Virchows Archiv A Pathol Anat (1989) 416:177–184

Virchows Archiv A
Pathological Anatomy and Histopathology

Case report

An epithelial and spindle cell breast tumour of myoepithelial origin

An immunohistochemical and ultrastructural study

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Summary. An infiltrating epithelial and spindle cell neoplasm developed in the breast of a 63-year-old female. An excisional biopsy was performed. Recurrence with rapid growth due to cyst development eventually resulted in more radical surgery. Interim fine needle aspirations had established its partially cystic nature. The unique microscopic appearance prompted the application of immunohistochemistry and electron microscopy. The tumour cells were found to exhibit characteristics denoting squamous and myoepithelial differentiation. Histopathological features of malignancy were absent. Our findings demonstrate the differentiation potential of breast epithelium. They are in concordance with the results of previous studies which delineate the histochemical and ultrastructural features of myoepithelia and establish the relationship of these cells to squamous metaplasia.

Key words: Myoepithelia – Myoepithelium – Myoepithelial differentiation – Myothelia – Squamous differentiation

Introduction

Myoepithelial cells are cells of epithelial origin with smooth muscle differentiation found in organs derived from ectoderm and entoderm. Peyron et al. (1926) presented cogent arguments based upon their own and preceding investigations supporting the dual glandular and myoepithelial differentiation of mammary epithelium and validated the concept of participation of myoepithelial cells in breast tumours. These cells have been implicated as participants and primary neoplastic elements in benign and malignant proliferative processes of breast as well as in tumours of skin, salivary gland, and lung (Hamperl 1939, 1970; Hübner et al. 1971; Ohtani and Sasano 1980; Strickler et al. 1987).

Studies using animal models have provided insight into the bidirectional maturation of the breast secretory unit. Based upon these findings, the existence of an undifferentiated progenitor cell for both the myoepithelium and columnar epithelial cells has been suggested, thus supporting the hypothesis of "dualisme primordiale" espoused by Peyron (Radnor 1972). Subsequently, evidence for the neoplastic transformation of myoepithelia was demonstrated with light-microscopic and ultrastructural studies (v. Bomhard and v. Sandersleben 1975).

One feature of myoepithelial cells is their capacity for squamous differentiation. The existence of squamous metaplasia with stromal-epithelial transition in a breast neoplasm was first documented by Kürsteiner (1894), and Salm (1957) first proposed the possibility of an epithelial-stromal transformation in a study of epidermoid metaplasia in a fibroadenoma. Ultrastructural evidence for such transformation now exists (Gersell and Katzenstein 1981; Kaufman et al. 1984; Reddick et al. 1985).

Thus far, tumours with dual glandular and spindle cell or pure spindle cell composition have been reported (Kermarec et al. 1973; Cameron et al. 1974; Tóth 1977; Erlandson and Rosen 1982; Zarbo and Oberman 1983; Kiaer et al. 1984; Daroca et al. 1985; Schürch et al. 1985; Thorner et al. 1986; Rosen 1987; Young and Clement 1988); and one clear cell myoepithelial neoplasm of the breast has been described (Cartagena et al. 1988). To our knowledge, no prior descriptions of benign tumours showing only squamous and myoepithelial

