Histol Histopathol (2008) 23: 531-538
DOI: 10.14670/HH-23.531

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Decreased expression of p16 in ovarian cancers represents an unfavourable prognostic factor

Pawel Surowiak[1,2,3], Verena Materna[1], Adam Maciejczyk[3], Marek Pudelko[3], Slawomir Suchocki[5], Witold Kedzia[4], Ewa Nowak-Markwitz[4], Malgorzata Dumanska[2], Marek Spaczynski[4], Maciej Zabel[2,6], Manfred Dietel[1] and Hermann Lage[1]

[1]Charité Campus Mitte, Institute of Pathology, Berlin, Germany, [2]Department of Histology and Embryology, University School of Medicine, Wroclaw, Poland, [3]Lower Silesian Centre of Oncology, Wroclaw, Poland, [4]Department of Obstetrics and Gynaecology, University School of Medicine, Poznan, Poland, [5]Department of Obstetrics and Gynaecology, Voivodship Hospital, Walbrzych, Poland and [6]Department of Histology and Embryology, University School of Medicine, Poznan, Poland

Summary. Decreased expression of p16 may result from hypermethylation of the promoter or from deletion of the gene. It can lead to intensified proliferation of neoplastic cells and to cytostatic drug resistance. The study was aimed at the examination of prognostic value of p16 expression in relation to Ki67 and caspase-3 in ovarian cancers using immunohistochemistry. The immunohistochemical studies were performed on 73 paraffin-embedded samples of ovarian cancers from 43 patients and samples from 6 healthy ovaries. We have used monoclonal antibodies against p16. ABC method and DAB were used for antigens visualisation. The intensity of the immunohistochemical reactions was appraised using the semi-quantitative IRS scale. In healthy ovaries we have shown strong reaction in the nuclei of surface epithelium. In the case of studied ovarian cancers, the reaction of a nuclear and cytoplasmic localization was obtained. The mean overall immunoreactivity score of nuclear p16 expression amounted to 5.30±3.44 SD in primary laparotomy material and 6.61±4.34 SD in secondary cytoreduction material. Statistical analysis demonstrated that lower p16 expression was typical of the younger patients and the patients who died. Kaplan-Meier's analysis proved that lower expression of p16 was characteristic of cases with shorter overall survival. In the present study we have demonstrated that lowered p16 expression represented an unfavourable prognostic index in ovarian cancer. Lowered p16 expression was also typical for chemotherapy-resistant ceases (cases of lower caspase-3 and higher Ki67 at secondary cytoreduction expression).

Offprint requests to: Prof. Dr. Hermann Lage, Charité Campus Mitte, Institute of Pathology, Charité platz, D-10117 Berlin, Germany. e-mail: hermann.lage@charite.de

Key words: p16, Ovarian cancer, Immunohistochemistry, Prognosis

Introduction

Malignant tumors of the ovary present one of the most difficult problems in gynaecologic oncology. In West and North Europe as well as in the United States ovarian cancer ranks fifth in incidence of all malignant tumors in females. Incidence of ovarian cancer in European Union is estimated at 12 to 17 new cases per 100.000 inhabitants annually, which corresponds to 27.000 to 30.000 new cases annually. Throughout the world, and in highly industrialized countries in particular, a continuous increase is observed in incidence of the tumor (Holschneider and Berek, 2000; Stewart and Kleihues, 2003; Cannistra, 2004).

Only around 35% patients with ovarian cancer survive 5 years without signs of the disease and the results of treatment have not clearly improved in the last 30 years. The poor results of therapy reflect first of all the low proportion of cancers diagnosed in I stage of advancement according to FIGO, due to location of the tumor and, hence, late development of clinical signs. Around 75% of cases are diagnosed at III and IV stages of clinical advancement according to FIGO. In such advanced cases only around 20% patients survive 5 years. Despite introduction of new cytostatic drugs, such as paclitaxel or topotecan, the proportion of patients surviving 5 years has not changed in the last 20 years (Holschneider and Berek, 2000; Stewart and Kleihues, 2003; Cannistra, 2004). The principal cause of failure in treatment of ovarian cancer involves resistance of neoplastic cells to chemotherapy (Gottesman, 2002).

For several years in centres all over the world studies have continued aimed at the discovery of new

prognostic and predictive indices in ovarian cancer. Until now, however, only such classical prognostic indices as degree of clinical advancement according to FIGO or radical character of cytoreduction during the operative procedure carry practical significance and high prognostic value. However, cases of the same advancement are frequently noted to demonstrate distinct clinical courses and to manifest different sensitivity to chemotherapy. Therefore, studies on ovarian cancer are focused on two principal problems: the search for new markers which would permit screening studies for early detection of the disease, and the search for new prognostic and predictive parameters in order to select cases of a more aggressive course among cases of similar clinical traits and/or cases with exponents of resistance or sensitivity to individual cytostatic drugs, which would permit individualised approach to the chemotherapy.

