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Human N-Acetylglucosamine-6-O-Sulfotransferase Involved in the Biosynthesis of 6-Sulfo Sialyl Lewis X: Molecular Cloning, Chromosomal Mapping, and Expression in Various Organs and Tumor Cells[1]

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N-Acetylglucosamine-6-O-sulfotransferase catalyzes the transfer of sulfate from 3′-phosphoadenosine 5′-phosphosulfate to position 6 of a non-reducing N-acetylglucosamine (GlcNAc) residue. We have cloned human GlcNAc-6-O-sulfotransferase cDNA, based on the sequence homology to cloned cDNA of mouse GlcNAc-6-O-sulfotransferase. The predicted protein sequence of the human enzyme was highly homologous to that of the mouse enzyme; in the 363 amino acid stretch of the catalytic region, the two proteins were nearly identical except for conservative changes in 3 amino acid residues. The expressed enzyme transferred sulfate to GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc. Co-transfection of the enzyme cDNA and fucosyltransferase VII cDNA into COS-7 cells resulted in cell surface expression of 6-sulfo sialyl Lewis X. Fluorescence *in situ* hybridization analysis revealed that the GlcNAc-6-O-sulfotransferase gene is located on human chromosome 7q31. mRNA of the human enzyme was strongly expressed in the bone marrow, peripheral blood leukocytes, spleen, brain, spinal cord, ovary, and placenta, and moderate levels of expression were observed in many organs including lymph nodes and thymus. *In situ* hybridization with the mouse system showed that the transcript was localized in specific regions of the brain, *i.e.* pyramidal cells in the CA3 subregion of the hippocampus, cerebellar nucleus and Purkinje cells. Among human tumor cells, strong expression of the mRNA was found in MOLT-4 and Jarkat lymphoblastic leukemia cells, Raji lymphoma cells, K-562 chronic myelogeneous leukemia cells, U251 glioma cells, and G361 melanoma cells. Carbohydrate structures synthesized by the sulfotransferase may be involved in various aspects of the differentiation and behavior of blood cells, their progenitor cells, and neurons in the central nervous system.

Key words: blood cells, central nervous systems, L-selectin ligand, 6-sulfo sialyl Lewis X, sulfotransferase.

The 6-O-sulfation of N-acetylglucosamine (GlcNAc) is found in keratan sulfate (*1*) and serine/threonine-linked as well as asparagine-linked oligosaccharides (*2-6*). GlcNAc-6-O-sulfotransferase has been recently studied using crude enzyme preparations (*7, 8*). The enzyme acts on GlcNAc exposed at non-reducing ends but not to internal GlcNAc. Therefore, the 6-sulfo N-acetyllactosamine structure is considered to be formed by the 6-O-sulfation followed through transfer of a galactosyl residue by β-1,4-galactosyltransferase (*7, 8*).

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Abbreviations: FACS, fluorescence-activated cell sorter; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; SSPE, sodium chloride/sodium phosphate/EDTA buffer; 6-sulfo sialyl Lewis X, NeuAcα2-3Galβ1-4(Fucα1-3)(SO4-6)GlcNAc; 6′-sulfo sialyl Lewis X, NeuAcα2-3(SO4-6)Galβ1-4(Fucα1-3)GlcNAc; 6,6′-bis-sulfo sialyl Lewis X, NeuAcα2-3(SO4-6)Galβ1-4(Fucα1-3)(SO4-6)GlcNAc.

GlcNAc-6-*O*-sulfation has recently gained much attention, since recognition by L-selectin requires sulfation of its ligand, GlyCAM-1, in high endothelial venules of lymph nodes (*9*). 6-Sulfo sialyl Lewis X and 6′-sulfo sialyl Lewis X have been identified as sulfated oligosaccharides in GlyCAM-1 (*10*). At the present time, it has not been established whether 6-sulfo sialyl Lewis X or 6′-sialyl Lewis X is important for L-selectin recognition (*11-17*). However, the results of enzymological (*14*), immunological (*15, 16*), and chemical (*13, 17*) studies strongly favor that 6-sulfo sialyl Lewis X is the ligand of L-selectin.

We recently cloned a mouse GlcNAc-6-*O*-sulfotransferase (*18*). The enzyme transferred sulfate to non-reducing GlcNAc in GlcNAcβ1-3Galβ1-4GlcNAc but not on Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc. GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc also served as a sulfate acceptor. Furthermore, the enzyme was involved in the formation of 6-sulfo sialyl *N*-acetyllactosamine and 6-sulfo Lewis X, and is expressed in the high endothelial venules of mesenteric lymph nodes, suggesting that the cloned enzyme may participate in the formation of the L-selectin ligand (*18*).

In this paper, we describe the molecular cloning and characterization of human GlcNAc-6-*O*-sulfotransferase. Because of the potential physiological and pathological significance of the sulfotransferase, information regarding the human enzyme may contribute to understanding of the etiologies of certain diseases and open a new way for their control. Furthermore, we provide new knowledge on GlcNAc-6-*O*-sulfotransferase in general, namely its chromosomal localization, direct evidence for the formation of 6-sulfo sialyl Lewis X, localization in specific regions of the brain, and mode of expression in tumor cells.

