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Molecular Cloning and Functional Expression of a Fifth-Type α2,3-sialyltransferase (mST3Gal V: GM3 synthase)[1]

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The cDNA encoding a new type of α2,3-sialyltransferase (mST3Gal V) was cloned from mouse brain cDNA library by PCR-based cloning approach using a pair of degenerate primers deduced from the nucleotide sequence information of mouse ST3Gal III and IV. The predicted amino acid sequence of mST3Gal V showed 27.3% and 26.4% identity to mST3Gal III and IV, respectively. The recombinant soluble mST3Gal V fused with protein-A, which expressed in the culture media of COS-7 cells, showed activity toward lactosylceramide (LacCer), and synthesized GM3. The apparent Km value for LacCer was 9.3 μM. mST3Gal V did not exhibit any activity toward other substrates we tested in this study, including glycoproteins and disaccharides. The mST3Gal V cDNA transfected F28-7 cells, which express large amount of lactosylceramide and very small amount of GM3 at native stage, expressed a large amount of GM3. The ST3Gal V gene was strongly expressed in mouse brain and liver, which contained a large amount of ganglioside. The gene expression seemed to be coincident with ganglioside expression in mouse. Thus, we conclude that mST3Gal V is the fifth-type α2,3-sialyltransferase carrying GM3 synthetic activity. © 1998 Academic Press

Key Words: **molecular cloning; gene expression; GM3 synthase; sialyltransferase.**

Gangliosides are a family of glycosphingolipids which contain sialic acid, and their structures are highly diversified. The gangliosides are widely detected at plasma membrane of higher vertebrates, especially in neural cells (3). In the past twenty years, evidences that gangliosides are functionally expressed have been accumulated, particularly in the case of cell differentiation, proliferation, adhesion and oncogenesis (4). For example, GQ1b ganglioside enhances outgrowth of neuroblastoma, GOTO and NB-1 cells (5); GM3 ganglioside induces HL-60 differentiation toward macrophage-like cells (6,7); GM1 or GM3 ganglioside inhibits the cellular proliferation through the decrease of phosphorylation activity of PDGF receptor of 3T3 cells and EGF receptor of A431 cells (8-10); oncogenesis changes cellular glycosphingolipid pattern.

Ganglioside is synthesized at the golgi apparatus. The biosynthesis is initiated from lactosylceramide and progresses with the stepwise addition of galactose, N-acetylgalactosamine and sialic acid. GM3 synthesis is the first step for ganglioside biosynthesis and all gangliosides are synthesized from GM3 by linkage specific glycosyltransferases (11-13). In recent years, the cloning of cDNAs encoding glycosyltransferases have been intensively progressed. Almost all glycosyltransferases involving ganglioside biosynthesis are successively cloned, ex-

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The nomenclature for gangliosides follows the system of Svennerholm (1). The abbreviated nomenclature for cloned sialyltransferases follows the system of Tsuji et al. (2). The abbreviations used are PCR, polymerase chain reaction; bp, base pair; kb, kilobase; BSM, mucin from bovine submaxillary gland; HPTLC, high performance thin layer chromatography; SDS-PAGE, polyacrylamide gel electrophoresis with an SDS-containing buffer system; G3PDH, glyceraldehyde 3-phosphate dehydrogenase gene; NeuAc, N-acetylneuraminic acid; CMP-NeuAc, cytidine 5′-monophospho-N-acetylneuraminic acid; SSC, saline-sodium citrate solution; SLS, sodium lauryl sarcosine.

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cept for GM3 synthase. To clarify the physiological function of gangliosides, it is necessary to clone, express and target GM3 synthase. Based on this consideration, we tried to clone the new type of sialyltransferase cDNA carrying GM3 synthetic activity, using the PCR-based approach. In order to clone GM3 synthase, which catalyzes α2-3 sialyltransfer to lactosylceramide (Galβ1-4Glc-cer), the sequence information of mouse ST3Gal III and IV, which exhibit α2-3 sialyltransfer activity toward Galβ1-4GlcNAc structure, was used to construct a pair of primers. Here, we describe the molecular cloning, gene expression and acceptor substrate specificity of newly cloned sialyltransferase.

