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The Pathological Response to Anthracycline is Associated with Topoisomerase IIα Gene Amplification in the HER2 Breast Cancer Subset

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***Abstract*— Background: HER2-positive breast cancer sensitivity to anthracyclines is enhanced when topoisomerase IIα (TOP2A) is co-amplified under both adjuvant and metastatic settings. However, the relationship between anthracycline sensitivity and TOP2A amplification in HER2-positive breast cancers in neoadjuvant settings is not known. Methods: The TOP2A gene status was examined by FISH in biopsies from 18 patients who received anthracycline and cyclophosphamide before surgery. Results: The TOP2A gene was amplified in 6/17 patients and was significantly associated with pathological response to the chemotherapy regimen. Conclusions: TOP2A amplification could predict anthracycline-sensitivity. Thus, the HER2/TOP2A co-amplified subtype may be effectively treated by anthracycline-containing regimens alone.**

***Keywords* — Breast Cancer; Neoadjuvant; Predictive factor; HER2; TOP2A; FISH**

I. INTRODUCTION

The monoclonal antibody trastuzumab, which interferes with HER2 activity, has proven to be indispensable for the treatment of HER2 type breast cancer. Now, the identification of the most effective anticancer agents to use with trastuzumab, and therefore further amplify treatment success, has become an important challenge for treating this aggressive subtype [1]. It is known that this subtype is sensitive to anthracyclines [2]. In addition, it was recently reported that the topoisomerase IIα (TOP2A) gene is co-amplified in approximately 40% of HER2 positive tumors in correlation with specific sensitivity to anthracycline-containing regimens [3-5]. However, because HER2 type breast cancer is usually treated by an anthracycline (A) regimen followed by taxan (T) and trastuzumab (H), it is difficult to identify a specific predictor for each drug in this subtype.

In our previous neoadjuvant study, in which HER2 subtype breast cancers were treated by epirubicin (E) and cyclophosphamide (C) alone, we failed to identify a specific predictive factor for this regimen [6]. However, in light of the TOP2A gene relationship with EC we have re-examined these samples to determine whether HER2/TOP2A co-amplification renders tumors more sensitive to EC.

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II. METHODS

TOP2A gene status and tumor specimens

Tumor biopsies of 18 patients with HER2 positive breast cancer were collected in our previous neoadjuvant study as published [6]. They consisted of eight HER2 and ten luminal-HER2 cases that were collected from patients after neoadjuvant treatment with four cycles of epirubicin (E, 90 mg/m2) and cyclophosphamide (C, 600 mg/m2), every 3 weeks prior to surgery. Pretreatment biopsies were also collected and stored as either formalin-fixed or paraffin embedded blocks. The study was approved by the Yokohama City University Medical Center in 2006.

The TOP2A gene status was examined by fluorescence in-situ hybridization (FISH) (TOP2A FISH pharmDx™ Kit; DAKO) in pre-treatment tumor biopsies. TOP2A copy number was then determined in a minimum of 20 interphase, non-overlapping, tumor cell nuclei and compared with that of chromosome 17 centromeres (CEP17) in the same nuclei. The ratio of TOP2A to CEP17 signals was then calculated. In

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accordance with previous reports, a TOP2A/CEP17 ratio greater than 2.0 was defined as gene amplification [4, 5].

Pathological evaluation

Preparation of specimens was described previously [6]. Briefly, H&E and cytokeratin-stained slides were prepared as 5-mm tissue sections from the primary tumor. The pathological breast tumor response was blindly assessed by three board-certified pathologists (T.S., A.N., and M.T.) according to the General Rules for Clinical and Pathological Recording of Breast Cancer of the Japanese Breast Cancer Society [7]. For analysis, the pathological effect was determined using the definition for quasi-pathological complete response (QpCR), which represents a combination of grades 2b and 3 [8]. In this setting grade 3 is defined as necrosis and/or the disappearance of all tumor cells and/or the replacement of cancer cells by granulation and/or fibrosis, and Grade 2b is defined as the presence of only a few remaining cancer cells.

