Tissue-Specific mRNA Expression Profiles of Human Carbohydrate Sulfotransferase and Tyrosylprotein Sulfotransferase

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Pairs of forward and reverse primers and TaqMan probes specific to each of 15 human sulfotransferases were prepared. The mRNA expression level of each target enzyme was analyzed in total RNA from single and pooled specimens of various human tissues (adrenal gland, bone marrow, brain, colon, heart, kidney, liver, lung, pancreas, peripheral leukocytes, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thymus, thyroid gland, trachea, and uterus) by real-time reverse transcription PCR using an ABI PRISM 7700 Sequence Detection System. The mRNA expression profiles of the sulfotransferases in these 23 different human tissues were used to identify the tissues exhibiting high transcriptional activity for these enzymes. These results provide valuable information for studies concerning the human carbohydrate sulfotransferase and tyrosylprotein sulfotransferase genes in various tissues.

Key words sulfotransferase; mRNA expression; tissue distribution; quantification

L-Selectin ligands require sulfation as a post-translational modification.¹⁾ Sulfotransferases potentially involved in the generation of selectin ligands have recently been reported.^{2–5)} These enzymes, such as carbohydrate sulfotransferase (CHST) and tyrosylprotein sulfotransferase (TPST), can sulfate the carbohydrate and protein moieties, respectively. Li *et al.* have reported that CHST1 and CHST2 contribute to the generation of optimal L-selectin ligands in vascular endothelial cells at sites of inflammation.⁶⁾ Hiraoka *et al.* have reported that CHST8 and CHST9 play critical roles in forming sulfo- $\rightarrow$ 4GalNAc β 1- $\rightarrow$ 4GlcNAc β 1- $\rightarrow$ R in both *N*-glycans and *O*-glycans in a tissue-specific manner.⁷⁾ Hiraoka *et al.* have also reported that CHST11 and CHST12 play complementary roles in chondroitin and dermatan sulfate synthesis in different tissues.⁸⁾ Ouyang and Moore have reported that TPST-1 and TPST-2 show distinct specificities toward peptide substrates.⁵⁾ Thus, the differences in substrate specificity of sulfotransferases have been demonstrated in a number of studies. However, there is only very limited information concerning the relationships between the expres-

sion level and function of human sulfotransferases because the molecular cloning of human sulfotransferases as reported in the present study was not achieved until 1997 to 2001^{2–5,7–16)} and studies on these sulfotransferases are therefore still in the early stages. The tissue distributions of many sulfotransferases such as CHST1,⁹⁾ CHST3,³⁾ CHST4,²⁾ CHST5,¹¹⁾ CHST7,¹³⁾ CHST9,⁷⁾ TPST1,⁴⁾ and TPST2⁵⁾ have also been reported. However, the tissue distribution of the mRNA expression of large numbers of human sulfotransferase genes has not been evaluated under the same experimental conditions using relatively quantitative data. The present study was therefore undertaken to investigate the mRNA expression levels of 15 human sulfotransferases using high-sensitivity real-time reverse transcription PCR (RT-PCR) in total RNA from single and pooled specimens of 23 adult human tissues. The nomenclature of these sulfotransferases is summarized in Table 1.

Table 1. Nomenclature of Sulfotransferases

Name	Abbreviation	GenBank accession No.	Trivial name(s)/synonym(s)
Carbohydrate (keratan sulfate Gal-6) sulfotransferase 1	CHST1	NM_003654	C6ST, KS6ST, KSGal6ST
Carbohydrate (<i>N</i> -acetylglucosamine-6- <i>O</i>) sulfotransferase 2	CHST2	NM_004267	C6ST
Carbohydrate (chondroitin 6) sulfotransferase 3	CHST3	NM_004273	C6ST, C6ST1
Carbohydrate (<i>N</i> -acetylglucosamine 6- <i>O</i>) sulfotransferase 4	CHST4	NM_005769	LSST, HEC-GlcNAc6ST
Carbohydrate (<i>N</i> -acetylglucosamine 6- <i>O</i>) sulfotransferase 5	CHST5	NM_012126	ALYE870, PRO1886, FLJ22167, MGC74625
Carbohydrate (<i>N</i> -acetylglucosamine 6- <i>O</i>) sulfotransferase 6	CHST6	NM_021615	MCDC1
Carbohydrate (<i>N</i> -acetylglucosamine 6- <i>O</i>) sulfotransferase 7	CHST7	NM_019886	C6ST-2
Carbohydrate (<i>N</i> -acetylgalactosamine 4- <i>O</i>) sulfotransferase 8	CHST8	NM_022467	GALNAC4ST-1
Carbohydrate (<i>N</i> -acetylgalactosamine 4- <i>O</i>) sulfotransferase 9	CHST9	NM_031422	GALNAC4ST-2
Carbohydrate sulfotransferase 10	CHST10	NM_004854	HNK1ST, HNK-1ST, MGC17148
Carbohydrate (chondroitin 4) sulfotransferase 11	CHST11	NM_018413	C4ST, C4ST1, C4ST-1, HSA269537
Carbohydrate (chondroitin 4) sulfotransferase 12	CHST12	NM_018641	C4S-2, C4ST2, C4ST-2
Galactose-3- <i>O</i> -sulfotransferase 1	GAL3ST1	NM_004861	CST
Tyrosylprotein sulfotransferase 1	TPST1	NM_003596	
Tyrosylprotein sulfotransferase 2	TPST2	NM_003595	

