Expression of MUC1 and MUC2 Mucin Gene Products in Human Ovarian Carcinomas

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Background: Aberrations in expression of mucin glycoproteins have been observed during malignant transformation of human ovarian epithelium. To date, several secretory mucin genes designated the MUC gene family have been identified, of which MUC1 encodes a mammary-type and MUC2 an intestinal-type epithelial mucin. However, information on the expression and potential value of MUC1 and MUC2 mucins in ovarian cancer is limited.

Methods: This study investigated immunohistochemical expressions of MUC1 and MUC2 mucins in 23 benign and 45 malignant human ovarian tumors to assess their clinicopathological relevance.

Results: All benign serous tumors and also associated normal-appearing epithelia expressed MUC1 mucin on the cell surfaces. Benign mucinous tumors occasionally expressed MUC1 and MUC2 mucins. Most serous carcinomas (19/21; 90%) expressed MUC1 but not MUC2 mucin. Of the 16 mucinous carcinomas, 10 (62%) and five (31%) expressed MUC1 and MUC2 mucins, respectively. Four of the five clear cell and the three endometroid type carcinomas expressed MUC-1 but not MUC-2 mucin. A significant association was found between a high expression of MUC1 and histological grade \( (P = 0.005) \) and also disease stage \( (P = 0.001) \).

Conclusion: These results suggest that a high expression of MUC1 may contribute to a poor prognosis in ovarian carcinoma.

Key words: mucins – MUC1 – MUC2 – immunohistochemistry – ovarian carcinoma – prognosis

INTRODUCTION

Human epithelial tumors express various mucin glycoproteins on their cell surfaces. Mucins comprise a family of high molecular weight glycoproteins with a large number of O-glycosylated tandem repeat domains varying in number, length and extent of O-glycosylation (1–4). Several human secretory mucin genes designated MUC genes have been identified, among which MUC1 gene encodes a membrane form of mucin-like O-glycoprotein or episialin, which is over-expressed in carcinoma of the breast and pancreas. The other MUC genes include MUC2 (prominent in the small and large intestine), MUC3 (predominant in the small intestine), MUC4 (universal for the epithelia), MUC5B (essentially in glandular acini in the submaxillary gland), MUC5C (present in respiratory and gastric tracts) (5,6), MUC6 (prominent in the stomach and gall bladder) (7) and MUC7 (mainly in the submandibular gland) (8). Protein products of MUC genes have been studied in tumors arising from various organs, including the breast, colon, pancreas and ovary (9–13). In breast and colon carcinomas, MUC1 gene products have been correlated with advanced disease stage (9,14,15). Furthermore, MUC1 mucin could facilitate dissemination of carcinoma cells by blocking cell–cell adhesion (16). However, little is known about the expression of glycoproteins encoded by MUC genes in ovarian carcinomas and information on their potential prognostic value in these tumors is lacking.

In the present study, we examined immunohistochemical expression of MUC1 and MUC2 mucins in 59 benign and malignant human ovarian tumors and correlated the results with the established clinicopathological factors of the disease to assess their potential relevance.

MATERIALS AND METHODS

Serial 4 \( \mu \)m sections from representative paraffin-embedded tissue specimens of 23 benign and 45 malignant human ovarian tumors were used after approval by the Institutional Review Board. The ages of the ovarian carcinoma patients...
ranged from 21 to 88 years (median: 54 years). A section from each tissue was stained with hematoxylin and eosin to confirm the diagnosis. Histological types and grades were determined using the World Health Organization Criteria (17) and the stage of tumors was assessed according to the International Federation of Gynecology and Obstetrics staging system (18). All carcinoma patients in this study received postoperative chemotherapy with various regimens for high-risk early stage (stage I, grade 3; stage IC; any stage II) or advanced disease (stages III and IV). The distribution of histological types and grades and the clinical stages of ovarian carcinomas are shown in Tables 1 and 2.

**ANTIBODIES**

Monoclonal antibodies against MUC1, MUC1-core and MUC2 mucins were obtained from Novocastra Laboratories (Newcastle upon Tyne, UK). Anti-MUC1 and MUC1-core antibodies had been raised against the human breast cancer cell line ZR75-1 and recognize a carbohydrate epitope of the MUC1 glycoprotein and a TRPAPG peptide in tandem repeat of MUC1 core glycoprotein, respectively. Anti-MUC2 antibody had been raised against a synthetic 29-amino-acid peptide of MUC2 glycoprotein (KYPTTTPISTTMVTPTPTGTQTTT) containing one repeat unit of 23 amino acids and part of the next repeat of four amino acids (19).

