Histol Histopathol (1999) 14: 351-357

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Comparison of different techniques for detection of Gal-GalNAc, an early marker of colonic neoplasia

I.T. Said[1,2], A.M. Shamsuddin[1,4], M.A. Sherief[3], S.G. Taleb[2], W.F. Aref[2] and D. Kumar[1]

[1]Department of Pathology, The University of Maryland School of Medicine, Baltimore, Maryland, USA,

[2]Department of Pathology, Minia University School of Medicine, Minia, Egypt and

[3]Department of Pathology, Ain Shams University School of Medicine. Cairo, Egypt

Summary. The tumor marker, D-galactose-ß[1-3]-*N*-acetyl-D-galactosamine (Gal-GalNAc, also known as T-antigen) can be identified by a very simple galactose oxidase-Schiff's (GOS) reaction either on tissues or on rectal mucus samples from patients with colorectal neoplasms. Gal-GalNAc is expressed in the neoplastic mucosa as well as the remote non-neoplastic mucosa. It is, however, not expressed in colonic mucosa of normal subjects. We studied the expression of Gal-GalNAc by GOS reaction, lectin reactivity and immunocytochemistry in 10 normal, 45 precancerous [5 Crohn's disease, 15 ulcerative colitis (5 without dysplasia and 10 with dysplasia), 25 tubular adenomas], and 25 adenocarcinoma cases. Normal mucosa remote from tubular adenoma and adenocarcinoma was also studied. The GOS method was compared with reactivity of the lectin jacalin and immunostaining with antibody to T antigen (Anti-Tag Ab). GOS reaction was negative in all of the 10 normal specimens. Of the 5 Crohn's disease specimens, 2 were positive and 3 negative. In the 5 ulcerative colitis cases without dysplasia, positive reaction was seen in 2 cases and negative in 3. Of the 10 cases of ulcerative colitis with dysplasia, 5 showed positivity in dysplastic areas, and 3 of these were also positive in remote non dysplastic mucosa. Twenty of 25 tubular adenomas yielded a positive reaction in the adenoma, 14 of them showing positivity also in remote mucosa; 3 cases showed a positive reaction only in remote mucosa. Of the 25 adenocarcinomas, 21 showed a positive reaction in the adenocarcinoma as well as the remote mucosa. GOS reaction was intense in well differentiated adenocarcinoma and weak in poorly differentiated adenocarcinoma. Intense reaction was also seen in the intracellular mucus of some aberrant crypts and morphologically normal crypts remote from adenocarcinoma and tubular adenoma. GOS reaction showed an overall sensitivity of 75.7% and specificity of 100% for cancer and precancerous lesions. Jacalin reactivity was slightly more sensitive (84.3%) but less specific (80%) and Tag Ab reactivity even less sensitive (50%) but as specific (100%) for neoplastic and dysplastic mucosa. We conclude that the detection of the carbohydrate moiety Gal-GalNAc varies with the technique used. Compared to other techniques, GOS reaction is extremely simple and has a high degree of sensitivity and specificity. It can be used for detection of this tumor marker in remote non-neoplastic mucosa of patients with neoplasia or at risk of developing neoplasia. It, therefore, could be used as a cost effective screening test in rectal biopsy specimens of such patients.

Key words: Tumor markers, T-Antigen, Jacalin, Cancer prevention

Offprint requests to: Dr. A.M. Shamsuddin, Department of Pathology ,10 S Pine Street, Baltimore, MD, USA 21201-1192. Fax: 410-706-8414

Introduction

Adenocarcinoma of the colon is the second most frequently diagnosed malignancy as well as the second most common cause of cancer death in the United States (Parker et al., 1997). It is treatable and often curable when localized within the bowel wall. As in other cancers, detection of colon cancer at a very early stage can reduce the mortality rate. It has been recommended that early detection by screening for colon cancer should be part of routine care for all adults starting at age 50 years, especially for those at high risk (those with first degree relatives with colon cancer, patients with panulcerative colitis, previous colon cancer, a family history of cancer, or a history of sporadic colon polyps) (Kim and Lance, 1997). Following treatment of colon cancer, periodic follow up by laboratory studies and physical examination may lead to earlier identification and management of recurrent cancer.

Prevention is an important method for cancer control, and primary prevention attempts to reverse precancerous lesion or in situ carcinoma to normal or stops them from progressing to invasive malignancies in population at high risk. Thus, for prevention and early detection, the key is to find the marker which is differentially expressed in high risk tissues (cancers and

precancerous) but not in normal (Yang and Shamsuddin, 1996).

