Molecular Cloning and Expression of Mouse GD1α/GT1αa/GQ1ba
Synthase (ST6GalNAc VI) Gene*

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A novel member of the mouse CMP-NeuAcβ-N-acetylglactosaminide 2,6-sialyltransferase (ST6GalNAc) subfamily, designated ST6GalNAc VI, was identified by BLAST analysis of expressed sequence tags. The sequence of the cDNA clone of ST6GalNAc VI encoded a type II membrane protein with 43 amino acids composing the cytoplasmic domain, 21 amino acids composing the transmembrane region, and 269 amino acids composing the catalytic domain. The predicted amino acid sequence showed homology to the previously cloned ST6GalNAc III, IV, and V, with common amino acid sequences in sialyl motif L and S among these four enzymes. A fusion protein with protein A and extracts from L cells transfected with ST6GalNAc VI in an expression vector showed enzyme activity of 2,6-sialyltransferase for GM1b, GT1b, and GD1a but not toward glycoproteins. Thin layer chromatography-immunostaining revealed that the products were GD1a, GQ1b, and GT1ac. Northern blotting revealed that this gene was expressed in a wide range of mouse tissues such as colon, liver, heart, spleen, and brain. It is concluded that this enzyme is a novel sialyltransferase involved in the synthesis of α-series gangliosides in the nervous system and many other tissues.

Sialic acid-containing glycosphingolipids are designated gangliosides and have been thought to play important roles in a wide variety of biological events such as cell-cell or cell-extracellular matrix interaction, protein targeting, and acceptance of extracellular molecules and particles (1). Among gangliosides that have been defined to date, four different linkages of sialic acids are identified, i.e. 2,3-galactose (Gal), 2,6Gal, 2,6-sialic acid (Sia), and 2,6-sialylgalactosaminide (GalNAc).

For the biosynthesis of sialyl compounds containing sialic acids in their carbohydrate moiety, a number of sialyltransferases should be present depending on the individual linkages and on the individual acceptor structures. Actually, more than 16 species of sialyltransferase genes utilizing glycoproteins and/or glycolipids as an acceptor have been cloned (2), i.e. 6 genes for 2,3Gal (ST3Gal), 1 gene for 2,6Gal (ST6Gal), 5 genes for 2,8Sia (ST8Sia), and 5 genes for 2,6GalNAc (ST6GalNAc). Many of these sialyltransferase genes were isolated by polymerase chain reaction based on similar sequences named sialyl motifs, which were first identified by Paulson and co-workers (3) in purified sialyltransferases, and in all sialyltransferases isolated thereafter (2).

Among gangliosides, α-series gangliosides that were defined as a new series of gangliosides containing NeuAc linked to the C6 position of GalNAc of the gangliotetraosyl backbone (4, 5) have been considered to be a minor component (6). Compared with O-glycan, 2,6-sialylated GalNAc structures are rarely detected in glycosphingolipids, and little is known about their expression and significance. Recently, we have isolated a unique member of the ST6GalNAc family designated ST6GalNAc V expressed in brain tissues in a restricted manner. ST6GalNAc V encoded by this gene utilized exclusively GM1b as an acceptor, resulting in the synthesis of GD1a but not of other α-series gangliosides such as GT1αa and GQ1b. Furthermore, α-series gangliosides have been reported to be expressed not only in brain but in lymphocytes (7), macrophages (8), mammary glands (9), and fibroblasts (10).

In the present study, we have isolated a novel member of the ST6GalNAc gene subfamily designated ST6GalNAc VI. This enzyme is specific for glycolipid acceptors and can synthesize all α-series gangliosides so far defined. The expression pattern of the gene is much broader than that of ST6GalNAc V and is distinct from that of other members of the ST6GalNAc subfamily.

**EXPERIMENTAL PROCEDURES**

Nomenclature of Cloned Sialyltransferase—Five members of the GalNAc 2,6-sialyltransferase (ST6GalNAc) subfamily have been cloned so far: ST6GalNAc I (11), ST6GalNAc II (12), ST6GalNAc III (13, 14), ST6GalNAc IV (14), and ST6GalNAc V (15). The GalNAc 2,6-sialyltransferase cloned in this study is referred to as ST6GalNAc VI according to Tsuji et al. (16).