In either of the contexts, studies on the p16-cyclinD1/CDK4-pRb pathway (G1 pathway) seem particularly interesting. In ovarian cancers disturbances in the system remain poorly recognised. Decreased expression of p16 may be typical for ovarian cancers of a more aggressive course (Kudoh et al., 2002; Hashiguchi et al., 2004; Katsaros et al., 2004). However, other authors failed to confirm it (Sui et al., 2000). Decreased expression of p16 is typical of ovarian cancer cells with higher proliferative potential (Ohtani et al., 2004). A few reports have described the relationship between disturbances in the G_1 pathway and resistance of ovarian cancer to chemotherapy (Kusume et al., 1999; Kudoh et al., 2002). Another important aspect involves the fact that expression of p16 in ovarian cancers is lowered, i.a., under effect of methylation of *p16* gene promoter (Ryan et al., 1998; Katsaros et al., 2004; Makaria et al., 2005). Considering the fact that hypermethylation of *p16* gene promoter plays a significant role in pathogenesis of numerous tumors (Esteller, 2005), it could also be typical for a proportion of ovarian cancers. A potential exists for detection of hypermethylation of gene promoters patients' blood (Fiegl et al., 2005), in order to detect the early presence of tumors or to monitor the course of the disease, the latter being particularly significant in ovarian cancers.

The present study was aimed at immunohistochemical examination of p16 expression in relation to Ki67, caspase-3 and clinical and pathological variables in 73 samples of ovarian cancers, originating from patients post-operatively treated with chemotherapy protocols based on platinum analogues.

Material and methods

Patients

Immunohistochemical examination was performed retrospectively on tissue samples taken for routine diagnostic purposes. Forty three patients operated in 1999-2002 due to ovarian carcinoma in the Department of Gynaecology and Obstetrics, University School of Medicine in Poznan, Poland were qualified to the studies. The cases were selected based on availability of tissue and were not stratified for known preoperative or pathological prognostic factors. The study was approved by an Institutional Review Board (IRB) and the patients gave their informed consent before their inclusion in the study. Following the first surgery (primary laparotomy – PL) all the patients were subjected to chemotherapy using platinum-based schemes (Table 1). Because merely two patients achieved optimal cytoreduction during PL, 36 patients from the same group were subjected also to the secondary cytoreduction (SCR). In 6 cases no tumor cells were detected in the material originating from the SCR. The patients were monitored by periodic medical check-ups, CA-125 serum levels, ultrasonographic and radiological examinations. During the follow-up period, 22 patients (51%) had a recurrent disease and 13 patients (30%) died of the disease. The mean progression-free survival time was 16.9 months (range 0 to 52 months), while the mean overall-free survival time was 24.6 months (range 6 to 52 months).

Fragments sampled from studied tumors were fixed in 10% buffered formalin and, then, embedded in paraffin. In each case, hematoxylin and eosin stained preparations were subjected to histopathological

Table 1. Patient and tumor characteristics.

Characteristics	No. (%)[3]
All patients	43 (100)
Age (mean 51.0)[1]	
≤ 50	20 (47)
50-60	16 (37)
>60	7 (16)
Grade[1]	
1	7 (16)
2	18 (42)
3	18 (42)
FIGO[1]	
I	1 (2)
II	1 (2)
III	41 (95)
Histology[1]	
Serous	37 (86)
Endometrioid	3 (7)
Other	3 (7)
Clinical response[2]	
Complete response	16 (37)
Stable disease	5 (12)
Progressive disease	22 (51)
Chemotherapy (in total)	
Cisplatin/Paclitaxel	31 (72)
Cisplatin/Cyclophosphamide/Adriblastin	6 (14)
Cisplatin/Cyclophosphamide/Paclitaxel	3 (7)
Cisplatin/Cyclophosphamide/Paclitaxel/Adriblastin	2 (5)
Carboplatin/Paclitaxel	1 (2)

[1]: Data are given for the first operation/diagnosis implemented; [2]: According to RECIST (Response Evaluation Criteria in Solid Tumors) (Therasse et al., 2000); [3]: Differences in the sum to 100 % in groups are due to rounding.

evaluation by two independent pathologists. The stage of the tumors was assessed according to the International Federation of Gynaecology and Obstetrics (Sobin and Wittekind, 2002). Tumors were graded according to the Silverberg grading system (Shimizu et al., 1998).

