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Differentiation between metaplastic carcinomas and sarcomas of the human female breast by fibronectin

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Summary. The distribution pattern of fibronectin in metaplastic carcinomas, stromal sarcomas, malignant cystosarcoma phyllodes tumours and histiocytic type lymphomas of the human female breast has been studied using the indirect immunoperoxidase technique on formalin fixed paraffin embedded tissue.

Fibronectin was demonstrated as intensely stained strands between tumour cells forming an irregular network in metaplastic carcinomas and lymphomas. Stromal sarcomas and the malignant stromal component of the phyllodes tumours exhibited, in contrast, a uniform staining throughout tumour cells and stroma which was weaker than in adjacent normal-looking connective tissue.

We suggest that the intense staining reaction of metaplastic carcinomas is due to the scirrhous reaction generally associated with invasive human breast carcinomas. The advantage of using fibronectin as a diagnostic tool in the differentiation of carcinoma/lymphoma versus sarcoma is the fact that the antigen is a stromal marker and its staining intensity is not influenced by the morphology or degree of differentiation of non-mesenchymal tumours.

Key words: Breast – Metaplastic carcinomas – Sarcomas – Immunoperoxidase – Fibronectin

Introduction

The glycoprotein fibronectin (FN) is one of the components of connective tissue demonstrated by immunohistochemical techniques at thin strands associated with collagen (Engvall et al. 1978; Jilek and Hörmann 1978; Stenman and Vaheri 1978; Hølund et al. 1982).

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A variety of mesenchymal cells such as fibroblasts, myoblasts, chondrocytes, Schwann cells and astroglial cells have been reported to synthesize and secrete FN in culture (for review see Mosesson and Amrani 1980) and it has been observed in fibroblasts and myoblasts of human tissues by indirect immunofluorescense and immunoperoxidase investigations (Stenman and Vaheri 1978; D'Ardenne et al. 1983; Hølund et al. 1982).

The presence of FN seems to be increased in inflammatory and benign proliferative tissues during embryonic development (Wartiovaara and Vaheri 1980) and tissue repair (Hølund et al. 1982; Repesh et al. 1982). In contrast FN in malignant mesenchymal tumours has been reported to occur in amounts comparable to normal connective tissue (Stenman and Vaheri 1981; DuBoulay 1983) or even less than that (Birembaut et al. 1981; Labat-Robert et al. 1981). In normal human breast tissue FN is present in the connective tissue within and between lobules, in basal laminas around ducts, glands, vessels and nerves (Stenman and Vaheri 1978; D'Ardenne et al. 1983) and along the luminal border of secretory cells (Labat-Robert et al. 1980; Christensen et al. in press).

Malignant mesenchymal tumours of the breast are rare, and their FN distribution pattern has not been described. Invasive human breast carcinomas, however, have been reported to show an intense stromal reaction for FN (Stenman and Vaheri 1981). Some breast carcinomas contain pseudosarcomatous areas of metaplastic epithelial origin, which may be impossible to differentiate from sarcomas by conventional staining techniques (Kaufmann et al. 1984). The present study introduces the demonstration of FN by the indirect immunoperoxidase technique as a valuable diagnostic tool in differentiating between sarcomas and metaplastic carcinomas of the human female breast.

Material and methods

Tissues. Formalin fixed paraffin embedded tissue from 8 metaplastic carcinomas, 7 stromal sarcomas, 3 malignant cystosarcoma phyllodes and 2 lymphomas of histiocytic type were obtained from the files of Frederiksberg hospital and the Glostrup hospital pathology department, University of Copenhagen, Denmark. Sections were cut 5 mym thick and for routine histological evaluation stained with HE, PAS, Alcian blue, Van Gieson connective stain and Gordon and Sweet's argyrophilic stain.

Specific diagnostic staining. For further classification of tumours, sections were stained with the following antibodies employing the indirect immunoperoxidase technique as described by Sternberger et al. (1974):

1) Mouse monoclonal antibody to human epithelial membrane antigen, EMA, Dakopatts, Code no. M 613, lot 074 A, dilution 1:10.

2) Rabbit immunoglobulin fraction to human Keratin, Dakopatts, Code no. A 575, lot 063, dilution 1:200.

3) Mouse monoclonal antibody to human leucocyte common antigen, L-C, Dakopatts, Code no. M 701, lot 175A, dilution 1:10.

