Insulin-like Growth Factor 1 Receptors in Human Breast Cancer and Their Relation to Estradiol and Progesterone Receptors

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ABSTRACT

Insulin-like growth factor 1 (IGF1) binding sites were characterized in breast cancer. We demonstrate the presence of one high affinity binding site. Chemical cross-linking of 

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1This work was supported by the Association pour la Recherche sur le Cancer (Villejuif) and by the Fédération Nationale des Centres de Lutte contre le Cancer.

2The abbreviations used are: IGF1, IGF2, insulin-like growth factors 1 and 2; IGF1-R, IGF1 receptor; ER, estradiol receptor; PgR, progesterone receptor.

have been characterized by competitive binding and cross-linking techniques on cultured human breast cancer cell lines (8) and on breast cancer cell line membranes (15). IGF1-R presents, as in normal tissue, close homology with insulin receptor with a Mr, 130,000 binding subunit. We have shown recently that the membranes extracted from breast cancer biopsies bind specifically IGF1 (15). These results indicate that IGF1 acting via the endocrine, paracrine, or autocrine pathways could be an important factor in the development of human breast cancer (16). It is therefore important for a better understanding of breast tumor biology to document the presence, absence, or alteration of IGF1-R.

In the present report IGF1-Rs in breast cancer are characterized and the level of these receptors in breast cancer biopsies is determined and related to the concentrations of estradiol and progesterone receptors in the same biopsies.

MATERIALS AND METHODS

Collection of Tumors. Tumor specimens, adenocarcinomas only, were obtained from 76 patients undergoing surgery for primary breast cancer in the Centre Oscar Lambret (Lille). At the time of collection, fat was removed and samples were divided into two parts: one was submitted for histological studies and the other was immediately frozen for receptor analysis. Our series was composed of 61 ductal, 9 lobular, 2 medullary, 1 ductallobular, 1 colloid, and 2 apocrine invasive carcinomas.

Tissue Processing. The frozen tissue was weighed and then pulverized (Spx-Bioblock, France). The tissues were homogenized in 20 mm Tris-3 mm EDTA-1 mm dithiothreitol-0.01% azide, pH 7.6.

The homogenate was centrifuged at 800 × g for 10 min and the supernatant was ultracentrifuged at 105,000 × g for 60 min. The supernatant (cytosol) was removed and the pellet ("microsomal" fraction) was resuspended in 25 mm Tris-HCl-10 mm MgCl2-10⁻⁴ M phenylmethylsulfonyl fluoride buffer, pH 7.6. The protein concentration was determined by the method of Lowry et al. (17) applied either directly in the cytosol fraction or after extraction from the membranes (with 1 N NaOH) in the microsomal fraction.

IGF1 Labeling. The human native IGF1 and IGF2 were a generous gift from Dr. Humbel (Zurich, Switzerland). Human synthetic IGF1 was purchased from Amersham (ARN 4010; Amersham- France, Paris, France). A modification of the method of Hunter and Greenwood (18), using 800 ng chloramine-T and 1 μg IGF1 with incubation for 50 s at 23°C, was used to iodinate IGF1. Iodinated IGF1 was purified on an ACA-54 column (LKB, France) and the tubes comprising the radioactive protein peak were diluted in assay buffer and could be stored as long as 2 weeks at 4°C. Specific activities, as calculated by isotope recovery, ranged between 160 and 220 μCi/μg. The quality of the preparation was checked after each iodination using a standard laboratory preparation of BT-20 breast cancer cell line membrane receptors. When 400 μg of the usual protein membrane preparation were utilized, at least 10% of the iodinated IGF1 was specifically bound when the tracer was considered acceptable.

