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Correlation Between Prolactin Receptors (PRL R), Estradiol (ER) and Progesterone Receptors (PgR) in Human Breast Cancer

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Abstract—*Free* (n = 432) *and total* (n = 387) *prolactin receptors* (*PRL R*) (*after* 3 M MgCl2 *desaturation*) *as well as estradiol* (*ER*) *and progesterone receptors* (*PgR*) *were measured in* 547 *breast cancer patients surgically treated in the Oscar Lambret Centre. Free PRL-R were found in* 43% *of the cases, total PRL R in* 72%, *ER in* 81% *and PgR in* 55%. *A statistically significant correlation was found by the Spearman test between ER on the one hand free PRL R* ($P < 0.02$) *and total PRL R* ($P < 0.001$) *on the other and between PgR on the one hand, free PRL-R* ($P < 0.05$) *and total PRL R* ($P < 0.01$) *on the other. A linear correlation test could be done on subgroups of values, excluding zero values, of each of the receptor type; a statistically significant correlation could be found between ER and total PRL R* ($P < 0.001$) *and between PgR and total PRL R* ($P < 0.05$). *These results confirm, on a large series, the relation between PRL R, ER and PgR in breast cancer.*

INTRODUCTION

THE INVOLVEMENT of prolactin in mammary tumors is well established in rodents either during the induction phase, in rats and mice, or during the induction and growth of the tumors in rats. As prolactin plasma levels were of poor value to study prolactin sensitivity of human breast cancer, we looked for PRL R on tumor plasma membranes [1, 2]; these receptors have been characterized [3]. In a first series of 92 primary breast cancers we had found a correlation between PRL R and ER as well as between PRL R and PgR [2]. We confirm our results on a greater series of 547 patients.

PATIENTS AND METHODS

Patients

All the patients included in that study were surgically treated for primary breast cancer. They all had local or regional disease. Some of them (T3 and T4 tumors according to the TNM classification) ($n = 27$) had two courses of chemotherapy (including Adriamycin, Cyclophosphamide, 5 Fluorouracile or Methotrexate) before surgery. No difference was found in either type of receptor positivity whether patients had prior chemotherapy or not.

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Methods

Prolactin receptors. PRL R assays were performed in 547 primary breast cancers according to Shiu [4]. Four hundred micrograms of membrane proteins were incubated with approximately 100 000 counts/min of iodinated hGH in the presence or absence of a 1000-fold excess of unlabeled oPRL (1 μg). The final incubation volume was adjusted to 0.5 ml with Tris–$MgCl_2$ buffer (pH 7.6) containing 0.1% bovine serum albumin (free PRL R). Since PRL does not appear to dissociate from its receptors during membrane preparation, when it was possible, desaturation of occupied receptors with $MgCl_2$ 3 M was performed before the assay of PRL sites [1] (total PRL R). Free PRL R (without *in vitro* desaturation) were measured in 432 tumors; total receptors (after *in vitro* desaturation) in 387 patients. Both determinations were carried out in 286 patients. Tumors were considered positive if the specific binding was more than 800 cpm.

Estradiol and progresterone receptors. ER was determined by the DCC method [5] as well as PgR [6]. The ER content of the diluted cytosol was determined by incubation of 100 μl cytosol (in duplicate) with 50 μl of four doses (10, 5, 1 and 0.5 mM) of (^{3}H) 2,4,7-estradiol with and without a 100-fold excess of diethylstilbestrol (DES). PgR