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Table 2. Total RNA Source Information for Various Tissues

Tissue	Pool size	Age	Sex	Race
Adrenal gland	61	15—61 years	Female, male	Caucasian
Bone marrow	8	18—56 years	Female, male	Caucasian
Brain	1	43 years	Male	Caucasian
Colon	1	35 years	Female	Caucasian
Heart	10	21—51 years	Female, male	Caucasian
Kidney	14	18—59 years	Female, male	Caucasian
Liver	1	51 years	Male	Caucasian
Lung	3	15—40 years	Female, male	Caucasian
Pancreas	2	50, 55 years	Male	Caucasian
Peripheral leukocytes	250	18—40 years	Female, male	Caucasian
Placenta	8	19—39 years	Female	Caucasian
Prostate	32	21—50 years	Male	Caucasian
Salivary gland	24	15—60 years	Female, male	Caucasian
Skeletal muscle	7	20—68 years	Female, male	Caucasian
Small intestine	5	20—61 years	Female, male	Caucasian
Spinal cord	49	15—66 years	Female, male	Caucasian
Spleen	14	30—66 years	Female, male	Caucasian
Stomach	1	50 years	Male	Caucasian
Testis	39	14—64 years	Male	Caucasian
Thymus	3	20—38 years	Male	Caucasian
Thyroid gland	65	15—61 years	Female, male	Caucasian
Trachea	—	18—54 years	Female, male	Caucasian
Uterus	3	40—61 years	Female	Caucasian

—, no data available.

MATERIALS AND METHODS

Materials Total RNA prepared from single and pooled specimens of adult human tissues (adrenal gland, bone marrow, brain, colon, heart, kidney, liver, lung, pancreas, peripheral leukocytes, placenta, prostate, salivary gland, skeletal muscle, small intestine, spinal cord, spleen, stomach, testis, thymus, thyroid gland, trachea, and uterus) was purchased from CLONTECH Laboratories, Inc. (Hilden, Palo Alto, CA, U.S.A.). The total RNA source information for these human tissues is shown in Table 2. The total RNA of the 23 adult human tissues (Table 2) used in the present study was the same as that used for the measurement of the mRNA expression levels of transporters in our previous studies.^{17,18)} The present study was approved by the Ethics Committee of Otsuka Pharmaceutical Factory, Inc. Yeast tRNA was purchased from Life Technologies, Inc. (Rockville, MD, U.S.A.), and the TaqMan One-Step RT-PCR Master Mix Reagents Kit was purchased from Applied Biosystems (Foster City, CA, U.S.A.). All other chemicals used in this study were of reagent grade.

Oligonucleotides The pair of forward and reverse primers and the TaqMan probe for peptidylprolyl isomerase A (PPIA) used for RT-PCR analysis employed sequences that have been reported previously.¹⁹⁾ The pairs of forward and reverse primers and the TaqMan probes for CHST1, CHST3, CHST4, CST, and TPST2 used for RT-PCR analysis also employed sequences that have been reported previously.²⁰⁾ The pair of forward and reverse primers and the TaqMan probe for TPST1 used for RT-PCR analysis also employed sequences that have been reported previously.²¹⁾ The pairs of primers and the TaqMan probes for the target mRNAs were designed based on the human mRNA sequence (Table 3) using Primer Express software (Applied Biosystems). The GenBank accession number and the position from the initia-