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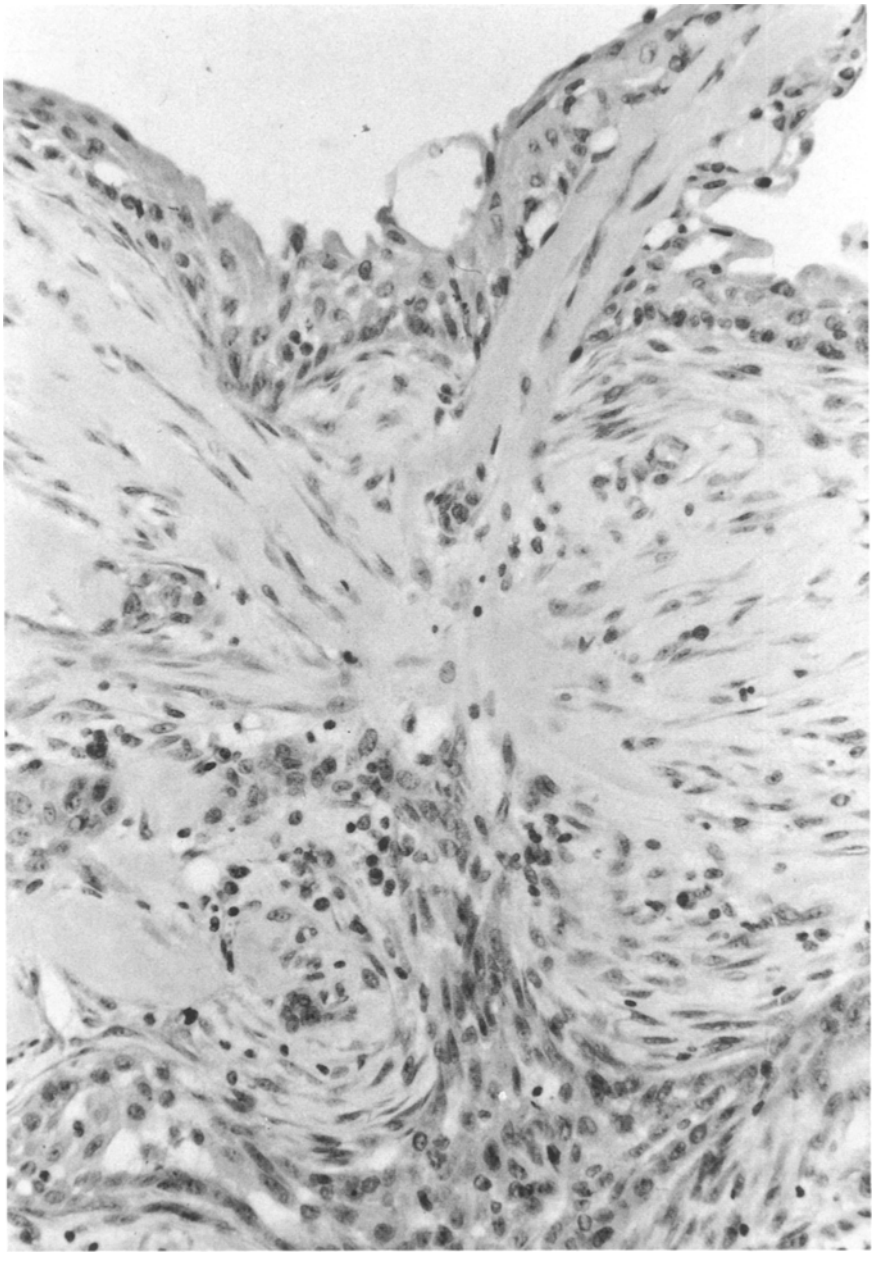

Fig. 1. Cystic spaces and clefts are lined with stratified epithelium which undergoes transition to spindle forms and merges with the stroma. Fusiform tumour cells grow in tapering trabeculae and condense into epithelioid aggregates within stroma. Epithelium lining larger spaces shows definite squamous change. H & E, ×40

differentiation have been presented in the literature.

Our study characterizes the histopathological features of such an epithelioid and spindle cell proliferative process. The findings of myoepithelial and squamous differentiation were confirmed using electron microscopy and immunohistochemistry.

Case report

A 63-year-old Caucasian female presented in April 1984 for evaluation of a painless breast mass located laterally above the nipple. A discrete mass could not be discerned; there were no changes in the skin and no axillary lymphadenopathy. A mammogram was interpreted as showing a single 13 mm asymmetric density without well-defined margins.

Fine needle aspiration showed a very cellular specimen indicative of an inflammatory and benign proliferative process. An excisional biopsy was performed. Because of the infiltrating nature of the tumour, the initial differential diagnosis included a reactive process, epitheliosis, sarcoma, and metaplastic carcinoma. Estrogen and progesterone receptor assays were negative.

An interim biopsy of the same breast one year later was interpreted as fibrocystic change. By May 1986, a large rounded mass measuring 3.8 cm in diameter had developed in the area of the preceding biopsy. It was of moderate density radiographically and had well-defined margins. Fine needle aspiration

