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Original Article

Characteristic of ER+/PR- and Ki67 value with breast cancer

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Abstract: Breast cancer subtype was defined by ER, PR, HER2 and Ki67 status since the definition of intrinsic subtypes for breast cancer was renovated in 2013 St. Gallen Consensus Conference. The level of ER, PR, HER2 and Ki67 are the main predictive and prognostic biomarkers in various breast carcinoma subtypes. We retrospectively analyzed clinic pathological parameters and immunohistochemical features of 398 breast cancer patients receiving surgery in our hospital from January 2012 to December 2015. Progress free survival was followed up, and logistic regression was applied to estimate the factors associated with high risk of progression. Among all women with breast cancer, recurrence was higher with low ER levels (HR: 5.59, 95% CI: 2.42-12.95, $P<0.001$), low PR levels (HR: 0.19, 95% CI: 0.04-0.90, $P = 0.036$), and high Ki-67 proliferation index (HR: 5.84, 95% CI: 1.91-17.85, $P = 0.002$). We found that the tumors were larger in patients with ER^+/PR^- than those with ER^+/PR^+ ($P<0.001$), and pathological staging II/III were more frequently found in ER^+/PR^- tumors ($P<0.001$). It was also shown that the high Ki67 level (≥20%) ($P<0.001$) and HER2-positive status ($P = 0.019$) were more frequently found in patients with ER^+/PR^- than ER^+/PR^+. Patients with higher Ki67 expression (≥20%) were younger than those with lower Ki67 expression ($P<0.001$), and Ki67 was higher in larger tumor size ($P = 0.012$). Tumor grade III was more easy to find higher Ki67 ($P<0.001$). Collectively, PR^- breast tumors were more likely to have an aggressive phenotype than PR^+ breast tumors by comparing the ER^+/PR^+ tumors with ER^+/PR^- tumors. We also found that higher Ki67 expression was associated with age, tumor size and tumor grade.

Keywords: Breast neoplasm, hormone receptors, HER2, Ki67

Introduction

Breast cancer is the most common female malignancy in the world, and the leading cause of cancer-associated mortalities among women globally. The breast cancers were defined as luminal A if Ki67 low and HER2-negative; as luminal B if Ki67 high or HER2-positive; and otherwise as HER2 positive or triple negative [1-3]. Hormone receptors (HR) is a major prognostic factor for breast cancer patients. Luminal A and luminal B breast cancer subtypes which ER are positive have less aggressive and better long-term prognoses than triple negative breast cancer (TNBC) [4]. Currently, it was reported that the level of PR determines the tumor response to endocrine treatment, PR^- breast tumors have less sensitivity to tamoxifen than PR^+ tumors in ER^+ breast cancer [5]. Therefore, it is necessary to estimate PR state to have a better prognosis and guide individual treatment.

As a nuclear antigen associated with cell proliferation, Ki67 has been identified as a molecular marker for the effective assessment of the cell proliferation index [6, 7]. In addition, luminal tumors have been classified into luminal A and luminal B subtypes based on the level of Ki67 expression.

We aimed to investigate the biologic character of ER^+/PR^- and Ki67 value expression, as

