Introduction of Sda\(^a\) Carbohydrate Antigen in Gastrointestinal Cancer Cells Eliminates Selectin Ligands and Inhibits Metastasis

Yuki I. Kawamura, Rei Kawashima, Ryuko Fukunaga, Kazunari Hirai, Noriko Toyama-Sorimachi, Makoto Tokuhara, Toshio Shimizu, and Taeko Dohi

1Department of Gastroenterology, Research Institute and 2Department of Surgery, International Medical Center of Japan; and 3GS platz Co., Ltd., Tokyo, Japan.

Abstract

The Sda\(^a\) blood group carbohydrate structure is expressed in the normal gastrointestinal mucosa. We reported previously that the expression of Sda carbohydrate structures and \(\beta 1,4\)-N-acetylgalactosaminyltransferase (\(\beta 1,4\)GalNAcT) activity responsible for Sda synthesis were remarkably decreased in cancer lesions of the gastrointestinal tract. In this study, we found that Sda antigen was expressed mainly in chief cells of normal stomach but not in cancer tissue by immunohistologic staining. In separated gastric mucosal cells, the Sda glycolipids and \(\beta 1,4\)GalNAcT activity were concentrated in a fraction that contained chief cells as a major population. We cloned the cDNA encoding the glycosyltransferase that catalyzes the synthesis of Sda (Sda-\(\beta 1,4\)GalNAcT). Introduction of this cloned cDNA into KATO III gastric or HT29 colonic cancer cell lines, which originally expressed the E-selectin ligands, sialyl Lewis\(^x\) and sialyl Lewis\(^a\), resulted in a marked increase in cell-surface expression of Sda along with the concomitant loss of both sialyl Lewis\(^x\) and sialyl Lewis\(^a\). Both KATO III and HT29 cells transfected with the Sda-\(\beta 1,4\)GalNAcT gene showed significantly decreased adhesion to activated human umbilical vein endothelial cells when compared with mock-transfected cells. Sda determinants showed no direct binding to Siglec-3, -5, -7, and -9. These Sda-\(\beta 1,4\)GalNAcT-transfected cells showed strikingly reduced metastatic potential in vivo when compared with mock-transfected cells. In summary, forced expression of Sda carbohydrate determinant caused remarkable elimination of carbohydrate ligands for selectin and reduced metastasis of human gastrointestinal tract cancer cells. (Cancer Res 2005; 65(14): 6220-27)

Introduction

A blood group Sda carbohydrate, GalNAc\(\beta 1-3\)(NeuAc\(\alpha 2-3\))Gal\(\beta 1-4\)GlcNAc, is abundantly expressed on glycolipids and glycoproteins in the normal gastrointestinal tract mucosa in the majority of humans (1–4). An important characteristic of this carbohydrate determinant is that it is expressed in cancer tissue is strikingly reduced or absent. In the stomach, this Sda antigen is expressed as a glycolipid [GalNAc\(\beta 1-3\)(NeuAc\(\alpha 2-3\))Gal\(\beta 1-4\)GlcNAc\(\beta 1-3\)Gal\(\beta 1-3\)Glc\(\beta 1-1\)Cer] localized in the oxyntic mucosa but not in antral mucosa or by gastric cancer tissues (3). In the colonic mucosa, Sda structures are expressed on glycoproteins but not on glycolipids. The last step in biosynthesis of Sda is catalyzed by \(\beta 1,4\)-N-acetylgalactosaminyltransferase (\(\beta 1,4\)GalNAcT). The Sda determinants are synthesized by addition of N-acetylgalactosame to terminal \(\alpha 2,3\)-sialylated galactose residue in the \(\beta 1,4\) linkage. The activity of \(\beta 1,4\)GalNAcT responsible for synthesizing the Sda determinant (Sda-\(\beta 1,4\)GalNAcT) dramatically decreases in gastric and colonic cancer tissues from an early stage onward (5). In the colon, the enzyme activity shows a proximal-distal gradient of expression and is attenuated in the cancer tissue itself (2, 6). The Sda structure is completely lost in established human gastrointestinal cancer cell lines, and this enzyme activity is not detected until the cells differentiate (7, 8). Thus, Sda is a differentiation-associated antigen, and the control system for its expression is significantly involved in the malignant changes in the gastrointestinal tract mucosa. However, at this point, the precise mechanisms for specific localization, differential expression on glycolipids and glycoproteins, cancer-associated alterations, and biological function of Sda in gastrointestinal tract remain unknown.

