RESEARCH COMMUNICATION

The Estrogen Receptor Negative-Progesterone Receptor Positive Breast Carcinoma is a Biological Entity and not a Technical Artifact

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Abstract

The ER-/PR+ breast tumor may be the result of a false ER negative result. The aim of this study was to investigate whether there is a difference in patient and tumor characteristics of the ER-/PR+ phenotype in an Asian setting. A total of 2629 breast cancer patients were categorized on the basis of their age, ethnicity, tumor hormonal receptor phenotype, grade and histological type. There were 1230 (46.8%) ER+/PR+, 306 (11.6%) ER+/PR-, 122 (4.6%) ER-/PR+ and 972 (37%) ER-/PR-. ER-/PR+ tumors were 2.5 times more likely to be younger than 50 years at diagnosis (OR: 2.52; 95% CI: 1.72-3.67). Compared to ER+/PR+ tumors, the ER-/PR+ phenotype was twice more likely to be associated with grade 3 tumors (OR:2.02; 95%CI: 1.00-4.10). In contrast, compared to ER-/PR- tumors, the ER-/PR+ phenotype was 90% less likely to be associated with a grade 3 tumor (OR: 0.12; 95%CI:0.05-0.26), and more likely to have invasive lobular than invasive ductal histology (OR: 3.66; 95%CI: 1.47-9.11). These results show that the ER-/PR+ phenotype occurs in a younger age group and is associated with intermediate histopathological characteristics compared to ER+/PR+ and ER-/PR- tumors. This may imply that it is a distinct entity and not a technical artifact.

Keywords: Breast cancer - hormonal receptors - ER-PR+ subtype - biological entity

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Introduction

Steroid hormone receptors (HR) are important prognostic and predictive markers for response to endocrine therapy in the management of breast cancer. Several studies have found that up to 10% of estrogen receptor-negative (ER-) breast cancers are progesterone receptor-positive (PR+) (Rakha et al., 2010) although recent evidence shows that the percentage is much lower when more sensitive immunohistochemical (IHC) methods for ER determination are used (Rakha et al., 2007; 2010; Rhodes & Jasani 2009) and the high proportion of ER-/PR+ may be due to a false-negative ER assay (Nadji et al., 2005; Nadji, 2008). There have been differing opinions on the status of ER- in the presence of PR+ status in breast tumors over the last few years. The ER-/PR+ phenotype is simply thought by some to be due to artifacts arising from the preparation or assay of the sample i.e. due to inadequate tissue fixation or technique failure of the IHC assay, and that these tumors are essentially positive for both receptors (Nadji et al., 2005; Nadji, 2008). If this is true, then it would be expected that the ER-/PR+ phenotype would have the same tumor characteristics as the ER+/PR+ phenotype. However, recent- studies have shown that ER-/PR+ tumors exhibit more aggressive behavior than double-hormone receptor-positive cancers (Rakha et al., 2007). The aim of this study was therefore to investigate whether there is a difference in patient and tumor characteristics between breast cancers of differing steroid hormone receptor status in an Asian setting.

Materials and Methods

The University Malaya Medical Centre (UMMC)'s prospective Breast Cancer Registry was used to identify 2629 patients newly diagnosed from 2003-2009 with known ER and PR status. Patients were divided into four categories namely ER+/PR+, ER+/PR+, ER-/PR+ and ER-/PR-. Factors studied in relation to these four groups were age, ethnicity (Chinese, Malay, Indian), tumor grade (Bloom-Richardson; grade 1, 2, 3) and histological type (invasive ductal, invasive lobular, others).

The estrogen and progesterone receptor status of each case was determined immunohistochemically utilizing the antibody clones 1D5 (Dako Ltd, Denmark) and PgR 636 (Dako Ltd Denmark), respectively. Briefly ,the slides were de-paraffinised and endogenous peroxidase blocked in 0.3% hydrogen peroxide prior to microwave antigen

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retrieval in 0.01M sodium citrate (pH 6.0) buffer for a duration of 30 minutes. Following incubation in primary antibody clones for 60 minutes, detection was achieved by placing in an EnVision polymer with HRP label (Dako, Ltd, Denmark) solution for a further30 min. Visualisation was achieved by using a hydrogen peroxide substrate a diaminobenzidine chromogen (Dako Ltd, Denmark). Nuclei were counterstained with Harris's Haematoxylin.

