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The Association Between the p53/ topoisomerase I and p53/ topoisomerase IIα Immunophenotypes and the Progression of Ovarian Carcinomas*

Zależność między p53/topoizomerazą I i p53/topoizomerazą IIα immunofenotypem a progresją raka jajnika

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Abstract

Background. In *in vitro* studies it has been revealed that p53 protein expression might regulate topoisomerase I (topo I) and topoisomerase IIα (topo IIα) levels in tumor cells. So far, the association between the p53 protein and topo I and topo IIα expression and its impact on ovarian carcinoma progression has not been analyzed.
Objectives. The aim of the study was to examine the association between topo I and topo IIα expression and p53 protein overexpression with respect to the morphological features and progressive growth of ovarian tumors.
Material and Methods. The expression of the studied biomarkers was estimated by immunohistochemical staining in tumor sections from 136 malignant and 30 benign ovarian neoplasms.
Results. Significant differences for topo I, topo IIα and p53 expression between malignant and benign tumors were observed ($p < 0.01$). The expression of topo IIα and p53 protein was associated with advanced stages of ovarian carcinomas ($p < 0.01$). Differences between topo I-positive cases and low (G1) and high (G3) tumor grade had only borderline significance ($p = 0.07$). In ovarian carcinomas, positive correlations between topo I and topo IIα, topo I and p53 and topo IIα and p53 protein expression were revealed ($p = 0.001$). No relationship between the studied biomarkers was found in benign ovarian tumors ($p > 0.05$). p53/topo I and p53/topo IIα immunophenotypes were associated with advanced stages of ovarian carcinoma ($p = 0.045$ and $p = 0.009$, respectively). p53/topo IIα positive ovarian carcinomas were more frequently observed in high than in low tumor grades and the differences were only of borderline significance ($p = 0.07$).
Conclusions. Current findings suggest that on the one hand, cooperation between topo I, topo IIα and p53 protein participates in the progressive growth of ovarian tumors. On the other hand, simultaneous expression of the studied proteins identifies the subgroup of ovarian cancers with aggressive biological features which might be considered in therapy (**Adv Clin Exp Med 2012, 21, 1, 35–42**).

Key words: ovarian neoplasms, topoisomerases, p53 protein, progression, immunohistochemistry.

Streszczenie

Wprowadzenie. Badania *in vitro* wykazały, że białko p53 reguluje stężenie topoizomerazy I (topo I) i topoizomerazy II (topo II) w komórkach nowotworowych. W rakach jajnika nie prowadzono analizy związków między topo I, topo IIα i białkiem p53, ani nie oceniano ich wpływu na progresywny wzrost nowotworów jajnika.
Cel pracy. Ocena zależności między występowaniem topoizomerazy I i topoizomerazy IIα oraz białka p53 w rakach jajnika z uwzględnieniem cech morfologicznych nowotworu i jego progresywnego wzrostu.
Materiał i metody. Obecność topo I i IIα oraz białka p53 oceniano w 136 rakach i 30 nowotworach łagodnych jajnika z użyciem przeciwciał monoklonalnych, stosując metodę immunohistochemiczną.

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Wyniki. Wykazano statystycznie istotne różnice w występowaniu topo I, topo IIα i białka p53 między rakami a łagodnymi nowotworami jajnika ($p < 0,01$). Obecność topo IIα i białka p53 była związana z wyższym stadium zaawansowania raka jajnika ($p < 0,01$). Topo I była częściej obserwowana w rakach o dużym stopniu złośliwości (G3) aniżeli w rakach o małym stopniu złośliwości (G1). Wykazane różnice były na granicy statystycznej istotności ($p = 0,07$). Zanotowano związek między występowaniem topo I i topo IIα oraz p53 i topo I lub topo IIα w rakach jajnika ($p = 0,001$). Takich zależności nie stwierdzono w łagodnych nowotworach jajnika ($p > 0,05$). Stwierdzono, ze w zaawansowanych rakach jajnika dominuje immunofenotyp p53/topo I+ oraz p53/topo IIα+ (odpowiednio: $p = 0,045$; $p = 0,009$). Na granicy statystycznej istotności były różnice w występowaniu p53/topo IIα+ przypadków z uwzględnieniem stopnia złośliwości nowotworu ($p = 0,07$).
Wnioski. Wyniki wskazują, że kooperacja między analizowanymi białkami może mieć istotne znacznie w progresywnym wzroście nowotworów jajnika. Jednoczesne występowanie białka p53 i topo I lub p53 i topo IIα może charakteryzować podgrupę raków jajnika o agresywnych biologicznych cechach, które mogą być uwzględniane w terapii nowotworów tego narządu (**Adv Clin Exp Med 2012, 21, 1, 35–42**).

