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CASE REPORT

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Intralobular growth of myoepithelial cell carcinoma of the breast

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Abstract Two cases of intralobular carcinoma of the breast showing myoepithelial cell differentiation are reported. One was an in situ lesion localized within a fibroadenoma; the second was predominantly in situ, but areas of invasion were present. The neoplastic cells had round to ovoid nuclei and were polygonal to spindle in shape displaying glycogen rich clear cytoplasm. Alpha-smooth muscle actin was present in the cytoplasm of the neoplastic cells in both cases. In one case the same cells displayed cytoplasmic microfilaments at electron microscopic level. Intralobular growth of neoplastic myoepithelial cells has never been described in the literature, and this line of differentiation has to be added to the endocrine and apocrine features occasionally observed in in situ lobular carcinomas of the breast.

Key words Breast · Lobular carcinoma in situ
Myoepithelial cells

Introduction

In the last decade, an increasing number of breast tumours showing myoepithelial cell differentiation have been described including spindle myoepithelial cell invasive carcinoma [8, 9, 21, 24] and adenomyoepithelioma [12, 17, 20, 23].

In contrast, in situ myoepithelial cell carcinomas are very rare. Recently one case of an intraductal carcinoma with myoepithelial cell differentiation was documented [22]. The purpose of this paper is to report on two cases of lobular carcinoma, predominantly in-situ, showing myoepithelial cell differentiation.

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Case reports

A 42-year-old female (case 1) presented with a 4 year history of a tender lump in the upper outer quadrant of the left breast. Fine needle revealed neoplastic cells. A quadrantectomy with axillary lymph node dissection was performed. The excised quadrant showed fibrofatty tissue macroscopically with an ill defined nodule measuring about 1.5 cm in its greatest axis in the centre. Fourteen lymph nodes were found in the axillary tissue. The patient has no sign of disease 12 months after the excision.

The second case (case 2) was a 47-year-old female who presented with a short history of a lump in the upper outer quadrant of the right breast. The lesion measured 0.8 cm in its greatest axis. A quadrantectomy was performed together with axillary lymph node dissection (9 lymph nodes). The patient has no sign of disease four months after quadrantectomy.

Materials and methods

Tissues were fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with haematoxylin and eosin (H & E), periodic acid-Schiff (PAS) before and after diastase digestion and Alcian blue (pH 2.5). For immunohistochemistry the avidin-biotin peroxidase method [18] was employed. Positive and negative controls were used. The antisera employed and their dilution are reported in Table 1. Thin blocks were retrieved from paraffin-embedded samples and embedded in epon-araldite for the electron microscopic study in case 1.

Results

The overall lesion in both cases consisted of expanded lobules grouped together in a scanty oedematous stroma in which numerous mature lymphocytes were present (Fig. 1). The acini were filled with neoplastic polygonal to spindle cells which displayed clear finely granular cytoplasm. The granules stained with PAS, but the stain disappeared with diastase pre-digestion. The nuclei of the neoplastic cells varied from round to ovoid and dis-

Table 1 Antisera employed in immunocytochemistry (*S.m. actin* alpha-smooth muscle actin; *EMA* epithelial membrane antigen, *GFAP* glial fibrillary acid protein, *LW keratin* low weight keratin, *HW keratin* high weight keratin, *GCDFP-15* gross cystic disease fluid protein-15, *P* polyclonal, *M* monoclonal)

Antisera	P/M	Source	Dilution
S.m. actin	M	Dr. G. Gabbiani (Geneve)	1:2000
CGDFP-15	P	Dr. D. E. Haagensen (Boston)	1:10000
Collagen IV	M	Dakopatts (Denmark)	1:100
Laminin	M	Dakopatts	1:20
EMA	M	Dakopatts	1:200
Factor VIII	P	Dakopatts	Kit
Chromogranin	M	Ortho Diagnostic (Milan)	1:20000
GFAP	M	Dakopatts	1:5000
LW keratin (EA 902)	M	Ortho Diagnostic	1:300
HW keratin (EA 903)	M	Ortho Diagnostic	1:300
S-100 protein	P	Dakopatts	1:1500
Vimentin	M	Dakopatts	1:200

played prominent nucleoli (Figs. 2, 3). Numerous, irregular mitoses were readily seen. Minute areas of patchy necrosis were present in the centre of some expanded solid acini in case 1. The acini although packed together were individually outlined by a neat PAS, laminin and collagen IV positive basal lamina (Fig. 4). At one edge of the lesion in case 1 numerous non neoplastic lobules