Treatment of sections with pepsin and hyaluronidase. Before immunoperoxidase staining for fibronectin deparaffinized sections were treated for 60 min at 37° C in 0,7% pepsin (SIGMA, Code no. P 7012) in 0,01 M HCL and then for 60 min at 37° C with testicular hyaluronidase, Hyalase^R, (2×10^6 units/l) (Hølund and Clemmensen 1982).

Antisera and control reagents. Specific rabbit immunoglobulin against human fibronectin was prepared as described by Clemmensen and Andersen (1982). Specific rabbit IgG against human fibronectin was obtained by affinity chromatography on human fibronectin linked to Sepharose as described by Hølund et al. (1981).

Immunoperoxidase staining for fibronectin. The indirect immunoperoxidase technique as described by Hølund et al. (1981) was used.

Control staining was performed by replacing the primary antiserum with a) antihuman fibronectin absorbed with fibronectin and b) phosphate-buffered saline.

Unfixed frozen sections of one of the metaplastic carcinomas (giant cell type) of this study, one myxoid sarcoma, 2 fibrosarcomas and 1 histiocytic type lymphoma of soft tissues other than the breast served as positive controls of the staining technique and pattern.

Results

The results of the indirect immunoperoxidase staining for EMA, Keratin and L-C are shown in Table 1.

Metaplastic carcinomas were EMA and/or Keratin positive in all epithelial-like tumour cells, which were not always recognizable in routine preparations. Metaplastic spindle cells were unreactive to both antibodies (Fig. 1). Only the lymphomas exhibited a distinct staining reaction for L-C on the surface of all tumour cells (Fig. 2). The stromal sarcomas and the malignant stromal component of the phyllodes tumours did not react with any of the 3 antibodies.

Staining reaction for fibronectin. In normal-looking breast tissue observed in conjunction with most sections FN was present as thin strands of connective tissue matrix, in basal laminas and in or around fibroblasts.

Table 1. Distribution of EMA, Keratin, L-C and FN in metaplastic carcinomas, stromal sarcomas, malignant cystosarcoma phyllodes tumours and histiocytic type lymphomas by the indirect immunoperoxidase technique

	EMA		Keratin		L-C		FN	
	T.C.	stroma	T.C.	stroma	T.C.	stroma	T.C.	stroma
Metaplastic carcinomas (Giant cell pattern)	+	−	+	−	−	−	(+)	+
Metaplastic carcinomas (Myxoid pattern)	−	−	−	−	−	−	−	+
Metaplastic carcinomas (Spindle cell pattern)	−	−	−	−	−	−	−	++
Stromal sarcoma	−	−	−	−	−	−	−	−
Malignant cystosarcoma phyllodes tumours (stroma)	−	−	−	−	−	−	−	−
Histiocytic type lymphomas	−	−	−	−	+	−	−	++

T.C.: Cytoplasmic or surface staining of tumour cells.
( ): just a few

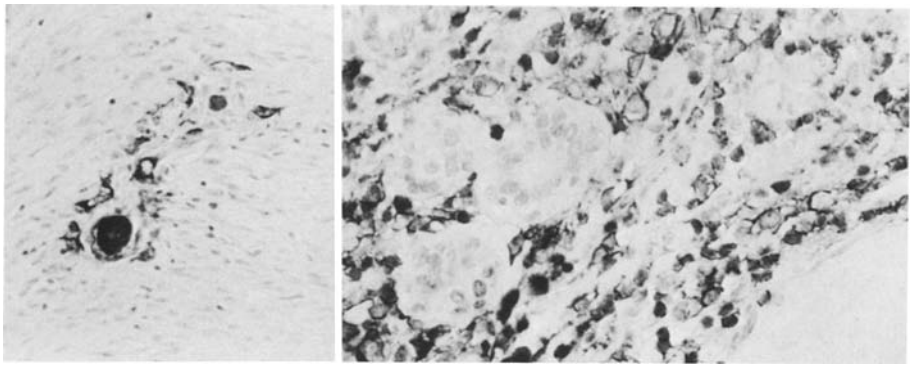

Fig. 1. Spindle cell metaplastic carcinoma. × 100. Immunoperoxidase staining for keratin. Epithelial-like tumour cells stain strongly, spindle cells are negative

Fig. 2. Histiocytic type lymphoma. × 150. Immunoperoxidase stain for leucocyte common antigen showing a distinct circumferential membrane staining of tumour cells

