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Molecular Cloning and Expression of Human ST6GalNAc III: Restricted Tissue Distribution and Substrate Specificity

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We isolated human ST6GalNAc III cDNA clones. The typical cDNA clones predicted a type II membrane protein of 305 amino acids with a short cytoplasmic transmembrane domain of sixteen amino acids and a catalytic domain of 280 amino acids. A short form clone predicted a protein of 240 amino acids lacking 65 amino acids including the transmembrane portion. The alternative usage of the second exon seemed to generate these two transcripts. Both had two common regions found among sialyltransferases cloned so far, *i.e.* sialyl motif L and sialyl motif S. Alignments of human, mouse and rat orthologs indicated that high homologies, *i.e.* 85–95% identity among these species at amino acid levels. We analyzed the expression pattern and substrate specificity of the product, demonstrating a very restricted expression pattern and a high substrate specificity. Northern blotting revealed that hST6GalNAc III is expressed in kidney and brain as a single band at 3.2 kb. In enzyme assay of the long form, the transfer of sialic acid onto α2,3-sialylated acceptor substrates, *i.e.* GM1b and sialyl lactotetraosylceramide, was observed. hST6GalNAc III also showed sialyltransferase activity toward *O*-glycans (but not *N*-glycans) in fetuin.

Key words: glycosphingolipid, kidney, *O*-glycan, sialyltransferase.

Abbreviations: Gal, galactose; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; Lc4, lactotetraosylceramide (Galβ1,3GlcNAcβ1,3Galβ1,4Glcβ1-Cer); nLc4, neolactotetraosylceramide (Galβ1,4GlcNAcβ1,3Galβ1,4Glcβ1-Cer); Le[a], Lewis a (Galβ1,3(Fucα1,4) GlcNAcβ1,3Galβ1,4Glcβ1-Cer); mAb, monoclonal antibody; D-MEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; CMP-NeuAc, cytidine 5′-monophospho-*N*-acetylneuraminic acid; TLC, thin layer chromatography; CDP-cholin, cytidine diphosphate cholin; PBS, phosphate-buffered saline; FITC, fluorescein isothiocyanate. The nomenclature of gangliosides is based on that of Svennerholm (*1*). The abbreviated nomenclature for cloned sialyltransferases follows Tsuji *et al.* (*2*).

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Sialic acids comprise a critical molecular family that modulates carbohydrate structures on various glycoproteins and glycolipids (*3*). A wide variety of functional complex carbohydrates are regulated their substantial natures and functions with sialylation catalyzed by a group of glycosyltransferases, *i.e*. sialyltransferases (*4*). To date, twenty sialyltransferase genes have been identified in mammals and birds (*2*). Their products transfer sialic acids from CMP-NeuAc (and/or CMP-NeuGc) usually to the terminal sites of the carbohydrate structures of glycoproteins and glycolipids.

Sialyltransferases have been classified into 4 subgroups based on linkages and acceptor structures (*2*). Among the 4 subfamilies of sialyltransferases, the GalNAcα2,6-sialyltransferase family contains 6 members that play roles in the synthesis of diverse sialyl-glycoconjugate structures. In particular, ST6GalNAc III–VI seem very unique in that they catalyze the synthesis of branched type disialyl structures by transfer of a sialic acid onto a GalNAc residue inside the backbone core chains. They also show somewhat phylogenetically separated linkages in a dendrogram of all sialyltransferases (*5*).

Among mouse/rat ST6GalNAc family members isolated to date, ST6GalNAc I and ST6GalNAc II were cloned as sialyl transferases that mainly utilize *O*-glycans containing no sialic acid at the non-reducing end as acceptors, *i.e*. GalNAc-Ser/Thr and Galβ1,3GalNAc-Ser/Thr. Then, mouse/rat ST6GalNAc III, ST6GalNAc IV, ST6GalNAc V, and ST6GalNAc VI were cloned, and found to be highly homologous in their primary structures (*6*, *7*) and similar in terms of substrate specificity; *i.e*., a terminal sialic acid with an α2,3 linkage on galactose is essential in their acceptor structures. Compared to ST6GalNAc IV, ST6GalNAc III prefers glycolipids to *O*-glycans as acceptors, and ST6GalNAc V/VI were further cloned as the sialyltransferases responsible for the synthesis of α-series gangliosides.

