Expression of Tn, Sialosyl-Tn, and T Antigens in Human Colon Cancer

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ABSTRACT

Mucin glycoproteins are major secretory products of the colon and contain O-linked oligosaccharides synthesized on a polypeptide backbone. The initial step in the synthesis of O-linked oligosaccharides is the addition of N-acetylgalactosamine to serine or threonine residues forming the Tn antigen. This substance can then receive additional carbohydrate residues such as sialic acid to form sialosyl-Tn antigen, or galactose to form T antigens. In the colon, the T antigen is an oncodevelopmental cancer-associated antigen but little is known about Tn and sialosyl-Tn expression. The present comparative immunohistochemical study was performed to analyze the expression of these antigens in fetal, normal adult, and malignant colorectal tissues with an aim toward elucidating whether Tn and sialosyl-Tn are also oncodevelopmental colon cancer-associated antigens and to gain insight into the earliest steps of mucin glycosylation in colonocytes. We used three reagents to detect Tn antigen (two monoclonal antibodies ETn-01 and CU-1, and one lectin Vicia villosa), two reagents to detect sialosyl-Tn (monoclonal antibodies TKH2 and B72.3) and one to detect T antigen (monoclonal antibody AH9-16).

Except for occasional reactivity with VVA and CU-1, cells of normal colon mucosa did not express Tn, sialosyl-Tn, or T antigens. However, in the transitional mucosa immediately adjacent to cancer, all three antigens were expressed (ranging from 35 to 67% of cases depending upon the reagent). In colon cancers, the percentage of cases expressing each antigen were as follows: Tn 72-81%, sialosyl-Tn 93-96%, and T 71%. Unlike T antigen, which was preferentially expressed by moderately well- and well-differentiated adenocarcinomas, both Tn and sialosyl-Tn antigens were expressed by most histological subsets of colon cancers, including poorly differentiated adenocarcinomas and mucinous (colloid and signet ring cell type) carcinomas. The majority of cancers expressed both Tn and sialosyl-Tn, usually in association with T antigen. Only one cancer lacked all three antigens. Fetal colon mucosal cells expressed all three antigens, particularly in goblet cell mucin.

These results indicate that like T antigen, Tn and sialosyl-Tn are oncodevelopmental cancer-associated antigens in the colon. Moreover, Tn and sialosyl-Tn antigens appear to be useful markers of poorly differentiated adenocarcinomas and mucinous carcinomas: two histological subsets that often fail to express other cancer-associated antigens and that are often associated with a poor clinical outcome. Finally, these results provide additional evidence for incomplete glycosylation in colon cancer cells and suggest that the regulation of mucin oligosaccharide synthesis is fundamentally different in normal and malignant colonocytes.

INTRODUCTION

Colonie epithelium is characterized by the production of mucus, the main component of which is mucin. Mucins are high molecular weight glycoproteins containing oligosaccharide side chains that are connected by O-glycosidic linkages to serine or threonine residues in the protein (apomucin) backbone. The first step in O-linked oligosaccharide synthesis is the addition of N-acetylgalactosamine to serine or threonine resulting in the formation of the Tn antigen (Fig. 1). Once synthesized, the further glycosylation of Tn antigen can proceed along different pathways. The factors that regulate the different glycosylation pathways are not well understood, but these reactions depend to a great extent upon the activity of specific glycosyltransferase enzymes, the availability of precursor substances, and other factors such as diverant cation requirements and availability of nucleotide sugar donors (1). The addition of sialic acid by an α2,6-sialyltransferase (Fig. 1, Pathway 1) forms the sialosyl-Tn antigen (SAα2,6GalNAcα-O-Ser/Thr) and this structure does not appear to undergo further glycosylation. The addition of galactose by a β1,3-galactosyltransferase (Pathway 2) results in the formation of the Thomsen-Friedenreich antigen (Galβ1,3GalNAcα-O-Ser/Thr) also known as T antigen. This disaccharide forms the so-called Core 1 structure which can be further glycosylated. Individuals with the Tn syndrome are deficient in this β1,3-galactosyltransferase resulting in the expression of Tn antigen on their hematopoietic cells (2). If N-acetylgalcosamine is added to the Tn antigen (Pathway 3), this forms the Core 3 structure which can also be further glycosylated. Other glycosylation reactions not shown in Fig. 1 can occur and have been described in recent reviews (1, 3).

