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Short communication

Molecular Cloning and Sequence Analysis of Human GM3 Synthase (hST3Gal V)

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The cDNA encoding CMP-NeuAc:lactosylceramide α2,3-sialyltransferase (GM3 synthase) was isolated from a human fetal brain cDNA library using sequence information obtained from amino acid sequences found in the conserved regions of the previously-cloned mouse GM3 synthase (mST3Gal V) and human sialyltransferases. The cDNA sequence included an open reading frame coding for 362 amino acids, and the primary structure of this enzyme predicted all the structural features characteristic of other sialyltransferases, including a type II membrane protein topology and both sialylmotifs. Comparative analysis of this cDNA with mST3Gal V showed 85% and 86% identity of the nucleotide and amino acid residues, respectively. The expression of this gene is highly restricted in both human fetal and adult tissues.

Keywords: cDNA, Human fetal brain, Sialyltransferase.

Introduction

Gangliosides are a family of sialic acid (NeuAc)-containing glycosphingolipids that are found in high concentration on the central nervous system (Svennerholm, 1980). They play important roles in a variety of biological processes, such as cell-cell interaction, adhesion, cell differentiation, growth control, and receptor function (Varki, 1993). The gangliosides are synthesized via four primary biosynthetic pathways by a family of glycosyltransferases in the Golgi apparatus (Iber *et al.*, 1992). The diversity of ganglioside composition among vertebrates reflects the differential regulation of ganglioside biosynthesis through different pathways. To date, four kinds of sialyltransferase cDNA (ST3Gal I-IV) responsible for ganglioside biosynthesis have been cloned and characterized (Lee *et al.*, 1993; 1994; Kono *et al.*, 1996; Tsuji, 1996; Nakayama *et al.*, 1996; Kim *et al.*, 1996; 1997).

GM3 is the first and the simplest of the gangliosides, and is synthesized by CMP-NeuAc:lactosylceramide α2,3-sialyltransferase (GM3 synthase, EC 2.4.99.9), which catalyzes the transfer of NeuAc from CMP-NeuAc to the nonreducing terminal sugar of lactosylceramide. GM3 synthase is a key regulatory enzyme for ganglioside biosynthesis (Fishman and Brady, 1976), catalyzing the first committed step in the synthesis of nearly all gangliosides. Isolation of a cDNA clone of GM3 synthase is critical for clarifying the molecular mechanism for ganglioside biosynthesis as well as to understand the biological roles of GM3 and other gangliosides. Very recently, GM3 synthase cDNA was cloned and characterized from mouse brain using a PCR-based cloning approach (Kono *et al.*, 1998).

cDNA encoding GM3 synthase obtained from nonhuman species is insufficient to study its biological functions in humans, including mRNA gene expression under various physiological and pathological conditions. To that end, we sought to isolate the human cDNA encoding GM3 synthase, and we report on the successful cloning of this target (hST3Gal V).

Materials and Methods

Polymerase chain reaction (PCR) To prepare the probe for screening the cDNA library, PCR was performed with degenerate primers (5′-primer, 5′-TG(C/T)CG(C/G)CG(C/G)TG(C/T)GTIGT(G/T)AG(C/T)GGIGGA-3′; 3′-primer, 5′-AAA(A/G)CC(C/T)GCIAA(A/G)CT(C/G)AC(C/T)TCITC(A/G)CA-3′)

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deduced from the conserved regions (sialylmotifs L and S) of the previously cloned mST3Gal V (Kono *et al.*, 1998) and human sialyltransferases (Kim *et al.*, 1996; 1997; Nakayama *et al.*, 1996). The cDNA synthesized from the mRNA of human fetal brain (Clontech, Palo Alto, USA) was used as a template for PCR. PCR amplification was carried out for 30 cycles at 94°C for 1 min, 37°C for 1 min, and 72°C for 2 min. The PCR products (0.5 kb) were subcloned into a TA cloning vector and sequenced.

Screening of the cDNA library Human fetal brain cDNA library in λgt10 (Clontech) was used for the cloning of human GM3 synthase cDNA. About 10^6 plaques were screened by plaque hybridization with the cloned PCR fragment (0.5 kb) as a probe. One positive clone was plaque-purified, and phage DNAs were digested with *Eco*RI. The *Eco*RI fragments of cDNA inserts were subcloned into pUC118 or 119 and sequenced.

Northern blot analysis Multiple tissue Northern blot of poly(A)$^+$RNAs was purchased from Clontech Laboratories for this analysis. The cDNA fragment (672 bp) digested with *Bam*HI/*Pst*I was purified, radiolabeled with [α-^{32}P]dCTP, and used as a probe.

