml^{-1} of biotinylated *Sambucus nigra* agglutinin (SNA lectin; Vector Laboratories, Burlingame, CA) for 30 min at room temperature. Glycoproteins recognized by SNA were identified using Vectastain ABC kits (avidin DH: biotinylated horseradish peroxidase H enzyme, Vector Laboratories, Burlingame, CA) and diaminobenzidine tetrahydrochloride (DAB)-nickel for visualization. Spot quantitation was performed using a Xerox Datacopy Scanner (model 730GS) connected to a Macintosh IIfx with MacImage 2.2 and Image 1.41 software (software was obtained independently from Wayne Rasband, NIMH, Building 36, Room 2A03, Bethesda, MD 20892). In order to estimate the linear range of this detection system and verify the use of integrated density values for quantitation of protein levels reflected in the various 'spots', a 'control' test plot was prepared using measured amounts of the glycoprotein standard BSA-fucosylamide (Sigma) and *Ulex Europeus* I lectin (UEA-1; Vector Laboratories, Burlingame, CA); none of the values used for data analyses of lectin blots of glycoproteins from treated or untreated cells exceeded this range.

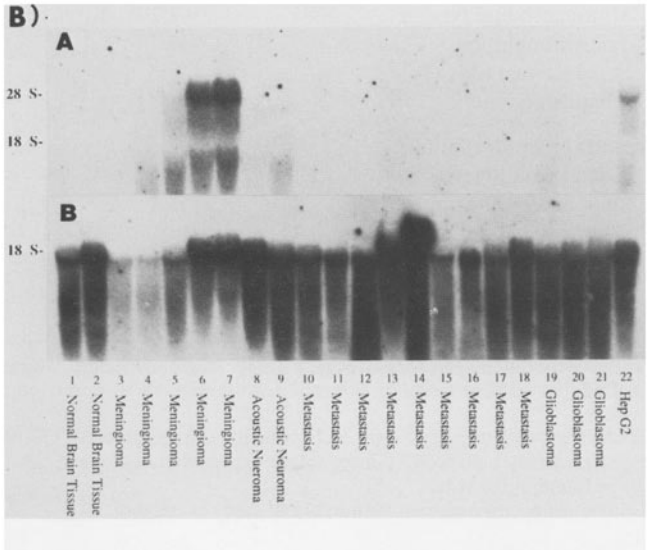
Results

We studied the expression of α2,6-ST by Northern analysis and by immunohistochemical analysis using an affinity purified α2,6-ST-specific polyclonal antibody. We also examined the expression of glycoconjugates

bearing α2,6-linked sialic acids using Western blotting with SNA lectin. The human hepatocarcinoma cell line, Hep G2 was used as a positive control in the Northern analyses [33].

The results of Northern analyses of primary brain tumours are shown in Fig. 1A–C. Total RNA was isolated from 56 fresh surgical specimens (24 meningiomas, 13 gliomas, 13 metastatic brain tumours, two acoustic neuromas, one pituitary adenoma, one oligodendrocytoma, and two normal brain tissue samples). Seventy-nine percent (19/24) of the meningiomas expressed α2,6-ST mRNA, 42% (10/24) of which showed very high expression. α2,6-ST mRNA expression was undetectable in normal brain tissue. In contrast, only 1/13 of the gliomas examined expressed detectable α2,6-ST mRNA. Metastases to the brain (in contrast to metastatic colon carcinomas [17, 18]) did not express measurable amounts of α2,6-ST mRNA. In all, thirteen metastatic brain tumours were examined. They arose from small cell lung carcinomas, uterine adenocarcinomas, clear cell carcinomas from the kidney, squamous cell carcinoma of unknown origin, and an adenocarcinoma of unknown origin. The brain tumour specimens used in these studies all expressed only the 4.7 kb transcript, which was expected based on the results of Wen *et al.* [9] and Paulson *et al.* [11]. Figure 2 depicts a Northern blot of a variety of tumour cell lines. SNB-19 and U87MG are established human glioblastoma cell lines, and like primary gliomas, contained no detectable α2,6-ST mRNA. SNB-19 cells could not be induced to express α2,6-ST mRNA by manipulating cell density, or by treatment with dibutyryl-cyclic AMP or retinoic acid. Lanes 13–15 contain RNA obtained from meningioma tumour tissues grown in primary culture. Three out of four were clearly positive, suggesting that α2,6-ST mRNA, at least in some cases, continues to be expressed in tissue culture. Interestingly, the human neuroblastoma cell line IMR-32 expresses substantial α2,6-ST mRNA