Of the alterations in glycosylation that have been described in cancer cells, incomplete glycosylation is a frequent event. This results in the appearance of precursor structures which in normal cells would remain cryptic or masked because of further glycosylation (4). For example, the T antigen is rarely expressed by normal colonocytes whereas cells of malignant, premalignant, and to some extent hyperplastic colon mucosa, frequently express this antigen (5-10). Moreover, expression of T antigen in premalignant adenomatous polyps seems to correlate with histologic criteria of malignant potential (10).

Previous biochemical studies have characterized oligosaccharide structures of normal human colon mucin as being primarily based on Core 3 structures (11, 12), and others have demonstrated Core 3 GlcNAc transferase activity in human (and rat) colon mucosa (13). This would suggest that in normal colonocytes, Pathway 3 of Fig. 1 might predominate over Pathways 1 and 2. Little is known about the oligosaccharide structures of mucin from colon cancer cells. We might postulate, based on the prevalence of T antigen expression, that in contrast to normal colonocytes, cancer cells preferentially synthesize oligosaccharides by Pathway 2 and perhaps Pathway 1, and that incomplete glycosylation beyond the initial steps might then result in the unmasking of these core-region structures.

The present immunohistochemical study was performed to investigate this hypothesis. This analysis was facilitated by the
Fig. 1. Simplified scheme of the biosynthesis of O-linked mucin glycoproteins. The synthesis of Tn antigen is catalyzed by a polypeptidyl-GalNAc transferase. Once formed, the Tn antigen has several biosynthetic fates, of which the three best characterized are indicated. Enzymes responsible for each of the three pathways are as follows: Pathway 1, α2,6-sialyltransferase; Pathway 2, β1,3-galactosyltransferase; and Pathway 3, β1,3-N-acetylgalactosaminyltransferase. Synthesis along Pathways 2 and 3 may proceed to further oligosaccharide elongation, whereas Pathway 1 is a terminal step (indicated by interrupted arrow). See text for details.

Recent availability of reagents that can identify Tn and sialosyl-Tn structures, although as yet, no reagent specific for the Core 3 structure is available. Our aims were threefold: (a) to define the expression of Tn and sialosyl-Tn antigens in fetal, normal adult, and malignant colorectal tissues; (b) to compare the expression of these antigens to T antigen in order to gain insight into the initial steps of glycosylation; and (c) to compare different anti-Tn and anti-sialosyl-Tn reagents to determine their relative reactivities.

MATERIALS AND METHODS

Tissues. Primary colorectal carcinoma tissues were obtained from 29 patients undergoing surgical resection. Nineteen were colonic adenocarcinomas, of which 14 were well differentiated or moderately differentiated, and five were poorly differentiated. The other 10 were mucinous (colloid) carcinomas in which more than 60% of tumor volume demonstrated abundant mucin production. In 25 cancer specimens, well-oriented mucosa immediately adjacent to the cancer was available for examination, and this region is referred to as TM. TM is grossly normal but usually exhibits microscopic abnormalities such as elongated and sometimes branched crypts, but no cellular atypia. 22 specimens of normal colonic mucosa were obtained at immediate autopsy from individuals without any colonic disease, according to the protocol established at the Department of Pathology, University of Maryland School of Medicine and kindly provided by Drs. Benjamin Trump and Abulkalam Shamsuddin. 11 cases of normal colonic mucosa were proximal to the splenic flexure, and 11 were distal. Fetal colonic tissue representing both proximal and distal regions was obtained from 10 second-trimester abortuses as approved by the Committee on Human Research, University of California San Francisco. All tissues used in this study were fixed in formalin, embedded in paraffin and cut into 5-μm serial sections for immunohistochemical staining.

