Abstract. Mucins are high molecular weight glycoproteins which are heavily glycosylated with many carbohydrate side chains. In epithelial cancers such as colorectal cancer, both qualitative and quantitative alterations in carbohydrate and polypeptide moieties of mucin glycoproteins occur. These changes in mucin glycoproteins are one of the most common phenotypic markers of colorectal carcinogenesis and may play an important pathobiological role. The expression of some of the sialylated carbohydrate antigens appears to correlate with a poor prognosis and increased metastatic potential in colorectal cancer. The increased exposure of peptide epitopes of mucin glycoproteins in colorectal cancer appears to be due to either abnormal glycosylation and/or altered levels of mucin gene transcription. In addition, dysregulation of tissue specific mucin genes occurs in colorectal cancers. This information is currently being exploited for further elucidation of the molecular mechanisms involved in carcinogenesis, tumor progression and metastasis, and the development of novel methods of colorectal cancer diagnosis and therapy. (Keio J Med 47 (1): 10-18, March 1998)

Key words: mucin, colorectal cancer, glycosylation, selectin

Introduction

It has long been known that one of the most common phenotypic changes in colonic neoplasia is a marked change in the glycosylation that occurs in colonocytes. Although the precise mechanism of these changes is not well understood, the qualitative and quantitative alterations in glycosylation appear to be caused primarily by the altered regulation of glycosyltransferases in cancer cells. The pattern of the relationship between the changes in glycosylation and their pathobiological role in various steps in colon carcinogenesis, progression and metastasis is emerging.

The observations that cancer associated carbohydrate epitopes such as sialyl Leα, sialyl Leα and sulfated carbohydrate structures serve as ligands for adhesion of cancer cells to E-, L- and P-selectins expressed on activated endothelial cells and platelets have suggested an important biological role for mucin glycoproteins in metastasis. The possibility that these changes are critical to some aspects of tumor cell behavior is strengthened by the demonstration that poorly metastatic cancer cells expressing low levels of sialyl Leα can be genetically engineered by transfection with α3 fucosyltransferase to increase the amount of sialyl Leα and that the resultant cells were found to adhere more strongly to E-selectins.

Diverse patterns of glycosylation changes that occur with malignant transformation of epithelial cells have been identified during the past decade due to considerable advances in monoclonal antibody, molecular cloning, sequencing and expression methods as well as sensitive analytical technology such as nuclear magnetic resonance and mass spectrometry. Recent advances in the molecular biology of mucins have also shown many mucin genes to be expressed in a highly tissue and cell specific manner, encoding distinct polypeptide backbone structures to which the carbohydrate moieties are attached.

Although changes in glycosylation have been demonstrated to occur in both O- and N-linked glycosylation of glycoproteins, as well as glycosphingolipids, the focus of this brief review is primarily O-linked mucin glycoproteins in colon cancer. In this review, the alter-
ations in both the carbohydrate and polypeptide moieties of mucin glycoproteins in colon cancer will be discussed first. Then, our recent work on the regulation of mucin genes in colon cancer will be presented, followed by a discussion of the possible role of mucin glycoproteins in colon cancer metastasis. Lastly, the potential clinical applications of mucin glycoproteins in the diagnosis and therapy of colon cancer will be discussed.

Biochemical Properties of Mucin Glycoproteins

Mucin glycoproteins consist of a protein backbone with many carbohydrate side chains of varying lengths, sequences, compositions and anomeric linkages. They have a very large molecular weight (400 to >1000 kDa), many O-glycosidically linked carbohydrate side chains which may constitute 50–85% of the total molecular weight, a high content of serine, threonine and proline in the protein backbone structure, and a buoyant density much higher than non-glycosylated proteins (1.35–1.50 g cm⁻³) due to their high carbohydrate content.