It has long been known that malignant transformation is associated with abnormal expression of carbohydrate determinants. In these altered carbohydrate structures, the expression of sialyl Lewis\(^x\) and sialyl Lewis\(^a\) [NeuAc\(\alpha 2-3\)Gal\(\beta 1-3\)Fuc\(\beta 1-3\)GlcNAc-R] by cancer tissues correlates with the progression of human carcinomas. Statistically significant correlations between the postoperative prognosis of the patients and the degree of expression of the sialyl Lewis\(^x\) and Lewis\(^a\) determinants in cancer tissues have been shown for both colon and stomach cancers (9–12). Further, sialyl Lewis\(^a\) and sialyl Lewis\(^x\) determinants in cancer cells serve as ligands for E-selectin, which is inducibly expressed by endothelial cells and functions as a cell adhesion molecule (13, 14). Thus, interactions between E-selectin and its specific carbohydrate ligands play a significant role in hematogenous metastasis. Sialyl Lewis\(^a\) determinants are synthesized by addition of fucose to GlcNAc in the \(\alpha 1,3\) linkage with \(\alpha 2,3\)-sialylated galactose residue. The \(\alpha 1,3\)-acetylated product of the same precursor yields the Sda\(^a\) antigen. Because the precursor of the sialyl Lewis\(^a\) determinant as well as sialyl Lewis\(^a\) are also the acceptor substrates for Sda\(\beta 1,4\)GalNAcT as shown previously (15), we postulated that Sda\(\beta 1,4\)GalNAcT may compete for the acceptor with \(\alpha 1,3/4\) fucosyltransferases, sialyl Lewis\(^a\) synthases, to produce Sda\(^a\) determinants.

To understand the significance of attenuated expression of Sda in gastrointestinal tract cancer tissues, we first determined the localization of Sda in the gastric oxyntic mucosa. Next, we introduced Sda\(^a\) determinant into tumor cells by forced expression of Sda\(\beta 1,4\)GalNAcT gene. To accomplish this, we obtained a cDNA of human Sda\(\beta 1,4\)GalNAcT from normal human stomach. In this study, we introduced the cDNA of Sda\(\beta 1,4\)GalNAcT into gastric...
and colorectal cancer cell lines and analyzed the alterations in carbohydrate chain expression and cell adhesion to E-selectin. We have also defined the effects of surface expression Sdα for metastatic potential of tumor cells in vivo.

Materials and Methods

Mice and reagents. Six- to 8-week-old male athymic BALB/c nude (nu/nu) mice were purchased from Japan SLC, Inc. (Hamamatsu, Shizuoka, Japan) and maintained in the animal facility under pathogen-free conditions at the International Medical Center of Japan (IMCJ; Tokyo, Japan). The present experiments had the prior approval of the Animal Experimentation Committee of the IMCJ. The human gastric (KATO III) and colorectal (HT29) cancer cell lines were maintained in DMEM supplemented with 10% FCS. Human umbilical vein endothelial cells (HVEC) were purchased from Cell Applications, Inc. (San Diego, CA) and maintained in cultures under standard conditions in 0.5% gelatin-coated flasks. The cells were maintained in endothelial cell basal medium (Cell Applications, Inc. San Diego, CA) and maintained in cultures under pathogen-free conditions at the International Medical Center of Japan (IMCJ; Tokyo, Japan) and maintained in the animal facility under pathogen-free conditions.

