Expression of Tn and Sialyl-Tn Antigens in the Neoplastic Transformation of Uterine Cervical Epithelial Cells

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ABSTRACT

The expression of simple mucin-type carbohydrate antigens, Tn and sialyl-Tn antigens, was evaluated by immunohistochemical staining with monoclonal antibodies in normal squamous epithelium, dysplasia, carcinoma in situ, and invasive squamous cell carcinoma of the uterine cervix. The expression of the Tn antigen detected by HB-Tn1 and B1.1 was found in 17 (20%) and 19 (23%) of the 83 invasive carcinomas, respectively, but was not found in the 36 normal squamous epithelia, 22 severe dysplasias, or 24 carcinomas in situ. The sialyl-Tn antigen was detected by HB-STn1 and TKH-2 in 14 (64%) and 11 (50%) of the 22 severe dysplasias, 13 (54%) and 10 (42%) of the 24 carcinomas in situ, and 48 (58%) and 42 (51%) of the 83 invasive carcinomas, respectively, but was completely absent in the 36 normal squamous epithelia. Coexpression of the sialyl-Tn antigen was observed in 89% of the cases expressing the Tn antigen. No significant difference was observed between the immunoreactivities of the antigens in the metastatic lymph nodes and primary tumors. No correlation was found between the expression of each antigen and clinical stage, histologic type, depth of invasion, lymphatic and vessel permeation, lymph node metastasis, or 5-year survival rate. The expression of Tn and sialyl-Tn demonstrates a specific change in the neoplastic progression from carcinoma in situ to invasive carcinoma and from normal to dysplasia, respectively, in squamous cell neoplastic lesions of the cervix. Tn and sialyl-Tn antigens may be useful markers for biologic investigation of neoplastic transformation in cervical squamous cell carcinoma.

INTRODUCTION

Aberrant glycosylation of glycoproteins or glycolipids, due to the incomplete synthesis of carbohydrate chains and accumulation of their precursors, is associated with neoplastic transformation (1, 2). Among such precursor glycoconjugates, Tn (GalNAcα1→6GalNAcα1→3Galβ1→4GlcNAc) and sialyl-Tn (neuraminic acidα1→6GalNAcα1→3Galβ1→4GlcNAc) antigens, which belong to simple mucin-type (O-linked) core carbohydrate antigens, are known to be onco developmental cancer-associated antigens that are expressed frequently in many tumors but rarely in normal tissues (3).

Many immunohistochemical studies using monoclonal antibodies have demonstrated the expression of Tn or sialyl-Tn antigen in normal and malignant tissues. Recently, a relationship between the expression of sialyl-Tn antigen, tumor differentiation, metastatic rate, and clinical outcome has been demonstrated in colorectal and gastric cancers (4–7). Previously, we investigated Tn antigen expression in uterine cervical cancer with histochemical staining using lectins and showed that Tn antigen expression is related to lymph vascular space invasion and lymph node metastasis. Therefore, Tn expression may be a useful marker of metastatic potential (8, 9). There are several reports of Tn and sialyl-Tn antigen expression in adenomas, borderline cancers, hyperplasias, and dysplasias in epithelial tissues (10–20). Unfortunately, no detailed study has been performed describing the changes of antigenic expression during the natural sequential history of neoplastic transformation from normal to metastatic carcinoma in uterine cervical tissues. Squamous cell carcinoma of the uterine cervix is the most representative neoplasm, in which the natural history of neoplastic transformation has become clear. The relationship between the antigenic expression and clinicopathologic aggressiveness in squamous cell carcinoma of the cervix is unclear.

Our immunohistochemical study using monoclonal antibodies was performed to investigate the change in expression of these antigens in normal squamous epithelium to squamous cell carcinoma of the cervix and metastatic lesions and the clinicopathological significance.

