Prognostic Value of Surface Antigens in Primary Human Breast Carcinomas, Detected by Monoclonal Antibodies

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ABSTRACT

Three monoclonal antibodies, raised against human milk fat globule membranes, have been applied to 194 primary human breast carcinomas. The detected antigenic sites were found to be heterogeneously distributed. A statistical association with estrogen receptor content and grade of anaplasia was found for two of the antigens, Mam 3a and Mam 3b. The presence of all three antigens was independent of menopausal status, age, primary lymph node metastases, and progesterone receptor status. Life table analysis showed a better survival for patients with tumors positive for Mam 3b.

The effect of these variables on recurrence-free survival has been analyzed using a Cox regression model. It is found that the most important prognostic factors are the number of positive lymph nodes, the estrogen receptor content, and the menopausal status of the high-risk patients. The ability of a model based on these factors to predict recurrence is not significantly improved by including any of the three surface antigens.

INTRODUCTION

Monoclonal antibodies raised against delipidated human milk fat globule membranes react with antigens on the cell surface of both normal and malignant cells of epithelial origin (3, 8–10, 15, 21). Since this method of antibody production is nonselective, numerous antibodies with different specificities are raised against antigens of unknown biological importance. However, in preliminary investigations, an association was observed between the presence of some of the antigens and the morphological grade of anaplasia (8, 18), suggesting that these antigens might be useful in the histological characterization of breast cancer tissue.

In the present study, possible relations between the presence of three surface antigens (Mam-3a, Mam-3b, and Mam-3c) in human breast tumors and other well-known prognostic factors have been investigated in 194 patients registered in the DBCG study. Furthermore, the value of these antigens in predicting recurrent disease has been examined using multivariate analysis enabling a comparison to other, and possibly more potent, prognostic factors.

MATERIALS AND METHODS

Patients. The present patient population is a part of the DBCG, which is a nationwide project for treatment of primary breast cancer in women. Approximately 95% of all such new patients in Denmark are registered in this project. The organization, design, and follow-up of the program have been described in detail elsewhere (1).

Following total mastectomy and partial axillary dissection, patients were divided into a low- and a high-risk group. If the tumor diameter was less than or equal to 5 cm and if there was no axillary lymph node involvement, patients were classified as low-risk patients (Protocol 77-a) and received no further treatment. The remaining patients were classified as high-risk patients; approximately 20% of these had tumors larger than 5 cm but no lymph node metastases. All high-risk patients were stratified according to menopausal status and randomized to protocols investigating the effect of systemic adjuvant therapy. They all received postoperative X-ray treatment. The premenopausal patients (Protocol 77-b) entered an adjuvant chemotherapy protocol, and the postmenopausal patients (Protocol 77-c) entered an adjuvant endocrine therapy protocol.

Patients are examined regularly for a period of 10 years. The end point in this study is recurrent disease or death regardless of cause of death.

Tumors investigated in the present study were from patients operated upon between September 1979 and February 1981. During this period, a total of 1795 patients entered the 3 DBCG protocols, and 398 (22%) had an estrogen receptor analysis of their tumor performed at a single laboratory (The Fibiger Institute, Division of Tumor Endocrinology). Of the 398 tumors, 205 biopsies (11%) were of a quality that permitted histological classification of the tissue, and 194 (10%) were available for the present study.

The clinical status of the patients has been evaluated as of June 1, 1983.

Histological Evaluation. From the frozen tissue used for receptor analysis, a central portion was fixed in formalin and paraffin embedded for histological investigation. The tumor type and the grade of anaplasia were evaluated by one of us on a hematoxylin-eosin-stained section. The tumor type was determined using the classification recommended by WHO (23). Infiltrating ductal carcinomas were graded according to anaplasia, using a modification of the method described by Bloom and Richardson (4), taking into account the formation of tubules, nuclear polymorphism, and mitotic activity.

Steroid Receptor Analysis. Analyses of estrogen and progesterone receptors were performed in a single laboratory. Estrogen receptor results were available for all patients, while progesterone receptor determinations were only available for 110 patients. The dextran-coated charcoal receptor analysis method was used in accord with the recommendation of the European Organization for Research and Treatment of Cancer (7), and receptor results were expressed in terms of fmol/mg of cytosol protein.

Monoclonal Antibodies. The 3 monoclonal antibodies 67 D11, 115 H10, and 116 C2, raised against delipidated human milk fat globule membranes (10), were applied to routinely formalin-fixed, paraffin-embedded sections of all 194 tumors. The presence of antigenic determinants was detected by a one-layer immunoperoxidase method (17). The histochemical antigen-antibody reaction was evaluated in 194, 184, and 171 cases for the 3 antibodies, respectively. Tumors were regarded to be positive when more than 10% of the epithelial cells gave a positive peroxidase reaction (17).