The KM694 mAb was initially selected by reaction with GM2 ganglioside but was subsequently shown to have greater binding affinity for the Sda with the same specificity as reported previously for anti-Sda mAb KM531 (3). Anti–sialyl Lewisx mAb (90/11; Biogenesis Ltd., Poole, United Kingdom), and pepsinogen II mAb (90/11; Biogenesis Ltd., Poole, United Kingdom), and KM93 (directed to sialyl Lewisx; ref. 16) were both provided by the Committee of the IMCJ. All studies using human samples had been approved by the Ethics Committee of the IMCJ. The human gastric (KATO III) and colorectal cancer cell lines and analyzed the alterations in carbohydrate chain expression and cell adhesion to E-selectin. We had also defined the effects of surface expression Sdα for metastatic potential of tumor cells in vivo.

Expression of Sdα Inhibits Metastasis

Table 1. β1,4GalNAcT activity (pmol/h/mg protein) in transfected KATO III cells

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Mock-KATO III</th>
<th>Sdαβ1,4GalNAcT-KATO III</th>
<th>Normal gastric mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>NeuAcα2-3Galβ1-4Glc-Pα</td>
<td>—</td>
<td>6.29</td>
<td>5,160</td>
</tr>
<tr>
<td>NeuAcα2-6Galβ1-4Glc-Pα</td>
<td>4</td>
<td>10</td>
<td>250</td>
</tr>
<tr>
<td>NeuAcα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-Pα</td>
<td>—</td>
<td>2.16</td>
<td>3,330</td>
</tr>
<tr>
<td>NeuAcα2-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Pα</td>
<td>—</td>
<td>2.39</td>
<td>3,410</td>
</tr>
<tr>
<td>NeuAcα2-6Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Pα</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*Activity was under the level of detection.
Binding assays to Siglec immunoglobulin chimera. Binding studies using recombinant Siglec-3, -5, -7, or -9 immunoglobulin chimera (R&D Systems, Inc., Minneapolis, MN) were done as described previously (20). In brief, recombinant Siglec immunoglobulin chimera was preincubated with affinity-purified rabbit anti-human IgG (DAKO, Glostrup, Denmark) followed by incubation with PE-conjugated streptavidin before application to the binding analyses with transfectants. Binding of recombinant molecules to the cells was evaluated by flow cytometry. For the inhibition experiments, the cells were incubated with blocking antibodies (10 μg/mL) for 30 minutes at 37°C.

In vivo metastasis assay. Exponentially growing KATO III cells were harvested and suspended in HBSS. Suspensions of KATO III cells (1 x 10⁷) were injected into the peritoneal cavity of individual male athymic mice. The mice were sacrificed 10 weeks later, and tumor lesions were examined. Twelve or 13 mice per group were used in this model. In some experiments, HT29 cells (1 x 10⁶) were injected intrasplenically, and nodule formation in the liver was numerated 8 weeks later. Three to five mice per group were used in this hepatic metastasis model.

Immunohistochemical analysis. This study was approved by the Ethics Committee of the IMCJ, and informed consent was obtained for the taking of all specimens. Frozen sections (8 μm thick) were prepared from a surgical specimen. After fixation in acetone and blocking with 3% bovine serum albumin (BSA) in PBS, sections were incubated with the indicated primary mAbs (5 μg/mL) for 2 hours. Bound mAbs were detected with FITC-conjugated goat anti-mouse IgM (1:800 dilution) or TRITC-conjugated goat anti-mouse IgG (1:800, Southern Biotechnology Associates).

Cell separation from gastric mucosa. Separation procedures followed a previously reported method (21) with some modifications. In brief, surgically obtained human gastric mucosa was washed, minced, and then incubated in HBSS with 0.1% BSA, 1 mmol/L EDTA, 3 mg/mL Dispase (Roche Diagnostics Corp., Indianapolis, IN), and 0.15 mg/mL collagenase (Sigma Chemical) for 20 minutes. The residue was further digested with Dispase and collagenase two more times, and the cell suspension was passed through a nylon mesh. Next, the cells were suspended in 40% Percoll and centrifuged with a vertical rotor at 30,000 x g for 15 minutes, which allowed separation of three cell fractions.

