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Topoisomerase II-α (*topoII*) and *HER2* amplification in breast cancers and response to preoperative doxorubicin chemotherapy

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Abstract

A significant proportion of breast cancers with *HER2* amplification have simultaneous amplification of topoisomerase II-α (*topoII*). Amplification of *HER2* and *topoII* was assayed using a novel chromogenic *in situ* hybridisation (CISH) method. *HER2* and *topoII* amplification status and the response to preoperative doxorubicin chemotherapy were analysed in 67 locally advanced breast cancer patients. Response to chemotherapy was increased in the cases with coamplification of *HER2* and *topoII* (18/19), whereas the response rate was significantly decreased in the cases without *HER2* and *topoII* amplification (17/36). The 12 cases with *HER2* amplification alone showed an intermediate response rate (9/12). The findings of the current study indicate that *topoII* amplification may play a role in determining chemosensitivity of breast cancers to doxorubicin chemotherapy.

Keywords: Breast cancer; Chemotherapy; Chromogenic *in situ* hybridisation; Doxorubicin; HER2; Topoisomerase II-α

1. Introduction

The HER2 (erbB2) protein is a 185-kd transmembrane tyrosine kinase and overexpression of HER2 arises from *HER2* gene amplification resulting in an increased gene copy number [1]. *HER2* amplification has been shown in 20–40% of human breast carcinomas and is associated with a poor clinical outcome, even following systemic chemotherapy [2–4]. Recent studies suggest that HER2 is a useful determinant of response to hormonal or cytotoxic chemotherapy. Data from the Cancer and Leukemia Group B (CALGB) 8869 and the National Surgical Adjuvant Breast and Bowel Project (NSABP) protocol B-11 have suggested that patients whose tumours overexpress HER2 may derive a preferential benefit from treatment with doxorubicin [5,6]. Doxorubicin is the single most effective drug for breast cancer, targeting topoisomeraseII-α (topoII). TopoII is a key enzyme in DNA replication and the *topoII* gene is located at chromosome band 17q12-21, close to the *HER2* gene. *In vitro* studies have indicated that sensitivity to topoII inhibitors is dependent on the expression level of topoII in the target cancer cells [7–9]. A significant proportion of breast cancers with *HER2* amplification show simultaneous amplification or deletion of *topoII* [10,11]. Amplification of *topoII* may lead to the overexpression of the topoII protein and ultimately to hypersensitivity to topoII inhibitors [11].

Most HER2 studies have been performed using immunohistochemistry (IHC) which detects HER2 protein overexpression. Measurement of *HER2* gene amplification is more accurate since protein overexpression is the result of gene amplification. Fluorescence *in situ* hybridisation (FISH) allows the assessment of the level of gene amplification and also provides information about the distribution of gene copies in histological sections [12]. A number of reports have verified its accuracy and apparent superiority over IHC in predicting response to trastuzumab in metastatic breast carcinoma [13,14]. The main difficulty for adopting FISH in the clinical setting is the need for additional equipment such as fluorescence microscopy and multiband fluorescence filters. Recently, novel technology to detect the DNA probe has been developed. Chromogenic *in situ* hybridisation (CISH) uses a simple IHC-like peroxidase reaction [15]. CISH is a

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promising method to overcome the practical limitations of FISH, although its standardisation has not yet been validated.

In this report, the amplification of *HER2* and *topoII* and the response to doxorubicin chemotherapy was analysed in samples taken from breast cancer patients.

2. Patients and methods

67 patients with locally advanced breast cancer underwent preoperative chemotherapy between March 1996 and December 2000 at the Inje University Sanggye Paik Hospital. The clinical characteristics of the patients are summarised in Table 1. All patients received four cycles of chemotherapy with doxorubicin prior to their operative treatment. Doxorubicin was administered at 3-weekly intervals at a dose of 50 mg/m^2. All patients underwent core needle biopsy (CNB) before the chemotherapy. CISH for the amplification of *HER2* and *topoII* was performed on CNB specimens. Tumour grade and oestrogen receptor (ER) status were also determined on the pretreatment specimens.

Physical examinations, complete blood counts and biochemistry profiles were performed every week. Toxicity was assessed according to the World Health Organization (WHO) criteria [16]. A number of side-effects such as neutropenia, nausea and stomatitis were observed during the chemotherapy, but no patient dropped out from the study. Magnetic Resonance Imaging (MRI) scan was performed on all patients before chemotherapy and prior to surgery after completion of the four cycles of chemotherapy. Response in terms of the change in tumour size was determined on the basis of MRI analyses. Responses were defined as a partial response (PR; more than 50% reduction of the primary tumour volume) and stable disease (SD; less than 50% reduction in tumour volume).

