Article is available online at http://www.webio.hu/por/2006/12/4/0223

ARTICLE

Estrogen Receptor α-Negative and Progesterone Receptor-Positive Breast Cancer: Lab Error or Real Entity?

Jawad KIANI, Afrasyab KHAN, Hina KHAWAR, Fawad SHUAIB, Shahid PERVEZ

Department of Pathology and Microbiology, The Aga Khan University, Medical Center, Karachi-74800, Pakistan

A retrospective study comparing the estrogen receptor (ER) α subtype and progesterone receptor (PR) profile of breast carcinomas amongst 1625 cases over 2.5 years was carried out. Strictly speaking it is generally believed that breast carcinomas can biochemically express PR only if they are ER-positive. However, a few ERα-PR+ cases do exist paradoxically. This class of tumors was the focus of our study in which we looked at the possible reasons for such an immunophenotype and compared it with a group of ERα+PR+ breast carcinomas. An internationally recognized immunohistochemical method employing monoclonal antibodies against estrogen and progesterone receptors was used. Correlations with established risk factors i.e. menopausal status, grade, tumor size and lymph node status were analyzed for our study group (ERα-PR+) and compared with a control (ERα+PR+). Out of the total 1625 cases, 29.91% (486) were ERα+PR+, 5.11% (83) were ERα+PR-, 56.86% (924) were ERα-PR- and 8.12% (132) were ERα-PR+. Patients' age was significantly lower in the ERα-PR+ group (P=0.002). Statistical analysis of the grading between the two study groups revealed no significant difference (P=0.091), although the ERα-PR+ group contained significantly more poorly differentiated tumors than the ERα+PR+ one (P=0.032). Tumor size was also significantly larger in the ERα-PR+ than in the ERα+PR+ group (P=0.046). The frequency of lymph node metastases was independent of receptor profile. In conclusion, our study group does exhibit characteristics which are suggestive of a distinct breast cancer phenotype (ERα-PR+) with a different etiology and prognosis.(Pathology Oncology Research Vol 12, No 4, 223–227)

Key words: estrogen receptor (ER) α, progesterone receptor (PR), breast carcinoma

Introduction

The measurement of estrogen receptor alpha (ERα) has become an important routine procedure in breast carcinoma, because the presence of ERα in the tumor indicates a higher chance of response to anti-estrogen hormonal therapy.[20] Positivity for ERα is therefore correlated with a better prognosis.[24] Biochemically, PR is one of the end products from estrogenic stimulation in the target tissues.[10,31,38] Thus, the demonstration of PR suggests the presence of ER by default. The presence of PR, in addition to ER suggests further the likelihood of response to hormonal therapy, presumably because the presence of PR indicates an intact and functioning ER pathway.[21] There is general agreement that approximately half of the women whose tumors have detectable ERα will obtain objective remission from some form of endocrine therapy.[5] This number increases to three quarters when PR, an estrogen-induced protein is included.[5] It has been suggested that since the synthesis of PR denotes a functional ER, its measurements with ER can be of greater value than the measurement of ER alone.[5] The prognostic impact of ER status is still under investigation but seems to indicate that significant amounts of ER in breast tumors are associated with a longer disease-free survival.[2,12] It is proposed that after some cell divisions and while still retaining some proliferative capacity the ER starts to get expressed.[1] This triggers the expression of PR. Once PR is synthesized and binds progesterone, it inhibits several genes related to cellular proliferation. This explains the growth characteristics of most breast cancers, e.g. in well-differentiated

Received: July 20, 2006; *accepted:* Oct 10, 2006

Correspondence: Prof. Shahid PERVEZ, MD, PhD, Departments of Pathology & Microbiology, Faculty of Health Sciences, Medical College, The Aga Khan University, Medical Center, Stadium Road, P.O. Box 3500, Karachi-74800, Pakistan. Fax: (92 21) 493-4294, 493-2095, Tel: (92 21) 485-91554/493-0051, ext. 1554, e-mail: shahid.pervez@aku.edu

ERα+PR+ tumors, PR expression would exhibit a feedback inhibition on ERα+ cells and would slow down the growth process. Since many poorly differentiated tumors do not express ER and, consequently, PR, they would remain insensitive to the blocking action of PR.