Assay for pepsinogen activity. Cells or various concentrations of standard porcine pepsinogen were incubated with 0.1 N HCl containing 1.8% hemoglobin and 6 mmol/L glycine for 15 minutes at 37°C. After stopping this reaction with 0.9% trichloroacetic acid, the solution was centrifuged and the supernatant was assessed at A280 nm.

Statistics. The data were expressed as the mean ± 1 SD, and the results were compared by the two-tailed, unpaired Mann-Whitney, Student’s t test or χ² test using the Statview II statistical program (Abacus Concepts, Berkeley, CA).

Results

Expression of Sda determinant in the normal human stomach and in gastric cancer. We found previously that Sda carbohydrate determinant was expressed in the oxyntic mucosa of
the human normal stomach. With the final aim of understanding the function of the Sd\(^a\) antigen, we initiated studies to determine the cell types that express Sd\(^a\) in the gastric body mucosa. In an immunohistologic study, cells at the base of the gastric glands were exclusively expressing the Sd\(^a\) determinant (Fig. 1A). The Sd\(^a\) was detected only in normal stomach but was not in gastric cancer tissue, confirming our earlier results (Fig. 1B). Most of the cells expressing Sd\(^a\) were positively stained with anti-pepsinogen mAb, which was used as a chief cell marker, but were not stained with anti–proton pump mAb, a parietal cell marker (Fig. 1C and D). To further confirm that Sd\(^a\) glycolipid is expressed in chief cells, we did cell separation from human stomach mucosa. Cells obtained from the gastric mucosa were divided into three fractions by centrifugation over 40% Percoll. Fraction 1 was rich in parietal cells having the typical morphology (Fig. 1E, top). Chief cells were enriched in the middle fraction (fraction 2; Fig. 1E, middle). Mucous cells were concentrated in fraction 3 (Fig. 1E, bottom). Pepsinogen activity was concentrated in fraction 2 (Fig. 1F). The Sd\(^a\) glycolipid was detected in chief cell–enriched fraction, whereas neither fraction 1 nor fraction 3 expressed Sd\(^a\) glycolipids (Fig. 1G). The cells in the chief cell–enriched fraction also contained higher levels of $\beta_{1,4}$GalNAcT activity than those in the parietal cell–rich or the mucous cell–enriched fractions (Fig. 1H). These results indicate that chief cells are the major source of the Sd\(^a\) glycolipid, which is formed by $\beta_{1,4}$GalNAcT.

**Cloning and characteristics of the human Sd\(^a\)-\(1,4\)GalNAcT cDNA.** In our next series of studies, we cloned a cDNA containing the full-length ORF of human $\beta_{1,4}$GalNAcT by the RACE method using a cDNA segment, which we had isolated previously as a human homologue of the murine Sda-1,4GalNAcT (18). The human $\beta_{1,4}$GalNAcT nucleotide sequence showed a 1,701-bp ORF encoding a predicted 566–amino acid protein with a typical type II topology, which is common in glycosyltransferases. It shares a 78.8% nucleotide identity with the murine Sd\(^a\)-\(1,4\)GalNAcT (17). This cDNA sequence was 100% identical to that in a recent report by Montiel et al. (ref. 22; AF510036 in Genbank nucleotide sequence database). When the human gastric cancer cell line KATO III was stably transfected with the human $\beta_{1,4}$GalNAcT cDNA, its homogenates transferred GalNAc at high efficiency to lactose or terminal lactosamine with sialic acid in $\alpha$2,3 linkage but not to those with sialic acid in $\alpha$2,6 linkage. No $\beta_{1,4}$GalNAcT activity was detected in mock-transfected cells (Table 1). This substrate specificity was the same as that seen in human gastric body mucosa (Table 1; ref. 15). Cell surface expression of the Sd\(^a\) structure was evidently increased in both KATO III and HT29 cancer cell lines after transfection with Sd\(^a\)-\(1,4\)GalNAcT, whereas cells transfected with the vector alone did not (Fig. 2A and B). These results show that the cDNA clone encodes a $\beta_{1,4}$GalNAcT identical to human Sd\(^a\)-\(1,4\)GalNAcT expressed in the human stomach. There was no alteration in cell growth or viability after Sd\(^a\)-\(1,4\)GalNAcT transfection.