EXPERIMENTAL PROCEDURES

Materials—^{35}S-PAPS (58.1 GBq/mmol) was from Dupont NEN, and α-^{32}P-dCTP (110 GBq/mmol) from Amersham. The human fetal brain 5′-STRETCH PLUS cDNA library and Human Multiple Tissue Northern (MTN) Blots were from Clontech. GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc was a generous gift from Dr. Keiichi Yoshida, Seikagaku Corporation, Tokyo. The following glycolipids were generous gifts from Dr. Makoto Kiso, Faculty of Agriculture, Gifu University; sialyl Lewis X ceramide, NeuAcα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glcβ1-Cer; 6-sulfo sialyl Lewis X ceramide, NeuAcα2-3Galβ1-4(Fucα1-3)(SO_4-6)GlcNAcβ1-3Galβ1-4Glcβ1-Cer; 6′-sulfo sialyl Lewis X ceramide, NeuAcα2-3(SO_4-6)Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glcβ1-Cer; and 6,6′-bis-sulfo sialyl Lewis X ceramide, NeuAcα2-3(SO_4-6)Galβ1-4(Fucα1-3)(SO_4-6)GlcNAcβ1-3Galβ1-4Glcβ1-Cer. The asialo compounds were prepared by digestion with neuraminidase from *Arthrobacter ureafaciens* (Nacalai Tesque).

Isolation of Human GlcNAc-6-O-Sulfotransferase cDNA—A cDNA fragment of mouse GlcNAc-6-*O*-sulfotransferase (*18*) was ^{32}P-labeled with a Megaprime™ DNA labeling system (Amersham), and then used to screen the λgt 11 human fetal brain cDNA library. Hybridization was carried out as described (*19*). A DNA insert was isolated from positive λgt 11 clones by digestion with *Eco*RI and then subcloned into the pBluescript II SK− (Stratagene). Then, its nucleotide sequence was determined by the dideoxy chain termination method (*20*) using an Applied Biosystems automated sequencer.

Construction of Expression Vectors—A cDNA fragment encoding the open reading frame of human GlcNAc-6-*O*-sulfotransferase was amplified by PCR using the primers, 5′-CTGAATTCGGAATGAAGGTGTTCCGTA-3′ and 5′-GAGAATTCTTAGAGACGGGGCTTCCGA-3′, and the cloned cDNA fragment as a template. PCR amplification was carried out at 94°C for 3 min, with 35 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min, in 5% (v/v) dimethylsulfoxide. The PCR product including the open reading frame of GlcNAc-6-*O*-sulfotransferase (nucleotide numbers 387-1844 in Fig. 1) was digested with *Eco*RI and then subcloned into the pcDNA3 expression vector (Invitrogen). A recombinant plasmid with the correct orientation, pcDNA3-hGlcNAc6ST, was used for expression. The plasmid containing the fragment in the reverse orientation, pcDNA3-hGlcNAc6STA, was used in control experiments. pCDM8-FucTVII, an expression vector of fucosyltransferase VII, was prepared as described previously (*21*).

Transient Expression of GlcNAc-6-O-Sulfotransferase cDNA—COS-7 cells (3×10^6 cells in a 10-cm dish) were transfected with 15 μg of a relevant plasmid by the DEAE-dextran method (*22*). After 65 h culture in Dulbecco-modified minimum essential medium containing 10% fetal calf serum, the cells were washed with phosphate-buffered saline (PBS), scraped off from the dishes, and then homogenized with a Dounce homogenizer in 1.5 ml/dish of 0.25 M sucrose, 20 mM Tris-HCl, pH 7.2, and 0.5% Triton X-100. The homogenates were centrifuged at $10,000\times g$ for 15 min, and the supernatants were saved as the extracts. For FACS analysis, the transfected cells were cultured for 48 h, introduced into 25 cm^2 culture flasks (3×10^5 cells per flask), and then further cultured for 36 h.

Assaying of Sulfotransferase Activity—The reaction mixture comprised 2.5 μmol of imidazole-HCl, pH 6.8, 0.5 μmol of $MnCl_2$, 0.1 μmol of AMP, 1.0 μmol of NaF, 25 nmol of GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc, 50 pmol of ^{35}S-PAPS (about 5×10^5 cpm), and 5 μl of an extract of COS-7 cells, in a final volume of 50 μl. The reaction mixture was incubated at 30°C for 5 h and the reaction was stopped by immersing the reaction tube in a boiling water bath for 1 min. ^{35}S-labeled oligosaccharides were separated from $^{35}SO_4$ and ^{35}S-PAPS by Superdex 30 gel chromatography (*18, 35*), and then the radioactivity was determined. The sulfotransferase reaction proceeded linearly up to 5 h under the assay conditions used.

Immunological Analysis—G152 murine IgM monoclonal antibody was produced as described previously (*16*), using 6-sulfo sialyl Lewis X ceramide as the immunogen. Upon ELISA (*16*), the G152 antibody reacted with 6-sulfo sialyl Lewis X ceramide, but not with 6′-sulfo sialyl Lewis X ceramide, 6,6′-bis-sulfo sialyl Lewis X ceramide, 6-sulfo Lewis X ceramide, Lewis X ceramide, or other glycolipids mentioned under "Materials." Therefore, the G152 antibody recognized the 6-sulfo sialyl Lewis X [NeuAcα2-3Galβ1-4(Fucα1-3)(SO_4-6)GlcNAc] antigen (*16*). G72 murine IgM antibody was similarly prepared and used (*16*); the antibody reacted with the 6-sulfo sialyl *N*-acetyllactosamine structure including 6-sulfo sialyl Lewis X (*16*). CSLEX-1 monoclonal antibody (*23*) was used to detect the sialyl Lewis X antigen. Cell surface expression of antigenic epitopes reactive with the monoclonal antibodies was

