Immunohistochemical expression of alpha-smooth muscle actin in infiltrating ductal carcinoma of the breast with productive fibrosis

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Summary

Myoepithelial cells are normally located between the epithelial cells and the basal lamina of secretory elements of exocrine glands. Their role in the histogenesis of breast tumours has been studied extensively, and a definite differentiation towards myoepithelial cells has been demonstrated in adenoid cystic carcinoma, adenomyoepithelioma, low-grade adenosquamous (syringomatous) carcinoma, pure malignant myoepithelioma and poorly differentiated myoepithelial-rich breast carcinoma. All these tumours are of low malignancy, with the exception of malignant myoepithelioma and poorly differentiated myoepithelial-rich carcinoma.

We examined the possibility that invasive ductal carcinoma of the breast might show differentiation towards both epithelial and myoepithelial cells because there is no reason to assume that one type of differentiation necessarily excludes the other. We performed the avidin-biotin immunohistochemical analysis of 20 cases of infiltrating ductal carcinomas (IDCs) with diffuse fibrosis, 20 cases of IDCs without fibrosis and five cases of metaplastic carcinomas, to detect myoepithelial differentiation of the tumour cells. Myoepithelial differentiation was determined by the expression of alpha-smooth muscle actin (alpha-SMA). We concluded that IDCs with diffuse fibrosis are associated with a myoepithelial immunophenotype of carcinoma cells.

Key words: Alpha-smooth muscle actin; Ductal carcinoma; Breast; Fibrosis.

Introduction

Since infiltrating ductal carcinoma of no special type or carcinoma NOS constitutes the bulk of infiltrating cancer of the breast, it is important to take stock of current views about the nature of this tumour. Murad and Scarpelli [1], while conceding that medullary carcinomas are epithelial tumours, put forward the interesting and revolutionary concept that the common "scirrhous" carcinoma represents a myoepithelial cancer. This conclusion was based on ultrastructural studies which showed that in medullary carcinoma there was a production of microvilli on the surface of the cell, and that these microvilli frequently abutted directly on to the surrounding stroma. This and other features led these authors to the conclusion that medullary carcinoma showed definite evidence of epithelial differentiation. There is a general agreement with the conclusion of Murad and Scarpelli [1] about the epithelial nature of medullary carcinomas. Medullary carcinoma and mucoid cacrinoma can justifiably be regarded as reflecting the opposite poles of differentiation, with the former showing almost exclusively absorptive surfaces and the latter demonstrating exaggerated secretory properties.

Murad and Scarpelli [1] went on further to conclude that "scirrhous" carcinoma was a myoepithelial cancer, basing this view mainly on their finding of basal lamina material around the clumps of infiltrating cancer cells. At first sight this appeared to be a very attractive hypothesis, especially if their findings were to receive independent confirmation. Unfortunately, these authors failed to consider all the existing evidence, including the formation of ducts with the presence of mucinous luminal secretion in these carcinomas, features which are hardly compatible with a purely myoepithelial type of tumour. Even the trabecular formations in infiltrating ductal carcinoma are much more suggestive of epithelial than of myoepithelial differentiation. There is no real question that there exists ample evidence of epithelial differentiation in infiltrating ductal carcinoma and that this tumour is most definitely not a pure myoepithelial cancer on well-established structural and histochemical grounds.

Materials and Methods

We studied 20 cases of IDCs with diffuse fibrosis covering more than 30% of each tumour area, 40 cases of IDCs without fibrosis as negative controls, and ten cases of metaplastic carcinomas as positive controls for myoepithelial differentiation.

The tissues were fixed in 10% neutral buffered formaldehyde at 4°C for 24 hours and processed for routine paraffin embedding. Paraffin blocks were available in all cases, and 3μm thick tissue sections were stained routinely with hematoxylin-eosin, and subsequently, using immunohistochemistry.

