Receptors for Epidermal Growth Factor and Insulin-like Growth Factor I and Their Relation to Steroid Receptors in Human Breast Cancer

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ABSTRACT

Levels of epidermal growth factor receptor (EGF-R) and insulin-like growth factor receptor (IGF-R) in breast cancer tissue were evaluated. The binding of growth factors was compared to the content of estrogen receptors (ER) and progesterone receptors (PgR).

EGF-R correlated negatively to the ER and PgR (Kendall correlation, $P < 0.001$), whereas the IGF-R correlated positively to ER and PgR (analysis of variance, $P < 0.001$). In contrast, no correlation was found between EGF-R and IGF-R. IGF-R binding was higher in tumor tissues than in adjacent normal tissues (Wilcoxon rank test, $P < 0.001$), whereas the EGF-R binding in normal tissue did not differ from that in cancer tissue. The degree of differentiation in ductal breast cancer correlated to EGF-R ($x^2$ test, $P = 0.018$), but not to IGF-R. The bindings of both growth factors were the same in metastases and primary breast tumors.

Our results show that EGF-R and IGF-R are present in normal breast tissue and breast cancer tissue. The growth factor receptors are related to steroid receptor content and their presence is associated with malignant transformation of breast cells and dedifferentiation of breast cancer.

INTRODUCTION

Estrogens are known to play an important role in breast cancer growth (1, 2). The mechanisms by which estrogen induces cell proliferation have recently been a focus of great interest. There is substantial evidence for the induction of autocrine growth factors by estrogen in breast cancer (1). The mammary tumor-promoting role of EGF and IGF in vivo have been shown in several models. Infusion of EGF and IGF into nude oophorectomized mice injected with MCF-7 breast cancer cells induced tumor formation (3). Tumor formation in a mouse species, highly susceptible to spontaneous mammary tumors, can be reduced by removing the main source of EGF, the submandibular gland, and can be stimulated by injecting EGF into the sialoadenectomized mice (4).

Both estrogen and progesterone as well as the growth factors act by binding to receptors specific for the respective hormones and growth factors. The estrogen and progesterone receptor contents of mammary tumors are considered important prognostic indicators. Patients with hormone receptor-positive tumors respond better to hormone therapy and survive longer (5). EGF receptors, a c-erb B protooncogene related product, have also been shown to be of importance for the tumorigenicity of breast cancer. High concentration of EGF receptors in A431 cells directly influenced the growth of these cells as solid tumors in nude mice (6). In human breast cancer biopsies high EGF receptor binding has been found in association with low content of estrogen receptors (7—9). A high EGF receptor content has been reported in mammary carcinoma metastases and tumors of estrogen receptors (7—9). A high EGF receptor content has been reported in mammary carcinoma metastases and tumors with poor prognosis (10). The results, however, are not uniform (11). IGF receptors have been identified in breast cancer cell lines (12), but their relation to steroid hormone receptors, EGF receptors or to the prognosis in patients with mammary tumors has not been established.

In this study, we have evaluated the relation between steroid hormone receptors and receptors for EGF and IGF as well as the relation between tumor differentiation and growth factor receptors. The presence of growth factor receptors in normal tissue, adjacent to carcinoma tissue, was also studied in an attempt to evaluate the role of growth factor receptors in malignant transformation of breast tissue.

MATERIALS AND METHODS

Patients and Tissue Samples. Patients who had breast cancer operations were included in the study with approval of the local ethical committee. Approximately 1-cm$^3$ samples of tumor tissue were frozen immediately after excision in liquid nitrogen and stored at —80°C until receptor analysis. Tissue samples were also fixed in formalin for histological evaluation by a pathologist not aware of the receptor content of the samples. EGF-R, ER, and PgR were tested in a total of 171 breast cancer samples (160 infiltrating ductal carcinoma, one medullary carcinoma, four medullary carcinomas, three papillary carcinoma, and three cystosarcoma). In 142 of these tissues IGF-R were determined as well. The stage of differentiation in 120 ductal carcinomas was divided into three categories according to WHO classification; low, intermediate, or high. In these 120 patients the tumor size was defined (less than 2 cm; equal or more than 2 cm), and the presence of axillary metastases was evaluated on the basis of surgical reports and histological examination. Thirteen metastases (four local and nine distant soft tissue metastases) were obtained from patients who underwent a second operation. The primary operation had been performed one-half to 14 years earlier. In 15 patients carcinoma tissue and samples from normal adjacent tissue were analyzed simultaneously. The normal tissue was prepared free from fat with scissors, and representative pieces were histologically verified. In two cases solely normal tissue adjacent to carcinoma tissue were studied, whereas the tumors were so small that only specimens for histological determination were available. In three cases carcinoma tissue and mastopathic surrounding tissue were studied. Biopsies from seven additional patients were also studied, five of these biopsies were mastopathic, whereas in two cases the biopsied material showed completely normal breast tissue.

