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Phenotypic alterations in breast cancer associated with neoadjuvant chemotherapy: A comparison with baseline rates of change

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A B S T R A C T

Several studies have documented phenotypic alterations in breast cancer associated with neoadjuvant chemotherapy [NACT], but many of these studies are limited by the fact that they did not account for the baseline rate of expected phenotypic change between biopsies and resections in the absence of NACT. Herein, we assess whether the NACT-associated rate of phenotypic change is significantly different than would be expected in a control population of patients that did not receive NACT. From a pathologic database, we documented the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER2/neu) phenotypes of consecutive invasive breast carcinomas (n = 826), as well as the subset in which at least one of these tests was assessed in both the biopsy and resection (n = 340). We then compared the rates of phenotypic change in the patients that did (n = 65) and did not (n = 275) receive NACT. Respectively, 49.2% and 36% of the NACT and non-NACT groups showed a biopsy-to-resection change in status for at least one biomarker (p = 0.0005). The NACT and non-NACT groups showed the following respective rates of a biopsy-to-resection change in phenotype: ER (9.2% vs 2.5%, p = 0.02); PR (30.7% vs 8%, p = 0.000006); Her2/neu-IHC (25% vs 22.3%, p = 0.7), Her2/neu-FISH (7% vs 3%, p = 0.6). The direction of change in the NACT group was *positive* in the biopsy to *negative* in the resection in > 70% of cases for all markers. For ER and PR, there was no statistically significant difference between cases that showed a biopsy-to-excision change in phenotype and those that were more phenotypically stable regarding a wide array of clinicopathologic variables. The average percentage of ER/PR-immunoreactive tumor cells in the pre-NACT biopsies was significantly lower in the phenotypically altered cases as compared to the phenotypically stable cases. Our findings confirm that phenotypic alterations in breast cancer occur after NACT, and that these changes are more pronounced for hormone receptors (especially PR); Significant NACT-associated alterations were not apparent for HER2/neu. A distinct pathologic profile for cases displaying a phenotypic change within the NACT group was not demonstrable. The pre-NACT levels of ER and PR may affect the likelihood of a phenotypic change. These results highlight the need for repeat testing in residual tumors after NACT.

1. Introduction

Neoadjuvant chemotherapy [NACT] has become an established approach in the management of a subset of patients with breast cancer. NACT serves to downstage locally-advanced tumors, thereby improving their operability and potentially reducing the extensiveness of surgery that is necessary to achieve an adequate excision [1-7]. For primarily operable cancers, NACT may also improve surgical options or eliminate the disease in its entirety [1-5]. For both groups, NACT facilitates an assessment of the tumoral responsiveness to the administered regimens, which may play a role in modulating the therapeutic approaches that are taken in the adjuvant setting for the same patient [8]. In some forms of breast cancer, such as inflammatory breast cancer, NACT is considered to be the standard of care, and there is evidence that NACT-based management approaches have improved overall patient outcomes for this notably aggressive tumor [9,10]. Similarly, for the larger group of patients with breast cancer, it is well established that patients with complete pathologic response to NACT show good long term outcomes [1,11-13]. More recently, however, immunohistochemically-defined “molecular subgroups” of the broader array of breast cancers has allowed for more accurate prediction of tumor responsiveness for each subgroup and accordingly, a more “personalized” management approach. For example, HER2-positive (nonluminal) and triple-negative tumors generally display high chemosensitivity rates, and complete pathologic response in such tumors have been associated with excellent prognosis [14,15]. However, a failure to achieve complete pathologic

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response in these 2 groups puts the patient at a substantial risk for relapses and have generally been associated with a poor prognosis [15,16]. These factors make the pathologic determination of NACT-responsiveness as well as biopsy/excision consistency in phenotype, of notable significance in these 2 subgroups.

Testing for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2/neu) are routinely performed on the core biopsy in which most initial diagnoses of breast cancer are made. These results are necessary to assess for the possibility of taking a neoadjuvant therapeutic-based approach to a given patient. Several previous studies have documented a significant rate of biopsy-to-resection change in ER, PR and HER2/neu status in breast cancers from NACT-treated patients, although the results have not been entirely congruent [17,18]. In their meta-analysis of 8 previously reported studies, Jabbour et al. [18] found the average biopsy-to-resection discordance rate for ER, PR, and HER2/neu to be 12.9%, 32.0%, and 8.9% respectively. With a few exceptions [19-29], many of the aforementioned studies are limited by the fact that they did not account for the baseline rate of expected phenotypic change between biopsies and resections in the absence of NACT. Herein, we assess whether the NACT-associated rate of phenotypic change is significantly different than would be expected in a control population of patients who did not receive NACT. We hypothesized that the use of a control group may be of analytic significance, since there is a known biopsy/excision discordance rate for ER, PR and HER2/neu that is related to tissue fixation, sampling errors, tumoral heterogeneity or other pre-analytic variables, independent of NACT use [30,31]. A secondary study goal is to determine whether residual tumors in the excision specimen that have undergone an NACT-associated change in phenotype for at least one marker are pathologically distinct from tumors that are more phenotypically stable. On this question, a finding in the affirmative may theoretically facilitate the morphologic delineation of patients with residual tumor after NACT that are more likely to have demonstrated a phenotypic change.