The disaccharide moiety D-galactose-ß-(1-3)-*N*-acetyl-D-galactosamine (Gal-GalNAc), also called Thomsen-Friedenreich antigen or T antigen, is a tumor associated carbohydrate that has been used as a biomarker of colonic cancer and other adenocarcinomas (Springer, 1984; Shamsuddin et al., 1995; Carter et el., 1997). Shamsuddin and Elsayed provided a new test for mass screening of colorectal cancer that is based on the enzymatic oxidation of Gal-GalNAc by the enzyme D-galactose oxidase followed by Schiff's reagent to produce a magenta coloration (Shamsuddin and Elsayed, 1988). The test was used in rectal mucus samples of patients with colorectal neoplasms. Studies done mostly in China and Japan confirmed the sensitivity and specificity of the test for detection of colorectal neoplasms and precancerous conditions (Sakamoto et al., 1990, 1993; Zhou et al., 1993; Shamsuddin, 1996). Other methods of detection of the T antigen have also been reported. These are reactivity with the lectin jacalin and immunoreactivity by using an antibody to it in an immunoperoxidase reaction (Hanisch and Baldus, 1997). In this study we investigated the sensitivity and specificity of detection of Gal-GalNAc by the above mentioned three techniques on formalin fixed, paraffin embedded tissue sections of colorectal cancers, precancerous lesions and normal colon specimens.

Materials and methods

One hundred and thirty colon samples were collected from surgical specimens and autopsies at the University of Maryland hospital. The samples comprised of 25 cases of colorectal adenocarcinoma (5 well differentiated, 8 moderately differantiated, 6 poorly differentiated, 6 mucinous adenocarcinoma), 25 cases of colorectal adenoma (20 with low grade dysplasia, and 5 with high grade dysplasia), 20 cases of inflammatory bowel disease [5 Crohn's disease, 15 ulcerative colitis(5 without dysplasia, 4 with low grade dysplasia, and 6 with high grade dysplasia)], 50 specimens of morphologically normal mucosa remote from cancers and adenoma; 10 specimens of colonic mucosa from normal subjects served as negative control. The tissues were fixed in 10% buffered formalin and embedded in paraffin. All sections were cut to 5 μm and stained routinely by hematoxylin and eosin (H&E) for histological diagnosis.

Galactose oxidase-Schiff's reaction procedure

Paraffin tissue sections were deparaffinized in xylene, rehydrated in graded alcohols according to standard procedure, and then transferred to PBS (pH 7.0) for 10 minutes. They were then flooded with D-galactose oxidase solution (100 units/ml; Sigma Chemical Co., St Louis, MO) for 1 hour at room temperature. The sections were then rinsed in distilled water for 10 min and stained with 2% Schiff's reagent (Sigma Chemical Co., St. Louis, MO) for 10 min at room temperature. The sections were rinsed in running tap water for 10 min, counterstained with haematoxylin for 2 secconds, rinsed with running tap water, dehydrated, cleared and mounted.

Jacalin Immunoreactivity

Tissue sections of 5 μm thickness were deparaffinized, rehydrated, and then treated with 0.1% trypsin (Sigma Chemical Co., St Louis, MO) and 0.1% calcium chloride in phosphate buffer, pH 7.5 at 37 °C for 10 min. Endogenous peroxidase activity was blocked with fresh 3% hydrogen peroxide in methanol for 30 min. Sections were incubated with biotinylated jacalin (1/5000 dilution; Vector Laboratories, Burlingame, CA) for 1 hour. Then the avidin-biotin complex (1:25 dilution; Vector Laboratories Burlingame, CA) was applied for 45 min at room temperature. The sections were washed in PBS for 5 minutes, and reacted with 3,3'-diaminobinzidine (0.02% Sigma Chemical Co., St Louis, MO) freshly prepared in 0.05M TRIS buffer (pH 7.6) containing 0.015M hydrogen peroxide for 7 minutes, counterstained with hematoxylin, dehydrated and mounted.

Immunohistochemistry with anti T-antigen antibody

The sections were deparffinized, rehydrated and treated with 3% hydrogen peroxide in methanol for 30 min to block endogenous peroxidase. To reduce non specific staining, the sections were incubated for 20 min. at room temperature with normal horse non immune serum (1:100 dilution; Vector Laboratories, Burlingame, CA). Excess normal serum was blotted from the slides. The sections were then incubated with the primary (mouse) antibody (1:50 dilution; DAKO, Co., Carpinteria, CA) for 1 hr at room temperature, washed 3 times for 5 min each in PBS (pH 7.2), and incubated with biotinylated secondary (horse anti mouse IgG) antibody (1:100 dilution; Vector Laboratories, Burlingame, CA) in PBS for 30 min at room temperature. Avidin-biotin complex (1:25 dilution; Vector Laboratories, Burlingame, CA) was then applied for 60 min. Sections were then incubated with 0.02% 3,3-diaminobenzidine, freshly prepared in 0.05M TRIS buffer (pH 7.6) containing 0.015M hydrogen peroxide, and then counterstained with haematoxylin before dehydrating and mounting.

