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Original Article

p53 Expression in Pretreatment Specimen Predicts Response to Neoadjuvant Chemotherapy Including Anthracycline and Taxane in Patients with Primary Breast Cancer

Tadahiko Shien[a]*, Takayuki Kinoshita[b], Kunihiko Seki[c], Miwa Yoshida[b], Takashi Hojo[b], Chikako Shimizu[d], Naruto Taira[a], Hiroyoshi Doihara[a], Sadako Akashi-Tanaka[b], Hitoshi Tsuda[c], and Yasuhiro Fujiwara[d]

[a]Department of Breast and Endocrine Surgery, Okayama University Hospital, Okayama 700–8558, Japan, Departments of [b]Surgical Oncology, [c]Pathology, [d]Medical Oncology, National Cancer Center Hospital, Chuo-ku, Tokyo 104–0045, Japan

While clinical and pathologic responses are important prognostic parameters, biological markers from core needle biopsy (CNB) are needed to predict neoadjuvant chemotherapy (NAC) response, to individualize treatment, and to achieve maximal efficacy. We retrospectively evaluated the cases of 183 patients with primary breast cancer who underwent surgery after NAC (anthracycline and taxane) at the National Cancer Center Hospital (NCCH). We analyzed EGFR, HER2, and p53 expression and common clinicopathological features from the CNB and surgical specimens of these patients. These biological markers were compared between sensitive patients (pathological complete response; pCR) and insensitive patients (clinical no change; cNC and clinical progressinve disease; cPD). In a comparison between the 9 (5%) sensitive patients and 30 (16%) insensitive patients, overexpression of p53 but not overexpression of either HER2 or EGFR was associated with a good response to NAC. p53 ($p = 0.045$) and histological grade 3 ($p = 0.011$) were important and significant predictors of the response to NAC. The correspondence rates for histological type, histological grade 3, ER, PgR, HER2, p53, and EGFR in insensitive patients between CNB and surgical specimens were 70%, 73%, 67%, 70%, 80%, 93%, and 73%. The pathologic response was significantly associated with p53 expression and histological grade 3. The correspondence rate of p53 expression between CNB and surgical specimens was higher than that of other factors. We conclude that the level of p53 expression in the CNB was an effective and reliable predictor of treatment response to NAC.

Key words: breast cancer, neoadjuvant chemotherapy, predictors

Neoadjuvant chemotherapy (NAC) is the standard therapy for patients with advanced local breast cancer and is used increasingly for operable disease. Clinical and pathologic responses are important prognostic parameters, but cannot be accurately predicted. Unfortunately, approximately 20% of breast cancer patients do not benefit from NAC (*i.e.*, they continue to show stable or progressive disease). One of the aims of NAC is to confirm the sensitivity of tumors to chemotherapy. Using NAC, we can directly determine the sensitivity to chemotherapy based on whether or not the primary tumor is diminished, whereas we

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*Corresponding author. Phone:+81–86–235–7265; Fax:+81–86–235–7269
E-mail:tshien@md.okayama-u.ac.jp (T. Shien)

cannot confirm the efficacy by adjuvant chemotherapy itself. However, non-sensitive patients have to endure relatively needless therapy for about 6 months, so it is very important to make the pre-diagnosis of sensitivity to chemotherapy if possible. Several biological markers that might predict response are under investigation [1–9]. Estrogen receptor, progesterone receptor, and HER2 are very useful markers for the selection of anticancer drugs and prediction of prognosis, but are not useful for predicting the response to chemotherapeutic agents such as anthracycline and taxane. Therefore, other biological markers from pretreatment core needle biopsy are needed to predict the response to NAC, to individualize treatment, and to achieve maximal efficacy.

In this study, we investigated biological markers from pre-treatment core needle biopsies of highly sensitive tumors and non-sensitive tumors and identified additional prognostic markers that might predict the response to NAC and aid in the selection of treatment strategy.

