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Original Article

TOP2A is an independent prognostic factor in patients with operable invasive breast cancer: a large-scale study

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Abstract: Purpose: The clinical role of TOP2A as a prognostic indicator for breast cancer has not been well examined. The aims of this study were to detect expression of the topoisomerase 2A (TOP2A) protein, to investigate the relationship between TOP2A andhuman epidermal growth factor receptor-2 (HER2) status or hormone receptor (HR) expression, and to further explorethe prognostic ability of TOP2A in resectable invasive breast cancer. Methods: Using an immunohistochemistry approach, TOP2A protein expression was assessed in 364 patients with invasive breast cancer stages I-III. Correlation between TOP2A and clinical and pathologic findings was evaluated by the X^2 test or Fisher exact test. The Kaplan-Meier method and multivariate Cox regression analysiswere used for survival analysis. Results: A total of 292 breast cancer patients were classified as overexpressing TOP2A. TOP2A overexpression was related to tumor grade (G3 vs. G2 vs. G1; P = 0.004) but showed no significant correlations with ER, PR, or HER-2 expression. Patients with high expression of TOP2A had a longer overall survival (OS) compared with patients with low TOP2A expression in both univariate and multivariate analyses. Conclusions: Our study showed a significant favorable predictive and prognostic effect of TOP2A protein overexpression in patients withoperable invasive breast cancer. Further prospective studies to validate the prognostic value of the TOP2A protein are urgently needed.

Keywords: Breast cancer, breast pathology, surgical pathology

Introduction

Breast cancer, the most frequently occurring cancer among women and the second most common cancer, is the fifth leading cause of cancer-related death worldwide [1, 2]. It is also the most frequent cause of cancer-related death in women in undeveloped regions such as Eastern Asia [1, 2]. Breast mastectomy represents one of the standard treatments for early disease [3], and the 21-gene Oncotype Dx test has been demonstrated in various clinical studies as an effective model that can predict prognosis and identify patients who can benefit from chemotherapy [4, 5]. Although 21-gene Oncotype Dx has a certain predictive value, identifying more specific tumor markers that could act as prognostic factors for patients receiving radical surgery is crucial for individualized treatment.

The topoisomerase II (TOP2A) gene, located on 17q21.2, is an essential DNA topoisomerase involved in the movement and untangling of DNA. Because of its involvement in cell division, TOP2A is widely considered to bea direct molecular target of topoisomerase inhibitors [6, 7] and chemotherapy based on anthracycline, which is considered fundamental to adjuvant chemotherapy after radical mastectomy [8].

The prognostic role of TOP2A overexpression has been investigated in various cancer types, including hepatocellular [9] and small cell lung [10] cancer. However, only a few studies with small sample sizes have investigated the prognostic properties of the TOP2A protein in resectable invasive breast cancer [11], with most of these studies reporting limited value for TOP2A as a prognostic marker [11, 12]. For instance, Qiao *et al.* [11, 12] found no prognos-

Table 1. Correlation between TOP2A expression and clinicopathological characteristics

Characteristics	All (number of patients)	Low TOP2A expression	High TOP2A expression	*P* value
Age				
< 55 years old	259	57	202	0.094
≥ 55 years old	105	15	90	
Pathological type				
Other types	37	9	28	0.464
Invasive ductal carcinoma	327	63	264	
Histological grade				
Grade 1	29	11	18	**0.004**
Grade 2	211	38	173	
Grade 3	71	7	64	
Venous/lymphatic invasion				
No	278	60	218	0.154
Yes	83	12	71	
Size of the primary tumor				
T1	162	34	128	0.618
T2-4	196	37	159	
Lymph node metastasis				
N0	162	38	156	0.945
N1-3	196	33	133	
Clinical stage				
I	107	21	86	0.877
II	178	34	144	
III	73	16	57	
Hormone therapy				
No	240	42	198	0.129
Yes	124	30	94	
Adjuvant radiotherapy				
No	212	38	174	0.294
Yes	152	34	118	
Adjuvant chemotherapy				
No	93	15	78	0.299
Yes	270	57	213	
ER				
Negative	131	22	109	0.284
Positive	233	50	183	
PR				
Negative	154	28	126	0.512
Positive	210	44	166	
HER-2				
Negative	277	65	212	0.074
Positive	87	7	80	