IGF1-R Assay. Unless otherwise specified, for the binding test 400 μg of membrane proteins were incubated for 5 h at 4°C with approximately 200,000 cpm of iodinated IGF1 in the presence or absence of an excess of IGF1 crude preparation (the crude mixture of IGF1 and IGF2 was a generous gift from Dr. Humbel). The final incubation volume was adjusted to 0.5 ml with Tris-MgCl2 buffer containing 0.1%...
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Results of chemical cross-linking of 125I-labeled IGF1 on breast cancer membranes. Competition for binding ($B/F$) of 125I [100% = binding in the absence of unlabeled growth factor (B)] in the presence of increasing amounts of unlabeled growth factors. INS, insulin.

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Fig. 3. Autoradiogram showing the size of \(^{125}\text{I}-\text{IGF1}\) binding unit complex in breast cancer membranes as analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions. Membrane proteins (800 \(\mu\)g) were incubated with \(^{125}\text{I}-\text{IGF1}\) in the absence (−) and in the presence (+) of unlabeled IGF1 (4 \(\mu\)g/ml). Cross-linking was performed with diisuccinimidyl suberate (500 \(\mu\)M). The radioligand-binding site complexes were solubilized with sodium dodecyl sulfate containing dithiothreitol and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. Arrow, position of Mr, 130,000 (130 K) complexes. In, BT-20 breast cancer cell membranes; 2, pooled breast cancer membranes; 3, 4, 5, individual breast cancer membranes. Ordinate, molecular weight in thousands.

in 72% (54 of 75) of the tumors when the characteristics of positivity defined above are considered.

Relation between IGF1-R and Clinical Features. Considering the tumor type, only 4 of 61 ductal carcinomas and 1 of 9 lobular carcinomas were negative and all other types were positive. The mean level of IGF1 in ductal carcinomas was 4.09% (3.49-4.80 ± 2 SEM) and 3.2% (2.2-4.71 ± 2 SEM) in lobular carcinomas; the difference between these two mean levels was not statistically significant. In the lobular carcinoma the specific binding was 9.8%, in the colloid carcinoma it was 13.8%, in the two apocrine carcinomas it was 1.5 and 6.3% and in the two medullary carcinomas it was 1.6 and 1.8%.

In our population no relation was found between IGF1-R and histoprognostic grading according to the studies of Scarff and Bloom, cellular density, stromal reaction, and node metastases.

IGF1-R positivity rate was higher in postmenopausal patients (\(\chi^2 = 6.779; P = 0.009\)). The mean level of IGF1-R was 4.3% (3.7-5.1 ± 2 SEM) in postmenopausal patients and 3.3% (2.3-4.6 ± 2 SEM) in premenopausal ones.

Relation between IGF1-R and ER or PgR. There was no relation (\(\chi^2\) test) between IGF1-R and ER positivity rates in the whole population; conversely there was a strong relation between IGF1-R and PgR positivity rates (\(P = 0.002\)). Fig. 5 shows that IGF1-R levels were significantly higher (\(P = 0.02\)) in ER+ than in ER− tumors. The IGF1-R level in PgR+ tumors was 4.2% (3.6-5 ± 2 SEM); it was lower, but not significantly, than in PgR-negative tumors: 3.2% (2.3-4.5 ± 2 SEM). The

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![Graph showing the distribution of IGF1-R as a function of ER status of the patients.](image)

Fig. 5. Mean value ± 2 SEM (bars) of IGF1-R according to the ER status of the patients.

![Graph showing the distribution of ln IGF1-R as a function of ln ER.](image)

Fig. 6. Distribution of ln IGF1-R as a function of ln ER. Three groups of value could be noted. When zero values were excluded, a statistically significant positive linear correlation could be found between ln IGF1-R and ln ER (\(r = 0.30; n = 58; P = 0.024\); ln IGF1-R = 0.154 ln ER + 0.674).
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IGF1-R level was higher in ER+ PgR+ tumors: 4.5% (3.7–5.3 ± 2 SEM) than in ER− PgR− tumors: 2.7% (1.9–3.7 ± 2 SEM) (P = 0.02).

