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Immunohistochemical profile of invasive lobular carcinoma of the breast: predominantly vimentin and p53 protein negative, cathepsin D and oestrogen receptor positive

Wenancjusz Domagala[1,3], Maciej Markiewski[1], Robert Kubiak[2], Jacek Bartkowiak[2], Mary Osborn[3]

[1] Department of Pathology, Medical Academy, Szczecin, Poland
[2] Department of Oncology, Medical Academy, Lodz, Poland
[3] Department of Biochemistry, Max Planck Institute for Biophysical Chemistry, Goettingen, Germany

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Abstract. Vimentin, p53 protein and cathepsin D positivity were assessed by immunohistochemistry, and oestrogen receptor (ER) by an enzyme immunoassay, in invasive lobular carcinomas (LC) of the breast. While vimentin was positive in only 5% (3/57) and p53 protein was positive only in 3% (2/63), cathepsin D was expressed in 86% (48/56) and ER in 78% (25/32). Classical LC were negative for p53 protein and all except one were cathepsin D positive. These results are in contrast to invasive ductal breast carcinomas (DC), where the reported average incidence of vimentin and p53 protein is much higher (19% and 33% respectively) and that of cathepsin D and ER lower (63% and 67% respectively). Thus lack of expression of vimentin and lack of p53 positivity together with high incidence of expression of cathepsin D and ER are more often associated with lobular than with ductal differentiation of invasive breast cancer. The results show that LC, distinguished morphologically, can further be defined by its immunohistochemical profile. This in turn may point to underlying biological differences between LC and DC.

Introduction

Although both invasive lobular carcinoma (LC) and invasive ductal carcinoma (DC) of the breast are thought to arise from the terminal duct lobular unit (Wellings et al. 1975), they differ in morphological appearance and in clinical behaviour. The principal histological criteria of classical LC include: single rows of widely separated malignant cells (Indian files), a "targetoid arrangement" of tumour cells and so called "skip areas". The tumour cells are less pleomorphic and usually smaller than those of DC (Millis and Girling 1989). In addition several variant forms of LC have been described (DiConstanzo et al. 1990; Millis and Girling 1989). The principal clinical difference between LC and DC is an increased frequency of multicentricity, bilaterality and bone metastases in LC (DiConstanzo et al. 1990; Millis and Girling 1989). It is not known whether the morphological differences between LC and DC are reflected by a different expression of biological markers which can be revealed by immunohistochemistry, since only a few such studies have been published. Reiner et al. (1988) have found that LC are more likely to be oestrogen receptor (ER) positive. Expression of alpha-lactalbumin, (Bailey et al. 1982; Nesland et al. 1985; Walker 1979) casein (Eusebi et al. 1977; Nesland et al. 1985) and carcinoembryonic antigen (Kuhajda et al. 1983; Nesland et al. 1985) have not been distinctive for LC.

While examining the prognostic significance of vimentin and p53 protein in breast carcinomas we were struck that the limited number of LC we studied were vimentin negative (Domagala et al. 1990a), and p53 protein was found only in 4% of LC (Domagala et al. 1993). This is in contrast to DC where both markers are often found (Cattoretti et al. 1988; Domagala et al. 1990a, b, 1993; Isola et al. 1992; Raymond and Leong 1989a, b; Thor et al. 1992). Here we have included not only vimentin and p53 protein but also cathepsin D and ER in order to see whether LC distinguished by morphological criteria can be further defined by markers reported to have prognostic significance in DC.

Materials and methods

Paraffin embedded tissues from 80 patients with primary LC of the breast and 7 patients with lobular carcinomas in situ (LCIS) were retrieved from the files of the Department of Oncology, Medical Academy, Lodz, and the Department of Tumour Pathology, Medical Academy, Szczecin, Poland. The specimens were collected between 1982–1992, and had been routinely fixed in formalin for 24 h. Histology of all cases was reviewed and representative sections were selected for immunohistochemistry.