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; TOP2A, topoisomerase 2A; HER2, human epidermal growth factor receptor-2. Bold values indicate statistical significance (P < 0.05).

ticvalue of changes in expression of either the TOP2A gene or protein in 256 cases of breast cancer. Other studies have reported contradictory conclusions [13, 14]. Therefore, the prognostic value of TOP2A expression in resectable invasive breast cancer remains controversial. This prompted us to apply immunohistochemistry (IHC) to evaluate the pro gnostic capacity of TOP2A expression at a large scale and in a well-defined cohort of breast cancer patients.

Patients and methods

Patients and treatments

The study group included 364 stage I-III breast cancer patientsat Zhejiang Cancer Hospital between 2006 and 2010 (**Table 1**). All 364 patients underwent primary surgery, including 36 breast-conserving surgeries and 328 modified radical operations. The patients did not receive any preoperative therapy. Among the patients, 245 individuals received postoperative chemotherapy based on anthracycline including fluorouracil + epirubicin + cyclophosphamide, docetaxel + epirubicin + cyclophosphamide, docetaxel + epirubicin, epirubicin + cyclophosphamide, epirubicin + cyclophosphamide follow docetaxel and doxorubicin + cyclophosphamide and so on. Another 34 patients received non-anthracycline-based regimens. Those patients accepting breast-conserving surgery with a tumor diameter greater than 5 cm or with lymph node metastasis received radiotherapy. Endocrine therapy was administered to patients who had hormone receptor-positive tumors. Two pathologists independently reviewed the

