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Altered Expression Pattern of Topoisomerase IIα in Ovarian Tumor Epithelial and Stromal Cells after Platinum-Based Chemotherapy[1]

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Abstract

OBJECTIVE: **The aim of this study was to evaluate the expression of topoisomerase IIα (*TOP2A*) in epithelial and stromal cells of ovarian cancer. *METHODS:* TOP2A expression was analyzed in normal ovarian tissue and in laser-microdissected ovarian tumor epithelial and adjacent stromal cells using quantitative real time RT-PCR (*n* = 38), RNA *in situ* hybridization (*n* = 13), and immunhistochemistry (*n* = 69). *Results: TOP2A* mRNA was detected by RNA *in situ* hybridization in all ovarian cancer samples, with stronger hybridization signals in tumor epithelial cells compared to adjacent stromal cells. The same expression pattern was found by immunohistochemistry (*P* = .0001). Very interestingly, specific changes of TOP2A were found in recurrent ovarian cancer after platinum-based chemotherapy: TOP2A expression decreased in tumor epithelial cells (*P* = .056) of recurrent ovarian cancer, whereas it increased in tumor adjacent stromal cells (*P* = .023) compared to primary ovarian cancer. *CONCLUSION: TOP2A* mRNA and protein expressions in ovarian cancer exhibit specific patterns in tumor epithelial and adjacent stromal cells, which are differentially modulated after platinum-based chemotherapy. These data support the possible importance of the stromal compartment in tumor progression and suggest that tumor stromal cells might be relevant to the development of chemotherapy resistance in ovarian cancer.**

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Keywords: *TOP2A*, ovarian cancer, RNA *in situ* hybridization, tumor stromal cells, chemotherapy resistance.

Introduction

Ovarian cancer is one of the most common malignancies in women and is the leading cause of death from all malignancies of the female genital tract in the western world [1]. The standard treatment is radical tumor-reductive surgery followed by platinum-based combination chemotherapy [2]. Although complete response is seen in 40% to 60% of cases, the majority of patients relapse and develop chemotherapy resistance, limiting the success of treatment [2]. Various drugs have demonstrated moderate efficacy under these circumstances, with response rates ranging from 14% to 34% [3]. However, remission observed with single-agent schedules is usually partial and has short duration, and this is especially true in cases of platinum-refractory tumors [3]. Better understanding of the mechanisms of chemotherapy resistance could potentially improve the management of recurrent ovarian cancer.

DNA topoisomerases are nuclear enzymes that are essential for DNA replication, RNA transcription, chromosomal condensation, and mitotic chromatid separation [4–7]. Type II topoisomerases are cellular targets of clinically active anticancer drugs, such as etoposide and doxorubicin, in the management of recurrent ovarian cancer [8–10]. Topoisomerase-targeted agents stabilize a transient covalent enzyme–DNA complex, which produces DNA strand cleavage and apoptosis [11]. It is known that alterations of topoisomerase enzymatic activity result in atypical multidrug resistance [12,13]. There are two different isoforms of topoisomerase II, namely topoisomerase IIα (TOP2A) and topoisomerase IIβ, which have distinct biochemical, pharmacological, and physiological properties [14].

To date, some studies have focused on TOP2A. Unlike topoisomerases I and IIβ, which are expressed constitutively throughout the cell cycle, *TOP2A* expression occurs only in the S and G_2–M phases [15]. The *TOP2A* gene encoding *TOP2A* is located on chromosome 17q21–22, and it is often coamplified in various tumor entities, including ovarian cancer, with the *HER2* oncogene, which is also located in 17q21.1 [16–22]. Furthermore, experimental data suggest that the tumor-suppressor gene *p53*, which is located on chromosome 17p13.1, regulates the minimal promoter of *TOP2A*, making *TOP2A* one of the downstream targets for *p53*-dependent regulation of cell cycle progression in human cells [23]. *TOP2A* has been previously

Abbreviations: *TOP2A*, topoisomerase IIα; RT-PCR, reverse transcription–polymerase chain reaction; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase

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implicated in contributing to drug resistance in ovarian cancer [21,22,24–27], but its expression does not seem to predict drug resistance as strong as other factors do [28].

