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Reciprocal control of colon-specific sulfomucin and sialosyl-Tn antigen expression in human colorectal neoplasia

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Abstract Histochemical reports claim that sulfomucins decrease and sialylated mucins increase during colon carcinogenesis. We examined the expression of colon-specific sulfomucins and sialosyl Tn antigen (STN) in normal small intestine, normal colorectal mucosa and colorectal tumours at different stages of progression immunohistochemically, using MAb 91.9H specific for colonic sulfomucins and MAb TKH-2 for STN. No expression of sulfomucins recognized by MAb 91.9H was found in normal small intestine, whereas STN staining was pronounced. The converse was the case in normal colorectal mucosa. Sulfomucins were still found in adenomas, but the amounts decreased with depth of invasion in cancers ($P<0.001$). In contrast, no STN could be detected in benign lesions, but staining became increasingly evident with invasion ($P<0.001$). This reciprocal control of expression of colon-specific sulfomucins and STN evident in tumour progression indicates that the mucous phenotype shifts from the colonic to the small intestinal type.

Key words Colon cancer · Colon-specific sulfomucin · Sialosyl-Tn · Immunohistochemistry · Cell differentiation

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Introduction

Colonic mucins are high-molecular-weight glycoproteins secreted mainly by goblet cells [5]. Their physiological functions include lubrication and modulation of water and electrolyte absorption, protecting colonocytes from a variety of damaging agents and invasion of potential pathogens [1, 6]. Recently, alterations of glycoproteins in mucin molecules occurring during malignant transformation have attracted attention in relation to oncogenesis [22], metastatic ability [9], immunogenicity [18] and prognosis [13].

Structurally the glycoproteins of the colon are extremely heterogeneous with regard to carbohydrate structure [23, 24], but in normal conditions they are histochemically designated as sulfomucins by their strong iron diamine staining [25]. It has been reported that there is a simultaneous decrease in sulfomucins and an increase in sialylated mucins during colonic carcinogenesis [3, 4].

In the gastrointestinal environment, one possible role of sulfomucin is protection of luminal surfaces against protease attack [21]. The structural characteristics and biosynthetic regulation of sulfated carbohydrate chains remain to be detailed, but Irimura et al. have generated a monoclonal antibody (MAb) specific to colonic sulfomucins and designated 91.9H, which allows localization at cell level [11, 28].

Sialosyl Tn antigen (STN) is not usually found in normal gastrointestinal tissues except for weak expression in goblet cells of the small intestine and parietal cells of the stomach [14, 17]. However, it is strongly expressed in a large number of adenocarcinomas, especially those of the stomach, colon and pancreas, and it has been reported that patients with STN-positive tumours have a worse prognosis than those with STN-negative lesions [13, 19].

In the present study, we investigated the expression of sulfomucins and STN in colorectal tumours at different stages of progression by immunohistochemistry using the MAb 91.9H noted above and MAb TKH-2 for STN,

we concentrated on cell differentiation as an aid to distinguishing benign and malignant lesions.

Materials and methods

Samples were taken from 5 normal small intestines, 10 colorectal adenomas and 127 colorectal cancers (20 cancers limited to the mucosa, 12 involving the submucosa, 12 the propria muscularis, 55 the subserosa and 28 invading beyond the subserosa) resected endoscopically or surgically at Aichi Cancer Center Hospital. The patients with colonic tumours were 81 men and 56 women, with a mean age of 61.6 years.

All specimens were fixed in 10% formalin and routinely embedded in paraffin. Tissue sections were examined after staining with haematoxylin-eosin (HE) and those through the longest tumour diameters were selected for immunohistochemical (91.9H and TKH-2) investigation. The UICC classification [8] was applied with regard to depth of invasion of cancers, and histological typing was performed with reference to the World Health Organization classification [15].

