Expression of Sialyl-Tn Antigens in Normal Squamous Epithelium, Dysplasia, and Squamous Cell Carcinoma in the Esophagus

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ABSTRACT

Two monoclonal antibodies, TKH2 and B72.3, directed toward the Sialyl-Tn antigen (SA 2, GalNAcα-3-Ser/Thr), were examined immunohistochemically to analyze the expression of these antigens in 20 areas of normal squamous epithelium, 12 lesions of dysplasia, and 86 cases of squamous cell carcinoma including 32 with superficial carcinoma in the esophagus. No expression of TKH2 or B72.3 was found in the normal squamous epithelium. Among the 12 lesions of dysplasia only one expressed TKH2. In carcinoma the expression of TKH2 and B72.3 was found in 40 (47%) and 21 (24%) of the 86 carcinomas, respectively; however, the number of positive malignant cells with TKH2 and B72.3 totaled less than half that in the tissue, and no relationship was found between either prognosis or lymph node metastasis and the expression of Sialyl-Tn antigen. These results indicate that Sialyl-Tn antigen appears in the process of malignant transformation or tumor progression in esophageal squamous cell carcinoma; however, the positive expression of Sialyl-Tn antigen was not directly connected to either prognosis or lymph node metastasis.

INTRODUCTION

Patients with esophageal squamous cell carcinoma show one of the most unfavorable prognoses among patients with carcinoma of gastrointestinal tract. The 5-yr survival rates of surgical treatment for patients with advanced esophageal carcinoma are 15.6%, and even in patients with early stage esophageal carcinoma, the 5-yr survival rates remain 56.7% (1). Therefore, a detailed study focusing on the biological behavior of esophageal carcinoma is needed to improve the prognosis.

Oncogenic transformation is often associated with changes in glycosylation in either glycolipids or glycoproteins in cell membranes (2-4). Among the variety of changes in glycosylation, Sialyl-Tn antigen, which is a mucin-associated antigen, is a result of premature sialylation of the Tn antigen by 2,6-sialyltransferase (5). This structure does not apparently undergo further glycosylation. In addition, it did not allow any further normal oligosaccharide elongation. Immunohistochemically monoclonal Sialyl-Tn antigen was detected by TKH2 which was developed against ovine submaxillary mucin (6). Recently Kjeldsen et al. (6) and Gold and Mattes (7) observed that B72.3, which is a murine-associated antigen, is a result of premature sialylation of the Tn antigen by 2,6-sialyltransferase (5). This structure was generated against human breast carcinoma cells (8), also recognizes Sialyl-Tn antigen. Sialyl-Tn antigen was studied in adenocarcinoma of the gastrointestinal tract, such as the colon, stomach, and pancreas (9-16), and was reported to be expressed strongly in malignant lesions. There have previously been no investigations of this antigen focusing on esophageal squamous cell carcinoma.

The aim of the present study was to define the expression of Sialyl-Tn antigen in the normal squamous cell epithelium, dysplasia, and carcinoma of esophagus by using immunohistochemical techniques, and, in addition, to examine the relationship between the expression of Sialyl-Tn antigen and either clinicopathological factors or the prognosis of esophageal carcinoma.