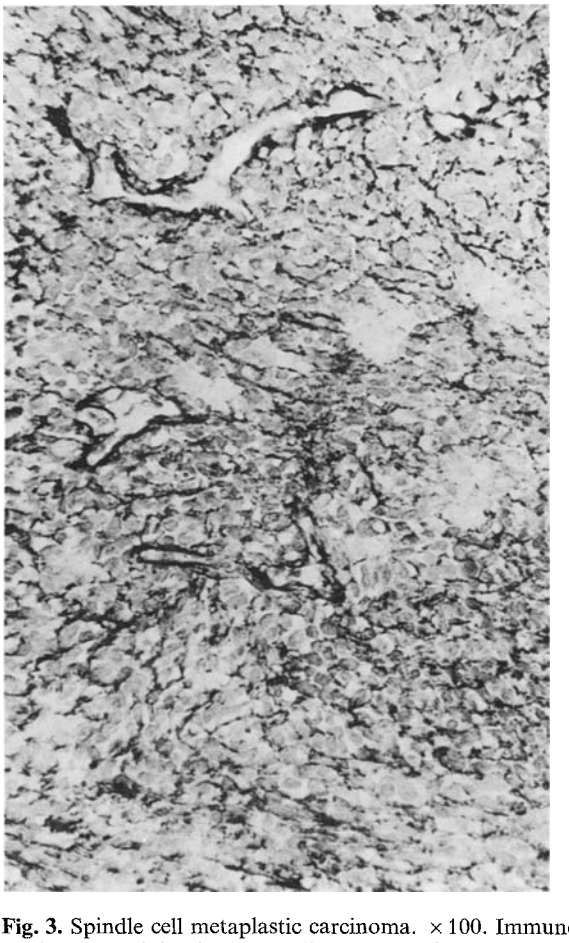

Fig. 3. Spindle cell metaplastic carcinoma. × 100. Immunoperoxidase staining for fibronectin. An intense staining between cells creates an irregular small-meshed network

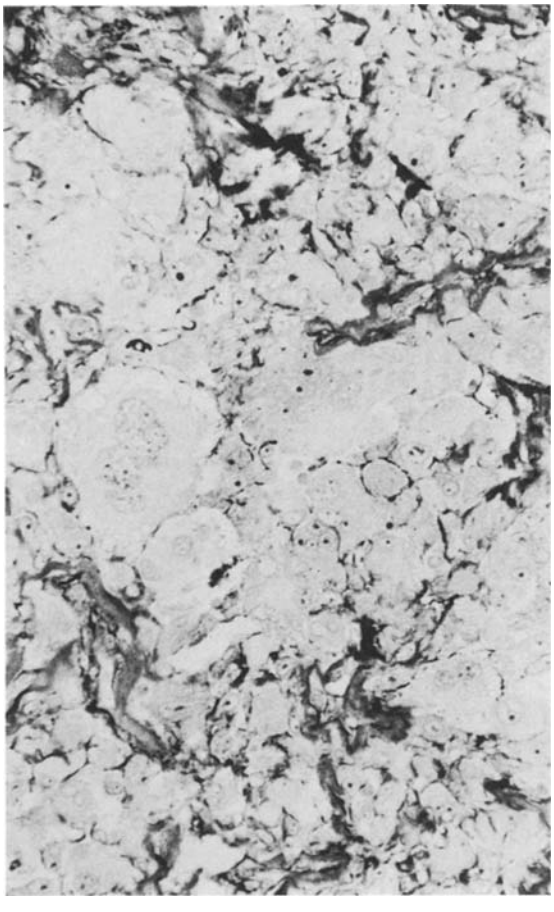

Fig. 4. Giant cell metaplastic carcinoma. ×200. A more irregular and less intense fibronectin positive network than in spindle cell variants is seen

Metaplastic carcinomas were represented by 5 duct carcinomas with a predominant spindle cell/myxomatous metaplasia, 2 with a predominant and one with a minor giant cell metaplasia. All of these tumours displayed a characteristic FN staining pattern. Distinctly FN positive strands or lumps surrounded individual tumour cells or small groups thus giving the appearance of a coarse, irregular network (Fig. 3). The staining seemed to be slightly more intense in spindle cell than in myxomatous and giant cell metaplastic foci (Fig. 4). The tumour stroma in general exhibited a more intense staining for FN than the connective tissue of adjacent normal-looking breast tissue. Most tumour cells were FN negative but a few pleomorphic or giant cells contained moderately or intensely FN positive coarse granules within their cytoplasm (Fig. 5).