Słowa kluczowe: nowotwory jajnika, topoizomerazy, białko p53, progresja, immunohistochemia.

Most patients with ovarian carcinoma are diagnosed in the advanced stage of the disease and the prognosis of these patients is generally poor [1, 2]. The biological characterization of ovarian carcinomas revealed that they represent heterogeneous group of tumors [3]. The pathomorphological features of ovarian cancers are often associated with abnormalities in proliferative activity and suppressor gene expression in tumor cells [4]. Therefore, an examination of the biological features of ovarian tumors with established morphological parameters might be useful for screening the subgroups of patients for selective therapy [4, 5]. Lately, attention has been focused on analysis of the association between biomarkers involved in different cellular mechanisms in ovarian tumors and their impact on the clinical outcome of patients [3, 5]. Now, among the biological factors, topoisomerases and p53 protein are being intensively investigated in many human tumors [3, 5–9].

Human DNA topoisomerases (topo) are essential nuclear enzymes that make structural changes in the topological state of DNA without changing the primary structure and play a crucial role in DNA replication, chromosome condensation, and chromosome segregation [10]. Topoisomerases are classified into type I and II according to their catalytic activity [10, 11]. Topoisomerase I makes a transient single-stranded break into DNA, passes a single strand of the DNA through the broken strand, and religates the break [10]. Topoisomerase II cleaves double-stranded breaks in one segment of replicated DNA, another double-strand DNA passes through the gate and religation restores the integrity of the cleaved DNA [11]. In ovarian carcinomas, topo I and topo IIα expression was observed in a high percentage of cases (30–70%) [2, 12, 13]. The topo I and IIα enzymes have been described as the molecular targets of anticancer agents such as topoisomerase I and II inhibitors e.g., irinotecan, topotecan, etoposide, teniposide and epirubicin [10]. Individual data revealed that on the one hand, the response of human tumors to topo I and II inhibitors partly depends on the levels of these enzymes in the tumor cells [7, 9]. On the other hand, there are reports suggesting that the effect of treatment using topo I- and II-targeted drugs may depend on DNA replication and the activity of the apoptotic pathway [9, 10].

The role of p53 protein overexpression in human tumors including ovarian carcinoma is well documented [11, 14]. p53 mutation or overexpression was observed in 40–80% of ovarian cancers, with dominance at the advanced stages of tumors [1, 14, 6]. The *in vitro* and *in vivo* studies suggested that the loss of functional p53 protein reflects a chemoresistant phenotype of tumors [10, 15].

The interaction of topo I with p53, which occurs through the C-terminus of p53, has been described by Mao et al. [16]. A model has been discussed where p53 activates topo I and this results in additional DNA damage. On the other side, topo I may assist the proofreading function of p53 and help in elimination of damaged tumor cells [16]. Individual data suggests that tumor cells which possess p53 mutations are significantly more sensitive to topo I inhibitors than p53 wild-type cells [17].

