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Original Article

Topoisomerase IIα and BRCA1 expression as predictive factors for anthracycline-based adjuvant chemotherapy response and prognosis in triple-negative breast cancers

Xiao-Ying Li[1], Mu Li[2], Feng Jin[1]

Departments of [1]Breast Surgery, [2]General Surgery, The First Affiliated Hospital of China Medical University, Shenyang, Liaoning Province, China

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Abstract: Triple-negative breast cancer (TNBC) is an aggressive histologically-defined cancer with a poor outcome for patients because there is a lack of targeted treatments. Anthracycline has an anti-tumor effect through binding to its target molecule, topoisomerase II alpha (TOP2A), and leads to DNA double-strand breaks. BRCA1 plays a critical role in repairing these breaks. This study investigated TOP2A and BRCA1 expression in TNBC correlated with disease-free survival (DFS) or overall survival (OS) in 105 primary TNBC patients treated with adjuvant anthracycline-based regimens. TOP2A and BRCA1 were evaluated by immunohistochemistry from 105 patients with TNBC in stages T1-T2, N0-N2 or M0. The DFS and OS rates for TOP2A-positive was significantly higher than TOP2A-negative patients, while the DFS and OS for BRCA1-negative was significantly increased than BRCA1-positive. The DFS and OS rates for TOP2A-positive and BRCA1-negative tumors were higher but they were not significantly compared with TOP2A-positive and BRCA1-positive tumors, but they were significantly higher than TOP2A-negative and BRCA1-positive tumors and TOP2A-negative and BRCA1-negative tumors. The combination of TOP2A-positive and BRCA1-negative phenotype might represent a favorable response in patients with TNBC who are treated with adjuvant anthracycline-based regimens.

Keywords: Topoisomerase II alpha, BRCA1, predictive marker, triple-negative breast cancer

Introduction

Triple-negative breast cancer (TNBC) is defined based on immunohistological criteria and characterized by estrogen receptor (ER), progesterone receptor (PR) and HER-2 receptor negativity; it is an aggressive tumor with a poor patient prognosis [1]. TNBC occurs in approximately 10-17% of all breast cancers. The tumors are often highly invasive, more frequently affect younger patients (<50 years) and are significantly more aggressive than other subtypes, although they initially respond well to chemotherapy. The aggressiveness is best illustrated by the fact that the peak risk of recurrence is within one to three years after diagnosis and the majority of deaths occur within the first 5 years following therapy [1, 2]. The development of personal targeted therapy based on molecular biology markers is the trend in current clinicalpractice, to identify the ideal treatment for an individual patient [3]. Because individualized treatment for TNBC does not currently exist, this mini-review focuses on discussion of the potential molecular targets and biomarkers for predicting the response in TNBC patients treated with targeted chemotherapy or/and biological therapy.

Topoisomerase II alpha (TOP2A) plays a pivotal role in DNA metabolism. It is a key enzyme that catalyzes transient double-strand breaks to resolve DNA topological problems during transcription, recombination, replication and chromosome partitioning [4, 5].

TOP2A is the target for anthracyclines, chemotherapeutic agents that bind and stabilize the