Materials—CMP-NeuAc, LacCer, asialo-GM2 (GA2), GM2, GM1, GD1a, GD1b, GT1b, fetuin, asialofetuin, bovine submaxillary mucin (BSM), and bovine submaxillary asialomucin (asialo-BSM) were purchased from Sigma. GM3 and GD3 were purchased from Snow Brand Milk Products Co. (Tokyo, Japan). α-32PdCTP was from ICN (Costa...
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Mesa, CA; GM1b, GD1α, and GT1αa were chemically synthesized as described previously (17). Asialo-GM1 (GA1) was prepared from GM1 by cleavage of a sialic acid with heating at 100 °C in 0.1 N HCl.

EST Data Base Search—A data base search was performed with the coding sequence of the GSTGalNAc IV gene (GenBank™ accession number Y15780) and a BLASTN algorithm against the EST database (dbEST) at The National Center for Biotechnology Information (NCBI). A mouse EST clone (GenBank™ accession number AA790409) was found, and the sequence (nucleotide number 804-1121) was obtained by the reverse transcription polymerase chain reaction (PCR) using mouse brain cDNA as a template.

5′ RACE Amplification of cDNAs Ends (RACE)—To obtain the 5′-end, cloning by RACE was performed according to the manufacturer's instructions (5′-RACE kit, Life Technologies, Inc.). Total RNA from mouse brain (0.4 μg) was used for first strand cDNA synthesis with gene-specific primer 1 (GSP1) 5′-GGTGCCTGCGTCGGA-TGTA-3′ (nucleotides 1017–1000). Nested PCR was performed with adaptor primer and GSP2 5′-ACACCTCTGCAGGCCCTTGG-3′ (nucleotides 993–797). A 995-base pair fragment with an initiation codon in agreement with the Kozak rule (18) was obtained.

Cell Culture—Mouse fibroblast L cells and human melanoma SK-MEL-37 cells were grown in Dulbecco's modified Eagle's minimum essential medium (DMEM) supplemented with 7.5% fetal calf serum at 37 °C in a 5% CO2 atmosphere. L cells were kindly provided by Dr. A. Amstuz at Menninger Hospital, Waco, Texas, and SK-MEL-37 cells were grown in Dulbecco's modified Eagle's minimum medium supplemented with 10% fetal calf serum. L cells were transfected with expression vectors using the QuiafectTM (Qiagen) according to the manufacturer's instructions. Approximately 24 h after the beginning of the transfection, the cells were trypsinized, and cell suspensions were transferred to 10-cm plates and cultured for 30 days with G418 (750 μg/ml).

Stable Transfection—SK-MEL-37 cells (10⁶ cells/6-cm plate) were transfected with 5 μg of pMIKneo-ST6GalNAc VI using 20 μl of SuperFect™ (Qiagen) according to the manufacturer's instructions. Approximately 24 h after the beginning of the transfection, the cells were trypsinized, and cell suspensions were transferred to 10-cm plates and cultured for 30 days with G418.

Flow Cytometric Analysis—The cell surface expression of GQ1bα was analyzed on a FACSCalibur with Cell Quest™ version 3.1f software (Becton Dickinson) using mouse anti-GQ1bα mAb GGR41 at a dilution of 1:2. The cells were incubated with mAbs for 45 min on ice and stained with fluorescein isothiocyanate-conjugated goat anti-mouse IgG (ICN/Cappel) at a 1:200 dilution. Control samples were prepared without mAbs.

Immunofluorescence Assay—SK-MEL-37 cells were transiently transfected with 5 μg of pMIKneo-ST6GalNAc VI or pMIKneo-ST6GalNAc V using SuperFect™ as described above. After trypsinization, cells were resuspended in complete medium, transferred onto cover glasses, and incubated at 37 °C for 24 h. The cells were fixed, with cold acetone for 10 min, air-dried, and processed for indirect immunofluorescence analysis as described (27).