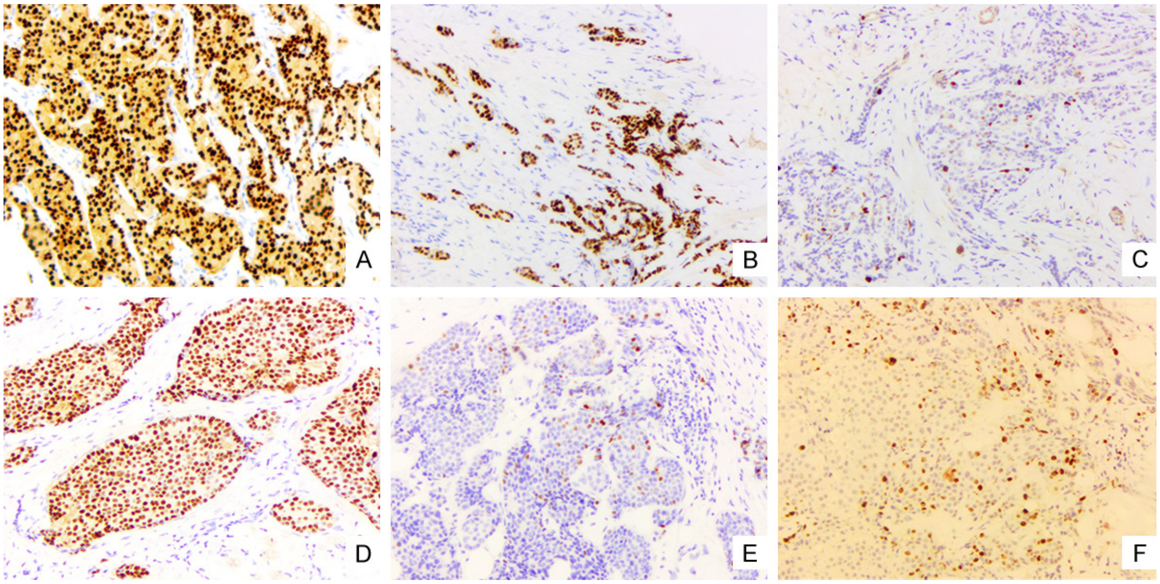

Figure 1. Photomicrographs of ER, PR, and Ki-67 in invasive breast cancer determined by immunohistochemistry (IHC, ×200). (A-C) come from one patient: (A) ER-positive (90%); (B) PR-positive (50%); (C) Ki-67-positive (3%); (D-F) Come from another patient: (D) ER-positive (90%); (E) PR-positive (<5%); (F) Ki-67-positive (30%).

well as the association with clinic pathological parameters in China.

Materials and methods

Patients

We retrospectively analyzed 398 breast cancer patients whose first tumor lumpectomies were in our hospital between January 2012 and December 2015. Patients receiving neoadjuvant therapy were removed from the study. The immunohistochemical indicators included ER, PR, HER2 and Ki67. Patients was treated with surgery by mastectomy or lumpectomy, and doing axillary node dissection according to the standard guideline. We collected clinical data such as age, menopausal status, tumor size, lymph node status, status of IHC (ER, PR, HER2 and Ki67). All of the pathologic and IHC outcomes were reviewed by two experienced pathologists in our hospital. The breast cancer was classified based on the 2015 St. Gallen Consensus Conference [8]. This study was approved by the Ethics Committee of Shandong Cancer Hospital. We obtained informed consent from the patients for publication of this report.

Pathologic definition

We used the streptavidin-peroxidase immunohistochemical method in this study. The DAB kit was from Fuzhou Maxim Biotechnology Co., Ltd. (Fujian, China). The primary antibodies used in this study included ER, PR, Ki67 (Beijing ZSGB Biotechnology Co., Ltd., Beijing, China), and HER2 (Maxim, Fujian, China). ER, PR, and Ki67 were assessed by immunohistochemistry (IHC), and HER2 overexpression due to amplification, was determined by IHC and fluorescent in situ hybridization (FISH) (**Figure 1**). The positive staining of the cells were determined according to their proportion among the total cells. ER-positive tumors were determined by at least 1% of nuclei positively stained, while PR^+ were determined by 20% of nuclei positively stained. More than 20% of Ki67 were regarded as high expression [3]. HER2 expression was classified as HER2-positive (score 3+), suspected HER2-positive (score 2+) and HER2-negative (score 0 or 1+). For those with suspected HER2-positive tumors, FISH should be used to confirm its expression [9]. Among the 398 patients, 72 patients with ER^+/PR^+ (18%), 153 patients with ER^+/PR^- (38%). According to the 2013 St. Gallen International Breast Cancer Conference, breast cancer was divided into four subtypes on the basis of their molecular markers (**Table 1**).

Statistical analysis

SPSS 17.0 software (SPSS Inc., USA) was used to analyze the dates. We used logistic regres-

Table 1. Breast carcinoma classification criteria

Subtypes	ER	PR	HER2	Ki-67
Luminal A	Positive	≥20%	Negative	<20%
Luminal B				
$HER2^-$	Positive	<20% or Ki-67 ≥20%	Negative	
$HER2^+$	Positive	Any	Positive	
HER2 overexpression	Negative	Negative	Positive	
TNBC	Negative	Negative	Negative	

Gnant M *et al*. (2015).