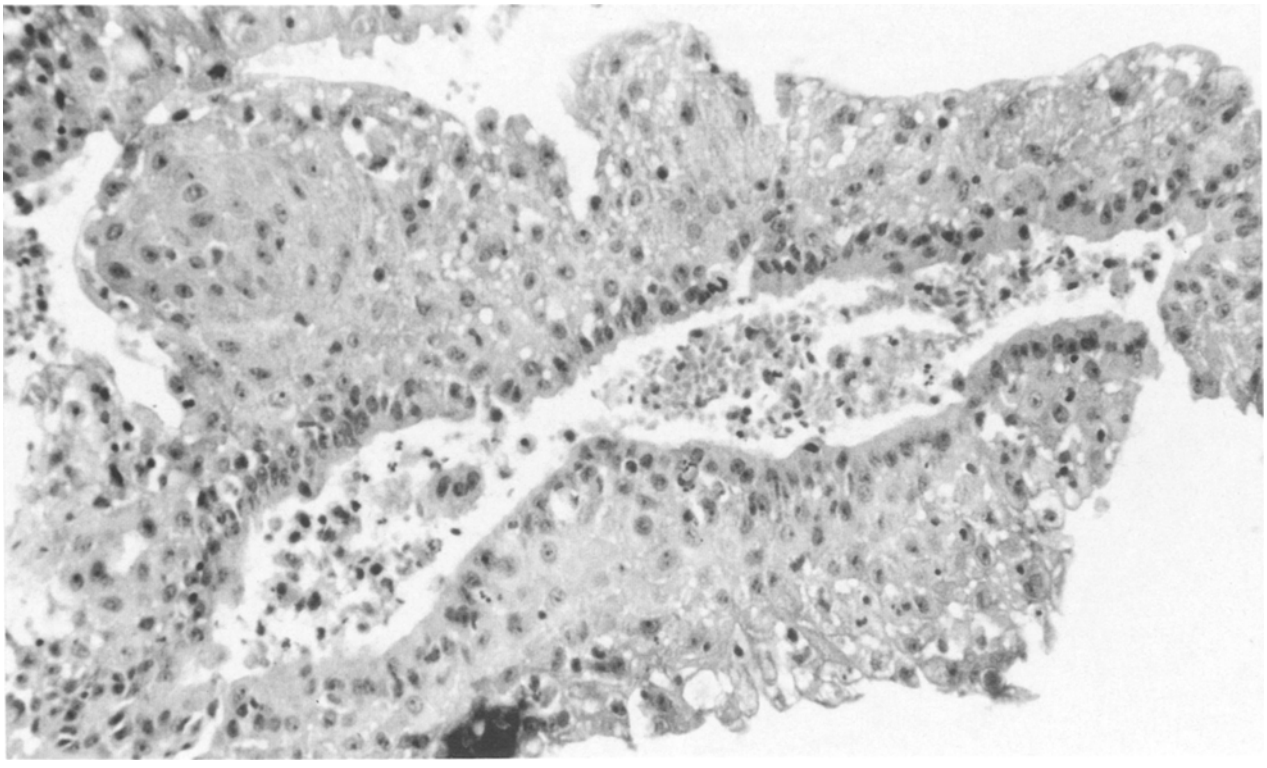

Fig. 2. FNA cell block. This depicts a portion of cyst lining consisting of stratified epithelium with a cuboidal basal layer. Intracytoplasmic mucin could not be demonstrated. Movat's pentachrome, ×40

yielded 12 cc of bloody fluid which proved to be a very cellular specimen consisting of an inflammatory and benign epithelial component. Aspiration resulted in the disappearance of the tumour on palpation.

After a tumour recurrence in August 1986, repeat aspiration yielded a similar specimen also composed of small tissue fragments consistent with cyst lining. Three months later, a third recurrence with enlargement to 5 cm prompted a critical multi-disciplinary review which recommended mastectomy. The lesion in the mastectomy specimen was interpreted as infiltrating epitheliosis with cystic change. No tumour was found in the axillary nodes. Post-operatively, the patient has remained free of disease.

Materials and methods

The specimens relevant to this study consisted of a cell block obtained via needle aspiration, the original biopsy, and the tumour in the left modified radical mastectomy. The excisional biopsy showed indistinctly demarcated dense fibrous tissue containing small cystic spaces and clefts; there were similar findings in the mastectomy specimen which also contained a large unicameral cyst 5 cm in diameter in contiguity with the solid portions of the tumour.

Tissues were all fixed in phosphate-buffered 10% formalin solution. Sections for routine light microscopy were examined with haematoxylin-eosin, Mayer's mucicarmine, and Movat's pentachrome.

Immunohistochemical staining was performed using the modified avidin-biotin peroxidase complex (ABC) method described by Hsu et al. (1981). The following antibodies were used: muscle specific actin (MSA), mouse monoclonal, 1:4000 (Enzo Biochem Inc., NY, USA); keratin, AE 1/3 mouse monoclonal, 1:200 (Hybritech Inc., San Diego, Ca., USA); vimentin, mouse monoclonal, 1:2000 (BioGenex Laboratories, Dublin, Ca., USA); S-100, rabbit polyclonal, 1:800 (Dakopatts Accurate Chemical and Scientific Co., Westbury, NY, USA); desmin, mouse monoclonal, 1:40 (Dakopatts); and epithelial membrane antigen (EMA), mouse monoclonal, 1:100 (Dakopatts). All sections were run in unison with the appropriate positive and negative controls.

Specimens selected from formaldehyde-fixed tissue for transmission electron microscopy were processed according to established procedures (Stempak and Ward 1964; Karnovsky 1965; Reynolds 1968; Spurr 1969) and examined under a Siemens IA electron microscope.

