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Detection of surface antigens defined by monoclonal antibodies in primary mucinous breast carcinomas

Relation to prognostic factors and recurrence-free survival

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Summary. Three monoclonal antibodies, 67D11, 115H10 and 115C2, raised against human milk fat globule membranes, have been applied to 207 primary mucinous breast carcinomas. The tumours reacted positively in 18% (67D11), 54% (115H10), and 20% (115C2) of the cases. The detected epitopes (MAM-3a (67D11), MAM-3b (115H10), and MAM-3c (115C2)) have formerly been shown to be markers of differentiation in infiltrating ductal carcinomas. In the present group of mucinous breast carcinomas, statistically significant correlation to high risk factors, such as occurrence of primary lymph node metastases, large tumour size, and local invasion of the tumour into overlying skin or deep fascie, were found. Furthermore, the antigen expression was less marked in pure mucinous carcinomas as compared to carcinomas also presenting with non-mucinous tumour areas. Thus, especially the antigen MAM-3b, is more frequently present in mixed tumours, in large tumours, in tumours with local invasion, and in tumours with primary lymph node metastases. However, no association could be demonstrated between expression of MAM-3b and recurrence-free survival.

Mucinous carcinomas of the breast apparently differ from other carcinomas not only with respect to morphology, but also in their pattern of antigenic expression in relation to other prognostic factors.

Key words: Mucinous breast carcinomas – Monoclonal antibodies – Prognostic factors

Introduction

Monoclonal antibodies that are raised against human milk fat globule membranes detect surface antigens in human breast tissue (Arklie et al. 1981;

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Foster et al. 1982; Hilkens et al. 1984). We have previously found (Rasmussen et al. 1985) that the presence of such antigens is associated with a high degree of differentiation in infiltrating ductal carcinomas Not Otherwise Specified (NOS), and that patients with breast tumours that express these antigens seem to have a slightly better prognosis.

Mucinous breast carcinomas are defined as tumours with excessive extracellular mucin production. This type is rare, and comprises approximately 2% of all breast carcinomas (Azzopardi 1979). Microscopically, islands of tumour cells are surrounded by large pools of mucin. If the whole tumour proces presents with this appearance it is regarded as a pure mucinous carcinoma, whereas a mixed mucinous carcinoma also consists of areas of tumour devoid of extracellular mucin (Norris and Taylor 1965; Rasmussen 1985).

Patients with this type of carcinoma have a better prognosis than patients with other histological diagnoses, (Lee 1984; McDivitt et al. 1968; Melamed et al. 1961; Silverberg et al. 1971; Wulsin and Schreiber 1962), and survival seems to improve with increasing amounts of extracellular mucin (Melamed et al. 1961; Norris and Taylor 1965; Rosen and Wang 1980; Silverberg et al. 1971). Since tumours with abundant extracellular mucin differ morphologically and prognostically from other breast carcinomas, we have investigated whether the pattern of antigen expression also differs in mucinous breast carcinomas compared to that found in infiltrating ductal carcinomas NOS.

Materials and methods

The criteria for inclusion in this investigation and the characteristics of the material are described in detail elsewhere (Rasmussen 1985). In summary, 207 primary mucinous breast carcinomas have been investigated. To enter the investigation, the following criteria had to be fullfilled:

1. At least 25% of the tumour should consist of areas with abundant extracellular mucin, and
2. In these areas, the mucin should comprise at least 33% of the total area (Rasmussen 1985).

The 207 patients with primary mucinous breast carcinomas fullfilling these criteria constitute a subgroup of patients registered in the Danish Breast Cancer Cooperative Group (DBCG). The DBCG organization conducts a nationwide program for treatment of primary breast cancer in women. The design and follow-up of the program has been described in detail by Andersen et al. (1981).

Clinical data. For all 207 patients, knowledge concerning age, menopausal status, tumour size, and the number of axillary lymph nodes with metastases at the time of primary operation are registered. Information concerning recurrence-free survival (RFS) is know for the 150 of the 207 patients that have entered the protocol for treatment regimens. The 207 patients in the present study have been operated between 01.01.77–31.10.82, and the median time of observation as of February 1, 1985 was 45 months.

Histological evaluation. The antigenic determinants under investigation are preserved in formalin-fixed, paraffin embedded material. (Rasmussen et al. 1984.) Four sections from each paraffin block containing malignant tumour tissue have been cut from all tumours. One section is stained with haemotoxylin-eosine for histological evaluation. The tumours have been divided into two groups:

1. Pure carcinomas where the whole mass consists of areas with tumour islands floating in abundant extracellular mucin, and

2. Mixed carcinomas where, in addition, areas with infiltrating tumour devoid of extracellular mucin are also seen. A tumour is regarded as mixed if only a few clusters of cells without extracellular mucin are present (Rasmussen 1985).