MATERIALS AND METHODS

Surgically resected esophageal squamous cell carcinoma from 86 cases was studied. All tissue specimens were registered during the period from 1965 to 1991 and were filed in the Department of Surgery II, Kyushu University Hospital. The 86 cases selected in this study did not receive any preoperative treatment, such as radiation or chemotherapy. All 86 cases were histologically diagnosed as squamous cell carcinoma of the esophagus. Of 86 cases 32 had carcinoma confined to either the intraepithelium, mucosa, or submucosa which fell under the classification of Tis and T1, by the new T-N-M classification (17). Of 86 cases 12 had dysplastic lesions in addition to carcinoma. Of 86 cases, 72 were men, and 14 were women. The mean age was 63 yr. For immunohistochemical staining 86 lesions of esophageal carcinoma, 12 lesions of dysplasia, and 20 areas of normal epithelium distant from carcinoma were used. The surgically resected esophageal tissues were fixed in 10% formalin and embedded in paraffin. The tissue blocks were cut in 5-μm slices, deparaffinized, and sent into the histochemical staining 86 lesions of esophageal carcinoma. The lesions of dysplasia, and 20 areas of normal epithelium distant from carcinoma were used. The sections were incubated with monoclonal B72.3 (Centcore) and monoclonal TKH2 immunoglobulin G1 against ovine submaxillary mucin (8) at 4°C overnight. The sections were treated with anti-mouse immunoglobulin G-biotin complex (Vector Laboratories) followed by avidin-peroxidase complex and were then stained with 3,3'-diaminobenzidine solution with 0.15% hydrogen peroxide.

Since the stained part of the Sialyl-Tn antigen in (a) the keratin layer in normal epithelium, (b) the keratinized area of the central part in cancer pearls, and (c) the necrotic areas in the tumor tissue was considered to represent a nonspecific reaction, it was thus judged as not having a positive expression.

RESULTS

Table 1 summarized the expression of B72.3 and TKH2 in the normal squamous epithelium (20 areas), dysplasia (12 lesions), and carcinoma (86 lesions). In normal squamous epithelium, neither B72.3 nor TKH2 was expressed in the cells of either the basal layer or the parabasal layer, although in a part of the esophageal glands of the submucosa and lamina propria, B72.3 and TKH2 were expressed in a few cases. In dysplasia none of lesions was stained with B72.3, while only one lesion was stained with TKH2.

In esophageal carcinoma TKH2 showed a more sensitive reaction than B72.3. B72.3 and TKH2 were expressed in 21 (24%) and 40 (47%) of the 86 lesions with carcinoma, respectively. All 21 lesions with a positive expression of B72.3 were involved in the lesions with a positive expression of TKH2. In positive lesions with either B72.3 or TKH2, antigens were located in the cell membranes and in the cytoplasm of malignant cells; however, they were not distributed in the surrounding stromal tissue adjacent to the malignant cells (Fig. 1). The number of stained cells with B72.3 or TKH2 in positive cases was less than 50% of the tumor cells.

The positive expression of B72.3 and TKH2 was compared with various clinicopathological factors in esophageal carcinoma (Table 2). The expression of both B72.3 and TKH2 correlated only to venous invasion; however, even in positive cases the cancer cells in the vessel did not always show positive expression. No other clinicopathological factors correlated to the positive expression of the two tumor-associated antigens.

The expression of B72.3 and TKH2 in the primary lesion was compared with that in metastatic lesions of the lymph node in 12 cases (Table 3). There was no case in which B72.3 was expressed in both

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containing oligosaccharide side chains that are connected by O-glycosidic linkage to either serine or threonine residues in the protein backbone. Sialyl-Tn antigen was not always specific for malignant tissue. This antigen was also expressed in a part of the mucin-producing normal glands such as goblet cells of the colon and parietal cells of the stomach (6, 12). In a few cases of this study the esophageal glands in the submucosa also expressed Sialyl-Tn antigen; however, no expression was found in the normal squamous epithelium in the esophagus. Considering that the normal squamous epithelium itself does not produce mucin or mucin-like substances, these results may be within normal expectations. In contrast to the nonexpression of Sialyl-Tn antigen in normal squamous epithelium, this antigen was expressed in squamous cell carcinoma. This result means that some part of malignant transformation in the esophagus was accompanied by the appearance of a premature sialylation of the O-linked carbohydrate core. It may also be possible to think that some percentage of carcinomas originate from the esophageal glands (18). In dysplasia, since none of the lesions except for one showed a positive expression, no premature sialylation of the O-linked carbohydrate core seemed to occur. Although it remains controversial as to whether dysplasia is a preneoplastic lesion or not (19–23), the positive expression of Sialyl-Tn antigen is able to distinguish carcinoma from dysplasia in the primary and metastatic lesions. However, in 5 cases with a TKH2 positive expression of primary lesions, 2 cases showed a positive expression in metastatic lesions, but 3 other cases showed a negative expression in metastatic lesions. In 5 cases with TKH2 positive expression in metastatic lesions, 3 cases did not show a positive expression in the primary lesions. These results indicated that the expression of Sialyl-Tn antigen had no correlation to lymph node metastasis.