Results

On light microscopy both surgical specimens exhibited similar features. Cystic spaces were lined with polygonal and flattened cells with a loose "flagstone" arrangement. The surface cells had abundant eosinophilic cytoplasm and oval, clefted nuclei with reticular chromatin, irregular small chromocenters, and small nucleoli. Intracellular mucin could not be shown. These cells merged with

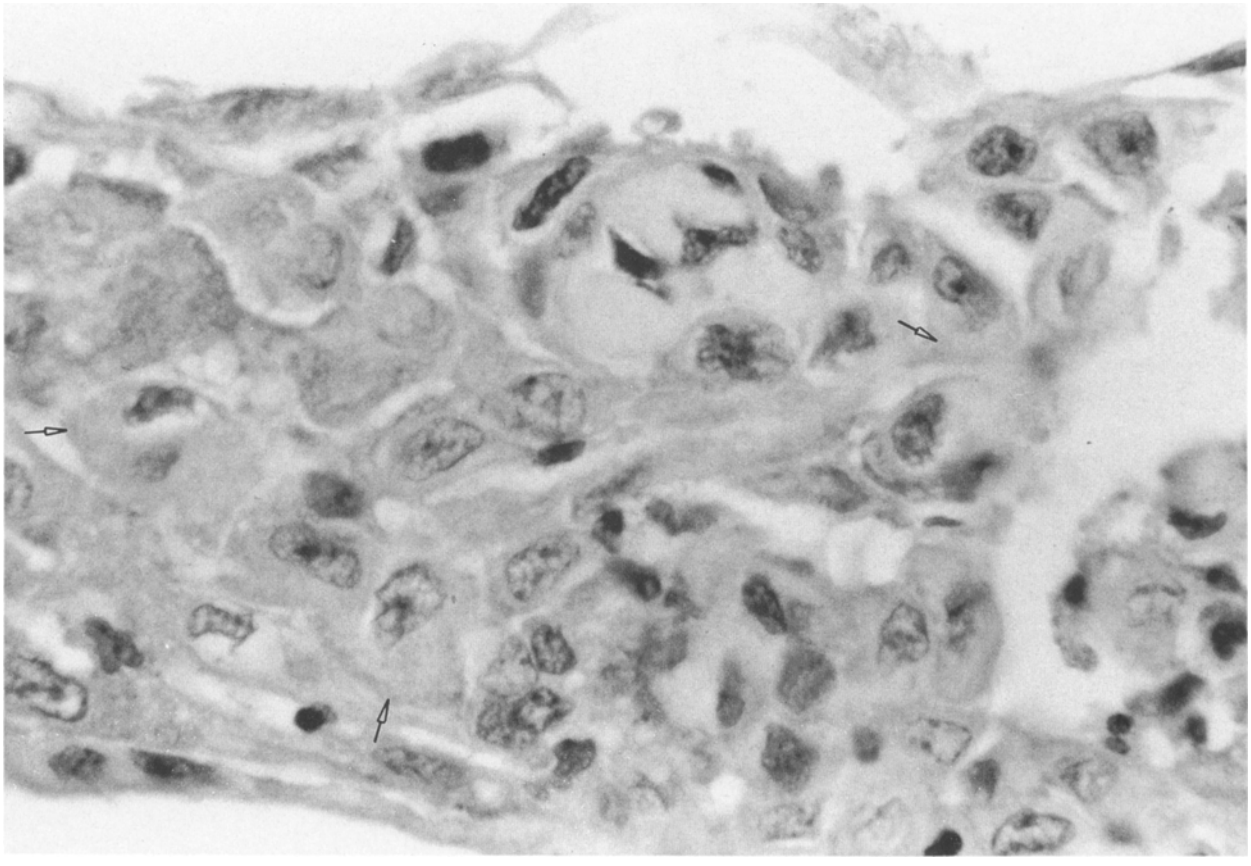

Fig. 3. Depiction of muscle specific actin shows the irregular cytoplasmic staining. Somewhat increased staining is frequently distributed in the more peripheral cytoplasmic zones (*arrows*). ABC-anti MSA, ×100

a cuboidal cell basal layer having similar tinctorial properties. In many areas, the cyst lining was easily distinguished from the underlying variably cellular stroma, but there were numerous foci where the basal cuboidal cells appeared to change to spindle-shaped forms, and these in turn merged with the adjacent fibrous tissue.

Deeper down, epithelioid and fusiform cells grew in anastomosing trabeculae and strands. Cleft spaces containing loosely-arranged polygonal cells formed in areas where the cell cords widened; tumour cells appeared to condense into epithelioid aggregates within the variably cellular and frequently hypocellular collagenized stroma. There was focal mitotic activity of normal configuration. Capillaries had proliferated in the fibrous tissue. In some peripheral portions of the neoplasm, tumour cells were found between preserved adipocytes with a resulting lace-like pattern. Remnants of well-preserved breast lobules were present in the tumour (Figs. 1, 4A, B).