A correlation was found between IGF1-R and ER (P = 0.0018) and between IGF1-R and PgR (P = 0.0011) by the Spearman test.

However, these relations were complex as shown in Figs. 6 and 7. By representing ln(IGF1-R) as a function of ln(ER) (Fig. 6) we found that three populations of results could be individualized: population 1, zero values of ER; population 2, zero values of IGF1-R; population 3, nonzero values of both ER and IGF1-R. Therefore we must limit the study of a linear correlation to that third population. A statistically significant linear positive correlation was found between ln ER and ln IGF1-R (r = 0.30; n = 58; P = 0.024). The bidimensional representations of ln IGF1-R as a function of ln PgR showed, as previously, that 3 populations could be individualized. The study of correlation was performed in the population of nonzero values of PgR and IGF1-R; a statistically significant positive correlation was found between ln PgR and ln IGF1-R (r = 0.41; n = 53; P = 0.0025).

Discussion

Research on IGF1-R has been hampered by the presence of a secreted, non-receptor binding protein; Clemmons et al. (25) have demonstrated that human fibroblast monolayers secrete a protein that binds IGF1, altering the IGF1-R binding.

In the present paper we demonstrate that IGF1 binds to membrane preparations from various tumors and that the binding is saturable, is specific, and corresponds to only one class of high affinity sites. Cross-linking experiments visualize one major band which corresponds to the expected size of the IGF1-R binding subunit. These results suggest that membrane preparations are devoid of non-receptor binding protein interference.

Almost all the human breast cancers that we studied contained IGF1 receptors (71 of 76). These findings suggest that most of the tumors could respond to IGF1 acting via either the endocrine, autocrine, or paracrine pathways.

Further studies would be useful to determine the exact tissular localization of IGF1-R in breast tumor; we cannot exclude the possibility that IGF1-Rs are present on normal cellular components of the tumor tissue like fibroblasts (7). However, indirect evidence suggests that the majority of IGF1-R might be on the epithelial cells: (a) it has been demonstrated that human breast cancer cell lines are rich in IGF1-R (3); (b) IGF1-Rs are low in benign breast diseases and undetectable in normal breasts while these tissues contain a large fibroblastic compartment (26). The fact that IGF1-R in breast cancers was significantly increased compared to normal tissue or benign breast disease (26) suggests that the expression of IGF1-R is associated with the malignant transformation of breast epithelial cells.

We showed, using the Spearman test, that IGF1-R and ER or PgR were linked in breast cancers (15). In the present study, a more detailed analysis shows that the positivity of IGF1-R was associated to the positivity of PgR; this relation did not exist between ER and IGF1-R. Conversely IGF1-R levels were significantly higher in ER-positive than in ER-negative tumors; it was not the case with PgR. These results are corroborated by our observation of high IGF1-R in postmenopausal patients' breast cancer, already known to be rich in steroid receptors (23). Finally a linear correlation was found between ER and IGF1-R and between PgR and IGF1-R; these results are in line with the paper recently published by Pekonen et al. (27).

The mechanisms by which IGF1-Rs are regulated in breast cancer are poorly understood. The observed coexpression of IGF1-R and steroid receptors could suggest a common regulator for these receptors. In vitro, it has been demonstrated that human breast cancer cell lines devoid of ER (ER negative) express and secrete higher amounts of IGF1 than do ER-positive lines (11). It has also been found that IGF1-R concentration is higher in ER-positive lines than in ER-negative lines (8). These results suggest that the lower IGF1-R concentration in ER-negative lines and ER-negative tumors could be due to an occupation of the receptors by endogenous ligand or receptor down-regulation; it is possible that a similar process occurs in breast cancer tissue.

In conclusion, the present study confirms that IGF1 could be an important regulator of human breast cancer growth and may indicate that lowering the IGF1 serum concentration or IGF1-R could be a beneficial treatment of breast cancer.

References

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