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Progesterone Receptors and Human Breast Cancer; 'Wassink Lecture' Presented at the 3rd EORTC Breast Cancer Working Conference*

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INTRODUCTION

THE DEVELOPMENT and widespread use of estrogen receptor (ER) assays to predict the endocrine dependence of a patient's tumor has changed the clinician's approach to the treatment of breast cancer. When it was discovered that cytoplasmic ER is responsible for the uptake of estrogen into target cells and is necessary for estrogen action, it was reasoned that ER might be useful in determining the endocrine responsiveness of breast cancers in patients with metastatic disease. This hypothesis has been confirmed, and it is now generally accepted that tumors lacking ER rarely respond to endocrine therapy whereas ER+ tumors frequently regress with hormonal manipulations. Determination of the ER status of the tumor is now standard practice in the United States.

The ER assay is most helpful when the tumor is ER–. These patients rarely respond to endocrine therapy. On the other hand, a positive ER does not guarantee a response to endocrine therapy, since about 40% of patients fail to respond despite a positive assay. Horwitz *et al.* [1] suggested that certain ER+ tumors do not regress with endocrine manipulation because of a defect in the estrogen response pathway distal to the binding step, leading to autonomous growth. What was needed was a way of testing the integrity of the entire metabolic sequence that should be triggered by the binding of estrogen to receptor. Based on the observation that progesterone receptor (PgR) is induced by estrogen in normal reproductive tissues and in human breast cancer cells in culture, they hypothesized that PgR might be a better marker than ER for an intact estrogen response pathway. We and others then began to routinely assay breast tumor tissue specimens for progesterone as well as estrogen receptor.

METHODOLOGY FOR PgR ASSAYS

Early attempts to measure PgR in human breast tumors were not successful due to the interference of progesterone binding to corticosteroid-binding globulin. Furthermore, the high dissociation rate of the receptor–progesterone complexes formed in the tumor cytosol often resulted in poor Scatchard plots. With the availability of R5020, a synthetic progestin which binds more tightly to PgR but only weakly to CBG, the assay technique has been considerably improved. The sucrose density gradient method has been a widely used procedure. But because this method is time-consuming and expensive, the dextran-coated charcoal method is often used, either as a single saturating dose assay or as a multi-point saturation analysis using a Scatchard plot. Namkung and Petra [2] have recently compared these methods and concluded that all of the procedures yield results with excellent agreement for the determination of total PgR. These results agree with those of Powell *et al.* [3], except that the latter found that extrapolation of Scatchard plots yielded receptor concentrations equal only to that calculated from the 8 S area of sucrose gradients rather than 8 S plus 4 S. We agree with the consensus report of DeSombre *et al.* [4] that laboratories involved in the measurement of steroid receptors should be monitored for quality control.

Even with the improvements in assay techniques, the PgR assay is still more difficult to perform than the ER assay. Hull *et al.* [5] recently reported that the occurrence of significant discordance between simultaneous breast tumor

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ER assays from the same patient was only 3%. For PgR, on the other hand, our preliminary findings are that the discordance rate for simultaneous assays is approximately 10%. Similarly, Klinga *et al.* [6] analyzed the PgR assay results from 34 women who had simultaneous biopsies from the primary tumor and an axillary lymph node. About 74% of the patients had identical PgR status in the tumor and in the involved lymph nodes, but PgR was apparently present in the tumor and not in the lymph nodes in 21% of the patients.

EFFECTS OF PRIOR THERAPY ON PgR CONCENTRATIONS

Allegra *et al.* [7] and Hull *et al.* [5] found that endocrine therapy, especially tamoxifen, within 2 months of biopsy resulted in a dramatic false *negative* ER rate. For PgR, however, the effects of prior therapy have only been studied in small series of patients. Mobbs [8] studied the effect of time and therapy on the hormone receptor status of breast carcinomas, but the number of patients with PgR assays was too few to permit statistical analysis. Bressot *et al.* [9] reported a significant reduction in PgR positivity when patients had been treated with radiotherapy prior to biopsy for PgR assay. Degenshein *et al.* [10] found that treatment with DES for 3 days prior to biopsy tended to increase PgR measurements in hormone-dependent tumors that were initially ER+/PgR−. Namer *et al.* [11] studied the effect of tamoxifen on PgR measurements before and after biopsy. In tumors containing ER, 6 of 14 patients showed an *increase* in PgR of greater than 30 fmol/mg cytosol protein, though the prior presence of PgR did not discriminate systematically between hormone-responsive and non-responsive tumors.