**IMMUNOHISTOCHEMISTRY**

Serial 4 µm paraffin tissue sections on silanized glass slides were stained using monoclonal antibodies to MUC1 and MUC2 mucins and the streptavidin–biotin–peroxidase complex method. The sections were deparaffinized in xylene and rehydrated in descending ethanol concentrations to distilled water. Subsequently, antigen retrieval in the tissue sections was achieved by placing them in a Coplin jar filled with 0.01 M citrate buffer, pH 6.0, and boiling for 5 min in a microwave oven at 600 W. The sections were then cooled to room temperature, equilibrated in PBS, pH 7.4, for 10 min and treated with 0.3% H₂O₂ in methanol to block endogenous peroxidase activity. Nonspecific antibody binding sites were blocked by 30 min of incubation with normal mouse serum. The primary antibodies were applied at 1:150 dilution overnight at 4°C. Secondary goat anti-mouse antibody (Dako, Japan) and streptavidin–biotin–peroxidase complex (Dako) were used at 1:600 and 1:800 dilutions, respectively, each for 1 h. The sections were washed thoroughly in PBS after each antibody incubation. Peroxidase activity was detected using 0.05% diaminobenzidine containing 0.01% H₂O₂ and counterstaining with hematoxylin.

Negative controls included the use of nonimmune normal mouse serum as the primary antibody. Positive controls consisted of sections from a breast carcinoma and a colon carcinoma previously known to be positive for MUC1 and MUC2, respectively. The interpretation of the stainings was made semiquantitatively by assessing the mean percentage of the stained tumor cells in five microscopic fields at 400× magnification and classified into four groups of 0, <10, 10–50 and >50% positive cells. The areas chosen for evaluation were free from central necrosis and had a relatively constant tumor cell/stromal cell ratio, thus reflecting the number of MUC1- or MUC2-positive tumor cells out of the total viable cancer cells. Tumors with less than 10% of positive cells were regarded as negative.

**Table 1. Expression of MUC1 and MUC2 mucins in human ovarian tumors**

<table>
<thead>
<tr>
<th></th>
<th>No. of cases</th>
<th>MUC1</th>
<th>MUC2</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Neg. &lt;10%</td>
<td>10–50%</td>
<td>&gt;50%</td>
</tr>
<tr>
<td></td>
<td>Neg. &lt;10%</td>
<td>10–50%</td>
<td>&gt;50%</td>
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<tr>
<td>Benign</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>15</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Mucinous</td>
<td>8</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Malignant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>21</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Mucinous</td>
<td>16</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Clear cell</td>
<td>5</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Endometroid</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>3</td>
<td>6</td>
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</table>

**Table 2. Association of histological grades with disease stages**

<table>
<thead>
<tr>
<th></th>
<th>No. of cases</th>
<th>Stage I–II</th>
<th>Stage III–IV</th>
<th>ρ value*</th>
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<tr>
<td>Grade 1–2</td>
<td>29</td>
<td>23</td>
<td>6</td>
<td>0.01</td>
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<tr>
<td>Grade 3</td>
<td>16</td>
<td>7</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>30</td>
<td>15</td>
<td></td>
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</tbody>
</table>

*Fisher’s exact test.
RESULTS

The immunohistochemical staining reactions for MUC1 were in general identical with that for MUC1-core peptide, although the latter showed somewhat limited reactivities. Therefore, the staining results with MUC1 mucin antibody were considered for evaluation. Representative examples of the stainings are demonstrated in Fig. 1. In normal-appearing ovarian epithelia associated with benign lesions, and also benign serous and mucinous ovarian tumors, MUC1 expression was confined to the cell surfaces. In serous, mucinous, clear cell and endometroid type adenocarcinomas, distinct cell membrane and occasional cytoplasmic stainings for MUC1 were observed.

Generally, the staining pattern appeared to be diffuse throughout the tumor areas, including loci of infiltrating cells and even single carcinoma cells. Benign mucinous tumors showed focal and less frequent expressions for MUC1 and

Figure 1. Expression of MUC1 and MUC2 mucins in various histological types of ovarian lesions. Cell surface staining for MUC1 is shown in (A) benign ovarian cystadenoma, (B) serous, (C) clear cell and (D) endometroid types of ovarian carcinomas. (E) Supranuclear staining for MUC2 in a mucinous carcinoma and (F) expression of MUC1 mucin by a single ovarian carcinoma cell released from the primary tumor site and infiltrated into the stroma are seen. Original magnifications: A, B and E, ×200; D, ×100; C and F, ×400.
had advanced-stage disease (low-stage (I–II) and nine of the 16 (56%) high-grade tumors stages. Most low-grade (1–2) tumors (23/29; 79%) also had a MUC1 but not MUC2 mucin. In the present series, the histological grades of tumors correlated well with the disease stages. Most low-grade (1–2) tumors (23/29; 79%) also had a low-stage (I–II) and nine of the 16 (56%) high-grade tumors had advanced-stage disease (P = 0.01) (Table 2). Significant correlations were found between MUC1 expression and histological grades (P = 0.005) and disease stages (P = 0.001) (Table 3). The majority of high-grade (14/16; 87%) and advanced-stage (14/15; 93%) tumors expressed MUC1 mucin in >50% of the tumor cells. Notably, infiltrating tumor cells in the stroma even though single cell in quantity demonstrated distinct MUC1 expression.