Extraction of Gangliosides—Gangliosides were extracted according to Furukawa et al. (22). Briefly, glycolipids were extracted from 125 mg of packed cells of transfectants and control cells transfected with a vector alone using chloroform/methanol (2:1, 1.1, and 1.2) sequentially. After a desalting, gangliosides were isolated by DEAE-Sephadex A-50 (Amersham Pharmacia Biotech) ion exchange chromatography. TLC was performed on high performance TLC plates using the solvent system of chloroform, methanol, 12 molar MeCl2, and 12 molar MeCl2. Northern Blot Analysis—mRNA was isolated using mRNA isolation kit (Milenyi Biotech, Bergisch, Germany) according to the manufacturer's instructions from C57BL/6 mouse tissues. Two μg of poly(A)+ RNA was separated on 1.2% agarose gel. The gel was transferred onto a GeneScreen Plus® membrane (DuPont). After baking, the filter was prehybridized for 2 h at 42 °C in a solution consisting of 5× saline/sodium phosphate/EDTA, 50% formamide, 5× Denhardt's solution, 1% SDS, and 10% dextran sulfate. Hybridization was carried out for 16 h at 42 °C in the same solution containing 5× saline/sodium phosphate/EDTA, 50% formamide, 5× Denhardt's solution, 1% SDS, and 10% dextran sulfate. Hybridization was carried out for 16 h at 42 °C in the same solution containing 5× saline/sodium phosphate/EDTA, 50% formamide, 5× Denhardt's solution, 1% SDS, and 10% dextran sulfate. Hybridization was carried out for 16 h at 42 °C in the same solution containing 5× saline/sodium phosphate/EDTA, 50% formamide, 5× Denhardt's solution, 1% SDS, and 10% dextran sulfate. Hybridization was carried out for 16 h at 42 °C in the same solution containing 5× saline/sodium phosphate/EDTA, 50% formamide, 5× Denhardt's solution, 1% SDS, and 10% dextran sulfate. Hybridization was carried out for 16 h at 42 °C in the same solution containing 5× saline/sodium phosphate/EDTA, 50% formamide, 5× Denhardt's solution, 1% SDS, and 10% dextran sulfate. Hybridization was carried out for 16 h at 42 °C in the same solution containing 5× saline/sodium phosphate/EDTA, 50% formamide, 5× Denhardt's solution, 1% SDS, and 10% dextran sulfate. Hybridization was carried out for 16 h at 42 °C in the same solution containing 5× saline/sodium phosphate/EDTA, 50% formamide, 5× Denhardt's solution, 1% SDS, and 10% dextran sulfate. Hybridization was carried out for 16 h at 42 °C in the same solution containing 5× saline/sodium phosphate/EDTA, 50% formamide, 5× Denhardt's solution, 1% SDS, and 10% dextran sulfate. Hybridization was carried out for 16 h at 42 °C in the same solution containing 5× saline/sodium phosphate/EDTA, 50% formamide, 5× Denhardt's solution, 1% SDS, and 10% dextran sulfate. Hybridization was carried out for 16 h at 42 °C in the same solution containing 5× saline/sodium phosphate/EDTA, 50% formamide, 5× Denhardt's solution, 1% SDS, and 10% dextran sulfate. Hybridization was carried out for 16 h at 42 °C in the same solution containing 5× saline/sodium phosphate/EDTA, 50% formamide, 5× Denhardt's solution, 1% SDS, and 10% dextran sulfate. Hybridization was carried out for 16 h at 42 °C in the same solution containing 5× saline/sodium phosphate/EDTA, 50% formamide, 5× Denhardt's solution, 1% SDS, and 10% dextran sulfate.

RESULTS

Isolation of ST6GalNAc VI cDNA—To clone GT1αa and GQ1bα synthase, the NCBI data bank of EST cDNA clones were probed with the deduced amino acid sequence of the mouse ST6GalNAc IV. A mouse EST clone (GenBank™ accession number AA790409) was found, and the sequence (nucleotide number 804-1121 in Fig. 1A) was obtained by reverse transcription PCR using mouse brain cDNA as a template. The sequence was similar to but distinct from that of the previously cloned sialyltransferases, suggesting that the clone encoded a novel member of the sialyltransferase gene family.