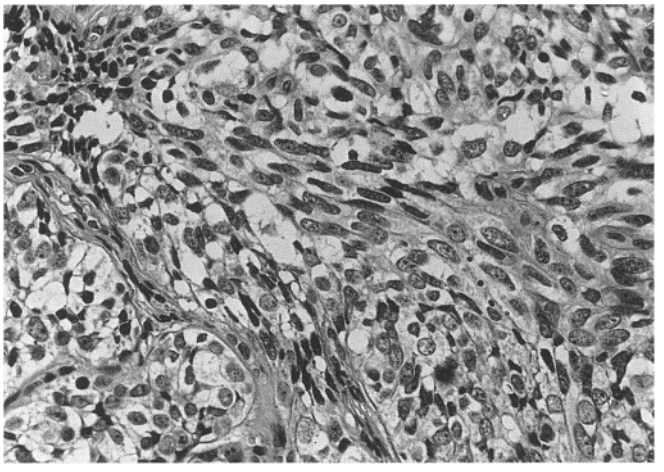

Fig. 2 Case 2; the neoplastic cells vary greatly in size. Some are polygonal in shape, others display a neat spindle shaped cytoplasm. (H & E ×350)

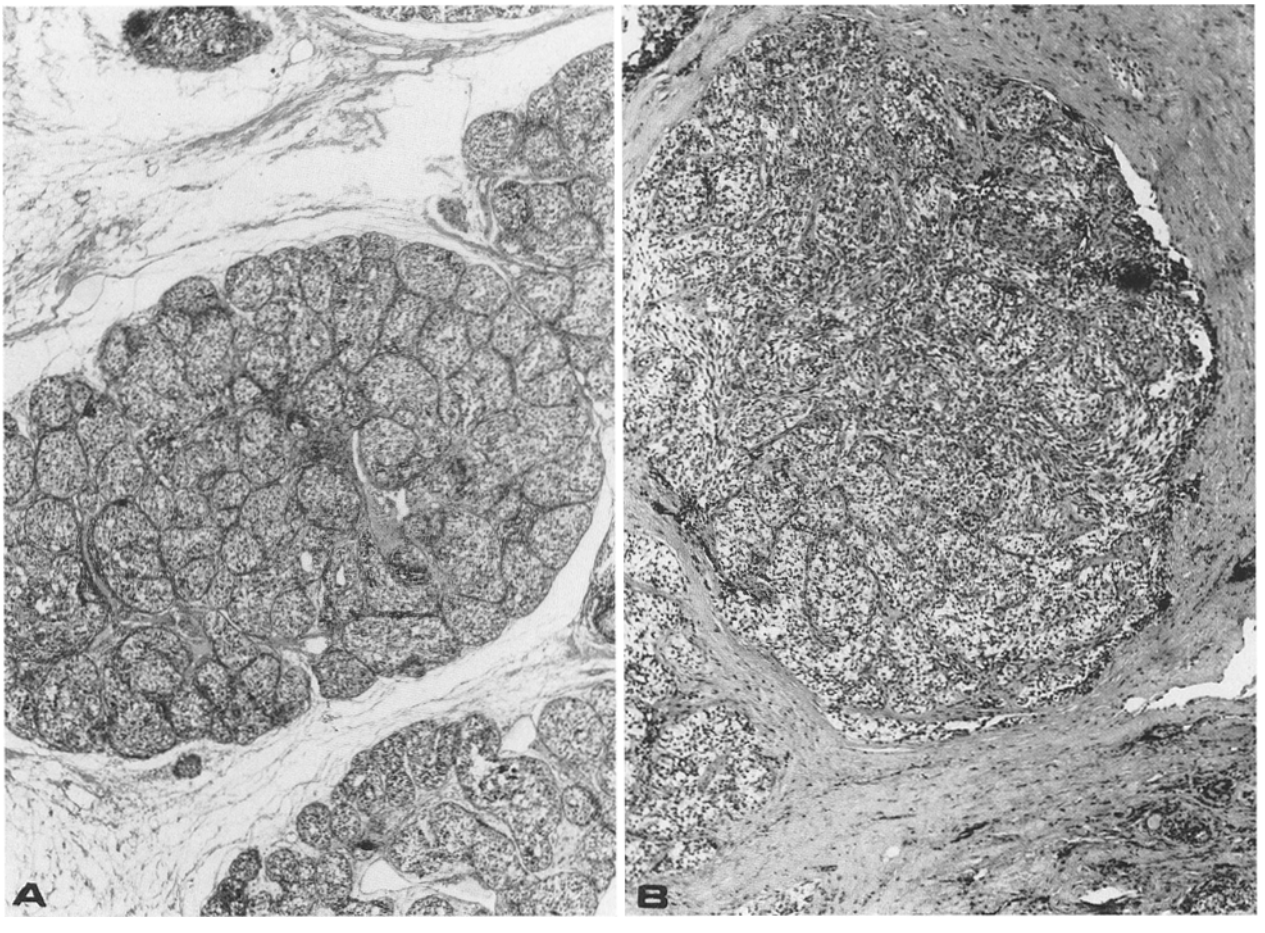

Fig. 1A, B Expanded lobules show acini filled with neoplastic cells. **A** Case 1; lobules are immersed in oedematous stroma. **B** Case 2; the neoplastic cells are spindle shaped. Haematoxylin and eosin (H & E) ×75

