ST6 *N*-Acetylgalactosaminide Alpha-2,6-Sialyltransferase 4 (ST6GALNAC4)

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Contents

Introduction

ST6GalNAc-III, ST6GalNAc-IV, ST6GalNAc-V, and ST6GalNAc-VI are members of NeuAcα2-3Galβ1-3GalNAc α2,6-sialyltransferases. These members except ST6GalNAc-IV can efficiently synthesize the ganglioside GD1α from GM1b (Sjoberg et al. 1996; Lee et al. 1999; Okajima et al. 1999, 2000; Ikehara et al. 1999; Harduin-Lepers et al. 2000; Tsuchida et al. 2005; Ko et al. 2010). Compared with other

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members, ST6GalNAc-IV prefers *O*-linked glycans of glycoproteins to glycolipids as substrates. Therefore, ST6GalNAc-IV may be the main candidate for synthesizing the NeuAcα2-3Galβ1-3(NeuAcα2-6)GalNAc residue, which is usually found in the *O*-glycans. ST6GalNAc-IV is also considered to be involved in the early alteration of the sialylation pattern of cell surface molecules in activated lymphocytes (Kaufmann et al. 1999). Like ST6GalNAc-III, ST6GalNAc-IV is a relatively small sialyltransferase (302 amino acids in length for human, mouse, and pig enzymes) compared with other animal sialyltransferases because of the short stem region. However, isoforms of ST6GalNAc-IV with different amino acid length are also reported (350 and 360 amino acids in length for mouse isoforms). The overall amino acid sequence identity of mouse ST6GalNAc-IV is 88.4 % to human ST6GalNAc-IV, 86.1 % to pig ST6GalNAc-IV, 43.0 % to mouse ST6GalNAc-III, 44.8 % to mouse ST6GalNAc-V, and 41.2 % to mouse ST6GalNAc-VI, respectively, but ST6GalNAc-IV shows no significant similarity to other sialyltransferases except in sialylmotifs.

Databanks

IUBMB enzyme nomenclature: E.C.2.4.99.7

ST6 *N*-acetylgalactosaminide alpha-2,6-sialyltransferase 4 (ST6GALNAC4)

Species	Gene symbol	GenBank accession number	UniProt ID	PDB accession number
Homo Sapiens	ST6GALNAC4	AF127142	Q9H4F1	N/A
	SIAT3C	AF162789 (genomic)		
	SIAT7D			
Mus musculus	St6galnac4	AJ007310	Q9R2B6 (long form)	N/A
		NM_011373		
		Y15779	Q8C3J2 (short form)	
	Siat7d	Y15780		
		Y19053- Y19057 (genomic)		
Sus scrofa	St6galnac4	EU643700	B2ZCZ7	N/A
	SIAT7B			

Name and History

Enzyme and Gene Names

Recommended enzyme names:
Alpha-*N*-acetyl-neuraminyl-2,3-beta-galactosyl-1,3-*N*-acetyl-galactosaminide alpha-2,6-sialyltransferase
Alternative enzyme name(s):
NeuAc-alpha-2,3-Gal-beta-1,3-GalNAc-alpha-2,6-sialyltransferase

ST6GalNAc-IV (short name = ST6GalNAcIV)
Sialyltransferase 7D (short name = SIAT7-D)
Sialyltransferase 3C (short name = SIAT3-C) (Human)
Recommended gene name:
Human: ST6GALNAC4 (synonyms: SIAT7D, SIAT3C)
Mouse: St6galnac4 (synonyms: Siat7d)

History

The mouse St6galnac4 cDNA has been independently cloned by two groups. Lee et al. (1999) cloned a fragment encoding a new sialylmotif by a polymerase chain reaction (PCR)-based approach using two primers deduced from the sialylmotif L sequences in mouse ST3Gal-I and ST3Gal-II with mouse brain cDNA as a template. A mouse brain cDNA library was then screened with this fragment as a probe. Among the positive clones, one clone included an open reading frame of 906 bp, encoding a protein of 302 amino acids in length. Homology searching and substrate specificities of the protein revealed that this is the fourth type of GalNAc α2,6-sialyltransferase (the second type of NeuAcα2-3Galβ1-3GalNAc α2,6-sialyltransferase). Therefore, it was designated ST6GalNAc-IV according to the abbreviated nomenclature system for cloned sialyltransferase (Tsuji et al. 1996).

