Evaluation of Transitional Mucosa of Colon with Special Reference to Sialylated Antigen and Nuclear DNA Content

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Evaluation of Transitional Mucosa of Colon with Special Reference to Sialylated Antigen and Nuclear DNA Content

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The expressions of glycosylated antigen SLX, STN and BM-1 were compared between the carcinoma-affected and transitional mucosa in the 69 patients with colon carcinoma (13 in carcinoma in adenoma and 21 in carcinoma in conjunction with adenoma).

In conclusion, the expression of SLX was specific of carcinoma, that of BM-1 was more often seen in an atypical mucosa and that of STN was intensified at the sites of either carcinoma including the part of adenomatous portion of carcinoma in adenoma or the transitional mucosa to colon carcinoma (TM) and carcinoma in adenoma by using HID-AB method (pH 2.5) with the serial sections.

The intensity of the expression of sialomucin in colon carcinoma showed twice as high as that in the transitional mucosa of carcinoma and/or carcinoma in adenoma.

In addition, the nuclear DNA content also was measured in accordance with the locations. In the transitional mucosa, all showed DNA diploidy pattern, but two out of the 13 patients with adenoma displayed DNA aneuploidy pattern which demonstrated strong positive STN expression.

In conclusion, it is suggested that high possibility of carcinoma should be taken into consideration in cases of adenoma with strong positive STN expression.

Introduction

Changes in mucin composition of the normal mucosa have been reported in comparison with the epithelium adjacent to carcinoma by means of the staining of high iron diamine-alcian blue-pH2.5 (HID-AB). It is well known that normal colon mucosa is mainly composed of sulphomucin which is dyed black-brown by HID staining. In contrast, the transitional mucosa is composed of sialomucin, which gets blue in color by AB staining. It also has been reported that the findings such as the thickness of the mucosa, proliferation of goblet cells and increased vascularity in lamina propria are prominent. However, the question under debate is as to whether or not these findings are precancerous states.

It has been focused on changes in glycosylated antigen on the surface of cancer cells with advances in the transformation process to carcinoma. The transformation process to carcinoma is divided into the two patterns. One is an abnormal accumulation of intermediate products generated by impairment of synthesis processes of glycosylated antigen, which names it inactivation of glycosylated antigen expression in which sialyl Tn (STN) belongs to this entity. The other is an abnormal generation of new glycosylated antigen which is not commonly seen in normal cells and is regarded as being neosynthesis. Le^- (BM-1) and sialyl Lewis^a^-i (SLX) are included in this entity.

The purpose of this study is to clarify the clinical significance of the expression of glycosylated antigens and to compare nuclear DNA ploidy pattern with the expression of these antigens (STN, SLX and BM-1).

Material and Methods

1. Subjects

Sixty-nine specimens resected for colon cancer were subjected to this study, consisting of carcinomas in adenoma 13 and adenomas in 21. The ages ranged from 35 to 84 with an average of 64 years. Men were predominant with a ratio of 41 to 28. According to histologic differentiation, well-differentiated adenocarcinoma was in 19, moderately differentiated in 48 and undifferentiated in two respectively. And in patients with carcinoma in adenoma, tubular adenoma in nine, adenovillous in two and villous adenoma in two, respectively. On the other hand, Stage I was seen in 16, Stage II in 20, Stage III in 23, Stage IV in seven and Stage V in three, respectively.

2. Methods

A) Mucin staining

Serial sections were fixed with formalin and embedded with paraffin. A 5 μm section was stained with high iron diamine-alcian blue-pH2.5 (HID-AB), by which sialomucin was dyed blue in color with AB.

The intensity of staining was classified as follows, (-): staining of less than 5%, (±): staining of five to 20%, (+):
staining of 20% to 50% and (++): staining of more than 50%.

The transitional mucosa (TM) was taken from the sites 2 cm apart from the visible margins of either carcinoma or adenoma.

B) The expression of glycosylated antigen

A 5 μm section was immunohistochemically stained by ABC method. Paraffin-embedded sections were heated to 60 °C for 30 min, treated for 5 min with 3% hydrogen peroxide in 0.05 m TBS (to block endogenous peroxidase), then rinsed three times with TBS. Slides were washed again with TBS and counter-stained with hematoxylin. Sections were evaluated using a light microscope with results expressed as a score based on the percentage of the total field positive staining. MoAbs used in this study were TKH-2, FH-2 were kindly supplied by Otsuka-Assay and BM-1 by Japan Ab Research Inst.

C) Measurement of nuclear DNA content

A 50 μm section taken from carcinoma, the transitional mucosa (TM) and the mucosa adjacent to carcinoma (NM) were all prepared for measurement of nuclear DNA content according to Schutte's method by using FACSCAN (Becton Dickinson Co). The DNA index (DI) was obtained from DNA histogram, classifying DNA diploidy patterns in which DI accounts for 1.0 and DNA aneuploidy pattern in which DI corresponds to more than one. The CV values over 8.0 were excluded from this study. The statistical analysis was made by x² test.

