Prognostic Significance of Insulin-like Growth Factor 1 Receptors in Human Breast Cancer

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INTRODUCTION

IGF1, also termed somatomedin-C, is a small polypeptide, the already well-known physiological role of which is to act, with growth hormone, on skeletal development via the endocrine pathway (1, 2). It has been demonstrated that IGF1 is synthesized in many tissues (3); the results have been confirmed using IGF1 complementary DNA probes (4, 5). These studies and the demonstration that IGF1 stimulates the growth of many cell types (6) suggest that this factor may also act via the autocrine or paracrine pathway. The first step of IGF1 action is its binding to membrane receptors the structure of which is remarkably similar to that of insulin, comprising a heterotetrameric structure with two α and two β subunits joined by disulfide bridges (7).

The IGF1 increase in human milk during lactation suggests that this factor is involved in growth control and normal human mammary gland differentiation (8). In breast cancer IGF1 stimulates the growth of various cell lines (9–13). Immunoreactive IGF1 had been found in the medium conditioned by breast cancer cell lines (11, 14, 15) but the very recent demonstration of the absence of IGF1 mRNA in these cells (16) suggests that this “immunoreactive IGF1” represents either an IGF1 related protein or IGF1 binding proteins (17, 18). These cell lines have been shown to produce binding proteins which can interfere with the RIA for IGF1 (19). It has also been proved that breast cancer cell lines bind IGF1; variations in the sensitivity to IGF1 correlate with IGF1 cell binding (9, 10). These results are confirmed using microsomal membrane preparation where the IGF1 receptor determination is not hampered by binding proteins (20–23).

We have recently shown that breast cancers (24) and benign breast diseases (25) contained IGF1 receptors. The aim of the present study was to determine the prognostic significance of IGF1-R in 297 breast cancer patients.

PATIENTS AND METHODS

Patients. Included in this study were 297 female breast cancer patients undergoing surgery for locoregional disease in the Centre Oscar Lambret (Lille) from January 1986. The mean age was 56 years (range, 28–86). Sixty-nine % of the patients were postmenoapausal. Clinical tumor diameter was less than 2 cm in 3%, between 2 and 5 cm in 82%, and more than 5 cm in 15% of the cases.

All patients were treated by segmentectomy when the tumor was less than 3 cm wide and by total mastectomy when the tumor was bigger or centrally located. An axillary dissection was carried out in all cases. Surgery was followed by radiotherapy on the chest wall after total mastectomy, or on the remaining breast tissue after segmentectomy, and on the internal mammary, subclavicular, and supraclavicular nodes. Only node-positive patients received adjuvant treatment, with chemotherapy (6 cycles) in premenopausal patients (cylophosphamide, 500 mg days 1, 3, and 5; 5-fluorouracil, 750 mg days 1–5; metotrexate, 20 mg days 2 and 4); and tamoxifen (30 mg/day for 2 years) in postmenopausal ones.

After completion of the treatment, the patients were examined every 4 months for 3 years, then every 6 months for 2 years, and then yearly. Mammography, chest X-rays, and plasma hepatic enzyme level measurements were carried out yearly; other X-rays, scans, or echographies were only symptom oriented.

Median duration of follow-up of living patients was 40 months.

Pathology. The type (lobular or ductular), differentiation, and histoprognostic grading according to the criteria of Scarff and Bloom (26) were noted together with the number of invaded axillary nodes and the number of excised nodes. Sixty-five % of the patients had positive axillary nodes; 66% were ductular, 11% were lobular, and 23% were of other types. Histoprognostic grading was done in 240 tumors: 21% were grade 1; 45% were grade 2; and 34% were grade 3.

Collection of Tumors. Tumor specimens consisted solely of adenocarcinomas. 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.

