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Blood Group-Related Carbohydrate Antigen Expression in Malignant and Premalignant Colonic Neoplasms

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Abstract Cell surface glycoconjugates of colonic epithelial cells carry certain carbohydrate antigens related to blood group substances. During the progression to malignancy, these oligosaccharide immunodeterminants undergo specific types of alterations. In colon cancers, the blood group antigens A, B, H, and Le^b, which are normally expressed only in the proximal colon, can be re-expressed in distal colon cancers or deleted in proximal colon cancers. Also, an antigen which is incompatible with the individual's blood type can be expressed. Similar alterations occur in adenomatous polyps, but with reduced frequency. The simple form of blood group-related Le^x and Le^y antigens found in normal mucosa can undergo modification by oligosaccharide elongation, internal fucosylation, and sialylation into novel structures found in carcinomas as well as in adenomas with greatest malignant potential. Finally, antigens representing the first steps of glycosylation, Tn, T, sialosyl-Tn (STn), which are normally cryptic in the colon, can be unmasked due to incomplete glycosylation in adenomatous polyps and cancers. Several of these antigens, such as extended Le^x, extended Le^y, T, and sialosyl-Tn, are quite cancer-specific in that they are rarely expressed in normal mucosa or hyperplastic polyps, but preferentially occur in adenomas of greatest malignant potential. As such, these antigens might be useful as candidate intermediate endpoint biomarkers.

Key words: adenomatous polyps, blood group antigens, carbohydrate antigens, chemoprevention, colorectal cancers, glycosylation, intermediate biomarker, Le^x antigen, Le^y antigen, sialosyl-Tn (STn) antigen, T antigen, Tn antigen

Epithelial cells throughout the body are rich in glycoconjugates (glycolipids and glycoproteins). The carbohydrate portion of these glycoconjugates contain a variety of oligosaccharide structures that are either identical to or closely related to blood group antigens found on red blood cells. This review will summarize the pattern of expression of blood group-related antigens in colonic tissues, with speculation on how these immunodeterminants might be used as intermediate endpoint biomarkers of colonic mucosa at risk for developing colonic neoplasia.

THE ABH AND LEWIS ANTIGEN SYSTEM

A, B, H and Lewis blood group substances are among the most prevalent carbohydrate antigens expressed by colonic epithelial cells. Expression of these antigens is developmentally regulated. During fetal development, the A,B,H, Le^a and Le^b antigens appropriate for the individual's blood type are expressed throughout the colon. However, at birth and persisting throughout adulthood, the A,B,H, and Le^b immunodeterminants become restricted to colonocytes of the proximal, but not distal, colon, although the Le^a antigen maintains a pan-colonic distribution (1). In cancers of the colon, blood group antigen expression can undergo three types of alterations (2). The most frequent alteration is *re-expression* in the distal colon of the A,B,H, or Le^b antigen that was present during fetal development. In the proximal colon, where colonocytes normally express blood group antigens, colon cancer cells may exhibit *deletion* of the relevant blood group substance. Occasionally, cancers of either the proximal or distal colon may express a blood group antigen which is *incompatible* with the host's blood type. Because these three types of

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alterations occur in cancerous but not normal colonic tissues, they are considered cancer-associated events. At a reduced frequency, these same alterations can take place in benign colonic polyps, particularly adenomatous polyps (3).

THE Le^x AND Le^y ANTIGENS

The Le^a and Le^b blood group antigens discussed above are synthesized on type 1 chain precursor structures. The Le^x and Le^y antigens are positional isomers of Le^a and Le^b respectively, and are synthesized on type 2 precursors. Some monoclonal antibodies recognize the Le^x trisaccharide in its simple form, whereas others preferentially recognize this structure when synthesized on an extended type 2 chain backbone with internal fucosylation (4,5) (Table I). Furthermore, the simple as well as the extended Le^x structures can be sialylated. The processes of chain elongation, internal fucosylation, and sialylation are cancer-associated alterations (6). Like the Le^x antigen, Le^y can occur as a simple immuno-determinant or on an extended type 2 chain with (or without) internal fucosylation (7,8).