Results and Discussion

The sequence of sialyltransferases cloned so far has shown highly conserved regions termed sialylmotifs L and S, which have not been found in other glycosyltransferases (Tsuji, 1996). Using human fetal brain cDNA as a template, PCR experiments with degenerate primers deduced from the sialyl motifs L and S in the cloned mST3Gal V and human sialyltransferases resulted in the amplification of the expected 0.5 kb band which was subcloned and sequenced. Among several clones, one clone named pGM3 encoded a peptide exhibiting 93% identity to mST3Gal V. In order to isolate a complete coding sequence of the gene, pGM3 was used as a probe to screen the human fetal brain cDNA library. One positive clone with an insert size of 2.3 kb was obtained (Fig. 1).

Sequence analysis showed that the 2.3 kb cDNA contains a 1089 bp open reading frame encoding a protein of 362 amino acids with 5′ and 3′-untranslated regions of 231 bp and 967 bp, respectively. The 3′-noncoding region has a poly(A) tail and is preceded by a typical polyadenylation signal.

The nucleotide and deduced amino acid sequences of this protein showed 85% and 86% identity with those of mST3Gal V, respectively, while, except for the sialylmotifs, there were no significant similarities between this protein and the other sialyltransferases cloned so far. As shown in Fig. 2, sialylmotif L in the center of these enzymes (45 or 46 amino acids in length) shows 60–82% sequence homology, whereas sialylmotif S in the COOH-terminal portion (23 amino acids) exhibits 61–83% homology. Among these sialyltransferases, this protein showed the highest homology with ST3Gal groups. These results suggest that this protein (designated as hST3Gal V)

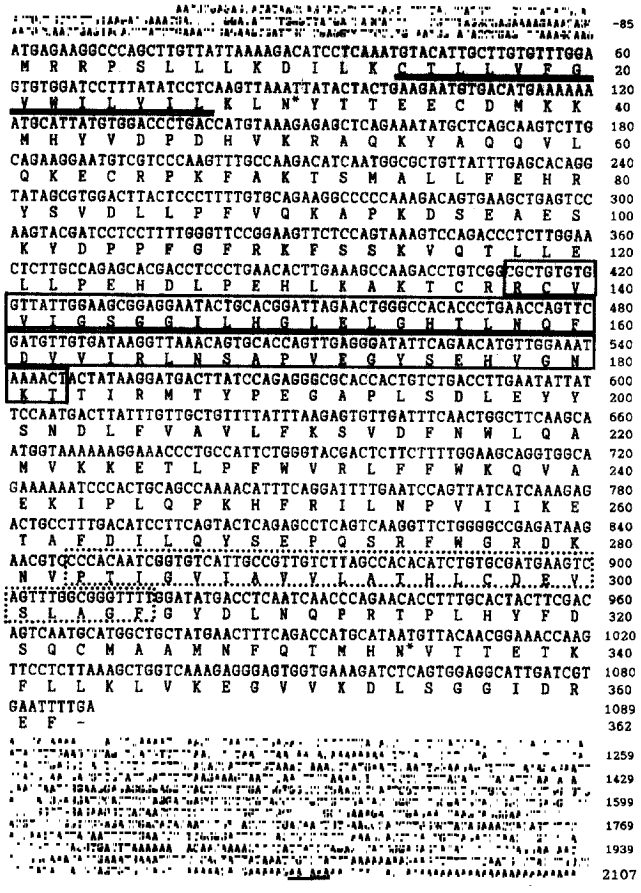

Fig. 1. Nucleotide and deduced amino acid sequences of human ST3Gal V. The nucleotide and amino acid sequences of human ST3Gal V are numbered from the presumed start codon and initiation methionine, respectively. The double-underlined amino acids correspond to a putative transmembrane domain. Sialylmotifs L and S are boxed by solid and dashed lines, respectively. The potential polyadenylation signal in the 3′ untranslated region is underlined. The potential N-linked glycosylation sites (Asn-Xaa-Ser/Thr) are indicated by asterisks.

belongs to the sialyltransferase family and is the human homolog of GM3 synthase.