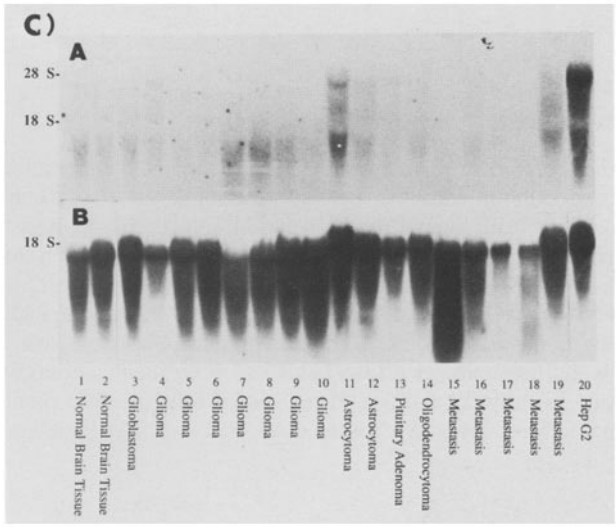


Figure 1A-C. Northern analysis of α2,6-ST mRNA expression in human brain tumours. Panel A: Brain tumour RNA probed with a cDNA coding for the human α2,6-ST as described in the Methods. Negligible α2,6-ST mRNA expression was observed in normal human brain. Meningiomas, but not glioblastomas, express α2,6-ST mRNA. A single transcript corresponding to 4.7 kb was seen in all α2,6-ST mRNA-expressing tumours. Hep G2, a human hepatocarcinoma cell line, was used as a positive control [37]. Panel B: The same blot probed with α-tubulin cDNA.

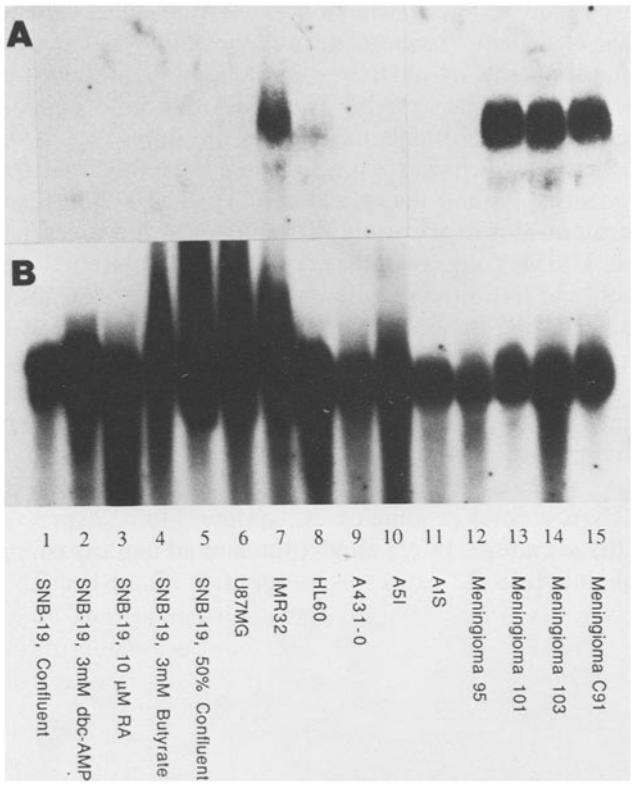


Figure 2. Northern analysis of α2,6-ST mRNA expression in human tumour cultures. Details describing the tissue culture conditions, cell lines, RNA isolation, Northern analysis are described in the text. The human, tumorigenic, glioblastoma cell line SNB-19, could not be induced to express α2,6-ST mRNA by retinoic acid, dibutyryl-cyclic AMP, butyric acid, or changes in cell density. The human neuroblastoma cell line IMR-32, does express α2,6-ST mRNA. Four meningiomas were placed in primary culture. Three out of four of these meningioma cultures robustly expressed α2,6-ST mRNA. Panels as in Fig. 1.