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 5 mM Tris-HCI-10 mM MgCl2-10− M PMSF buffer, pH 7.6. The protein concentration was determined by the method of Lowry et al. (27), 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 the Ligue du Nord Contre le Cancer (Lille) and Association pour la Recherche sur le Cancer (Villejuif). The abbreviations used are: IGF1, insulin-like growth factor 1; IGF1-R, insulin-like growth factor 1 receptor(s); BT, total binding; NS, nonspecific binding; ER, estrogen receptor; PR, progesterone receptor; OS, overall survival; RFS, relapse-free survival; GHP, histoprognostic grading.

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1 This work was supported by the Ligue Nationale Contre le Cancer (Paris), Ligue du Nord Contre le Cancer (Lille) and Association pour la Recherche sur le Cancer (Villejuif).

2 The abbreviations used are: IGF1, insulin-like growth factor 1; IGF1-R, insulin-like growth factor 1 receptor(s); BT, total binding; NS, nonspecific binding; ER, estrogen receptor; PR, progesterone receptor; OS, overall survival; RFS, relapse-free survival; GHP, histoprognostic grading.
gift from Dr. Humbel (Zurich, Switzerland). Human synthetic IGF1 (ARN 40110) was purchased from Amersham-France, Paris, France. A modification of the method of Hunter and Greenwood (28), with 800 ng chloramine-T and 1 ng IGF1 with incubation for 50 s at 23°C, was used to iodinate IGF1. The latter was purified on an ACA-54 column (LKB, France) and the tubes containing the radioactive protein peak were diluted in assay buffer and could be stored for 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 (total radioactivity) of iodinated IGF1 in the absence (BT) or presence (NS) 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% bovine serum albumin (fraction V, ref. A3912; Sigma Chemical Company, St. Louis, MO). The binding was separated by a 30-min 3,000 × g centrifugation. Duplicates were used for total and nonspecific determinations. The specific binding was the difference between BT and NS. The intraassay reproducibility was less than 5%; the interassay reproducibility was less than 8%. In each series, a characterized pool of cell membrane receptors (BT-20) was included to ensure the assay quality control. A tumor was considered positive (IGF1-R+) when the specific binding was higher than 1% for the positive threshold since when the assay had been performed on boiled membranes, i.e., on membranes with denatured receptors, the difference between the binding in the absence or presence of an excess of IGF1 was always less than 1%. The mean NS/total radioactivity ratio (in percentage) was 1.2%, ranging from 0.6 to 2.5%. The mean NS/BT ratio in positive tumors was 20%, ranging from 12 to 47%. In negative tumors the NS/BT ratio was always greater than 50%.

Estradiol and Progesterone Receptor Assay. [3H]-17ß-Estradiol (specific activity, 101 Ci/mmol) and [3H]-Org 2058 (specific activity, 53 Ci/mmol) were purchased from Amersham-France; the nonradioactive diethylstilbestrol and cortisol were purchased from Steraloids, Inc. (Pawling, NJ); and the cold Org 2058 from Amersham-France. Both ER and PgR were determined by the dextran-coated charcoal method (29, 30). Our laboratory is affiliated with the European Organization of Research and Treatment of Cancer Receptor Study Group which organizes quality controls of the assays (31). Tumors with more than 10 fmol/mg protein ER were considered positive and tumors with more than 10 fmol/mg protein PgR were considered positive.

Statistical Analysis. Statistical analyses were realized using the SAS statistical Software on VAX VMS 6320. The distribution of the parameters (ER, PgR, IGF1-R) were lognormal after excluding zero values, confirming what we had found in previous studies (32).

Relations between qualitative variables were determined using the χ² test (with Yates correction when necessary). Correlations between parameters were assessed according to the Spearman R nonparametric test; moreover, in order to make these correlations explicit, linear regressions were performed after excluding zero values (Pearson coefficient as done in a previous paper) (24).

OS and RFS were studied by actuarial method analysis. Comparison between curves was carried out by the logrank test.

The proportional hazards regression method of Cox (33, 34) was used to assess the prognostic significance of different clinical, pathological, hormonal factors taken individually and in association, assuming that the covariates act multiplicatively on the hazard function. No time-dependent variable was introduced. These last analyses were performed with DASH Software (Dash Software Development Group, Boston, MA).