Histological typing was performed according to published criteria (DiConstanzo et al. 1990; Millis and Girling 1989). There

Correspondence to: Mary Osborn, Department of Biochemistry, Max Planck Institute for Biophysical Chemistry, Postfach 2841, D-37018 Goettingen, Germany

were 31 "classic" LC and 49 "variant" forms. Forty-one lobular breast carcinomas included in this study were used in previous studies on vimentin (Domagala et al. 1990a, b) and 47 such tumours in a study on p53 (Domagala et al. 1993) in breast carcinomas.

For immunohistochemistry sections were deparaffinized in xylene, transferred to 100% and 95% ethanol and air-dried. The mouse monoclonal V9 antibody (Osborn et al. 1984), the rabbit polyclonal p53 antiserum CM-1 diluted 1:70 (Medac, Hamburg, FRG), mouse monoclonal p53 antibody DO1 (Oncogene Science, Manhasset, N.Y.; USA) and rabbit polyclonal cathepsin D antiserum diluted 1:100 (Medac, original supplier Novocastra, Newcastle, England) were used as primary antibodies. For the cathepsin D assay, deparaffinized sections were treated with 0.1% trypsin for 5 min at room temperature prior to immunohistochemistry. Staining was revealed with the streptavidin-biotin-peroxidase kit (Histostain-SP kit, Zymed Laboratory, San Francisco, Calif., USA). The sections were slightly counterstained with haematoxylin.

Cytoplasmic immunostaining, especially around the nucleus of tumour cells, was seen in 15/47 (32%) of LCs treated with CM-1 antibody. The DO1 antibody did not show cytoplasmic staining of tumour cells and was therefore the preferred reagent.

The ER enzyme immunoassay (EIA) was used as recommended by the manufacturers (Abbott Laboratories, Chicago, Ill., USA). ER concentrations were expressed in fmol/mg cytosol protein. They were quantified in the range of 0 to 500 fmol/mg cytosol. A cut-off value of 60 fmol/mg cytosol protein was chosen to divide high and low ER cancers (Domagala et al. 1990a).

Results

Tumours were considered positive for vimentin when there was cytoplasmic staining with V9 antibody in >10% of tumour cells as assessed semi-quantitatively (Fig. 1b). Negative staining of benign epithelial cells and positive staining of macrophages, fibroblasts and endothelial cells constituted built-in positive and negative controls. No classic LC expressed vimentin (Fig. 1a). Tumour cells in 3 of 57 LC (5%) expressed vimentin (Table 1). In one tumour approximately 25% of tumour cells expressed vimentin and in two tumours over 50% of tumour cells were vimentin positive. Three vimentin positive tumours were solid type variant LCs. All three were cathepsin D positive and p53 protein negative.

Wild type nuclear p53 protein cannot be detected by immunohistochemistry in normal cells because of its very short half-life and low abundance. However, many mutant p53 proteins accumulate in the nuclei of tumour cells where they can be detected immunohistochemically (Fig. 2b) and immunohistochemical detection of nuclear p53 protein is thus regarded as synononymous with mutation (Lane and Benchimol 1990). In this study we used a 1% cutoff level, that is, tumours were considered positive for p53 if there was strong nuclear staining in >1% of tumour cells as assessed semi-quantitatively. Davidoff et al. (1991) have shown that no mutations of the highly conserved regions of the p53 gene were present in tissues containing rare cells with nuclear staining.

There were 55 tumours with no nuclear staining (Fig. 2a) and 6 tumours in which a few (<1%) tumour cells were stained. Thus there were 61 LC that were scored as negative for nuclear p53. Of 63 LC, only 2 (3%) were positive for nuclear p53: 1 accumulated p53 in more than 75% of tumour cell nuclei, the other in 25–30% of tumour cell nuclei. Histologically, both tumours were variant forms of LC.

