

Implications of Topoisomerase (TOP1 and TOP2 α) Expression in Patients With Breast Cancer

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Abstract. *Background/Aim:* We evaluated the usefulness of topoisomerases (TOPs) expression as prognostic predictors in breast cancer. *Patients and Methods:* We retrospectively investigated sixty cases with primary breast cancer. We evaluated the tumor and non-tumor mRNA levels of TOP1 and TOP2 α using quantitative reverse-transcription polymerase chain reaction. TOP1/TOP2 α positivity was defined as the ratio of the mRNA expression of cancer/normal tissue of >1 for both TOP1 and TOP2 α . *Results:* TOP1 and TOP2 α were markedly overexpressed in breast cancer tissues compared to normal breast tissues. Of the 60 cases, 46 (76.7%) were positive for TOP1/TOP2 α . The relapse-free survival was relatively shorter for patients with positive TOP1/TOP2 α . There was no recurrent disease among the 14 patients who were negative for TOP1/TOP2 α , whereas four of the 46 TOP1/TOP2 α -positive patients had disease recurrence. *Conclusion:* Negative TOP1 or TOP2 α expression may be useful for predicting better prognoses in breast cancer patients.

Breast cancer (BC) is the most common cancer in women and it is a leading cause of mortality worldwide (1, 2). BC is a heterogeneous group of tumors with large variations in

prognosis and sensitivity to treatments (3, 4). Identification of additional biomarkers is needed to stratify BC for individualized treatment. The topoisomerases (TOP) are able to change DNA topology to facilitate replication and transcription (5-7). There are two types of TOPs: topoisomerase I (TOP1) and topoisomerase II (TOP2), classified according to their ability to make transient single- or double-stranded breaks in DNA (5-8). TOPs play key roles in DNA function and are potential targets for cancer therapy. Several TOP inhibitors are used in clinical setting (8, 9). For example, TOP2 α (TOP2 α) is a molecular target of anthracycline, which is one of the key drugs in BC therapy (3, 10-15). Although TOP1 and TOP2 α have been reported to play key roles in tumor progression in various types of cancer (8), the clinicopathological features and significance of TOP1 and TOP2 α in BC have not been well elucidated. In this study, we investigated the correlations between the expression of TOP1 and TOP2 α and the clinicopathological features of cases with BC, and we evaluated the usefulness of their expression for the prognosis of BC, focusing on the differential expression between cancer tissue and non-cancerous tissue.

Patients and Methods

We retrospectively investigated sixty cases with primary BC who had undergone radical breast surgery. Breast cancer tissue and paired normal tissue were obtained at the Division of Breast and Endocrine Surgery, Gunma University between March 2009 and January 2012. Cases who received neoadjuvant chemotherapy, cases with synchronous bilateral breast cancer, male cases, and cases with incomplete clinical information were excluded. Written informed consent was obtained from all cases for the use of their records, samples and images in future studies, and this was approved by the Clinical Ethics Committee of Gunma University.

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Key Words: Topoisomerase I, topoisomerase II α , breast cancer.

The patient's age, histology, size of invasive primary tumor, lymphatic (ly) or vascular invasion (v), nuclear grade, estrogen receptor (ER) and progesterone receptor (PgR) expression status, human epidermal growth factor receptor 2 (HER2) score of the primary tumor, axillary lymph node status, and the use of adjuvant chemotherapy or hormone-therapy were extracted from the database. The ER and PgR statuses and the HER2 scores of the primary tumors were assessed as previously described (16). We evaluated the tumor and non-tumor mRNA levels of TOP1 and TOP2 α using quantitative reverse-transcription polymerase chain reaction (RT-qPCR) (17).

Analysis of mRNA expression. Immediately after breast surgery, breast cancer tissue and paired normal breast tissue were obtained for RNA extraction. RNA was isolated from the tumor tissue and non-tumorous tissue separately. cDNA was prepared using the High Capacity Reverse Transcription kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The TOP1 and TOP2 α expression levels were determined by TaqMan real-time PCR (TaqMan array card; Life Technologies) after TaqMan assay-based pre-amplification, as described (17). Beta-actin was used as an internal control for normalization. The gene expression levels are expressed as ratios (*i.e.*, the differences between the Ct values) between the target gene and the control gene.

The assay IDs used in this study are as follows. TOP1 (mRNA: TOPOI, assay ID: Hs0023259_m1, gene symbol: TOP1, gene name: DNA topoisomerase I) and TOP2 α (mRNA: TOPOII, assay ID: Hs003573333_g1, gene symbol: TOP2 α , gene name: DNA topoisomerase II alpha). We calculated the TOP1 and TOP2 α expression ratios of paired cancer tissue and non-cancerous tissue. Samples were TOP1/TOP2 α positive when the ratio of mRNA expression in cancer vs. normal tissue was >1 for both TOP1 and TOP2 α . The samples were negative for TOP1/TOP2 α when either TOP1 or TOP2 α was negative.