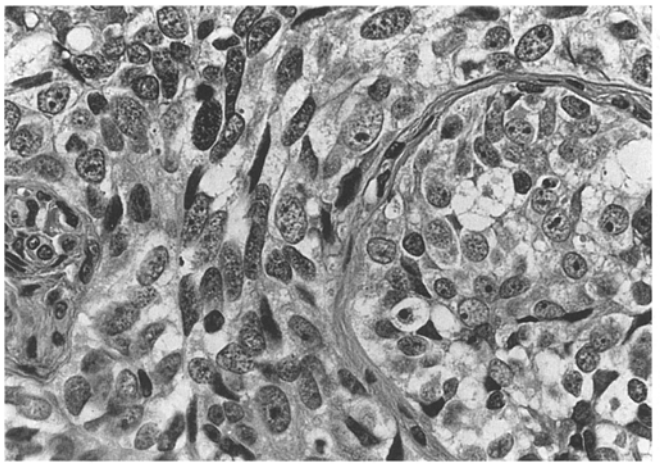

Fig. 3 Case 2; irregular nuclei are readily visible: they vary from round to ovoid and display a prominent nucleolus. The cytoplasm is clear. (H & E ×450)

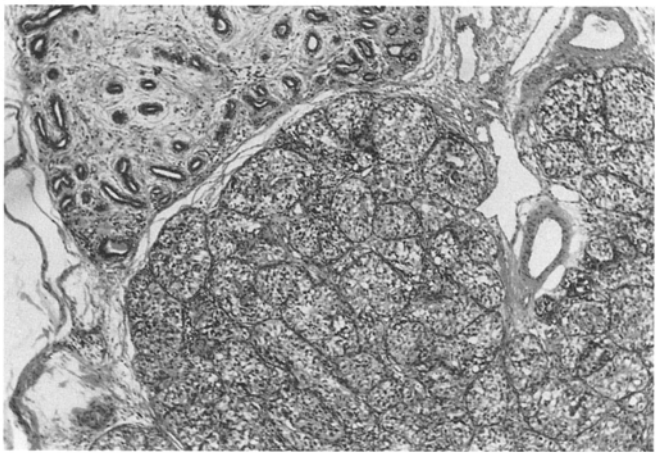

Fig. 5 Case 1; part of the lesion is constituted by a pericanalicular fibroadenoma. (H & E ×100)