Materials and Methods

All patients with operable breast cancer who were treated between May 1998 and July 2006 at the National Cancer Center Hospital with anthracycline and/or taxane as NAC were included in this retrospective study. NAC was indicated for clinical stage II breast cancer patients with tumors larger than 3 cm and stage III breast cancer patients. Core needle biopsy was performed before NAC to allow pathological diagnosis. Doxorubicin (DOX, 50 mg/m^2) and docetaxel (DTX, 60 mg/m^2) were administered for four 3-week cycles before surgery. Additional adjuvant treatment with DOX/DTX was given if patients achieved complete or partial remission after NAC. Otherwise, patients were treated with four cycles of iv cyclophosphamide, methotrexate, and 5FU. Trastuzumab was not administered to the patients with HER2-overexpressing tumors. Tamoxifen (20 mg/day) or anastrozole (10 mg/day) was administered for 5 years after surgery if either the pretreatment biopsy specimen or the surgical specimen post-chemotherapy was positive for estrogen-receptor or progesterone receptor.

Pretreatment diagnosis was established by our pathologists using samples from core needle biopsy or surgical resection. Overexpression of hormone receptors, p53, HER2 and EGFR was examined by immunohistology. Surgical specimens were sectioned at about 7–10 mm and classified for pathological response. Pathological features were described and invasive ductal carcinomas were classified into 3 subtypes (papillotubular, solid tubular, and scirrhous) according to the General and Pathological Recording of Breast Cancer guidelines established by the Japanese Breast Cancer Society [10]. The criteria for histological grading of IDC were based on a modification of those recommended by the WHO [11, 12]. The response criteria used in this study include Fisher's system [13], complete pCR denotes no histological evidence of tumor cells, pCR with DCIS denotes no histological evidence of invasive tumor cells (specimens with only noninvasive cells included), and pINV denotes the presence of invasive tumor cells. Overexpression of ER (1D5, Dako Cytomation, Baltimore, MD, USA), PgR (1A6, Novocastra), HER2 (Herceptest, Dako), p53 (DO7, Dako), and EGFR (2-18C9, Dako) were examined by immunohistology using the noted antibodies. The criterion for ER, PgR, and p53 was staining of more than 10% of cancer cell nuclei, regardless of intensity. HER2 and EGFR grading is as follows: 0: negative, 1+: slightly positive in more than 10% of cancer cells, 2+: moderately positive in more than 10% of cancer cells, 3+: markedly positive in more than 10% of cancer cells. 2+ and 3+ were considered positive for HER2 and EGFR.

Clinical response to NAC was decided from the 2 greatest perpendicular diameters (before each chemotherapy treatment and before surgery) of tumors in the breast and axillary lymph nodes. Absence of clinical evidence of palpable tumors in the breast and axillary lymph nodes was defined as a clinical complete response (cCR). Reduction in total tumor size of 30% or greater was graded as clinical partial response (cPR). An increase in total tumor size of more than 20% or appearance of new suspicious ipsilateral axillary adenopathy was considered progressive disease (cPD). Tumors that did not meet the criteria for objective response or progression were classified as stable disease (cSD). In this study, we analyzed biological markers from core needle biopsies before NAC in complete pCR cases and non-sensitive tumors (clinical SD and PD), and demonstrated bio-

logical predictors of pathological response to PST.

Statistical analysis was carried out using JMP version 6.0 (SAS Institute Inc., Cary, NC, USA). Associations between ordinal variables were assessed using χ^2 analyses or the Fisher exact test for two-by-two variables. The statistical significance (P) was taken as a measure of the strength of evidence against the null hypothesis, and $p \leq .05$ was considered statistically significant.

Results

One hundred and eighty-three patients with operable breast cancer were treated with NAC at National Cancer Center Hospital between May 1998 and October 2001. Table 1 lists the patient and tumor characteristics. The median age was 50 years (range: 29–70). At diagnosis, 41 (22%) patients were in stage IIA, 63 (34%) were in stage IIB, 37 (20%) were in stage IIIA, and 42 (23%) were in stage IIIB. Breast conserving surgery was performed for 55 (30%) patients after NAC. The overall clinical response rate to NAC was 83% (cCR+cPR) and the pCR rate was 13%. 30 (17%) patients were insensitive to NAC (cSD or cPD). Among the responsive patients, 9 (5%) exhibited complete pCR (pathologically no tumor in the breast) and 14 (8%) exhibited pCR with DCIS.

