

ORIGINAL ARTICLE

Does single receptor positive breast carcinoma differ with regard to age, tumor grade, and HER2 amplification status?

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Summary

Purpose: To investigate whether there is a difference in patient and tumor characteristics in cases with single receptor positive (SRP) (ER-/PR+ and ER+/PR-) breast carcinoma in comparison with the double receptor positive (DRP) (ER+/PR+) and double receptor negative (DRN) (ER-/PR-) tumors.

Methods: A total of 255 breast cancer patients were categorized on the basis of their tumor hormonal receptor phenotype, age, grade, and HER2 amplification status. The study focused on the SRP phenotype (ER+/PR- and ER-/PR+) and compared it with the DRP (ER+/PR+) and DRN (ER-/PR-) tumors.

Results: There were 103 (40.3%) DRP tumors, 98 (38.4%) DRN tumors and 54 (21%) SRP tumors, 41 (16.1%) of which were ER+/PR- and 13 (5.1%) were ER-/PR+. Compared to DRP tumors, the SRP group was more likely

to be associated with grade 3 tumors and higher frequency of HER2 amplification status. ER-/PR+ tumors were more likely to be associated with younger age at diagnosis compared to ER+/PR- tumors. HER2 amplification, age, and grade were not significantly different between ER-/PR+ and DRN groups. Compared to the DRN group, the ER+/PR- group had lower grade.

Conclusions: Our findings demonstrated that SRP phenotype including ER+/PR- and ER-/PR+ tumors is different from DRP group with regard to age, grade, and HER2 amplification status. Moreover, our data showed that ER-/PR+ tumors are associated with younger age.

Key words: breast carcinoma, hormonal receptors, single receptor

Introduction

Prognosis and survival rates of breast carcinoma vary and are correlated with a large number of characteristics such as age, tumor stage, and the inherent properties of the tumor [1]. Histological grade and histological type are two of the morphologic findings on which the classification of breast carcinomas is based [2].

Estrogen receptor (ER), progesterone receptor (PR), and HER2 are the three markers used in routine clinical practice for their predictive significance on the response or resistance to treatment and the potential use of new drugs [3-6].

Compared to HER2- tumors, which are typically connected with ER+ tumors, HER2+ tumors

may be more aggressive and have a worse prognosis [7]. HER2 positivity has also been shown to contribute to a worse response to hormone blockade [8,9]. Moreover, a negative correlation has been shown between HER2 positivity and hormone receptor positivity [10].

ER status is associated with response to hormonal blockade, such as tamoxifen [11,12]. Although the prognostic value of PR expression does not depend on ER status [13], and immunohistochemistry (IHC) determination of PR expression has been clinically validated, PR is reported together with ER [14]. It has also been shown that tamoxifen is less effective in ER+/PR- tumors than

in ER+/PR+ tumors [9,15].

The significance of ER+/PR- tumors as a distinct subset of breast cancer has been well documented [13,14,16,17]. However, there is still a debate over the significance of ER-/PR+ tumors as a clinically and biologically distinct group of breast cancer. Some authors reported that there are no true ER-/PR+ breast tumors in the sense that they are technical artifacts [18,19] or very rare to be in clinical use [20] while some others claimed that they are clinically and biologically distinct tumors [15,21].

The aim of this study was to investigate whether there is a difference in patient and tumor characteristics of the single receptor positive (SRP) (ER-/PR+, ER+/PR-) breast carcinoma phenotype in comparison with DRP and DRN groups.

Methods

Patients selection

Primary invasive ductal type breast carcinoma cases (N=255) referred to the Department of Pathology, Tepecik Training and Research Hospital, Izmir, Turkey between 2011 and 2014 were investigated in this study. HER2, IHC, and fluorescent in situ hybridization (FISH) analyses were performed successfully for each case as part of the routine diagnostic workup. Histological features of each tumor were obtained from pathology reports. The following variables were assessed: tumor hormonal receptor phenotype, age, grade, and HER2 amplification status.

Histological examination

Histological assessment of tumor type and grade were performed routinely on formalin-fixed and paraffin embedded specimens according to the 2010 WHO classification [22].

