**Name of Journal: *World Journal of Clinical Oncology***

**ESPS Manuscript NO: 19749**

**Manuscript Type: Editorial**

**Tumor biology in estrogen receptor-positive, human epidermal growth factor receptor type 2-negative breast cancer: Mind the menopausal status**

Yamashita H. Estrogen receptor-positive breast cancer

**Hiroko Yamashita**

**Hiroko Yamashita,** Breast Surgery, Hokkaido University Hospital, Sapporo 060-8648, Japan

**Author contributions:** Yamashita H conceived the issues which formed the content of the manuscript and wrote the manuscript.

**Conflict-of-interest** **statement:** The author has no conflict of interest.

**Data sharing statement:** No additional data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to:** **Hiroko Yamashita, MD, PhD,** Breast Surgery, Hokkaido University Hospital, Kita 14, Nishi 5, Kita-ku, Sapporo 060-8648, Japan. [hirokoy@huhp.hokudai.ac.jp](mailto:hirokoy@huhp.hokudai.ac.jp)

**Telephone:** +81-11-7067381

**Fax:** +81-11-7067384

**Received:** May 20, 2015

**Peer-review started:** May 20, 2015

**First decision:** August 19, 2015

**Revised:** September 28, 2015

**Accepted:** October 23, 2015

**Article in press:**

**Published online:**

**Abstract**

Breast cancer is not one disease, but can be categorized into four major molecular subtypes according to hormone receptor (HR) [estrogen receptor (ER) and progesterone receptor (PgR)] and human epidermal growth factor receptor type 2 (HER2) expression status. Ki67 labeling index and/or multigene assays are used to classify ER-positive, HER2-negative breast cancer into luminal A and luminal B (HER2-negative) subtypes. To date, most studies analyzing predictive or prognostic factors in ER-positive breast cancer have been performed in postmenopausal women, mainly using patients and samples in adjuvant aromatase inhibitor trials. In contrast, even the clinical roles of PgR and Ki67 have been little analyzed so far in premenopausal women. PgR is one of the estrogen-responsive genes, and it has been reported that plasma estradiol levels are related to expression levels of estrogen-responsive genes including PGRin ER-positive breast cancer. In this article, biological differences, especially differences in expression of PgR and Ki67 in ER-positive breast cancer between pre- and postmenopausal women are discussed. Clinical roles of PgR and Ki67 in ER-positive breast cancer differ between pre- and postmenopausal women. We suggest that the mechanisms of development and estrogen-dependent growth of ER-positive breast cancer might differ according to menopausal status.

**Key words:** Breast cancer; Estrogen receptor; Progesterone receptor; Ki67; Menopausal status

**© The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Progesterone receptor (PgR) is one of the estrogen-responsive genes, and it has been reported that plasma estradiol levels are related to expression levels of estrogen-responsive genes including PGRin estrogen receptor (ER)-positive breast cancer. In this article, biological differences, especially differences in expression of PgR and Ki67 in ER-positive breast cancer between pre- and postmenopausal women are discussed. Clinical roles of PgR and Ki67 in ER-positive breast cancer differ between pre- and postmenopausal women. We suggest that the mechanisms of development and estrogen-dependent growth of ER-positive breast cancer might differ according to menopausal status.

Yamashita H. Tumor biology in estrogen receptor-positive, human epidermal growth factor receptor type 2-negative breast cancer: Mind the menopausal status. *World J Clin Oncol* 2015; In press

**INTRODUCTION**

Breast cancer is not one disease, but a group of diseases that can be categorized into four major molecular subtypes according to their expression of hormone receptors (HR) [estrogen receptor (ER) and progesterone receptor (PgR)] and human epidermal growth factor receptor type 2 (HER2). Thus they are classified as: HR+/HER2-, HR+/HER2+, HR-/HER2+, and triple negative (HR-/HER2-). Treatments need to be tailored to a patient’s particular subtype, so that endocrine therapies for HR-positive breast cancer and anti-HER2 therapies for HER2-positive breast cancer are recommended as first choice regardless of whether the disease is in the early stages or has become metastatic.