In the analyses of sialylated tumor-associated antigens in human colon cancers, pancreatic cancers and renal cancers, disialyl-glycosphingolipid antigens have been considered to have implications for the malignant properties of individual tumors (*8*). Recently, we identified the sialyltransferase that is responsible for the synthesis of disialyl Lewis[a] (Le[a]) antigen in human colon cancer cells, *i.e*. ST6GalNAc VI (*9*). As for other members of the ST6GalNAc family, only rat (*10*) and/or mouse cDNA clones (*5*) of ST6GalNAc III–V have been isolated and characterized.

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In this study, we isolated human ST6GalNAc III cDNA clones and analyzed their expression and substrate specificity, demonstrating a restricted expression pattern and substrate specificity. These data should contribute to the functional analysis of various disialyl-compounds in human normal and cancer tissues.

MATERIALS AND METHODS

Nomenclature of Cloned Sialyltransferase—Five members of the human GalNAc α2,6-sialyltransferase (ST6-GalNAc) subfamily have been cloned so far: ST6GalNAc I, ST6GalNAc II, ST6GalNAc IV, ST6GalNAc V, and ST6GalNAc VI. The GalNAc α2,6-sialyltransferase cloned in this study is referred to as ST6GalNAc III according to Tsuji *et al*. (*11*).

Isolation of Human ST6GalNAc III—To isolate cDNA clones, a reverse transcription polymerase chain reaction (RT-PCR) using a human brain cDNA library (ResGen (Invitrogen Corporation)) as a template was performed. A sense primer (5′-GAATGTGGGCTGGAGAGGTC-3′) and antisense primer (5′-GCAGAGTCACCATCCACATC-3′) were used for PCR amplification, which was carried out using TITANIUM™ Taq DNA Polymerase (Advantage2 Kits, Clontech) as follows: 95°C for 3 min, 28 cycles of (95°C for 0.5 min and 68°C for 3 min), and 72°C for 10 min. A RT-PCR-amplified 1,182-base pair cDNA was subcloned into pT-Adv vector using an AdvanTage™ Cloning Kit (Conthech). The nucleotide sequence was determined by the dideoxy termination method using an ABI PRISM 310 genetic analyzer (Applied Biosystems, Foster City, CA).

Construction of Expression Vectors—An expression vector pcDNA3.1(+)-hST6GalNAc III was prepared by inserting a *Hin*dIII and *Xho*I fragment from pT-Adv-hST6GalNAc III into the *Hin*dIII and *Xho*I sites of pcDNA3.1(+) vector. To prepare a soluble fusion enzyme, a truncated form of ST6GalNAc III lacking 21 amino acids from the NH_2 terminus was prepared by PCR using a 5′ primer containing an *Eco*RI site, 5′-ATCCTCGAATTCCTGCTGGTTGTGCGTCTT-3′ (nucleotides 175–192), and a 3′ primer containing a *Xho*I site, 5′-GGCGGCTCGAGTCAGGAAAACCATTATCA-3′ (nucleotides 1021–1038), and the cloned cDNA fragment as a template. The product was digested with *Eco*RI and *Xho*I and subcloned into these sites of the pCDSA vector (presented by S. Tsuji).

Preparation of Membrane Fraction—Mouse fibroblast L cells were grown in Dulbecco's modified Eagle's medium (D-MEM) supplemented with 7.5% fetal calf serum (FCS). L cells (3×10^6) were plated in 10-cm dishes (Greiner Bio-One) at least 48 h prior to transfection. Cells were transiently transfected with an expression plasmid (4 μg) by the DEAE-dextran method (*12*). After 48 h of culture in D-MEM containing 7.5% FCS, the cells were harvested by trypsinization. The cells were pelleted, washed with phosphate-buffered saline (PBS), and lysed in ice-cold PBS containing 1 mM phenylmethylsulfonylfluoride using a nitrogen cavitation apparatus (Parr Instrument Co., Moline, IL) at 400 p.s.i. for 30 min. Nuclei were removed by low speed centrifugation, and the supernatant was centrifuged at $100,000 \times g$ for 1 h at 4°C. The pellet was resuspended in ice-cold 100 mM sodium cacodylate buffer, pH 6.0, and used as an enzyme source.