To obtain the 5′-end, the cloning strategy of 5′-RACE was employed. The revealed sequence of the overlapping cDNA fragments indicated a single open reading frame of 999 base pairs coding for a protein of 333 amino acids containing three potential N-glycosylation sites (Fig. 1A). The deduced amino acid sequence corresponds to a 38,165 Da polypeptide. The position of the AUG start codon was determined according to the Kozak consensus sequence (18). Hydroyphathy (28) indicated one prominent hydrophobic segment of 21 residues in length in the amino-terminal region, predicting that the protein has type II transmembrane topology characteristic of many other glycosyltransferases cloned to date (Fig. 1B). The primary structure of the identified cDNA showed 29, 30, and 40% amino acid sequence identity to mouse ST6GalNAc III. ST6GalNAc IV,
and ST6GalNAc V, respectively (Fig. 2). The newly cloned gene was designated ST6GalNAc VI based on results described below.

Sialyltransferase Activity of the Newly Cloned Enzyme—To analyze the sialyltransferase activity of ST6GalNAc VI, a fusion gene consisting of the IgM signal peptide sequence, the protein A IgG binding domain, and the putative active domain of ST6GalNAc VI (residue number 25-333) was constructed and transfected into L cells. In this system, the soluble enzyme (ProtA-ST6GalNAc VI) would be secreted. Using this soluble form of ProtA-ST6GalNAc VI as the enzyme source, we examined the sialyltransferase activity toward various glycolipids. As shown in Fig. 3, [14C]NeuAc was incorporated into GM1b, GD1a, and GT1b commonly containing the NeuAc\(^{\alpha}2,3\)Gal\(^{\beta}1,3\)GalNAc sequence at the nonreducing end. ProtA-ST6GalNAc VI exhibited little activity toward GA1 and GD1b and no activity toward GM1, indicating that a sialic acid linked to galactose at the nonreducing end by an \(\alpha^{2,3}\) linkage was required for the substrate activity. Then we determined the acceptor specificity of the enzyme toward glycoproteins. As summarized in Table I, ProtA-ST6GalNAc VI showed minimal activity toward fetuin and no activity toward asialofetuin, BSM, and asialo-BSM. Similar results were obtained using the L cell extracts transfected with pMIKneo-ST6GalNAc VI (data not shown).

Characterization of the Enzyme Product—As shown in Fig. 3, the TLC mobilities of the enzyme products synthesized from GM1b and GD1a were identical to those of GD1a and GT1a, respectively. When GT1b was used as a substrate, the enzyme product was detected at a position slightly lower than authentic GQ1b, suggesting that this band is GQ1b\(^{\alpha}\). GD1a sialylated with ST6GalNAc VI (lane 3) as well as GT1a\(^{\alpha}\) in the standard (lane 1) were weakly stained with mAb GGR-41 as reported previously (24), indicating that they had an identical structure.

Determination of the Enzymatic Activity in Cell Culture—To explore the enzymatic activity of the ST6GalNAc VI gene in vivo, we transiently transfected a ST6GalNAc VI expression vector into SK-MEL-37 cells, which synthesize GT1b but not GQ1b\(^{\alpha}\) (data not shown). As shown in Fig. 5B, indirect immunofluorescence assays using mAb GGR-41 revealed that the overexpression of the ST6GalNAc VI cDNA in SK-MEL-37 cells resulted in the expression of a novel antigen localized in the cytoplasm and probably in Golgi apparatus. This staining was specific for GQ1b\(^{\alpha}\) (or possibly GT1a\(^{\alpha}\)), since no significant immunofluorescence was observed in cells transfected with the vector alone (Fig. 5A). On the other hand, SK-MEL-37 cells transiently transfected with an expression vector of ST6GalNAc V (GD1a synthase) cDNA exhibited no immunofluorescence, confirming that the enzyme could not utilize GD1a nor GT1b as substrates (Fig. 5C).

To test the ability of the ST6GalNAc VI gene to determine...
Expression on the cell surface, we attempted to establish stable transfectant lines of SK-MEL-37 cells. As shown in Fig. 5E, SK-MEL-37 cells stably transfected with ST6GalNAc VI (designated SK-MEL-37/ST6GalNAc-VI) gave a positive peak with mAb GGR-41. Then glycosphingolipids extracted from the stable line were analyzed by TLC. Because we could not visualize the newly synthesized gangliosides with resorcinol/HCl reagent, TLC-immunostaining was conducted using GQ1bα synthesized in vitro from GT1b as a control. As shown in Fig. 5F, the acidic glycolipids extracted from SK-MEL-37/ST6GalNAc-VI clearly gave a band like the control GQ1bα at the same migration site, indicating that the newly synthesized gangliosides contained GQ1bα.