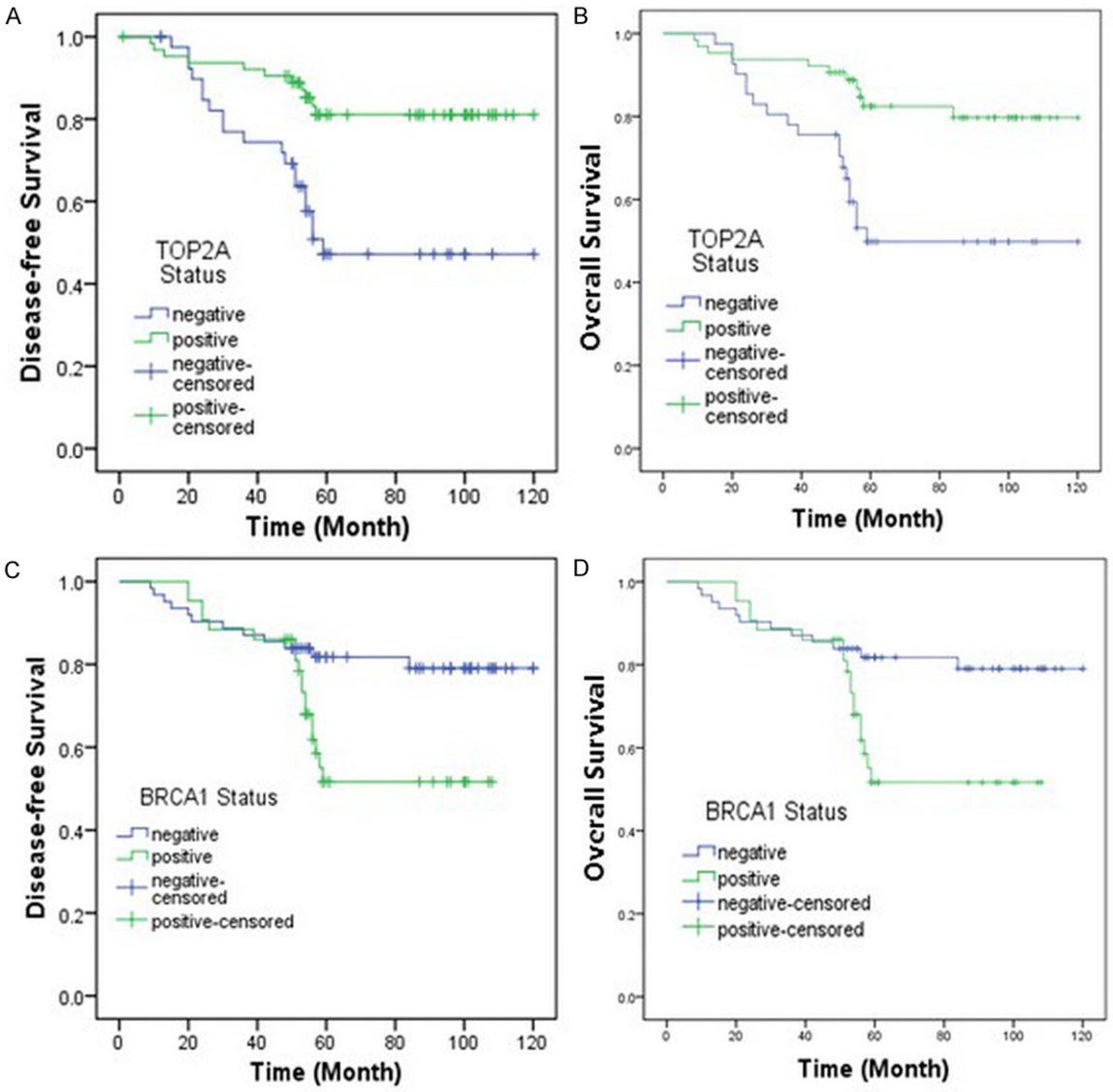

Figure 1. Effects of TOP2A or BRCA1 expression status of TNBC on the DFS and OS. A. Kaplan-Meier curve representation of disease-free survival in TNBC according to TOP2A positive and TOP2A negative. B. Kaplan-Meier curve representation of overall survival in TNBC according to TOP2A positive and TOP2A negative. C. Kaplan-Meier curve representation of disease-free survival in TNBC according to BRCA1 positive and BRCA1 negative. D. Kaplan-Meier curve representation of overall survival in TNBC according to BRCA1 positive and BRCA1 negative.

topoisomerase-DNA complex and cause double-strand DNA breaks that can lead to apoptosis [6]. An in vitro study showed that breast cancer cells with the overexpression of TOP2A are more sensitive to doxorubicin [7]. It has been reported that overexpression of TOP2A is observed in 20-62% of breast cancers and the TOP2A gene amplification in 12-24% of breast cancers [6, 8-10]. Approximately 79.3% of TNBC tumors were TOP2A-positive [11]. Antitumor activity of the anthracycline-based regimens was associated with TOP2A overexpression or TOP2A gene amplification, although the opposite results have also been reported [6, 12].

BRCA1 plays an important role in double-strand DNA repair [13]. It is reasonable to suggest that the loss of BRCA1 might lead to increased sensitivity of cancers to DNA-damaging agents. DNA-damaging agents include the following types of drugs: 1) DNA strand cross-linkers, including alkylating agents that cause DNA double-strand breaks; 2) topoisomerase I and II inhibitors, such as etoposide and anthracyclines; 3) platinum-based compounds that form

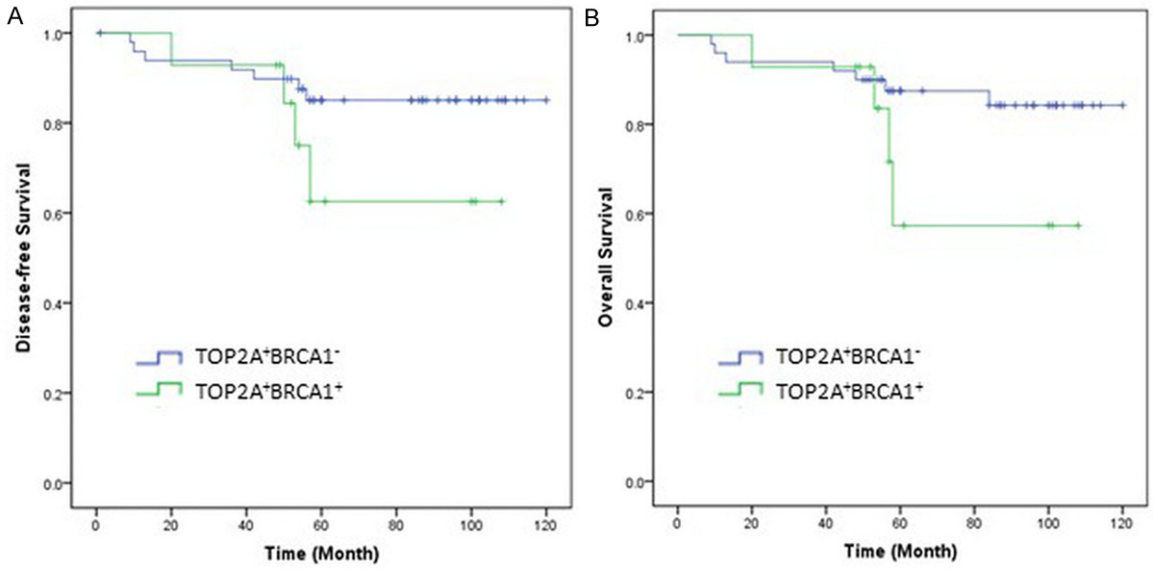

Figure 2. Effects of combined TOP2A and BRCA1 expression status of TNBC on the DFS and OS. A. Kaplan-Meier curve representation of disease-free survival in TNBC according to combined TOP2A positive and BRCA1 negative compared to combined TOP2A positive and BRCA1 positive. B. Kaplan-Meier curve representation of overall survival in TNBC according to combined TOP2A positive and BRCA1 negative compared to combined TOP2A positive and BRCA1 positive.

