

Tumor- and stromal cell-specific expression of topoisomerase II α and HER-2/neu in primary and recurrent ovarian cancer: Results of a prospective study

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Abstract. The aim of the present study was to evaluate the expression of topoisomerase II α (TOP2A) and HER-2 in tumor epithelial and adjacent stromal cells in ovarian cancer (OvCa). Immunohistochemistry for TOP2A and HER-2 was performed on 50 OvCa specimens from the Tumor Bank of Ovarian Cancer, and was correlated to established clinicopathological parameters. TOP2A was expressed in 98% and HER-2 in 52% of the OvCa specimens. Moderate expression of TOP2A was detected in 72% of the adjacent stromal cells. TOP2A and HER-2 were strongly expressed in the tumor epithelial cells of primary OvCa, but reduced in recurrent OvCa. Stromal expression of TOP2A increased in recurrent OvCa after platinum-based chemotherapy. In conclusion, distinct epithelial and stromal cell expression of TOP2A and HER-2 is a novel feature in the tumor biology of OvCa. Differential cellular expression of TOP2A in relation to previous chemotherapy probably reflects a modified activity of the 'stromal compartment' in drug resistance. Thus, analysis of TOP2A expression in tumor and stromal OvCa cells can aid in iden-

tifying subgroups of patients who may have a more favorable response to chemotherapy.

Introduction

Ovarian cancer (OvCa) has the highest mortality rate of all female gynaecological cancers, with an overall 5-year survival rate of approximately 30% (1,2). At the time of primary diagnosis, most patients have advanced stage disease (FIGO III/IV) (3,4). Cytoreductive surgery is known to have a strong prognostic effect on survival (4-6). Despite the initial high response rates of current standard chemotherapy with carboplatin and paclitaxel, most patients relapse and soon succumb to the disease due to tumor progression (1). Unfortunately, the complex mechanisms of platinum resistance are still poorly understood (7). Thus, the confirmation of novel molecular factors is a promising strategy that may help to individualize chemotherapy for OvCa.

Topoisomerase II α (TOP2A) is an important proliferation marker in solid cancer (8,9), with significant expression during the S and G2-M phases (9,10). TOP2A is also the cellular target of anticancer drugs such as etoposide and doxorubicin (11), and has been implicated in contributing to atypical multidrug resistance in OvCa (12,13). Nonetheless, it does not appear to predict resistance as strongly as other factors. Only a few studies have evaluated TOP2A expression in OvCa cells, most describing increased amplification in advanced tumor stages associated with poor clinical outcome (8).

The HER-2 proto-oncogene is essential for tumor growth and proliferation, with a wide range of amplification in solid tumors (14-16). Different ligands can bind through its extracellular segments, and can mediate intracellular signal transduction through tyrosine kinase activity. HER-2 is also a target for novel therapeutic approaches, such as trastuzumab, a recombinant antibody designed to block signaling through the HER-2 receptor. HER2 expression in OvCa shows rates of 1.8-76%, but data related to its prognostic value are controversial (16-18).

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Abbreviations: FIGO, Federation of Obstetrics and Gynecology; IRS, immunoreactive score, TOP2A, topoisomerase II α , OvCa, ovarian cancer

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The TOP2A gene is located adjacent to the HER-2 gene on chromosome 17q, and is either amplified or deleted in a great majority of HER-2-amplified primary tumors (6,19). Recent trials suggest that amplification (and/or deletion) of TOP2A may lead to sensitivity or resistance to clinically used TOP2A inhibitors (14). Nevertheless, data regarding the expression of TOP2A and HER-2 in OvCa are limited.

The aim of this prospective study was to analyze the expression of TOP2A and HER-2/neu in malignant epithelial cells and adjacent stromal cells in primary and recurrent OvCa.