MATERIALS AND METHODS

Materials

Some glycosphingolipids (galactosylceramide, lactosylceramide, asialo-GM1, GM1, GD1a and GM3), glycoproteins (fetuin, asialofetuin, mucin from bovine submaxillary gland, asialomucin, α1-acid glycoprotein), disaccharides (Galβ1-3GlcNAc (Type I), Galβ1-4GlcNAc (Type II), Galβ1-3GalNAc (Type III) and Galβ1-4Glc (lactose)) and Triton-CF-54 were purchased from Sigma, paragloboside was from Wako Co. (Japan), CMP-[^{14}C]NeuAc (11.8 GBq/mmol) was from Amersham Corp, IgG-Sepharose was from Pharmacia, LipofectAMINE and Macrophage-SFM Medium were from GIBCO BRL, NANase I, II and III were from GLYCO (USA), pRc/CMV vector was from Invitrogen. Asialo-α1-acid glycoprotein was prepared by digestion of α1-acid glycoprotein with Vibrio cholerae sialidase. F28-7 cells were kindly provided by Dr. Hakomori (14).

Polymerase chain reaction (PCR) cloning with degenerate oligonucleotides. PCR was performed with degenerate primers (5′-primer N1Q1-L, 5′-CGCTG(C/T)GTTITNGTIGGNAATGG-3′; 3′-primer, N1Q1-S, 5′-(A/G)TCATAGCCAAAGCC(A/C)G-3′) deduced from conserved regions, sialylmotifs L and S in mouse ST3Gal III and IV. The cDNA synthesized from mRNA of adult mouse livers was used as a template for PCR. The cycling parameters for PCR were 94°C for 40 sec, 37°C for 40 sec, and 72°C for 1 min for the first 5 cycles, and 94°C for 40 sec, 55°C for 40 sec, and 72°C for 1 min for the following 35 cycles. The 0.5-kb PCR products were blunt-ended, kinated and then subcloned into the *Eco*RV site of pBluescript SK+ (Stratagene). The subclones were characterized by sequencing. Approximately 10^6 plaques of a adult mouse brain cDNA library were screened with the 0.5-kb-PCR fragment. Standard molecular cloning techniques described by Maniatis et al. were used (15).

Preparation of soluble mST3Gal V fused with protein A. Truncated form of mST3Gal V, lacking the first 60 amino acids from the deduced initiation methionine, was prepared by PCR amplification using isolated cDNA clone (mST3Gal V) as template with 5′-primer containing an *Eco*RI site and 3′-primer containing a *Xho*I site, i.e., 5′-primer M1-Sec, 5′-GAA***GAATTC***GACATGAAAAGAATG-3′ (nucleotides 184-207) and 3′-primer M1-Tail, 5′-CAG***CTCGAG***GGT-GAAGTCCCTGTG-3′ (complementary to the mST3Gal V coding strand nucleotide 1300-1323). The resulting amplified 1140-bp fragment was blunt-ended, kinated and then subcloned into the *Eco*RV site of pBluescript SK+. Then inserted fragment was cut out by digestion of *Eco*RI and *Xho*I and inserted into the *Eco*RI and *Xho*I sites of expression vector pcDSA (16). The insert junctions were confirmed by restriction enzyme and DNA sequencing. The resulting plasmid consisted of the IgM signal peptide sequence, the protein A IgG binding domain and the N-terminal truncated form of mST3Gal V. Expression plasmid (20 μg) was transiently transfected into COS-7 cells on a 150-mm plate using LipofectAMINE reagent. After 5 h and 24 h of transfection, the cell culture media were changed to Dulbecco's Modified Eagle's Medium containing 2% fetal calf serum followed by Macrophage-SFM Medium. The fusion protein of protein A and N-terminal truncated mST3Gal V expressed in media was absorbed to IgG-sepharose gel (50 μl of resin / 50 ml of culture media) at 4°C for 16 h. The fusion protein absorbed to IgG-Sepharose gel was used as the enzyme source.