The three monoclonal antibodies, 67D11, 115H10 and 115C2 (Hilkens et al. 1984), that detect the corresponding antigens MAM-3a, MAM-3b and MAM-3c, are applied to the other three sections. For the detection of a positive antigen-antibody reaction, a one-layer immunoperoxidase method has been utilized. The sections were used as their own positive controls. This could be done since the antigen-antibody reaction proved always to be heterogenous, both intra- and intertumourally (Rasmussen et al. 1984; Rasmussen et al. 1985). The carcinomas are classified as positive if 10% of the total number of tumour cells present exhibit a positive peroxidase reaction (Rasmussen et al. 1984). This cut-off level was chosen arbitrarily. If the presence of antigen represents a marker of differentiation, it seems relevant to demand that a resonable number of tumour cells express the antigens. Higher cut-off levels have, therefore, been applied and together with the 10% level semiquantitatively evaluated. No difference in results were obtained (Rasmussen et al. 1985).

Statistical methods. Clinical data and macroscopic tumour characteristics have been obtained from the DBCG-registrar. The comparison of characteristics between groups of patients were performed by the Chi-square tests with Yates correction in the relevant contigency tables. The recurrence-free survival (RFS) was analyzed by use of the life-table method, and compared by the log-rank test. A two tailed p-value of less than 5% is considered significant.

Results

Of the 207 mucinous carcinomas, 95 were of the pure type and 112 of the mixed type. The antigen-antibody reaction was technically evaluable with

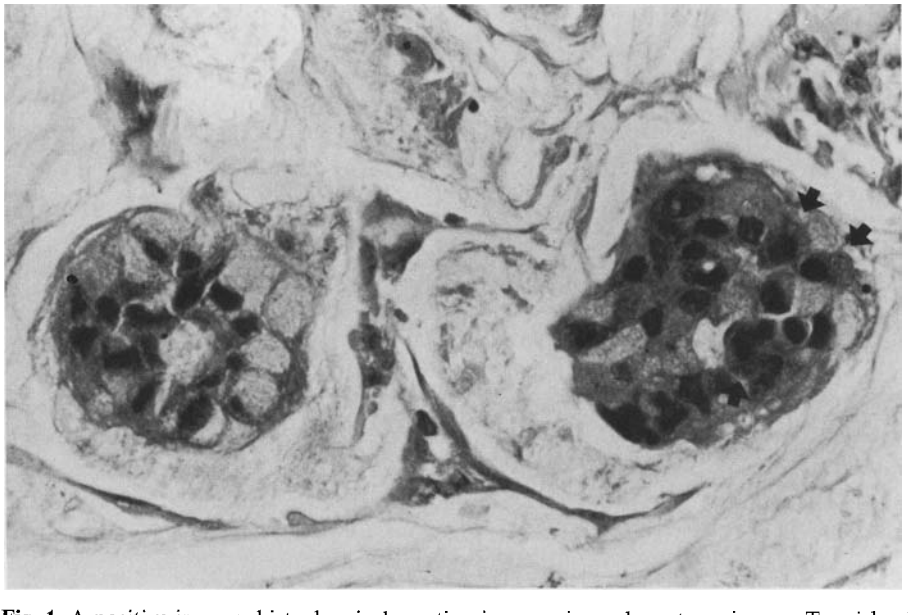

Fig. 1. A positive immunohistochemical reaction in a mucinous breast carcinoma. Two islands of tumour cells are seen lying in abundant extracellular mucin. The right hand group of cells are predominantly positive (*arrows*), whereas the tumour cells to the left are mainly negative. Staining with antibody 115H10, × 400

Table 1. Clinical and histopathological characteristics of 207 mucinous breast carcinomas (N = number of patients)

Characteristics	N	%
Menopausal status		
Pre	27	13
Post	180	87
Lymph node metastases		
−	160	78
+	46	22
Unknown	1	
Tumour size		
$\leqslant 5$ cm	175	87
>5 cm	26	13
Unknown	6	
Local invasion		
−	177	87
+	27	13
Unknown	3	
Morphology		
Pure	95	46
Mixed	112	54
Antigenic expression		
MAM-3a		
−	164	82
+	36	18
Unknown	7	
MAM-3b		
−	98	46
+	107	54
Unknown	2	
MAM-3c		
−	158	80
+	41	20
Unknown	8	

the three antibodies in 200 (67D11), 205 (115H10), and 199 (115C2) cases respectively.