Immunohistochemistry

Formalin-fixed paraffin embedded tissue was freshly cut (4 μm). The sections were mounted on Superfrost slides (Menzel Gläser, Germany), dewaxed with xylene, and gradually rehydrated. The activity of endogenous peroxidase was blocked by 30 min incubation in 1% H_2O_2. The sections were boiled for 10 min in a microwave oven, in Antigen Retrieval Solution (DakoCytomation, Denmark) at 500W. This was followed by immunohistochemical reactions using monoclonal (ZJ11) mouse antibodies directed against p16 (Chemicon International, Temecula, CA, USA). The antibodies were diluted 1:100 in the Antibody Diluent, Background Reducing (DakoCytomation, Denmark). The sections were incubated with an antibody for 1 hour at room temperature. Subsequently, they were incubated with biotinylated antibodies (15 min, room temperature) and with the streptavidin-biotinylated peroxidase complex (15 min, room temperature) (LSAB+, HRP, DakoCytomation, Denmark). DAB (DakoCytomation, Denmark) was used as a chromogen, employing 7 min incubation at room temperature. All the sections were counterstained using Meyer's hematoxylin. Every reaction was accompanied by the negative control in which specific antibody was substituted by the Primary Mouse Negative Control (DakoCytomation, Denmark).

The procedures of immunohistochemical detection of Ki67 (Surowiak et al., 2006) and caspase-3 (Materna et al., 2007) expressions were described previously.

Control reactions

We also performed control reactions on six samples of healthy human ovaries (from the archive of Dept. of Histology and Embryology, University School of Medicine, Wroclaw, Poland).

Scoring of immunostaining results

The intensity of the immunohistochemical reactions was appraised using the semi-quantitative Immuno-Reactive Score (IRS) scale, in which intensity of the reaction and percentage of positive cells was scored (Table 2). The final result represented a product of scores given for individual traits and ranged between 0 and 12 (Remmele and Stegner, 1987). The intensity of immunohistochemical reactions was evaluated independently by two pathologists. In cases of divergencies, the evaluation was repeated using a double-headed microscope.

Table 2. Evaluation criteria of nuclear p16 expression using the immunoreactive score (10).

Percentage of positive cells	Points	Intensity of reaction	Points
No positive cells	0	No reaction	0
<10% of positive cells	1	Weak reaction	1
10–50% of positive cells	2	Moderate reaction	2
51–80% of positive cells	3	Intense reaction	3
>80 of positive cells	4		

Statistical analysis

Statistical analysis of the results took advantage of Statistica 98 PL software (Statsoft, Poland). The employed tests included U Mann-Whitney's, Spearman's rank correlation and Chi^2 tests. Kaplan-Meier's statistics and log-rank tests were performed using SPSS software (release 10.0; SPSS Inc., Chicago, IL, USA) to estimate the significance of differences in survival times. The length of progression-free survival was defined as the time between the primary surgical treatment and diagnosis of a recurrent tumor or death. Since using an univariate analysis no significant relationships were disclosed between studied clinicopathological parameters (age, histology, grade, CA-125 at PL level) and overall survival and progression free time of studied patients ($P>0.05$), no multivariate analysis was performed. Since 95% of the studied patients were at the stage FIGO III, we did not investigate relationships between stage and survival data.

We have also performed Kaplan-Meier's statistics and log-rank tests on the subgroup of 35 FIGO III patients receiving post-surgical platinum and paclitaxel containing combination therapy.

Results

p16 immunostaining in control preparations and in ovarian cancers

In healthy ovaries we have shown a strong reaction in the nuclei of surface epithelium (Fig. 1A). In the case of studied ovarian cancers, the reaction of a nuclear and cytoplasmic localization was obtained, of variable intensity in individual cases (Fig. 1B). The mean overall immunoreactivity score of nuclear p16 expression amounted to 5.30±3.44 SD (min. 0, max. 12) in PL material and 6.61±4.34 SD (min. 0, max. 12) in SCR material.

At the first stage of statistical analysis the Mann-Whitney's U test was employed to compare the overall immunoreactivity score of p16 expression at PL and SCR. The test demonstrated no significant differences (P=0.21).