adducts with DNA; and 4) bleomycin, which directly damages DNA by inducing double-strand breaks [14-16]. BRCA1 was recently shown to be a predictive factor in response to anthracycline-based regimens. Because anthracycline-based treatment induces DNA double-strand breaks, it is possible that BRCA1 might modulate the response to anthracycline-based therapy. It has been reported that BRCA1 overexpression or wild-type BRCA1 caused a decrease in breast cancer sensitivity to anthracyclines [17]. Therefore, the BRCA1 expression level might influence the cancer sensitivity to anthracycline-based regimens [17, 18]. As described above, it has been postulated that TOP2A and BRCA1 expression might be associated with sensitivity to anthracycline-based regimens and might be potential predictors for the response to individualized anthracycline treatment regimens for patients with TNBC. Thus, we investigated TOP2A and BRCA1 expression simultaneously in TNBC patient tissues, and their relationship with disease-free survival (DFS) and overall survival (OS).

Materials and methods

Patients

This study was approved by the Institutional Review Board of the China Medical University, according to the Declaration of Helsinki. All participations provided written informed consent.

There were 105 patients with TNBC who were recruited at the First Affiliated Hospital of the China Medical University between April 2004 and September 2010. The group of 105 patients enrolled into our study met the following criteria: (1) invasive ductal breast cancer in clinical stages T1-T2, N0-N2 or M0; (2) no expression of ER, PR and HER-2; (3) underwent radical surgery (mastectomy or breast-conserving therapy); (4) adjuvant anthracycline-based chemotherapy (EC or CEF regimens: 60 mg/m^2 epirubicin, 50 mg/m^2 doxorubicin or 50 mg/m^2 pirarubicin plus cyclophosphamide or plus cyclophosphamide plus 5-fluorouracil); (5) patients with N2 treated with radiotherapy only; and (6) did not receive endocrine therapy. All 105 patients were followed up for a median of 67.5 months (range, 9-120 months), and during follow-up, there were 27 patients who relapsed, 6 patients with metastasis and 30 deaths related to relapsed or distant metastases. DFS was defined as the time between surgery and the date of first appearance of either local recurrence, distant metastasis, second primary cancer on another organ or death from any cause during follow-up. Patients known to be alive with no evidence of disease were censored at

