Triple-negative breast cancer exhibits a favorable response to neoadjuvant chemotherapy independent of the expression of topoisomerase IIα

HIROKO NOGI[1], KEN UCHIDA[1], MAKIKO KAMIO[1], KUMIKO KATO[1], YASUO TORIUMI[1], TADASHI AKIBA[2], TOSHIAKI MORIKAWA[3], MASAAKI SUZUKI[4], TADASHI KOBAYASHI[5] and HIROSHI TAKEYAMA[1]

Departments of [1]Breast and Endocrine Surgery, [2]Surgery, [3]Thoracic Surgery, [4]Pathology and [5]Medical Oncology and Hematology, The Jikei University School of Medicine, Minato-ku, Tokyo 105-8461, Japan

Abstract. The present study retrospectively analyzed the utility of topoisomerase IIα expression as a prognostic marker to predict the neoadjuvant chemotherapeutic response and survival among different breast cancer subtypes. The patients were subtyped and the expression of topoisomerase IIα was determined using immunohistochemistry. All patients (n=147) received an anthracycline-containing regimen preoperatively, and 139 (95%) patients also received docetaxel. Of the 147 patients, 25 (17%) were triple-negative and 20 (17%) were human epidermal growth factor receptor 2 (HER2)-positive. Among these subtypes, a significantly higher a rate (P<0.0001) and higher incidence of topoisomerase IIα expression (P=0.036) were observed compared with that in the hormone receptor-positive and HER2-negative breast cancer types. However, the expression of topoisomerase IIα revealed no correlation with the treatment response or survival in any of the subtypes. Therefore, these results indicated that the favorable response to anthracycline-containing chemotherapy among triple-negative and HER2-positive breast cancer was independent of the expression of topoisomerase IIα.

Introduction

Breast cancer is the most common malignant disease among women in the Western world and Japan. However, advances in the systemic treatment of breast cancer, particularly in chemotherapy, have contributed to declines in the breast cancer mortality rates (1). Anthracycline-containing regimens are the most widely used in the adjuvant and neoadjuvant settings for patients with breast cancer (2). Several previous

clinical investigations have revealed that the use of neoadjuvant chemotherapy for patients with locally advanced breast cancer increased the surgical resectability rates and that the response to therapy correlated with the patients' ultimate disease-free survival (3-5). In addition, significant tumor volume reduction following neoadjuvant chemotherapy may permit subsequent breast-conserving surgical treatment (6,7) and a unique advantage of neoadjuvant chemotherapy is the possibility to take serial measurements of the primary tumor, therefore, allowing *in vivo* assessment of factors predictive of the sensitivity to the treatment (8).

Anthracyclines act via several mechanisms, however, the interaction with the nuclear enzyme topoisomerase IIα appears to be the most prominent mechanism (9). Topoisomerase IIα, which is a critical nuclear DNA binding enzyme, functions by reducing DNA twisting and supercoiling by cutting both strands of the DNA helix simultaneously, allowing selected regions of the DNA to untangle and to consequently engage in transcription, replication or repair processes. Disruption of topoisomerase IIα has been demonstrated to lead to double-stranded DNA breaks and cell death, and topoisomerase IIα is, therefore, also a proliferation marker of tumor cells, in addition to a target of anthracycline-based chemotherapy (10).

However, previous studies have reported variable expression levels of topoisomerase IIα and responses to anthracycline-containing chemotherapy in breast cancer, and while *in vivo* and *in vitro* studies each demonstrate that there is indeed an association between the expression levels of topoisomerase IIα and chemosensitivity to anthracyclines, these results remain controversial (11-14).

Gene expression profiling has identified distinct breast cancer molecular subtypes associated with different clinical outcomes. Breast cancer is a molecularly heterogeneous disease, which can be divided into ≥4 or 5 groups based on the expression profiles, including luminal A and B, normal breast-like, human epidermal growth factor receptor 2 (HER2)-positive, and basal-like (predominantly triple negative) breast cancer (15,16). Previous studies, including our previous study, revealed that triple negative breast cancer is associated with an improved pathological complete response rate compared with the other subtypes (17-19).

