Prognostic Significance of Prolactin Receptors in Human Breast Cancer

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ABSTRACT

Overall survival and relapse free survival (RFS) were studied in 547 patients according to the presence of prolactin receptors (PRL-R), either free or total (after 3 M MgCl₂ desaturation). All these patients were surgically treated for locoregional disease in the same institution between 1978 and 1984. In actuarial survival studies, RFS was higher in total PRL-R positive patients in the whole population (P < 0.02). When the population was divided into two groups, according to either the presence or the absence of node metastasis or the presence or absence of estradiol receptor, the higher RFS was restricted to node positive (P < 0.001) and to estradiol receptor positive patients (P < 0.01). The Cox analysis on RFS showed that free PRL-R alone was a significant prognostic factor in estradiol receptor positive patients; total PRL-R alone was never significant; when considered together with steroid receptors, free as well as total PRL-Rs were significant prognostic factors in some subgroups of patients.

INTRODUCTION

The role of lactogenic hormones in the induction and growth of rodent mammary tumors is well known (1). However, it is not established if these hormones have any connection with the development of human mammary cancer (2, 3). Because prolactin action is mediated by specific membrane receptors (4), we and others sought PRL-R in tumor tissue (5-13). In most studies PRL-R were found in about 50% of the cases. However, the presence of PRL-R does not automatically imply prolactin sensitivity. We showed that not all PRL-R+ tumors respond to PRL with an increase in DNA synthesis (14). However, PRL-R- tumors never respond to prolactin. α-Lactalbumin production in vivo was noted in patients with PRL-R+ or PRL-R- tumors (15).

The aim of this study was to determine the clinical significance of PRL-R; in this work we present our results on the OS and RFS as a function of either free or total PRL-R in 547 patients in whom PRL-R assays were carried out at the time of curative surgery.

PATIENTS AND METHODS

Patients

Included in this study were 547 female breast cancer patients. Of these 65% were postmenopausal and all had locoregional disease. 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 radiation therapy on the chest wall after total mastectomy, or on the remaining breast tissue after segmentectomy, and on the internal mammary, subcutaneous, and supraclavicular nodes.

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1 This work was supported by the Ligue Nationale de Lutte Contre le Cancer du Nord et du Pas-de-Calais and by the Association pour la Recherche sur le Cancer (Villejuif).

2 The abbreviations used are: PRL-R, prolactin receptor(s); PRL-R+, PRL-R-, prolactin receptor positive or negative (other receptor statuses are similarly designated); OS, overall survival; RFS, relapse free survival; N+, N-, node positive or negative; ER, estradiol receptor; PgR, progesterone receptor; FPRL-R, free PRL-R; TPRL-R, total PRL-R. Only node positive patients received adjuvant treatment with chemotherapy (cyclodiphosphamide, 5-fluorouracil, Adriamycin, or methotrexate) and, in ER or PgR positive patients, hormone treatment (radiation castration in premenopausal patients and tamoxifen after the menopause). The chemotherapy protocols were similar to those in common use in other centers.

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 done yearly; other x-rays, scans, or echographies were only symptom oriented. The duration of patient follow-up ranged from 1 month to 6 years; the number of remaining patients at the beginning of each 6-month period is noted in Fig. 2.

Pathology

The type (lobular or ductular), differentiation, and histoprognostic grading were noted together with the number of invaded nodes and the number of excised nodes.

Methods

Prolactin Receptors. PRL-R assays were performed according to the method of Shiu et al. (16) in 547 primary breast cancers between 1979 and 1984. The methods used were described in previous papers (12, 13). The frozen tissues were weighted, pulverized with a Thermovac tissue pulverizer (Thermovac Industries Corp., NY), and homogenized in 0.02 M 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 resulting supernatant (cytosol) was carefully removed and used for the steroid receptor assay (12). The pellet (microsomal fraction) was resuspended in 25 mM Tris-HCl-10 mM MgCl₂, pH 7.6. FPRL-R were determined by incubating for 16 h at room temperature 400 μg of membrane proteins with approximately 100,000 cpm of iodinated human growth hormone (17), in the presence or absence of a 1,000-fold excess of unlabeled ovine prolactin (1 μg). The final incubation volume was adjusted to 0.5 ml with Tris-MgCl₂ buffer containing 0.1% bovine serum albumin. Since prolactin does not dissociate from its receptors during membrane preparation, TPRL-R was also assayed. To accomplish this, membranes normally used in the assay were preincubated with 0.5 ml of 3 M MgCl₂ for 5 min and 4 ml of cold Tris-HCl buffer (pH 7.6) containing 0.1% bovine serum albumin were then added to each tube (17). Following centrifugation at 2,200 × g, the pellets were resuspended in Tris-HCl buffer and binding of the labeled hormone was assayed as described above. Specific binding was calculated as the difference between the cpm bound in the absence and the presence of the excess unlabeled hormone and expressed as the percentage of the total radioactivity added to the tube.