Sialyltransferase assay and product characterization. The reaction mixture comprising 0.125 M MES buffer (pH 6.4), 12.5 mM $MgCl_2$, 2.5 mM $CaCl_2$, 0.38% Triton CF-54, 0.25 mM CMP-[^{14}C]NeuAc (900 Bq) and different amounts of glycosphingolipid, glycoprotein or disaccharide in total volume of 16 μl was preincubated at 37°C for 10 min. The four microlitter suspension of enzyme bound IgG-Sepharose gel was added to the prewarmed reaction mixture. The final concentration of MES, $MgCl_2$, $CaCl_2$, Triton CF-54, CMP-[^{14}C]NeuAc in the reaction mixture were 0.1 M, 10 mM, 2 mM 0.3% and 0.2 mM, respectively. Each reaction mixture was incubated at 37°C for an appropriate time with gentle agitation using a microtube mixer (MT-360; TOMY, Japan). In the case of glycosphingolipid substrates, the reaction was terminated by the addition of 200 μl Dulbecco's PBS (D-PBS). For the separation of [^{14}C]NeuAc conjugated glycosphingolipids and donor substrate CMP-[^{14}C]NeuAc, the reaction mixtures were applied on C-18 columns (Sep-Pak Vac, 100 mg; Waters, USA), which had been washed with water and 0.1 M KCl. The C-18 columns were then washed with water and the glycosphingolipids were eluted with methanol. The methanol solutions were dried up, and then the residues were subjected to HPTLC with two solvent systems of chloroform : methanol : 0.2% $CaCl_2$ = 55:45:10 and chloroform : methanol : 2.5 N NH_4OH = 60:40:9. When glycoprotein substrates were used, the reaction was terminated by the addition of 10 μl of 3 times loading buffer for SDS-PAGE. The reaction mixtures were applied on SDS-PAGE (5-20% acrylamide gel) to separate [^{14}C]NeuAc conjugated glycoproteins from CMP-[^{14}C]NeuAc. For the separation of [^{14}C]NeuAc conjugated disaccharides and CMP-[^{14}C]NeuAc, the reaction mixtures were subjected to HPTLC with a solvent system of ethanol : pyridine : 1-buthanol : acetate : water = 100:10:10:3:30. The radioactive materials were visualized with a BAS 2000 radio image analyzer (Fuji Film, Japan).

For linkage analysis, [^{14}C]NeuAc was incorporated to lactosylceramide using the fusion protein of protein A and N-terminal truncated mST3Gal V expressed in media of mST3Gal V transfected COS-7 cells. [^{14}C]NeuAc incorporated to lactosylceramide was digested with linkage-specific sialidases, NANase I (specific for α2,3-linked sialic acids), NANase II (specific for α2,3-, α2,6-linked sialic acids) and NANase III (specific for α2,3-, α2,6-, α2,8-linked sialic acids). After digestion, desialylated glycolipid was analyzed by HPTLC with two solvent systems of chloroform : methanol : 0.2% $CaCl_2$ = 55:45:10 and chloroform : methanol : water = 65:25:4.

Construction of the stable transfectants of mST3Gal V into F28-7 cells. The protein coding region of mST3Gal V (nucleotides -16-1323) was amplified by PCR using isolated cDNA clone (mST3Gal V) as template with 5′- and 3′-primers i.e., 5′-primer M1-ATG, 5′-ACGCGGCGCTGCGGCAATGCCA-3′ (nucleotides -16-6) and 3′-primer M1-Tail (described above). The resulting amplified 1339-bp fragment was blunt-ended, kinated and then subcloned into the *Eco*RI site of pBluescript SK+. The inserted fragment was cut out by digestion of *Not*I and *Apa*I and inserted into the *Not*I and *Apa*I sites of expression vector pRc/CMV. Expression plasmid (10 μg) was transfected into F28-7 cells by electroporation (Gene pulser, BIO-RAD). After 48 h of transfection, the 5000 cells were divided into each whole of 96 culture plate and 0.3 mg/ml of G418 was added to each whole. The G418 resistant colonies were proliferated and the gangliosides were extracted. The ganglioside was analyzed by HPTLC and detected by resorcinol reagent.