Expression of the ST6GalNAc VI Gene—To determine the size of ST6GalNAc VI mRNA and its expression pattern, Northern blot analysis was conducted using the full-length cDNA as a probe. As shown in Fig. 6, a transcript of 2.5 kilobase pairs was detected in all the tissues examined. The gene expression is abundant in colon, brain, liver, and heart. An additional band at approximately 7.5 kilobase pairs was detected only in mouse colon.

We previously reported that the ST6GalNAc V (GD1α synthase) gene is expressed exclusively in the brain tissue and is involved in the synthesis of GD1α in the nervous tissues (15). To compare the transcription levels of ST6GalNAc VI and ST6GalNAc V in mouse brain, poly (A)1 RNA prepared from adult cerebrum, cerebellum, or 16-day postcoitum mouse embryo was analyzed by Northern blot hybridization. As shown in Fig. 7, both ST6GalNAc V and VI are expressed in cerebrum.
Various acceptor substrates were incubated in the standard assay mixture using Prota-ST6GalNAc VI as an enzyme source. Each substrate was used at the concentration of 0.2 mM for glycolipids and 0.4 mg/ml for glycoproteins. Relative rates are calculated as a percentage of the incorporation obtained with GM1b.

| Acceptor | Structure(s) | Relative rate |
|----------|--------------|---------------|
| Fetuin   | NeuAc2,3Galβ1,3GalNAc-Ser/Thr | 0.3 |
| BSM      | NeuAc2,3Galβ1,3GalNAc,Ser/Thr | 0 |
| Asialo-fetuin | NeuAc2,3Galβ1,4GlcNAc-R | 0 |
| Asialo-BSM | NeuAc2,3Galβ1,4Glcβ1-Cer | 0 |
| LactCer  | Galβ1,4Glcβ1-Cer | 0 |
| GA2      | GalNAcβ1,4Galβ1,4Glcβ1-Cer | 0.3 |
| GA1      | Galβ1,3GalNAcβ1,4Galβ1,4Glcβ1-Cer | 0 |
| GM1b     | NeuAc2,3Galβ1,3GalNAcβ1,4Galβ1,4Glcβ1-Cer | 100.0 |
| GM3      | NeuAc2,3Galβ1,4Glcβ1-Cer | 0 |
| GM2      | GalNAcβ1,4NeuAc2,3Galβ1,4Glcβ1-Cer | 0 |
| GM1      | Galβ1,3GalNAcβ1,4NeuAc2,3Galβ1,4Glcβ1-Cer | 0 |
| GD1a     | NeuAc2,3Galβ1,3GalNAcβ1,4NeuAc2,3Galβ1,4Glcβ1-Cer | 3.3 |
| GD3      | NeuAc2,3NeuAc2,3Galβ1,4Glcβ1-Cer | 0 |
| GD1b     | Galβ1,3GalNAcβ1,4NeuAc2,3NeuAc2,3Galβ1,4Glcβ1-Cer | 0.2 |
| GT1b     | NeuAc2,3Galβ1,3GalNAcβ1,4NeuAc2,3NeuAc2,3Galβ1,4Glcβ1-Cer | 56.2 |

\(^a\) Data are from Spiro and Bhoyroo (37) for fetuin and Tsuji and Osawa (38) for BSM.

\(^b\) R represents the remainder of the N-linked oligosaccharide chain.

\(^c\) 8.96 nmol/h/μl of an enzyme solution.

**FIG. 4. TLC-immunostaining of the products of the enzyme assay.** Glycolipids (5 μg) were sialylated with Prota-ST6GalNAc VI (10 μl) for 6 h and purified by C	extsubscript{18} Sep-Pak cartridge, dried, and subjected to TLC with a solvent system of chloroform, methanol, 12 mM MgCl	extsubscript{2} (40:50:10). The complete conversion of each substrate to a less migrating compound was confirmed by TLC followed by visualization with 0.1% premulin reagent. A, TLC-immunostaining with mAb KA-17. Lane 1 contained acidic glycosphingolipids (2.5 μg) extracted from bovine brain (BB) consisting of GM1, GD1a, GD1b, and GT1b as major components. Lane 2, standard GD1a (2.5 μg); lane 3, GM1b sialylated with ST6GalNAc VI. As a control, the same reaction was performed without the enzyme (lane 3). B, TLC-immunostaining with mAb GGR-41. Lane 1, standard GT1α (2.5 μg); lane 3, GD1a sialylated with ST6GalNAc VI; lane 5, GT1b sialylated with ST6GalNAc VI. As a control, the same reaction was performed without the enzyme (lanes 2 and 4). Lane 6 was acidic glycosphingolipids (2.5 μg) extracted from bovine brain (BB). Ori, origin.

