Original Article

Implications of Progesterone Receptor Status for the Biology and Prognosis of Breast Cancers

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Background: It remains unknown whether knowledge of the status of progesterone receptor (PR) expression is useful for distinguishing between luminal A and B breast cancer subtypes and for providing an accurate prognosis for patients with estrogen receptor-positive (ER+) and human epidermal growth factor receptor 2-negative (HER2–) breast cancer. We aimed to assess the role of PR status in determining the biology and prognosis of early ER+ and HER2– breast cancer.

Methods: This was a retrospective study of 105 patients with ER+ and HER2– breast cancer who underwent surgery between 2005 and 2013. Relapse-free survival (RFS), distant-metastasis-free survival (DFS) and overall survival (OS) were evaluated. The prognostic value of PR was evaluated using a Cox regression model.

Results: Apart from the menstruation status, there were no significant differences between the PR+ and PR– patients. PR– patients exhibited a tendency towards a higher nuclear grade and Ki-67 labeling index. In particular, postmenopausal patients with PR expression status < 10% exhibited a significantly higher nuclear grade and tendency towards a higher Ki-67 labeling index. After a median follow-up of about 5 years, the 5-year RFS, DFS and OS rates of the PR+ and PR– patients were not significantly different. PR was not a significant prognostic factor by multivariate analysis.

Conclusion: PR status does not have prognostic value for patients with early ER+ and HER2– breast cancer who received adequate therapies. However, it may be reasonable to use PR status for distinguishing between luminal A and B breast cancer subtypes.

Key words: progesterone receptor, breast cancer, prognosis

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Introduction

For patients with breast cancer, assessment of the PR status of their tumor is considered important for differentiating between luminal A and B subtypes and for predicting responsiveness to endocrine therapy. Based on a study by Prat et al., the 2013 St. Gallen guidelines confirmed that PR has added value for distinguishing between luminal A and B breast cancer subtypes that are determined by immunohistochemical (IHC) analysis, as described in the 2011 St. Gallen guidelines^{1–3)}. Tumors with decreased or no expression of PR have poorer response to selective ER modulators such as tamoxifen (TAM)⁴⁾ and better response to aromatase inhibitors (AI)⁵⁾.

However, PR status has also been reported to be not significant for predicting response to endocrine therapy. The Early breast cancer trialists' collaborative group (EBCTCG) overview of all randomized trials of adjuvant TAM therapy for early breast cancer reported that PR status did not predict response to TAM⁶⁾. In addition the AI exemestane was reported to be equally effective for ER+/PR+ and ER+/PR– breast cancer⁷⁾.

Thus, the role of PR status in determining the

prognostic and biologic characteristics of breast cancers has been controversial. We aimed to assess the role of PR status in determining the biology and prognosis of breast cancers, by retrospectively analyzing patients who underwent surgery and adjuvant therapy for early ER+ and HER2– breast cancer.

Patients and Methods

Patients

A total of 105 patients with invasive breast cancers who underwent surgery in our hospital between September 2005 and September 2013 were investigated retrospectively. Men with breast cancer and women with *in situ* carcinoma, bilateral breast carcinoma, tumors less than 0.5 cm, or who underwent neoadjuvant chemotherapy or chemotherapy for another disease were excluded from the study. Informed consent was obtained from every patient. Decisions regarding treatment were made by the treating physician on the basis of patient preference, type and stage of disease and recurrence risk factors. Patients were treated with breast-conserving surgery or mastectomy. Most patients who underwent breast-

conserving surgery received radiotherapy to the whole breast after surgery. Most patients received post-operative endocrine therapy. Some patients received post-operative chemotherapy.

