Prognostically Significant Protein Components of Human Breast Cancer Tissues

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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 tissues. 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 RII 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, 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 antigenic 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 RII-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°C. 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 x g for 60 min at 4°C) 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°C.

MuMTV Proteins. The isolation of virus from RII 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 sufficient...
Pathological characteristics of those breast cancers whose eluates showed a p50 band in SDS-PGE were not significant different from breast cancers lacking this band in regard to the relative frequency of pathologically 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 shown in Table 1.
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 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

<table>
<thead>
<tr>
<th>Series</th>
<th>Total</th>
<th>Stage I</th>
<th>Stage II4+</th>
<th>NG III</th>
<th>PVI + SH</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-p50</td>
<td>24 (100)</td>
<td>14 (58)</td>
<td>4* (17)</td>
<td>8 (33)</td>
<td>7 (29)</td>
</tr>
<tr>
<td>I-p50</td>
<td>10 (100)</td>
<td>4 (40)</td>
<td>3 (30)</td>
<td>3 (30)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>F-p50</td>
<td>9 (100)</td>
<td>1 (11)</td>
<td>8 (89)</td>
<td>1 (11)</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Others-p50</td>
<td>30 (100)</td>
<td>7 (23)</td>
<td>18 (60)</td>
<td>2 (7)</td>
<td>5 (17)</td>
</tr>
<tr>
<td>Total</td>
<td>73 (100)</td>
<td>26 (36)</td>
<td>33 (45)</td>
<td>14 (19)</td>
<td>14 (19)</td>
</tr>
</tbody>
</table>

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, χ² Yates, 11.88, p < 0.001; and the others-p50 series, χ² Yates, 8.53, p < 0.005.
cancer patients’ leukocytes are preferentially responsive 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.

<table>
<thead>
<tr>
<th>Stage</th>
<th>S-p50</th>
<th>I-p50</th>
<th>F-p50</th>
<th>Others-p50</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8 (62%)</td>
<td>2 (33%)</td>
<td>3 (25%)</td>
<td></td>
</tr>
<tr>
<td>II &lt; 4</td>
<td>3 (23%)</td>
<td>2 (33%)</td>
<td>1 (8%)</td>
<td></td>
</tr>
<tr>
<td>II4+</td>
<td>2a (15%)</td>
<td>2 (33%)</td>
<td>4 (100%)</td>
<td>8 (67%)</td>
</tr>
<tr>
<td>Total</td>
<td>13 (100%)</td>
<td>6 (100%)</td>
<td>4 (100%)</td>
<td>12 (100%)</td>
</tr>
</tbody>
</table>

a Numbers in parentheses, percentages.

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; χ² 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 particularly 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.

REFERENCES


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