Abstract. The role of tumor suppressor p21WAF1 expression in epithelial ovarian cancer has not been definitely explained and the clarification of mutual p53 and p21WAF1 relations concerning proliferative activity seems to be very important for understanding of a functional link between p53 and cell-cycle control. Therefore the expression of p53 and p21WAF1 was assessed immunohistochemically in a series of 50 ovarian carcinomas considering clinicopathological variables. The reactivity of three anti-p53 monoclonal antibodies (DO-7, PAb240, PAb1620) recognizing immunologically distinct forms of p53 were analysed in relation to p21WAF1 level in individual patients. p21WAF1 was expressed in 24 (48%) of all cases. The detection of p53 protein was related to the antibody applied and DO-7 antibody appears to be better than both PAb240 and PAb1620. However, independently of antibody used significant inter- and intratumoral heterogeneity in p53 and p21WAF1 expression was revealed. The identification of different p53/p21WAF1 phenotypes reflect the complex and multiple relations between these two cell-cycle regulators indicating that in ovarian carcinomas p21WAF1 activation may be both p53-dependent and p53-independent. High cell proliferation was usually accompanied by undetectable or weak p21WAF1 staining. There was no significant correlation between p53 and p21WAF1 expression and histology, stage and grade of ovarian carcinomas (p>0.05).

Introduction

Defects in the regulatory mechanisms responsible for cell-cycle control are crucial in malignant transformation and tumor progression. The control of the cell-cycle is closely associated with interactions of cyclines, cyclin-dependent kinases and cyclin-dependent kinase inhibitors. When the DNA of cells is damaged wild-type p53 protein is stabilized and activates the expression of p21WAF1, a universal inhibitor of cyclin-dependent kinases causing growth arrest of cells (1,2). In several studies strong associations between DNA damage, p53 abnormalities and lack of detectable p21WAF1 expression have been found (2-5). Although elevated levels of wild-type p53 protein can transcriptionally induce p21WAF1, several pathways of p53-independent activation of p21WAF1 have been described (6,7) and confirmed by frequently observed lack of correlation between p53 and p21WAF1 expression (7-11).

It is also known that p21WAF1 protein can bind to proliferating cell nuclear antigen (PCNA) and blocks the DNA replication (9,11). However, the possible association between p21WAF1 and cell proliferation remains controversial (10,12-16).

Clarification of a mutual p53 and p21WAF1 relation considering proliferative activity seems to be very important for the understanding of a functional link between p53 and cell-cycle control in malignant neoplasms.

The relation between the p53 and p21WAF1 expression in ovarian carcinomas was described in several reports but the results are not uniform. The majority of authors were unable to detect clear inverse correlation between p53 functional status and p21WAF1 expression (6,7,10,17) while some reports indicate that in ovarian epithelial cancers p21WAF1 expression is mostly p53-dependent (3,12,18).

The present studies were undertaken to examine the relationship between immunohistochemically detected p53 accumulation and p21WAF1 expression in a series of epithelial ovarian carcinomas, considering clinicopathological variables and PCNA activity. For the first time the reactivity of three anti-p53 monoclonal antibodies recognizing immunologically different p53 epitopes were analyzed in relation to the p21WAF1 level in tumor material of individual patients.

Materials and methods

Tissue specimens. Tumor tissue sections were derived at the time of initial surgery from 50 ovarian carcinomas and 10 ovarian cystadenomas. No low malignant potential tumors were included. All patients were hospitalized at the First Department of Gynecology, Medical University, Wroclaw,
Poland (Head Dr M. Gabrys). For each case routine hematoxylin and eosin staining determined histopathologic diagnosis. As can be seen in Table I serous carcinomas were most common (27 tumors) followed by endometrioid (12 cases), undifferentiated (7 cases) and mucinous tumors (4 cases) (54, 24, 14, 8% respectively). All carcinomas were staged according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO). Nine patients were classified as FIGO stage I (18%), 4 as stage II (8%), 17 as stage III (34%) and 20 as stage IV (40%). According to routine WHO histological grading system (19), 17 tumors were poorly differentiated (39.5%), 16 moderately (37.2%), 10 well differentiated (23.2%), and 7 cases were classified as undifferentiated carcinomas (Table II). Among benign ovarian neoplasms 7 serous and 3 mucinous cystadenomas were recognized. Ten histologically normal ovaries removed from patients undergoing surgery for non-neoplastic diseases were also included in the study.