Mucosa from colloid carcinoma patient was used as a positive control in each staining run.

Results

The staining pattern by GOS, jacalin, and anti T-antigen antibody was similar. The positive reaction was seen at the apical cell membrane, goblet cell vacuoles extracellular mucus, intraluminal mucus, and in the

cytoplasm of signet ring cells.

Normal colon

No expression of Gal-GalNAc was detected by GO-Schiff's (GOS) and anti-Tag Ab in the epithelial cells in the 10 normal colon specimens. Weak staining was observed with the lectin jacalin in two of the 10 cases.

Ulcerative colitis and Crohn's disease

The expression of Gal-GalNAc was seen by GOS in 2 of 5 (40%) specimens of ulcerative colitis without dysplasia, 2 of 6 (30%) specimens of ulcerative colitis with low grade dysplasia and 3 of 4 (75%) specimens with high grade dysplasia. GOS reactivity was seen in the dilated and distorted crypts or even in normal appearing crypts (Fig. 1) and focally in dysplastic crypts. Two of 5 (40%) specimens of Crohn's disease stained positive with GOS. Staining with lectin jacalin was seen in 2 of 5 (40%) specimens of ulcerative colitis with no dysplasia, 5 of 6 (83%) specimens with low grade dysplasia, 3 of 4 (75%) specimens of ulcerative colitis with high grade dysplasia the reaction was seen in the dysplastic and non dysplastic areas in ulcerative colitis, and 2 of 5 cases of Crohn's disease. Gal-GalNAc expression was seen by anti-Tag Ab only in 4 of 15 (26%) specimens of ulcerative colitis, in 1 case without dysplasia and in 3 cases with high grade dysplasia, and in 1 of 5 cases of Crohn's disease.

Tubular adenomas

Expression of Gal-GalNAc was seen by GOS in 15 of the 20 (75%) tubular adenomas with low grade dysplasia and in all 5 tubular adenomas with high grade dysplasia. The intensity of staining was strong in all cases and was observed in the mucus cells of normal as well as dysplastic crypts (Fig. 2) The lectin jacalin stained 19 of 20 (95%) tubular adenomas with low grade dysplasia, and all five cases (100%) of adenomatous polyps with high grade dysplasia. However, the stromal cells also showed a non specific weak positive reaction (Fig. 3). The expression of Gal-GalNAc was detected by anti-Tag Ab only in 12 of 20 (60%) adenomatous polyps with low grade dysplasia and in 3 of 5 (60%) adenomatous polyps with high grade dysplasia.

Table 1. The expression of Gal-GalNAc in tubular adenomas, adenocarcinomas, and remote mucosa

Specimen	Technique		
	GOS	Jacalin	Anti-T antigen
Adenoma =25	20 (80%)	24 (96%)	15 (60%)
Remote mucosa	17 (68%)	24 (96%)	0 (0%)
Carcinoma =25	21 (84%)	23 (92%)	15 (60%)
Remote mucosa	21 (84%)	23 (92%)	15 (60%)

Colorectal carcinoma

The GOS reaction was positive in 21 of 25 (84%) cancer specimens. The well differentiated and moderately differentiated adenocarcinomas showed more intense staining than poorly differentiated adenocarcinoma. The GOS reaction was strong in 5 of the 5 (100%) well differentiated carcinomas and 6 of 8 (75%) moderately differentiated carcinomas (Fig. 4). Reactivity was observed in all 6 cases (100%) of mucinous carcinoma (Fig. 5). A moderate to weak cytoplasmic staining was observed in 4 of 6 (66%) poorly differentiated carcinomas. The expression of Gal-GalNAc with lectin jacalin was seen in 23 of the 25 (92%) cases of adenocarcinoma (Fig. 6). Intense reactivity was observed in all of cases of mucinous adenocarcinoma as well as all cases of well differentiated and moderately differentiated adenocarcinomas. Two of the poorly differentiated adenocarcinomas did not show staining. Once again, the neoplastic glandular elements and to a lesser degree, the stromal cells showed positive reaction.