Processing of Breast Tissue. The tissue was thawed and cut into small pieces with a razor blade, washed repeatedly with 0.04 M Tris-HCl, 1.5 mM EDTA, 0.02% NaN$_3$, pH 7.4, and homogenized with an Ultraturrax homogenizer in the same buffer at 4°C. After centrifugation at 800 $x$ g for 5 min, the pellet was discharged and the supernatant was further centrifuged at 30,000 $x$ g for 15 min. The 30,000 $x$ g supernatant was further centrifuged at 105,000 $x$ g for 60 min, and the supernatant fraction, designated cytosol fraction, was immediately used for ER and PgR determination. The 30,000 $x$ g pellet was resuspended in 10 mM Tris-HCl, 50 mM NaCl, 0.1% bovine serum albumin, 0.02% NaN$_3$, (Tris-NaCl-buffer) pH 7.4, and was centrifuged at 30,000 $x$ g for 15 min. The pellet was rehomogenized in Tris-NaCl-buffer, and its protein content was adjusted to 1.7 mg/ml. These membrane samples were stored at —80°C until analysis for IGF-R and EGF-R.

Growth Factors. Mouse EGF (Sigma, St. Louis, MO) and human recombinant IGF-I (Amgen, Thousand Oaks, CA) were iodinated by chloramine T (13). The specific activities were 50—150 $\mu$Ci/$\mu$g. The

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3 The abbreviations used are: EGF, epidermal growth factor; IGF, insulin-like growth factor; EGF-R, epidermal growth factor receptor; IGF-R, insulin-like growth factor receptor; ER, estrogen; PgR, progesterone receptors.

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iodinated growth factor remained stable for at least 2 months, as tested by trichloroacetic acid precipitation.

Receptor Determinations. ER and PgR were determined as described by Vihko et al. (14). Briefly, the cytosol fractions were incubated with various concentrations of estrogen or progesterone for 18 h at 4°C. Bound and unbound steroids were separated by dextran-coated charcoal. The affinity and capacity of the steroid receptors were determined by Scatchard analysis (15), and receptor content was expressed as fmoI/mg cytosol protein.

For EGF receptor determination, a 100 µl membrane suspension was incubated with [125I]EGF, 30,000 cpm, in the presence or absence of 1 x 10^-9 M unlabeled EGF in a final volume of 250 µl Tris-NaCl buffer, pH 7.4, for 60 min at 37°C. The incubation mixture was layered on top of 300-µl ice-cold Tris-NaCl buffer and centrifuged at 30,000 x g for 15 min. The supernatant was aspirated, and the pellet counted in an autogamma counter. The binding depicting EGF-R activity was expressed as specific binding (total [125I]EGF binding minus binding in the presence of 1.6 x 10^-4 M EGF) in percentage of total [125I]EGF added (B/T %).

For IGF receptor determination, a 100 µl membrane suspension was incubated with [125I]IGF-I, 30,000 cpm, in the presence or absence of 0.7 x 10^-7 M unlabeled IGF-I in a final volume of 250 µl Tris-NaCl buffer, pH 7.6, for 18 h at 4°C. The binding assay was then processed as described for EGF. The binding depicting IGF-R activity was expressed as specific binding (total [125I]IGF-I binding minus binding in the presence of 0.7 x 10^-7 M IGF-I) in percentage of total [125I]IGF-I added (B/T %).

The presence of a 34,000 IGF-binding protein, identical with placental protein 12 (16), was measured in the cytosol fraction of breast tissues by a specific radioimmunoassay as previously described (17). The sensitivity of this assay is 9.8 ng/ml. The data were expressed as ng/mg cytosol protein.

Analysis of variance and Kendall correlation were used to study correlation between steroid receptors and growth factor receptors in cancer tissue. For comparison of growth factor binding in cancer tissue and adjacent normal tissue Wilcoxon signed rank test was used. An EGF and IGF binding higher than 2% was arbitrarily called high binding. Comparisons of high and low EGF and IGF binding to cancer tissue differentiation, to presence of metastases, to tumor size, to benign mammary lesions, and to normal mammary tissue was carried out by the χ² test.

RESULTS

The EGF binding to breast tumor tissue was specific and had an apparent affinity of 1.3 x 10^10 M^-1 (Figs. 1 and 2). The specificity of the IGF binding suggests a type I receptor, with less than 10% cross-reactivity by multiplication stimulating activity (rat IGF-II) and approximately 0.1% cross-reactivity by insulin (Fig. 1). The Kₜ value for the IGF-R was 0.5−1.7 x 10^10 M^-1 (Fig. 2).