Analysis of ST3Gal V gene expression. Messenger RNA (10 μg) was fractionated on a denaturing formaldehyde-agarose gel (1%) and

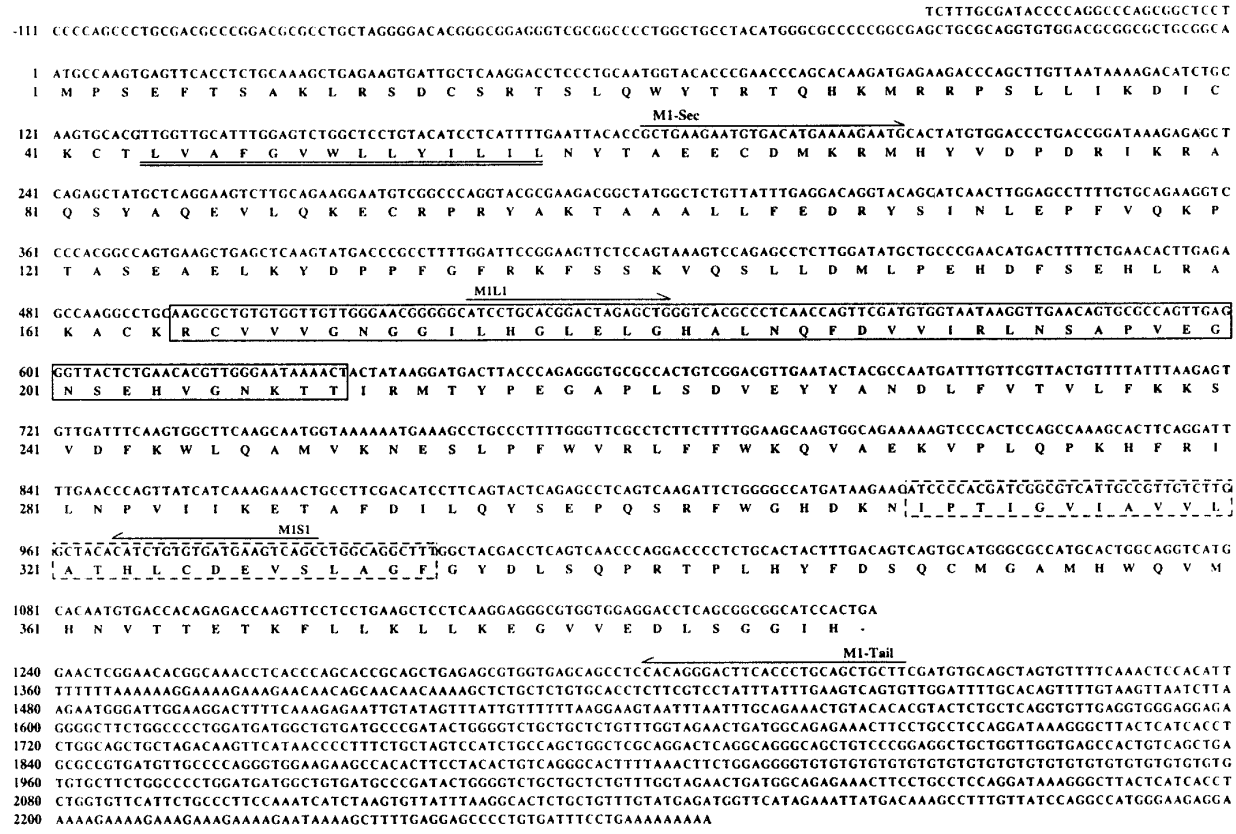

FIG. 1. Nucleotide and deduced amino acid sequences of mouse ST3Gal V. The nucleotide and amino acid sequences of mouse ST3Gal V are numbered from the presumed start codon and initiation methionine, respectively. The double underlined amino acids are correspond to a putative transmembrane domain. Sialylmotifs L and S are boxed by solid and dashed lines, respectively. The positions of the PCR primers are indicated by arrows.

