Cellular localization of MUC1 in Benign and Malignant Breast Lesions with the Histological Correlation and the Prognostic Significance

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**Purpose:** MUC1 is a large transmembrane glycoprotein, which is overexpressed in the majority of carcinomas. The high expression of MUC1 is associated with aggressive tumors, with the MUC1 antigen used as a marker to monitor disease progression in breast cancer patients. Although the MUC1 tumor marker is both sensitive and specific for predicting a relapse in breast cancer, it is not commonly used during the follow-up of breast cancer patients. The aim of this study was to evaluate whether the differential patterns of MUC1 expression in different histological types of breast carcinoma could be used to distinguish tumors from benign lesions, and determine its prognostic relevance with other biological parameters.

**Methods:** 22 normal breast, 7 intraductal hyperplasia (IDH) and 307 malignant lesions were selected and immunostained with MUC1. The patterns of reaction were classified as intraluminal border (ILB), cytoplasmic, intercellular membrane (ICM), intranuclear or mixed staining.

**Results:** All the normal breast samples showed weak cytoplasmic staining in the ducts and lobules. All the IDH samples showed moderate cytoplasmic and ILB staining. Of the 307 malignant lesions, only 2 (0.8%) showed negative staining; MUC1 positivity was observed in 4 (1.3%), with only ILB staining; 8 (2.6%) with weak cytoplasmic staining, 16 (5.2%) with weak cytoplasmic and intranuclear staining, 168 (54.7%) with moderate cytoplasmic and ILB staining, and 109 (35.5%) with strong cytoplasmic and ICM staining. MUC1 positivity with a moderate to strong staining intensity was observed in 90.6% of the infiltrating ductal carcinomas (221/244), 96.5% of the intraductal carcinomas (28/29), 87.5% of the infiltrating lobular carcinomas (7/8), 66.6% of the mucinous and secretory carcinomas (10/15), 100.0% of the apocrine carcinomas (5/5) and 100.0% of the medullary carcinomas (6/6). The expression of MUC1 was statistically significant between the histological tumor types ($p = 0.034$), tumor size ($p = 0.046$), and HER-2/neu ($p = 0.004$).

**Conclusion:** These data suggest the expression of MUC1 was different in normal breast, IDH and malignant breast tumors, and was significantly correlated with the histological tumor types, tumor...
size and HER-2/neu oncogene.

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**Key Words**: Breast neoplasms, Immunohistochemistry, MUC1 mucin peptide, Prognosis

**INTRODUCTION**

Mucins are classified into two main forms: the membrane-associated form and the secreted form. (1) The main membrane-associated mucin, MUC1, was first cloned from human breast cancer and mammary epithelium. (1) MUC1 has long been recognized as a tumor-associated antigen. The MUC1 is widely expressed by breast epithelium, and the amount and cellular localization of its expression is variable. Some of the MUC1 expression has been associated with malignant transformation, but the significance of this is controversial. (2-4) This mucin is expressed more intensely in cancers than in normal tissues, and this is the result of upregulated mucin synthesis and/or mucin accumulation that's caused by abnormal transport within the tumor cells. (5) MUC1 is an integral part of the cell membrane in normal breast epithelium, and it is expressed on the apical borders of normal mammary epithelial cells, whereas carcinoma cells exhibit an increased MUC1 expression with different patterns, mainly expressed over the entire cell surface. (2-5)

MUC1 is a heavily glycosylated transmembrane member of the mucin family and it is overexpressed in many tumor types including breast, pancreatic, lung, prostate and ovarian tumors. (6-8) The incidence of MUC1 overexpression in breast and pancreatic tumors has been reported to be as high as 90%. (9) Much interest has been focused on the expression of its members in breast cancer because of their potential roles as prognostic indicators and their involvement in cancer therapy. (10, 11) Elevated MUC1 expression has been associated with an increased metastatic potential and poor survival. (6, 7, 11, 12) On the other hand, MUC1 antibodies are mainly associated with lactation and with a non-pregnant status. These observations are in line with the importance of pregnancy for the risk of breast cancer, and this creates opportunities to use MUC1 for risk assessment. (13) MUC1 is a promising tumor antigen for performing target-directed immunotherapy and creating vaccines against human breast cancer cells. (14) Humoral and cellular responses to MUC1 have been reported in breast cancer patients, therefore, MUC1 is being evaluated as a target for immune intervention therapy. (11, 14-17)

In the present study, we examined the expressions of MUC1 in 307 patients with breast carcinoma, and these subjects' clinical features and pathologic types had been characterized, we also included 22 cases of normal breast and 7 cases of IDH. The objective of the present work was to determine the distribution pattern of MUC1 in each type of carcinoma and to evaluate whether the differential patterns of mucin expression could be used to distinguish malignant tumors from benign lesions. Further we evaluated its correlation with the other prognostic variables, as well as the clinicopathologic data and biological parameters, including tumor recurrences.