Kaufmann et al. (1999) used mRNA differential display PCR to search for genes in activated T cells, and they obtained a 3.6 kb cDNA clone named MK45, encoding a protein having sialylmotifs L and S. The deduced amino acid sequence of MK45 was found to be identical with that of mouse ST6GalNAc-IV.

The human ST6GALNAC4 was identified by a basic local alignment search tool (BLAST) analysis of the human expression sequence tag (EST) database using the rat St6galnac3 cDNA sequence as a query, and the cDNA encoding the entire open reading frame of human ST6GALNAC4 was amplified by PCR using cDNAs of HepG2 cells as templates (Harduin-Lepers et al. 2000).

The pig St6galnac4 cDNA was amplified with PCR using gene-specific primers and the pig liver cDNAs as templates (Ko et al. 2010).

Analyses of the genomic structure and transcription regulation of the mouse and human ST6GALNAC4/St6galnac4 genes have been performed (Takashima et al. 2000; Harduin-Lepers et al. 2000; Kim et al. 2003; Kang et al. 2004). The International System for Gene Nomenclature named this gene as *Siat7d*.

Structure

Mouse, human, and pig sialyltransferases corresponding to ST6GalNAc-IV consist of 302 amino acids and their amino acid sequences predict a type II transmembrane topology. Animal sialyltransferases share common structural features, including a short N-terminal cytoplasmic tail, a transmembrane domain, a stem region, and a catalytic domain (see the ▶ Chap. 75, "ST8 Alpha-*N*-Acetyl-Neuraminide

Alpha-2,8-Sialyltransferase 6 (ST8SIA6)"). The amino acid sequence length of these enzymes ranges from about 300 to 600 residues. This range can primarily be attributed to differences in the length of the stem regions. Like ST6GalNAc-III, ST6GalNAc-IV is a relatively small sialyltransferase compared with other animal sialyltransferases because of the short stem region. The catalytic domain of animal sialyltransferases contains highly conserved motifs termed sialylmotifs L (long), S (short), III (third position in the sequence), and VS (very short), and the importance of the conserved histidine residue in sialylmotif VS, which is involved in the catalytic activity, was examined by using the mouse ST6GalNAc-IV, ST8Sia-II, and ST8Sia-IV mutants (Kitazume-Kawaguchi 2001).

The mouse St6galnac4 gene spans over 12 kb of genomic DNA with six exons (Takashima et al. 2000). The human ST6GALNAC4 gene also consists of six exons, which spans over approximately 9 kb of genomic DNA (Kim et al. 2003). The exon–intron boundaries of the ST6GalNAc-IV gene and those of the ST6GalNAc-III, ST6GalNAc-V, and ST6GalNAc-VI genes are very similar, suggesting that these genes arose from a common ancestral gene through gene duplication (see the ▶ Chap. 67, "ST6 *N*-Acetylgalactosaminide Alpha-2,6-Sialyltransferase 3 (ST6GALNAC3)").

