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Increased Expression of UDP-Galactose Transporter Messenger RNA in Human Colon Cancer Tissues and Its Implication in Synthesis of Thomsen-Friedenreich Antigen and Sialyl Lewis A/X Determinants[1]

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ABSTRACT

A series of human nucleotide sugar transporters of the Golgi apparatus was recently cloned, including the transporters for UDP-galactose (UDP-Gal), UDP-*N*-acetylglucosamine (UDP-GlcNAc) and CMP-sialic acid (CMP-SA). We have examined the mRNA expression of these three transporters in human colon cancer tissues by reverse transcription-PCR analysis and compared it with that in nonmalignant colonic mucosa prepared from the same patients. The amount of mRNA for UDP-Gal transporter was significantly increased in colon cancer tissues compared with nonmalignant mucosa tissues ($P = 0.035$; $n = 20$). The increase was more prominent in patients with advanced colorectal cancer of Dukes' stages C and D, in which the amount of UDP-Gal transporter mRNA in cancer tissues showed on average about a 3.6-fold increase over the paired nonmalignant mucosa (statistically significant at $P = 0.004$; $n = 14$). The mRNA content of the other two transporters showed no significant difference between the paired cancer and normal tissues. When UDP-Gal transporter cDNA was stably transfected to cultured human colon cancer cells, the expression of Thomsen-Friedenreich (TF) antigen and of sialyl Lewis A (NeuAcα2→3Galβ1→3[Fucα1→4]GlcNAcβ1→R) and sialyl Lewis X (NeuAcα2→3Galβ1→4[Fucα1→3]GlcNAcβ1→R) determinants was significantly induced on transfectant cells, which resulted in markedly enhanced cell adhesion to vascular E-selectin. These findings suggest that the increase of UDP-Gal transporter mRNA is involved in the enhanced expression of cancer-associated carbohydrate determinants such as TF and sialyl Lewis A/X antigens in colon cancers.

INTRODUCTION

Malignant transformation is associated with abnormal glycosylation, resulting in the synthesis and expression of altered carbohydrate antigens on cancerous mucins and glycolipids (1, 2). Sialyl Lewis A[3] and sialyl Lewis X determinants, for example, serve as ligands for the adhesion molecule E-selectin expressed in vascular endothelial cells and play important roles in hematogenous metastasis of colon cancer cells (3–5). TF antigen is known to be expressed also on many human carcinomas including colon cancers (6, 7), and was proposed to be a marker of carcinogenesis during progression of the adenoma-carcinoma sequence (8–12). The expression of TF antigen in primary colorectal carcinomas was found to correlate with an enhanced risk of liver metastasis in a clinical study (13). It is important to understand the mechanism for the enhanced synthesis of these carbohydrate determinants in cancers.

Many studies have been published regarding the alteration of glycosyltransferase mRNAs in cancer tissues on the assumption that the alteration of some glycosyltransferases would predominantly influence the synthesis of these carbohydrate determinants (14–18). Some of these reports describe alterations of glycosyltransferases fairly relevant to the enhanced expression of these carbohydrate determinants in cancer. As to the biosynthesis of these carbohydrate determinants, however, the supply of nucleotide sugars in the Golgi apparatus must also be taken into consideration as well as the alteration in glycosyltransferases. The molecular cloning of human sugar-nucleotide transporters has remarkably progressed recently. For example, human transporters for UDP-Gal (19), UDP-GlcNAc (20), and CMP-SA (21, 22) were all cloned in the last few years. The nucleotide-sugar transporters are located in the Golgi membranes, and transport the substrates essential for glycosyltransferases from the cytosol to the Golgi lumen (23–26). The alteration in nucleotide-sugar transport activity is expected to greatly affect the synthesis of glycoconjugates under certain conditions (23–26), but the expression of mRNAs for nucleotide-sugar transporters in cancer tissues has not been studied to date. In the study of glycosyltransferases in cancer tissues, substantial evidence had been accumulated by assaying their enzymatic activities before their mRNAs started to be evaluated in cancers. However, a molecular biological approach has long been awaited for the study of nucleotide sugar transporters because it is difficult to functionally assess their activity.

In the present study, we examined the expression of mRNAs for several nucleotide sugar transporters in colon cancers and found that the message for UDP-Gal transporter is significantly increased in cancers compared with nonmalignant colonic epithelia. We will also present evidence indicating that UDP-Gal transporter significantly affects the synthesis of several important carbohydrate determinants including those of TF, sialyl Lewis A, and sialyl Lewis X, and that it would also affect the cell adhesive activity of cancer cells mediated by these determinants.

MATERIALS AND METHODS

Clinical Samples and RNA Extraction. Surgical specimens were obtained from 20 patients with colorectal cancer at surgical operation and processed as described previously (15). The median age of patients was 59.8 years. The carcinomas were staged according to the Astler-Coller modification of Dukes' classification (27). Malignant and nonmalignant portions of each specimen were used for RNA extraction. Nonmalignant mucosa was scraped off using slide glasses, and tissue specimens of cancer were carefully excised so as to eliminate noncancerous tissue components. Samples were frozen rapidly and stored at −80°C until RNA extraction. Specimens were powdered

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[3] The abbreviations used are: sialyl Lewis A, NeuAcα2→3Galβ1→3[Fucα1→4]GlcNAcβ1→R; sialyl Lewis X, NeuAcα2→3Galβ1→4[Fucα1→3]GlcNAcβ1→R; G3PDH, glyceraldehyde 3-phosphate dehydrogenase; CMP-SA, CMP-sialic acid; GlcNAc, *N*-acetylglucosamine; Gal, galactose; hUGT, human UDP-Gal transporter; TF, Thomsen-Friedenreich (antigen), or Galβ1–3GalNAcα1-Ser/Thr; RT-PCR, reverse transcription-PCR; Tn (antigen), GalNAcα1-Ser/Thr; sialyl Tn (antigen), NeuAcα2→6 GalNAcα1-Ser/Thr.