Despite such remarkable progress in this area, there remain a number of unanswered questions regarding the role of the hormone receptor in the regulation of breast cancer cells. One of these questions concerns the existence of a small percentage of patients with ERα-negative but PR-positive tumor. This is so because it is generally accepted that the level of PR in mammary gland and its tumors is regulated by estrogens, presumably through the estrogen receptors.[16-18] It is believed, therefore, that the detection of both receptors in breast cancers indicates that the complex molecular events that are involved in the regulation of protein synthesis and cell growth by estrogens are intact.[16-18] So theoretically speaking, tumors that do not contain ER, but contain PR, should not exist. However, some studies have identified a small percentage of such tumors, usually between 2% to 6%.[13,21,25,28,37] Others reported the incidence for this subgroup of breast cancer to be 5% to 10%.[14,17] The reasons for such a receptor profile are unclear. In this study, we looked at the relative frequency, morphologic characteristics and possible reasons for such an immunophenotype by using an internationally recognized robust immunohistochemical method. With the recent development of monoclonal antibodies against nuclear estrogen and progestin receptor epitopes,[9,23] immunohistochemical measurements have increasingly gained momentum, and several groups have studied their prognostic relevance.[24,26] Clear benefits of this technique are visualization of the receptor protein to disclose tumor heterogeneity, independence of receptor-masking estrogens of endogenous and exogenous origin, no interference of data with receptor-blocking substances, and minimal tumor quantity required for analysis. We looked at the group with ERα-PR+ phenotype in a major tertiary care referral center and compared its frequency and demographics with those of the ERα+PR+ group to explain the possible reasons for the former phenotype.

Materials and Methods

Sample size

This study group consisted of 1625 consecutive breast biopsy and mastectomy specimens received for ERα/PR evaluation by immunohistochemistry in the years 2001-2003 (over 2.66 years) at The Aga Khan University Hospital, Department of Pathology, Karachi. In this study only classic infiltrating ductal carcinomas (NOS) were included and the histological grading was performed according to the modified method described by Bloom and Richardson,[4] taking 3 criteria into consideration, i.e. tubule formation, nuclear pleomorphism and mitosis, each given a point ranging from 1-3. Grades were allocated as follows: 3-5 points (grade 1, well-differentiated), 6-7 points (grade 2, moderately differentiated) and 8-9 points (grade 3, poorly differentiated).

Immunohistochemistry

Immunohistochemical (IHC) analysis of ERα and PR was performed on formalin-fixed, paraffin-embedded tumor tissue. Three to four micrometer thick sections were cut from one representative block for each case and control. A microwave antigen retrieval method was applied using citrate buffer (pH 6.0) or target retrieval solution. Endogenous peroxidase activity was blocked with 3% H_2O_2. The slides were then incubated with monoclonal antibodies against estrogen and progesterone receptor (ER clone ID5 and PR clone IA6, ready-to-use; DAKO, Denmark). Envision system was used to detect the reaction (*Figure 1*). All cases were run with known positive controls for each antibody and, where possible, with normal