Cathepsin D immunostaining was seen as red, coarse, or tiny cytoplasmic granules in tumour cells (Fig. 3a, b). Cathepsin D positive macrophages served as a built-

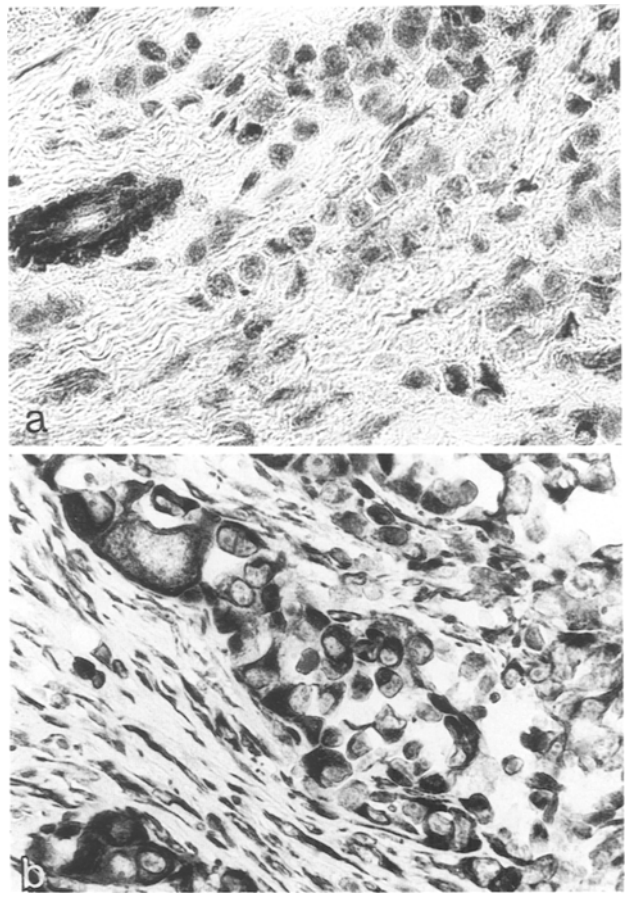

Fig. 1a, b. Vimentin in lobular (**a**) and in ductal NOS (**b**) breast carcinoma. **a** All tumour cells are vimentin negative. Note strongly vimentin positive cells of the wall of the blood vessel. **b** Strong vimentin expression is seen in the cytoplasm of almost all cancer cells. Connective tissue cells are also vimentin positive. Indirect immunoperoxidase, light nuclear counterstain

Table 1. Expression of vimentin, p53 protein, cathepsin D and oestrogen receptor (ER) in invasive (LC) and in situ lobular breast carcinomas (LCIS)

Marker	LC	LCIS
vimentin[a]	3/57 (5%)[c]	0/3
p53[b]	2/63 (3%)	0/7
Cathepsin D	48/56 (86%)	3/5
ER	25/32 (78%)	

[a] 41 cases reported in Domagala et al. 1990a, b
[b] 47 cases reported in Domagala et al. 1993
[c] 3 vimentin positive tumours were also positive for cathepsin D and p53 protein
Number of positive tumours/number of tumours tested

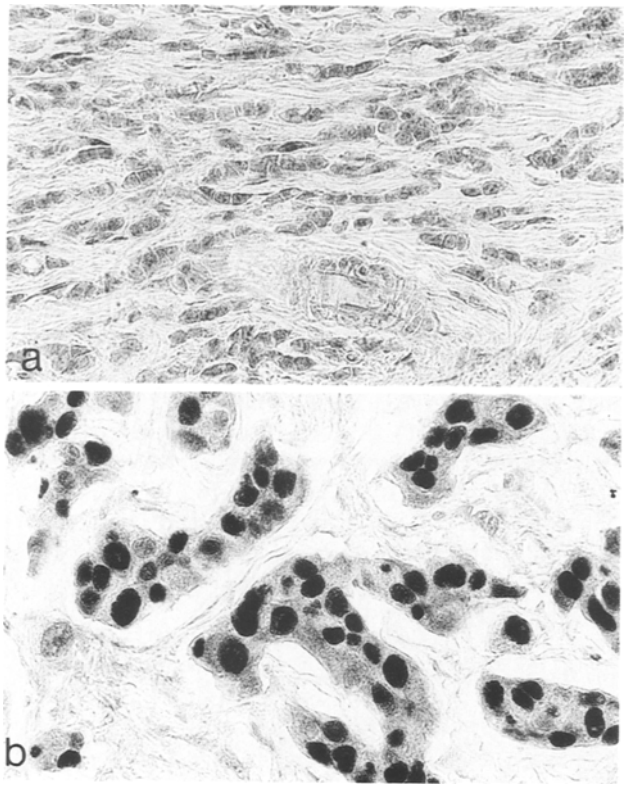

Fig. 2a, b. p53 protein in lobular (**a**) and in ductal (**b**) breast carcinoma. **a** The nuclei of all tumour cells are negative for p53 protein. **b** Almost all tumour cell nuclei accumulate p53 protein. Streptavidin-biotin-peroxidase, light nuclear counterstain

