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Immunohistochemical localization of myoepithelial cells and basement membrane in normal, benign and malignant human breast lesions *

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Summary. Distributions of actin and type IV collagen were investigated immunohistochemically as markers for myoepithelial cells and basement membranes. Carnoy's and Methacarn-fixed, paraffin-embedded tissues from 103 human breast lesions from 103 patients were examined; 65 with carcinomas, 27 with mastopathies, 9 with fibroadenomas and 2 with phyllodes tumours. Fifty-five samples of the normal mammary gland tissue adjacent to tumours were also included for comparison. In normal breast and benign breast diseases, type IV collagen was identified around the mammary glandular cells and actin-positive cells were demonstrated to attach to basement membranes. In noninvasive carcinomas, type IV collagen was found as a continuous lining around a cell nest, while actin-positive cells were usually absent in ductal but quite numerous in lobular carcinomas. In invasive carcinomas, type IV collagen was fragmented or absent and actin-positive cells were very uncommon around the fragmentary basement membranes. These results suggest that the different distributions of myoepithelial cells and basement membrane material is useful in the differential diagnosis of surgical pathology of the breast.

Key words: Breast – Carcinoma – Actin – Type IV collagen – Immunohistochemistry

Introduction

The precise diagnosis and classification of breast lesions is sometimes difficult when based on morphology alone. Epitheliosis is an abnormal intraglandular cell proliferation and mammary carcinomas may arise from progression of atypical epitheliosis, thus histological distinction between benign or atypical epitheliosis and carcinoma is of great importance. It is often difficult to distinguish noninvasive from invasive carcinomas and in addition, certain types of carcinomas may be confused with benign proliferative conditions. Examination of cellular form and intercellular matrices may help to solve these diagnostic problems.

The presence and behavior of myoepithelial cells and basement membranes have not been extensively investigated in breast lesions. The identification of myoepithelial cells is difficult by morphology also and stains for poorly characterized enzymes or on non-specific staining techniques have been used in earlier studies (Puchtler et al. 1966; Pulley et al. 1973). Electron microscopy has also been used for this purpose (Ahmed 1974a, 1974b; Ozzello 1974, 1984). Since myoepithelial cells are very rich in 50–80 Å-thick actin filaments, they can be identified in fixed and embedded tissues by an immunohistochemical method using anti-actin antibody (Bussolati et al. 1980a). Basement membranes have been detected by the periodic acid-Schiff (PAS) staining (Flotte et al. 1980) and by silver impregnation. Recently, type IV collagen, a major component of the basement membrane restricted to the basal lamina of the basement membrane (Yaoita et al. 1978) has been stained to localize the membranes in paraffin-embedded tissues (Albrechtsen et al. 1981; Gusterson et al. 1982). Antibodies to basement membrane proteins and myoepithelial cells are now available in diagnostic pathology and will be more valuable when they can be applied to paraffin embedded tissues.

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To evaluate the significance of the expression of a biological marker in breast tissues, it is imperative to use a large series of morphologically well-characterized specimens. Therefore, the cases reported in this study were carefully examined histologically in haematoxylin and eosin sections before resorting to immunohistochemistry. The present study was conducted to investigate immunohistochemically the topographical localization, distribution and morphological characteristics of myoepithelial cells and basement membranes in normal, benign and malignant mammary tissues of surgically obtained human breast specimens.

Materials and methods

Samples of 103 human breast lesions derived from the same number of patients were examined. Tissues were fixed in either Carnoy's (60% ethanol, 30% chloroform and 10% acetic acid) solution or Methacarn (60% methanol instead of ethanol) fixative (Puchtler et al. 1970) overnight at room temperature, and then routinely embedded in paraffin. For histological examination, 4 μm-thick serial sections were prepared and stained with haematoxylin and eosin (H-E), PAS, silver impregnation and Puchtler's azophloxine stain (Puchtler et al. 1966). The samples came from 65 cases of invasive carcinomas of various types (47 ductal carcinomas including 20 associated with a noninvasive component, 11 scirrhous, 3 mucinous, 1 apocrine, 1 tubular and 2 lobular, both being associated with an in situ component), 9 cases of fibroadenoma, 2 of phyllodes tumour and 27 of mastopathy (Table 1).