In the present study, *TOP2A* expression has been carefully analyzed in stromal and tumor epithelial cells of primary and recurrent ovarian cancers—the latter after platinum-based chemotherapy. To overcome the problem of histologic heterogeneity of ovarian malignancies, due to variable amounts of stromal cells, tumor epithelial cells, and large areas of necrosis [29], we applied laser-supported microdissection to obtain pure subpopulations of tumor epithelial and stromal cells. Our data demonstrate a change in the *TOP2A* expression of tumor epithelial and adjacent stromal cells in recurrent ovarian cancer after platinum-based chemotherapy, which may be important for the development of chemoresistance in ovarian cancer.

Materials and Methods

Tissue Specimens

Ovarian cancer samples were obtained from 62 patients after surgical treatment at the Department of Gynecology of the Charité University Hospital (Tumor Bank Ovarian Cancer, Berlin, Germany). Thirty-four of 62 patients had primary tumors and had never been treated with chemotherapy prior to surgery, whereas the remaining 28 had recurrent disease and had been previously treated with platinum-based chemotherapy (platinum/paclitaxel, $n=24$; platinum/cyclophosphamide, $n=4$). After excision, one part of the tissue sample was immediately stored in liquid nitrogen, whereas a second part was transferred into 4% formalin and embedded in paraffin. All samples were histologically examined on hematoxylin–eosin sections by a pathologist. Patient details, including tumor histology, grade and stage, ascites, and residual tumor, are summarized in Table 1. The International Federation of Obstetrics and Gynecology (FIGO) classification was used for clinical staging, and Gynecologic Oncology Group criteria were used for histologic grading [30]. The Ethics Committee of the Charité University Hospital approved the study protocol. Preoperative consent was obtained from each patient using written basic information. Borderline ovarian tumors were excluded from the study because they represent a distinct biologic entity [31].

Laser Microdissection and RNA Extraction

To isolate pure cell subpopulations from tumor specimens, laser-captured microdissection was used following standard protocol [32,33] with some modifications. Serial 7-μm frozen tissue sections were cut in a cryostat and mounted on a sterile 2.5-μm membrane, fixed immediately in 70% ethanol, counterstained with hematoxylin–eosin, and air-dried. The membrane was turned upside-down and fixed with adhesive tape on a second sterile slide. The first slide served as a template, on which the areas of tumor were marked. Areas of interest were selected under microscopy, aided by visual control software [Molecular Machines and Industries (MMI), Glattbrugg, Switzerland]. On consecutive sections, tumor epithelial cells and adjacent stromal cells were microdissected using a UV laser microdissection system (MMI) and capture transfer films (Arcturus GmbH, Moerfelden-Walldorf, Germany). Microdissected groups of cells were pooled and collected in ice-cold tubes, containing 50 μl of guanidine isothiocyanate buffer with 2% β-mercaptoethanol. Special care was taken during all steps of preparation to avoid contamination. Total RNA was extracted from 30 mm^2 of microdissected tissues for each sample using the guanidine isothiocyanate/cesium chloride method [34]. PicoGreen (Molecular Probes, Eugene, OR) was used for RNA quality control.

Table 1. Patients' Clinicopathological Characteristics.

Parameter	Primary Cancer (Untreated Patients)	Recurrent Cancer (Platinum-Treated Patients)
Ovarian cancer samples	34 (54.8%)	28 (45.2%)
Age at treatment [median (range)] (years)	56 (30–86)	50.5 (26–68)
Histology		
Serous–papillary	21 (61.8%)	25 (89.3%)
Endometrioid	8 (23.5%)	2 (7.1%)
Mucinous	2 (5.9%)	1 (3.6%)
Mixed/others	3 (8.8%)	0
FIGO stage		
I	8 (23.5%)	2 (7.1%)
II	1 (2.9%)	0
III	13 (38.2%)	19 (67.9%)
IV	12 (35.3%)	7 (25%)
Histologic grade		
I	5 (14.7%)	6 (21.4%)
II	10 (29.4%)	9 (32.1%)
III	19 (55.9%)	13 (46.4%)
Ascites		
None	9 (26.5%)	18 (64.3%)
<500 ml	13 (38.2%)	5 (17.9%)
>500 ml	12 (35.3%)	5 (17.9%)
Postoperative residual tumor mass		
Macroscopically tumor-free	23 (67.6%)	11 (39.3%)
≤2 cm	6 (17.7%)	12 (42.9%)
>2 cm	5 (14.7%)	5 (17.9%)

Quantitative Reverse Transcription–Polymerase Chain Reaction (RT-PCR)