The expression of Gal-GalNAc by anti-Tag Ab in 15 of the 25 (60%) cases of adenocarcinoma intense staining was observed in only 4 of these; all of them being mucinous carcinoma. The well differentiated and moderate differentiated adenocarcinoma showed positive reaction in 8 of the 13 (61%) cases (Fig. 7), and 3 of the 6 cases (50%) of poorly differentiated carcinoma were positive.

Crypts adjacent to and remote from carcinoma

We studied the expression of Gal-GalNAc in normal crypts, dilated crypts and distorted crypts (abnormal or aberrant crypts) remote from 25 adenocarcinomas and 25 tubular adenomas. Remote mucosa was stained in 17 of 25 cases of tubular adenoma by the GOS reaction. Fourteen of these cases showed positive reaction in the adenomatous epithelium. In 3 cases only the remote mucosa stained. The staining was seen in the goblet cell vacuoles and intraluminal mucin of morphologically normal, dilated and distorted crypts remote from cancer. All 21 adenocarcinoma cases that had a positive reaction with the GOS sequence also showed positive staining of the remote mucosa. GOS reactivity was seen in 2 specimens of ulcerative colitis with dysplasia and in 1 without dysplasia. Jacalin staining was observed in the remote mucosa of all 24 cases and that stained positively in the tubular adenoma and in all 23 cases of adenocarcinoma that stained positively in their tumor. Jacalin reactivity was seen in 3 specimens of ulcerative colitis with dysplasia and in 2 without dysplasia. The anti-Tag antibody did not stain the remote mucosa in any case of adenoma or adenocarcinoma (Table 1).

Discussion

The disaccharide Gal-GalNAc, also known as Tag, is a precursor of the M and N blood group substances. Gal-

GalNAc normally contains a sialic acid that is attached to the terminal galactose. It is believed that removal of sialic acid allows the sugar moiety to be oxidized by the enzyme galactose oxidase (Shamsuddin, 1991; Hanisch and Baldus, 1997). Schulte and Spicer (1983) first demonstrated the use of the enzyme D-galactose oxidase to study Tag in rat tracheal gland secretory glycoproteins. The enzyme D-galactose oxidase specifically oxidizes C-6 hydroxyl groups of D-galactopyranose and N-acetylgalactosamine residues of Gal-GalNAc, generating two vicinal aldehyde groups, which react with basic fuchsin to give magenta/purple coloration, called galactose-oxidase-Schiff or GOS reaction (Shamsuddin and Elsayed, 1988). It is believed that sialic acid free Gal-GalNAc is present in mucus of colorectal cancer and precancer of both human and experimental animals, but not in normal mucin (Shamsuddin, 1991, 1996). In 1981, Shamsuddin et al. first proposed that the mucin composition is altered throughout the entire colon in patients with colonic cancer and precancer by the way of the generalized field effect of carcinogens. One abnormality may be the loss of the terminal sialic acid residues, leaving Gal-GalNAc exposed. This alteration may be due to reduction of glycosyltransferases, leading to aberrant or incomplete glycoprotein synthesis (Shamsuddin et al., 1981). Based on this hypothesis, Shamsuddin developed a simple test, using the GOS technique, to detect the marker Gal-GalNAc in the rectal mucus of patients with precancerous and cancerous lesions of the colon (Shamsuddin, 1994; Shamsuddin and Sakamoto, 1994).

Jacalin is an IgA specific lectin isolated from dried seeds of jack fruit, *Artocarpus intergrifolia* and interacts with D-galactose (Urdiales-Viedma et al., 1995). It is able to bind to Gal-GalNAc residues that are O-glycosically linked to serine or threonine. The major component of the active lectin seems to be a molecule of about 54,000 dalton and probably made up of three non-glycosylated non covalently linked subunits (Aucouturier et al., 1987). There are few reports about reactivity of the lectin jacalin on tissue sections. Muscle fibers in ten days-old rats were stained with jacalin (Kirkeby et al., 1992) and it also binds selectively to goblet cells in conjuctival tissue (Prause et al., 1989). Urdiales-Viedma et al. (1995) observed that jacalin stains histiocytes in paraffin embedded tissues in all cases of reactive sinus histiocytosis, and macrophages in follicular hyperplasia and in granulomas of tuberculosis. They failed to find any staining in cases of eosinophilic granulomas, giant cell tumors of tendon sheath, pleomorphic fibrous histiocytomas, and in Hodgkin's disease. They concluded that jacalin could be used as a marker for detection and differentiation of histiocytes/macrophages from histiocyte mimicking cells. We could not find any report of use of jacalin in colorectal tumors. Thus, this is the first study of jacalin reactivity in colorectal tumors. However, its usefulness is limited by its non-specific reactivity or cross reactivity to the stromal cells perhaps containing sialylated Gal-GalNAc.