The carbohydrate moieties of glycoproteins and glycolipids are capable of generation diverse recognition signals because of the variety of the type of sugars, diverse glycosidic and anomeric linkages, and also due to extensive branching. Because of structural diversity, carbohydrate containing molecules have been implicated in many important biological functions of cells. These include receptor functions for growth factors, hormones, toxins, bacteria, and virus lectins, growth regulation, cellular differentiation, homotypic and heterotypic, cell-cell interactions, cell-substratum or cell-basement membrane interactions and various immunological functions.¹⁻⁴ In addition, carbohydrates contribute to the unique physiochemical properties of the molecules which provide a protective function for mucosal cells.

Recently, the structures of 9 human mucin polypeptide backbone structures have been identified using molecular cloning techniques.¹ These mucin genes are expressed in a highly tissue and cell specific fashion and may be membrane associated or secreted. An example of membrane mucin is MUC1, which is highly expressed in normal mammary and pancreatic ductal cells (Fig 1).¹,⁹,¹¹ It has a central tandem repeat region which is highly glycosylated with a transmembrane domain and a cytoplasmic tail which interacts with actin cytoskeletal components.

An example of secretory mucin is MUC2, one of the major intestinal mucins (Fig 1). MUC2 also has a central tandem repeat region which is more heavily glycosylated than that of MUC1.¹²,¹³ Furthermore, it has a high cysteine content in the non-repetitive regions flanking the tandem repeats, which may be involved in polymerization into the large multimers characteristic of secretory mucins. MUC5AC and MUC6 are two gastric mucins, that we have recently cloned and sequenced, which have a structural motif similar to that of MUC2.¹⁴,¹⁹,²⁰ These are normally expressed in the stomach but not in the colon.

Alteration of Mucin Glycoproteins in Colon Cancer

The changes in mucin glycoproteins that occur in gastrointestinal cancers may be broadly divided into two general types, aberrant glycosylation and altered
expression of mucin polypeptide epitopes (Table 1). Aberrant glycosylation includes changes in the expression of core region carbohydrates which arise due mainly to incomplete synthesis, and of backbone region and peripheral region carbohydrates that occur mainly due to elongation and modification of existing structures. Thus, in colon cancer cells, the carbohydrate side chains of mucin glycoproteins may exhibit multiple cancer-associated carbohydrate antigenic epitopes in either the core region or the peripheral and backbone regions of the carbohydrate side chains. In cancer cells, altered expression of mucin polypeptide antigens occurs due either to sparse and/or incomplete glycosylation, altered transcriptional regulation of tissue specific mucin genes, or to dysregulation of mucin genes resulting in the expression of inappropriate or ectopic gene products.1-7

Figure 2 shows a simplified scheme summarizing the available data on the biosynthesis of the core region carbohydrates of the carbohydrate side chains of mucin glycoproteins in normal and colon cancer cells. When the first sugar, GaINAc, is attached to the protein backbone apomucin by the action of a specific glycosyltransferase, Tn antigenic activity appears. Once formed, the Tn antigen can follow several biosynthetic pathways, of which the three best characterized are shown here. Synthesis of sialyl Tn in this pathway is a terminal step since no further glycosylation occurs once a sialic acid is added to the Tn antigen α2,6 linkage. In contrast, synthesis along these two pathways may proceed to further elongation of carbohydrate chains with or without branching.

Although much more work is necessary, the available data support the hypothesis that in colon cancer cells, the pathway resulting in the increased synthesis of shorter carbohydrate side chains such as Sialyl Tn antigen and Sialyl T antigens is a preferred one. Recent studies also indicate that decreased O-acetylation of sialic acid in colon cancer cells may in part be responsible for the increased expression of sialylated antigens including sialyl Tn antigen.21-23