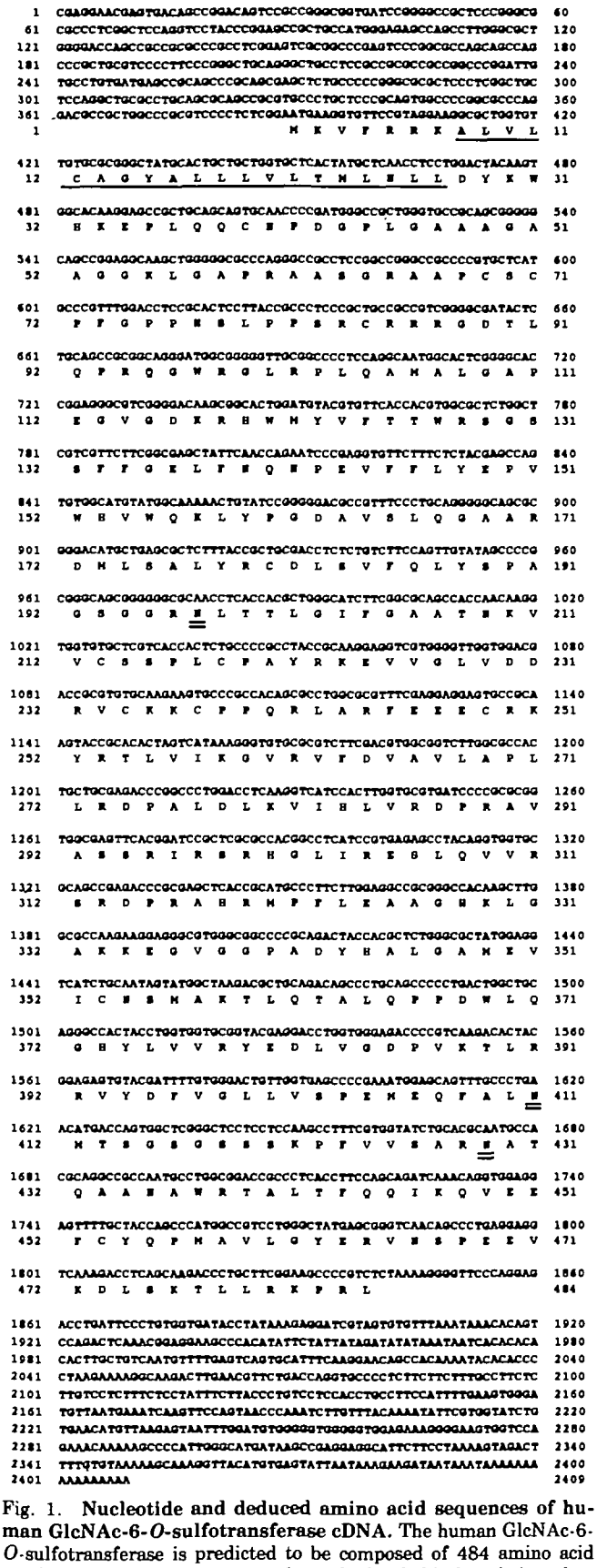

Fig. 1. **Nucleotide and deduced amino acid sequences of human GlcNAc-6-*O*-sulfotransferase cDNA.** The human GlcNAc-6-*O*-sulfotransferase is predicted to be composed of 484 amino acid residues. The putative transmembrane hydrophobic domain is underlined, and potential *N*-linked glycosylation sites are doubly underlined.

surveyed by FACS as described (*15*) using a FACScan (Becton Dickinson).

Northern Blot Analysis—The *Bpu*1102I-*Bam*HI 368 bp fragment (nucleotide numbers 910-1277 in Fig. 1) of the cloned human cDNA was used for Northern hybridization as a probe. The blots were washed at 55°C in 2×SSPE, 0.1% SDS, and finally in 0.1×SSPE, 0.1% SDS at 55°C. The membranes were exposed to a BAS-imaging plate and then the radioactivity on the membrane was quantified with a BAS 2000 radioimage analyzer (Fuji Film). Northern blot analysis of samples from neuroblastomas was also performed as described elsewhere (*24*).

In Situ Hybridization Analysis—Specimens from C57 BL/6J mice were subjected to *in situ* hybridization as described previously (*25*). As the GlcNAc-6-*O*-sulfotransferase probe, a 0.6 kbp *Pst*I fragment of the previously cloned mouse cDNA (*18*) was subcloned into pBluescript II SK−. Sense and antisense cRNA probes were prepared by *in vitro* transcription with a DIG RNA labeling kit (Boehringer Mannheim, Germany).

Fluorescence In Situ Hybridization (FISH) Analysis—FISH was performed as described previously (*25, 26*) using the cloned human GlcNAc-6-*O*-sulfotransferase cDNA, 2,409 bp, as a probe.

Cell Lines—The G401, Jurkat, MCF-7, Neuro2a, and NB41A3 cell lines were from the American Type Culture Collection. The U251 cell line was from the Riken Gene Bank. The Ishikawa and NS-1 cell lines were donated by Japan Immunoresearch Laboratories, and Dr. R. Ueda, Nagoya City University, respectively. The neuroblastoma cell lines, SK-N-SH, NGP (*27*), and CHP901 (established by A.N.), were cultured as described previously (*27*).

Patients and Tumor Tissues—Colon carcinoma specimens and adjacent normal mucosa specimens were ob-

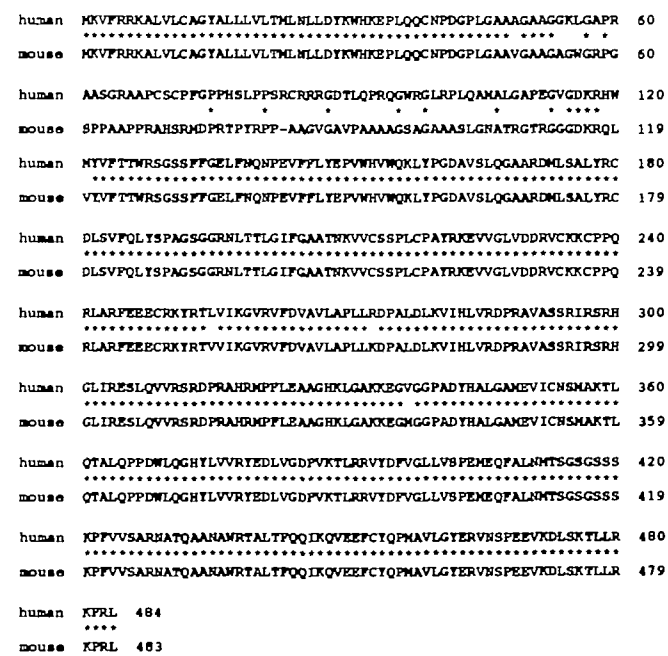

Fig. 2. **Comparison of the amino acid sequences of the human and mouse GlcNAc-6-*O*-sulfotransferases.** The alignment was performed using the GENETYX program (Software Development). Identical residues between the two sequences are shown by asterisks.

