

Identification and Functional Characterization of a Human GalNAc α 2,6-Sialyltransferase with Altered Expression in Breast Cancer

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Abstract

Background: We sought to identify genes with altered expression during human breast cancer progression by applying mRNA comparisons of normal and tumor mammary cell lines with increasingly malignant phenotypes. The gene encoding a new sialyltransferase (STM) was found to be down-regulated in tumor cells. Abnormal expression and enzymatic activities of sialyltransferases in tumor cells result in the formation of tumor-associated carbohydrate antigens that can be used for the better understanding of the disease process and are applied for tumor diagnosis and immunotherapy. Altered glycosylation patterns of the MUC1 mucin, in particular, is a target antigen for immunotherapy of breast and other cancers.

Materials and Methods: Total RNAs from multiple normal mammary epithelial cell strains and tumor cell lines were compared by differential display and the differential expression of selected cDNAs was confirmed by Northern analyses. Recombinant STM was expressed in COS-7 cells. The substrate and linkage specificity of STM was examined using various oligosaccharides and *O*-glycosylated proteins as acceptor substrates. The chromosomal localization of the SIATL1 gene was assigned by somatic cell hybrid analysis.

Results: A human sialyltransferase gene was identified by differential display as being down-regulated in breast tumor cell lines as compared to normal mammary epithelial

cell strains, and the corresponding full-length cDNA (*stm*) was cloned. The encoded protein of 374 amino acid residues contained the *L*- and *S*-sialylmotifs, two catalytic regions conserved in all functional sialyltransferases. Recombinant STM is an active GalNAc α 2,6-sialyltransferase with Gal β 1,3 GalNAc-*O*-Ser/Thr and (+/–Neu5Ac α 2,3) Gal β 1,3GalNAc-*O*-Ser/Thr acceptor specificity. The SIATL1 gene, encoding STM, was mapped to the long arm of human chromosome 17 at q23-qter, a region that is non-randomly deleted in human breast cancers. However, Southern analyses indicated that SIATL1 is usually not grossly rearranged in breast tumors. Northern analyses showed that the gene was widely expressed in normal human tissues, as well as in normal breast and prostate epithelial cell lines, but significantly down-regulated or absent in corresponding tumor cell lines.

Conclusions: Our findings suggest that aberrant expression of STM sialyltransferase in tumors could be a feature of the malignant phenotype. In breast cancers, the MUC1 mucin is overexpressed and contains shorter *O*-glycans as compared to the normal mucin. Because STM catalyzes the synthesis of *O*-glycans, cloning and characterization of its substrate specificity will contribute to the understanding of the molecular mechanisms underlying the aberrant glycosylation patterns of *O*-glycans and the formation of mucin-related antigens in human breast cancers.

Introduction

The growth and dissemination of human tumors is a multistage process involving aberrant functions of the tumor cell, which result from changes in the expression of multiple genes. The concept of RNA genetics in cancer (1) underscores that the identification

of specific changes in gene expression, underlying the switch of a normal cell to a malignant phenotype, is essential for the prognosis and staging of malignant tumors, as well as for the design of effective anti-cancer therapies. Subtractive hybridization and differential display (2) methods of differential expression cloning were used to identify genes with aberrant expression during human mammary carcinogenesis. Genes with various cellular functions and altered expression in normal mammary epithelial cells versus corresponding tumor cells were cloned and functionally characterized (3–5). The gene encoding STM, a new human sialyltransferase, was identified among the differentially expressed

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This manuscript is dedicated (by G.S., A.A., and G.S.) to the memory of our mentor and friend Professor Ruth Sager.

genes based on its decreased or absent mRNA expression in breast tumor cell lines.

Sialyltransferases constitute a family of glycosyltransferases that catalyze the posttranslational transfer of sialic acid (*N*-acetylneuraminic acid) to acceptor oligosaccharide substrates at terminal positions on glycoproteins and glycolipids (6). It is estimated that the human genome encodes more than 20 different sialyltransferases required to synthesize all known sialo-oligosaccharide structures present in mammalian cells, but only 16 distinct human sialyltransferase cDNAs have been cloned (7–9). Originally, sialyltransferases were biochemically purified and their cDNAs were cloned using N-terminal sequences (10,11). Comparison of the obtained cDNA sequences revealed two highly conserved regions, termed the *L*- and *S*-sialylmotifs, that participate in substrate binding (12,13). Subsequently, several sialyltransferases were cloned by PCR using degenerate primers designed within the sialylmotifs (14,15) or by expression cloning (16–18). Cloning of the gene encoding STM by differential display adds an entirely different approach to the identification of novel sialyltransferases with putative functional significance in disease-related processes.

Sialyltransferases differ in their substrate specificity and tissue distribution, and they are classified into four families according to the carbohydrate linkages they synthesize: the ST3Gal-, ST6Gal-, ST6GalNAc-, and ST8Sia-families. The members of each family exhibit strong activity toward certain acceptor groups, although the substrate specificities of these enzymes overlap; one linkage can be synthesized by multiple enzymes (8,9).

Specific terminal glycosylation sequences are important recognition determinants in cell–cell interactions, protein targeting, and host–pathogen interactions (19,20), as well as important regulators of cell growth, cell maturation, and differentiation (21,22). Pathologic conditions are often associated with modified terminal sugar structures induced by changes in expression of the glycosyltransferases responsible for their synthesis. Thus, tumor cells are characterized by aberrant expression and enzymatic activities of sialyltransferases (23–27). The appearance of new glycoconjugate structures on the surface of tumor cells and the accumulation of precursors due to blocked synthesis result in the formation of tumor-associated carbohydrate antigens (24–27). In particular, sialic acid has a masking effect on tumor antigens, because hypersialylation of the tumor cell surface results in decreased susceptibility of tumor cells to natural killer cells with a compromising effect on the host immune system. In addition, sialic acids are involved in a variety of biological processes (28,29). Specific recognition of sialoglycoconjugates by adhesion molecules underlies their involvement in determining the metastatic potential of malignant cells (30,31).

We describe the identification and cDNA cloning of the gene encoding the STM sialyltransferase on the basis of its down-regulated expression in mammary

tumor cells, as well as the biochemical characterization of the encoded enzyme. Cloning and characterization of human sialyltransferases will enable studies on their normal cellular role(s), as well as on the mechanism of their aberrant regulation in malignant cells and its functional consequences.

Materials and Methods

Cell Lines and Media

Normal human mammary epithelial cell strains (81N, 76N, and 70N) derived from reduction mammaplasty specimens, as well as primary (21NT, 21PT), and metastatic (21MT-1, 21MT-2) tumor cell lines were established in long-term culture and characterized as described previously (32). Metastatic mammary tumor cell lines MCF-7, BT-474, BT-549, T-47D, ZR-75-1, MDA-MB-157, MDA-MB-231, MDA-MB-361, MDA-MB-435, and MDA-MB-436 were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and were routinely grown in DFCI-1 medium (33). Normal-immortalized prostate epithelial cell lines: CF3 (HPV-immortalized), CF91, and MLC (SV40-immortalized) were provided by Dr. Johng Rhim and were cultured in KGM medium (Difco, Beckton Dickinson, Sparks, MD, USA). Prostate metastatic tumor cell lines DU145, LNCaP, and PC3 were obtained from the ATCC. All cells were plated at a density of 1×10^6 cells per 100-mm diameter plate, cultured for 3–5 days, and harvested when the cultures were about 75% confluent for RNA isolation and near confluent for DNA isolation. Tissue culture medium components were purchased from Life Technologies, Inc., Gaithersburg, MD, USA or Hyclone, Logan, UT, USA.

Differential Display of mRNAs

Total cellular RNAs (50 ng) from exponentially growing cells were treated with DNase I in the presence of RNasin ribonuclease inhibitor, in order to remove residual DNA contamination (4). RNAs were extracted with phenol/chloroform, precipitated with ethanol, and redissolved in DEPC-treated water. Subsequently, RNAs were reverse-transcribed with a 3'-anchoring oligo dT primer T₁₂MA (where M is degenerate for G, C, or A). The resultant cDNAs were amplified by polymerase chain reaction (PCR) using T₁₂MA and an arbitrary 10-mer (OPA3:AGTCAGC-CAC) as 5'-primer, and compared side by side on a sequencing gel as ³⁵S-labelled partial cDNA fragments corresponding to the 3'-end of the mRNAs (4).

Cloning, Sequencing of cDNAs, and Database Analysis

The partial *stm* cDNA obtained from differential display was reamplified by PCR, cloned into the PCR II vector using the TA cloning system (Invitrogen, Carlsbad, CA, USA), and sequenced on both strands with T7 and SP6 primers. Full-length cDNA clones were isolated from a 76N cDNA library constructed in λ Zap (Stratagene, La Jolla, CA, USA), which was screened

using the cloned PCR product as a probe. A full-length cDNA was sequenced on both strands using multiple internal primers (data not shown). Sequencing was performed with an ABI automated sequencer (model 373A). Oligonucleotides were synthesized by Amifof, Cambridge, MA, USA. The BLAST algorithm was used for nucleic acid sequence comparisons (34). Protein sequence comparisons were performed on GCG with final alignments on PILEUP and PRETTYPLOT.