Assay methods

IHC

The following antibodies (ER dilution: 1/800: clone 1D5, Dako, Denmark; PR dilution 1/1000: clone PgR 636, Dako, Denmark; clone: A0485, dilution 1/300, Dako, Denmark) were used to determine the ER and PR status of each case according to the manufacturer's instructions. All single-receptor positive cases were reevaluated for ER and PR stains. Repeat ER and PR stains were reviewed by a pathologist blinded to clinical characteristics and tumor histology. Nuclear staining of any intensity was considered positive in all PR and ER IHC staining cases.

FISH

FISH analysis was carried out on tissue mi-

croassays using the IQ FISH pharmDxKit (Dako, Denmark) as previously described [23]. After incubation at 60°C for 60 min, the paraffin sections were deparaffinized in two series of xylol, and rehydrated with ethanol series. Pretreatment of the slides with EnVision Flex (20x) solution (Dako, Denmark) in a water bath at 99°C for 10 min was followed by enzymatic digestion with ready-to-use pepsin for 4 min at 37°C on hybridizer (Dako, Denmark). Then, the slides were dehydrated and 10 µl of HER2/cen17 probe was incubated to each tissue sections.

Before the hybridization period for 120 min at 45°C, the slides and probe were denatured at 66°C for 10 min. Then, the slides were washed with stringent wash buffer at 63°C for 10 min in a water bath. After dehydration, tissue sections were counterstained with 10 µl of fluorescence mounting medium containing 4,6-diamino-2-phenylindole (DAPI).

Scoring method of FISH

The samples were classified after counting 20 tumor cell nuclei with a fluorescence microscope (Olympus BX51, Japan) equipped with a DAPI/Spectrum Red/Spectrum Green filter set using a ×100 oil immersion objective lens. A sample was considered to be amplified when the ratio of HER2/cen17 was ≥ 2 [24].

Statistics

Clinicopathologic features of each group were statistically analyzed by Pearson's chi-square test and Mann-Whitney U test. A p value <0.05 was considered statistically significant. Statistical tests were carried out using the SPSS version 15.0 for Windows (SPSS Inc., Chicago, Ill, USA).

Results

There were 103 (40.3%) DRP tumors, 98 (38.4%) DRN tumors and 54 (21%) SRP tumors, 41 (16%) of which were ER+/PR- and 13 (5%) were ER-/PR+. Table 1 presents the clinicopathological characteristics of the 4 groups.

Compared to the DRP group, both ER+/PR- and ER-/PR+ tumors were likely to have higher HER2 amplification (10.7 vs 29.3%, $p < 0.01$ and 10.7 vs 38.5%, $p < 0.05$). However, compared to the DRN group, neither ER+/PR- group nor ER-/PR+ group was significantly different in relation to HER2 amplification (44.9 vs 29.3%, $p = 0.0893$ and 44.9 vs 38.5%, $p = 0.6612$). There was no significant difference between ER+/PR- and ER-/PR+ groups in relation to HER2 amplification status (29.3 vs 38.5%, $p = 0.5354$).

ER+/PR- group was shown to have higher grade than DRP tumors (36.6 vs 20.4%, $p < 0.05$) and lower grade than DRN tumors (36.6 vs 81.6%,

Table 1. Clinicopathological characteristics of the four groups. HER2 estimation by FISH

Variables	Total N (%)	DRP N (%)	ER+/PR- N (%)	ER-/PR+ N (%)	DRN N (%)
Amplified*	72 (28.2)	11 (10.7)	12 (29.3)	5 (38.5)	44 (44.9)
Non-amplified	183 (71.8)	92 (89.3)	29 (70.7)	8 (61.5)	54 (55.1)
Grade					
Low (I -II)	130 (51)	82 (79.6%)	26 (63.4)	4 (30.8)	18 (18.4)
High** (III)	125 (49)	21 (20.4)	15 (36.6)	9 (69.2)	80 (81.6)
Age, years					
<40***	102 (40)	32 (31)	12 (29.3)	8 (61.5)	50 (51)
≥40	153 (60)	71 (69)	29 (70.7)	5 (38.5)	48 (49)
Total	255 (100)	103 (40.4)	41 (16.1)	13 (5.1)	98 (38.4)