Anti-type IV collagen antibody was produced by immunizing rabbits with type IV collagen purified from human kidneys, as described previously for anti-mouse type IV collagen antibody (Oikawa et al. 1986). This antibody did not cross-react with proteoglycans, laminin or entactin. Anti-actin antibody, purchased from Biochemical Technologies Inc., Stoughton MA, USA, was produced by immunizing rabbits with homogeneous chicken gizzard actin. This anti-actin antibody bound to F and G actins from both muscle and non-muscle tissues of all vertebrate and invertebrate species, and showed no detectable cross-reactivity with tubulin, keratin or myosin.

The ABC method using peroxidase-conjugated antibodies was employed for immunocytochemistry (Hsu et al. 1981). Normal goat serum, biotinylated anti-rabbit IgG and avidin-biotin complex were purchased from Vector Lab., Burlingame, CA, USA. For detection of type IV collagen, tissue sections were digested with pronase before incubation with the antiserum, since no reaction occurred without the pretreatment. In brief, sections were incubated with phosphate-buffered saline (PBS), pH 7.2 for 15 min and further with 0.1 mg pronase (Actinase; Kaken Pharmacol. Co., Tokyo, Japan) in 1 ml PBS for 15 min at 37° C, and then rinsed in Tris-HCl buffer, pH 7.2. Antigenic sites were visualized using a freshly prepared solution of 3, 3′ diaminobenzidine (DAB) and hydrogen peroxide in the usual way. Sections were weakly counterstained with haematoxylin, cleared and mounted in balsam. The diluent for all antisera was Tris-HCl buffer at pH 7.2, and all steps took place at room temperature unless otherwise specified. Immunohistochemical controls involved substitution of the specific antiserum with normal rabbit serum.

Results

Several different fixatives and enzymatic pretreatments were tested to achieve maximum staining in the preliminary experiment. The best staining with the antisera used here was accomplished by fixation in either Carnoy's or Methacarn fixative, both giving similar satisfactory results. Formaldehyde fixation abolished the immunoreactivity of actin partially and that of type IV collagen completely. Regardless of the fixation procedures used,

Table 1. Histological classification, number of cases and staining patterns of actin and type IV collagen in normal and pathological breast

Types	No. of cases	Immunohistochemical findings	
		Actin	Type IV collagen
Normal	55	Contiguous positive in ducts, discontiguous in acinus	Linearly positive around epithelial cell nests
Fibroadenoma	9	Positive cells vary in different areas	Same as normal
Phyllodes tumour	2	Same as fibroadenoma; myofibroblasts also positive	Same as normal
Mastopathy	27	Regularly positive	Same as normal
Carcinoma			
Invasive ductal	47	Almost all negative	Negative or discontinuous
(non-invasive component)	(20)	Usually negative	Positive
Scirrhous	11	Negative	Negative
Mucinous	3	Negative	Negative
Apocrine	1	Negative	Negative
Tubular	1	Negative	Negative
Invasive lobular	2	Negative	Negative
(in situ component)	(2)	Prominently positive	Linearly positive around lobules

