

Prognostically Significant Protein Components of Human Breast Cancer Tissues¹

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SUMMARY

Cryostat sections of clinicopathologically characterized breast cancer tissues were eluted with phosphate-buffered 0.9% sodium chloride solution, pH 7.2. The proteins were then characterized by polyacrylamide gel electrophoresis with and without prior treatment with sodium dodecyl sulfate. Approximately 65% of the breast cancer tissue eluates contained a prominent protein fraction with a molecular weight of 47,000 to 55,000 (p50). No such component was found in 15 of 17 eluates of benign breast tissue. Charge density studies disclosed that the p50 component included three populations of proteins that could be characterized according to the migration relative to gp55 derived from RIII murine mammary tumor virus, namely, fast (F-p50), intermediate (I-p50), and slow (S-p50).

Prognostically favorable pathological characteristics, *i.e.*, stage, nuclear grade, and lymphoreticuloendothelial responses, were proportionately most frequently found among S-p50 breast cancers and were least frequently found among F-p50 breast cancers. It appears that the S-p50 component acts *in vivo* as a prognostically significant immunogen. Further knowledge of the relationship between protein characteristics and clinicopathological features of human breast cancers would contribute to our understanding of mammary carcinogenesis and biological behavior.

INTRODUCTION

It has been shown that breast cancer patients' leukocytes, which are responsive, *in vitro*, to autologous and homologous immunogenic breast cancer tissues, commonly cross-react with MuMTV,³ but not with normotypic breast tissues from control or cancerous breasts (2, 4, 5). It thus appears that some breast cancer tissues contain MuMTV-like anti-

genic components which are lacking in benign breast tissues. The presence of distinctive components in breast cancer tissues was also suggested by studies of breast tissue proteins characterized by PGE (3, 4, 8).

We now report that most breast cancer tissues contain protein components that are uncommon in normotypic breast tissues derived from control and cancerous breast specimens. More particularly, the electrophoretic protein patterns associated with individual breast cancer tissues are correlated with prognostically significant pathological characteristics. It further appears that a prognostically significant cancer-associated protein component appears to be similar in molecular weight and charge to gp55 of RIII-MuMTV.

MATERIALS AND METHODS

Tissue Eluates. Cryostat sections were prepared from microscopically characterized benign and malignant breast tissues. In all instances, information was available regarding the stage of the disease and prognostically significant histopathological characteristics, *i.e.*, NG, PVI, and SH. Twelve to 15 cryostat sections, 100 μm thick and approximately 1 sq cm in face area, were incubated in 1.0 ml of 0.157 M NaCl, pH 7.2, for 16 hr at 4°. A proportionally smaller volume of 0.157 M NaCl, pH 7.2, or, when available, a larger number of sections was used whenever the face area of the tissue block was significantly smaller than 1 sq cm. Following incubation, the eluates were cleared by centrifugation ($32,000 \times g$ for 60 min at 4°) and subsequent Millipore filtration (0.45 μm pore size). The protein concentrations in such preparations, as determined by the method of Lowry *et al.* (10), ranged between 8 and 12 mg/ml. The eluates were stored at -70°.

MuMTV Proteins. The isolation of virus from RIII milk and the techniques for virus disruption have been described elsewhere (6, 9, 11).

PGE. Aliquots of tissue eluates containing 50 to 60 μg protein and of an MuMTV protein preparation containing 30 to 50 μg protein were studied by SDS-PGE and by electrophoresis in 8.5% polyacrylamide gels without prior SDS denaturation of the proteins. The SDS-PGE procedure has already been described (9, 12). The SDS gels were stained with Coomassie brilliant blue. The 8.5% PGE studies were performed in tube gels using a continuous pH 8.92 Tris-glycine buffer system (0.043 M in regard to Tris with suffi-

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³ The abbreviations used are: MuMTV, murine mammary tumor virus; PGE, polyacrylamide gel electrophoresis; gp55, glycoprotein, 55,000 MW (also designated as gp52 by some authors); NG, nuclear grade (primary tumor); PVI, perivascular lymphoid cellular infiltration (primary tumor); SH, sinus histiocytosis (lymph nodes); SDS, sodium dodecyl sulfate; pps, pulses/sec; L-RE, lymphoreticuloendothelial.