Preparation of Soluble Forms hST6GalNAc III—L cells were transfected with pCDSA-hST6GalNAc III by the DEAE-dextran method and cultured for 16 h in D-MEM containing ITS culture supplement (Becton Dickinson, Bedford, MA), and the cells were cultured for another 4 days. The culture medium was then collected, concentrated 100-fold, and dialyzed against 100 mM sodium cacodylate buffer (pH 6.0) as described previously (*13*).

Sialyltransferase Assay Using Glycolipids as Acceptors—The sialyltransferase assay was performed in a mixture containing 100 mM sodium cacodylate buffer, pH 6.0, 10 mM $MgCl_2$, 0.3% Triton CF-54, 0.64 mM CMP-NeuAc (Sigma), 4,500 dpm/μl CMP-[^{14}C]NeuAc (Amersham Pharmacia Biotech), the enzyme solution (10 μg as protein), 5 μg of acceptors, 8 mM 2,3-dehydro-2-deoxy-*N*-acetyl-neuraminic acid as ganglioside neuraminidase inhibitor in a total volume of 25 μl. The reaction mixture was incubated at 37°C for 2 h. The products were isolated using a C_{18} Sep-Pak cartridge (Waters, Milford, MA) and analyzed by TLC with a solvent system of chloroform/methanol/0.2% $CaCl_2$ (55:45:10). The radioactivity on each plate was visualized with a BAS 2000 image analyzer (Fuji Film, Tokyo, Japan). For kinetic analysis, incubation was performed using various concentrations of the acceptor substrate GM1b (0–0.2 mM).

Sialyltransferase Assay Using Glycoproteins as Acceptors—The sialyltransferase assay was performed in a reaction mixture as described above except that 25 μg of acceptors were added. The reaction mixture was incubated at 37°C for 2 h, and the reaction was terminated by the addition of 3 μl of trichloroacetic acid. The precipitates obtained were washed twice with 1ml of ethanol and dried up. The precipitates were then dissolved in 50 μl of 0.2% SDS and processed for scintillation counting.

Digestion of Sialylated Glycoproteins with Glycopeptidase F (PNGase F)—To remove *N*-glycans, glycoproteins were digested with glycopeptidase F (TaKaRa, Japan). ^{14}C-labeled samples were digested with PNGase F according to the manufacturer's instructions. In brief, sialylated samples were denatured in PNGase F-denaturing buffer (0.5% SDS, 1% 2-mercaptoethanol, 0.5 M Tris-HCl, pH 8.6) by incubation for 3 min at 100°C. The samples were mixed with 5 μl of 5% NP-40, 26 μl of H_2O, and 1 mU of PNGase F, and then samples were digested for 18 h at 37°C. Subsequently, the reaction solution was diluted with SDS/PAGE sample buffer, and SDS/PAGE was performed in 10% (w/v) acrylamide gel. The radioactive bands were visualized and quantified with a BAS 2000 image analyzer (Fuji Film, Tokyo, Japan).

Analysis of hST6GalNAc III Gene Expression—The expression levels of the hST6GalNAc III gene in human tissues was examined by Northern blotting using Human Multiple Tissue Northern (MTN™) Blot (CLONTECH). It was hybridized with a [^{32}P]dCTP-labeled ST6GalNAc III cDNA probe as previously described (*6*).

RESULTS

Isolation of Human ST6GalNAc III cDNA—Using the human expressed sequence tag database, we identified a sequence (GeneBank™ accession number NM_152996)

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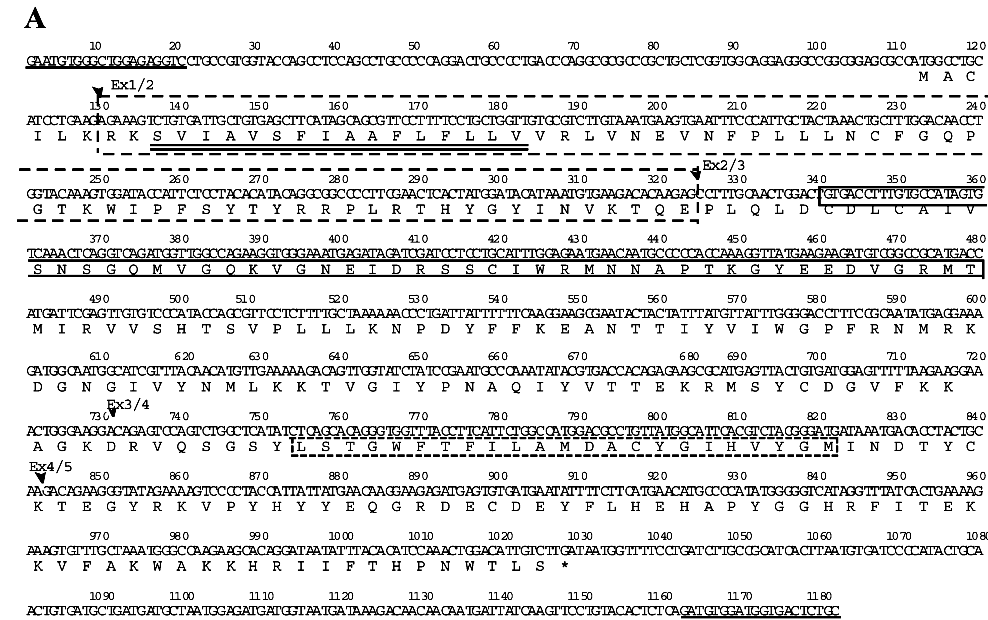
A



