Evaluation of the Ca1 antibody in the diagnosis of invasive breast cancer

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SUMMARY An evaluation of Ca1 antibody staining was performed on paraffin sections from 136 breast lesions (64 benign and 72 malignant). Although cytoplasmic staining was encountered significantly more often in malignant lesions, the false negative rate was 6.9% and the false positive rate 56.2%. Benign lesions which showed positive staining included gynaecomastia, cystic mastopathy and fibroadenomata.

Various other monoclonal antibodies showed staining similar to Ca1 antibody. Ca1 antibody was observed to bind to epithelial membrane antigen-coated sepharose beads.

The Ca antigen was extracted by Ashall *et al* from a segregant malignant hybridoma produced by fusion of diploid fibroblasts and malignant cells from a human cervical carcinoma. The monoclonal antibody (Ca1) to this antigen was reported to identify malignant cells.¹ Binding of the antibody using the indirect immunoperoxidase technique was encountered in the majority of malignant tumours tested but not in benign tumours although some normal tissues exhibited binding of Ca1.² The present study was designed to evaluate the efficacy of Ca1 antibody staining in differentiating benign from malignant lesions of the breast. In addition, other monoclonal antibodies were tested.

Material and methods

The study was conducted on a series of 136 paraffin sections from breast lesions (64 benign and 72 malignant). Sections were stained with the Ca1 monoclonal antibody (Wellcome Diagnostics) using the indirect immunoperoxidase technique.

STAINING PROCEDURE

Lillie's buffered formalin fixed, paraffin embedded 5 μ m sections were mounted on poly-L-lysine coated slides and taken to water. Endogenous peroxidase activity was abolished by treating with methanol peroxide solution (30% hydrogen peroxide 1 part: methanol 19 parts). The sections were treated with solution A (fetal calf serum, bovine albumen, sodium azide, phosphate-buffered saline). This was

followed by overnight treatment with the Ca1 antibody diluted in solution A and subsequent treatment with peroxidase conjugated antimouse serum (Serotec) diluted in solution B (solution A 95 ml—human serum group AB 5 ml). The peroxidase was developed by the use of diaminobenzidine.³ Sections were allowed to sit in water for 10 min to avoid clouding of slides caused by solution A and B. Nuclei were counterstained using Mayer's Haemalum and blued using Scott's tap water substitute. The slides were then dehydrated, cleared and mounted in butyl phthalate styrene.

Staining sequence

Reagent	Duration (min)
Methanol peroxide	30
Tris-buffered saline	3 $\times$ 10
Solution A	30
Ca1 (1/50 in solution A)	Overnight
Solution A	3 $\times$ 10
Peroxidase conjugated antimouse IgM (1/30 in solution B)	30
Tris-buffered saline	3 $\times$ 10
DAB/H ₂ O ₂	10
H ₂ O	10

Other antibodies tested using indirect immunoperoxidase technique were HMFG1 and HMFG2 (monoclonal antibodies to human milk fat globule membrane, Seward Laboratory); four mouse IgM monoclonal antibodies (supplied by Dr M Kerr, Ninewells Hospital); OKT6 which binds to common thymocytes and OKT8 which stains common thymocytes, mature T helper thymocytes and T helper lymphocytes (Ortho Diagnostics Ltd).

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Table 1 Staining of paraffin sections with Ca1

	No of cases	Cytoplasmic staining	Membrane staining	Intraduct staining	Interstitial staining
Malignant					
Invasive duct carcinoma	42	40	40	17	33
Duct carcinoma in situ	17	14	15	12	14
Invasive lobular carcinoma	12	12	12	3	6
Lobular carcinoma in situ	1	1	1	1	1
Total malignant	72	67*	68	33	54
Benign					
Fibroadenoma	36	13	26	26	15
Intraduct Papilloma	11	6	11	11	5
Gynaecomastia	17	17	17	16	14
Total benign	64	36	54	53	34

*Cytoplasmic staining significantly more common in malignant group ($p < 0.001$, χ^2 test).

Results

CA1 ANTIBODY

The staining data for the 136 lesions are shown in Table 1. Cell membrane staining was the most common type. It was observed in both benign (Figs. 1 and 2) and malignant lesions (Fig. 3). The intensity of the cell membrane binding of Ca1 showed considerable variation and was pronounced and irregular on the luminal membranes of duct cells in benign lesions (Fig. 1), normal sebaceous glands

(Fig. 4) and normal breast ducts. Intraductal staining was encountered in benign breast lesions, particularly fibroadenomata and less frequently in malignant lesions. The intraduct material encountered in fibroadenomata stained deep blue with the Alcian blue/PAS staining method⁴ indicating its acid mucosubstance nature. Methylation at 60°C followed by saponification abolished the Ca1 antibody staining in both fibroadenomata and malignant controls. This suggests that the Ca antigen is a sulphated glycoprotein and that the mucin in the ducts of the fibroadenomata is the same or a very similar antigen.