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Table 1 *PCR primers used for amplifying nucleotide sugar transporter messages*

mRNA species	Size of fragments (bp)	Primers
UDP-Gal transporter	538	U[a] 5′-GGGGACCGCCAGTGCGGCTCACAG-3′[b]
		L 5′-TGCCAGGCCTGCCCCAGGGTTCTG-3′
UDP-Gal transporter 1	1019	U 5′-GGTGACATACCAGCTGAAGA-3′
		L 5′-TGCCTCCAAAGAGGTTAGAG-3′
UDP-Gal transporter 2	548	U 5′-GCTTCGCAGGTGTCTACT-3′
		L 5′-GGAACCCTTCACCTTGGT-3′
UDP-GlcNAc transporter	753	U 5′-ACAATGTTCGCCAACCTAAAATAC-3′
		L 5′-AAGAACAACTACTATCCAGGTCAG-3′
CMP-SA transporter	412	U 5′-TGTGCTGGGAGTTACGGTTGTTAC-3′
		L 5′-TTACTGAAGCAATGGTGGAAAGGA-3′
G3PDH	983	U 5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′
		L 5′-CATGTGGGCCATGAGGTCCACCAC-3′

[a] U, upper strand primers; L, lower strand primers.
[b] These primers detected UDP-Gal transporters both 1 and 2.

in liquid N_2, and total cellular RNA was extracted with guanidine isothiocyanate and purified by cesium chloride gradient centrifugation.

RT-PCR Analysis. Total RNA (5 μg) was incubated at 70°C for 10 min and placed on ice for at least 1 min. Reverse transcription into cDNA was achieved using the First Strand cDNA Synthesis kit (Life Technologies, Inc., Rockville, MD), according to the manufacturer's protocol using oligo d(T) as initiation primer in a final reaction volume of 21 μl. One μl of the retrotranscription reaction was subjected to PCR amplification using nucleotide-sugar transporters and human G3PDH-specific primers. The primers for RT-PCR analysis of nucleotide sugar transporters and G3PDH used in this study are summarized in Table 1. The cycle numbers most suitable for quantitative RT-PCR analysis on tissue samples were determined in preliminary experiments, *i.e.*, 30 cycles for UDP-Gal transporter primers recognizing common sequence of UDP-Gal transporter 1 and UDP-Gal transporter 2, 28 cycles for UDP-GlcNAc T, 35 cycles for CMP-SA transporter primers, 30 cycles for UDP-Gal transporter 1, and 40 cycles for UDP-Gal transporter 2. Each cycle consisted of 1 min at 94°C, 45 s at 55°C, and 1 min at 72°C. A reaction without cDNA or reverse transcriptase product was performed as negative control to exclude the possibility of amplification of contaminating genomic DNA. The G3PDH transcripts were amplified from the same cDNA samples (35 cycles, Tm = 64°C), as an internal control for the RT-PCR analysis. Aliquots of each reaction were fractionated by electrophoresis through a 2% agarose gel including ethidium bromide. After electrophoresis, the intensities of the bands were quantified by the Densitograph apparatus and software (AE-6920WLSA; ATTO, Tokyo).

Cells, Antibodies, and Flow Cytometric Analysis. Cultured human colon cancer cell lines, WiDr, SW1083, SW480, LoVo, HT29, Caco-2, HCT116, HCT15, CoR-1, Colo320, Colo201, and C-1 were maintained in Dulbecco's modified MEM (Life Technologies, Inc., Rockville, MD) supplemented with 10% FCS (Biowhittaker, Gaithersburg, MD) and cultured at 37°C in a humidified atmosphere of 5% CO_2 in air. Total RNA was isolated from 1×10^6 cells according to the acid guanidinium thiocyanate-chloroform extraction method using an Isogen kit (Nippon-Gene, Tokyo, Japan) and the amount of RNA was quantitated by spectrophotometry at 260 nm. In some experiments, SW1083 and SW480 cells were cultured in a glucose-free Gal-containing medium for 2 weeks. The medium was glucose-free RPMI 1640 supplemented with 2.0 g/liter D^+-Gal (Wako, Osaka, Japan) and 10% FCS.

The anti-sialyl Lewis A antibody 2D3 was prepared as described previously (3, 28). The anti-sialyl Lewis X antibody CSLEX-1 was obtained from American Type Culture Collection (Manassas, VA; Ref. 29), and SNH-3 was a gift from Dr. Sen-itiroh Hakomori (Pacific Northwest Research Foundation, Seattle, WA; Refs. 3, 28). Monoclonal antibodies against TF (HB-T1, IgM, κ), Tn (HB-Tn1, IgM, κ) and sialyl Tn (HB-STn1, IgG1, κ) antigens were purchased from DAKO (Glostrup, Denmark). Flow cytometric analysis was performed by FACScan (Becton Dickinson, Mountain View, CA). The cells were harvested at a semiconfluent stage and stained with respective monoclonal antibody using purified antibody at 1 μg/ml or culture supernatant at a dilution of 1:10 at 4°C for 30 min. The cells were then washed three times with PBS containing 0.5% BSA and stained with a 1:200 dilution of FITC-conjugated goat anti-mouse immunoglobulin (Silenus Laboratories, Boronia, Australia) at 4°C for 30 min.

Transfection Experiments. The UDP-Gal transporter transfectant cell lines SW1083/hUGT1 and SW480/hUGT1 were established by the transfection of expression vector pMKIT-*neo*/hUGT1 containing cDNA of hUGT1 (19) to the cultured human colon cancer cell lines SW1083 and SW480. The expression vector pMKIT*neo* was a kind gift from Dr. Kazuo Maruyama (Department of Hygiene, Tokyo Medical and Dental University, Tokyo, Japan). DNA (4 μg) was transfected to the SW1083 or SW480 cells (1×10^6) by the lipofection method using the Lipofectamine Plus reagent (Invitrogen Corp., Carlsbad, CA), according to the manufacturer's protocol. The cells were allowed to grow for 2 days before being subjected to selection for the ability to grow in medium containing 600 μg/ml geneticin (G418-sulfate, Invitrogen Corp.). Two weeks of selection were required for stable expression. Cells grown in selecting medium were used as the UDP-Gal transporter 1 transfectant cells for flow cytometric analysis. Later, typical weak, moderate, or strong expressers of UDP-Gal transporter 1 mRNA were cloned from these SW1083/hUGT1 or SW480/hUGT1 cells by limiting dilution, and were designated as clones 1–6, respectively.