Gal-GalNAc can also be detected by using monoclonal or polyclonal antibodies to T antigen (Hanisch and Baldus, 1997). Using monoclonal antibodies, conflicting results have been published; most studies show positive reactivity of cancer with Tag antibodies except monoclonal antibodies HH8 and HT8 which did not stain colorectal carcinoma (Orntoft et al., 1990). Whereas monoclonal antibody BW 835 has recently been characterized to bind to T antigen (Hanisch et al.,

Table 2. Sensitivity and specificity of different methods of Gal-GalNAc detection

Technique	Sensitivity – Specificity			
	Overall	IBD	Adenoma	Carcinoma
GOS	75.7-100	45-100	92-100	84-100
Jacalin	84.3-80	60-80	96-80	92-80
T-ag	50-100	25-100	60-100	60-100

IBD: Inflammatory bowel disease.

Table 3. Comparison of time and steps taken to perform the three tests for Gal-GalNAc detection on tissue samples.

Technique	Number of steps	Total time for reaction
GOS	2	1.5 hour
Jacalin	5	2.5 hour
Anti-Tag Ab	6	3.5 hour

Fig. 1. GOS reaction in ulcerative colitis showing crypt abscess. The abnormal and dilated crypt shows magenta coloration indicating expression of Cal-GalNAc. x 400

Fig. 2. GOS reaction in tubular adenoma. A crypt with mild dysplasia shows magenta reaction in goblet cells. x 960

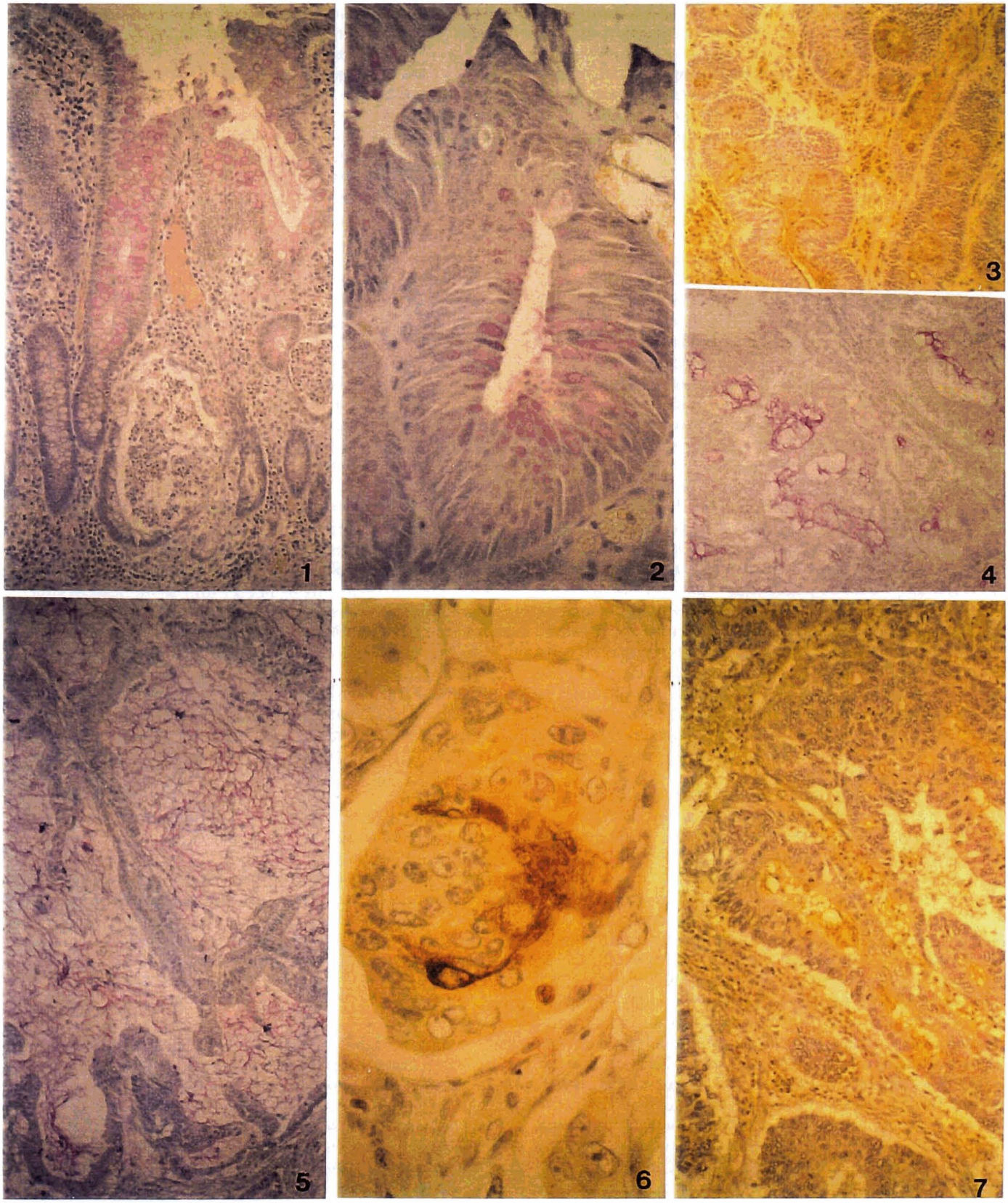
Fig. 3. Jacalin staining in tubular adenoma showing positive reaction in adenomatous epithelium and non specific reaction in stroma. x 400