Table 2. The baseline features of 398 breast cancer patients

Variables	No. of cases	Proportion (%)
Diagnosed age		
<48 years	175	44.0
≥48 years	223	56.0
Menopausal status		
Yes	168	42.2
No	230	57.8
Pathological type		
Invasive ductal carcinoma	356	89.5
Invasive lobular carcinoma	18	4.5
Others	24	6.0
Histological grade		
I	11	2.8
II	219	55.0
III	98	24.6
Not applicable	70	17.6
Tumor size		
T_1 (≤2 cm)	158	37.4
T_2 (2~5 cm)	216	51.8
T_3 (>5 cm)	24	10.8
Lymph node metastasis		
Positive	216	56.0
Negative	182	44.0
Pathological staging		
I	69	17.3
II	313	78.6
III	16	4.1
ER^+	225	56.5
PR^+	72	18.1
$HER2^+$	135	33.9
Ki-67^+ (≥20%)	249	62.6

sion analysis to estimate the relation of progression with the ER, PR, Ki67, age, menopausal status, tumor size, and lymph node metastasis. We used the Chi-square test to analyze the difference between two groups. All tests were regarded as statistically significant when the *P* value was <0.05.

Results

Patient characteristics

The clinical characteristics and pathological features were shown in **Table 2**. Ages of the 398 patients ranged from 18 to 81 years (mean, 47.9 ± 9.7 years; median, 48 years). More than 90% of patients were diagnosed with invasive carcinoma and grade III tumors were 24.6%. ER and PR positive cases were 225 (56.5%) and 72 (18.1%). There were 135 (33.9%) of cases had $HER2^+$ breast cancer. 62.6% of patients had high expression of Ki67, which were classified as ≥20%.

There were 40 (10.0%), 132 (33.2%), 53 (13.3%), 82 (20.6%), and 91 (22.9%) cases classified as Luminal A, Luminal B-$HER2^-$, Luminal B-$HER2^+$, TNBC, and $HER2^+$ subtype, respectively. Among which luminal B subtype accounted for the highest proportion of the 398 cases of breast cancer. There were no association between molecular subtypes and diagnosed age or menopausal status of breast cancer patients. Pathological type, histological grade, tumor size, lymph node metastasis, pathological staging and $Ki67^+$ were remarkably different in the various luminal subtypes (P<0.05, all) (**Table 3**).

ER, PR, and Ki67 are associated with high risk of progression

Table 4 shows the ORs and 95% CIs for the ER, PR, Ki67, age, menopausal status, tumor size, and lymph node metastasis. We tracked down the patients, and whether progress was used to complete the logistic regression. ER levels (HR: 5.59, 95% CI: 2.42-12.95, P<0.001), PR levels (HR: 0.19, 95% CI: 0.04-0.90, P = 0.036), Ki-67 proliferation index (HR: 5.84, 95% CI: 1.91-17.85, P = 0.002), tumor size (HR: 1.61, 95% CI: 1.26-2.04, P<0.001), and lymph node metastasis (HR: 2.84, 95% CI: 1.27-6.36, P = 0.011) demonstrated the high risk of progression.

Lack of PR expression in ER^+ tumors may be a surrogate marker of aggressive

As shown in **Table 5**, we summarized the clinical and biologic tumor characteristics in ER^+/

Table 3. Relationship between the molecular subtypes of breast cancer and the clinicopathological features