Single-stranded cDNA was synthesized with Omniscript Reverse Transcriptase Kit (Qiagen, Hilden, Germany), according to the protocol supplied by the manufacturer. Real-time PCR was performed using Gen Amp 5700 sequence detection system (PE Applied Biosystems, Weiterstadt, Germany) using intron-spanning primers and Fam (5′ end)–labeled and Tamra (3′ end)–labeled specific oligonucleotides. The housekeeping gene *GAPDH* was used as reference. Primers and the TaqMan probe were designed using the Primer Express software (PE Applied Biosystems). The sequences for the: 1) forward primer, 2) TaqMan probe, and 3) reverse primer were as follows: for *TOP2A*: 1) 5′-AAG GAA CTA AAA GGG ATC CAG CTT-3′, 2) Fam-5′-TGT CTC TCA AAA GCC TGA TCC TGC CAA-3′-Tamra, and 3) 5′-TTT GCG GCG ATT CTT GGT-3′; for *GAPDH*: 1) 5′-GAA GGT GAA GGT CGG AGT C-3′, 2) Fam-5′-CAA GCT TCC CGT TCT CAG CC-3′-Tamra, and 3) 5′-GAA GAT GGT GAT GGG ATT TC-3′.

The following PCR reaction mixture in a total volume of 25 μl was used: 1 ng of total RNA; 0.2 μM forward and reverse primers; 0.2 μM TaqMan probe; 500 μM dATP, dCTP, dGTP, and dUTP; 3.5 mM $MgCl_2$; 1.25 U of AmpliTaq Gold; and 12.5 U of AmpErase UNG in 1× TaqMan buffer. Signals were detected with a fluorescence-measuring instrument in real-time (ABI Prism 7700 Sequence Detector; PE Applied Biosystems). Relative RNA quantification was performed with the C_T method, as described by the manufacturer, normalizing C_T values to the housekeeping gene *GAPDH* [35] and calculating the relative expression values of tumor tissues with respect to nonmalignant, noninflammatory ovarian tissue RNA (Clontech, Heidelberg, Germany), which was used as normal. All real-time RT-PCR experiments were performed in triplicate standard. Only RNA samples from laser-microdissected tissues with good overall RNA quality ($C_T < 31$ for the housekeeping gene *GAPDH*) were included in the final analysis.

Nonradioisotopic RNA In Situ *Hybridization*

TOP2A expression on the mRNA level was analyzed with nonradioisotopic *in situ* hybridization in tissue sections from 13 ovarian tumors. An IMAGE cDNA clone for *TOP2A* (GenBank accession no. AA047124) was obtained from Incyte Genomics (St. Louis, MO) and verified by sequencing. Labeling, quantification, hybridization, and detection for DIG mRNA probes were performed according to supplier protocol (Roche Diagnostics, Mannheim, Germany). *TOP2A* antisense probe was labeled with DIG UTP by *in vitro* transcription, precipitated with ethanol, and dissolved in DEPC–H_2O. In a prehybridization step, 5-μm paraffin-embedded ovarian cancer slides were deparaffinized, alcohol-rehydrated, fixed in 4% PFA, and pretreated with Proteinase K. For hybridization, slides were incubated with 2.5 ng/μl *TOP2A* RNA probe for 12 to 14 hours at 65°C. Stringent posthybridization washes were performed at 60°C using 20% (wt/vol) formamide and 0.5× SSC. Signal detection was achieved by incubation with alkaline phosphatase–labeled anti-DIG antibody, developed with BM purple substrate, and counterstained with nuclear fast red. Hybridization with *TOP2A* mRNA sense probe was performed in parallel for each slide as negative control.

Immunohistochemistry

The avidin–biotin complex (ABC) method was used for the staining of TOP2A in formalin-fixed, paraffin-embedded tissues. Series from each tissue sample were cut into 4-μm sections. During deparaffinization, endogenous peroxidase activity was quenched with a hydrogen peroxide solution. For antigen retrieval, slides were incubated in citrate buffer (pH 6.0) and heated for 20 minutes in a pressure cooker. Nonspecific binding sites were blocked with CAS-blocking solution (Dako, Hamburg, Germany). After cooling at room temperature, slides were incubated with TOP2A primary antibody (Ki-S1 clone; Roche, Penzberg, Germany) for 1 hour. Detection was accomplished by incubation of biotinylated secondary antibody, followed by the tertiary ABC complex, which bound to biotin on the secondary antibody. Slides were then developed with an AEC Substrate Kit (Zymed Laboratories Inc., San Francisco, CA). Sections were counterstained with hematoxylin. Negative controls were performed using water, instead of antibodies. A total of 62 samples from ovarian tumors and 7 normal ovarian tissue samples used as controls were analyzed immunohistochemically for TOP2A with Ki-S1 antibody. Hematoxylin–eosin staining was performed for basic histology, and one slide was stained without primary antibody for negative control.

