Correlation between Estrogen Receptor Protein and Carcinoembryonic Antigen in Normal and Carcinomatous Human Breast Tissue

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We determined estrogen receptor protein and carcinoembryonic antigen in cytosols prepared from 189 human breast carcinoma tissues, 85 benign or normal breast biopsies, and 101 tissue specimens metastatic from breast carcinoma. Carcinoembryonic antigen was observed in 70% of the primary carcinomatous tissues, 15% of the benign or normal specimens, and 51% of the metastases. Ninety-six of the 189 primary carcinomatous specimens with increased concentrations of carcinoembryonic antigen were also positive for estrogen receptor protein, whereas 67 of the 72 benign or normal biopsies with low concentrations of carcinoembryonic antigen were also negative for estrogen receptor protein. All five benign specimens with positive estrogen receptor protein and normal carcinoembryonic antigen concentrations were from fibroadenomas. The concordance between estrogen receptor protein and carcinoembryonic antigen in the primary carcinomatous tissue was 66%, in metastatic carcinoma 51%, and in benign and normal tissue 85%.

Additional Keyphrases: cancer, diagnosis and monitoring of treatment • relative merits of the two determinations

There have been numerous attempts to devise tests to establish whether a patient with breast carcinoma will benefit from hormone therapy or ablation of the endocrine glands (1). In this regard the binding of estrogen to receptor protein has received wide attention (2–10). In target tissue the estrogen receptor protein (ERP) is located in the cytoplasm, and is involved in estrogen transport to the nucleus and thereby influences nucleic acid synthesis (11). Estrogen receptor protein has been primarily observed in sex-hormone target tissue, but it has also been reported to be present in human pancreatic carcinoma (12) and in rat kidney (13). A long-term study of estrogen receptor protein in tissue of patients with mammary carcinoma is underway in our laboratory. Results of plasma carcinoembryonic antigen (CEA)1 assays have been particularly studied in patients with cancer of the colon, but increases have been observed by us and others in patients with breast cancer. Steward et al. (14) observed increased plasma CEA concentrations (exceeding 2.5 μg/liter) in one of 17 patients with benign breast disease, two of 10 patients with primary breast carcinoma, four of 12 patients with carcinoma and local spread, and 37 of 47 patients with distant metastases. They found that CEA concentrations decreased in eight patients whose disease showed objective remission after treatment and concluded that serially determined CEA values correlated with response to treatment. Chu and Nemoto (15) observed similar elevations in single assays but concluded that CEA assays did not provide an adequate index to progression or regression of breast cancer.

The purpose of this report is to describe our experience in measuring ERP and CEA in breast tissues and to discuss the relationship of ERP and CEA.

Materials and Methods

Apparatus

Tissue was homogenized with a Polytron homogenizer (Brinkman Instruments, Inc., Westbury, N. Y. 11590) using a PT 10 ST generator. A Beckman-Spinco L2 Ultracentrifuge with a 50 Ti Rotor (Beckman Instruments, Inc., Mountainside, N. J. 07091) was used to prepare the cytosol, and protein was measured with a Gilford spectrophotometer (Gilford Instrument Laboratories Inc., Oberlin, Ohio 44074).

Radioactivity was measured in a Tri-Carb Liquid Scintillation Spectrometer 3380 (Packard Instrument Co., Downers Grove, Ill. 60515).

Reagents and Solutions

Homogenization buffer: The composition of this, per liter, was 100 mmol of N-tris(hydroxymethyl)methyl-2-aminoethan sulfonic acid (TES), 120 mmol of thio-

1 Nonstandard abbreviations used: ERP, estrogen receptor protein; CEA, carcinoembryonic antigen; TES, N-tris(hydroxymethyl)methyl-2-aminoethan sulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid.
N,N,N',N'-tetraacetic acid solution, 100 mmol/liter: Dissolve 3.8 g of EGTA (Sigma) in 50 ml of water, adjust to pH 8.0 with 0.1 mol/liter KOH and dilute with distilled water to 100 ml.

**Assay buffer:** The assay buffer is the same as the homogenization buffer except that it also contains 10 g of ovalbumin (Sigma) per liter of the buffer.

**Ethylene glycol bis(β-aminomethyl ether)-N,N,N',N'-tetraacetic acid solution, 100 mmol/liter:**

- Dissolve 3.8 g of EGTA (Sigma) in 50 ml of water, adjust to pH 8.0 with 0.1 mol/liter KOH, and dilute to 100 ml.