(lane 7), however no primary neuroblastomas were included in these studies so it remains unclear whether these tumours express α2,6-ST mRNA *in vivo*. HL-60 cells (lane 8) expressed α2,6-ST mRNA as expected since leukaemia cell lines have been reported to express α2,6-ST activity [10, 33]. Glioblastomas have been reported to overexpress a truncated form of epidermal growth factor receptor which may play a significant role in regulation of their growth in an autocrine fashion [49, 50]. The human epidermoid carcinoma cell line, A431-0, has been a useful model to study the structure and function of epidermal growth factor receptors [51]. Two subclones of this cell line were used in these studies: (1) A5I, which are growth inhibited by epidermal growth factor; and (2) A1S, which are growth stimulated by EGF [52]. It can be seen in lanes 9–11 that no α2,6-ST mRNA expression was detected in these cell lines under the conditions described in the Methods.

Northern analyses of clinical specimens are useful to evaluate mRNA expression, but cell-type expression cannot be examined since brain tumours can contain cells of mixed morphology undergoing cell division at different rates as well as non-tumour material such as blood cells and vascular endothelial cells. Consequently, we used an affinity purified, monospecific antibody directed against α2,6-ST. As can be seen in Table 1, these studies closely paralleled the results obtained by Northern analysis: glioblastomas were found to be effectively negative, with only one showing faint positive staining, and no metastatic brain tumours stained with anti-α2,6-ST. Among the different meningioma sub-types, benign meningothelial and transitional meningiomas stained strongly with the anti-α2,6-ST antibody, while aggressive anaplastic meningiomas were negative. These results suggest the existence of meningioma-associated glycoproteins with α2,6-linked sialic acids. Consequently, Western blots of glioma and meningioma proteins were performed using SNA lectin which recognizes α2,6-linked sialic acid residues.

Figure 3 depicts two Western blots made by performing two-dimensional SDS-PAGE on proteins obtained

Table 1. Histochemical examination of human brain tumours

Tumour Type	*α2,6-ST*[a]	*SNA*[b]
Gliomas		
Glioblastomas	0/8[c]	0/8
Anaplastic astrocytomas	0/8	0/8
Fibrillary astrocytomas	0/8	0/8
Pilocytic astrocytoma	0/1	0/1
Mixed glioma	0/1	0/1
Oligodendrogliomas	0/5	0/5
Ependymomas	0/5	0/5
Choroid plexus neoplasms	3/3	3/3
Medulloblastomas	0/5	0/5
Meningiomas		
Meningothelial type	9/10	9/10
Transitional type	4/7	4/7
Fibroblastic type	0/3	0/3
Anaplastic type	1/2	0/2
Metastatic brain tumours		
Adenocarcinomas	0/4	0/4
Squamous cell carcinoma	1/1	1/1
Clear cell carcinoma	0/1	0/1
Mixed mesodermal tumour	0/1	0/1
Adenosquamous carcinoma	1/1	1/1
Poorly differentiated-malignant neoplasm	0/1	0/1

[a]These experiments were performed using a monospecific, affinity purified, polycolonal antibody directed against α2,6-ST as described in the text.
[b]SNA refers to *Sambuscus nigra* lectin which has been reported [39, 40] to preferentially bind to α2,6-linked sialic acids. Methodological details can be found in the text.
[c]Number of positive cases/total number of cases examined.