Transcriptional activation was observed of topo IIα inhibited by wild-type p53 protein as a result of the binding of this protein to the specific p53 sites in the promoter of topo IIα [11]. Tumors overexpressing mutant forms of p53 protein theoretically do not have the capacity to negatively regulate topo IIα transcriptional activity, so the tumors should possess a high level of topo IIα expression [18]. However, tumors containing wild-type p53 protein cause transcriptional interference with the topo IIα promoter, and as a result, they should reflect a low level of this enzyme [11, 19]. So far, the role of p53 protein in the regulation of topo I and topo IIα expression in human ovarian tumors, with regard to the morphological features of the cells and the progressive growth of tumors, has not been estimated.

The aim of the study was to investigate the simultaneous expression of topoisomerase I, topoisomerase IIα expression and p53 protein overexpression as biological biomarkers of aggressive growth in ovarian carcinomas.

Material and Methods

Patients

The study was performed on tissue sections from one hundred and thirty six patients diagnosed with primary ovarian carcinoma and thirty with benign ovarian tumors, hospitalized in the First Clinic of Gynecology, Wroclaw Medical University, Wrocław, Poland, between 2000 and 2010. Their ages ranged from 21 to 78 years (mean, 54 years). Tumor tissues were obtained during the initial surgery. Tumor specimens were frozen at –80°C according to frozen tissue procedure. According to the International Federation of Gynecology and Obstetrics (FIGO) classification, thirty six of those with primary ovarian carcinoma were in stage I/II and one hundred in stage III/IV. The tumors were histologically verified and graded according to World Health Organization criteria (Jerzy Rabczyński PhD, Department of Pathomorphology, Wroclaw Medical University). The study included 73 serous, 32 endometrioid, 5 mucinous and 26 undifferentiated ovarian carcinomas as well as 20 serous and 10 mucinous cystadenomas. Twenty seven were well (G1), forty six moderately (G2) and sixty three poorly (G3) differentiated.

Immunohistochemistry

Immunohistochemical staining (IHC) for the analyzed proteins was performed on frozen five-µm tissue sections using the Universal DakoCytomation LSAB + Kit, a peroxidase procedure (LSAB+ Kit:HRP, Dako, Copenhagen, Denmark) and the following primary monoclonal antibodies: anti-p53 protein, which reacts with both wild-type and mutant forms of unphosphorylated human p53 protein recognizing an epitope between amino acids 20 and 25 (clone DO-7, Novocastra, Newcastle, UK), anti-topoisomerase I, which binds to a region within the middle of the topoisomerase I molecule (clone 1D6, Novocastra), and anti-topoisomerase IIα, which reacts with human topoisomerase II alfa (clone 3F6, Novocastra). Endogenous peroxidase reactivity was blocked with 3% H_2O_2 and nonspecific tissue reactions with 10% BSA (Bovine Serum Albumin). Tissue specimens were incubated with primary antibodies (anti-p53 protein, anti-topo I and anti-topo IIα) 60 minutes at room temperature. Following a wash with 0.1 M Tris-buffer, pH = 7.4 (TBS), the tissue specimens were incubated with a secondary biotinylated rabbit antibody, anti-mouse IgG (Dako, Denmark, Copenhagen) and streptavidin horseradish peroxidase-conjugated (Dako), both for 15 minutes at room temperature. After a wash with TBS, the antigen-antibody reaction was visualized by DAB (3,3 diaminobenzidine) (Dako) as a chromogen (8 minutes, room temperature). The sections were counterstained with hematoxylin and mounted. An incubation buffer (TBS) without a primary antibody was used as a negative control. The internal positive controls were normal tonsil tissues for topo I and topo IIα and ovarian carcinoma tissue with p53 gene mutation for p53 protein expression.

Interpretation of Immunohistochemical Staining

The evaluation of immunohistochemical data was performed without the knowledge of the clinical data. Sections were scored semiquantitatively, taking into account the intensity of immunostaining and the number of tumor cells exhibiting staining for p53 protein, topo I and topo IIα antibodies. In each case, at least 1000 tumor cells were counted in randomly chosen fields, p53 protein overexpression and expression of topo I and topo IIα was calculated as a percentage of the positive cells in relation to the total number of cells using a semiquantative scale. The cases were scored as positive for p53 protein, topo I, topo IIα expression when more than 10% of the tumor cells showed nuclear immunostaining [6, 20].