**Elimination of selectin ligands by introduction of the human Sd\(^a\)-\(1,4\)GalNAcT.** Sd\(^a\)-\(1,4\)GalNAcT and fucosyltransferases, which produce sialyl Lewis\(^a\) and sialyl Lewis\(^x\), share acceptor carbohydrates. As expected, transfection of KATO III cells with Sd\(^a\)-\(1,4\)GalNAcT caused loss of expression of sialyl Lewis\(^a\) (Fig. 2A). These results were confirmed by TLC immunostaining and Western blot analysis. In mock-transfected KATO III cells, the Sd\(^a\) was present in only trace amounts in the glycolipid extracts (Fig. 3A), although GM2 ganglioside was visible because of its weak cross-reactivity. In contrast, KATO III cells transfected with Sd\(^a\)-\(1,4\)GalNAcT expressed Sd\(^a\) glycolipids, with concomitant elimination of sialyl Lewis\(^a\) (Fig. 3A). As expected from the substrate specificity where Sd\(^a\)-\(1,4\)GalNAcT uses both glycolipids and oligosaccharide as acceptor substrates, these changes were also evident in glycoproteins (Fig. 3C). The anti–sialyl Lewis\(^a\) mAb

---

**Figure 2.** Flow cytometric analysis of Sd\(^a\), sialyl Lewis\(^a\), and sialyl Lewis\(^x\) on Sd\(^a\)-\(1,4\)GalNAcT-transfected cells. Human gastric cancer KATO III cells (A) and colon cancer HT29 (B) cells were stably transfected with the human Sd\(^a\)-\(1,4\)GalNAcT gene (bottom) or mock-transfected with the vector alone (top) and were stained with mAb KM694 (specific for Sd\(^a\)), KM93 (specific for sialyl Lewis\(^a\)), or 116-NS-19-9 (specific for sialyl Lewis\(^x\)). Filled histograms, control staining without mAbs. Clones of KATO III (C) and HT29 (D) stably transfected with the Sd\(^a\)-\(1,4\)GalNAcT gene were assessed for expression levels of Sd\(^a\)-\(1,4\)GalNAcT by quantitative RT-PCR (top). These Sd\(^a\)-\(1,4\)GalNAcT-transfected clones (Hi, Int, and Lo) were also analyzed as in (A and B) by flow cytometry. Data are mean fluorescence intensities (MFI) of stained cells. Representative of four separate experiments, which gave similar results.
bound to many glycoprotein components in mock-transfected KATO III of >100 kDa, mostly mucins; however, no Sdα was detected. After introduction of Sdα,1,4GalNAcT into KATO III cells, all sialyl Lewisx-positive glycoprotein bands disappeared, and glycoproteins bearing the Sdα determinant concomitantly appeared. Because the expression of sialyl Lewisα antigen by KATO III cells was originally low, we could not examine the effect of Sdα,1,4GalNAcT transfection on the expression of sialyl Lewisα in this cell line (Fig. 2A). Introduction of the Sdα,1,4GalNAcT gene into HT29 cells resulted in the loss of expression of sialyl Lewisα in glycolipids and glycoproteins (Fig. 3B and D). Sialyl Lewisα was not expressed on glycolipids in the original HT29 cells. Furthermore, we obtained several clones of stably Sdα,1,4GalNAcT-transfected KATO III cells (Fig. 2C) and HT29 cells (Fig. 2D), which expressed Sdα,1,4GalNAcT mRNA at different levels: high, intermediate, and low. These clones also expressed different levels of cell surface Sdα, which paralleled the mRNA produced (Fig. 2C and D). Of note, expression of both sialyl Lewisα and sialyl Lewisα was significantly diminished in all of these Sdα,1,4GalNAcT-transfected clones, suggesting that Sdα,1,4GalNAcT has the potential to eliminate the expression of sialyl Lewisα and sialyl Lewisα efficiently, essentially irrespective of its translational level in the cell.

