Mammary Cancer Antigen Recognized by Monoclonal Antibody B72.3 in Apocrine Metaplasia of the Human Breast

Maura Castagna, Marianna Nuti, and Francesco Squartini

ABSTRACT

Monoclonal antibody B72.3 recognizing a pan-associated carcinoma antigen expressed also in metastatic human breast cancer cells has been tested using the avidin-biotin peroxidase method applied to paraffin-embedded sections in 50 samples of mammary tissue showing apocrine metaplasia and in 58 cases of other mild or severe focal epithelial proliferative changes of the breast, including mostly *in situ* lobular or ductal carcinomas collateral to clinical cancer removed after radical mastectomy. The antigen detected by this antibody was present in the apocrine cells of 48 cases (96%). In the majority of these cases the reactivity was localized on the luminal border of the apocrine cells and in the luminal secretion. But ten cases showed positive staining also in the cell cytoplasm either focal or diffuse. The normal structures and mild focal hyperplastic changes collateral to clinical cancer were, in the majority of the cases (43 of 55), negative, and, when positive, displayed positivity only at the luminal border. By contrast, the independent foci of *in situ* carcinoma (17 of 31 examined), the intraduct papillomas (seven cases of 14), and the intraductal component of breast carcinoma (seven cases of 17) were positive, displaying a cytoplasmic focal or diffuse staining. In conclusion, mammary apocrine metaplasia, a metaplastic change of the normal epithelium that has been associated with increased breast cancer risk, shares antigens in common with breast cancer cells and/or with cells showing severe atypia. The possible clinical significance of the site of antigenic expression (cytoplasm or luminal border) needs further investigation.

INTRODUCTION

Apocrine metaplasia of the human breast is a benign elementary change included in the “gross cystic disease complex.” This change is characterized by a modified epithelium with cells displaying a granular acidophilic cytoplasm. Apocrine metaplasia is a pathological change when seen in the breast duct branching system, and it is associated with an increased risk of developing breast cancer (1–6).

In a previous study on the expression of different TAA in breast lesions, we observed the expression of a mucin-like pan-associated carcinoma antigen with a molecular weight of over 10^6, recognized by the monoclonal antibody B72.3 (7–10), in the apocrine cells of all of the 5 mammary tissue samples showing apocrine metaplasia. We report here the study of the expression of these TAA in 50 cases of apocrine metaplasia and in 58 cases of mild or severe focal epithelial proliferative changes of the breast, including mostly *in situ* carcinoma and intraduct carcinoma.

MATERIALS AND METHODS

Tissue paraffin-embedded blocks were obtained from surgical mastectomy material (breast removed for clinical cancer) sent to the Institute of Pathological Anatomy and Histology, University of Pisa, in the years 1983 and 1984. Tissues were formalin fixed and paraffin embedded following standard procedures. Hematoxylin-eosin sections were reviewed in advance in order to select cases for the presence of apocrine metaplasia and apocrine cysts (50 cases) or focal proliferative changes including *in situ* carcinomas (58 cases). Additional 5-μm sections were made of the selected blocks, and these were tested by the immunoperoxidase avidin-biotin method (Vector Laboratories). A total number of 108 mammary tissue specimens from 108 different cases of mastectomy for clinical cancer were tested. The age range of the patients was between 25 and 79 yr. The reactivity of normal-looking breast structures, apocrine metaplasia, focal proliferative changes, *in situ* carcinomas, and intraductal component of clinical carcinoma has been investigated. Data concerning the reactivity of the infiltrative component of clinical cancer and lymphomatomatous metastases in the same cases explored in this study have been previously reported (10, 11).

The B72.3 monoclonal antibody ascitic fluid was used at a 1:500 dilution. Overnight incubation was carried out in a humidified chamber at 4°C. Negative controls were made for each specimen in every experiment. In the control slides the primary antibody was omitted, and the sections were incubated in phosphate-buffered saline containing 0.1% bovine serum albumin, which was also used to dilute the ascitic fluid. Immunoperoxidase-stained slides were reviewed independently by two separate investigators, and the results were then discussed.

Hybridoma methodology and details of the general reactivity of the B72.3 antibody are described elsewhere (7, 8). Briefly the antibody was generated by immunizing mice with membrane-enriched fractions of metastatic breast carcinoma cells. This antibody reacts with 84% of breast cancers (after an overnight incubation), 94% of colon cancers, 96% of non-small cell lung carcinomas, and 100% of common epithelial ovarian cancers. It appears to have a reactivity restricted to neoplastic cells versus the normal counterpart, since a variety of assays including immunohistochemistry failed to demonstrate any reactivity with normal tissues. (For complete review of the immunohistochemical reactivity of the B72.3 monoclonal antibody, see Ref. 9.) This monoclonal antibody was generated in the Laboratory of Tumor Immunology and Biology of the National Cancer Institute, NIH, Bethesda, MD, and was supplied by Dr. J. Schlom, Chief of the Laboratory.