Statistical Analysis

Correlations between p53 protein overexpression, topo I and topo IIα expression with clinicopathological parameters of ovarian carcinomas were statistically studied using a Chi-square test. To compare the expression of p53, topo I and topo IIα between benign and malignant ovarian tumors, a Chi-square test was also used. Associations between p53 protein overexpression and topo I, topo IIα expression were analyzed using a Spearman rank correlation coefficient test. A Chi-square test was used to assess the association between clinico-pathological parameters and topo I/p53, topo IIα/p53 positive *vs* topo I/p53, topo IIα/p53 negative cases. Differences were considered as significant when $p < 0.05$.

Results

The studied biomarkers showed heterogeneous distribution in the tumor specimens. Figure

Table 1. Association between topoisomerase I, topoisomerase IIα, p53 protein expression and clinicopathological parameters of ovarian carcinomas

Tabela 1. Związek między występowaniem topoizomerazy I, topoizomerazy IIα, białka p53 a parametrami kliniczno-patologicznymi raków jajnika

Histological types of ovarian cancers (Typy histologiczne raka jajnika)	Immunopositivity – percentage [%] of positive cases (Immunoreaktywność – odsetek [%] dodatnich przypadków)			
	N	topoisomerase I	topoisomerase II α	p53-protein
		N (%)	N (%)	N (%)
Serous (Surowicze)	73	50 (68.4)	54 (74.0)	51 (69.9)
Non-serous (Niesurowicze)	63	47 (74.6)	52 (82.5)	39 (61.9)
FIGO I/II III/IV	 36 100	 23 (63.9) 74 (74.0)	 23 (63.9)* 83 (83.0)	 16 (44.4)* 74 (74.0)
Grade (Stopień zróżnicowania) G1 G2 G3	 27 46 63	 15 (55.5) 32 (69.5) 50 (79.4)	 18 (66.7) 35 (76.1) 53 (84.1)	 15 (55.5) 23 (63.0) 46 (73.0)
Benign tumors (Nowotwory łagodne) vs Ovarian carcinomas (Raki jajnika)	30 136	5 (16.7)* 97 (71.3)	6 (20.0)* 106 (77.9)	0 (0.0)* 90 (66.1)

N – number of positive cases.
N – liczba dodatnich przypadków.

* Statistically significant.
* Różnica statystycznie istotna.

Table 2. Relationship between mean value of topoisomerase I, topoisomerase IIα and p53 protein expression and clinicopathological variables of ovarian carcinomas

Tabela 2. Zależność między wartościami średnimi immunohistochemicznego występowania topoizomerazy I, topoizomerazy IIα i białka p53 a parametrami kliniczno-patologicznymi raków jajnika

Histological types of ovarian cancers (Typy histologiczne raka jajnika)	Immunopositivity – percentage [%] of positive cases (Immunoreaktywność – odsetek [%] dodatnich przypadków)			
	N	topoisomerase I	topoisomerase IIα	p53-protein
		mean (%) ± SD (%) średnia	mean (%) ± SD (%) średnia	mean (%) ± SD (%) średnia
Serous (Surowicze)	73	36.02 ± 28.99	33.69 ± 29.27	35.34 + 30.46
Non-serous (Niesurowicze)	63	32.69 ± 25.91	34.44 ± 26.62	28.57 + 27.81
FIGO I/II III/IV	 36 100	 29.44 ± 28.48 36.30 ± 27.14	 25.83 ± 31.92 * 37.00 ± 25.95	 20.83 + 28.01* 36.30 + 28.87
Grade (Stopień zróżnicowania) G1 G2 G3	 27 46 63	 23.33 ± 24.65* 35.43 ± 28.72 38.57 ± 26.99	 25.92 ± 30.79* 32.39 ± 27.17 38.73 ± 26.79	 23.70 + 27.33* 30.00 + 29.88 37.46 + 29.18

N – number of positive cases.
N – liczba dodatnich przypadków.