Control frozen sections of the metaplastic carcinoma with a minor giant cell pattern confirmed the presence of a strongly stained FN positive tumour stroma, but in addition many of the cells showed a distinct cytoplasmic

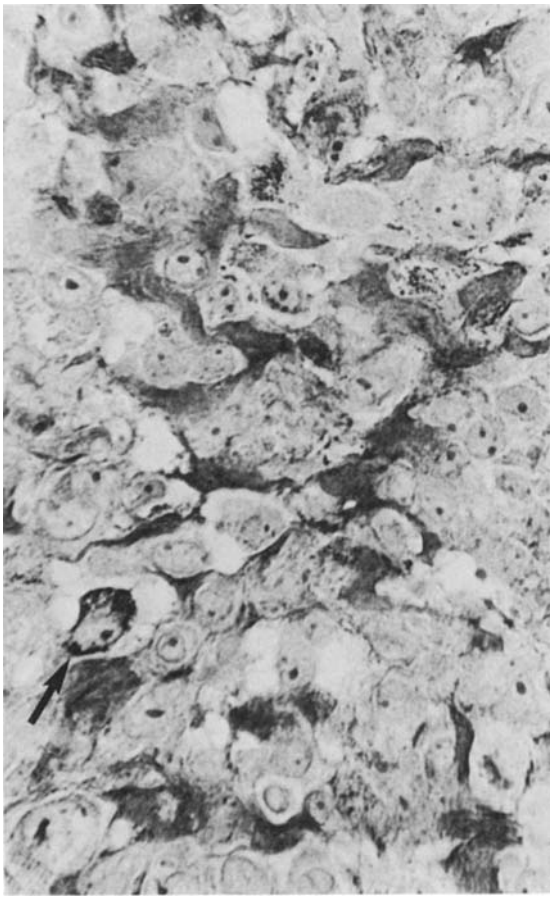

Fig. 5. Pleomorphic tumour cells of a metaplastic carcinoma. ×200. A few of the tumour cells contain coarse granules of fibronectin positive material within their cytoplasm (*arrow*).

reaction, which was greatly reduced both quantitatively and qualitatively in formalin fixed samples of the tumour.

All 7 stromal sarcomas were composed of spindle cells occasionally forming interlacing bundles. Five of the tumours contained myxoid areas with stellate cells and a stroma rich in acid mycopolysaccharide. The FN staining was weak and in both spindle cell and myxomatous areas less intense than in normal-looking connective tissue (Fig. 6). Only the vessels, abundantly present in these tumours, presented a ring of strongly FN positive material subendothelially. FN was evenly distributed within tumour cells and stroma complicating the recognition of cell borders.

The malignant cystosarcomata phyllodes were characterized by a malignant mesenchymal stroma interspersed by elongated, cleft-like ducts lined by benign epithelium (Norris and Taylor 1967).

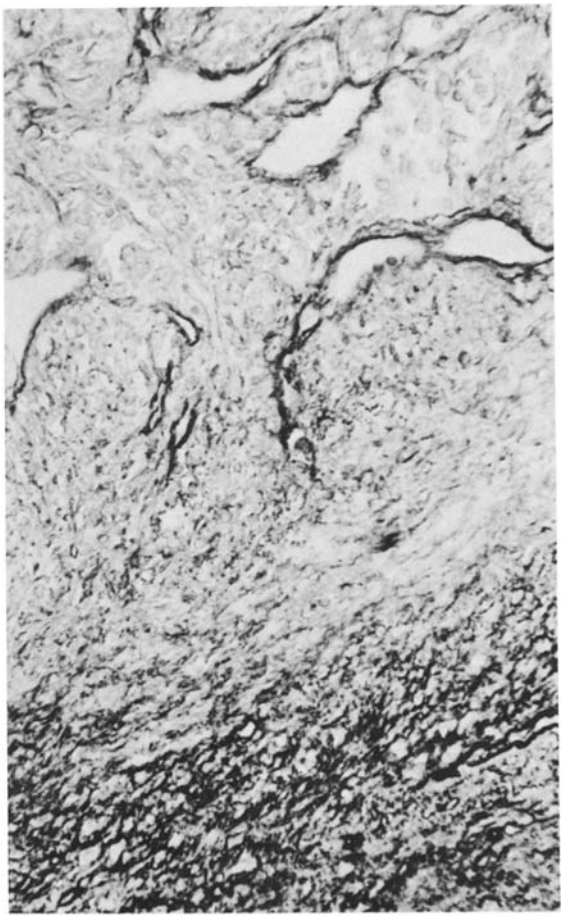

Fig. 6. Spindle cell stromal sarcoma. × 100. Immunoperoxidase stain for fibronectin. The mesenchymal tumour tissue is weakly stained compared to normal-looking connective tissue (bottom). Only vessels are clearly outlined by fibronectin located subendothelially

The morphology and FN staining pattern of the stromal component were indistinguishable from those of the stromal sarcomas with the exception of one tumour with liposarcomatous foci. The fat vacuoles appeared completely FN negative. The benign epithelial component of the tumours was unreactive to FN as well, but at the stromal-epithelial junction a staining similar to the one around vessels was observed (Fig. 7).