Semiquantitative Scoring Interpretation

A semiquantitative scoring system was used for the evaluation of immunohistochemical and *in situ* hybridization data, as described previously [36]. A minimum of 1000 cells (approximately 10 high-power fields) were counted to determine the percentage of positive cells. Mean dye intensity was assessed using the following scale: 0 = negative, 1 = low, 2 = middle, and 3 = strong. The percentages of stained cells varied as follows: 0, negative; 1, <10%; 2, 10% to 50%; 3, 51% to 80%; 4, >80% positive cells. According to the scores, tissues were classified as having low (0–2 points), middle (3–6 points), or strong (7–12 points) TOP2A expression.

Statistical Analysis

Statistical analysis was performed using SPSS statistical software for Windows (SPSS, Inc., Chicago, IL). To compare expression levels between different cell groups (tumor *versus* stroma, and primary *versus* recurrent), the Mann-Whitney *U* test was used. The correlation of *TOP2A* expression with histopathological type, FIGO stage, histologic grade, residual tumor, ascites, age at diagnosis, and overall survival was evaluated with univariate and multivariate analyses with the Cox regression model. $P < .05$ was considered statistically significant.

Results

Quantitative RT-PCR of TOP2A mRNA in Ovarian Cancer

TOP2A expression in laser-microdissected tumor epithelial and adjacent stromal cells from ovarian tumors was analyzed by real-time RT-PCR. Only microdissected tissue samples with optimal RNA quality ($C_T < 31$ for the housekeeping gene *GAPDH*; see Materials and Methods section) were included in the final analysis. This criterion was fulfilled by 38 RNA preparations, representing 16 RNA samples from microdissected tumor stromal cells and 22 RNA samples from microdissected ovarian tumor epithelial cells.

Figure 1*A* illustrates real-time RT-PCR data for *TOP2A* mRNA expression in stromal cells of primary and recurrent ovarian cancers. Compared to a control RNA from a normal nonmicrodissected ovary mainly containing normal stromal cells (Clontech; named "Ref" in Figure 1*A*), *TOP2A* mRNA was upregulated in 13 of 16 (81%) preparations of tumor stromal cells, indicating that *TOP2A* overexpression in tumor stroma is a general feature of primary and recurrent ovarian cancers. Comparing *TOP2A* mRNA expression between stromal cells of primary and recurrent ovarian cancers, we found a more abundant expression in stromal cells from platinum-treated patients compared to nontreated patients