17β-[2,4,6,7-3H(N)] Estradiol (nominal label), 110 kCi/ml, 98% pure, was obtained from New England Nuclear, Boston, Mass. 02118, and 17β-estradiol was obtained from Sigma. The anti-uterotropic agent CI628 was a gift from Parke-Davis & Co., Detroit, Mich. 48232.

**Charcoal (acid washed) preparation:** Norit A (Fisher Scientific Co., Pittsburgh, Pa. 15219) was suspended for 2 h in 10 mmol/liter HCl (400 g/liter), washed repeatedly with large volumes of distilled water until the pH was neutral, and dried at 110 °C.

**Charcoal-dextran preparation:** 1.25 g of Norit A (acid-washed) and 12.5 mg of dextran ("Dextran T-40"; Pharmacia Inc., Piscataway, N. J. 08854) were suspended in 50 ml of the assay buffer and the pH was adjusted to pH 7.4.

**Procedure**

**Tissue procurement and storage.** Tissues for ERP and CEA analysis were portions of surgical specimens received in the Department of Pathology for frozen-section study. All tissues were received within 5 min after surgical excision and were placed in labeled clean Petri dishes on ice in a closed polystyrene (Styrofoam) container, in a refrigerator. Specimens were delivered to the laboratory, processed immediately, or kept at -20 °C.

One of us (P.P.R.), made the final pathological description of the tissues based on examination of the routine slides, including the frozen section made at the time the sample was submitted for analysis, and the gross description of the specimen. The histology of these specimens has been considered in another publication (16). About 80% of the specimens were described as infiltrating ductal carcinoma.

**Cytosol preparation.** Samples were trimmed of fat, weighed, minced, and homogenized in four volumes of homogenization buffer, and centrifuged (105 000 × g, 1 h, 2 °C). The homogenization was done in an ice bath, with use of three 15-s pulses, with 45-s pauses being allowed between pulses for cooling. This schedule of homogenization is essential to prevent heat denaturation of the protein. We analyzed aliquots of the cytosol for ERP, CEA, and protein concentration.

**Estrogen receptor assay.** ERP was determined by a competitive inhibition assay in which the cytosol was incubated in a mixture of the assay buffer, EGTA, and [3H]estradiol in the presence or absence of either unlabeled estradiol or an anti-uterotropic agent, CI628. Unbound [3H]estradiol was removed from the mixture with dextran-coated charcoal by centrifugation (60 min, 4 °C, 1200 × g). The radioactivity of a 150-μl aliquot of the supernate was measured. The concentration of ERP was calculated from the data on binding of 1 nmol of [3H]estradiol, both in the absence and presence of 1 μmol of cold estradiol or 20 μmol of the estrogen antagonist CI628, and was expressed as fmol/mg of cytosol protein. Similar results were obtained whether excess unlabeled estradiol or CI628 were used as the inhibitor.

The cytosol was additionally incubated with 2 and 5 nmol of [3H]estradiol and the dissociation constant of the estradiol/ERP complex was obtained from a three-point Scatchard plot (17). Based on empirical findings with normal tissue and the findings of others (18), we stated the specimen to be positive when it contained more than 10 fmol of ERP per milligram of cytosol protein and was inhibited more than 60% by the excess unlabeled estradiol or anti-uterotropic substance. This is equal to 6 fmol of estrogen-specific receptor protein per milligram of cytosol protein. Borderline specimens exhibited one or the other of the positive characteristics.

**Carcinoembryonic antigen assay.** CEA was determined in an aliquot of the cytosol by the modified (19) plasma CEA method of Hansen et al. (20). In this procedure, an appropriate volume of cytosol (50 to 500 μl) was diluted to 2.5 ml with a 155 mmol/liter sodium chloride solution and treated with an equal volume of 1.2 mol/liter perchloric acid. After 30 min, the mixture was centrifuged and the supernate was dialyzed overnight. The dialyzed protein was then incubated with CEA antiserum and 125I-labeled CEA as in the CEA radioimmunoassay described by Hansen et al. (20). After incubation the free 125I-labeled CEA was separated from the material bound with zirconyl phosphate gel by centrifugation, and the radioactivity of gel measured. Based on empirical findings with normal tissue we used a normal/abnormal cutoff value of 3.0 ng/mg of cytosol protein.