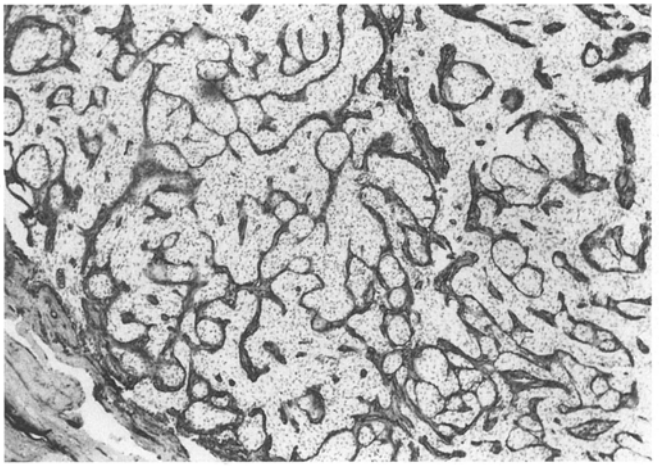

Fig. 4 Case 1; the acini are individually outlined by anti-collagen IV antiserum. Avidin-biotin complex (ABC) peroxidase ×100

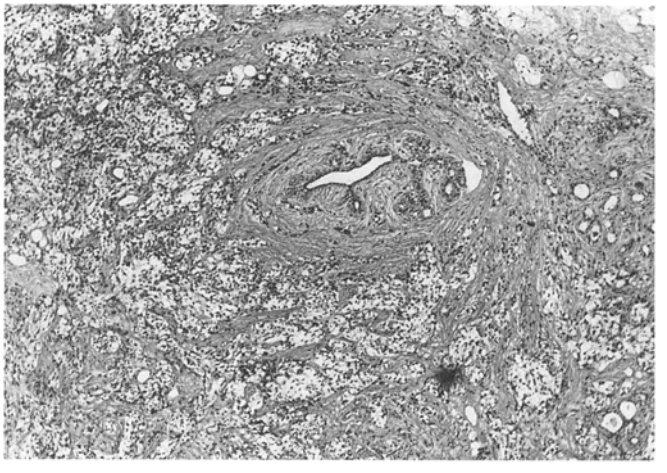

Fig. 6 Case 2; nests of invasive cells with clear cytoplasm surround a residual small duct. Some cells show a periductal single file pattern of invasion. (H & E ×100)

were seen immersed in an intralobular stroma showing prominent myxoid features which are reminiscent of a pericanalicular fibroadenoma (Fig. 5). In case 2 the lobules merged with irregular sheets of neoplastic elements, occasionally showing an indian file invasive pattern (Fig. 6). In these areas no hint of laminin positive basal lamina was seen. The cells in these areas were often spindle shaped and displayed a clear cytoplasm (Fig. 7). About 10% of the total neoplastic proliferation, mostly of the spindle cells type, was immunostained by anti-alpha-actin antiserum in case 1. Alpha-smooth muscle actin was immunolocalized in at least 50% of the total neoplastic proliferation of case 2. The same antiserum strongly stained the myoepithelial cells which outlined the neoplastic acini as well as those encircling normal acini (Figs. 8–10). Most of the clear cells in the invasive component were strongly immunoreactive with anti-alpha-smooth muscle actin antiserum (Fig. 11). A small number of neoplastic cells, mainly located in the centre of the

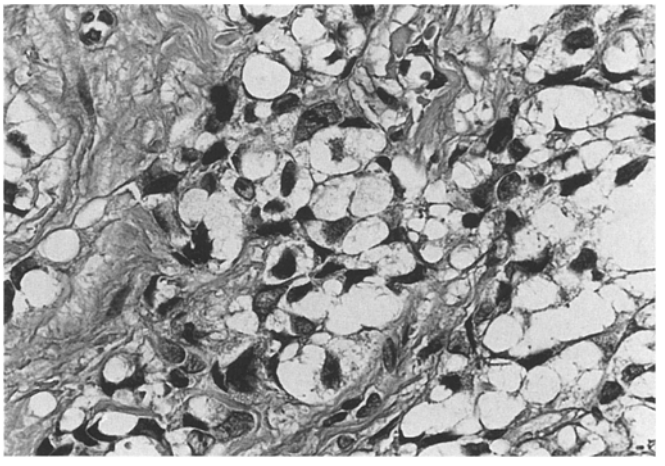

Fig. 7 Case 2; at higher power the neoplastic invasive cells show hyperchromatic nuclei and clear cytoplasm. (H & E ×450)