* Statistically significant.
* Różnica statystycznie istotna.

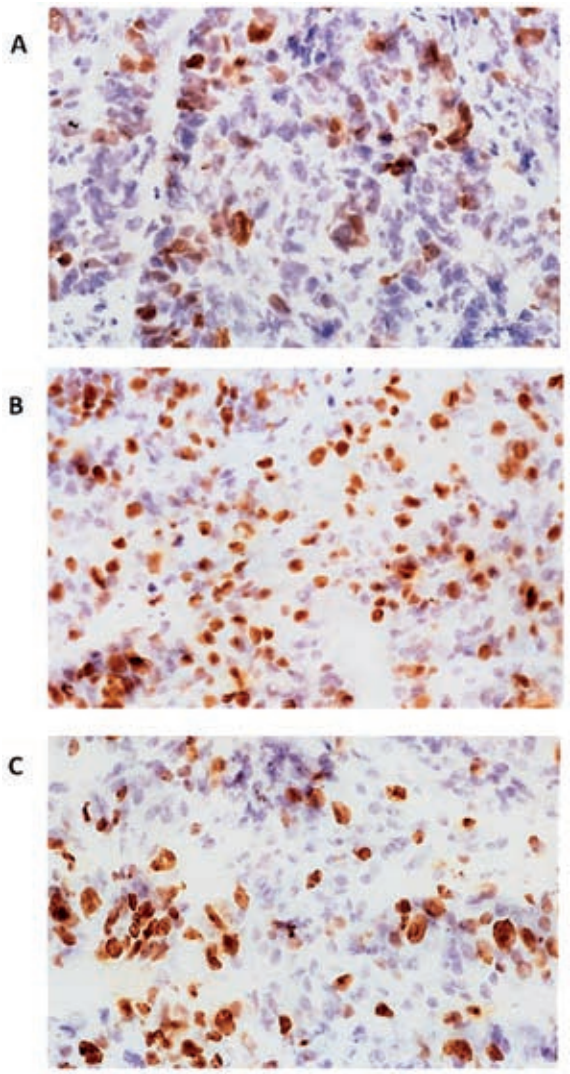

Fig. 1. Immunohistochemical staining for topo I, topo IIα and p53 protein in ovarian carcinoma.
A) topo I expression was detected in a high percentage of tumor cells; B) strong nuclear topo IIα expression in tumor cells; C) many tumor cells display p53 protein overexpression (ABC technique × 200)

Ryc. 1. Immunohistochemiczne barwienie dla topo I, topo IIα i białka p53 w rakach jajnika. A) obecność topo I wykryto w dużym odsetku komórek nowotworowych; B) znaczny odsetek komórek nowotworowych z jądrową akumulacją topo IIα; C) intensywna jądrowa lokalizacja białka p53 w komórkach raka jajnika (technika ABC × 200)