Using antibodies that distinguish the simple from the extended forms of Le^x and Le^y antigens, several observations have been made with respect to the expression of these antigens in colonic tissues (9-11) (Table I). The simple Le^x and Le^y antigens are expressed in normal colonic mucosa, typically by cells at the base of the crypts. Hyperplastic polyps (which are believed to have no intrinsic malignant potential) and adenomatous polyps (which are the premalignant precursors to colon cancer), as well as colon carcinomas, express simple Le^x and Le^y antigens in the majority of instances. Thus, these antigens assume a promiscuous distribution and do

TABLE I. Expression of Le^x and Le^y Antigens in Colonic Tissues

Antigen (MAb)	Antigenic Structure	N	HP	AP	C
Le^x (SSEA-1)	Galβ1,4GlcNAc-R ↑α1,3 Fuc	+	+	+	+
Difucosyl-Le^x (FH4)	Galβ1,4GlcNAcβ1,3Galβ1,4GlcNAc-R ↑α1,3 ↑α1,3 Fuc Fuc	-	-	+	+
Sialyl-Le^x (FH6)	Galβ1,4GlcNAcβ1,3Galβ1,4GlcNAc-R ↑α2,3 ↑α1,3 ↑α1,3 Sia Fuc Fuc	-	-	+	+
Le^y (AH-6)	Galβ1,4GlcNAc-R ↑α1,2 ↑α1,3 Fuc Fuc	+	+	+	+
Extended Le^y (CC-1, CC-2)	Galβ1,4GlcNAcβ1,3Galβ1,4GlcNAc-R ↑α1,2 ↑α1,3 Fuc Fuc	+p	-	+	+
Trifucosyl Le^y (KH1)	Galβ1,4GlcNAcβ1,3Galβ1,4GlcNAc-R ↑α1,2 ↑α1,3 ↑α1,3 Fuc Fuc Fuc	-	-	+	+

N: normal; HP: hyperplastic polyps; AP: adenomatous polyps; C: carcinoma; p: proximal colon

not distinguish colonic tissues according to malignant potential.

In contrast, the extended forms of Le^x and Le^y are not expressed in normal mucosa or hyperplastic polyps, but are preferentially expressed by adenomatous polyps and colon cancers. Moreover, in adenomas, expression of these more complex structures tends to correlate with the histopathological features of malignant potential, namely larger adenoma size, increased villous histology, and more severe grades of dysplasia (10,11).

THE Tn, T, AND SIALOSYL-Tn ANTIGENS

The blood group-related carbohydrate antigens mentioned above can be synthesized on glycolipids as well as glycoproteins. Others such as the Tn, T, and sialosyl-Tn antigens are preferentially synthesized on glycoproteins and, in particular, mucin-type glycoproteins (12). Mucins are high molecular weight glycoproteins consisting of numerous oligosaccharide side chains that are attached by O-glycosidic linkages to serine and threonine residues on a polypeptide (apomucin) backbone. The initial step in mucin O-linked glycosylation is the addition of N-acetyl-galactosamine (GalNAc) to serine or threonine residues. This structure (GalNAc-O-Ser/Thr) is the Tn antigen (Table II). Once formed, the Tn structure can undergo further glycosylation. If galactose is added, the T antigen (Galβ1,3GalNAc-O-Ser/Thr) is formed. However, if sialic acid is added, this creates the sialosyl-Tn antigen (Siaα2,6GalNAc-O-Ser/Thr).

Results of immunohistochemical staining of colonic tissues with monoclonal antibodies and lectins to these three antigens are summarized in Table II (13-15). Normal colonic mucosa typically does not express any of these mucin-associated carbohydrate antigens, whereas the vast majority of colon cancer tissues do. In colonic polyps, the Tn antigen is expressed by essentially all hyperplastic and adenomatous lesions, therefore it does not distinguish benign mucosa according to malignant potential (15). The T antigen is also expressed by many hyperplastic and adenomatous polyps, but among the adenomas, the frequency of T antigen expression is greater in those lesions with more malignant potential (13,16). This is particularly true when using a monoclonal antibody rather than a lectin (peanut agglutinin) to identify the T antigen in tissues (13). Unlike the Tn and T antigens, sialosyl-Tn antigen is only rarely expressed in hyperplastic polyps, and when it occurs in these lesions, only a few cells weakly express the antigen. However, approximately 56% of adenomas express sialosyl-Tn antigen and those that do tend to be the larger, more villous, and more severely dysplastic lesions (15). Thus, sialosyl-Tn antigen appears to be a rather sensitive and specific marker for colonic neoplasia that correlates with the progression of adenomas to carcinoma.