Fig. 4. GOS reaction in moderate differentiated adenocarcinoma showing magenta reaction in secreted mucus in the lumen. x 400

Fig. 5. GOS reaction in mucinous adenocarcinoma showing Gal-GalNAc expression in mucus lakes. x 400

Fig. 6. Jacalin staining in well differentiated adenocarcinoma. x 960

Fig. 7. Anti T-antigen antibody staining in moderate differentiated adenocarcinoma. x 400


1
2
3
4
5
6
7

1995).

The present study was undertaken to localize the Gal-GalNAc residue on tissue specimens of pre-neoplastic and neoplastic colorectal mucosa by GO-Schiff's sequence and to compare the results with Jacalin and Tag Ab binding reaction. Our results showed the following sensitivity and specificity data (Table 2). Overall sensitivity for the GOS reaction was 75.7% and specificity 100%; for jacalin, 84.3% and 80%, and for anti-Tag Ab 50% and 100% respectively. For pre-cancerous lesions, the sensitivity and specificity figures were 71.1% and 100% for GOS, 80% and 80% for jacalin, and 44.4% and 100% for anti-Tag Ab. For carcinomas these figures were 84% and 100% for GOS, 92% and 80% for Jacalin and 60% and 100% for anti-Tag Ab. For inflammatory bowel disease: 45% and 100% for GOS, 60% and 80% for jacalin, 25% and 100% for Tag Ab. For tubular adenoma: 92% and 100% for GOS, 96% and 80% for jacalin and 60% and 100% for anti-Tag Ab. We found no expression of Gal-GalNAc by GOS reaction or anti-Tag Ab in 10 normal colonic mucosa specimens. Weak staining was observed in two cases with Jacalin. This weak staining was probably due to the ability of Jacalin to bind to sialylated Gal-GalNAc which may be expressed in normal colonic mucosa (Hagiwara et al., 1988; Corbeau et al., 1995).

We also observed that the expression of Gal-GalNAc was more frequent and intense in mucinous and well differentiated adenocarcinomas than in poorly differentiated adenocarcinomas. This finding supports the earlier observation of Springer (1984) that anaplastic carcinoma often has greater Tn antigen (a precursor of Tag) and T antigen ratio than well differentiated adenocarcinoma. Glands, or cells react with GOS, but not with jacalin or anti-Tag Ab and *vice versa* may be due to the fact that the Gal-GalNAc detected by GOS is different, perhaps they have a different intercarbohydrate bond, different degree, and position of sialylation or another yet unknown structure.

The morphologically normal, dilated and distorted crypts remote from cancer and adenoma showed expression of Gal-GalNAc by GOS in 38 of the 50 cases (76%) and by jacalin staining in 47 of 50 (94%) cases. The correspondence between increased GOS and jacalin reactivity in morphologically normal mucosa remote from cancer and the presence of colonic neoplasm suggests that the alterations in mucin resulting in increased GOS and jacalin reactivity are associated with the carcinogenic process (Carter et al., 1997).