Nonstatic Monolayer Cell Adhesion Assay. Cell adhesion experiments were performed as described previously (3, 30). Parental SW480 cells, mock-transfected SW480, and SW480/hUGT1 clones 4–6 were cultured in monolayers in 24-well plates. To this culture, 2′7′-bis-(carboxyethyl)-5-(and-6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM)-labeled 300.19/E-selectin cells (1×10^6 cells/0.5 ml/well, murine B lymphoma 300.19 cells transfected with human E-selectin cDNA; Ref. 31) were added; and the plate was placed on a rotating platform for incubation under shear (90 rpm) for 20 min at room temperature. The 300.19/E-selectin cells were kindly supplied by Dr. Geoffrey S. Kansas (Department of Microbiology-Immunology, Northwestern University, Medical School, Chicago, IL). After nonadherent cells were washed out three times with PBS, the cells were lysed with 0.5% NP40, and the attached cells were counted by measuring fluorescence intensity using an Arvo 1420 multilabel counter (Wallac, Gaithersburg, MD).

RESULTS

RT-PCR Analysis of Nucleotide Sugar Transporter Gene Expression in Human Colon Cancer Tissues. Fig. 1 shows typical examples of RT-PCR analyses of UDP-Gal, UDP-GlcNAc, and CMP-SA transporter transcripts in colorectal cancer tissues, indicating a marked increase of UDP-Gal transporter mRNA in cancer tissues compared with nonmalignant colonic epithelia. The UDP-GlcNAc and CMP-SA transporter mRNAs showed a much less prominent change. The mRNA for G3PDH was expressed almost equally in all of the samples and served as an internal control. Fig. 2 summarizes the results of densitometric analyses of 20 cases. The amount of mRNA for hUGT was significantly increased in cancer tissues compared with nonmalignant mucosa ($P = 0.035$; $n = 20$). Furthermore, in 14 patients with advanced colorectal cancer with lymph node- or distant metastasis (Dukes' C and D cases), the amount of UDP-Gal transporter mRNA in colon cancer tissues was increased 0.9- to 20.1-fold, on average about 3.6-fold compared with the amount in paired nonmalignant mucosa, and the difference was statistically significant at $P = 0.004$ (Fig. 2*A*). No significant difference was observed between cancer tissues and nonmalignant mucosa in Dukes' A and B cases

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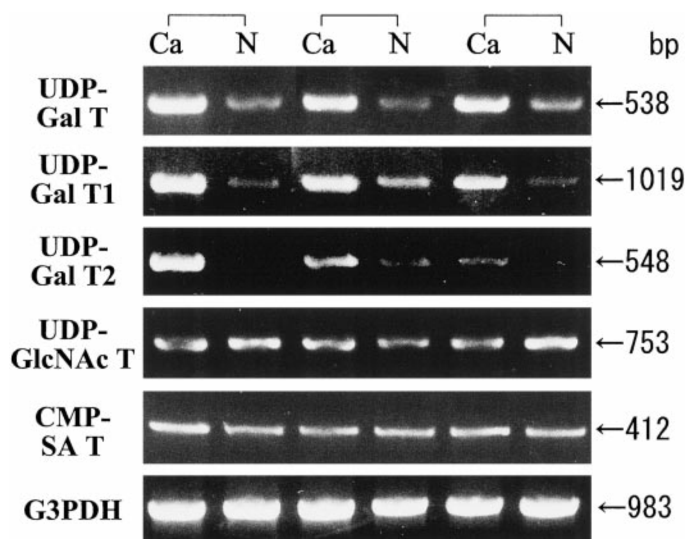

Fig. 1. Typical examples of RT-PCR analyses of nucleotide sugar transporter gene expression in human colon cancer tissues and nonmalignant mucosa. After PCR reaction using the specific primers, the aliquots of products were electrophoresed in 2% agarose gel and were stained with ethidium bromide. Sizes of each band are indicated in bp. For primers used, see Table 1. *Ca*, cancerous tissues; *N*, nonmalignant mucosa prepared from the same patient; *UDP-Gal T*, UDP-Gal transporter, 30 cycles; *UDP-Gal T1*, UDP-Gal transporter 1, 30 cycles; *UDP-Gal T2*, UDP-Gal transporter 2, 40 cycles; *UDP-GlcNAc T*, UDP-GlcNAc transporter, 28 cycles; *CMP-SA T*, CMP-SA transporter, 35 cycles.

($n = 6$). The UDP-GlcNAc transporter and CMP-SA transporter mRNA were present in all of the colonic tissues, and their levels in the paired cancer and nonmalignant tissues were not significantly different.

The hUGT is known to occur in two isoforms, UDP-Gal transporter 1 and 2, which were suggested to be produced from the same gene through alternative splicing (19, 22). When specific primers for UDP-Gal transporters 1 and 2 were applied, the transcripts for both isotypes were found to be significantly increased in cancer tissues compared with the paired nonmalignant mucosa (Figs. 1 and 2*B*). UDP-Gal transporter 1 turned out to be the major molecular species, because significant bands appeared with 30 cycles, whereas UDP-Gal transporter 2 seemed to be a minor species in colonic tissues because as many as 40 cycles of PCR were needed to obtain detectable bands (Fig. 1).

Induction of TF Antigen Expression in Cultured Human Colon Cancer Cells SW1083 Transfected with UDP-Gal Transporter cDNA. When cultured human colon cancer cell lines were subjected to RT-PCR analysis for hUGT gene expression, its mRNA was found in all of the cell lines. SW480 had the weakest expression, and the level of expression in the cells was comparable with that in nonmalignant mucosa prepared from patients with colon cancer.