Northern and Southern Blots

Total cellular RNAs were purified by standard guanidinium isothiocyanate and cesium chloride centrifugation as described elsewhere (4). Equal amounts of 20 μ g RNA per lane were loaded and transferred onto nylon membrane. Following hybridization, RNA blots were washed in $2 \times$ SSC containing 0.1% SDS at 65°C for 1 hr, dried, and exposed to Kodak films. After stripping at high stringency the blots were re-hybridized with a 32 P-labeled 36B4 probe as a loading control. The 36B4 gene encodes a ribosomal protein, whose expression is not affected by growth conditions or estrogen receptor expression (35). Genomic DNA was isolated and hybridized by standard methods (4). Densitometric scans of autoradiographs were obtained with an imaging densitometer (BioRad GS-700, BioRad Laboratories, Hercules, CA, USA) using the Molecular Analyst software.

Somatic Cell Hybrid and Southern Blot Analyses

Two panels of human-rodent somatic cell hybrids, the monochromosome hybrid mapping panel 2 and the regional mapping panel for chromosome 17 (obtained from the National Institute of General Medical Sciences [NIGMS]), were used to map the SIATL1 gene. The chromosome 17 content of the six hybrids in the regional mapping panel is shown in Fig. 6. DNA isolated from hybrid and parental cell lines were digested to completion with *Hind*III, separated electrophoretically on 0.8% agarose gels, and transferred onto nylon filters. Blots were hybridized with a 32 P-labeled 2.0-kb full-length *stm* cDNA probe.

Expression of Recombinant Protein and Sialyltransferase Assay

The cDNA encoding the catalytic domain of STM (Arg⁴²-Arg³⁷⁴) was amplified by PCR, using the primers A, 5'-GGAGCCTCGAGGGACACCACATCATTTG-3' (nt 157-181) and B, 5'-GCCGCAACTCGAGAAGAAGCAAAGCG-3' (nt 1180-1205), with the *Xho*I restriction site being underlined. The amplified and *Xho*I-digested 1.05-kb fragment was inserted into the *Xho*I site of pcDSA vector (36). The single insertion in the correct orientation was analyzed by restriction digests and DNA sequencing, and the resulting expression construct was designated pcDSA-STM, which consisted of the IgM signal peptide sequence, a protein A IgG binding domain, and the catalytic domain of STM. COS-7 cells (5×10^6) were transiently transfected with 10 μ g of

pcDSA-STM using the DEAE-dextran procedure and cultured as described previously (37). Following a 48-hr transfection, the culture medium was collected and the A-STM fusion protein secreted into the medium was adsorbed on IgG-Sepharose (60 μ L of resin/30 ml of medium) at 4°C for 16 hr. The resin was collected by centrifugation, washed three times with PBS, suspended in a final volume of 50 μ L of Dulbecco's Modified Eagle medium without fetal bovine serum, and used as immobilized enzyme.

Enzymatic Assays

Enzyme activity assays were performed as described previously (36,37). Each reaction mixture contained 0.1 M of MES buffer (pH 6.4), 10 mM of MgCl₂, 2 mM of CaCl₂, 0.3% Triton CF-54, 0.1 M of CMP-[¹⁴C]NeuAc (3.6 kBq), 0.15 mM of acceptor substrate, and 5 μ L of enzyme preparation, in a total volume of 20 μ L. After a 4-hr incubation at 37°C, the reaction was terminated by adding SDS-polyacrylamide gel electrophoresis loading buffer (10 μ L) and boiling at 100°C for 15 min. Subsequently, the incubation mixture was subjected to SDS-PAGE for glycoprotein acceptors. For glycolipid acceptors, the incubation mixtures were applied on a C-18 column (Sep-Pak Vac, 100 mg; Waters, Milford, MA, USA) (37). The radioactive materials in glycoproteins or glycolipids were visualized with a BAS2000 radio image analyzer (Fuji Film, Tokyo, Japan), and the radioactivity incorporated into the acceptor was counted. The NDV sialidase was purchased from Oxford, Abingdon, Oxon, UK, NANase III from Glyco, Novato, CA, USA, and *N*-Glycanase from Genzyme, Tokyo, Japan. For linkage analysis of sialic acids, [¹⁴C]NeuAc-incorporated fetuin and asialofetuin were synthesized with STM. After incubation with STM, [¹⁴C]sialylated glycoproteins were collected by ethanol precipitation and washed 10 times with 70% ethanol to remove the substrate CMP-[¹⁴C]NeuAc. To obtain the oligosaccharide portion of [¹⁴C]sialylated fetuin and asialofetuin, the [¹⁴C]sialylated glycoproteins were treated with 0.1 N NaOH/1 M of NaBH₄ at 37°C for 48 hr, and neutralized by the gradual addition of acetic acid in an ice-bath. Samples were desalted by gel filtration on Sephadex G-25 (1.3 \times 25 cm). Then, [¹⁴C]sialylated oligosaccharide alditols were purified by preparative TLC. The purified [¹⁴C]sialylated oligosaccharide alditols were treated with various sialidases (NANase I, specific for α 2,3-linked sialic acids; Newcastle Disease Virus sialidase, specific for α 2,3- and α 2,8-linked sialic acids; *Vibrio cholerae* sialidase, specific for α 2,3-, α 2,6-, and α 2,8-linked sialic acids). Samples were subjected to HPTLC with silica gel sorbant and a solvent system of 1-propanol:aqueous ammonia:water = 6:1:2.5. The chromatogram was visualized with a BAS2000 radio image analyzer. The reference oligosaccharide alditol [Gal β 1,3(NeuAc α 2,6) GalNAc-ol (Oligo 1) and NeuAc α 2,3 Gal β 1,3 (NeuAc α 2,6)GalNAc-ol (Oligo 2)] were detected with the resorcinol reagent.

Results

Identification of the *STM* Sialyltransferase by Differential Display

Differential display allows simultaneous comparisons of gene expression between multiple, closely related cell populations. In an attempt to isolate genes whose expression is reduced or lost during human mammary tumor progression, total RNA from normal mammary epithelial cell strains (76N, 70N), which senesce in culture, were displayed and compared with primary (21PT, 21NT), and metastatic ER+ (MCF-7) and ER- (21MT-1, 21MT-2, MDA-MB-435) malignant tumor cell lines (Fig. 1A), which all replicate indefinitely in culture. The 21T tumor progression series of cell lines (21PT→21NT→21MT-2→21MT-1) was established from one patient with infiltrating and intraductal breast carcinoma (32). A partial cDNA of 125 bp was identified as being down-regulated or absent in tumor cells (Fig. 1A) and was named *stm*. The differential expression of *stm* was confirmed by Northern hybridizations using the cloned and 32 P-labeled partial *stm* cDNA as a probe

(Fig. 1B). The two differentially expressed cDNAs of ~125 bp shown in Figure 1A were cloned and sequenced independently and both corresponded to *stm*. The *stm* cDNA displayed no homology to any nucleotide sequence in the Genbank/EMBL databases.

Cloning and Sequencing of a Full-Length cDNA

The *stm* partial cDNA was used as a probe to screen 300,000 plaques of a cDNA library constructed from 76N normal mammary epithelial cells. Positive clones were selected and their differential expression was confirmed on Northern blots (Fig. 1B). A full-length cDNA (1930 bp) was sequenced on both strands, and contained a single open reading frame (40–1161 bp) with a consensus AUG start codon, a 5'-untranslated region (39 bp), and a 3'-untranslated region (765 bp) containing a polyadenylation signal AATAAA (nt 1890–1895) and a polyA tail. The nucleotide sequence as well as the deduced primary sequence are shown in Fig. 2. The encoded protein of 374 amino acid residues was named STM (locus name SIATL1), because it displayed homology to known sialyltransferases, and represents a new member of