MATERIALS AND METHODS

Cervical tissue was obtained from patients who underwent surgical resection in the Department of Obstetrics and Gynecology at Tokushima University Hospital. All specimens were fixed in 10% formalin, embedded in paraffin, and cut into 4-μm serial sections. They consisted of 22 severe dysplasias; 24 carcinomas in situ; 83 invasive squamous cell carcinomas (stage Ia, 17; stage Ib, 21; stage Ia, 14; and stage IIb, 31), including 27 metastatic pelvic lymph nodes respective to primary tumors; and 36 normal morphologic squamous epithelia at least 1 cm remote from the neoplastic lesions. Thirty-one cases of cervical adenocarcinoma also were examined. The histologies of invasive squamous cell carcinomas were 13 keratinizing, 47 large cell nonkeratinizing, and 6 small cell nonkeratinizing types. The clinical stage and histological classification were determined according to the systems of the International Federation of Gynecology and Obstetrics and WHO. The degree of lymphatic and vessel permeation was categorized into two groups: none to mild, in which few if any cancer cells were found in the capillary spaces in H&E-stained sections; and moderate to severe, in which appreciable numbers of cancer cells were observed. The depth of invasion was measured from the surface at the thickest area of the malignant tissue.

Tn and sialyl-Tn antigens were detected by immunohistochemical staining by the avidin-biotin-peroxidase complex method using two monoclonal antibodies for each antigen (HB-Tn1 and B1.1 specific for Tn antigen, HB-STn1 and TKH-2 specific for sialyl-Tn antigen). The immunogens and isotypes of these antibodies were as follows: HB-Tn1, asialo-ovine submaxillary mucin, mouse IgM (DAKO, Glostrup, Denmark); B1.1, synthetic GalNAcα1→3Galβ1→4GlcNAc; HB-STn1, ovine submaxillary mucin, mouse IgG1 (DAKO); TKH-2, ovine submaxillary mucin, mouse IgG1 (Otsuka, Tokushima, Japan).

After deparaffinization and rehydration, the sections were immersed in 0.3% H2O2 in absolute methanol for 20 min to block endogenous peroxidase activity and then washed three times with 0.1 M PBS (pH 7.4). Normal nonimmune serum was applied for 20 min to eliminate nonspecific staining and then removed by blotting. Each primary antibody, at a dilution of 1:75 in PBS, was incubated for 60 min at room temperature and washed with PBS. A biotinylated secondary antibody was added for 30 min and then washed with PBS. Sections were treated with avidin-biotin-peroxidase complex solution (Vector, Burlingame, CA) for 45 min and then washed with PBS. The slides were treated with 0.05% diaminobenzidine and 0.01% H2O2 in PBS for 5–7 min, counterstained with Mayer’s hematoxylin, dehydrated, and mounted. In each experiment, negative controls were treated by substituting normal mouse serum for primary monoclonal antibody.

Staining was considered positive when more than 5% of the cancer and related cells were stained. Results were assessed and scored by two independent observers. Life table analysis was calculated by the Kaplan-Meier method and the generalized Wilcoxon test. Other statistical analyses using the χ2 test

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The abbreviations used are: GalNAc, N-acetyl-D-galactosamine; VVA, Vicia villosa agglutinin.
Table 1: Expression of Tn and sialyl-Tn antigens in the uterine cervical tissues and metastatic lymph node of squamous cell carcinoma

