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Original contribution

Characterization of estrogen receptor–negative/progesterone receptor–positive breast cancer☆,☆☆

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Summary Despite the controversies, estrogen receptor–negative/progesterone receptor–positive (ER−/PR+) breast cancers have a reported incidence of 1% to 4%. These tumors are less well defined, and it is unclear whether ER−/PR+ represents a distinct subtype. Thus, we analyzed 5374 consecutive breast cancers to characterize the clinicopathological features of this underrecognized subset of tumors. The ER−/PR+ tumors, constituting 2.3% of the total, were mostly high grade and significantly seen in younger patients and African American women when compared with the ER+/PR+ and ER+/PR− groups, but similar to that of ER−/PR− phenotype ($P < .0001$). A significantly prolonged relapse-free survival (RFS) was associated with the ER+/PR+ subtype when compared with the ER+/PR− ($P = .0002$) or ER−/PR+ ($P = .0004$) tumors, whereas all 3 groups showed a superior outcome to that of the ER−/PR− phenotype. In the subset of patients receiving endocrine therapy, those with ER+/PR+ tumors had a significantly prolonged RFS ($P = .001$) and disease-specific survival ($P = .005$) when compared with the group with an ER+/PR− phenotype, but did not significantly differ from those with ER−/PR+ tumors. No significant survival advantage was found between the ER+/PR− and ER−/PR+ tumors in any group of patients analyzed. Furthermore, a higher PR expression was associated with a favorable RFS and disease-specific survival in the patients with ER−/PR+ tumors. Therefore, the ER−/PR+ tumors demonstrate a similar, if not higher than, response rate to endocrine therapy when compared with the ER+/PR− tumors and thus are important to identify. Routine PR testing remains necessary in assisting clinical decision making in the pursuit of precision medicine.

1. Introduction

The expression of hormonal receptors in breast cancer has proven to be a powerful predictive factor and also to have a significant prognostic value [1,2]. It has been shown that 75% to 85% of breast cancers with an estrogen receptor–positive/progesterone receptor–positive (ER+/PR+) phenotype respond

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to endocrine therapy, in contrast to less than 10% response rate in those with a double-negative phenotype (ER−/PR−), demonstrating the critical value of evaluation of hormonal receptor status in predicting response to endocrine therapy [3]. Thus, assessment of ER and PR status, along with the HER2 oncogene/oncoprotein, is mandatory in the routine care of all patients with breast cancer, and endocrine response is now the first consideration for selection of adjuvant systemic therapy [4].

Although the clinical significance of assessing ER expression is undisputed, there has been an ongoing debate over the value of PR as an independent predictive and prognostic marker due to conflicting observations in the literature. As an ER-dependent gene product, PR expression is theoretically a surrogate marker for a functional ER pathway, and thus, assessment of PR should assist in predicting response to endocrine therapy more accurately. Some studies demonstrated that PR levels were independently associated with disease-free and overall survival in the adjuvant setting as well as in patients with ER+ metastatic disease [5,6]. In contrast, others showed that PR status was not a strong factor for predicting endocrine response or for survival outcome and thus did not add either diagnostic information or have a therapeutic impact on breast cancer [7].

Although approximately 70% of breast cancers are ER+, these tumors do not always harbor an ER+/PR+ phenotype. ER+/PR+ tumors reportedly comprised 57% of early breast cancers, whereas 25% of tumors exhibited an ER+/PR− phenotype in one large cohort study [8]. Although the current treatment for ER+/PR+ and ER+/PR− tumors is similar, this latter group displayed a more aggressive biological behavior than ER+/PR+ tumors [8], likely due to the fact that ER+/PR− tumors share gene expression patterns with both ER+/PR+ and ER−/PR− phenotypes [9].

Even more controversial in this regard are the ER−/PR+ breast cancers. This subtype has a reported incidence of 1% to 4% [10–13]. Although some authorities deemed it as a technical artifact arising from inadequate tissue fixation or failure of the immunohistochemical assay [14,15], others argued that even using optimally fixed tissues and any level of nuclear immunoreactivity of tumor cells as a positive result, the ER−/PR+ was still retained as a unique entity [10,16], although some contended that the ER−/PR+ classification was too rare to be of clinical use [7]. In this study, we sought to characterize the pathologic features and prognostic significances of ER−/PR+ tumors and to evaluate the importance of PR testing in routine breast cancer management.

2. Materials and methods

This retrospective study was performed after approval by the University of Alabama at Birmingham Institutional Review Board. The Tumor Registry of the authors' institution was searched to identify invasive breast cancer cases between 1997 and 2013. The patients' demographic information, clinical outcomes, and the pathologic features of the primary tumor were recorded. The accuracy of the data collected was further confirmed using the electronic medical record. Given the aim of the study, the cases without ER and/or PR status were excluded from the analysis. This led to a total of 5374 patients meeting the inclusion criteria. The clinical stage was based on imaging modality for breast cancer surveillance with or without confirmation by tissue biopsy.

The original ER and PR immunohistochemical slides from the tissue specimens obtained prior to the 2010 American Society of Clinical Oncology and the College of American Pathologists guidelines [17], particularly those from the single-positive (ER+/PR− or ER−/PR+) tumors, were reviewed. These specimens were fixed for variable times but were typically within the 6- to 72-hour window. All specimens used for hormonal receptor testing obtained after the 2010 American Society of Clinical Oncology–College of American Pathologists guidelines were fixed 6 to 72 hours before commencing the immunohistochemistry protocol. Immunostaining for ER and PR was performed using the streptavidin-biotin method on an automated immunostainer (Benchmark XT; Ventana Medical Systems, Tucson, AZ) for all cases irrespective of age, as previously described [18]. In brief, the deparaffinized sections were incubated in sodium citrate buffer for 60 minutes at 100°C for antigen retrieval. Hydrogen peroxide was applied for 4 minutes at 37°C to block endogenous peroxidase. After rinsing, ER (clone 6F11 [until 2008] and clone SP1 [after 2008], prediluted; Ventana) or PR (clone 1A6 [until 2008] and clone 1E2 [after 2008], prediluted; Ventana) antibody was then applied for 36 minutes at 37°C, followed by a biotinylated secondary antibody for 8 minutes. Sections were then rinsed, visualized with diaminobenzoic acid, counterstained with hematoxylin, and then taken offline to be dehydrated and coverslipped. A case of breast cancer known to express ER and PR was used as a positive control, whereas negative controls were performed by replacing the primary antibodies by normal serum.

A positive ER or PR was defined as at least 1% of tumor cell nuclei with immunoreactivity. In cases of an ER−/PR+ phenotype, the ER and PR assays were repeated as appropriate, that is, in conditions such as lack of staining of internal or external controls, or when the external controls were not as expected or artifacts involving most of sample were detected, to rule out a false-negative ER or false-positive PR. The original slides from ER−/PR+ tumors were reviewed to assess the extent of PR expression in ER−/PR+ tumors using an *H* score as determined by multiplying the intensity (0, 1+, 2+, and 3+) by the percentage of tumor cell nuclei stained, giving a range of 0 to 300.

The categorical data obtained were statistically evaluated using the χ^2 test, whereas continuous data were evaluated using the Student *t* test. Distant relapse-free survival (calculated from the date of diagnosis to the date of distant recurrence) and disease-specific survival (from the date of diagnosis to the date of death) were mapped on Kaplan-Meier curves. Patients who survived or were lost to follow-up were considered as censored data in the analysis. The log-rank test was used to compare