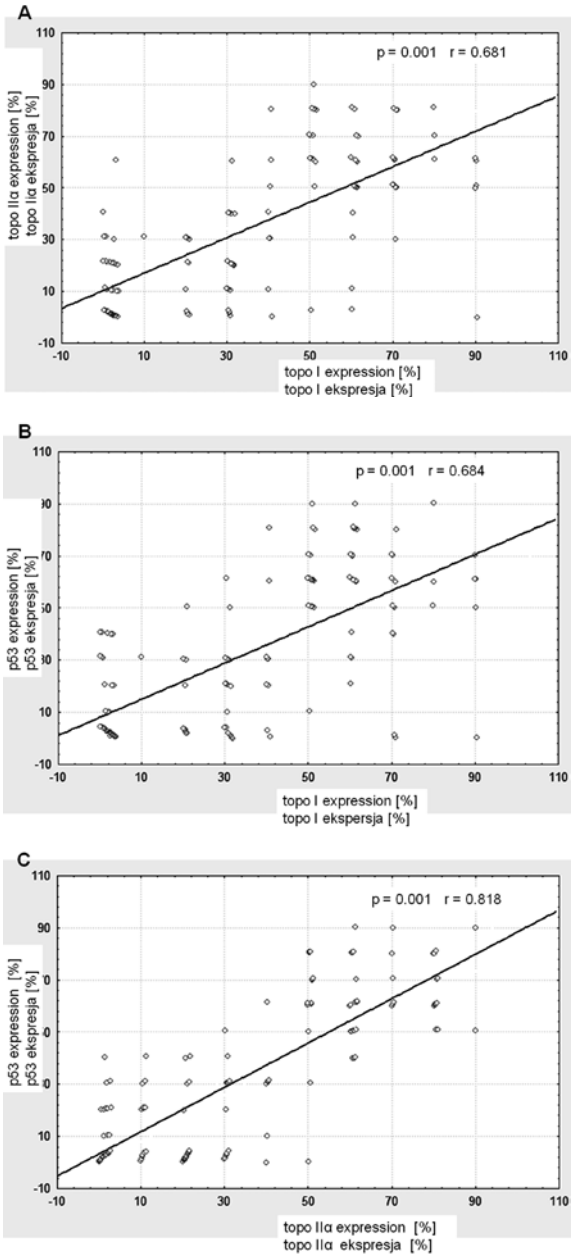

Fig. 2. Correlation between topo I, topo IIα and p53 protein expression in ovarian carcinomas. There was a significant correlation between: A) topo I and topo IIα (r = 0.681, p = 0.001); B) topo I and p53 protein (r = 0.684, p = 0.001); C) topo IIα and p53 overexpression (r = 0.818, p = 0.001)

Ryc. 2 Analiza zależności między występowaniem topo I, topo IIα i białkiem p53 w rakach jajnika. Wykazano związek między A) topo I i topo IIα (r = 0,681; p = 0,001); B) topo I i białkiem p53 (r = 0,684; p = 0,001); C) topo IIα i białkiem p53 (r = 0,818; p = 0,001)

1A, B and C illustrate immunostaining for topo I, topo IIα and p53 protein in ovarian carcinomas respectively. Tables 1 and 2 show the association between topo I, topo IIα and p53 protein expression and the clinicopathological parameters. The differences between topo I-positive cases and low (G1) and high (G3) tumor grades had only borderline significance (p = 0.07). However, the immunohistochemical staining for topo I considered using the mean +/- standard deviation showed significant differences between low (G1) and high (G3) tumor grades (p < 0.01; Table 2). Topo IIα expression was associated with advanced FIGO stages of tumors (p < 0.01; Table 1). Likewise, the mean value of topo IIα expression increased significantly from low to high FIGO stages (p < 0.01, Table 2). Significant differences were also observed between the mean value of topo IIα expression in low (G1) *vs.* high