Attenuated cell adhesion to endothelial cells after introduction of Sdα,1,4GalNAcT. Because both sialyl Lewisα and sialyl Lewisα on the cancer cell surface are ligands for E-selectin (13, 14), we next examined the effects of directed expression of Sdα,1,4GalNAcT on adhesion to endothelial cells. Whereas mock-transfected KATO III cells strongly adhered to activated HUVEC, the Sdα,1,4GalNAcT-transfected KATO III cells showed significantly reduced adhesion (Fig. 4A and B). The same results were obtained with HT29 Sdα,1,4GalNAcT transfectants (Fig. 4C). The contribution of E-selectin was established by the blocking effect of anti-E-selectin mAb (Fig. 4C). These results clearly show that the introduction of Sdα,1,4GalNAcT abolished E-selectin-dependent adhesion of cancer cells to endothelial cells in vitro.

Sdα does not bind to Siglec family molecules. Siglec family molecules are sialic acid-dependent cell adhesion molecules. Siglec-7 has been reported to have a binding activity to a certain carbohydrate (disialyl Lewisα), which is expressed preferentially on nonmalignant colonic epithelial cells (20). Assuming that Sdα may be ligand for the Siglec family; we next tested the binding of Sdα,1,4GalNAcT transfectants to recombinant Siglec-3, -5, -7, and -9 immunoglobulin chimera. However, direct binding of Sdα determinant to Siglec proteins was not detected (data not shown).

Inhibition of tumor metastasis by introduction of Sdα,1,4GalNAcT. We next evaluated the effects of directed expression of Sdα,1,4GalNAcT in gastrointestinal cancer cells on their metastatic potential. Mock-transfected KATO III cells metastasized to the spleen, liver, peritoneum, and seminal vesicles when injected into the peritoneal cavity of nude mice (Fig. 5; Table 2). In contrast, mice inoculated with Sdα,1,4GalNAcT-transfected KATO III cells developed no macroscopic tumor lesions (Table 2). Although several sections of the spleen, liver, and...
semenal vesicles were prepared and examined histologically, there were no metastatic foci in mice given Sda\(^{-}\)β1,4GalNAcT-transfected KATO III cells. The colorectal cancer cell line HT29 was also used to test hematogenic metastasis in the liver. Eight weeks after intrasplenic injection, tumor nodules were established in the primary site of all mice injected with both mock-transfected and Sda\(^{-}\)β1,4GalNAcT-transfected HT29 cells, indicating that there was no difference in cell growth or viability in vivo between them. Metastatic nodules were seen in the liver of all mice injected with mock-transfected HT29 cells; however, Sda\(^{-}\)β1,4GalNAcT-transfected metastatic foci occurred in only one of the mice tested (Table 2). These results clearly show that the introduction of Sda\(^{-}\)β1,4GalNAcT efficiently inhibited tumor metastasis of sialyl Lewis\(^{x/a}\)-positive cancer cells.