Enzyme Activity Assay and Substrate Specificity

The enzyme activity of mouse ST6GalNAc-IV was measured using the soluble form of this enzyme fused with *Staphylococcus aureus* protein A (Lee et al. 1999). Enzyme activity was measured in 50 mM MES buffer, pH 6.0, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5 % Triton CF-54, 100 μM CMP-[^{14}C]NeuAc (10.2 kBq), an acceptor substrate, and an enzyme preparation, in a total volume of 10 μl. As acceptor substrates, 10 μg proteins, 5 μg glycolipids, or 10 μg oligosaccharides were used. The enzyme reaction was performed at 37 °C for 2 h. For glycoprotein acceptors, the reaction was terminated by the addition of SDS-polyacrylamide gel electrophoresis loading buffer (10 μl), and then the incubation mixtures were directly subjected to SDS-polyacrylamide gel electrophoresis. For glycolipid acceptors, the incubation mixtures were applied on a C-18 column (Sep-Pak Vac, 100 mg; Waters, Milford, MA), which had been washed with water. The glycolipids were eluted from the column with methanol, dried, and then subjected to chromatography on a high-performance thin-layer chromatography (HPTLC) plate (Merck, Germany) with a solvent system of chloroform, methanol, and 0.02 % $CaCl_2$ (55:45:10). Acceptor substrates were visualized by staining with Coomassie Brilliant Blue (for glycoproteins) or by the orcinol-H_2SO_4 method (for glycolipids). The radioactive materials in glycoproteins or glycolipids were visualized with a BAS2000 radio image analyzer (Fuji Film Tokyo, Japan), and the radioactivity incorporated into acceptors was counted.

The enzyme activity of human ST6GalNAc-IV was measured using both the soluble and full-length forms of this enzyme (Harduin-Lepers et al. 2000). The soluble enzyme consisted of the preprotrypsin leader sequence, an N-terminal FLAG-tag (DYKDDDDK), and a truncated form of human ST6GalNAc-IV lacking

the first 36 amino acids. Enzyme activity was measured in 0.1 M cacodylate buffer; pH 6.2; 10 mM $MnCl_2$; 0.2 % Triton CF-54; 50 μM CMP-[^{14}C]Neu5Ac (1.85 kBq), with one acceptor substrate; and 20 μl of the enzyme source in a final volume of 50 μl. As acceptor substrates, 2 mg/ml glycoproteins, or 1 mM aryl glycosides or free oligosaccharides, was used and the enzyme reaction was performed at 33 °C for 3 h. Reaction products were separated from CMP-[^{14}C]Neu5Ac, depending on the acceptor substrate. For glycoproteins, the reaction was terminated by precipitation with 1 % (w/v) phosphotungstic acid in 0.5 M HCl, followed by filtration on glass microfiber filters (GF/A; Whatman Biosystems Ltd., Maidstone, Kent, U. K.). For aryl glycosides, the reaction was stopped with 450 μl of H_2O and heating at 100 °C for 5 min. Products were applied on to a C_{18} Sep-Pak cartridge (Waters Millipore Corp.) and eluted with 30 % (v/v) acetonitrile in water. For glycolipids, reactions were stopped by adding 1 ml of methanol; samples were centrifuged at 3,000 g for 5 min, diluted with 1 ml of water, and applied on to a Sep-Pak C_{18} cartridge.

Glycolipids were eluted with 5 ml of chloroform/methanol (1:1, v/v). For free oligosaccharides, the reaction mixture was heated at 100 °C for 5 min and then centrifuged. The supernatant was subjected to Whatman 3 paper chromatography and then developed with pyridine/ethyl acetate/acetic acid/water (5:5:1:3, by vol.) for 26 h.