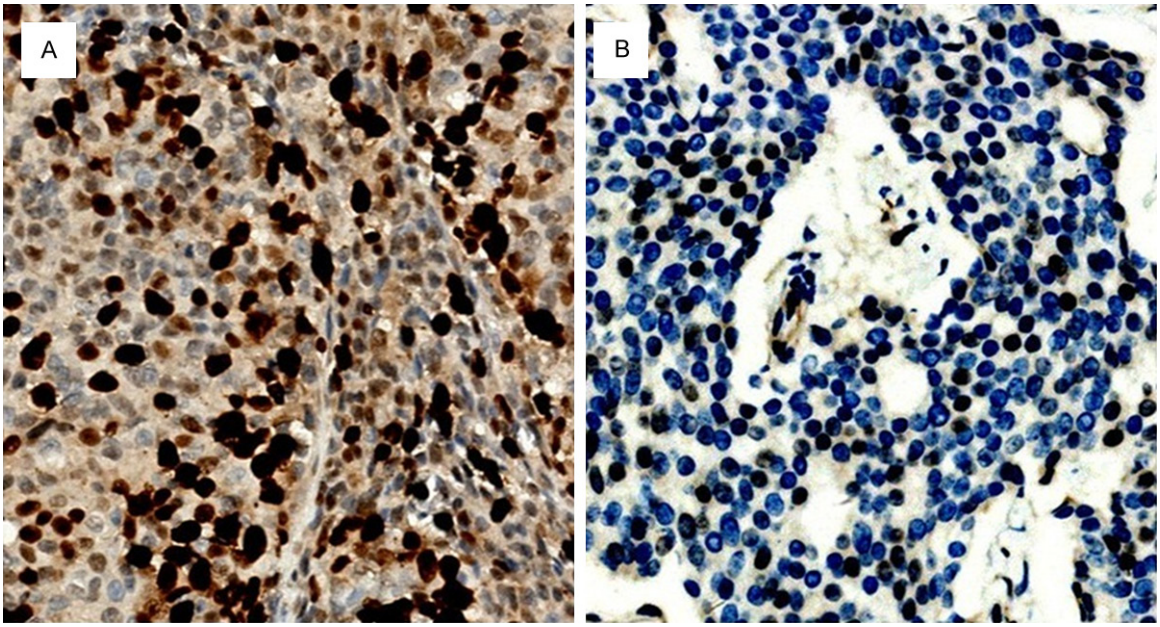

Figure 1. Immunohistochemical staining of the TOP2A protein in human breast carcinoma tissue. Positive expression (original magnification, ×200) (A) and negative expression (original magnification, ×200) (B).

histological subtypes according to the 7th American Joint of Cancer Committee (AJCC) system [15] without any knowledge of the patient clinical data. All patients signed informed consent forms before being enrolled in this study. Our study was approved by the Ethics Committee of Zhejiang Cancer Hospital.

Immunohistochemical staining

Expression of TOP2A, human epidermal growth factor receptor 2 (HER-2), estrogen receptor (ER) and progesterone receptor (PR) was detected by IHC performed using a standard avidin-biotin peroxidase technique (UltraView DAB Detection Kit, Ventana, Germany). Paraffin sections of 4-μm thickness were prepared and fixed in 10% formalin. The paraffin-embedded tumor tissues were incubated with primary antibodies, as follows: rabbit monoclonal anti-ER/PR antibodies, anti-Topoisomerase IIα (JS5B4) antibodies and anti-HER-2/neu antibodies. All primary antibodies applied at working concentration, were added for 34 min at room temperature using the VENTANA platform (Roche, Mannheim, Germany). All slides were washed before incubation with a secondary antibody, followed by detection using an UltraView DAB Detection Kit (Roche, Mannheim, Germany).

Evaluation of immunostaining

For TOP2A expression, the percentage score was recorded from 0 to 4 according to the extent of nuclear-stained tumor cells (0 ≤ 1%, 1 = 1-10%, 2 = 11-25%, 3 = 26-50% and 4 ≥ 51%. The intensity score was graded from 0 to 3 (0 = negative; 1 = weak staining; 2 = moderate staining; 3 = strong staining). Finally, TOP2A expression was scored semi-quantitatively according to the 0-3+ score (0 = 0+; 1~4 = 1+; 5~8 = 2+; 9~12 = 3+), which was calculated by multiplying the scores for positive cells and staining intensity. For statistical analysis, scores of ≥ 1+ and 0 were considered as positive and negative expression, respectively. HER-2 was scored from 0 to 3+ [5, 16]. In addition, ER and PR positive staining was defined as staining in >1% of tumor cells. Two experienced pathologists, blinded to the clinical information, evaluated the results independently.

Statistical and survival analyses

Correlations between TOP2A, ER, PR, HER-2, and clinicopathological characteristics were assessed using the X^2 testor Fisher's exact test (two-sided). The primary endpoints for this study were disease-free survival (DFS) and overall survival (OS). DFS was defined as the

Table 2. Univariate analysis of factors that influence disease-free survival and overall survival in 364 invasive breast cancer patients

Factors	Patients (n) in analysis	*P* value of DFS	*P* value of OS
Age			
< 55 years old	259	0.641	0.505
≥ 55 years old	105		
Pathological type			
Other types	37	0.790	0.213
Invasive ductal carcinoma	327		
Histological grade			
Grade 1	25	0.282	0.650
Grade 2	156		
Grade 3	23		
Venous/lymphatic invasion			
No	278	0.120	0.132
Yes	83		
Size of the primary tumor			
T1	162	**0.004**	**0.047**
T2-4	196		
Lymph node metastasis			
N0	194	**0.009**	**0.033**
N1-3	166		
Clinical stage			
I	107	**< 0.001**	**< 0.001**
II	178		
III	73		
Hormone therapy			
No	240	0.269	**0.019**
Yes	124		
Adjuvant radiotherapy			
No	212	0.359	0.937
Yes	152		
Adjuvant chemotherapy			
No	93	0.804	0.785
Yes	270		
ER expression			
Negative	131	0.187	0.214
Positive	233		
PR expression			
Negative	154	**0.037**	**0.004**
Positive	210		
HER expression			
Negative	277	0.543	0.279
Positive	87		
TOP2A expression			
Negative	72	0.839	**0.032**
Positive	292		