MAb 91.9H and MAb TKH-2 were raised as described previously [11, 17, 28]. The MAb TKH-2 used in this study was kindly donated by Dr. Itzkowitz SH (Gastrointestinal Research Laboratory, Division of Gastroenterology, Mount Sinai School of Medicine), but it is commercially available. MAb 91.9H is not commercially available at present. After deparaffinization and dehydration, sections were incubated with fresh 3% hydrogen peroxide in methanol and treated sequentially with normal horse serum, TKH-2 (1:1000) or 91.9H (1:400000), biotin-labelled horse anti-mouse IgG, and the avidin-biotin-peroxidase complex (ABC) [10]. The sites of peroxidase binding were visualized with the aid of diaminobenzidine. Sections were lightly counterstained with haematoxylin for microscopic examination.

The degree of MAb staining in each tissue sections was classified according to the percentage of positive tumour cells as follows: +1, under 10% of the tumour area; +2, between 10% and 50%; +3, between 50% and 90%; +4, over 90%. Variation in MAb reactivity in serial sections was investigated with tumour progression, from adenomas to cancers involving the mucosa, the submucosa, the muscularis propria, the subserosa and below the serosa.

The positivity of MAb 91.9H and MAb TKH-2 was also scored based on the degree of staining, the +1, +2, +3 and +4 described above being assigned corresponding scores of 1, 2, 3 and 4 for 91.9H and 4, 3, 2 and 1 for TKH-2. That is to say that high scores were given to samples having strong characteristics of colonic mucosa. Scores were compared for each tumour category for MAb 91.9H and TKH-2 and their multiplication product.

The Chi-square test for trend was applied to compare the incidences of each degree of MAb staining with depth of invasion.

Results

In the normal small intestine, sulfomucins recognized by MAb 91.9H were not found, whereas STN binding MAb TKH-2 was present in goblet cells of all samples (Fig. 1). Conversely, in normal colorectal mucosa, sulfomucins were present in all goblet cells, whereas STN was hardly detectable (Fig. 2). However, in transitional mucosa adjacent to cancers some 91.9H-negative and TKH-2-positive sites were noted.

In colorectal tumours, sulfomucins detected by MAb 91.9H and STN by MAb TKH-2 were distributed in the apical cytoplasm or at the cell membranes rather than diffusely (Figs. 3, 4). There were no differences between tumour areas for sulfomucins, but expression of STN appeared stronger at sites of invasion.

Data for incidences of each degree of MAb 91.9H and TKH-2 reactivity in lesions with different depths of invasion are shown in Fig. 5a and b. Expression of sulfomucins, almost ubiquitous in adenomas, decreased with increasing depth of invasion in cancers ($P<0.001$). The opposite was the case for STN, staining becoming more pronounced with increasing extent of invasion ($P<0.001$).

Sample numbers for each progression stage, classified according to scores for MAb stainings, are summarized in Fig. 6. All adenomas and half of the mucosal cancers (Tis) were found to have multiplication products of 12 or 16 for the 91.9H and TKH-2 scores (white zone). Inclusion of tumours in this white zone indicates that they are adenomas or mucosal cancers has a sensitivity of 66.7% and specificity of 98.1%. The positive predictive value is 90.9%, and the negative predictive value is 91.3%. Furthermore, most of the invasive cancers involving the submucosa or below (T1, T2, T3, T4) had scores of 6 or under (dark grey zone). Inclusion of tumours in this dark grey zone marks them out as invasive cancers with a sensitivity of 85.0% and specificity of 86.7%. The positive predictive value is 95.8% and the negative predictive value is 61.9%. The light grey boxes form an intermediate zone.

