EDITORIAL

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

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Abstract

Breast cancer is not one disease, but can be categorized into four major molecular subtypes according to hormone receptor [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 *PGR* in 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; Progesterone receptor; Estrogen receptor; Ki67; Menopausal status

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 *PGR* in 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.

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]. 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].

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]. 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[6,7]. 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]. 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]. It is clear that PgR expression does not fully reflect estrogen dependence: Many PgR-negative tumors respond to tamoxifen or aromatase inhibitors[9-12]. Furthermore, it has been reported that plasma estradiol levels are related to expression levels of estrogen-responsive genes, such as *PGR* and trefoil factor 1 (*TFF1*)/*pS2*, in ER-positive breast cancer in both pre- and postmenopausal women[6,7]. Dunbier *et al*[7] 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. They demonstrated that plasma estradiol levels were significantly associated with expression of estrogen-responsive genes in ER-positive breast cancer.

In premenopausal women, Haynes *et al*[6,13] 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. 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.

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 posttreatment 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].

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]. 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]. 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]. 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].

PgR expression has been reported to be a prognostic factor for postmenopausal ER-positive breast cancer patients in adjuvant aromatase inhibitor trials[3-5]. 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]. In contrast, in premenopausal women, PgR expression was not associated with disease-free survival[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]. 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]. 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]. 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]. 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].

In terms of a predictive value for Ki67, Dowsett et al[21] 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. 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[9,22].

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]. 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]. We therefore suggest that the mechanisms of development and estrogen-dependent growth of ER-positive breast cancer might differ according to menopausal status.

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