Table 3. Primers and Probes Used for RT-PCR Analysis

mRNA (GenBank No.)	Forward primer (Position)	Reverse primer (Position)	Probe (Position)
CHST2 (NM_004267)	5'-CCGTCAAGACACTACGGAGAGT-3' (1295—1316)	5'-GGCAAACTGCTCCATTTCG-3' (1365—1347)	5'-TACGATTTTGTGGGACTGTTGGTAGCC-3' (1318—1345)
CHST5 (NM_012126)	5'-CGCTCTATCTTTTGTGCGACA-3' (292—313)	5'-AGTTGAAAAGGGGACAGGTT-3' (370—349)	5'-TTTGATGCCTACATGCCACAGAGCC-3' (322—346)
CHST6 (NM_021615)	5'-TTCCTGTGCGACATGGACG-3' (298—316)	5'-GAAAGAGGTCGGACAGGTTGC-3' (363—344)	5'-TTTGATGCCTATCTGCTTGGCGC-3' (319—342)
CHST7 (NM_019886)	5'-TGGGCGAAGCTCTTAACCAAGC-3' (350—370)	5'-GCTTGCCATAGATGCCACA-3' (419—401)	5'-CCCGGAGCTTTCTACTTGTATGAGCCC-3' (372—399)
CHST8 (NM_022467)	5'-CCTGCGACCTGGAACAATG-3' (6—24)	5'-CTGATGAAGAGGAGAGGCCT-3' (89—69)	5'-CTGGCCTGCATGTTCTCTTCCATCC-3' (28—52)
CHST9 (NM_031422)	5'-ATGATCGGTGCTCCAAAGAG-3' (1132—1152)	5'-TCACGACTTGAGCATGGTTC-3' (1216—1196)	5'-TTTCCCACCTTAAAGATAGGACTCTTCCG-3' (1159—1189)
CHST10 (NM_004854)	5'-AAATACAGGAGGAACCGACA-3' (669—690)	5'-CTGTTATACACGGTAATGCCCG-3' (950—929)	5'-ATCCAGTTTGAAGATTCTGCGGTACTC-3' (703—732)
CHST11 (NM_018413)	5'-AACGAACACTGGCAACCGC-3' (766—784)	5'-ACTTGCCCAAGAGTCATATG-3' (835—815)	5'-TACTCACTCTGCCATCCCTGCCACAT-3' (787—812)
CHST12 (NM_018641)	5'-GGGTGGTGTTCATGATCC-3' (40—58)	5'-TGC AAGTAGAAGTGGCGCGG-3' (110—92)	5'-CTGATCATCTGTACTGGGACAGCGC-3' (61—86)

The positions of the primers and probes were counted from the position of the initiation codon.

Table 4. Expression of Human Sulfotransferase mRNAs in Various Tissues

Tissue	CHST1	CHST2	CHST3	CHST4	CHST5	CHST6	CHST7	CHST8
Adrenal gland	0.0213	0.0531	0.278	0.0157	0.00302	0.0839	0.0508	0.00975
Bone marrow	0.00392	0.667	0.0226	0.000292	0.000681	BLQ	0.0211	0.00351
Brain	0.149	0.340	0.0661	0.000802	0.0171	0.167	0.0597	0.0542
Colon	0.00275	0.0492	0.0477	0.00299	0.304	0.0157	0.0162	0.00221
Heart	0.0137	0.0563	0.144	0.000868	0.00335	0.0300	0.245	0.00358
Kidney	0.00498	0.0462	0.108	0.00337	0.00357	0.00153	0.0160	0.000644
Liver	0.00221	0.0101	0.0122	0.0307	0.000167	BLQ	0.123	0.000669
Lung	0.0205	0.146	0.0918	0.00939	0.00498	0.0370	0.0451	0.00202
Pancreas	0.00146	0.00834	0.0344	0.0557	0.00115	BLQ	0.0139	0.00248
Peripheral leukocytes	0.0000778	0.379	0.0000749	0.000329	0.000122	BLQ	0.105	0.00260
Placenta	0.00858	0.639	0.251	0.000178	0.000950	0.0151	0.143	0.00211
Prostate	0.00512	0.193	0.159	0.00259	0.00303	0.00923	0.0298	0.00992
Salivary gland	0.0397	0.0670	0.0728	0.00414	0.000745	0.0822	0.0192	0.00131
Skeletal muscle	0.0138	0.0715	0.511	0.000155	0.00282	BLQ	0.109	0.00776
Small intestine	0.00641	0.119	0.0358	0.000656	0.380	0.0225	0.0188	0.00220
Spinal cord	0.0393	0.681	0.269	0.00172	0.0129	0.568	0.0417	0.0689
Spleen	0.0172	0.434	0.0370	0.00102	0.00124	BLQ	0.161	0.00965
Stomach	0.00450	0.140	0.0759	0.00213	0.0196	0.00252	0.0247	0.00167
Testis	0.0780	0.0718	0.655	0.00628	0.0156	0.0645	0.0649	0.0391
Thymus	0.00354	0.243	0.0415	0.00105	0.000614	0.0102	0.0288	0.00207
Thyroid gland	0.0918	0.130	0.0780	0.00843	0.00460	BLQ	0.0309	0.00238
Trachea	0.0652	0.103	0.179	0.0170	0.0121	0.688	0.0431	0.00309
Uterus	0.0125	0.138	0.259	0.00243	0.00483	0.0394	0.0925	0.00666

