Monoclonal antibody B72.3 in benign breast lesions

S Soomro, S Shousha

Abstract

It has been suggested that the monoclonal antibody B72.3 may be useful as a diagnostic tool in fine needle aspirates of breast masses because it recognises “tumour associated glycoprotein (TAG)-72”. The antigen was sought in paraffin wax sections of 43 normal and benign breast biopsy specimens, using the avidin-biotin complex technique, to assess the extent of its presence in non-malignant tissue. Strong focal staining was seen in 21 (49%) cases. In 29 cases of fibrocystic change staining was present in 17 (59%). All areas of apocrine metaplasia were positive, as well as a few normal ducts and acini and occasional areas of adenosin. Focal positivity was present in five out of 12 foci of ductal epithelial hyperplasia and in three out of seven radial scars. Staining was absent in two areas of lobular hyperplasia, three areas of sclerosing adenosis, and in a focus of lactational change. Focal positivity was also seen in two out of five fibroadenomas and in two out of three intraduct papillomas. Five normal subareolar sections and a section of normal lactating breast were negative.

It is concluded that B72.3 monoclonal antibody can show focal reactivity with a variety of normal and benign epithelial mammmary structures, and it is doubtful that its use would be of any help in differentiating benign from malignant cells in fine needle aspirates.

B72.3 is a murine IgG monoclonal antibody which was generated by using metastatic tumour tissue from a human mammary carcinoma. The antibody recognises a high molecular weight antigen, designated tumour associated glycoprotein (TAG)-72, which is rich in carbohydrate and has mucin-like biochemical and biophysical properties. The antigen has been identified in most carcinomas of a variety of organs including breast, ovaries, oesophagus, stomach, colon, pancreas, as well as in non-small cell bronchogenic carcinoma.

It has been suggested that immunostaining with this antibody may be useful in fine needle aspirates of breast masses to differentiate between malignant and benign cells. But there have also been reports which suggest that the antigen is present in some benign breast lesions. This study aimed at assessing the extent of the antigen in histological sections of a variety of non-malignant breast tissues. The assumption was that any positive staining seen in these tissues would have been reflected in the aspirates if these were taken. On the other hand, studying histological sections rather than aspirates provides more definitive and specific diagnosis which would allow the type of lesion or cell that is more likely to give rise to positive staining to be identified more accurately.

Methods

Paraffin wax sections of 43 routinely processed, formalin fixed normal and benign breast biopsy specimens were examined. The cases were selected so as to represent a variety of benign lesions, and some were derived from non-neoplastic areas of breasts with malignant disease elsewhere. The benign lesions included 29 cases of fibrocystic change, five fibroadenomas, and three intraduct papillomas. The normal tissue was derived from mastectomy specimens in five cases and from a lactating breast in the sixth case.

Sections 5 μm thick were cut from each case, dewaxed in xylene, and rehydrated in graded alcohols. Endogenous peroxidase activity was blocked by 0.3% hydrogen peroxide in methanol for 30 minutes. After rinsing three times for five minutes each in 0.1M TRIS-buffered saline (TBS), pH 7.6, sections were incubated for 30 minutes in 10% normal rabbit serum in TBS. Excess rabbit serum was then tipped off and sections were incubated overnight with the monoclonal antibody B72.3 (Cell Tech UK) at 4°C. Sections were then rinsed in TBS as before and incubated for 30 minutes in biotinylated rabbit anti-mouse immunoglobulin at a dilution of 1 in 250 (Dakopatts). After three rinses in TBS sections were covered for 60 minutes with avidin-biotin complex horseradish

Results of histological diagnosis and B72.3 staining

<table>
<thead>
<tr>
<th>Histological diagnosis</th>
<th>No of cases</th>
<th>No of positive results (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrocystic change (FCC)</td>
<td>29</td>
<td>17 (59)</td>
</tr>
<tr>
<td>Ductal epithelial hyperplasia</td>
<td>12</td>
<td>5 (42)</td>
</tr>
<tr>
<td>Mild</td>
<td>2</td>
<td>2 (100)</td>
</tr>
<tr>
<td>Moderate and florid</td>
<td>7</td>
<td>2 (29)</td>
</tr>
<tr>
<td>Apocrine</td>
<td>3</td>
<td>1 (33)</td>
</tr>
<tr>
<td>Lobular hyperplasia</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Radial scar</td>
<td>7</td>
<td>3 (43)</td>
</tr>
<tr>
<td>Sclerosing adenosis</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Lactational change (in FCC)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Intraduct papillomas</td>
<td>3</td>
<td>2 (67)</td>
</tr>
<tr>
<td>Fibroadenoma</td>
<td>5</td>
<td>2 (40)</td>
</tr>
<tr>
<td>Normal subareolar tissue</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Normal lactating breast</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
peroxidase (Dakopatts). After rinsing with TBS the sites of peroxidase activity were visualised by incubating the sections in 0.05% diaminobenzidine (Sigma UK) and 0.01% hydrogen peroxide for six minutes. Sections were then counterstained with haematoxylin, dehydrated in graded alcohols, cleared in xylene and mounted with Permount.