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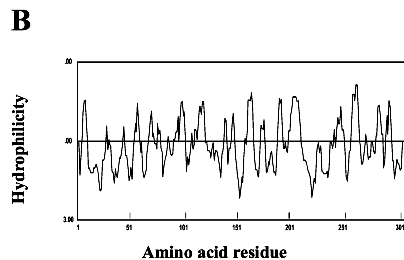

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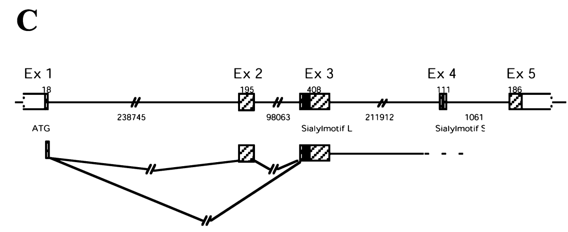

D

Sialylmotif L

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hST6GalNAc III    77  C-DLCAIVSNSGQMVGQKVGNEIDRSSCIWRMN-NA-PTKGYEEDVGRMT  123
hST6GalNAc IV     73  CR-SCAVVSSSGQMLGSGLGAEIDSAECVFRMN-QA-PTVGFEADVGQRT  119
hST6GalNAc V      93  CR-DCALVTSSGHILHSRQGSQIDQTECVIRMN-DA-PTRGYGRDVGNRT  139
hST6GalNAc VI    105  C-HQCVIVSSSSHLLGTKLGPEIERAECTIRMN-DA-PTTGYSADVGNKT  151
hST6GalNAc I     362  CI-TCAVVGNGGILNNSHMGQEIDSHDYVFRL-SGALI-KGYEQDVGTRT  408
hST6GalNAc II    148  CI-RCAVVGNGGILNGSRQGPNIDAHDYVFRL-NGAVI-KGFERDVGTKT  194
hST3Gal I        139  CR-RCAVVGNSGNLRESSYGPEIDSHDFVLRMNKGAPTAHGFEADVGTKT  185
hST6Gal I        181  -WGRCAVVSSAGSLKSSQLGREIDDHDAVLRFN-GAPTAN-FQQDVGTKT  227
hST8Sia I        135  -LKKCAVVGNGGILKKSGCGRQIDEANFVMRCN-LAPLSSEYTKDVGSKS  182
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Sialylmotif S