DISTRIBUTION OF PgR CONCENTRATIONS

The cutoffs used to distinguish ER-positive and ER-negative tumors are fairly standard. Values greater than 10 fmol/mg cytosol protein are generally considered positive, while those less than 3 fmol/mg are considered negative. ER concentrations between 3 and 10 fmol/mg are usually considered to be intermediate, but are often combined with positive values for statistical analyses. Comparable cutoffs for PgR are not nearly so standard. Various authors have used cutoffs ranging from 1 to 20 fmol/mg cytosol protein. The most common values have been either 10 or 5 fmol/mg.

Since PgR is known to be a result of estrogen action on ER, it might be expected that breast tumors would less often contain PgR than ER. Table 1 shows that this is, indeed, the case. If 10 fmol/mg cytosol protein is used as a cutoff for both ER and PgR, then 58% of the tumors were ER+ compared to only 39% which were PgR+. Using a cutoff of 5 fmol/mg increases PgR positivity to about 48%.

Table 1. Cumulative frequency distributions of steroid receptors

fmol/mg cytosol protein	ER (%) ($n = 7197$)	PgR (%) ($n = 5877$)
<3	30	44
<5	35	52
<10	42	61
<20	50	69
<50	65	79
<100	76	86
<500	99	98
<1000	100	99

CORRELATION WITH PATHOLOGIC FEATURES

PgR status has been correlated with certain pathologic features. Millis [12] reported that well-differentiated tumors were more frequently receptor-positive than poorly differentiated tumors. McCarty *et al.* [13] observed the same relationship, and noted that the relationship between receptor content and histologic grade was enhanced by considering ER and PgR simultaneously. No correlations could be demonstrated between the relative cellularity of the tumor or the apparent invasive character of the tumor and the receptor levels. Delarue *et al.* [14] found that inflammatory tumors showed a higher proportion of PgR negativity than operable tumors.

ADVANCED BREAST CANCER

In order to test the hypothesis that PgR is an effective predictor of endocrine responsiveness, we pooled the results of several investigators around the world (Table 2). Even though receptor assays were performed by different laboratories, treatments administered by several investigators and responses determined by different clinicians, the results are extremely promising.

As predicted, tumors lacking both ER and PgR regressed infrequently (9%) with endocrine therapy, while the highest response rate, 71%, was observed in the group of tumors containing both ER and PgR. This lends credence to the hypothesis that PgR may be a good marker for endocrine dependence.

A 53% response rate was found for ER−/PgR+ tumors. These tumors are rare, and it is possible that inadequacy of assay techniques might account for a portion of this group. Sarrif and

Table 2. Response to endocrine therapy

Reference	ER+/PgR+	ER+/PgR-	ER-/PgR+	ER-/PgR-
[23]	12/20	9/25	0/1	2/20
[10]	26/33	3/14	1/1	0/14
[22]	20/29	3/14	1/2	2/9
[24]	4/6	2/7	-	-
[25]	2/6	0/7	-	0/7
[26]	15/24	3/5	-	0/2
[27]	33/40	2/20	1/3	3/35
[28]	7/10	8/12	-	-
[29]	16/20	14/45	-	3/20
[30]	9/12	2/6	2/3	3/30
[31]	23/30	3/14	1/1	0/10
[32]	7/10	11/15	1/1	2/16
[33]	14/23	1/5	1/3	1/8
Total	188/263 (71%)	61/189 (32%)	8/15 (53%)	16/171 (9%)

Durant [15] reported evidence that some apparently ER-/PgR+ tumors do in fact contain ER, and suggested that ER assays be repeated for these patients after pretreatment of the cytosol with dextran-coated charcoal.

According to the hypothesis, ER+/PgR- tumors should not regress with endocrine therapy, and yet 32% did respond. Perhaps the hypothesis is imperfect, and the PgR synthesis may not be linked sufficiently closely to the pathway of estrogen control of tumor growth to be an accurate predictor in all cases [16].