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; TOP2A, topoisomerase 2A; HER2, human epidermal growth factor receptor-2; PFS, disease-free survival; OS overall survival. Bold values indicate statistical significance (P < 0.05).

time from the date of surgery to the first documentation of relapse or death. OS was defined as the time from the date of surgery to death. Univariate analysis was performed using the Kaplan-Meier method and log-rank tests to evaluate prognostic differences between groups. Multivariate analysis using the Cox hazards model was implemented to identify factors of independent significance. For all analyses, a *P* value < 0.05 was considered significant by two-sided tests. All statistical analyses were performed using SPSS 18.0 software (IBM SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

Table 1 presents the main clinicopathological characteristics of the patients. The mean age of the patients was 50 years (ranging from 27 to 83 years). The last follow-up evaluation was December 30, 2015, with a median follow-up of 58 months (1.8-111.0 months). Thirty-seven patients (10.2%) developed tumor recurrence, and 16 of those patients (4.4%) died. There were 364 females enrolled in this study. Most of the patients were diagnosed with invasive ductal carcinoma. Among all patients, 29 (8.0%) had histological grade 1, 211 (58.0%) histological grade 2, and 71 (19.5%) histological grade 3. One hundred and sixty-two patients (44.5%) were at pathologic stage T1, 135 (45.9%) at pathologic stage T2, 21 (5.8%) at pathologic stage T3, and 8 (2.2%) at pathologic stage T4. In addition, 194 (53.3%) were at pathologic stage N0, 104 patients (28.6%) at pathologic stage N1, 34 (9.3%) at pathologic stage N2,

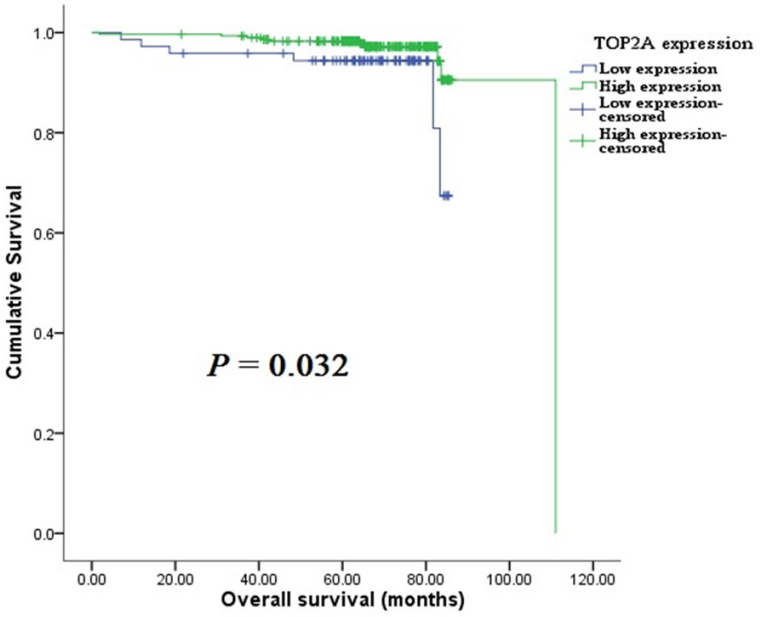

Figure 2. Kaplan-Meier plot for overall survival (OS) according to the level of TOP2A expression.

and 28 (7.7%) at pathologic stage N3. Moreover, 107 (7.5%), 178 (40.4%), and 73 (52.2%) patients were categorized as stages I, II, and III, respectively. Unfortunately, the TNM stage and histological grade were unavailable for some of the patients.

