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The extracellular matrix in sarcomatoid carcinomas of the breast

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Abstract. The distribution of the main extracellular matrix components has been investigated immunohistochemically in four cases of breast sarcomatoid carcinomas. The histogenesis of these tumours is still unclear, but most evidence suggests that the sarcomatous component originates from mesenchymal conversion of carcinomatous cells. We found that carcinomatous portions of the tumours were associated with linear basement membrane-like deposition of laminin, type IV collagen and heparan sulphate proteoglycan that partially circumscribed the epithelial nests. The sarcomatous components produced an extracellular matrix immunoreactive to fibronectin, type I, III and VI collagens and tenascin. However, in two cases, in some sarcomatous areas, focal pericellular staining for antibodies to laminin and type IV collagen was seen. These results indicate that modifications observed in the double tissue component of these tumours involve not only the cell shape and the cytoskeleton, but also the components of the extracellular matrix. The significance of these findings and their relevance in the understanding of the phenotypic pattern changes of these biphasic tumours are discussed.

Key words: Sarcomatoid carcinoma – Breast – Extracellular matrix – Immunohistochemistry – Epithelial-mesenchymal conversion

Introduction

Sarcomatoid carcinomas are unusual tumours composed of both carcinomatous and sarcomatous tissue (Meis et al. 1987). Their histogenesis has long been the object of controversy and many theories on the origin of the double cell component have been proposed (Eusebi et al. 1989; Harris 1982; for review see: Weidner 1987). However, the most commonly accepted view is that the sarcomatous component originates from mesenchymal conversion of the carcinomatous epithelial cells (Battifora 1976; Hall and Levison 1989; Meis et al. 1987). Epithelial-mesenchymal conversion has been observed during embryonic development (Franke et al. 1982; Hay 1989), in carcinoma cell lines (Boyer et al. 1989a, b, Dulbecco et al. 1981), and can be induced, in experimental conditions, in adult differentiated epithelia (Greenburg and Hay 1982, 1986, 1988). The cells lose their epithelial shape and acquire a mesenchymal phenotype, but the change is not merely morphological, but also involves profound modifications in the cytoskeleton and desmosomal proteins (Boyer et al. 1989a, b; Franke et al. 1982; Greenburg and Hay 1988), as well as in the extracellular matrix (ECM) components (Dulbecco et al. 1981; Greenburg and Hay 1986; Hay 1989). Tissue phenotype depends on a complex, reciprocal interplay between cell-cell and cell-matrix interactions (McDonald 1989; Watt 1986). It has been suggested that a disruption of these relationships may result in the phenotypic changes observed in sarcomatoid carcinomas (Hall and Levison 1989). The ECM greatly affects cell behaviour, either stabilizing the epithelial phenotype or favouring a mesenchymal differentiation and some ECM components seem to trigger epithelial-mesenchymal conversions (Greenburg and Hay 1982, 1986, 1988; Hay 1989). Moreover, some changes in the ECM composition might be expected as a result of an epithelial-mesenchymal conversion (Greenburg and Hay 1986; Hay 1989) and in some sarcomatoid carcinomas, production of extracellular fibrous components has been observed by conventional microscopy (Battifora 1976; Gould et al. 1981). Against this background, we have studied four cases of breast sarcomatoid carcinomas by immunohistochemistry. Our aim was to analyse the distribution of the main ECM components, as well as the changes of the cytokeratin and vimentin cytoskeleton, in both carcinomatous and sarcomatous components of these tumours.

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Table 1. Clinical and pathological features of breast sarcomatoid carcinomas

Case no.	Age (years)	Size (cm)	Histology		Surgical treatment	Follow-up
			Carcinoma	Sarcoma		
1	45	1.7	DC	Spindle, myxoid and pleomorphic	EB	Alive, 1 year
2	81	3	DC and SCC	Spindle and pleomorphic	SM	Dead, 7 years
3	76	4	DC	Spindle, myxoid and chondroid	RM	Dead, 3 years
4	50	14	DC	Spindle and myxoid	SM	Alive, 4 years

DC, Ductal carcinoma; SCC, squamous cell carcinoma; EB, excisional biopsy; SM, simple mastectomy; RM, radical mastectomy