then transferred to a nylon membrane (Nytran; Schleicher & Schuell, Germany). The membrane was prehybridized in a solution comprising of 50% formamide, 5 × SSC, 5 × Denhart's, 1% SLS and 100 μg/ml denatured salmon sperm DNA at 37°C for 1 h. Hybridization was performed overnight at 37°C with a ^{32}P-labeled DNA probe made by the random priming method. The 0.5-kb-PCR fragment was used as a probe. The membrane was washed twice in 2 × SSC, 0.1% SDS at 65°C, and finally in 0.5 × SSC, 0.1% SDS at 65°C.

RT-PCR (Reverse transcripts-PCR) was performed with cDNAs synthesized from mRNAs (1 μg) of various cell lines (F11, P19, neuronal differentiated P19, Neuro2a, B16, LLL). Primers used for RT-PCR were 5′- and 3′-primers of ST3Gal V and G3PDH; 5′-primer for ST3Gal V, M1L1, 5′-ATCCTGCACGGACTGAGCTC-3′ (nucleotides 523-543); 3′-primer for ST3Gal V, M1S1, 5′-GCTGACTTC-ATCACACAGATG-3′ (complementary to the ST3Gal V coding strand nucleotide 967-987); 5′-primer for G3PDH 5′-ACCACA-GTCCATGCCATCAC-3′ (nucleotides 526-547); 3′-primer for G3PDH, 5′-TCCACCACCCTGTTGCTGTA-3′ (complementary to the G3PDH coding strand nucleotide 958-977) (17). The cycling parameters for PCR of ST3Gal V and G3PDH were 94°C for 40 sec, 55°C for 40 sec, and 72°C for 1 min for 30 and 14 cycles, respectively.

RESULTS

Cloning and Nucleotide Sequencing of a New Type of Sialyltransferase cDNA

Recently, four types of α2,3-sialyltransferase cDNAs were cloned from mouse (18-20). To isolate the cDNA encoding a new type of sialyltransferase, which exhibits α2,3-sialyltransfer activity toward Galβ1-Glc structure, we conducted PCR cloning experiments with two degenerate primers based on two highly conserved regions, sialylmotifs L and S, of mouse ST3Gal III and IV exhibiting α2,3-sialyltransfer activity toward Galβ1-4GlcNAc (20). The PCR product corresponding to a 0.5-kb fragment was subcloned and sequenced. Among several clones, one clone pCRM1, encoded a peptide exhibiting 53.2% and 48.0% identity to mouse ST3Gal III and IV, respectively. This clone was used for further investigation.

pCRM1 was used as the probe to screen the adult mouse brain cDNA library. Several overlapping clones were obtained and sequenced. Among them, one cDNA named M1 encoded a putative protein of 387 amino acids and exhibited type II transmembrane topology (Fig. 1). M1 consisted a NH_2-terminal cytoplasmic tail, a transmembrane domain, a stem region and a large COOH-terminal active domain including two highly conserved regions, sialylmotifs L and S, same as all sialyltransferases so far cloned. The predicted amino acid sequence of M1 exhibited 27.3% and 26.4% identity to mST3Gal III and IV, respectively. M1 turned out