*DRP vs ER+/PR-, $p<0.01$; DRP vs ER-/PR+, $p<0.05$; ER+/PR- vs ER-/PR+, $p=0.5354$;

DRN vs ER+/PR-, $p=0.0893$; DRN vs ER-/PR+, $p=0.6612$

**ER+/PR- vs DRP, $p<0.05$; ER-/PR+ vs DRP, $p<0.05$; ER-/PR+ vs ER+/PR-, $p<0.05$;

DRN vs ER+/PR-, $p<0.001$; ER-/PR+ vs DRN, $p=0.298$

***ER+/PR- vs DRP, $p=0.1149$; ER-/PR+ vs DRP, $p<0.05$; ER-/PR+ vs ER+/PR-, $p<0.05$;

ER-/PR+ vs DRN, $p=0.478$; DRN vs ER+/PR-, $p=0.205$.

ER: estrogen receptor, PR: progesterone receptor, DRP: double receptor positive, DRN: double receptor negative

$p<0.001$). ER-/PR+ group had also significantly higher grade than DRP group (69.2 vs 20.4 %, $p<0.001$). ER-/PR+ tumors had non-significantly higher grade than DRN tumors (69.2% vs 81.6%, $p=0.298$ and 69.2 vs 59.1%, $p=0.4891$). Moreover, ER-/PR+ tumors were shown to have higher grade than ER+/PR- tumors (69.2% vs 36.6%, $p<0.05$).

Compared to the patients with DRP and ER+/PR- tumors, those with ER-/PR+ tumors were significantly younger (less than 40 years) (31 vs 61.5%, $p<0.05$; 29.3 vs 61.5%, $p<0.05$, respectively). Yet, age was not significantly different between ER-/PR+ and DRN groups (61.5 vs 55.1%, $p=0.478$).

In summary, SRP phenotype had higher HER2 amplification status and higher grade compared to the DRP group. Patients with ER-/PR+ were significantly younger and were more likely to have higher tumor grade than those with ER+/PR- tumors. There were no significant differences between ER-/PR+ and DRN groups in relation to HER2 amplification, age, and grade. Compared to the DRN group, ER+/PR- group had lower tumor grade.

Discussion

Determining ER and PR status of breast carcinomas has been a standard practice because of the significant positive correlation of ER and PR with

tumor differentiation [25].

The American Society of Clinical Oncology and the College of American Pathologists advocated that both ER and PR should be examined on all newly diagnosed cases of invasive breast cancer [26].

Although there has been an established consensus over the prognostic and predictive significance of testing ER expression, the additional benefit from the assessment of PR receptor remains controversial [15,20,27-29].

In 2004, Olivotto et al. reported the percentage of ER-/PR+ cases as 0.1%, suggesting that PR testing in breast cancer should be stopped [20]. In 2005, Colomer et al. claimed that PR assessment is useful in identifying a distinct breast cancer subtype which is likely to respond positively to adjuvant hormonal therapy [29].

Basing their research on large and well-characterized series of breast cancers, in 2007 Rakha et al. showed that ER+/PR- and ER-PR+ tumors are distinct breast cancer groups compared to the DRP and DRN breast cancers. The authors demonstrated also that SRP breast tumors appear independent of age, with high grades and HER2 positivity, leading to worse patient outcomes compared to DRP tumors [15].

SRP group is comprised predominantly of ER+/PR- tumors, which accounts for 10-16% of all breast cancers [15,18,21,29]. The significance of

ER+/PR- tumors as a distinct disease subtype is well-established. [13,14,16,17]. On the other hand, ER-PR+ group, which accounts for only 2-8% of all breast cancers, is uncommon, and its natural history and responsiveness to hormone therapy remains uncertain [13,15,29-33]. While some reports claimed that ER-/PR+ tumors represent a distinct biologic entity [15,21,34,35], some others suggest that ER-PR+ group is a technical artifact or very rare to be of clinical use [18,19,36].