Table 2. Antibodies employed for immunohistochemistry

Antibody	Type	Dilution	Tissue specificity	Source
Anti-laminin	P	1:800	All basement membranes	Heyl, Berlin, Germany
Anti-collagen IV	M	1:50	All basement membranes	Dakopatts, Glostrup, Denmark
Anti-HSP	M	1:25	All basement membranes	Chemicon International, Temecula, Calif
Anti-fibronectin	P	1:10000	Interstitial connective tissue, some basement membranes	Dakopatts
Anti-collagen I	P	1:700	Interstitial connective tissue	Sera-Lab, Sussex, England
Anti-collagen III	M	1:20	Interstitial connective tissue	Heyl
Anti-collagen VI	P	1:2000	Interstitial connective tissue	Heyl
Anti-tenascin	M	1:100	Some mesenchymal extracellular matrices	Dakopatts
Anti-keratin AE1/3	M	Prediluted	Epithelial cells	Ortho Diagnostic Systems, Milan, Italy
Anti-keratin CAM 5.2	M	Undiluted	Epithelial cells	Becton Dickinson Mountain View, Calif
Anti-vimentin	M	1:6	Mesenchymal cells	Boehringer, Mannheim, Germany
Anti-desmin	M	1:50	Striated and smooth muscle cells	Dakopatts
Anti-S-100 protein	P	Prediluted	Melanocytes, adipocytes, Schwann cells	Ortho Diagnostic Systems

P, Polyclonal; M, monoclonal; HSP, heparan sulphate proteoglycan

Materials and methods

Four sarcomatoid carcinomas of the female breast were identified. In these tumours, carcinomatous tissue together with a sarcomatous component were seen in routinely stained haemotoxylin and eosin sections. The main clinical and pathological data are provided in Table 1. In all cases paraffin blocks of 10% buffered formalin-fixed tissue were available, and some samples of methacarn-fixed tissue were also available in one case.

For immunohistochemical staining, 3-μm-thick serial sections were treated with the peroxidase-antiperoxidase procedure, according to Sternberger (1974). The antibodies employed in this study, together with their specificities, dilutions and sources, are indicated in Table 2. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in absolute methanol for 30 min. Enzymatic digestion for ECM molecule immunostaining (Kirkpatrick and D'Ardenne 1984) was performed as follows. Sections to be stained for laminin, heparan sulphate proteoglycan, and type I, III, IV, and VI collagen were pretreated with 0.4% pepsin (Sigma, St. Louis, Mo.), in 0.01M hydrochloric acid (pH 2.0), for 120 min at 37° C. Staining for fibronectin and tenascin was performed after digestion with 0.05% protease XXIV (Sigma) in phosphate-buffered saline, pH 7.3, for 20 min, at 37° C. Sections of methacarn-fixed tissue were pretreated in a similar way, but only for 15 min (laminin, heparan sulphate proteoglycan, and type I, III, IV and VI collagen) or 2 min (fibronectin and tenascin). Trypsin (Sigma) was employed for sections to be stained with CAM 5.2 and desmin, and protease XIV (Sigma) for AE1/AE3 and anti-S100 protein antibodies. Then, the sections were incubated with the primary antibody, at room temperature, for 2 h (CAM 5.2, desmin, AE1/AE3 and S-100 protein) or overnight (all other antibodies). To develop a colour reaction 3-3′-diaminobenzidine was used. Sections, of known immunoreactivity were also stained as positive controls.

Results

In all cases, carcinomatous epithelial nests were partially surrounded by linear immunoreactivity for antibodies to the basement membrane components: laminin, type IV collagen and heparan sulphate proteoglycan (Abrahamson 1986; Timpl 1989). Sarcomatous tissue was generally devoid of these ECM components, except for the blood vessel basement membranes (Fig. 1A). However, in cases

