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Chromosome 17 Centromere Duplication and Responsiveness to Anthracycline-Based Neoadjuvant Chemotherapy in Breast Cancer[1,2]

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Abstract

Human epidermal growth factor receptor 2 (*HER2*) and *topoisomerase II alpha* (*TOP2A*) genes have been proposed as predictive biomarkers of sensitivity to anthracycline chemotherapy. Recently, chromosome 17 centromere enumeration probe (CEP17) duplication has also been associated with increased responsiveness to anthracyclines. However, reports are conflicting and none of these tumor markers can yet be considered a clinically reliable predictor of response to anthracyclines. We studied the association of *TOP2A* gene alterations, *HER2* gene amplification, and CEP17 duplication with response to anthracycline-based neoadjuvant chemotherapy in 140 patients with operable or locally advanced breast cancer. *HER2* was tested by fluorescence *in situ* hybridization and *TOP2A* and CEP17 by chromogenic *in situ* hybridization. Thirteen patients (9.3%) achieved pathologic complete response (pCR). *HER2* amplification was present in 24 (17.5%) of the tumors. *TOP2A* amplification occurred in seven tumors (5.1%). CEP17 duplication was detected in 13 patients (9.5%). CEP17 duplication correlated with a higher rate of pCR [odds ratio (OR) 6.55, 95% confidence interval (95% CI) 1.25-34.29, $P = .026$], and analysis of *TOP2A* amplification showed a trend bordering on statistical significance (OR 6.97, 95% CI 0.96-50.12, $P = .054$). *TOP2A* amplification and CEP17 duplication combined were strongly associated with pCR (OR 6.71, 95% CI 1.66-27.01, $P = .007$). *HER2* amplification did not correlate with pCR. Our results suggest that CEP17 duplication predicts pCR to primary anthracycline-based chemotherapy. CEP17 duplication, *TOP2A* amplifications, and *HER2* amplifications were not associated with prognosis.

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Abbreviations: CEP17, chromosome 17 centromere enumeration probe; CI, confidence interval; CISH, chromogenic *in situ* hybridization; DFS, disease-free survival; EC-D, epirubicin (90 mg/m^2) and cyclophosphamide (600 mg/m^2) followed by docetaxel (100 mg/m^2); ER, estrogen receptor; FEC75, fluorouracil (600 mg/m^2), epirubicin (75 mg/m^2), and cyclophosphamide (600 mg/m^2); FISH, fluorescence *in situ* hybridization; HR, hazard ratio; HER2, human epidermal growth factor receptor 2; OR, odds ratio; OS, overall survival; pCR, pathologic complete response; PR, progesterone receptor; TOP2A, topoisomerase II alpha

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Introduction

Predicting response to anthracycline-based therapy is a central challenge in patients with breast cancer. Several randomized studies have shown that anthracycline-based adjuvant therapy produces a modest improvement in survival in patients with early-stage breast cancer [1]. In addition, it has been shown that incorporation of taxanes further improves pathologic complete response (pCR) in the neoadjuvant setting [2–7]. However, the risk of serious side effects must be considered and predictive factors are needed to help clinicians select the most appropriate drug for each patient. To date, no specific biomarkers have been identified to predict tumor response to anthracycline-based chemotherapy.

The *human epidermal growth factor receptor 2* (*HER2*) and *topoisomerase II alpha* (*TOP2A*) genes have been proposed as markers of sensitivity to anthracycline chemotherapy. Although several studies have reported an association between *HER2* amplification with anthracycline sensitivity [8–13], only two of these were statistically significant [10,13]. *In vitro* and *in vivo* studies indicate that *HER2* positivity alone does not alter anthracycline sensitivity [14] and the underlying mechanism remains elusive. The *TOP2A* gene, located at 17q12-q21, close to the *HER2* gene, encodes TOP2A, a key enzyme in DNA replication and the molecular target of anthracyclines [15,16]. When *HER2* is amplified, genes situated around 17q21, such as *TOP2A* gene, may be either co-amplified or deleted [17]. Because of this physical proximity, some researchers have proposed that the link between *HER2*-positive disease and anthracycline sensitivity is the presence of *TOP2A* alterations (amplifications and deletions) rather than *HER2* amplifications [18,19]. However, the results of these studies have not always been consistent [20].

Given the location of *HER2* and *TOP2A* on chromosome 17, recent reports have focused on the predictive role of other molecular alterations localized within the 17q21 region, including alterations in key genes such as *HER1-3* [21], *p53* [22], and *BRCA1* [23], and variations in the copy number of subchromosomal regions, including the chromosome 17 centromere (CEP17) duplication [21]. On the basis of recent data from array comparative genomic hybridization, CEP17 duplication is defined as increased copy number of CEP17 [21] rather than polysomies of the whole chromosome 17 [24,25]. CEP17 duplication has been described as a marker of genomic instability [26] and has received a great deal of attention as a potential predictor of anthracycline benefit [21,27].