When the SW1083 cells that moderately expressed endogenous UDP-Gal transporter mRNA were further transfected with UDP-Gal transporter 1 gene, a significant induction of TF antigen was observed in the transfectant cells, which was essentially not expressed in parental and mock-transfected cells (Fig. 3*A*). A significant increase of sialyl Lewis A was also noted in the transfected cells (Fig. 3*A*). Expression of Tn, sialyl Tn, or sialyl Lewis X in UDP-Gal transporter 1 transfectant cells showed no remarkable change compared with that in parental and mock-transfectant cells (Fig. 3*A*).

The clones that were obtained by limiting the dilution of the SW1083/hUGT1 transfectant cells showed a variable expression of TF antigen and sialyl Lewis A determinant on the cell surface (Fig. 3*B*), which was well correlated with the content of UDP-Gal trans-

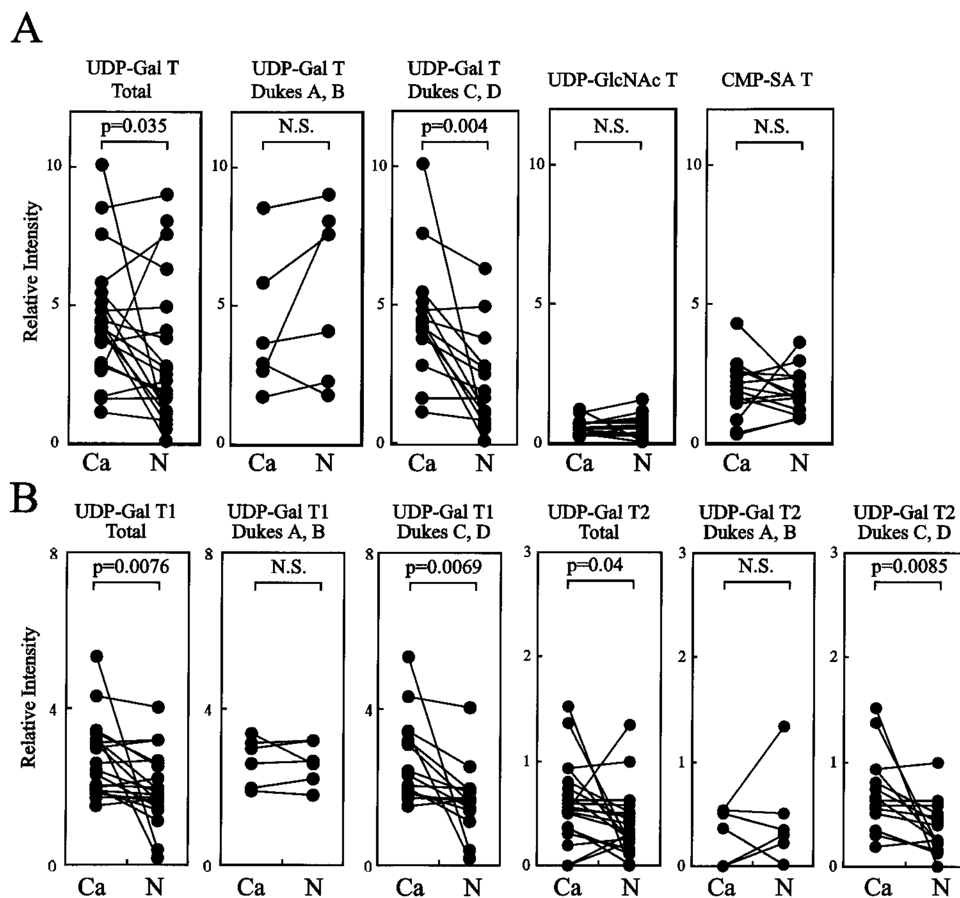

Fig. 2. Results of RT-PCR analyses of mRNA levels of nucleotide sugar transporters in human colon cancer tissues and nonmalignant mucosa. *Ca*, cancerous tissues; *N*, nonmalignant mucosa prepared from the same patient. Paired *t* test was performed to ascertain statistical significance between the amount in cancer tissue and in nonmalignant mucosa. *A*, *UDP-Gal T*, UDP-Gal transporter, 30 cycles; *UDP-GlcNAc T*, UDP-GlcNAc transporter, 28 cycles; *CMP-SA T*, CMP-SA transporter, 35 cycles. *B*, *UDP-Gal T1*, UDP-Gal transporter 1, 30 cycles; *UDP-Gal T2*, UDP-Gal transporter 2, 40 cycles. For primers used, see Table 1.