FPRL-R (without in vitro desaturation) were measured in 432 tumors; TPRL-R (after in vitro desaturation) were measured in 387 patients. The receptor assays were carried out at different times. In each assay, 70 to 100 patients were included. Duplicates were used for total and nonspecific binding determination. In each series a pool of human mammary cancer membranes and of T-47D cells (PRL-R rich breast cancer cell in long term tissue culture) was included to assess the reproducibility of the assay. The within assay coefficient of variation was 7%; the between assay coefficient of variation was 9%. There was no significant variation between assays, either in the range or in the mean level of PRL-R. A tumor was considered F- or TPRL-R+ when the specific binding was greater than 0.8%. We used 0.8% for the technical cutoff because when the assay was 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

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prolactin was always less than 0.8% (12, 13). There was no significant variation in the percentage of PRL-R positivity between these assays. ER and PgR. ER as well as PgR were determined by the dextran coated charcoal method (18, 19). The laboratory performing those receptor determinations is affiliated to the European Organization of Research and Treatment of Cancer which organizes quality controls of the assays (20). Tumors with more than 3 fmol/mg protein ER were considered ER+ and tumors with more than 25 fmol/mg protein PgR were considered PgR+. The PgR threshold had been determined 8 years ago at a time when methodological difficulties still existed and was maintained for homogeneity of the results.

Statistical Analysis

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 (21) was used to assess the prognostic significance of different clinical, pathological, and hormonal factors taken individually and in association. Statistical analysis was performed using DASH Software (Dash Software Development Group, Boston, MA) on a Vax 11/780 computer.

RESULTS

Receptor Determinations

ER and PgR

ER were found in 81% and PgR in 57% of the patients.

PRL-R

Positivity Rate of PRL-R. FPRL-R were found in 43% and TPRL-R in 72% of the tumors.

Both assays were done in 285 patients; F- and TPRL-R were found in 38% of the cases, neither F- nor TPRL-R in 20%, only TPRL-R in 36%, and only FPRL-R in 6%. This last group could be explained by the loss of proteins during desaturation (17).

PRL-R Levels. PRL-R levels are shown in Fig. 1 for F- and TPRL-R. When considering the subgroups of TPRL-R+ tumors, the median value of the difference between T- and FPRL-R was 1.2% range, (0–13.7%).

Actuarial Survival Studies as a Function of ER and PgR

OS was better in ER+ (P = 0.0001) and PgR+ patients (P = 0.02); when the study was restricted to N+ patients, OS was better in ER+ (P = 0.0001) and in PgR+ (P = 0.0008) patients. No prognostic significance of ER and PgR was found for OS in N− patients.

RFS was better in ER+ (P = 0.03) but not in PgR+ patients. When the study was restricted to N+ patients, RFS was better in ER+ (P = 0.0001) and in PgR+ (P = 0.008) patients. In N− patients neither ER nor PgR had a prognostic significance for RFS.

Actuarial Survival Studies as a Function of PRL-R

All the patients could be included for OS studies; for the RFS studies, 365 patients could be included in the TPRL-R group and 410 in the FPRL-R group because no information on relapse could be obtained in the other patients.

Neither F- nor TPRL-R had any prognostic significance on OS in the whole population. Similarly, no prognostic value was noted when the population was divided into two groups according to either nodal status or ER.

Conversely, RFS was better in patients with TPRL-R (Fig. 2a) (χ² = 6.57; P < 0.02). The repartition of the prognostic factors between TPRL-R+ and TPRL-R− patients is shown in Table 1. Axillary node metastasis has been divided into 4 groups (node negative, less than 3 nodes, between 3 and 10, and more than 10 invaded nodes) since in our population, patients without node metastasis and patients with one or two nodes had the same OS or RFS. TPRL-R positive patients more often had between 3 and 10 invaded nodes (P < 0.03). No difference in the other prognostic factors was found between these two groups.

