

SHORT REPORT

P-cadherin as a marker in the differential diagnosis of breast lesions

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Aims: To assess the value of the calcium dependent cell adhesion molecule P-cadherin as a myoepithelial marker in the differential diagnosis of benign and malignant breast lesions.

Methods: Immunohistochemical analysis of normal breast, sclerotic breast lesions, tubular carcinomas, and ductal carcinoma in situ using a P-cadherin specific antibody and comparison with smooth muscle actin.

Results: All myoepithelial cells in normal breast ducts, ductules, and lobules and sclerotic lesions showed strong staining for P-cadherin. There was no staining of tubular carcinomas; myoepithelial cells were demonstrated around in situ carcinomas. Weaker reactivity was seen in a proportion of cells in some hyperplasias and in situ carcinomas. This weak reactivity in these tissues was not seen for smooth muscle actin but in radial scars, tubular carcinomas, and ductal carcinoma in situ staining of stromal cells caused difficulties in the identification of myoepithelial cells.

Conclusion: P-cadherin is a useful marker in the differential diagnosis of breast lesions.

There are various benign and malignant breast lesions that can be difficult to differentiate histologically and which, therefore, can cause diagnostic problems. The frequency of this occurring has increased with the introduction of mammographic screening—for example, differentiating between tubular carcinoma and radial scar. Another problem area is the determination of the presence or absence of invasion in cases of ductal carcinoma in situ.

“We screened a range of benign and malignant breast lesions to evaluate the role of P-cadherin in aiding the differential diagnosis of potential diagnostic problems”

The presence or not of myoepithelial cells has been recognised to be of value in the assessment of these diagnostic problems.¹ The markers used for their identification include smooth muscle actin (SMA), S100, and cytokeratin 14, but some problems have been reported with these—for example, SMA is also present in myofibroblasts² and S100 can be present in normal hyperplastic and neoplastic epithelium, in addition to myoepithelial cells.³

P-cadherin is a calcium dependent cellular adhesion molecule that was first identified in mouse placenta.⁴ It has subsequently been identified in a variety of human tissues including myoepithelial cells.⁵

In our study, we screened a range of benign and malignant breast lesions to evaluate the role of P-cadherin in aiding the differential diagnosis of potential diagnostic problems and compared it with SMA.

MATERIALS AND METHODS

Tissues

Cases were selected from the files of the department of histopathology, University Hospitals of Leicester NHS Trust. They had been fixed in 4% formaldehyde in saline for 18 to 36 hours and processed through to paraffin wax. They included normal breast tissue from 10 reduction mammoplasty specimens, seven cases with the features of radial scar/complex sclerosing lesion, seven tubular carcinomas, and 10 cases of ductal carcinoma in situ, three of which had either small foci of infiltrating ductal carcinoma or microinvasion.

Antibodies

Mouse monoclonal antibody (clone 56) directed against P-cadherin (Transduction Laboratories, BD Biosciences, Oxford, UK) had been generated against a peptide comprising amino acids 72 to 269 of human P-cadherin. One band of molecular mass 120 kDa has been reported on western blotting. Mouse monoclonal antibody (M0851) against SMA was from Dako Ltd (Ely, UK), as were biotinylated rabbit anti-mouse immunoglobulin antiserum and streptavidin–biotin–peroxidase complex.

Immunohistochemistry

Heat induced antigen retrieval was used with pressure cooking for two minutes in 0.1M citrate buffer (pH 6.0). The primary antibodies were applied at a dilution of 1/25 (anti-SMA) and 1/50 (anti-P-cadherin) and incubated for 18 hours at 4°C, followed by incubation with biotinylated rabbit anti-mouse immunoglobulin antiserum at 1/400 and streptavidin–biotin–peroxidase complex. Peroxidase was developed with diaminobenzidine/hydrogen peroxide and sections were counterstained with Mayer's haematoxylin. Normal human skin was used as a positive control for each batch of staining for P-cadherin and the negative control was omission of the primary antibody.

RESULTS

P-cadherin was detected in the myoepithelial cells of ducts and lobules of all 10 samples from reduction mammoplasties. There was no difference in reactivity between large and small ducts or lobules and no differences between different tissues. Staining was generally strong and clearly defined and there was no reactivity of the epithelium. There were similar findings for normal breast associated with breast lesions. One of the 10 samples showed weaker staining for SMA in myoepithelium around acini showing microcystic change but no other differences were noted and there was strong, clearly defined staining of all myoepithelia. There was also staining of small and large vessels.