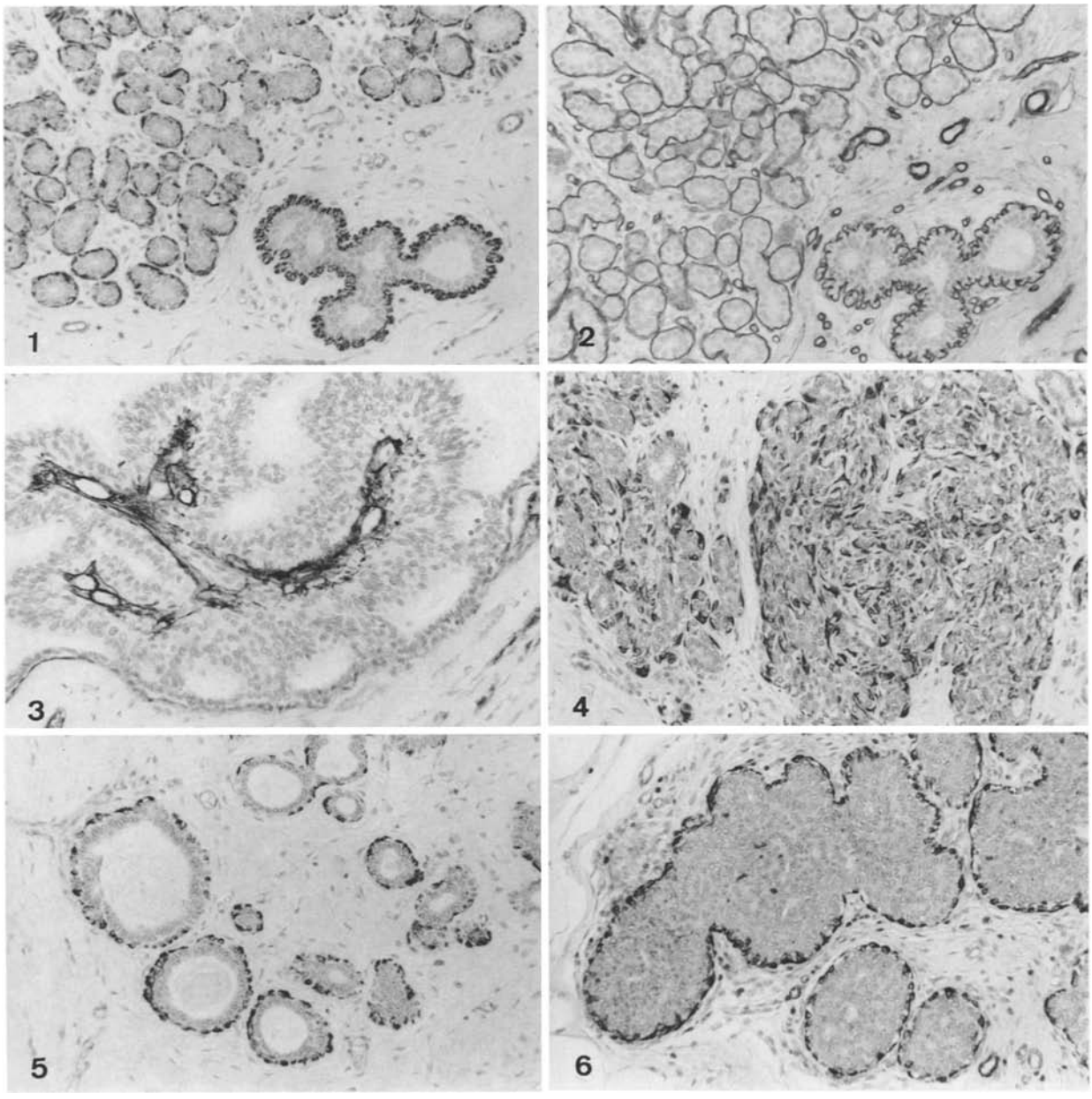

Fig. 1. Normal breast stained with anti-actin serum. Peripheral myoepithelial cells are contiguous in ducts and discontinuous in acini. × 200

Fig. 2. Normal breast stained with anti-type IV collagen serum. Basement membranes adjacent to myoepithelial cells and blood vessel walls are clearly delineated. × 200

Fig. 3. Intraductal papilloma stained with anti-type IV collagen serum. Note the presence of basement membranes along papillary projections. × 200

Fig. 4. Sclerosing adenosis stained with anti-actin serum. Note prominent cytoplasmic staining of myoepithelial cells. × 200