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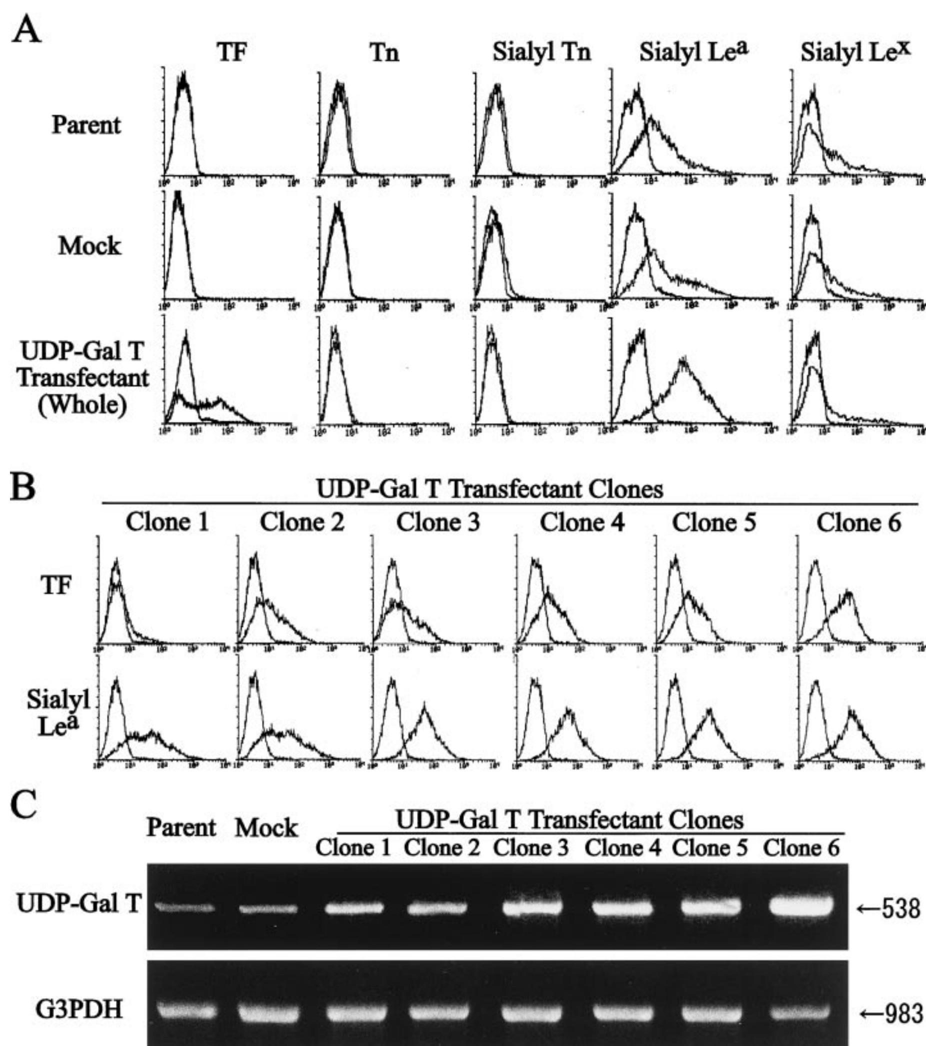

Fig. 3. Expression of carbohydrate determinants in UDP-Gal transporter transfectant clones of cultured human colon cancer cells SW1083. *A*, expression of TF antigen (*TF*), Tn, sialyl Tn, sialyl Lewis A (*sialyl Le^a*), and sialyl Lewis X (*sialyl Le^x*) determinants on parental, mock-transfected, and UDP-Gal transporter 1-transfected SW1083 cells (SW1083/hUGT1 cells). The SW1083 cells that were transfected with UDP-Gal transporter 1 cDNA were used for flow cytometric analysis without further cloning. *B* and *C*, expression of TF antigen (*TF*), sialyl Lewis A (*sialyl Le^a*) (*B*), and UDP-Gal transporter 1 (*UDP-Gal T*) mRNA (*C*) in the six clones derived from the SW1083 cells transfected with UDP-Gal transporter 1 cDNA by limiting dilution.

porter 1 mRNA as ascertained by RT-PCR (Fig. 3*C*). These findings suggested that the increase of UDP-Gal transporter mRNA levels was actually involved in the induction of TF antigen in these cells.

Induction of TF, Sialyl Lewis A, and Sialyl Lewis X Expression in Cultured Human Colon Cancer Cells SW480 Transfected with UDP-Gal Transporter cDNA. When UDP-Gal transporter 1 gene was transfected to SW480 cells, the weakest expresser, the induction of TF antigen was only minute, whereas a moderate increase of sialyl Lewis A and a remarkable induction of sialyl Lewis X were observed as shown in Fig. 4*A*. Expression of Tn and sialyl Tn remained unchanged compared with parental and mock-transfected cells.

When the clones of SW480/hUGT1 transfectant cells were examined, the level of expression of TF, sialyl Lewis A, and sialyl Lewis X again correlated well with the mRNA content in the clones as shown in Fig. 4, *B* and *C*. It was notable that the significant induction of sialyl Lewis X expression is overt in all transfectant clones, even in clones 1 or 2, which expresses UGP-Gal transporter 1 mRNA only weakly. On the other hand, the enhanced expression of sialyl Lewis A was noted only in clones 4, 5, and 6, which contain relatively higher amounts of UGP-Gal transporter 1 mRNA. The induction of TF antigen expression was seen only in clones 5 and 6, which express UGP-Gal transporter 1 mRNA most strongly.

E-selectin-mediated Cell Adhesion of Cultured Human Colon Cancer SW480 Cells Transfected with UDP-Gal Transporter cDNA. The sialyl Lewis A and sialyl Lewis X determinants are known to serve as ligands for E-selectin and to mediate adhesion of cancer cells to vascular endothelial cells (3–5). This adhesion is proposed to be involved in hematogenous metastasis of colon cancer cells (3–5). Because significant expressions of these determinants were induced in the SW1083 or SW480 cells transfected with UDP-Gal transporter 1 cDNA, we tested adhesion of E-selectin-expressing cells to these transfectant clones. As shown in Fig. 5, an approximately 2-fold increase of E-selectin-mediated cell adhesion was noted in the SW1083 clones transfected with UDP-Gal transporter 1 cDNA, and adhesion of the SW480 clones transfected with UDP-Gal transporter 1 cDNA was enhanced 2.6- to 4.2-fold compared with adhesion of the mock-transfected cells.

Expression of TF Antigen and Sialyl Lewis A Determinant in Colon Cancer Cells Cultured in Gal-Rich Medium. To investigate whether a change in the supply of monosaccharide would also affect expression of the carbohydrate determinants, cultured human colon cancer cells were transferred from a conventional glucose-containing medium to a glucose-free Gal-rich medium. After being cultured in Gal-rich medium, parental SW1083 cells showed an increased expression of TF antigen and sialyl Lewis A determinant (Fig. 6). When SW1083/hUGT1 clone 6 was cultured in Gal-rich medium, a modest increase of TF antigen and a moderate increase of sialyl Lewis A were still observed (Fig. 6), which indicated that the effect of the Gal-rich medium treatment and the transfection of UDP-Gal transporter cDNA were additive. This suggested that some factors other than UDP-Gal transporter also limit the supply of UDP-Gal for glycosyltransferases in this clone. HCT15 cells showed only a modest increase in TF