P-cadherin was present at the periphery of glands within the dense sclerotic tissue of all radial scars/complex sclerosing lesions. There was no staining of stroma or stromal cells. SMA was detected in myoepithelial cells of the glands but in four of the seven cases there was prominent stromal cell reactivity,

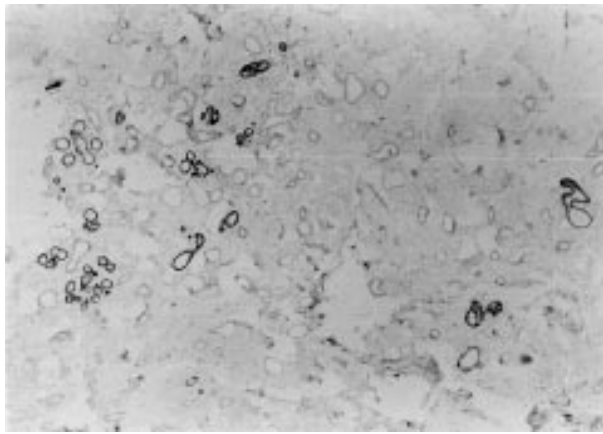


Figure 1 Tubular carcinoma with a small number of normal acini present. No staining of neoplastic tubules for P-cadherin; myoepithelial cells demonstrated in normal acini.

with lesser reactivity in the other three. Both P-cadherin and SMA were detected in myoepithelial cells in associated sclerosing adenosis. Five of the seven cases had associated usual-type ductal hyperplasia and in four of these there was weak staining for P-cadherin in varying proportions of cells within the ductal proliferation, with strong staining of surrounding myoepithelial cells, but no staining of other epithelial cells. There was no staining of ductal proliferations by the anti-SMA antibody.

For all of the tubular carcinomas there was a clear discrimination between normal glands and neoplastic tubular structures for P-cadherin, with staining of myoepithelial cells in the normal glands, but no staining around the neoplastic structures (fig 1). SMA showed the lack of myoepithelial cells around neoplastic tubules, but in five of the seven cases there was prominent stromal cell reactivity, which made interpretation more difficult.

Myoepithelial cells were detected in all cases of ductal carcinoma in situ with anti-P-cadherin (fig 2). This was seen in those cases with invasion, but in some ducts staining was discontinuous. In three of the 10 cases there was cytoplasmic staining of a proportion of the malignant cells within ducts, with similar staining in the small foci of infiltrating ductal carcinoma in one case. The intensity was less than that seen in adjacent myoepithelial cells. This was not seen with SMA, but the staining of stromal cells adjacent to ducts made identification of the myoepithelial cell layer more difficult in four of the seven cases (fig 3). In one other case there was a

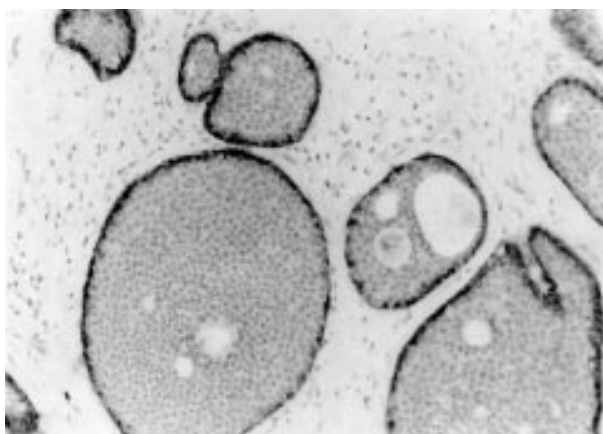


Figure 2 Ductal carcinoma in situ with clearly defined staining of myoepithelial cells for P-cadherin.

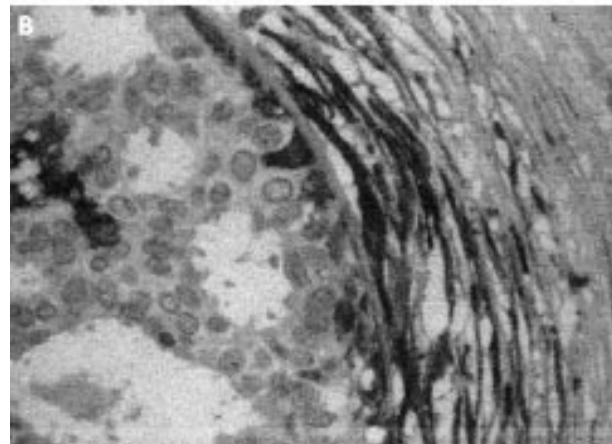
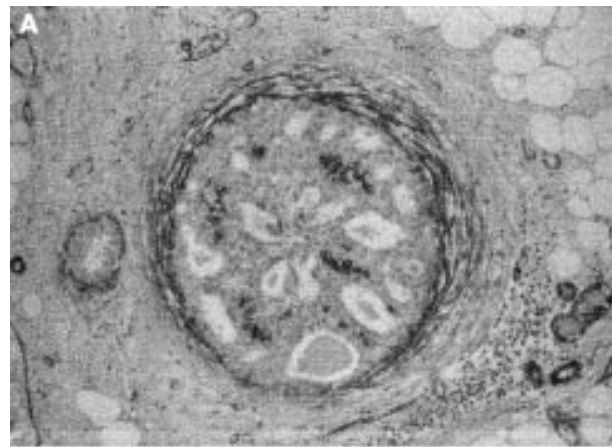