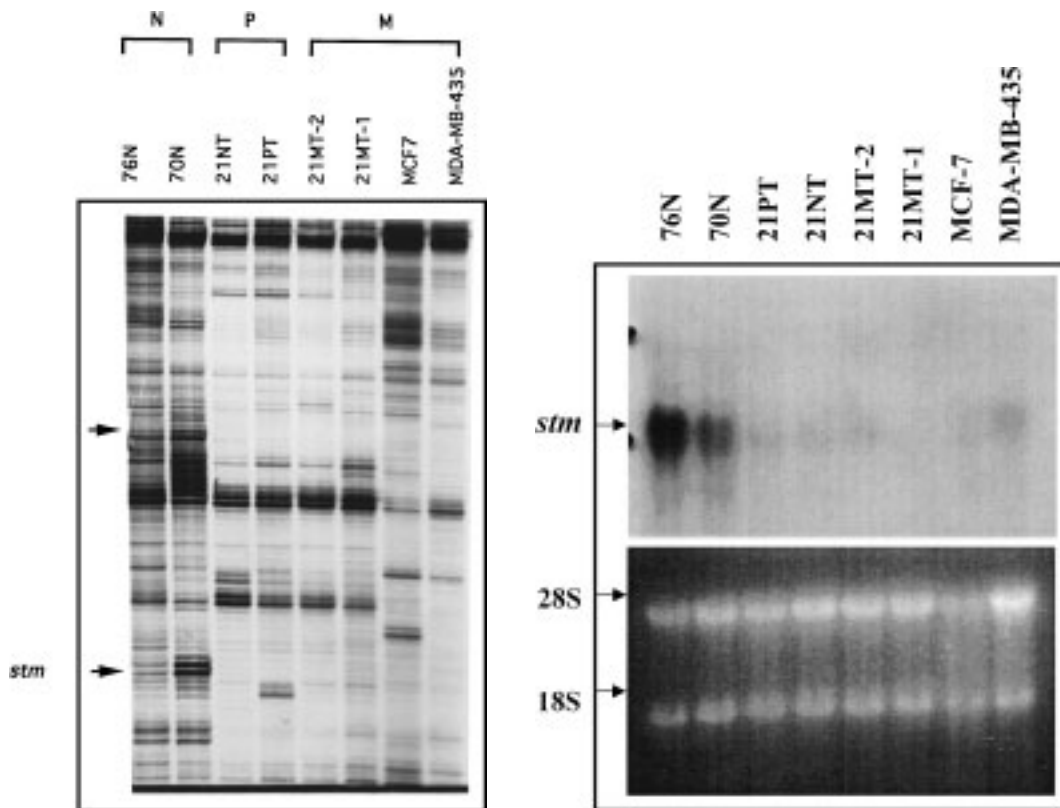


Fig. 1. Discovery of STM sialyltransferase by multiway differential display. (A) Total RNAs were isolated from normal human mammary epithelial cell strains (N, 76N, 70N; lanes 1 and 2), primary (P, 21NT, 21PT; lanes 3 and 4) and metastatic mammary tumor cell lines (M, 21MT-1, 21MT-2, MCF-7, and MDA-MB-435; lanes 5, 6, 7, and 8). The RNAs were reverse-transcribed with the T₁₂MA 3'-end anchored primer and amplified with T₁₂MA and OPA3 (AGTCAGCCAC), a 5'-end random primer. The position of the *stm* partial cDNA (125 bp), and another example of a differentially displayed cDNA, are indicated with arrows. (B) Northern blot analysis of differential *stm* gene expression. A cloned and 32 P-labeled partial *stm* cDNA obtained from differential display was used as a probe (upper). Ethidium bromide-staining of the gel used for the Northern blot (lower). Each lane contains 20 μ g of total RNA. The 28S and 18S subunits of ribosomal RNA are shown.

1	GGGACGTCAGCGGACGGGGCGCTCGGGGCCGGGGCTGT	39
40	ATG GGG CTC CCG CGC GGG TCG TTC TTC TGG GTG CTG CTC CTG CTC ACG GCT GCC TGC TCG	99
1	Met Gly Leu Pro Arg Gly Ser Phe Phe Trp Val Leu Leu Leu Leu Thr Ala Ala Cys Ser	20
100	GGG CTC CTC TTT GCC CTG TAC TTC TCG GCG GTG CAG CGG TAC CCG GGG CCA GCG GCC GGA	159
21	Gly Leu Leu Phe Ala Leu Tyr Phe Ser Ala Val Gln Arg Tyr Pro Gly Pro Ala Ala Gly	40
160	GCC AGG GAC ACC ACA TCA TTT GAA GCA TTC TTT CAA TCC AAG GCA TCG AAT TCT TGG ACA	219
41	Ala Arg Asp Thr Thr Ser Phe Glu Ala Phe Phe Gln Ser Lys Ala Ser Asn Ser Trp Thr	60
220	GGA AAG GGC CAG GCC TGC CGA CAC CTG CTT CAC CTG GCC ATT CAG CGG CAC CCC CAC TTC	279
61	Gly Lys Gly Gln Ala Cys Arg His Leu Leu His Leu Ala Ile Gln Arg His Pro His Phe	80
280	CGT GGC CTG TTC AAT CTC TCC ATT CCA GTG CTG CTG TGG GGG GAC CTC TTC ACC CCA GCG	339
81	Arg Gly Leu Phe Asn Leu Ser Ile Pro Val Leu Leu Trp Gly Asp Leu Phe Thr Pro Ala	100
340	CTC TGG GAC CGC CTG AGC CAA CAC AAA GCC CCG TAT GGC TGG CGG GGG CTC TCT CAC CAA	399
101	Leu Trp Asp Arg Leu Ser Gln His Lys Ala Pro Tyr Gly Trp Arg Gly Leu Ser His Gln	120
400	GTC ATC GCC TCC ACC CTG AGC CTT CTG AAC GGC TCA GAG AGT GCC AAG CTG TTT GCC CCG	459
121	Val Ile Ala Ser Thr Leu Ser Leu Leu Asn Gly Ser Glu Ser Ala Lys Leu Phe Ala Pro	140
460	CCC AGG GAC ACC CCT CCA AAG TGT ATC CCG TGT GCC GTG GTG GGC AAC GGA GGC ATT CTG	519
141	Pro Arg Asp Thr Pro Pro Lys Cys Ile Arg Cys Ala Val Val Gly Asn Gly Gly Ile Leu	160
520	AAT GGG TCC CGC CAG GGT CCC AAC ATC GAT GCC CAT GAC TAT GTA TTC AGA CTC AAT GGA	579
161	Asn Gly Ser Arg Gln Gly Pro Asn Ile Asp Ala His Asp Tyr Val Phe Arg Leu Asn Gly	180
580	GCT GTG ATC AAA GGC TTC GAG CGC GAT GTG GGC ACC AAG ACT TCC TTC TAT GGT TTC ACT	639
181	Ala Val Ile Lys Gly Phe Glu Arg Asp Val Gly Thr Lys Thr Ser Phe Tyr Gly Phe Thr	200
640	GTG AAC ACG ATG AAG AAC TCC CTC GTC TCC TAC TGG AAT CTG GGC TTC ACC TCC GTG CCA	699
201	Val Asn Thr Met Lys Asn Ser Leu Val Ser Tyr Trp Asn Leu Gly Phe Thr Ser Val Pro	220
700	CAA GGA CAG GAC CTG CAG TAT ATC TTC ATC CCC TCA GAC ATC CGC GAC TAT GTG ATG CTG	759
221	Gln Gly Gln Asp Leu Gln Tyr Ile Phe Ile Pro Ser Asp Ile Arg Asp Tyr Val Met Leu	240
760	AGA TCG GCC ATT CTG GGC GTG CCT GTC CCT GAG GGC CTA GAT AAA GGG GAC AGG CCG CAC	819
241	Arg Ser Ala Ile Leu Gly Val Pro Val Pro Glu Gly Leu Asp Lys Gly Asp Arg Pro His	260
820	GCC TAT TTT GGA CCA GAA GCC TCT GCC AGT AAA TTC AAG CTG CTA CAT CCG GAC TTC ATC	879
261	Ala Tyr Phe Gly Pro Glu Ala Ser Ala Ser Lys Phe Lys Leu Leu His Pro Asp Phe Ile	280
880	AGC TAC CTG ACA GAA AGG TTC TTG AAA TCA AAG TTG ATT AAC ACA CAT TTT GGA GAC CTA	939
281	Ser Tyr Leu Thr Glu Arg Phe Leu Lys Ser Lys Leu Ile Asn Thr His Phe Gly Asp Leu	300
940	TAT ATG CCT AGT ACC GGG GCT CTC ATG CTG CTG ACA GCT TTG CAT ACC TGT GAC CAG GTC	999
301	Tyr Met Pro Ser Thr Gly Ala Leu Met Leu Leu Thr Ala Leu His Thr Cys Asp Gln Val	320
1000	AGT GCC TAT GGA TTC ATC ACA AGC AAC TAC TGG AAA TTT TCC GAC CAC TAT TTC GAA CGA	1059
321	Ser Ala Tyr Gly Phe Ile Thr Ser Asn Tyr Trp Lys Phe Ser Asp His Tyr Phe Glu Arg	340
1060	AAA ATG AAG CCA TTG ATA TTT TAT GCA AAC CAC GAT CTG TCC CTG GAA GCT GCC CTG TGG	1119
341	Lys Met Lys Pro Leu Ile Phe Tyr Ala Asn His Asp Leu Ser Leu Glu Ala Ala Leu Trp	360
1120	AGG GAC CTG CAC AAG GCC GGC ATC CTT CAG CTG TAC CAG CGC	1161
361	Arg Asp Leu His Lys Ala Gly Ile Leu Gln Leu Tyr Gln Arg	374
1162	TGACCCCAATGCACAGGCGCTTGGCTTCTTCAAGACTTGGGGCCCTGATCCCTCAAGTGGCCAAAAGCTTTTTFACCT	1241
1242	TTTCAATCTTCACCTTCCCTTGCCAAACAGAGGGCACTGGGGTGAATCAAGATTTTCATCGAGGTCTGTTCATATAGGA	1321
1322	CACCCAGCTTGTCCCTTGGCTCATCCAAGAACTCTCTGTATCTTAAACAAATACATCTCAATCTTGGCCAAAGGAAATG	1401
1402	GACTGCTTGGCTGGATGGCACTGAGCAACTTTAGGAAATCTCGGTGGAGTGTTCAGCAAGATCAGACAGCAGTCCAGGT	1481
1482	CAAAGGCAACACACAGCTCCAGCCAAATCCCTCGGTGGCACATCCACCCAGATGCTAAAGTGATTCAAGGACTC	1561
1562	CAGGACACCTTAAAGAGCCTTTCTAAGAACATGATAGGCCTTACTTCTGCTCCATAATAAAGTGGGAGAAAAAGCCAGA	1641
1642	ATATAACTTAAGACTAGATAAATGCGTACATGATGGACCAATTTTCTTTTGGCTGGGTAGAGAAATCATATAAAAC	1721
1722	GCAGGCTGTTTTCAGCATGGAGATGACTCTCAGAACACTGGGAGGGTCTGGCACTTGATGGGGTTAGTTGCTTGGCAGCC	1801
1802	GCCTGGCCACTGAGGGAAGTCCCACTTTCAGAGATGTATCACCACCTTGTCCACCAACAGGATGATGTCACCAACAGGATGATGT	1881
1882	CACCAGGTAATAAACCTTTCATCCTCACAAAAAATAAAAAAAAAA	1926