Using the Chi^2 test, relationships were examined between the overall immunoreactivity score of p16 expression on one hand and histological type of the tumor and grade on the other. No significant

relationships were detected (Table 3). Using the Spearman's rank correlation, relationships were examined between the overall immunoreactivity score of p16 expression on one hand and patient age and CA-125 concentrations on the other (Table 3). We have shown a positive correlation between overall immunoreactivity score of p16 at SCR expression and patients age (*P=0.03*) (Table 3).

p16 expression in relation to Ki67 and caspase-3

Intensity of the Ki67 and caspase-3 expression in the sections from the studied cases have been described in our previous studies (Surowiak et al., 2006; Materna et al., 2007). We have also described the prognostic predictive value of Ki67 and caspase-3 expression. In this study we have analysed relationships between p16 on the one hand and Ki67 and caspase-3 on the other. The computations have shown the following relationships: negative correlation between p16 at PL and caspase-3 at PL, negative correlation between p16 at PL and Ki67 at SCR, positive correlation between p16 at PL and caspase-3 at SCR and a positive correlation between p16 at SCR and caspase-3 at SCR (Table 4).

p16 expression and patients survival

At the first stage of statistical analysis of relationships between p16 expression and survival of the patients, Chi^2 test was used. The relationships were examined between the overall immunoreactivity score of p16 expression on one hand and clinical response to chemotherapy, relapses and patient deaths on the other. We have shown that patients who died had a significantly lower overall immunoreactivity score of p16 at PL expression (P<0.001) (Table 3) (Fig. 2).

In Kaplan-Meier's analysis overall survival time and

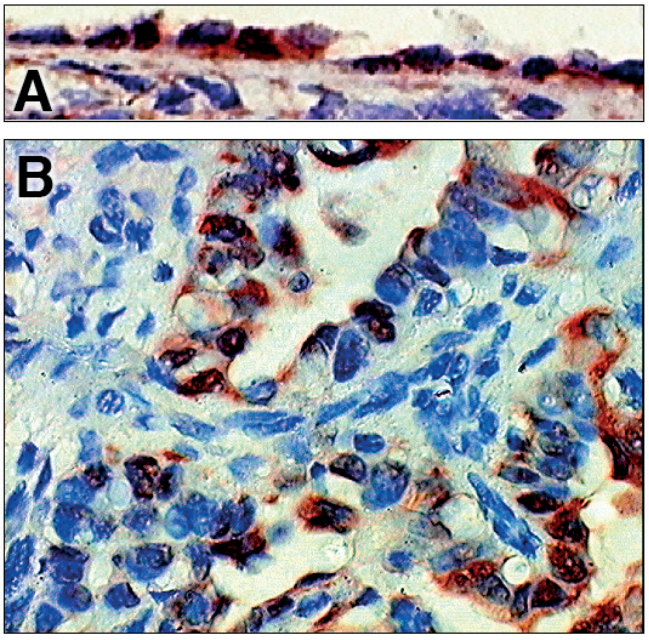

Fig. 1. Immunohistochemical p16 localization in: **(A)** healthy human ovarian surface epithelium (hematoxylin, x 400), **(B)** ovarian carcinoma (hematoxylin, x 400).

Table 3. Correlation between p16 expression and various clinicopathological factors.

Characteristics	p16 at PL expression *P* value / test	p16 at SCR expression *P* value / test
Histologic type[a]	0.6289 Chi^2 test	-
Grade[a]	0.7198 Chi^2 test	-
Age	0.6268 Spearman's rank correlation	0.0325, R=0.38 Spearman's rank correlation
CA-125 serum levels at PL	0.7139 Spearman's rank correlation	0.2646 Spearman's rank correlation
CA-125 serum levels at SCR	0.5194 Spearman's rank correlation	0.3204 Spearman's rank correlation
Clinical response	0.2126 Chi^2 test	0.7149 Chi^2 test
Relapses	0.1321 Chi^2 test	0.4197 Chi^2 test
Deaths	0.0008 Chi^2 test	0.1363 Chi^2 test

PL: primary laparotomy; SCR: secondary cytoreduction; [a]: Relationships between p16 expression at SCR on one hand and histological type and grade on the other were not examined.

Table 4. Realationships between p16 and Ki67 and caspase-3 expression (Spearman's rank correlation).

	p16 at PL	p16 at SCR
p16 at PL	-	P=0.267403 R=0.205503
Ki67 at PL	P=0.498075 R=-0.106157	P=0.783796 R=0.051357
caspase-3 at PL	P=0.001878 R=-0.460706	P=0.685310 R=-0.088608
p16 at SCR	P=0.267403 R=0.205503	-
Ki67 at SCR	P=0.000102 R=-0.649852	P=0.756220 R=-0.059143
caspase-3 at SCR	P=0.020004 R=0.422559	P=0.018606 R=0.437176

PL: primary laparotomy; SCR: secondary cytoreduction.

progression-free time were compared between groups showing lower (IRS 0-4) and higher (IRS 6-12) overall immunoreactivity score of p16 expression at PL and SCR. The computations demonstrated that lower expression of p16 at PL was typical for cases of a shorter overall survival time (P=0.015) (Fig. 3).

In the subgroup of 35 FIGO III patients receiving

Table 5. Relationships between overall survival time (OS) and progression-free survival (PFS) and expression of p16 in the subgroup of FIGO stage III patients treated with platinum-based drugs and paclitaxel.