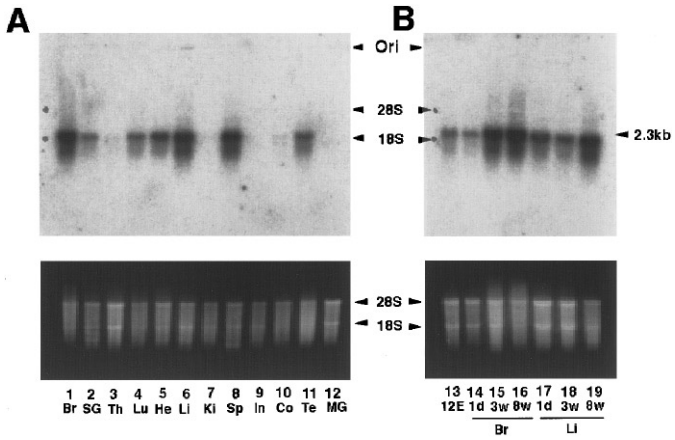

FIG. 2. Expression of the ST3Gal V gene. A, B, Northern blot analysis. Messenger RNAs (10 μg) were prepared from various mouse adult tissues (A) and different stage mouse brains and livers (B): 1, 14-16, brain; 2, salivary gland (SG); 3, thymus; 4, lung; 5, heart; 6, 17-19, liver; 7, kidney; 8, spleen; 9, small intestine; 10, colon; 11, testis; 12, mammary gland (MG); 13, 12-day postcoital mouse embryo. The hybridization probe was made from pCRM1 fragment (519-bp) by the random priming method.

to be another type of α2,3-sialyltransferase, which exhibited the GM3 synthetic activity and it was designated as mST3Gal V.

Expression of the ST3Gal V Gene in Mouse Tissues and Various Cell Lines

The expressions of ST3Gal V gene in several mouse tissues were examined by Northern blot analysis. In adult mouse tissues, a strong band corresponding to 2.3-kb was observed in brain and liver. Moderate levels of signals in heart, spleen, testis and week signals in salivary gland (SG) and lung were detected (Fig. 2A). The expression level of ST3Gal V was not changed from embryonic stage to 8-week-old mouse in the brain and liver (Fig. 2B). The gene expression of ST3Gal V in 6 cell lines were analyzed by RT-PCR. All cell lines we tested expressed ST3Gal V gene (data not shown).

Sialyltransferase Activity of the Enzyme Encoded by M1

To determine the enzymatic activity of the new sialyltransferase, we constructed an expression plasmid, pcDSA-mST3Gal V, which allowed expression of the COOH-terminal portion of mST3Gal V as a secretable protein fused with the IgG-binding domain of *Staphylococcus aureus* protein A (21). The plasmid was then transfected into COS-7 cells, and the protein A-fused mST3Gal V expressed in the media was absorbed to IgG-Sepharose gel, which was used as the enzyme source. In view of the amino acid sequence similarity between ST3Gal V and III, we examined the sialyltransferase activity toward several glycosphingolipids, glycoproteins and disaccharides. [^{14}C]NeuAc was incorporated into lactosylceramide by the activity of mST3Gal V and the product comigrated with authentic GM3 on HPTLC using two solvent systems chloroform : methanol : 0.2% $CaCl_2$ = 55:45:10 (Fig. 3) and chloroform : methanol : 2.5 N NH_4OH = 60:40:9 (data not shown). mST3Gal V did not exhibit any other activity toward glycolipids, glycoproteins and disaccharides we tested (galactosylceramide, paragloboside, asialo-GM1, GM1, GD1a, fetuin, asialofetuin, mucin from bovine submaxillary gland, asialomucin, α1-acid glycoprotein, asialo-α1-acid glycoprotein, type I, II, III disaccharides and lactose) (Table I).

The apparent Km and Vmax values for lactosylceramide were 9.3 μM and 10.2 nmol/h/ml.

For linkage analysis, [^{14}C]NeuAc was incorporated to lactosylceramide by the activity of mST3Gal V and the product was digested with linkage-specific sialidases, NANase I (specific for α2,3-linked sialic acids), NANase II (specific for α2,3-, α2,6-linked sialic acids) and NANase III (specific for α2,3-, α2,6-, α2,8-linked sialic acids). The sialylated lactosylceramide was sensitive for all types of NANase (data not shown). This result strongly suggested that the incorporated sialic acids contained α2,3 linkage.