Fig. 2. Sequence and structural features of STM. Primary amino acid sequence of the STM sialyltransferase inferred from the cDNA sequence. The L-sialylmotif (aa 148–194) and the S-sialylmotif (aa 303–324) are defined in brackets. Overlined sequence indicates a potential membrane anchor domain. Conserved cysteines are underlined. Potential N-linked glycosylation sites are marked with an asterisk. Upper numbers on the right and left represent nucleotides and lower numbers represent amino acid residues. The OPA3 priming position (nt 1801–1810) is underlined. A polyadenylation signal is present (nt 1890–1896).

the sialyltransferase gene family. The initiator ATG indicated in Fig. 2 lies within a relatively strong Kozak consensus (38) and is probably the translation start site, because the resulting protein sequence aligns optimally with other sialyltransferases (data not shown). The downstream sequence contains no AUG codons in proximity to the initiation site, and no AUG was found in the additional 288-bp upstream of the predicted start codon when a longer cDNA was sequenced (data not shown). In Figure 2, the sequence of the 5'-arbitrary OPA3 primer was underlined (nt 1801–1811) and contained two mismatches. The original partial cDNA obtained from differential display corresponds to bases 1801–1926 (Fig. 2), where sialyltransferases do not display any significant homology and, therefore, it gave no clues to the identity of the differentially displayed gene.

Structure of STM and Sequence Comparison to Other Sialyltransferases

A Hoop and Woods hydrophilicity plot revealed a hydrophobic region Phe⁸-Phe²⁸ at the N-terminus, which could serve as a noncleavable anchor domain, a common structural feature of sialyltransferases (6). A potential membrane-spanning region suggests that STM has a type II membrane orientation and a predicted molecular weight of 42 kDa. A potential site for casein kinase II (CK-2) phosphorylation is present in STM (Thr⁴⁵-Ser-Phe-Glu⁴⁸), and three consensus motifs for N-glycosylation at positions Asn⁸⁵, Asn¹³⁰, and Asn¹⁶¹ (see Fig. 2). Glycosylation as well as phosphorylation/dephosphorylation are plausible mechanisms that regulate the activity of glycosyltransferases. A primary sequence alignment of STM with related sialyltransferases revealed that STM contains two catalytic domains, the L-sialylmotif (aa 148–194) and S-sialylmotif (aa 303–324), of 47 and 23 amino acid residues, respectively. All sialyltransferases share these functional motifs, which are unique to the sialyltransferase gene family, are indispensable for sialyltransferase activity, and likely contain binding sites for donor and acceptor sugars. In both sialylmotifs, about 60% of the amino acids are conserved, which are mostly charged or polar. A third homology domain shared by sialyltransferases is the VS-sialylmotif, a short stretch of four highly conserved residues (His³³⁶-Tyr-Phe-Glu³³⁹) and Leu³⁶³ located near the C-terminus (39). Also present in STM are the cysteine residues (Cys¹⁵¹ and Cys³¹⁷) involved in the formation of a disulfide bond, which is essential for the active conformation of sialyltransferases (40). Recently, it was shown by site-directed mutagenesis that the invariant residues Cys¹⁸¹ or Cys³³² in the L- and S-sialylmotifs participate in the formation of an additional intradisulfide linkage that is essential for proper conformation and activity of ST6Gal I (13). STM displayed the highest sequence similarity to mouse ST6GalNAc II (41) with 229 identical and 274 conserved amino acids, whereas 229 amino acids were identical and 242 amino acids

were conserved between STM and chicken ST6GalNAc II (42). As shown in Fig. 3, sequence similarity between STM, mouse and chicken ST6GalNAc II is not restricted to sialylmotifs. It should be noted that, although the overall amino acid identity between mouse and human ST6GalNAc II is only 61%, it is 82.3% in the region from the N-terminus of the L-sialylmotif to the C-terminus of the enzyme, which is considered to contain the catalytic site and substrate binding sites. The corresponding region displays a 75.2% identity between chicken and human ST6GalNAc II. Therefore, these three enzymes will probably display similar acceptor specificities.

Down-Regulated Expression of *stm* in Human Cancer Cell Lines

The differential expression of *stm* was confirmed by Northern blot analysis employing a series of normal and tumor mammary epithelial cell lines. Two mRNA species with approximate sizes of 2.5 and 1.8 kb were detected in 81N, 76N, and 70N normal mammary epithelial cell strains. Northern blots were hybridized to a full-length *stm* cDNA probe. The same expression pattern was obtained when blots were hybridized to probes corresponding to different fragments of the cDNA (data not shown), indicating that the two transcripts more likely represent alternatively processed messages of the same gene, as reported for the liver α 2,6-sialyltransferase (43). Expression of *stm* was markedly down-regulated in the T-47D, BT-474, and MDA-MB-435 tumor cell lines, while traces or no *stm* message was detected in all other tumor cell lines (Fig. 4A). However, the MDA-MB-361 metastatic tumor cell line expressed *stm* mRNA levels which were as high as in 76N and higher than in 81N and 70N normal cell strains (Fig. 4A). MDA-MB-361 differs from other metastatic breast tumor cell lines in karyology in that it was isolated from a brain metastasis and not from pleural effusions, and has a lower malignant potential (44). In addition, down-regulation of *stm* expression was observed in prostate malignant tumor cells. As shown in Fig. 4B, *stm* was expressed at similar levels in all three normal-immortalized prostate epithelial cell lines (CF3, CF91, MLC) but down-regulated in PC3 and completely absent in the DU145 and LNCaP prostate metastatic tumor cell lines. These results indicate that *stm* could be down-regulated in human tumors, although this should be confirmed by studies employing matched normal and tumor tissue specimens.