Expression of ER, PgR, HER2 and the proliferation marker Ki67 in breast cancer tissues is routinely assessed by immunohistochemistry, and multigene assays have recently been introduced for estimating prognosis and treatment efficacy[[1](#_ENREF_1)]. The choice of appropriate drug therapies, especially the indication of adjuvant chemotherapy for ER-positive, HER2-negative early breast cancer, the subtype which is diagnosed in almost 80% of breast cancer cases, is sometimes controversial. Ki67 labeling index and/or multigene assays, such as 21-gene recurrence score (Oncotype Dx), 70-gene signature (Manmaprint) and PAM50 risk of recurrence score, that classify ER-positive, HER2-negative breast cancer into luminal A and luminal B (HER2-negative) subtypes are commonly used in practice, and adjuvant chemotherapy in addition to endocrine therapy is recommended for luminal B subtype[[2](#_ENREF_2)].

To date, most studies analyzing predictive or prognostic factors in ER-positive breast cancer have been performed in postmenopausal women, mainly using patients and samples in adjuvant aromatase inhibitor trials[[3-5](#_ENREF_3)]. In contrast, even the clinical roles of PgR and Ki67 have been little analyzed so far in premenopausal women.

PgR is one of the estrogen-responsive genes, and it has been reported that plasma estradiol levels are related to expression levels of estrogen-responsive genes including *PGR* in ER-positive breast cancer in both pre- and postmenopausal women[[6](#_ENREF_6),[7](#_ENREF_7)]. We previously investigated the expression of estrogen-responsive genes (PgR and TFF1), a progesterone-responsive gene (RANKL), ER-related genes and Ki67 in ER-positive, HER2-negative breast cancer samples, and compared the correlations between expression levels of these molecular markers and clinicopathological factors, including prognosis, between pre- and postmenopausal women. Our results suggested that the mechanisms of development and estrogen-dependent growth of ER-positive breast cancer might differ according to menopausal status[[8](#_ENREF_8)]. Thus, host factors, such as serum levels of estrogen and progesterone might affect the expression of multiple genes in ER-positive breast cancer tissues.

In this article, biological differences, especially in PgR expression and Ki67 labeling index in ER-positive, HER2-negative breast cancer between pre- and postmenopausal women are discussed.

***PgR expression in ER-positive breast cancer tissues correlates with serum estrogen levels***

PgR is an estrogen-responsive gene, and its expression, together with that of ER, is routinely examined in breast cancer tissues. We previously reported that expression levels of PgR in pretreatment biopsies were not predictive of the response to the neoadjuvant aromatase inhibitor exemestane, and that expression levels of PgR were decreased in posttreatment tumors compared to their levels in pretreatment specimens regardless of the treatment response[[9](#_ENREF_9)]. It is clear that PgR expression does not fully reflect estrogen dependence: Many PgR-negative tumors respond to tamoxifen or aromatase inhibitors[[9-12](#_ENREF_9)]. Furthermore, it has been reported that plasma estradiol levels are related to expression levels of estrogen-responsive genes, such as *PGR* andtrefoil factor 1 (*TFF1*)/*pS2*, in ER-positive breast cancer in both pre- and postmenopausal women[[6](#_ENREF_6),[7](#_ENREF_7)]. Dumbier and colleagues examined mRNA expression of estrogen-responsive genes including PgR in pretreatment tumor biopsies from postmenopausal patients with ER-positive breast cancer treated with the neoadjuvant anastrozole, and pretreatment plasma estradiol levels were determined by highly sensitive radioimmunoassay[[7](#_ENREF_7)]. They demonstrated that plasma estradiol levels were significantly associated with expression of estrogen-responsive genes in ER-positive breast cancer.

In premenopausal women, Haynes and colleagues reported significant differences in the expression of estrogen-related genes including PgR in ER-positive breast tumors across the menstrual cycle: Gene expression of estrogen-related genes was higher when serum estradiol levels were high[[6](#_ENREF_6)]. They also demonstrated that expression of the progesterone-regulated gene *RANKL* was almost three-fold higher when serum progesterone levels were at their highest point of the menstrual cycle[[13](#_ENREF_13)].