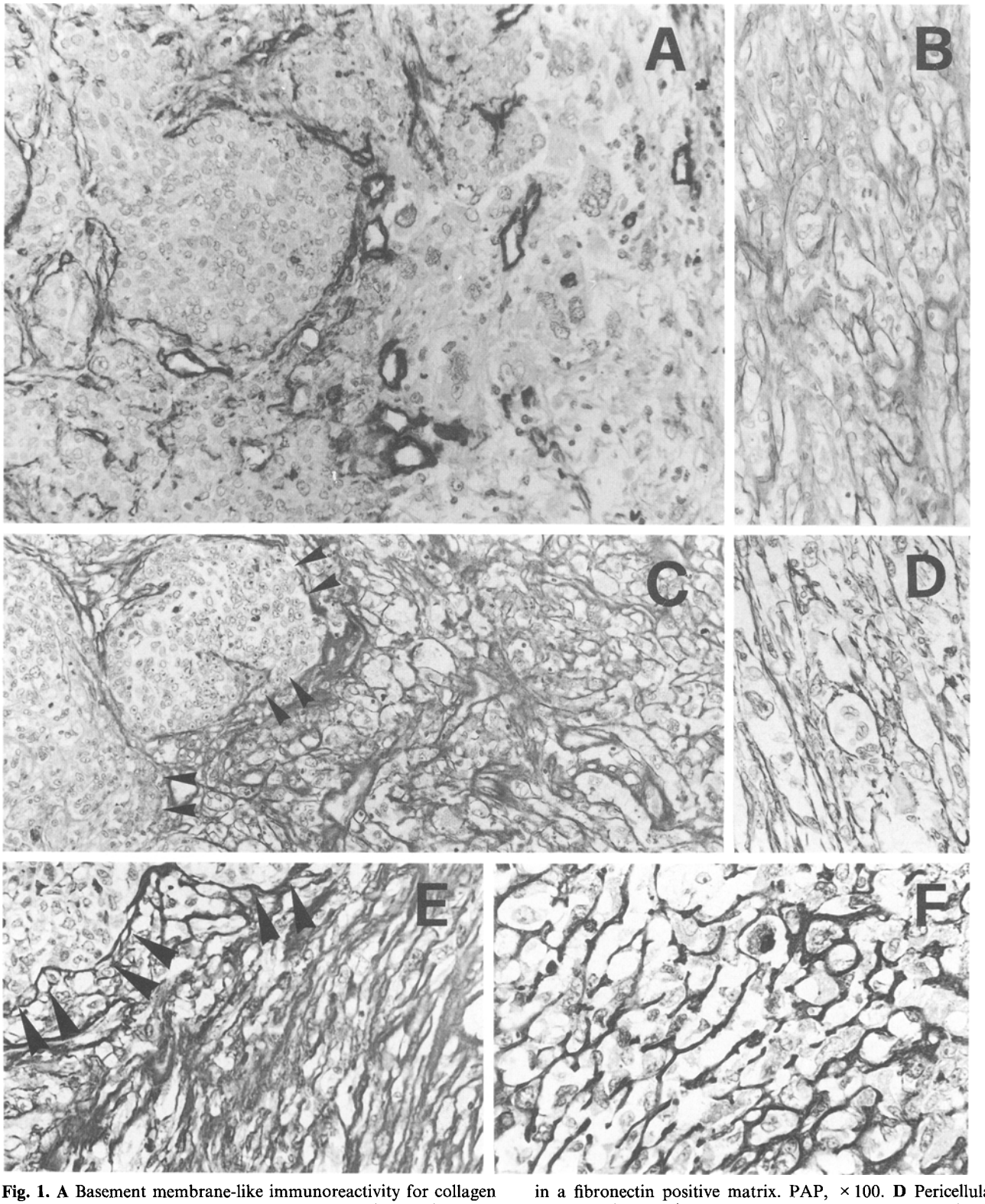

Fig. 1. **A** Basement membrane-like immunoreactivity for collagen type IV is seen among carcinomatous nests *(left)*, but not in sarcomatous areas *(right)*. Peroxidase-antiperoxidase (PAP), × 100. **B** A sarcomatous field with pericellular labelling for laminin. PAP, × 160. **C** No immunoreactivity for fibronectin is observed within carcinoma *(arrow heads)*. Sarcomatous cells *(right)* are wrapped in a fibronectin positive matrix. PAP, × 100. **D** Pericellular immunoreactivity for fibronectin among sarcomatous cells. PAP, × 160. **E** Negative immunostaining in carcinoma **(arrow heads),** but positive immunoreaction in sarcomatous area *(bottom)* for collagen I. PAP, × 100. **F** Strong staining for collagen I among sarcomatous cells. PAP, × 160