The cell block preparation from a fine needle aspiration specimen contained fragments of stratified epithelium consistent with cyst lining. A basal layer was formed by cuboidal cells which transformed into less tightly arranged larger epithelioid forms, some of which had abundant clear cytoplasm (Fig. 2).

For immunocytochemistry normal breast tissue and stromal elements served as internal controls. We interpreted all cases of questionably positive reactions as negative.

Lumen surfaces and adjacent cytoplasm of residual duct epithelial cells are highlighted by EMA MAbs, but normal myothelium and the neoplastic epithelium were non-reactive. There was an irregular staining among the tumour cells for MSA, and its cytoplasmic distribution was uneven (Fig. 3). Actin positivity was strong in periductal myoepithelium and present in stromal cells. S-100 antigen was demonstrated only focally in nuclei and cytoplasm of the epithelioid and spindle-shaped tumour cells as opposed to the relatively greater reaction in normal myoepithelium. Stromal spindle

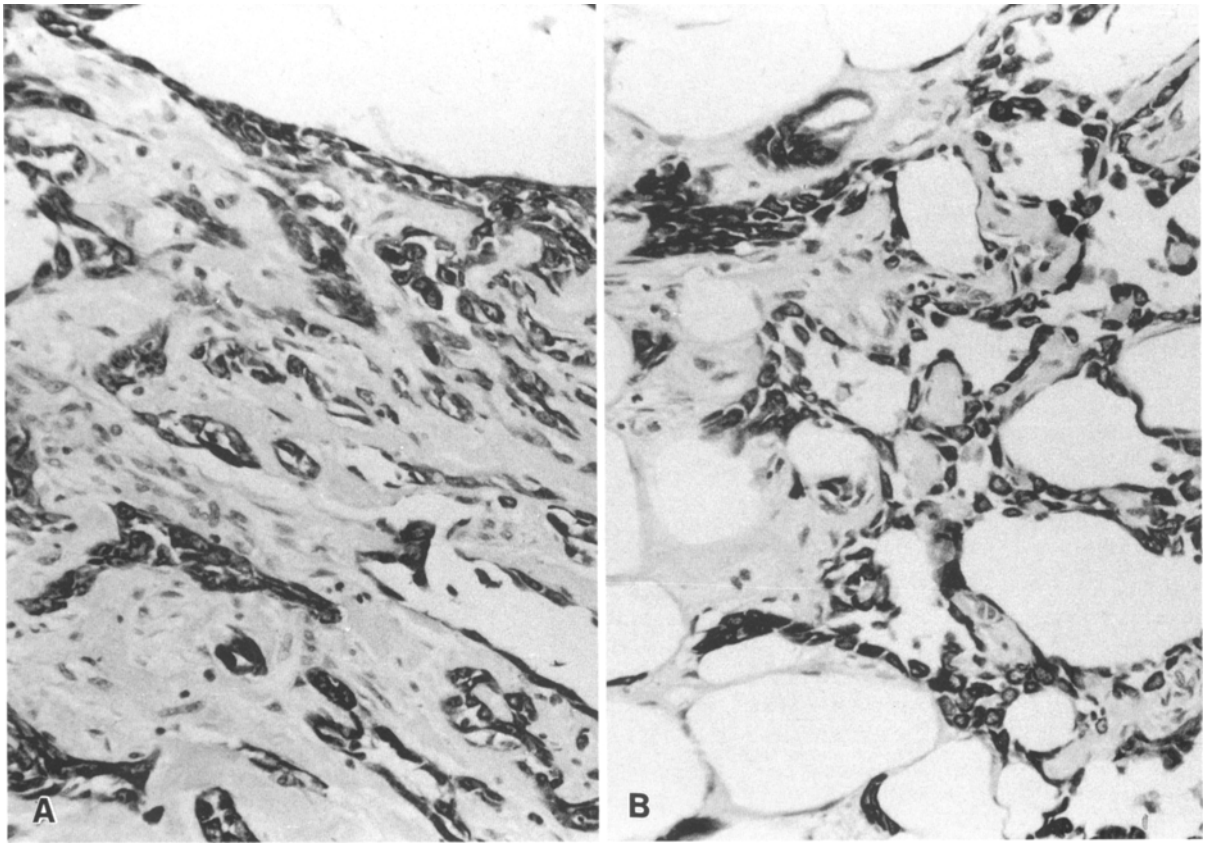

Fig. 4. (**A, B**) Strong reactivity for cytokeratin is evident throughout. In peripheral regions of the tumour (**B**), the cells are found between preserved adipocytes without the development of significant fibroplasia. ABC-antikeratin AE 1/3, (**A, B**) ×40