**METHODS**

Twenty-two samples of normal breast tissue, 7 cases of IDH and 307 cases of malignant lesions were selected at the Our Lady Mercy Hospital and Uijeongbu St. Mary's Hospital, from 1998 and 2004. The paraffin embedded tissue blocks were stained by using immunohistochemical techniques with mouse monoclonal anti-MUC1 antibodies. The tumor types were classified as follows: infiltrating ductal carcinomas including atypical

<table>
<thead>
<tr>
<th>Table 1. The distribution patterns of MUC1 expression in normal breast tissue and intraductal hyperplasia.</th>
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<tbody>
<tr>
<td><strong>MUC1 patterns</strong></td>
</tr>
<tr>
<td>Negative ILB Weak C Weak C &amp; nuclear Moderate C &amp; ILB Strong C &amp; ICM</td>
</tr>
<tr>
<td>Normal</td>
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<tr>
<td>IDH</td>
</tr>
</tbody>
</table>

ILB = intraluminal border; C = cytoplasmic; ICM = intercellular membranous; IDH = intraductal hyperplasia.
medullary carcinomas, intraductal carcinomas, lobular tumors including infiltrating lobular carcinomas and lobular carcinomas in situ, mucinous and secretory carcinomas, apocrine carcinomas and medullary carcinoma. The following information was obtained from the patients’ medical records: age, the type, grade and size of their tumors, the lymph node status and tumor recurrences. The tumors were graded according to the modified Scarff-Bloom-Richardson system.\(^{(18)}\) The in situ lesions, mucinous carcinomas, secretory carcinomas and etc. were categorized as being nongraded. Four cases of intraductal apocrine carcinomas were categorized as being nongraded, with one invasive apocrine carcinoma being categorized as grade II.\(^{(19)}\)

**Immunohistochemical (IHC) analyses**

In order to evaluate a large number of tumors, low-density tissue microarray blocks were made at the hospital. One block contained 20 cores (a single core per tumor), with each core measured 4mm in diameter. The four-micrometer sections were mounted onto the positively charged slides.

Immunohistochemical analysis for MUC1 (clone: VU-4-H5, 1:100, Zymed, South San Francisco, CA, USA) was performed on the formalin-fixed, paraffin-embedded materials. After deparaffinization and blocking the endogenous peroxidase, the tissue sections were steamed in 0.1M, pH 8.0, EDTA buffer for 10 min. in a microwave oven. The sections were incubated overnight at 4 °C with MUC1 antibody diluted 1:100. A standard avidin-biotin-peroxidase complex (ABC) technique was used for visualization with diaminobenzidine being used as the chromogen (Histostain Plus-kit, 1:100, Zymed, South San Francisco, CA, USA). The sections were counterstained with hematoxylin. Benign and neoplastic breast tissues were used as controls.

**Immunohistochemical (IHC) interpretation**

The stained sections were reviewed by two authors for the type, pattern and amount of mucin positivity. The sections were scored for the percentage of cell that stained in a semiquantitative manner and also the location of staining within the positive cells. The cells were considered to be stained positive if the brown pigment of diaminobenzidine could be readily detected at the scanning magnification (x100). The staining intensity was graded as negative (−), weak (+), moderate (+++) or strong (+++).\(^{(4)}\) The pattern of reaction was classified as intraluminal border (ILB), intercellular membrane (ICM), cytoplasmic, intranuclear or mixed staining.\(^{(4, 20)}\) The ILB of the staining consisted of staining on the apical membrane of the duct luminal surface. ICM staining consisted of highlighting