Antibodies and Lectins. For the detection of Tn antigen, 2 Mabs\(^4\) and one lectin were used. Mab ETn 1.01 (IgM isotype) was developed by immunizing a Biozzi mouse with erythrocytes from an individual with the Tn syndrome.\(^5\) This antibody requires the Tn antigen GalNAc monosaccharide and does not bind to normal cells presenting MN or T antigens. The immunogen for the CU-1 Mab was a high-molecular weight glycoprotein Tn antigen secreted by a human squamous lung carcinoma cell line LU-65 (14). This IgG, Mab does not cross-react with blood group A antigen, unlike other Tn Mabs that have also been raised against the LU-65 glycoprotein Tn antigen (15). The binding of CU-1 to Tn antigen can be inhibited by monosaccharide GalNAc and specifically by p-nitrophenoxy-α-D-GalNAc but not by p-nitrophenoxy-β-D-GalNAc. The B4 lectin from Vicia villosa seeds specifically agglutinates Tn-exposed erythrocytes, a reaction which can be inhibited by saccharides or glycoproteins containing GalNAc (16).

Two Mabs were used to detect sialosyl-Tn. TKH2 (IgG\(_2\)) was raised against ovine submaxillary mucin as immunogen (17). The B72.3 Mab (IgG\(_1\)) was developed several years ago using membranes from a human breast carcinoma metastasis as immunogen (18). This antibody has undergone considerable testing both as a diagnostic and therapeutic reagent (19). The high molecular weight mucin-like glycoprotein recognized by B72.3 has been termed TAG-72, and recent biochemical studies suggest that the epitope involved is sialosyl-Tn (17, 20).

Monoclonal anti-T antibody (AH9-16) was obtained from Chemibiomd, Ltd. (Edmonton, Canada) and kindly provided by Dr. R. Murray Ratcliffe. We have used this IgM antibody in previous studies of colonic tissues (10).

Reagents. Biotinylated B4 lectin from Vicia villosa was obtained from Sigma Chemical Co. (St. Louis, MO). Histostain SP, provided in a kit with biotinylated rabbit anti-mouse IgG + IgA + IgM and streptavidin-peroxidase conjugate, was obtained from Zymed Laboratories (South San Francisco, CA). The buffer used was PBS (0.1 M phosphate, 1.5 M NaCl, pH 7.4) containing 1% bovine serum albumin.

Tissue Staining. The Mabs CU-1, TKH2, and B72.3 were used as undiluted hybridoma supernatant. Other working concentrations were as follows: Mab ETn 1.01, 1.3 μg/ml; VVA, 5 μg/ml; Mab AH9-16, 2 μg/ml.

The streptavidin-peroxidase technique of immunohistochemistry was performed as described previously (21), with all steps conducted at room temperature. Briefly, after deparaffinization and rehydration, sections were incubated with fresh 3% hydrogen peroxide in methanol for 10 min and then washed three times with buffer. Next, 5% normal rabbit serum in PBS was applied for 20 min and removed by blotting. Primary antibody or biotinylated-VVA were incubated for 90 min and washed three times with buffer. Then, for antibody-stained slides, biotinylated secondary antibody (1:75 in PBS) was added for 20 min and washed three times with buffer. All sections then received streptavidin-peroxidase conjugate (10 μg/ml) for 30 min and were washed with buffer three times. Finally, slides were reacted with diaminobenzidine substrate for 10 minutes, rinsed with tap water, and mounted.

Negative controls consisted of substituting normal mouse serum for Mabs and PBS for VVA, which resulted in negative staining.

Scoring. Staining intensity was graded as follows: absent (0), weak (1+), moderate (2+), strong (3+). For carcinomas, the number of low power (×10) optical fields in a specimen which were positively stained was expressed as a percentage of the total number of optical fields containing tumor. For normal mucosa and TM, the percentage of total crypts which stained positive were scored.

RESULTS

Normal Adult Colon. In normal adult colonic epithelium, Tn expression was essentially absent (Table 1). Only three specimens reacted weakly with VVA in some of the goblet cells, and with CU-1 in the supranuclear cytoplasm (Fig. 2). The lack of staining of normal colonic mucosa with anti-Tn reagents is consistent with the lack of any cross-reactivity with blood group A antigen.

In these same tissues, sialosyl-Tn and T antigens were not expressed at all.