Shamsudddin hypothesized that the histochemically altered foci of morphologically normal appearing mucosa were perhaps areas of initiated foci, and that these may be predictors of cancer away from their site of sampling (McKenzie et al., 1987; Shamsuddin, 1991, 1996). In other words, as a result of generalized effect of the carcinogens throughout the entire field of the target tissue, it is most likely that the mucosa away from an obvious cancer would be abnormal and expressing Gal-GalNAc. Shamsuddin also rationalized that: (a) the presence of cancer in large intestine implies previous exposure of the host to carcinogens; (b) most carcinogens act by field effect, where the entire target tissue is subjected to carcinogenic stimuli; (c) carcinogens induce multi focal changes throughout the entire target tissue viz. colorectal cancer; and (d) of the many initiated sites, only some of them may be promoted to recognizable cancer (Shamsuddin, 1996). Thus the alterations in normal appearing, initiated but not promoted mucosa may express some of the markers of cancer and pre-cancer, such as Gal-GalNAc. Table 1 and 2 shows that GOS is the best technique for detection of Gal-GalNAc in remote mucosa.

Gal-GalNAc expression was also demonstrated in other known precancerous conditions of the colonic mucosa e.g. ulcerative colitis with and without dysplasia and Crohn's disease. This indicates that expression of Gal-GalNAc may be one of the earliest phenotypic abnormality which may precede morphological changes of colorectal neoplasia (McKenzie et al., 1987; Xu et al., 1992; Yang and Shamsuddin, 1995) and establishes the role of Gal-GalNac as a tumor marker. Its presence in morphologically normal colonic crypts may be useful in determining the malignant potential of that colonic mucosa.

In this study we have shown that the GO-Schiff's reaction is a sensitive and specific way for detection of this marker. The lectin jacalin, although more sensitive, is less specific, and the anti-Tag Ab (clone HT8) is much less sensitive (Orntoft et al., 1990). Table 3 shows the comparison between the 3 different techniques for detection Gal-GalNAc. Note that GOS is the simplest and the fastest of all 3 reactions yet yielding the best overall sensitivity.

In conclusion, The GOS reaction has the potential to identify the tumor marker Gal-GalNAc either at the tissue level or by mucin test. This test, simplest of all the three, could be used for screening and early detection of colorectal cancer and precancerous lesions (Yang and Shamsuddin, 1996; Shamsuddin 1996). Furthermore, studies have shown that Gal-GalNAc expression can be suppressed by cancer chemopreventive and chemotherapeutic agent IP_6. Following IP_6 treatment, HT-29 human colon carcinoma cells terminally differentiate and produce mucin, yet not Gal-GalNAc (Sakamoto et al., 1993; Yang and Shamsuddin, 1995). Thus Gal-GalNAc can also serve as biomarker to monitor the efficacy of chemoprevention.

References

Aucouturier P., Mihaesco E., Mihaesco C. and Preud'Homme J-L. (1987). Characterization of Jacalin, the human IgA and IgD binding lectin from jackfruit. Mol. Immunol. 24, 503-511.

Carter J.H., Deddens J.A., Pullman J.L., Colligan M.B., Whiteley L.O. and Carter H.W. (1997). Validation of Galactose oxidase Schiff's reagent sequence for early detection and prognosis of human colorectal adenocarcinoma. Clini. Cancer Res. 3, 1479-1489.

Corbeau P., Pasquali J-L. and Devaux C. (1995). Jacalin, a lectin

interacting with O-linked sugars and mediating protection of $CD4^+$ cells against HIV-1, bind to the external envelope glycoprotein gp120. Immunol. Lett. 47, 141-143.

Hanisch F-G., Stadie T. and Bosslet K. (1995). Monoclonal antibody BW835 defines a site-specific Thomsen-Friedenreich disaccharide linked to threonine with VTSA Motif of MUC1 tandem repeats. Cancer Res. 55, 4036-4040.

Hanisch F-G. and Baldus S.E. (1997). The Thomsen-Friedenreich (TF) antigen: a critical review on the structural, biosynthetic and histochemical aspects of pancarcinoma-associated antigen. Histol. Histopathol. 12, 263-28.

Hagiwara K., Collet-Cassart D., Kobayashi K. and Vaerman J.P. (1988). Jacalin: Isolation, characterization and influence of various factors on its interaction with human IgA1, as assessed by precipitation and latex agglutination. Mol. Immunol. 25, 69-83.

Kirkeby S., Bog-Hansen T.C. and Moe D. (1992). Mosaic lectin and enzyme staining patterns in rat skeletal muscle. J. Histochem. Cytochem. 40, 1511-1516.

Kim E.C. and Lance P. (1997). Colorectal polyps and their relationship to cancer. Gastroentrol. Clin. N. Am. 26, 1-17.

McKenzie K.J., Purnell D.M. and Shamsuddin A.M. (1987). Expression of carcinoembryonic antigen, T-antigen and oncogene products as a markers of neoplastic and preneoplastic colonic mucosa. Hum. Pathol. 18, 1282-1286.