<table>
<thead>
<tr>
<th>Tumor types</th>
<th>Negative</th>
<th>ILB</th>
<th>Weak C</th>
<th>Weak C &amp; nuclear</th>
<th>Moderate C &amp; ILB</th>
<th>Strong C &amp; ICM</th>
<th>Total</th>
<th>(p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFD</td>
<td>2 (0.8)</td>
<td>1 (0.4)</td>
<td>6 (2.5)</td>
<td>14 (5.7)</td>
<td>135 (55.3)</td>
<td>86 (35.3)</td>
<td>244 (79.5)</td>
<td>0.002</td>
</tr>
<tr>
<td>DCIS</td>
<td>0</td>
<td>0</td>
<td>1 (3.5)</td>
<td>0</td>
<td>17 (58.6)</td>
<td>11 (37.9)</td>
<td>29 (9.4)</td>
<td></td>
</tr>
<tr>
<td>Lobular</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (12.5)</td>
<td>5 (62.5)</td>
<td>2 (25.0)</td>
<td>8 (2.6)</td>
<td></td>
</tr>
<tr>
<td>Muc&amp;Sec</td>
<td>0</td>
<td>3 (20.0)</td>
<td>1 (6.7)</td>
<td>1 (6.7)</td>
<td>5 (33.3)</td>
<td>5 (33.3)</td>
<td>15 (4.9)</td>
<td></td>
</tr>
<tr>
<td>Apocrine(^1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (60.0)</td>
<td>2 (40.0)</td>
<td>5 (1.6)</td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (50.0)</td>
<td>3 (50.0)</td>
<td>6 (2.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2 (0.7)</td>
<td>4 (1.3)</td>
<td>8 (2.6)</td>
<td>16 (5.2)</td>
<td>168 (54.7)</td>
<td>109 (35.5)</td>
<td>307</td>
<td></td>
</tr>
</tbody>
</table>

ILB = intraluminal border; C = cytoplasmic; ICM = intercellular membranous; IFD = infiltrating ductal carcinoma, no specific type; DCIS = intraductal carcinoma; Lobular = infiltrating lobular carcinoma and lobular carcinoma in situ; Muc&Sec = mucinous carcinoma and secretory carcinoma; AC = apocrine carcinoma; MC = medullary carcinoma.

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the intercellular aspect of the cytoplasmic border, and cytoplasmic staining consisted of granular staining of the cell cytoplasm. The arbitrary divisions were low, i.e., <50% (any cells with up to 50% of the tumor cells) and high, i.e., >50% (greater than 50% of the tumor cells), and the percentages of cells showing MUC1 expression were used for the statistical analyses. (3, 5, 21) The tumor cells were compared to the epithelial staining that was present in the adjacent benign breast tissue.

Statistical analysis
A statistical analysis was performed using SPSS for Windows software (Version 8.0; SPSS, Chicago, IL). The Pearson X2-test was used to compare the categorical variables. Two-sided p values <0.05 were considered as statistically significant.

RESULTS
IHC expression for MUC1
All cases of normal breast showed weak positive staining in the cytoplasm of the ducts and lobules (Fig 1a). All the cases of IDH showed moderately positive cytoplasmic and ILB staining (Fig 1b) (Table 1). MUC1 was mostly expressed within the cytoplasm, along the ILB or on the ICM of the tumor cells. Of the 307 cases, only 2 cases (0.8%) showed as being negative (Table 2). summarizes the staining patterns along with the histological correlation for MUC1 in a number of the 307 cases of breast carcinomas. The MUC1 staining was less intense in the benign epithelium when compared to the malignant cells under the same staining condition. MUC1 positivity was observed in the 4 cases (1.3%) that had only ILB staining (Fig 2a & b), 8 cases (2.6%) had weak cytoplasmic staining, 16 cases (5.2%) had weak cytoplasmic and intranuclear staining, 168 cases (54.7%) had moderate cytoplasmic and ILB staining, and 109 cases (35.5%) had strong cytoplasmic and ICM staining (Fig 2c). MUC1 positivity with moderate to strong staining intensities was observed in 90.6% of the infiltrating ductal carcinoma (221/244), 96.5% of the intraductal carcinoma (28/29), 87.5% of the infiltrating lobular carcinoma (7/8), 66.6% of the mucinous and secretory carcinoma (10/15), 100.0% of the apocrine carcinoma (5/5) and 100.0% of the medullary carcinoma (6/6). The staining pattern of the MUC1 expression was statistically significant according to the histological types (p = 0.002).
### Relationships between MUC1 expression and the clinicopathological characteristics, the ER, PR and HER-2/neu oncogene

The MUC1 expression was significantly correlated with the histological tumor types ($p = 0.034$), tumor size ($p = 0.046$), and the HER-2/neu ($p = 0.004$), but it was not correlated with the patient's age ($p = 0.926$), the tumor grade ($p = 0.368$), the lymph node status ($p = 0.854$), the ER ($p = 0.412$), PR ($p = 0.136$) and tumor recurrence ($p = 0.469$) (Table 3).