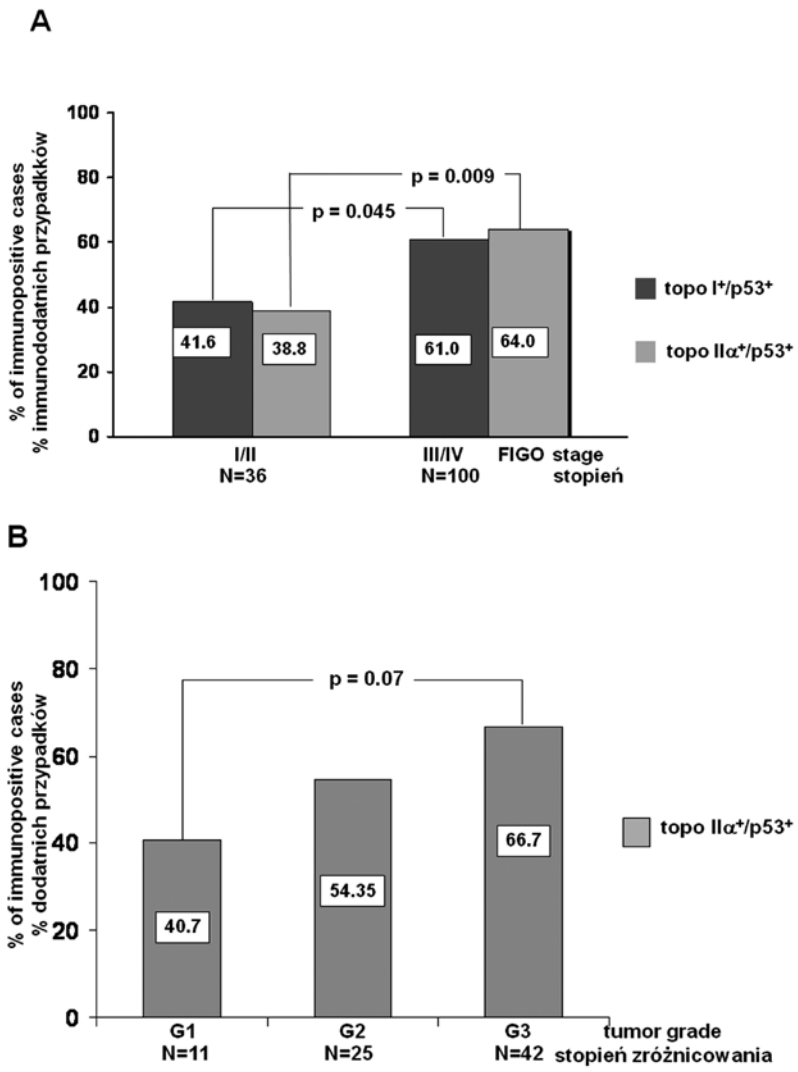

Fig. 3. Correlation between topo I/p53 and topo IIα/p53 immunophenotypes and clinicopathological parameters of ovarian carcinomas. A) topo I/p53 and topo IIα/p53 immunophenotypes were associated with advanced FIGO stages (p = 0.045 and p = 0.009, respectively); B) topo IIα/p53 immunophenotype was observed more frequently in high grade (G3) than in low grade (G1) tumors. The differences were on the statistical borderline (p = 0.07)

Ryc. 3. Analiza związków między topoI/p53 i topo IIα/p53 immunofenotypami a kliniczno-patologicznymi parametrami raków jajnika. A) topo I/p53 i topo IIα/p53 immunofenotypy były związane z zaawansowanym rakiem jajnika (p = 0,045; p = 0,009, odpowiednio); B) topo IIα/p53 immunofenotyp był częściej obserwowany w rakach o dużym stopniu złośliwości (G3) aniżeli o małym stopniu złośliwości (G1). Wykazana różnica była na granicy statystycznej istotności (p = 0,07)

(G3) tumor grades (p < 0.01; Table 2). p53-positive ovarian carcinomas were associated with advanced stages of tumors (p < 0.001; Table 1). Similarly, the mean value of p53 protein overexpression correlated with advanced FIGO stages (p < 0.01; Table 2). The mean value of p53 overexpression was significantly higher (mean; 37.46, SD, 29.18) in poor (G3) than in well (G1) differentiated ovarian carcinomas (mean; 23.70, SD, 27.33) (p < 0.01; Table 2). There was a significant increase of topo I, topo IIα and p53 protein expression from benign ovarian tumors to ovarian carcinomas (p < 0.01; Table 1). Correlations between the expressions of topo I and topo IIα (p = 0.001; r = 0.681), topo I and p53 protein (p = 0.001; r = 0.684) and topo IIα and p53 (p = 0.001; r = 0.818) were found in ovarian carcinomas (Fig. 2A, B, C). No correlations were found between the analyzed biomarkers in benign ovarian tumors. Topo I/p53 and topo IIα/p53 immunopositive-ovarian carcinomas were associated with the advanced stages of the disease (p = 0.045 and p = 0.009 respectively; Fig. 3A). However, ovarian carcinomas expressing both topo IIα and p53 protein were more frequently observed in high (G3) than in low (G1) tumor grades but the differences were of borderline significance (p = 0.07; Fig. 3B).