A Kyte and Doolittle hydropathy analysis (Kyte *et al.*, 1982) revealed one potential membrane-spanning region consisting of 14 hydrophobic residues, located 13 residues from the amino terminus (Fig. 1). This structural feature suggests that the the hST3Gal V protein, like other cloned sialyltransferases, has a type II membrane topology (Paulson *et al.*, 1989; Tsuji, 1996; Kim *et al.*, 1997). Compared with mST3Gal V, hST3Gal V shows a similar domain structure consisting of an NH_2-terminal cytoplasmic domain (13 residues), a hydrophobic transmembrane domain (14 residues), and a COOH-terminal active domain (335 residues) including two highly conserved regions, sialylmotifs L and S, like all so-far cloned sialyltransferases. A remarkable difference between

(Sialylmotif L)

		Residues	Homology
ST3Gal I	RCAVVGNSGNLRESSYGPEIDSHDFVLRMNKAP-TAGFEADVGTKT	(141~185)	(80%)
ST3Gal II	RCAVVGNSGNLRGSGYGQDVDGHNFIMRMNQAP-TVGFEQDVGSRT	(151~195)	(71%)
ST3Gal III	RCIIVGNGGVLANKSLGSRIDDYDIVVRLNSAP-VKGFEKDVGSKT	(158~203)	(62%)
ST3Gal IV	RCVVVGNGHRLRNSSLGDAINKYDVVIRLNNAP-VAGYEGDVGSKT	(117~162)	(82%)
ST3Gal V	RCVVIGSGGILHGLELGHTLNQFDVVIRLNSAP-VEGYSEHVGNKT	(138~182)	(100%)
ST6Gal I	RCAVVSSAGSLKSSQLGREIDDHDAVLRFNGAP-TANFQQDVGTKT	(183~227)	(64%)
ST8Sia I	KCAVVGNGGILKKSGCGRQIDEANFVMRCNLPPLSSEVTKDVGSKS	(122~167)	(65%)
ST8Sia II	TCAIVGNSGVLLNSGCGQEIDAHSFVIRCNLAP-VQEYARDVGLKT	(156~200)	(60%)
ST8Sia III	ICAVVGNSGILTFIQCGREIDKSDFVFRCNFAP-SEAFQRDVGRKT	(161~204)	(67%)
ST8Sia IV	TCAVVGNSGILLDSECGKEIDSHNFVIRCNLAP-VVEFAADVGTKS	(141~185)	(62%)
ST8Sia V	KCAVVGNGGILKNSRCGREINSADFVFRCNLPPISEKYTMDVGVKT	(163~208)	(62%)
CONSENSUS	-CAVVGN-G-L--S--G--ID--DFV-R-N-AP---GF--DVG-KT		

(Sialylmotif S)

		Residues	Homology
ST3Gal I	PSTGILSVIFSMHVCDEVDLYGF	(267~289)	(83%)
ST3Gal II	PSTGMLVLFFALHVCDEVNVYGF	(227~249)	(78%)
ST3Gal III	PTLGSVAVTMALHGCDEVAVAGF	(300~322)	(83%)
ST3Gal IV	PTTGLLAITLALHLCDLVHIAGF	(258~280)	(78%)
ST3Gal V	PTIGVIAVVLATHLCDEVSLAGF	(284~306)	(100%)
ST6Gal I	PSSGMLGIIIMMTLCDQVDIYEF	(221~243)	(61%)
ST8Sia I	LSTGLFLVSAALGLCEEVAIYGF	(258~280)	(67%)
ST8Sia II	PTTGLLMYTLATRFCKQIYLYGF	(293~315)	(70%)
ST8Sia III	LSTGILMYTLASAICEEIHLYGF	(299~321)	(67%)
ST8Sia IV	PSTGLLMYTLATRFCDEIHLYGF	(278~300)	(74%)
ST8Sia V	ISTGLILVTAALELCEEVHLFGF	(299~321)	(67%)
CONSENSUS	PSTG-L-----AL--CDE----YGF		

Fig. 2. Comparison of sialylmotifs L and S of hST3Gal V with those of previously-cloned human sialyltransferases. The human sialyltransferases are hST3Gal I (Kitagawa *et al.*, 1994), hST3Gal II (Kim *et al.*, 1996), hST3Gal III (Kitagawa *et al.*, 1993), hST3Gal IV (Kitagawa *et al.*, 1994), hST3Gal V (this publication), hST6Gal I (Grundmann *et al.*, 1990), hST8Sia I (Nara *et al.*, 1994), hST8Sia II (Angata *et al.*, 1997), hST8Sia III (Lee *et al.*, 1998), hST8Sia IV (Nakayama *et al.*, 1995) and hST8Sia V (Kim *et al.*, 1997).