RESULTS

The results are reported in Table 1. Of the 50 cases tested containing apocrine metaplasia, 48 were positive corresponding to 96% of reactivity.

The pattern of distribution of the TAA on apocrine metaplastic changes has also been evaluated. In all the cases no heterogeneity of expression was observed; samples were either completely positive or totally negative. However, different sites of expression were observed, and the stain localization was therefore classified as follows: (a) only on the luminal border of the “apocrine” epithelial cells, i.e., the portion of the plasma membrane which faces directly the glandular lumen, 38 of the 48 positive cases showing only this pattern of expression of the TAA; (b) on the luminal border as well as in the cytoplasm with a diffuse pattern of staining, 5 cases displaying this antigen distribution; and (c) on the luminal border and in the cytoplasm but with granular, focal cytoplasmic concentration of the antigen, 5 other cases falling in this group.

Luminal secretion was observed in 18 of the 50 specimens...
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Table 1 Reactivity to monoclonal antibody B72.3 of apocrine metaplasia of the breast

<table>
<thead>
<tr>
<th>No. of cases tested</th>
<th>No. of positive cases</th>
<th>%</th>
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<tbody>
<tr>
<td>50</td>
<td>48</td>
<td>96</td>
</tr>
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</table>

Table 2 Localization of monoclonal antibody B72.3 in mammary apocrine metaplasia

<table>
<thead>
<tr>
<th>Localization</th>
<th>No. of positive cases/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal cell border</td>
<td>48/50</td>
</tr>
<tr>
<td>Cytoplasm, diffuse</td>
<td>5/50</td>
</tr>
<tr>
<td>Cytoplasm, focal</td>
<td>5/50</td>
</tr>
<tr>
<td>Luminal secretion</td>
<td>15/18</td>
</tr>
</tbody>
</table>

studied, and 15 of them were positive after the immunoperoxidase reaction with monoclonal antibody B72.3. A summary of these observations is illustrated in Table 2.

A typical staining of the apical border of apocrine metaplasia is shown in Figs. 1 to 3. Staining of the luminal secretion is shown in Fig. 4. The cytoplasmic focal or diffuse staining is shown in Figs. 5 and 6.

The normal-looking glandular structures and focal hyperplastic changes collateral to clinical breast cancer were also analyzed in a total of 108 cases, and data on their reactivity with monoclonal antibody B72.3 are summarized in Table 3. The normal-looking glandular structures and mild focal hyperplastic changes were usually negative, but on occasion a few normal or hyperplastic structures showed a weak positivity, the antigen being expressed only on the apical border of the epithelial cells. In particular, changes such as atypical lobules (Fig. 7) (5, 6) and sclerosing adenosis were negative.

On the other hand, the B72.3 TAA were clearly expressed in 13 of 24 in situ ductal carcinomas, in 4 of 7 in situ lobular carcinomas (total: 17 of 31 or 55%), and in 7 of 17 intraductal components of clinical carcinomas (41%). In addition, 7 of 14 intraduct papillomas examined were positive (50%). In all of the positive cases the antigen was localized in both the plasma membrane and the cytoplasm of the cells. Both focal and diffuse patterns of cytoplasmic distribution were found (Fig. 8).

DISCUSSION

This immunoperoxidase study demonstrates the expression of a mammary tumor-associated antigen recognized by the monoclonal antibody B72.3 on apocrine metaplasia of the breast. It is important to remember that the B72.3 antigen is selectively expressed in the neoplastic cells of breast, colon, non-small cell lung, and ovarian carcinomas (9). Therefore this antibody recognizes a broadly associated tumor antigen. In breast carcinomas the antigen is expressed in 84% of cases, and the distribution is highly heterogeneous (12), whereas in apocrine metaplasia the antigen is almost always present, and the overall distribution is homogeneous. The tentative conclusion is that an antigen peculiar to the luminal border of apocrine metaplasia is consistently, although not invariably, expressed in the malignant cells of breast as well as other body sites.