Results

A) Positive expression (more than (+) intensity of glyco-sialyl antigen in mucin)

1) The expression in mucin generated by colon carcinoma (Fig. 1):
The positive expression rates of sulphomucin were seen in three carcinomas out of 69 (4%), in 26 transitional mucosa (TM) out of 68, all of the adjacent mucosas (NM) to carcinomas. The expression rate of sulphomucin in the adjacent mucosas to carcinomas was significantly higher (p < 0.01) rather than those in the other lesions. The positive expression rates of sialomucin were seen in five (7%) of carcinomas, 65 (94%) of the transitional mucosas and 20 (29%) of the adjacent mucosas to carcinomas with statistically significant difference (p < 0.01) in the transitional mucosa as compared with those in carcinoma and NM.

2) The expression in mucin generated by carcinoma in adenoma (Fig. 2):
The positive expression rates of sialomucin were 0% in carcinoma, 77% in adenoma, 92% in TM and 16% in NM.
The positive expression rates of sialomucin in adenoma and TM were significantly higher than that in NM.

3) The expression of mucin generated by adenoma (Fig. 3)
The positive expression rates of sulphomucin were seen in 15 (71%) of adenoma, in 20 (95%) of TM and in 21 (100%) of NM.
The positive expression rates of sulphomucin in TM and NM were significantly higher than that in adenoma (p < 0.05). The positive expression rates of sialomucin were 48% in both adenoma and TM, whereas 38% in NT without significant difference.
**B) The expression of glycosialy lantigen that demonstrates the (+) or more intensity of the staining**

1) The expression of glycosialyl antigen in colon cancer (Fig. 1).

The positive SLX expression rates were 61% in carcinoma, 3% in TM, and 1% in NM, indicating significantly high positive rate in carcinoma \( (p < 0.01) \). In addition, the positive STN antigen expression showed 58% in carcinoma and 71% in TM, demonstrating significantly higher positive rates in carcinoma and TM rather than that in NM \( (p < 0.01) \). The positive BM-1 antigen expression rates revealed 100% in carcinoma, 6% in TM and 2% in NM, showing significantly high rate in carcinoma \( (p < 0.01) \) as shown in SLX antigen expression.

![Fig. 1. SLX Staining in colon carcinoma the grade of staining (++)](image)

2) The expression of glycosialyl antigen in carcinoma-in-adenoma (Fig. 2).

The expression of SLX antigen: The expression was positive in 10 patients (77%) with carcinoma, in 2 (15%) with adenoma and in no patients (0%) with transitional or healthy mucosas. A significant differences was seen between carcinoma and the others \( (p < 0.01) \).

The expression of STN antigen: Positive expression was seen in one (8%) of carcinoma, 7 (54%) of adenoma, 8 (61%) of transitional mucosa: and 0% of normal mucosa. There was a statistical difference between carcinoma and adenoma.

The expression of BH-1 antigen: The expression of BM-1 antigen was positive in 11 (85%) of carcinoma, 8 (62%) of adenoma, 0% of transitional and healthy mucosas, respectively. The positive expression rates were higher \( (p < 0.01) \) in carcinoma and adenoma than those in transitional and healthy mucosas and also those in carcinomas were significantly higher \( (p < 0.05) \) than those in adenoma.

![Fig. 2. STN antigen expression in transitional mucosa the grade (+++)](image)

3) The glycosialyl antigen expression in adenoma (Fig. 3).

The positive SLX antigen expression rates were 24% in adenoma, 5% in TM and 0% in NM with significantly high expression rate in adenoma \( (p < 0.05) \). The positive STN antigen expression rates were 29% in adenoma, 24% in TM and 0% in NM, indicating a higher expression rate in adenoma and TM than that in NM \( (p < 0.05) \). The BM-1 antigen expression rates were 62% in adenoma, 15% in TM and 0% in NM with a significantly high rate in adenoma \( (p < 0.01) \).

![Fig. 3. The sialomucin expression in transitional mucosa HID-AB staining](image)

**C) Nuclear DNA content (Table 1)**

In carcinoma, aneuploidy pattern was revealed in 26 (41%), whereas all of TM and NM indicated diploidy pattern. Four (31%) of patients with carcinoma in adenoma showed aneuploidy pattern, on the other hand, two (13%) of patients with adenoma revealed aneuploidy pattern.
Table 1. Nuclear DNA content by location

<table>
<thead>
<tr>
<th>Location</th>
<th>DNA content</th>
<th>Aneuploidy</th>
<th>Diploidy</th>
<th>Undetermined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma</td>
<td>26/63 (41%)</td>
<td>37/63 (59%)</td>
<td>6/69 (9%)</td>
<td></td>
</tr>
<tr>
<td>Transitional</td>
<td>0/65 (0%)</td>
<td>65/65 (100%)</td>
<td>4/69 (6%)</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0/67 (0%)</td>
<td>67/67 (100%)</td>
<td>2/69 (3%)</td>
<td></td>
</tr>
<tr>
<td>Ca. in adenoma</td>
<td>4/11 (31%)</td>
<td>7/11 (69%)</td>
<td>2/13 (15%)</td>
<td></td>
</tr>
<tr>
<td>Adenoma</td>
<td>2/15 (13%)</td>
<td>13/15 (87%)</td>
<td>6/21 (28%)</td>
<td></td>
</tr>
</tbody>
</table>

