EXPRESSION OF P53, DCC, AND HER-2/NEU IN MUCINOUS CARCINOMA OF THE BREAST

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We investigated the clinicopathologic and oncoprotein expression characteristics of 11 pure mucinous and 76 non-mucinous infiltrating ductal carcinomas in the human female breast. We compared patient age, tumor size, axillary lymph node status, and the expression of estrogen receptor (ER), progesterone receptor (PR), deleted-in-colon cancer (DCC), HER-2/neu, and p53. Mucinous carcinoma with axillary lymph node metastasis occurs less frequently than non-mucinous carcinoma (0% vs 63.1%; \( p = 0.0018 \)). Compared with the non-mucinous type, mucinous carcinoma specimens have more DCC expression (100% vs 48.7%; \( p = 0.0027 \)) and more ER expression (90.9% vs 26.9%; \( p = 0.0023 \)), but less HER-2/neu overexpression (0% vs 38.1%; \( p = 0.0302 \)). We confirmed that mucinous carcinoma samples from the breast reveal distinct clinicopathologic and oncoprotein expression features compared with non-mucinous carcinoma and, therefore, it seems reasonable to suggest different biologic characteristics and manifestations.


Genetic alterations are frequently associated with neoplasia. The activation of proto-oncogenes and the inactivation of tumor-suppressor genes may be responsible for oncogenesis, and many of the tumor-suppressor genes that have been isolated have demonstrated a loss of heterozygosity (LOH) in several types of human neoplasia [1].

The candidate tumor-suppressor gene DCC (deleted-in-colon) was isolated by Fearon et al, who mapped an allelic deletion to chromosome 18q 21.3 in colorectal carcinoma [2]. DCC expression is reduced or absent in 88% of various colorectal carcinoma cell lines and in 70% of colorectal cancers [2,3], which is consistent with a tumor-suppressive function of the gene product. There is decreased expression of DCC mRNA in human colorectal cancers and an association with both prognosis and the probability of distant metastasis [4,5].

LOH in 18q is reported in approximately 40% of breast cancers, but this does not always include the DCC locus. LOH at the DCC locus was detected in 52% of patients with breast cancer, and 77% of these had a distinct reduction in, or total loss of, DCC expression [6]. The reduction in the expression of the DCC-gene protein in breast cancer is approximately 50% [7,8]. These findings indicate that DCC plays an important role in the oncogenesis of breast cancer.

The p53 tumor-suppressor gene encodes a DNA-binding nuclear protein that appears to inhibit the progression of a cell from G1 to S phase [9]. Expression of the p53 protein in breast cancer has prognostic significance [10]. The HER-2/neu proto-oncogene, located on chromosome 17q 21.2–q12, codes for a 185-kDa transmembrane growth factor receptor and is amplified and/or overexpressed in approximately 25% of breast carcinomas; it is associated with poor patient prognosis [11].

Breast cancer is a heterogeneous disease with regards to morphology, invasive behavior, metastatic capacity, hormone expression, oncogene and tumor-suppressor gene expression, and clinical outcome. Mucinous carcinoma is a specific histologic type of breast cancer characterized by abundant extracellular mucin. The reported frequency of
mucinous carcinoma of the breast is about 1–6% of all breast cancers [12,13]. It is generally thought that the tumor is more prevalent in older women and has a better prognosis than the more common ductal breast cancer [14,15].

To evaluate and compare the clinicopathologic and oncoprotein expression characteristics of mucinous and non-mucinous invasive breast cancers, we used immunohistochemical staining with monoclonal antibodies to estrogen receptor (ER), progesterone receptor (PR), and p53 and polyclonal antibodies to HER-2/neu and DCC. The results revealed that mucinous carcinoma has distinct histopathologic and oncoprotein expression characteristics compared with non-mucinous carcinoma.

**MATERIALS AND METHODS**

Clinical records for patients who had been diagnosed with primary mucinous carcinoma of the breast between 1990 and 1999 were accessed from Department of Pathology files at the Buddhist Tzu Chi General Hospital. Carcinomas were histologically verified as mucinous when nearly all the tumor cells were suspended in abundant extracellular mucin. In total, there were 11 pure mucinous carcinomas. Seventy-six non-mucinous infiltrating ductal carcinomas were randomly selected for comparison. Clinicopathologic factors recorded were age, tumor size, and pathologically verified lymph node status.