Both lymphomas were of histiocytic type not otherwise specified (The non-Hodgkin's lymphoma pathological classification project, 1982). The rounded tumour cells infiltrated the breast tissue diffusely and surrounded rather than destroyed normal ducts and glands. These tumours contained scattered, intensely stained strands of FN positive material, which might be concentrically arranged around FN negative proliferation nodules of

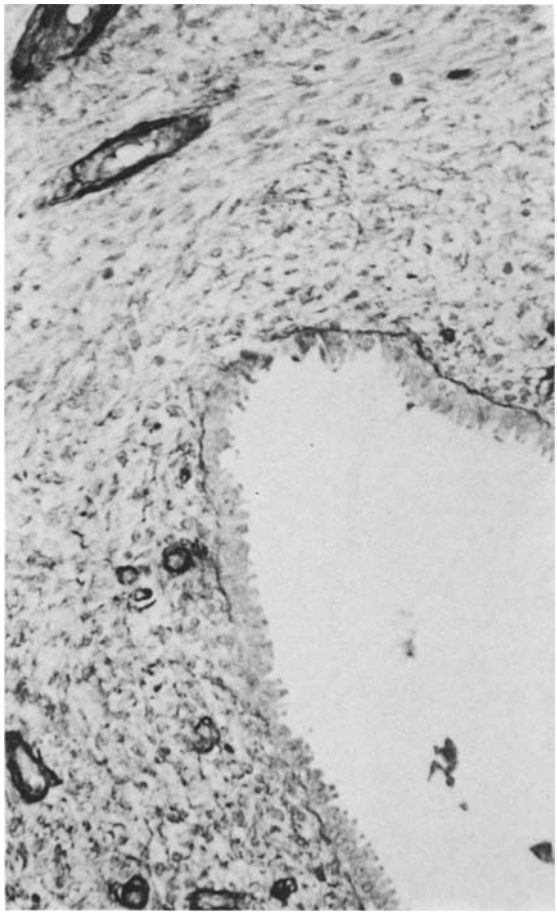

Fig. 7. Malignant cystosarcoma phyllodes. × 100. Stroma and epithelium are fibronectin negative but a distinct reaction is observed at the stromal-epithelial junction.

cells (Fig. 8). In general, however, the FN staining pattern showed a great resemblance to that observed in the metaplastic carcinomas.

Discussion

Fibronectin is considered to be a structuring glycoprotein responsible for tissue organization and is found in increased amounts in newly formed connective tissue (Stenman et al. 1977; Hølund et al. 1982; Repesh et al. 1982).

The irregular, strongly FN positive network observed around cells in the metaplastic carcinomas of the present study might be due to a benign fibrotic response – the so-called desmoplastic or scirrhous reaction – associated with most human invasive breast carcinomas (Kao et al. 1984). Another possibility, however, might be that the pericellular staining represents