The substrate specificity of mouse ST6GalNAc-IV is similar to that of ST6GalNAc-III, although ST6GalNAc-IV prefers *O*-glycans to glycolipids as substrates (Lee et al. 1999). Among the glycolipids examined, only GM1b served as an acceptor substrate for mouse ST6GalNAc-IV. ST6GalNAc-IV exhibited higher activity toward fetuin than GM1b but very low activity toward asialofetuin. These results suggest that ST6GalNAc-IV requires the NeuAcα2-3Galβ1-3GalNAc sequence in fetuin and GM1b just like ST6GalNAc-III. On the other hand, GD1a, which has the NeuAcα2-3Galβ1-3GalNAc sequence and an additional NeuAc residue at the internal galactose, did not serve as an acceptor substrate. Just as for ST6GalNAc-III, the α2-3-linked sialic acid attached to the internal galactose of GD1a seems to abolish the ability of ST6GalNAc-IV to transfer sialic acid to the NeuAcα2-3Galβ1-3GalNAc sequence. It should be noted that the oligosaccharide NeuAcα2-3Galβ1-3GalNAc was a good acceptor substrate for ST6GalNAc-IV, while such an oligosaccharide was a poor substrate for mouse ST6GalNAc-III. The activities of ST6GalNAc-IV toward nonsialylated Galβ1-3GalNAc and desialylated NeuAcα2-3Galβ1-3(NeuAcα2-6)GalNAc were almost negligible. Thin-layer chromatography analysis revealed that the oligosaccharide alditol derived from [^{14}C]NeuAc-incorporated fetuin synthesized with ST6GalNAc-IV migrated to the same position as NeuAcα2-3Galβ1-3(NeuAcα2-6)GalNAc-ol. From these results, ST6GalNAc-IV is considered to be the second type of NeuAcα2-3Galβ1-3GalNAc α2,6-sialyltransferase which can synthesize the NeuAcα2-3Galβ1-3(NeuAcα2-6)GalNAc structure.

The substrate specificity of human ST6GalNAc-IV is basically similar to that of mouse ST6GalNAc-IV; however, human ST6GalNAc-IV did not exhibit activity toward GM1b (Harduin-Lepers et al. 2000). The K_m values of human ST6GalNAc-IV for NeuGcα2-3Galβ1-3GalNAc, NeuAcα2-3Galβ1-3GalNAcα-*O*-Bz, and CMP-NeuAc were estimated to be 1.10, 2.21, and 0.07 (mM), respectively.

The glycosylation pattern of pig St6galnac4-transfected cells was detected by flow cytometry and immunofluorescence analysis with specific lectins, and NeuAcα2-3Galβ1-3(NeuAcα2-6)GalNAc or NeuAcα2-6GalNAc structures were revealed to be newly synthesized by pig ST6GalNAc-IV (Ko et al. 2010).

Preparation

The enzymatic properties of ST6GalNAc-IV were characterized by using recombinant enzymes. To prepare the soluble form of mouse ST6GalNAc-IV, the DNA fragment encoding a truncated form of mouse ST6GalNAc-IV, lacking the first 36 amino acids of the open reading frame, was prepared by PCR amplification, and this fragment was inserted into a pcDSA vector (Lee et al. 1999). The resulting plasmid encoded a protein consisting of the mouse IgM signal peptide sequence, the *Staphylococcus aureus* protein A IgG-binding domain, and a truncated form of mouse ST6GalNAc-IV. This expression vector was transiently transfected into COS-7 cells on a 150 mm plate using Lipofectamine™ reagent (Life Technologies, Inc.). The protein A-fused ST6GalNAc-IV expressed in the medium was adsorbed to an IgG-Sepharose gel (Amersham Pharmacia Biotech, Piscatway, NJ, USA; 50 μl resin/50 ml culture medium) at 4 °C for 16 h. The resin was collected by centrifugation, washed three times with phosphate-buffered saline, suspended in 50 μl (final volume) of Dulbecco's modified Eagle medium without fetal bovine serum, and used as the soluble enzyme.