Tissue	CHST9	CHST10	CHST11	CHST12	GAL3ST1	TPST1	TPST2
Adrenal gland	0.000384	0.000270	0.0791	0.237	0.000242	0.0974	0.214
Bone marrow	BLQ	BLQ	0.191	0.444	0.0000476	0.0881	0.334
Brain	0.00768	0.00222	0.0593	0.253	0.0209	0.143	0.103
Colon	0.00191	BLQ	0.0255	0.125	0.00673	0.0448	0.0696
Heart	0.0379	0.00100	0.0244	0.337	0.00141	0.0696	0.182
Kidney	0.00447	0.000129	0.0202	0.182	0.0915	0.0863	0.176
Liver	0.0162	BLQ	0.0134	0.104	0.00141	0.102	0.406
Lung	0.0231	0.000233	0.0921	0.292	0.00175	0.118	0.413
Pancreas	0.0172	BLQ	0.0257	0.133	0.00737	0.0778	3.97
Peripheral leukocytes	BLQ	0.000107	0.297	0.416	0.0000751	0.0297	0.363
Placenta	BLQ	0.000799	0.176	0.338	0.00226	0.150	0.434
Prostate	0.0254	0.00141	0.0453	0.278	0.00261	0.119	0.0921
Salivary gland	0.0391	0.000317	0.0120	0.256	0.000804	0.0314	0.0743
Skeletal muscle	0.00419	0.00209	0.0159	0.674	0.00800	0.136	0.635
Small intestine	0.000616	0.000127	0.0341	0.140	0.0183	0.0408	0.0884
Spinal cord	0.0270	0.00109	0.0754	0.267	0.0869	0.221	0.0408
Spleen	0.0186	0.000295	0.119	0.538	0.0000869	0.109	0.247
Stomach	0.00167	0.000198	0.0571	0.189	0.00611	0.0860	0.104
Testis	0.0273	0.00242	0.0368	0.363	0.0179	0.576	0.279
Thymus	0.00151	0.000303	0.104	0.151	0.000140	0.0188	0.0819
Thyroid gland	0.00441	0.000886	0.0197	0.207	0.0000612	0.158	0.457
Trachea	0.163	0.000743	0.0698	0.322	0.00133	0.163	0.166
Uterus	0.00112	0.00243	0.0957	0.301	0.00449	0.255	0.154

BLQ, below the limit of quantification. Data are expressed as the ratio of the target mRNA to PPIA mRNA. Experiments were performed in duplicate. The highest values among the various tissues are shown in boxes.

tion codon are also shown in Table 3. Each primer and/or probe was homology searched by an NCBI BLAST search to ensure that it was specific for the target mRNA transcript. The primers and TaqMan probes were synthesized by QIAGEN Japan (Tokyo, Japan) and the Genosys Division of SIGMA-ALDRICH JAPAN K.K. (Ishikari, Hokkaido, Japan). The TaqMan probes contained 6-carboxyfluorescein (FAM) at the 5' end and 6-carboxytetramethylrhodamine (TAMRA) at the 3' end and were designed to hybridize to a sequence located between the PCR primers.

