Lewis Type 1 Antigen Synthase (β3Gal-T5) Is Transcriptionally Regulated by Homeoproteins

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The type 1 carbohydrate chain, Galβ1-3GlcNAc, is synthesized by UDP-galactose:β-N-acetylgalcosamine β1,3-galactosyltransferase (β3Gal-T). Among six β3Gal-Ts cloned to date, β3Gal-T5 is an essential enzyme for the synthesis of type 1 chain in epithelium of digestive tracts or pancreatic tissue. It forms the type 1 structure on glycoproteins produced from such tissues. In the present study, we found that the transcriptional regulation of the β3Gal-T5 gene is controlled by homeoproteins, i.e. members of caudal-related homeobox protein (Cdx) and hepatocyte nuclear factor (HNF) families. We found an important region (−151 to −121 from the transcription initiation site), named the β3Gal-T5 control element (GCE), for the promoter activity. GCE contained the consensus sequences for members of the Cdx and HNF families. Mutations introduced into this sequence abolished the transcriptional activity. Four factors, Cdx1, Cdx2, HNF1α, and HNF1β, could bind to GCE and transcriptionally activate the β3Gal-T5 gene. Transcriptional regulation of the β3Gal-T5 gene was consistent with that of members of the Cdx and HNF1 families in two in vitro systems. 1) During in vitro differentiation of Caco-2 cells, transcriptional up-regulation of β3Gal-T5 was observed in correlation with the increase in transcripts for Cdx2 and HNF1α. 2) Both transcript and protein levels of β3Gal-T5 were determined to be significantly reduced in colon cancer. This down-regulation was correlated with the decrease of Cdx1 and HNF1β expression in cancer tissue. This is the first finding that a glycosyltransferase gene is transcriptionally regulated under the control of homeoproteins in a tissue-specific manner. β3Gal-T5, controlled by the intestinal homeoproteins, may play an important role in the specific function of intestinal cells by modifying the carbohydrate structure of glycoproteins.

Carbohydrate antigens are involved in many biological events. Among the antigens, Lewis antigens, which have Galβ1-3GlcNAcβ- as a backbone structure, are the major ones expressed in human tissue. Whereas the type 2 (Galβ1-4GlcNAcβ-) structure is distributed ubiquitously, the type 1 (Galβ1-3GlcNAcβ-) structure is synthesized only in certain tissues, such as epithelium of the digestive tracts or pancreatic tissue. This restricted distribution of Lewis type 1 antigens has been considered to be the result of the limited expression of UDP-Gal:GlcNAc β1,3-galactosyltransferases (β3Gal-Ts), which synthesize the type 1 structure. Sialyl Lewis a (sLeα), known as CA19-9 antigen in the clinical field, belongs to the Lewis type 1 antigens. CA19-9 is one of the most useful tumor markers and shown to be frequently accumulated in sera of colonic and pancreatic carcinomas.

We reported previously (2) the molecular cloning of β3Gal-T5 and presented evidence that this enzyme is responsible for the synthesis of Lewis type 1 antigens in colonic mucosa, pancreatic tissue, and cell lines derived from them. Moreover, we showed that the amount of type 1 antigen, such as CA19-9, on glycoproteins was well correlated with the β3Gal-T5 transcript level in these cell lines, suggesting that the transcriptional control of β3Gal-T5 might be the major regulatory factor for the synthesis of the Lewis type 1 structure. This enzyme efficiently acts on glycolipids as well as N-linked glycoproteins such as carcinoembryonic antigen, a well known tumor marker, and arranges its carbohydrate composition (3). In addition to the activity for synthesizing the type 1 structure, β3Gal-T5 can transfer a galactose in the β1,3-linkage to the mucin core-3 structure (GlcNAcβ1-3GlcNAcβ-) which is abundantly expressed in colonic epithelium (4).