Fig. 5. Blunt duct adenosis stained with anti-actin serum. Note regularly arranged myoepithelial cells. × 200

Fig. 6. Duct papillomatosis stained with anti-actin serum. Note myoepithelial cells regularly distributed at the periphery of the lesion. × 200

prior pronase treatment was found to be essential for visualization of type IV collagen and to give better results than trypsin treatment. In pronase (Actinase)-treated breast tissue sections, type IV collagen was clearly stained in a line around the matrix of epithelial cells and vessel walls, while it was weakly stained in endo- and perineurium of peripheral nerves and on adipose cell mem-

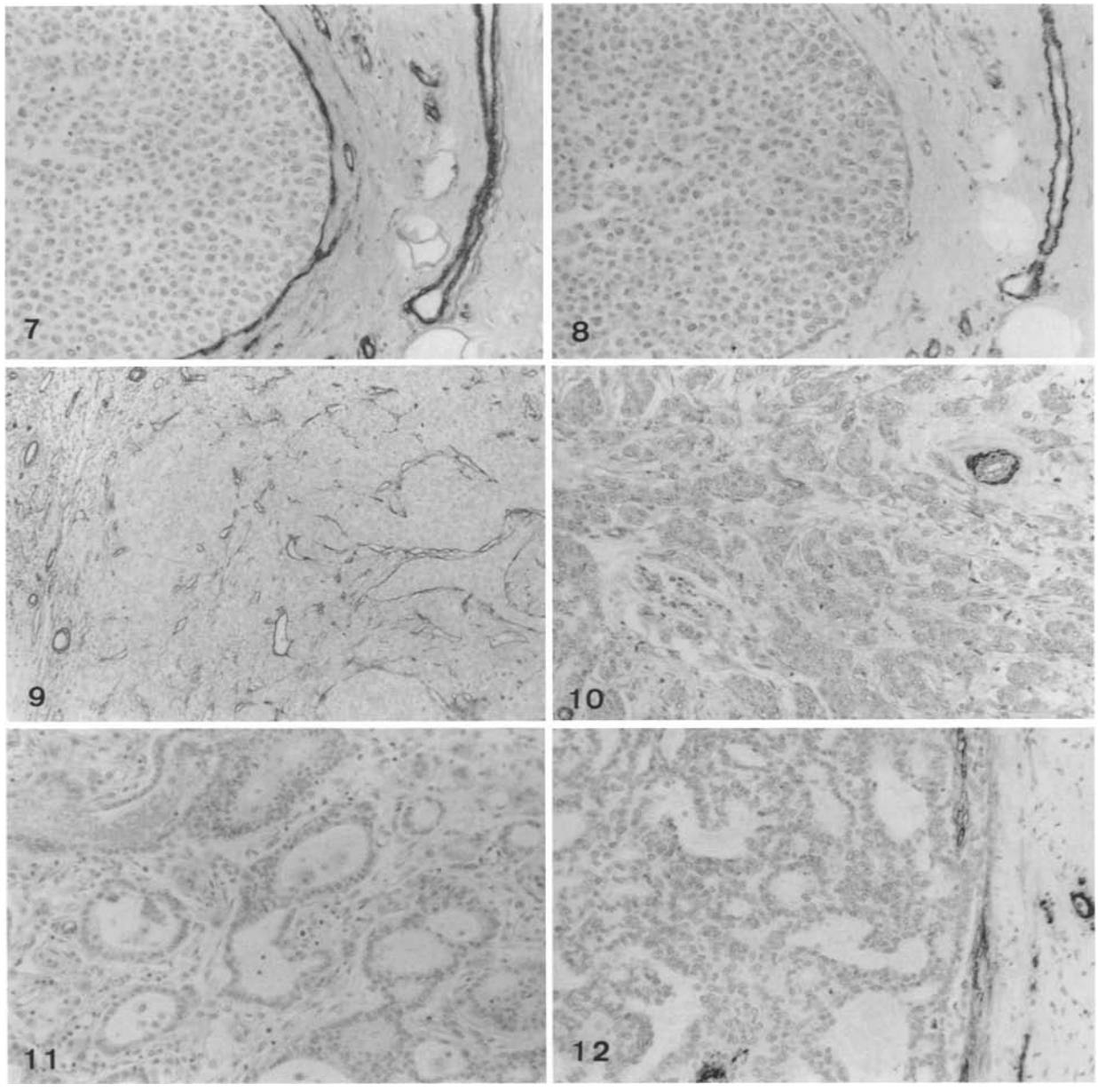

Fig. 7. Non-invasive ductal carcinoma stained with anti-type IV collagen serum. Note cancerous foci completely bordered with basement membranes, and vessel walls also bordered with them. ×200

Fig. 8. Non-invasive ductal carcinoma stained with anti-actin serum. Note the absence of myoepithelial cells at the periphery of the lesion. ×200

Fig. 9. Invasive ductal carcinoma stained with anti-type IV collagen serum. Note lacking or fragmentation of basement membranes. ×100

Fig. 10. Scirrhous carcinoma stained with anti-actin serum. Note actin positive cells present in residual normal ducts but not in tumourous foci. Compare with Fig. 4 ×200