RESULTS

ER and PgR

ER and PgR were found in, respectively, 71 and 61% of the cases.

IGF1-R

Positivity Rate of IGF1-R

IGF1-R were found in 87% of the cases.

IGF1-R Levels

IGF1-R levels are shown in Fig. 1. The mean geometric value was 3.87% (range, 1-23%).

Relation between IGF1-R and Clinical Features

A relation between IGF1-R and age (older patients having higher levels) was found (χ² test, P = 0.026; Spearman test, ρ = 0.0152). No difference was found between node-positive and node-negative patients, nor was variation according to tumor diameter found.

IGF1-R were less frequently found in GHP3 than in GHP1 or GHP2 (χ² test, P < 0.0001) and in poorly (or moderately) differentiated tumors rather than in well differentiated ones (χ² test, P = 0.05). No difference was observed according to ductular or lobular origin of the tumor.

Relation between IGF1-R and ER or PgR

There was a relation between IGF1-R and ER positivity rate (P < 0.001) and between IGF1-R and PgR positivity rate (P < 0.001) in the whole population. These positive relations were found in postmenopausal patients (P < 0.001); in premenopausal ones a relation was found only between IGF1-R and ER (P = 0.003). A highly significant correlation was found between IGF1-R and ER on the one hand (P = 0.0001) and PgR on the other (P = 0.0001) (Spearman test). When excluding zero values (n = 76), a linear correlation was found only between IGF1-R and ER (P = 0.0004). The correlation was found in premenopausal as well as in postmenopausal patients (respectively, for ER and PgR, for premenopausal P = 0.0001 and 0.05 and for postmenopausal P = 0.0001 and 0.0001) (Spearman test).

Fig. 1. Distribution of human breast cancers (%) as a function of their IGF1-R levels [in percentage of total (7) counts/400 μg of membrane proteins]. Bs, specific binding.
When excluding zero values in these subgroups (respectively for pre- and postmenopausal patients, \( n = 29 \) and \( 47 \) for the relation between ER and IGF1-R; \( n = 22 \) and \( 62 \) for the relation between PgR and IGF1-R), a linear correlation was found in premenopausal patients between IGF1-R and ER (\( P = 0.009 \)) but not between IGF1-R and PgR. In postmenopausal patients, a correlation was obtained with ER (\( P = 0.004 \)) and PgR (\( P = 0.049 \)).

**Prognosis Studies**

**RFS. Actuarial Survival (Table I; Fig. 2).** Two hundred seventy-six patients were included in this study. A longer RFS was found in patients with IGF1-R than in those without (\( P = 0.014 \)). ER and PgR were prognostic on RFS also (respectively, \( P = 0.002 \) and \( 0.02 \)); IGF1-R was a prognostic factor in node-positive (\( P = 0.0003 \)) but not in node-negative patients; IGF1-R was not a prognostic factor in ER-positive (\( P = 0.46 \)) or ER-negative patients (\( P = 0.23 \)). Conversely IGF1-R was a prognostic factor in PgR-negative patients (\( P = 0.011 \)), but not in PgR-positive ones (\( P = 0.53 \)).

**Cox Analysis (Table 2).** By Cox analysis IGF1-R was a significant prognostic factor (\( P = 0.016 \)); the other ones were the number of positive nodes (\( P = 0.032 \)), ER (\( P = 0.002 \)), PgR (\( P = 0.002 \)), histop prognostic grading according to the criteria of Scarff and Bloom (\( P = 0.004 \)), and the tumor diameter (\( P = 0.019 \)). When combining IGF1-R with other prognostic factors, the relative weight of each parameter can be measured: IGF1-R and node metastases (\( P = 0.005 \)), ER (\( P = 0.008 \)), PgR (\( P = 0.007 \)), GHP (\( P = 0.0007 \)), \( P = 0.13 \) and \( 0.01 \)), GHP (\( P = 0.0007 \)), \( P = 0.20 \) and \( 0.019 \)), and PgR (\( P = 0.011 \)), \( P = 0.005 \) and \( 0.060 \)). When combining IGF1-R, ER, and PgR the \( P \) value was \( 0.006 \) (\( P = 0.06 \), \( 0.17 \), and \( 0.33 \), respectively); when combining IGF1-R, GHP, and nodes the \( P \) value was \( 0.0002 \) (respectively, \( 0.015 \), \( 0.035 \), and \( 0.010 \)).