Figure 3 Ductal carcinoma in situ with staining of both myoepithelial cells and myofibroblasts for smooth muscle actin. (A) Low power; (B) high power.

variation between the ducts. The remaining two were low grade ductal carcinoma in situ and there was little stromal reactivity.

DISCUSSION

P-cadherin was identified in all myoepithelial cells of all breast tissues studied, with no differences between ducts and lobules, and overall strong staining. This consistency of reactivity is better than that described for cytokeratins, which may have a high specificity for myoepithelial cells (for example, cytokeratin 14 is specific for basal cells), but a low sensitivity, with not all myoepithelial cells reacting.^{3,6,7} Variation in sensitivity has been described for other myoepithelial markers, such as smooth muscle myosin heavy chain and SMA,⁷ but we found little variation with SMA. Calponin has a high sensitivity, but myofibroblasts may stain,⁸ which can be a problem when assessing invasion. The difference found between P-cadherin and SMA related to the reactivity of stromal cells (myofibroblasts). There was no P-cadherin reactivity in these cells, which resulted in the clear delineation of myoepithelial cells, whereas the SMA positivity in myofibroblasts, as reported previously,² caused problems with interpretation in radial scars, tubular carcinomas, and all but low grade ductal carcinomas in situ. P-cadherin staining was seen in a proportion of usual-type ductal hyperplasias and ductal carcinoma in situ, with reactivity in small areas of an invasive carcinoma. P-cadherin can be detected in approximately 40% of invasive carcinomas and is associated with high grade.⁹ The staining in hyperplasias related to areas of so called intermediate differentiation and in both situations was not as intense as that seen in myoepithelial cells. There was no staining of

Take home messages

- P-cadherin is a highly sensitive marker for myoepithelial cells, although a proportion of benign and malignant proliferations are also stained
- When differentiating between a radial scar and a tubular carcinoma, the distinct staining of myoepithelial cells for P-cadherin and the lack of myofibroblast reactivity make it preferable to smooth muscle actin
- Thus, P-cadherin should be considered as a helpful tool in the differential diagnosis of breast lesions

normal epithelial cells as has been reported for S100,³ which can also stain ductal proliferations.

“There was no P-cadherin reactivity in myofibroblasts, which resulted in the clear delineation of these cells, whereas the smooth muscle actin positivity in myofibroblasts caused problems”

On balance, P-cadherin has a high sensitivity for myoepithelial cells; its specificity problems relate to staining of a proportion of benign and malignant proliferations. In instances such as differentiating between a radial scar and a tubular carcinoma the distinct staining of myoepithelial cells with anti-P-cadherin and the lack of myofibroblast reactivity make it preferable to SMA. It is certainly a marker that should be considered as a helpful tool in the differential diagnosis of breast lesions.

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ECHO**TGF- β and transglutaminase 2 work together to cause cataracts**

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A molecular study has shown for the first time that transglutaminase 2 (TGase 2) overexpression induced by transforming growth factor β (TGF- β) is a key step in the development of some cataracts.

The investigators showed TGase 2 mRNA and TGase 2 protein in human lens epithelial cells taken from anterior polar cataracts but not in cells from nuclear cataracts or healthy lens cells.

When they added TGF- β to cultures of human lens epithelial cells (HLE B-3) TGase 2 expression increased in a time and dose dependent way, compared with untreated cell cultures.

The investigators then treated cultures unprimed or primed by TGF- β with fluorescein cadaverine to see whether fibronectin cross linking seen in cataracts was due to increased TGase 2 activity. Fluorescent foci on the surface of the primed cells confirmed that it was. Separate staining with antibody to fibronectin showed abundant fibrils on the surface of primed cells but hardly any on unprimed cells. Superimposed images obtained with each label showed coincident surface labelling of the primed cells, indicating that TGF- β induces TGase 2 cross linking of fibronectin. Finally, double staining with fluorescein cadaverine and fibronectin antibody showed greater, co-localised staining of HLE B-3 cells transfected with TGase 2 gene compared with control cultures transfected with vector only.

TGase 2 activity has been suspected in cataract formation as it can cross link extracellular matrix proteins with very stable isopeptide bonds. TGF- β is another key factor in cataract formation, but exactly how it interacts with TGase 2 was not known.

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