Southern Analysis

Restriction digests of total genomic DNAs isolated from normal and tumor mammary cell lines were hybridized to a full-length *stm* cDNA probe. The *Eco*RI digest revealed two major hybridizing fragments of 7.8 and 4.4 kb, respectively, and a minor fragment of 6.2 kb that, upon prolonged exposure, was detected in all lanes (Fig. 5). However, the 4.4-kb band was absent in MDA-MB-231 and

cST6GalNAc II	1:	MGSPRWKRF	FCFL	LLAAFTSS	LLLYGH	YATY	-D	VRS	GPR	VVTS	LLQ	PELL	F	L	V	R	P	D	T	P	H	P	59																																							
hST6GalNAc II	1:	MGLPR	GSFF	FW	LLLL	TAACS	GL	-L	FAL	Y	FS	AV	Q	R	-----	Y	-----	P	G	P	A	A	G	A	41																																					
mST6GalNAc II	1:	MDLPR	R	W	L	F	R	M	L	L	V	A	T	S	S	G	-I	L	L	M	-L	Y	S	S	A	G	Q	Q	-----	S	-----	P	E	T	Q	V	P	A	40																							
cST6GalNAc II	60:	D	N	S	H	K	E	L	R	G	T	V	K	S	R	E	F	F	S	Q	P	S	S	E	L	E	K	P	K	P	S	G	K	Q	P	T	P	C	R	S	V	A	A	T	A	K	A	D	P	T	F	G	E	L	F	Q	F	D	I	119		
hST6GalNAc II	42:	---	R	D	T	T	S	F	E	---	A	F	F	Q	S	K	A	S	N	S	W	T	G	---	K	G	Q	A	-	C	R	H	L	L	H	L	A	I	Q	R	H	P	H	R	G	L	F	N	L	S	I	88										
mST6GalNAc II	41:	---	R	N	M	A	-	Y	P	R	---	A	F	F	D	P	K	P	P	N	S	E	-	N	---	R	K	S	R	L	-	C	H	S	L	S	L	A	I	Q	K	D	R	R	F	R	S	L	F	D	L	S	T	87								
cST6GalNAc II	120:	P	V	L	M	W	D	H	F	N	P	E	T	W	D	R	L	K	A	R	R	V	P	Y	G	W	Q	G	L	S	Q	A	A	V	G	S	T	L	R	L	L	N	T	S	S	N	T	R	L	F	D	R	H	L	F	-	P	G	G	C	178	
hST6GalNAc II	89:	P	V	L	L	W	G	D	L	F	T	P	A	L	W	D	R	L	S	O	H	K	A	P	Y	G	W	R	G	L	S	H	Q	V	I	A	S	T	L	S	L	L	N	G	S	E	S	A	K	L	F	A	P	P	R	D	T	P	P	K	C	148
mST6GalNAc II	88:	P	V	L	L	W	E	G	L	F	T	O	E	L	W	N	L	S	O	H	K	V	P	Y	G	W	Q	G	L	S	H	E	V	I	A	S	T	L	R	L	L	K	S	P	E	S	G	E	L	F	G	A	P	R	K	L	P	L	S	C	147	
cST6GalNAc II	179:	I	R	C	A	V	V	G	N	G	G	I	L	N	G	S	R	Q	G	R	A	I	D	A	H	D	L	V	F	R	L	N	G	A	I	T	K	G	F	E	D	V	G	S	K	V	S	F	Y	G	F	T	V	N	T	M	K	N	S	L	238	
hST6GalNAc II	149:	I	R	C	A	V	V	G	N	G	G	I	L	N	G	S	R	Q	G	P	N	I	D	A	H	D	V	F	R	L	N	G	A	V	I	K	G	F	E	R	D	V	G	T	K	T	S	F	Y	G	F	T	V	N	T	M	K	N	S	L	208	
mST6GalNAc II	148:	I	R	C	A	V	V	G	N	G	G	I	L	N	G	S	R	Q	G	K	I	D	A	H	D	V	F	R	L	N	G	A	I	T	E	A	F	E	R	D	V	G	T	K	T	S	F	Y	G	F	T	V	N	T	M	K	N	S	L	207		
cST6GalNAc II	239:	I	A	Y	E	A	Y	G	F	T	R	T	P	Q	K	D	L	K	Y	I	F	I	P	S	D	A	R	D	I	M	L	R	S	A	I	Q	G	S	P	V	P	E	G	L	D	K	G	D	E	P	P	K	Y	F	G	L	E	A	S	298		
hST6GalNAc II	209:	Y	S	Y	W	N	L	G	F	T	S	V	P	Q	G	D	L	Q	Y	I	F	I	P	S	D	I	R	D	Y	V	M	L	R	S	A	I	L	G	V	P	V	P	E	G	L	D	K	G	D	R	P	H	A	Y	F	G	P	E	A	S	268	
mST6GalNAc II	208:	I	S	Y	A	K	L	G	F	T	S	V	P	Q	G	N	L	R	Y	I	F	I	P	S	S	I	R	D	Y	L	M	L	R	S	A	I	L	G	V	P	V	P	E	G	P	D	K	G	D	R	P	H	T	Y	F	G	P	E	T	S	267	
cST6GalNAc II	299:	A	E	K	F	K	L	L	H	P	D	F	L	H	Y	L	T	T	R	F	L	S	E	L	L	D	M	Q	Y	G	H	L	Y	M	P	S	T	G	A	L	M	L	L	T	A	L	H	T	C	D	Q	V	S	A	Y	G	F	I	T	A	358	
hST6GalNAc II	269:	A	S	K	F	K	L	L	H	P	D	F	I	S	Y	L	T	E	R	F	L	K	S	K	L	I	N	T	H	F	G	D	L	Y	M	P	S	T	G	A	L	M	L	L	T	A	L	H	T	C	D	Q	V	S	A	Y	G	F	I	T	S	328
mST6GalNAc II	268:	A	S	K	F	K	L	L	H	P	D	F	I	S	Y	L	T	E	R	F	L	K	S	K	L	I	N	T	R	F	G	D	M	Y	M	P	S	T	G	A	L	M	L	L	T	A	L	H	T	C	D	Q	V	S	A	Y	G	F	I	T	N	327
cST6GalNAc II	359:	N	Y	E	Q	F	S	D	H	Y	E	P	E	K	K	P	L	I	F	Y	A	N	H	D	L	L	E	A	L	W	R	S	L	H	R	A	G	I	M	E	L	Y	Q	R	404																	
hST6GalNAc II	329:	N	Y	W	K	F	S	D	H	Y	F	E	R	K	M	P	L	I	F	Y	A	N	H	D	L	S	L	E	A	L	W	R	D	L	H	K	A	G	I	L	Q	L	Y	Q	R	374																
mST6GalNAc II	328:	N	Y	Q	Y	S	D	H	Y	F	E	R	E	K	K	P	L	I	F	Y	A	N	H	D	L	S	L	E	A	S	L	W	R	D	L	H	N	A	G	I	L	W	L	Y	Q	R	373															

Fig. 3. Primary sequence alignment of STM with mouse and chicken GalNAc II α 2,6-sialyltransferases. Sequences appear in Genbank/EMBL with the accession numbers STM, hST6GalNAc II (U14550); mouse, mST6GalNAc II (X93999); and chicken, cST6GalNAc II (X77775). Amino acid sequences are boxed according to the 3/3 score. Amino acids are identified by the single-letter code.

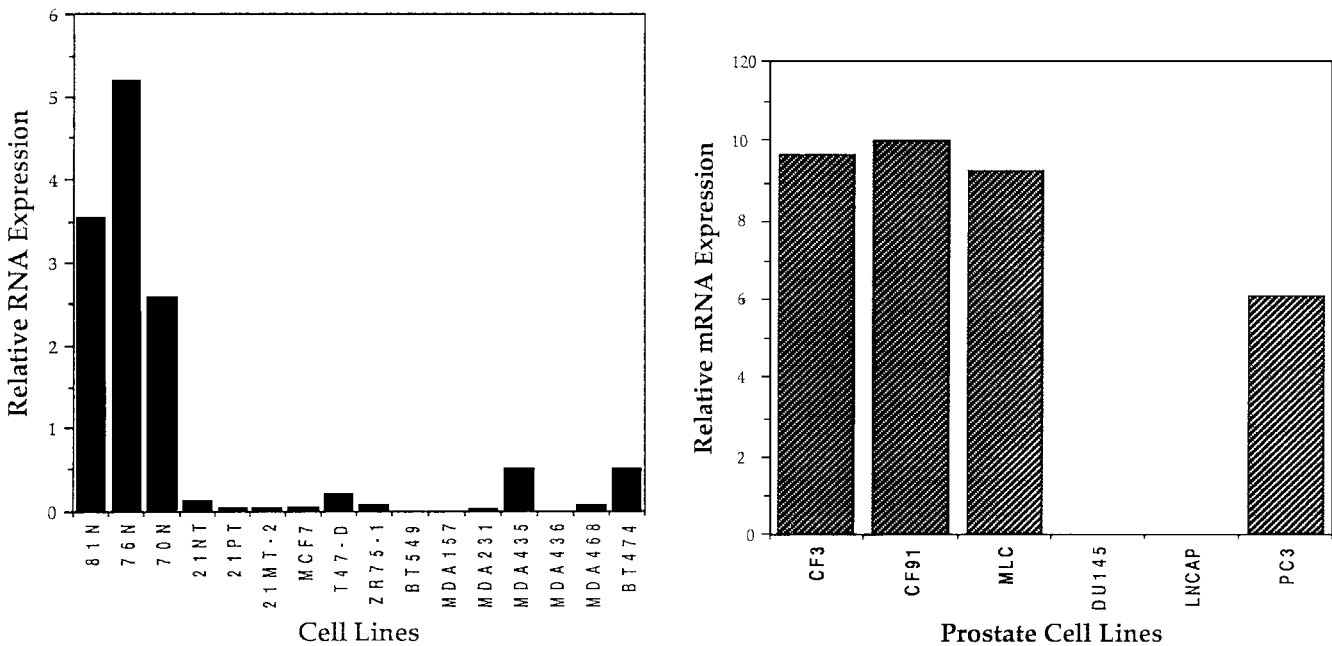


Fig. 4. Differential expression of STM sialyltransferase in human normal and tumor cell lines. Densitometric scans of Northern blot autoradiographs of left, human mammary epithelial normal cell strains, primary, and metastatic tumor cell lines. Right, human prostate epithelial normal-immortalized and metastatic tumor cell lines. Northern blots contained 20 μ g of total RNA in each lane and were hybridized to a 32 P-labeled full-length *stm* cDNA probe. For an internal standard, blots were hybridized to a 32 P-labeled 36B4 cDNA probe (35). Ratios of STM/36B4 RNA levels are presented. Details on cell lines are given in Materials and Methods.