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cient anion added to achieve pH 8.92 at 25°). The protein aliquots were diluted 1:3 with Tris-glycine buffer of one-half the ionic strength of the tank buffer to improve zone sharpening as suggested by Hjerten *et al.* (7). Prior to application to the gels, aqueous bromophenol blue (0.1%) and sucrose were added to the samples. After preelectrophoresis for 1 hr, sample electrophoresis with 16 gels/experiment was performed at room temperature with precooled (4°) buffer (tank Model 4200; Ortec, Inc., Oak Ridge, Tenn.). A pulsed constant power supply, Ortec, Inc., Model 4100, was used. For the 1st 30 min, we used 90 V and 75 pps, resulting in a current reading of 10 ma, and subsequently, until completion of the run, 250 V and 75 pps with a 2-step increase of the pulse frequency to 225 pps, resulting in a final current reading of 42 ma. The discharge capacitance was set at 1.0 μ F throughout the run. The gels were stained in 0.5% naphthol blue black (C.I. No. 20470, Allied Chemicals) in 3.5% acetic acid for 30 min and destained either by diffusion or electrophoretically (destainer Model 4216, Ortec, Inc.) in 7.5% acetic acid. Both the Coomassie brilliant blue-stained SDS-gels and the naphthol blue-black-stained gels were scanned on a Densicord 5099, Photovolt recording densitometer with a 610-nm filter.

PGE of the tissue eluates and of the MuMTV proteins was performed simultaneously in the same system.

RESULTS

SDS-PGE (Molecular-Weight Determinations). Chart 1 depicts densitometer tracings of SDS-PGE preparations of MuMTV proteins and of representative eluates from malignant and benign breast tissues. It is evident that the breast cancer tissue eluates contain a distinct protein fraction in the 47,000 to 55,000-molecular weight region which is not demonstrable in the benign breast tissue sample. Of 64 breast cancer tissue eluates, 41 (64%) showed a sharply defined protein fraction with a molecular weight of 47,000 to 55,000. We have designated this fraction as p50. In approximately one-half of the breast cancer tissue eluates, protein fractions were demonstrable in the region of 40,000 and 34,000 while most of the eluates had a protein band in the 28,000-molecular weight region. The pattern shown for the benign breast tissue (Chart 1) is representative of the findings in the normotypic areas of 9 of 9 cancerous and 6 of 8 noncancerous breast specimens. Two samples of benign breast tissue had a protein band in the 50,000-molecular weight region. None of the samples had protein bands in the 40,000-molecular weight region. It is emphasized that the benign breast tissues chosen for study were characterized by abundant parenchymal structures. Their epithelial cellularity was equal to or greater than that of most of the cancer tissues.

Since a p50 band was preferentially found in the eluates of cancerous as compared with benign breast tissues, it was of interest to determine whether the prominence of this protein was correlated with clinicopathological characteristics of individual breast cancer tissues. As seen in Table 1, the pathological characteristics of those breast cancers whose eluates showed a p50 band in SDS-PGE were not significantly different from breast cancers lacking this band

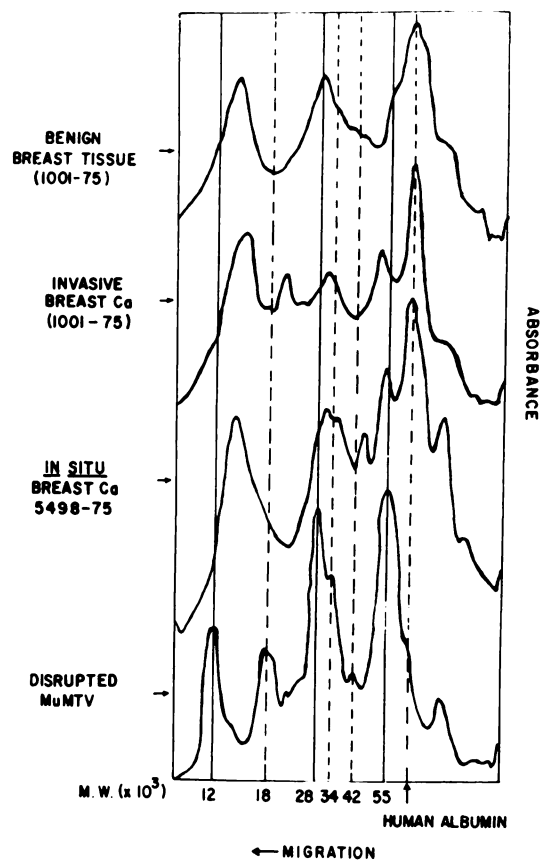


Chart 1. Densitometric tracings of protein components of MuMTV and eluates of human breast tissues separated simultaneously by SDS-PGE. The breast cancer proteins include a component which migrates similarly (5498-75) or somewhat faster (1001-75) than gp55 of MuMTV. Note that the proteins of normotypic breast tissue from a cancerous breast (1001-75) lack a spike in this region.