	PL n=35	SCR n=24
	Score 0-4 n=16 Score 6-12 n=19	Score 0-4 n=10 Score 6-12 n=14
Overall survival	P=0.1002	P=0.1801
Progression-free survival	P=0.9793	P=0.6006

A

	p16 [IRS score]	FIGO	Grade	Age	Death	Progression	Response
Lower p16 expression (IRS 0-4)	0	3	3	53	1	1	PD
	0	3	3	40	1	1	PD
	0	3	2	46	1	1	PD
	0	3	3	51	1	1	PD
	1	3	2	56	1	1	PD
	1	3	2	49	1	1	PD
	2	3	3	37	1	1	PD
	2	3	1	51	1	1	PD
	2	3	3	45	1	1	PD
	2	3	2	54	0	0	SD
	3	3	3	47	0	1	PD
	3	2	1	48	1	1	PD
	3	3	2	40	0	0	CR
	3	3	1	54	0	0	CR
	3	3	3	74	0	0	SD
	3	3	2	52	0	0	SD
	4	3	1	52	0	0	CR
	4	3	3	58	0	0	CR
	4	3	2	56	0	0	CR
	4	3	2	44	0	0	CR
	4	3	3	67	0	1	PD
Higher p16 expression (IRS 6-12)	6	3	2	37	0	1	PD
	6	3	2	51	0	0	CR
	6	3	3	49	0	0	CR
	6	3	3	50	1	1	PD
	6	3	3	50	0	0	CR
	6	3	3	72	0	1	PD
	6	3	2	58	0	0	SD
	8	3	2	56	0	1	PD
	8	3	3	71	0	0	CR
	8	3	3	51	0	1	PD
	8	3	3	47	0	0	CR
	8	3	2	48	0	0	CR
	8	1	1	30	0	0	CR
	9	3	2	34	0	0	CR
	9	3	2	42	1	1	PD
	9	3	3	50	0	0	CR
	9	3	2	51	1	1	PD
	9	3	1	66	0	1	PD
	9	3	2	33	0	1	PD
	12	3	2	61	0	0	CR
	12	3	3	62	0	1	PD
	12	3	1	52	0	0	SD

B

	p16 [IRS score]	FIGO	Grade	Age	Death	Progression	Response
Lower p16 expression (IRS 0-4)	0	3	2	42	1	1	PD
	0	3	3	53	1	1	PD
	0	3	3	40	1	1	PD
	0	3	2	46	1	1	PD
	2	3	2	34	0	0	CR
	2	3	2	48	0	0	CR
	3	3	1	52	0	0	CR
	3	3	1	51	1	1	PD
	3	3	2	49	1	1	PD
	4	3	3	50	0	0	CR
	4	3	1	52	0	0	SD
	4	3	2	54	0	0	SD
	4	3	2	44	0	0	CR
Higher p16 expression (IRS 6-12)	6	3	2	56	1	1	PD
	6	3	3	47	0	1	PD
	6	3	2	58	0	0	SD
	8	3	3	58	0	0	CR
	8	3	3	37	1	1	PD
	8	3	3	71	0	0	CR
	8	3	2	52	0	0	SD
	9	3	2	37	0	1	PD
	9	3	2	51	0	0	CR
	12	2	1	48	1	1	PD
	12	3	3	49	0	0	CR
	12	3	2	51	1	1	PD
	12	3	3	45	1	1	PD
	12	3	2	61	0	0	CR
	12	3	2	56	0	0	CR
	12	3	1	54	0	0	CR
	12	3	3	74	0	0	SD
	12	3	3	67	0	1	PD

Fig. 2. Expression of p16 **(A)** at primary laparotomy and **(B)** at secondary cytoreduction and clinical and pathological data on the patients. CR: complete response, SD: stable disease, PD: progressive disease.