Histopathological and immunohistochemical evaluations

All histopathological and immunohistopathological diagnoses were determined by pathologists at our facility or at another laboratory facility within our partnership. Surgical specimens were embedded in paraffin, sectioned and stained with hematoxylin and eosin. ER positivity, PR positivity and Ki-67 labeling index were determined by immunohistochemical (IHC) analysis. Tumors were considered ER+ if $\geq 1\%$ of tumors cells were stained positive for ER expression. Tumors were considered PR+ if $>20\%$ of tumors cells were stained positive for PR expression. HER2 expression status was determined by IHC analysis, with scores of 0, 1+, 2+ indicating the following: no cells with membrane staining and $<10\%$ of cells with membrane staining, $\geq 10\%$ of cells with slight membrane staining, $\geq 10\%$ of cells with low or medium membrane staining, respectively. Tumors that were 2+ by IHC analysis were also examined by fluorescence *in situ* hybridization, with an amplification ratio < 2.0 indicating negative status.

Statistics

Relapse-free survival (RFS) was defined as the period from the date of operation to the date of the first confirmation of relapse (i.e., local relapse or metastasis) or death from any cause, whichever came first. Distant-metastasis-free survival (DFS) was defined as the period from the date of operation to the date of the first confirmation of distant metastasis or death from any cause, whichever came first. Overall survival (OS) was defined as the period from the date of operation to the date of death from any cause. RFS, DFS and OS were estimated using the Kaplan-Meier method and compared using the log-rank test. The Mann-Whitney *U* test was used for comparisons of continuous outcomes, while the chi-square or Fishers' exact test was used for comparisons of categorical variables. Risk factors affecting prognosis were assessed by multivariate analysis using Cox proportional-hazards model. Differences were considered significant for $P < 0.05$.

Results

Patient characteristics

Table 1 shows the characteristics of 105 breast cancer patients. There were no significant differences in age, body mass index, surgical procedure, tumor size, number of metastatic axillary lymph nodes, proportion of patients with ER+ tumors, nuclear grade, Ki-67 labeling index, proportion of patients

undergoing radiation therapy after-breast conserving surgery, proportion of patients undergoing post-operative chemotherapy, and proportion of patients undergoing post-operative endocrine therapy between the PR+ and PR- patients. Among the PR+ and PR- patients, menstruation status was significantly different. The proportion of postmenopausal patients was higher among PR- than PR+ patients. PR- patients had a tendency to higher nuclear grade and Ki-67 labeling index, although the differences were not significant.

Assessment of the Ki-67 labeling index was initiated in 2009, and the number of patients with Ki-67 labeling index values was limited. Chemotherapy was significantly more frequently administered to patients who were younger, with larger tumors, more metastatic lymph nodes, higher Ki-67 labeling indices, and tumors with higher nuclear grade. Among patients who did or did not undergo chemotherapy, there were no statistical differences in the proportions of those with ER+ and PR+ tumors.

Table 2 shows the clinicopathological features between premenopausal and postmenopausal patients. The proportion of ER was significantly higher among postmenopausal than premenopausal patients, while the proportion of PR was significantly lower among postmenopausal than premenopausal patients. Postmenopausal patients had a tendency to higher nuclear grade and Ki-67 labeling index, although the differences were not significant.

Table 3 shows the nuclear grade and Ki-67 labeling index in postmenopausal patients. When 10% was used for the cut-point of PR, lower PR expression was significantly associated with higher nuclear grade and had a tendency to higher Ki-67 labeling index.

Relapse-free, distant-metastases-free, and overall survival

The RFS rates were estimated for PR+ and PR- patients. The median follow-up period was 61 months. The estimated 5-year RFS rates of PR+ and PR- patients were 93.6% and 90.1%, respectively ($P = 0.742$, Fig. 1). There were 10 patients who developed relapse, including 2 with local and regional lymph node recurrences and 8 with distant metastases. The median time to relapse was 54 months (range 9–77 months).