Transitional Mucosa. Unlike normal mucosa, colonocytes of the mucosa immediately next to cancer often expressed Tn and sialosyl-Tn (Table 1, Fig. 3). All three anti-Tn reagents stained

\(^4\) The abbreviations used are: Mab, monoclonal antibody; VVA, Vicia villosa agglutinin; TM, transitional mucosa; PBS, phosphate buffered saline.

Table 1  Expression of Tn, sialosyl-Tn, and T antigens in human colonic tissues

<table>
<thead>
<tr>
<th></th>
<th>Tn</th>
<th>Sialosyl Tn</th>
<th>T</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1.01</td>
<td>VVA</td>
<td>CU-1</td>
</tr>
<tr>
<td>Normal colon (n = 22)</td>
<td>0</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Transitional mucosa (n = 25)</td>
<td>35</td>
<td>67</td>
<td>61</td>
</tr>
<tr>
<td>Colon cancer (n = 29)</td>
<td>72</td>
<td>72</td>
<td>81</td>
</tr>
<tr>
<td>Well differentiated (n = 4)</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Moderately differentiated (n = 10)</td>
<td>60</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>Poorly differentiated (n = 5)</td>
<td>80</td>
<td>80</td>
<td>75</td>
</tr>
<tr>
<td>Mucinous (n = 10)</td>
<td>80</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>Fetal Colon (n = 10)</td>
<td>10</td>
<td>100</td>
<td>50</td>
</tr>
</tbody>
</table>

Fig. 2. Tn expression in normal colonic mucosa. In general, Tn was not expressed in normal colonic mucosa. However, in a few cases, VVA (A) bound weakly to some goblet cells (arrow) and Mab CU-1 (B) bound weakly to supranuclear cytoplasm (arrow). (Original magnification, ×50)

The T antigen was found only in the supranuclear cytoplasm of cells in TCM.

Colon Cancer. The majority of colon cancers expressed Tn antigen although the percentage of total positive cases varied depending upon the reagent used (Table 1). With each of the three anti-Tn reagents, the negative cases were mostly among the well- and moderately well-differentiated adenocarcinomas. All mucinous carcinomas expressed Tn antigen except for one case not stained with VVA and two cases negative with Mab 1.01. There were some differences at the cellular level between the three anti-Tn reagents. In general, the staining intensity was moderate to strong with Mab CU-1 and VVA, but was weak to moderate with Mab 1.01. In well- and moderately differentiated adenocarcinomas, all three reagents stained cell cytoplasm and cancer gland luminal contents, whereas cell membranes were stained with Mabs CU-1 and 1.01 but only rarely with VVA (Fig. 4A). All three reagents stained cell cytoplasm in poorly differentiated adenocarcinomas (Fig. 5). In mucinous colon cancers, both Mab CU-1 and VVA binding were particularly prevalent and intense in the mucin within the cells and in extracellular mucin lakes (Fig. 6A). However, Mab 1.01 staining was often weaker and rarely found in extracellular mucin.

The expression of sialosyl-Tn was nearly ubiquitous in colon cancer. Only one poorly differentiated cancer specimen did not bind TKH2 and two were negative with B72.3. The prevalence,
cellular location, and staining intensity of TKH2 and B72.3 were strikingly similar. In well-differentiated adenocarcinomas, both Mabs reacted strongly, and preferentially stained cell membranes and cancer gland contents, with only occasional cytoplasmic staining (Fig. 4B). In poorly differentiated adenocarcinomas, cytoplasmic staining could be found (Fig. 5B). In mucinous carcinomas, practically all of the extracellular mucin lakes were markedly positive, with weaker staining of intracellular mucin and virtually no membrane staining (Fig. 6A).

T antigen was expressed in 71% of colon cancers, usually among the moderate- and well-differentiated adenocarcinomas. Compared to Tn and sialosyl-Tn, T antigen was found in fewer mucinous carcinomas. Anti-T staining of adenocarcinomas occurred mainly on cell membranes and glandular contents, but in mucinous carcinomas staining was mostly intracellular with rare expression in mucin lakes.