Categorical variables were compared using the chi-square test –and continuous variables using one way analysis of variance (ANOVA). Univariable and multivariable logistic regression analyses were used to determine the association between patient and tumor characteristics (independent) and hormone receptor status (dependent). All analyses were performed using SPSS version 16.0 (SPSS Inc, Chicago, USA).

Results

There were 1230 (46.8%) patients with ER+/PR+, 306 (11.6%) ER+/PR-, 122 (4.6%) ER-/PR+ and 972 (37%) ER-/PR- tumors. The mean age was 53, 55, 48 and 52 years old respectively (p<0.001). There was no significant difference in distribution of hormone receptor status between the different ethnic groups (Table 1).

In the univariable logistic regression, patients with ER-/PR+ tumors were 2.5 times more likely to be younger than 50 years at diagnosis compared to all other phenotypes; OR: 2.52; 95% CI: 1.72 – 3.67.

The ER-/PR+ phenotypes were twice more likely to be grade 3 tumors, compared to ER+/PR+ cases, (OR:2.02; 95%CI: 1.00-4.10) (Table 2) and 90% less likely to be associated with a grade 3 tumor compared to the ER-/PR-, phenotypes (OR: 0.12; 95%CI:0.05-0.26). The ER-/PR+ tumors were also highly likely to have invasive lobular histology than invasive ductal histology (OR: 3.66; 95%CI: 1.47-9.11), compared to ER-/PR- tumors.

Following multivariable analysis, age less than 50 years (OR:2.23; 95%CI: 1.46-3.40) and grade 3 tumors (OR:2.02; 95%CI: 0.98-4.19) were twice as likely to be associated with ER-/PR+ phenotypes when compared to ER+/PR+ tumors. Compared with ER-/PR- phenotypes, ER-/PR+ tumors were more likely to be associated with younger age (<50 years); OR: 2.90; 95%CI: 1.86-4.53), but 90% less likely to be associated with grade 3 tumors (OR: 0.10; 95%CI: 0.04-0.23).

Table 1. Comparison of Four Hormonal Receptor Phenotypes in Relation to Other Clinicopathological Variables.

	ER+/PR+	ER+/PR-	ER-/PR+	ER-/PR-	
No of patients					
	1230	306	122	972	p<0.001
Total n=2629					
	47%	12%	5%	37%	
Mean age	53	55	48	52	p<0.001
Ethnicity					p=0.10
Malay	229 (12%)	45 (14%)	23 (15%)	210 (14%)	
Chinese	853 (69%)	217 (71%)	81 (66%)	628 (65%)	
Indian	148 (19%)	44 (15%)	18 (19%)	134 (22%)	
Grade					p<0.001
1	146 (15%)	20 (8%)	11 (11%)	18 (2%)	
2	596 (61%)	134 (56%)	58 (56%)	256 (33%)	
3	230 (24%)	85 (36%)	35 (34%)	498 (65%)	
Type					p<0.001
IDC	1050 (85%)	277 (91%)	107 (88%)	896 (92%)	
ILC	71 (6%)	15 (5%)	7 (6%)	16 (2%)	
Others	109 (9%)	14 (5%)	8 (6%)	60 (6%)	

* 'IDC; invasive ductal carcinoma, ILC; invasive lobular carcinoma

Table 2. Association Between Patient/Tumor Characteristics and the ER-/PR+ Hormonal Receptor Phenotype

	ER-/PR+ vs ER+/PR+		ER-/PR+ vs ER-/PR-	
	Uni OR	Multi OR*	Uni OR	Multi OR*
Age				
<50	2.28(1.6-3.4)+	2.23(1.5-3.4)+	2.44(1.7-3.6)+	2.90(1.9-4.5)+
≥50	1.00	1.00	1.00	1.00
Race				
Chinese	1.00	1.00	1.00	1.00
Malay	1.06(0.7-1.7)	1.12(0.7-1.9)	1.04(0.6-1.8)	1.15(0.6-2.1)
Indian	1.28(0.8-2.2)	1.44(0.8-2.6)	0.85(0.5-1.4)	0.87(0.5-1.5)
Tumor grade				
1	1.00	1.00	1.00	1.00
2	1.29(0.7-2.5)	1.30(0.7-2.6)	0.37(0.2-0.8)+	0.36(0.2-0.8)+
3	2.02(1.0-4.1)+	2.02(0.9-4.2)	0.12(0.0-0.3)+	0.10(0.0-0.2)+
Histology				
IDC	1.00	1.00	1.00	1.00
ILC	0.97(0.4-2.2)	0.73(0.2-3.1)	3.66(1.5-9.1)+	1.10(0.2-5.9)
Others	0.72(0.3-1.5)	1.45(0.5-4.3)	1.12(0.5-2.4)+	1.47(0.5-4.8)