The EGF binding in breast cancer did not correlate to the IGF binding in the same tumor (N = 142, variance analysis, R = 0.114, P = 0.113. Kendall correlation, T = −0.047, P = 0.207). There was, however, a nonlinear negative correlation both between EGF-R binding and ER content (Kendall correlation: N = 170, T = −0.259, P < 0.001), and between EGF-R binding and PgR (Kendall correlation: N = 170, T = −0.206, P < 0.001), indicating an inverse relationship between EGF-R and steroid receptors in breast cancer (Figs. 3 and 4). There was a linear correlation between IGF-R binding and ER content (N = 142, R = 0.505, P < 0.001), and between IGF-R and PgR content (N = 142, R = 0.346, P < 0.001), showing that increased steroid receptor content is associated with increased IGF-R binding (Figs. 5 and 6). The correlation between ER and PgR was significant (N = 170, R = 0.407, P < 0.001) (data not shown).

There was no correlation between growth factor binding and age of the patients (mean age, 60 years; range, 28–89 years). Neither was any association observed between growth factor binding and breast tumor size (<2 cm or ≥2 cm), or presence of axillary metastases at the time of the primary operation in 120 patients with ductal breast cancer (data not shown). In ductal carcinoma with low differentiation, EGF binding was high in 26%, and IGF binding was high in 64% (Fig. 7). In intermediate-differentiated ductal carcinoma, high EGF-R binding was observed in 13% and high IGF-R binding in 67%. In highly differentiated cases the EGF-R binding was high in 7% and the IGF-R binding was high in 50%. Thus, a trend for higher EGF binding in less-differentiated tumors was evident. The χ² test resulted in P = 0.018 for EGF binding in high versus low-differentiated ductal carcinoma. The IGF binding was similar in all three groups.

In metastases, high EGF binding was observed in 7% and high IGF binding in 46% (Fig. 7). The growth factor binding in metastases was not different from that in primary tumors.

In one of eight cases of proliferative mastopathia (14%) EGF...
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binding exceeded 2%, and in four of eight of the tissues (50%) the IGF binding was above 2%, but lower than 4% (Fig. 8). In 5% (one of 19) of normal breast tissues EGF and IGF binding exceeded 2%. The IGF binding in normal breast tissues displayed in Fig. 8 was significantly lower than that in the ductal carcinomas in Fig. 7 ($\chi^2$ test, $P < 0.001$), whereas no difference was found between EGF binding to normal and to cancer tissues. The only normal tissue with high EGF and IGF binding was adjacent to a carcinoma which was so small that only samples for histological examination were obtained, but no receptor determinations were possible.

When growth factor receptor binding was compared in paired samples obtained from breast cancer tissue and adjacent normal tissue, the binding of IGF to cancer tissue was higher than that to normal tissue (Wilcoxon signed rank test, $P < 0.001$; Fig. 9). In the same 15 pairs of tissues, no difference in EGF-R binding was evident. Only one cancer tissue showed EGF-R binding exceeding 2%, whereas the binding was low in all normal adjacent tissues.

Breast tissues were also tested for 34,000 IGF-binding protein (placental protein 12). The binding protein was detectable in two of 73 breast cancer tissues (6.6 ng/mg cytosol protein and 2.6 ng/mg cytosol protein) and in one of 19 normal breast tissues (4.0 ng/mg cytosol protein). The latter was adjacent to a cancer tissue without detectable binding protein.

**DISCUSSION**

The observation that breast tumor cell lines produce growth factors in response to estrogen stimulation (1), and that breast tumor cell lines have receptors for growth factors including EGF receptors (1, 18) and IGF receptors (12), raised the question of a possible relationship between steroid receptors and growth factor receptors. In this work we show that such relations exist, both between ERs, EGF-Rs, and IGF-Rs as well as between PgRs, EGF-Rs, and IGF-Rs. The relation between steroid receptors and EGF-Rs is, however, distinct from that between steroid receptors and IGF-Rs, and no relation between EGF-Rs and IGF-Rs was observed.

Our results on an inverse relation between EGF receptors and steroid receptors are consistent with previous reports (7–10, 19). The reason why high ER and PgR excludes the presence
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Fig. 6. IGF receptors versus progesterone receptors in 142 breast cancer tissues. Analysis of variance, $R = 0.346$, $P < 0.001$. $B$, bound tracer; $T$, total tracer added.

Fig. 7. The EGF receptors and IGF receptors in ductal breast cancer of low, intermediate, and high differentiation, and in secondary metastases. $B$, bound tracer; $T$, total tracer added.