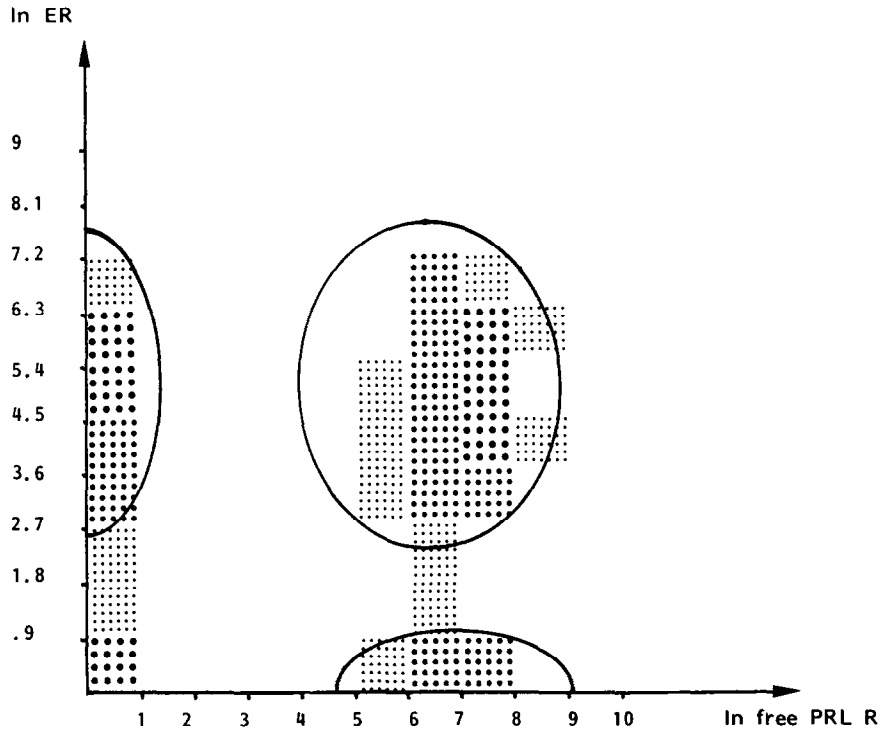

Fig. 1. Distribution of ln free PRL R values as a function of ln ER values. Three groups of values could be noted. □ < 3 patients, ⁘ 11–20 patients; ⁙ 4–10 patients; ⁘ > 21 patients.

was assayed with four doses (10, 5, 1 and 0.5 mM) of (^{3}H)R_{5020} with and without a 100-fold excess of non-labeled R_{5020}.

The radioactive ligands and cytosol were incubated at 4° C for 16 hr. A 500 µl suspension of dextran-coated charcoal (DCC) (2,5 mg Norit A-activated charcoal, 250 µg dextran in 1 ml Tris buffer, pH 8,0, 4° C) was added to each tube. Tubes were then shaken for 45 min at 0–4° C and then sedimented by centrifugation at 4° C for 20 min at 3000 rev/min. The DCC-treated supernatant was combined with 3 ml of Beckman Ready-Solv scintillator and counted in a Beckman Model LS 6800 liquid scintillation counter. The ER and PgR data were analyzed by the method of Scatchard to determine the dissociation constant (K_d) and concentration of ER and PgR in each tumor specimen, expressed as fmol of receptor/mg cytosol protein. The laboratory performing those receptor determinations is affiliated to the EORTC (European Organization of Research and Treatment of Cancer) which organizes quality controls of the assays. 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+.

Statistical analysis

The distributions of the parameters (ER, PgR, PRL R) were previously studied. They were log normal (ln) after excluding zero values. Relation between variables were carried out according to Spearman R non-parametric correlation test. In order to explicit these relations, linear regression analysis was performed on subsets of the statistical population when it was possible.

RESULTS

Frequence of positivity of ER, PgR, PRL R

ER was found in 81% of the cases and PgR in 55%. Free PRL R were found in 43% of the patients and total PRL R in 72% of the cases.

Relation between ER and PRL R

The study has been carried out with the 2 types of PRL R. A relation was found by the Spearman test with free PRL R ($P = 0.02$), and total PRL R ($P < 0.001$).

However this relation was complex as shown in Figs. 1 and 2. By representing ln (ER) as a function of ln (PRL R) (either free or total) we found that three populations of results could be individualized: (1) zero values of ER, (2) zero values of PRL R (either free or total), (3) non-zero values of both ER and PRL R (either free or total). Therefore, we must limit the study of a linear correlation to that third population. No linear correlation was found between ER and free PRL R expressed as ln-values ($r = 0.074$; $n = 260$) but a significant correlation was demonstrated between ln ER and