The study of neoadjuvant endocrine therapy in premenopausal women with ER-positive breast cancer showed that positive PgR expression status by immunohistochemistry dramatically decreased in post-treatment specimens (34.4%) compared to the values in pretreatment biopsies (98.9%) in patients treated with neoadjuvant anastrozole plus the LHRH agonist goserelin for 24 wk, whereas the percentage of patients with positive PgR status did not change significantly from baseline (91.9%) to 24 wk (89.5%) in patients treated with neoadjuvant tamoxifen plus goserelin[[14](#_ENREF_14)].

Taken together, these data suggest that expression levels of PgR in ER-positive breast cancer tissues are associated with serum estrogen levels in both pre- and postmenopausal women.

***Biological differences between pre- and postmenopausal women with ER-positive, HER2-negative breast cancer –PgR***

A study analyzing clinicopathological characteristics of breast cancer in patients registered to the Japanese Breast Cancer Registry in 2011 showed that the ER-positive rate was approximately 90% in patients in their 40s and approximately 80% in those over 50 years old, while the PgR-positive rate was approximately 85% in patients in their 40s but less than 70% in those over 50 years old[[15](#_ENREF_15)]. We previously showed that the incidence of ER-positive, PgR-negative breast cancer in women aged 50 years or younger and in those older than 50 years were 6% and 15%, respectively, whereas for ER-positive, PgR-positive tumors, incidences were 81% and 64%, respectively[[16](#_ENREF_16)]. Moreover, most tumors had high PgR expression in women aged 50 or younger or in premenopausal women, while the distribution of PgR expression levels was evenly spread in tumors in women over 50 years of age or in postmenopausal women[[16](#_ENREF_16)]. This suggests that reduced circulating estrogens after menopause could be the cause in the incidence of ER-positive/PgR-negative or ER-positive/low-PgR tumors in postmenopausal women[[17](#_ENREF_17)].

PgR expression has been reported to be a prognostic factor for postmenopausal ER-positive breast cancer patients in adjuvant aromatase inhibitor trials[[3-5](#_ENREF_3)]. Our retrospective studies also demonstrated that high expression of PgR significantly correlated with improved disease-free survival in postmenopausal women with ER-positive, HER2-negative breast cancer[[8](#_ENREF_8)]. In contrast, in premenopausal women, PgR expression was not associated with disease-free survival[[8](#_ENREF_8)].

***Biological differences between pre- and postmenopausal women with ER-positive, HER2-negative breast cancer –Ki67***

Ki67 is a nuclear protein that is expressed during all phases of the cell cycle except the G0 phase, and is a marker of tumor proliferation[[18](#_ENREF_18)]. Recent studies have shown that the so called “luminal A” subtype—characterized by low histological grade, low proliferation as measured by Ki67, high hormone receptor status, and negative HER2 status—is less responsive to chemotherapy, and that no preferable chemotherapy regimen could be defined for treatment of this subtype[2].

The prognostic significance of Ki67 was examined in postmenopausal women who were treated with letroszole or tamoxifen in the BIG1-98 trial[[19](#_ENREF_19)]. It was reported that higher values (> 11%) of Ki67 labeling index were associated with worse disease-free survival. Our previous study showed that when the cutoff point for determining the division between low and high Ki67 labeling index was set at 14%, low Ki67 labeling index was strongly associated with increased disease-free survival in postmenopausal women with ER-positive breast cancer[[8](#_ENREF_8)]. We also indicated that high expression of Ki67 (≥ 14%) was significantly associated with decreased disease-free survival in postmenopausal patients treated with adjuvant aromatase inhibitors[[20](#_ENREF_20)]. In contrast, the best cutoff points of Ki67 labeling index for disease-free survival were 30% for premenopausal women with ER-positive breast cancer[[8](#_ENREF_8)].

In terms of a predictive value for Ki67, Dowsett and colleagues measured the expression of Ki67 in tumor biopsy samples taken before and after 2 wk of presurgical endocrine treatment in postmenopausal hormone receptor-positive breast cancer. They showed that a change in Ki67 labeling index between levels before and after 2 wk of endocrine treatment was significantly associated with clinical response[[21](#_ENREF_21)]. On the other hand, we demonstrated that Ki67 level in a tumor biopsy before treatment with the neoadjuvant aromatase inhibitor exemestane did not correlate with response to the therapy[[9](#_ENREF_9),[22](#_ENREF_22)].