4) The glycosialyl antigen expression according to the locations (Table 2)

Table 2 shows SLX antigen expression rates which indicated over 60% in advanced carcinoma and carcinoma in adenoma irrespective of the diagnosis. In contrast, the SLX antigen expression rates in adenoma and/or adenoma in carcinoma showed 15% and 24%, respectively. Nevertheless, the STN antigen expression varied with the diagnosis in spite of the same location. The STN antigen expression rate was 54% in the site of adenoma of carcinoma whereas 29% in benign adenoma which corresponded to one half of that in adenoma of carcinoma. The STN antigen expression rate of TM to adenoma was 24% which accorded with half of 61% in carcinoma-in-adenoma and 71% in advanced carcinoma, exceptionally indicating 8% in carcinoma in adenoma.

The BM-1 antigen expression rates were almost the same as the SLX antigen, indicating more than 50% of the BMI expression rate in carcinoma and adenoma, whereas less than 15% in TM and NM.

The expression modes of sulphomucin corresponded to the locations except for TM in advanced carcinoma. However, no expression of sulphomucin was noted in TM of advanced carcinoma, whereas a 70% or more expression rate of sulphomucin was defined in TM and NM with a low expression rate of 38% in TM of advanced carcinoma. The sialomucin antigen expression was the same as the STN-one, indicating 77% in carcinoma in adenoma, while 48% in benign adenoma. There was no close correlation between sialomucin and STN antigen expressions.

Discussion

It is well accepted that sulphomucin contained in mucin of the normal colon mucosa is a predominant content. In this study, it is defined that the sulphomucin content in the adjacent mucosa to carcinoma is decreased, on the contrary, the sialomucin content is predominantly increased. The similar tendency is recognized on the transitional mucosa of dimethylhydrazine-induced colon carcinoma as well as neoplastic polyp. In addition, it is reported that these alternations of mucin compositions are quantitative regardless of cell atypism, so that it is of great use in evaluation of biological behavior of malignant cells for malignant tumors. Furthermore, it is often seen that sialomucin is predominantly contained in the transitional mucosa to adenoma. It means that some of the transitional mucosas to adenoma are changing into the carcinogenic process.

From the above findings, it is sure that an adenoma accompanying the transitional mucosa with strong positive...
expression of sialomucin must be carcinoma or has been already in part carcinoma.

Recently, much attention has been directed toward the cell surface antigens. It also has become apparent that sialylated antigen plays an important role in carcinogenesis. Changes in sialylated antigens in relation to carcinogenesis are divided into the two patterns, one is incomplete sign of sialylated antigens, the other is new formation of sialylated antigens. It is well known that STN belongs to the former and SLX, BM-1 belong to the latter.

It is defined from the result of this study that SLX antigen is newly generated in association with carcinogenesis and is not present in non-cancer cells. Furthermore, BM-1 antigen is not present in the normal cells and generated in accordance with atypism of cells. On the other hand, STN antigen has been exaggerated in advanced cancer cells, meanwhile it has been expressed in the presence of carcinoma regardless the grades of cell atypism.

The expression of STN in carcinoma is three times greater than that in benign adenoma. Therefore, it is speculated that STN antigens are accumulated by impaired synthesis of sialylated antigen in the cells adjacent to carcinoma.

Recent development of FCM made it possible to measure nuclear DNA content quickly and objectively and great strides in the development of cell biology have been achieved.

Aneuploidy pattern is one of the tumor markers and a tool of prediction of the prognosis for malignant diseases. On the other hand, the more the sizes of adenoma increase, the higher the incidences of aneuploidy pattern become. Therefore, measurement of nuclear DNA content is of great value to know transformation of carcinoma from adenoma prior to morphologically definitive alteration. It is, however, reported that DI in adenoma is commonly lower than that in carcinoma.

In this study, it was defined that two out of 15 patients with adenoma showed aneuploidy pattern, ranging 1.3 cm and 1.2 cm in size, that revealed villous adenoma and tubulo-villous adenoma in histology. The mucin composition was composed of a mixture of sulphomucin and sialomucin. In addition, tubulo-villous adenoma showed a predominant composition of sulphomucin, demonstrating moderately positive expression (+) of SLX, STN and BM-1 of sialy-lated antigens.

The epitheliums adjacent to or apart from adenoma were evaluated in terms of nuclear DNA content and the expression of STN.

In this series, all adenomas revealed diploidy pattern and DNA analysis in the neighboring mucosa clarified that a diploidy pattern was commonly revealed in all but two that showed an aneuploidy pattern with highly strong expression of STN antigen.

In conclusion, a strong expression of STN in the transitional and neighboring mucosas to adenoma is suggestive of a presence of carcinogenesis and/or carcinoma. Physicians should be aware of carcinogenetic changes from adenoma in the follow-up of adenoma-bearing patients.

Acknowledgement

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