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

Kensuke Kumamoto, Yoshiko Goto, Koji Sekikawa, Seiichi Takenoshita, Nobuhiro Ishida, Masao Kawakita, and Reiji Kannagi

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-acetylgalactosamine (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 colon 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 (antigen) 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 A3 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...
in liquid N₂, and total cellular RNA was extracted with guanidine isothiocya-
nate 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 retrotranscrip-
tion 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 experi-
ments, 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 includ-
ing 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 humid-
ified atmosphere of 5% CO₂ in air. Total RNA was isolated from 1×10⁶ cells
classified as an internal control for the RT-PCR analysis. Aliquots of each
reaction were fractionated by electrophoresis through a 2% agarose gel includ-
ing ethidium bromide. After electrophoresis, the intensities of the bands were
quantified by the Densitograph apparatus and software (AE-6920WLSA; ATTO, Tokyo).

**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 samples of the and served as an internal control. Fig. 2 summarizes the results of densitometric analyses of 20 cases. The amount of mRNA for hUGT1 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 trans-
porter 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 non-
malignant mucosa, and the difference was statistically significant at P = 0.004 (Fig. 2A). No significant difference was observed between cancer tissues and nonmalignant mucosa in Dukes’ A and B cases

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### Table 1.

<table>
<thead>
<tr>
<th>mRNA species</th>
<th>Size of fragments (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UDP-Gal transporter</td>
<td>538</td>
</tr>
<tr>
<td>UDP-Gal transporter 1</td>
<td>1019</td>
</tr>
<tr>
<td>UDP-Gal transporter 2</td>
<td>548</td>
</tr>
<tr>
<td>UDP-GlCNac transporter</td>
<td>753</td>
</tr>
<tr>
<td>CMP-SA transporter</td>
<td>412</td>
</tr>
<tr>
<td>G3PDH</td>
<td>983</td>
</tr>
</tbody>
</table>

*U, upper strand primers; L, lower strand primers.

These primers detected UDP-Gal transporters both 1 and 2.

**UDP-Gal TRANSPORTER IN COLON CANCER**
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 2B). 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. 3A). A significant increase of sialyl Lewis A was also noted in the transfected cells (Fig. 3A). Expression of Tn, sialyl Tn, or sialyl Lewis X in UDP-Gal transporter 1 transfecant cells showed no remarkable change compared with that in parental and mock-transfected cells (Fig. 3A).

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. 3B), which was well correlated with the content of UDP-Gal trans-
porter 1 mRNA as ascertained by RT-PCR (Fig. 3C). 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. 4A. 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, 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, 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, parental SW1083 cells showed an increased expression of TF antigen and sialyl Lewis A determinant (Fig. 6).
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 non-malignant 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 transfecant cells. The results indicated that the increase of
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-
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:1,3-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), and glycolipids (47), which could be corrected by a profound abnormality in the expression of proteoglycans (44, 45), 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 Km value for sugar nucleotides of the glycosyltransferases involved in their synthesis is high. In this context, it is noteworthy that the Km 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 Km 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 Km 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 Km values is compatible with our current findings, especially with SW480/hUGT1 transfectedant 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 Km 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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REFERENCES


4626
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