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Immunohistochemical analysis: The presence of α-smooth muscle actin was examined in our samples by means of the avidin-biotin complex (ABC) peroxidase method using the monoclonal antibody anti-asm-1. Sections were pretreated with H_2O_2/methanol and subsequently with 0.1 M periodic acid, 0.005 M NaBH4 and normal horse serum. They were incubated for 20h with anti-asm-1 hybridoma supernatant containing 5 μg/ml of IgG diluted 1:600. This first incubation was followed by ABC-peroxidase staining using the Vectastain Kit anti-mouse IgG (Vector Laboratories, Burlingame, Ca). Peroxidase activity was revealed with 30% DAB (3,3´-diaminobenzidine, Serva Heidelberg, FRG) in PBS containing 0.015% H_2O_2. Slides were weakly counterstained with Mayer's hematoxylin and mounted in Eukitt. Controls were performed by using a mouse IgG or by omitting the primary antibody.

The immunostained sections were examined with a x 40 objective and the distribution of alpha-SMA within the cell was recorded. Every stained cell was scored as positive regardless of staining intensity. To count the number of cells with alpha-SMA staining, a 10 x 10 square calibrated grid was inserted into the eyepiece of an Olympus binocular microscope.

Five-to-ten fields were examined for each section, and at least 1,000 cells were scored, depending on cellularity. The percentage of positive cells was recorded as the alpha-SMA index.

$$\text{Alpha-SMA index} = \frac{\text{no. of positive cells}}{\text{no. of total (positive+negative cells)}}$$

The alpha-SMA index ranged from 0-100%, with a mean of 18%. The mean alpha-SMA index was evaluated in three ranges: low alpha-SMA index (under 18%), grade I; moderate alpha-SMA index (from18 to 50%), grade II; and high alpha-SMA index (from 51 to 100%), grade III.

Results

The sections were examined independently by two observers, and positive cellular staining for anti-asm-1 antibody was manifested as fine red cytoplasmic granularity and/or surface membrane expression (Figure 1).

The results of the immunostaining are summarized in Table 1. Alpha-SMA was expressed in 16 of 20 IDCs with diffuse fibrosis (80%), in seven of 40 IDCs without fibrosis (18%) and in all ten (100%) metaplastic carcinomas. Of 16 positive IDCs with diffuse fibrosis six were scored as alpha-SMA grade II and ten as alpha-SMA grade III. Of seven positive IDCs without fibrosis two were scored as alpha-SMA grade I, three as alpha-SMA grade II and two as alpha-SMA grade III. Of ten positive metaplastic carcinomas one was scored as alpha-SMA grade I, four as alpha-SMA grade II, and five as alpha-SMA grade III.

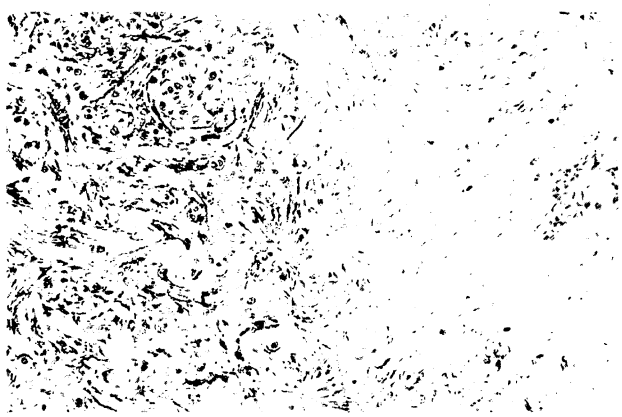

Figure 1. — Immunohistochemical staining for alpha-SMA in infiltrating ductal carcinoma of the breast with diffuse fibrosis. anti-asm-1 x100.

Table 1. — *Results of alpha-SMA Immunostaining.*

	Positive cases	Reactivity		
		grade I	grade II	grade III
IDCs with fibrosis	16/20 (80%)	-	6	10
IDCs without fibrosis	7/40 (18%)	2	3	2
Metaplastic	10/10 (100%)	1	4	5