This pathway involving core 3 structures appears to be the predominant pathway in the synthesis of mucin carbohydrate side chains in normal colonic mucosa and appears to be blocked in colon cancer.24 Thus, the increased expression of core region carbohydrate anti-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Mucin Glycoproteins In Colon Cancer</th>
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<tbody>
<tr>
<td>Aberrant Glycosylation</td>
<td></td>
</tr>
<tr>
<td>Core region carbohydrate changes: Tn, T, Sialyl T, Sialyl T</td>
<td>Incomplete glycosylation</td>
</tr>
<tr>
<td>Shift in core type synthesis (core 3 → core 1)</td>
<td>De-O-acetylation of O-acetyl sialic acid</td>
</tr>
<tr>
<td>Peripheral and backbone region carbohydrate changes:</td>
<td></td>
</tr>
<tr>
<td>A, B, H, Leα, Leβ</td>
<td>Sialyl Leα (CA 19-9), Sialyl-type 1 chain (CA 50), S-Pan-1</td>
</tr>
<tr>
<td>Sialyl Leα, Extended Leα, Polymeric Leα, Extended Leβ</td>
<td>Polylactosamine (type 2 chain) (i)</td>
</tr>
<tr>
<td>Elongation of backbone</td>
<td>Modification of existing structure</td>
</tr>
<tr>
<td>De-O-acetylation of O-acetyl sialic acid</td>
<td></td>
</tr>
<tr>
<td>Altered Expression of Mucin Polypeptide Epitopes (altered level or pattern)</td>
<td>MUC1, MUC2, MUC3, MUC5, MUC6</td>
</tr>
<tr>
<td>Sparse and/or incomplete glycosylation</td>
<td>Altered transcription</td>
</tr>
<tr>
<td>Altered methylation of the promoter of mucin genes</td>
<td>Dysregulation of mucin gene (inappropriate or ectopic expression)</td>
</tr>
</tbody>
</table>

Further elongation and branching by the addition of Gal, GlcNAc, Fuc, NeuAc, GalNAc and Sulfate

Fig 2 Biosynthesis of some core region carbohydrates of mucin glycoproteins. Gal, galactose; Fuc, fucose; GalNAc, N-acetylglalactosamine; GlcNAc, N-acetylglucosamine; NeuAc, N-acetylneuraminic acid; NeuAc[OAc]; O-acetyl, N-acetylneuraminic acid; +, increased in colon cancer.
gases such as T, Tn and sialyl Tn antigens, as well as sialyl T and disialyl T antigens, appears to provide an example of incomplete glycosylation in colon cancer.

When the immunohistochemical expression of core region carbohydrate antigens Tn, Sialyl Tn and T was compared between normal and cancerous colonic tissue using monoclonal antibodies, increased expressions of all three antigens were observed in colon cancer tissues with high sensitivity and specificity. The expression of these antigens in adenomatous polyps of different histologic types and the degree of dysplasia have also shown positive correlations with parameters of malignant potential. In addition, select core region carbohydrates such as T, Tn and Sialyl Tn antigens are currently being tested as potential tumor vaccines as active immunotherapy for patients with colon cancer and in experimental animal models. Phase II clinical trials being conducted by several groups in the U.S. show good promise.

**Changes in the Backbone and Peripheral Region Carbohydrates of Mucin Glycoproteins in Colon Cancer**

The biosynthesis of the backbone and peripheral region carbohydrates of mucin glycoproteins in colon cancer cells can be summarized as follows. As mentioned before, in colon cancer cells, the synthesis along the core 1 carbohydrate structure predominates over that of the core 3 structure, in contrast to normal colonic cells. In colon cancer cells, the elongation of the carbohydrate side chain occurs through the Core 1 disaccharide pathway. The critical step in this pathway is the addition of GlcNAc to GalNAc by the action of β6GlcNAcT or core 2 GlcNAT. Once GlcNAc is added, the sequential addition of additional carbohydrates occurs by the action of a series of glycosyltransferases. In colon cancer cells, the increase in the length of a polyactosamine structure consisting of this disaccharide unit occurs mainly due to an increase in β3GlcNAc transferase activity which is rate limiting. At least one sialyl transferase (α3NeuAc T) and one fucosyl transferase (α3Fuc T) are thought to be able to generate tumor associated antigenic epitopes, sialyl Le^a^ or sialyl Le^x^ epitopes respectively. However, as mentioned before, the increased expression of sialylated antigens in colon cancer may be partly due to decreased O-acetylation of sialic acids in colon cancer mucins.