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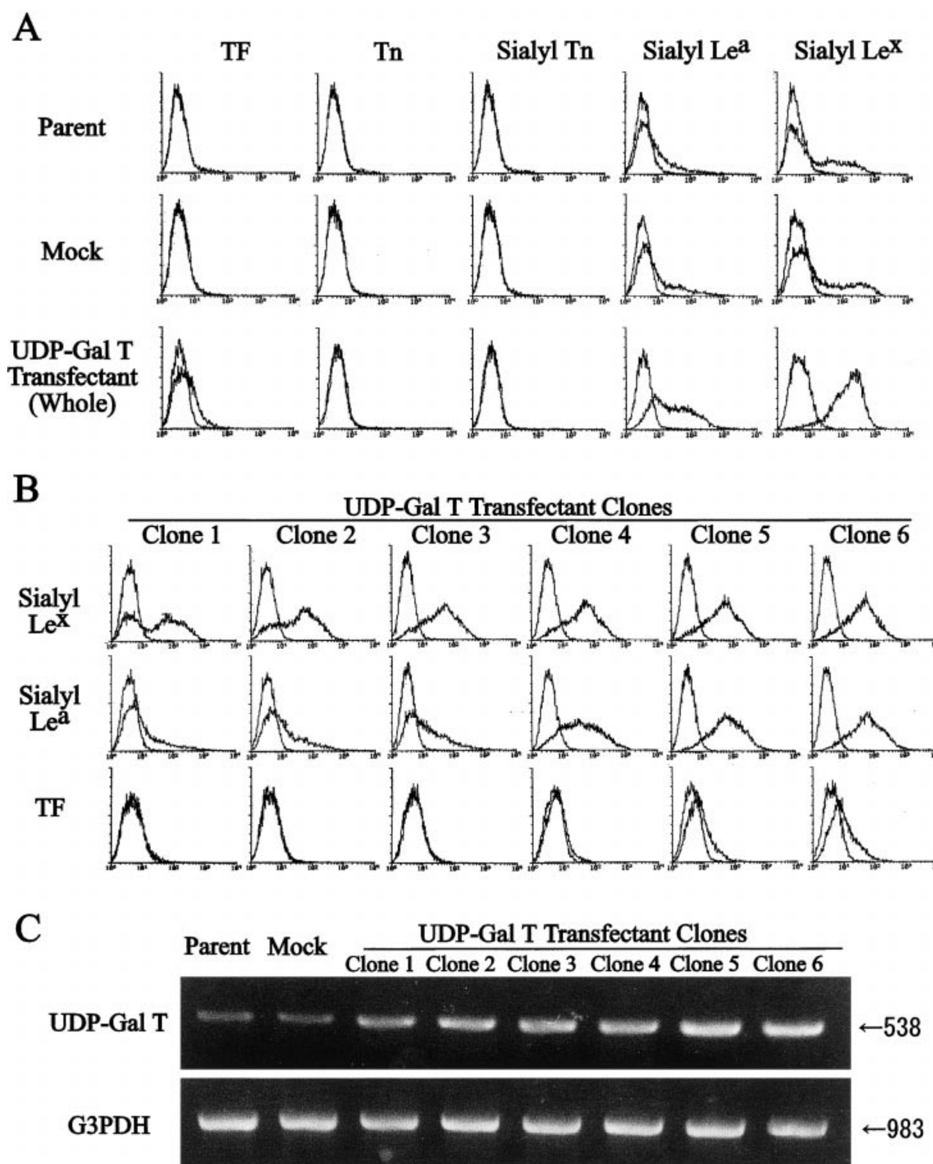

Fig. 4. Expression of carbohydrate determinants in UDP-Gal transporter transfectant clones of cultured human colon cancer cells SW480. *A*, expression of TF antigen (*TF*), Tn, sialyl Tn, sialyl Lewis A (*sialyl Le^a*), and sialyl Lewis X (*sialyl Le^x*) determinants on parental, mock-transfected, and UDP-Gal transporter 1-transfected SW480 cells (SW480/hUGT1 cells). The SW480 cells transfected with UDP-Gal transporter 1 cDNA were used for flow cytometric analysis without further cloning procedure. *B* and *C*, expression of sialyl Lewis X, sialyl Lewis A, and TF antigens (*B*) and UDP-Gal transporter 1 mRNA (*C*) in the six clones derived from the SW480 cells transfected with UDP-Gal transporter 1 cDNA by limiting dilution. *UDP-Gal T*, UDP-Gal transporter.

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antigen but a marked increase in sialyl Lewis A expression, whereas Colo320 showed a moderate increase in TF antigen but no induction of sialyl Lewis A when cultured in Gal-rich medium (Fig. 6). These results indicated that Gal-rich medium treatment frequently results in the enhanced expression of TF or sialyl Lewis A determinant. These results also indicated that the effect of Gal-rich medium treatment on carbohydrate determinant expression considerably varies from cell to cell depending on the set of glycosyltransferases inherent in the treated cells, as exemplified in the lack of sialyl Lewis A expression in Colo320, or the lack of TF antigen expression in HCT15 cells. The same treatment of SW480 cells yielded only a minute change in the expression of TF and sialyl Lewis A determinants, which suggested that the modest availability of UDP-Gal transporter in the cells limits the effect of Gal-rich medium treatment. The treatment of SW480 cells transfected with UDP-Gal transporter cDNA exhibited a weak but significant induction of TF accompanied by an enhancement of sialyl Lewis A expression, which showed a 2-fold increase in terms of mean fluorescence intensity (Fig. 6). This again indicated that the effect of the Gal-rich medium treatment and the transfection of UDP-Gal transporter cDNA were additive when the cells contained a sufficient amount of UDP-Gal transporter. No remarkable change was observed in the expression of sialyl Lewis X by the Gal-rich medium treatment of these cell lines.