Variables	Luminal A	Luminal B		HER2 enriched	TNBC	χ^2	P
		HER2 (-)	HER2 (+)				
No. of cases (%)	40 (10.0)	132 (33.2)	53 (13.3)	82 (20.6)	91 (22.9)		
Diagnosed age							
<48 years	18 (4.5)	62 (15.6)	25 (6.3)	28 (7.0)	42 (10.6)	4.108	0.392
≥48 years	22 (5.50	70 (17.6)	28 (7.0)	54 (13.6)	49 (12.3)		
Menopausal status							
Yes	15 (3.8)	53 (13.3)	24 (6.0)	40 (10.1)	36 (9.1)	2.511	0.643
No	25 (6.2)	79 (19.9)	29 (7.3)	42 (10.5)	55 (13.8)		
Pathological type							
Invasive ductal carcinoma	37 (9.3)	110 (27.6)	45 (11.3)	75 (18.8)	89 (22.4)	21.296	0.006
Invasive lobular carcinoma	3 (0.7)	10 (2.6)	4 (1.0)	0 (0.0)	1 (0.25)		
Others	0 (0.0)	12 (3.0)	4 (1.0)	7 (1.8)	1 (0.25)		
Histological grade							
I	2 (0.5)	5 (1.3)	0 (0.0)	1 (0.3)	3 (0.7)	39.741	0.001
II	33 (8.3)	68 (17.1)	27 (6.8)	43 (10.8)	48 (12.1)		
III	2 (0.5)	26 (6.5)	15 (3.8)	20 (5.0)	35 (8.8)		
Not applicable	3 (0.7)	33 (8.3)	11 (2.7)	18 (4.5)	5 (1.3)		
Tumor size							
T1 (≤2 cm)	37 (9.3)	37 (9.3)	21 (5.3)	31 (7.7)	32 (8.1)	62.976	0.001
T2 (2~5 cm)	3 (0.7)	87 (21.9)	27 (6.8)	42 (10.6)	57 (14.3)		
T3 (>5 cm)	0 (0.0)	8 (2.0)	5 (1.2)	9 (2.3)	2 (0.5)		
Lymph node metastasis							
Positive	16 (4.0)	61 (15.4)	25 (6.3)	49 (12.3)	65 (16.3)	19.602	0.001
Negative	24 (6.0)	71 (17.8)	28 (7.0)	33 (8.3)	26 (6.6)		
Pathological staging							
I	14 (3.5)	17 (4.3)	10 (2.5)	13 (3.3)	15 (3.8)	15.688	0.047
II	26 (6.5)	106 (26.6)	42 (10.5)	65 (16.3)	74 (18.6)		
III	0	9 (2.3)	1 (0.3)	4 (1.0)	2 (0.5)		
Ki-67 states							
≥20%	0	92 (23.2)	37 (9.3)	47 (11.8)	73 (18.4)	66.045	0.001
<20%	40 (10)	40 (10)	16 (4.0)	35 (8.8)	18 (4.5)		

Table 4. Analysis of factors related to progress

Variable	HR	95% CI	P
ER	5.59	2.42-12.95	<0.001
PR	0.19	0.04-0.90	0.036
Ki67	5.84	1.91-17.85	0.002
Age	1.00	0.95-1.01	0.969
Menopausal status	0.51	0.18-1.44	0.205
Tumor size	1.61	1.26-2.04	<0.001
Lymph node metastasis	2.84	1.27-6.36	0.011

PR^+ and ER^+/PR^- early breast cancer patients. PR level was not associated with diagnosed age, menopausal status, histological grade and lymph node metastasis (P>0.05, all). However, larger size were found more frequently in ER^+/PR^- tumors than in ER^+/PR^+ tumors (T2 and T3, P<0.001). Pathological staging were found statistically significant difference in ER^+/PR^+ and ER^+/PR^- early breast cancer patients (P<0.001).

ER^+/PR^- tumors displayed more aggressive features than ER^+/PR^+ tumors. HER2 overexpression are markers of tumor aggressiveness with poor survival in breast cancer (P<0.01) [10-12]. HER2 positive state were more frequent in ER^+/PR^- tumors than ER^+/PR^+ tumors. Higher Ki67 expression was strongly predictive marker of poor overall survival [13]. Compared with $ER^+/$

Table 5. Clinical and biological characteristics in patients with ER^+/PR^+ and ER^+/PR^- breast cancer

	Total 225	ER^+/PR^+ 72	ER^+/PR^- 153	*P*-value
Diagnosed age				
<48	105	36	69	0.492
≥48	120	36	84	
Menopausal status				
Yes	92	26	66	0.317
No	133	46	87	
Histological grade				
I		2	5	0.535
II		46	82	
III		12	31	
Not applicable		12	35	
Tumor size				
T1 (≤2 cm)	95	61	34	0.001
T2 (2~5 cm)	117	11	106	
T3 (>5 cm)	13	0	13	
Lymph node metastasis				
Positive	102	30	72	0.449
Negative	123	42	81	
Pathological staging				
I	57	36	21	0.001
II	162	36	126	
III	6	0	6	
Ki67 status				
≥20%	129	24	105	0.001
<20%	96	48	48	
HER-2 status				
Positive	53	10	43	0.019
Negative	172	62	110	

PR^+ tumors, high Ki67 expression (≥20%) was more common in ER^+/PR^- tumors than ER^+/PR^+ tumors ($P<0.01$).