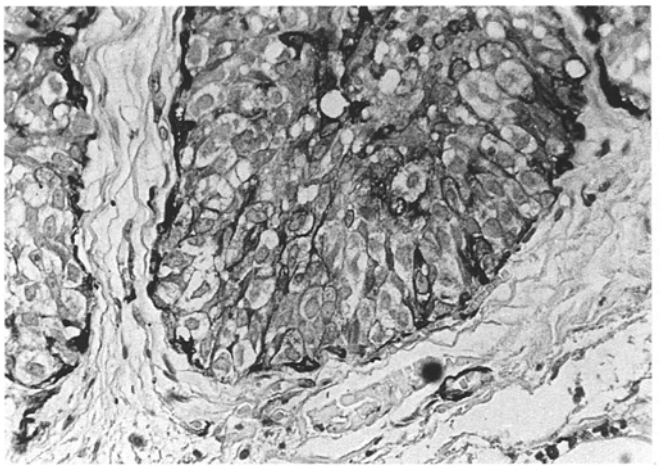

Fig. 8 Case 1; numerous neoplastic elements are immunostained by the anti-alpha-actin antiserum. In the outer cell layer residual myoepithelial cells are strongly stained. (ABC) peroxidase ×350)

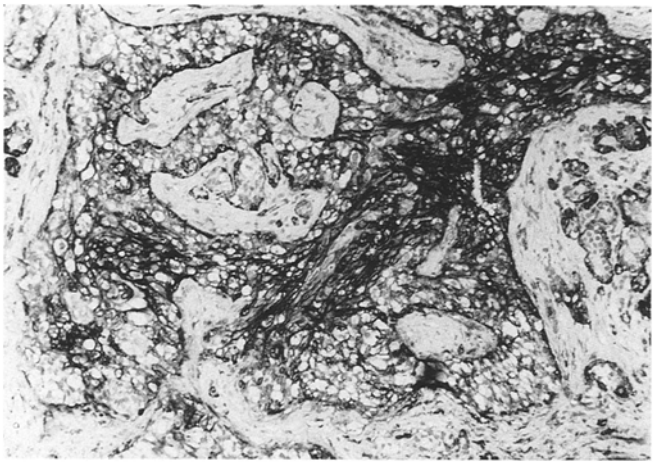

Fig. 9 Case 2; several neoplastic cells in this in situ part, show an intense staining of their cytoplasm. The myoepithelial outer cell layer is well evident. (ABC peroxidase, anti-alpha-actin antiserum ×200)

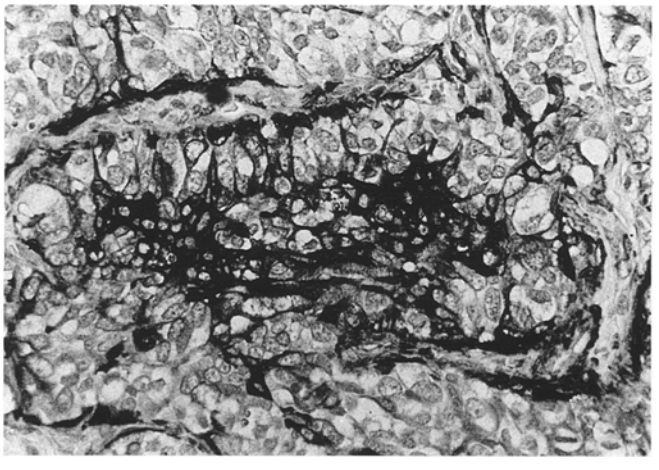

Fig. 10 Case 2; the central part of this acinar structure is constituted by strongly immunoreactive neoplastic elements. (ABC peroxidase, anti-alpha-actin antiserum ×350)