Discussion

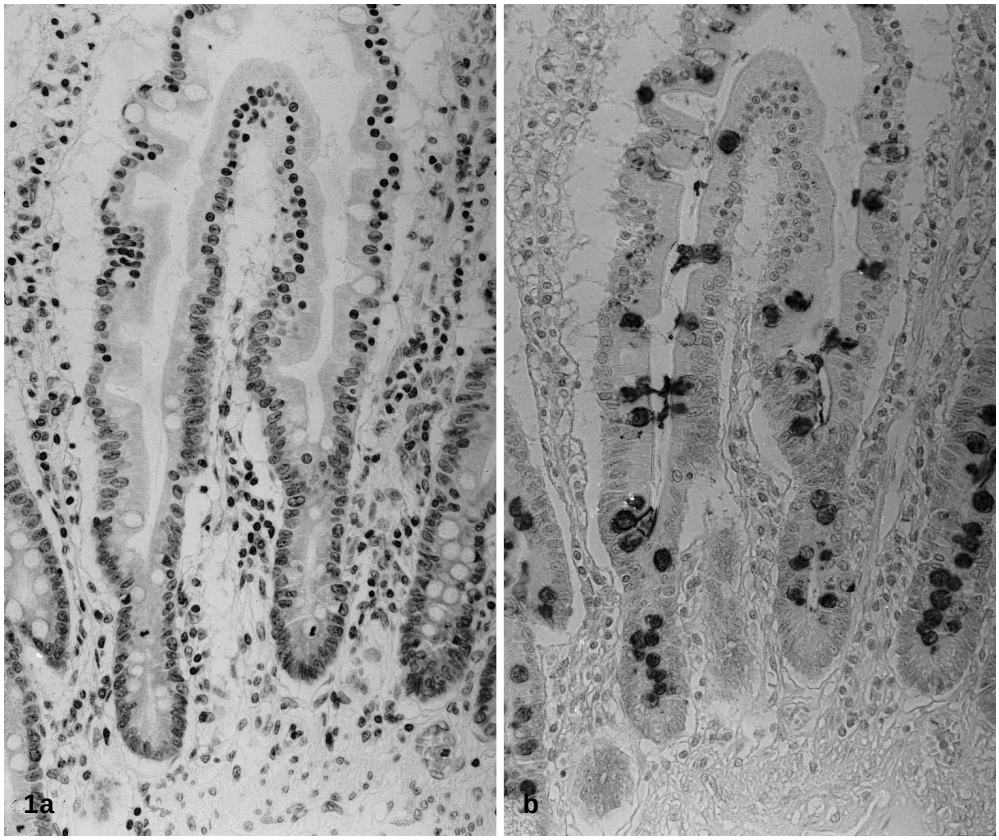
The finding that sulfomucins decrease and STN increases with tumour progression is consistent with previous reports [12, 19, 20]. The details of why this occurs remain unclear, although it clearly involves progressive reduction in sulfation of carbohydrate chains during colon carcinogenesis. As regards STN, its expression in cancers is generally thought to be due to incomplete synthesis and/or premature $\alpha 2 \rightarrow 6$ sialylation [7, 17, 26]. However, Jass's group have reported its presence in pseudocryptic form (due to *O*-acetylation of sialic acid) in normal colorectal goblet cells and indicated that its expression in colorectal cancers is due to alteration of sialic acid structure (loss of *O*-acetyl substituents) [16].

The reciprocal changes in colon-specific sulfomucins and STN could clearly have a direct clinical value, especially for distinguishing submucosal cancers from more benign lesions. The use of both monoclonal antibodies 91.9H and TKH-2 allowed a quantitative separation of submucosal and mucosal malignancies. Thus, in cases where the morphological features are borderline or biopsy samples are too limited for reliable evaluation of invasive depth, simultaneous examination of the expression of colon-specific sulfomucins and STN might be useful as a supplemental diagnostic procedure.

Previous papers on changes in sulfomucins or STN in cancers have been concerned mainly with aspects of ma-

Fig. 1a, b Serial sections of normal small intestine. Note the lack of staining with MAb 91.9H (**a**) but the MAb TKH-2 reactivity in the goblet cells (**b**). **a** 91.9H immunohistochemistry, ×66, **b** TKH-2 immunohistochemistry, ×66

Fig. 2a, b Serial sections of normal colorectal mucosa. Note reactivity for MAb 91.9H in the goblet cells (**a**), but the lack of MAb TKH-2 binding (**b**). **a** 91.9H immunohistochemistry, ×50, **b** TKH-2 immunohistochemistry, ×50