**Discussion**

There are significant correlations between the degree of expression of sialyl Lewis\(^{x/a}\) determinants in cancer tissues and the postoperative prognosis of patients with colon, lung, breast, stomach, prostate, and urinary bladder cancers (10–12, 23–26). Therefore, elimination of the sialyl Lewis\(^{x/a}\) determinants from cancer cells has been an important goal for the control of cancer metastasis. The present study provides direct evidence that the production of this Sda\(^{a}\) determinant by the action of a specific glycosyltransferase, Sda\(^{-}\)β1,4GalNAcT, efficiently eliminated sialyl Lewis\(^{x/a}\) from cancer cells, reduced the E-selectin-mediated adhesion of these cells to the endothelium, and efficiently abolished metastasis in vivo. Through this analysis, we found that introduction of the Sda\(^{-}\)β1,4GalNAcT gene essentially inhibited the expression of sialyl Lewis\(^{x}\) and sialyl Lewis\(^{a}\) from both glycoprotein and glycolipid components. Further, these effects were essentially independent of the number of copies of the Sda\(^{-}\)β1,4GalNAcT gene expressed. It is of note that the presence of this single glycosyltransferase led to profound changes in the types of carbohydrate determinants displayed by the cancer cells.

Many glycosyltransferases participate in the total biosynthesis of sialyl Lewis\(^{x}\) and sialyl Lewis\(^{a}\), and their synthesis is completed by the final addition of sialic acid and fucose by the action of α2,3 sialyltransferases and α1,3/4 fucosyltransferases (Fig. 6). To our knowledge, even for this final step, seven genes encoding the α2,3 sialyltransferase family and six human α1,3/4 fucosyltransferase genes are likely involved (27). Extensive studies on the expression levels of these sialyltransferases and fucosyltransferases in gastrointestinal tract cancers have been carried out; however, the precise mechanisms for up-regulation of sialyl Lewis\(^{x/a}\) has not yet been determined (28–30). Apparently, the final expression levels of sialyl Lewis\(^{x/a}\) epitopes in colon cancer tissues are not controlled by a single glycosyltransferase but through a combinatorial effect of multiple glycosyltransferases. Based on this fact, the interruption of a certain fucosyltransferase [e.g., FUT3 (31)] may not always result in the elimination of sialyl Lewis\(^{x/a}\) in human cancers, because other fucosyltransferases, such as FUT4-FUT7, may be also involved in the up-regulation of sialyl Lewis\(^{x/a}\) in cancer tissues. In the present study, we showed that introduction of a single glycosyltransferase, Sda\(^{-}\)β1,4GalNAcT, resulted in the total loss of both sialyl Lewis\(^{x}\) and sialyl Lewis\(^{a}\) epitopes from both glycolipids and glycoproteins. Because Sda\(^{-}\)β1,4GalNAcT uses substrates that are required in the very last step in the synthesis of sialyl Lewis\(^{x/a}\) as shown in Fig. 6, this effect should be seen in any cancer cells that express high levels of sialyl Lewis\(^{x/a}\) irrespective of the underlying mechanism for expression. Thus, it is a great advantage that introducing a single
gene for one enzyme is able to alter the expression of functional carbohydrates dramatically, whose expression is actually under multifactorial control. It is also notable that the elimination of sialyl Lewisx/a occurred irrespective of the expression levels of the Sdα-β1,4GalNAcT, which suggests that Sdα-β1,4GalNAcT has a strong potential to compete for substrates with other glycosyltransferases at the site of glycosylation of proteins and glycolipid synthesis. If Sdα-β1,4GalNAcT is a dominant enzyme in the terminal modification of carbohydrates as our data clearly suggest, the loss of its activity in gastrointestinal tract cancers may contribute to the up-regulation of sialyl Lewisx/a as a part of the malignant transformation progress. The nature of the mechanisms regulating the tissue-specific expression and cancer-associated down-regulation of Sdα-β1,4GalNAcT remains a major question to be answered. Clearly, this approach is important because it will provide the tools necessary to manipulate the expression of Sdα by modifying the regulation system at the translational level. Defining the details of this mechanism for expression of the Sdα antigen in relation to that of sialyl Lewisx/a is also a challenge for future studies of the regulation of glycosylation by glycosyltransferase activities.