It has been reported that alterations of glycosyltransferase activity give rise to a dramatic modification of carbohydrate structures in several biological processes, such as differentiation and carcinogenesis. Caco-2 cells, derived from colon adenocarcinoma, have been frequently used as a model for intestinal differentiation because of their unique character, i.e. spontaneous differentiation into enteroocyte-like cells as defined morphologically and enzymologically. In this process, β3Gal-T

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1 The abbreviations used are: β3Gal-T, β1,3-galactosyltransferase; β4Gal-T, β1,4-galactosyltransferase; EMSA, electrophoretic mobility shift assay; HNF, hepatocyte nuclear factor; sLeα, sialyl Lewis a; Leα, Lewis a; Leβ, Lewis b; Leβ, Lewis a; Leγ, Lewis γ; SI, sucrase-isomaltase; LPH, lactase-phlorizin hydrolase; GCE, β3Gal-T5 control element; RT, reverse transcriptase; PBS, phosphate-buffered saline; Cdx, caudal-related homeobox protein.
activity is up-regulated (5), resulting in an increase in the type 1 structure (6). It is not yet clarified which β3Gal-T is responsible for the β3Gal-T activity in Caco-2 cells. In colon cancer tissue, β3Gal-T activity toward N-glycan and glycolipids significantly decreased as compared with normal mucosa, whereas β4Gal-T activity was essentially unchanged (7). Down-regulation of β3Gal-T5 transcription in colon cancer was also reported (3). These reports suggest that β3Gal-T5 directs β3Gal-T activity in colonic epithelium and is transcriptionally down-regulated in cancer.

The tissue-specific expression and unique acceptor substrate specificity of β3Gal-T5 and the alteration in β3Gal-T activity during carcinogenesis and intestinal cell differentiation suggested its biological importance, which prompted us to examine the molecular basis for the transcriptional regulation of the β3Gal-T5. In a previous study, we determined the organization of β3Gal-T5 gene and the transcription initiation site by the 5′-rapid amplification of cDNA ends method, and we found several consensus sequences for known transcription factors within about 200 bp upstream of the transcription initiation site by computer analysis (2). Thus, we carried out the present study to identify essential transcription factors for the β3Gal-T5 gene, and we found that the intestine-specific transcription factors, Cdx and HNF1, were functional in transcriptional activation for β3Gal-T5. We also examined the alteration of β3Gal-T5 expression upon intestinal differentiation and carcinogenesis both in vitro and in vivo.

EXPERIMENTAL PROCEDURES

Cell Cultures—All cell lines were obtained from American Type Culture Collection (Manassas, VA) and maintained in Dulbecco’s minimal essential medium supplemented with 10% fetal calf serum at 37 °C in a 5% CO2, 95% air atmosphere. For differentiation experiments using Caco-2 cells, 2 mM sodium butyrate (Sigma) was added to the medium during carcinogenesis and intestinal cell differentiation suggesting its biological importance. The tissue samples were immediately frozen in liquid nitrogen and stored at –80 °C until total RNA isolation. Preparation of cDNA and Quantification of Transcription—Total RNA was isolated from tissue samples and cell lines with the acid/guanidinium/phenol/chloroform extraction method. Complementary DNAs were synthesized with an oligo(dT) primer from 5 μg of total RNA in a 20-μl (total volume) reaction mixture using a Superscript Preamplification System for First Strand cDNA Synthesis (Invitrogen). After cDNA synthesis, the reaction mixture was diluted 50-fold with water and then stored at –80 °C until use.

The quantitative RT-PCR using the LightCycler real time PCR instrument (Roche Applied Science) was performed according to the supplier’s manual. Standard plasmids of Cdx1, Cdx2, HNF1α, and HNF1β were prepared with PCR cloning as described above. The sequences of primers and fluorescent (fluorescein isothiocyanate and LC-Red 640) probes for quantitative RT-PCR are listed in Table I. Primers were designed to be located in different exons and not to detect contaminating genomic DNA. After 5 min of preheating, 50 cycles of PCR were performed. Each cycle consisted of 0 s of denaturing at 95 °C, 5 s of annealing at 55 °C, and 10 s of extension at 72 °C.

Reporter Plasmid Constructs—The cosmID human genomic library, kindly provided by Dr. H. Inoko (Tokai University, Kanagawa, Japan), was screened by hybridization with a full-length β3Gal-T5 cDNA as a probe. Two clones out of three positives were found to contain the β3Gal-T5 exon 1 by restriction mapping and Southern blotting. A 3.8-kbp XbaI fragment containing exon 1 was subcloned into pBluescript SK+ (Stratagene). The 3′-end of this fragment was located within the first intron, a SalI/EcoRI fragment, containing the 2.9-kbp 5′-flanking region and 5′-half of the first exon, was subcloned into pBluescript and subsequently subcloned into the KpnI and SacI sites of the pGL3 basic vector (Promega) for promoter assay. Several deletion mutants were prepared by appropriate restriction enzyme digestion and self-ligation. Smaller mutants (~200 bp) were cloned by PCR, and entire sequences were verified by sequencing. A site-directed mutation in the homeoprotein consensus sequence was introduced into a 200-bp HincII/EcoRI construct (containing from -151 to +49 of the promoter region) with a PCR-based mutagenesis kit (Takara).