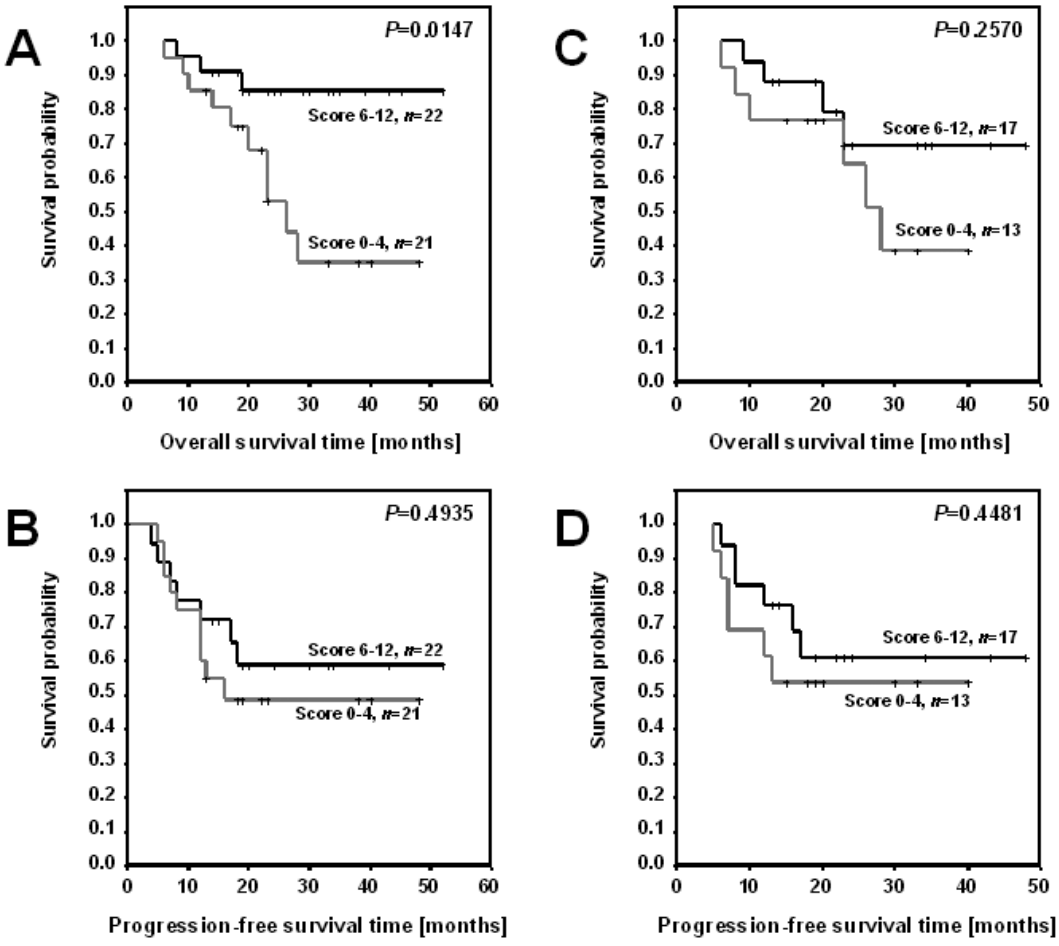

Fig. 3. Kaplan-Meier curves for survival and expression of p16 in the studied group of 43 ovarian cancer patients: p16 expression at primary laparotomy and **(A)** overall survival and **(B)** progression-free survival. p16 expression at secondary cytoreduction and **(C)** overall survival and **(D)** progression-free survival.

post-surgical platinum and paclitaxel containing combination therapy the analysis has shown no significant relationships between p16 expression and overall survival time and progression-free time (Table 5).

Discussion

In this study we have described the expression of p16, detected by immunohistochemistry in malignant epithelial ovarian tumors, in the samples from primary laparotomies (PL) and secondary cytoreductions (SCR) in the group of Polish patients. We have demonstrated that normal surface epithelium of ovaries manifests strong nuclear expression of p16 (Fig. 1A). In primary tumors of ovaries in four cases no p16 expression was detected, similarly to tumor samples originating from SCR. In samples originating either from PL or from SCR a decreased (smaller than mean) p16 expression has been observed in around 50% of cases (Fig. 2A,B). Thus, ovarian cancers have been shown to manifest inhibited expression of p16. We have shown no significant relationships between p16 expression at PL and at SCR. In the present study, expression of p16 was examined by immunohistochemistry. Thus, decreased expression of the marker in the studied cases could be linked either with hypermethylation or, as described by Kudoh et al. (2002), with deletion of the gene.

The significance of disturbances in the p16-cyclin D1/CDK4-pRb pathway (G_1 pathway) in ovarian cancers has already been described in several studies. Cyclin D1 is regulated by p16, whereas the cyclin D1-activated CDK4 phosphorylates pRb. As a sequel, this leads to the release of proteins such as E2F, which show the potential to activate genes indispensable for cell cycle progression through the G1 phase. p16 controls cell cycle at the G1 phase, inhibiting the capacity of complexes consisting of D/CDK4 cyclins and D/CDK6 cyclins to phosphorylate pRb. Thus, decreased expression of p16 leads to intensified proliferation of tumor cells (Ohtani et al., 2004). In this work we have studied the relationship between p16 expression and proliferation markers Ki67. We have show (in the PL and SCR material), that there is no correlation between p16 and Ki67 expression. It suggests that in the case of ovarian cancers, p16-dependent influence on the cell