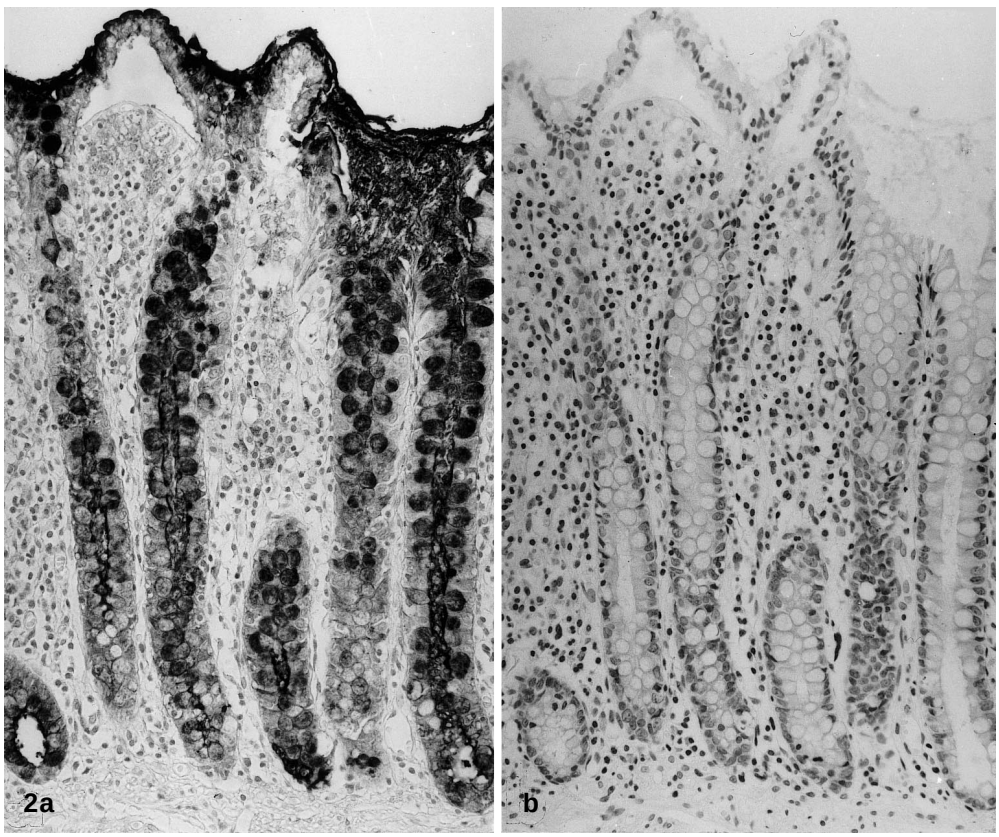