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hST6GalNAc III   215  LSTGWFTFLLAMDACYGIHVVGM  237
hST6GalNAc IV    211  LSTGWFTMILALELCEEIVVVGM  233
hST6GalNAc V     231  LSTGWFTMTIALELCDRINVVGM  253
hST6GalNAc VI    242  LSTGWFTMVIAVELCDHVHVVGM  264
hST6GalNAc I     520  PTTGALLLLTALQLCDQVSAVGF  541
hST6GalNAc II    303  PSTGALMLLTALHICDQVSAVGF  325
hST3Gal I        267  PSTGILSVIFSMHVCDEVDLYGF  289
hST6Gal I        321  PSSGMLGIIIMMILCDQVDIYEF  343
hST8Sia I        273  LSTGLFLVSAALGLCEEVAIYGF  295
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Fig. 1. **Nucleotide and deduced amino acid sequences, hydropathy plot of human ST6GalNAc III (long form), a schematic diagram of the genomic organization, and comparison of sialyl motif L and sialyl motif S.** A: The putative transmembrane domain of human ST6GalNAc III is double underlined. Sialyl motifs L and S are boxed in solid and dashed lines, respectively. The arrowheads indicate exon junctions. The position of the primers used for cloning are indicated by underlining. The region boxed in the bold dashed line does not exist in the short form of human ST6GalNAc III. B: Hydropathy profile of human ST6GalNAc III determined according to Hopp and Woods. C: Shaded boxes represent coding sequences and open boxes represent untranslated sequences of the human ST6GalNAc III gene. Exons are denoted Ex 1 to 5 with their sizes (in bp) indicated above the boxes, and the intron sequences, determined from genomic databases, are shown by the solid lines with their sizes (in bp) indicated below. The sizes of Ex 1 and 5 refer to the coding regions only. A schematic representation of the 5′ end of the two transcripts is shown below the gene. D: The human sialyltransferase motifs are grouped by the linkages that they form. The sequences are from ST6GalNAc III (this publication), ST6GalNAc I/II/IV/V/VI, ST3Gal I, ST6Gal I, and ST8Sia I. Highly conserved amino acids among many sialyltransferases are boxed in black. Gray boxes indicate conserved amino acids residues among ST6GalNAc III/IV/V/VI.

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with similarity to mouse ST6GalNAc III. Two corresponding cDNA fragments were obtained by RT-PCR using a human brain cDNA library. These two cDNA clones seemed to be derived based on the alternative splicing. The nucleotide sequence of the longer form predicted the cDNA to contain an open reading frame encoding a protein of 305 amino acids (Fig. 1A). Hydropathy of the predicted protein sequence suggested that the protein has the structural organization of a membrane protein with type II topology, which is commonly detected in glycosyltransferases. A hydrophobic segment with 16 amino acids is present near the amino terminus. This putative signal anchor sequence would place 9 amino acid residues within the cytosolic compartment and 280 amino acids within the Golgi lumen as a catalytic domain (Fig. 1B). The nucleotide sequence of the other clone predicted a shorter form containing 240 amino acids lacking the transmembrane portion. Both forms of the newly cloned sialyltransferase possessed two typical common regions present among sialyltransferases cloned so far, *i.e.* sialyl motif L and sialyl motif S (Fig. 1C). The longer clone is composed of 5 exons sharing a common genomic

Fig. 2. **Multiple amino acid sequence alignment of rat, mouse and human ST6GalNAc IIIs by the ClustalW program.** The sequences are of rat ST6GalNAc III, mouse ST6GalNAc III and human ST6GalNAc III (this article). The L and S sialyl motifs are boxed in black and gray, respectively.

structure among ST6GalNAc IV/V/VI (Ex1: 4–6 a.a., Ex2: 59–82 a.a., Ex3: 136 a.a., Ex4: 37 a.a, Ex5: 61–75 a.a.) (*14*). A comparison of the primary structure of the identified cDNA and those of other human sialyltransferases in the sialyl motif L and sialyl motif S regions is shown (Fig. 1D). The predicted amino acid sequence of this cDNA shows higher amino acid sequence identity to human ST6GalNAc IV, ST6GalNAc V and ST6GalNAc VI (57, 46 and 53% for sialyl motif L and 65, 60 and 60% for sialyl motif S, respectively) than to ST6GalNAc I and II, or other ST families. Even the identity in the whole a.a. sequence between ST6GalNAc III and ST6GalNAc IV–VI is 34–41%, indicating that these four family members are closely related. Alignments of human, mouse and rat orthologs indicated high homologies, *i.e*. 95% identity between rat and mouse, 86% between human and mouse, and 85% between human and rat (Fig. 2).

Expression of the Human ST6GalNAc III Gene—To examine the expression pattern and the size of the hST6GalNAc III mRNA, Northern blotting was performed (Fig. 3). The expression of hST6GalNAc III was restricted to kidney and brain, showing a single band at 3.2 kb.