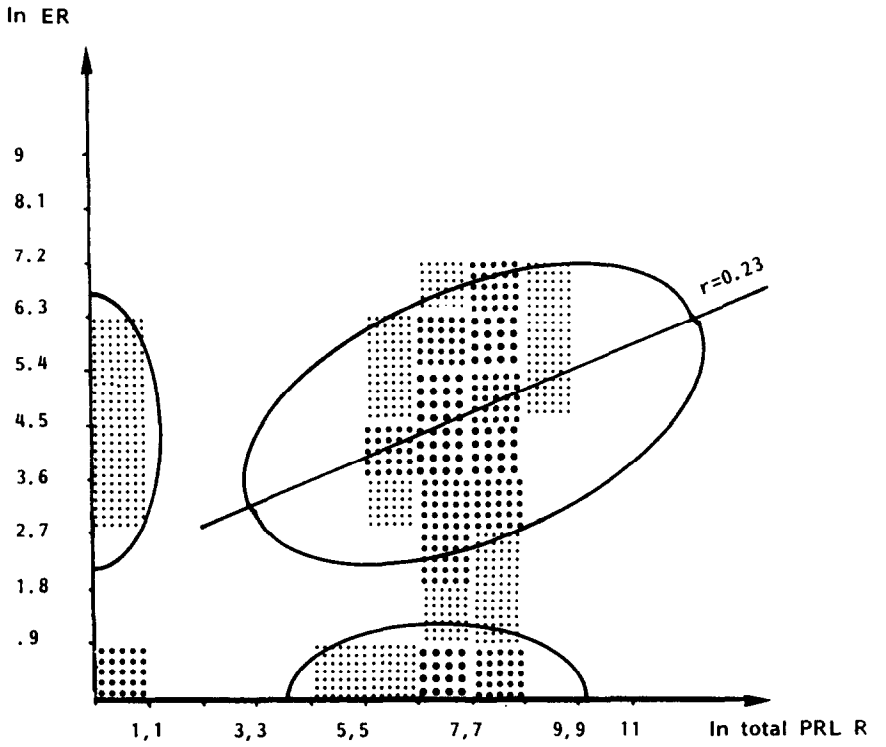

Fig. 2. Distribution of ln total PRL R values as a function of ln ER values. Three groups of values could be noted. When zero values were excluded, a statistically significant linear correlation could be found between ln total PRL R and ln ER (r = 0.23, $P < 0.001$).

ln total PRL R ($r = 0.23$; $n = 289$; $P < 0.001$).

The relations between ER and PRL R were studied separately for pre- and post-menopausal patients. No correlation was found for pre-menopausal women; in post-menopausal ones a relation was found by the Spearman test with total PRL R ($P < 0.001$) and by the linear correlation test ($r = 0.21$; $n = 254$; $P < 0.001$).

Relation between PgR and PRL R

A relation was found between PgR and total PRL R ($P < 0.01$) free PRL R ($P < 0.05$) by the Spearman test.

The bidimensional representations of PgR and PRL R (Figs. 3 and 4) showed as previously that 3 populations could be individualized: (1) zero values of PgR, (2) zero values of PRL R (either free or total), (3) non-zero values of both ER and PRL R (either free or total). The study of correlation was performed in that third population: no significant correlation was found between free PRL R and PgR expressed as ln values ($r = 0.55$; $n = 202$) but a significant correlation was noted between ln PgR and ln total PRL R ($r = 0.123$; $n = 226$; $P < 0.05$).

The relations between PgR and PRL R were studied separately for pre- and post-menopausal patients. No correlation was found for pre-menopausal women; in post-menopausal ones, a relation was found by the Spearman test with free PRL R ($P < 0.05$) and total PRL R ($P < 0.001$) and by the linear correlation test with total PRL R ($r = 0.29$; $n = 250$; $P < 0.001$).

DISCUSSION

In this study we have confirmed the correlation between PRL R on one hand, ER and PgR on the other. The relation between ER and PRL R was stronger with total PRL R than with free PRL R. This could be explained by the fact that among $MgCl_2$ desaturated receptors there were receptors involved in prolactin action. The correlations were found only in post-menopausal patients.