Materials and methods

Tissue specimens. OvCa samples were obtained intra-operatively from 50 patients (24 with primary and 26 with relapsed cancers) at the Department of Gynecology, Charité University of Berlin, Germany. Specimens were collected and documented according to the surgical and pathological standard operating procedures of the Tumor Bank Ovarian Cancer using a systematic intraoperative documentation tool (3). The control group included 4 cystadenomas and 7 normal ovaries from 11 women who underwent surgery for benign disease (4 cystadenomas, 3 leiomyomas and 4 benign ovarian cysts). After excision, the tissues were transferred to 4% formalin and paraffin embedded. All samples underwent standard histological examination by an experienced pathologist (I.K.). The Ethics Committee of the Charité University Hospital approved the study protocol. Pre-operative informed consent was obtained from each patient.

Immunohistochemistry. Immunohistochemistry was performed on paraffin sections using the standard avidin-biotin complex method. Endogenous peroxidase activity was quenched with a hydrogen peroxide solution. Non-specific binding sites were blocked with CAS solution (Zymed Laboratories, CA). Primary antibodies included Ki-S1 for TOP2A (IgG2a, 1:80) and A0485 for HER-2 (IgG1, 1:200) (Dako Cytomation, Glostrup, Denmark). Biotinylated secondary antibody, the tertiary avidin-biotin complex and the AEC Substrate Kit from Biocarta (Hamburg, Germany) were used for detection. For each sample, a negative control was performed. All histological features were evaluated separately by two investigators (R.C. and I.K.).

For the evaluation of TOP2A, the semiquantitative immunoreactive score (IRS) was applied. In each case, a minimum of 1000 cells were counted in five to ten high-power fields (x400) to determine dye intensity and the percentage of positive cells. Mean dye intensity was assessed as follows: 0, negative; 1, low; 2, moderate; and 3, strong intensity. The percentages of stained cells were classified as follows: 0%, negative; 1, <0%; 2, 10-50%; 3, 51-80%; and 4, >80% positively stained cells. An IRS score of 0-2 was considered negative, whereas moderate (IRS 3-6), or strong (IRS 7-12) immunostaining indicated TOP2A overexpression.

HER-2 immunostaining was evaluated following the Dako HercepTest™ protocol: score 0, no staining or membrane staining of <10% of the cells; score 1+, partial staining in >10% of the cells; score 2+, moderate or 3+, strong membrane staining in >10% of the cells. A score of 0 and 1+ was considered negative, and a score of 2+ and 3+, positive.

Table I. Clinicopathological characteristics of the patients.

Parameter	Primary cancer	Recurrent cancer
Ovarian cancer samples	24 (48.0%)	26 (52.0%)
Histology		
Serous-papillary	14 (58.3%)	23 (88.5%)
Endometrioid	5 (20.8%)	2 (7.7%)
Mucinous	2 (8.4%)	1 (3.8%)
Serous-mucinous	3 (12.5%)	0
FIGO stage		
I	5 (20.8%)	2 (7.7%)
II	1 (4.2%)	0
III	8 (33.3%)	18 (69.2%)
IV	10 (41.7%)	6 (23.1%)
Histological grade		
I	2 (8.3%)	6 (23.1%)
II	10 (41.7%)	8 (30.8%)
III	12 (50.0%)	12 (46.1%)
Ascites		
≤500 ml	13 (54.2%)	21 (80.8%)
>500 ml	11 (45.8%)	5 (19.2%)
Postoperative residual tumor mass		
Macroscopically tumor-free	15 (62.5%)	9 (34.6%)
Tumor residuals	9 (37.5%)	17 (65.4%)
FIGO, Federation of Obstetrics and Gynecology.		

Statistical analysis. For statistical analysis, the χ^2 test was used to compare immunostaining with clinicopathological characteristics. Evaluations were performed using SPSS 15.0 statistical software (SPSS Inc., Chicago, IL). For survival analysis, Kaplan-Meier analysis and Cox regression were applied. A p-value <0.05 was considered to indicate statistical significance.