Immunohistological characteristics from core needle biopsy before NAC are listed in Table 2. There were 62 (34%) cases of solid tubular primary tumor, 65 (36%) scirrhous, 34 (19%) papillotubular, 9 (5%) ILC, and 3 (2%) mucinous carcinomas. 88 (48%) cases were histological grade 3. 66 (36%) were ER positive and 72 (39%) were PgR positive. 73 (40%) were HER-2 positive (2+ and 3+ in immunohistological examination).

We evaluated age, histological type, histological grade, ER, PgR, HER2, EGFR, and p53 as predictive factors for response to NAC by comparing 9 (5%) sensitive (complete pCR) and 30 (17%) insensitive (cSD and cPD) tumors (Table 3). In univariate analysis, histological grade 3 ($p = 0.011$) and p53 ($p = 0.045$) were significant predictors of complete pCR. However, EGFR and HER2 were not predic-

Table 1 Patient and tumor characteristics

Parameter	No. of patients (%)
Total	183
Age (median)	50 (29–70)
Clinical stage	
Stage IIA	41 (22%)
Stage IIB	63 (34%)
Stage IIIA	37 (20%)
Stage IIIB	42 (23%)
Operation	
Bt + Ax	128 (70%)
Bp + Ax	55 (30%)
Clinical response	
cCR	32 (17%)
cPR	121 (66%)
cNC	29 (16%)
cPD	1 (1%)
Pathological response	
complete pCR	9 (5%)
pCR with DCIS	14 (8%)
pINV	160 (87%)

Bt, total mastectomy; Bp, partial mastectomy; Ax, axillary lymph node dissection.

Table 2 Immunohistological characteristics of CNB before PST

Parameter	No. of patients (%)
Histological type	
IDC	161 (88)
Solid tubular	62 (34)
Scirrhous	65 (36)
Papillotubular	34 (19)
ILC	9 (5)
mucinous	3 (2)
others	10 (5)
Histological grade	
3	88 (48)
2	88 (48)
1	7 (4)
ER	
positive	66 (36)
negative	117 (64)
PgR	
positive	72 (39)
negative	111 (61)
HER2	
positive (2 + and 3 +)	73 (40)

tors.

We analyzed the immunohistological features of CNB specimens. The correspondence rates of these features in insensitive patients between CNB and surgical specimens are shown in Table 4. The correspondence rates for histological type, histological grade 3, ER, PgR, HER2, p53, and EGFR were 70%, 73%, 67%, 70%, 80%, 93%, and 73%. The correspondence rate of EGFR was not low; however, in almost all patients with a discrepancy between CNB and surgical specimens, EGFR overexpression changed from negative to positive.

Discussion

The identification of predictive factors for NAC is very important for order made cancer treatment. The development of new medicines has diversified chemotherapeutic regimens, and the selection of treatment strategy according to individual cancer characteristics has become more difficult. To aid in selection, translational research has begun to demonstrate important correlations between prognostic factors and sensitivity to chemotherapy.

Table 4 Correspondence rates of biological markers in insensitive patients between CNB and surgical specimens

Parameter	%
Histological type	70
Histological grade 3	73
ER	67
PgR	70
HER2	80
p53	93
EGFR	73

In this study, we retrospectively evaluated response to NAC including anthracycline and taxane and a number of biomarkers. We found that pathologic response significantly associated with p53 expression and histological grade 3.

In our analysis, p53 could predict response of NAC. p53 accumulation was reported to be associated with a poor response to anthracycline in node-negative breast cancer patients [14], and may compromise the efficacy of anthracycline but not of taxane [15]. All patients in this study received both anthracycline and taxane, and p53 was an independent predictive factor of response to NAC similar to these reports. We cannot analyze the response of anthracycline and taxane respectively. However commonly we use both drugs in NAC. If the tumor has p53 mutation before NAC, we should check the response of anthracycline tightly and change to taxane when the response is wrong.