Fig. 11. Invasive ductal (Papillo-tubular) carcinoma-stained with anti-actin serum. Note the absence of actin-positive cells. Compare with Fig. 5 ×200

Fig. 12. Papillary carcinoma stained with anti-type IV collagen serum. Note the absence of basement membranes around cancerous nests. Compare with Fig. 3 ×400

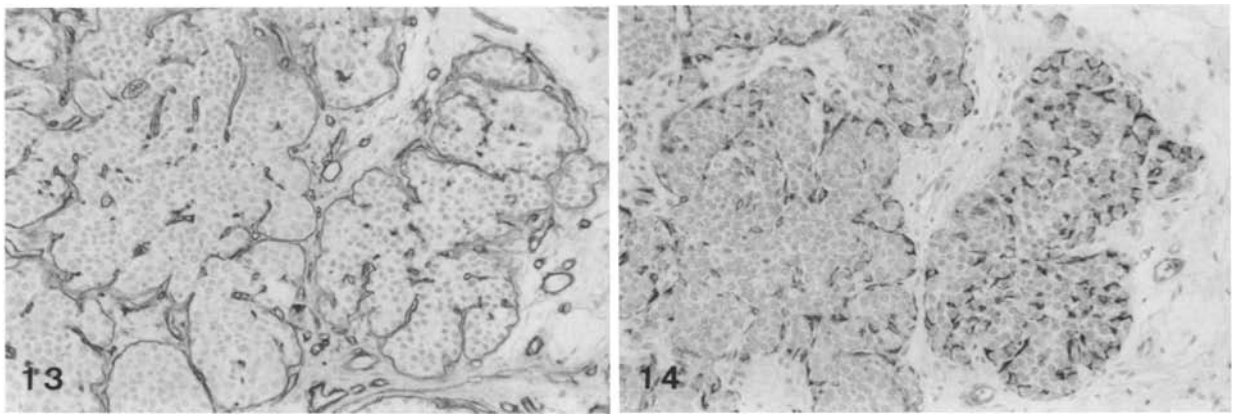

Fig. 13. Lobular carcinoma in situ stained with anti-type IV collagen serum. Note affected lobules completely surrounded by basement membranes. × 200

Fig. 14. Lobular carcinoma in situ stained with anti-actin serum. Note frequent appearance of myoepithelial cells with triangular or flattened cytoplasm. × 200

branes. Epithelial components and connective tissue elements showed no positive staining in the cytoplasm. Anti-actin antibody marked the cytoplasm of myoepithelial cells and the smooth muscles of vessel walls in a selective manner. Secretory epithelia were not positively stained. Control sections stained with normal rabbit serum instead of the specific antibodies were completely negative. Immunostaining for actin and type IV collagen was compared with conventional PAS or azophloxine staining with regard to the ability to recognize basement membranes and myoepithelial cells, confirming that the immunological method was more selective and more reliable.