Results

Patient and tumor characteristics. The mean age of the patients was 54.0 years (range 26-86). Most patients presented advanced FIGO stage III (n=26) and IV (n=16) disease, with predominantly serous histology (n=37). At the time of surgery, ≤500 ml malignant ascites were found in 34 patients, while 16 patients had >500 ml ascites. In 24 women, complete tumor resection (macroscopically tumor-free) was achieved. The main clinicopathological characteristics of the patients are summarized in Table I.

Twenty-four of the 50 women with primary disease had never been treated with chemotherapy prior to surgery (platinum naïve), whereas 26 with recurrent OvCa had been previously treated with platinum-based chemotherapy (platinum/paclitaxel, n=24; platinum/cyclophosphamide, n=2). Thirteen women underwent surgery after the first relapse, 10 after the second and 3 after the third.

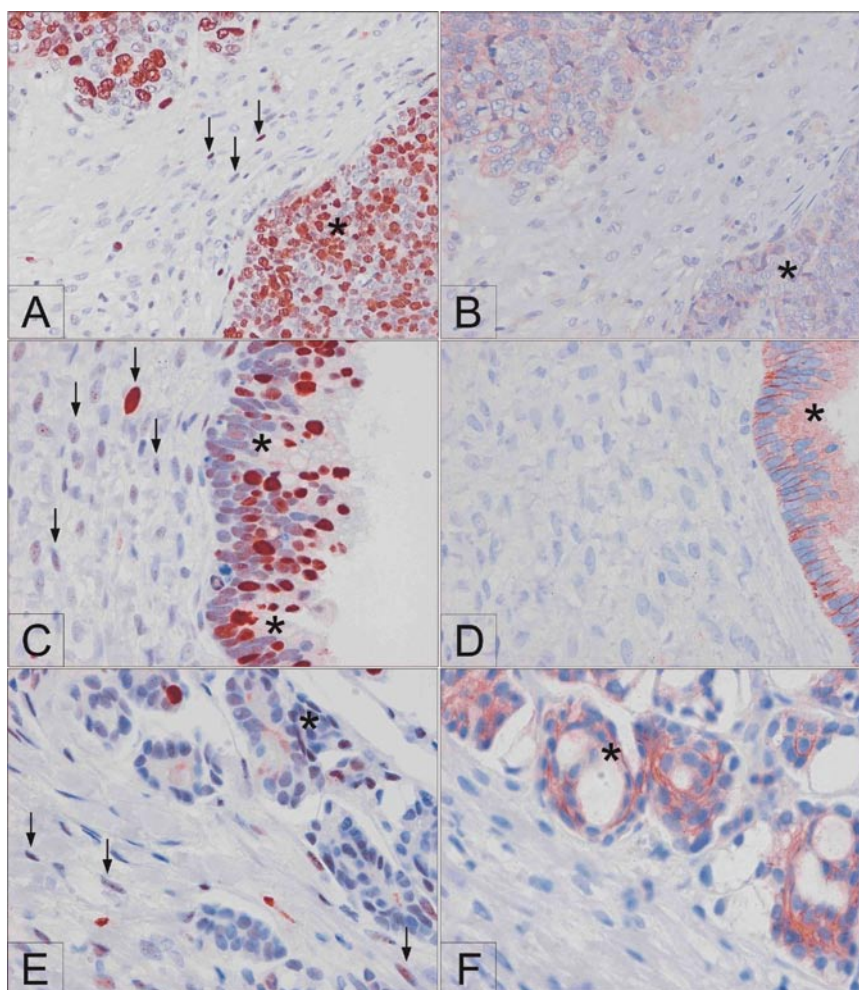


Figure 1. Immunohistochemical labeling of TOP2A protein expression (A, C and E) and HER-2 activity (B, D and F) in primary endometrioid ovarian cancer (A and B) (x200), primary mucinous ovarian cancer (C and D) (x400) and recurrent serous ovarian cancer (E and F) (x400). Expression of TOP2A is nuclear, and that of HER-2 is on the membrane of tumor cells (asterisks). Significant immunostaining in the adjacent stromal cells evaluated for TOP2A is barely detectable (arrows). HER-2 exhibits no activity in stromal fibroblasts (B, D and F).