One of the difficulties with retrospective studies is that patients are frequently treated with a variety of therapies. The Southwest Oncology Group has recently initiated a prospective clinical trial designed to study the relationship between PgR and response to tamoxifen in patients with newly diagnosed metastatic breast disease. Patients who have received prior hormonal adjuvant therapy are eligible, provided they have not failed during therapy and that therapy has been stopped for at least 3 months. Patients must be ER+; PgR may be either positive or negative. All patients will be treated with tamoxifen, 10 mg p.o. twice daily, given alone, until there is documented progression of the disease. The objectives of the study are to relate the response rates and response durations to the amount of PgR and ER in the patients' tumors. It is anticipated that the results of this clinical trial will be available within 2-3 yr. Only with prospective studies such as this will we be able to accurately evaluate the prognostic value of PgR for endocrine responsiveness in advanced breast cancer.

PRIMARY BREAST CANCER

We recently examined the prognostic value of PgR in a series of patients with primary breast cancer and discovered that not only was it a significant factor, but it actually surpassed ER in its ability to predict disease-free survival. The study population consisted of patients who were enrolled on a randomized adjuvant therapy protocol conducted in Cleveland, OH [17]. All patients had a radical or modified radical mastectomy and documented stage II breast cancer. Patients were stratified according to their ER status and the number of positive axillary lymph nodes and were then randomized to one of three adjuvant therapies: (1) cytoxan, methotrexate and 5-fluorouracil (CMF); (2) CMF plus tamoxifen (CMFT); (3) CMFT plus bacillus Calmette-Guerin vaccination. We found that PgR+ patients had longer disease-free intervals than PgR- patients, regardless of treatment

*Table 3. PgR and percentage of patients free of disease**

fmol of PgR/mg cytosol protein	No. of patients	12 months (%)	18 months (%)	24 months (%)	36 months (%)
<5	41	79	68	59	50
5-49	68	100	88	85	65
≥50	80	100	100	97	90

*Ref. [17]. Percentages estimated by actuarial survival analyses of disease-free intervals for stage II patients.

regimen. We also found that the more PgR in the tumor, the more likely a prolonged disease-free survival (Table 3).

We examined several multivariate models for predicting time to recurrence in this population. When PgR was considered in combination with other patient characteristics, both the number of positive lymph nodes and the PgR level were significant prognostic factors. The surprising result was the lack of significance of ER. If the number of positive nodes and the PgR status were known, then the ER status did not provide any additional prognostic power for predicting disease-free survival. On the other hand, PgR always retained its significance no matter what other factors were included in the model.

Other investigators have also concluded that PgR is a significant predictor of disease-free survival. Saez *et al.* [18] reported that the recurrence rate for ER+/PgR+ patients with resectable breast cancer was significantly less than for patients who lacked these receptors. Pichon *et al.* [19] concluded that the presence of PgR in a small group of patients with primary tumors was associated with a markedly lower frequency of metastases. Bertuzzi *et al.* [20] found that PgR was a better discriminator of recurrence than ER for node-negative patients.

ER has been considered an independent prognostic factor for early breast cancer [21]. Based on nearly 4000 patients with primary disease, we have found this to be true also for PgR. PgR status does not correlate with several other known prognostic factors: the number of positive axillary nodes, the location of the primary tumor or a family history of breast cancer. When analyzed univariately, PgR correlates with neither age nor menopausal status, though when age and menopausal status are considered together, premenopausal women have higher PgR levels than postmenopausal women of the same age. Large primary tumors are more likely to be PgR- than smaller tumors, perhaps due to tumor necrosis or perhaps simply as an indication of more aggressive disease.

In summary, the ER assay has become a standard practice in the management of breast cancer. ER negativity effectively identifies patients who will not benefit from endocrine therapy. However, response rates of only 50–60% are observed in ER+ tumors. There is increasing evidence that PgR is also a powerful prognostic factor and may be a better marker of tumor hormone dependence than ER. We suggest that, in addition to ER, PgR be routinely measured in breast cancer tumors. Only with carefully designed prospective clinical trials utilizing both ER and PgR will we be able to properly evaluate the utility of these two important prognostic factors.

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