Correspondence to: Dr Hiroko Nogi, Department of Breast and Endocrine Surgery, The Jikei University School of Medicine, 3-25-8 Nishi-shinbashi, Minato-ku, Tokyo 105-8461, Japan
E-mail: nogi_h@jikei.ac.jp

In addition, several biomarkers and intrinsic subtypes have been reported as predictors of the neoadjuvant response (20,21). However, no basis for selecting the optimal chemotherapy for individual patients has been determined, and the association between the expression of topoisomerase IIα and the different subtypes remains to be elucidated.

With this in mind, the present study aimed to retrospectively analyze whether the protein expression levels of topoisomerase IIα assisted in predicting the response to anthracycline-containing neoadjuvant chemotherapy among each breast cancer subtype and whether it is a prognostic marker of survival.

Patients and methods

Patients. A prospective database of 147 Japanese women with stage II or III breast cancer who received neoadjuvant chemotherapy between May 1985 and January 2008 was analyzed. All patients received standard anthracycline-containing neoadjuvant chemotherapy. Adjuvant endocrine therapy for 5 years was prescribed for patients with hormone receptor (HR) -positive tumors, whereas adjuvant trastuzumab for 1 year was prescribed for patients with HER2-amplified/overexpressed tumors from 2001 onwards. Systemic and breast examinations were performed prior to neoadjuvant chemotherapy, prior to surgery, and every 12 months postoperatively using chest and abdominal computed tomography, mammograms, breast ultrasonography and bone scans. The present study was approved by the Ethics Committee of the Jikei University School of Medicine and written informed consent was obtained from the patients.

Immunohistochemistry (IHC) and defining breast cancer subtypes. IHC was performed, according to the standard protocol using 3 μm sections of paraffin-embedded tissues and the rabbit monoclonal antibody, anti-estrogen receptor (ER; SP1; Roche Diagnostics, Ltd., West Sussex, UK), for ER staining, and the rabbit monoclonal antibody, anti-progesterone receptor (PgR; 1E2; Roche Diagnostics, Ltd.), for PgR staining. Nuclear staining of ≥10% was considered positive. Tumors with ER and/or PgR positive expression were considered hormone receptor (HR)-positive. The expression of HER2 was determined using IHC with a rabbit polyclonal antibody (Dako, Glostrup, Denmark) on 4 μm sections of paraffin-embedded tissue. A staining score of 3[+], according to the HercepTest criteria (22), was considered positive and a 2[+] result was only considered positive if confirmed by fluorescence *in situ* hybridization with an amplification ratio of ≥2.0. The expression of topoisomerase IIα was determined by IHC using a mouse monoclonal antibody (M7186; 1:100; Dako) on 3 μm sections of paraffin-embedded tissue. The topoisomerase IIα staining was considered positive if nuclear staining ≥20% was observed (Fig. 1). Immunohistochemical proxies were used for subtyping and the tumors were classified into three subtypes, HR[-]/HER2^{-} (triple-negative), any HR/HER2^{+} (HER2-positive) and HR[+]/HER2^{-}.

Statistics. The response to chemotherapy was assessed, according to the Response Evaluation Criteria in Solid Tumors guidelines. The overall survival was measured from the date of diagnosis to the date of mortality, or the last follow-up.
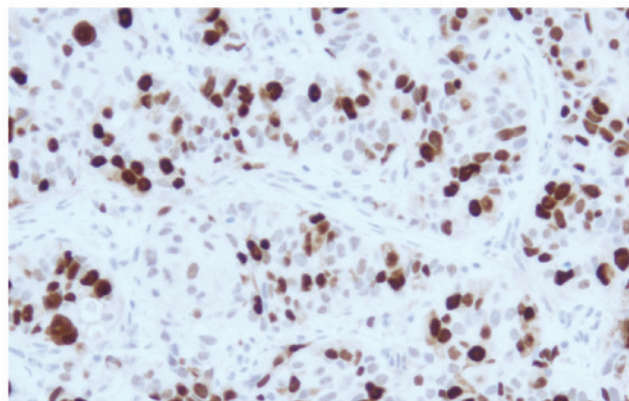

Figure 1. Positive immunostaining for topoisomerase IIα.