*OR, Odd ratio, Derived using logistic regression model with age, race, tumor grade and tumor histology as independent variables and the ER-/PR+ phenotype as dependent variable, +Indicates that OR is statistically significant; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma

Discussion

The presence of ER expression in breast cancer is well known to be used as the main determinant of response in the adjuvant hormone treatment of patients with breast cancer. PR is an estrogen-regulated gene and its expression is therefore thought to indicate a functioning ER pathway (Rakha et al., 2010). Four subgroups are derived when the combined expressions of ER and PR are considered, namely double HR+ (ER+/PR+), single HR + (ER+/PR- and ER-/PR+) and double HR- (ER-/PR-). Double HR+ is associated with older age, smaller tumor size and lower grade (Dunnwald et al., 2007; Rakha et al., 2010) and has been shown to have good response to hormonal therapy in comparison to double HR- where the reported response rate is negligible (Rakha et al., 2007; 2010). Western studies have shown that the percentages of ER+/PR+ were in the region of 55-65% and ER-/PR- were 18-25%. In this Asian breast cancer cohort, we found a lower proportion of double HR+ (46.8%) and higher proportion of double HR- (37 %) tumors when compared to the West (Rhodes, Jasani et al. 2000; Bhoo Pathy, Yip et al. 2011) which is consistent with other Asian studies (Chow & Ho 2000; Yip 2009). This is probably due to the fact that compared to Caucasian women, Asian patients are relatively younger (below 50 years) at diagnosis(Yip 2009) and also use less hormone replacement therapy (Bhoo et al., 2011). It has been previously shown that hormone replacement therapy use is associated with higher rates

of ER positivity (Bhoo et al., 2011).

The significance of the single HR+ phenotype that includes ER+/PR- and ER-/PR+ is still poorly understood. The percentage of ER+/PR- (11.6%) and ER-/PR+ (4.6%) tumors in our cohort is found to be consistent to those quoted in other studies. These tumors are often of higher grade, larger size and hormonal therapies are less likely to be effective than in the ER+/PR+ phenotype(Rakha, et al., 2007; De et al., 2008; Rakha et al., 2010). In this study we found that the ER-/PR+ tumor was significantly associated with a younger age at diagnosis compared to all other phenotypes. A similar finding was found (Rakha et al., 2007) who showed that the majority of the ER+/PR- phenotypes were significantly older as compared to the ER-/PR+ phenotype. Our result is also consistent with previous studies that showed the ER-/PR+ phenotype occurring twice as often in the less than 50-year age group as compared to those 50 years of age and above (De Maeyer et al. 2008; Rhodes and Jasani 2009). If the ER-/PR+ phenotype does not exist and is indeed an artifact of the ER or PR assay then there should be no difference in the age at diagnosis.

Studies have shown that single hormone receptor positive phenotype which include ER+/PR- and ER-/PR+ were more often of higher histology grade and larger in size, more likely to be aneuploid and show higher expression of proliferation-related genes than ER+/PR+ and both single HR+ groups are similar and they both might have biological characteristics somewhere in between ER+/PR+ and ER-/PR- (De et al., 2008; Rakha et al., 2010). We found that higher grades of tumors are more likely to be associated with ER-/PR+ as compared with ER+/PR+ tumors but less likely to be associated with ER-/PR- tumors in this series and more likely to have invasive lobular than invasive ductal histology. This is the first time that the ER-/PR+ has been shown to be associated with invasive lobular carcinoma (ILC). However this finding has to be interpreted with caution because ILC comprised only 8.9% of the breast cancers in this study. It is known that infiltrating lobular carcinomas are less common in Asians compared to Caucasians (Yip 2009).

In conclusions, the ER-/PR+ phenotype is significantly associated with a younger age at diagnosis compared to all other phenotypes. It also is associated with intermediate histopathological characteristics compared to ER+/PR+ and ER-/PR- tumors, whereby it is more aggressive than ER+/PR+ tumor but less aggressive than ER-/PR- tumors and therefore unlikely to be a technical artifact.

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