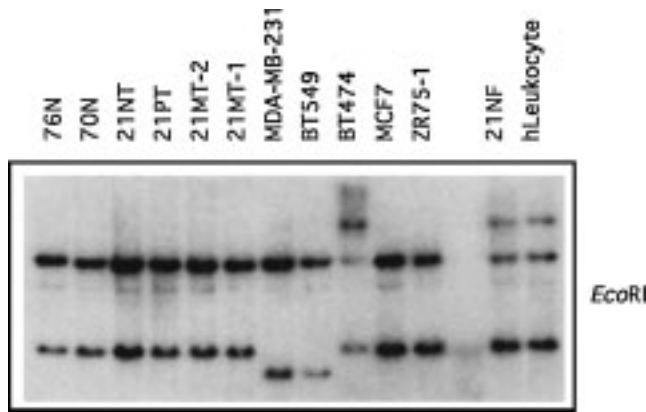


Fig. 5. Southern blot analysis of the gene encoding STM sialyltransferase in normal and tumor human mammary epithelial cell lines. A full-length *stm* cDNA was 32 P-labeled and used as a probe. Each lane contained 20 μ g of total *Eco*RI-restricted genomic DNA. The sizes of hybridizing fragments were 7.8, 6.2, and 4.4 kb. The size of the higher band present only in BT-474, 21NF, and leukocyte was 9.9 kb, and that of the lower band present only in MDA-MB-231 and BT-549 was 4.1 kb.

BT-549 tumor cell lines, which instead contained a lower band of 4.1-kb. In the BT-474 tumor cell line, as well as in the 21NF, normal fibroblasts and leukocytes, a band of approximately 9.9 kb was detected. In *Hind*III digests of the same DNAs, uniform bands of 6.0, 5.5, and 4.3 kb were detected (data not shown). The uniform restriction patterns obtained on Southern blots suggest that the *SIATL1* gene does not appear to be consistently rearranged in breast tumor cell lines, although genomic alterations might be present in a subset of breast tumors. Down-regulation of *stm* expression could be regulated at the transcriptional level in some tumors or by the presence of genomic mutations in others. Alternatively, the differences detected in the patterns of hybridizing restriction fragments could arise from polymorphisms.

Mapping of the *SIATL1* Gene to Human Chromosome 17q23-qter

The chromosomal localization of *SIATL1* was initially determined by analysis of its segregation in a monochromosome hybrid mapping panel. The human *SIATL1*-specific *Hind*III fragments, which could clearly be resolved from the hamster and mouse fragments, segregated with human chromosome 17 (data not shown). There were no discordancies for *SIATL1* localization to this chromosome. By analysis of a regional mapping panel for chromosome 17, *SIATL1* was subsequently regionally mapped to an interval between 17q23 and 17qter, defined by the breakpoint in hybrid GM10502 (Fig. 6).

Tissue Distribution of *STM*

A Northern blot containing polyA⁺ RNA from different normal human tissues was hybridized to a *stm* cDNA probe corresponding to the 3'-untranslated sequence. This probe is expected to specifically

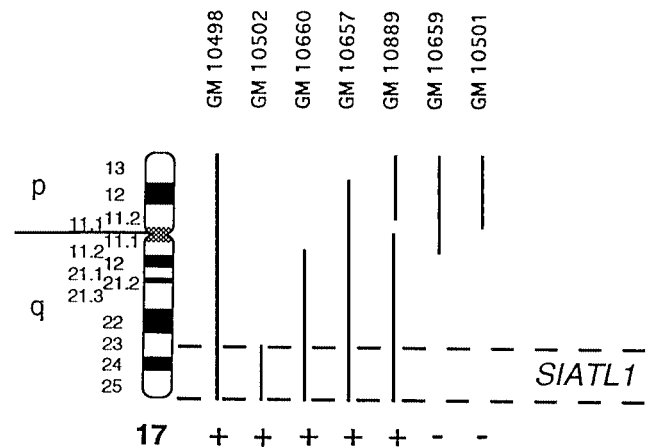


Fig. 6. Chromosomal localization of the *SIATL1* gene. Ideogram showing the chromosome 17 content of the regional mapping panel used for sublocalization of the *SIATL1* gene. This panel allows assignment of genes to at least six different intervals on chromosome 17. Southern blot analysis showed that the presence of *SIATL1*-specific sequences correlated with the presence of the region 17q23-qter. The presence (+) or absence (-) of *SIATL1* sequences in the hybrid clones are indicated.

detect only *stm*-specific transcripts because sialyltransferases display no significant homologies in the 3'-untranslated regions of their cDNAs. The signal intensities are roughly proportional to the abundance of the transcripts. The expression of *stm* displayed a wide tissue distribution. As shown in Fig. 7, abundant expression of *stm* was detected in heart, lung, skeletal muscle, kidney, and pancreas, whereas lower *stm* levels were detected in placenta. The liver contained trace amounts of *stm* mRNA, which was not detected in brain tissue. The same transcripts were detected when this tissue blot was hybridized to a full-length probe.

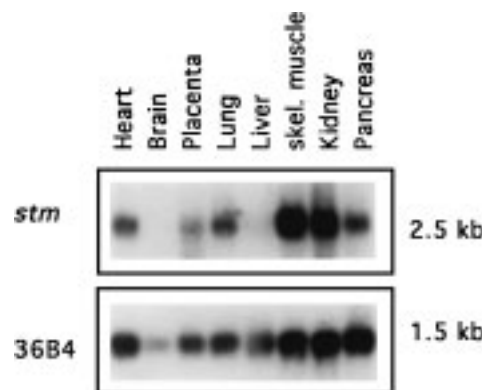


Fig. 7. Expression of STM sialyltransferase in normal human tissues. The Northern blot was probed with the 0.7-kb *Eco*RI + *Xho*I restriction fragment that corresponds to the 3'-untranslated end of the *stm* cDNA (upper) and 36B4 as an internal standard (lower). Each lane contained approximately 2 μ g of pure polyA⁺ RNA. The RNAs were run on a denaturing formaldehyde/1.2% agarose gel and blotted onto a nylon membrane (Human MTN Blot, Clontech, #7760-1).

Table 1. Acceptor substrate specificity of recombinant STM sialyltransferase

Acceptor	Representative Structures of Carbohydrates	STM	mST6GalNAc II (%)	mST6GalNAc I
Fetuin	NeuAc α 2-3Gal β 1-3GalNAc-Ser/Thr NeuAc α 2-3Gal β 1-3(NeuAc α 2-6) GalNAc-Ser/Thr NeuAc α 2-6(3)Gal β 1-4GlcNAc-R	83.3	76.0	84.0
Asialofetuin		100.0	100.0	100.0
Asialo-agalacto-fetuin		9.12	12.0	91.0
BSM	NeuAc α 2-3Gal β 1-3GalNAc-Ser/Thr NeuAc α 2-6GalNAc-Ser/Thr	4.73	7.32	19.0
Asialo-BSM		9.12	15.0	169.0
α 1 acid glycoprotein	NeuAc α 2-6(3)Gal β 1-4GlcNAc-R	0	0	14.0
Asialo- α 1 acid glycoprotein		0	0	8.0
Ovomucoid		3.38	9.41	n.t.
Gal β 1-3GalNAc-benzyl		0	0	0
NeuAc α 2-3Gal β 1-3GalNAc-benzyl		0	2.50	n.t.
Asialo-GM1	Gal β 1-3GalNAc β 1-4Gal β 1-4Glc1-1Cer	0	0	0
GM1b	NeuAc α 2-3Gal β 1-3GalNAc β 1-4Gal β 1-4Glc1-1Cer	0	0	n.t.
Paragloboside	Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc1-1Cer	0	0	n.t.