Coexpression of Tn, Sialosyl-Tn, and T Antigens in Colon Cancer. Table 2 summarizes the patterns of simultaneous expression of Tn, sialosyl-Tn, and T antigens that were found. Most often, tumors expressed all three antigens although there was heterogeneity in cellular location and staining intensity between antigens. In six tumors (25%), T antigen was absent but Tn and sialosyl-Tn were expressed. This suggests that either the T antigen is not synthesized (due to insufficient galactosyltransferase activity), or it is masked by additional carbohydrate residues. Two cancers did not express Tn but did express both sialosyl-Tn and T suggesting that all of the Tn precursor substance was further glycosylated. In one poorly differentiated adenocarcinoma, all three antigens were absent, representing in all likelihood, the inability of these tumor cells to synthesize GalNAc onto the protein backbone or possibly a lack of synthesis of the mucin polypeptide. In no case was Tn expressed without concomitant sialosyl-Tn or T expression.

Fetal Colon. In fetal colonic mucosa, Tn, sialosyl-Tn, and T were all expressed but with considerable variation depending upon the reagent used (Table 1). In the case of the anti-Tn reagents, Mab 1.01 staining was essentially absent from mucosal cells and meconium (luminal secretions), Mab CU-1 reacted with half of the cases in the cells but not in meconium, and VVA bound strongly to both cells and meconium in all cases. A striking finding was the strong and prevalent goblet cell staining with both VVA and Mab CU-1 (Fig. 7, A and B).

In terms of sialosyl-Tn expression, both TKH2 and B72.3 reacted strongly with goblet cells of the mucosa in about half the cases (Fig. 7C). Meconium staining was frequently positive with TKH2 but weak or negative with B72.3. Three fetal
Fig. 6. Mucinous carcinoma, colloid type, stained with VVA (A) and Mab B72.3 (B). VVA strongly stains mucin in both intracellular (arrow) and extracellular (arrowhead) locations. B72.3 preferentially stains extracellular mucin with only weak staining of intracellular mucin. (Original magnification, × 50)

Table 2 Simultaneous expression of Tn, sialosyl-Tn and T antigens in colon cancer tissues

<table>
<thead>
<tr>
<th>Tn</th>
<th>Sialosyl Tn</th>
<th>T</th>
<th>No. (total, 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>15</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

*In five of the 29 cancers, sections were unavailable for staining with one of the antibodies, so these cases are omitted from this simultaneous comparison.

specimens expressed T antigen weakly in cell cytoplasm and secretions.

The five fetal specimens that expressed CU-1-defined Tn also expressed sialosyl-Tn and two of them expressed T. However, since VVA stained all of the fetal colonic specimens, there were four cases where only VVA-defined Tn occurred without sialosyl-Tn or T.

**DISCUSSION**

A common feature of colon cancer is the production of mucin. However, it is not known if mucin expression simply reflects the lineage of the cells that give rise to the cancer, or whether it may play a role in the biological behavior of the cancer cell.

In support of the latter concept, patients with mucinous carcinomas of the colon (a histological subset in which most of the tumor volume consists of mucin) have been found to have a particularly poor outcome in some reports (22, 23). However, since other series have not confirmed this poor prognosis (24, 25), it raises the possibility as to whether qualitative rather than
quantitative mucin alterations might be important in the biology of colon cancer.

To gain insight into qualitative mucin alterations that might occur in colon cancer, we have examined the expression of carbohydrate antigens associated with the earliest steps of mucin glycosylation. By simultaneously comparing the expression of three antigens (Tn, sialosyl-Tn, T) in normal and malignant colonic tissues, several observations emerged. Normal colonicocytes did not express sialosyl-Tn or T and only rarely expressed Tn antigen. The occasional expression of Tn suggests that in some cases this structure may be in the process of being further glycosylated. In contrast to normal mucosa, three-quarters of the colon cancers expressed Tn, T antigens, and practically all of them expressed sialosyl-Tn. Therefore the appearance of these core-region antigens provides further evidence for incomplete glycosylation in colon cancer cells. The considerable expression of Tn antigen represents another example of precursor accumulation in colon cancer much like the expression of blood group H substance represents accumulation of precursor for the A and B substances (26), and T antigen represents accumulation of precursor for the MN blood group antigens (27). While Tn expression in some colon cancer cells might suggest that glycosylation is blocked just after the addition of the initial GalNAc residue, the fact that there is frequent coexpression of the sialosyl-Tn and T along with Tn (Table 2) indicates that the biosynthetic machinery necessary for sialylation and further glycosylation of Tn exists in these tissues.