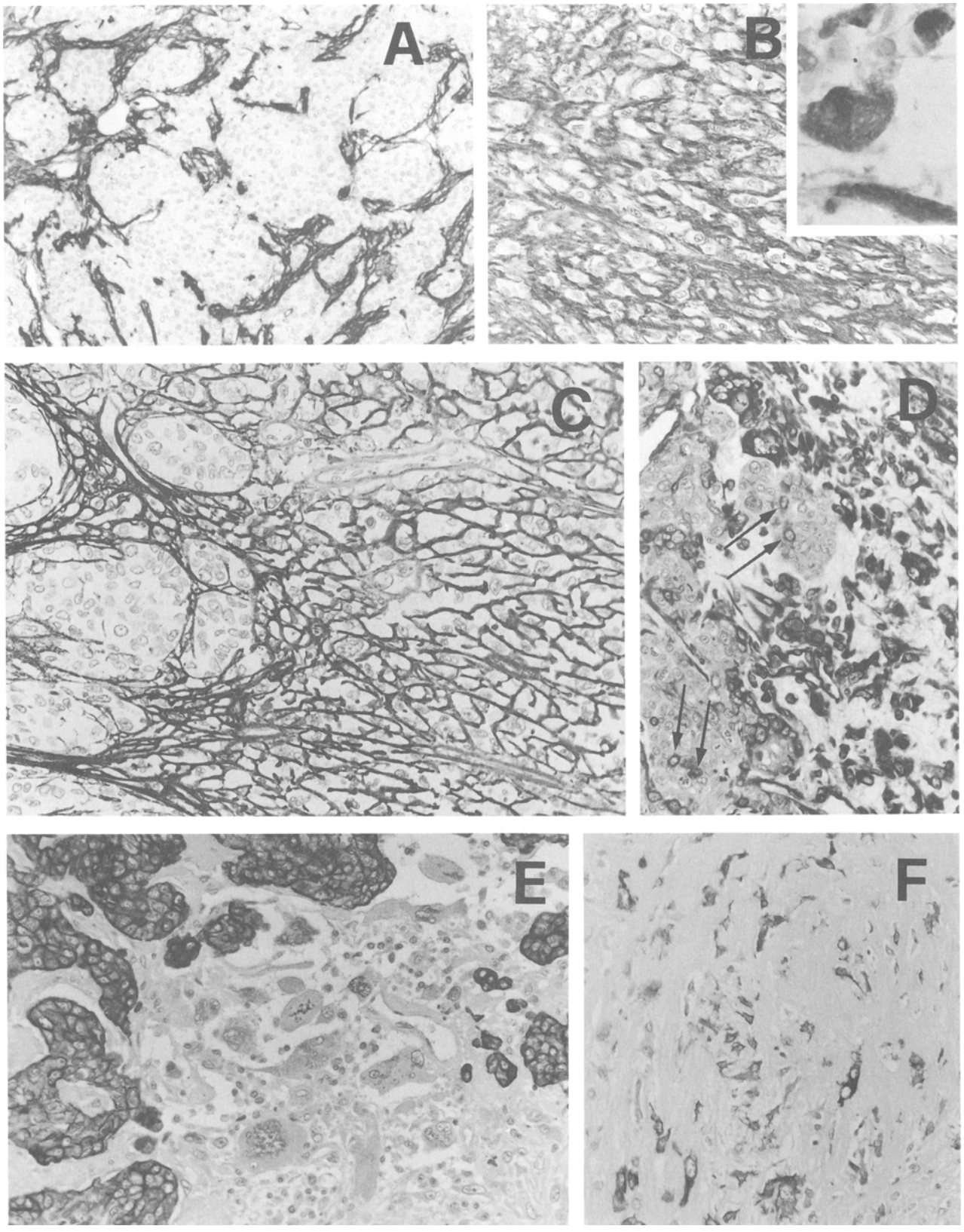

Fig. 2. **A** Only supporting connective tissue is reactive to collagen III, within the carcinoma. Peroxidase-antiperoxidase (PAP), ×40. **B** Extracellular staining for collagen III in sarcomatous area. PAP, ×40. *Inset:* strong intracytoplasmic reactivity to collagen III in some sarcomatous cells. PAP, ×400. **C** No immunoreactivity for tenascin is found within carcinoma *(left)*. However, a strong positivity is seen within sarcomatous tissue *(right)* and at the boundary between carcinoma and sarcoma. PAP, ×100. **D** Sarcomatous cells *(right)* are strongly labelled by anti-vimentin antibody. However, some carcinomatous cells *(arrows)* also are reactive. PAP, ×100. **E** Carcinomatous cells are reactive to anti-cytokeratin antibodies. Sarcomatous cells *(center)* are unreactive. PAP, ×100. **F** Many sarcomatous cells are labelled by antibodies to cytokeratin. PAP, ×100

2 and 4, pericellular staining for laminin and type IV collagen was observed in some fields among the sarcomatous cells (Fig. 1B). Antibodies to the glycoprotein fibronectin (Linder et al. 1978) diffusely stained the ECM around sarcomatous cells in all tumours with a fibrillar pattern (Fig. 1C, D); carcinomatous portions were negative, only blood vessel walls and supporting connective tissue being immunoreactive (Fig. 1C). The ECM of the sarcomatous tissue was strongly immunoreactive for the interstitial collagen types I, III and VI (Engel et al. 1985; Nowack et al. 1976) (Figs. 1E, F, 2A, B). In addition to a fibrillar staining of the ECM, in three cases an intracellular cytoplasmatic reactivity of some sarcomatous cells was observed with antibodies to types I, III and VI collagen (Fig. 2B, inset), suggesting that tumour cells were synthesizing these ECM components. Blood vessel walls, and their supporting connective tissue, were also stained by these antibodies, in carcinomatous as well as adjacent normal breast tissue, but no staining was seen inside the carcinomatous nests (Figs. 1E, 2A). Immunoreactivity for the glycoprotein tenascin (Chiquet-Ehrismann et al. 1986) was detected in benign