ER-/PR+ tumors could represent false-negative ER assay resulting from methodological problems with ER detection analysis. The presence of ER could also be at such a low level to be detected by the assay. Alternatively, ER-/PR+ tumors could reflect false-positive PR due to cross-reaction of monoclonal antibodies with other antigens [37,38]. Thus, in order to rule out a false result, ER testing of patients with ER-/PR+ classification has been recommended to be repeated [26].

This paper analyzed in detail the clinopathological characteristics of SRP phenotype in terms of HER2 amplification status, grade, and age through comparison with DRP and DRN groups. In our cohort, 16.1% of the cases were ER+/PR- and 5.1% were ER-/PR+. These figures are consistent with previous studies.

Receptor - HER2

Women with HER2 positive breast cancer have lower hormone receptors due to the negative relationship between hormone receptors and HER2 [39]. It is postulated that in these cases tamoxifen functions as an estrogen agonist to stimulate growth of breast cancer cells, which express an increased level of HER2 and ER co-activation resulting in de novo resistance for endocrine therapies [40,41]. HER2 positivity is thought to contribute to relative resistance to endocrine therapies [10].

Previous studies showed that SRP tumors exhibit higher expression in HER2 than DRP tumors [9,15]. In our study, similar to the findings in the literature, SRP breast carcinoma showed higher amplification of HER2 than DRP tumors.

Receptor - Age

In 2005, Huang et al. reported that the presence of a negative relationship between HER2

and PR was confirmed only in women older than 45, which might explain the sensitivity of breast cancers to anti-oestrogens in young women compared to those beyond 45 years [42].

Prior studies showed that ER-/PR+ breast cancer occurs more commonly in younger patients compared to other phenotypes [18,21,34,35]. Similarly, significant dominance of older age in ER+/PR- group compared to the ER-/PR+ group has been shown [15].

Our findings confirmed the findings of previous studies on the association between ER-/PR+ tumors and younger age at diagnosis by demonstrating that ER-/PR+ group was likely to occur more commonly in patients younger than 40 years.

Receptor-Grade

Compared to the DRP group, SRP breast cancers are more likely to be high grade, large-sized and aneuploid, and show higher expression of proliferation-related genes [15,18,44].

In our study, SRP phenotype also exhibited higher grade compared to DRP. Compared to DRN tumors, the SRP phenotype showed non-significantly lower grade.

Limitations

The limitations of this study include the small sample size and the limited number of ER-/PR+ cases. Insignificant differences between ER-/PR+ and DRN groups might result from these limitations. Yet, to the best of our knowledge, this is the first case study in Turkish patients that scrutinized clinical and biological differences of SRP phenotype compared to other groups of breast carcinoma, in which all SRP cases were reviewed by a pathologist blinded to clinical characteristics and tumor histology, and HER2 amplification was confirmed by FISH analysis.

In conclusion, our findings demonstrated that SRP phenotype including ER+/PR- and ER-/PR+ tumors is different from DRP group with regard to age, grade, and HER2 amplification status. Moreover, our data showed that ER-/PR+ tumors are associated with younger age. Further work with larger sample size is clearly needed to confirm and extend the present findings.