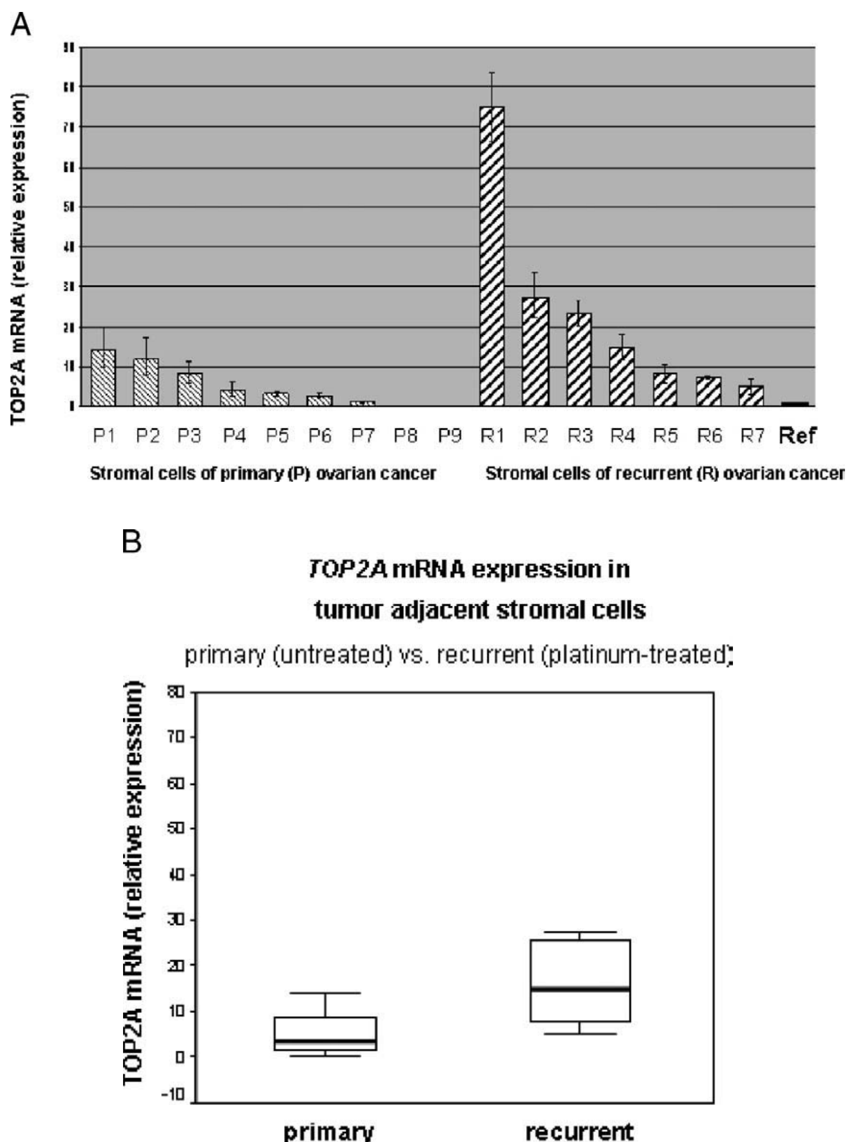

Figure 1. *(A) Real-time RT-PCR data showing the upregulation of TOP2A mRNA expression in tumor-adjacent stromal cells after platinum-based chemotherapy. Relative TOP2A expression levels in tumor stroma of primary ovarian cancer (P) and recurrent ovarian cancer (R) are compared. Ref = Reference tissues (normal ovary). (B) TOP2A expression in tumor-adjacent stromal cells of primary and recurrent ovarian cancers. Box plot presentation of real-time RT-PCR data. TOP2A mRNA expression was more abundant in stromal cells from platinum-treated recurrent tumors compared to expression of primary ovarian cancer in stromal cells (P = .017).*

(P = .017) (Figure 1*B*); however, the opposite was found for *TOP2A* expression in tumor epithelial cells (i.e., more abundant expression in primary ovarian cancer than in recurrent ovarian cancer, but this was not statistically significant) (P = .97).

Cellular Localization of TOP2A *mRNA and Protein*

TOP2A mRNA expression was detected with nonradioisotopic *in situ* hybridization in tissue sections from ovarian tumors (n = 13). Representative staining patterns of *TOP2A* mRNA in primary and recurrent ovarian cancers are shown in Figure 2. Abundant *TOP2A* mRNA expression was detectable in tumor cells from primary serous papillary tumors (Figure 2*A*, *arrows*); weak *TOP2A* expression was also detectable in adjacent stromal cells (Figure 2*A*, *arrowheads*). In recurrent serous papillary ovarian cancer, *TOP2A* mRNA expression was detectable in tumors (Figure 2*D*, *arrows*), but it was less abundant than the expression found in primary tumors (*cf.* Figure 2, *A* and *D*). However, *TOP2A* mRNA expression increased in stromal cells of recurrent ovarian cancer (Figure 2*D*, *arrowheads*), both with respect to the number of stained cells and staining intensity. Hybridization with the *TOP2A* sense (control) probe did not lead to any nonspecific signals in the control sections (Figure 2, *B* and *E*).

Next, we analyzed TOP2A protein expression by immunohistochemistry using the well-characterized TOP2A-specific antibody Ki-S1 (Figure 3). Immunohistochemical analysis showed strong nuclear localization of the TOP2A protein, consistently with previously reported findings for the Ki-S1 antibody [37]. We analyzed TOP2A protein expression in the surface epithelium of normal ovary and inclusion cysts (derivatives of normal surface epithelium) (n = 7), primary