Disease-free survival was measured from the date of operation until the date of recurrence or the last follow-up. The association between each subtype and the age of the patients was evaluated using the Kruskal-Wallis test. The association between each subtype and the clinical factors, response rate to neoadjuvant chemotherapy and topoisomerase IIα expression in the patients, were evaluated using the Fisher's exact test. Cumulative survival probabilities were calculated using the Kaplan-Meier method, and differences between the survival rates were tested using the log-rank test. Logistic regression analyses were performed to evaluate the association between the expression of topoisomerase IIα, and the response to chemotherapy and survival among each breast cancer subtype. All statistical analyses were performed using Stata® software (Version 13; StataCorp LP, College Station, TX, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

Patients and tumor characteristics. The performed chemotherapeutic regimens, which have changed over time since the first cases were obtained in 1985, were as follows: 6 cycles of doxorubicin (50 mg/m^2), 5-fluorouracil (500 mg/m^2) and cyclophosphamide (500 mg/m^2) in 8 patients (5%); 6 cycles of alternate administration of epirubicin (60 mg/m^2), 5-fluorouracil (500 mg/m^2) and cyclophosphamide (500 mg/m^2) with docetaxel (75 mg/m^2) in 6 patients (4%); 6 cycles of concurrent administration of doxorubicin (50 mg/m^2) and docetaxel (60 mg/m^2) in 41 patients (28%); 4 cycles of epirubicin (100 mg/m^2), 5-fluorouracil (500 mg/m^2) and cyclophosphamide (500 mg/m^2), followed by 4 cycles of docetaxel (100 mg/m^2) in 92 patients (63%). Therefore, all patients received an anthracycline-based regimen and 139 patients (95%) also received docetaxel. The regimens did not differ according to the subtype. The median patient age was 51 years (range, 27-71 years). Table I lists the demographic, tumor characteristics, and the results of the Fisher's exact and Kruskal-Wallis tests among each subtype. The age of the patients with HR/HER2^{+} tumors was significantly higher compared with that of patients with HR[-]/HER2^{-} (P=0.04) and HR[+]/HER2^{-} tumors (P=0.03), and the menopausal status significantly differed between patients with any HR/HER2^{+} and the other two subtypes (P=0.02). By contrast, the tumor size and nodal status were similar among the three subtypes (Table I).

Table I. Demographic and tumor characteristics.

Characteristic	All patients n=147	HR⁻/HER2⁻ n=25	Any HR/HER2⁺ n=20	HR⁺/HER2⁻ n=102	P-value
Age (years)					
Median	51.0	49.5[a]	55.4	50.5[b]	0.04[a], 0.03[b]
Range	27-71	34-68	39-70	27-71	
Menopause, n (%)					
Pre	83 (57)	13 (52)	6 (30)	64 (63)	0.02
Post	64 (43)	12 (48)	14 (70)	38 (37)	
Pretreatment tumor size, n (%)					
≤5 cm	90 (61)	18 (72)	13 (65)	59 (58)	NS
>5 cm	57 (39)	7 (28)	7 (35)	43 (42)	
Pretreatment lymph node status, n (%)					
Negative	84 (57)	13 (52)	9 (45)	62 (61)	NS
Positive	63 (43)	12 (48)	11 (55)	40 (39)	

Chi-square and Fisher's exact tests; NS, not statistically significant; HR, hormone receptor; HER2, human epidermal growth factor receptor 2. [a]P-value between any HR/HER2⁺ and HR⁻/HER2⁻, [b]P-value between any HR/HER2⁺ and HR⁺/HER2⁻.

Table II. Responses to chemotherapy according to the breast cancer subtypes.

Response	All patients n=147	HR⁻/HER2⁻ n=25	Any HR/HER2⁺ n=20	HR⁺/HER2⁻ n=102	P-value
Clinical response, n (%)					
Complete/partial response	132 (90)	22 (88)	19 (95)	91 (89)	NS
Stable disease	15 (10)	3 (12)	1 (5)	11 (11)	
Pathological response, n (%)					
Complete response	26 (18)	10 (40)	8 (40)	8 (8)	<0.0001
Residual disease	121 (82)	15 (60)	12 (60)	94 (92)	

NS, not statistically significant; HR, hormone receptor; HER2, human epidermal growth factor receptor 2.