Discussion

The present immunohistochemical study concerning topo I and topo IIα expression in malignant and benign ovarian tumors is in agreement with other results [2, 8, 21], which showed significantly higher levels of topo I and topo IIα expression in ovarian carcinomas than in benign tumors. In our and other authors' opinions, expression of topo I and topo IIα is associated with biologically aggressive tumor behavior [2, 21]. According to present data and other authors' studies [20–22], the association of topo IIα expression with advanced stages of tumors might reflect a high progressive growth of ovarian cancer. However, the higher mean of topo IIα expression found in poorly differentiated ovarian carcinomas suggests that this enzyme might be

involved in the maturation of tumor cells. Similarly to previous data, no correlation was observed between topo I expression and the staging and grading of ovarian tumors [2, 23]. However, the mean value of topo I expression was associated with poorly differentiated ovarian carcinomas in the current study. These results are in keeping with other data in which topo I expression was associated with poorly differentiated oral squamous cell carcinomas [24]. Several reports have indicated that topo I expression in human solid tumors is a marker of sensitivity to topo I-inhibitors [2, 9, 24]. Only individual data revealed the prognostic value of topo I expression in ovarian cancer [25]. Current rate of p53 immunopositivity (68.3%) in ovarian carcinomas is in line with other studies [1, 6, 26]. Taking into account the data from Hogdall et al. [26] and Lee et al. [6] and authors own results, the association between p53 protein overexpression and high FIGO stages of ovarian carcinoma suggest that p53 protein overexpression indicates a more aggressive biological phenotype of ovarian cancers. The lack of association between p53 protein overexpression and tumor grades observed in the current study is in agreement with some reports [22] but contrary to others [6]. However, the association revealed between the mean value of p53 overexpression and poorly differentiated ovarian cancers in present study is consistent with other data [26]. The observed discrepancies between present data and other investigators' may reflect differences in the scoring of p53 immunopositivity as no international consensus exists [26].

There is no data concerning the association between topo I, topo IIα and p53 protein overexpression with respect to morphological features and the progressive growth of ovarian carcinomas. The positive correlation found between topo I and topo IIα expression and high nuclear accumulation of p53 protein in ovarian carcinomas indicates that the levels of both enzymes might be regulated by the overexpression of p53 protein, and suggests that this subgroup of tumors expresses a mutant form of p53 protein [9], and so does not have the capacity to act as a negative regulator of topo I and topo IIα expression [11]. Similarly to previous data [24], the authors postulate that high levels of topo I and topo IIα expression in p53-positive ovarian carcinomas might be considered as biological parameters for therapy. The correlation revealed between topo I/p53 and topo IIα/p53-positive cases and advanced FIGO stages of ovarian cancers suggests that coexpression of topoisomerases and p53 protein determine the worse clinical features of the tumors [13] and, on the other side, identify the subgroup of patients with ovarian cancers which possess aggressive biological features. The trend to higher coexpression of topo IIα/p53+ in poorly differentiated ovarian carcinomas seems to demonstrate that the maturation of tumor cells might be regulated by the parallel levels of p53 and topo IIα expression. The lack of association between the studied biomarkers in benign ovarian tumors clearly indicates that cooperation between these biomarkers might facilitate the progression of ovarian tumors.

In conclusion, present findings suggest that cooperation between topo I, topo IIα and p53 protein is important in the progressive growth of ovarian tumors. On the other hand, simultaneous expression of the studied proteins identifies the subgroup of ovarian cancers with aggressive biological features which might be considered in therapy.

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