In contrast, in premenopausal women, overall tumor response was better in patients who had a baseline Ki67 index of ≥ 20% compared with those whose baseline Ki67 index was < 20% in a study of patients treated with neoadjuvant anastrozole or tamoxifen who also received goserelin for 24 wk[[14](#_ENREF_14)]. It is possible that Ki67 may be positively stained in ER-positive breast cancer cells with estrogen-dependent growth, and that neoadjuvant endocrine treatment may be effective for Ki67-positive, estrogen-dependent tumor cells in premenopausal women.

**CONCLUSION**

Clinical roles of PgR and Ki67 in ER-positive breast cancer differ between pre- and postmenopausal women. Of the available multigene assays, PgR and Ki67 are included in Oncotype DX and PAM50, and genes related to ER-signaling are included in EndoPredict. Care should be taken when these assays are introduced for premenopausal women, because most studies involved in the development of multigene assays for ER-positive breast cancer were performed in postmenopausal women. We previously analyzed genetic and environmental factors, endogenous hormones and growth factors to identify risk factors for ER-positive breast cancer, and showed that risk factors differ between women of different menopausal status[[23](#_ENREF_23)]. We therefore suggest that the mechanisms of development and estrogen-dependent growth of ER-positive breast cancer might differ according to menopausal status.

**REFERENCES**

1 **Wazir U**, Mokbel K. Emerging gene-based prognostic tools in early breast cancer: First steps to personalised medicine. *World J Clin Oncol* 2014; **5**: 795-799 [PMID: 25493218 DOI: 10.5306/wjco.v5.i5.795]

2 **Goldhirsch A**, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, Senn HJ. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Ann Oncol* 2013; **24**: 2206-2223 [PMID: 23917950 DOI: 10.1093/annonc/mdt303]

3 **Viale G**, Regan MM, Maiorano E, Mastropasqua MG, Dell'Orto P, Rasmussen BB, Raffoul J, Neven P, Orosz Z, Braye S, Ohlschlegel C, Thürlimann B, Gelber RD, Castiglione-Gertsch M, Price KN, Goldhirsch A, Gusterson BA, Coates AS. Prognostic and predictive value of centrally reviewed expression of estrogen and progesterone receptors in a randomized trial comparing letrozole and tamoxifen adjuvant therapy for postmenopausal early breast cancer: BIG 1-98. *J Clin Oncol* 2007; **25**: 3846-3852 [PMID: 17679725 DOI: 10.1200/JCO.2007.11.9453]

4 **Dowsett M**, Allred C, Knox J, Quinn E, Salter J, Wale C, Cuzick J, Houghton J, Williams N, Mallon E, Bishop H, Ellis I, Larsimont D, Sasano H, Carder P, Cussac AL, Knox F, Speirs V, Forbes J, Buzdar A. Relationship between quantitative estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER-2) status with recurrence in the Arimidex, Tamoxifen, Alone or in Combination trial. *J Clin Oncol* 2008; **26**: 1059-1065 [PMID: 18227529]

5 **Bartlett JM**, Brookes CL, Robson T, van de Velde CJ, Billingham LJ, Campbell FM, Grant M, Hasenburg A, Hille ET, Kay C, Kieback DG, Putter H, Markopoulos C, Kranenbarg EM, Mallon EA, Dirix L, Seynaeve C, Rea D. Estrogen receptor and progesterone receptor as predictive biomarkers of response to endocrine therapy: a prospectively powered pathology study in the Tamoxifen and Exemestane Adjuvant Multinational trial. *J Clin Oncol* 2011; **29**: 1531-1538 [PMID: 21422407 DOI: 10.1200/JCO.2010.30.3677]

6 **Haynes BP**, Viale G, Galimberti V, Rotmensz N, Gibelli B, A'Hern R, Smith IE, Dowsett M. Expression of key oestrogen-regulated genes differs substantially across the menstrual cycle in oestrogen receptor-positive primary breast cancer. *Breast Cancer Res Treat* 2013; **138**: 157-165 [PMID: 23378065 DOI: 10.1007/s10549-013-2426-0]