The antigens are heterogeneously distributed throughout the tumour, and positive staining is located either in the cytoplasm or on the surface membrane of the cells (Fig. 1). In six cases, an additional positive reaction was seen in the extra-cellular mucin. However, extra-cellular mucin staining was never found without the presence of positive tumour cells.

The mean age of the patients are 68 years, range 39–89 years. Table 1 shows the menopausal status of the patients, and the histopathological and antigenic characteristics of their tumours. The three antigens are present in 18% (MAM-3a), 54% (MAM-3b) and 20% (MAM-3c) of the cases respectively. Approximately one fourth of the patients have primary lymph

Table 2. Relationships between the presence of the three antigens and clinical and histopathological characteristics

Characteristics	MAM-3a		MAM-3b		MAM-3c	
	pos./total	p	pos./total	p	pos./total	p
Menopausal status						
Pre	5/24	0.92	11/25	0.51	5/24	0.81
Post	31/176		96/180		36/175	
Lymph node metastases						
−	21/153	0.007	72/158	0.0005	26/154	0.023
+	15/46		35/46		15/44	
Tumor size						
$\leqslant 5$ cm	31/168	0.86	86/173	0.044	35/169	0.83
>5 cm	5/26		19/26		6/24	
Local invasion						
−	30/170	0.86	85/175	0.009	34/170	0.58
+	6/27		21/27		7/26	
Pure	13/90	0.25	34/93	0.0005	9/90	0.0008
Mixed	23/110		73/112		32/109	

node metastases, tumours larger than 5 cm, or local invasion into overlying skin or deep fascia.

The relationship between the presence of antigens and clinical and histopathological variables known to be associated with prognosis are shown in Table 2. The antigens are equally expressed in tumours from patients of different menopausal status.

Tumours that have spread to the axillary nodes at the time of the primary operation have a significantly higher proportion of antigenic expression. All three antigens are detected twice as frequently in tumours from node-positive patients.

The presence of MAM-3b is also associated with large tumours ($p=0.04$) and with local invasion ($p=0.009$). The antigen pattern is also related to whether the tumours are of the mixed or the pure type. Significantly fewer pure carcinomas than mixed carcinomas express the antigens MAM-3b and 3c. The antigen MAM-3a is, however, equally often expressed in both types of mucinous carcinomas. Similarly, no significant relationship is found between the expression of MAM-3a and 3c, and tumour size and local invasion, although these antigens are found more frequently among large tumours and tumours with local invasion.

The impact of expression of antigen on RFS is analyzed in Fig. 2. In the 150 protocol entered patients, the presence of the three antigens is evaluable in 144 (MAM-3a), 148 (MAM-3b), and 144 (MAM-3c) cases. Approximately 75% of the patients are alive and disease-free, and, as can be seen, the RFS is in the same order in antigen-positive and -negative patients. This is also the case if the RFS for patients positive for all three antigens

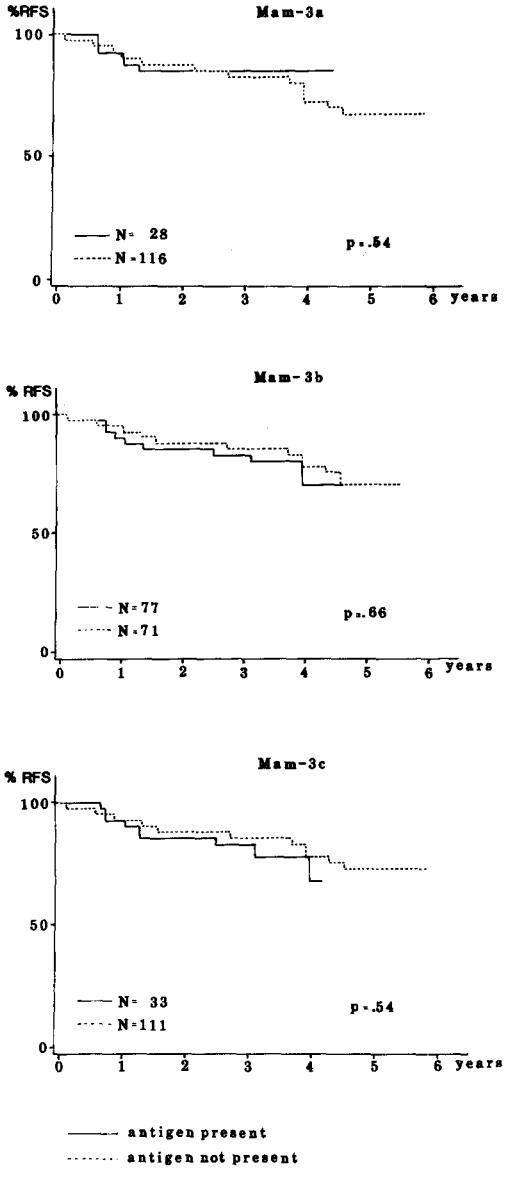

Fig. 2. The relation between recurrence-free survival (RFS) and the presence of surface antigens in 150 primary mucinous breast carcinomas. (N = number of patients in the different groups)

is compared with that of patients negative for all three antigens (data not shown).