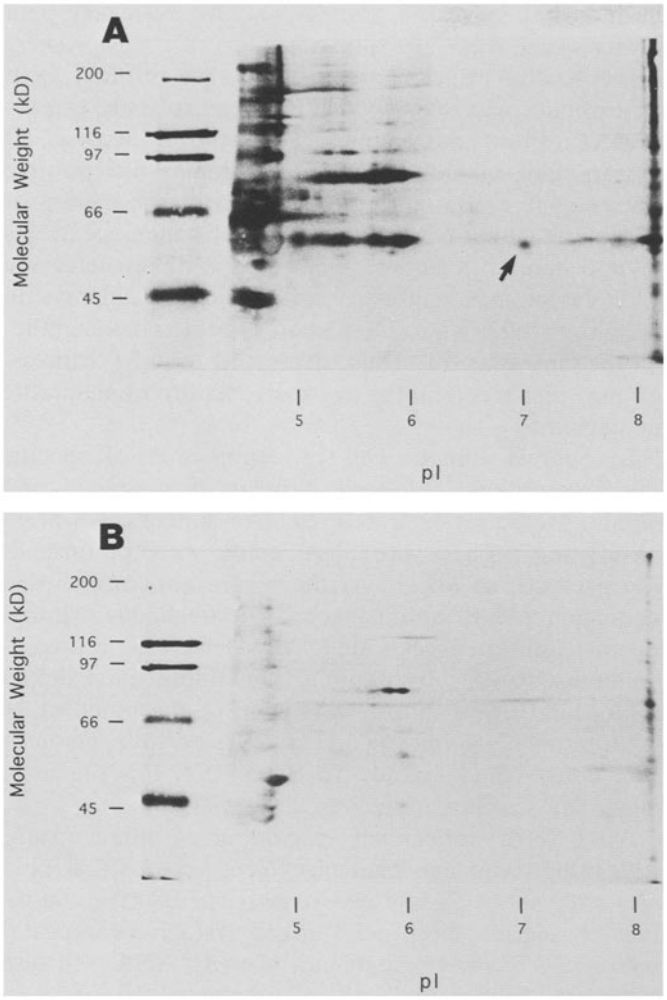


Figure 3. Western blots of meningioma and glioblastoma proteins separated by two-dimensional SDS-PAGE and probed with SNA lectin. The details of the Western blotting conditions are contained in the Methods. It can be seen in (A) that the meningioma blot displayed abundant SNA-positive spots compared to the glioblastoma blot (B). Most of the staining occurred in the acidic region of the blots, most likely due to glycoproteins containing oligosaccharide chains bearing sialic acids. Since neither α2,6-ST mRNA expression nor anti-α2,6-ST antibody staining was observed in glioblastomas, the weakly stained, SNA-positive, spots may reflect the presence of O-linked glycoproteins containing sialic acids with α2,6 linkages or α2,3-linked sialoglycoproteins. SNA staining of the meningioma Western blot revealed the presence of a M_r = 53 kDa, pI ~7.0 spot (MEN-1) not found in gliomas (arrow).

from (Fig. 3A) one representative primary meningioma and (Fig. 3B) one representative primary glioblastoma specimen. Each sample contained an identical amount of protein. Thus, the comparatively low staining observed in Fig. 3B is not due to less protein applied to the gels, but reflects less SNA-positive protein expression. In general, meningiomas reacted much more strongly with SNA lectin than did the gliomas examined. Typically there were more spots in the meningioma blots and they were different with respect to M_r and pI than the glioma blots, with most of the staining occurring in the acidic region of the blots, most likely due to glycoproteins containing oligosaccharide chains bearing sialic acids. In Fig. 3A, the arrow points out one of the meningioma-associated, SNA-positive spots with an M_r = 53 kDa and a pI = 7.0 (MEN-1) that appeared in all seven of the meningiomas examined, but was expressed at barely detectable levels, if at all, in seven out of the seven glioblastomas examined. A summary of these results is shown in Fig. 4.

Because we found much more abundant expression of α2,6-ST mRNA in some of the meningiomas compared to the Hep G2 positive control, Southern analyses were performed to examine whether this overexpression was due to chromosomal abnormalities in meningiomas [36]. Using a 0.4 kb cDNA fragment obtained from the coding region of *α2,6-ST* gene (as described in the Methods), it can be seen in Fig. 5 that all of the samples tested gave the same band. Thus, it does not appear that the overexpression of α2,6-ST mRNA in some meningiomas is likely due to alterations of the *α2,6-ST* gene.

Discussion

α2,6-ST activity and the expression of glycoconjugates with α2,6-linked sialic acids by tumour cells has been linked to metastatic potential in colon carcinomas [17–20]. In addition, α2,6-ST appears to regulate the