The pattern of distribution of this antigen in the benign changes may be important. Therefore, the site of antigenic expression should be extensively evaluated, since it might have clinical significance. The B72.3 TAA are accumulated in the cytoplasm of malignant breast cells, whereas in those few cases of normal or hyperplastic mammary structures that are B72.3 positive, the antigen is expressed only in the apical border of the cells. In apocrine metaplasia the majority of cases show a pattern of reactivity in the apical membrane except for 10 of 48 cases where reactivity is also in the cytoplasm. Therefore, the question arises of whether these few cases may have a different clinical course in terms of increased risk of developing breast carcinoma. In fact, the antigenic expression on the luminal cell membrane and/or into the luminal secretion is a phenomenon apparently distinct from the cytoplasmic expression, since it has been shown for the human breast that the patterns of expression of the T-antigen (one of the MN blood groups system) in normal and malignant cells are different, being of the "luminal-secretory" type in normal glands or in benign lesions and of the "cytoplasmic" type in mammary cancer (13).

The relationship between apocrine metaplastic epithelium and breast cancer has been reported by several investigators (1-6). Haagensen has shown a strong positive correlation, with 10-fold increased risk of carcinoma, in patients with microscopic apocrine metaplasia in biopptic material (14). An antiserum named GCDFP-15 (gross cystic disease fluid protein 15) has been described (3, 14, 15). This antiserum, although it looks like B72.3, is different in respect to the molecular weight of the antigen recognized (M, 15,000 versus 100,000), the percentage of reactive breast cancers (47 versus 84%), and the reactivity with colon cancers (0 versus 94%).

Mazoujian et al. (16) have described the histochemical reactivity of GCDFP-15 with other apocrine glands of the axilla, vulva, eyelid, and ear canal. We have also tested axillary and perineal apocrine glands with B72.3 monoclonal antibody, and
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Fig. 2. Apocrine metaplasia. Intense staining of the apical border. The staining is homogeneous (arrows). Immunoperoxidase staining with monoclonal antibody B72.3, x 80.

Fig. 3. Apocrine cyst. Intense, linear, homogeneous staining of the apical cell border, x 80.

Fig. 4. Apocrine cyst with luminal secretion. Note the dark luminal cell border and the staining of the luminal secretion. x 200.

Fig. 5. Apocrine cyst. Apical and focal cytoplasmic staining (arrows) of the "pale" epithelial cells. x 200.
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Fig. 6. In situ ductal carcinoma. Focal cytoplasmic staining (arrows) of the epithelial cells. × 200.

Table 3 Reactivity to monoclonal antibody B72.3 of normal glandular structures and other focal changes collateral to clinical breast cancer

<table>
<thead>
<tr>
<th>Structures and focal changes</th>
<th>No. of cases</th>
<th>Positive cases</th>
<th>Localization of reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ducts</td>
<td>27</td>
<td>4</td>
<td>Luminal border*</td>
</tr>
<tr>
<td>Normal lobules</td>
<td>5</td>
<td>1</td>
<td>Luminal border* + secretion</td>
</tr>
<tr>
<td>Persistent lobules*</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperplastic ducts</td>
<td>9</td>
<td>5</td>
<td>Luminal border</td>
</tr>
<tr>
<td>Cystic ducts</td>
<td>4</td>
<td>2</td>
<td>Luminal border</td>
</tr>
<tr>
<td>Sclerosing adenosis</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atypical lobules</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intraduct papillomas</td>
<td>14</td>
<td>7</td>
<td>Cytoplasm, diffuse or focal</td>
</tr>
<tr>
<td>In situ carcinomas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ductal</td>
<td>24</td>
<td>13</td>
<td>Cytoplasm, focal or diffuse</td>
</tr>
<tr>
<td>Lobular</td>
<td>7</td>
<td>4</td>
<td>Cytoplasm, diffuse</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

* Only few ducts or alveoli.
* In otherwise atrophic breasts.

they resulted positive at the luminal border (17, 18). Therefore we can similarly conclude that the “pale epithelium” of apocrine metaplasia of the breast and the epithelium of other apocrine glands in the body share antigens in common. This, in turn, supports the hypothesis that mammary apocrine epithelium derives through a true metaplastic process.

Breast fluids obtained by needle aspiration of cysts are now being examined in order to study the possible shedding of the antigen recognized by the B72.3 monoclonal antibody in the secretions of the cysts. A common antigen between breast cancer cells and apocrine metaplasia of the breast with different sites of localization stimulates further investigations in which

Fig. 7. Atypical lobule with intrinsic epithelial proliferation (epitheliosis). Staining with B72.3 antigen is negative. × 22.

Fig. 8. In situ ductal mammary carcinoma independent from and collateral to the clinical cancer. Intense cytoplasmic staining of the transformed cells either focal or diffuse. × 200.
the clinical course of patients with a known antigenic pattern is followed, in order to assess any predictive or prognostic value.

REFERENCES

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