DISCUSSION

In this study we found a significant increase in the expression of UDP-Gal transporter mRNA in cancer tissues compared with nonmalignant mucosa in patients with colon cancer. This increase was frequently observed in cancer tissues from patients with lymph node- or distant metastasis, whereas it was not significantly increased in cancer tissues in patients without lymph node metastasis. This suggests that an increase of UDP-Gal transporter mRNA would affect the metastatic behavior of cancer cells. However, it was not easy to predict the functional results of the increase of UDP-Gal transporter mRNA because UDP-Gal is involved in so many synthetic pathways of cellular glycoconjugates.

To investigate the functional consequences of the increase of hUGT mRNA detected in cancerous tissues, we chose two colon cancer cell lines that moderately (SW1083) or only weakly (SW480) express endogenous UDP-Gal transporter mRNA, and we prepared two series of stable transfectant cells. The results indicated that the increase of

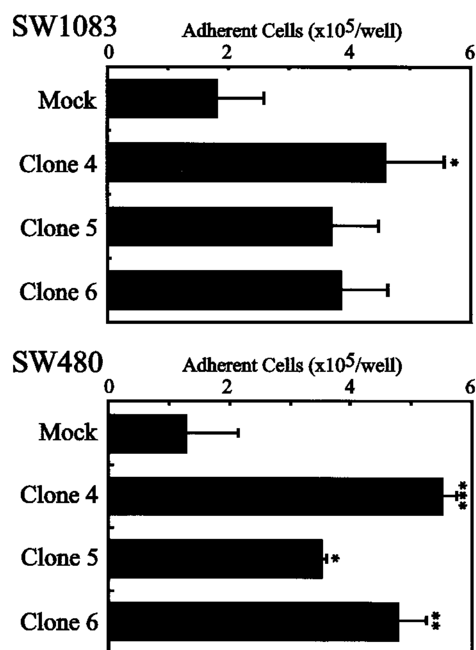

Fig. 5. Adhesion of UDP-Gal transporter transfectant clones of cultured human colon cancer cells SW1083 or SW480 to E-selectin. Results of nonstatic monolayer cell adhesion experiments using murine B lymphoma cells 300.19 transfected with human E-selectin cDNA (300.19/E-selectin) are shown. *Top panel*, *mock*, mock-transfected SW1083 cells; *Clones 4–6*, clones of SW1083 cells transfected with UDP-Gal transporter 1 cDNA (SW1083/hUGT1) obtained by limiting dilution. *Bottom panel*, *mock*, mock-transfected SW480 cells; *Clones 4–6*, clones of SW480 cells transfected with UDP-Gal transporter 1 cDNA (SW480/hUGT1) obtained by limiting dilution. *, statistically different at $P < 0.05$; **, statistically different at $P < 0.01$; ***, statistically different at $P < 0.001$. For experimental details see "Materials and Methods."

UDP-Gal transporter significantly induces the surface expression of TF antigen and sialyl Lewis A and sialyl Lewis X determinants in colon cancer cells. The increased expression of sialyl Lewis A and sialyl Lewis X determinants led to a remarkably enhanced adhesion of cancer cells to vascular E-selectin, which is involved in cancer metastasis.

With regard to the mechanism leading to aberrant expressions of the cancer-associated carbohydrate determinants, abnormalities in glycosyltransferase activities and transcripts have been studied extensively to date (14–18). However, it is obvious that the expression of carbohydrate determinants is regulated by various factors other than glycosyltransferases, including cytoplasmic synthesis of appropriate sugar nucleotide substrates and their transportation into the Golgi lumen.

Increased expression of TF antigen is associated with colon cancers as well as cancers of other origins. TF antigen expression is correlated with liver metastasis of colon cancers (13). TF antigen is known to be able to bind to galectins and asialoglycoprotein receptors in hepatocytes and is implicated in the molecular mechanism of liver metastasis (32). Significant alteration of its expression was detected in a cultured cell line, which lacks synthetic enzyme for the determinant (33). However, it is notable that the activity of GalNAcα (β1,3-galactosyltransferase), the synthetic enzyme for TF antigen, in colon cancerous tissues was not significantly different from that in its normal colonic mucosa (14, 34). If the activity of the sialyltransferase that sialylates TF antigen were to decrease in cancer tissues, the expression of TF antigen would be enhanced. In fact, however, the sialyltransferase is increased in colon cancers (15, 35), and the TF antigen will be detectable only when the synthesis of TF antigen exceeds the rate of sialylation by the sialyltransferase. Similarly, if the activities of *N*-acetylglucosaminyltransferases, which use TF antigen as an acceptor were decreased in cancer tissues, the expression of TF antigen would be enhanced. Although the core 3 and 4 *N*-acetylglucosaminyltransferases are decreased (14), the core 2 *N*-acetylglucosaminyltransferase is elevated in cancers (36). Thus far, changes in glycosyltransferases do not unequivocally explain the increased expression of TF antigen in cancer.

The carbohydrate determinants sialyl Lewis A and sialyl Lewis X serve as ligands for the adhesion molecule E-selectin when cancer cells adhere to vascular endothelial cells during the course of hema-