Another important feature of Sdα-β1,4GalNAcT is its relatively broad substrate specificity. It is known to catalyze reactions that add GalNAc in β1,4 linkage to both glycolipids and glycoproteins with both type 1 and 2 core chains as acceptor oligosaccharide substrates (Fig. 6). This characteristic substrate usage resulted in total elimination of sialyl Lewisx/a from both glycolipids and glycoproteins. Previously, two research groups introduced FUT1, which catalyzes the addition of a terminal fucose in α1,2 linkage to a lactosamine residue, into HT29 cancer cells and succeeded in reducing the level of sialyl Lewisx (32, 33). However, their results with sialyl Lewisx were inconsistent. The discrepancy between these two studies seems to be derived from the heterogeneity of expression patterns of carbohydrates that can occur in a cancer cell line (33). This again indicates the difficulty in applying gene delivery of FUT1 for the treatment of cancer cells in the human gastrointestinal tract, which displays immense heterogeneity and variation. In addition, FUT1 activity is already up-regulated in human colon cancer (34). In contrast, our approach using Sdα-β1,4GalNAcT could be more beneficial than that used in the above trials to ensure reduction of both sialyl Lewisx and sialyl Lewisa, when safe and sufficient methods to transduce genes for clinical application are developed. In addition, Sdα-β1,4GalNAcT would be applicable for not only gastrointestinal tract cancer but also cancer of the pancreas and biliary tracts, where the expression of sialyl Lewisx is significantly correlated with the postoperative prognosis of patients with these cancers (34, 35).

Because the regulation system for Sdα expression and its function is still unknown, it is difficult to predict any possible adverse effects of the forced expression of Sdα in vivo. In this study, we attempted to assume a function for the Sdα antigen by determining its cellular localization in the stomach. We found that Sdα glycolipid was expressed in chief cells. It explained in part that both Sdα antigen and Sdα-β1,4GalNAcT activity were strikingly reduced or absent in the antral mucosa, intestinal metaplasia, and gastric cancer tissue because of the absence of chief cells in these

![Figure 6. Biosynthetic pathway of sialyl Lewisx/a and Sdα. Filled diamonds, sialic acid (N-acetylneuraminic acid); open circles, galactose; filled squares, N-acetylglucosamine; filled circles, glucose; hatched square, N-acetylgalactosamine; open triangle, fucose; R, core carbohydrate structure.](image-url)
tissues. However, the relation of Sd with the function of chief cells remains to be elucidated. The Sd carbohydrate showed no apparent binding to the Siglec family molecules, adhesion molecules that recognize sialic acid residues. The Sd glycolipids were not detected in gastrointestinal tract cancers, and in vitro Sd expression was associated with growth inhibition caused by induction of differentiation by dimethylformamide (7). We did not see changes in cell growth or viability after Sd-1,4GalNAcT transfection in this study. These results at least tell us that Sd is not expressed where malignant changes occurs in the gastrointestinal tract. Our results clearly show that the normalisation of surface carbohydrates by directed expression of a single glycosyltransferase, Sd-[1,4]GalNAcT, efficiently decreased the metastatic potential of sialyl Lewis x+−positive cancer cells. We feel that manipulation of gastrointestinal cancer cells to make them express Sd is a promising approach for controlling metastasis. Certainly, we need to gain further knowledge about the regulation of their expression system and their biological functions.

Acknowledgments


The costs of publication of this article were defrayed in part by the payment of page charges. This article must not be interpreted as reflecting the official position of any funding agency.

We thank Miyuki Nakasui for her technical assistance, Eri Watanabe (National Institute of Infectious Diseases, Tokyo, Japan) for her help with cell sorting, and Dr. Yutaka J. Kawamura for his helpful advice and discussion.

References


Introduction of Sdα Carbohydrate Antigen in Gastrointestinal Cancer Cells Eliminates Selectin Ligands and Inhibits Metastasis

Yuki I. Kawamura, Rei Kawashima, Ryuko Fukunaga, et al.


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/65/14/6220

Cited articles  This article cites 33 articles, 15 of which you can access for free at:
http://cancerres.aacrjournals.org/content/65/14/6220.full.html#ref-list-1

Citing articles  This article has been cited by 6 HighWire-hosted articles. Access the articles at:
/content/65/14/6220.full.html#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.