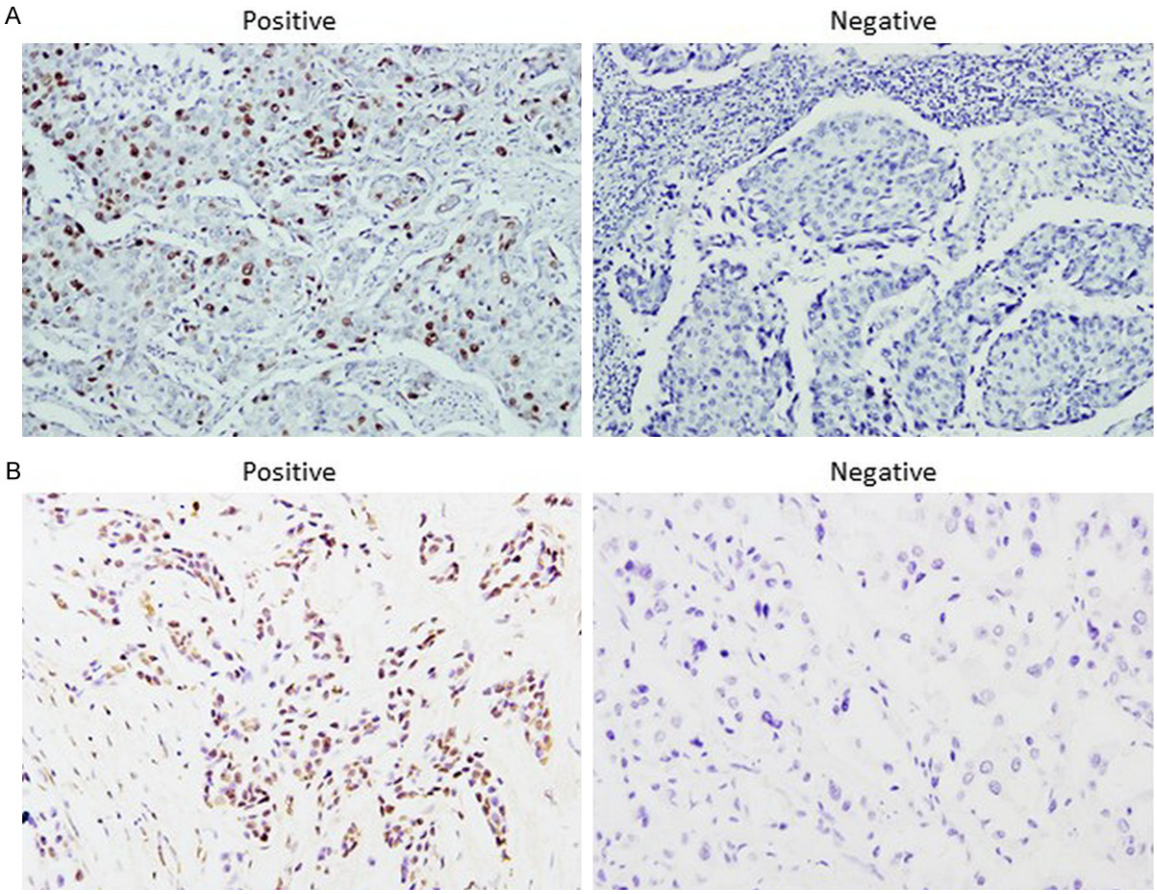

Figure 3. Immunohistochemical staining of TOP2A and BRCA1.Representative results of immunohistochemical staining of TOP2A and BRCA1 (200×). A. Nuclear staining of TOP2A-positive (left), TOP2A-negative (right) is seen in tumor cells. B. Nuclear staining of BRCA1-positive (left), BRCA1-negative (right) is seen in tumor cells.

the last follow-up date. OS was defined as the time from surgery to death from any cause, and patients who were alive or lost to follow-up were censored at the date of their last follow-up visit.

Immunohistochemistry of TOP2A and BRCA1 expression

Surgically excised tumor specimens were fixed with 10% neutral formalin and embedded in paraffin, and 4-μm-thick sections were prepared. Immunostaining was performed using the avidin-bioyin-peroxidase complex method (Ultrasensitive™, MaiXin, Fuzhou, China). The sections were deparaffinized in xylene, rehydrated with graded alcohol, and then boiled in 0.01 M citrate buffer (pH 6.0) for 2 min in an autoclave. Hydrogen peroxide (0.3%) was applied to block endogenous peroxide activity and the sections were incubated with normal goat serum for 20 min at room temperature to reduce nonspecific binding. The samples were then incubated overnight at 4°C with a mouse monoclonal anti-TopoIIα antibody (1:200 dilution; KiS1, MaiXin, Fuzhou, China) for TOP2A, or with a mouse monoclonal anti-BRCA1 antibody (1:70 dilution; Ab-1, MaiXin, Fuzhou, China) for BRCA1. They were subsequently incubated at room temperature for 30 min with ABC Kit (MaiXin, Fuzhou, China) using biotinylated anti-mouse IgG antibody for BRCA1 and TOP2A.

The nuclei in 50 tumor cells were counted under a microscope by two independent examiners, and the proportion of stained cells was recorded.

Staining, using immunohistochemistry (IHC), was evaluated semi-quantitatively following the immunoreactive score, as described by Remmele and Stegner [19]. Briefly, optical staining intensity was graded as 0 = none, 1 = weak

Table 1. Relationship between TOP2A or BRCA1 expression and clinicopathological characteristics in TNBC

		Top2A		P	BRCA1		P
		Positive	Negative		Positive	Negative	
Age	≥50	27	18		19	26	
	<50	37	23	0.511	24	36	0.488
Tumor size	T1	28	12		16	24	
	T2	33	26		25	34	
	T3	3	3	0.316	2	4	0.901
Lymph node metastasis	No	31	12		17	26	
	Yes	33	29	0.04	26	36	0.483
Nuclear grade	II	60	39		42	57	
	III	4	2	0.565	1	5	0.211

Table 2. Relationship between TOP2A or BRCA1 expression and DFS, OS in TNBC treated with Anthracycline-based regimens

	TOP2A				BRCA1			
	DFS	P	OS	P	DFS	P	OS	P
Positive	81.00%	0.001	79.70%	0.001	52.80%	0.03	51.70%	0.018
Negative	47.20%		49.80%		79%		79%	

staining, 2 = moderate staining and 3 = strong staining. The score was multiplied by the percentage of positively-stained cells (0 = no staining; 1, ≤10% of cells stained; 2, 11-50% of cells stained; 3, 51-80% of cells stained; and 4, ≥81% cells stained). In this study, 11.9% was used as the cut-off value (median value) for TOP2A, and 10% was used as the cut-off value for BRCA1.

Statistical analysis

All analysis was performed using SPSS version 20.0 (IBM SPSS Statistics). Categorical data are presented as numbers and the corresponding percentage, while continuous data are presented as the median and range values. Univariate analysis was performed using the Chi-squared test followed by a multivariable logistic regression analysis. DFS and OS were estimated using the Kaplan-Meier method and Cox regression analysis. A *P* value of 0.05 or less was considered to be significant.