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. of cases</th>
<th>HB-Tn1 (%)</th>
<th>B1.1 (%)</th>
<th>VVA (%)</th>
<th>HB-STn1 (%)</th>
<th>TKH-2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal squamous epithelium</td>
<td>36</td>
<td>0 (0)*</td>
<td>0 (0)*</td>
<td>0 (0)*</td>
<td>0 (0)*</td>
<td>0 (0)*</td>
</tr>
<tr>
<td>Severe dysplasia</td>
<td>22</td>
<td>0 (0)*</td>
<td>0 (0)*</td>
<td>0 (0)*</td>
<td>14 (64)</td>
<td>11 (50)</td>
</tr>
<tr>
<td>Carcinoma in situ</td>
<td>24</td>
<td>0 (0)*</td>
<td>0 (0)*</td>
<td>0 (0)*</td>
<td>13 (54)</td>
<td>10 (42)</td>
</tr>
<tr>
<td>Invasive squamous cell carcinoma</td>
<td>83</td>
<td>17 (20)</td>
<td>19 (23)</td>
<td>11/25 (44)</td>
<td>48 (58)</td>
<td>42 (51)</td>
</tr>
<tr>
<td>Metastatic lymph node</td>
<td>27</td>
<td>9 (23)</td>
<td>7 (26)</td>
<td>13/14 (93)</td>
<td>16 (59)</td>
<td>13 (48)</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>31</td>
<td>20 (65)</td>
<td>23 (74)</td>
<td></td>
<td>23 (74)</td>
<td>20 (65)</td>
</tr>
</tbody>
</table>

* Percentages of positive cases.

The staining data of VVA were quoted from our previous studies (Refs. 8 and 9). The positive incidence of the same samples are shown.

P < 0.01 compared with the invasive squamous cell carcinoma group.

P < 0.01 compared with the neoplastic groups.

P < 0.05 compared with the invasive squamous cell carcinoma group.

RESULTS

Staining results are summarized in Table 1. Our previous data of histochemical staining with VVA lectin are also shown in Table 1 for comparison. In separate experiments using two monoclonal antibodies (HB-Tn1 and B1.1), the Tn antigen was detected only in invasive squamous cell carcinomas and not in normal squamous epithelium or intraepithelial neoplasias. The positive incidence (20% by HB-Tn1 and 23% by B1.1) in invasive cancer was significantly higher than in normal (P < 0.01) and intraepithelial neoplastic epithelium (P < 0.05). The sialyl-Tn antigen was expressed in approximately one-half of the cases of all neoplastic cells, including invasive squamous cell carcinomas, but was completely absent in normal squamous epithelium. The positive incidence of sialyl-Tn antigen in severe dysplasia (64% by HB-STn1 and 50% by TKH-2), carcinoma in situ (54% and 42%, respectively, and invasive squamous cell carcinoma (58% and 51%, respectively) was significantly higher than in normal squamous epithelium (P < 0.01). In adenocarcinoma, the antigens were expressed more frequently than in squamous cell carcinoma (Table 1). The antigen-staining patterns by the two different antibodies were similar. The antigens were dispersed on the cell membrane, in the cytoplasm, and in central and peripheral areas of the neoplastic cell nest; however, they were expressed predominantly in the cytoplasm, supranuclear (Golgi) area, and peripheral nest area. The antigen patterns were highly variable with staining, ranging from every neoplastic cell to small foci of staining; however, the expression rate was under 30% in most of the neoplastic cells. Coexpression of the sialyl-Tn antigen was observed in 89% of the cases expressing the Tn antigen. A representative case for Tn and sialyl Tn antigen expression is shown in Fig. 1.

The expression of both antigens was compared with various clinicopathological features in invasive cervical carcinoma. No significant difference was observed between the expression of each antigen, clinical stage, histological type, depth of invasion, parametrial spread, lymphatic and vessel permeation, lymph node metastasis, or 5-year survival rate (Table 2). Furthermore, no significant difference in the immunoreactivities of these antigens was seen between the primary tumor and their respective metastatic lymph nodes. No statistical difference in the 5-year survival curves (Fig. 2) when compared with antigen expression was demonstrated.