**DISCUSSION**

The results of this prospective study show that IGF1-R have a prognostic significance on RFS as well as on OS whatever the statistical method used (actuarial survival or Cox analysis). IGF1 receptors have already been characterized in microso-
nal membrane from breast cancer cell lines (20–23) as well as from human breast tumors (24, 35); the IGF1 binding as shown by competitive binding studies and cross-linking experiments corresponds to the insulin inhibitable type I receptor previously described (7, 36) and was not contaminated by a nonreceptor binding protein.

Most breast cancers contain IGF1-R, as shown by other authors (37, 38) as well as ourselves (24); a highly significant correlation was found by all authors between IGF1-R and ER or PgR (24, 37, 38). IGF1-R was found in the differentiated tumors (GHP1 or 2) with steroid receptors.

Since IGF1-R are related to ER and PgR, it was not unexpected to observe a prognostic significance of IGF1-R. The importance of its prognostic value was important whatever the test used, in RFS as well as in OS studies. The median duration of follow-up was too short to allow any definite conclusion on subgroup analysis. Nevertheless IGF1-R was a significant prognostic factor on OS and RFS in PgR-negative patients. The number of IGF1-R-negative, node negative patients was too low to allow the study of the prognostic significance in that subgroup of patients where new prognostic factors are needed. In multiparameter Cox analysis combining IGF1-R with the most important biological and clinical factors to evaluate their respective weight, IGF1-R was an important biological parameter in RFS as well as OS studies. When combining IGF1-R with the pathological prognostic factors, each individual factor remained prognostic; IGF1-R cannot replace GHP or axillary node metastases. Their association was a very significant prognostic factor.

It might seem paradoxical that a tumor containing receptors for a growth factor, IGF1-R, had a better prognosis than a tumor without. One speculation could be that IGF1-R were measurable mostly when they were not occupied by endogenous IGF1 and thus not stimulated by this growth factor; in fact the binding of IGF1 in our experience was partly reversible (23) and thus the former speculation is unlikely to be correct. Another hypothesis could be that tumors with IGF1-R have retained some physiological growth control by IGF1 which could explain the better prognosis. This hypothesis is sustained by the close links between IGF1-R, ER, PgR, and differentiation. It is important to know the regulation of IGF1-R to allow the study of the results to be interpreted. In vitro it has been demonstrated recently that IGF1 was not produced by breast cancer cells (16) excluding an autocrine regulation of IGF1-R; however, these cells can modulate the exogenous IGF1 action through the production of an IGF1-binding protein secretion (17, 18). IGF1 or steroid action on IGF1-R is not known.

When dividing the patients into 3 groups according to IGF1-R levels, negative (<1%), borderline (1–2%), and positive (>2%), RFS (P = 0.011) as well as OS (P = 0.005) were significantly different between these 3 groups when studied by actuarial survival analysis. We looked for a special prognostic significance of very high IGF1-R levels (25% highest levels) but there was no significant difference with 75% lowest IGF1-R levels. Patients with very high IGF1-R levels do not have either a better or a worse prognosis. In the experience of Fockens et al. (38), no prognostic significance of IGF1-R could be found when dividing the patients in 3 groups. We did not find any prognostic significance of IGF1-R in this case either. Our results confirm the clinical value of the biochemical threshold of IGF1-R.

Our results show that patients with IGF1-R positive tumors had a better prognosis on RFS as well as on OS than patients without.

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