TOP2A expression and clinicopathological characteristics

Correlations between clinicopathological characteristics and TOP2A expression were investigated. A total of 292 patients were classified as overexpressing TOP2A (**Figure 1**), and TOP2A overexpression was related to tumor grade (G3 vs. G2 vs. G1; P = 0.004). However, there were no significant differences between the level of TOP2A expression and other clinicopathological features such as age, depth of invasion, lymph node metastasis, venous/lymphatic invasion, clinical stage, or adjuvant chemotherapy. Additionally, TOP2A expression showed no significant correlations with HER-2, ER, or PR expression. **Table 1** presents correlations between TOP2A expression and clinicopathological features. In total, 193 patients (66.7%) with high TOP2A expression received anthracycline-based chemotherapy; 53 patients (73.6%) with low TOP2A expression received anthracycline-based chemotherapy (P = 0.203). Most HER-2 positive patients refused trastuzumab targeted therapy due to economic reasons, with only 12 HER-2 positive patients receivingthis therapy.

Survival analysis

Using the log-rank test and Kaplan-Meier method, patients with high TOP2A expression had longer OS (P = 0.032) compared with patients with low TOP2A expression (**Table 2** and **Figure 2**). However, no significant difference was found for DFS according to TOP2A expression. Survival benefits for DFS and OS were also apparent in those with significant depth of invasion (P = 0.004 and 0.047, respectively), lymphatic metastasis (P = 0.009 and 0.033, respectively), clinical stages (both P < 0.001), and PR expression (P = 0.037 and 0.004) (**Table 2**). In addition, hormone therapy was correlated with breast cancer DFS (P = 0.032) (**Table 2**).

Cox analysis was performed toassess the impact of the variables on survival. The Cox proportional hazard model confirmed that TOP2A expression (HR = 0.19, 95% CI 0.06-0.69, P = 0.011), PR expression (HR = 0.13, 95% CI 0.03-0.61, P = 0.010), and adjuvant radiotherapy (HR = 0.23, 95% CI 0.06-0.99, P = 0.050) were independent prognostic factors of favorable OS in breast cancer. In contrast, clinical stage was an independent unfavorable factor for both DFS and OS (P < 0.001 and 0.004, respectively) (**Table 3**).

Discussion

TOP2A expression and its involvement inpromoting the initiation and progression of cancer havebeen widely studied. However, its value in the prognosis of operable invasive breast cancer patients remains under debate. A significant association between high TOP2A expression and poor prognosis [17, 18] as well as suggestions of an association between high TOP2A mRNA expression and poor prognosis [19-21] have been reported, where as other studies [22, 23], including the present study,

Table 3. Multivariate analysis of disease-free survival and overall survival in invasive breast cancer

Variables	Disease-free survival			Overall survival		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Clinical stage						
I	Reference		**0.004**	Reference		**< 0.001**
II	0.52	0.09-3.05	0.468	2.64	0.23-30.61	0.438
III	1.90	0.34-10.60	0.464	32.99	3.78-287.45	**0.002**
Hormone therapy						
No				Reference		
Yes				0.17	0.02-1.69	0.132
Size of the primary tumor						
T1	Reference					
T2-4	3.71	0.88-15.69	0.075			
Adjuvant radiotherapy						
No				Reference		
Yes				0.23	0.06-0.99	**0.050**
PR expression						
Negative	Reference			Reference		
Positive	0.07	0.51-0.25	0.065	0.13	0.03-0.61	**0.010**
TOP2A expression						
Negative				Reference		
Positive				0.19	0.05-0.69	**0.011**

Abbreviations: PR, progesterone receptor; TOP2A, topoisomerase 2A. Bold values indicate statistical significance (P < 0.05).

found opposite results. The main reasons for the discordant results are as follows: (1) small sample sizes; (2) differences in clinical stage and pathologic type; (3) differences in the methods used to assess TOP2A; (4) patientselection bias.