Sialyltransferase Activity of the Cloned cDNA Product—To analyze the sialyltransferase activity of human ST6GalNAc III, the expression vector of the cloned cDNA, pcDNA 3.1-hST6GalNAc III, was transfected into L cells by the DEAE-dextran method, and the extracts were used for sialyltransferase assay using CMP-[^{14}C]NeuAc as a donor. The enzyme utilized GM1b almost exclusively, and no other gangliosides were significantly utilized as an acceptor. The apparent K_m value for GM1b was 0.02 mM. The relative incorporation rates to various acceptors are summarized in Table 1. The incorporation of [^{14}C]NeuAc toward sialyl lactotetraosylceramide was

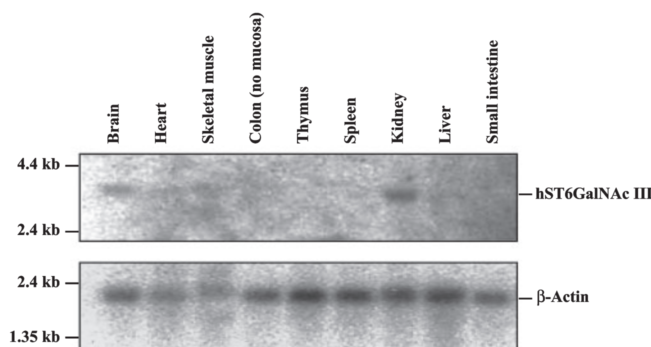

Fig. 3. **Expression pattern of human ST6GalNAc III gene in various human tissues.** Northern blot analysis was carried out using a commercially prepared membrane as described in Materials and Methods. They were hybridized with a [^{32}P]dCTP-labeled ST6GalNAc III cDNA probe. After the dehybridization, the same filter was re-probed with a human β-actin cDNA probe.

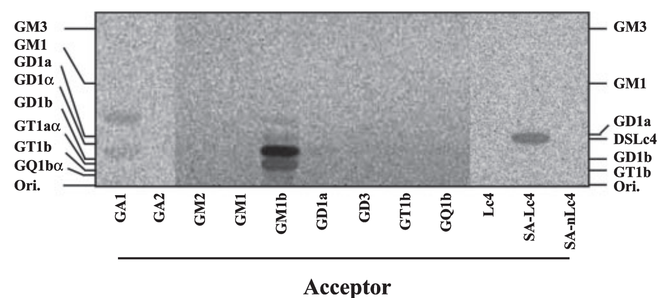

Fig. 4. **Acceptor specificities of human ST6GalNAc III.** Five micrograms each of glycolipid was used as an acceptor in the enzyme assay with hST6GalNAc III expressed in L cells transfected with the cDNA. Radiolabeled sialylated products resulting from the hST6GalNAc III activity were purified on C_{18}-Sep-Pak columns and further separated by HPTLC. Acceptors used are indicated in the figure.

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Table 1. **Acceptor substrate specificity of ST6GalNAc III**[a]

Acceptor	Structure	Relative rate (%)[b]
Fetuin	NeuAcα2,3Galβ1,3GalNAc-Ser/Thr	58
	NeuAcα2,3Galβ1,3(NeuAcα2,6)GalNAc-Ser/Thr	
	NeuAcα2,6(3)Galβ1,4GlcNAc-R[c]	
Asialo-fetuin		3.2
α^1-acid glycoprotein	NeuAcα2,6(3)Galβ1,4GlcNAc-R	0
GA2	GalNAcβ1,4Galβ1,4Glcβ1-Cer	0
GA1	Galβ1,3GalNAcβ1,4Galβ1,4Glcβ1-Cer	3.6
GM1b	NeuAcα2,3Galβ1,3GalNAcβ1,4Galβ1,4Glcβ1-Cer	100[d]
GM2	GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glcβ1-Cer	0
GM1	Galβ1,3GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glcβ1-Cer	0
GD1a	NeuAcα2,3Galβ1,3GalNAcβ1,4(NeuAcα2,3)Galβ1,4Glcβ1-Cer	3.8
GD3	NeuAcα2,8NeuAcα2,3Galβ1,4Glcβ1-Cer	0
GT1b	Galβ1,3GalNAcβ1,4(NeuAcα2,8NeuAcα2,3)Galβ1,4Glcβ1-Cer	0
GQ1b	NeuAcα2,8NeuAcα2,3Galβ1,3GalNAcβ1,4(NeuAcα2,8NeuAcα2,3)Galβ1,4Glcβ1-Cer	0
Lc4	Galβ1,3GlcNAcβ1,3Galβ1,4Glcβ1-Cer	0
Sialyl Lc4	NeuAcα2,3Galβ1,3GlcNAcβ1,3Galβ1,4Glcβ1-Cer[e]	6
Sialyl Le[a]	NeuAcα2,3Galβ1,3(Fucα1,4)GlcNAcβ1,3Galβ1,4Glcβ1-Cer	0