Reporter Assay—Approximately 24 h before transfection, cells were removed from transfection, cells were removed, and 5 μg of DNA was used in a 24-well plate. Cells were transfected using the lipofection method with LipofectAMINE 2000 Reagent (Invitrogen), 0.5 μg of reporter plasmid (pGL3), and 10 ng of internal reference plasmid pRL-CMV, according to the manufacturer’s protocol. For co-transfection experiments, 0.5 μg of the expression vector was transfected together with reporter vectors.
Twenty four hours later, reporter activity was quantified with a Dual luciferase assay kit (Promega). The luciferase activity was normalized to an internal reference activity for each well, and the average for at least three experiments was shown as relative light units.

Nuclear Protein Extraction and Electrophoretic Mobility Shift Assay (EMSA)—Nuclear extracts were prepared as described previously (8). Radiolabeling of probe oligonucleotides and protein-DNA binding reactions were performed using a BandShift kit (Amersham Biosciences) according to the manufacturer’s directions. Briefly, protein-DNA binding reactions were performed in the reaction buffer (10% glycerol, 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.05% Nonidet P-40, and 0.5 mM dithiothreitol), including 1 μg of poly(dI-dC)-poly(dI-dC) and 50,000 cpm of probe. Reactions were initiated by the addition of nuclear extract and run for 30 min at room temperature to form protein-nucleotide complexes that were separated on a 5% nondenaturing polyacrylamide gel in 100 mM Tris acetate, 1 mM EDTA (pH 8.3). Gels were dried before the visualization of radiolabeled complexes with a BAS2000 image analyzer. For competition assays, unlabeled competitor was added to the reaction mixtures at a 100-fold molar excess of the concentration of labeled probe before the addition of the nuclear extract. Supershift assays were performed by adding 1/250 antibody (anti-HNF1; sc-6547, anti-HNF1; sc-7411, purchased from Santa Cruz Biotechnology) after an initial incubation period of 30 min. The incubation was then continued for an additional 30 min.

Preparation of Anti β3Gal-T5 Monoclonal Antibody—A partial cDNA encoding a truncated β3Gal-T5, which lacks a cytoplasmic tail and a transmembrane domain, was amplified by RT-PCR and subcloned in a pBluescript vector. The insert was subsequently subcloned in pET14b bacterial expression vector (Promega). The recombinant protein expressed in Escherichia coli accumulated in the insoluble fraction. The inclusion body was solubilized and dialyzed; rats were immunized with dialyzed protein, and the production and screening of hybridomas were performed as described previously. Finally, a monoclonal antibody reacting to the recombinant β3Gal-T5 protein was established. Immunological specificity was confirmed by immunostaining and Western blotting using Namalwa cells which express β3Gal-T1 to -T5 (2).

Immunohistochemical Staining—Small intestine, stomach, and nor-
Identification of the cis-Element Required for Activation of \(3\text{Gal-T5}\)—To determine the region essential for the transcriptional regulation of \(3\text{Gal-T5}\), we isolated a genomic clone encompassing the \(3\text{Gal-T5}\) promoter region (about 2.9 kbp in length) for a reporter assay. We constructed several deletion plasmids containing various lengths of the 5'-flanking region of \(3\text{Gal-T5}\) gene and examined the reporter activity with the SW1116 cells (\(3\text{Gal-T5}\) expressing cells). This result revealed that the promoter region, from –151 to –1, contains several consensus binding sites for known transcription factors, such as Cdx, HNF1, C/EBP, AP-1, and GATA etc. (Fig. 2). Two Cdx consensus sequences (ATAAA and ATTAT in Fig. 2), named as Cdx(A) and Cdx(B), were found in this region.

Results

Identification of the cis-Element Required for Activation of \(3\text{Gal-T5}\)—For further characterization of transcription factors that bind to GCE, EMSA was performed with the radiolabeled GCE probe and nuclear extracts of SW1116 cells and COS-1 cells transiently expressing Cdx or HNF1 protein. The bands corresponding to Cdx and HNF1 are indicated by open arrowheads. Nonspecific bands are indicated with asterisks.