**Protein determination.** Protein concentration was determined in an aliquot of cytosol by the method of Waddell (21), which relates protein concentration to the difference in ultraviolet absorption at 215 and 225 nm, with solutions of human serum albumin as standards.

**Results**

Estrogen Receptor Protein in Normal, Benign, and Carcinoma Tissue

In Figure 1 are shown the values of specific ERP in the specimens in which both ERP and CEA were determined. The specific ERP is the product of the total ERP per milligram of cytosol protein and the inhibition, expressed as a decimal (e.g., 20 fmol/mg × 0.60 inhibition = 12 fmol/mg). In the histologically normal tissues the range was 0–13.6 fmol of ERP per milligram of cytosol protein. The mean value was 1.8 fmol/mg of cytosol protein (SD, 2.9). If the specimen with 13.6 fmol of ERP
Per milligram of cytosol protein is eliminated from the statistical evaluation, the mean value is 1.5 fmol/mg of cytosol protein (SD, 2.0). The values in benign specimens ranged from 0 to 12.4 fmol/mg of cytosol protein and in the cancer tissue from 0 to 286 fmol/mg of cytosol protein.

Table 1 summarizes our total experience in determining ERP based on our previously described positive/negative cutoff criteria. Of the 264 primary breast carcinomas, 143 (54%) were positive for ERP, 38% were found to be negative, and 8% were borderline. In 56 patients, normal tissue was obtained from the same or opposite breast of women with breast cancer. In none of these could we detect positive ERP, but one was considered borderline. Only seven of 97 specimens of benign breast tissue were found to contain detectable ERP, and five were in the borderline range. The positive benign specimens included six fibroadenomas and one specimen from a patient with gynecomastia. Five of the six fibroadenomas were from 10- to 27-year-old females, and contained a prominent epithelial-cell component; the other fibroadenoma was from a 51-year-old woman who also had atypical hyperplasia in her breast tissue.

The percentage of positive ERP in breast tissue metastatic to other sites was less than that observed in primary carcinomas. Of the 136 such tissues, 57 (42%) were positive. It was observed in 14 cases that lymph node and skin nodules removed surgically at the same time as primary breast cancer almost always yielded the same ERP pattern (11 were positive in both tissues, two were negative in both, and in one case the primary carcinoma was ERP-positive and the metastasis ERP-negative).

Relationship of Dissociation Constant (Kd) and Total ERP to Specific Inhibition

Table 2 shows the relationship between the percent inhibition of tritiated estradiol binding to ERP by a 1000-fold excess of exogenous nonradioactive estradiol or a 20 000-fold excess of CI628 and the dissociation constant (Kd) of binding or the total concentration of estrogen receptor. ERP-positive tissues (those with inhibition greater than 60%) usually exhibited a Kd of less than 5 × 10^{-9} mol/liter (193 of 221, or 87% of the specimens). Tissues that were ERP negative (less than 60% inhibition) had a higher Kd, 74 of 259 or 29.9% has a Kd of less than 5 × 10^{-8} mol/liter. Although most specimens that were positive for ERP had a low Kd, there was no significant correlation between these two parameters.

Table 2 shows that there are two distinct groups relating ERP and specific inhibition, one corresponding to ERP-positive (>10 fmol), the other to ERP-negative (<10 fmol), with a small scatter of borderline cases. Among the latter, there were 34 cases with greater than 60% inhibition but less than 10 fmol of ERP per milligram of cytosol protein and five specimens with greater than 20 fmol/mg, but less than 60% inhibition. Most of the specimens with a high inhibition also had a large amount of ERP. However, there were a sufficient number of specimens with low inhibition and a large concentration of ERP to make both assays necessary for the evaluation of positivity.

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Table 1. Estrogen Receptor Protein in Normal and Carcinomatous Human Breast Tissue