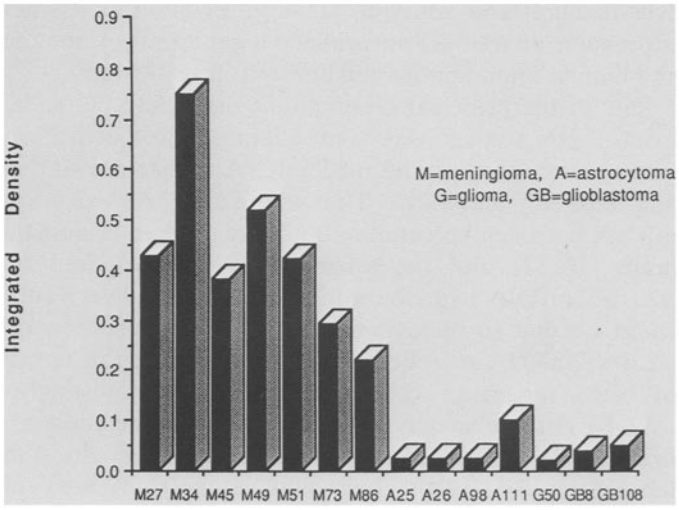


Figure 4. The expression of an α2,6-linked, sialic acid bearing, M_r = 53 kDa, pI 7.0 glycoprotein (MEN-1) by Western blotting of primary brain tumour tissues. This figure summarizes a series of Western blot analyses of primary brain tumour tissues with SNA lectin. Details of the Western blotting techniques are described in the Methods. MEN-1 was expressed in all seven meningioma tumours examined but was, at best, negligible in gliomas.

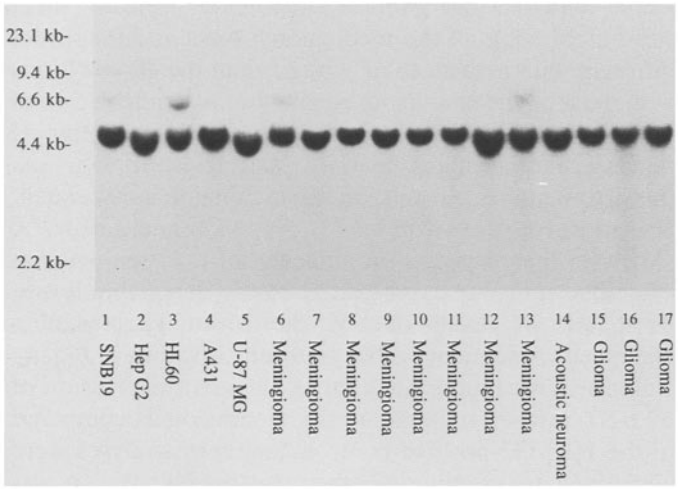


Figure 5. Southern analysis of β-galactoside α2,6-sialyltransferase in brain tumours. Twenty μg of genomic DNA, isolated from each primary brain tumour specimen and cell line, was digested by *Eco* R1 and electrophoresed in a 0.8% agarose gel and transferred to a Duralon nylon membrane (Stratagene, La Jolla, CA). After UV cross-linking, blots were hybridized and radiolabelled cDNA probe synthesized using a random priming kit (Boehringer Mannheim, Indianapolis, IN). The 0.4 kb cDNA of the human α2,6-ST was used as template (as described above). Under the conditions employed, no apparent alterations in the coding region of the *α2,6-ST* gene could be detected.

expression of cell-surface antigens necessary for lymphocyte function and adhesion [12–14]. In this report, the expression of α2,6-ST in primary human brain tumours and human brain tumour cell lines was investigated.

One of the principal observations that could be made from these studies was that gliomas, irrespective of grade, neither express α2,6-ST mRNA nor stain with the anti-α2,6-ST antibody. The expression of α2,6-ST mRNA has been reported in both fetal and adult human brain [36, 37], but the levels were low compared to α2,6-ST mRNA expression in such tissues as liver. This could be due to an overall low level of expression of α2,6-ST mRNA or reflect expression restricted in terms of brain region or cell type, or both. Interestingly, α2,6-ST enzyme activity was undetectable in the adult rat brain [37]. Immunohistochemical experiments in our laboratory [38] have shown that, at best, α2,6-ST is weakly expressed in some subsets of adult human and rat neurons. Glial staining was not observed in either species. Thus, it seems likely that normal human glial cells do not express appreciable amounts of α2,6-ST. Moreover, the transformation of astrocytes into gliomas appears to have no effect on α2,6-ST induction either. Unlike gliomas, the human neuroblastoma cell line IMR-32 does express α2,6-ST mRNA, suggesting that the induction of neural tumours may be associated with an increase in α2,6-ST expression.

The second principal observation that resulted from these studies was that meningiomas can robustly express α2,6-ST. From the immunohistochemical analysis, it appears that meningothelial meningiomas are positive whereas fibroblastic and aggressive anaplastic meningiomas are negative. Normal human and rat meningeal cells do not stain with anti-α2,6-ST [38]. Unlike metastatic colon carcinomas, aggressive anaplastic meningiomas do not express α2,6-ST mRNA whereas benign meningothelial meningiomas do. Thus, decreased α2,6-ST expression may play a role in the aggressive nature of anaplastic meningiomas [41].