Control sections were treated in the same way except that the specific antisera was replaced by TBS.

**Results**

Strong focal positive staining was seen in 21 of the 43 (49%) cases examined. The staining was mostly seen in epithelial cells, and occasionally in luminal secretions of cysts or normal-looking ducts and acini. The only stromal staining seen was observed in the central part of a radial scar.

Two patterns of staining were noted in epithelial cells: localised lumina surface staining in cells showing apocrine metaplasia, and diffuse cytoplasmic staining in all other positive epithelial cells.

The detailed results are summarised in the table.

**FIBROCYSTIC CHANGE**

Positive focal staining was seen in 17 out of the 29 (59%) cases examined. Positive staining was seen in cases with and without associated carcinoma elsewhere in the breast. All areas of apocrine metaplasia were positive (fig 1). Staining was also seen in a few normal ducts and acini (13 cases) (fig 2), in luminal secretions of some normal ducts and acini (eight cases), as well as in cysts (two cases), and in occasional areas of adenosis (one case).

The reactivity of ductal epithelial hyperplasia was variable. Thus while both foci of mild hyperplasia seen were positive (fig 1), only about a third of cases with moderate and florid hyperplasia (two out of seven) and atypical hyperplasia (one out of three) showed focal positivity (fig 3). Both cases of lobular hyperplasia examined were negative.

Focal positivity was also detected in three out of seven (43%) radial scars. The staining was usually present in a few ducts, but in one case was also seen in the stroma occupying the central part of the lesion (fig 4). No staining was seen in three cases with sclerosing adenosis, nor in a case showing a focus of lactational change.

**INTRADUCT PAPILLOMA**

Two of the three (67%) cases examined showed positive staining which was mainly seen in areas of apocrine metaplasia and occasionally in luminal secretions. The most extensive staining was seen in a papilloma which consisted almost entirely of metaplastic apocrine cells (fig 5).

**FIBROADENOMA**

Two out of the five (40%) fibroadenomas examined showed focal positivity which was usually associated with the glandular structures.
in the pericanalicular rather than the intra-
canalicular component of the lesions (fig 6).

NORMAL BREAST TISSUE

The normal large subareolar ducts were always negative for staining but patchy staining of the
overlying normal stratified squamous epider-
thelium was often present. Normal lactating
breast tissue did not stain.

Discussion

The presence of TAG-72, shown by the mono-
clonal antibody B72.3, was originally thought to be a tumour specific antigen 1 hence the name. Later, however, it became clear that, like
many other similar antigens, it may occasion-
ally be expressed in non-malignant cells. 7 In
the breast this was particularly true in cells showing apocrine metaplasia. 8 In spite of this
several recent articles have suggested that
immunostaining with this antibody may help in
differentiating benign from malignant cells in
difficult breast needle aspirates. 9-11 This sugges-
tion was based on the assumption that
positivity in non-malignant cells is only seen in
metaplastic apocrine cells, 6 or in a few benign
cells. 12

The findings of our study suggest that strong
focal immunostaining for monoclonal antibody
B72.3 may be more widespread than is cur-
rently thought. It was found in around half the
benign breast lesions examined. Staining was
seen not only in metaplastic apocrine cells, which
may be recognised as such in cytological
smears, but was also seen in cells from normal-
looking ducts and acini, hyperplastic ducts,
radial scars and fibroadenomas. Some of these
findings have been confirmed in a recent report. 10

Admittedly, only a minority of cells stained in
the positive cases. This may explain why the
stain was originally thought to be useful as a
diagnostic tool in needle aspirates. As almost
half the benign lesions examined can provide
false positive results, however, this is probably
even enough to deter us from depending on this
particular immunoreactivity to guide us in
deciding whether a cell is benign or malignant,
especially as many malignant cells can be B72.3
negative. 16-19

The clinical importance of the presence of
positively stained cells in benign lesions is
unclear. Our findings and those of Castagna et
al 6 suggest that almost all metaplastic apocrine
cells express the antigen on their surfaces. The
association between apocrine metaplasia and
malignancy is controversial, but it is unlikely
that metaplastic cells have a higher malignant
potential than non-metaplastic cells. 11, 12

The situation is even more difficult to
explain in B72.3 positive non-metaplastic cells.
In haematoxylin and eosin stained sections
most of these cells do not show any atypical or
unusual features, but this is probably not
equivalent to exclude the possibility that such cells
may have a malignant potential that is distinct
from B72.3 negative cells. In this respect we
have noticed a direct association between B72.3
immunostaining and the presence of mucin in
some malignant breast tumours. 13 It would be
interesting to investigate the possible existence
of a similar relation in benign breast lesions,
but this is beyond the purpose of this study.

It is concluded that B72.3 can show focal
reactivity with a wide variety of normal and
benign epithelial mammary structures, and it is
doubtful that its use would be of any help in
differentiating benign from malignant cells in
fine needle aspirates.

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**Notes**