in positive control. Of 56 LC, 48 (86%) were cathepsin D positive, and 4 were negative. In the remaining 4 cases cathepsin D positive tumour cells appeared focally in a section, in less than 10% of cells, and these were included in the negative group. In 36/48 (75%) there was very strong positivity; the cytoplasm of tumour cells was full of coarse cathepsin D positive granules. All except 1 classical LC expressed cathepsin D.

We chose a higher cut-off value of 60 fmol/mg of cytosol protein for ER since the ER EIA assay is more sensitive than the dextran-coated charcoal method (Thorpe 1987). Thus a value of 53 fmol/mg cytosol protein was the highest ER EIA level designated as ER

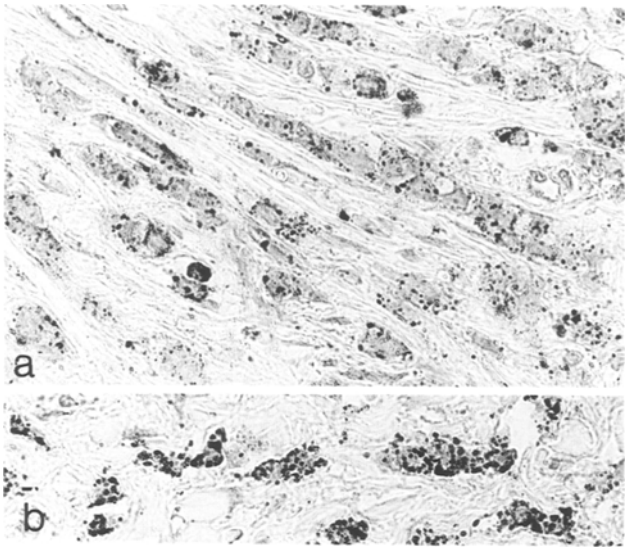

Fig. 3a, b. Cathepsin D in lobular carcinoma. **a** Note numerous coarse cytoplasmic granules positive for cathepsin D. **b** Strong cathepsin D expression in the cytoplasm of tumour cells in a different case. Streptavidin-biotin-peroxidase, light nuclear counterstain

negative by the dextran-coated charcoal method (Thorpe 1987). Therefore by adopting the higher cut-off ER value the comparison of our results with previous reports on ER in LC which used the dextran-coated charcoal method and 10 fmol/mg as the cut-off level is more meaningful.

Of 32 LC tested for ER, 25 (78%) were ER positive. Fifteen of these had ER values exceeding 300 fmol/mg cytosol protein.

Discussion

The results of our study show that LC distinguished morphologically can be further defined using immunohistochemical markers. We found that the vast majority of LC was cathepsin D positive (86%), ER positive (78%), vimentin negative (95%) and p53 protein negative (97%) (Table 1). These results are in contrast to DC where the reported average incidence of vimentin and p53 protein is much higher (19% and 33% respec-

Table 2. Vimentin, p53 protein, cathepsin D and ER in invasive ductal breast carcinomas (DC)[a]

Marker	Number and % positive	References
vimentin	116/606 (19%) (range: 13–25%, 37% in grade III DC)	Cattoretti et al. 1988 Domagala et al. 1990a, b Raymond and Leong 1989a, b [c]
p53 protein	595/1823 (33%) (range: 14–52%)	
cathepsin D	292/462 (63%) (range: 60–66%)	Domagala et al. 1992 Garcia et al. 1987 Henry et al. 1990
ER[b]	117/265 (67%)	Reiner et al. 1988

[a] Only studies with over 100 cases included. Values depend partially on cut off levels and antibodies used. Data taken from the references indicated for each marker