The biochemical mechanism by which Tn, T, and sialosyl-Tn antigens are not expressed in normal, but become expressed in cancerous, colonic epithelium is not established. Studies that have biochemically characterized the oligosaccharide structures residing on normal colonic mucin indicate that the initial GalNAc (Tn) and Siaα2,6GalNAc (sialosyl-Tn) structures indeed do occur (17,18). However, since these carbohydrates

TABLE II. Expression of Tn, T and Sialosyl-Tn Antigens in Colonic Tissues

Antigen (Mab, lectin)	Antigenic Structure	N	HP	AP	C
Tn (CU-1; VVA)	GalNAc-O-ser/thr	-	+	+	+
T (AH9-16)	Galβ1,3GalNAc-O-ser/thr	-	+	+	+
Sialosyl-Tn (TKH2)	Siaα2,6GalNAc-O-ser/thr	-	-	+	+

are found in the innermost region of the oligosaccharides, it is likely that they are masked by more elaborate oligosaccharides in the normal mucin and are thereby prevented from reacting with lectins or antibodies. Then, in the course of malignant transformation, *incomplete glycosylation* causes many of these carbohydrate side chains to become truncated, resulting in an unmasking of these cryptic structures (6). Exposure of the T antigen in colonic neoplasms probably also occurs by incomplete glycosylation, despite the fact that the Galβ1,3GalNAc linkage has not yet been described in normal colonic mucin (17,18).

A paradoxical situation exists in that certain carbohydrate antigens such as Le^x and Le^y become better tumor markers when synthesized on elongated oligosaccharides whereas others such as Tn, T, and sialosyl-Tn are revealed in tumors as a consequence of oligosaccharide truncation. Although the reason for this apparent paradox is not yet clear, chain elongation may be a relatively specific process only for lacto series (type 1 and type 2) structures (19), and differences may also relate to whether the carbohydrate antigen is on a mucin or glycolipid.

CARBOHYDRATE ANTIGENS AS INTERMEDIATE ENDPOINT BIOMARKERS

It is possible that some of the aforementioned carbohydrate antigens might be useful as intermediate endpoint biomarkers for predicting the subsequent development of colonic neoplasia. Conceptually, the better candidate markers would be those that are not expressed in normal mucosa or hyperplastic polyps but become expressed in adenomatous epithelium. Thus, extended Le^x and Le^y antigens, as well as sialosyl-Tn and to a lesser extent the T antigen, meet these criteria. Ideally however, besides these attributes, a promising marker should be expressed by histologically normal mucosa before an adenoma develops. At the present time, it is difficult to predict which of these antigens might perform well for this purpose. To date, this question could not be addressed in a patient population at risk for developing recurrent adenomas because these patients are usually not entered into a standardized follow-up program, and even in those that have surveillance colonoscopies, it is not common for normal appearing mucosa to be biopsied.

Chronic ulcerative colitis, however, can be considered a surrogate model for this clinical situation. Individuals with chronic idiopathic ulcerative colitis involving the entire colon for more than 8-10 years duration are at high risk for developing dysplasia and colon cancer (20). Colonic tissues of some patients with chronic ulcerative colitis express sialosyl-Tn antigen (21) as determined by immunohistochemistry with monoclonal antibody B72.3 which recognizes this epitope (22,23). However, the frequency of antigen expression increases when dysplasia and certainly carcinoma are present in ulcerative colitis tissues (21).

Because of the increased cancer risk, patients with long-standing ulcerative colitis are advised to undergo periodic surveillance colonoscopy to obtain biopsies of macroscopically abnormal as well as normal mucosa with the intent of detecting dysplasia in its earliest phases. Taking advantage of this clinical situation, a retrospective study was performed in which sialosyl-Tn antigen expression was analyzed in serial colonoscopic biopsies of histologically normal mucosa in patients who subsequently developed colon carcinoma (24). A control group consisted of age, sex, and disease-duration matched individuals who had not yet developed cancer. In 86% of the cases who developed subsequent cancer or high-grade dysplasia, sialosyl-Tn antigen was found in at least one prior surveillance biopsy, compared to 38% of control patients. In some cases, sialosyl-Tn antigen expression was found as many as 4-9 years prior to the development of the neoplasm. Similar findings using peanut agglutinin to detect T antigen have been reported in rectal biopsies of ulcerative colitis patients (25).

These observations suggest that certain carbohydrate cancer-associated antigens may serve as intermediate endpoint biomarkers for common types of colorectal neoplasia. Now that clinical trials have been set up to investigate the role of dietary intervention on polyp recurrence rates, some of these markers can be tested.

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