Results

The expression of TOP2A and BRCA1 in Immunohistochemical staining are shown in **Figure 3**. The relationship between TOP2A or BRCA1 expression, and clinical parameters and status is shown in **Table 1**. There was no difference between TOP2A or BRCA1 expression and age, tumor size or nuclear grade status. Significantly higher TOP2A expression is related to lymph node metastasis (P = 0.040). The relationship between TOP2A or BRCA1 expression and DFS or OS in 105 TNBC patients is shown in **Table 2** and **Figure 1**. DFS and OS were significantly improved in TOP2A-positive patients compared with TOP2A-negative patients (P = 0.001). The DFS and OS in BRCA1-negative patients was significantly increased compared with BRCA1-positive patients (P = 0.03 and P = 0.018, respectively). Multivariate analysis of TOP2A and BRCA1 expression was adjusted for age, tumor size, lymph node metastasis and nuclear grade, and showed that TOP2A expression was a significant factor in patients with TNBC, which was associated with DFS and independent of the other factors (HR = 0.318, 95% CI, 0.131-0.769; P = 0.011) in **Table 3**. In addition, multivariate analysis showed that lymph node metastasis status was an independent predictive factor for DFS, and nuclear grade status was an independent predictive factor for DFS and OS in TNBC (**Table 3**). Results of the combined analysis of TOP2A and BRCA1 expression are shown in **Table 4** and **Figure 2**. The DFS and OS rates for TOP2A-positive and BRCA1-negative tumors (85% and 84.3%, respectively) were higher but they were not significantly compared with TOP2A-positive and BRCA1-positive tumors (62.5% and 57.3%, P = 0.174 and P = 0.141, respectively), but they were significantly higher than TOP2A-negative and BRCA1-positive tumors (48.8% and 48.6%, P = 0.002 and P = 0.001, respectively) and TOP2A-negative and BRCA1-negative tumors (50% and 58.3%, P = 0.004 and P = 0.015, respectively). The 3-year DFS and OS rates for patients with TOP2A-positive and BRCA1-negative tumors (91.8 % and 92%) was similar to that for TOP2A positive and BRCA1 positive tumors (92.9% and 92.9%, P = 0.5). The 5-year DFS and OS rates for TOP2A-positive and BRCA1-negative tumors (85% and 84.3%), how-

Table 3. Multivariate Cox regression analysis on DFS and OS in 105 TNBC treated with Anthracycline-based regimens

		DFS			OS		
		HR	95% CI	P	HR	95% CI	P
Age	≥50	1		0.904	1		0.614
	<50	1.047	0.494-2.219		1.215	0.570-2.588	
Tumor Size	>2 cm	1		0.146	1		0.199
	≤2 cm	0.545	0.241-1.236		0.580	0.253-1.332	
Lymph nodes metastasis	No	1		0.033	1		0.101
	Yes	2.56	1.077-6.087		1.994	0.874-4.551	
Nuclear Grade	III	1		0.001	1		0.001
	II	0.047	0.013-0.176		0.128	0.036-0.453	
TOP2A Status	Negative	1		0.011	1		0.062
	Positive	0.318	0.131-0.769		0.441	0.186-1.044	
BRCA1 Status	Negative	1		0.472	1		0.161
	Positive	1.393	0.564-3.442		1.913	0.772-4.738	

Table 4. Relationship between combined TOP2A or BRCA1 expression and DFS, OS in TNBC treated with Anthracycline-based regimens

Top2A	BRCA1	DFS	P	OS	P
Positive	Negative	85%		84.30%	
Positive	Positive	62.50%	0.174	57.30%	0.141
Negative	Positive	48.80%	0.002	48.60%	0.001
Negative	Negative	50%	0.004	58.30%	0.015

ever, were significantly higher than TOP2A-positive and BRCA1-positive tumors (62.5% and 57.3%, P<0.001).

Discussion

TNBC are associated with aggressive tumors and a poor patient prognosis. There are currently no specific predictors for targeted treatment of these tumors [20]. TOP2A is a target molecule of the anthracyclines [21, 22], and it has been speculated that TOP2A status could predict the tumor's sensitivity to anthracycline-based therapy in patients with TNBC. Our study showed a significantly longer DFS for patients with TOP2A-positive tumors than for those with TOP2A-negative tumors. This might indicate a significant association between TOP2A positivity and a preferable outcome, and a favorable prognosis in patients with TNBC who are treated with anthracycline-based regimens. Recently, TOP2A as a predictive factor for anthracycline-based regimens has been studied. In our study, multivariate analysis revealed TOP2A expression as an independent prognostic factor for anthracycline-based adjuvant chemotherapy in TNBC. Knoop et al. reported that patients with a TOP2A gene amplification showed an enhanced recurrence-free survival when treated with CEF or CMF (cyclophosphamide plus methotrexate plus 5-fluorouracil), but not in patients with normal TOP2A gene [6]. A similar finding was also reported by Tanner et al., who detected a better relapse-free survival for patients with TOP2A gene amplification who were treated with a tailored and dose-escalated CEF regimen compared with those treated with low-dose CEF followed by CTCb (cyclophosphamide, thiotepa and carboplatin) [12]. Recent reports have shown that TOP2A overexpression is significantly associated with a higher pathological complete response (pCR) rate and favorable DFS in patients treated with anthracycline-based regimens [8, 9, 23, 24]. Data suggested that TOP2A overexpression or TOP2A gene amplification might be a predictive marker of sensitivity to anthracycline-based regimens [8]. In contrast to HER2, TOP2A gene levels do not correlate with protein levels [23, 25, 26], because of the multifactorial complexity in the TOP2A translation pathway and the transcriptional regulatory role of proliferation signals [27]. Moreover whether TOP2A overexpression determined by immunohistochemistry or TOP2A gene amplification determined by FISH might be more closely associated with response to the anthracycline-based treatment for patients, some authors found a stronger association for TOP2A overexpression, because

the drug target is TOP2A protein, but not its gene or RNA [27, 28]. TOP2A is a key enzyme during cell division and it is most strongly expressed in the S and G2/M phases [29]. TOP2A-positive tumors are thought to have a higher rate of proliferation and a higher proportion of cells in the S or G2/M phases than TOP2A-negative tumors. Thus, it seems reasonable to suggest that TOP2A-positive tumors are more likely to have a mitotic score of III or to be ER-negative because both types of tumors are highly proliferative. High levels of TOP2A mRNA and protein expression were recently observed in TNBC [30, 31]. Our study found that 64 of 105 TNBC was TOP2A-positive, the proportion of TOP2A-positive cells in patients with TNBC was approximately 61%.