tained from 5 patients operated on at the University Hospital, Nagoya University. Twenty-two fresh neuroblastoma samples were obtained from patients at Japanese Neuroblastoma Study Group institutions before starting the treatment. All diagnoses of neuroblastomas were confirmed by histological assessment of surgically resected tumor specimens. The tumors were staged according to the International Neuroblastoma Staging System (*28*). Six were in stage 1, 5 in stage 2, 2 in stage 4S, 4 in stage 3, and 5 in stage 4. N-myc was amplified in 4 tumors.

RESULTS

Predicted Primary Structure of Human GlcNAc-6-O-Sulfotransferase—Using mouse GlcNAc-6-*O*-sulfotransferase as a probe, we screened a human fetal brain cDNA library, and obtained a cDNA clone consisting of 2,409 nucleotides. Sequence analysis of the clone yielded a predicted protein sequence of 484 amino acids, which is highly homologous to that of mouse GlcNAc-6-*O*-sulfotransferase. The protein has a transmembrane domain near the N-terminus, indicating that the protein has a type II transmembrane topology. The nucleotide sequence around the putative initiation codon (nucleotide numbers 390-393 in Fig. 1) agrees with Kozak's rule (*29*). We also noted that there was another ATG 41 bases upstream from the assumed initiation codon. This site also agrees with Kozak's rule, and ATG is present at the corresponding position of the mouse cDNA. There were no stop codons between the two ATGs in either the human or mouse cDNA. Therefore, in addition to the predicted protein with a short cytoplasmic tail, another form of the enzyme with a long cytoplasmic tail may be present. The presence of short and long cytoplasmic tails has also been found for β-1,4-galactosyltransferase; the one with a long tail is located on the cell surface, and that with a short tail in the Golgi (*30*).

The human protein is highly homologous to mouse GlcNAc-6-*O*-sulfotransferase (Fig. 2). Although the region corresponding to the stem region is less homologous, the expected catalytic regions are nearly identical; in the 363 amino acid segments, only 3 amino acid residues are different, and all the changes are conservative ones.

Expression of GlcNAc-6-O-Sulfotransferase Activity—To confirm that the cloned cDNA encodes human GlcNAc-6-*O*-sulfotransferase, the cDNA encoding the protein with a short cytoplasmic tail was inserted into a mammalian expression vector, pcDNA3, and then transfected into COS-7 cells. The extract of the transfected cells transferred sulfate to GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNac (Table I), but not to Galβ1-4GlcNacβ1-3Galβ1-4GlcNAc (data not shown). GlcNAc-6-*O*-sulfotransferase activity in the transfected cells was 5-fold above that in the control transfections, of which extracts were prepared from untransfected cells or from antisense transfected cells.

The GlcNAc-6-O-Sulfotransferase Is Involved in the Biosynthesis of 6-Sulfo Sialyl Lewis X Antigen—When COS-7 cells were transfected with sulfotransferase cDNA together with fucosyltransferase VII cDNA, the cells became able to react with the G152 antigen, whose epitope is 6-sulfo sialyl Lewis X (Fig. 3). Parental COS-7 cells as well as cells transfected with only one of the cDNAs or cells transfected with antisense cDNA did not express the antigen. COS-7 cells transfected only with the sulfotransferase cDNA became positive for the G72 antigen, whose epitope is 6-sulfo sialyl *N*-acetyllactosamine. Therefore, the cloned protein is human GlcNAc-6-*O*-sulfotransferase involved in the synthesis of 6-sulfo sialyl Lewis X.

Expression of GlcNAc-6-O-Sulfotransferase in Human Organs—Northern blot analysis revealed that the enzyme was expressed in many organs (Fig. 4A). The size of the major mRNA was 3.6 kb, while a minor band of 5.6 kb was observed for some organs. Strong expression was found in the bone marrow, peripheral blood leukocytes, spleen, brain, spinal cord, ovary, and placenta. Moderate levels of expression were noted in the lymph node, thymus, heart, lung, trachea, stomach, small intestine, colon, thyroid, prostate, and adrenal gland. The expression in the liver, testis and pancreas was low. In various regions of the brain, the levels of expression were not dramatically different

TABLE I. **Overexpression of human GlcNAc-6-*O*-sulfotransferase in COS-7 cells.** Sulfotransferase activities in extracts of cells transfected with pcDNA3-hGlcNAc6ST (Sense) or pcDNA3-hGlcNAc6STA (Antisense), or no plasmid (None) were determined using GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc as a sulfate acceptor. The activity was calculated from the radioactivity contained in fraction numbers 79 to 82 on Superdex 30 chromatography (*35*). Values obtained in the absence of the acceptor were subtracted from the values for experimental runs. Values shown are averages ± SD for triplicate cultures.