Table 1
Clinical characteristics of the patients ($n = 67$)

	No. (%)
Age (years)	
< 50	55 (82)
$\geqslant 50$	12 (18)
Tumour size (cm)	
5–10	61 (91)
> 10	6 (9)
Oestrogen receptor	
Negative	36 (54)
Positive	31 (46)
Histological grade	
I	15 (22)
II	30 (45)
III	22 (33)
Operation	
Mastectomy	18 (27)
Breast conservation surgery	49 (73)

2.1. CISH analysis for HER2 and topoII

CISH was done on 3 μm-thick histological sections. The slides were deparaffinised and incubated in a SPOT-Light™ heat pretreatment-buffer (Zymed Inc., South San Francisco, CA, USA) at 92–100 °C for 15 min. After washing with phosphate buffered saline (PBS), 100 μl SPOT-Light™ tissue pretreatment enzyme (Zymed) was applied at 37° for 5 min. The slides were washed with PBS and dehydrated with graded ethanols. A cover slip was applied onto the slide after application of 15 μl of a digoxigenin-labelled *HER2* probe (Zymed). The slides were treated on 95 °C hot plate for 5–10 min and incubated at 37 °C for 16–24 h. After incubation, the slides were treated in 0.5× sodium chloride citrate (SCC) for 5 min and washed with PBS/Tween solution. The slides were treated with 3% hydrogen peroxide for 10 min. 100 μl fluorescein isothiocyanate (FITC)-sheep antidigoxigenin (Zymed) was applied for 30–60 min after the application of 100 μl CAS-Block™ (Zymed) for 10 min. After washing with PBS, 100 μl horse radish peroxidase (HRP)-goat anti-FITC (Zymed) was applied for 30–60 min. After washing with PBS, 150 μl 3,3-diaminobenzidine tetrahydrochloride (DAB) was applied for 20–30 min. After washing with purified water and dehydration with ethanol and xylene, the slides were counterstained with haematoxylin-eosin. CISH for *topoII* was performed using the same method and SPOT-Light Topoisomerase II (Zymed). Amplification of *HER2* and *topoII* was determined as a gene copy number of more than 4 or when a large gene copy cluster was seen in over 50% of the nuclei in the cancer cells.

2.2. Statistics

Comparisons of responses among groups were assessed with the Fisher's Exact test. Differences in clinical responses according to the amplification of *HER2* and *topoII* were compared using a linear regression analysis. P values less than 0.05 were considered statistically significant.

3. Results

Before the current study, we performed CISH on a tissue array of 188 breast cancers. *HER2* was amplified in 43 (23%) and *topoII* was amplified in 23 tumours (12%). *TopoII* amplification was significantly associated with *HER2* amplification (Table 2).

On the basis of this preliminary result, response to preoperative doxorubicin chemotherapy was analysed

Table 2
Correlation between amplification of *HER2* and topoisomerase II (topoII) by chromogenic *in situ* hybridisation (CISH) in 188 breast carcinomas

TopoII amplification	HER2/neu amplification		Total *n*
	n (%)	*n* (%)	
No amplification	138 (84)	27 (16)	165
Amplification	7 (30)	16 (70)	23

$P < 0.001$ for correlation between *HER2* and *topoII* amplifications.

according to the amplification status of *topoII* and *HER2* in 67 breast cancers. *HER2* was amplified in 31 (46%) and *topoII* was amplified in 19 tumours (28%). All those with *topoII* amplification also showed *HER2* amplification. Clinical responses were observed in 44 cases (66%). Clinical complete responses were not observed whereas 5 cases were confirmed as having complete pathological responses in surgically resected specimens. These 5 cases were *topoII*-amplified tumours and had an *in situ* cancer component in the surgical specimens, but had no invasive cancer. Due to the small study sample size, we included these 5 cases in the responder group in the statistical analysis. In univariate analysis, *HER2* and *topoII* amplification were significantly associated with the response to chemotherapy (Table 3). When we subcategorised the tumours according to their *HER2* and *topoII* amplification status, 95% of the *HER2+/topoII+* group were responders ($P = 0.038$) (Table 4).