TOP2A may also prospectively serve as a predictive biomarker of beneficial effects of anthracycline treatment of breast cancer [22, 24]. The long-term follow-up results of the BCIRG0-06 Study [24] revealed that breast cancer patients with HER2 and TOP2A gene co-amplificationexhibit greater benefit from an anthracycline-based regimen than from trastuzumab treatment. However, the results for TOP2A gene status/protein expression detected by FISH, qPCR or IHC are inconsistent [21, 23]. TOP2A protein expression, rather than gene amplification, has been shown to account for a greater proportion ofthe beneficial properties of adjuvant anthracycline-based therapy in breast cancer [25, 26]. Thereare several explanations for this. One reason concerns variations in mRNA splicing that consequently generate different TOP2A protein isomers with varying activity and subcellular localization. Second, TOP2A protein expression is strongly influenced by multiple factors [27], including the cellular proliferative rateand protein phosphorylation. Overall, the TOP2A gene status cannot predict protein expression. Therefore, in this study, we examined TOP2A protein expression by IHC.

We explored the level of TOP2A expression using IHC and its associations with clinicopathological variables and survival. Previous studies [11, 14, 20, 26] have shown that TOP2A expression correlates with ER, HER-2, Ki-67 positivity rates and nodal status, which is inconsistent with our study. Notably, in this study, TOP2A protein overexpression was significantly associated with alower degree of differentiation.

Interestingly, TOP2A overexpression served as an independent favorable predictor for OS but not for DFS in our study.In addition, HER and ER expression failed to demonstrate statistically significant predictive ability in our survival analysis. The currentstudy, which to the best of our knowledge is the largest study to date, further confirmed that TOP2A protein expression determined by IHC is a favorable prognostic predictor for invasive breast cancer. The observed

prolongation of OS may be because patients with TOP2A protein overexpression experiencegreater benefit from anthracycline-based therapy.

In the NEAT/BR9601 trial, Bartlett *et al.* [22] investigated prospective predictive biomarkers for patients who would benefit from anthracycline. TOP2A amplification and deletion in 1762 breast patients were analyzedusing tissue microarrays. The results indicated that TOP2A amplification is a favorable prognostic marker, whereas TOP2A deletion is an adverse prognostic marker. TOP2A amplification was associated with increased TOP2A protein expression, but its deletion decreased protein expression in vitro [28]. Our results, as detected using a more convenient and economical assay, are highly consistent with those of Bartlett *et al.* [22]. However, opposite results were observed by Engstrom *et al*. [29], whereby TOP2A amplification patients experienced poor survival outcomescompared to TOP2A deletion patients.

TOP2A gene amplification occurs in more than 30% of tumors with concomitant HER2 gene amplification, even though deletions are also frequently detected [30]. A previous study [23] revealed a favorable prognostic capacity of HER2/TOP2A co-amplification in high-risk, early-stage breast cancerpatients treated with anthracycline-based adjuvant chemotherapy. However, we found no connection between HER2 and TOP2A protein expression. One possible explanation for this difference could be the inconsistency in TOP2A gene application and protein expression.

In summary, our study is the largest one to detect the prognostic utility of TOP2A in resectable invasive breast cancer based on IHC. TOP2A protein expression can serve as a significant prognostic factor in patients with invasive breast cancer. The main limitation in this study is that it wasa single-hospital, retrospective research study. Thus, further multicenter and prospective studies are necessary to validate the prognostic role of TOP2A. Moreover, alternative chemotherapeutic regimens and/or novel topoisomerase inhibitors need to be investigated.

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Disclosure of conflict of interest

None.

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