Plasmid	Sulfotransferase activity (pmol/h/mg protein)
None	1.33±0.62
Antisense	1.29±0.23
Sense	6.67±0.51

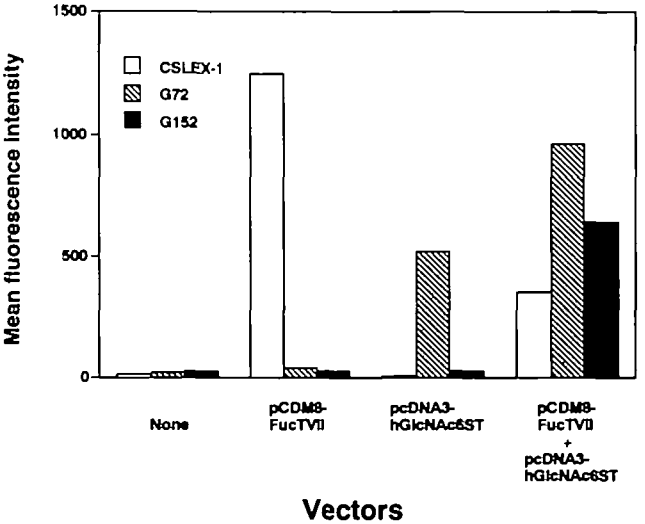

Fig. 3. **The GlcNAc-6-*O*-sulfotransferase is involved in the synthesis of 6-sulfo sialyl Lewis X.** COS-7 cells were transfected with the fucosyltransferase VII expression vector, pCDM8-FucTVII (*21*), or the sulfotransferase expression vector, pcDNA3-hGlcNAc-6ST, or both the transferases vectors, pCDM8-FucTVII and pcDNA3-hGlcNAc6ST, or without vectors as described under "EXPERIMENTAL PROCEDURES." The transfected cells were reacted with anti-sialyl Lewis X monoclonal antibody, CSLEX-1 (*23*), anti-6-sulfo sialyl *N*-acetyllactosamine monoclonal antibody, G72 (*16*), and anti-6-sulfo sialyl Lewis X monoclonal antibody, G152 (*16*), respectively. The stained cells were analyzed by FACS. The data presented here are the mean fluorescence intensities of the antigen-positive populations of transfected cells.

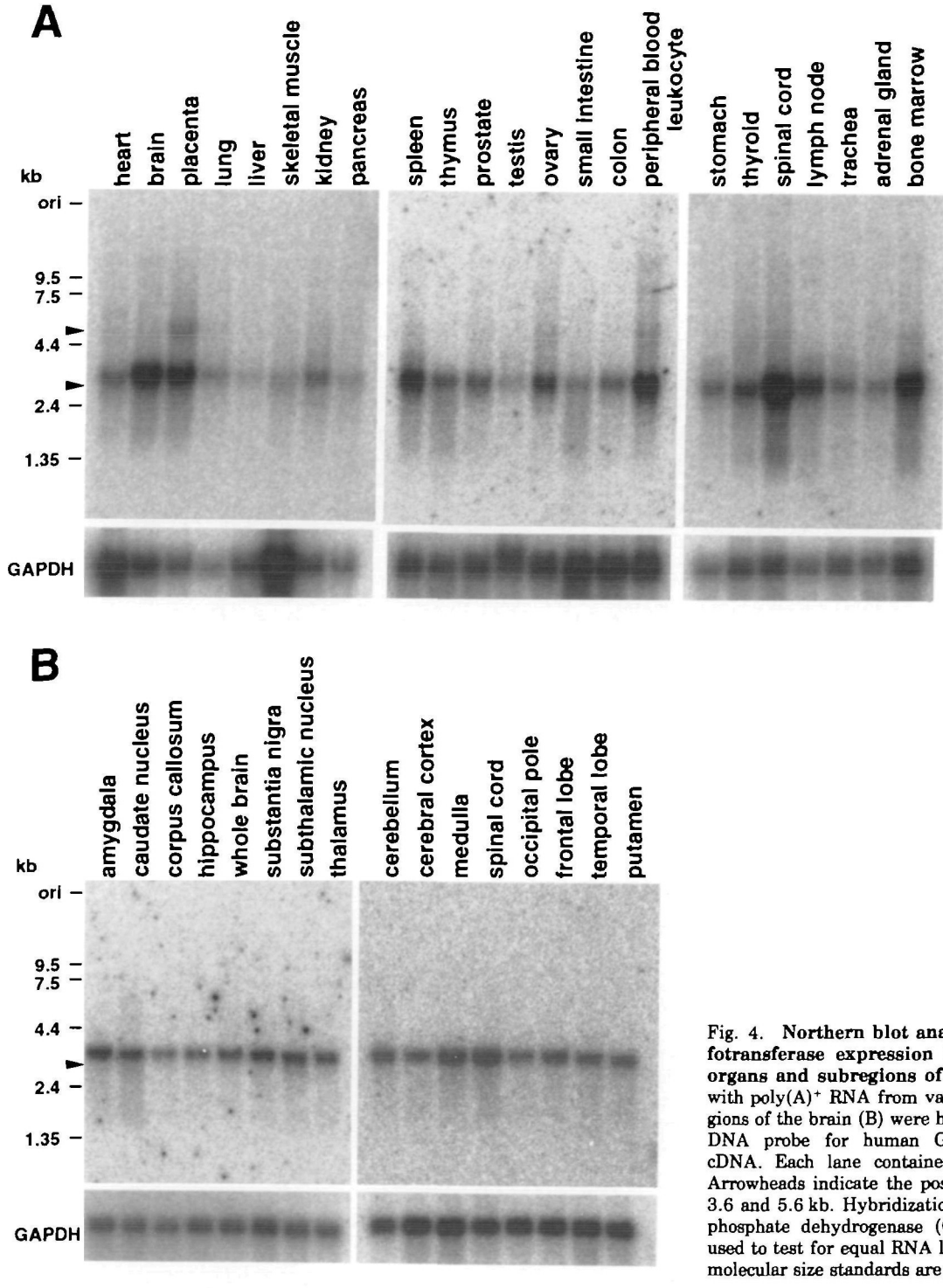

Fig. 4. **Northern blot analysis of GlcNAc-6-*O*-sulfotransferase expression in various adult human organs and subregions of the brain.** Northern blots with poly(A)$^+$ RNA from various organs (A) and subregions of the brain (B) were hybridized with a ^{32}P-labeled DNA probe for human GlcNAc-6-*O*-sulfotransferase cDNA. Each lane contained 2 μg of poly(A)$^+$ RNA. Arrowheads indicate the positions of different mRNAs: 3.6 and 5.6 kb. Hybridization with a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA probe was used to test for equal RNA loading. The positions of the molecular size standards are indicated at the left.