To examine the *in vivo* activity of mST3Gal V, we established the transformed cells by stable transfection of mST3Gal V gene into F28-7 cells, which express a large amount of lactosylceramide and very small amount of GM3. As shown in Fig. 4, mST3Gal V stable transfected F28-7 cells but not native F28-7 expressed

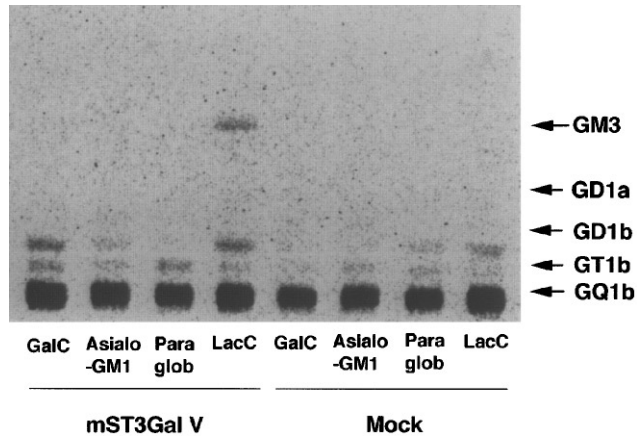

FIG. 3. Incorporation of [^{14}C]NeuAc into glycosphingolipids by cloned sialyltransferase. The glycosphingolipids (0.2 mM) indicated in the panels were incubated with the cloned sialyltransferase (GalC, galactosylceramide; LacC, lactosylceramide, paraglob, paragloboside). The resulting glycosphingolipids were analyzed by HPTLC with a solvent system of chloroform : methanol : 0.2% $CaCl_2$ = 55:45:10. The culture media from pcDSA (Mock) and pcDSA-mST3Gal V (mST3Gal V) transfected COS-7 cells were used as enzyme sources. The radioactive materials were visualized with a BAS 2000 radio image analyzer.

TABLE I

Acceptor Substrate Specificity of mST3Gal V

Acceptor	Relative activity (%)
Galactosylceramide	0
Lactosylceramide	100
Paragloboside	0
Asialo-GM1	0
GM1	0
GD1a	0
Asialofetuin	0
Fetuin	0
AsialoBSM	0
BSM	0
Asialo-α1-acid glycoprotein	0
α1-acid glycoprotein	0
Galβ1-4Glc (lactose)	0
Galβ1-3GlcNAc (type I)	0
Galβ1-4GlcNAc (type II)	0
Galβ1-3GalNAc (type III)	0

Note. The table shows the relative activities as to the incorporation of sialic acids into lactosylceramide as a substrate. Activity of ST3Gal V for lactosylceramide was 10.2 nmol/h/ml Each acceptor substrate was used at concentration of 0.2 mM. A value of 0 indicates less than 0.1%

GM3. Transfection of mST3Gal V gene into F28-7 cells led to the GM3 expression.

DISCUSSION

In this study, we successively cloned the fifth type of α2,3-sialyltransferase (mST3Gal V). We examined its substrate specificity and the gene expression pattern in mice.

mST3Gal V cDNA was cloned by PCR approach with two degenerate primers deduced from the sequence information of mST3Gal III and IV, which exhibit α2,3-sialyltransfer activity toward Galβ1-4GlcNAc. We searched mST3Gal V homologues in dbEST database. Among these sequences, one exhibited 98% identity to mST3Gal V, which should be a human counterpart (EST58727). mST3Gal V exhibited 27.3% and 26.4% identity at the amino acid level to mST3Gal III and IV, respectively. A dendrogram constructed according to Higgins and Sharp (22) revealed that mST3Gal V belongs to the ST3-Gal family, especially closely related to ST3Gal III (data not shown). ST3Gal family is classified into two subfamilies. ST3Gal I and II comprise one subfamily, and III, IV and V the other. mST3Gal V cDNA encoded a protein of 387 amino acids, which contained two highly conserved regions, sialylmotifs L and S, same as 14 other studies so far cloned (23-27). α2,3-sialyltransferases, including the new mST3Gal V, contain the four conserved cysteins (**C**X$^{72\text{-}78}$**C**XR-**C**S^{2}VGNX$^{140-154}$**C**) in their sequences, named as Kurosawa motif (27). This consensus sequence including four cystein may be important to maintain the functional protein conformation.

