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Altered Glycosylation of Mucin Glycoproteins in Colonic Neoplasia

Young S. Kim

Gastrointestinal Research Laboratory (151M2), VA Medical Center, Department of Medicine, University of California, San Francisco, California 94121

Abstract Considerable alteration of cellular carbohydrates such as glycolipids and glycoproteins occurs in colonic neoplasia. Some of these changes are also observed at certain embryonic stages of differentiation and are, therefore, considered onco-developmental changes. In colon cancer cells, many of the phenotypic markers for malignancy have been found on carbohydrate moieties, and some have been found on the peptide portion of mucin glycoproteins. The changes in carbohydrate antigens include altered expression of core region carbohydrates, extension of backbone structures and modification of peripheral carbohydrate structures that may arise due to abnormal glycosylation processes. Altered glycosylation may also result in the exposure of the peptide moiety of the mucin glycoprotein. Therefore, these altered mucin glycoprotein structures may serve as tumor markers. However, it remains to be determined whether they will be useful as intermediate endpoint markers.

Key words: carbohydrate antigens, chemoprevention, colorectal cancer antigens, intermediate biomarker, mucin glycoproteins

The earliest changes in carcinogenesis are impaired growth regulation and differentiation. Thus, phenotypic or genotypic markers that reflect these altered states in the various stages of carcinogenesis are not only important in identifying subjects at high risk, but may also be useful in assessing the efficacy of chemopreventive measures. Considerable data have recently become available on the molecular genetics and proliferative activity of mucosal cells at some of these stages. However, it is unclear at present what other phenotypic markers are available, particularly for early stages in carcinogenesis, and to what extent these markers and stages are reversible.

It is now well accepted that neoplasia arises from disordered regulation of growth and differentiation (1). The growth and differentiation signals are mediated by various factors which bind to specific receptors resulting in postreceptor intracellular signaling through diverse and complex mechanisms. Signals enter the nucleus to affect the expression of various genes involved in cell division or differentiation programs. In normal colon cells, the balance between growth and terminal differentiation program is tightly controlled, while there is imbalance between the pathways of growth versus differentiation in preneoplastic and neoplastic colon cells.

CELL LINEAGE-ASSOCIATED DIFFERENTIATION MARKERS IN NORMAL AND CANCEROUS COLON

What are cell differentiation (biochemical and immunological) markers in normal, premalignant and malignant cells? First, the phenotypic markers that are expressed by cells of a particular lineage in an advanced stage of differentiation should be considered. Second, one has to also consider those phenotypic markers that are expressed in a stage-specific manner during development in fetal colon and also in premalignant and malignant

Address reprint requests to Young S. Kim, VA Medical Center, GI Research Lab (151M2), 4150 Clement Street, San Francisco CA 94121

colon, but not in normal adult colon, as so-called oncodevelopmental markers. Lastly, there can be ectopic expression of markers for other cell lineages resulting from maldifferentiation (2).

CHANGES IN CELLULAR CARBOHYDRATES

Neoplastic transformation of colonic cells is accompanied by diverse phenotypic changes in morphological, functional, biochemical and immunological changes (3). Among these, changes in cellular carbohydrates in the surface membranes and intracytoplasmic compartment have been the most prominent ones. Cellular carbohydrates may be broadly classified into glycoproteins and glycolipids. Glycoprotein is a complex protein with carbohydrate side chains attached to the protein backbone structure, while glycolipid is a complex lipid with carbohydrate side chains linked to the lipid moiety called ceramide, which consists of sphingosine and fatty acid.

These carbohydrate moieties of glycoproteins and glycolipids are capable of generating diverse recognition signals because of the variety of carbohydrates, diverse glycosidic and anomeric linkages, and extensive branching. Therefore, in addition to providing many antigenic epitopes on the cell surface, they have been implicated in many important biological functions such as binding of hormones, growth factors, bacteria, toxins and viruses, cell-cell and cell-substratum interactions and diverse immunological processes.

GLYCOLIPID ANTIGENS AS DIFFERENTIATION MARKERS IN CANCER

Glycolipids may be broadly classified into three types, depending on the basic core structure of their oligosaccharides. Globo-, lacto- and ganglio-series glycolipids are derived from lactosyl ceramide by alternate pathways of chain elongation. Mammalian embryos and embryonal carcinoma cells have been found to express predominantly globo-series glycolipids such as globoside, Forssman, SSEA-3, SSEA-4 and globo-ABH antigens. During development and differentiation, globo-series glycolipids are decreased, while lacto- and ganglio-series glycolipids increase (3,4).