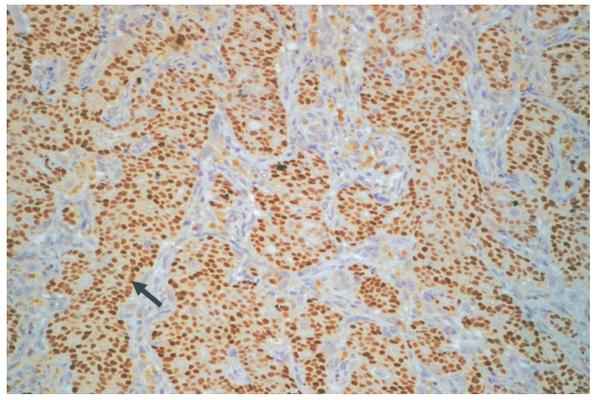

Figure 1. *Grade 2 infiltrating ductal carcinoma of the breast stained with a monoclonal antibody against ERα subtype (clone ID5). Note a diffuse strong nuclear staining consistent with strong positive ER status. x40*

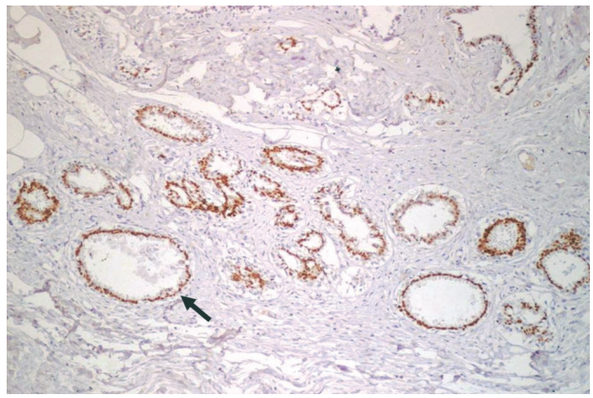

Figure 2. *Normal breast tissue in the same tumor block was used as internal built-in control at the time of block selection. x10*

breast lobules adjacent to tumor to assess built-in control (*Figure 2*). For negative control, the primary antibody was omitted for each run.

Staining and semi-quantitative scoring

The immunohistochemical localization of ERα and PR was scored in a semi-quantitative fashion, incorporating both the intensity and the distribution of specific staining as described elsewhere.[13] Evaluation was recorded as percentage of positively stained tumor cells in each of five intensity categories denoted as zero (no staining), 1+ (weak but detectable), 2+ (mildly distinct), 3+ (moderately distinct) and 4+ (strong). For each tissue, a value designated as HSCORE was derived by summing up the percentages of cell staining, each intensity multiplied by the weighted intensity of staining. An H score of ≤74 was taken as negative, 75-99 as weakly positive, 100-119 as intermediate positive, while 120 and above was interpreted as strongly positive.

Correlations with established risk factors as tumor size, grade, lymph node status and menopausal status, were analyzed for our study group (ERα-PR+) in comparison with those for the ERα+PR+ group. Statistical analysis was done using the SPSS (11.0) software.

Results

Out of the total 1625 cases, 29.91% (486) were ERα+PR+, 5.11% (83) ERα+PR-, 56.86% (924) ERα-PR- and 8.12% (132) ERα-PR+ (*Table 1*). The median and mean ages of the first study group (ERα-PR+) were 47 and 47.04, and those of the second study group (ERα+PR+) were 50 and 51.0, respectively. Patients' age was significantly lower in the ERα-PR+ group (P=0.002). Only 44.27% (58) of patients in the ERα-PR+ group, while 55.35% (269) of those in the ERα+PR+ group were diagnosed after the age of 50 (post-menopausal). As far as tumor grades were concerned, in the first study group (ERα-PR+) 6.06% (8) of tumors were graded as grade 1 (well-differentiated), 67.42% (89) as grade 2 (moderately differentiated) and 26.52% (35) as grade 3 (poorly differentiated). In the second group (ERα+PR+) 9.46% (46) of tumors were grade 1 (well-differentiated), 76.95% (374) grade 2 (moderately differentiated) and 12.96% (63) grade 3 (poorly differentiated). A χ^2 statistical analysis of grading revealed no significant difference between the two study groups (P=0.091), although the ERα-PR+ tumors were intermediate, containing significantly more poorly differentiated tumors than the ERα+PR+ group (P= 0.032). Tumor size was significantly larger in the ERα-PR+ group than in the ERα+ PR+ group (P=0.046). The mean tumor size in the first study group (ERα-PR+) was found to be 4.84 cm (SD=3.593) compared to 3.83 cm (SD=2.76) in the second group (ERα+PR+). 22.7% (30/132) of patients in the first group and 16.6% (81/486) of the second group had evidence of axillary lymph node involvement with a mean number of lymph nodes involved being 7.3 (SD=7.13) and 8.3 (SD=8.48), respectively (P=0.107). The frequency of lymph node metastases was therefore independent of receptor profile (*Table 1*).