The following evidence strongly suggests that mST3Gal V is a candidate for GM3 synthase. First of all, the substrate specificity was very restricted to lactosylceramide. The recombinant soluble mST3Gal V fused with protein-A expressed in culture media of COS-7 cells, exhibited activity toward lactosylceramide and the product was comigrated with authentic GM3 on HPTLC. Linkage analysis using linkage-specific sialidases elucidated sialic acids of the product from lactosylceramide were bound with α2,3 linkage. The recombinant soluble mST3Gal V did not show any activity toward other acceptor substrates we tested, including glycolipids, glycoproteins and disaccharides. The Km value for lactosylceramide was very low (9.3 μM). So far, four α2,3-sialyltransferases were cloned from mouse (mST3Gal I-IV). mST3Gal I, II, III and IV showed activities toward Galβ1-3GlcNAc (type I), Galβ1-4GlcNAc (type II) and Galβ1-3GalNAc (type III) structures but most preferable substrates for mST3Gal I, II, III and IV were types III, III, I and II, respectively (20), and did not exhibit activity toward lactose and lactosylceramide. mST3Gal V is the first case exhibiting activity toward lactosylceramide. Secondly, ganglioside expression level was correlated well with the ST3Gal V gene expression. In all the mouse tissues we examined, brain and liver expressed ST3Gal V gene most highly. Interestingly, gangliosides are also exist in great amounts in these two tissues (28). All gangliosides are synthesized through GM3, resumably synthesized by ST3Gal V. In F28-7 cells, large amounts of lactosylceramide and negligible amount of GM3 was detected. The ST3Gal V stable transfected F28-7 cells,

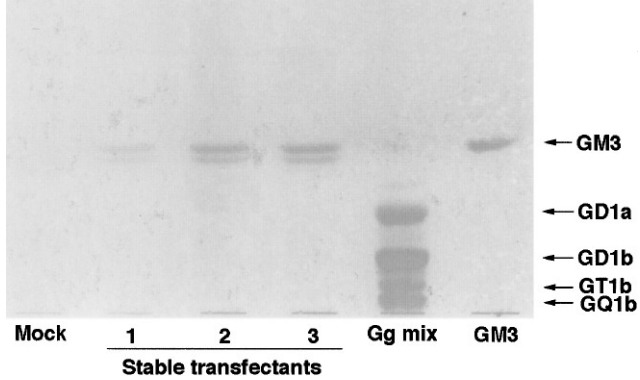

FIG. 4. Ganglioside pattern of stable transfectants of mST3Gal V. The plasmids, Rc/CMV (Mock) and Rc/CMV-mST3Gal V(mST3Gal V), were transfected into F28-7 cells using lipofectAMINE reagent. After 48 h incubation, 0.3 mg/ml G418 (neomycin) was added to the culture media and the 3 resistant colonies (1, 2, 3) for G417 were proliferated. Gangliosides from cells were extracted, separated by HPTLC with a solvent system of chloroform : methanol : 0.2% $CaCl_2$ = 55:45:10 and detected by resorcinol reagent. The standard gangliosides (bovine brain ganglioside(Gg mix), GM3) were applied in the same HPTLC plate.

on the other hand, expressed a large amount of GM3. This result strongly suggested that mST3Gal V acts as GM3 synthase *in vivo*.

We are now studying the transcriptional regulation of ST3Gal V gene. Our preliminary results indicated that three 5′-untranslated regions of ST3Gal V gene may be involved in the regulation of ST3Gal V gene expression in tissue specific manner. The exact mechanism for gene expression is still under investigation. The finding of this new ST3Gal V sialyltransferase, possibly a GM3 synthase, will help us to understand more about the ganglioside synthesis and their regulations.

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