Similarly, when the population was divided into two groups according to ER status, the prognostic value was restricted to ER+ patients (Fig. 2b) (χ² = 9.1; P < 0.01). Other prognostic factors of patients in these two subgroups are listed in Table 2. ER+ TPRL-R+ patients were less likely to have less than 3 invaded nodes (P < 0.04); they more often had between 3 and 10 invaded nodes (P < 0.03).

When the population was divided into two groups according to nodal status (N+ or N−), the prognostic significance of TPRL-R was restricted to N+ patients (Fig. 2c) (χ² = 12.7; P < 0.001); characteristics of patients in these 2 subgroups are listed in Table 3. N+, PRL-R+ patients were more likely to have a grade 2 tumor (P < 0.05) and to be ER+ (P < 0.01).

Cox Analysis

Overall Survival (Table 4). In our experience, the only prognostic factors for OS (Table 4) were the tumor diameter (P < 0.0001) and the number of invaded nodes (P < 0.0001). Neither ER, nor PgR, nor PRL-R (either free or total) had a significant prognostic value; the association ER and PgR was of borderline significance (P = 0.08). In N+ patients, the number of invaded nodes (P < 0.003) and the tumor diameter (P = 0.02) had a prognostic value. However, no hormone receptor either alone or in association had any prognostic significance. In N− patients, the age of the patients (P = 0.02), the associations ER, PgR, and PRL-R (either free or total) (P = 0.04), ER and TPRL-R (P = 0.05) had a prognostic significance. The prognostic factors for OS in ER+ patients were the number of invaded nodes (P = 0.0002) and the tumor diameter (P = 0.02).
The only prognostic factor for OS in ER− patients was the number of metastatic nodes (P = 0.005).

Relapse-free Survival (Table 5). The prognostic factors for RFS were the number of invaded nodes (P < 0.0001) and the association ER, PgR, and FPRL-R (P = 0.04); the association of ER, PgR, and TPRL-R was of borderline significance (P = 0.08). In N+ patients the number of invaded nodes (P = 0.01) and PgR (P = 0.03) had a prognostic significance for RFS. In N− patients the association of ER, PgR, and FPRL-R was a prognostic factor (P = 0.04) as well as the age of the patients (P = 0.05). The only prognostic factor in ER− patients was the number of invaded nodes (P = 0.005). In ER+ patients FPRL-R (P = 0.02) were a significant prognostic factor as well as the...
DISCUSSION

The actuarial survival analysis indicates that in our patients ER had a prognostic significance for OS. ER and PgR were prognostic factors for OS and RFS in N+ patients; these results are in agreement with those of Hähnel et al. (22) and Hubay et al. (23) for ER and Mason et al. (24) for PgR. Our results show that PRL-R are also prognostic factors. Patients with TPRL-R had a better RFS. This difference was found in the whole population in spite of a more frequent nodal involvement in TPRL-R+ patients. The number of patients by the end of the study was obviously very small, so it is not possible to be sure that there was a plateau. However, by the logrank test comparing two curves, it was thus possible to stress that differences in RFS were significant at least during the first years after surgery. Similarly the number of patients in the ER− group is too small to draw any definite conclusion on the prognostic significance of PRL-R in that subgroup. More patients in the TPRL-R+ group had nodal involvement and thus received adjuvant treatment (52.3% versus 42.6%, not significant); conversely more ER+ TPRL-R− patients had nodal involvement and thus received adjuvant treatment (55.3% versus 40.4%) (P < 0.03). The role of treatment on the prognosis cannot be excluded, but it would then have affected the RFS in the whole population and in the ER+ group in the opposite way. The absence of prognostic significance of PRL-R in N− patients may be explained by the length of follow-up which is too short for a study of prognostic factors in that subgroup. To our knowledge only Waseda et al. (25) have published results obtained by the actuarial method on the prognostic significance of PRL-R; this author found PRL-R in only 13% of the tumors, which is considerably less than the frequency generally found; since the number of patients in his study was not very important, such a small percentage led to a great difference between the numbers of PRL-R+ and PRL-R− patients. The poor prognosis of PRL-R+ patients could be explained by this fact.