The TOP2A gene is located on 17q21-22, close to the HER2 gene. Therefore, some authors suggest that the TOP2A gene amplification is relevant to HER2 gene amplification [32]. Current results are also in agreement with the majority of published research, which showed 40-50% co-amplification [28, 33-35]. Previous studies reported that the association between HER2 expression and sensitivity to anthracycline-based regimens might be an indirect association that is mediated through TOP2A [36, 37].

In addition to the importance of TOP2A as a predictive factor for a favorable prognosis in patients treated with anthracycline-based regimens, we also investigated the influence of BRCA1 expression on predicting the response to anthracycline-based regimens in patients with TNBC. Most TNBC and basal-like cancers overlap [38], and basal-like cancers usually carry the BRCA1 mutation, leading to a deficiency in BRCA1 expression [39-41]. The loss of BRCA1 expression is frequently recognized in both types of tumors, resulting in a lack of the repair function for DNA double-strand breaks and thus leading to apoptosis. In addition, it was found that an adjuvant or neo-adjuvant anthracyclin-based treatment improves the prognosis and enhances the pCR rate of patients carrying BRCA1 mutations [42]. An in vitro study showed that a mouse cell line that was deficient in BRCA1 was highly sensitive to doxorubicin, and that induction of wild-type BRCA1 resulted in a reduced level of apoptotic cell death after treatment with this agent [43]. In this study, the proportion of BRCA1 loss was around 59% in TNBC. The loss of BRCA1 expression alone was significantly associated with a favorable prognosis and longer DFS and OS. In a combined analysis of TOP2A and BRCA1 expression, the TOP2A positive and BRCA1 negative tumors showed a significantly longer DFS and OS compared with other TOP2A and BRCA1 combination groups. Moreover, the TOP2A-positive and BRCA1-negative phenotype also has a higher rate of DFS and OS (85.00% and 84.30%) than TOP2A-positive tumors alone (81.00% and 79.70%) and the BRCA1-negative tumors alone (52.80% and 51.70%). These results suggest that BRCA1 plays a role in double-strand DNA repair, thus modulating sensitivity to anthracycline-based regimens in patients with TNBC. However, the exact reason why loss of BRCA1 expression confers sensitivity to anthracycline-based regimens remains unknown. We speculated that DNA double-strand breaks that result from anthracycline-based regimens are less likely to be repaired in tumor cells that are defective in BRCA1 expression, resulting in cell cycle arrest and apoptosis. Thus, combined analysis of TOP2A and BRCA1 expression may be beneficial for predicting the response to anthracycline-based regimens in patients with TNBC.

In conclusion, our study suggests that a TOP2A-positive and BRCA1-negative phenotype in patients with TNBC might be used to predict of an increased sensitivity to anthracycline-based regimens, and might be related to a longer survival time and a more favorable prognosis. Combined TOP2A and BRCA1 expression, assessed by IHC, might be useful for the predicting the response to anthracycline-based therapy in patients with TNBC. Although a TOP2A-positive and BRCA1-negative phenotype in patients with TNBC is generally considered to be a biological aggressive subtype, leading to a high recurrence rate, our findings might indicate that OS and DFS could be prolonged and a better prognosis could be achieved if the patient was appropriately treated using anthracycline-based regimens. In addition, use of TOP2A expression as an independent predictive biomarker for anthracycline-based chemotherapy needs to be verified using a larger number of patients with TNBC. In the present study, the dose of anthracyclines was lower than the standard treatment. However,

an association between biomarkers and the response to anthracycline-based regimens was observed in patients with TNBC. This different observation might be obtained if patients were treated with a standard dose of anthracycline-based regimens. Further studies are needed in a larger number of patients with TNBC and treated with higher dose of anthracycline-based regimens.

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Disclosure of conflict of interest

None.

Address correspondence to: Feng Jin, Department of Breast Surgery, First Affiliated Hospital of China Medical University, No. 155 North Nanjing Street, Heping District, Shenyang 110001, Liaoning Province, China. Tel: 86-24-83283555; Fax: 86-24-22703576; E-mail: cmu_13998866946@hotmail.com

References

[1] Reis-Filho JS, Tutt AN. Triple negative tumours: a critical review. Histopathology 2008; 52: 108-118.

[2] Shiu KK, Tan DS, Reis-Filho JS. Development of therapeutic approaches to 'triple negative' phenotype breast cancer. Expert Opin Ther Targets 2008; 12: 1123-1137.

[3] Ahn S, Cho J, Sung J, Lee JE, Nam SJ, Kim KM, Cho EY. The prognostic significance of tumor-associated stroma in invasive breast carcinoma. Tumour Biol 2012; 33: 1573-1580.

[4] Wang JC. Cellular roles of DNA topoisomerases: a molecular perspective. Nat Rev Mol Cell Biol 2002; 3: 430-440.

[5] Watt PM, Hickson ID. Structure and function of type II DNA topoisomerases. Biochem J 1994; 303: 681-695.

[6] Knoop AS, Knudsen H, Balslev E, Rasmussen BB, Overgaard J, Nielsen KV, Schonau A, Gunnarsdóttir K, Olsen KE, Mouridsen H, Ejlertsen B. Retrospective analysis of topoisomerase IIa amplifications and deletions as predictive markers in primary breast cancer patients randomly assigned to cyclophosphamide, methotrexate, and fluorouracil or cyclophosphamide, epirubicin, and fluorouracil: Danish Breast Cancer Cooperative Group. J Clin Oncol 2005; 23: 7483-7490.