Table 1

| nuclear extract | SW1116 |
|-----------------|--------|
| probe           | GCE    |
| competitor      | (–)   |
| antibody        | (–)   |
| HNF1α           |        |
| HNF1β           |        |
| HNF1β           |        |
| Cdx1/2          |        |

Fig. 3. Analysis of GCE-binding protein in nuclear extract of SW1116 cells. EMSA was performed using nuclear proteins of SW1116 cells and a GCE double-stranded radiolabeled probe. Competition assays were carried out with a 100-fold excess of GCE and SIF1 oligonucleotide. Supershift assays were done by addition of 1 \(\mu\)l of anti-HNF1α or anti-HNF1β antibody. The bands corresponding to HNF1α and Cdx1 and/or Cdx2 are indicated by open arrowheads.

Fig. 4. Binding of recombinant Cdx and HNF1 protein to GCE oligoprobe. EMSA was performed with the radiolabeled GCE probe and nuclear extracts of SW1116 cells and COS-1 cells transiently expressing Cdx or HNF1 protein. The bands corresponding to Cdx and HNF1 are indicated by open arrowheads. Nonspecific bands are indicated with asterisks.
Four homeoproteins, Cdx1, Cdx2, HNF1α, and HNF1β, were cloned in an expression vector in this study, and each of them was transiently expressed in COS-1 cells. The nuclear extracts of COS-1 cells expressing Cdx1 or Cdx2 formed a complex with the GCE probe, which appeared as a band almost at the same migration length as the lower band in the previous SW1116 experiment (Fig. 4). These results further indicated that the lower band in the SW1116 EMSA experiment is a GCE-Cdx1 and/or Cdx2 complex.

Specific protein-DNA complexes were also observed when reactions were performed with nuclear extract of COS-1 cells expressing HNF1α or HNF1β protein, and the complex of GCE-HNF1α appeared at the same position as the upper band in the SW1116 experiment (Fig. 4). The complex of GCE-HNF1β appeared at a position slightly lower than that of GCE-HNF1α. As shown with asterisks in Fig. 4, nonspecific bands were observed, which also appeared when the reaction was carried out with nuclear extract of COS-1 cells transfected with a null expression vector as a negative control. Thus, four homeoproteins, Cdx1, Cdx2, HNF1α, and HNF1β, were demonstrated to bind to GCE.

Each of four homeoproteins, Cdx1, Cdx2, HNF1α, and HNF1β, was co-transfected into COS-1 cells with the reporter construct containing −151 to +49 of the 5′-flanking region of the β3Gal-T5 promoter (Fig. 5). The reporter activity directed by Cdx1, Cdx2, HNF1α, or HNF1β increased 3-, 5-, or 11-fold, respectively, compared with that of the null expression plasmid. Thus, all homeoproteins were demonstrated to be capable of exerting the promoter activity for the β3Gal-T5 gene.

β3Gal-T5 Was Up-regulated during Enterocytic Differentiation of Caco-2 Cells Correlating to Up-regulation of HNF1α and Cdx2—Changes of transcript levels for β3Gal-T5 and homeoproteins were examined during differentiation of Caco-2 cells, which have been used as an in vitro model for intestinal differentiation. As shown in Fig. 6, when the enterocytic differentiation of Caco-2 cells was induced by sodium butyrate, the transcript levels for β3Gal-T5, Cdx2 and HNF1α were elevated in 2 or 4 weeks after confluence, although the Cdx1 transcript was not detected and the HNF1β transcript level did not change. Whereas the transcript levels for Cdx2 and HNF1α peaked 2 weeks after confluence, the β3Gal-T5 transcripts gradually increased in 4 weeks after confluence. These results indicated that the β3Gal-T5 gene is transcriptionally regulated by Cdx2 and/or HNF1α during the differentiation of Caco-2 cells.