Holdaway [7] on 7 breast cancers noted that PRL R positive tumors were generally ER+. Partridge [8] related, on a small number of cases, that the affinity of PRL R was higher in the ER+ tumors. A relation between ER and PRL R had been found by Stagner [9]; his results have been published in an abstract form and, to our knowledge, have not been confirmed later on. He found that of the six tumors that lacked lactogenic hormone binding, none had ER and of 8 tumors with ER, all had receptors for lactogenic hormones. Rae Venter [10] in a series of 55 tumors, did not find any correlation between ER and PRL R; they found that the tumors with ER concentration of 6 to 100 fM/mg of protein had a higher mean level of PRL R than ER− tumors. The absence of correlation could be due to the low levels of PRL R

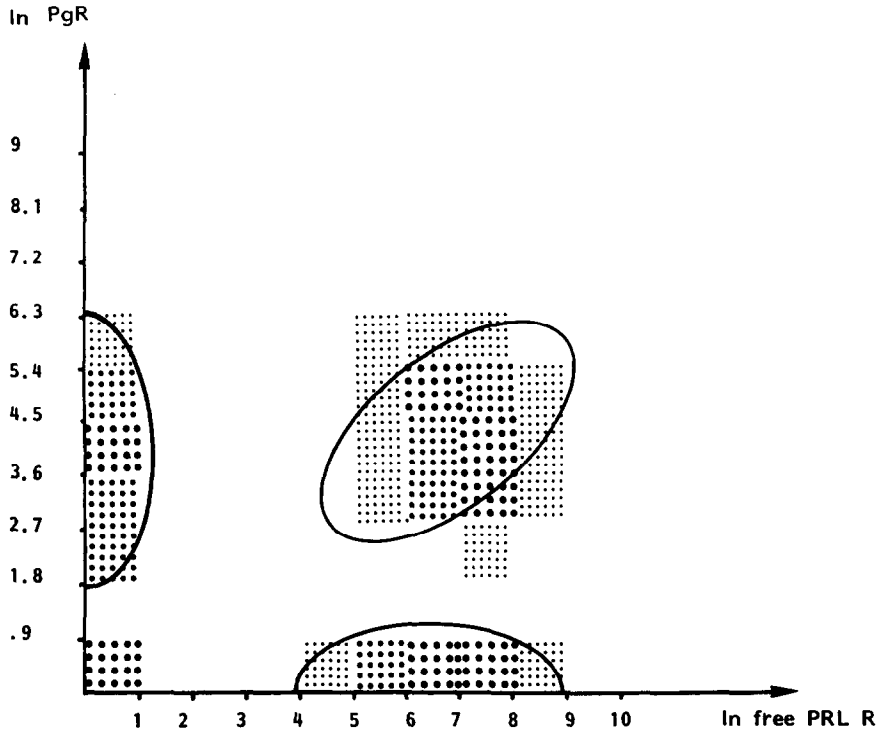

Fig. 3. Distribution of ln free PRL R values as a function of ln PgR values. Three groups of values could be noted.