Fig. 8. The EGF receptors and IGF receptors in mastopathic and normal breast tissue. $B$, Tissue adjacent to carcinoma tissue; $\Delta$, tissue with no adjacent malignancy. $B$, bound tracer; $T$, total tracer added.

Fig. 9. The IGF receptors (top) and EGF receptors (bottom) in 15 breast cancer tissues ($\bullet$) and adjacent normal tissues ($\circ$). $B$, bound tracer; $T$, total tracer added.

of high EGF-R and vice versa is not evident. The relation is further complicated by observations that both estrogen (20) and progesterone (21) increase EGF-R content in hormone-responsive tissue, and that EGF (22) induces its own receptor.

Recently, the HER-2/neu oncogene which is related to the EGF receptor was shown to be amplified in human breast tumors, and the gene amplification was a significant predictor of relapse and survival (23). Similarly, the content of EGF receptors which is related to the c-erb B oncogene has been reported to be correlated to metastases and dedifferentiation (6, 10). In support of such a dedifferentiating role for EGF-R we observed a higher EGF-R binding in tumors with low histological differentiation than in tumors with high differentiation. Walker et al. (11), in contrast, found no correlation between tumor differentiation or DNA ploidy and EGF-R. Similarly, no correlation between EGF-R and differentiation was seen by Skoog et al. (19). Sainsbury et al. (9) reported that tumors with a higher EGF binding have a higher potential for metastases and that metastases have a higher EGF-R binding. Our results, however, do not support such a conclusion; we found an EGF-R binding in metastases similar to that in primary breast tumors, and we found no correlation between EGF-R binding in breast tumors and the presence of metastases at primary operation.

Comparing the EGF-R binding in normal breast tissue and adjacent carcinoma tissue no statistically significant difference was seen, although the highest EGF-R binding in the paired samples was seen in tumor tissue. The only normal tissue with high EGF-R binding was adjacent to a carcinoma tissue which due to its small size was not available for receptor determination. Therefore, in this particular case it remained unclear whether the binding in the tumor exceeded that in the normal tissue. The presence of EGF-R in normal murine breast cells has been reported (24), supporting our observation on measurable EGF-R in normal human breast tissue.

The presence of IGF-R in breast cancer, as shown in this study, and their presence in breast tumor cell lines (12), as well as the production of IGF by breast tumors (1), suggest a role for IGF in breast tumor growth. The IGF-R expression was
different from that of the EGF-R in breast tumors, and correlated positively to both ER and PgR. The mechanisms possibly regulating the IGF-R expression in breast tumors are not known, but the observed relation to steroid receptors suggest that there might be a common regulator for these receptors. Estrogen, which induces the progesterone receptor, has been shown to increase the IGF-I production in breast cancer (1), but the effect of estrogen on the IGF receptor has not, to our knowledge, been evaluated.

The IGF binding in breast tumors was significantly increased compared to that in the adjacent normal tissue, suggesting that the expression of IGF-R is associated with malignant transformation of breast epithelial cells. The difference between normal and tumor tissue was much more consistent as regards to IGF-R than to EGF-R. Interestingly, the only normal breast tissue exhibiting high IGF binding was the same tissue sample exhibiting high EGF binding. It is tempting to speculate that the tissue in close proximity to mammary cancer tissue, in spite of its normal histological appearance, possesses malignant biochemical features. The growth factor receptors in normal breast tissue need, however, to be studied in more detail before the normal range for growth factor binding in breast tissue can be established.

When differentiation of ductal carcinoma was compared to IGF binding, no trend for increased receptor binding with decreasing differentiation was evident. The total number of mammary tumors with high IGF binding was, however, greater than the total number of tumors with high EGF binding. Thus, the increased expression of IGF-R seems to be a more general property for breast tumors than the expression of EGF-R.

It has been reported that IGF-R determinations might be hampered by the presence of secreted nonreceptor binding proteins. A 34,000 IGF-binding protein (identical to placental protein 12) has indeed been shown in breast cancer tissue (25). It was, therefore, of interest to determine what role this binding protein might play. Our result, however, show that the amount of 34,000 IGF-binding protein in breast tissue is low and exceeded the detection limit of our assay only in three out of 80 breast tissue samples.

In conclusion, we have shown that the EGF-R and IGF-R in breast cancer are both correlated to the ER and the PgR. The EGF-R is negatively and nonlinearly related to the steroid receptors so that a high EGF-R binding excludes high ER and PgR contents, and high level of ERs and PgRs excludes high EGF-R binding. In contrast, IGF-R is positively correlated to both ER and PgR in breast cancer. Both EGF-R and IGF-R are also present in normal breast tissue.

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