When the expression of several types of extended Le^a^ and Le^x^ antigens using monoclonal antibodies was compared, all types of Le^a^ and Le^x^ antigens with or without sialylation or fucosylation including sialyl Le^a^ on the extended backbone are absent from normal colonic mucosa and from hyperplastic polyps. However, they are expressed in adenomatous polyps and colon cancer. Thus, these antigens are colon cancer associated antigens. The expression of these antigens also correlates positively with the malignant potential of adenomatous polyps.

The increased expression of extended Le^x^ antigens in colon cancer cells has recently been explored in the site-specific delivery of an anti Le^x^ adriamycin drug conjugate to colon cancer xenografts in nude mouse models.

In addition, both sialyl Tn and extended sialyl Le^x^ antigens may serve as prognostic markers. The primary tumors expressing these antigens have a worse prognosis than those with tumors that do not express them.

The changes in carbohydrate structures of mucin glycoproteins that occur in colon cancer cells may be summarized as follows. In normal mucin glycoprotein, the tandem repeats in the central tandem repeat region are heavily glycosylated, there are many carbohydrate side chains per molecule and each chain is long. With malignant transformation, the tandem repeats are more sparsely glycosylated, and carbohydrate chains may be much shorter and/or modified in the outer region. These changes may be due to altered carbohydrate metabolism or to changes in glycosyltransferases or in O-acetylation of sialic acids or to altered processing of mucin in cancer cells. Thus, the modified sugar structures, or exposed inner sugar core structures, or protein backbone moiety may serve as tumor markers and tumor vaccines and may also be involved in various biological properties of cancer.

**Changes in the Mucin Polypeptide Backbone Structures**

At least 9 different human mucin genes have been identified to date, MUC1 through MUC8 (there are two MUC5s – AC and B). The predominant structural feature of mucin is the central region consisting of repeat peptide sequences which become heavily glycosylated. The tandem repeat units of each mucin gene have distinct amino acid sequences, but they all have a high content of threonine and/or serine, potential O-glycosylation sites. Mucins are also expressed in an extremely tissue and cell specific fashion. For example, MUC2 and 3 are highly expressed in the intestine, while MUC5AC and 6 are highly expressed in normal stomach but not expressed in normal intestine and colon.

The changes in the expression of mucin polypeptide antigens and in the regulation of mucin genes in colon cancer may be summarized as follows: 1) an increased expression of mucin peptide antigens (e.g. MUC1 and MUC2 in mucinous cancer) caused by either a) sparse glycosylation with increased exposure of peptide epi-
topes, or b) incomplete glycosylation with increased exposure of peptide epitopes or c) increased transcription of the mucin gene. 2) Decreased expression of mucin polypeptide antigens caused by decreased transcription of mucin genes (MUC2, MUC3), or. 3) De novo expression or inappropriate or ectopic expression of mucin peptides (e.g. MUC5AC, MUC6), caused by inappropriate or ectopic expression of mucin genes due to dysregulation. When the immunohistochemical expressions of MUC1, 2, and 3 peptide epitopes and mRNA levels were compared between normal and colon adenocarcinoma tissues using specific mucin peptide antibodies and Northern blotting, increased expressions of MUC1, MUC2 and MUC3 mucin peptide epitopes were observed in colonic adenocarcinomas of all histological subtypes. In mucinous colon cancers, the expression of MUC2 mucin peptide epitopes was greatly increased. MUC2 mRNA levels were decreased in colon adenocarcinoma as compared to normal colonic tissues while being increased in mucinous carcinoma.42,43

When one considers the mRNA levels of colonic adenocarcinoma, the observed increases in MUC1, 2 and 3 mucin polypeptide expressions are likely due to decreased glycosylation. In mucinous cancers, increased expression of MUC2 mucin epitopes is likely due to upregulation of the MUC2 mucin gene. Interestingly, when the expressions of stomach associated MUC5 and MUC6 mucin polypeptide epitopes were examined in colonic adenocarcinomas, both mucin polypeptide epitopes which are not expressed in normal colonic mucosa were found to be expressed ectopically in most colon cancer patients.