This has been illustrated by glycosylation changes during pre-implantation mouse development (4). They observed that two globo-series glycolipids, stage-specific embryonic antigens 3 and 4 disappear, while lactosamine glycan antigens, which may be present both on glycolipids and glycoproteins, SSEA-1 (Le^x) and Le^y appear at the 8 cell and 16 cell stage respectively. SSEA-1 or Le^x antigen disappears from the outer tier of cells following compaction, as these cells differentiate into trophectoderm. Thus, carbohydrate antigen expression varies a great deal during development and differentiation. Because SSEA-1 is found both in cancer and in normal fetal development, it is called an oncofetal or oncodevelopmental antigen.

ALTERED MUCIN GLYCOPROTEIN ANTIGEN EXPRESSION IN COLONIC NEOPLASIA

Glycoproteins may be broadly classified into two major types, "N-glycosidic" and "O-glycosidic", depending on the type of linkages between the protein and the carbohydrate side chains and on their carbohydrate compositions. Most serum and membrane glycoproteins have N-glycosidic linkages between asparagine of the protein backbone and N-acetylglucosamine, while mucin glycoproteins have O-glycosidic linkages between serine or threonine and N-acetylgalactosamine. Of 6 sugars commonly found in glycoproteins, mannose occurs, in general, only in "N-glycosidic" type glycoproteins, whereas other sugars occur in both types. Both types of glycoproteins are found in intestinal secretions such as mucins and immunoglobulin, and both are also present as structural components of the intestinal mucosal membrane. In colorectal cancer cells it has long been observed that both quantitative and qualitative alterations in O-glycosidic mucin glycoproteins occur. With malignant transformation in other cell types, considerable alteration in oligosaccharide side chains of N-glycosidic glycoproteins have been reported to occur, but much less is known about the changes in N-glycosidic glycoproteins in colon cancer cells (5,6).

Mucins are very large glycoproteins which are heavily glycosylated up to approximately 85% carbohydrates which are linked O-glycosidically to the polypeptide backbone structure. The heavy

glycosylation gives them the high density, hydrodynamic volume and viscosity necessary for some of their biological functions, such as protection and lubrication of the gastrointestinal tract. However, this heavy glycosylation has also made determination of their peptide structure very difficult using classical protein sequencing methods, since conditions which deglycosylate mucin glycoprotein also cause a significant amount of proteolysis. In the biosynthesis of membrane and secreted O-linked mucin glycoproteins, the polypeptide backbone of mucin glycoprotein is synthesized in the rough endoplasmic reticulum and carbohydrates are added sequentially by the action of a series of specific glycosyltransferases in the Golgi apparatus. Once fully glycosylated, completed mucin glycoproteins are either stored and secreted or become incorporated into the plasma membranes through membrane vesicles (5,6).

The structure of mucin glycoproteins with the polypeptide backbone linked to many oligosaccharide side chains of varying composition, length and linkage are shown schematically in Figure 1. Two composite structures of mucin oligosaccharides are shown. Mucin oligosaccharides are rarely this complex or complete, but are always linked O-glycosidically between N-acetyl-galactosamine, designated as GalNAc and threonine or serine residues of the polypeptide backbone. In cancer cells, the carbohydrate side chains may exhibit multiple cancer-associated carbohydrate antigenic epitopes. These include core region carbohydrates such as Tn, sialosyl Tn or T antigens which become exposed due to incomplete glycosylation, and peripheral and backbone region carbohydrates such as extended and/or polyfucosylated Le^x or Le^y antigens or sialylated Le^x antigens, which represent modification of existing structures (6).

With the recent availability of monoclonal antibodies of defined specificities, structural alterations in mucin glycoproteins in epithelial cancer cells are beginning to be defined. Three groups of mucin glycoprotein tumor markers with different specificities are summarized in Table 1. These tumor markers include those whose epitopes are on carbohydrates, either in the peripheral and backbone region or in the inner core region of carbohydrate side chains, those specific for peptide moieties of mucin glycoproteins, and those for which the epitope specificities are, as yet, undetermined.