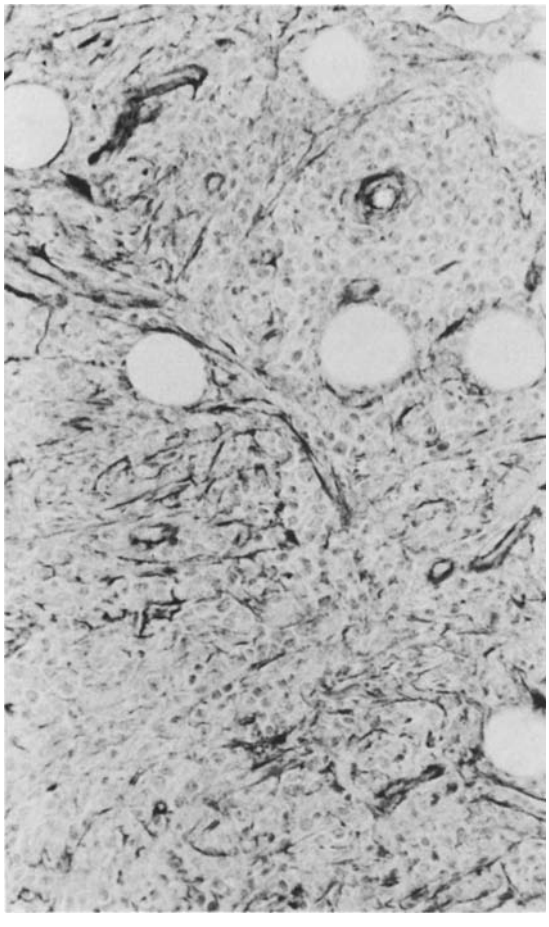

Fig. 8. Malignant lymphoma of histiocytic type. × 100. Scattered strands of fibronectin positive material are seen between tumour cells. Top right is a proliferation nodule without staining apart from a vessel in the center

the efforts of the tumour cells to produce basement membrane FN, which in normal breast tissue is considered to be a product of the epithelial cells (Asch et al. 1981; Stampfer et al. 1981; Taylor-Papadimitriou et al. 1981).

Stampfer et al. (1981) observed a faint FN positive powdery stippling all around the surface of both cultured and tissue-bound tumour cells of 14 primary human breast carcinomas and considered that this supported this latter theory, but most other immunofluorescence investigations have demonstrated a loss of pericellular FN in non-medullary invasive human breast carcinomas (Labat-Robert 1980; Stenman and Vaheri 1981; Birembaut et al. 1981; Noel et al. 1982).

The FN positive network around metaplastic spindle cells displayed a greater staining intensity than the matrix of myxomatous – and giant cell metaplasia. This might indicate that invasive breast carcinomas with a pro-

nounced scirrhous reaction are especially prone to develop spindle cell metaplasia, a theory which is in accordance with the observation that FN matrix supports the morphological conversion of rounded cells into spindle shaped (Chen et al. 1978b).

A moderate to strong cytoplasmic staining reaction was observed in just a few pleomorphic and giant tumour cells of epithelial origin. On frozen section controls this cytoplasmic staining was much more extensive and intense just as we in a previous study have found it to be in poorly differentiated and predominantly individually growing breast carcinoma cells, including those of one of the metaplastic giant cell carcinomas of the present study (Christensen et al. in press). In that study, however, the tissue samples had been treated with Sainte Marie's cold ethanol fixation (Ethanol/acetic acid 1%) recommended by Hølund et al. (1981) and Szendröi et al. (1983) as the most gentle method for preservation of FN.

In accordance with previous observations on soft tissue tumours (Birembaut et al. 1981; Labat-Robert et al. 1981), and consistant with the theory that fibroblasts loose their surface-bound FN during malignant transformation (Hynes 1976; Chen et al. 1978a; Vaheri and Mosher 1978; Rouslahti 1984), our stromal sarcomas and the malignant stromal component of our phyllodes tumours stained considerably more weakly for FN than the adjacent normal-looking connective tissue.

The lymphomas of the breast included in this study for comparison could not be convincingly differentiated from metaplastic carcinomas by their FN distribution pattern alone. Some proliferation foci lacked FN completely just as it has been recorded absent in germinal centers of normal lymphatic tissue (Stenman and Vaheri 1978; D'Ardenne et al. 1983). In the bulk of tumour tissue, however, fine fibres of FN positive material created a network comparable to the one observed in normal lymphatic tissue (Stenman and Vaheri 1978; D'Ardenne et al. 1983) and in the metaplastic carcinomas of the present study.

Lymphomas, on the other hand, can be succesfully diagnosed by monoclonal leucocyte common antibody using the indirect immunoperoxidase technique on formalin fixed tissue (Warnke et al. 1983).

Epithelial membrane antigen (EMA) and Keratin gave, as expected, more unpredictable staining results (Sloane and Ormerod 1981; Schlegel et al. 1980). We found in work controlled by frozen section, that the expression of both antibodies was dependent of the degree of differentiation of the tumour cells.

FN has proven to be a valuable tool by this study in differentiating between metaplastic carcinomas and true sarcomas of the human female breast. The fact that identification of metaplastic carcinomas is based on the demonstration of a strong FN positive staining of the matrix interwoven between tumour cells and independent of the morphological type and degree of differentiation of the tumour itself increases the opportunity of achieving uniform and reliable results. It also suggests that FN staining might prove useful in distinguishing between metaplastic carcinomas and soft tissue sarcomas of organs other than the breast.

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