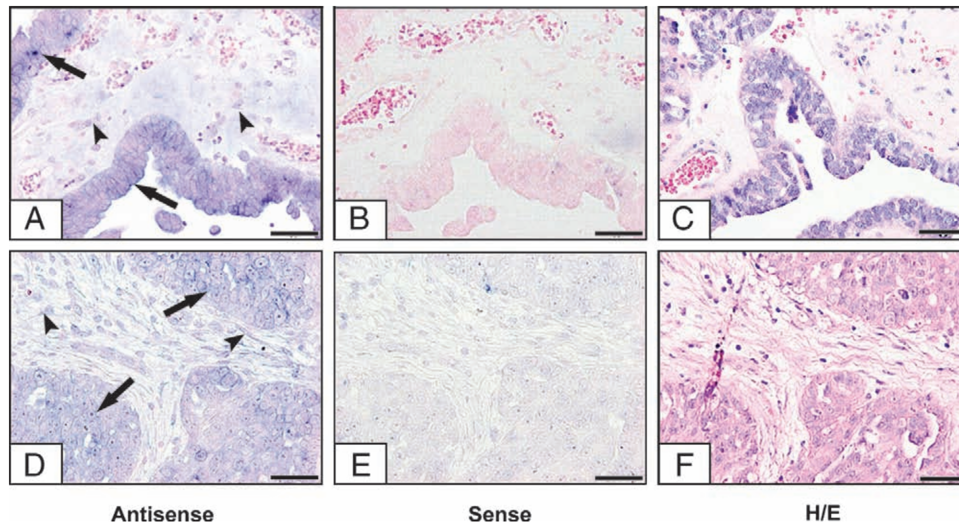

Figure 2. *TOP2A mRNA detection using nonradioactive mRNA* in situ *hybridization. (A–C) Primary ovarian cancer. (D–F) Recurrent ovarian cancer. (A + D) Antisense probe. (B + E) Sense (control) probe. (C + F) Hematoxylin–eosin staining. TOP2A mRNA is detected in tumor cells (arrows) and tumor-adjacent stromal cells (arrowheads). The sense probes in (B) + (E) do not show any signal. Scale bar = 50 μm.*

ovarian cancer (n = 34), and recurrent ovarian cancer after platinum-based chemotherapy (n = 28). In ovarian inclusion cysts (Figure 3, *A–C*) and ovary surface epithelium (data not shown), TOP2A protein was hardly detectable in epithelial cells and surrounding stromal tissues (Figure 3*A*; Figure 3*B*, *scale up of stromal cell area*). Very strong TOP2A protein expression was detected in tumor cells of primary ovarian cancer (Figure 3*D*, *arrows*). A smaller number of adjacent