Axial Gradient Expression of β3Gal-T5 and HNF1β in Colon and Significant Decrease of β3Gal-T5 and Cdx1 in Colon Cancer—To examine the alteration of β3Gal-T5 expression in cancer in vivo, we quantified the amounts of transcripts for β3Gal-T5 and four homeoproteins in 29 pairs of tissue samples from colon cancer and the surrounding normal mucosa using real time RT-PCR. The samples were divided into two groups based on the location of the lesion, i.e. “left colon,” descending to the sigmoid colon and rectum, and “right colon,” including the cecum, ascending, and transverse colon. No significant difference was observed between the two groups in sex, age, clinical stage, nodal metastasis, depth of invasion, and pathological features such as histological type or vascular/lymphatic invasion (data not shown). As shown in Fig. 7, the β3Gal-T5 transcript level of both groups decreased in cancer compared with normal mucosa with significance (p < 0.05), and interestingly, the β3Gal-T5 transcript level was significantly higher in the left colon than the right colon.
Regarding the homeoproteins, the transcript levels for Cdx1 and HNF1 were also significantly lower in the left colon than the right colon. The amounts of Cdx1 and HNF1 transcript decreased significantly in cancer (p < 0.05). In contrast, those of Cdx2 and HNF1 presented no significant difference between the left colon and right colon. In some cases, the serum CA19-9 level was determined; however, no significant relation was found between the CA19-9 level and 3Gal-T5 transcript (data not shown).

Detection of 3Gal-T5 in Normal, Metaplastic, and Cancerous Tissue by Immunohistochemical Analysis—We newly raised an anti-3Gal-T5 monoclonal antibody and examined normal and cancerous tissues by immunohistochemistry. The specificity of the antibody was confirmed by Western blot analysis using the lysate of Namalwa cells transfected with each of the 3Gal-T1 to -T5 genes (Fig. 8). This antibody specifically reacted to the lysate of 3Gal-T5 Namalwa cells alone and not to the other cell lysates. It detected the specific band of 3Gal-T5, 42 kDa in size (Fig. 8). As shown in Fig. 9, perinuclear staining was observed in columnar cells of both small intestine (in Fig. 9, A and E) and colon (B and F). In colon, strong signals were observed especially at the top of villi rather than crypt, whereas the signal strength was almost equal through the top to the bottom of villi in small intestine. In colon cancer tissue, the signals were apparently weakened as compared with those of normal tissue (Fig. 9, C and G). However, in several cancer samples, which were immunohistochemically confirmed to express CA19-9 antigen, the signals for 3Gal-T5 were also weakened as compared with those of the corresponding normal tissue (data not shown). Whereas 3Gal-T5 was barely expressed in normal gastric foveolar epithelium and proper glands, strong signals were detected in absorptive cells of intestinal metaplasia in the stomach (Fig. 9, D and H).

DISCUSSION

More than 120 genes for glycosyltransferases have been cloned to date, whereas only a few genes have been analyzed for their transcriptional mechanism. In this study, we analyzed the molecular mechanism for transcriptional regulation of the 3Gal-T5 gene. The members of two independent homeoprotein families, the Cdx and HNF1 families, were demonstrated to form a specific complex with the 3Gal-T5 promoter region and activate its gene transcription. This is the first report to demonstrate that homeoproteins are involved in the regulation of glycosyltransferases at the molecular level. In fact, our results indicated that four factors were differentially expressed depending on the system. Cdx1, Cdx2, and HNF1α were expressed in SW1116 cells and probably functioned as transcriptional factors. Cdx2 and HNF1α expression were correlated with 3Gal-T5 expression in the Caco-2 system. Finally, Cdx1 and HNF1β expression were correlated with 3Gal-T5 expression in actual colon cancer tissue. To explain this discrepancy, we speculate as follows. SW1116 and Caco-2 cells are cell lines...
transcripts for Cdx1, Cdx2, and HNF1 proteins in SW1116 cells by the real time RT-PCR method. The GCE-HNF1 complex as a lower band. The lower band expression. The expression of each factor may be regulated by an oligonucleotide, and the co-transfection assay in Fig. 5 demonstrated that they are also able to transactivate the cis-element for transcriptional regulation of this gene, and it revealed that a 30-bp region, which is located about 150 bp upstream of the transcription initiation site, is an essential factor involved in the regulation of homeobox genes and/or homeobox speculations in SW1116 and Caco-2 cells, some genes share certain features, namely HNF1-, Cdx-, and GATA-binding sites within 200 bp upstream of their transcription initiation site (Fig. 10). Two closely located Cdx-binding sites, but only the first site Cdx(A), overlapped by the HNF1-binding site, was demonstrated to be functional in this study. It was demonstrated that both HNF1 and Cdx proteins can bind to this site, whereas we did not observe a decrease of the Cdx2 transcripts in cancer tissue (Fig. 7). This discrepancy might be due to a difference of methodology.