Statistical analysis. In order to examine whether the clinicopathological features associated with the TOP1/TOP2 α expression, we divided the BC cases into two groups based on the positive/negative TOP1/TOP2 α status. We performed a univariate statistical analysis using the χ^2 test with Yates' correction or Fisher's exact test. Student's *t*-test was used for comparisons of the two groups. For the correlation between TOP1/TOP2 α status and prognosis, Kaplan–Meier curves of disease-free survival (DFS) were drawn using the log-rank test. DFS was defined from the day of surgery to the day of initial locoregional and/or distant BC recurrence in the follow up term. A difference with a *p*-value<0.05 was considered significant.

Results

In total, 60 cases with paired cancer and normal tissue were included in the analysis. The expression levels of TOP1 and TOP2 α in cancer and non-cancerous tissues are shown in Figure 1. TOP1 and TOP2 α were both markedly overexpressed in the cancerous tissue compared to the non-cancerous tissue in cases with BC. The characteristics of the two patient groups based on positive/negative TOP1/TOP2 α status are summarized in Table I, which also provides the results of the univariate analysis conducted to investigate the

Table I. Clinicopathological features associated with relative expression of TOP1/TOP2 α .

	TOP1/TOPO2 α negative (n=14)	TOP1/TOP2 α positive (n=46)	<i>p</i> -Value
Age (y.o., median)	64 (30-87)	59 (37-87)	0.534
Histology (n)			0.623
Invasive ductal carcinoma	11	38	
Invasive lobular carcinoma	2	3	
Others	1	5	
Tumor size (\pm SD) (mm)	2.5 \pm 0.9	2.7 \pm 2.1	0.716
Lymph node metastasis (n)	4	21	0.206
ER positive (n)	12	37	0.498
PgR positive (n)	12	33	0.247
HER2 positive (n)	3	5	0.272
Nuclear grade 3 (n)	6	24	0.381
ly positive (n)	9	36	0.918
v positive (n)	1	13	0.096
Adjuvant chemotherapy	5	21	0.367
Adjuvant hormonotherapy	11	35	0.580

TOP: Topoisomerase; n: number; ER: estrogen receptor; PgR: progesterone receptor; HER2: human epidermal growth factor receptor 2; ly: lymphatic invasion; v: vascular invasion.

relationships between TOP1/TOP2 α expression and various clinicopathologic variables.

Of the 60 eligible patients, 46 (76.7%) had positive TOP1 and TOP2 α expressions; *i.e.*, both TOP1 and TOP2 α mRNA expression levels in cancer tissue were higher than those in normal tissue. There was no significant correlation between positive TOP1/TOP2 α status and any of the patients' clinicopathological variables. The Kaplan–Meier curves showed that the DFS was relatively shorter for the cases with positive TOP1/TOP2 α , but there was no significant difference (*p*=0.276) (Figure 2). The overall median follow-up period was 73.1 months (range=3.2-90.0 months). There was no recurrent disease among the 14 patients with negative TOP1/TOP2 α , whereas of the 46 patients with positive TOP1/TOP2, four patients had disease recurrence. It is interesting that the negative TOP1/TOP2 α patients had no recurrent disease although the differences were not significant in any clinicopathological features between positive and negative TOP1/TOP2 α cases.

Discussion

Topoisomerases (TOP1 and TOP2 α) are potential targets for cancer treatment. TOP1 and TOP2 α are expressed in various tumors, including breast cancer (6). Several studies have reported that TOP1 and TOP2 α are tumor drivers in various cancers (8). The key observations obtained in the present study were as follows: 1) TOP1/TOP2 α expression was not

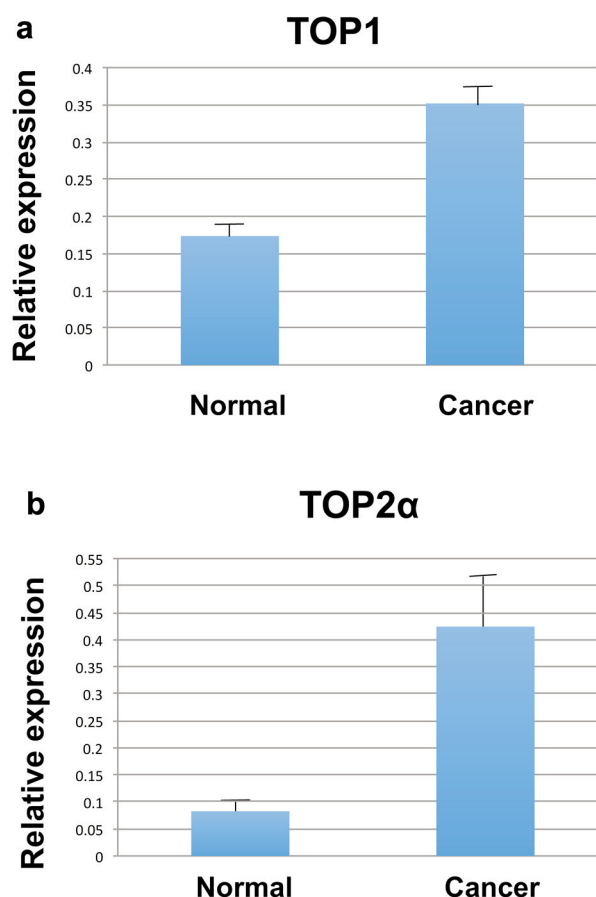


Figure 1. The expression levels of *TOP1* and *TOP2α* in cancer and non-cancerous tissue are shown. (a) *TOP1* and (b) *TOP2α* are markedly overexpressed in the cancer tissue compared to the non-cancerous tissue in cases with breast cancer.