The DFS rates were estimated for PR+ and PR- patients. The median follow-up period was 62 months. The estimated 5-year DFS rates of PR+ and PR- patients were 93.5% and 100%, respectively ($P = 0.094$, Fig. 2). There were 8 PR+ and no PR- patients with distant metastasis. The median time to distant metastasis was 56 months (range 21–77 months). There were 4 patients with pulmonary metastasis, 3 with bone metastasis, 2 with ovarian metastasis, and 1

Table 1 Patients characteristics

Characteristic	PR+ group n = 79	PR- group n = 26	P-value
Age-year			
Median	55	59	0.262
Range	32–95	36–81	
Menstruation status-no. (%)			
Premenopause	31 (39)	4 (15)	0.030
Postmenopause	48 (61)	22 (85)	
BMI			
Median	22.4	22.9	0.803
Range	15.7–34.4	16.6–29.8	
Surgical procedure-no. (%)			
mastectomy	14 (18)	2 (8)	0.357
breast-conserving surgery	65 (82)	24 (92)	
Tumor size-cm			
Median	1.5	1.5	0.797
Range	0.5–4.5	0.6–3.5	
Lymph node metastasis number			
Median	0	0	0.479
Range	0–4	0–1	
ER positivity proportion-%			
Median	90	90	0.429
Range	60–100	1–100	
Nuclear grade-no. (%)			
Grade 1	48 (61)	14 (54)	0.534
Grade 2 or 3	31 (39)	12 (46)	
Ki67-%			
Median	17	34	0.799
Range	1–85	5–76	
not examined	55	16	
Radiation therapy after breast conserving surgery-no. (%)	60 (92)	23 (96)	0.556
Post-operative chemotherapy-no. (%)	29 (37)	13 (50)	0.230
Post-operative endocrine therapy-no. (%)	76 (96)	25 (96)	0.991

Table 2 Clinicopathological features between premenopausal and postmenopausal patients

Features	Premenopuase n = 35	Postmenopause n = 70	P-value
ER positivity proportion-%			
Median	90	100	0.003
Range	60–100	1–100	
PR positivity proportion-%			
Median	80	60	0.014
Range	1–100	0–100	
Nuclear grade-no. (%)			
Grade 1	22 (63)	40 (57)	0.574
Grade 2 or 3	13 (37)	30 (43)	
Ki67-%			
Median	17.1	17.8	0.732
Average	18.7	24.3	
Range	1–58	1–84	
not examined	23	48	

Table 3 Nuclear grade and Ki-67 labeling index in postmenopausal patients

	PR $\geq$ 10% n = 56	PR < 10% n = 14	P-value
Nuclear grade-no. (%)			
Median	1	2	0.011
Range	1–3	1–3	
Ki-67 labeling index-%			
Median	17	45	0.130
Range	1–84	4–76	
not examined	40	8	

with hepatic metastasis.

The OS rates were estimated for PR+ and PR– patients. The median follow-up period was 63 months. The estimated 5-year OS rates of PR+ and PR– patients were 96.8% and 100%, respectively ($P = 0.221$, Fig. 3). There were 4 PR+ patients and no PR– patients who died. The median time to death was 66 months (range 23–92 months). Two patients died of breast-cancer-related causes, 1 died of senility-related causes, and 1 died of acute pyelitis.

Fig. 1 Relapse-free survival.
There is no significant difference between PR+ and PR– patients.

Fig. 2 Distant-metastasis-free survival.
There is no significant difference between PR+ and PR– patients.

Fig. 3 Overall survival.

There is no significant difference between PR+ and PR- patients.

Table 4 Relapse free survival, distant metastases free survival and overall survival (Multivariate analysis)

Relapse free survival	Multivariate analysis		
	Hazard ratio	95% Confidence interval	P-value
Menstruation status (postmenopause vs. premenopuase)	0.93	0.59–1.45	0.763
Tumor size (>1 cm vs. ≤1 cm)	0.78	0.48–1.24	0.301
Lymph node involvement (positive vs. negative)	0.88	0.52–1.50	0.657
PR positivity (positive vs. negative)	1.04	0.64–1.67	0.872