III. Results

The TOP2A gene was detected by FISH in 17/18 cases of HER2-positive breast cancer. From these, the gene was determined to be amplified in 6 cases (35.3%) as indicated by a ratio greater than 2 when TOP2A expression was compared to that of CEP17. Comparison of the gene status to pathological grades found that TOP2A gene amplification was significantly correlated with pathological response (P<0.001) (Figure). In 4 of 5 cases with QpCR, the TOP2A/CEP17 ratio was greater than 2.0, while the remaining case of Grade 2b was 1.89. At a cut-off value of 2.0, the positive- and negative-predictive values and accuracy were 80% (4/5 cases), 81.8% (10/12 cases), and 81.2% (14/17 cases), respectively.

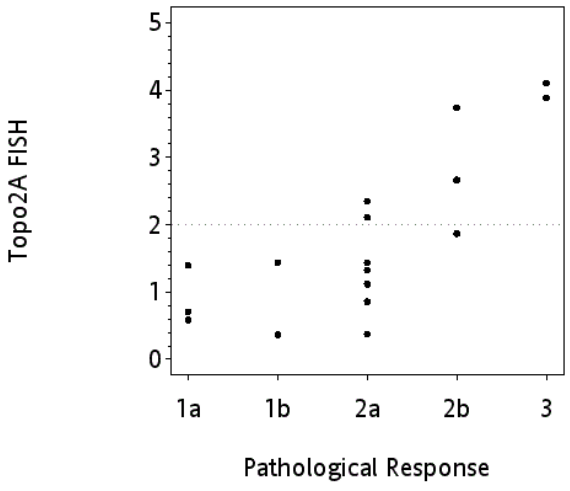

Figure. The association between TOP2A gene status and pathological response after 4 cycles of AC in HER2-positive breast cancer. The ratio of TOP2A/CEP17 was plotted against the pathological response (grades 1a to 3). Means of TOP2A/CEP17 gene status were statistically compared among pathological responses in terms of the linear trend using one-way ANOVA. A threshold ratio for gene amplification was set at 2 (indicated by dotted line).

IV. Discussion

In our study, four cycles of EC, a minimal chemotherapy regimen, resulted in 27.8% of patients exhibiting pathology of the QpCR grade within the HER2 subtype. Among them, the pathological response was significantly associated with TOP2A gene amplification. Of note, QpCR was used for calculation in this study because pathological examination was thoroughly performed by 5-mm serial dissection of entire surgical specimens. Thus, the result of this neoadjuvant study supports the findings of previous reports conducted under adjuvant and metastatic settings, which found that the TOP2A gene is amplified in approximately 40% of HER2 subtypes and predicts the efficacy of AC-based chemotherapies [4, 5]. However, conflicting data exist on this issue, which could be account technical rather than biologic factors. Negative results were from the studies with other methods than FISH like polymerase chain reaction [9] or different thresholds for TOP2A amplification like TOP2A/CEP17 $\geqslant$ 1.5 [10]. Our data analysis supports the definition of amplification as a TOP2A/CEP17>2.0 by FISH, as this cut-off value resulted in robust data with respect to positive- and negative-predictive values, as well as accuracy.

In our previous study, TOP2A protein expression did not associate with gene alteration (p=0.98, data not shown), nor did it predict the efficacy of EC [6]. The TOP2A protein is a key enzyme in DNA replication and RNA transcription. It receives many translational and transcriptional signals, and therefore it might not be directly associated with AC-sensitivity, although the target of this drug is not the protein but the gene [11].

Recent studies have shown that clinical outcomes were equivalent between AC-T and AC-TH treatment regimens in the HER2 and TOP2A-coamplified breast cancer subset, suggesting that trastuzumab could be omitted from treatments of this subpopulation [4, 5]. Moreover, results of this study suggest that even taxan could be refrained for this subset and therefore its related adverse effects.

In summary, our findings suggest that for breast cancers with HER2 and TOP2A co-amplification, it would be reasonable to treat patients with AC preoperatively and to consider adding TH according to the pathology of surgical specimens. Prospective studies on the value of TOP2A analysis by FISH will be of clinical relevance to confirm its predictive value for anthracycline-sensitivity in this breast cancer subset.

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