<table>
<thead>
<tr>
<th>Source of tissue</th>
<th>No. samples</th>
<th>Positive</th>
<th>Negative</th>
<th>Borderline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary carcinoma</td>
<td>265</td>
<td>143 (54%)</td>
<td>100 (38%)</td>
<td>22 (8%)</td>
</tr>
<tr>
<td>Breast benign and normal</td>
<td>153</td>
<td>7 (5%)</td>
<td>141 (92%)</td>
<td>5 (3%)</td>
</tr>
<tr>
<td>Metastatic carcinoma</td>
<td>136</td>
<td>57 (42%)</td>
<td>67 (49%)</td>
<td>12 (9%)</td>
</tr>
<tr>
<td>Total</td>
<td>554</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Positive > 60% inhibition > 10 fmol/mg protein.
b Negative < 60% inhibition < 10 fmol/mg protein.
c Borderline > 60% inhibition < 10 fmol/mg protein. 
< 60% inhibition > 20 fmol/mg protein.
d 6 fibroadenomas, 1 gynecomastia 
# 2 fibroadenomas, 2 gynecomastias, 1 normal
fMetastatic sites: skin nodules, lymph nodes, ovaries, pleural fluid cells, pleura, liver, omentum, lung, muscle, bone, and endometrium.
Table 2. Relation between Inhibition, Dissociation Constant, and Estrogen Receptor Protein

<table>
<thead>
<tr>
<th>Dissociation constant (K_d), mol/liter</th>
<th>Total estrogen receptor protein</th>
</tr>
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<tbody>
<tr>
<td>(&lt; 5.0 \times 10^{-10})</td>
<td>(5 \times 10^{-9})</td>
</tr>
<tr>
<td>% Inhibition(^a)</td>
<td>No. specimens</td>
</tr>
<tr>
<td>0-25</td>
<td>0</td>
</tr>
<tr>
<td>26-50</td>
<td>3</td>
</tr>
<tr>
<td>51-60</td>
<td>0</td>
</tr>
<tr>
<td>61-80</td>
<td>4</td>
</tr>
<tr>
<td>81-100</td>
<td>78</td>
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</table>

\(^a\)by exogenous estradiol

Table 3. Carcinoembryonic Antigen in Normal and Carcinomatous Human Breast Tissue

<table>
<thead>
<tr>
<th>Source of tissue</th>
<th>No. samples</th>
<th>Positive (%)</th>
<th>Negative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary carcinoma</td>
<td>189</td>
<td>133 (70%)</td>
<td>56 (30%)</td>
</tr>
<tr>
<td>Breast, benign and normal</td>
<td>85</td>
<td>13 (15%)(^a)</td>
<td>72 (85%)</td>
</tr>
<tr>
<td>Metastatic carcinoma(^b)</td>
<td>101</td>
<td>52 (51%)</td>
<td>49 (49%)</td>
</tr>
</tbody>
</table>

\(^a\)Three normal tissues (adjacent to cancer tissue), six fibroadenomas, four gynecomastias.
\(^b\)Sites: skin nodules, lymph nodes, pleural fluid cells, ovaries, omentum, bone, liver, muscle, and endometrium.

Table 3 summarizes our results for CEA in cytosols obtained from primary and metastatic carcinomas. Of the 188 breast carcinomas, 133 (70%) were positive for CEA. In contrast, of the 85 benign or normal breast tissues, only 13 (15%) were positive. Fifty-one percent of metastases from breast carcinoma were positive for CEA.

No attempt was made to determine plasma CEA in these patients. However, data on such assays were available for 16 individuals. Of these 16 patients, five had elevated breast cytosol CEA, and of these two had elevated plasma CEA. In four others the plasma CEA was elevated and the tumor negative. In seven others, both plasma and tumor CEA were within normal limits.

Concordance Data Between ERP and CEA

In Figure 3 are plotted the CEA and the specific ERP concentrations of all the specimens. As can be seen, there is no direct relationship between the absolute concentrations of these two constituents in breast cytosol.

Table 4 lists the relationships between positive and negative values for CEA and ERP in the specimens when the previously described positive/negative cutoff criteria are used. There was 66% concordance between CEA and ERP for carcinomatous breast tissues: 96 primary cancer specimens were positive for ERP and CEA and 28 were negative. In 65 primary cancer specimens there was nonagreement between ERP and CEA,

CEA in Breast Tissue

Figure 2 shows the concentrations of CEA in the specimens in which both ERP and CEA were determined. The CEA concentration in cytosols of 30 of the 31 specimens of normal breast tissue ranged from 0 to 3.7 ng of CEA per milligram of cytosol protein (2 SD range, 0.81 ± 2.2 ng/mg). Thus, we used a normal/abnormal cutoff value of 3.0 ng/mg of cytosol protein. In one histologically normal specimen the CEA concentration was 116.2 ng/mg of cytosol protein. It is of interest that the total ERP in this specimen was 13.9 fmol/mg of cytosol protein, with an inhibition of 57% (specific ERP of 7.9 fmol/mg of cytosol protein). The values in benign specimens ranged from 0 to 30.7 ng/mg of cytosol protein and in the cancer tissue from 0 to 11 780 ng/mg of cytosol protein.
28 being positive for ERP and negative for CEA and 37 positive for CEA and negative for ERP.