**Immunohistochemical staining**

Rabbit polyclonal anti-chicken DCC antibody (anti-cDCC), donated by Dr. Chuong, was developed using in vitro immunization with synthesized peptide as an antigen, as described previously [16]. The antigenic peptide was designed from codons 721–730 (DESVPDQPS) of the third fibronectin type III domain of the DCC gene.

Rabbit polyclonal anti-HER-2/neu antibody and mouse monoclonal anti-ER, anti-PR, and anti-p53 antibodies were ordered from Dako (Copenhagen, Denmark).

Individual tissue sections of 4–5 µm were deparaffinized and heated in a 10-mM citric acid monophosphate buffer (pH 6.0) for 30 minutes in a 1.35-kW microwave oven at high power [17]. This method of enhancing the recognition of antigen in archival tissue is termed antigen retrieval. To minimize the evaporation of buffer during heating, the tissue slides were microwaved in a nonmetallic kitchen pressure cooker. Immunohistochemical staining was performed with labeled streptavidin from a complex kit (Dako). This immunohistochemical technique involved the sequential application of the following antibodies: primary rabbit anti-DCC (1:300), mouse anti-ER (1:100), mouse anti-PR (1:50), rabbit anti-HER-2/neu (1:200), mouse anti-p53 (1:100), biotinylated anti-rabbit and anti-mouse secondary antibodies (Dako; 30 minutes), and a tertiary streptavidin peroxidase (Dako; 30 minutes). Antibody incubation was followed by tissue-section rinse in phosphate-buffered saline, repeated three times (5 minutes each). All slides were stained with all antibodies simultaneously, as described here.

Following treatment with chromogen-3-amino-9-ethyl carbazole (AEC), the sites of immunoprecipitate formation were identified by light microscopy. Positive and negative control sections were included with each assay. Samples were regarded as positive for DCC when at least 25% of tumor cells were assessed as granular cytoplasmic immunoreactive, but this classification proved redundant, since staining for DCC turned out to be an all-or-nothing phenomenon [18]. Membrane staining was interpreted as HER-2/neu oncoprotein expression, with the amount of staining scored in a blinded fashion as negative (no immunostaining), trace positive (a few immunostained cells scattered throughout the tumor or located along one edge of the specimen), moderate (distinct membrane staining in most cells), or strong (intense membrane staining in most cells) [19]. Overexpression was defined as moderate or strong membrane immunostaining, as previously described [11]. Immunostaining for ER, PR, and p53 was regarded as positive when at least 25% of tumor cells were nuclear immunoreactive.

**Statistical analysis**

Associations of mucinous and non-mucinous carcinomas with DCC expression and other tumor characteristics were calculated using Chi-squared or Fisher’s exact tests. A two-tailed \( p \) of less than 0.05 was considered statistically significant.

**RESULTS**

The clinicopathologic and oncoprotein expression characteristics of 11 mucinous carcinomas and 76 non-mucinous infiltrating ductal carcinomas are listed in the Table. Age and tumor size were not significantly different in the two carcinoma types. Mucinous carcinoma with axillary lymph node metastasis occurred less frequently than non-mucinous carcinoma (0% vs 63.1%; \( p = 0.0018 \)). There was greater ER expression in mucinous carcinoma.
Oncoprotein expression in mucinous breast cancer

Evidence has been accumulating that tumors show alterations in oncoprotein expression, which may be caused by genetic mutation or modulation of oncogenesis [1]. We found a very high frequency (100%) of strong DCC protein expression in mucinous carcinoma compared with non-mucinous infiltrating ductal carcinoma of the breast. This implies that there is no loss of DCC protein expression in mucinous carcinoma.

### DISCUSSION

Evidence has been accumulating that tumors show alterations in oncoprotein expression, which may be caused by genetic mutation or modulation of oncogenesis [1]. We found a very high frequency (100%) of strong DCC protein expression in mucinous carcinoma compared with non-mucinous infiltrating ductal carcinoma of the breast. This implies that there is no loss of DCC protein expression in mucinous carcinoma.