Response rate to neoadjuvant chemotherapy. The clinical and pathological response rates did not differ among the regimes (data not shown). Table II lists the clinical and pathological response rates to neoadjuvant chemotherapy. A total of 132 patients (90%) showed an objective clinical response. The objective clinical response rate revealed no difference among the subtypes. A total of 26 patients (18%) achieved a pathological complete response; 10 patients (40%) with HR⁻/HER2⁻ tumors and 8 patients (40%) with any HR/HER2⁺ tumors achieved favorable pathological complete response rates, and these rates were significantly higher compared with the response rate of patients with HR⁺/HER2⁻ tumors (8%; P<0.0001).

Expression levels of topoisomerase IIα in the subtypes. Table III shows the expression levels of topoisomerase IIα among the subtypes. It was demonstrated that 88/147 tumors (60%), including 19/25 (76%) HR⁻/HER2⁻ tumors, 15/20 (75%)

any HR/HER2⁺ tumors and 54/102 (52%) HR⁺/HER2⁻ tumors, overexpressed topoisomerase IIα. The frequency of topoisomerase IIα overexpression was significantly higher in any HR/HER2⁺ and HR⁻/HER2⁻ tumors compared with in the HR⁺/HER2⁻ tumors (P=0.036).

Correlation between the expression of topoisomerase IIα and the response to neoadjuvant chemotherapy among the subtypes. Table IV shows the association between the expression of topoisomerase IIα and the pathological complete response rates. It was demonstrated that 19/88 (22%) topoisomerase IIα-positive tumors and 7/59 (12%) topoisomerase IIα-negative tumors achieved a pathological complete response. Topoisomerase IIα-positive expression was associated with a favorable response. Additionally, 8/19 (42%) topoisomerase IIα-positive and 2/6 (33%) topoisomerase IIα-negative HR⁻/HER2⁻ tumors achieved a pathological complete response. Furthermore, 6/15 (40%) topoisomerase IIα-positive

Table III. Expression of topoisomerase IIα according to the breast cancer subtypes.

Topoisomerase IIα expression	Overall n=127	HR⁻/HER2⁻ n=25	Any HR/HER2⁺ n=20	HR⁺/HER2⁻ n=102	P-value
Positive[a]	88 (60%)	19 (76%)	15 (75%)	54 (52%)	0.036
Negative	59 (40%)	6 (24%)	5 (25%)	48 (47%)	

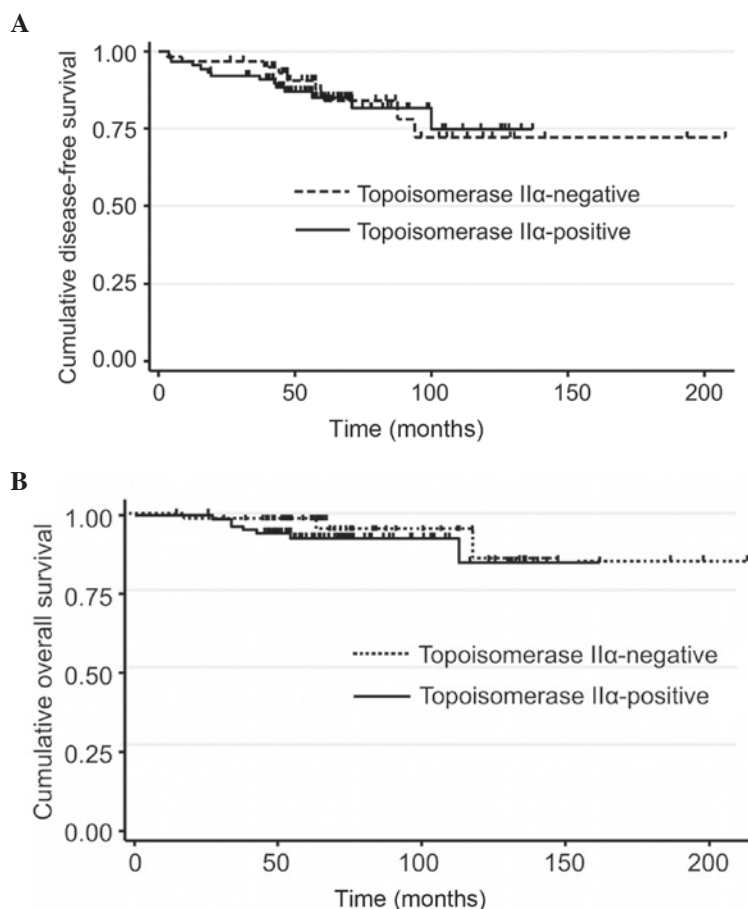HR, hormone receptor; HER2, human epidermal growth factor receptor 2. [a]Defined as >20% positive staining.