To prepare the soluble form of human ST6GalNAc-IV, the DNA fragment encoding a truncated form of human ST6GalNAc-IV, lacking the first 36 amino acids of the open reading frame, was prepared by PCR amplification, and this fragment was inserted into a pFLAG-CMV-1 vector (Harduin-Lepers et al. 2000). The resulting plasmid encoded a protein consisting of the preprotrypsin leader sequence, the FLAG tag, and a truncated form of human ST6GalNAc-IV. To prepare the full-length form of human ST6GalNAc-IV, the DNA fragment containing the human ST6GALNAC4 coding region in its entirety was prepared by PCR amplification, and this fragment was inserted into a pFLAG-CMV-1 vector. The resulting plasmid encoded the N-terminal FLAG-tagged human ST6GalNAc-IV. Each expression vector (10 μg) was transiently transfected into COS-7 cells on a 100 mm^2 tissue-culture dish using Lipofectamine™ and Plus reagent (Life Technologies, Inc.). The transfected cells were harvested 48 h after transfection by scraping into phosphate-buffered saline and were pelleted by low-speed centrifugation. The recombinant human ST6GalNAc-IVwas found to be expressed within the cells and in the culture media that was thus collected; ultrafiltered 10-fold against 20 mM MES, pH 6.2, 50 mM NaCl, and 1 mM dithioerythritol; and used as the enzyme source.

Biological Aspects

Northern blot analysis of the mouse St6galnac4 gene in several tissues revealed that this gene is highly expressed in the colon and brain, moderately expressed in the lung,

heart, thymus, and spleen (Lee et al. 1999). Three major transcripts (1.6–1.9, 2.0–2.2, and 3.6–3.7 kb), which mainly differed in the length of their 3′-untranslated regions, were generated from this gene (Kaufmann et al. 1999; Lee et al. 1999; Takashima et al. 2000). Expression of the mouse St6galnac4 gene in embryonal stage (E12) and 1-day-old (P1) brain was relatively low, whereas expression in 3-week-old and 8-week-old brain was high, indicating that the expression in brain is developmentally regulated (Lee et al. 1999). On the other hand, Northern blot analysis of the human ST6GALNAC4 gene revealed that this gene is to be expressed at low levels as a 2.2 kb transcript in almost all human tissues examined, indicating a possible constitutive expression (Harduin-Lepers et al. 2000). In addition to this constitutive expression, a 2.5 kb transcript was detected in the heart, brain, and skeletal muscles. The expression of the human ST6GALNAC4 gene was also detected in various human cancer cell lines. In human fetal tissues, the level of ST6GALNAC4 gene expression was highest in the liver, a very low level being observed in the kidney and lung, while it was not detected in brain (Kim et al. 2003). The reverse transcription PCR (RT-PCR) analysis showed that the pig St6galnac4 gene was constitutively expressed in most tissues (Ko et al. 2010). Especially, it was abundantly expressed in the tongue, muscle, and heart, whereas not expressed in pancreas at all. The precise biological functions of ST6GalNAc-IV in vivo has not been demonstrated yet, but one potential function of ST6GalNAc-IV seems to be to synthesize NeuAcα2-3Galβ1-3(NeuAcα2-6)GalNAc residue on glycoproteins.

Interestingly, it has been shown that the mouse St6galnac4 expression is rapidly induced in activated CD8 T cells in vivo (Kaufmann et al. 1999). The expression reached the highest levels 4 h after antigen triggering and then declined rapidly to nearly base levels within 45 h. It should be noted that the induced expression level is much higher than the normal expression levels in mouse tissues. Moreover, the mouse St6galnac4 expression was also induced in lipopolysaccharide-activated B cells and antigen-triggered CD4 T cells in vitro. The rapidly induced expression in activated lymphocytes is specific for St6galnac4, since mRNA expression levels of other sialyltransferases remained largely unchanged during the early stage of lymphocyte activation. Thus, ST6GalNAc-IV is considered to be a potent candidate sialyltransferase that is involved in the early alteration of the sialylation pattern of cell surface molecules in activated lymphocytes.