[7] Järvinen TA, Tanner M, Rantanen V, Bärlund M, Borg A, Grénman S, Isola J. Amplification and deletion of topoisomerase IIalpha associate with ErbB-2 amplification and affect sensitivity to topoisomerase II inhibitor doxorubicin in breast cancer. Am J Pathol 2000; 156: 839-847.

[8] Cardoso F, Durbecq V, Larsimont D, Paesmans M, Leroy JY, Rouas G, Sotiriou C, Renard N, Richard V, Piccart MJ, Di Leo A. Correlation between complete response to anthracycline-based chemotherapy and topoisomerase II-alpha gene amplification and protein overexpression in locally advanced/metastatic breast cancer. Int J Oncol 2004; 24: 201-209.

[9] Coon JS, Marcus E, Gupta-Burt S, Seelig S, Jacobson K, Chen S, Renta V, Fronda G, Preisler HD. Amplification and overexpression of topoisomerase IIalpha predict response to anthracycline-based therapy in locally advanced breast cancer. Clin Cancer Res 2002; 8: 1061-1067.

[10] Fritz P, Cabrera CM, Dippon J, Gerteis A, Simon W, Aulitzky WE, van der Kuip H. c-erbB2 and topoisomerase IIalpha protein expression independently predict poor survival in primary human breast cancer: a retrospective study. Breast Cancer Res 2005; 7: 374-384.

[11] Tan DS, Marchió C, Jones RL, Savage K, Smith IE, Dowsett M, Reis-Filho JS. Triple negative breast cancer: molecular profiling and prognostic impact in adjuvant anthracycline-treated patients. Breast Cancer Res Treat 2008; 111: 27-44.

[12] Tanner M, Isola J, Wiklund T, Erikstein B, Kellokumpu-Lehtinen P, Malmström P, Wilking N, Nilsson J, Bergh J. Topoisomerase IIalpha gene amplification predicts favorable treatment response to tailored and dose-escalated anthracycline-based adjuvant chemotherapy in HER-2/neu-amplified breast cancer: Scandinavian Breast Group Trial 9401. J Clin Oncol 2006; 24: 2428-2436.

[13] Zhong Q, Chen CF, Li S, Chen Y, Wang CC, Xiao J, Chen PL, Sharp ZD, Lee WH. Association of BRCA1 with the hRad50-hMre11-p95 complex and the DNA damage response. Science 1999; 285: 747-750.

[14] Cleator S, Heller W, Coombes RC. Triple-negative breast cancer: therapeutic options. Lancet Oncol 2007; 8: 235-244.

[15] Kennedy RD, Quinn JE, Mullan PB, Johnston PG, Harkin DP. The role of BRCA1 in the cellular response to chemotherapy. J Natl Cancer Inst 2004; 96: 1659-1668.

[16] Zdraveski ZZ, Mello JA, Marinus MG, Essigmann JM. Multiple pathways of recombination define cellular responses to cisplatin. Chem Biol 2002; 7: 39-50.