Orntoft T.F., Harving N. and Langkilde N.C. (1990). O-linked mucin-type glycoproteins in normal and malignant colon mucosa: lack of T-antigen expression and accumulation of TN and sialosyl-Tn antigens in carcinomas. Int. J. Cancer 45, 666-672.

Parker S.L., Tong T., Bolden S. and Wingo P.A. (1997). Cancer statistics. CA Cancer J. Clin. 47, 5-25.

Prause J.U., Jensen O.A., Paschides K., Stouhase A. and Vangsted P. (1989). Conjunctival cell glycoprotein pattern of healthy persons and of patients with primary Sjogren's syndrome. Light microscopical investigation using lectin probes. J. Autoimmun. 2, 495-500.

Sakamoto K., Nakano G. and Nagamachi Y. (1990). A Pilot study on the usefulness of a new test for mass screening of colorectal cancer in Japan. Gastroentrol. Jpn. 25, 432-436.

Sakamoto K., Muratani M., Ogawa T. and Nagamachi Y. (1993a). Evaluation of a new test for colorectal neoplasms: a prospective study of asymptomatic population. Cancer Biotherapy 8, 49-55.

Sakamoto K., Venkatraman G. and Shamsuddin A.M. (1993b). Growth inhibition and differentiation of HT-29 cells in vitro by inositol hexaphosphate (phytic acid). Carcinogenesis 14, 1815-1819.

Schulte B.A. and Spicer S.S. (1983). Light microscopic histochemical detection of sugar residues in secretory glycoproteins of rodent and human tracheal glands with lectin-horseradish peroxidase conjugates and the galactose oxidase-Schiff's sequence. J. Histochem. Cytochem. 31, 391-403.

Shamsuddin A.M. (1991). Diagnostic assay for colon cancer. CRC. Press. Boca Raton, FL.

Shamsuddin A.M. (1994). Screening test and kit for cancerous and precancerous conditions. US patent No. 5, 348, 860.

Shamsuddin A.M. (1996). A simple mucus test for cancer screening. Anticancer Res. 16, 2193-2200.

Shamsuddin A.M. and Elsayed A.M. (1988). A test for detection of colorectal cancer. Hum. Pathol. 19, 7-10.

Shamsuddin A.M. and Sakamoto K. (1994). Carbohydrate tumor marker: basis for a simple test for colorectal cancer. Adv. Exp. Med. Biol. 354, 85-99.

Shamsuddin A.M., Weiss L., Phelps P.C. and Trump B.F. (1981). Colon epithelium, IV: Human colon carcinogenesis: Changes in human colon mucosa adjacent to and remote from carcinoma of the colon. J. Natl. Cancer Inst. 66, 413-419.

Shamsuddin A.M., Tyner G.T. and Yang G.Y., (1995). Common expression of the tumor marker D-galactose-ß(1-3)-N-acetyl-D-galactosamine by different adenocarcinomas. Evidence of field effect phenomenon. Cancer Res. 55, 149-152.

Springer G.F. (1984). T and Tn, general carcinoma antigens. Science 224, 1198-1206.

Urdiales-Viedma M., De Haro-Munoz T., Martos-Padilla S., Abad-Ortega J.M., Varela-Duran J. and Granda-Paez R. (1995). Jacalin, another marker for histiocytes in paraffin embedded tissues. Histol. Histopathol. 10, 597-602.

Yang G.Y. and Shamsuddin A.M. (1995). IP6-induced growth inhibition and differentiation of HT-29 human colonic cancer cells: involvement of intracellular inositol phosphates. Anticancer Res. 15, 2479-2488.

Yang G.Y. and Shamsuddin A.M. (1996). Gal-GalNAc: a biomarker of colon carcinogenesis. Histol. Histopathol. 11, 801-806.

Xu H., Sakamoto K. and Shamsuddin A.M. (1992) Detection of tumor marker D-galactose-ß-(1-3)-N-acetyl-D-galactosamine in colonic cancer and precancer. Arch. Pathol. Lab. Med. 116, 1234-1238.

Zhou D-Y., Feng F-C., Zhang Y-L., Lai Z-S., Zhang W-D., Li L-B., Xu G-L., Wan T-M., Pan D-S., Zhou D., Zhang Y-C. and Li S-B. (1993). Comparison of Shams' test for rectal mucus to an immunological test for fecal occult blood in large intestinal cancer screening. Analysis of a check-up of 6480 asymptomatic subjects. Chin. Med. J. 106, 739-742.

Accepted July 26, 1998