Measurement of the promoter activity of the mouse St6galnac4 gene demonstrated that the first 441 bp upstream sequence from the translational initiation codon contains the minimum promoter for the expression of this gene by a mouse fibroblast cell line, NIH3T3 (Takashima et al. 2000). Like the mouse St6galnac3 promoter, the minimum promoter region does not contain a TATA or CAAT box but has three putative Sp1-binding sites. Mobility shift assaying and mutational analysis of the promoter region indicated that all three Sp1-binding sites are independently involved in the transcription regulation of the mouse St6galnac4 gene in NIH3T3 cells. The differences between the basic transcription regulation of the mouse St6galnac3 and St6galnac4 genes by Sp1 may be related to the expression levels of these genes.

Similar to the mouse St6galnac4 gene, the 5′-flanking region of the human ST6GALNAC4 gene lacks canonical TATA and CAAT boxes. However, it contains

putative binding sites for transcription factors such as Sp1 and MZF1, which are involved in the transcriptional regulation of the human ST6GALNAC4 gene in Jurkat T cells (Kang et al. 2004). The human ST6GALNAC3 and ST6GALNAC4 genes are constitutively expressed in a colon adenocarcinoma cell line HT-29 (Higai et al. 2006). However, its expression was suppressed by tumor necrosis factor α (TNFα) stimulation, while TNFα induced the increase in the expression of ST6GALNAC3 mRNA in HT-29 cells. On the other hand, NFκB-p65 siRNA treatment enhanced the constitutive expression levels of ST6GALNAC4 mRNA that was suppressed by TNFα.

Knockout and Transgenic Mice

There is no report regarding St6galnac4 knockout and transgenic mice. However, the St6galnac4-targeted ES clones are available from the MMRRC (Mutant Mouse Regional Resource Centers supported by NIH).

Human Disease

Association of ST6GALNAC4 with human disease has not been reported yet.

Future Perspective

ST6GalNAc-III, ST6GalNAc-IV, ST6GalNAc-V, and ST6GalNAc-VI are the members of one ST6GalNAc subfamily and some of their enzymatic properties are overlapped. Each gene has characteristic and different expression patterns, suggesting that the expression of these genes is regulated in a different manner. Although promoter regions of these genes have been analyzed, the mechanisms of cell-, tissue-, and stage-specific expressions of these genes have not yet been clarified. In addition, the mechanism of rapid induction of mouse ST6GalNAc-IV in activated lymphocytes and its biological significance in immune response have not been elucidated. At present, the precise biological functions of these enzymes in vivo cannot be distinguished. To solve this problem, it is necessary to know the mechanisms of cell-, tissue- and stage-specific expressions of these genes. Identification of transcription factors that are involved in cell-, tissue- and stage-specific expressions may help to understand the different expression patterns of these genes. Generating knockout mice of these genes will also help to reveal the precise biological functions of these enzymes.

Cross-References

- ST6 *N*-Acetylgalactosaminide Alpha-2,6-Sialyltransferase 3 (ST6GALNAC3)
- ST6 *N*-Acetylgalactosaminide Alpha-2,6-Sialyltransferase 5,6 (ST6GALNAC5,6)

Further Reading

Harduin-Lepers et al. (2000): Report the cDNA cloning of human ST6GALNAC4, the enzymatic properties, the gene structure, and the expression patterns of the ST6GALNAC4 gene.

Kaufmann et al. (1999): Report the cDNA cloning of mouse St6galnac4 by the mRNA differential display approach and the rapid induction of the St6galnac4 gene expression in lymphocytes after activation.

Kim et al. (2003): Report the genomic structure and promoter analysis of human ST6GALNAC4 gene.

Lee et al. (1999): Report the cDNA cloning of mouse St6galnac3 and St6galnac4, the substrate specificities of these enzymes, and the expression patterns of the St6galnac3 and St6galnac4 genes.

Takashima et al. (2000): Compare the genomic structures of mouse St6galnac3 and St6galnac4 genes and report on an analysis of promoter activities and the identification of Sp1-binding sites that are involved in the transcription regulation of these genes.

Takashima and Tsuji (2011): Give a short review of the functional diversity of mammalian sialyltransferases.

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