(Fig. 4B).

Specific Localization of the Transcript in the Brain, as Revealed by In Situ Hybridization—Because of the overall strong expression of the transcript in various regions of the brain, we attempted to determine its localization, and thus performed *in situ* hybridization analysis. We employed the mouse brain for this purpose, since it was difficult to obtain fresh human brains. When an adult mouse brain was fixed and used for the hybridization, strong signals were detected in pyramidal cells in the CA3 subregion of the hippocampus (Fig. 5, A and B), cerebellar nucleus and Purkinje cells (Fig. 5C). Moderate signals were observed in other subregions including the CA1 subregion of the hippocampus (Fig. 5A), thalamus, piriform cortex, pontine nucleus, dorsal cochlear nucleus, olfactory tubercle, and olfactory bulb (data not shown).

Expression of Human GlcNAc-6-O-Sulfotransferase in Tumors—Among human tumor cells, high levels of the

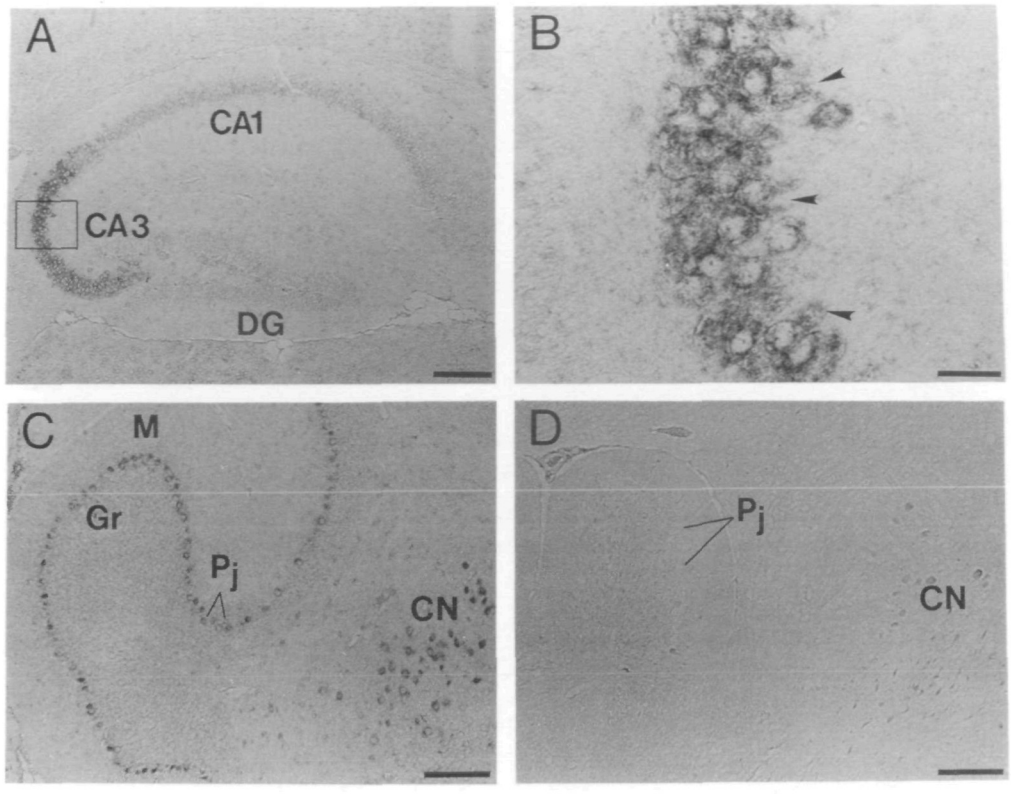

Fig. 5. ***In situ* localization of GlcNAc-6-*O*-sulfotransferase transcripts in the brain.** A sagittal section of adult mouse brain hybridized with the antisense probe exhibited specific signals in pyramidal cells in the CA3 subregion of the hippocampus (A, B), and in the Purkinje cells and the cerebellar nucleus (C). Hybridization with the sense probe yielded no signal (D). The boxed area in A is enlarged in B. CA1, CA3; CA1 and CA3 subregions of the hippocampus; DG, dentate gyrus; Gr, granular layer; Pj, Purkinje cells; M, molecular layer; CN, cerebellar nucleus. Arrowheads indicate pyramidal cells. Bars: 100 μm, except for B, in which it is 25 μm.