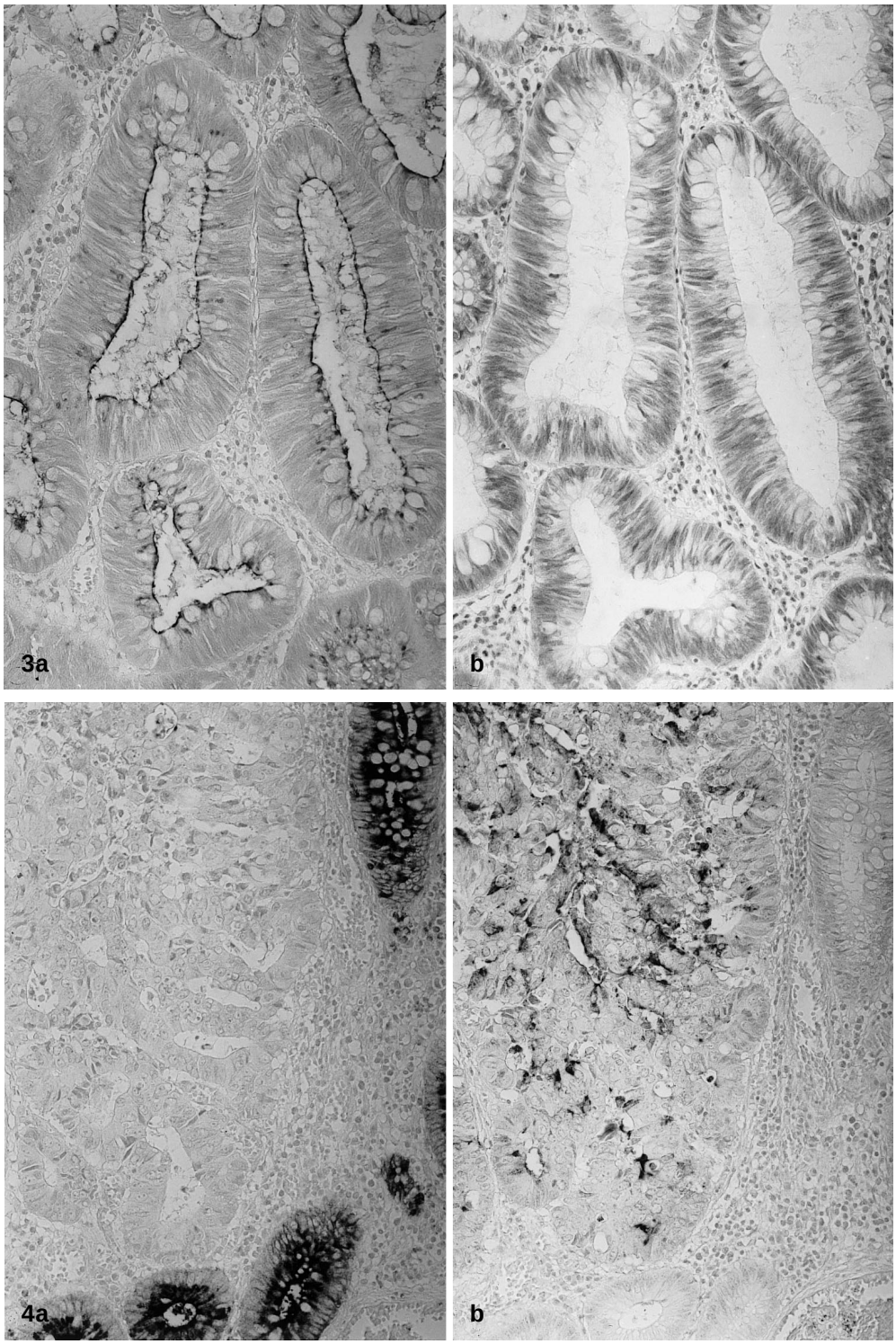