and 16-day postcoitum mouse embryo. In contrast, only the ST6GalNAc VI transcript was found in cerebellum.

**DISCUSSION**

Among five ST6GalNAc cDNAs isolated to date, ST6GalNAc I and ST6GalNAc II were cloned as sialyltransferases that mainly utilize O-glycans as an acceptor. ST6GalNAc I prefers GalNAc-Ser/Thr, and ST6GalNAc II acts on Galβ1,3GalNAc-Ser/Thr. ST6GalNAc III, ST6GalNAc IV, and ST6GalNAc V showed high homology in their primary structures (15), and a similar substrate specificity, i.e. a terminal sialic acid with an α2,3 linkage on galactose was essential as an acceptor structure. The amino acid sequence alignment of these six ST6GalNAc demonstrated that ST6GalNAc VI is closer to ST6GalNAc III, IV, and V in the primary structure (Fig. 2). However, ST6GalNAc IV preferred O-glycans as acceptors (14), whereas ST6GalNAc III and V better utilize glycolipid acceptors. Therefore, the ST6GalNAc VI reported in this study is more similar to ST6GalNAc III and V in terms of the preferred substrate structure than ST6GalNAc I, II, and IV.

In the fine substrate specificities of ST6GalNAc III, V, and VI, ST6GalNAc III utilized both O-glycan and glycolipids as an acceptor when analyzed by cDNA of mouse (14) and rat (13). In contrast, ST6GalNAc V acts only toward a glycolipid acceptor (15). However, these two enzymes are very similar in terms of the fine substrate structure, i.e. they utilize only GM1b in glycolipid acceptors, resulting in the synthesis of GD1α. ST6GalNAc VI is almost specific for glycolipid acceptors but is distinct from the other two enzymes in its unique substrate specificity. ST6GalNAc VI could catalyze the transfer of NeuAc by α2,6 linkage not only onto GalNAc in GM1b but that in GT1b and GD1a, resulting in the synthesis of GQ1bα and GT1α, respectively. Therefore, we have identified here for the first time a member of the ST6GalNAc subfamily that can synthesize GQ1bα and GT1α as well as GD1α.

Furthermore, the expression of ST6GalNAc VI was different from that of ST6GalNAc III or V. The ST6GalNAc III gene was expressed mainly in heart, lung, and brain (14). In contrast, ST6GalNAc V was almost specifically expressed in the brain as previously reported by us (15). ST6GalNAc VI was expressed very strongly in colon tissue, at moderate levels in heart, liver, brain, and spleen and weakly in all other tissues examined. Therefore, ST6GalNAc VI seems to be a widely expressed novel ST6GalNAc with a broad substrate specificity for glycolipid structures.

α-Series gangliosides have been thought to be minor components. GD1α was reported as a minor ganglioside in bovine brain tissues (6) and as an accumulated structure in the proximal dendrites and cell bodies of Purkinje cells in murine cerebellum (24). On the other hand, GT1α and GQ1bα were found in the rat brain and spinal cord in the nerve terminals of a certain population of cholinergic fibers (29) and in the dorsal and lateral horn of human thoracic cord (25, 30). The distribution of GD1α and that of GT1α and GQ1bα are not necessarily identical. ST6GalNAc VI may synthesize GD1α or GT1α and GQ1bα depending on the alternative synthesis of asialo-series
gangliosides or ganglio-series gangliosides, respectively. This
should be determined by the expression level of GM3 synthase
as described previously by our group (20). For the expression of
GT1a and GQ1b, ST6GalNAc VI should be responsible, since
only ST6GalNAc VI can synthesize GT1a and GQ1b to our
knowledge. However, for the synthesis of GD1α, it is difficult to
decide which enzyme is responsible in the individual tissues at
this moment. There may be several tissue-specific ST6GalNAc