To address tumour cell-type expression of specific sialoglycoproteins, Western blots of meningioma and glioma proteins, separated by two-dimensional SDS-PAGE and probed with SNA lectin, were performed. The presence of MEN-1 in the seven randomly chosen meningioma blots, and its very low to negligible expression in gliomas, suggests that MEN-1 may be a meningioma-associated glycoprotein containing α2,6-linked sialic acids. Upon careful inspection of the blots, other apparent meningioma-associated, SNA-positive, glyoproteins could be identified. To date MEN-1 is the most conspicuous and reproducible.

While SNA is effectively specific for α2,6-linked sialic acids, this lectin can recognize Neu5Ac(α2,3)Gal linkages with about 50-fold less sensitivity [39] (Glioblastomas do express both α2,3-linked sialoglycoconjugates and α2,3-ST mRNA: data not shown.) SNA will also recognize sialic acids with α2,6-linkages on oligosaccharides that are O-linked to proteins, and neural tissues do contain appreciable amounts of O-linked oligosaccharides bearing α2,6-linked sialic acids [40]. Glycoproteins bearing these carbohydrate side chains would also react with SNA lectin. This, too, could explain the SNA-positive spots observed in the glioma Western blots in spite of the fact that neither α2,6-ST mRNA nor anti-α2,6-ST-reactive material was found in gliomas.

In order to begin to study how α2,6-ST mRNA expression is regulated in gliomas and meningiomas, we performed Southern analyses to test the hypothesis that the *α2,6-ST* gene is altered in some brain tumours. We used a 0.4 kB cDNA probe that spans the N-terminal region of the coding sequence of the human *α2,6-ST* gene, and found that all of the samples gave the same pattern, demonstrating, in the region that we were able to probe, no alterations in the *α2,6-ST* gene. The human *α2,6-ST* gene spans at least 45 kb, contains at least 6 exons and 3 upstream promoters. Thus, while there were no apparent tumour-associated alterations in the region of the *α2,6-ST* gene examined in these experiments, clearly other parts of the gene need to be probed before a final conclusion can be drawn.

Le Marer *et al.* [34] have reported that the transfection of fat fibroblasts with the *c-Ha-ras* oncogene induced an increase in α2,6-ST activity due to increased protein brought about by an increase in α2,6-ST mRNA. They also reported that a number of other oncogenic transfections failed to have this effect. We examined the presence of *c-Ha-ras* in brain tumours by Northern analysis. *c-Ha-ras* was found in meningiomas, gliomas, and normal brain (data not shown). We also examined the mutation of *c-Ha-ras* [35] and found no point mutations in meningiomas. Furthermore, there was no correlation between tissue and expression levels of *c-Ha-ras* in the tissues examined. Thus, it did not appear that *c-Ha-ras* oncogene itself was responsible for the expression of α2,6-ST in meningiomas. Neither could the lack of *c-Ha-ras* account for the apparent lack of α2,6-ST mRNA expression found in glioblastomas and normal brain. However, this does not exclude the possibility that other oncogenes do play a role in the modulation of the expression of α2,6-ST as well as other glycosyltransferases in brain tumours.

In the thirteen metastatic brain tumours examined, no α2,6-ST mRNA expression was detected. If the primary tumours do express this mRNA, then these data would strongly suggest that α2,6-ST activity is suppressed as part of the process of successful metastasis to the brain. CD22 is a lymphocyte cell-surface glycoprotein that recognizes NeuAcα2,6Gal determinants and is believed to play a role in B-cell/B-cell and B-cell/T-cell adhesion [42, 43]. Adhesion molecules expressed on endothelial cells, platelets, and leukocytes (E-, P-, and L-Selectins, respectively) bind to ligands containing sialic acid (e.g. Sialyl-Lewis^X) [44–46]. Perhaps suppression of glycoconjugates bearing α2,6-linked sialic acid inhibits the kind of adhesive events that allow tumour cells to migrate to the brain and proliferate. Alternatively, inhibition of α2,6-ST activity may be part of some types of tumorigenesis. However, at least in the case of colon carcinoma, α2,6-ST activity and the expression of α2,6-linked sialic acids is increased [18–20]. Immunohistochemical studies of the primary tumours of these brain metastases suggested that most of them neither expressed α2,6-ST nor glycoconjugates bearing α2,6-linked sialic acids (unpublished observations).