Statistical Methods. Comparisons of characteristics between groups of patients were made using the likelihood ratio test for homogeneity in the relevant contingency table. For continuous variables, this was supplemented by a graphical inspection. Recurrence-free survival was ana-
SURFACE ANTIGENS IN BREAST CANCER

lyzed by the life-table method using the log rank test for comparison of
survival curves (16). The prognostic value of the presence of antigens
was evaluated analyzing their significance in a Cox regression model (6).

The assumption of proportional hazards was checked by grouping
patients into strata according to the values of the variable under inves-
tigation. Plots of the estimated cumulative hazard function were there-
after compared for each stratum as described by Andersen (2). Since
the assumption of proportional hazards was not fulfilled in the model
identified, a stratified version of the Cox model was used (13).

RESULTS

To determine whether the group of patients in the present
investigation was representative of all patients in DBCG proto-
cols during the same period of time, characteristics from the 2
populations were compared. No significant differences were
found. At the time of primary operation, 30% of the patients
were premenopausal, while 70% were postmenopausal. One or
more axillary lymph nodes were found to be positive for tumor
metastases in 43% of the patients. The estrogen receptor level
was higher than 10 fmol/mg of cytosol protein in 77%, and in
39%, it was higher than 100 fmol/mg of cytosol protein. The
progesterone receptor concentration was higher than 10 fmol/
mg of cytosol protein in 40%. As of June 1, 1983, the median
observation time of these patients was 30 months. One hundred
thirty-five patients were alive and disease free.

Table 1 shows the types of carcinoma and the distribution
according to the grade of anaplasia of the infiltrating ductal
carcinomas in this study.

The frequency of expression of the 3 antigens tested in the
194 tumors varies from 19% (Mam-3c) to 54% (Mam-3b), as can
be seen in Table 2. While some tumors may contain all 3 antigens,
others have only one or none. The amount of detectable antigen
differs, both intra- and intertumorally.

The statistical association between the presence of the 3
surface antigens and other patient characteristics is shown in
Table 3. A statistically significant relation is found between the
estrogen receptor content and the presence of the antigens
Mam-3a and Mam-3b, as well as between the grade of anaplasia
and Mam-3b. The frequency of tumors with Mam-3b is high
among the highly differentiated tumors and in estrogen receptor-
positive tumors. In most cases, however, no associations are
found between the presence of the antigens in the tumor and
the clinical or histopathological factors investigated.

The relation between recurrence-free survival and the presence
of each of the 3 antigens is shown in Chart 1. The
recurrence-free survival is longer in patients with tumor antigens.
However, this difference is not significant in any of the cases ($p$
= 0.06, $p$ = 0.19, $p$ = 0.57).

In order to more precisely assess the importance of the
presence of antigens in predicting recurrence-free survival, a
multivariate analysis was carried out. A list of the variables
considered is given in Table 4. The first column of the Table

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Table 4  List of variables included in the analysis of recurrence-free survival and results of univariate Cox analysis and of inclusion in the final model

<table>
<thead>
<tr>
<th>p value</th>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Inclusion in final model</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.20</td>
<td>Menopausal status</td>
<td></td>
<td>0.71</td>
</tr>
<tr>
<td>0.57</td>
<td>Protocol</td>
<td>b, others</td>
<td>+*</td>
</tr>
<tr>
<td>0.001</td>
<td>c, others</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>0.54</td>
<td>Age at mastectomy</td>
<td></td>
<td>0.95</td>
</tr>
<tr>
<td>0.03</td>
<td>Size of primary tumor</td>
<td>25 cm, &gt;5 cm</td>
<td></td>
</tr>
<tr>
<td>0.006</td>
<td>Logarithm</td>
<td></td>
<td>0.78</td>
</tr>
<tr>
<td>&lt;0.0001</td>
<td>No. of positive nodes</td>
<td>0, 21</td>
<td>+</td>
</tr>
<tr>
<td>&lt;0.0001</td>
<td>Grade of anaplasia</td>
<td>(n = 169)</td>
<td>+</td>
</tr>
<tr>
<td>0.02</td>
<td>Grade of anaplasia</td>
<td>(n = 180)</td>
<td>0.58</td>
</tr>
<tr>
<td>0.03</td>
<td>Estrogen receptor</td>
<td>&lt;10 fmol, &gt;10 fmol</td>
<td>0.87</td>
</tr>
<tr>
<td>0.0003</td>
<td>Progesterone receptor</td>
<td>&lt;10 fmol, &gt;10 fmol</td>
<td>+</td>
</tr>
<tr>
<td>0.22</td>
<td>Mamm-3a (n = 184)</td>
<td></td>
<td>0.36</td>
</tr>
<tr>
<td>0.07</td>
<td>Mamm-3b (n = 184)</td>
<td></td>
<td>0.77</td>
</tr>
<tr>
<td>0.17</td>
<td>Mamm-3c (n = 171)</td>
<td></td>
<td>0.29</td>
</tr>
</tbody>
</table>

* +, included in the final model; −, cannot be estimated in the final model.
* By one pathologist in the present investigation.
* By pathologist participating in the DBCG.