**DISCUSSION**

Molecular studies have shown that several different MUC1 isoforms can be generated from the MUC1 gene. The most extensively studied MUC1 isoform is a polymorphic mucin-like type 1 transmembrane protein containing extracellular, transmembrane and cytoplasmic domains. MUC1 is expressed by almost all human glandular epithelial tissues and throughout all regions of the gastrointestinal tract. MUC2 is also a polymorphic mucin but is mostly expressed in the intestinal mucosal cells.

Ovarian cancer is a highly lethal disease. Approximately two-thirds of patients with ovarian carcinoma eventually develop recurrence and die of the disease. Early detection of such patients who may benefit from additional therapies could improve their survival times. In the present study, we demonstrated that a high expression of MUC1 was frequently associated with ovarian carcinomas compared with benign ovarian tumors. In addition, a high expression of MUC1 mucin was significantly associated with a high histological grade and an advanced disease stage, suggesting a potential role in the prognosis of ovarian carcinoma. These results could be supported in part by the findings of a previous study that showed positive reactivity for MUC1 in all of the 13 serous and 20 mucinous ovarian carcinomas (100%) compared with 28 of the 67 (42%) benign serous and mucinous ovarian tumors, although no attempt was made to identify the clinicopathological correlates (12). In the borderline tumors, MUC1 was positive in all seven serous (100%) and 12 of the 16 (75%) mucinous tumors. Another study (13) reported that a minority of the 29 benign ovarian epithelial tumors (34%) but most of the 21 low malignant potential (86%) and 128 invasive ovarian tumors (81%), showed high MUC1 expression on the cell membrane.

Membrane mucins have several functions in epithelial cells, including cytoprotection, extravasation during metastases, maintenance of luminal structure and signal transduction. It has been shown that MUC1 mucin undergoes phosphorylation on both tyrosine and serine residues and, as a consequence, can potentially bind second messenger or adaptor proteins such as Grb2 (20). Moreover, expression of MUC1 protein can enhance tumor initiation and progression in vivo (21). Also, the cytoplasmic domain of the MUC1 protein, depending on cell adhesion, interacts directly with β-catenin (22). These results suggest that MUC1 protein participates in signal transduction and may well play an active role in the oncogenic process.

We found that a high expression of MUC-1 mucin was associated with a high grade and an advanced stage of ovarian carcinoma. In this regard, *in vitro* studies have shown that increasing MUC1 expression results in decreased integrin-mediated cell adhesion to extracellular matrix components (16). Furthermore, Kondo et al. (23), using breast cancer cell lines, reported that overexpression of MUC1 suppressed E-cadherin-mediated intercellular adhesion. They also showed that following treatment of the cells with antisense oligonucleotides, reduced expression of MUC1 led to the enhancement of cell–cell adhesion. In ovarian cancers, low expression of MUC1 in the apical membrane was associated with early stage and good outcome for invasive tumors (13). In addition, low cytoplasmic expression of MUC1 was a predictor for good prognosis, particularly within stage III tumors. It was concluded that MUC1 influences the metastatic ability of ovarian cancer. Interestingly, we noted a distinct positive staining for MUC1 in microinvasive ovarian carcinoma cells and even in single carcinoma cells spreading in the stroma. This finding may point to the potential application of MUC1 mucin expression as a marker to identify micrometastatic cells from ovarian carcinoma in the lymph nodes or other organs which could be otherwise easily missed in routine histological examination.

MUC2 is predominantly expressed in the small and large intestine and is therefore considered as an intestinal mucin. In a previous study (13), it was found that most benign and low malignant potential tumors showed MUC2 expression. In contrast to this finding, we rarely observed MUC2 expression in benign ovarian tumors. Further studies are required to determine the role of MUC2 expression in ovarian tumors.

Overall, the above observations demonstrate that a high expression of MUC1 mucin is frequently associated with epithelial ovarian cancers, which may provide an important

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**Table 3. Association of MUC1 expression with histological grades and disease stages**

<table>
<thead>
<tr>
<th>Grade</th>
<th>No. of cases</th>
<th>MUC1 expression</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;50%</td>
<td>≥50%</td>
</tr>
<tr>
<td>I–II</td>
<td>30</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>III–IV</td>
<td>15</td>
<td>1</td>
<td>14</td>
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*Fisher’s exact test.
insight into new approaches for early detection and therapy. Recently, mucins have attracted interest as potential targets for immunotherapy of cancers of several organs, including the ovaries, and MUC1 mucin may well be a candidate for developing immunotherapy/vaccines for ovarian cancer.

References