DISCUSSION

In the present immunohistochemical study, we demonstrate for the first time that the expression of the Tn antigen appears in invasive cervical squamous cell carcinoma but not in normal squamous epithelium, severe dysplasia, or carcinoma in situ. The sialyl-Tn antigen is expressed in severe dysplasia, carcinoma in situ, and invasive cervical squamous cell carcinoma but not in normal squamous epithelium. These results indicate that both antigens are neoplastic markers with high specificities. The expression of Tn and sialyl-Tn antigens is closely linked to the neoplastic change from carcinoma in situ to invasive squamous cell carcinoma and from normal squamous epithelium to severe dysplasia, respectively. Our results suggest that shorter or more immature carbohydrate chains such as the Tn antigen tend to appear in the more advanced stages of neoplastic transformation. Clinically, analysis of these two antigens may allow us to distinguish each stage of neoplastic transformation in the squamous epithelium of the cervix.
Tn AND SIALYL-Tn EXPRESSION IN NEOPLASTIC TRANSFORMATION

Table 2 Clinicopathological features and expression of Tn and sialyl-Tn antigens in invasive squamous cell carcinoma of the uterine cervix

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of cases</th>
<th>Tn (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>HB-Tn1</th>
<th>B1.1</th>
<th>HB-STn1</th>
<th>TKH-2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ia</td>
<td>17</td>
<td>2 (12)</td>
<td>2 (12)</td>
<td>10 (59)</td>
<td>8 (47)</td>
<td></td>
</tr>
<tr>
<td>Ib</td>
<td>21</td>
<td>5 (24)</td>
<td>6 (29)</td>
<td>11 (52)</td>
<td>10 (48)</td>
<td></td>
</tr>
<tr>
<td>IIa</td>
<td>14</td>
<td>2 (14)</td>
<td>2 (14)</td>
<td>7 (50)</td>
<td>5 (36)</td>
<td></td>
</tr>
<tr>
<td>IIb</td>
<td>31</td>
<td>8 (26)</td>
<td>9 (29)</td>
<td>20 (65)</td>
<td>19 (61)</td>
<td></td>
</tr>
<tr>
<td><strong>Histological type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Keratinizing</td>
<td>13</td>
<td>2 (15)</td>
<td>3 (23)</td>
<td>9 (69)</td>
<td>6 (46)</td>
<td></td>
</tr>
<tr>
<td>Large cell nonkeratinizing</td>
<td>47</td>
<td>11 (23)</td>
<td>11 (23)</td>
<td>26 (55)</td>
<td>25 (53)</td>
<td></td>
</tr>
<tr>
<td>Small cell nonkeratinizing</td>
<td>6</td>
<td>2 (33)</td>
<td>3 (50)</td>
<td>3 (50)</td>
<td>3 (50)</td>
<td></td>
</tr>
<tr>
<td><strong>Depth of invasion</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;5 mm</td>
<td>17</td>
<td>2 (12)</td>
<td>2 (12)</td>
<td>10 (59)</td>
<td>8 (47)</td>
<td></td>
</tr>
<tr>
<td>&gt;5 mm</td>
<td>66</td>
<td>15 (23)</td>
<td>17 (26)</td>
<td>38 (58)</td>
<td>34 (52)</td>
<td></td>
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<tr>
<td><strong>Lymphatic and vessel permeation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>50</td>
<td>9 (18)</td>
<td>9 (18)</td>
<td>28 (56)</td>
<td>24 (48)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>33</td>
<td>8 (24)</td>
<td>10 (30)</td>
<td>20 (61)</td>
<td>18 (55)</td>
<td></td>
</tr>
<tr>
<td>Lympathic and vessel permeation</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>None to mild</td>
<td>59</td>
<td>11 (19)</td>
<td>13 (22)</td>
<td>33 (56)</td>
<td>30 (51)</td>
<td></td>
</tr>
<tr>
<td>Moderate to severe</td>
<td>24</td>
<td>6 (25)</td>
<td>6 (25)</td>
<td>15 (63)</td>
<td>12 (50)</td>
<td></td>
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<tr>
<td><strong>Lymph node metastasis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>54</td>
<td>11 (20)</td>
<td>11 (20)</td>
<td>30 (56)</td>
<td>28 (52)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>29</td>
<td>6 (21)</td>
<td>8 (28)</td>
<td>18 (62)</td>
<td>14 (48)</td>
<td></td>
</tr>
<tr>
<td><strong>5-yr survival</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥5 yr</td>
<td>67</td>
<td>15 (22)</td>
<td>17 (25)</td>
<td>39 (58)</td>
<td>33 (49)</td>
<td></td>
</tr>
<tr>
<td>&lt; 5 yr</td>
<td>16</td>
<td>2 (13)</td>
<td>2 (13)</td>
<td>9 (56)</td>
<td>9 (56)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Percentages of positive cases.