In summary, the decreased expression of α2,6-ST in malignant (i.e. anaplastic) compared to benign (i.e. meningothelial) meningiomas is converse to that found in colon carcinomas and may be related to the malignant potential of meningiomas (albeit less α2,6-ST expression, rather than more, may be associated with increased malignancy) and glioblastomas, on the other hand, express virtually no α2,6-ST mRNA. It has been reported that glycoproteins in brain primarily contain sialic acid linked α2,3 whereas α2,6 linkages are found predominantly in serum glycoproteins [47, 48]. These results are consistent with the findings presented here. They also suggest that the regulation of α2,3-linked sialic acid-bearing glycoconjugates may be important in neuro-oncogenesis and invasivity.

The heterogeneity in the expression of the glycosyltransferase examined in these studies suggests that model systems can be created in which glycosyltransferase or glycosidase genes of interest could be transfected into established or primary tumour cell lines lacking their expression. Clearly, such model systems could be useful in further characterizing the function of cell-surface glycoconjugates in tumorigenesis as well as studying the regulation of their expression.

Acknowledgements

The authors thank Dr Eric Bremer for his helpful comments and discussion and Ms Bernice Bonilla for her help in preparing this manuscript. We are also indebted to Mr Kevin Cramer for his assistance in obtaining fresh tumour specimens.

References

1. Weinstein J, de Souza-e-Silva U, Paulson JC (1982) *J Biol Chem* **257**: 13835–44.
2. Weinstein J, Lee EU, McEntee K, Lai P, Paulson JC (1987) *J Biol Chem* **262**: 17735–43.
3. Roth J, Taatjes DJ, Lucocq JM, Weinstein J, Paulson JC (1985) *Cell* **43**: 287–295.
4. Colley KJ, Lee EU, Paulson JC (1992) *J Biol Chem* **267**: 7784–93.
5. Paulson JC, Beranek WE, Hill RL (1977) *J Biol Chem* **252**: 2356–62.
6. Weinstein J, Souza-e-Silva U, Paulson JC (1982) *J Biol Chem* **257**: 13835–44.
7. Weinstein J, Lee EU, McEntee K, Lai PH, Paulson JC (1987) *J Biol Chem* **262**: 17735–43.
8. Svensson EC, Soreghan B, Paulson JC (1990) *J Biol Chem* **265**: 20863–68.
9. Wen DX, Svensson EC, Paulson JC (1992) *J Biol Chem* **267**: 2512–18.
10. Svensson EC, Conley PB, Paulson JC (1992) *J Biol Chem* **267**: 3466–72.
11. Paulson JC, Weinstein J, Schauer A (1989) *J Biol Chem* **264**: 10931–34.
12. Bast BJE, Zhou L-J, Freeman G, Colley K, Ernst TJ, Munro JM, Tedder TF (1992) *J Cell Biol* **116**: 423–57.
13. Erikstein BK, Funderud S, Aas-Eng A, De Lange Davies C, Blomhoff HK, Smeland EB (1992) *Eur J Immunol* **22**: 1149–55.
14. Braesch-Andersen S, Stamenkovic I (1994) *J Biol Chem* **269**: 11783–86.
15. Hanasaki K, Varki A, Stamenkovic I, Bevilacqua MP (1994) *J Biol Chem* **269**: 10637–43.
16. LeMarer N, Laudet V, Svensson EC, Cazlaris H, Hille BV, Lagrou C, Stehelin D, Montreuil J, Verbert A, Delannoy P (1992) *Glycobiology* **2**: 49–56.