cycle can be compensated by other cell cycle regulators. The negative correlation between p16 at PL expression and Ki67 at SCR shows that in the cases of lower p16 at PL expression, proliferation of tumor cells after chemotherapy is more pronounced as compared to the cases of higher p16 at PL expression. It suggests that a decrease in p16 expression can be typical for chemotherapy-resistant ovarian cancer cases. Kusume et al. (1999) demonstrated that patients with ovarian cancer who showed no disturbances in the G_1 pathway tended to achieve a higher complete response rate to chemotherapy. The patients also manifested longer overall survival time. Similarly, Kudoh et al. (2002) examined disturbances in the G_1 pathway in ovarian cancer. They showed that homozygous deletion of *P16/CDKN2* was characteristic of patients who did not respond to chemotherapy by remission, and of patients with shorter overall survival. In this work we have also shown that lowered p16 at PL expression is typical for the cases of higher at PL and of lower at SCR caspase-3 expression. The above mentioned patients are known to be chemotherapy-resistant (Materna et al., 2007). So, the data confirm suggestions that lowered p16 at PL expression can be typical for chemotherapy-resistant ovarian cancer cases.

There are a lot of discrepancies regarding p16 expression and its relation to histological type and grade of the tumor. Milde-Langosch et al. (1998) have shown that loss of p16 expression is typical for endometroid and mucinous ovarian cancer. Armes et al. (2005) have shown similar p16 expression intensity in serous and endometroid tumors. Also, other authors (Fujita et al., 1997; Kommos et al., 2007) have shown no significant differences in p16 expression between different histological types. O'Neill et al. (2007) have shown that high-grade ovarian serous carcinoma exhibit significantly higher p16 expression than low-grade serous carcinoma. In our study we have shown, that p16 expression intensity is independent of histological type or grade of the tumor.

The present study has shown that patients who deceased manifested a significantly lower expression of p16 in samples from primary laparotomies. Analysis of Kaplan-Meier demonstrated that lowered expression of p16 in samples from primary laparotomies characterized cases of a shorter overall survival time. Hashiguchi et al. (2004) documented that the status of the G_1 pathway was an independent prognostic factor in ovarian carcinomas. Sui et al. (2000) examined expression of CDK4 and p16 in samples of ovarian cancer. They found no relationship between expression of the proteins and duration of the patients' survival. Kommoss et al. (2007) have shown that among p16 positive ovarian cancer cases, survival was better for patients with intermediate expression as compared to low or high expression levels.

In conclusion, the present study demonstrated that lowered expression of p16 was typical of ovarian cancer patients with shorter survival. Lowered p16 expression was also typical of tumors resistant to cytostatic drugs. Thus, immunohistochemical estimation of a decreased p16 expression, linked to promoter hypermethylation or deletion of the gene, was shown to exhibit a real prognostic significance and to be more universal than analysis of several genetic disturbances (methylation, deletion, polymorphism etc.). Further studies on disturbances in the G_1 pathway may allow development of a new prognostic panel and better understanding of pathogenesis in ovarian cancers.

References

Armes J.E., Lourie R., de Silva M., Stamaratis G., Boyd A., Kumar B., Proce G., Hyde S., Allen D., Grant P. and Venter D.J. (2005). Abnormalities of the RB1 pathway in ovarian serous papillary carcinoma as determined by overexpression of the p16(INK4A) protein. Int. J. Gynecol. Pathol. 24, 363-368.

Cannistra S.A. (2004). Cancer of the ovary. N. Engl. J. Med. 351, 2519-2529.

Esteller M. (2005). Aberrant DNA methylation as a cancer-inducing mechanism. Annu. Rev. Pharmacol. Toxicol. 45, 629-656.

Fiegl H., Millinger S., Mueller-Holzner E., Marth C., Ensinger C., Berger A., Klocker H., Goebel G. and Widschwendter M. (2005). Circulating tumor-specific DNA: A marker for monitoring efficacy of adjuvant therapy in cancer patients. Cancer Res. 65, 1141-1145.

Fujita M., Enomoto T., Haba T., Nakashima R., Sasaki M., Yoshino K., Wada H., Buzurd G.S., Matsuzaki N., Wakasa K. and Murata Y. (1997). Alteration of p16 and p15 genes in common epithelial ovarian tumors. Int. J. Cancer 74, 148-155.

Gottesman M.M. (2002). Mechanisms of cancer drug resistance. Annu. Rev. Med. 53, 615-627.

Hashiguchi Y., Tsuda H., Inoue T., Nishimura S., Suzuki T. and Kawamura N. (2004). Alteration of cell cycle regulators correlates with survival in epithelial ovarian cancer patients. Hum. Pathol. 35, 165-175.