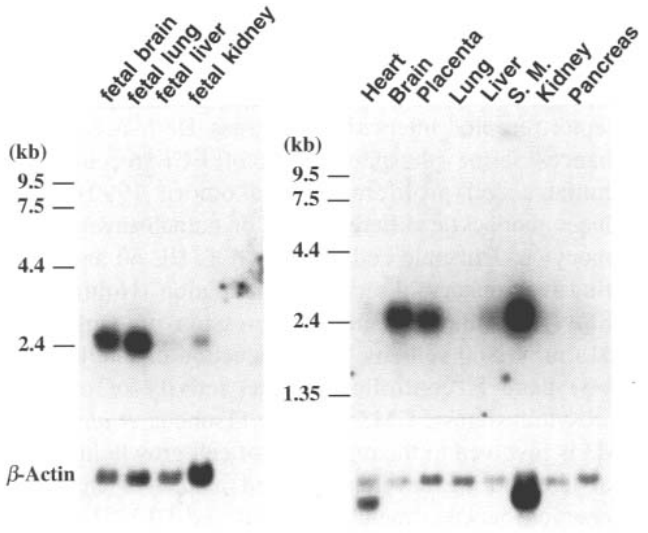

Fig. 3. Expression of hST3Gal V in various human tissues. Northern blots with poly(A)$^+$RNA from various human tissues were hybridized with ^{32}P-labeled DNA probe for hST3Gal V. The same filters were rehybridized with a probe corresponding to β-actin to estimate the amounts of RNA present in each lane. S.M., skeletal muscle.

the two enzymes was found in the cytoplasmic domain which was composed of 43 and 13 amino acids in mST3Gal V and hST3Gal V, respectively. All sialyltransferases cloned to-date have 7–12 amino acids in the cytoplasmic domain (Tsuji, 1996).

To determine the size of human ST8Sia V mRNA and its tissue distribution, Northern blots with poly(A)$^+$RNA derived from human fetal and adult tissues were probed with a 672 bp fragment isolated from hST3Gal V cDNA. As shown in Fig. 3, the probe detected an mRNA band of approximately 2.4 kb. In fetal tissues, the level of expression was highest in the brain and lung, and lowest in the kidney, while the gene was abundantly expressed in adult brain, placenta, and skeletal muscle. These results are distinct from those previously shown in the case of mST3Gal V where the transcript of 2.3 kb was detected in the liver and heart as well as in the brain (Kono *et al.*, 1998).

Based on the amino acid sequences of the highly-conserved regions of the previously cloned mouse GM3 synthase (mST3Gal V) and human sialyltransferases, we have isolated and characterized a full-length cDNA encoding GM3 synthase from human fetal brain cDNA library. The following evidence represents that this enzyme corresponds to the human homolog of mST3Gal V which transfers CMP-NeuAc with an α2,3 linkage to a galactose residue of lactosylceramide. First, this enzyme has a high homology with mST3Gal V at the nucleotide and amino acid levels. Second, the deduced amino acid sequence of this enzyme contains the characteristic sequences found in all the sialyltransferases so far cloned, sialylmotifs L and S (Tsuji, 1996). Site-directed mutagenesis of sialylmotif L indicated that it participates in the binding of CMP-NeuAc, a common donor substrate for all the sialyltransferases (Datta and Paulson, 1995). Very recently, mutations in sialylmotif S were found to cause a change of K_m values for both the donor and the acceptor substrates, indicating that this motif participates in the binding of both substrates (Datta *et al.*, 1998). Third, this enzyme has a type II membrane topology characteristic of all other sialyltransferases cloned to date (Tsuji, 1996).

Sialyltransferases cloned so far have been shown to exhibit remarkable tissue-specific expression, which is correlated with the existence of cell type-specific carbohydrate structure. In general, their expression appears to be regulated at the transcriptional level. Northern blot analysis revealed that the human GM3 synthase gene was expressed in a tissue-specific manner, like other sialyltransferase genes.

Ganglioside GM3 is known to play important roles in the modulation of cell growth through the modified signal

transduction and cell differentiation. GM3 inhibits tyrosine phosphorylation of the epidermal growth factor (EGF) receptor and EGF-dependent cell growth, independent of receptor-receptor interaction, whereas De-*N*-acetyl-GM3 enhances serine phosphorylation of EGF receptor and stimulates cell proliferation (Hakomori, 1990). GM3 induces monocytic differentiation of human myeloid and monocytoid leukemic cell lines such as HL-60 and U937 during macrophage-like cell differentiation (Nojiri *et al.*, 1986). The tumor-associated expression of ganglioside GD1a in NFS60 cells by the introduction of interleukin-3 (IL-3) gene is controlled by the activity of a single glycosyltransferase, GM3 synthase (Tsunoda *et al.*, 1995). GM3 is involved in the regulation of cell growth in human fetal liver during development and induces a change in interaction between membrane lipid and GLUT1 (Lee *et al.*, 1995; Yoon *et al.*, 1997).

Analysis of the *in vivo* expression of ganglioside GM3 in human cell lines using the hST3Gal V gene will be useful for elucidation of its biological function. Furthermore, the acquisition of hST3Gal V cDNA will pave the way for studies on human ganglioside biosynthesis through genomic and transcriptional analysis.

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