Immunohistochemistry. Positive TOP2A immunostaining (IRS ≥ 3) was observed in tumor cells (primary and recurrent) in 49 of the 50 patients; 66% (n=33) were strongly and 32% (n=16) were moderately positive (Figs. 1 and 2). In adjacent stromal cells of primary and recurrent OvCa, TOP2A expression was observed to be strong in 12% (n=6), moderate in 60% (n=30) and negative in 28% (n=14) of the specimens (Fig. 2). Antibody staining was usually nuclear. Thus, in only one patient with serous recurrent OvCa were both epithelium and stromal cells negative for TOP2A.

Expression of HER-2 was detected in the tumor epithelium of primary and recurrent OvCa as membrane staining. A strong positive score (3+) was determined in 9 patients (18%) and a moderate positive score (2+) in 17 patients (34%) (Fig. 2). Negative HER-2 immunostaining (0 or 1+) was observed in 24 patients (48%). No HER-2 membrane staining was observed in the stromal cells of any of the 50 OvCa samples.

Normal ovarian tissues had negative (85.7%) or low (14.3%) expression of TOP2A in the epithelial cells, and all adjacent stromal cells were negative (Table II). TOP2A expression was slightly stronger in the epithelial cells of cystadenomas: 25% had strong, 50% moderate and 25% negative expression, while most stromal cells (75%) were negative.

In all 50 adenocarcinomas, TOP2A was significantly overexpressed in 98% of the tumor epithelial cells (median IRS=9, range 2-12) ($p<0.01$) and moderately expressed in 72% of the adjacent stroma (median IRS=4, range 0-12) ($p<0.05$) (Fig. 2). HER-2 expression was also stronger in the tumor epithelium compared to weak activity in cystadenomas and negative expression in normal epithelial cells ($p<0.05$). In 52% of the tumor cells, the median positive HER-2 score was 2+ (range 0-3+), whereas 48% of the cells were negative (Fig. 2). Tumor epithelium expressed TOP2A more strongly than it did HER-2 (98 vs. 52%, $p<0.05$, Fig. 2).

The expression of TOP2A and HER-2 in the tumor epithelial and adjacent stromal cells was further analyzed according to pre-treatment status (Table II). Tumor cells of primary OvCa exhibited mostly moderate (25%) or strong (75%) positive expression of TOP2A. In the epithelial cells of primary OvCa, weak to strong membrane expression of HER-2 was detected, with a score of 2+ (41.7%) or 3+ (20.8%), whereas 37.5% of cells were negative (Table II; Fig. 1). Moreover, in primary cancers, a significant number of adjacent stromal cells (50%) clearly demonstrated moderate expression, while in several specimens (12.5%) strong TOP2A expression was also observed in the nuclei (Table II; Fig. 1A and C). Negative stromal TOP2A