In addition, a recent analysis of 5 year survival of patients with Dukes’ B1, B2 and C1 colon cancer showed that all 10 patients with negative fundic mucin peptide expression and all 7 patients with negative MUC5 mucin peptide expression survived 5 years, while the patients with positive expression of these peptide antigens showed 65–70% 5 year survival.46 This result indicates the expression of MUC5 and gastric mucin peptide epitopes may serve as prognostic indicators.

Regulation of Mucin Genes in Colon Cancer

To date, only MUC1 and MUC2 promoters have been fully sequenced. The analysis of the promoter region of MUC2 indicates that immediately upstream from the cap site, the MUC2 promoter contains a TATA box and a CACCC box.44 Transient transfection experiments utilizing deletion constructs containing varying lengths of the promoter indicate that the CACCC box is important for MUC2 gene transcriptions. Electrophoretic mobility shift assay (EMSA) experiments showed that Sp1 is capable of binding this motif. In addition, this element appears to be able to bind other factors since only one band is supershifted with anti-Sp1. The region between bases −171 and −228 may be important for specific expression from the MUC2 promoter as it significantly enhances promoter activity in Cla colon cancer cells with a relatively high expression of MUC2. Recently, the importance of the 5' flanking region in goblet-cell-specific regulation has been postulated for intestinal trefoil factor with the identification of so-called goblet cell response elements with a 9 base sequence in the intestinal trefoil factor promoter gene. The identical 9 base sequence, CCCCTCCCC, is present between bases −289 and −299 of the MUC2 promoter. We are currently examining the possible role of this sequence in goblet cell specific expression of the MUC2 gene.

Our preliminary study indicates that possible mechanisms of upregulation of MUC2 mucin genes involve the signal transduction pathway involving PKC in the activation of transcription factors NFkB and possibly Sp1 and other transcription factors which may bind to a positive regulatory element in the region −2864/−1386 and possibly a CACCC box in the MUC2 promoter.45 We have also observed that a tumor promoter, TPA, upregulates MUC2 mRNA in colon cancer cells through the protein kinase C (PKC) pathway.46 However, the precise mechanism of activation and the transcription factors involved in the activation by PKC pathways are not yet known.

In addition, our preliminary study indicates that hypermethylation of CpG islands in the MUC2 5' flanking region may cause lower expression of the MUC2 gene while hypomethylation may cause upregulation of the MUC2 mucin gene in colon cancer cells. However, we do not know whether this is a primary or secondary event.

The Role of Mucin Glycoproteins in Colon Cancer Metastasis

Cancer metastasis is a complex process consisting of a cascade of multiple, sequential, selective and interdependent events. In colon cancer metastasis, the cancer cells must detach from the primary tumor, invade the extracellular matrix (ECM) and enter the circulation, interact with lymphocytes and other blood components, form multicellular aggregates and metastasize to preferential organ sites where they adhere to the capillary bed and, once access has been gained, cross the basement membranes as they extravasate to establish secondary parenchymal tumor foci by proliferation, induction of angiogenesis and evasion of host defense mechanisms.

There are several experimental models for studying each step in the metastatic cascade. In our study, we
used two models. One is a cecal wall injection model that closely mimics the actual metastatic process of colon cancer in man and another model is a splenic injection system, which represents the terminal stages of metastasis, namely the ability of colon cancer cells to colonize the liver, the most frequent site of colon cancer metastasis.47,48