[17] Fedier A, Steiner RA, Schwarz VA, Lenherr L, Haller U, Fink D. The effect of loss of Brcal on the sensitivity to anticancer angets in p53-deficient cells. Int J Oncol 2003; 22: 1169-1173.
[18] Lafarge S, Sylvain V, Ferrara M, Biqnon YJ. Inhibition of BRCA1 leads to increased chemoresistance to microtubule-interfering agents, an effect that involves the JNK pathway. Oncogene 2001; 20: 6597-6606.
[19] Remmele W, Stegner HE. Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. Pathologe 1987; 8: 138-140.
[20] Rakha EA, El-Sayed ME, Green AR, Lee AH, Robertson JF, Ellis IO. Prognostic markers in triple-negative breast cancer. Cancer 2007; 109: 25-32.
[21] Wang JC. DNA topoisomerases. Annu Rev Biochem 1996; 65: 635-692.
[22] Cummings J, Smyth JF. DNA topoisomerase I and II as targets for rational design of new anticancer drugs. Ann Oncol 1993; 4: 533-543.
[23] O'Malley FP, Chia S, Tu D, Shepherd LE, Levine MN, Huntsman D, Bramwell VH, Andrulis IL, Pritchard KI. Topoisomerase II alpha protein and responsiveness of breast cancer to adjuvant chemotherapy with CEF compared to CMF in the NCIC CTG randomized MA.5 adjuvant trial. Breast cancer Res Treat 2011; 128: 401-409.
[24] Almeida D, Gerhard R, Leitão D, Davilla C, Damasceno M, Schmitt F. Topoisomerase II-alfa gene as a predictive marker of response to anthracyclines in breast cancer. Pathol Res Pract 2014; 210: 675-679.
[25] Mueller RE, Parkes RK, Andrulis I, O'Malley FP. Amplication of the TOP2A gene does not predict high levels of topoisomerase II alpha protein in human breast tumor samples. Genes Chromosomes Cancer 2004; 39: 288-297.
[26] Romero A, Martín M, Cheang MC, López García-Asenjo JA, Oliva B, He X, de la Hoya M, García Sáenz JÁ, Arroyo Fernández M, Díaz Rubio E, Perou CM, Caldés Llopis T. Assessment PCR, gene expression microarrays, immunohistochemistry, and fluorescence in situ hybridization. Am J Pathol 2011; 178: 1453-1460.
[27] Oakman C, Moretti E, Sotiriou C, Viale G, Di Leo A. Topoisomerase II alpha and responsiveness of breast cancer to adjuvant chemotherapy. J Natl Cancer Inst 2009; 101: 1735-1736.
[28] Schindlbeck C, Mayr D, Olivier C, Rack B, Engelstaedter V, Jueckstock J, Jenderek C, Andergassen U, Jeschke U, Friese K. Topoisomerase IIalpha expression rather than gene amplification predicts responsiveness of adjuvant anthracycline-based chemotherapy in women with primary breast cancer. J Cancer Res Clin Oncol 2010; 136: 1029-1037.
[29] Meyer KN, Kjeldsen E, Straub T, Knudsen BR, Hickson ID, Kikuchi A, Kreipe H, Boege F. Cell cycle-coupled relocation of types I and II topoisomerases and modulation of catalytic enzyme activities. J Cell Biol 1997; 136: 775-788.
[30] Sayed-Ahmed MM, Hafez MM, Al-Shabanah OA, Al-Rejaie SS, Aleisa AM, Al-Yahya AA, Alsheikh A, Al Diab AI, Al-Akeely MH. Increased expression of biological markers as potential therapeutic targets in Saudi women with triple-negative breast cancer. Tumori 2013; 99: 545-554.
[31] Mrklic I, Pogorelic Z, Capkun V, Tomic S. Expression of topoisomerase II-αin triple negative breast cancer.Appl Immunohistochem. Appl Immunohistochem Mol Morphol 2014; 22: 182-187.
[32] Schindlbeck C, Mayr D, Olivier C, Rack B, Engelstaedter V, Jueckstock J, Jenderek C, Andergassen U, Jeschke U, Friese K. Topoisomerase IIalpha expression rather than gene amplification predicts responsiveness of adjuvant anthracycline-based chemotherapy in women with primary breast cancer. J Cancer Res Clin Oncol 2010; 136: 1029-1037.
[33] Press MF, Sauter G, Buyse M, Bernstein L, Guzman R, Santiago A, Villalobos IE, Eiermann W, Pienkowski T, Martin M, Robert N, Crown J, Bee V, Taupin H, Flom KJ, Tabah-Fisch I, Pauletti G, Lindsay MA, Riva A, Slamon DJ. Alteration of Topoisomerase II-Alpha Gene in Human Breast Cancer: Association With Responsiveness to Anthracycline-Based Chemotherapy. J Clin Oncol 2011; 29: 859-867.
[34] Arriola E, Moreno A, Varela M, Serra JM, Falo C, Benito E, Escobedo AP. Predictive value of HER-2 and Topoisomerase II alpha in response to primary doxorubicin in breast cancer. Eur J Cancer 2006; 42: 2954-2960.
[35] Engstrøm MJ, Ytterhus B, Vatten LJ, Opdahl S, Bofin AM. TOP2A gene copy number change in breast cancer. J Clin Pathol 2014; 67: 420-425.
[36] Penault-Llorca F, Cayre A, Bouchet Mishellany F, Amat S, Feillel V, Le Bouedec G, Ferrière JP, De Latour M, Chollet P. Induction chemotherapy for breast carcinoma: predictive markers and relation with outcome. Int J Oncol 2003; 22: 1319-1325.
[37] Konecny GE, Pauletti G, Untch M, Wang HJ, Möbus V, Kuhn W, Thomssen C, Harbeck N, Wang L, Apple S, Jänicke F, Slamon DJ. Association between HER2, TOP2A, and response to anthracycline-based preoperative chemotherapy in high-risk primary breast cancer. Breast Cancer Res Treat 2010; 120: 481-489.

[38] Banerjee S, Reis-Filho JS, Ashley S, Steele D, Ashworth A, Lakhani SR, Smith IE. Basal-like breast carcnomas: clinical outcome and response to chemotherapy. J Clin Pathol 2006; 59: 729-735.

[39] Foulkes WD, Brunet JS, Stefansson IM, Straume O, Chappuis PO, Bégin LR, Hamel N, Goffin JR, Wong N, Trudel M, Kapusta L, Porter P, Akslen LA. The prognostic implication of the basal-like (cyclinEhigh/p27low/p53+/glomeruloid-microvascular-proliferation+) pheotype of BRCA1-related breast cancer. Cancer Res 2004; 64: 830-835.

[40] Turner NC, Reis-Filho JS. Basal-like breast cancer and the BRCA1 phenotype. Oncogene 2006; 25: 5846-5853.

[41] Liedtke C, Mazouni C, Hess KR, André F, Tordai A, Mejia JA, Symmans WF, Gonzalez-Angulo AM, Hennessy B, Green M, Cristofanilli M, Hortobagyi GN, Pusztai L. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol 2008; 26: 1275-1281.

[42] Goffin JR, Chappuis PO, Bégin LR, Wong N, Brunet JS, Hamel N, Paradis AJ, Boyd J, Foulkes WD. Impact of germline BRCA1 mutations and overexpression of p53 on prognosis and response to treatment following breast carcinoma: 10-year follow up data. Cancer 2003; 97: 527-536.

[43] Fedier A, Steiner RA, Schwarz VA, Lenherr L, Haller U, Fink D. The effect of loss of Brca1 on the sensitivity to anticancer agents in p53-deficient cells. Int J Oncol 2003; 22: 1169-1173.