Mucin is usually produced by glandular cells. Normal squamous epithelium does not express these mucin-type carbohydrate antigens; therefore, their expression by neoplastic transformation strongly supports the hypothesis that cervical squamous cell carcinoma derives from bipotential reserve cells, which can differentiate to either squamous or glandular cells. In this regard, cervical adenocarcinoma should have a prevalence for expression of these antigens compared with other carcinomas. Indeed, the frequency of expression of these antigens was found to be higher in adenocarcinoma of the uterine cervix (Table 1).

Because cell surface carbohydrate antigens are thought to be important in cell-cell interactions such as cell recognition and response, the atavistic expression of Tn and sialyl-Tn antigens may have clinicopathological implications. In a previous histochemical study using VVA lectin, which has a high specificity for Tn antigen (21), we demonstrated that Tn antigen expression correlated with vascular permeation, parametrial spread, metastasis to pelvic lymph nodes, and a decreased 5-year survival rate in cervical squamous cell carcinoma (8, 9). In the present study, neither Tn nor sialyl-Tn antigen expression correlated with aggressive tumor biology as invasion, metastasis, or survival. The complete lack of correlation of Tn antigen expression with metastatic potential is different from our previous study. This indicates that the lectin staining by VVA is more indicative than the anti-Tn monoclonal staining for estimating the prognosis of invasive cervical cancer. This contradiction is due to the probes used for the detection of the Tn antigen. No correlation of Tn antigen expression with aggressive metastatic behavior has been observed in breast, gastric, or urinary bladder carcinoma (16, 22, 23), although the clinicopathological significance of sialyl-Tn antigen expression remains controversial (4-7, 16, 20, 22-25). A report of VVA lectin histochemistry demonstrated that elevated Tn antigen expression is related to an overall poor prognosis in urinary bladder cancer (26). In breast, gastric, and colorectal carcinoma, several reports describe a positive correlation between tumor metastasis and the expression of the ligand for Helix pomatia agglutinin lectin (27-31), which binds specifically to α- and β-linked GalNAc residues (21). Anti-Tn mono-

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Fig. 2. Five-year survival curves of patients according to the expression of Tn (HB-Tn1 and B1.1) and sialyl-Tn (HB-STn1 and TKH-2) antigens.
clonal antibody is specific for a Tn epitope that has three consecutive GalNAc-O-Ser/Thr residues (32). VVA is believed to be specific for GalNAc-O-Ser/Thr but may cross-react with antigens possessing the α- or β-GalNAc structure. From comparisons of their carbohydrate-binding specificities, some carbohydrate structures, not recognized by anti-Tn monoclonal antibody but recognized by VVA lectin, may correlate with metastasis and poor clinical outcome. Some GalNAc-O-Ser/Thr glycoproteins not having three consecutive GalNAc-O-Ser/Thr residues essential for Tn antigenicity, or another monosaccharide correlate with metastasis and poor clinical outcome. Some GalNAc-O-Ser/Thr glycoproteins not having three consecutive GalNAc-O-Ser/Thr residues essential for Tn antigenicity, or another monosaccharide such as a unique terminal α- or β-GalNAc unit may play an important role in the development of metastasis through alterations of cell-cell or cell-matrix interactions. The molecular basis for these differential binding properties awaits further study.

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