7 **Dunbier AK**, Anderson H, Ghazoui Z, Folkerd EJ, A'hern R, Crowder RJ, Hoog J, Smith IE, Osin P, Nerurkar A, Parker JS, Perou CM, Ellis MJ, Dowsett M. Relationship between plasma estradiol levels and estrogen-responsive gene expression in estrogen receptor-positive breast cancer in postmenopausal women. *J Clin Oncol* 2010; **28**: 1161-1167 [PMID: 20124184 DOI: 10.1200/JCO.2009.23.9616]

8 **Hosoda M**, Yamamoto M, Nakano K, Hatanaka KC, Takakuwa E, Hatanaka Y, Matsuno Y, Yamashita H. Differential expression of progesterone receptor, FOXA1, GATA3, and p53 between pre- and postmenopausal women with estrogen receptor-positive breast cancer. *Breast Cancer Res Treat* 2014; **144**: 249-261 [PMID: 24549642 DOI: 10.1007/s10549-014-2867-0]

9 **Yamashita H**, Takahashi S, Ito Y, Yamashita T, Ando Y, Toyama T, Sugiura H, Yoshimoto N, Kobayashi S, Fujii Y, Iwase H. Predictors of response to exemestane as primary endocrine therapy in estrogen receptor-positive breast cancer. *Cancer Sci* 2009; **100**: 2028-2033 [PMID: 19659610 DOI: 10.1111/j.1349-7006.2009.01274.x]

10 **Anderson H**, Bulun S, Smith I, Dowsett M. Predictors of response to aromatase inhibitors. *J Steroid Biochem Mol Biol* 2007; **106**: 49-54 [DOI: 10.1016/j.jsbmb.2007.05.024]

11 **Elledge RM**, Green S, Pugh R, Allred DC, Clark GM, Hill J, Ravdin P, Martino S, Osborne CK. Estrogen receptor (ER) and progesterone receptor (PgR), by ligand-binding assay compared with ER, PgR and pS2, by immuno-histochemistry in predicting response to tamoxifen in metastatic breast cancer: a Southwest Oncology Group Study. *Int J Cancer* 2000; **89**: 111-117 [PMID: 10754487 DOI: 10.1002/(SICI)1097-0215(20000320)89: 2<111: : AID-IJC2>3.0.CO; 2-W]

12 **Ellis MJ**, Coop A, Singh B, Mauriac L, Llombert-Cussac A, Jänicke F, Miller WR, Evans DB, Dugan M, Brady C, Quebe-Fehling E, Borgs M. Letrozole is more effective neoadjuvant endocrine therapy than tamoxifen for ErbB-1- and/or ErbB-2-positive, estrogen receptor-positive primary breast cancer: evidence from a phase III randomized trial. *J Clin Oncol* 2001; **19**: 3808-3816 [PMID: 11559718]

13 **Haynes BP**, Viale G, Galimberti V, Rotmensz N, Gibelli B, Smith IE, Dowsett M. Differences in expression of proliferation-associated genes and RANKL across the menstrual cycle in estrogen receptor-positive primary breast cancer. *Breast Cancer Res Treat* 2014; **148**: 327-335 [PMID: 25367875 DOI: 10.1007/s10549-014-3181-6]

14 **Iwata H**, Masuda N, Sagara Y, Kinoshita T, Nakamura S, Yanagita Y, Nishimura R, Iwase H, Kamigaki S, Takei H, Tsuda H, Hayashi N, Noguchi S. Analysis of Ki-67 expression with neoadjuvant anastrozole or tamoxifen in patients receiving goserelin for premenopausal breast cancer. *Cancer* 2013; **119**: 704-713 [PMID: 22972694 DOI: 10.1002/cncr.27818]

15 **Kurebayashi J**, Miyoshi Y, Ishikawa T, Saji S, Sugie T, Suzuki T, Takahashi S, Nozaki M, Yamashita H, Tokuda Y, Nakamura S. Clinicopathological characteristics of breast cancer and trends in the management of breast cancer patients in Japan: Based on the Breast Cancer Registry of the Japanese Breast Cancer Society between 2004 and 2011. *Breast Cancer* 2015; **22**: 235-244 [PMID: 25758809 DOI: 10.1007/s12282-015-0599-6]