Discussion

There is a great diversity of opinion in regard to the cell of origin of mammary carcinomas. It should be pointed out here that the evidence for the cells of origin is scanty. As Ozzello [2] emphasized, epithelial and myoepithelial cells probably share the same ancestry and hence they may represent different forms of differentiation of the same parent cell. Likewise, the same cell type, or 'stem cell', may give rise to most or almost all mammary carcinomas, the different tumour varieties representing different lines of differentiation. In other words it is the direction of differentiation which can best be assessed and analysed; the actual cell of origin is frequently speculative and may be very similar, if not identical, in different tumour types. Some authors have seen a link between the cytoplasmic filaments of tumour cells and those of myoepithelial cells and have suggested that ductal carcinomas may arise from myoepithelium [3, 4, 5]. In contrast, other workers have concluded that there is no evidence that the cytoplasmic filaments, present in breast cancer cells, are an indication of myoepithelial derivation. According to these other workers, cytoplasmic filaments with similar features can be found in numerous neoplastic and non-neoplastic cells unrelated to myoepithelium [1, 6]. Goldenberg *et al.* [6], in one of the best early ultrastructural studies, emphasized that the presence in breast carcinomas of cells rich in cytoplasmic filaments does not necessarily guarantee their myoepithelial origin. However, they did feel that the more loosely arranged filaments they had observed were at least suggestive of myoepithelial differentiation. In view of what is now known about myofibroblasts in the stroma of certain breast carcinomas [7], one may have to reappraise whether this cell type could not have been stromal rather than neoplastic. Goldenberg, and co-workers [6] concluded that "the coexistence in the same tumour of cells with abundant filaments and cells with secretory granules, and such surface membrane specializations as microvilli and canaliculi, would indicate that both cell types participate", i.e. both epithelial and myoepithelial

cells. Like Ozzello [2], Goldenberg *et al.* [6] found evidence to suggest that these two cell types have a common histogenetic lineage. The concept of a myoepithelial cancer has, however, maintained its advocates. On the basis of enzymatic patterns, Murad [8] postulated that most infiltrating ductal (scirrhous) cancers are of a myoepithelial nature. This conclusion was largely based on the remarkable finding of abundant ATPase activity on the surface of the cancer cells, a finding which boosted this hypothesis. In 1971 Murad [9] put forward a tentative new classification of breast carcinoma based on enzymatic reactions. He recognized three groups: "ductal epithelial" (medullary) cancer, "ductular carcinoma" (LCIS or CLIS) and "myoepithelial cell carcinoma". Murad proposed that "scirrhous" (infiltrating duct) carcinoma be renamed a myoepithelial carcinoma on the grounds of the presence of ATPase on the surface of the carcinoma cells. His findings were of great interest but to identify infiltrating ductal carcinoma as a myoepithelial cancer constituted a big leap which did not take the whole range of evidence into account. Ahmed [10] was the first worker to demonstrate convincingly that ATPase is localized to the plasma membrane of breast cancer cells which showed unequivocal evidence of epithelial differentiation on other well-established grounds. Murad [11] identified both intraductal and papillary carcinoma as myoepithelial, again based mainly on surface localization of ATPase activity. The ultrastructural evidence he put forward at this time was, however, inconclusive and unacceptable. Buell, Tremblay and Rowden [12] investigated the distribution of adenosine triphosphatase in infiltrating ductal carcinoma as well as in the non-neoplastic breast. Discussing discrepancies in the literature they stressed that, for technical and other reasons, the histochemical identification of ATPase is not an entirely reliable method for differentiating myoepithelial from myoepithelial cells in the breast. They found that the majority of infiltrating ductal carcinomas do not possess uniform ATPase activity and they therefore doubted the validity of the proposed new classification of Murad [9]. In a subsequent communication, Murad *et al.* [13] again identified papillary carcinoma with myoepithelial cells, a view which is a priori so unlikely that it is surprising that it should be reiterated so forcefully without stronger evidence to support it, and without any reference to prior work which puts the opposite viewpoint so well. They appear to have based their conclusion solely on the ultrastructural findings in the 1975 work but these are, as already indicated, quite inconclusive.

The breast myoepithelial cell is the 'Cinderella' of mammary biology. Although its contribution to benign and some malignant pathologies is recognised, it has been largely neglected in molecular and biological studies. The reason for this has been the perception that its role in normal physiology is confined to lactation and the belief that most breast cancers arise from luminal epithelial cells. Its broader biological significance and its potential use as a model system for understanding breast carcinogenesis has drawn the attention of many investigators [14-21].

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