[a]Various acceptor substrates were incubated in the standard assay mixture using the membrane fraction of cells transfected with ST6GalNAc III as an enzyme source. Each substrate was used at a concentration of 0.1 mM for glycolipids and 0.4 mg/μl for glycoproteins. [b]Relative rates are calculated as a percentage of the incorporated NeuAc to that onto GM1b. [c]R represents the remainder of the *N*-linked oligosaccharide chain. [d]7.0 nmol/h/μl of an enzyme solution. [e]Ceramide mimic $-CH_2(C_{14}H_{29})_2$ are chemically bonded to the oligosaccharides in stead of ceramide.

less than that toward GM1b. Furthermore, this enzyme showed activity toward fetuin, which is a glycoprotein with both *N*-glycans and *O*-glycans.

Substrate Specificity of Cloned ST6GalNAc III—We analyzed the sialyltransferase activity for various glycolipids and glycoproteins. Previously, we reported that hST6GalNAc V and VI show sialyltransferase activity for sialyl Lc4, leading to the synthesis of disialyl Lc4 (and disialyl Le[a]). As shown in Fig. 4, it was found that hST6GalNAc III also synthesizes disialyl Lc4 by utilizing sialyl Lc4 as a substrate. This identity was confirmed by its migration just below GD1a on TLC.

To investigate the activity toward glycoproteins, various glycproteins were incubated with the expressed sialyltransferase, and the reaction products were analyzed by SDS-PAGE/autoradiogram. Among glycoproteins, sialic acids were transferred onto fetuin, but not onto asialofetuin, BSA, or α_1-acid glycoprotein by the action of this enzyme.

Effects of N-Glycanase Treatment on [^{14}C]NeuAc-Labeled Fetuin with hST6GalNAc III—Fetuin contains both *O*-glycans and *N*-glycans. To examine the preference of this enzyme for these glycans, we performed *N*-glycanase treatment on [^{14}C]NeuAc-incorporated fetuin with hST6GalNAc III. *N*-Glycanase (glycopeptidase F) releases *N*-linked oligosaccharides from glycoproteins. When the sialylated product was treated with *N*-glycanase, most of the incorporated sialic acids were still

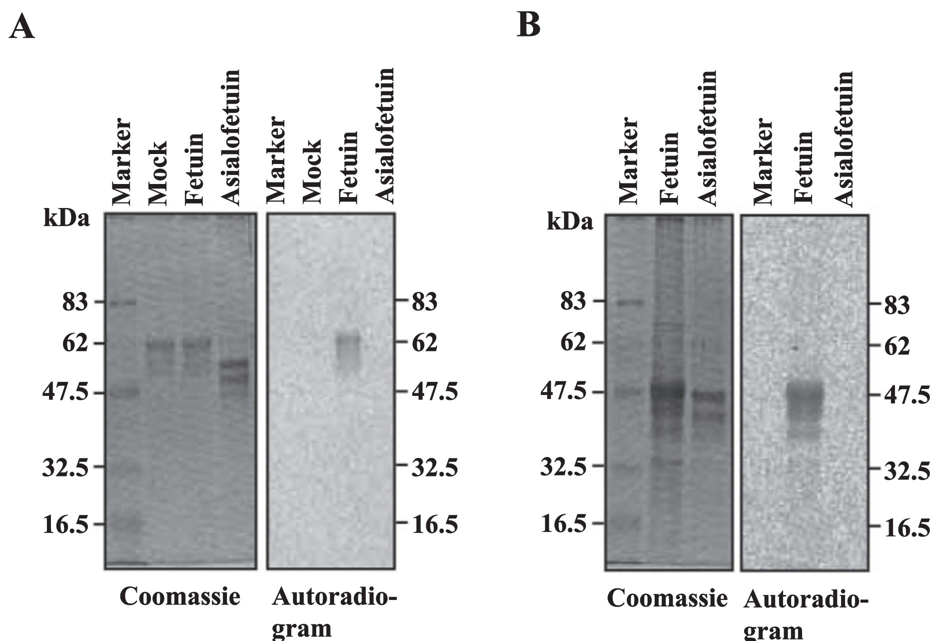

Fig. 5. **Glycanase F treatment of the enzyme products to determine the glycans into which sialic acid is incorporated.** The sialylated products of fetuin and asialo-fetuin were subjected to SDS–polyacrylamide gel electrophoresis (A, left), and radioactivity was detected with a BAS2000 radioimage analyzer (Fuji Film) (A, right). After Glycanase F treatment, the resulting products were separated by SDS-PAGE (B, left), and the radioactive bands were detected by autoradiography (B, right).