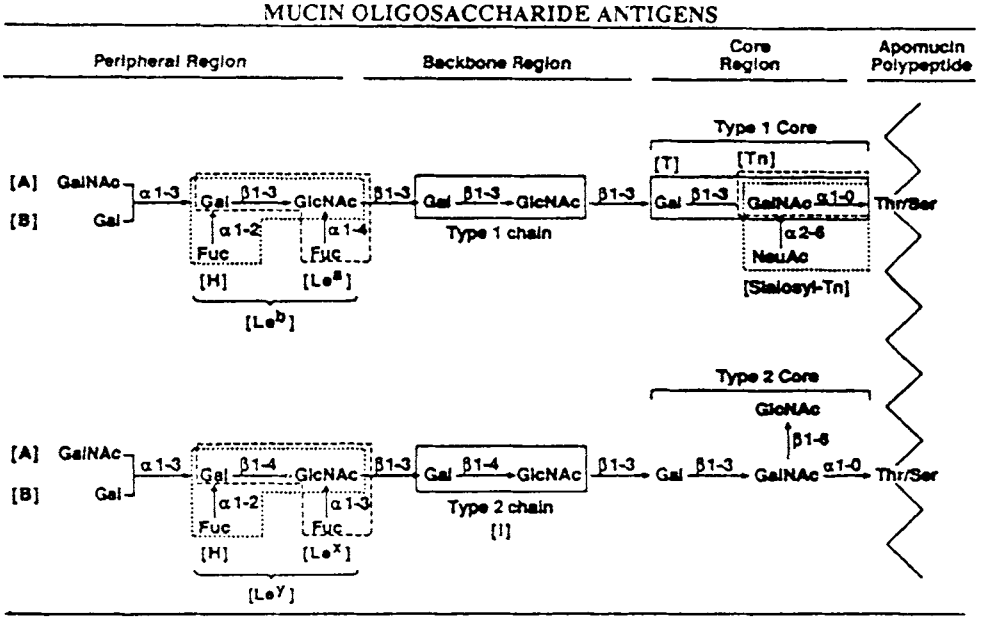

Figure 1

Table 1

Mucin Glycoprotein Tumor Markers of Colorectal Cancer

Carbohydrate epitopes
Peripheral and backbone-region carbohydrate changes
A,B,H, Le^a, Le^b
Sialyl Le^a (CA19-9)
Sialyl Le^x, Extended Le^x, Extended Le^y
Polyfucosylated -Le^x, -Le^y
Unbranched Type 2 polylactosamine (i)
Core-region carbohydrate changes
T, Tn, Sialyl Tn
Peptide epitopes
Apomucins
MUC1 (PUM, PEM, HMFG, Episialyn)
MUC2, MUC3
Indeterminate epitopes
M1 antigen, crypt cell antigens, cora antigen
MCC-CO-450 antigen

CORE REGION CARBOHYDRATE ANTIGEN EXPRESSION

The expression of usually cryptic core region carbohydrate antigens illustrates one of the more common carbohydrate structural changes in epithelial cancers. The core region carbohydrate antigens that we studied are the Tn antigen, which has N-acetylgalactosamine linked to apomucin, and two disaccharide antigens, sialyl Tn and T antigens, which have additional sialic acid and galactose added to GalNAc, respectively (Fig. 1). Recently, several monoclonal antibodies have been produced having varying specificities against these antigens as well as to other core region carbohydrate structures.

Tn, Sialyl Tn and T antigen expression in normal and cancerous colonic mucosa using monoclonal antibodies demonstrated that these antigens have high sensitivity and specificity.

Fetal colonic mucosal cells expressed all three antigens indicating that they are oncodevelopmental antigens. In addition, adenomatous polyps of high grade dysplasia expressed these antigens more frequently (7,8). Thus, these core region carbohydrate antigens appear to be premalignant and malignant markers.

Although the precise biochemical basis for the expression of these usually cryptic core region carbohydrate antigens of mucin glycoprotein is not clear, it would appear that in colon cancer cells, the pathway of synthesis of sialyl Tn antigen is a preferred one since no further glycosylation occurs once sialic acid is added to N-acetylgalactosamine linked to apomucin (Tn antigen). In addition, in colon cancer cells, glycosyltransferases involved in further elongation of T antigen seem to be blocked resulting in the accumulation of precursors T and Tn antigens (Figure 1).

Thus, the appearance of T, Tn and sialyl Tn antigens provide an example of incomplete glycosylation in premalignant and malignant colon cells.