Apparently normal mammary tissues adjacent to tumours were used as normal mammary gland. Staining for actin revealed two distinct configurations of epithelial cells: positive myoepithelial cells were pronounced and contiguous in the ducts but attenuated and separated in the acini (Fig. 1). Secretory epithelia and fibrous stroma were uniformly negative. Serial sections stained with anti-type IV collagen antibody showed continuous single lines around both acini and ducts, which demarcated the epithelial components from the mesenchyme completely (Fig. 2). There were no obvious difference in stainability between pre- and postmenopausal specimens detected by 2 antibodies.

In benign tumours and proliferative disorders the staining patterns obtained with the antisera to actin and type IV collagen were similar to those found in the normal breast with only minor changes. In fibroadenomas, the distribution of actin-positive cells varied from tumour to tumour and from area to area in an individual tumour, in spite of the presence of easily recognized double-cell layers of epithelium. In phyllodes tumours, actin-positive cells showed a similar distribution to that seen in fibroadenomas, although stromal cells corresponding to myofibroblasts were also stained with anti-actin antibody. In benign papillomas, the actin-positive cells were regularly positioned between the secretory cells and continuous basement membranes (Fig. 3). In mastopathies, sclerosing adenosis was surrounded by continuous intact basement membranes with pronounced actin-positive cells (Fig. 4). Blunt duct adenosis (Fig. 5), cysts and apocrine metaplasia contained myoepithelial cells in a regular manner. Duct papillomatosis and lobular hyperplasia contained actin-positive cells at the external margins, whereas no actin-positive cells were found in the centers (Fig. 6). In these conditions, therefore, a layer of myoepithelial cells was regularly distributed, without proliferation along the base of the secretory epithelium, and the epithelial components were surrounded by a continuous intact basement membrane.

In the intraductal components of ductal carcinomas, tumour cell nests were bordered with continuous type IV collagen-positive basement membranes (Fig. 7). Most of the lesions were devoid of actin-positive cells (Fig. 8), and had tumour cells directly attaching to the basement membranes. In some of the smaller lesions contiguous or non-contiguous compressed actin-positive cells attached to basement membranes at the outermost margins of the tumour cell nests were sometimes seen. In these cases, the actin-positive cells never detached from the basement membrane and were never found near the centers of the neoplastic foci. In the inva-

sive areas of ductal carcinomas, type IV collagen was invariably either fragmented or absent (Fig. 9). Actin-positive cells were sometimes seen around these discontinuous basement membranes without accompanying any proliferative changes. In scirrhous carcinomas, cells infiltrating into the abundant fibrous stroma were negative for both actin (Fig. 10) and type IV collagen. Papillary, tubular, mucinous and apocrine carcinomas were completely free from actin-positive myoepithelial cells (Fig. 11) and type IV collagen-positive basement membranes (Fig. 12).

In lobular carcinoma in situ, each acinus was surrounded by continuous type IV collagen (Fig. 13), and actin-positive cells were more frequent than in non-invasive ductal carcinomas. Most of the myoepithelial cells were found either in flattened shapes along the basement membrane or in triangular shapes perpendicularly to them (Fig. 14). In invasive lobular carcinomas, neither type IV collagen nor actin-positive cells were demonstrable.