Comparison of the substrate specificity of STM (hST6GalNAc II), mST6GalNAc I (46) and mST6GalNAc II (41) sialyltransferases. The relative activity of incorporation of sialic acids into asialofetuin as a substrate is shown. Each substrate was used at the concentration of 0.15 mM. A value of 0 indicates less than 0.1%. R represents the remainder of the *N*-linked oligosaccharide chain.

GalNAc, *N*-acetylgalactosamine; GlcNAc, *N*-acetylglucosamine; NeuAc, *N*-acetylneuraminic acid.

Acceptor Substrate Specificity

The cDNA sequence that encodes the putative enzymatically active domain of STM (Arg⁴²-Arg³⁷⁴) was fused to the IgG binding domain of protein A and transiently expressed in COS-7 cells. The fusion protein efficiently mediated the transfer of sialic acids to glycosidically *O*-linked oligosaccharides of fetuin and asialofetuin because sialic acids were incorporated into *N*-glycanase resistant materials. The relative activity of incorporation of sialic acids into the asialofetuin substrate is shown in Table 1. Native fetuin contains three glycosidically *O*-linked oligosaccharides, two of which are NeuAc α 2,3Gal β 1,3GalNAc and NeuAc α 2,3Gal β 1,3(NeuAc α 2,6)GalNAc. Therefore, in native fetuin, the GalNAc residues in two of the three *O*-linked oligosaccharides can serve as acceptors. As shown in Table 1, the incorporation of NeuAc residues for the asialofetuin was increased about 20% from that of native fetuin. The incorporation of NeuAc residues for the agalactoasialofetuin (galactosidase-treated asialofetuin) was dramatically decreased. The activity toward asialo-BSM, in which only 5% of the total carbohydrate chains contain the Gal β 1,3GalNAc sequence, was almost negligible. No significant activity was observed toward α 1 acid glycoprotein having only glycosidically *N*-linked

oligosaccharides. In addition, glycosphingolipids could not serve as acceptors for this enzyme. The acceptor specificity of STM was similar to that observed for mouse and chicken ST6GalNAc II (41,42), but significantly different from that of chicken and mouse ST6GalNAc I (45,46). The incorporated NeuAc residues are resistant to treatment with NDV sialidase, but sensitive to treatment with NANase III (Table 2).

Table 2. Linkage analysis of incorporated sialic acids

Treated by	Relative Count (%)
NANase III	0.0
NDV sialidase	85.0
<i>N</i> -glycanase	96.3
Nontreated fetuin	100.0

[¹⁴C] sialylated fetuin was prepared as described in Materials and Methods. Subsequent to sialidase and *N*-glycanase treatment, each sample was subjected to SDS-PAGE. The radioactive materials in fetuin were visualized with a BAS2000 radio image analyzer and the incorporated radioactivity was counted.

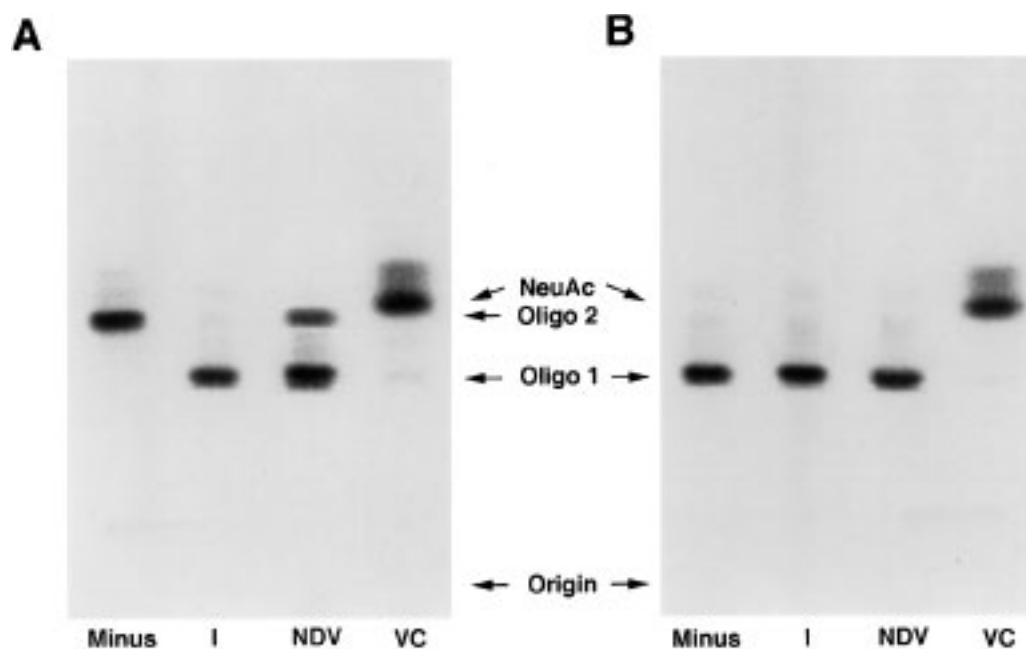


Fig. 8. Linkage analysis of incorporated sialic acids. Oligosaccharide alditols derived on 0.1 N NaOH/1 M NaBH₄ treatment of [¹⁴C]sialylated (A) fetuin, and (B) asialofetuin, were treated in the absence of sialidase (minus) or the presence of NANase I (I), Newcastle Disease Virus sialidase (NDV), and *Vibrio cholerae* sialidase (VC). Oligo 2, NeuAca_{2,3}Gal β 1,3(NeuAca_{2,6})GalNAc-ol; Oligo 1, Gal β 1,3(NeuAca_{2,6})GalNAc-ol.

The former sialidase cleaves α 2,3- and α 2,8-linkage but not α 2,6-sialyl-linkage, and the latter cleaves α 2,3-, α 2,6- and α 2,8-sialyl linkage. These results show that the cloned gene encodes an enzymatically active GalNAc α 2,6-sialyltransferase. To determine the linkage specificity of STM, [¹⁴C]sialylated oligosaccharide alditols were prepared by β -elimination of [¹⁴C]sialylated fetuin and asialofetuin (Fig. 8). Subsequently, a desalted sample was subjected to HPTLC. All of the radioactive product migrated as a low molecular compound, that is, no radioactivity remained at the origin, suggesting that [¹⁴C]sialylation occurred exclusively on *O*-linked glycan chains of fetuin (not shown). In Fig. 8A, NDV lane shows two bands resulting from partial digestion with NDV sialidase. The upper and lower bands correspond to Oligo 2 (NeuAca_{2,3}Gal β 1,3(NeuAca_{2,6})GalNAc-ol) and Oligo 1 (Gal β 1,3(NeuAca_{2,6})GalNAc-ol), respectively. Linkage analysis using linkage-specific sialidases revealed that the [¹⁴C]sialylated oligosaccharide alditols contained α 2,6-linked sialic acids.

Discussion

We sought to identify genes with aberrant expression in human breast tumors by applying differential display comparisons of multiple normal mammary cell strains and tumor cell lines with increasingly malignant phenotypes. A gene encoding a new sialyltransferase (named STM) was identified as being down-regulated in breast tumor cells and the corresponding full-length cDNA was cloned. STM

has a putative type II transmembrane topology and contains the *L*- and *S*-sialylmotifs, two functional domains present in all enzymatically active sialyltransferases. The catalytic domain of STM was expressed in COS-7 cells as a fusion protein with the IgG binding domain of protein A, and its enzymatic activity was characterized. STM could efficiently mediate the transfer of sialic acids to *O*-linked oligosaccharides in native fetuin and asialofetuin but not in agalactoasialofetuin, whereas *N*-linked oligosaccharides and glycosphingolipids could not serve as acceptors. Therefore, STM is an active GalNAc α 2,6-sialyltransferase with Gal β 1,3GalNAc-*O*-Ser/Thr and (+/-Neu5Ac α 2,3) Gal β 1,3GalNAc-*O*-Ser/Thr acceptor specificity and represents the human ST6GalNAc II. Nonetheless, STM displayed the highest primary sequence similarity to mouse (41) and chicken (42) ST6GalNAc II, which also form the linkage Neu5Ac α 2,6GalNAc on the Gal β 1,3GalNAc acceptor. Given that sequence homologies of STM to mouse and chicken ST6GalNAc II are 75% and 77%, respectively, STM is likely encoded by a unique sialyltransferase gene. Unique sialyltransferase genes from one species typically exhibit <50% primary sequence homology, and the same gene exhibits >95% homology in different species (7). Overall primary sequence homologies of STM to known sialyltransferases range from 15–23% for amino acid identities and 20–30% for conservative replacements.