The HNF1 family was first cloned as liver-specific transcription factors but recently shown to be involved in the expression of several genes in the intestine, such as the SI (14, 17, 25, 26), LPH (16, 17, 27), claudin-2 (19), and dipeptidyl peptidase IV genes (28). The involvement of HNF1 in carcinogenesis is not clearly understood; however, recent genetic analysis revealed that frequent mutations in the HNF1 gene occur in colon cancers (10) and hepatic adenomas (11). Another speculation is that, in SW1116 and Caco-2 cells, some genes involved in the regulation of homeobox genes and/or homeobox genes themselves may be lost or mutated.

The luciferase reporter assay using the β3Gal-T5 promoter revealed that a 30-bp region, which is located about 150 bp upstream of the transcription initiation site, is an essential cis-element for transcriptional regulation of this gene, and it was named GCE. The EMSA experiment in Fig. 4 demonstrated that four homeoproteins, Cdx1, Cdx2, HNF1α, and HNF1β, are able to form a specific complex with the GCE oligonucleotide, and the co-transfection assay in Fig. 5 demonstrated that they are also able to transactivate the β3Gal-T5 promoter.

We determined the transcript levels for the four homeoproteins in SW1116 cells by the real time RT-PCR method. The transcripts for Cdx1, Cdx2, and HNF1α were found to be abundantly expressed in SW1116 cells, but the transcript for HNF1β could not be detected (data not shown). This is consistent with the EMSA experiment (Fig. 4), in which we detected the GCE-HNF1α complex as an upper band and probably the GCE-Cdx1 and/or 2 complex as a lower band. The lower band was strongly indicated to be the complex of GCE-Cdx, because 1) it appeared at the same position as that of the complex of GCE-Cdx protein expressed in COS-1 cells, and 2) it was competed by an excess of unlabeled SIF1 that specifically binds to the Cdx protein. These results suggested the direct involvement of Cdx and HNF1 homeobox family proteins in the transcriptional regulation of β3Gal-T5.

Cdx homeoproteins, human orthologs of Drosophila caudal, are transcription factors that are expressed exclusively in the small intestine and colon (12). Many intestine-specific genes, such as those for sucrase-isomaltase (SI) (13, 14), lactase-phlorizin hydrolase (LPH) (13, 15–17), intestine phospholipase A/lipase (18), and claudin-2 (19) have been demonstrated to be transcriptionally regulated by Cdx proteins. It has also been reported that the Cdx family is involved in intestinal carcinogenesis, differentiation, and morphogenesis using several experimental approaches (20–23). These results suggest, in general, that Cdx family proteins induce intestinal differentiation, and their down-regulation gives rise to cancer. Down-regulation of Cdx1 expression in colon cancer tissue has been demonstrated with Northern blotting (12) and immunostaining (24), being compatible with our results using the quantitative RT-PCR method in this study. Mallo et al. (12) also documented a significant decrease of Cdx2 transcripts in human colon cancer, whereas we did not observe a decrease of the Cdx2 transcripts in cancer tissue (Fig. 7). This discrepancy might be due to a difference of methodology.

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Interestingly, the transcription for the β3Gal-T5 gene is under the cooperative control of Cdx and HNF1 as the transcription for SI (14), LPH (16), and claudin-2 (19), which are well known proteins specifically expressed in intestine, is controlled in the same manner. The intestine phospholipase A/lipase promoter also has binding sites for Cdx and HNF1 (18), although it has yet to be reported whether the HNF1 site is functional or not. The promoter regions of these genes share certain features, namely HNF1-, Cdx-, and GATA-binding sites within 200 bp upstream of their transcription initiation site (Fig. 10). Two closely located Cdx-binding sites were contained in the claudin-2 and SI promoters. The β3Gal-T5 promoter also has two putative Cdx-binding sites, but only the first site Cdx(A), overlapped by the HNF1-binding site, was demonstrated to be functional in this study. It was demonstrated that both HNF1 and Cdx proteins can bind to this site, although the question of whether Cdx and HNF1 act synergistically or exclusively remains to be answered.