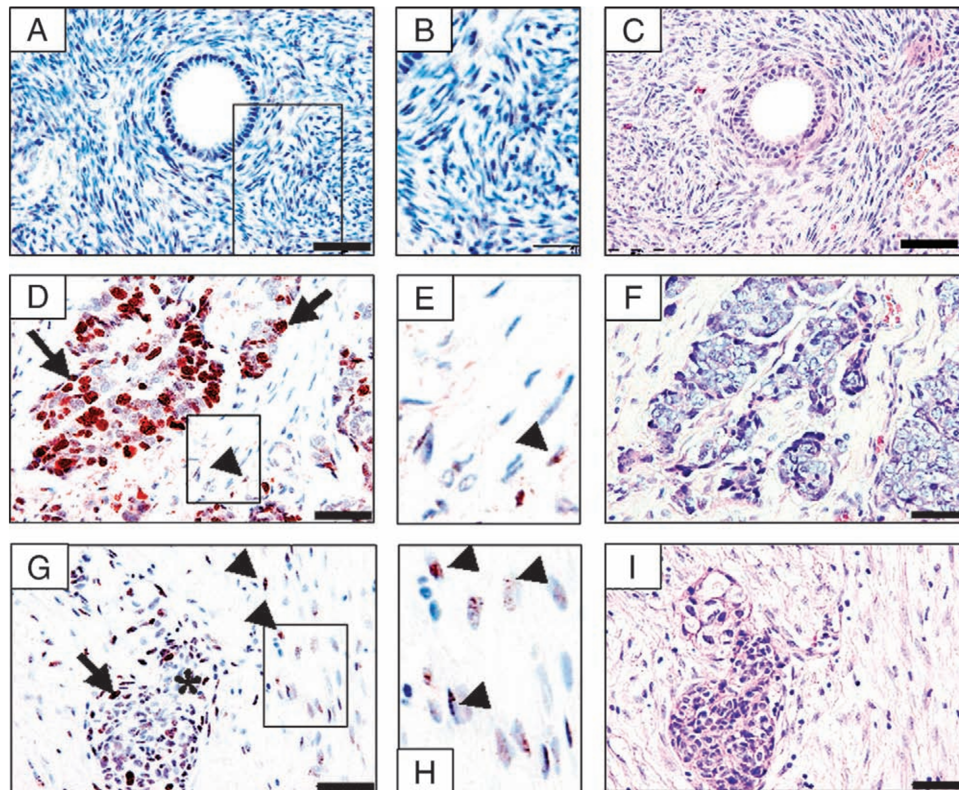

Figure 3. *Immunohistochemical analysis of TOP2A protein expression in nonmalignant ovarian tissues (A–C), primary ovarian cancer (D–F), and recurrent tumor (G–I). (B), (E), and (H) are scale up of the boxed regions shown in (A), (D), and (G), respectively. (C), (F), and (I) are hematoxylin–eosin stainings of consecutive sections. (A) TOP2A protein expression in the epithelium of inclusion cysts and in surrounding stromal cells is hardly detectable. (D) Primary ovarian cancer is characterized by very abundant TOP2A expression in tumor epithelial cells (arrows) and a clearly detectable TOP2A expression in tumor-adjacent stromal cells (arrowheads). (G) TOP2A protein expression in recurrent ovarian cancer after platinum-based chemotherapy. Expression in tumor cells decreased (asterisk) compared to that in primary cancer, whereas TOP2A expression in tumor-adjacent stromal cells (arrowheads) increased. Scale bar = 50 μm.*

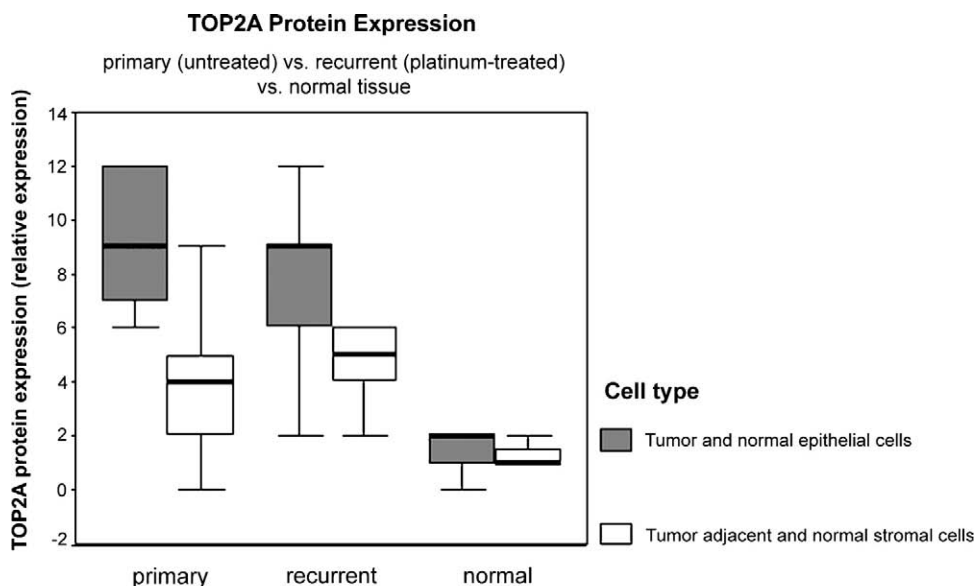

Figure 4. *Box plot presentation of immunohistochemical TOP2A expression data. TOP2A protein expression in primary (nontreated) and recurrent (platinum-treated) ovarian tumors, as well as in normal ovarian tissues, is shown. TOP2A protein expression is reduced in tumor epithelial cells from platinum-treated patients (P = .023), compared to its expression in epithelial tumor cells of untreated patients. In the tumor stroma, the opposite was found: TOP2A protein was more abundantly expressed in the stromal cells of recurrent ovarian cancer after platinum-based chemotherapy compared to stromal cells of primary tumors (P = .056).*

stromal cells clearly demonstrated TOP2A protein expression in the nucleus as well (Figure 3*D*, *arrowhead*; Figure 3*E*, *scale up of boxed stromal cell region*). In recurrent tumors after platinum-based chemotherapy, TOP2A protein expression was still highly abundant in a proportion of tumor cells (Figure 3*G*, *arrow*), whereas other tumor cells expressed TOP2A protein only moderately (asterisk), indicating a decrease in overall TOP2A expression in these tumor cells. However, TOP2A protein expression in the stromal compartment of recurrent ovarian cancer increased (Figure 3*G*, *arrowheads*; Figure 3*H*, *scale up of boxed stromal cell area*), both with respect to the number of stained cells and staining intensity.