While the absence of exposed T, sialosyl-Tn, and Tn antigens in normal mucosa can be interpreted to mean that these structures are not synthesized, there is some evidence that indeed these determinants are present in normal colonicocytes. For example, peanut lectin binding sites (T antigen) can be observed in normal colonic mucosa after neuraminidase treatment (5, 7).

In addition, oligosaccharides of normal colonic mucin often have sialic acid conjugated to the linkage GalNAc (11, 12). However, although sialosyl-Tn was found as such, the Sia2,6GalNAc structure was most frequently found as an internal structure of longer oligosaccharides based on a Core 3 structure. This would therefore suggest that the linkage GalNAc of normal colonic mucin preferentially receives a GlcNAc residue first (forming the Core 3 structure), because adding a sialic acid first would prevent further oligosaccharide elongation (Reference 1; Fig. 1).

Based on the present observations and available literature, we would propose the following: in normal colonicocytes, the β1,3 GlcNAc-transferase (Fig. 1, Pathway 3) is highly active, converting the Tn substance down the Core 3 glycosylation pathway. This is performed efficiently since very little Tn antigen remains exposed in normal cells. In contrast, colon cancer cells have highly active β1,3 Gal transferase (Pathway 2) and α2,6 sialyltransferase (Pathway 1) enzymes and/or insufficient β1,3 GlcNAc transferase activity. This would allow the galactosyltransferase and sialyltransferase enzymes to compete favorably with the GlcNAc transferase for the Tn precursor, but perhaps the efficiency of these reactions is suboptimal since considerable Tn antigen remains exposed in cancer cells. In rectal carcinoma, other investigators have identified a GalNAc-GalNAc linkage (28) which has been referred to as Core 5 (3) and implies the activity of an α1,3GalNAc transferase that might be different from the blood group A GalNAc transferase. Clearly, therefore, more work is needed to further clarify glycosylation pathways in colon cancer, and to substantiate the above hypothesis.

The observation that T and Tn antigens are carcinoma-associated was made by Springer and coworkers over a decade ago (29). Much of that work involved the use of human anti-T and anti-Tn antisera by immunohistochemical and some immunohistochemical methods primarily in nongastrointestinal malignancies. The present immunohistochemical study, using murine Mabs and a lectin, extends these observations to colon cancer tissues, confirming that T, Tn, and sialosyl-Tn are carcinoma-associated antigens in this organ. The cancer-associated nature of T antigen in the colon has been known for several years based on studies using peanut agglutinin, as well as polyclonal and monoclonal antibodies (5–10). A recent immunohistochemical report used Mabs raised against synthetic T antigen in its α or β configuration on frozen sections (30). Those results suggested that the carrier molecule of the Galβ1,3GalNAc disaccharide of colon cancers might by glycolipid (β configuration) rather than glycoprotein (α configuration). While we cannot completely exclude the possibility that we too are detecting T antigen on glycolipid instead of glycoprotein, the fact that we still observe T antigen after dewaxing our paraffin-embedded tissues, coupled with the knowledge that the expression of many carbohydrate antigens is shared by both glycoproteins and glycolipids (31), supports our above hypothesis concerning core-region antigen expression in colonic mucin glycoprotein.