Holschneider C.H. and Berek J.S. (2000). Ovarian cancer: epidemiology, biology, and prognostic factors. Seminars. Surgical Oncol. 19, 3-10.

Katsaros D., Cho W., Singal R., Fracchioli S., Rigault de la Longrais I.A., Arisio R., Massobrio M., Smith M., Zheng W., Glass J. and Yu H. (2004). Methylation of tumor supressor gene *p16* and prognosis of epithelial ovarian cancer. Gynecol. Oncol. 94, 685-692.

Kommoss S., du Bois A., Ridder R., Trunk M.J., Schmidt D., Pfisterer J. and Kommoss F. the AGO-OVAR. (2007). Independent prognostic significance of cell cycle regulator proteins p16(INK4a) and pRb in advanced-stage ovarian carcinoma including optimally debulked patients: a translational research subprotocol of a randomised study of the Arbeitsgemeinschaft Gynaekologische Onkologie Ovarian Cancer Study Group. Br. J. Cancer 96, 306-313.

Kudoh K., Ichikawa Y., Yoshida S., Hirai M., Kikuchi Y., Nagata I., Miwa M. and Ichida K. (2002). Inactivation of *p16/CDKN2* and *p15/MTS2* is associated with prognosis and response to chemotherapy in ovarian cancer. Int. J. Cancer 99, 579-582.

Kusume T., Tsuda H., Kawabata M., Inoue T., Umesaki N., Suzuki T. and Yamamoto K. (1999). The p16-Cyclin D1/CDK4-pRb pathway and clinical outcome in epithelial ovarian cancer. Clin. Cancer Res. 5, 4152-4157.

Makaria P.B., Saboorian M.H., Ashfag R., Toyooka K.O., Minna J.D., Gazdar A.F. and Schorge J.O. (2005). Promoter hypermetylation

profile of ovarian epithelial neoplasms. Clin. Cancer Res. 11, 5365-5369.

Materna V., Surowiak P., Markwitz E., Spaczynski M., Drag-Zalesinska M., Zabel M. and Lage H. (2007). Expression of factors involved in regulation of DNA mismatch repair- and apoptosis pathways in ovarian cancer patients. Oncol. Rep. 17, 505-516.

Milde-Langosch K., Ocon E., Becker G. and Loning T. (1998). p16/MTS1 inactivation in ovarian carcinomas: high frequency of reduced protein expression associated with hyper-methylation of mutation in endometrioid and mucinous tumors. Int. J. Cancer 79, 61-65.

O'Neill C.J., McBride H.A., Connolly L.E., Deavers M.T., Malpica A. and McCluggage W.G. (2007). High-grade ovarian serous carcinoma exhibits significantly higher p16 expression than low-grade serous carcinoma and serous borderline tumor. Histopathology 50, 773-779.

Ohtani N., Yamakoshi K., Takahashi A. and Hara E. (2004). The *p16INK4a-RB* pathway: molecular link between cellular senescence and tumor supression. J. Med. Invest. 51, 146-153.

Remmele W. and Stegner H.E. (1987). Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. Pathologe 8, 138-140. (in German).

Ryan A., Al-Jehani R.M., Mulligan K.T. and Jacobs I.J. (1998). No evidence exists for methylation inactivation of the p16 tumor suppressor gene in ovarian carcinogenesis. Gynecol. Oncol. 68, 14-17.

Shimizu Y., Kamoi S., Amada S., Akiyama F. and Silverberg S.G. (1998). Toward the development of a universal grading system for ovarian epithelial carcinoma: testing of a proposed system in a series of 461 patients with uniform treatment and follow-up. Cancer 82, 893-901.

Sobin L.H. and Wittekind C. (2002). TNM classification of malignant tumors. Wiley-Liss. New York.

Stewart W. and Kleihues P. (2003). World cancer report. IARC Press, Lyon. pp 220-222.

Sui L., Dong Y., Ohno M., Goto M., Inohara T., Sugimoto K., Tai Y., Hando T. and Tokuda M. (2000). Inverse expression of Cdk4 and p16 in epithelial ovarian tumors. Gynecol. Oncol. 79, 230-237.

Surowiak P., Materna V., Kaplenko I., Spaczy*ƒ*ski M., Dietel M., Lage H. and Zabel M. (2006). Topoisomerase 1A, HER-2/neu and Ki67 expression in paired primary and relapse ovarian cancer tissue samples. Histol. Histopathol. 21, 713-720.

Therasse P., Arbuck S.G., Eisenhauer E.A., Wanders J., Kaplan R.S., Rubinstein L., Verweij J., Van Glabbeke M., van Oosterom A.T., Christian M.C. and Gwyther S.G. (2000). New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J. Natl. Cancer Inst. 92, 205-216.

Accepted November 5, 2007