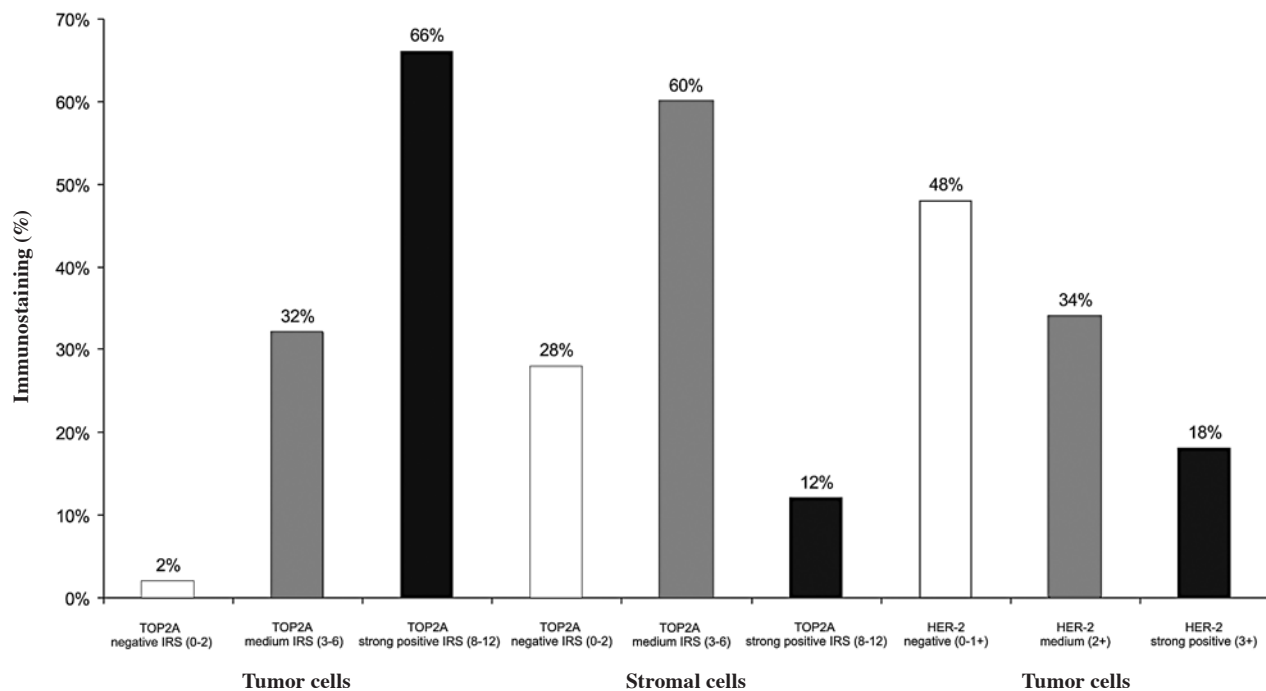


Figure 2. Distinct expression of TOP2A and HER-2 in tumor epithelium and stromal cells. TOP2A was negative (IRS score 0-2) in 2% of the tumor cells and 28% of the adjacent stromal cells, while HER-2 was negative (0-1+) in 48% of the tumor cells (white bars). TOP2A was moderately expressed (IRS score 3-6) in 32% of the tumor cells and 60% of the stromal cells, while HER-2 was moderately expressed in 34% of the tumor cells (grey bars). TOP2A was strongly expressed (IRS score 8-12) in 66% of the tumor cells and only 12% of the adjacent stromal cells, while HER-2 was strongly expressed (3+) in 18% of the tumor cells (black bars).

Table II. Tumor and stromal cell expression of TOP2A and HER-2 and correlation with chemotherapy status (platinum pre-treatment).

Target	Cell type	Tissue	Expression			p-value
			Negative	Moderate	Strong	
TOP2A (IRS score)	Epithelium	Normal ovary	6 (85.7%)	1 (14.3%)	0	p<0.01
		Benign cystadenoma	1 (25.0%)	2 (50.0%)	1 (25.0%)	
		Primary cancer (platinum naïve)	0	6 (25.0%)	18 (75.0%)	
		Recurrent cancer (platinum pre-treated)	1 (3.8%)	10 (38.5%)	15 (57.7%)	
	Stroma	Normal ovary	7 (100.0%)	0	0	p<0.05
		Benign cystadenoma	3 (75.0%)	1 (25.0%)	0	
		Primary cancer (platinum naïve)	9 (37.5%)	12 (50.0%)	3 (12.5%)	
		Recurrent cancer (platinum pre-treated)	5 (19.2%)	18 (69.2%)	3 (11.6%)	
HER-2 (Hercep score)	Epithelium	Normal ovary	7 (100.0%)	0	0	p<0.05
		Benign cystadenoma	4 (100.0%)	0	0	
		Primary cancer (platinum naïve)	9 (37.5%)	10 (41.7%)	5 (20.8%)	
		Recurrent cancer (platinum pre-treated)	15 (57.7%)	7 (26.9%)	4 (15.4%)	