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detected as shifted bands (Fig. 5B, right), indicating that they were not released from the protein. This result suggests that hST6GalNAc III preferentially acts on *O*-glycans (but not *N*-glycans) in fetuin (Fig. 5).

DISCUSSION

Although there have been a number of sialyltransferases cloned so far, many of them play roles in the substitution of terminal portions of carbohydrate structures with sialic acids. In contrast, ST6GalNAc III–ST6GalNAc VI catalyze sialylation onto inside GalNAc residues in ganglio-series and GlcNAc residues in lacto-series glycosphingolipids (*5–7*, *9*). We previously demonstrated that ST6GalNAc V/VI transfer sialic acid to GlcNAc residues in lacto-series glycosphingolipids using a corresponding antibody (*9*). Sialyltransferases require the presence of a sialic acid on galactose at the non-reducing end of the carbohydrate structures. Eventually, they form branched type disialyl-structures regardless of the core structures.

ST6GalNAc III was first isolated from a rat brain cDNA library (*10*), and then from a mouse cDNA library (*5*) by PCR using degenerative primer sets. In adult rat tissues, ST6GalNAc III was found to be expressed in spleen, kidney, lung and brain in this order. It was more strongly expressed in both newborn brain and kidney (*10*). In adult mice, ST6GalNAc III was found to be expressed strongly in brain, lung and heart, and weakly in kidney, mammary gland, spleen, thymus and testis (*5*). Therefore, the expression patterns of ST6GalNAc III differ among species, and expression in human tissues appears more restricted than in rat and mouse. In both rat and mouse, the ST6GalNAc III gene is expressed during the developmental stages of the brain, suggesting this might be also the case in humans.

Disialyl-glycosphingolipids have been considered to have some implications in the malignant properties of various human cancers. Disialyl Le^a was reported to be a possible serum marker in colon cancer patients (*8*). In human renal cancers, disialyl Lc4 and GalNAc-disialyl Lc4 have also been claimed to be associated with malignant transformation and metastatic potential (*15*). If sialyltransferases and genes responsible for the synthesis of these disialyl-structures are identified, this information would strongly promote studies on the roles of the disialyl-carbohydrate structures in human cancers and in physiological processes such as tissue development.

During the cDNA cloninig of human ST6GalNAc III, we isolated a short form of the cDNA lacking the transmembrane portion. Since the product of this short form cDNA was expected to be secreted as a soluble enzyme in the culture medium of transfectant cells, we tried to measure enzyme activity in the supernatant of transfectant cells, but none could be detected. The short form might act to inhibit of the long form of the enzyme. This issue remains to be clarified.

Recently, sialic acid-reactive proteins, named the siglec family, have been identified (*16*). In particular, siglec-7 is detected in natural killer cells and macrophages, and it has been reported to act specifically on disialyl-carbohydrates, such as GD3, GD1b, GD2, as well as disialyl Le^a and disialyl Lc4 (*17*). The cytoplasmic portion of siglec-7 contains an interesting motif named ITIM (immunoreceptor tyrosine-based inhibitory motif) that transduces inhibitory signals. Therefore, the expression of disialyl-glycosphingolipids derived from the action of ST6GalNAc III might have implications in the protection of expressing cells from NK cell attack. Whether ST6GalNAc III is really involved in the synthesis of disialyl-glycosphingolipids in human cancer cells is quite interesting, and now under investigation in our laboratory.

The NIH Image program was developed at the National Institutes of Health and is available through the internet by anonymous FTP from ftp://zippy.nimb.gov or on a floppy disk from the National Technical Information Service, Springfield, VA (part no. PB95-500195GED). We thank Ms. T. Mizuno and Ms. M. Nakayasu for technical assistance. This study was supported by the New Energy and Industrial Technology Development Organization, and by Grants-in-aid for the Center of Excellence Research (*10*CE2006), Scientific Research B (13470021), and Scientific Research on Priority Areas C (12670111 and 14013028) from the Ministry of Education, Science, Sports, and Culture of Japan.

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