Table 5  Final model of a multivariate analysis of recurrence-free survival (stratified according to node-positive/node-negative patients)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficient</th>
<th>Relative risk</th>
<th>Further reduction p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol (b, a, and c)</td>
<td>-1.33</td>
<td>0.26</td>
<td>0.0005</td>
</tr>
<tr>
<td>No. of positive nodes</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>0, 21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–3, &gt;4</td>
<td>0.83</td>
<td>2.3</td>
<td>0.01</td>
</tr>
<tr>
<td>Estrogen receptor (&lt;100 fmol, &gt;100 fmol)</td>
<td>-1.33</td>
<td>0.26</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

* +, variable used for stratification.

The monoclonal antibodies used in this study have been raised against delipidated human milk fat globule membranes. The secretion of milk is a pinocytotic process in which the milk fat droplets are surrounded by membrane from the apical part of the cell and pinched off into the lumen of the ducts (5). The antigens used to generate these antibodies are thus from a highly specialized and differentiated cell; it is, therefore, reasonable to assume that expression of some of these antigens on tumor cells would reflect differentiation of the tumor tissue. Since it is known that a high degree of differentiation indicates a low potential of cancer, tumors expressing such antigens could have a better prognosis than tumors without the antigen.

In an evaluation of the potential value of these antigens as prognostic factors, the validity of the results depends upon the reproducibility of the methods used. Several possible methodological problems can be envisaged in this investigation. The grading found by the pathologist in...
this investigation appears to be valid, because the degree of anaplasia is found to be significantly related to recurrence-free survival ($p = 0.02$), which is in accord with the literature (4, 20, 22). This was not the case for the routine grading in the various departments of pathology throughout the country on optimally fixed material. When included in the final Cox regression model, however, neither gradings contribute significant prognostic information.

Another problem is whether the antibodies used react with formalin-fixed, paraffin-embedded tissue. In this respect, it may be noted that both the reactivity of the antigens with antibodies and the intensity of staining have earlier been demonstrated to be the same in both frozen and formalin-fixed, paraffin-embedded tissues for the particular antibodies investigated here (17).

A third problem resides in selection of the criterion used to distinguish between tumors with positive and negative staining. The selection of the requirement that 10% of the cells are positively stained to distinguish between tumors with and without antigens has been arbitrary. Increasing this criterion to a level of 50% has also been investigated with respect to recurrence-free survival, and the results were essentially the same as those obtained using the 10% limit.

In the present study, Mam-3b is found to be significantly related to grade of anaplasia and estrogen receptor status, thus confirming the results from our preliminary investigation. Furthermore, it distinguishes between rates of recurrence-free survival, with a $p$ value of 0.06. The presence of Mam-3a is also found to be significantly associated with estrogen receptor status. Since both grade of anaplasia and estrogen receptor content are prognostic factors (12, 19, 22), the demonstrated effect on recurrence-free survival of the presence of antigen may merely reflect a relation of these antigens to the other 2 prognostic factors. Using a multivariate Cox analysis, it became clear that none of the 3 antigens is in itself an important prognostic factor in predicting recurrence-free survival. It is of particular interest that the variables of degree on anaplasia and tumor size also lose their importance in the model identified.

The disappearance in the final model of the demonstrable correlation between presence of Mam-3b and recurrence-free survival is, maybe, understandable in light of the technique used to raise the antibodies. Monoclonal antibodies are characterized by their high specificity for epitopes (14). The character of the antigens is unknown and may represent anything from quite unspecific building blocks in the cell membrane to molecules important for the biological function of the differentiated cell. It is notable in this regard that the antigens investigated here are found only in cell membranes from a number of different tissues of epithelial origin, including the mammary gland (11, 18). However, the heterogeneity that characterizes the presence of the antigens in mammary tumor tissue (6, 7, 9) supports the assumption that the presence of these antigens may be a marker of differentiation in this tissue. The relation found between the surface antigen Mam-3b and a morphological differentiation factor (the grade of anaplasia) and a biological differentiation factor (the estrogen receptor content) implies that this could be the case for this particular antigen.

ACKNOWLEDGMENTS

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