When the biologic properties of high and low metastatic variants of human colon cancer cells, LS174T48,49 were compared, the high metastatic variant cells showed a much higher incidence of liver metastasis after cecal wall injection and liver colonization activity after intrasplenic injection than did low metastatic variants or parental cells.47,48 They also showed higher in vitro invasive property and higher type IV collagenase activity. When the ability of these cells to bind to ECM components was compared, these cells showed much stronger adhesion to matrigel, laminin, fibronectin and type IV collagen than the metastatic variants. However, no difference in their ability to bind to type 1 collagen was observed. The high metastatic variant cells showed higher ability to bind to E-selectin expressed on vascular endothelial cells than to low metastatic cells. These cells also showed higher mucin synthesis and secretion as well as higher levels of MUC2 message. They also expressed higher levels of sialyl Tn thought to play a role in adhesion to ECM and of sialyl LeX antigens which are a ligand for binding to selectins.

Treatment of high metastatic variant cells with either Benzyl α-GaINAc, a mucin, O-glycosylation inhibitor, or with neuraminidase not only significantly reduced the binding of high metastatic cells to ECM components and to E-selectins, but also reduced their liver colonizing activity as well as the in vitro invasive property and type IV collagenase activity.47,48 These studies indicate that mucin type carbohydrate side chains and N-acetylneuraminic acid are involved in increased adhesion to ECM and the increased metastatic activity of these variant cells.

To further examine the role of mucin glycoproteins in colon cancer metastasis, we examined the expression of mucin associated carbohydrate antigens in primary and metastatic human colon cancer tissues using immunohistochemical methods. Our study showed that the level of expression of all the sialylated antigens examined, sialyl T, sialyl Tn and sialyl LeX antigens, was increased in the metastases while the desialylated counterparts, Tn, T and LeX antigen, showed decreased expression in the metastases compared to primary colon cancer49 (Table 2).

Taken together these data indicate that increased sialylation of mucin type glycoproteins correlates with increased metastatic properties of cancer cells.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Structure</th>
<th>Metastatic/Primary Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tn</td>
<td>GalNAc-α-Thr/Ser</td>
<td>↓</td>
</tr>
<tr>
<td>T</td>
<td>Galβ1,3GalNAc-α-Thr/Ser</td>
<td>↑</td>
</tr>
<tr>
<td>Sialyl Tn</td>
<td>GalNAc-α-Thr/Ser</td>
<td>↑</td>
</tr>
<tr>
<td>Sialyl T</td>
<td>Galβ1,3GalNAc-α-Thr/Ser</td>
<td>↑</td>
</tr>
<tr>
<td>Sialyl LeX</td>
<td>Galβ1,4GlcNAcβ1,3Galβ1,4GlcNAc-R</td>
<td>↑</td>
</tr>
<tr>
<td>↑ α2,6</td>
<td>↑ α2,6</td>
<td>↑ α2,6</td>
</tr>
<tr>
<td>↑ α2,3</td>
<td>↑ α2,3</td>
<td>↑ α2,3</td>
</tr>
<tr>
<td>↑ a1,3</td>
<td>↑ a1,3</td>
<td>↑ a1,3</td>
</tr>
<tr>
<td>NeuAc</td>
<td>NeuAc</td>
<td>NeuAc</td>
</tr>
<tr>
<td>Fuc</td>
<td>Fuc</td>
<td>Fuc</td>
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**Mucin Glycoprotein Binding to Selectins**

Recently, a new family of molecules known as the selectins containing NH2 terminal lectin-like domain have been identified. These are L, E, and P selectins which are located in lymphocytes and leukocytes, activated endothelial cells and platelets. These molecules have been thought to be involved in inflammatory processes and lymphocyte homing. The ligand specificities for L-selectin are sulfated and sialylated mucin glycoproteins while the ligands for E and P selectins have been demonstrated to be sLeX and sLeX antigenic determinant structures, as well as sulfated glycoproteins. Many types of cancer cells also express high levels of sialyl LeX and/or sialyl LeX epitopes and sulfated oligosaccharides, and the selectins have been suggested to play a role in cancer cell adhesion to endothelial cells and aggregation of cancer cells with platelets through these ligands.1,3-5,50

The presence of sialyl LeX and sialyl LeX epitopes in human colon cancer mucins has been demonstrated immunohistochemically and by the immunochemical method using specific MAbs. We have recently revealed the presence of sulfated LeX epitope in colon cancer mucins by immunochemical methods and the presence of large amounts of various types of sulfated LeX oligosaccharides in human colon cancer xenograft mucins by protein NMR/mass spectroscopy.51 Clearly, further studies are necessary to elucidate the mechanisms by which these ligands are involved in tumor cell and selectin binding.