BACKBONE REGION CARBOHYDRATES

The core region carbohydrates are elongated by two major types of backbone structures (Figure 1). The type 1 chain consists of (Gal 1-3GlcNAc 1-3Gal -R), while the type 2 chain unit consists of Gal 1-4 GlcNAc- 1-3 Gal -R (N-acetyllactosamine). The type 1 chain is usually repeated only a few times and results in a shorter oligosaccharide. However, the type 2 chain may be repeated in the same oligosaccharide side chain many times to form long poly-N-acetyl-lactosamine backbone structures.

In normal mucosal cells, the type 2 polylactosamine backbone structures can also be branched by the addition of N-acetylglucosamine indicated by the solid circles in a beta 1-6 linkage to galactose as a branch point (forming big I antigen).

However, in the oncofetal pathway in most mammalian cells, there is increased

Table 2.

Cancer-Associated Carbohydrate Changes in Mucin Glycoproteins in Colonic Neoplasia

1.	Reexpression of antigens that are usually present in fetal tissues (A,B,H and Le^b in distal colon).
2.	Expression of antigens incompatible with the patients blood type (A,B,H, Le^b).
3.	Incompletion or blocked synthesis of oligosaccharide side chains resulting in deletion of antigens or exposure of inner core sugar antigenic structures (A,Le^b)(T,Tn, Sialyl Tn).
4.	Increased levels of antigen expression compared with normal tissues (short chain Le^x, Le^y).
5.	Neosynthesis
	a) Modification of existing structures involving elongation, fucosylation, and sialylation (extended polyfucosylated Le^x or Le^y, sialyl Le^x).
	b) New antigenic structures (?).

straight chain elongation with unbranched repeating type 2 chain polylactosamine units forming so-called small i antigen. This type of unbranched type 2 chain is highly expressed in colon and hepatocellular carcinoma compared to normal tissues (9).

PERIPHERAL AND BACKBONE REGION CARBOHYDRATES

As mentioned previously, there can also be modification of existing structures in the peripheral or backbone regions of carbohydrate side chains.

The immunodeterminant structure for Le^x is the trisaccharide structure with a fucose linked alpha 1,3 to the N-acetylglucosamine. Le^y antigen is a tetrasaccharide with additional fucose linked to galactose. Both antigens are expressed only on type 2 lactosamine backbone structure (Figure 1).

To summarize our data on Le^x and Le^y antigen expression in normal and cancerous colonic cells, in normal colon cells, Le^x and Le^y antigens are expressed predominantly on short chain type 2 backbone structures. In premalignant and malignant colon cells, the extended form of these antigens as well as type 2 polylactosamine chain with or without terminal sialylation or internal fucosylation can occur (10,11). Thus, elongation of the backbone structure of Le^x and Le^y antigens appears to be a cancer-associated phenomenon in colon cells. Since these antigens are also expressed in fetal colon, these are oncodevelopmental antigens.

These cancer associated carbohydrate changes in mucin glycoproteins, as well as others I have not discussed, are summarized in Table 2.

POLYPEPTIDE BACKBONE STRUCTURE OF MUCIN (Apomucin)

Recently four types of apomucin have been described (MUC1, 2, 3 & 4)(12-16). Although only MUC1 cDNA has been fully sequenced, the general structural motif of the apomucin structures is that the protein is large and there is a central core region consisting of many tandem repeat units flanked on either side by the unique sequences. Each apomucin contains tandem repeat units of different sizes and different amino acid compositions. In normal mucosal cells, there appears to be organ specificity, e.g. MUC1 for normal breast and pancreatic cells, MUC2 and MUC3 for small intestine and colon, and MUC4 for bronchial cells. However, in epithelial cancer cells, these apomucins are expressed to a varying degree regardless of the organ of origin. The biochemical and molecular basis of the expression of these usually cryptic peptide epitopes of the protein backbone structure is not known but appears to be due either to impaired glycosylation or altered processing or abnormal gene expression in cancer cells (17).