17. Sata T, Roth J, Zuber C, Stamm B, Heitz PU (1991) *Am J Pathol* **139**: 1435–48.
18. Bresalier RS, Rockwell RW, Dahiya R, Duh Q, Kim YS (1990) *Cancer Res* **50**: 1299–307.
19. Nakagawa T, Nakao Y, Matsui T, Koizumi T, Matsuda S, Maeda S, Fujita T (1985) *Br J Cancer* **51**: 357–63.
20. Toribara NW, Sack TL, Gum JR, Ho SB, Shively JE, Wilson JKV, Kim YS (1989) *Cancer Res* **49**: 3321–27.
21. Yates AJ, Thompson DK, Boesel CP, Albrightson C, Hart RW (1979) *J Lipid Res* **20**: 428–36.
22. Traylor TD, Hogan EL (1980) *J Neurochem* **34**: 126–31.
23. Berra B, Riboni L, De Gasperi R, Gaini SM, Ragnotti G (1983) **40**: 777–82.
24. Davidsson P, Fredman P, Collins VP, von Holst H, Mansson J-E, Svennerholm L (1989) *J Neurochem* **53**: 705–90.
25. Wikstrand CJ, Longee DC, McLendon RE, Fuller GN, Friedman HS, Fredman P, Svennerholm L, Bigner DD (1993) *Cancer Res* **53**: 120–26.
26. Grundmann U, Nerlich C, Rein T, Zettlmeissl G (1990) *Nucleic Acids Res* **18**: 66.
27. Chomczynski P, Sacchi N (1987) *Anal Biochem* **162**: 156–59.
28. Chirgwin JM, Przbyla AE, MacDonald RJ, Rutter WJ (1979) *Biochemistry* **18**: 5294–99.
29. Ramagli LS, Rodiriguez LV (1985) *Electrophoresis* **6**: 559–63.
30. O'Farrell PH (1975) *J Biol Chem* **250**: 4007–11.
31. LeGendre N (1990) *Biotechniques* **9**(Suppl.): 788–805.
32. Towbin H, Staehelin T, Gordon J (1979) *Proc Nat Acad Sci USA* **76**: 4350–54.
33. Wang X, Vertino A, Eddy RL, Byers MG, Jani-Sait SN, Shows TB, Lau JTY (1993) *J Biol Chem* **268**: 4355–61.
34. LeMarer N, Laudet V, Svensson EC, Cazlaris H, Van Hille B, Lagrou C, Stehelin D, Montreuil J, Verbert A, Delannoy P (1992) *Glycobiology* **2**: 49–56.
35. Bos JL (1989) *Cancer Res* **49**: 4682–89.
36. Robbins SL, Cotran RS (1979) *Pathological Basis of Disease*, 2nd Ed. p. 165. Philadelphia, PA: W.B. Saunders.
37. Kitagawa H, Paulson JC (1994) *J Biol Chem* **269**: 17872–78.
38. Kaneko Y, Yamamoto H, Colley KJ, Moskal JR (1995) *J Histochem Cytochem* **43**: 945–54.
39. Shibuya N, Goldstein IJ, Broekaert WF, Nsimba-Lubaki M, Peeters B, Peumans WJ (1987) *J Biol Chem* **262**: 1596–601.
40. Sata T, Lackie PM, Taatjes DJ, Peumans W, Roth J (1989) *J Histochem Cytochem* **37**: 1577–88.
41. Kepes JJ (1986) *J Neuropath Exp Neurol* **45**: 95–107.
42. Stamenkovic I, Sgroi D, Aruffo A, Sun Sy M, Anderson T (1991) *Cell* **66**: 1133–44.
43. Wilson GL, Fox CH, Fauci AS, Kehrl J (1991) *J Exp Med* **173**: 137–46.
44. Varki A (1992) *Curr Opin Cell Biol* **4**: 257–66.
45. Lasky LA (1992) *Science* **258**: 964–69.
46. Bevilacqua MP, Nelson RM (1993) *J Clin Invest* **91**: 379–87.
47. Krusius T, Finne J (1977) *Eur J Biochem* **78**: 369–79.
48. Margolis RU, Margolis RK (1989) *Neurobiology of Glycoconjugates*, pp. 86–88. New York: Plenum Press.
49. Libermann TA, Razon N, Bartal AD, Yarden Y, Schlessinger J, Soreq H (1984) *Cancer Res* **44**: 753–60.
50. Wong AJ, Bigner SH, Bigner DD, Kinzler KW, Hamilton SR, Vogelstein B (1987) *Proc Natl Acad Sci USA* **84**: 6899–903.
51. Yamazaki H, Fukui Y, Ueyama Y, Tamaoki N, Kawamoto T, Taniguchi S, Shibuya M (1988) *Mol Cell Biol* **8**: 1816–20.
52. Hanai N, Nores GA, MacLeod C, Torres-Mendez C-R, Hakomori S (1988) *J Biol Chem* **263**: 10915–21.