There was a concordance rate of 57% for metastatic carcinoma, with the instances of lack of agreement about equal in kind.

Benign and normal breast tissue showed a higher correlation between ERP and CEA. Of 85 tissues, 72 (85%) were in concordance, with 67 being negative for ERP and CEA and five being positive for both.

The overall concordance between ERP and CEA in all specimens was 68% (254/375).

Discussion

Numerous workers have reported that ERP measurement can be helpful in determining the appropriate treatment for patients with breast carcinoma (18, 22). Individuals who do not demonstrate positive ERP evidently are not likely to benefit from ablative or hormonal chemotherapy and may be treated initially by chemotherapy or radiation (10). Our data support the conclusions of other workers that specific ERP must be determined and the assay of $K_d$ is necessary to monitor the specificity of the assay and to improve its sensitivity (10).

Unlike ERP, CEA can be measured in the peripheral blood and it has been reported that sequential CEA assays can be useful in detecting early evidence of recurrent disease (23). Because the material being measured in the plasma presumably originates in the tumor, the appearance of detectable or increasing concentrations depends not only on the presence of tumor tissue but also on how much CEA it is producing. Therefore, information about the amount (rather than simply the presence) of CEA produced by a given tumor could prove extremely helpful when one is evaluating plasma CEA for evidence of recurrent disease.

The concentration of ERP is not directly related to the absolute concentration of CEA; rather, the relationship is based on the normal/abnormal cut-off values of the two constituents. The presence of CEA in the cancer tissue cytosols may merely reflect increased protein synthesis in the malignant tissue or increased epithelial cellularity in the specimen. Other proteins, including enzymes, have been observed in abnormally high concentration in human breast cancers (24, 25).

Recently an antigenically distinct IgA-like protein has been found to be a normal product of glandular epithelium, and to be present in increased amounts (based on immunofluorescence examination) in the cytoplasm of mammary carcinoma cells (26). The relationship of this glycoprotein (which has a reported molecular weight of 58 000) to CEA has not been studied. Sialyltransferase (EC 2.4.99.1) has been found to be increased amounts in breast cancer tissue and abnormally high sialyltransferase activity in plasma has been reported in some women with breast cancer (27). This enzyme is involved in the biosynthesis of glycoproteins and it would be of importance to relate its activity in breast cancer tissue to that of CEA.

The physiological function of carcinoembryonic proteins is unknown. $\alpha$-Fetoprotein has been reported (in rats) to serve as an estrogen-binding protein (28, 29), and an estradiol affinity column was used to prepare antisera to $\alpha$-fetoprotein (30). These findings could not be confirmed in humans (31). It would be attractive to hypothesize that physiological CEA has estrogen-binding properties. However, we find (unpublished data) that 19 ng of the "purified" CEA provided as a standard in the Hoffmann-La Roche CEA assay kit or

<table>
<thead>
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<th>Table 4. Concordance of the ERP and CEA Assays</th>
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<tr>
<td><strong>No. specimens</strong></td>
</tr>
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<td>Primary breast (189)</td>
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<td></td>
</tr>
<tr>
<td>Benign and normal (85)</td>
</tr>
<tr>
<td>Metastatic breast (101)</td>
</tr>
</tbody>
</table>

<sup>a</sup> One normal breast (ERP borderline), one fibroadenoma, one gynecomastia, two gynecomastias (ERP borderline).

<sup>b</sup> Five fibroadenomas.

<sup>c</sup> Four normal breast (ERP borderline), three fibroadenomas, one gynecomastia.
5 mg amount of CEA extracted from colon washings or from urine do not bind estrogen in the assay described in this paper. It seems that ERP and CEA appear in the tissue independently of each other. Long-term follow-up studies of these patients are needed, to determine if CEA assays in the cytosol obtained from mammary carcinoma yields information that is of diagnostic, prognostic, or epidemiologic value. Our data seem to indicate that the less-complex CEA assay cannot be substituted for ERP analysis. However, analysis of CEA in the cytosol may prove to be helpful in evaluating plasma CEA as a marker for recurrence subsequently if tumors with low CEA cytosol values must produce bulky metastases before blood CEA concentration increases measurably.

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References