***Table 1.* Immunohistochemical hormone receptor profile**

Parameters	*ERα+PR+*	*ERα-PR+*	*P value*
No. (%)	486 (29.91)	132(8.12)	
Age (mean)	51.0	47.04	0.002*
Tumor grade (%)			
1	9.46	6.06	0.576
2	76.95	67.42	0.174
3	12.96	26.52	0.032*
Overall significance			0.091
Tumor size (cm)	3.83	4.84	0.046*
Axillary lymph node involvement (%)	16.6	22.7	0.107

*p <0.05=significant difference

Discussion

The steroid hormone estrogen has important functions in target tissues and plays a major role in proliferation of tumor cells in breast and uterus among other organs. Its receptor has two subtypes, ER-alpha (α) and ER-beta (β),[19] the alpha subtype being more expressed than the beta subtype.[15] ERα and ERβ both belong to the nuclear receptor superfamily, members of which share a common structural architecture. In the mature mammary gland, ERα is spontaneously observed in epithelial cells whereas ERβ is more broadly expressed in epithelial and stromal cells, including fibroblasts and endothelial cells.[33] It is noteworthy that about 50% of breast cancer patients express both ERα and ERβ.[32] The function of ERβ is thought to counteract that of ERα, with the α subtype expression leading to an increase in estrogen-stimulated proliferation and the β subtype expression resulting in a decrease in proliferation and thus a favorable prognosis in breast cancer patients. Shaaban et al carried out an immunohistochemical analysis of 283 samples of breast tissue with 94.3% of normal breast lobules and 60.0% of invasive cancers showing subtype β expression.[30] In terms of predictive factors for treatment, studies have been done evaluating ERβ mRNA expression and immunohistochemical staining and also the clinical response to endocrine therapy, but the studies so far on the β subtype have been inconclusive and controversial. Hence, we have focused completely on the α subtype in our study and the discussion here will also be in this context.

Previous studies using complex binding assays to search for ER in ER-PR+ tumors have reported some positive cases.[28,35] More recently, immunologic, rather than func-

tional, assays for ER have been developed, using antibodies that can measure both occupied and unoccupied receptors,[8] but their definitive role has not yet been established.

The frequency of immunohistochemically ERα-PR+ breast carcinomas is generally 2% to 6% of all cases.[13,21,25,28,37] The frequency in the present series (8.12%, 132 patients) is slightly higher than previously reported. Previous studies have also suggested that this receptor profile occurs more commonly in tumors from younger women. In the present series, the mean age (47.04) of our patients with ERα-PR+ tumors was significantly lower than that of patients with other receptor profiles.

The other prognostic factors examined in this study also suggest that ERα-PR+ tumors differ from ERα+PR+ ones. These tumors were found to be larger and more aggressive (grade 3) compared to the control group (ERα+PR+). The higher tumor grade in ERα-PR+ tumors compared with ERα+PR+ tumors confirms previous reports.[21] The presence of lymph node metastases did not correlate with any hormone receptor profile and was therefore also consistent with previous reports.[21]