The activity of several glycosyltransferases changed during the enterocytic differentiation of Caco-2 cells (5, 29), resulting in a wide variety of alterations in carbohydrate structure such as a decrease in polylactosaminylglycans (6, 30) and increase at the type 1 antigen (6). Once the type 1 structure is formed on the nonreducing end of the polylactosamine chain through the activity of β3Gal-T5, it probably works as a terminating signal for elongation of the chain, resulting in the shortening of the chain. Amano et al. (5) reported that the increase of type 1 antigen is well correlated with up-regulation of the β3Gal-T.
activity. In this study, we demonstrated a gradual elevation of β3Gal-T5 transcript levels upon the differentiation of Caco-2 cells, indicating that β3Gal-T5 directs the major part of β3Gal-T activity in Caco-2 cells. This study also demonstrated that transcription for Cdx2 and HNF1α was augmented, whereas the transcript for Cdx1 or HNF1β was absent or did not change, respectively, during this process. These results matched well the previous reports that documented up-regulation of Cdx2 (31) and HNF1α (32) expression during the differentiation of Caco-2 cells. These results strongly suggested that the up-regulation of β3Gal-T activity during the differentiation of Caco-2 cells is controlled by Cdx2 and/or HNF1β.

Immunohistochemical study using anti-β3Gal-T5 monoclonal antibody showed strong signals in epithelial cells of the small intestine and colon, whereas signals in colon adenocarcinoma cells were weakened as compared with those of normal mucosa. This finding is consistent with the transcript levels in this study and the β3Gal-T activity level in the previous study (7). In normal colon, the β3Gal-T5 protein was detected in both goblet cells and absorptive cells, especially in highly differentiated epithelial cells in the top of villi. This observation is compatible with the use of β3Gal-T5 as a marker for intestinal differentiation. In stomach, β3Gal-T5 is markedly expressed in intestinal metaplasia, comparable with that in small intestine but barely expressed in normal gastric mucosa. We have reported the strong expression of Leα antigen in intestinal metaplasia, correlating with a marked augmentation of the expression of α1,3-fucosyltransferase III (FUT3), which is involved in the synthesis of Leα (33). The results of the present study suggested that β3Gal-T5 was also involved in this phenomenon. It is noteworthy that both Cdx1 and Cdx2 are barely detectable in normal gastric mucosa but strongly expressed in intestinal metaplasia (24, 34, 35). Recently, Cdx2 transgenic mice were shown to develop intestinal metaplasia in the stomach, which indicated Cdx2 was essential for metaplastic formation (36). Taken together, we assume that intestinal metaplasia arising in the gastric mucosa is induced by the up-regulation of Cdx1 and Cdx2 expression, resulting in

Figure 9. Expression of β3Gal-T5 protein in small intestine, colon, colon cancer, and stomach. The tissue expression of β3Gal-T5 protein in small intestine (A and E), colon (B and F), colon cancer (C and G), and stomach (D and H) was examined by immunohistochemistry using anti-human β3Gal-T5 monoclonal antibody and paraffin-embedded human tissue. Magnified views of the open boxes in the left panels (A–D) are shown in the right panels (E–H). Metaplastic glands arising in normal gastric epithelium are indicated by solid arrowheads (D). FE, foveolar epithelium; IM, intestinal metaplasia. Scale bars indicate 50 μm.
transcriptional activation of β3Gal-T5, followed by the augmented expression of Leα antigen.

It is of interest what kind of modification in the carbohydrate structure and population is induced by the alteration of β3Gal-T5 during several biological processes such as differentiation, metaplastic formation, and carcinogenesis. Salvani et al. (3) reported that transfection of β3Gal-T5 in CHO-FT3 cells gave rise to the expression of Lewis type 1 antigens, reduction of Lewis type 2 antigens, and shortening of the polylactosamine chain. In our previous study, we introduced Lewis type 2 antigens, and shortening of the polylactosamine chain. In our previous study, we introduced Lewis type 2 antigens, and shortening of the polylactosamine chain. In our previous study, we introduced Lewis type 2 antigens, and shortening of the polylactosamine chain. In our previous study, we introduced Lewis type 2 antigens, and shortening of the polylactosamine chain.

It is quite interesting that homeoproteins essential for the cell differentiation in the intestine transcriptionally regulate the β3Gal-T5 gene, leading to the specific formation of the type 1 carbohydrate structure. β3Gal-T5 may play a crucial role in the maintenance of the intestinal phenotype and function through the modification of carbohydrate antigens.

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J. Biol. Chem. 2003, 278:36611-36620.
doi: 10.1074/jbc.M302681200 originally published online July 10, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M302681200

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