When the binding of a colon cancer cell to human umbilical vein endothelial cells was examined, activation of E-selectin by pretreatment of endothelial cells with interleukin 1 (or TNFα) resulted in markedly increased binding of cancer cells. The finding that the ligand for E-selectin binding is a mucin glycoprotein
is supported by the observation that pretreatment of cancer cells with Bzl-α-GalNAc, a mucin glycosylation inhibitor, virtually abolished the cancer cell binding. Similar results were obtained when colon cancer cell binding to E-selectin coated wells was examined.\textsuperscript{52} Both sialyl Le\textsuperscript{a} and sialyl Le\textsuperscript{b} structures are involved in the binding of colon cancer cells to E-selectin. Recent studies also indicate that some colon cancer cells bind to L and P selectins.\textsuperscript{51} However, the nature of the ligands involved in the binding to these selectins remains to be elucidated.

### Potential Clinical Application

Potential clinical applications of cancer associated carbohydrate and peptide moieties of mucin glycoproteins in colorectal cancer are listed in Table 3. Although high levels of mucin glycoprotein antigens (e.g. sialyl Le\textsuperscript{a}, sialyl Le\textsuperscript{b}, sialyl Tn, T, and MUC1 apomucin) are observed in the serum of patients with epithelial cancers, no mucin glycoprotein antigenic markers have yet been shown to be sensitive or specific enough to be clinically useful as diagnostic serological markers for cancers.\textsuperscript{1,7}

Monoclonal antibodies against mucin-associated antigens (sialyl Tn, T, Tn, sialyl Le\textsuperscript{a}, Le\textsuperscript{b}) may also be used for targeting radioisotopes (\textsuperscript{131}I, \textsuperscript{111}In, \textsuperscript{131}Tc) for imaging and therapy or for targeting cytotoxic drugs and toxins. Cytotoxic T lymphocytes obtained from draining lymph nodes of patients with breast and pancreatic cancers recognize a cell surface MUC1 tandem repeat peptide sequence and kill the target tumor cells expressing this epitope in a non-major histocompatibility complex-restricted fashion.\textsuperscript{53} These MAbs and the synthetic mucin fragments (carbohydrates and mucin polypeptides) are currently being evaluated for their potential as tumor vaccines.\textsuperscript{28–31,54}

### Future Directions

Alterations in both the carbohydrate and protein moieties of mucin glycoproteins in colorectal cancer cells have been amply documented. However, limited data are available on the biochemical and molecular mechanisms involved in regulation of the mucin gene family and of the multiglycosyltransferase system in colorectal carcinogenesis. In addition, further studies are needed to clarify the structure-function relationships of carbohydrate and peptide moieties of mucin glycoproteins of colorectal cancer cells. Currently, mucin associated carbohydrates and peptides in epithelial cancer cells are being exploited for immunohistochemical diagnosis and prognostic assessment, serological detection, radioimmunolocalization and immunotherapy. The synthesis of cancer associated oligosaccharides and polypeptide backbone structures or their mimetics as glycan interaction inhibitors or tumor vaccines may play an important role in the treatment of colorectal cancer. Thus, further elucidation of the structure, biology and molecular mechanisms involved in the regulation of the expression of cancer-associated mucin glycoproteins and the development of transgenic or knockout animals of mucin genes and/or glycosyltransferases will yield very important and useful data leading not only to furthering our knowledge of colorectal carcinogenesis, but also to development of effective diagnostic and therapeutic methods for colorectal cancer.

### Acknowledgements

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