After counting 10 microscopic high-power fields, with at least 1000 counted cells per specimen, immunoreactive scores (see Materials and Methods section) were determined and statistically analyzed. The results are shown as box plots in Figure 4. TOP2A protein was overexpressed in both stromal and tumor cells from tumor tissues (Figure 4, *four boxes on the left*) compared to normal ovarian tissues (Figure 4, *two boxes on the right*) (P = .0001). Consistent with RNA *in situ* hybridization data, TOP2A protein expression in primary and recurrent ovarian cancers was clearly more abundant in tumor cells compared to adjacent stromal cells ($P < .01$). Consistent with our quantitative RT-PCR data, TOP2A protein expression increased in stromal cells from platinum-treated tumors compared to stromal cells of primary tumors (P = .056). An inverse correlation was found for tumor cells (i.e., decreased expression in tumor cells from platinum-treated patients compared to nontreated patients) (P = .023). No significant correlation was found between TOP2A expression in tumor and stromal cells on the mRNA and protein levels and in clinicopathological data (histopathological type, FIGO stage, histologic grade, residual tumor, ascites, age at diagnosis.

Discussion

Systematic gene expression analysis presented in this study shows, for the first time, distinct *TOP2A* expression patterns in tumor and adjacent stromal cells in ovarian cancer. *TOP2A* expression was much more abundant in tumor cells compared to adjacent stromal cells on both the protein and mRNA levels. Moreover, a specific pattern of *TOP2A* expression was found on the mRNA and protein levels with respect to platinum-based chemotherapy: *TOP2A* expression was clearly more abundant in tumor cells from nontreated patients compared to platinum-treated patients, whereas the inverse pattern was found in stromal cells (i.e., more abundant *TOP2A* expression in stromal cells from tumor relapses after platinum chemotherapy compared to stromal cells in tumors of nontreated patients).

These differences in *TOP2A* expression in tumor epithelium and adjacent stroma cells may reflect different effects of platinum chemotherapy on the two cell subpopulations and underscore a possible role of stromal cells in ovarian tumor progression and failure of chemotherapy. To date, various studies have focused on the mechanisms governing the development of chemotherapy resistance in different tumor entities, implicating a role for *TOP2A* [6,21,22,24–27,38,39] and suggesting that *TOP2A* expression in tumor cells could be used to predict responses to chemotherapy [18,19,21–24]. Our results suggest, for the first time, a possible role for tumor stroma in the development of drug resistance, and this possibility should be evaluated in future studies. Furthermore, analysis of *TOP2A* expression in subpopulations of tumor and adjacent stromal cells could lead to the development of methods that allow a more accurate prediction of response to therapy.

The significantly higher expression of *TOP2A* in tumor stroma after platinum-based chemotherapy is an important

finding from a biologic prospective. Stromal changes, together with DNA mutations and tissue disorders, are considered to be important factors contributing to the initiation and progression of epithelial cancers [40]. It has been previously shown that stromal fibroblasts play an important role in tumorigenesis, by taking part in tissue repair and remodeling through the activation and production of epithelial growth factors and degrading enzymes [41–43]. Furthermore, there exists evidence suggesting that epithelial tumors can activate adjacent stromal fibroblasts, which in turn can promote the growth and invasion of tumor cells [44]. Moreover, stromal cells can be activated by different factors, such as stromelysin-1, platelet-derived growth factor, or irradiation, and then promote malignant progression of epithelial cells [45–47]. Particularly in ovarian cancer, stromal activity has been previously implicated in a hypothesis supporting an ovarian cancer-prone preneoplastic phenotype [48]. This theory is based on findings on ovaries from women with an inherited risk for ovarian cancer, which contained two or more of the following histologic characteristics: surface epithelial pseudostratification, surface epithelial papillomatosis, deep cortical invaginations with epithelial inclusion cysts, and increased stromal activity [48].

In conclusion, systematic gene expression analysis, with three independent methods, showed distinct *TOP2A* expression patterns: a chemotherapy-independent higher *TOP2A* expression in primary tumor compared to adjacent stromal cells, a platinum-chemotherapy–associated pattern of *TOP2A* downregulation in tumor cells, and upregulation in adjacent stromal cells. These distinct expression patterns support the role of tumor stroma interface in tumor progression and suggest that it might play a role in mechanisms leading to drug resistance and failure of response to chemotherapy.

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