Distant metastases free survival	Multivariate analysis		
	Hazard ratio	95% Confidence interval	P-value
Menstruation status (postmenopause vs. premenopuase)	0.93	0.60–1.45	0.768
Tumor size (>1 cm vs. ≤1 cm)	0.82	0.52–1.31	0.423
Lymph node involvement (positive vs. negative)	0.87	0.51–1.47	0.616
PR positivity (positive vs. negative)	1.05	0.66–1.67	0.816

Overall survival	Multivariate analysis		
	Hazard ratio	95% Confidence interval	P-value
Menstruation status (postmenopause vs. premenopuase)	0.98	0.63–1.53	0.958
Tumor size (>1 cm vs. ≤1 cm)	0.81	0.51–1.28	0.370
Lymph node involvement (positive vs. negative)	0.66	0.39–1.09	0.108
PR positivity (positive vs. negative)	0.93	0.59–1.47	0.776

Risk factors associated with relapse, distant metastasis, and death

Risk factors for relapse, distant metastases and death were investigated by multivariate analysis. Independent variables included menstruation status, tumor size, lymph node involvement and PR positivity. No significant independent risk factors were identified for occurrence of relapse, distant metastasis, or death (Table 4).

Discussion

The predictive significance of the PR status of a breast malignancy has been controversial with regard to response to endocrine and/or chemotherapy. In a study that found that PR status was independent predictive factor for identifying breast cancer patients who would benefit from adjuvant endocrine therapy, the relative risk of recurrence was reported to be reduced by 53% for ER+/PR+ patients and 25% for ER+/PR- patients among those who received

adjuvant endocrine therapy⁴⁾. In that study, about 50% of patients had positive axillary lymph nodes or tumors larger than 2 cm. Eighty-six percent of study patients were older than 50 years, suggesting that postmenopausal women were the main population of the study. However, they were treated using TAM, but not AI. Results of anastrozole, TAM alone or in combination (ATAC) trial suggested that patients with ER+/PR– tumors were best treated by estrogen withdrawal using AI⁵⁾. In our study, 23% of patients had positive axillary lymph nodes, and 32% of patients had tumors larger than 2 cm. Sixty-six percent of study patients were postmenopausal women and they received AI, which are superior to TAM for the treatment of postmenopausal breast cancer⁸⁾. The earlier stage of prognostic factors and higher recurrent inhibition effect by AI in our study could result in no significant difference of RFS, DFS, and OS between PR+ and PR– patients.

The significance of PR expression status for distinguishing between luminal A and B breast cancer subtypes has also been a question. In two independent cohorts data sets, patients with IHC-luminal A tumors having low positive PR+ tumor cells ($\leq 20\%$) showed significantly poorer survival compared with tumors with $>20\%$ of PR+ tumor cells^{3,9,10)}. Especially, the low PR expression has had a high correlation with poor prognosis in postmenopausal patients¹¹⁾. PR-low or negative luminal B-like breast cancer showed significantly higher histological grade than that of PR-high luminal B-like breast cancer¹²⁾. In our study, nuclear grade and Ki-67 labeling index were not significant different between PR+ group and PR– group, but showed tendency to higher nuclear grade and Ki-67 labeling index in PR– group. In postmenopausal women, PR expression was inversely correlated with nuclear grade significantly. Ki-67 labeling index also had similar tendency. PR is a marker of a functional ER, and the expression of PR is induced by a sufficient level of estrogen. The lower estrogen milieu in postmenopausal women leads to lower ER activity, and the prognostic effect of PR could be more important than that of the premenopausal women. These results suggest that low and negative PR expression levels reflects the worse property of breast cancer and the additional assessment of PR status for differentiating between luminal A and B subtypes is reasonable. The statistically insignificant results of Ki-67 labeling index might be accounted for by the inadequate power of our study.

In conclusion, PR status does not have prognostic value for patients with low-risk ER+ and HER2– breast cancer who received adequate therapy.

However, it may be reasonable to use PR status for distinguishing between luminal A and B subtypes.

Conflict of Interest

The authors declare that they have no conflict of interest.

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