References

- Azzopardi JG, Ahmed A, Millis RR. Problems in breast pathology. *Major Probl Pathol* 1979; 11, i-xvi: 1-466.
- Weigelt B, Geyer FC, Reis-Filho JS. Histological types of breast cancer: how special are they? *Mol Oncol* 2010;4:192-208.
- Kariya S, Ogawa Y, Nishioka A et al. Relationship between hormonal receptors, HER-2, p53 protein, bcl-2, and MIB-1 status and the antitumor effects of neoadjuvant anthracycline-based chemotherapy in invasive breast cancer patients. *Radiat Med* 2005;23:189-194.
- Pinto AE, Andre S, Pareira T, Nobrega S, Soares J. C-erbB-2 onco- protein overexpression identifies a subgroup of estrogen receptor positive (ER+) breast cancer patients with poor prognosis. *Ann Oncol* 2001; 12:525-533.
- Looi LM, Cheah PL. C-erbB-2 oncoprotein amplification in infiltrating ductal carcinoma of breast correlates to high histologic grade and loss of estrogen receptor protein. *Malays J Pathol* 1998;20:19-23.
- Kaptain S, Tan LK, Chen B. Her-2/neu and breast cancer. *Diagn Mol Pathol* 2001;10:139-152.
- Sørli T, Perou CM, Tibshirani R et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci USA* 2001;98:10869-10874.
- Arpino G, Weiss H, Lee AV et al. Estrogen receptor-positive, progesterone receptor-negative breast cancer: Association with growth factor receptor expression and tamoxifen resistance. *J Natl Cancer Inst* 2005;97:1254-1261.
- Arpino G, Wiechmann L, Osborne CK et al. Crosstalk between the estrogen receptor and the HER tyrosine kinase receptor family: Molecular mechanism and clinical implications for endocrine therapy resistance. *Endocr Rev* 2008;29:217-233.
- Konecny G, Pauletti G, Pegram M et al. Quantitative association between HER-2/neu and steroid hormone receptors in hormone receptor- positive primary breast cancer. *J Natl Cancer Inst* 2003;95:142-153.
- Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. *Mod Pathol* 1998;11:155-168.
- Ciocca DR, Elledge R. Molecular markers for predicting response to tamoxifen in breast cancer patients. *Endocr* 2000;13:1-10.
- Bardou VJ, Grazia Arpino, Richard ME et al. Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. *J Clin Oncol* 2003;21:1973-1979.
- Mohsin SK, Weiss H, Havighurst T et al. Progesterone receptor by immunohistochemistry and clinical outcome in breast cancer: a validation study. *Mod Pathol* 2004;17:1545-1554.
- Rakha EA, El-Sayed ME, Green AR et al. Biologic and clinical characteristics of breast cancer with single hormone receptor positive phenotype. *J Clin Oncol* 2007;25:4772-4778.
- Liu S, Chia SK, Mehl E et al. Progesterone receptor is a significant factor associated with clinical outcomes and effect of adjuvant tamoxifen therapy in breast cancer patients. *Breast Cancer Res Treat* 2010;119:53-61.
- Punglia RS, Kuntz KM, Winer EP, Weeks JC, Burstein HJ. The impact of tumor progesterone receptor status on optimal adjuvant endocrine therapy for postmenopausal patients with early-stage breast cancer: a decision analysis. *Cancer* 2006;106:2576-2582.
- De Maeyer L, Van Limbergen E, De Nys K et al. Does estrogen receptor negative/progesterone receptor positive breast carcinoma exist? *J Clin Oncol* 2008;26:335-336. Author's reply 336-338.
- Nadji M, Gomez-Fernandez C, Ganjei-Azar P, Morales AR. Immunohistochemistry of estrogen and progesterone receptors reconsidered: experience with 5,993 breast cancers. *Am J Clin Pathol* 2005;123:21-27.
- Olivotto IA, Truong PT, Speers CH et al. Time to stop progesterone receptor testing in breast cancer management. *J Clin Oncol* 2004;22:1769-1770.
- Rhodes A, Jasani B. The oestrogen receptor-negative/progesterone receptor-positive breast tumour: a biological entity or a technical artifact? *J Clin Pathol* 2009;62:95-96.
- Ellis IO, Schnitt SJ, Sastre-Garau X et al. Invasive breast carcinoma. In: Tavassoli FA, Devilee P (Eds): *World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of the Breast and Female Genital Organs*. Lyon, France: IARC Press, 2003, pp:13-59.
- Pala EE, Bayol U, Ozguzer A, Akman O. HER2 status in gastric cancer: a comparison of two novel in situ hybridization methods (IQ FISH and dual color SISH) and two immunohistochemistry methods (A0485 and HercepTest™). *Pathol Res Pract* 2013;209:548-554.
- Wolff AC, Hammond ME, Hicks DG et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update. *J Clin Oncol* 2013;31:3997-4013.
- Nisa A, Bhurgri Y, R Farrukh, Kayani N. Comparison of ER, PR & HER-2/ neu (C-erb B2) reactivity pattern with histologic grade, tumor size and lymph node status in breast cancer. *Asian Pac J Cancer Prev* 2008;9:553-556.
- Hammond ME, Hayes DF, Dowsett M et al. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer. *J Clin Oncol* 2010;28:2784-2795.
- Colozza M, Larsimont D, Piccart MJ. Progesterone receptor testing: not the right time to be buried. *J Clin Oncol* 2005;23:3867-3868. Author's reply 3869-3870.
- Fuqua SA, Cui Y, Lee AV, Osborne CK, Horwitz KB. Insights into the role of progesterone receptors in breast cancer. *J Clin Oncol* 2005; 23:931-932. Author's