Discussion

Several non-specific staining methods have been used to identify the myoepithelium and the basement membrane (Puchtler et al. 1966; Flotte et al. 1980). However, immunohistochemistry is considered to provide more reliable results. Myoepithelial cells can be identified by their contractile proteins, actin and myosin (Bussolati 1980; Bussolati et al. 1980a, 1980b, 1981; Gabbiani et al. 1976; Gabbiani 1979; Hayashi et al. 1984; Mitchell et al. 1985), or by the S-100 protein (Egan et al. 1987). Basement membranes can be identified by their content of type IV collagen and laminin (Albrechtsen et al. 1981; Gusterson et al. 1982; Warburton et al. 1982; Ekblom et al. 1984; Mitchell et al. 1985). Methacarn and Carnoy's fixatives, non-aqueous protein-precipitating fixatives, were suitable for immunohistochemical detection of actin and type IV collagen, because of excellent preservation of immunoreactivity and tissue structures in paraffin sections. Formaldehyde caused reduction or abolition of the staining: cross-linking fixative might affect antigenicities for certain antibodies (Mitchell et al. 1985). Large numbers of formalin-fixed paraffin embedded blocks are readily available from surgical files, although Carnoy's and Methacarn-fixed tissues are rarely obtained (it may be useful to fix specimens in different fixatives for future retrospective immunohistochemical studies). Detection of actin did not require any enzymatic pretreatment, whereas pronase digestion was essential to visualize type IV collagen (Mitchell et al. 1985). Anti-type IV collagen antibody stained basement membranes linearly without cytoplasmic activity, a pattern suggestive of active synthesis of this protein (Warburton et al. 1982), in the tissues studied.

In benign lesions, mammary parenchyma was completely separated from surrounding mesenchyme by type IV collagen, just as in normal glands (Figs. 2, 3). Myoepthelial cells were clearly revealed by staining with anti-actin serum, present between the basement membrane and secretory cells (Figs. 1, 4, 5, 6). It is often difficult to differentiate duct papillomatosis from non-infiltrating ductal carcinoma, but in these preparations myoepithelial cells always occurred at the periphery in the lesion. In contrast, however, they were undetectable (Fig. 8) or were only found at the periphery of cell nests demarcated by type IV collagen in a solid line (Fig. 7) in the majority of non-infiltrating ductal carcinomas. In such instances myoepithelial cells might be interpreted as a residual, non-neoplastic component (Hayashi et al. 1984). Thus, the presence of myoepithelial cells does not lead to the diagnosis of the benign condition, but their absence at the periphery of cell nests supports the diagnosis of a malignant proliferation. Finally, the fragmentation and disappearance of type IV collagen during tumour invasion is useful in differentiating invasive from non-invasive carcinomas (Fig. 9).

Some type of carcinoma can be confused with benign proliferative conditions, for example well-differentiated tubular (Fig. 11) and papillary (Fig. 12) carcinomas are often indistinguishable from blunt duct adenosis (Fig. 4) and benign papillary lesions (Fig. 3). Lack of basement membrane and myoepithelial cells may provide strong evidence for malignancy (Flotte et al. 1980; Gusterson et al. 1982; Papotti et al. 1983).

The staining patterns of actin in the lobular carcinoma in situ seem to fit those observed by Bussolati (1980, 1981). In contrast to non-invasive ductal carcinomas, comparatively good preservation of the myoepithelium (Fig. 14) makes the presence or absence of myoepithelial cells unsuitable for discrimination between lobular carcinoma in situ and lobular hyperplasia.

In general, our results on the staining of normal, benign and malignant breast lesions of actin and type IV collagen are in agreement with those reported by Ahmed (1974a, 1974b), Gabbiani et al. (1976), Gabbiani (1979) and Ozzello (1974, 1984) dealing with histochemical, immunofluorescent and ultrastructural observations. However,

Gabbiani et al. (1976) and Gabbiani (1979) showed strong actin positivity in breast cancer cells with invasive activity. As we found no actin positivity in cancer cells, we can not exclude that technical differences may account for the discrepancy between the results.

Consequently, although immunohistochemical findings may not establish the final diagnosis, the present study indicates that the presence of actin can help to differentiate dysplastic from neoplastic conditions of the breast, while type IV collagen is useful for differentiating noninvasive from invasive carcinomas. When combined, they may offer valuable information in the differential diagnosis in breast pathology.

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