The survival curves are shown with respect to the expression of either B72.3 or TKH2 in Fig. 2. The 3-yr survival rates were 32% with a positive expression of B72.3 and 37% with a negative expression of B72.3. There was no statistical difference. The 3-yr survival rates were 29% with a positive expression of TKH2 and B72.3 and 42% with a negative expression of TKH2. In addition, there was no statistical difference.

**DISCUSSION**

Sialyl-Tn antigen, which is a mucin-associated antigen, has been known to be expressed strongly in adenocarcinomas which are generated from mucin-producing tissue, such as the colon, pancreas, and stomach (9–16). Mucins are high-molecular-weight glycoproteins

<table>
<thead>
<tr>
<th>B72.3</th>
<th>TKH2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Dysplasia</td>
</tr>
<tr>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>0</td>
<td>46</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

**Table 2 Clinicopathological factors and expression of B72.3 and TKH2 in 86 cases of esophageal carcinoma**

<table>
<thead>
<tr>
<th>Factors</th>
<th>B72.3</th>
<th>TKH2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>62.7</td>
<td>63.5</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Location</td>
<td>Upper</td>
<td>Middle</td>
</tr>
<tr>
<td>Differentiation</td>
<td>Well</td>
<td>Moderate</td>
</tr>
<tr>
<td>Depth</td>
<td>ep-sm</td>
<td>pm-a</td>
</tr>
<tr>
<td>Lymphatic invasion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous invasion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

**Table 3 Expression of B72.3 and TKH2 in primary lesion and metastatic lymph node of esophageal carcinoma (12 cases)**

<table>
<thead>
<tr>
<th>Case</th>
<th>B72.3</th>
<th>TKH2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>Metastasis</td>
<td>Primary</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.
SIALYL-TN ANTIGEN IN ESOPHAGEAL CARCINOMA

It was reported that Sialyl-Tn antigen was expressed in noncarcinomatous tissues such as the transitional mucosa in the colon (12, 15) or chronic pancreatitis in the pancreas (10, 13). Compared with mucin-producing tissue such as the colon or pancreas, Sialyl-Tn antigen in the esophagus may be useful for detecting malignant lesions.

The sensitivity of Sialyl-Tn antigen for esophageal carcinoma is low, and even in positive cases expression was seen in less than half of the malignant cells in the tissue. In other tissues such as the colon, pancreas, or stomach, Sialyl-Tn antigen was expressed more frequently and seemed to be closely correlated to malignant transformation (9, 14-16, 24, 25). However, this was also controversial (12, 13). In this study, the expression of Sialyl-Tn antigen did not correlate to prognosis. The expression of Sialyl-Tn antigen in the primary lesion was also not associated with that in metastatic lesions of the lymph nodes. These results indicate that the positive expression of Sialyl-Tn antigen does not represent either the tumor progression or the metastatic potential of the esophageal carcinoma. Nakasaki et al. (11) reported that a variety of tumor-associated antigens were expressed in a single tumor of either the colon or stomach. Such diversity may develop gradually during tumor progression, resulting eventually in a complex mosaic pattern of antigen expression. In esophageal carcinoma a variety of tumor-associated antigens were also expressed (26). Sialyl-Tn antigen was mucin-associated antigen and does not exist in the normal squamous epithelium. However, during the process of malignant transformation or tumor progression, the Sialyl-Tn antigen does begin to be expressed as a part of the heterogeneous expression of tumor-associated antigens.

REFERENCES


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