Previous studies reported poor prognosis for patients with HER2-overexpression. Several studies indicate that HER2 expression can predict sensitivity to anthracycline chemotherapy [16]; however, in this study, HER2 was not a predictor of pCR to NAC. HER2 negative patients rate were 22% of good responders and 33% of poor responders. In this study trastuzumab was not administered to patients with HER2 overexpression tumors. However, in these days, trastuzumab significantly improved the prognosis and the response to chemotherapy in these patients [17]. It was reported that the rate of pCR patients administered trastuzumab was significantly high. HER2 expression was not predictor of response to anthracycline and taxane in this study. We need to examine the relationship between HER2-overexpression and response to chemotherapy with trastuzumab.

Table 3 Univariate analysis of clinicopathological features between sensitive (pCR) and insensitive cases (cNC + cPD)

Parameter	Sensitive (n = 9) (%)	Non-sensitive (n = 30) (%)	*p*-value
Age < 50	3 (33)	19 (63)	N.S.
Histological type (so.)	6 (67)	12 (40)	N.S.
Histological grade 3	8 (89)	13 (43)	0.011
ER negative	8 (89)	17 (57)	N.S.
PgR negative	6 (67)	17 (57)	N.S.
HER2 positive	2 (22)	10 (33)	N.S.
p53 positive	5 (56)	6 (20)	0.045
EGFR positive	3 (33)	7 (23)	N.S.

so, solid tubular carcinoma

A previous study observed EGFR expression in 37–80% of basal-like tumors, as identified by DNA microarray, and reported poorer prognosis for this phenotype [18–20]. We hypothesized that EGFR expression might distinguish the basal-like phenotype and predict poorer response to NAC. However, in this study, EGFR was not an independent predictive factor of response to NAC. It was reported that EGFR is expressed in 7–36% of breast carcinomas with high grade conventional invasive ductal carcinoma (IDC) [21–24] and EGFR expression was seen in 272 (20%) of 1388 cases. In a univariate analysis, Tsutsui *et al.* showed a significantly poorer clinical outcome for patients with EGFR-positive tumors compared with those who were EGFR-negative, both for overall survival and disease-free survival [21]. The correspondence rate of EGFR overexpression between core needle biopsy and surgical specimens was higher than the correspondence rates of common predictive factors (ER, PgR, and HER2) between the 2 types of specimens. However, the rates of EGFR expression were relatively low in both sensitive (33%) and insensitive patients (23%). In addition, in cases in which EGFR expression did not correspond between CNB and surgical specimens, EGFR was always negative in CNB, but positive in the surgical specimen. Therefore, it is possible that core needle biopsy specimens are inadequate to evaluate EGFR overexpression, or that EGFR expression was stimulated by chemotherapy. Following NAC, highly malignant EGFR-positive tumor cells increased in number, while EGFR-negative cells decreased in number. In these specimens, other common predictive factors did not change pre- and post-NAC; therefore it is not certain that all of the CNB specimens were inadequate. Indeed, it may be that NAC changed the characteristics of some tumors.

We evaluated EGFR, HER2, p53 and other common markers in specimens from pretreatment core needle biopsies as predictors of response to NAC. p53 was a more significant predictor than ER and histological grade, factors that have been previously reported. These results may have been influenced by the uncertainty of core needle biopsy results and the heterogeneity of cancer cells in the tumors. The correspondence rates of these common markers between CNB and surgical specimens were relatively low. However, the correspondence rate of p53 was significantly high. This result indicates that p53 is a stable parameter and suitable for predicting the response to neoadjuvant chemotherapy and for pretreatment diagnosis from CNB specimens.

Pretreatment diagnosis from CNB specimens is necessary to decide the strategy for primary breast cancer treatment. Therefore, identifying prognostic factors is very important, and we need a greater sample size to establish a classification system to predict patient outcome.

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