[b] Determined by ER-ICA, for other reports see Discussion

[c] See references cited in Domagala et al. 1993

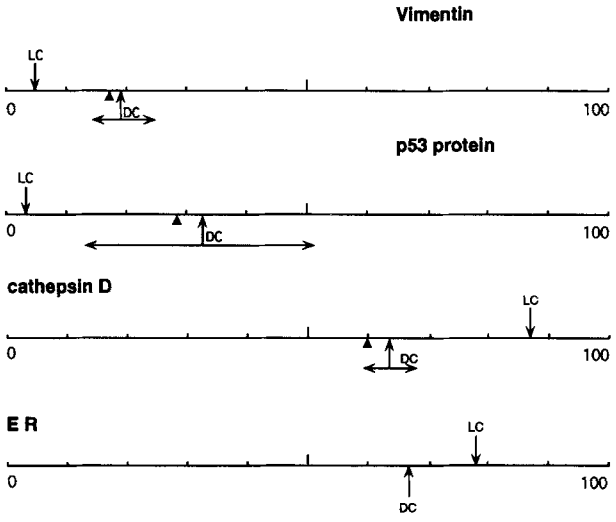

Fig. 4. Vimentin, p53 protein, cathepsin D and ER in invasive lobular carcinomas (LC) and invasive ductal carcinomas (DC) of the breast. For each marker the percentage of positive cases is shown on a scale from 0 (*left*) to 100% (*right*), with LC above, and DC below the line. ↓LC: average percentages for LC taken from Table 1 of this study; ↑DC: average percentages and ↔: ranges taken for each marker from all reports cited in Table 2; ▲ our results on DC published in Domagala et al. 1990a, b, 1992, 1993. The LC results are outside the range seen for each of the four markers in DC. Thus lower percentages of LC than DC are vimentin and p53 positive, while higher percentages of LC than DC are cathepsin D and ER positive

tively) and that of cathepsin D and ER lower (63% and 67% respectively) (Table 2).

In recent studies all LCs (0/41) were negative for vimentin (Domagala et al. 1990a, b). Here we have tested an additional 16 cases and again all were vimentin negative except for three solid type variant forms. Raymond and Leong (1989a, b) reported 7 vimentin negative LCs and Gould et al. (1990) found scattered vimentin positive cells in 1 of 7 LCs. In summary, only 4 of 71 (6%) LCs expressed vimentin. No classical LC was vimentin positive. Although Gould et al. (1990) did not specify the histological subtype of the 1 vimentin positive LC in their study, it is clear that vimentin expression in LC is a very rare event. This is in sharp contrast to DC, where 116 of 606 (19%) cases analysed in five studies (Cattoretti et al. 1988; Domagala et al. 1990a, b; Raymond and Leong 1989a, b) were vimentin positive. In grade III DC this percentage increased to 37% (53/142) (Table 2).

In a recent report we found only 2 of 47 LC with p53 protein accumulation (Domagala et al. 1993). All 16 additional LC tested in the present study were p53 protein negative. In five separate reports a further 27 LC were tested and only 5 were found to be positive for p53 protein (references in Domagala et al. 1993). Thus, p53 protein accumulation was found in 8% (7/90) of LC. In contrast, in invasive breast DC the reported range of incidence of p53 protein accumulation is from 14% to 52% with an average of 33% (references in Domagala et al. 1993).

We found cathepsin D expression in 86% of LC. Seventy-five percent of positive tumours were very strongly positive. All classic LC except 1 were cathepsin D positive. The incidence of cathepsin D expression in LC has not been reported, however in three immunohistochemical studies the enzyme has been found in 60% (81/136) (Domagala et al. 1992), 64% (149/232) (Garcia et al. 1987) and 66% (62/94) (Henry et al. 1990) of invasive breast DC. Thus cathepsin D expression is found with higher frequency among LC than in invasive ductal carcinomas. This is particularly striking if classic LC and invasive DC are compared.