connective tissue around carcinomatous nests and at boundaries between carcinomatous and sarcomatous tissue. The latter also showed a rather diffuse pericellular or fibrillar staining for this antibody (Fig. 2C). No immunolabelling for tenascin was observed in the interstitial connective tissue of the normal breast, far from the tumours. In general, the intensity of ECM staining for fibronectin, types I, III and VI collagen and tenascin was greater in spindle or pleomorphic sarcomatous areas than in myxoid or chondroid ones.

Mesenchymal-like cells of the sarcomatous tissues showed a uniformly strong immunoreactivity for anti-vimentin antibodies. In three tumours (cases 1, 3 and 4) some carcinomatous nests were also reactive for vimentin, albeit less intensely stained (Fig. 2D). Immunostaining for cytokeratins was always positive in those areas where a carcinomatous differentiation was seen in routine haematoxylin and eosin sections (Fig. 2E). Moreover, in the sarcomatous tissue of all cases, several tumour cells with a mesenchymal morphology were found to be immunoreactive to cytokeratins (Fig. 2F). In adjacent sections, these cells were also labelled by anti-vimentin antibodies, thus suggesting a co-expression of these intermediate filaments. Instead, no relation could be found between expression of cytokeratin and immunostaining of the surrounding ECM for laminin or type IV collagen in sarcomatous tissue, indicating that expression of these epithelial-type markers was not directly related.

Since some mesenchymal cells, such as adipocytes, muscle and Schwann cells, produce basement membranes (Abrahamson 1986), we performed an immunostaining with antibodies to desmin and S-100 protein, (labelling muscle cells, adipocytes and Schwann cells respectively), in order to investigate the possibility of a differentiation pathway towards these cell lineages. However, no staining of sarcomatous cells was observed in the tumours with pericellular labelling for laminin or type IV collagen. No morphological feature suggesting these specific mesenchymal differentiations was seen in haematoxylin and eosin sections.