<table>
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<th>Table. Expression of p53, DCC, and HER-2/neu in mucinous carcinoma of the breast</th>
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<tbody>
<tr>
<td>Mucinous carcinoma (n = 11)</td>
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<td>Tumor size (cm), n (%)</td>
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<td>Axillary node status, n (%)</td>
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<td>Estrogen receptor, n (%)</td>
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<td>Progesterone receptor, n (%)</td>
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<td>p53 overexpression, n (%)</td>
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<td>DCC expression, n (%)</td>
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<td>HER-2/neu overexpression, n (%)</td>
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* Mucinous vs non-mucinous carcinoma, Chi-squared test.

\( 90.9\% \ vs \ 26.9\%; \ p = 0.0023 \) but there was no difference in PR expression (54.5% mucinous vs 47.3% non-mucinous; \( p = 0.6562 \)).

p53 protein expression was lower in mucinous than non-mucinous carcinomas (9.1% vs 34.2%) (Figure 1), but the result was not significant (\( p = 0.1821 \)). The most significant finding was that all 11 mucinous carcinomas had DCC protein expression (Figure 2), compared with 48.7% of non-mucinous carcinomas. In addition, none of the 11 mucinous carcinomas had HER-2/neu overexpression, while 38.1% of non-mucinous carcinomas revealed HER-2/neu oncoprotein overexpression.

![Immunohistochemical staining for p53 reveals nuclear staining in mucinous carcinoma (3-amino-9-ethyl carbazole, × 400).](image-url)
mucinous breast cancer and suggests that loss of DCC protein is unlikely to play a major role in the carcinogenesis of mucinous carcinoma of the breast. This finding is in agreement with an earlier study of mucinous carcinoma of the colorectum [20]. In addition, both breast and colorectal mucinous carcinomas do not exhibit 18q LOH, suggesting that mucinous carcinoma may differ genetically from non-mucinous carcinoma [20].

In another study, strong DCC expression was found in mucinous adenocarcinoma of the cervix [21], indicating that DCC expression is related to mucinous differentiation. Our results reconfirm this association. Research examining DCC expression in mucinous carcinoma of different organs (ovary and pancreas) is planned for the near future to further investigate this association.

To address such a scenario, a genetic model for multistep alterations in relevant oncogenes and associated allelic losses in colorectal tumorigenesis has been advocated [1]. Concordant p53, DCC, and c-ki-ras alterations are associated with increased frequency of metastasis in colorectal carcinoma. A variety of genetic changes in breast cancer, including oncogene amplification (HER-2/neu, int-2, c-myc, H-ras) and deletion or mutation in tumor-suppressor genes (RB, p53) have also been reported [22,23]. The results of this study revealed no overexpression of HER-2/neu and a low frequency (9.1%) of p53 expression, further suggesting that these two genes are rarely involved in tumorigenesis of mucinous breast cancer, which may contribute to the better prognosis in these cancers [10,11]. Our research confirms findings from previous studies demonstrating a high frequency of ER expression (90.9%) in mucinous breast cancer [24,25].

In conclusion, mucinous carcinomas of the breast present less axillary lymph node metastasis, retain expression of DCC protein, demonstrate frequent expression of ER and a low frequency of p53 overexpression, and are negative for HER-2/neu overexpression. We suggest that these characteristics may offer an explanation for the better prognosis established for patients diagnosed with mucinous carcinoma of the breast compared with non-mucinous infiltrating ductal carcinoma.

ACKNOWLEDGMENTS

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REFERENCES


p53, DCC 及 HER-2/neu
腫瘤蛋白在黏液性乳癌之表達

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我們觀察 11 個單純黏液性乳癌及 79 個非黏液性侵犯性乳腺管乳癌比較病人年齡、
腫瘤大小、腋下淋巴狀態，動情激素受體、黃體激素受體、HER-2/neu 及 p53 之表
達。黏液性乳癌合併腋下淋巴轉移比非黏液性乳癌低 (0% 比 63.1%；p = 0.0018)，
另外，黏液性乳癌比非黏液性乳癌更多 DCC 表達 (100% 比 48.7%；p = 0.0027)，
更多動情激素受體表達 (90.9% 比 26.9%；p = 0.0023)。但較少 HER-2/neu 表達
(0% 比 38.1%；p = 0.0302)。我們證實黏液性乳癌在臨床病理及腫瘤蛋白的表現上
與非黏液性乳癌不同，因此合理解釋為黏液性乳癌有不同的生物特性及表現。

關鍵詞：乳癌，DCC，p53，HER-2/neu，黏液性乳癌
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