Do tumors that truly do not contain ER but contain PR exist? This remains the central question about steroid receptors in breast carcinomas. Explanations that have been proposed include a high level of circulating estrogen in young women that either causes binding sites on the receptor to be occupied with endogenous ligands or competes with exogenous ligands in the binding assay.[13,25,28,34] The first is based on the observation that in younger patients the high levels of circulating estrogens may cause transfer of the cytoplasmic receptor to the nucleus, causing false low levels of ER.[36] However, several studies have failed to find an inverse correlation between either circulating or tumor-cell levels of estrogen and levels of ER.[6,7,27,37] The second is based on the observation of Zava et al[39] and Panko and Macleod[22] that certain tumors may have uncharged ER in the nucleus that may function without estrogens. A third possible explanation for this phenomenon would be that such patients were actually ERα+ but endogenous or exogenous estrogens (e.g. birth control pills) might result in competition for the radioactive estrogen used in the assay and so provide a falsely negative ER result. However, there is evidence to indicate that false-negative ER was not due to the presence of endogenous estrogens.[6,7] One theory postulates that the ER is abnormal and therefore activates the estrogen pathway, but does not bind estrogen.[11] Still other possibilities are laboratory error and even the presence of a true ER-negative, PR-positive state. Evidence from experimental studies suggests that defective ERs may exist, which may not contain estrogen- or antibody-binding sites, but which are, nevertheless, capable of stimulating PR synthesis.[3,11] Such variant malignant cells would not be estrogen-responsive. The presence of a PR gene with abnormal regulation that functions in the absence of estrogen has also been reported in a breast carcinoma cell line.[29] An investigator[34] has argued that all ERα- PR+ tumors should be regarded as biologically equivalent to ERα+PR+ tumors. However, the response rate to hormonal therapy for ERα-PR+ tumors is substantially lower than that of ERα+PR+ ones,[21] suggesting real differences between the two hormone receptor profiles.

In conclusion, whatever the cause of ERα-PR+ tumors, it is apparent that tumors with this hormone receptor profile are heterogeneous. They do not merely reflect tumors that are actually ERα+PR+, whose ER is masked in binding assays by endogenous estrogen in younger women. The present data suggest differences in prognostic factors compared with ERα+PR+ tumors. The ERα-PR+ tumors appear to be heterogeneous and biologically different from ERα+PR+ ones.

These results also have important clinical implications. Most clinicians elect to proceed directly to chemotherapy when the results of estrogen receptor assay are negative. However, there are a small percentage of women with ERα- tumor who have been reported to respond to hormones.[6] If ERα is negative then it could be falsely assumed that PR is also negative, but the case could be of our study group (ERα-PR+). Hence in a clinical setting both ER and PR should be tested. This could otherwise result in unnecessary chemotherapy or may deprive the patient of anti-estrogenic therapy. This should be given special attention in the case of pre-menopausal women. Our results suggest that one should be careful to determine that such women are truly estrogen-receptor-negative, especially if their progesterone receptors are positive. This observation stresses the need for better laboratory methods including increased affinity antibodies to detect these possibly masked estrogen receptors.

Another important possibility is the presence of ER β subtype instead of the α subtype. This could lead to an ERα- and PR+ status in a breast cancer patient. But as mentioned before, the prognostic indication of the β subtype is still not well characterized, nor is its response to hormonal treatment (the α subtype has a well established response to hormonal therapy). Thus, this observation underlies the importance of β subtype detection and the further studying of its prognostic value and its potential as a novel target for pharmacological therapy.

These results suggest that information regarding the menopausal status and the possible administration of exogenous hormones should be requested by laboratories before doing estrogen and progesterone receptor evaluations in breast cancer. Finally, and most importantly, we believe that standardized assays of ER (both α and β subtypes) and PR should be used internationally to overcome the artifact problems that already exist among the variety of current methods and that in all cases both ER (α/β) and PR should be estimated rather than ER alone.