Ki67 was correlation with age, tumor size and tumor grade

As a proliferation marker, Ki-67 is associated with the extent of tumor differentiation, invasion and metastasis as well as prognosis [14]. In this study, the level of Ki67 expression was higher in younger patients and larger tumors ($P<0.05$, both). Tumor grade III was more easily to find high Ki67 ($P<0.05$). Lymph node metastasis and HER2 state had no correlation with Ki67 ($P>0.05$). There were more possibility to have a higher Ki67 in patients with ER^- or PR^- (**Table 6**).

Discussion

The biological characteristics of breast cancer and clinical prognosis are closely inter-linked. The reduced mortality was well-documented for breast cancer over the last few decades, with the development of more effective treatments for different molecular features. Molecular biomarkers including ER, PR, HER2 and Ki67 are related to classification on the basis of four major breast cancer subtypes. The ER positive cancers were defined as luminal A with higher PR (≥20%), low Ki67 index; and Luminal B-HER2 negative if Ki67 high; or HER2-positive [2, 15].

Hormonal receptors, which are acknowledged predictive and prognostic biomarkers, were used to determine benefit from endocrine therapies in breast cancers. The expression of receptors are routinely measured by immunohistochemistry [16]. Although standard therapy to HR^+ breast cancer is endocrine treatment, evidence reported that breast cancers lack of PR expression are less sensitive to tamoxifen than positive PR expression in ER^+ tumors. Gradishar *et al.* hypothesized that the loss of PR is a marker for increased growth factor receptor tyrosine kinase activity that causes lower PR expression and tamoxifen resistance in ER^+ breast cancer patients [17, 18]. In this study, we tried to find out the association with markers such as ER, PR, Ki67 and HER2 expression which are most useful in predicting response to therapy, and compare them with other prognostic parameters including diagnosed age, menopausal status, histological grade, tumor size, and lymph node metastasis in breast cancer patients. We found that breast cancers with PR^- had larger tumor size, more advanced pathological stage, more HER2 positive location, and higher Ki67 expression, which are more aggressive ($P<0.001$, all).

Ki67, a nuclear antigen related to cell proliferation, is a molecular biomarker for the evaluation of the cell proliferation. Evidences reported that the proliferative activity of Ki67 is related to the tumor invasion, differentiation, metastasis, and prognosis [19-21]. The level of Ki67

Table 6. Baseline characteristic of Ki67 Index

	Total 398	Ki67<20% 149	Ki67≥20% 249	*P*-value
Age				
<48	203	59	144	0.001
≥48	195	90	105	
Tumor size				
≤2 cm	158	71	87	0.012
>2 cm	240	78	162	
Tumor grade				
I-II	230	113	117	0.001
III	99	15	84	
NA	69	21	48	
Lymph node metastasis				
Negative	182	70	112	0.698
Positive	216	79	137	
HER2 state				
Negative	263	100	163	0.736
Positive	135	49	86	
ER				
Negative	173	53	120	0.014
Positive	225	96	129	
PR				
Negative	326	101	225	0.001
Positive	72	48	24	

expression lead to diverse subtypes including luminal A and luminal B since 2013 St. Gallen Consensus Conference. In this study, we investigated the relationship between Ki67 and the clinicopathology of breast cancer. We found that younger patients have more possibility to have a higher Ki67. Higher Ki67 was accompanied with larger size tumors, advanced tumor grade, and negative PR state ($P<0.001$, all).

Our research demonstrated the association with ER, PR, HER2 and Ki67 index in breast cancer in different molecular subtype status. More importantly, we found that the lack of PR expression in ER^+ tumors may be a surrogate marker of aggressiveness which were related with Ki67 and HER2. A significant Ki67 increase was more common in younger patient and larger size tumors, and patients with ER^- or PR^- are more possibility to have a higher Ki67. Follow-up prognosis is required to investigate the association with molecular biomarkers and disease-free survival (DFS), and further exploration is needed to clarify the mechanism.

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Disclosure of conflict of interest

None.

Abbreviations

HR, hormone receptor; TNBC, triple negative breast cancer; IHC, immunohistochemistry; DFS, disease-free survival; FISH, fluorescent in situ hybridization.

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