Cytoplasmic staining was encountered in the majority of malignant lesions and less commonly in benign lesions. A noticeable feature of cytoplasmic staining as it affected benign lesions was its frequent localisation of the luminal side of the cell. In sections of cystic mastopathy, cells showing apocrine meta-

Fig. 1 Simple mastopathy, showing strong staining of cell membranes and intraduct material and weak staining of cell cytoplasm $\times 400$.

Fig. 2 Gynaecomastia, showing strong staining of cell cytoplasm, cell luminal membranes and intraduct material $\times 400$.

Fig. 3 *Infiltrating lobular carcinoma, showing strong staining of cell cytoplasm and cell membranes $\times 400$.*

Fig. 4 *Nipple region, showing staining of sebaceous glands $\times 100$.*

plasia showed granular staining of the cytoplasm on the luminal side of the cell (Fig. 5).

Interstitial staining was observed in both benign and malignant lesions. It consisted of a few discrete clumps of material showing no definite distribution or form.

OTHER ANTIBODIES

HMFG1 produced a very similar pattern of staining to Ca1, HMFG2 binding being more diffuse. Both HMFG1 and HMFG2 stained the intraductal mucin of fibroadenomata. All the four mouse IgM monoclonal antibodies produced strong staining of the cytoplasm in the majority of sections from breast carcinoma. Both OKT6 and OKT8 resulted in very weak staining of carcinoma cells.

The affinity of Ca1 antibody for epithelial membrane antigen (EMA) was tested using EMA-coated sepharose beads (Sera-Lab). Two hundred μ l of Ca1 solution at 1/50 were placed in a tube with the

Fig. 5 *Apocrine metaplastic duct epithelium, showing granular staining in the cytoplasm on the luminal side of the nucleus and cell membrane staining $\times 400$.*

Fig. 6 Duct carcinoma in situ, showing variations in staining between cells and areas of the lesion $\times 65$.

EMA-sepharose beads, agitated for 3 h at room temperature and then centrifuged at 500 g for 5 min. The supernatant was then aspirated off and used to stain paraffin sections of both invasive duct carcinoma and fibroadenomata. No staining was shown by any of the paraffin sections tested.

Discussion

It is clear that the results of the present study do not support the previously reported data with Ca1 as a specific marker for malignant cells.² Although cytoplasmic staining with Ca1 is encountered significantly more often in malignant lesions than in benign disease ($p < 0.001$), it is not a reliable indicator of malignancy. Thus, the false negative rate of the test was 6.9% and the false positive rate 56.2%. The true predictive value of cytoplasmic staining with Ca1 when positive was 65% and when negative, 85%. Thus positive Ca1 antibody cytoplasmic staining would only be due to cancer in 65% of cases and negative cytoplasmic staining reliably excludes cancer in only 85%.

Within sections from both benign and malignant lesions, considerable variation in Ca1 binding was encountered between adjacent cells and areas (Fig. 6). One possible explanation for this phenomenon

may be a variable expression of the antigen by different cell lines within the lesion. This has been reported in other studies with monoclonal antibodies raised against human breast tissue.⁵

Another reason for the heterogeneous nature of the staining could be that the Ca antigen is contained in a secretory product of the cells. Thus cells loaded with secretory granules would exhibit strong cytoplasmic staining. This would explain the strong staining with Ca1 of the luminal membranes of cells and the staining of intraductal mucin of fibroadenomata. The granular staining with Ca1 observed in cells showing apocrine metaplasia further supports this hypothesis. The appearance and location of these glycolipid granules⁶ are very similar to those stained by Ca1 antibody in areas of apocrine metaplasia and suggest that they are the same or closely allied granules.

There is also a strong possibility that some of the observed staining with Ca1 antibody may be the result of non-specific binding. Thus the other antibodies studied showed a pattern of staining similar to that of Ca1 strongly suggesting that there is non-specific binding of mouse IgM. Finally we have observed that Ca1 antibody binds to the EMA which has a wide distribution in normal and neoplastic epithelium.^{7,8}

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