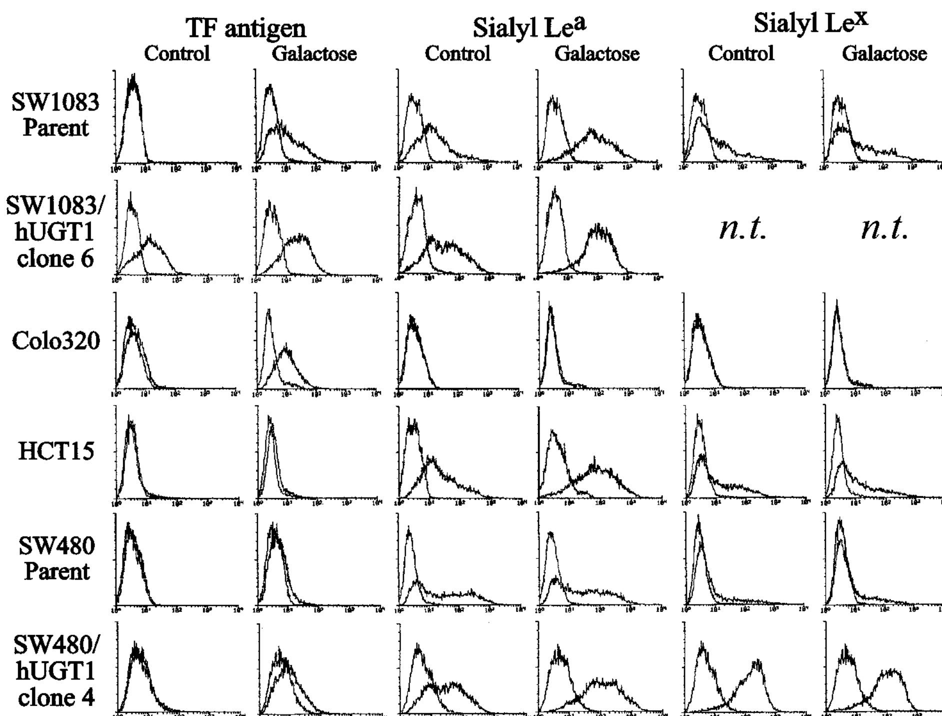

Fig. 6. Expression of carbohydrate determinants in human colon cancer cells cultured in Gal-rich medium. Results of flow cytometric analyses of expression of TF (*TF antigen*), sialyl Lewis A (*sialyl Le*^{a}), and sialyl Lewis X (*sialyl Le*^{x}) determinants in parental SW1083 (*SW1083 Parent*), clone 6 of SW1083 cells transfected with UDP-Gal transporter 1 cDNA (*SW1083/hUGT1 clone 6*), Colo320, HCT15, parental SW480 (*SW480 parent*), and clones of SW480 cells transfected with UDP-Gal transporter 1 cDNA (*SW480/hUGT1 clone 4*) are shown. Cells were cultured in glucose-free Gal-rich (2.0 g/liter) medium. *n.t.*, not tested. For experimental details see "Materials and Methods."

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togenous metastasis (5). Clinical statistics also indicate that patients with colorectal cancer cells that strongly express sialyl Lewis A/X have a significantly higher risk of developing hematogenous metastasis (4, 37–39). The expression of sialyl Lewis A/X is increased in colon cancer tissues compared with nonmalignant mucosa. With regard to the increased sialyl Lewis A expression in cancer, the activity of GlcNAcβ: β1,3-galactosyltransferase responsible for the synthesis of type 1 chain sialyl Lewis A precursors is not increased in cancer but is even significantly decreased (40, 41). No significant increase in fucosyltransferase activities or their transcripts was detected in colon cancers (15, 42, 43), and only an increase of the sialyltransferase activity and its mRNA was noted in colon cancers with a relatively weak statistical correlation with sialyl Lewis A expression (15, 42). As for the increased expression of sialyl Lewis X in colon cancer, enzymatic activity and mRNAs of fucosyltransferases or sialyltransferases specific to its synthesis have been studied intensively, but no relevant change in glycosyltransferases that would unequivocally explain the increased expression of sialyl Lewis X in colon cancer is known to date (15, 18).

Availability of nucleotide sugars in the Golgi lumen is suggested to greatly influence the expression of carbohydrate determinants (24–26). UDP-Gal transporter deficient mutant cells are known to exhibit a profound abnormality in the expression of proteoglycans (44, 45), glycoproteins (46), and glycolipids (47), which could be corrected by the transfection of UDP-Gal transporter cDNA (19, 22). The activity of nucleotide sugar transporters would significantly affect expression of the carbohydrate determinants, especially when the K_m value for sugar nucleotides of the glycosyltransferases involved in their synthesis is high. In this context, it is noteworthy that the K_m value for UDP-Gal of the β1,3-galactosyltransferase responsible for the synthesis of TF antigen is reported to be as high as 0.152 mM (48). The K_m for UDP-Gal of the β1,3-galactosyltransferase involved in the synthesis of type 1 chain sialyl Lewis A precursor is reported to be 0.20 mM in human colon (40), and 0.269 mM in a cultured human colon cancer cell line (16). These values are comparable with the value calculated earlier for a β1,3-galactosyltransferase from pig trachea, which was 0.223 mM (49). On the other hand, the K_m value for UDP-Gal of the β1,4-galactosyltransferase involved in the synthesis of type 2 chain lactosamine, the precursor for sialyl Lewis X, is reported to be in the range of 0.012–0.014 mM (40).

This difference in K_m values is compatible with our current findings, especially with SW480/hUGT1 transfectant cells, which indicated that only a slight increase of UDP-Gal transporter cDNA expression was enough for the full induction of sialyl Lewis X synthesis, whereas a much stronger expression of UDP-Gal transporter cDNA was necessary for the significant induction of TF antigen and sialyl Lewis A synthesis. Expressions of TF antigen and sialyl Lewis A were more frequently induced than those of sialyl Lewis X by the transfection of UDP-Gal transporter cDNA or the treatment with Gal-rich medium. These findings are also compatible with the notion that the synthesis of TF antigen and sialyl Lewis A is more strongly limited by the availability of UDP-Gal than is the synthesis of sialyl Lewis X.

To our knowledge, there is no information on the UDP-Gal concentration in the Golgi apparatus of any mammalian cells. We could find only one report describing an intracellular concentration of UDP-Gal in rat mammary tissues, which was calculated to be 20–33 μM (50). Because the K_m value of UDP-Gal transporter for UDP-Gal is much less than this concentration (51), the transporter would be fully active physiologically, and the concentration of UDP-Gal in the Golgi apparatus would be highly dependent on the amount of UDP-Gal transporter available in the Golgi membranes. Our results on the Gal-rich medium treatment suggested that some other factors would also affect the UDP-Gal concentration when cells had a sufficient amount of UDP-Gal transporter.

This study first indicated that a significant change in the sugar nucleotide transporter occurs along with malignant transformation, and that this eventually leads to the abnormal expression of carbohydrate determinants. The Golgi transport of UDP-Gal is one of the important factors affecting expression of the important cancer-associated carbohydrate determinants involved in cell adhesion, far more important than expected.

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