IRS score: 0-2, negative; 3-6, moderate; 8-12, strong expression. Hercep test score: 0-1+, negative; 2+, moderate; 3+, strong expression.

immunostaining was observed in one-third of these cases (37.5%). In women with recurrent OvCa, tumor epithelial cells presented moderate (38.5%) to strong (57.7%) expression of TOP2A, whereas 3.8% of cells were negative. In this group, the tumor cells were predominantly HER-2 negative (57.7%), while 26.9% had moderate and 15.4% strong expression (Table II;

Fig. 1). Thus, in recurrent OvCa, immunostaining showed a significant decrease in overall TOP2A and HER-2 expression in the tumor cells ($p<0.01$ and <0.05 , respectively). Notably, TOP2A expression in the stromal cells of recurrent OvCa increased, with 19.2% showing negative, 69.2% moderate and 11.6% strong immunostaining ($p<0.05$) (Table II; Fig. 1E).

Table III. Correlation between simultaneous TOP2A and HER-2 expression in tumor and stromal cells.^a

Chemotherapy status (platinum pre-treatment)	TOP2A and HER-2/neu expression			Percentage
	TOP2A epithelial cells	TOP2A stromal cells	HER-2 epithelial cells	
Primary cancer (platinum naïve)	+	+	+	8 (33.3%)
	+	-	+	7 (29.2%)
	+	+	-	5 (20.8%)
	+	-	-	4 (16.7 %)
	-	-	-	0
Recurrent cancer (platinum pre-treated)	+	+	+	10 (38.5%)
	+	-	+	1 (3.8%)
	+	+	-	11 (42.3%)
	+	-	-	3 (11.6%)
	-	-	-	1 (3.8%)

^aTumor cells presenting expression of both targets in 15 samples (62.5 %) of primary and 21 samples (42.3%) of relapsed ovarian cancers ($p<0.05$). Simultaneous tumor and stromal TOP2A expression was observed in 54.1% of primary cancers compared to 80.8% of recurrent disease. +/-, positive IRS score (≥ 3)/negative IRS score (0-2). +/-, positive Hercep score (≥ 2 +)/negative Hercep score (0-1).

Correlation between TOP2A and HER-2 expression. A direct correlation was observed between HER-2 and TOP2A immunostaining in all tumor epithelial cells ($p<0.05$); TOP2A had a median IRS of 9 (range 2-12) and HER-2 a median score of 2+ (range 0-3+).

Primary OvCa had simultaneous TOP2A and HER-2 expression in 62.5% of the tumor cells. Eight samples (33.3%) also showed simultaneous TOP2A stromal activity (Table III). In 14 of 24 primary OvCa patients (54.1%), positive TOP2A activity was observed in both the tumor and stromal cells. In recurrent OvCa, only 42.3% of the patients presented simultaneous TOP2A and HER-2 activity in the tumor epithelial cells, while 38.5% showed additional TOP2A stromal activity. Increased simultaneous TOP2A tumor and stromal cell expression was detected in 21 of the 26 recurrent patients (80.8%). Only one recurrent adenocarcinoma was negative for both targets in tumor and stromal cells.

Correlation between classical clinical parameters and survival analysis. After a median follow-up of 22 months (0-74 months), 18 patients (36%) (11 with primary and 7 with recurrent cancer) remained alive. Thirty-two patients (64%) (13 with primary, 19 with relapsed cancer) succumbed to the disease due to tumor-specific causes during the observation period.