TaqMan RT-PCR Conditions Total RNA samples obtained from adult human tissues were diluted under the same conditions as in our previous studies.^{17,18)} Total RNA at 25000 pg per 50 μ l of reaction mixture was used for measurement of the target mRNA in each tissue. The RT-PCR assay was performed using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems) under the same conditions as in our previous studies.^{17,18)}

Data Analysis Samples were deemed positive at any given cycle when the value of the emitted fluorescence was

greater than the threshold value calculated by the instrument's software (Sequence Detector Ver. 1.9.1). The threshold cycle (Ct), which is defined as the cycle at which PCR amplification reaches a significant value (*i.e.*, usually 15 times greater than the standard deviation of the baseline), is given as the mean value. The relative expression of each mRNA was calculated by the ΔCt method (where ΔCt is the value obtained by subtracting the Ct value of PPIA mRNA from the Ct value of the target mRNA), as employed in our previous studies.^{17,18)} Specifically, the amount of target mRNA relative to PPIA mRNA is expressed as $2^{-(\Delta\text{Ct})}$. Data are expressed as the ratio of the target mRNA to PPIA mRNA.

RESULTS AND DISCUSSION

In this study, we investigated the mRNA expression levels of 15 human sulfotransferases in adult human tissues. Analysis was conducted by RT-PCR using the ABI PRISM 7700 Sequence Detection System in the presence of the TaqMan probe. We selected PPIA as the endogenous control for mRNA measurements. The rationale used to select the most suitable housekeeping gene has been described in detail in our previous studies.^{17,19,22)} The mRNA expression levels of human CHSTs, GAL3ST1, and TPSTs in various tissues are shown in Table 4. Consistent with the findings of another study in human subjects,⁹⁾ CHST1 mRNA was expressed ubiquitously, with the highest levels of expression in the brain and very low levels of expression in peripheral leukocytes. CHST2 mRNA was expressed ubiquitously, with the highest levels of expression in the spinal cord. Similar to the findings of another study in human subjects,³⁾ CHST3 mRNA was expressed ubiquitously, with the highest levels of expression in the testis and very low levels of expression in peripheral leukocytes. Similar to the findings of another study in human subjects,²⁾ CHST4 mRNA was expressed ubiquitously, with high levels of expression in the liver and pancreas as compared with other tissues. Consistent with the findings of another study in human subjects,¹¹⁾ CHST5 mRNA was expressed ubiquitously, with high levels of expression in the colon and small intestine. CHST6 mRNA was expressed at high levels in the brain, spinal cord, and trachea and was detectable in the adrenal gland, colon, heart, kidney, lung, placenta, prostate, salivary gland, small intestine, stomach, testis, thymus, and uterus. Similar to the findings of another study in human subjects,¹³⁾ CHST7 mRNA was expressed ubiquitously, with the highest levels of expression in the heart. Consistent with the findings of other studies in human subjects,^{7,14)} CHST8 mRNA was expressed ubiquitously, with high levels of expression in the brain, spinal cord, and testis. Consistent with the findings of another study in human subjects,⁷⁾ CHST9 mRNA was expressed at the highest levels in the trachea and was detectable in other tissues except for the bone marrow, peripheral leukocytes, and placenta. Similar to the findings of another study in human subjects,¹⁵⁾ CHST10 mRNA was expressed at high levels in the brain, skeletal muscle, testis, and uterus and was detectable in other tissues except for the bone marrow, colon, liver, and pancreas. Consistent with the findings of another study in human subjects,⁸⁾ CHST11 and CHST12 mRNA were expressed ubiquitously, with the highest levels of ex-

pression in peripheral leukocytes and skeletal muscle, respectively. GAL3ST1 mRNA was expressed ubiquitously, with the highest levels of expression in the kidney and very low levels of expression in the bone marrow, peripheral leukocytes, spleen, and thyroid gland. Consistent with the findings of another study in human subjects and mice,⁴⁾ TPST1 mRNA was expressed ubiquitously, with the highest levels of expression in the testis. Consistent with the findings of another study in human subjects and mice,⁵⁾ TPST2 mRNA was expressed ubiquitously, with the highest levels of expression in the pancreas. The results for the tissue-specific mRNA expression profiles of each human CHST and TPST are summarized by comparison of qualitative data such as that obtained by Northern blotting analysis against data reported in the literature. However, there is only very limited information concerning the relationships between the expression site and function because studies on these sulfotransferases are still in the early stages. We therefore hope that other researchers will find our results helpful in conducting studies to clarify the relationships between the expression site and function.

In conclusion, the results of the present study provide valuable information concerning the tissue-specific profiles of mRNA expression of human carbohydrate sulfotransferase and tyrosylprotein sulfotransferase in various tissues. The information obtained in this study may be important in assessing the feasibility of measuring the mRNA expression levels of target genes in target tissues.

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