Fig. 3a, b Serial sections of a colon adenoma. MAb 91.9H binding is present at the luminal surface of the adenoma cells (**a**) which are not stained with MAb TKH-2 (**b**). **a** 91.9H immunohistochemistry, ×66, **b** TKH-2 immunohistochemistry, ×66

Fig. 4a, b Serial sections of a colon cancer invading the serosa. **a** MAb 91.9H reactivity is present the residual normal colon mucosa (*right* and *bottom*) but not in the cancer cells 91.9H immunohistochemistry, ×50). **b** In contrast, MAb TKH-2 binding is limited to the cancer. TKH-2 immunohistochemistry, ×50



lignant behaviour, such as metastatic ability, and prognosis [13, 19, 20]. In the present study, we focused on the cell differentiation of colorectal tumours. Phylogenetically, small intestinal epithelium is found in all levels of vertebrates, whereas the gastric and colonic epithelium appears only in higher animals [2]. When cells lose their differentiation on treatment with various agents, they occasionally show phylogenetic reversion. Intestinal metaplasia in normal pyloric mucosa might thus be considered a reversion to a more primitive state. In gastric cancers, we have previously shown that the phenotypic expression changes from gastric type to

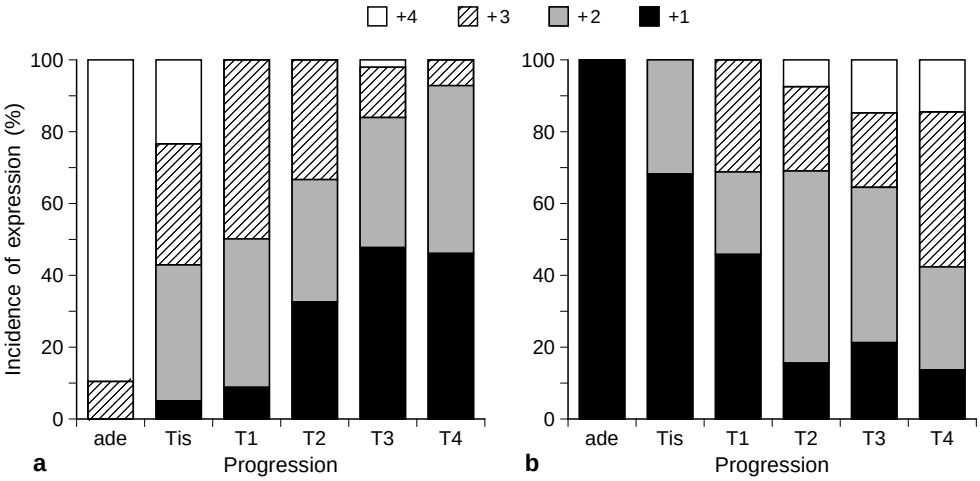


Fig. 5 Incidences of the different degrees of **a** MAb 91.9H and **b** MAb TKH-2 reactivity in adenomas and colorectal cancers with various depths of invasion. Note the increase in STN (TKH-2 reactivity) with progression and the decrease in sulfomucin (91.9H) (both $P<0.001$). The UICC classification was used for depth of invasion of cancers: *ade* adenoma (n=10), Tis (n=20), T1 (n=12), T2 (n=12), T3 (n=55), T4 (n=28). Reactivity of tumour: *+1* under 10% of the tumour area, *+2* between 10% and 50% of the tumour area, *+3* between 50% and 90% of the tumour area, *+4* over 90% of the tumour area

TKH2 (score)	91.9H (score) 4	3	2	1
4	ade 9 Tis 4 T1 0 T2 0 T4 0 T4 0	ade 1 Tis 5 T1 2 T2 0 T4 0 T4 0	ade 0 Tis 4 T1 2 T2 0 T4 2 T4 1	ade 0 Tis 1 T1 1 T2 2 T4 9 T4 3
3	ade 0 Tis 1 T1 0 T2 0 T4 0 T4 0	ade 0 Tis 2 T1 2 T2 2 T4 5 T4 0	ade 0 Tis 3 T1 1 T2 3 T4 8 T4 5	ade 0 Tis 0 T1 0 T2 1 T4 1 2 T4 3
2	ade 0 Tis 0 T1 0 T2 0 T4 0 T4 0	ade 0 Tis 0 T1 2 T2 1 T4 4 T4 2	ade 0 Tis 0 T1 2 T2 1 T4 5 T4 6	ade 0 Tis 0 T1 0 T2 1 T4 2 T4 4
1	ade 0 Tis 0 T1 0 T2 0 T4 1 T4 0	ade 0 Tis 0 T1 0 T2 1 T4 1 T4 0	ade 0 Tis 0 T1 0 T2 0 T4 3 T4 1	ade 0 Tis 0 T1 0 T2 0 T4 3 T4 3

Fig. 6 Relationship between sample numbers for each progression stage and scores for MAb stainings. The UICC classification was used for the depth of invasion of cancers. (*ade* adenoma)

small intestinal type with growth [27]. The results of this study suggest to us the possibility that the mucous phenotype shifts from the colon to the small intestine type.

In conclusion, the reciprocal control of expression of colon-specific sulfomucins and STN evident with tumour progression is consistent with a process of intestinalization of small-intestine type.

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