Analysis of two independent mapping panels allowed us to assign the SIATL1 gene encoding STM

to the long arm of human chromosome 17 at q23-qter. This localization differs from those described for mapped sialyltransferases (e.g., the Gal β 1,4GlcNAc α 2,6-sialyltransferase gene was mapped to 3q27-28 [47], Gal β 1,3 GalNAc/Gal β 1,4 GlcNAc α 2,3-sialyltransferase to 11q23-24 [47], Gal β 1,3(4) GlcNAc α 2,3-sialyltransferase to 1p33-34 (47), and NeuA α 2,3Gal β 1,4Glc β 1-1/B4Cer α 2,8-sialyltransferase to 12p11.2-12.1 [48]). The region of 17q to which SIATL1 maps is syntenic with mouse chromosome 11. At least 28 genes on human 17q11.2-qter are known to map on mouse chromosome 11 (49). Localization of SIATL1 to this region suggests that SIATL1 might be part of this linkage group and thus resides on mouse chromosome 11. Chromosome 17q is frequently affected in human breast cancers. Three breast cancer-associated genes have been mapped to 17q: *BRCA1* at 17q21, *NME1* (*NM23*) at 17q21-22, and *ERBB2* at 17q11.2-12 (50,51). In addition, three separate regions on 17q are nonrandomly deleted in breast cancer: a region surrounding *BRCA1* at 17q21, a central region flanked by markers D17S86 and D17S21, and a distal region including markers D17S4 and D17S24 at 17q23-q25 (52,53). Interestingly, the latter region coincides with the region to which SIATL1 was mapped. Whether or not SIATL1 is deleted in a subgroup of breast cancers remains to be established.

The SIATL1 gene specifies two transcripts of approximately 2.5 and 1.8 kb, probably generated through alternative splicing or alternative promoter utilization, as shown for other sialyltransferases (54,55). Northern analyses of normal and tumor cell lines showed that both transcripts are significantly down-regulated and mostly absent in mammary and prostate tumor cell lines (see Fig. 4A, 4B). Of all breast tumor cell lines tested, only MDA-MB-361 expressed *stm* at levels similar (and even higher) than normal mammary epithelial cells. The MDA-MB-361 cell line was isolated from a brain metastasis; it has a lower malignant potential than cell lines isolated from pleural effusions (e.g., MDA-MB-435, MDA-MB-231, and MDA-MB-468 [44]), which express much less or no *stm* message. Down-regulation of *stm* expression in tumor cells could arise from genomic alterations. Because Southern analysis did not reveal consistent gross genomic rearrangements in mammary tumor cells, we speculate that down-regulation of *stm* in tumor cells occurs at the level of transcription, although deletions or point mutations cannot be excluded in a subset of breast tumors. Regulation of sialyltransferases at the level of transcription was described previously (56,57). It should be mentioned that the expression of *stm* could not be induced by cycloheximide in MDA-MB-231 breast tumor cells, suggesting that negative regulation of *stm* transcription in tumor cells does not involve protein factors with short half-life times. In 76N normal breast cells, *stm* mRNA was enhanced 1.5- to 2.0-fold, probably due to the stabilizing effect

of cycloheximide. Furthermore, down-regulation of *stm* in MDA-MB-231 tumor cells does not result from altered RNA stability (data not shown).

The putative functional implication(s) of STM inactivation in tumor cells have not been established. Normally, surface sialic acids play important roles as receptors for molecules that regulate cellular growth, differentiation, cell-cell communication, and adhesion. Nonetheless, aberrant glycosylation of glycoproteins and glycolipids in human cancers has been associated with an invasive phenotype (23,28,29). Neoplastic cells often express a larger amount of multiantennary and hypersialylated *N*-linked glycans than their normal counterparts, due to incomplete synthesis of carbohydrate chains and accumulation of their precursors (58). Hypersialylation results in increased surface charge that affects the interactions of tumor cells with host cells, and increases their metastatic potential (23), as well as tumor cell immunogenicity (59). Thus, aberrant sialic acid structures are recognized as tumor-associated surface antigens and result, at least in part, from the aberrant regulation of glycosyltransferases (25-27). For example, elevated α 2,3-sialyltransferase activity is involved in the accumulation of the α 2,3-sialylated Lec antigen (NeuA α 2,3 Gal β 1,3GlcNAc), precursor for the CA19-9 antigen that is elevated in pancreatic and colorectal malignant tumors (25). In breast cancer patients, the expression of ST3Gal III is often highly increased and positively correlates with the expression of ST6Gal I and ST3Gal IV (60), leading to the formation of the Sialyl-Lewis α and Sialyl-Lewis \times determinants. In human breast tumors, high expression of ST3Gal III and ST6Gal I is associated with a poor prognosis. In addition, the Tn antigen (GalNAc α 1-*O*-Ser/Thr) and Sialyl-Tn antigen (NeuA α 2,6GalNAc α 1-*O*-Ser/Thr) are mucin-type (*O*-linked) carbohydrate epitopes expressed in many human tumors. The Sialyl-Tn antigen is synthesized by ST6GalNAc I (61) and is related to poor prognosis for colorectal and breast cancer patients (26). A long cDNA of 2.46 kb encoding an active ST6GalNAc I enzyme, as well as a shorter splice variant of 2.23 kb encoding an inactive enzyme, have been identified (61). Interestingly, we have cloned different splice variants of STM lacking the *S*-sialylmotif due to premature stop codons (data not shown). The long form of the human ST6GalNAc I gene encodes the candidate synthase for the sialyl-Tn antigen (61). It should be noted that mouse ST6GalNAc II also exhibits activity toward GalNAc-*O*-Ser/Thr (62), but chick and human ST6GalNAc II have a very weak activity and no activity, respectively, toward GalNAc-*O*-Ser/Thr (61,63).

Two linkage patterns are common in *O*-linked oligosaccharides: the α 2,3 (Neu5A α 2,3 Gal-R and NeuA α 2,3GlcNAc-R) and the α 2,6 (NeuA α 2,6GalNAc-*O*-Ser/Thr). Of all known sialyltransferases, the synthesis of *O*-linked sialylated structures is

catalyzed by GalNAc α 2,6-sialyltransferases, namely ST6GalNAc I (43,46), ST6GalNAc II (41,42), ST6GalNAc III (15,64), ST6GalNAc IV (64), and ST6GalNAc V (64,65), as well as by Gal β 1,3GalNAc α 2,3-sialyltransferases, namely ST3Gal I (11,66) and ST3Gal II (67,68). In breast tumor cells, aberrant glycosyltransferase activities engaged in assembling, elongating, and terminating *O*-glycan core 1 (Gal β 1,3GalNAc α -R) and core 2 (Gal β 1,3 (GlcNAc β 1,6)GalNAc α -R) result in altered glycosylation of the polymorphic epithelial mucin (product of the MUC1 gene) and the selective exposure of a cryptic epitope in the tandem repeat (69,70). In breast and ovarian carcinomas, MUC1 is overexpressed and contains a large amount of *O*-linked glycans, which in normal mammary epithelial cells consist of core 2-based structures. The mucin expressed by breast carcinomas has shorter side chains, often consisting of sialylated core 1 structures (Gal β 1,3GalNAc) synthesized by ST3Gal I, which adds sialic acid to core 1 and terminates chain extension (71). ST3Gal I expression is elevated in primary breast carcinomas when compared to normal or benign tissue (70). Although Gal β 1,3GalNAc α 2,3-sialyltransferase activity is increased by several fold in breast tumor cells (69), the Gal β 1,3 GalNAc(GlcNAc to GalNAc) β 1,6-GlcNAc-transferase activity, which is responsible for core 2 synthesis, is absent (69,70). Loss of core 2 branching in tumor cells leads to shorter, sialylated *O*-glycans, adjacent to peptide epitopes. Thus, aberrant glycosylation of MUC1 in breast cancer results, at least in part, from increased activity of the ST3Gal I sialyltransferase (70). Increased α 2,3 sialylation of core 1 inhibits core 2 branching. Recently, it was shown that it is primarily the elevation of ST3Gal I in breast tumors that leads to the dominant expression of core 1 *O*-glycans on MUC1, because ST3Gal I can compete effectively with C2GnT1 β 6GlcNAc-transferase *in vivo*, and inhibit the formation of core 2 structures on MUC1 (72).

In normal cells, sialyltransferases that use the same substrate but add different sugars can compete for the common substrate providing that they overlap in the Golgi. Down-regulation or inactivation of certain sialyltransferases, like STM, in breast tumor cells could enhance the activity of other sialyltransferases that catalyze linkages associated with hypersialylated cell surfaces. Understanding the sialylation mechanisms will be facilitated by the identification of all sialyltransferases present in human cells, and by the elucidation of the mechanism of their aberrant regulation in tumor cells. Cloning of STM and determination of its differential expression and substrate specificity should contribute to the elucidation of pathways involved in biosynthesis of *O*-glycan chains of mucins in normal versus tumor cells, and should enable a complete analysis, as well as clinical applications of mammary cell mucins and mucin-related antigenicity.

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The nucleotide sequence reported in this manuscript appears in Genbank/EMBL with the accession number U14550. The HUGO/GDB designation for the gene symbol is SIATL1.

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