In 1975 Rosen et al. found a significantly higher frequency of ER positive tumours among LC (92%, 12/13) than DC (24%, 5/21). Antoniades and Spector (1979) reported for LC that 85% were ER positive and 69% ER-rich as compared to DC where 61% were ER positive and 21% were ER rich. Rasmussen et al. (1981) and Smith et al. (1987) reported 81% and 90% of LC as ER positive compared to 61% and 67% ER positive DC respectively. The alveolar variant of LC was reported as particularly rich in ER (Nesland et al. 1992; Shousha et al. 1986). McCarty et al. (1980) found that the ER values in LC were higher than those of DC by 20–25%. All LC (16/16) studied by Nesland et al. (1985) were ER positive. However, several reports based on biochemical ER determination do not show a correlation between ER and histological type of breast cancer (see Mohammed et al. 1986; Lesser et al. 1981; Silfversward et al. 1980). Reiner et al. (1988) reported no difference for LC and DC with the ER dextran coated charcoal assay but observed a clearly higher frequency of ER positivity in LC (53/63, 84%) versus DC (177/265, 67%) when a ER- immunocytochemical assay was used. Our results, based on a sensitive ER EIA technique with which 78% of LC were found to be ER positive and 60% of these were also ER-rich (i.e. >300 fmol/mg cytosol protein), are in agreement with that report.

Immunohistochemistry with several additional markers in breast LC has not proved distinctive for LC. Alpha-lactalbumin has been found in 0% (Bailey et al. 1982), 17% (Walker 1979) and 19% (Nesland et al. 1985) of LC. Casein has been reported in 81% (Nesland et al. 1985) and 100% (Eusebi et al. 1977) of LC. Carcinoembryonic antigen was expressed in 33% (Kuhajda et al. 1983) and 65% (Nesland et al. 1985) of LC.

In summary, although no single immunohistochemical marker appears specific for LC these tumours are predominantly vimentin negative, p53 protein negative, and cathepsin D and ER positive. Examination of Fig. 4 shows that lack of expression of vimentin and of p53 protein together with a high incidence of expression of cathepsin D and ER seem to be more often associated with lobular than with ductal differentiation of invasive breast carcinoma. Thus the results in LC appear outside the range of DC with vimentin and p53 on the lower side and cathepsin D and ER on the higher side (Fig. 4).

There have been several studies relating the markers used in this study to prognosis for patients with DC. Thus node negative patients with vimentin negative tumours have a better prognosis than do those with vimen-

tin positive tumours (Domagala et al. 1990c). Likewise for node negative DC patients p53 protein positivity is associated with a poorer prognosis (Allred et al. 1993; Isola et al. 1992; Thor et al. 1992). The data for cathepsin D and prognosis is not clear. Several immunoenzymatic studies have found that high concentrations of cathepsin D in the cytosol of breast carcinomas were associated with poor prognosis and an increased risk of metastasis (for a review see Rochefort et al. 1990). There have been two immunohistochemical studies. In one, positive cathepsin D immunostaining was associated with a significant prognostic advantage in lymph node positive breast cancer patients, while in the subgroup of ER positive tumours cathepsin D staining was associated with significantly prolonged survival (Henry et al. 1990). However, in the second we found no correlation of positive cathepsin D immunostaining with survival at 5 years for patients with invasive DC (Domagala et al. 1992).

When survival of patients with LC and DC are compared "most studies have not observed dramatic differences" (DiConstanzo et al. 1990). However in an extensive study based on 230 patients with invasive LC (DiConstanzo et al. 1990) it was possible to identify a subgroup of patients with node negative classical LC for whom the disease free survival was significantly better than for node negative patients with DC, although it should be noted that there were no differences in overall survival of the two groups. It remains to be seen whether the different frequency of expression of vimentin, p53 protein, cathepsin D and ER in LC and DC may help to explain such prognostic differences, as well as differences in their clinical behaviour. Future research may also determine whether those cases of LC positive for vimentin or p53 protein or negative for cathepsin D or ER are different in biological behaviour to the remaining LC cases which share a more common immunophenotype. Finally the fact that LC differ from DC with respect to incidence of expression of vimentin, p53 protein, cathepsin D and ER should be taken into account in any studies looking for an association between those markers and other pathological factors or prognosis. In such studies DC and LC should be analysed separately.

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