associated with various clinicopathological characteristics or with the prediction of poor prognosis in breast cancer patients, and 2) the cases with negative *TOP1/TOP2α* had no recurrent disease. Our results suggest that *TOP1/TOP2α* expression may be an indicator of disease recurrence in cases with operable BC, and cases with negative *TOP1/TOP2* may have a lower risk of disease recurrence. In some types of cancers, elevated *TOP1* and *TOP2α* expressions were significantly associated with poor survival (7), which is consistent with our present findings.

Elevated expression of *TOP1* and *TOP2α* is used as a potent biomarker for the response to chemotherapy drugs in multiple cancers. In BC, *TOP2α* is well known as a biomarker of anthracyclines treatment, which are some of the most widely used chemotherapeutic agents for BC. However, the evidence of a prognostic role of *TOP2α* in BC is controversial (14, 15), as is that for *TOP1* (18, 19). The topology of DNA is regulated by both *TOP1* and *TOP2α*.

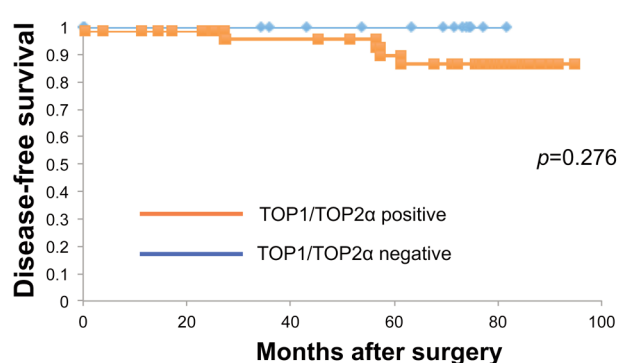


Figure 2. The disease-free survival (DFS) shown by the Kaplan-Meier curves is relatively but not significantly shorter for the cases with positive *TOP1/TOP2α* ($p=0.276$). Among the negative *TOP1/TOP2α* cases, there was no recurrent disease. The overall median follow-up period was 73.1 months (range=3.2-90.0 months).

We, thus, assessed the value of the combination of *TOP1* and *TOP2α* in this study, and our analyses revealed that there was no recurrence of disease when either *TOP1* or *TOP2α* expression was negative. Therefore, an elevated expression of either *TOP1* or *TOP2α* may not reflect an increased risk for recurrent disease in cases with BC.

The DNA topoisomerases *TOP1* and *TOP2α* regulate the topology of DNA in cancer tissue and normal tissue (5). Herein, we focused on the differential expressions between tumor tissue and non-tumor tissue. The gene expression ratio of cancerous tissue to non-cancerous tissue from the same case provides more information and reduces the effects of individuality (20). Gene expressions in cancer tissue alone may be insufficient for indicating clinicopathological features. In fact, several comparisons of gene expression profiling between cancer tissue and paired normal tissue have suggested that the gene expression ratio of cancer and normal tissue predicts clinical outcome more efficiently (20-22).

This study has some potential limitations, the major ones is that it is a retrospective analysis with a relatively small number of cases. However, to the best of our knowledge, this is the first report to describe *TOP1* and *TOP2α* expression focused on the ratios of tumor vs. non-tumorous tissue in cases with BC. The expression rate may provide more information even with small numbers of patients. The negative *TOP1/TOP2α* status in breast cancer patients may be predictor of a lower risk of disease recurrence. Additional research is needed to explore possible benefits of determining the *TOP1* and *TOP2α* expression of primary BC.

In conclusion, the results of our present analyses demonstrated that negative *TOP1* or *TOP2α* expression in primary BC may be effective for predicting better prognoses among cases with BC, and this finding could be useful in identifying the subset of cases with negative *TOP1/TOP2α*.

expression for the purpose of improving prognostic accuracy. Large studies are warranted to further evaluate the relationship between the combination of these two factors and BC recurrence.

Conflicts of Interest

The Authors declare that they have no competing interests in relation to this study.

Authors' Contributions

MO and TF analyzed data and TF wrote the initial draft of the manuscript. YN, TH, YK, TO and JH collected data and were involved in the initial study conception and design. TF and KS interpreted the results and were involved in drafting the work and revising it critically for important intellectual content. TF approved the final version to be published. All Authors have read and approved the final manuscript.

Acknowledgements

The Authors would like to thank Ms. Fumie Takada and Ms. Harumi Kanai for their secretarial assistance. FT and HK belong to Department of General Surgical Science, Gunma University, Maebashi, Japan.

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Received July 7, 2020

Revised July 28, 2020

Accepted July 29, 2020