cells and duct columnar epithelium were also S-100 positive. Both normal myoepithelium and tumour cells showed variable staining for vimentin. Other tissue elements in which this was demonstrated included adipocytes, stromal spindle cells, lymphocytes, neutrophils, endothelium, and vascular smooth muscle. Desmin could not be shown in normal myoepithelia and tumour cells, and was present only in vascular smooth muscle. Striking reaction was seen for keratin (Fig. 4A, B). Whereas tumour cells were strongly positive, normal myoepithelium expressed this antigen to a lesser degree.

Ultrastructurally apposing tumour cells were joined by mature desmosomes. The undulations of their cell membranes were frequently accentuated. Adjacent short processes from the same cell occasionally adhered to each other via desmosomes. Thin, short microvilli projected from some free surfaces. Separated cells of the surface epithelium did not possess desmosomal structures, but dense filamentous condensations were found next to the cell membrane. A fine fibrillar or granular substance suggestive of basal lamina was noted present in close proximity to their surfaces. Intracytoplasmic lumina were not evident. Varying numbers of small vesicular invaginations, or caveolae, indented cell membranes. Many free cell surfaces were in close contact with collagen bundles.

Nuclei were large and were characterized by oval or angular shapes with occasional cytoplasmic indentation. Their chromatin was of moderately coarse granularity, finely dispersed, aggregated peripherally, and also in conspicuous chromocenters; the usually single nucleoli varied in size.

The distribution of the cytoplasmic organelles varied considerably from cell to cell. In some, there were numerous mitochondria; rough endoplasmic reticulum was prominent. Two types of filamentous material were present. Larger filaments measuring from 10 to 12 nm were arranged in dense, irregular, often curvilinear sheafs and fascicles; a predilection for perinuclear distribution was noted in some cells. Smaller diameter fibers of 6 to 8 nm diameter formed somewhat haphazardly arranged densities. These structures often appeared localized