### DISCUSSION

In the present study, the MUC1 staining was less intense in the benign epithelium when compared to the malignancy under the same staining condition. This finding supported that the membrane expression of this mucin often changes from apical to circumferential, which is coincidental with the loss of polarity of the epithelial cells during malignant transformation, and this often results in large amounts of mucin being either shed or secreted by tumor cells. However, it is not clear what factors regulate the endogenous mucin expression in the breast and what factors are responsible for this change.

The subcellular localization of MUC1 appears to play a significant role in determining the disease outcome. One study analyzed that the cytoplasmic staining found that increased staining was associated with decreased survival. The associations between the cytoplasmic expression and a poor outcome and between the apical expression and a favorable outcome have also been reported. The presence of apical membrane staining within the tumor cells indicates that the MUC1 targeting pathways are intact and this is associated with a better prognosis. Conversely, the presence of diffuse cytoplasmic staining may indicate that the processing and targeting pathways are defective and this is associated with a worse prognosis. The presence of ICM (circumferential membrane) staining may indicate the greatest undifferentiated regulation of MUC1.
tern of staining is interesting because the size of the MUC1 molecule (300-500 nm) appears to be incompatible with the normal intercellular space ≈ 30 nm. (3) In the present study, we examined the different patterns between the benignity and malignancy of the tumor cells that showed apical membrane and cytoplasmic staining. The finding of high expression of cytoplasmic mucin in infiltrating ductal carcinomas is consistent with the poor prognosis in those cases. We found that there was significant correlation for the different histological types of tumor. However, another study reported that the pattern and extent of MUC1 positivity do not vary according to the histopathological subtype of breast cancer. (4) Two characteristics are suggested with respect to the relationship of the MUC1 expression and histological types of tumor: the first is the altered mucin expression pattern present in the tumor cells and the second is a distinct distribution difference between the more differentiated tumor types and the poorly differentiated tumor types.

When performing a multivariate statistical analysis, we did not find a significant correlation among the higher (> 50%) and lower (<50%) proportions of MUC1 expression and the patient's age, tumor grade, lymph node status, ER, PR and tumor recurrence. We found that there was significant correlation for the tumor types, the tumor size and the HER-2/neu oncogene and the result was consistent with part of other report. (22) This association may be linked to tumor differentiation, the biologic behavior and the clinical prognosis. Another study concluded that among the various mucins expressed in breast cancer, MUC1 and MUC3 are the potential prognostic indicators, with MUC1 having the strongest relationship with the patients' outcomes. (11)

MUC1 overexpression in cancer is thought to influence adhesion, invasion and immune surveillance. (3, 23) Recent research has found that abundant production of mucin protein was well correlated with tumor cell metastasis. (12, 24) It might play a role in metastasis by inhibiting the adhesion of the tumor cells and for the tumor cells escaping from the immune surveillance. (10) Cell surface mucins are complex glycoproteins that are expressed on the apical membrane surface of the mucosal epithelial cells. They are thought to influence cell adhesion in malignant epithelial cells. (25) The overexpression of MUC1 leads to a decrease in the cell-to-cell and cell-to-matrix contacts, and this causes inhibition of killing the cancer cells by the cytotoxic T lymphocytes. (10) MUC1 has been intensely studied as a candidate antigen for a cancer vaccine. It appears to have some unique properties for immune stimulation and recognition. MUC1-specific cytotoxic T lymphocyte activity can be detected in MUC1-immunized patients. (16)

CONCLUSIONS

We found a different expression of MUC1 in normal breast, IDH and malignant breast tumors, and MUC1 was significantly correlated with the histological types of tumor, the tumor size and the HER-2/neu oncogene. The presence of MUC1 is associated with the more better differentiated tumors and may have a prognostic value in the predicting the patient outcome.

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