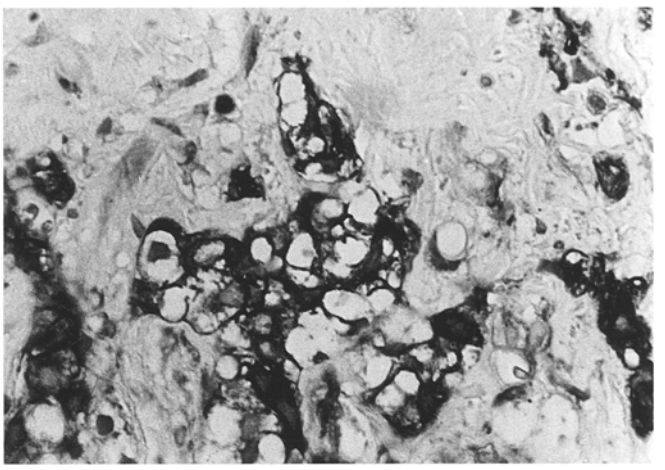

Fig. 11 Case 2; numerous invasive elements are immunostained by anti-alpha-actin antiserum. (ABC peroxidase, ×450)

acini appeared positive with anti-low-weight cytokeratin and epithelial membrane antigen (EMA) antisera. EMA antiserum also outlined small irregular spaces which were hardly visible at the H & E level. High-weight cytokeratin, glial fibrillary acid protein (GFAP), S-100 protein, chromogranin and vimentin antisera were consistently negative in the neoplastic cells, as well as in the normal acinar tissue surrounding the lesion, including myoepithelial cells of the acini. Gross cystic disease fluid protein-15 stained occasional entrapped residual glandular structures in case 2. The breast tissue sampled from quadrantectomy in both cases showed normal medium to small size ducts as well as normal lobules. Occasional cysts lined by apocrine epithelium were also present. The axillary lymph nodes showed reactive changes.

By electron microscopy the neoplastic cells showed ovoid to elongated nuclei. The nuclear membrane was often indented and peripheral nucleoli were seen. The cytoplasm although generally poorly preserved contained bundles of thin filaments (3.5–4 nm) readily visible (Fig. 12).

Discussion

The present cases were characterized by a proliferation of pleomorphic cells showing a lobular growth pattern which was localized within a fibroadenoma in case 1. Although the neoplastic acini were packed together, these were outlined by a well defined layer of actin positive myoepithelial cells and neatly delineated by a PAS, laminin and collagen IV positive basal lamina which are strongly suggestive signs of an in situ lesion [4, 13, 15]. These features were lacking in the areas of invasion as seen in case 2. The intralobular proliferating cells, although of varying shape, appeared similar to one another and displayed a clear glycogen rich cytoplasm. Their nuclei were irregular with prominent nucleoli and atypical mitoses were seen. In addition minute foci of necrosis

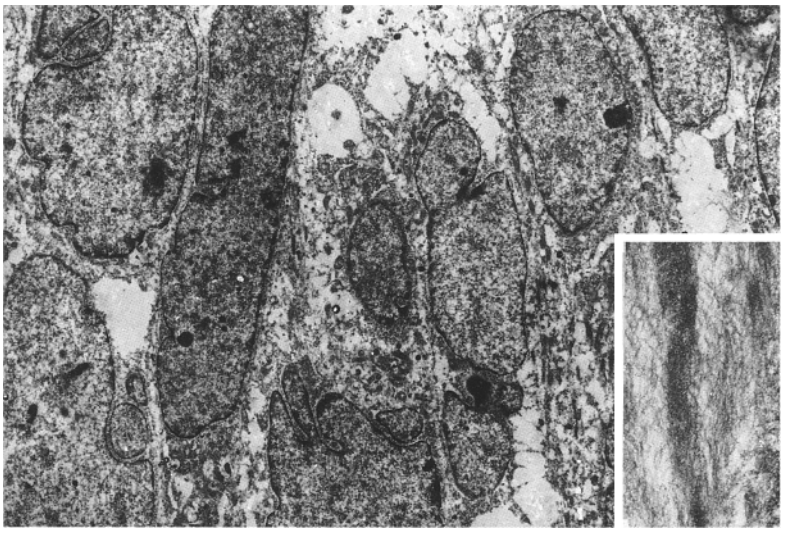

Fig. 12 Case 1; polygonal and spindle neoplastic cells show nuclei with irregular prophiles. Insert: at higher magnification microfilaments are visible in the cytoplasm. Electron microscopy ×3000; *insert* ×35000

were present in case 1. All these features are consistent with an intralobular malignant neoplastic lesion. The malignant nature of the process is further emphasized by the areas of invasion as seen in case 2. Hyperplastic lobular epithelial proliferations are usually constituted by two types of cells with eosinophilic cytoplasm and do not show atypical mitoses nor necrotic areas [2].