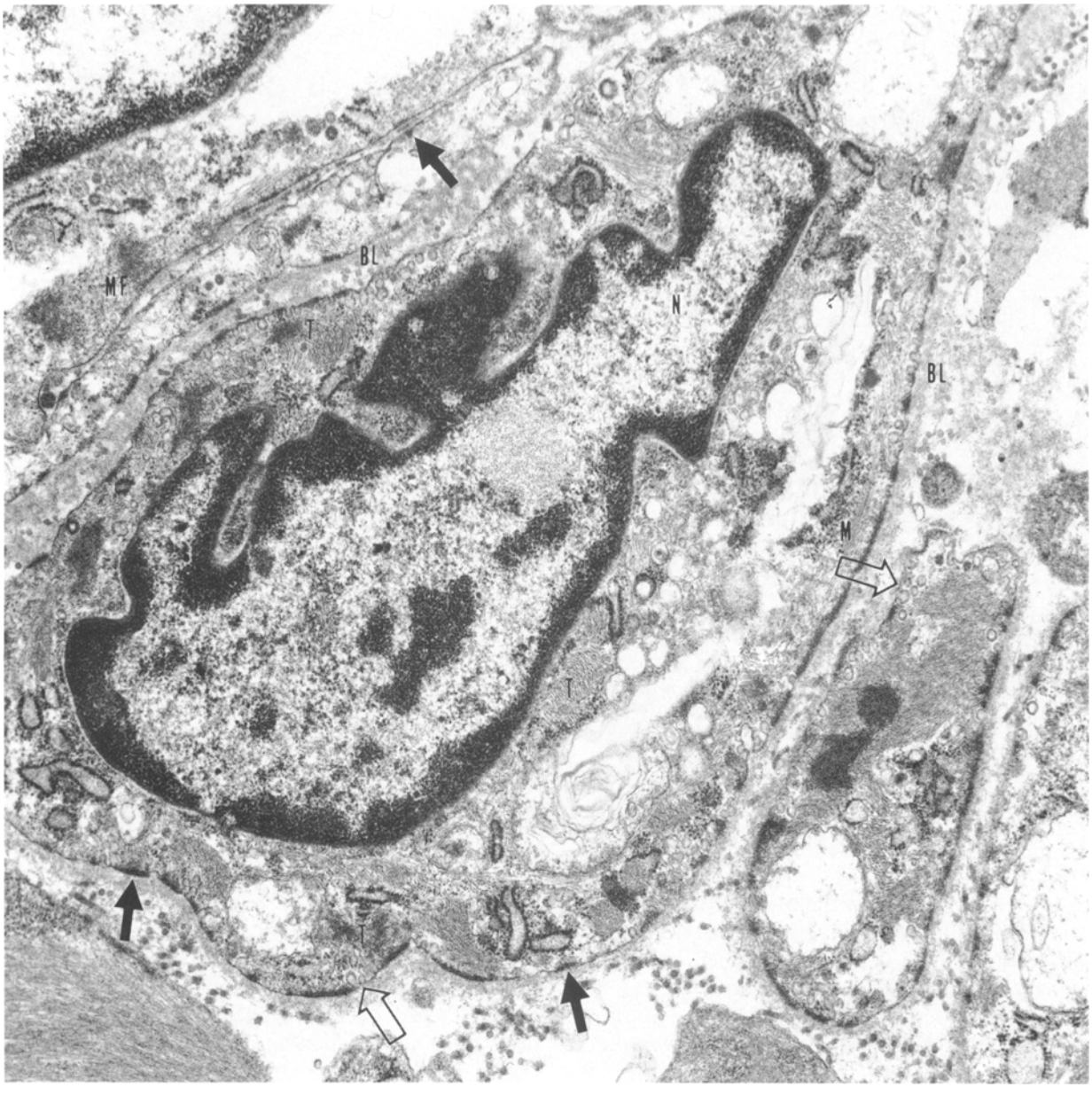

Fig. 5. A more separated epithelioid tumour cell. Micropinocytic invaginations are numerous (*open arrows*). Organelles are well-developed. The cell is virtually surrounded by a basal lamina (BL). Marginal plaques are prominent (*solid arrows*); some assume the shape of hemidesmosomes, but complete desmosomal structures are not formed in the absence of continuity between cell membranes. There is a patchy distribution of microfilaments (MF) and tonofilaments (T). The large nucleus (N) has an angulated shape and cytoplasmic indentations; the chromatin is centrally well dispersed and condensed along the nuclear membrane. ×4000

in more peripheral zones within the cytoplasm (Figs. 5, 6).

Lymphoid cells and small blood vessels were frequently encountered in the stroma. Smaller fusiform cells were found close to tumour cells. Golgi zones and the endoplasmic reticulum of these were well-developed, and fine cytoplasmic filaments were grouped peripherally; pinocytic vesicles invaginated cell surfaces. These cells were incompletely invested with a basement membrane, and closely approximated collagen bundles merged with their borders.

Discussion

There are several points worthy of comment concerning the routine light-microscopic findings. Fine needle aspiration proved to be a valuable tool