According to the Cox analysis of the RFS, FPRL-R values were of borderline significance in the whole population (P = 0.08) as well as in N+ patients (P = 0.06); in ER+ patients FPRL-R were statistically significant (P = 0.02). The association ER, PgR, and FPRL-R was significant in the whole population and in the N− patients (P = 0.04). FPRL-R were stronger prognostic factors in the Cox analysis and TPRL-R in the actuarial studies; PRL-R (except FPRL-R in ER+ patients) were never prognostic factors when considered alone. The differences between actuarial and Cox studies could be accounted for by the fact that in the former only the presence of receptors is studied whereas in the latter a continuum of values is taken into consideration; the level of the TPRL-R may be less useful to determine prognosis than the presence of these receptors. In our experience, the level of TPRL-R did not appear to have a prognostic significance for RFS. A study had been carried out using a cutoff value of 2% (data not shown). Another explanation could be that PRL-R were not independent from the other prognostic factors.

According to Cox analysis, ER and PgR never appeared to be prognostic factors (nor did steroid receptors). The prognostic significance of the combination ER, PgR, and PRL-R in N− patients (a similar result was noted on FPRL-R on RFS studies) must be interpreted with caution since the duration of follow-up was rather short to assess prognostic factors in N− patients. These results suggest a selection of highly differentiated tumors by these 3 receptors.

According to Cox analysis, ER and PgR never appeared to be prognostic indicators influencing survival; PgR was the only individual factor of prognostic importance for RFS in N+ patients. ER had no impact on RFS. Our observation confirms the earlier work of Clark et al. (26).

Relationships between PRL-R and steroid receptors are suggested in the literature. We showed in 92 patients (13) and confirmed in this population (27) that a statistically significant correlation existed between ER and PgR, between ER and T- or FPRL-R, and between PgR and T- or FPRL-R. This had been suggested by Holdaway and Friesen (6) and Stagner et al. (10) and confirmed by Murphy et al. (28) for cell lines as well as for mammary cancer. Partridge and Hähnel (7) found that the affinity of PRL-R was higher in ER+ tumors. Conversely neither Rae-Venter et al. (9) nor Waseda et al. (25) found a relationship between PRL-R and steroid receptors. Our present results are in favor of a hormonal system involving ER, PgR, and PRL-R in human breast cancer. Experimentally, estradiol stimulates PRL-R biosynthesis in vitro in EFM-19 cells (29) or in vivo (30). Conversely, prolactin stimulates the appearance of ER in human breast cancer cells (31); antiprolactin treatment leads to a decrease in ER levels in rat mammary tumors (32). The better prognosis found in ER+ PRL-R+ patients could be explained by a selection of patients with the most differentiated tumors.

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Table 4 Prognostic factors for overall survival (Cox analysis)

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<tr>
<th>Variable</th>
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<th>N+</th>
<th>N−</th>
<th>ER+</th>
<th>ER−</th>
</tr>
</thead>
<tbody>
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<td>Tumor diameter</td>
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<td>0.02</td>
<td>0.11</td>
<td>0.02</td>
<td>0.60</td>
</tr>
<tr>
<td>N+ no.</td>
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<td>0.003</td>
<td>0.0002</td>
<td>0.0005</td>
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<tr>
<td>Age</td>
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<td>0.94</td>
<td>0.02</td>
<td>0.33</td>
<td>0.75</td>
</tr>
<tr>
<td>GHP*</td>
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<td>0.98</td>
<td>0.30</td>
<td>0.66</td>
<td>0.34</td>
</tr>
<tr>
<td>ER</td>
<td>0.23</td>
<td>0.79</td>
<td>0.07</td>
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<tr>
<td>PgR</td>
<td>0.11</td>
<td>0.20</td>
<td>0.34</td>
<td>0.14</td>
<td>0.07</td>
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<tr>
<td>FPRL-R</td>
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<td>0.45</td>
<td>0.67</td>
<td>0.36</td>
<td>0.68</td>
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<tr>
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<td>ER, PgR, FPRL-R</td>
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<td>0.19</td>
<td>0.04</td>
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<tr>
<td>ER, FPRL-R</td>
<td>0.35</td>
<td>0.60</td>
<td>0.07</td>
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</tr>
<tr>
<td>ER, TPRL-R</td>
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<td>0.28</td>
<td>0.05</td>
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</tr>
<tr>
<td>PgR, FPRL-R</td>
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<td>0.25</td>
<td>0.66</td>
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* GHP, histoprognostic grading (33).

Table 5 Prognostic factors for relapse free survival (Cox analysis)

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<td>0.50</td>
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<tr>
<td>PgR</td>
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* GHP, histoprognostic grading (33).
REFERENCES


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