Table 3
Relationship between response and patients characteristics

	Response (%)	*P* value
Age (years)		
50 ($n = 55$)	35 (64)	0.24
$\geqslant 50$ ($n = 12$)	9 (75)	
Tumour size (cm)		
5–10 ($n = 61$)	41 (67)	0.17
> 10 cm ($n = 6$)	3 (50)	
Oestrogen receptor		0.69
Negative ($n = 36$)	25 (69)	
Positive ($n = 31$)	19 (61)	
Histological grade		0.11
I ($n = 15$)	8 (53)	
II ($n = 30$)	20 (66)	
III ($n = 22$)	16 (73)	
HER2 amplification		0.013
Absent ($n = 36$)	17 (47)	
Present ($n = 31$)	27 (87)	
Topoisomerase IIα (*topoII*) amplification		0.011
Absent ($n = 48$)	26 (54)	
Present ($n = 19$)	18 (95)	

Table 4
Responses to doxorubicin preoperative chemotherapy according to *HER2*/topoisomerase II-α (*topoII*) amplification status

	Response *n* (%)	Total *n*
HER2−/topo II−	17 (47)	36
HER2+/topo II−	9 (75)	12
HER2+/topo II+	18 (95)	19

$P = 0.038$.

4. Discussion

The results of the current study indicate that preoperative doxorubicin chemotherapy is highly effective in breast cancers that have coamplification of *HER2* and *topoII*. In contrast, the clinical response was significantly decreased in breast cancers without *HER2* and *topoII* amplification. A number of studies in the adjuvant setting have suggested that anthracycline-based chemotherapy is particularly effective for women with HER2-positive breast cancers [5,17]. The biological mechanism for this finding has not yet been fully explained, but topoII is believed to be involved in the altered chemosensitivity to topoII inhibitors including doxorubicin in breast cancers [18]. Chromosomal location of the *topoII* gene is close to the *HER2* gene at 17q21-22 and *topoII* has been shown to be coamplified in 12% of *HER2*-amplified cases [11]. A recent study reported that *HER2* and *topoII* coamplification was observed in 44% and physical deletion of *topoII* was also observed by FISH in 42% of *HER2*-amplified primary breast cancers [10]. The investigators reported that no *topoII* copy number aberrations were found in breast cancers without *HER2* amplification. In the current study, *topoII* was amplified only in HER2-amplified tumours, which agrees with the results from the aforementioned study. The finding from the current study, that breast cancers with a coamplification of *HER2* and *topoII* showed a good response to doxorubicin chemotherapy, suggests that *topoII* amplification may explain the altered chemosensitivity to topoII inhibitors in *HER2*-amplified breast cancers. However, *topoII* amplification cannot solely explain this chemosensitivity because the clinical response also appeared to be significantly increased in *HER2*-amplified tumours without *topoII* amplification in our study.

A number of studies have indicated that response to preoperative chemotherapy is increased in smaller tumours [19,20]. The relationship between tumour size and clinical response could not be confirmed in our study, possibly as our study included mostly large tumours. Clinical or pathological complete responses have been reported in 4–20% of patients treated with various preoperative chemotherapies. We observed no clinical complete responses and a pathological complete response was confirmed in only five tumours with a

combined *HER2*/*topoII*-amplification status. The relatively low response rate of the current study might be explained by either a large tumour size and/or the low dose of doxorubicin used.

The frequency of *HER2* and *topoII* amplification in the studied patients was much higher than that from our series of 188 patients. This finding can be explained by the fact that doxorubicin treatment was given based on a positive IHC for HER2 protein. Although IHC is the method most commonly used in clinical practice to assess HER2 status, FISH seems to be more accurate in that DNA is less affected by variations in tissue fixation and processing than protein. A major obstacle to FISH analysis is the need to use special fluorescence microscopy with multibandpass fluorescence filters and this makes it difficult for most institutes to integrate FISH into routine clinical diagnostics. The results of the current study indicate the feasibility of CISH in the assessment of gene amplification. CISH does not require any additional equipment that is not already present in most pathological laboratories. Moreover, most pathologists are familiar with peroxidase-based immunostaining. Another advantage of CISH in routine practice is that simultaneous verification of histology can be done. In the panel of 188 breast cancers, we observed a concordance of 94% by CISH and FISH for *HER2* amplification. High concordance between the two techniques validates CISH analysis to assess the gene amplification status. One study has reported a high level of concordance between CISH and FISH in determining *HER2* gene copy number. However, CISH is presently regarded as an investigational technique as the clinical value of CISH has not yet been assessed.

In summary, response to preoperative doxorubicin chemotherapy was apparently increased in *HER2*-amplified breast cancer and the therapeutic efficacy was further increased by *topoII*-coamplification. This indicates that *topoII* status may have a role to play in the chemosensitivity of breast cancers to doxorubicin chemotherapy. CISH appears to be a promising method to assess gene amplification status, being both a feasible and relatively low-cost analysis.

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