Table 1

Pathological characteristics of breast cancers in relation to p50 protein (SDS-PGE)

Prognostically favorable characteristics are only minimally more frequent among p50+ breast cancers. Breast cancer tissues with and without a p50 protein spike are similar in regard to the relative frequency of prognostically favorable NG III, perivenous lymphocytic infiltration, sinus histiocytosis and stage distribution.

p50	Total	Stage I	Stage II4+	NG III	PVI + SH
+	41	17 (42) ^a	17 (42)	9 (22)	9 (22)
-	23	8 (35)	12 (52)	3 (13)	3 (13)

^a Numbers in parentheses, percentages.

in regard to the relative frequency of prognostically favorable characteristics, namely, stage, nuclear grade, and the presence of PVI in the primary tumor and SH in the axillary nodes.

8.5% PGE (Charge Density Determinations). Densitometric tracings of protein fractions of acetone-disrupted MuMTV and representative eluates of control and cancerous breast tissues are shown in Chart 2. The slow-moving prominent double band seen in the MuMTV preparation represents gp55 as proven by parallel studies using isolated gp55. Only 1 of 25 benign breast tissue eluates demonstrated protein bands in this region. In contrast, most of the breast cancer tissue eluates contained protein components

that migrated similarly to MuMTV gp55. The latter finding is particularly representative of those breast cancer tissue eluates in which a p50 band is demonstrable by SDS-PGE, namely, 35 out of 42 (85%). Of 23 breast tissue eluates that did not show a prominent p50 band in SDS preparations, only 4 demonstrated protein bands in the gp55 region in 8.5% PGE preparations. It appears that breast cancer protein(s) that comprise the p50 SDS-PGE band migrate in 8.5% PGE to the region of MuMTV gp55, *i.e.*, a range of 0.45 to 0.37 relative to human serum albumin.

Chart 2 demonstrates that some eluates (*e.g.*, 2843-75) are characterized by a prominent protein fraction which migrates slightly faster than the forward spike of MuMTV gp55 (0.45 relative to albumin, designated as F-p50). In other instances, a prominent band is seen between the gp55 peaks (0.41 relative to albumin, designated as I-p50), while

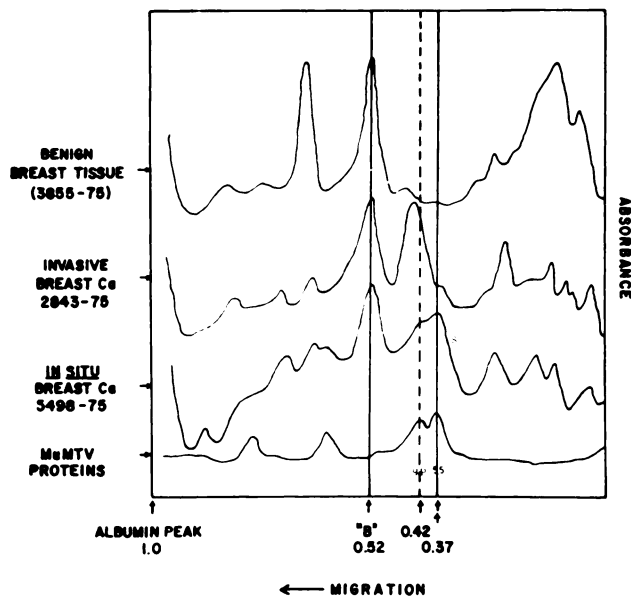


Chart 2. Densitometric tracings of protein components of MuMTV and eluates of human breast tissues separated simultaneously by 8.5% PGE. The migration of various components may be characterized in relation to serum albumin. Benign breast tissue eluates typically lack protein components in the region of gp55 of MuMTV. Breast cancer tissue eluates commonly contain prominent protein components with migrations similar (5498-75) or faster (2843-75) than gp55 of MuMTV. The "B" band is a serum component found regularly in eluates of benign and malignant breast tissues.

some eluates contain a prominent component the migration of which corresponds to the slower-moving gp55 peak (0.37 relative to albumin, designated as S-p50). Approximately 40% of the breast cancer tissue eluates lacked a single or dominant band in the above regions. Instead, they contained a mixture of the described protein components. Such eluates were designated as "others-p50." In such mixtures, the fast-moving component (F) tended to predominate, while the intermediate (I) and, particularly, the slow (S) components were minimally represented. Another group of breast cancer tissues resembles the electrophoretic pattern of benign breast tissues in that there are no demonstrable bands in the region between 0.45 and 0.37 relative to the albumin. The point to be emphasized is that breast cancer tissues differ from one another in regard to p50 protein content. The particular protein patterns associated with individual breast cancers were reproducibly demonstrable in repeated tests.

Table 2 presents the pathological characteristics of breast cancers in relation to the p50 protein patterns found in 8.5% PGE. The prognostically favorable characteristics (Stage I, NG III, PVI, and SH reactivity) are proportionately most frequent in the S-p50 group of breast cancers and proportionately least frequent in the F-p50 group. A reverse relationship is found in regard to prognostically unfavorable Stage II4+ breast cancers.