In the survival analysis, patients with primary disease who underwent optimal tumor resection (14 tumor-free patients vs. 11 patients with residual tumor, $p=0.002$) or who presented initially low amounts of ascites (13 cases ≤ 500 ml vs. 11 cases >500 ml ascites, $p=0.002$) achieved an improved overall survival. For patients with recurrent disease (13 with first, 10 with second and 3 with third relapse), no survival benefit was ascertained according to the tumor residual mass (8 tumor-free patients vs. 16 patients with residual tumor, n.s.) or ascite volume (19 cases ≤ 500 ml vs. 5 cases >500 ml ascites, n.s.). TOP2A tumor and stromal expression had no influence on overall survival.

Discussion

In this prospective study, we identified a distinct cellular expression for TOP2A and HER-2 in 50 primary and recurrent ovarian adenocarcinomas. Our results are in agreement with previous immunohistochemical studies, which found that TOP2A (8,20) and HER-2 (16,21) presented increased activity in OvCa compared to benign and normal ovaries. Notably, we evaluated specific activity for TOP2A in both the tumor epithelium as well as in stromal cells in the majority of the analyzed samples. TOP2A immunostaining was much more abundant in the tumor epithelium than in the adjacent stroma (9,22). TOP2A amplification was correlated with HER-2 activity in the tumor epithelial cells. It is known that TOP2A appears to be a sensitive marker in OvCa, and its activity is commonly associated with HER2 expression (13,14, 23).

Although the complex heterogeneity of OvCa tissue is well known, no studies have separately confirmed target expression in the tumor epithelium and adjacent stromal cells. Moreover, most studies have evaluated only the tumor compartment, whereas data regarding target expression in the stromal cells of OvCa are limited (22). Our results demonstrate clear expression of TOP2A at the protein level in both cell types.

The development of platinum resistance is a major clinical problem encountered in OvCa chemotherapy (7,12). A number of studies discuss the potential role of TOP2A as a predictor of response to chemotherapy (13,14,20, 24, 25). Although limited to a small patient group, we found stronger expression of TOP2A and HER-2 in the tumor cells of chemotherapy-naïve patients than in patients previously treated with platinum. Interestingly, inverse patterns were found in the adjacent stromal cells of a few patients; TOP2A expression in relapsed samples after platinum pre-treatment was higher than TOP2A expression in the stroma of primary disease patients. These results indicate for the first time the complex aspects of TOP2A expression in epithelial and stromal OvCa cells with respect to previous

treatment. Additional multicenter trials with a large number of patients are needed to support these findings.

Tumor invasion is thought to be highly dependent on dynamic interactions with the surrounding extracellular matrix and neighboring cells (26-28). Stromal changes and tissue remodeling are well-known factors that induce the invasion of epithelial cancers (22,27). Here, stromal fibroblasts play a crucial role (29,30, 31). In this context, the expression of TOP2A in adjacent stromal cells after platinum-based chemotherapy might also reflect some anatomic features of the excised tissue site (i.e., native stroma to the anatomic location). Evidence suggests that invasive epithelial tumor cells can activate so-called 'carcinoma-associated fibroblasts' as part of the desmoplastic reaction, which in turn can promote the growth and invasion of tumor cells (32-35). In OvCa, stromal activation has been implicated in a hypothesis supporting an OvCa-prone pre-neoplastic phenotype (36).

The stratification of subgroups of patients who may benefit from specific targeted therapies is a powerful clinical strategy (6,37). At present, approved agents such as topotecan and doxorubicine constitute an important element of therapy for recurrent OvCa (11,14,38). Nevertheless, only a few patients achieve long-term stabilization, and most responses are temporary. Although our understanding of tumor biology is expanding, the clinical benefits achieved have been disappointing, and there are no available tumor-associated factors that can predict the cohort of patients who may benefit from specific therapy regimes in this patient setting (18,24). In this context, the analysis of target expression in adjacent stromal cells of relapsed OvCa adds to our understanding of drug resistance and warrants further study in a multicenter setting.

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