We hypothesized that CEP17 duplication is associated with a higher response to neoadjuvant chemotherapy. We explored the association of CEP17 duplication and *TOP2A* alterations with response to anthracycline-based neoadjuvant therapy. We studied a cohort of patients with early or locally advanced breast cancer treated with anthracycline-based primary chemotherapy, and we chose pCR as a surrogate marker of chemosensitivity.

Materials and Methods

Ethics Statement

This study was conducted according to the Declaration of Helsinki principles, with approval from the Clinical Research Ethics Committee at Institut d'Investigacions Biomèdiques Sant Pau. Written informed consent was obtained from all patients.

Study Design and Patients

We retrospectively studied 140 consecutive patients with stage II or III breast cancer who received anthracycline-based neoadjuvant chemotherapy in our hospital between 1993 and 2010. All patients had confirmed diagnosis based on histopathology of biopsy and none of them had prior treatment with surgery, chemotherapy, or radiation. Our study included patients with *HER2*-positive carcinomas treated before the approval of trastuzumab in 2006. The study excluded patients with bilateral or inflammatory tumors. Fifty-five patients received neoadjuvant treatment with anthracyclines alone [FEC75: fluorouracil (600 mg/m^2), epirubicin (75 mg/m^2), and cyclophosphamide (600 mg/m^2) given every 3 weeks for four to six cycles, n = 40, or FAC60: fluorouracil (500 mg/m^2), doxorubicin (60 mg/m^2), and cyclophosphamide (500 mg/m^2) given every 3 weeks for four to six cycles, n = 15] between 1993 and 2002. Eighty-five patients received neoadjuvant treatment with anthracyclines in combination with taxanes [EC-D: epirubicin (90 mg/m^2) and cyclophosphamide (600 mg/m^2) given every 3 weeks for four cycles followed by docetaxel (100 mg/m^2) every 3 weeks for four cycles] from 2003 to 2010. Patients were staged according to the tumor-node-metastasis (TNM) system. Clinical response was assessed by palpation, breast ultrasound, mammography, and/or magnetic resonance imaging before systemic therapy and before curative surgery. Clinical responses were evaluated according to the response evaluation criteria in solid tumors (RECIST) criteria [28] every two cycles. Patients treated with FEC75 or FAC60 with partial response received surgical treatment after four cycles of chemotherapy and two additional cycles of FEC75 or FAC60 were administered after surgery. Patients treated with EC-D underwent breast surgery after completion of chemotherapy. Patients with positive hormone receptor tumors received radiotherapy and endocrine therapy. The extent of residual disease was measured in the surgical specimen. The primary endpoint for this study was pCR defined as the absence of invasive cancer in the breast and axillary lymph nodes at the time of definitive surgery. The secondary endpoints for the study were disease-free survival (DFS) and overall survival (OS). Patients were followed up according to the breast cancer guidelines.

Tumor Samples and Tissue Microarrays

We analyzed 140 representative formalin-fixed, paraffin-embedded tumor core biopsies obtained before neoadjuvant treatment. Paraffin blocks were stored at room temperature. Samples were identified only by an identification number assigned to each patient. A stained section of each tumor sample was prepared to confirm the diagnosis and to identify representative tumor areas. Tissue microarrays were prepared from formalin-fixed, paraffin-embedded tissue taken from three representative areas of each tumor. Serial 5-μm sections were obtained for immunohistochemical, fluorescence *in situ* hybridization (FISH), and chromogenic *in situ* hybridization (CISH) analyses.

Immunohistochemistry

Prediluted antibodies for estrogen receptor (ER; clone EP1), progesterone receptor (PR; clone 636) and Ki67 (clone MIB-I) were obtained from Dako (Glostrup, Denmark). Sections were processed in a PT Module using Dako high pH buffer (Dako) for deparaffinization and antigen retrieval. Sections for the Ki-67 study were processed with Dako low pH buffer. All immunohistochemical stains were performed in an Autostainer Link using the EnVision method (Dako). *HER2* overexpression was analyzed using the HercepTest assay (Dako). Tumors were classified as ER or PR positive when at least 1% of the tumor cells showed staining in the nuclei cells [29]. *HER2* was considered overexpressed when a uniform intense (3+) membrane staining was present in >30% of invasive tumor cells [30]. The percentage of Ki67-stained nuclei was evaluated independently of the intensity and its positivity cutoff value