Only recently has Tn expression been reported in colon. Hirohashi et al. developed two Mabs that recognize Tn but cross-react with blood group A antigen, and these reagents stained most colon cancers but not normal colonic mucosa (15). The CU-1 antibody used in the present study has previously been shown not to cross-react with the A antigen, and in that study, 88% of colon cancers but also 33% of normal mucosa stained positive (14). Longenecker et al. (30) developed murine Mabs to synthetic Tn antigen and found that by using immunoperoxidase staining of frozen sections, all of seven colon cancers expressed Tn whereas normal tissues (including colon) did not. In terms of sialosyl-Tn expression in the colon, Kurosaka and coworkers first described this structure on glycoproteins of a human rectal adenocarcinoma (28) and later developed a Mab specific for this carbohydrate structure using LS 180 colon cancer cells (32). Recently, Jkeldsen et al. (17) and Gold and Mattes (20) observed that Mab B72.3 recognizes sialosyl-Tn. Therefore, based on previous immunohistochemical reports using the B72.3 antibody, sialosyl-Tn is absent in normal colonic mucosa but quite prevalent in colon cancer (33, 34). Our study confirms this finding not only using B72.3 but also a new Mab TKH2 developed against ovine submaxillary mucin known to express sialosyl-Tn. Moreover, the strength of the present study is the simultaneous comparison of Tn, sialosyl-Tn, and T in the same tissues, allowing at least tentative conclusions to be drawn concerning the biosynthesis of mucin-associated antigen expression in the colon. As far as the histological type of colon cancer is concerned, T antigen expression was more frequent in the well- and moderately differentiated tumors than in the poorly differentiated adenocarcinomas or mucinous carcinomas. However, with Tn antigen, the inverse relationship was found. This supports earlier observations by Springer that anaplastic carcinomas often have a greater Tn/T ratio than well-differentiated adenocarcinomas (29). In mucinous carcinomas, the strong staining intensity and high proportion of cells expressing Tn and sialosyl-Tn were striking. Thor et al. have also described B72.3 staining of mucinous colon carcinomas (33). Thus, these antigens appear to be good markers of colon cancer mucin elaborated both by signet ring cell carcinomas (a rare histological subset character-
ized by intracellular mucin accumulation) and colloid carcinomas (characterized by large extracellular lakes of mucin). Since patients with mucinous carcinomas (particularly signet ring cell type) and poorly differentiated adenocarcinomas often have a poor prognosis, the Tn and sialosyl-Tn antigens may be useful markers for these subsets of patients.

The phenomenon of enhanced expression of all three antigens in TM is one that is shared by many other tumor-associated antigens that are not usually expressed in normal colonic mucosa. In this study, when antigens were expressed in TM, they were usually found in the supranuclear cytoplasm, but several cases showed strong goblet cell staining with VVA and TKH2, suggesting perhaps a premalignant mucin alteration in these cells. Filipe and coworkers have noted enhanced sialomucin expression in goblet cells of TM using histochemical techniques, so perhaps sialosyl-Tn contributes to sialomucin expression. The observation of sialosyl-Tn expression defined by Mab TKH2 in mucosal capillaries of TM is intriguing. This might represent blood-borne sialosyl-Tn antigen since mucosal capillaries furthest from the cancer and those in truly normal colonic mucosa did not express the antigen.

Fetal colonic mucosa was examined with an aim toward determining if Tn and sialosyl-Tn are oncodevelopmental antigens. Indeed, both of these antigens were expressed by second-trimester fetuses and usually in goblet cells. This suggests that fetal colonocytes, like cancer cells, possess the enzymatic machinery to synthesize structures of Pathways 1 and 2. Other studies of core-region oligosaccharides found on human meconium glycoproteins have identified these antigens and several others, indicating a diverse repertoire of mucin oligosaccharide synthesis in the fetus (35). Although all of the fetal specimens bound VVA, only half bound CU-1, TKH2, and B72.3. This may reflect subtle differences in epitope structure recognized by the different reagents.

We purposely chose three different anti-Tn and two anti-sialosyl-Tn reagents for this study to examine their relative reactivities. The two sialosyl-Tn reagents were quite similar in reactivity. The two sialosyl-Tn reagents for this study to examine their relative reactivities. The two sialosyl-Tn reagents were quite similar in reactivity. The two sialosyl-Tn reagents were quite similar in reactivity. The two sialosyl-Tn reagents were quite similar in reactivity. The two sialosyl-Tn reagents were quite similar in reactivity. The two sialosyl-Tn reagents were quite similar in reactivity. The two sialosyl-Tn reagents were quite similar in reactivity. The two sialosyl-Tn reagents were quite similar in reactivity. The two sialosyl-Tn reagents were quite similar in reactivity. The two sialosyl-Tn reagents were quite similar in reactivity.


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