Previous studies had shown that L-RE responses to foci of *in situ* carcinoma were prognostically significant (1). It appeared that prognostically significant immunogenicity was present during the *in situ* phase of the disease. The biological behavior of breast cancer seemed to reflect the retention of such *in situ*-associated immunogens by the invasive cancer tissue and specific hypersensitivity against such immunogens. It was therefore of interest to examine the relationship between stage and p50 proteins in those breast cancers showing L-RE responses to foci of *in situ* cancer. These data are shown in Table 3. It is evident that, among breast cancer patients having L-RE responsiveness to *in situ* breast cancers, the stage distribution is prognostically most favorable among the S-p50 cases. These data provide further evidence of biologically significant variability among breast cancer tissues, independent of the immunological competence of the host, and suggest that S-p50 is a correlate of prognostically favorable behavior.

Table 2
Heterogeneity of human breast cancer tissues in regard to p50 protein characteristics (8.5% PGE)

S-p50 breast cancers have the lowest frequency of prognostically unfavorable Stage II4+ cases and the highest frequency of prognostically favorable NG, perivascular lymphoid cell infiltrations, sinus histiocytosis, and Stage I cases. A reverse distribution is seen in F-p50 breast cancers.

Series	Total	Stage I	Stage II4+	NG III	PVI + SH
S-p50	24 (100) ^a	14 (58)	4 ^b (17)	8 (33)	7 (29)
I-p50	10 (100)	4 (40)	3 (30)	3 (30)	1 (10)
F-p50	9 (100)	1 (11)	8 (89)	1 (11)	1 (11)
Others-p50	30 (100)	7 (23)	18 (60)	2 (7)	5 (17)
Total	73 (100)	26 (36)	33 (45)	14 (19)	14 (19)

^a Numbers in parentheses, percentages.

^b The relative proportion of Stage II4+ cases is significantly less in the S-p50 series, compared with the F-p50 series, χ^2 Yates, 11.88, $p < 0.001$; and the others-p50 series, χ^2 Yates, 8.53, $p < 0.005$.

Table 3

Stage distribution of breast cancer cases having L-RE responses to areas of *in situ* carcinoma; relation to p50 proteins

The stage distribution is prognostically most favorable among S-p50 breast cancers. The relative frequency of Stage II4+ cases is significantly lower among the latter cases than among the combined data of the F-p50 and others-p50 cases.

Stage	S-p50	I-p50	F-p50	Others-p50
I	8 (62) ^a	2 (33)		3 (25)
II < 4	3 (23)	2 (33)		1 (8)
II4+	2 ^b (15)	2 (33)	4 (100)	8 (67)
Total	13 (100)	6 (100)	4 (100)	12 (100)

^a Numbers in parentheses, percentages.

^b The relative proportion of Stage II4+ cases is significantly less in the S-p50 series, compared with the combined F-p50 and others-p50 series; χ^2 Yates, 7.98, $p < 0.005$.

DISCUSSION

This study has demonstrated that most breast cancer tissues contain protein components that are uncommon in normotypic tissues from cancerous and noncancerous breast specimens. Such differences are demonstrable in both SDS- and non-SDS-PGE preparations. These observations confirm earlier reports from this laboratory (3, 4). Equally interesting is the finding that breast cancer tissues are heterogeneous in regard to quantitative and/or qualitative variations in protein patterns as seen in 8.5% PGE preparations. Such categorization appears to be biologically significant, as judged by prognostically significant correlations with the stage of the disease. In short, it appears that the protein characteristics of breast cancer tissues provide an additional indicator of biologically significant variability of breast cancers.

The biological behavior of individual breast cancers reflects tumor-host interactions wherein the intrinsic aggressive potential of the cancer cell is opposed by immunologically mediated responses of the host (1). The former is correlated with the degree of tumor differentiation (NG), while the latter is a function of the immunogenicity of the cancer tissues and the specific hypersensitivity responsiveness of the host. The finding that PVI and SH responses are preferentially associated with breast cancers having S-p50 protein suggests that this component is immunogenic. This possibility is further supported by the finding that breast cancer patients' leukocytes are preferentially responsive to breast cancer tissues containing S-p50 protein (3). Such reactivity to S-p50-containing breast cancer tissues is par-

ticularly manifest by leukocytes that are simultaneously responsive to MuMTV.

Whether the physicochemical and antigenic similarities between protein components of MuMTV and breast cancer tissues will ultimately prove to reflect identity or merely coincidence does not detract from the biologically significant correlates of the various protein patterns of breast cancer tissues. The ability to recognize prognostically significant variability among breast cancers is in itself pertinent to a better understanding of mammary carcinogenesis and behavior. It would seem to be of clinical and conceptual importance to extend our knowledge of the relationship between protein characteristics and clinicopathological features of human breast cancers.

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