16 **Yamashita H**, Iwase H, Toyama T, Takahashi S, Sugiura H, Yoshimoto N, Endo Y, Fujii Y, Kobayashi S. Estrogen receptor-positive breast cancer in Japanese women: trends in incidence, characteristics, and prognosis. *Ann Oncol* 2011; **22**: 1318-1325 [PMID: 21119029 DOI: 10.1093/annonc/mdq596]

17 **Yamamoto Y**, Yamamoto-Ibusuki M, Iwase H. Menopausal status should be taken into consideration for patients with luminal a breast cancer in terms of the effect of differential biology on prognosis. *J Clin Oncol* 2013; **31**: 2516 [PMID: 23690409 DOI: 10.1200/JCO.2013.49.4062]

18 **Yerushalmi R**, Woods R, Ravdin PM, Hayes MM, Gelmon KA. Ki67 in breast cancer: prognostic and predictive potential. *Lancet Oncol* 2010; **11**: 174-183 [PMID: 20152769 DOI: 10.1016/S1470-2045(09)70262-1]

19 **Viale G**, Giobbie-Hurder A, Regan MM, Coates AS, Mastropasqua MG, Dell'Orto P, Maiorano E, MacGrogan G, Braye SG, Ohlschlegel C, Neven P, Orosz Z, Olszewski WP, Knox F, Thürlimann B, Price KN, Castiglione-Gertsch M, Gelber RD, Gusterson BA, Goldhirsch A. Prognostic and predictive value of centrally reviewed Ki-67 labeling index in postmenopausal women with endocrine-responsive breast cancer: results from Breast International Group Trial 1-98 comparing adjuvant tamoxifen with letrozole. *J Clin Oncol* 2008; **26**: 5569-5575 [PMID: 18981464 DOI: 10.1200/JCO.2008.17.0829]

20 **Yamamoto M**, Hosoda M, Nakano K, Jia S, Hatanaka KC, Takakuwa E, Hatanaka Y, Matsuno Y, Yamashita H. p53 accumulation is a strong predictor of recurrence in estrogen receptor-positive breast cancer patients treated with aromatase inhibitors. *Cancer Sci* 2014; **105**: 81-88 [PMID: 24118529 DOI: 10.1111/cas.12302]

21 **Dowsett M**, Smith IE, Ebbs SR, Dixon JM, Skene A, Griffith C, Boeddinghaus I, Salter J, Detre S, Hills M, Ashley S, Francis S, Walsh G, A'Hern R. Proliferation and apoptosis as markers of benefit in neoadjuvant endocrine therapy of breast cancer. *Clin Cancer Res* 2006; **12**: 1024s-1030s [PMID: 16467120 DOI: 10.1158/1078-0432.CCR-05-2127]

22 **Toi M**, Saji S, Masuda N, Kuroi K, Sato N, Takei H, Yamamoto Y, Ohno S, Yamashita H, Hisamatsu K, Aogi K, Iwata H, Takada M, Ueno T, Saji S, Chanplakorn N, Suzuki T, Sasano H. Ki67 index changes, pathological response and clinical benefits in primary breast cancer patients treated with 24 weeks of aromatase inhibition. *Cancer Sci* 2011; **102**: 858-865 [PMID: 21231986 DOI: 10.1111/j.1349-7006.2011.01867.x]

23 **Yoshimoto N**, Nishiyama T, Toyama T, Takahashi S, Shiraki N, Sugiura H, Endo Y, Iwasa M, Fujii Y, Yamashita H. Genetic and environmental predictors, endogenous hormones and growth factors, and risk of estrogen receptor-positive breast cancer in Japanese women. *Cancer Sci* 2011; **102**: 2065-2072 [PMID: 21790896 DOI: 10.1111/j.1349-7006.2011.02047.x]

**P-Reviewer:** Geok CT, Menelaos Z, Tsikouras P **S-Editor:** Qiu S **L-Editor: E-Editor:**