References

1. *Ballare C, Bravo, AI, Sorin I, et al:* The expression of progesterone receptors coincides with an arrest of DNA synthesis in human breast cancer. Cancer 67: 1352-1358, 1991
2. *Berger U, McLelland RA, Wilson P, et al:* Immunohistochemical determination of estrogen receptor, progesterone receptor and 1, 25-dihydroxvitamin D3 receptor in breast cancer and relationship to prognosis. Cancer Res 51: 239-244, 1991
3. *Berkenstam A, Glaumann H, Martin M, et al:* Hormonal regulation of estrogen receptor messenger ribonucleic acid in $T47D_{co}$ and MCF-7 breast cancer cells. Mol Endocrinol 3: 22-28, 1989
4. *Bloom HJG, Richardson WW:* Histological grading and prognosis in breast cancer. Br J Cancer 11:359-377, 1957
5. *deSombre, ER:* Steroid receptors in breast cancer. In: The Breast. (Eds: McDivitt RW, Oberman HA, Ozello L and Kaufman N), Williams and Wilkins, Baltimore, 1984, pp149-174
6. *Edery M, Goussard J, Dehennin L, et al:* Endogenous oestradiol-17β concentration in breast tumors determined by mass fragmentography and by radioimmunoassay: relationship to receptor content. Eur J Cancer 17: 115-120, 1981
7. *Fishman J, Nisselbaum JS, Menendez-Botet CJ, Schwartz MK:* Estrogen and estradiol content in human breast tumors: relationship to estradiol receptors. J Steroid Biochem 8: 893-896, 1977
8. *Greene GL, Press MF:* Steroid receptor structure (including monoclonal antibodies and new methods of determination): structure and dynamics of the estrogen receptor. J Steroid Biochem 24: 1-7, 1986
9. *Greene GM, Fitch FW, Jensen EV*: Monoclonal antibodies to trophilin: probes for the study of estrogen receptors. Proc Natl Acad Sci USA 77: 157-161, 1980
10. *Horwitz KB, McGuire WL:* Estrogen control of progesterone receptor in human breast cancer. Correlation with nuclear processing of estrogen receptor. J Biol Chem 253: 2223-2228, 1978
11. *Horwitz KB:* Cellular heterogeneity and mutant oestrogen receptors in hormone resistant breast cancer. Cancer Surv 14: 41-54, 1992
12. *Howat JMT, Harris M, Swindell R, Barnes DM:* The effect of oestrogen and progesterone receptors on recurrence and survival in patients with carcinoma of the breast. Br J Cancer 51: 263-270, 1985
13. *Kiang DT, Kollander R:* Breast cancers negative for estrogen receptor but positive for progesterone receptor, a true entity? J Clin Oncol 5: 662-666, 1987
14. *Leclercq G, Heuson JC, Deboel MC, et al:* Estrogen and progesterone receptors in human breast cancer. In: Progesterone Receptors in Normal and Neoplastic Tissues. (Eds: McGuire WL, Raynaud J-P, Baulieu E-E), Raven, New York, 1977, pp 141-153
15. *Leygue E, Dotzlaw H, Watson PH, Murphy LC:* Altered estrogen receptor alpha and beta messenger RNA expression during human breast tumorigenesis. Cancer Res 58: 3197-3201, 1998
16. *McGuire WL:* Hormone receptors. Their role in predicting prognosis and response to endocrine therapy. Semin Oncol 5: 428-433, 1979
17. *McGuire WL, Horwitz KB, Pearson DH, Segaloff A:* Current status of estrogen and progesterone receptors in breast cancer. Cancer 39: 2934-2947, 1977
18. *McGuire WL:* Steroid receptors in human breast cancer. Cancer Res 38: 4289-4291, 1978
19. *Nuclear Receptors Nomenclature Committee:* A unified nomenclature system for the nuclear receptor superfamily. Cell 97:161-163, 1999