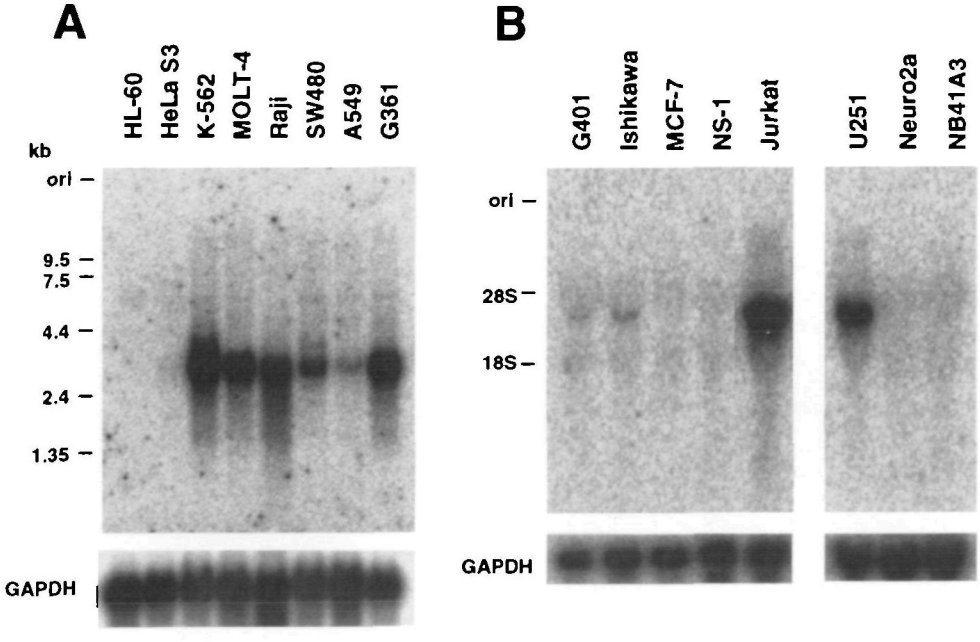

Fig. 6. **Northern blot analysis of GlcNAc-6-*O*-sulfotransferase expression in tumor cells.** Northern blots with 2 μg of poly(A)$^+$ RNA (A) and 10 μg of total RNA (B) from various tumor cells were hybridized with a ^{32}P-labeled DNA probe for human GlcNAc-6-*O*-sulfotransferase cDNA as described under "EXPERIMENTAL PROCEDURES." Hybridization with a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA probe was used to test for equal RNA loading. The positions of the molecular size standards (A), and those of ribosomal RNAs (B) are indicated at the left.

mRNA were found in K-562 chronic myelogeneous leukemia cells, MOLT-4 lymphoblastic leukemia cells, Jurkat T cell leukemia cells, Raji Burkit lymphoma cells, G361 melanoma cells, and U251 glioma cells (Fig. 6, A and B). A moderate level of the signal was found in SW480 colon adenocarcinoma cells. Weak expression was observed in A549 lung adenocarcinoma cells, G401 Wilms tumor cells, and Ishikawa uterine endometrial adenocarcinoma cells. SMS-KCN and SMS-KAN neuroblastoma cells also weakly expressed the mRNA (data not shown). The following cells did not express the mRNA: HL-60 promyelocytic leukemia cells, S3 Hela cells, MCF-7 breast carcinoma cells (Fig. 6), and SH-SY5Y, SK-N-AS, NGP, and CHP901 neuroblastoma cells (data not shown). Mouse Neuro2a and NB41A3 neuroblastoma cells also did not express the mRNA. In five surgically removed specimens of colon carcinomas, Glc-

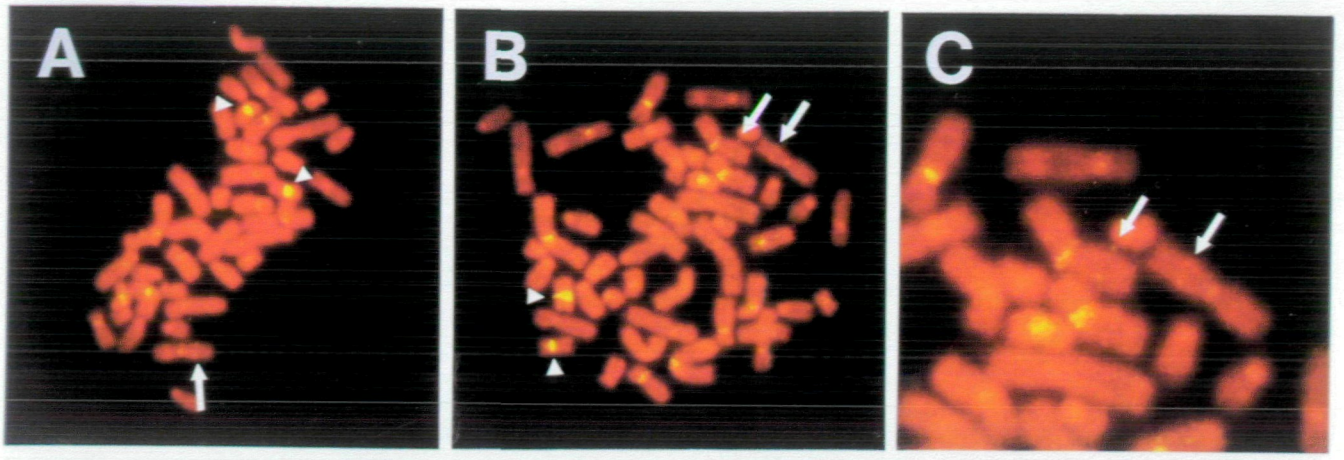

Fig. 7. **Fluorescence *in situ* hybridization analysis to determine the chromosome localization of human GlcNAc-6-*O*-sulfotransferase.** Two sections of normal human metaphase spreads are shown (A, B). Arrows indicate the fluorescence signals specific for human GlcNAc-6-*O*-sulfotransferase on chromosome 7q31 (A, B, C), and arrowheads the hybridization signals of α-microsattelite DNA located on chromosome 16 (A, B). The area indicated by arrows in B was magnified (C).

NAc-6-*O*-sulfotransferase mRNA levels were not different from those in adjacent colonic mucosal tissues (data not shown). The GlcNAc-6-*O*-sulfotransferase mRNA was not detected in 22 neuroblastomas isolated from the patients (data not shown).