Figure 2. (A) The disease-free survival according to topoisomerase IIα the status. (B) The overall survival according to the topoisomerase IIα status.

and 2/5 (40%) topoisomerase IIα-negative any HR/HER2⁺ tumors, 5/54 (9%) topoisomerase IIα-positive and 3/48 (6%) topoisomerase IIα-negative HR⁺/HER2⁻ tumors achieved a pathological complete response. Topoisomerase IIα-positive expression was not significantly associated with a favorable response among all subtypes.

Association between the expression of topoisomerase IIα and survival. Fig. 2 shows the association between the expression of topoisomerase IIα and survival. It was revealed that 7/88 (8%) patients with topoisomerase IIα-positive and 3/59 (5%) patients with topoisomerase IIα-negative tumors succumbed to mortality, while 14/88 (16%) patients with topoisomerase IIα-positive and 9/59 (15%) patients with topoisomerase IIα-negative tumors exhibited recurrence. The expression of topoisomerase IIα was not associated with the overall and disease-free survival.

Discussion

The present study used IHC to evaluate the expression levels of topoisomerase IIα, HER2, ER and PgR in tumor samples obtained from the pretreatment biopsies of breast cancer patients receiving an anthracycline-containing regimen as neoadjuvant chemotherapy. This retrospective data analysis suggested that the favorable response to anthracycline-containing neoadjuvant chemotherapy among the triple-negative and HER2-positive subtypes was independent of the expression of topoisomerase IIα.

Anthracyclines, including doxorubicin and epirubicin, which is less cardiotoxic compared with doxorubicin, are extensively used for the treatment of breast cancer, and anthracycline-containing polychemotherapy regimens have reduced breast cancer mortality by ~1/3 (1). The cardiac toxicity of

Table IV. Association between the expression of topoisomerase IIα and the pathological complete response rate.

Topoisomerase IIα expression	Pathological complete response rate				
	Overall	HR⁻/HER2⁻	Any HR/HER2⁺	HR⁺/HER2⁻	P-value
Positive	19/88 (22%)	8/19 (42%)	6/5 (40%)	5/54 (9%)	0.051
Negative	7/59 (12%)	2/6 (33%)	2/5 (40%)	3/48 (6%)	0.019

HR, hormone receptor; HER2, human epidermal growth factor receptor 2.

anthracyclines is well described and the most common form, congestive heart failure, is known to be closely associated with the cumulative dose. Although limiting the cumulative dose to ~240-360 mg/m² doxorubicin has assisted in reducing the incidence of congestive heart failure to ~1.6-2.1%, data from long-term survivors of childhood cancer indicate that there is no true threshold for anthracycline-assoicated cardiotoxicity, and that cardiac damage may become apparent years later. However, studies from adjuvant breast cancer trials have shown that the likelihood of late cardiac effects in women who receive adjuvant anthracycline is low (2). However, since not all patients benefit from anthracyclines, a means of selecting the appropriate patients for the treatment is clearly of great interest. Anthracyclines have three major mechanisms of action: i) Inhibition of DNA and RNA synthesis by intercalating between the base pairs of the DNA/RNA strand; ii) enhancement of catalysis of oxidation-reduction reactions and iii) inhibition of topoisomerase IIα (9). Notably, the first mechanism also appears to be dependent on the inhibition of topoisomerase IIα for cytotoxicity.