20. *Osborne CK, Yochmowitz MG, Knight WA, McGuire WL:* The value of estrogen and progesterone receptors in the treatment of breast cancer. Cancer 46: 2884-2888, 1980
21. *Osborne CK:* Receptors. In: Breast Diseases. (Eds: Harris JR, Hellman S, Henderson IC, Kinne DW), 2nd ed, JB Lippincott Co, Philadelphia, PA, 1991, pp 301-325
22. *Panko WB, MacLeod RM:* Uncharged nuclear receptors for estrogen in breast cancers. Cancer Res 38: 1948-1951, 1978
23. *Perrot-Applanat M, Logeat F, Croyer-Picard MT, Milgrom E:* Immunocytochemical study of mammalian progesterone receptor using monoclonal antibodies. Endocrinology 116: 147-1483, 1985
24. *Pertschuk LP. Kim DS, Kamran Nayer MA, et al*: Immunocytochemical estrogen and progestin receptor assays in breast cancer with monoclonal antibodies. Cancer 66: 1663-1670, 1990
25. *Pichon MF, Milgrom* E: Oestrogen receptor negative-progesterone receptor positive phenotype in 1,211 breast tumors. Br J Cancer 65: 895-897, 1992
26. *Reiner A, Neumeister B, Spona J, et al:* Immunocytochemical localization of estrogen and progestin and prognosis in human primary breast cancer. Cancer Res 50: 7057-7061, 1990
27. *Saez S, Martin PM, Chouvet CD:* Estradiol and progesterone receptors in human breast adenocarcinoma in relation to plasma estrogen and progesterone levels. Cancer Res 38: 3468-3473, 1978
28. *Sarrif AM, Durant JR:* Evidence that estrogen-receptor-negative progesterone-receptor-positive breast and ovarian carcinomas contain estrogen receptor. Cancer 48: 1215-1220, 1982
29. *Savouret J-F, Fridlanski F, Atger M, et al:* Origin of the high constitutive level of progesterone receptor in T47-D breast cancer cells. Mol Cell Endocrinol 75: 157-162, 1991
30. *Shaaban AM, O'Neill PA, Davies MP, et al:* Declining estrogen receptor beta expression defines malignant progression of human breast neoplasia. Am J Surg Pathol 27: 1502-1512, 2003
31. *Shapiro CM, Schiffeling D, Bitran JD, et al*: Prognostic value of estrogen receptor value in pathologic stage 1 and 2 adenocarcinoma of the breast. J Surg Oncol 19: 119-121, 1982
32. *Shigehira S, Makiko H, Masakazu T:* Clinical significance of estrogen receptor β in breast cancer. Cancer Chemother Pharmacol 56: s21-s26, 2005
33. *Speirs V, Skliris GP, Burdall SE, Carder PJ:* Distinct expression patterns of ER alpha and ER beta in normal human mammary gland. J Clin Pathol 55: 371-374, 2002
34. *Thorpe SM:* Immunological quantitation of nuclear receptors in human breast cancers: relation to cytosolic estrogen and progesterone receptors. Cancer Res 47: 1830-1835, 1987
35. *Thorsen T:* Occupied and unoccupied oestradiol receptor in human breast tumor cytosol. J Steroid Biochem. 24: 1-7, 1986
36. *Toft DO, O'Malley BW:* Target tissue receptors for progesterone: The influence of estrogen treatment. Endocrinology 90: 1041-1045, 1972
37. *Vihko R, Janne O, Kontula K, Syrjala P:* Female sex steroid receptor status in primary and metastatic breast carcinoma and its relationship to serum steroid and peptide hormone levels. Int J Cancer 26: 13-21, 1980
38. *Watson CS, Medina D, Clark JH:* Characterization and estrogen stimulation of cytoplasmic progesterone receptor in the ovarian-dependent MXT-3590 mammary tumour line. Cancer Res 39: 4098-4104, 1979
39. *Zava DT, Chamness GC, Horwitz KB, McGuire WL:* Biologically active estrogen receptor in the absence of estrogen. Science 196: 663-664, 1977