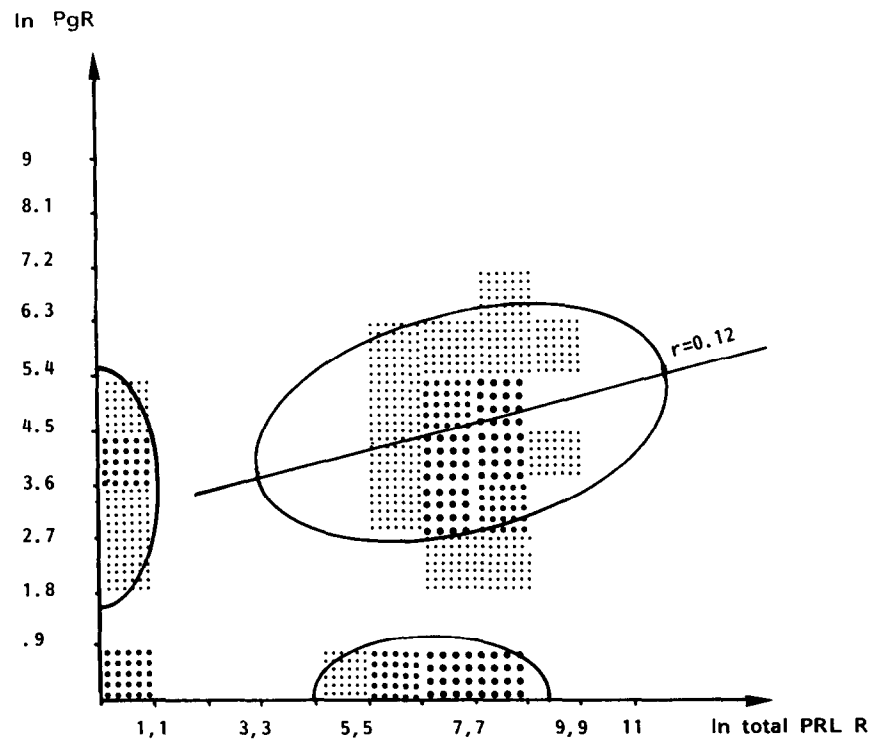

Fig. 4. Distribution of ln total PR R values as a function of ln ER values. Three groups of values could be noted. When zero values were excluded, a statistically significant linear correlation could be found between ln total PRL R and ln PgR ($r = 0.12$, $P < 0.05$).

in the tumors where the ER level was high (> 250 fM/mg protein). Murphy [11] has also found a correlation between PRL R and ER in 30 tumors. To our knowledge, no other author has reported a correlation between PgR and PRL R. However since PgR is linked to ER that is linked to PRL R, such an association was not unexpected. The absence of correlation between PRL R and steroid receptors in premenopausal patients was surprising. An explanation could be that high PRL R levels were more frequently found in post-menopausal patients. It cannot be excluded that the hormonal system involving prolactin, estradiol and progesterone is not functional in premenopausal women.

A relation between ER and PRL R had recently been found by Murphy in 1984 [11] on cell lines of human origin. He could not find PRL R binding in ER− cells. However, Shiu [12] had found low levels in some ER− cells.

The positive correlation between PRL R and ER in breast cancer biopsies and in breast epithelial cell lines in long term tissue culture suggests that the expression of these two receptors is linked. From a theoretical point of view, this relation is not surprising since many studies of experimental mammary tumors as well as normal tissues have shown that prolactin, estradiol and progesterone as well as their receptors were interlinked [13]. Experimentally, estradiol stimulates the biosynthesis of PRL R either *in vitro* in T-47 D or MCF-7 cells [14] and in EFM 19 cell line [15] or *in vivo* [16]. Posner [17] has shown that estradiol has a direct or indirect effect on the expression of PRL R in rat liver tissue. Conversely, prolactin stimulates the appearance of ER [13] and anti-prolactinic treatment lowers ER levels [18] or ER and PgR levels [19]. Prolactin is able to increase ER concentrations in DMBA induced tumors *in vivo* [20, 21] and *in vitro* [22]. In one breast cancer epithelial cell line in long term tissue culture [23] and in the mammary gland of the normal mouse [24], prolactin induced the transformation of the ER from its inactive to its active form and increased the level of nuclear receptors; this might explain that we have observed an increase in the number of positive ER tumors in breast cancer in premenopausal women after bromocriptine treatment [25].

A relation between the receptor levels seems thus probable. Furthermore, De Sombre [26] had noted that DMBA induced mammary tumors in rats were more likey to regress after ovariectomy if they had ER and PRL R. Powell [27] obtained better regression of MTW9 tumors in rats with higher levels of PRL R. Holdaway [28] has pointed out that the tumors that contained PRL R also had significant levels of steroid receptors.

The presence of PRL R does not automatically imply prolactin sensitivity. The receptor machinery may not be functional. It is probably the case in some patients. This has been shown experimentally [28]; in our experience not all the tumors with PRL R synthetise DNA after stimulation with prolactin [29]. Similarly alpha-lactalbumin production seems to be independent of the presence of PRL R in patients [30]. An association between ER and PRL R might thus only exist in prolactin sensitive tumors and that could explain why several authors could not find a relation in smaller group of patients.

Another alternative could be that ER and PRL R genes are simultaneously, but independently, expressed in differentiated cancer cells.

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