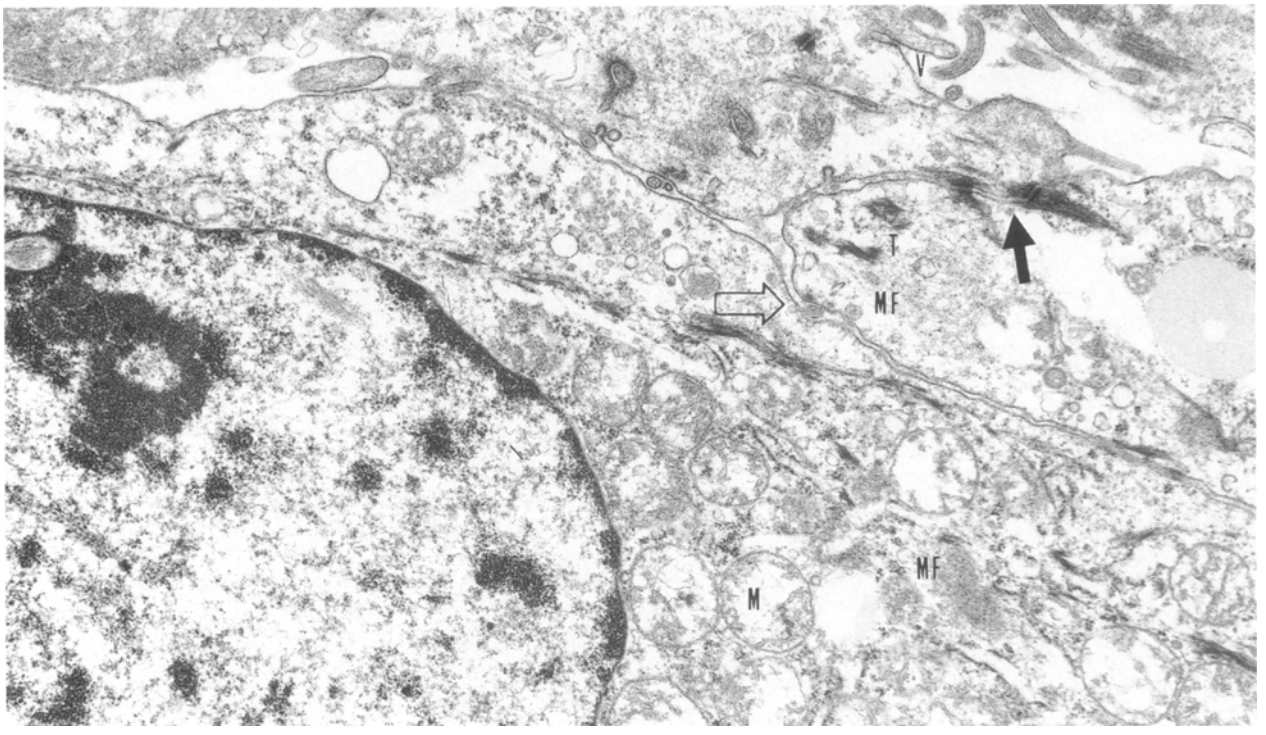

Fig. 6. Adjoining spindle cells are shown in this illustration. Desmosomal attachments bond the cell membranes (*solid arrow*). Aggregates of tonofilaments (T) and microfilaments (MF) are present. Organelles, especially mitochondria (M), are well developed; their distribution and concentration vary considerably from cell to cell. Only few invaginations of the cell membrane can be seen (*open arrow*). Cytoplasmic processes and microvilli (V) project from the free surfaces of the elongated cells. A condensation of granular material suggestive of basal lamina has formed on the surface of the epithelioid variant. × 6000

in the making of two interim evaluations and assessing the cytomorphology of the tumour epithelium. Mucin histochemistry indicative of secretory activity was completely negative. Features associated with malignant change were absent: The nuclear-cytoplasmic ratio was not increased. Mitotic activity was focally observed; but mitotic figures were normal, and there was no nuclear anaplasia.

The pattern of immunostaining agreed with the published criteria for myoepithelial differentiation (Kahn et al. 1985), i.e. variable staining for actin, S-100, keratin, and vimentin. Although there was variability in strength of staining between neoplastic cells and normal myoepithelium, an identical pattern could be demonstrated. A strong positiveness for EMA which is associated with duct epithelium was absent in tumour cells.

Ultrastructural characteristics which have been shown to indicate myoepithelial differentiation include mature desmosomes, remnants of basal lamina, pinocytic invaginations of the plasmalemma, tonofilaments, and microfilaments (Ohtani and Sasano 1980; Erlandson and Rosen 1982); these were demonstrated in our study. Actin filaments were often sparsely developed and irregularly distributed, and this correlates well with the degree of intensity and distribution of the respective immunostaining for MSA. The frequent profusion of larger diameter intermediate filaments correlates with the strong keratin positiveness. Depiction of microvilli on free surfaces of cells represents a feature which has also been described in association with areas of squamous differentiation in spindle cell carcinoma (Gersell and Katzenstein 1981). The stromal spindle cells containing microfilaments and cell membrane invaginations but lacking tonofilaments and desmosomal components represent myofibroblasts (Seemayer et al. 1979).

Are there other neoplasms of the breast whose features bear resemblance to those of our case? One candidate which has been noted to exhibit a limited light-microscopic similarity and shares ultrastructural characteristics is the spindle cell carcinoma (Gersell and Katzenstein 1981); however, its cytomorphology is unequivocally malignant. Also, a similar pattern has been demonstrated to partially involve the interior and periphery of an intracystic papilloma (Reddick et al. 1985); our case was devoid of a glandular component. Of all the reported composite tumours con-

taining glandular and spindle cell myoepithelial elements none showed light-microscopic evidence of squamous differentiation.

In conclusion, we have presented a case whose histopathological features are unique. The tumour shows cellular dimorphism, and our investigations have established the presence of squamous and myoepithelial differentiation. Our paper should serve to broaden the current understanding of the histogenesis of breast neoplasms and the differentiating capacity of duct epithelium. Both the clinical course and histopathology suggest that this is a benign process.

Acknowledgement. We are indebted to Paul McMillan, Ph.D., Director, Medical Electron Microscopy and Ramakrishna Nayak, M.D., Director, Immunohistochemistry of the Rhode Island Hospital, Providence, Rhode Island for their assistance. We thank Mr. Anthony J. McIntyre, Sr. for his support in the preparation of photographic material and Mrs. Winifred Jacome, Librarian, for her diligent efforts in securing bibliographic material. Drs. M.A. Chernow and I.S. Leja of the Newport Hospital, Newport, Rhode Island made available tissues and reports from the patient's final surgical procedure.

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Received October 20, 1989 / Accepted June 5, 1989