REFERENCES

1. Harris, CC. Human tissues and cells in carcinogenesis research. Cancer Res., 47:1-10, 1987.
2. Kim, YS. Differentiation of normal and cancerious colon cells. In "The status of differentiation therapy of cancer". Waxman S, Rossi, GB & Takaku F (eds.) Raven Press, pp 29-44, 1988.
3. Hakomori, SI. Aberrant glycosylation in tumors and tumor associated carbohydrate antigens. Adv. Cancer Res., 52:257-331, 1989.
4. Rosenman, SJ, Fenderson, BA, Hakomori, SI. The role of glycoconjugates in embryogenesis. In "Glycoconjugates in Medicine". Ohyama, M & Muramatsu, T. Professional Postgraduate Services. pp 43-50, 1988.
5. Neutra, MR, Forstner, JF. Gastrointestinal mucus: synthesis, secretion and function. In "Physiology of the Gastrointestinal Tract". Johnson, LR (ed.) Raven Press, pp 975-1009, New York.
6. Kim, YS, Byrd, JC. Colonic and pancreatic mucin glycoproteins expressed in neoplasia. In "Biochemical and Molecular Aspects of Selected Cancers". Vol. 1. Academic Press, Inc. pp 277-311, 1991.
7. Yuan, M, Itzkowitz, SH, Boland, CR, Kim, YD, Tomita JT, Palekar, A, Bennington, JL, Trump, BF, Kim, YS. Comparison of T antigen expression in normal, premalignant and malignant human colonic tissue using lectin and antibody immunohistochemistry. Cancer Res., 46:4841-4847, 1986.
8. Itzkowitz, SH, Yuan, M, Montgomery, CK, Kjeldsen, T, Takahashi, HK, Bigbee, WL, Kim, YS. Expression of Tn, sialosyl Tn and T antigens in human colon cancer. Cancer Res., 49:197-204, 1989.
9. Miyake, M, Kohno, N, Nudelman, ED, Hakomori, SI. Human IgG3 monoclonal antibody directed to an unbranched repeating type 2 chain (Gal 1-4 GlcNAc 1-3 Gal 1-4 GlcNAc 1-3 Gal 1-R) which is highly expressed in colonic and hepatocellular carcinoma. Cancer Res., 49:5689-5695, 1989.
10. Itzkowitz, SH, Yuan, M, Fukushi, Y, Palekar, A, Phelps, PC, Shamsuddin, AM, Trump, BF, Hakomori, SI, Kim, YS. $Lewis^x$ and sialylated $Lewis^x$ related antigen expression in human malignant and non-malignant colonic tissues. Cancer Res., 46:2627-2632, 1986.
11. Kim, YS, Yuan, M, Itzkowitz, SH, Sun, Q, Kaizer, T, Palekar, A, Trump, BF, Hakomori, SI. Expression of Le^y and extended Le^y blood group related antigens in human malignant, premalignant and nonmalignant colonic tissues. Cancer Res., 46:5985-5992, 1986.
12. Gendler, SF, Lancaster, CA, Taylor-Papadimitriou, J, Duhig, T, Peat, N, Burchell, J, Pemberton, L, Lalani, E-N, Wilson, D. Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. J. Biol. Chem., 265:15286-15293, 1990.
13. Gum, JR, Byrd, JC, Hicks, JW, Toribara, NW, Lamport DTA, Kim, YS. Molecular cloning of human intestinal mucin cDNAs. J. Biol. Chem., 264:6480-6487, 1989.
14. Lan, MS, Batra, SK, Qi, W, Metzgar, RS, Hollingsworth, MA. Cloning and sequencing of a human pancreatic tumor mucin cDNA. J. Biol. Chem., 265:15294-15299, 1990.
15. Gum, JR, Hicks, JW, Swallow, DM, Lagace, RL, Byrd, JC, Lamport, DTA, Siddiki, B, Kim, YS. Molecular cloning of cDNAs derived from a novel human intestinal mucin gene. Biochem. Biophys. Res. Commun., 171:407-415, 1990.
16. Porchet, N, VanCong, N, Dufosse, J, Audie, JP, Guyonnet-Duperat, V, Gross, MS, Denis, C, Degand, P, Bernheim, A, Aubert, JP. Molecular cloning and chromosomal localization of a novel human tracheo-bronchial mucin cDNA containing tandemly repeated sequences of 48 base pairs. Biochem. Biophys. Res. Commun., 175:414-422, 1991.
17. Yonezawa, S, Byrd, JC, Dahiya, R, Ho, JJL, Gum, JR, Sqallow, DM, Kim, YS. Differential mucin gene expression in human pancreatic and colon cancer cells. Biochem. J., 276:599-605, 1991.