2. Materials and methods

This study was approved by the Human Research Protections Program at the University of California San Diego (Project number: 161362). A pathologic database was queried for consecutive patients with invasive breast carcinoma and who received a resection (mastectomy or breast conserving surgery) at our institution during a 6.5-year period. For each patient, pathologic reports were reviewed, including those for the preceding core biopsies. We then determined the number of patients who that had ER, PR and/or HER2/neu testing was performed on both the biopsy and the resection, and recorded these results for both specimen types. The rate of biopsy-to-resection change in phenotype for each marker was determined. For ER and PR, a change in phenotype was determined to be present if a case changed from a *positive* result in the biopsy to *negative* result in the resection, or vice versa. All cases were scored using 2010 criteria of the American Society of Clinical Oncology and College of American Pathologists [32]; the percentage of immunoreactive cells that displayed any immunoreactivity was also documented for each case. For HER/2neu, change was assessed at the *negative* (scores 0 or 1 +) versus *equivocal* or *positive* (scores 2 + or 3 +) threshold for immunohistochemistry (IHC) and at the *amplified* versus *not amplified* threshold for fluorescence in situ hybridization (FISH), using applicable scoring criteria [33]. The medical records for patients whose tumors received ER, PR and HER2/neu testing on both the biopsy and resection were also searched, and the proportion of these patients who received NACT was determined. Patients who received NACT thereby formed the study group (NACT group) and patients who did not receive NACT (but whose tumors still had testing on both the biopsy and the resection) formed the control group (non-NACT group). The NACT and non-NACT groups were compared regarding their rates of a biopsy-to-resection change in phenotype for each biomarker, using the Fisher's exact test.

A wide array of clinicopathologic features were recorded for all patients within the NACT group, including patient age, tumor size, tumor histotype (ductal versus non-ductal), histologic grade (modified Scarff Bloom-Richardson grading system, recorded as grade I versus grade II/III), stage (TNM staging system), ductal carcinoma in situ (DCIS) component (present or absent), lymphovascular invasion (present or absent), lymph node status (positive or negative), tumor necrosis (present or absent) and margins status (positive or negative), all as was determined from the resection specimen. Cases that showed no biopsy-to-resection change in phenotype for any of the biomarkers were classified as phenotypically stable (PS). Cases showing such change for at least one biomarker were classified as phenotypically-altered (PA). PA and PS cases were then statistically compared regarding the aforementioned variables, using the Fisher's exact and Student *t*-tests. An alpha of 0.05 was used for all statistical analyses.

For all of the study period, it was an institutional policy to perform ER, PR and HER2/neu testing by immunohistochemistry on all newly diagnosed cases of breast cancer. For the latter part of the study period, a dual testing HER2/neu testing approach, with both IHC and FISH on all cases [34], was employed. Additionally, for the last 2.5 years of the study period, it was an institutional policy to perform all of the aforementioned tests on the residual tumor after NACT.

Immunohistochemical studies at the UC San Diego laboratory are performed with the Ventana Benchmark automation and the Ultra View detection kit (Ventana Medical Systems, Tucson, AZ) using the following primary antibodies: ER (Clone SP1; prediluted, Ventana), PR (clone IE2; prediluted; Ventana) and HER2/neu (clone 4B5; prediluted; Ventana). FISH is performed using the dual-color HER2/CEP17 probe (PathVysion Her2/neu DNA Probe Kit, Abbott Molecular, Inc.). The alternative dual-color HER2/LIS1 (17p13) probe is used only if equivocal results are obtained with HER2/CEP17.

3. Results

826 consecutive cases of invasive breast cancer were accessioned during the study period. ER, PR and HER2/neu testing were performed on both the biopsy and the resection in 340 cases (41%). 65 (19%) of these 340 patients received NACT, and accordingly formed the study (NACT) group. The control (non-NACT) group was composed of the remaining 275 patients who did not receive NACT. 32 (49.2%) of the 65 cases in the NACT group had a biopsy-to-resection change in status for at least one biomarker, as compared with 72 (36%) of the 275 cases in the non-NACT group ($p = 0.0005$) (Tables 1).

ER: There was a biopsy-to-resection change in ER status in 6 (9.2%) of 65 cases in the NACT group and in 7 (2.5%) of 275 cases in the non-NACT group ($p = 0.02$). The direction of biopsy-to-resection phenotype change was positive to negative in 83.3% of the NACT group and 71.4% in the non-NACT group ($p = 1$). Within the NACT group, there was no significant difference between PA and PS cases regarding patient age, tumor size, lymph node status, tumor histotype, histologic grade, stage,

Table 1
Rates of biopsy-to-resection change in phenotype.

Phenotype	Number of cases tested in both biopsy and resection		Change in phenotype (NACT group) n (%)	Change in phenotype (non-NACT group) n (%)	p Value
	NACT	Non-NACT			
ER	65	275	6 (9.2%)	7 (2.5%)	0.02
PR	65	275	20 (30.7%)	22 (8%)	0.000006
HER2/neu by FISH	28	83	2 (7%)	3 (3.6%)	0.6
HER2/neu by IHC	60	229	15 (25%)	51 (22.3%)	0.71