It is well known that most carcinomas in situ which occur within fibroadenomas, as the present case 1, have a lobular origin [2]. As the neoplastic cells showed pleomorphic nuclei and spotty necrosis was present, the possibility of an extension to lobular structures (lobular cancerization) from a duct carcinoma was taken into consideration. In spite of a careful search of the residual tissue of both quadrantectomies, no neoplastic ducts were observed. Monomorphic cells are no longer a prerequisite for in situ lobular carcinomas as cases of in situ and invasive lobular carcinomas constituted by pleomorphic elements have been reported [14].

Ten per cent of the total neoplastic proliferation in case 1 and 50% in case 2 was immunoreactive with anti-alpha-actin serum and ultrastructurally displayed in case 1 cytoplasmic microfilaments which are all features indicative of myoepithelial cell differentiation [1, 16]. The same cells were negative with anti-S100 protein, GFAP and vimentin antisera, as were the myoepithelial cells outlining the normal acini present in the same sections. The lack of staining for these latter markers which are considered to be pertinent to myoepithelial cells, is probably due to the existence of more than one type of myoepithelial element [3] or alternatively a consequence of the fact that formalin is not the optimal fixative for myoepithelial cells [7]. It appears that the neoplastic cells of the present cases share many similarities in common to those constituting the outer cell layers of adenomyoepitheliomas [12], including the clear glycogen rich cytoplasm and the lack of staining with anti-vimentin and anti-GFAP antisera.

The neoplastic cells constituting ordinary in situ lobular carcinoma do not usually stain with actin antisera [4, 13] although entrapped residual myoepithelial cells can occasionally be seen within the lobular neoplastic proliferation [5]. The latter are easily identified even at the H & E level by their hyperchromatic nuclei. In contrast in the present cases the actin positive elements showed irregular nuclei and were identical to the non actin stained neoplastic cells in all respects.

The neoplastic elements were not stained by chromogranin and GCDFP-15 antisera, the latter is a well established marker of apocrine differentiation [19]. This excludes both a differentiation towards endocrine and apocrine elements which have been occasionally reported in lobular carcinoma in situ [10, 11]. The variable positivity of the neoplastic cells with EMA antiserum further emphasizes the existence of different lines of differentiation in carcinomas including those rich in myoepithelial cells [12].

The present cases have to be distinguished from adenomyoepitheliomas as the latter are constituted by two different neoplastic cell types. Apocrine differentiated cells are "normal" constituents of adenomyoepitheliomas [12]. These are totally lacking in the present cases. Occasional residual "secretory" epithelium was seen within the neoplastic acini only in case 2, a phenomenon probably due to the fact that it was overrun by the neoplastic cellular population.

The present cases have many similarities with the intraductal actin-rich myoepithelial cell carcinoma reported by Tamai [22] including the irregular nuclei of the neoplastic cells, the atypical mytoses, the clear glycogen rich cytoplasm, the variable EMA positivity, the lack of apocrine differentiation of the neoplastic elements and the focal areas of invasion as seen in case 2. The only difference in the present cases is that no ductal structures appear to be involved.

Cartagena et a. [6] recently reported a case of invasive clear cell myoepithelial cell carcinoma showing an indian file type of growth, strongly reminiscent of the invasive parts seen in some areas of case 2. Therefore it is possible that the present cases are the in situ equivalent

of clear cell invasive myoepithelial lobular carcinomas, as defined by Cartagena et al. [6].

The purpose of this paper was to report the first cases of in situ lobular carcinoma showing myoepithelial cells; a further line of differentiation to be added to endocrine and apocrine lobular carcinomas. In both cases follow-up was short and further studies must be made to understand the biological behaviour of these lesions.

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