Topoisomerase IIα is the only enzyme able to cleave and relegate double-stranded DNA. This enzyme acts during the relaxation of DNA supercoils, which accumulate during gene transcription and along with the progression of the replication fork. In addition, only topoisomerase IIα can perform the decatenation of replicated circular double-stranded DNA, and it is obligatorily involved in the remodeling of chromatin during mitosis. There are two highly homologous isoforms of topoisomerase II in humans, which are encoded by different genes. The gene for topoisomerase IIα is located on chromosome 17q21-22, while the gene for topoisomerase IIβ is located on chromosome 3q24 (10,23).

Drugs that interfere with topoisomerase IIα include anthracyclines (doxorubicin and epirubicin), etoposide, teniposide and amsacrine. These agents act by binding covalently with topoisomerase IIα following the occurrence of double-strand breaks, inducing lethal cellular damage by inhibition of relegation. An increase in the expression of topoisomerase IIα is associated with the sensitivity to these agents as a result of the increased substrate on which the drug may act.

Gene expression profiling has identified distinct breast cancer molecular subtypes (15,16) and previous studies have shown that triple-negative breast cancer is associated with an improved pathological complete response rate compared with the other subtypes (17-19). Nevertheless, the predictive role of topoisomerase IIα in each subtype remains to be elucidated. By contrast, HER2 amplification and overexpression

have been reported as predictive markers of the benefit of anthracycline treatment in the adjuvant setting (14). Because of its location in the identical amplicon on chromosome 17, the gene encoding topoisomerase IIα (TOP2A) is frequently co-amplified with that of HER2 (24,25), which in turn leads to the overexpression of its protein product and possibly, to a greater sensitivity to anthracyclines (25-27). In 2011, Di Leo et al (14) performed a meta-analysis, in which they identified that HER2 amplification and TOP2A amplification and deletion may have certain value in the prediction of responsiveness to anthracycline-containing chemotherapy. However, non-HER2 amplified and non-TOP2A altered tumor types also appear to derive benefits from treatment with anthracyclines. Furthermore, in their meta-analysis, triple-negative breast cancer and moderately hormone-sensitive tumor types appeared to exhibit and improved response to anthracycline treatment compared with treatment with the cyclophosphamide, methotrexate and fluorouracil regimen. Therefore, a differential benefit from anthracyclines may exist within these subtypes. Since all triple-negative tumors, and ~90% of moderately hormone-sensitive tumors, from that previous study revealed no TOP2A gene amplification, other mechanisms of increased anthracycline sensitivity may exist. Du et al (13) suggested that topoisomerase IIα is a predictive factor for breast cancer patients who received anthracycline-containing neoadjuvant chemotherapy using fluorescence in situ hybridization in another meta-analysis. However, the authors could not detect an association between the expression of topoisomerase IIα and sensitivity to anthracycline-containing regimens using IHC, which is similar to the results of the present study.

Notably, the target of anthracycline is the topoisomerase IIα protein as opposed to the gene, and it is known that there is a lack of correlation between gene status and protein expression (28-30). Proliferation signals can lead to overexpression of the topoisomerase IIα protein independently of the TOP2 gene status (29,31). In normal cells, the expression of topoisomerase IIα is regulated according to the cell cycle. In proliferating cells, topoisomerase IIα becomes detectable in the late G1 phase, and the quantity gradually increases, peaking in G2/M. By contrast, increased expression of topoisomerase IIα is commonly observed in malignant tumors, irrespective of the cell cycle stage (10).

There are certain limitations to the present study. Triple-negative, moderately hormone-sensitive and HER2-positive tumors are characterized by high proliferation (15,32,33) and this data further confirmed that topoisomerase IIα overexpression was more frequently

observed among the triple-negative and HER2-positive subtypes. However, ideally, quantification of nuclear concentrations of topoisomerase IIα protein may be a more appropriate way to investigate its predictive value as opposed to IHC alone. Furthermore, the present study included breast cancer patients treated with anthracycline combinations, as well as other drugs. Therefore, the use of these other drugs, including taxanes, cyclophosphamide and fluorouracil, may have influenced the activity of topoisomerase IIα and obscured any existing association.

In conclusion, the present findings do not justify the routine use of immunohistochemical staining of topoisomeras IIα as a predictive marker of the response to anthracycline-containing regimens. Women with triple-negative and HER2-positive tumors appear to derive benefits from anthracycline-containing chemotherapy independently of the expression of topoisomerase IIα.

Acknowledgements

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