FIG. 5. Expression of GQ1bα on SK-MEL-37 cells transfected with ST6GalNAc VI cDNA. The expression of GQ1bα on SK-MEL-37 cells
was detected with mAb GGR41 at a 1:2 dilution as a primary antibody. A, B, and C are confocal microscopy fluorescence images of SK-MEL-37 cells transiently transfected with pMIKneo vector (A), pMIKneo-ST6GalNAc VI (B), or pMIKneo-ST6GalNAc V (C). The bar indicates 50 μm. D and E are
flow cytometric patterns of SK-MEL-37 cells stably transfected with pMIKneo vector (D) or pMIKneo-ST6GalNAc VI (E). The expression of
GQ1bα was analyzed as described under “Experimental Procedures.” The ordinate and abscissa represent cell numbers (Counts) and relative
fluorescence intensity (FL1-H), respectively. Thin lines represent anti-GQ1bα antibody, and solid lines represent controls with the second antibody alone. F, acidic glycosphingolipids were extracted from SK-MEL-37 cells stably transfected with pMIKneo vector or pMIKneo-ST6GalNAc VI and
were subjected to TLC-immunostaining as described under “Experimental Procedures.” BB, a ganglioside standard of bovine brain gangliosides (5 μg); GQ1bα, 5 μg of GQ1bα synthesized in vitro from GT1b using ProtA-ST6GalNAc VI; BD/Mock, gangliosides from 125 μl of mock-transfected
SK-MEL-37 cells; BD/ST6GalNAc VI, gangliosides from 125 μl of SK-MEL-37 cells transfected with pMIKneo-ST6GalNAc VI.

FIG. 6. Expression pattern of the ST6GalNAc VI gene in vari-
ous mouse tissues. Northern blots with 2 μg of poly (A)+ RNA from
various adult mouse tissues were probed with 32P-labeled mouse
ST6GalNAc VI full-length cDNA as described under “Experimental
Procedures.” The same filters were probed with glyceraldehyde-phos-
phate dehydrogenase (GAPDH) cDNA after removing the radioactivity.
The positions of ribosomal RNAs are indicated at the right.

FIG. 7. Differential expression of ST6GalNAc V and
ST6GalNAc VI genes in mouse brain. Northern blots with 5 μg of poly (A)+ RNA from mouse cerebrum, cerebellum, and 16-day postco-
titum mouse embryo (E16) were probed with mouse ST6GalNAc VI
full-length cDNA, ST6GalNAc V cDNA as described (15), and glyceral-
dehyde-phosphate dehydrogenase (GAPDH). The positions of ribosomal
RNAs are indicated at the right.
members capable of synthesizing GD1α. In the expression analysis of the ST6GalNAc V gene, there was a controversial finding that the transcript could not be found in mouse cerebellum, whereas GD1α was strongly stained in Purkinje cells by Furuya et al. (24). As shown in Fig. 7, ST6GalNAc VI was definitely expressed in cerebellum, suggesting that this gene, not ST6GalNAc V, is responsible for the GD1α expression in mouse cerebellum.

α-Series gangliosides have been reported as cholinergic nerve-specific molecules (Chol-1) (31, 32) or binding molecules to myelin-associated glycoprotein in vitro (33). These studies indicate that α-series gangliosides play critical roles in the interaction and communication between neuronal cells and their supportive cells. Furthermore, the immunostaining pattern of the cDNA-transfectant cells indicated that GQ1b is also localized in the cytoplasm and Golgi apparatus, suggesting that GQ1b plays roles not only as a ligand for extracellular molecules but as a regulatory factor of intracellular events (34, 35). The availability of the ST6GalNAc VI gene would enable us to clearly demonstrate the roles of α-series gangliosides in the neuronal development and functions.

Most studies on the α-series of gangliosides have been done with animal tissues or cells. The fact that no human studies have been reported to date suggests that these gangliosides are also localized in the cytoplasm and Golgi apparatus, suggesting that GQ1b plays roles not only as a ligand for extracellular molecules but as a regulatory factor of intracellular events (34, 35). The availability of the ST6GalNAc VI gene would enable us to clearly demonstrate the roles of α-series gangliosides in the neuronal development and functions.

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