Discussion

Sarcomatoid carcinomas are uncommon tumours of controversial histogenesis. The most likely pathogenetic mechanism is that proposed by Battifora (1976), who suggests that in these tumours some carcinoma cells undergo a conversion to sarcoma cells. Although the tissue phenotype in adult organisms is stable, and epithelia and mesenchymal cells form distinct tissues, in several circumstances some degree of plasticity of the differentiation state is possible. Indeed, conversion of cells with epithelial characteristics to mesenchymal-type cells is a phenomenon observed during embryogenesis (Franke et al. 1982; Hay 1989) and in vitro (Boyer et al. 1989a, b; Dulbecco et al. 1981; Greenburg and Hay 1982, 1986, 1988; Zuk et al. 1989). It has been suggested that these conversions involve a change of differentiation resulting in the loss of the epithelial phenotype and in the acquisition of the mesenchymal one (Hay 1989). Therefore, the expression of cytokeratin, laminin and type IV collagen, typical of epithelium, is replaced by expression of vimentin, fibronectin and type I collagen typical of the mesenchymal phenotype (Hay 1989).

In this study it has been shown that a set of changes in the distribution of both ECM components and intermediate filaments takes place in sarcomatoid carcinoma of the breast. We have observed that carcinomatous nests were partially surrounded by a linear immunoreactivity for the basement membrane laminin, type IV collagen and heparan sulphate proteoglycan. In contrast the sarcomatous tissue-associated ECM stained for fibronectin and the interstitial collagen types I, III and VI. These findings indicate that the changes of tissue differentiation observed histologically correspond to a definite change in the ECM.

As true sarcomas are, in general, negative for cytokeratin, the expression of this marker in sarcomatous cells of sarcomatoid carcinomas has been regarded as an argument supporting the epithelial origin of these cells (Ellis et al. 1988; Eusebi et al. 1989). In agreement with these studies, we too have observed a positive immunoreaction for cytokeratin of several sarcomatous cells. In a similar way, in some fields of the sarcomatous areas, together with expression of fibronectin and interstitial type collagens, we have observed pericellular staining for laminin and type IV collagen, ECM components typically associated with epithelial tissues.

Several explanations for these findings are possible. According to the theory of epithelial-mesenchymal conversion, one might speculate that variable degrees of mesenchymal conversion in carcinomas are possible and that tissue with a sarcomatous morphology may retain expression of some epithelial-type markers. This has been observed by several groups and might result from a partial or incomplete activation of the mesenchymal differentiation program (Boyer et al. 1989a, b; Dulbecco et al. 1981; Zuk et al. 1989). Alternatively, it is possible that mesenchymal conversion has been complete, but persistence of some epithelial markers in the sarcomatous tissue occurs due to slow turnover of these molecules (Greenburg and Hay 1988). These possibilities deserve further investigation.

Finally, we have observed a convincing extracellular staining for monoclonal antibodies to tenascin in the sarcomatous portions of all our cases. Tenascin is a mesenchymal extracellular glycoprotein, involved in epithelial-mesenchymal interactions (Chiquet-Ehrismann 1990; Chiquet-Ehrismann et al. 1986; Ekblom and Aufderheide 1989). Although its role in developmental and neoplastic processes is yet to be defined, it has been found to induce loss of cell-cell contact and cell detachment (Chiquet-Ehrismann 1990). A loss of cell-cell adhesion is a feature of sarcomatoid carcinomas, and its importance in the pathogenesis of these tumours has been emphasized (Sherwin et al. 1963). An involvement of tenascin in the process leading to the sarcomatous modifications of these lesions is possible. Whether some ECM molecules may have a role in promoting or maintaining these changes in sarcomatoid carcinoma remains to be investigated.

Our immunohistochemical study has shown a change in ECM component distribution that parallels the morphological differentiation of the tissue components of sarcomatoid carcinomas. Carcinomatous nests were surrounded by basement membrane-type matrix whereas sarcomatous areas were mainly associated with a mesenchymal matrix. Although these data do not allow us to draw conclusions about the histogenesis of breast sarcomatoid carcinomas, our results suggest that immunohistochemistry for ECM components may be valuable in studying the changes in differentiation in these biphasic tumours.

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