- reply 932-933.
29. Colomer R, Beltran M, Dorcas J et al. It is not time to stop progesterone receptor testing in breast cancer. *J Clin Oncol* 2005;23:3868-3869. Author's reply 3869-3870.
 30. Dowsett M, Cuzick J, Wale C et al. Retrospective analysis of time to recurrence in the ATAC trial according to hormone receptor status: An hypothesis-generating study. *J Clin Oncol* 2005;23:7512-7517.
 31. Fern M, Stal O, Baldetorp B et al. Results of two or five years of adjuvant tamoxifen correlated to steroid receptor and S-phase levels. South Sweden Breast Cancer Group, and South-East Sweden Breast Cancer Group. *Breast Cancer Res Treat* 2000;59:69-76.
 32. Colditz GA, Rosner BA, Chen WY et al. Risk factors for breast cancer according to estrogen and progesterone receptor status. *J Natl Cancer Inst* 2004;96:218-228.
 33. Bernoux A, de Cremoux P, Laine-Bidron C et al. Estrogen receptor negative and progesterone receptor positive primary breast cancer: Pathological characteristics and clinical outcome. Institut Curie Breast Cancer Study Group. *Breast Cancer Res Treat* 1998;49:219-225.
 34. Ng CH, Pathy NB, Taib NA, Mun KS, Rhodes A, Yip CH. The estrogen receptor negative-progesterone receptor positive breast carcinoma is a biological entity and not a technical artifact. *Asian Pac J Cancer Prev* 2012;13:1111-1113.
 35. Chan M, Chang MC, González R et al. Outcomes of Estrogen Receptor Negative and Progesterone Receptor Positive Breast Cancer. *PloS One* 2015;10:e0132449. doi:10.1371/journal.pone.0132449.
 36. Hefti MM, Hu R, Knoblauch NW et al. Estrogen receptor negative/progesterone receptor positive breast cancer is not a reproducible subtype. *Breast Cancer Res* 2013;15:R68.
 37. Fuqua SA, Fitzgerald SD, Chamness GC et al. Variant human breast tumor estrogen receptor with constitutive transcriptional activity. *Cancer Res* 1991;51:105-109.
 38. McGuire WL. Breast cancer prognostic factors: Evaluation guide- lines. *J Natl Cancer Inst* 1991;83:154-155.
 39. Huang HJ, Neven P, Drijckoningen M et al. Association between tumor characteristic and HER2/neu by immunohistochemistry in 1362 women with primary operable breast cancer. *J Clin Pathol* 2005;58:611-616.
 40. Shou J, Massarweh S, Osborne CK et al. Mechanisms of tamoxifen resistance; increased estrogen receptor – her-2/neu cross talk in ER/Her-2 positive breast cancer. *J Natl Cancer Inst* 2004;96:926-935.
 41. Rakha EA, Reis-Filho JS, Ellis IO. Basal-like breast cancer: a critical review. *J Clin Oncol* 2008;26:2568-2581.
 42. Huang HJ, Neven P, Drijckoningen M et al. Association between HER-2/neu and the progesterone receptor in oestrogen-dependent breast cancer is age-related. *Breast Cancer Res Treat* 2005;91:81-87.
 43. Love RR, Duc NB, Havighurst TC et al. Her-2/neu overexpression and response to oophorectomy plus tamoxifen adjuvant therapy in estrogen receptor-positive premenopausal women with operable breast cancer. *J Clin Oncol* 2003;21:453-457.
 44. Sundblad AS, Caprarulo L. Immunohistochemical characteristics of mammary carcinomas with estrogen-negative and progesterone-positive receptors. *Medicina (B Aires)* 1996;56:683-689.3870.