Chromosomal Localization of Human GlcNAc-6-O-Sulfotransferase—Fluorescence *in situ* hybridization analysis revealed that the human GlcNAc-6-*O*-sulfotransferase gene was in the chromosome 7q31 region (Fig. 7). Therefore, the sulfotransferase gene is different from the gene on the 16th chromosome whose abnormality causes macular corneal dystrophy with the characteristic of poor sulfation in keratan sulfate (*31*).

DISCUSSION

Human GlcNAc-6-*O*-sulfotransferase was found to exhibit extensive sequence similarity to mouse GlcNAc-6-*O*-sulfotransferase. In particular, in the putative catalytic domain encompassing 363 amino acid residues, 99% amino acids were identical between the human and mouse enzymes, and all the amino acid changes were conserved ones. This high degree of homology is unusual. For example the mouse and human (Fukuta, M., Kobayashi, Y., Uchimura, K., Kimata, K., and Habuchi, O., submitted for publication) chondroitin 6-sulfotransferases are 90% identical in the corresponding region. The primary sequence of fucosyltransferase IV, which forms the Lewis X antigen, is also 90% conserved in the catalytic regions in man and mouse (*32*). The high degree of evolutional conservation of the human and mouse GlcNAc-6-*O*-sulfotransferases implies the rigid requirement of the protein structure for the transferase action.

In spite of the evolutionary conserved protein structure of GlcNAc-6-*O*-sulfotransferase, the mode of expression of the enzyme differs between man and mouse. In the mouse, the enzyme is not expressed in the bone marrow or spleen, but in man it is strongly expressed in these organs. On the other hand, the pancreas strongly expresses the mRNA in the mouse, but expresses it only weakly in man.

Lymph nodes moderately expressed the sulfotransferase mRNA in both species. In the mouse, the mRNA was detected in high endothelial venules (HEV) of lymph nodes. Although we did not perform *in situ* hybridization analysis in man, we can infer that in man, the enzyme is also expressed in HEV. Furthermore, we provided, for the first time, direct evidence that the human enzyme is involved in the synthesis of 6-sulfo sialyl Lewis X. It is possible that the enzyme also participates in the synthesis of the L-selectin ligand.

Sulfotransferase is strongly expressed in the brain in both man and the mouse. Therefore, precise localization in the mouse brain was determined by *in situ* hybridization. We found that the transcript was localized in specific regions, such as the pyramidal cells in the CA3 subregion of the hippocampus, cerebellar nucleus and Purkinje cells. The product of the sulfotransferase may also be involved in neural communication.

We performed a rather extensive survey of GlcNAc-6-*O*-sulfotransferase expression in tumor cells. So far, we have observed that strong expression of the mRNA is restricted to certain tumors and that a change in the expression is not a general phenomenon associated with tumorigenesis. Generally, the weak expression of sulfotransferase in neuroblastomas in spite of strong expression in brain neurons, and strong expression of the signal in a melanoma cell line are interesting observations and should prompt further studies in this area.

The mode of expression of human GlcNAc-6-*O*-sulfotransferase in organs and tumor cells indicates its potential role as a cell surface signal in blood cells and probably their progenitor cells. The enzyme mRNA is highly expressed in the bone marrow, spleen and peripheral leukocytes, and moderately so in the thymus. Furthermore, higher levels of mRNA expression were observed in four lines of leukemia/lymphoma cells. Since the ligands of E-selectin and P-selectin are on leukocytes, one obvious question is whether the sulfation of the E- and P-selectin ligands increases their affinity to selectins, as in the case of the ligand of L-selectin. However, the 6-sulfation and 6′-sulfation of sialyl Lewis X hexasaccharide ceramide do not change the binding affinity to P-selectin, but decrease or abolish its binding to E-selectin (*33*), although the 3′-sulfation of the Lewis a structure

enhances the binding to E-selectin (*34*). Therefore, the role of GlcNAc-6-*O*-sulfotransferase in blood cells and their progenitor cells is not likely to be enhancement of the binding affinity to the E- and P-selectin ligands. Precise identification of leukocyte subpopulations and hematopoietic progenitor cells based on the enzyme expression will provide clues as to the biological significance of the high level expression of the mRNA in these cells.

The sulfotransferase gene has been assigned to chromosome 7q31. Previously, we assigned the location of mouse chondroitin 6-sulfotransferase as chromosome 9 (*25*). Mouse chromosome 9 does not have segments homologous to human chromosome 7. Thus, in spite of the extensive sequence homology (Fukuta, M., Kobayashi, Y., Uchimura, K., Kimata, K., and Habuchi, O., submitted for publication), the chondroitin 6-sulfotransferase and GlcNAc-6-*O*-sulfotransferase genes do not exist on the same chromosome as a multigene complex of related genes.

Macular corneal dystrophy is a genetic disease involving poorly sulfated keratan sulfate. Since GlcNAc-6-*O*-sulfation precedes galactose sulfation (*19*), the poor sulfation might be due to an abnormal GlcNAc-6-*O*-sulfotransferase. The gene causing the disease is located on the 16th chromosome (*31*). Thus, so far we have not been able to obtain evidence that the cloned human GlcNAc-6-*O*-sulfotransferase is involved in the biosynthesis of keratan sulfate. Together with the mode of expression of the sulfotransferase in human organs, these results may imply that this newly cloned sulfotransferase is the enzyme involved in the formation of the 6-sulfo-*N*-acetyllactosamine structure in glycoproteins and/or glycolipids, and that there is another GlcNAc-6-*O*-sulfotransferase involved in keratan sulfate biosynthesis. However, we can not rule out the possibility that the present sulfotransferase participates in keratan sulfate biosynthesis in a specific organ such as the brain.

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