The antigen MAM-3b has formerly been shown to have an impact on prognosis (Rasmussen et al. 1985). In Table 3 the relation between the presence of MAM-3b and RFS is shown for node-negative and node-positive patients. The number of patients in the different groups are too small for any valid statistical analysis, but it is notable that the percentage of recurrences or deaths is higher among patients with antigen-negative tumours

Table 3. Relation between number of recurrences or deaths and the presence of MAM-3b in node-positive and node-negative patients

Node-negative				Node-positive			
MAM-3b	No. pts.[a]	recurrence/ death	%	MAM-3b	No. pts.[a]	recurrence/ death	%
+	51	8	16	+	26	10	38
–	65	14	22	–	6	4	75

[a] Abbrivation for number of patients

than patients with antigen-positive tumours, irrespective of lymph node status.

Discussion

The antigens in question are surface membrane antigens, that originate from the apical epithelial cell membrane (Ceriani et al. 1977). The MAM-3c antigen has recently been identified, and is a novel carbohydrate moiety related to the Lewis blood group and the SSEA-1 oncodevelopmental antigen (Gooi et al., in press). The extra-cellular mucin in mucinous breast carcinomas is known to consist of acid or neutral mucopolysaccarides (Walker 1982). The observation that the mucin exhibits a positive peroxidase reaction in a few cases, may be due to diffusion of antigens from cell membranes into the surroundings.

Several investigators have examined the relation between the presence of surface antigens in breast cancer and other tumour and patients characteristics, (Arklie et al. 1981; Ellis et al. 1985; Mariani-Costantini et al. 1984; Sawtell et al. 1984; Wilkinson et al. 1984). Recently, we analyzed 200 primary tumours (Rasmussen et al. 1984), mainly representing infiltrating ductal carcinomas NOS for the presence of the three antigens, and found that the presence of two of them, MAM-3a and MAM-3b, was correlated to the degree of differentiation, oestrogen receptor content, and tumour size. Moreover, there was a trend toward a better prognosis in patients with a positive reaction for MAM-3b, but this association disappeared in a multivariate analysis, that also considered lymph node status and oestrogen receptor content.

In the present investigation the monoclonal antibodies have been applied to mucinous breast carcinomas, which have a better prognosis than other carcinomas (Lee 1984; McDivitt et al. 1968; Melamed et al. 1961; Norris and Taylor 1965; Silverberg 1971; Wulsin and Schreiber 1962). In view of the recent associations found for ductal carcinomas between presence of the antigens and known prognostic factors, we expected to find a positive correlation not only between the antigen expression and prognostic factors indicative of a good prognosis, but also between the presence of antigen and RFS. In contrast, however, we found that the antigenic expression

was far less marked in the pure mucinous carcinomas than in the mixed carcinomas. The presence of antigen was also correlated to large tumour size and occurrence of lymph node metastases. Nevertheless, with a median time of follow-up of approximately 4 years this close relation between poor prognostic factors and antigen expression was not reflected in the RFS of the mucinous breast tumour patients. On the contrary, a higher frequency of recurrences was found among antigen negative patients than among antigen positive patients – regardless of lymph node status –, when the RFS data was considered in relation to the presence of the antigen MAM-3b. The difference is marked in the small group of node-positive patients; 62% of the antigen-positive patients were alive and disease-free versus only 25% of the antigen-negative patients. In the node-negative patients, only a slight difference is found; there was 84% RFS in the antigen-positive patients versus 78% in the antigen-negative patients. The lack of correlation between antigen expression and RFS that is illustrated in Fig. 2 may, therefore, be attributed either to the fact that the majority of patients, namely 116, are node-negative, or to the close relation between antigen expression and the presence of positive lymph nodes, neutralizing the prognostic effect of antigen expression.

The main characteristic of the pure and mixed mucinous breast carcinomas is the presence of areas with large amounts of extracellular mucin (Azzopardi 1979). Since this characteristic morphological appearance seems to be associated with the antigen expression as well as with prognostic factors such as lymph node status (Silverberg et al. 1971) and nuclear grade (Fisher et al. 1975), in a manner that is different from that found for other types of breast carcinomas, it is worthwhile to analyse the relationship between these factors in an multivariate analysis. Such a study is, therefore, currently in progress.

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