**Table I. Relations between p53, p21\(^{WAF1}\) and PCNA overexpression and histological type and FIGO stages of ovarian carcinomas.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>p53 positive with</th>
<th>p21(^{WAF1}) positive</th>
<th>PCNA positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>p53 positive</td>
<td>p21(^{WAF1}) positive</td>
<td>PCNA positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DO-7</td>
<td>PAb240</td>
<td>PAb1620</td>
</tr>
<tr>
<td>Histological type*</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Serous</td>
<td>27</td>
<td>19 (70.4)</td>
<td>9 (33.3)</td>
<td>9 (33.3)</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>12</td>
<td>8 (66.7)</td>
<td>2 (16.6)</td>
<td>3 (25.0)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>7</td>
<td>2 (28.6)</td>
<td>1 (14.3)</td>
<td>3 (42.8)</td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I/II</td>
<td>13</td>
<td>5 (38.5)</td>
<td>1 (7.7)</td>
<td>1 (7.7)</td>
</tr>
<tr>
<td>III/IV</td>
<td>37</td>
<td>24 (64.9)</td>
<td>11 (29.7)</td>
<td>14 (37.8)</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>29 (58.0)</td>
<td>12 (24.0)</td>
<td>15 (30.0)</td>
</tr>
</tbody>
</table>

*Not statistically significant, p>0.05.

<table>
<thead>
<tr>
<th>Grade of differentiation</th>
<th>Total</th>
<th>p53 positive with</th>
<th>p21(^{WAF1}) positive</th>
<th>PCNA positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>p53 positive</td>
<td>p21(^{WAF1}) positive</td>
<td>PCNA positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DO-7</td>
<td>PAb240</td>
<td>PAb1620</td>
</tr>
<tr>
<td>Well (G1)</td>
<td>10</td>
<td>4 (40.0)</td>
<td>1 (10.0)</td>
<td>1 (10.0)</td>
</tr>
<tr>
<td>Moderate (G2)</td>
<td>16</td>
<td>12 (75.0)</td>
<td>7 (43.7)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>Poor (G3)</td>
<td>17</td>
<td>10 (58.8)</td>
<td>3 (17.6)</td>
<td>7 (41.2)</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>26 (61.9)</td>
<td>11 (26.1)</td>
<td>13 (30.9)</td>
</tr>
</tbody>
</table>

*Not statistically significant, p>0.05.

**Antibodies.** For immunohistochemical staining the three following monoclonal antibodies against p53 protein were applied: DO-7 (Dako, Denmark); original IgG concentration: 0.3 ng/ml dilution 1:25; PAb240 and PAb1620 (Medical Biochemistry and Molecular Biology, University of the Saarland, Homburg, Germany), original IgG concentration of both antibodies was 1 mg/ml, dilution 1:25. DO-7 antibody reacts both with wild-type and mutant p53 protein, recognizing an epitope between amino acids 21 and 25 (20). PAb240 antibody recognizes a conformation-dependent epitope expressed in the majority of mutant p53 protein. It is a well defined linear sequence localized between residues 213 and 217 on the human p53 protein (21,22). The epitope detected by PAb1620 remains not well defined and seems to react with the wild-type p53 conformation (21). For detection of p21\(^{WAF1}\) protein mouse monoclonal antibody, clone 4D10; (NCL-WAF1, Novocastra Lab. Ltd. UK) at a working dilution of 1:20 was...
PAb1620 staining was detected in 15 of 50 (30%) cases and cells were higher with DO-7 than with PAb240 antibody. PAb240. The intensity of staining and percentage of reactive cases, whereas 12 (24%) of carcinomas reacted with antibodies showed a clear nuclear staining. p53 expression localization were found. p53 detectable by DO-7 and PAb240 antibodies used benign ovarian neoplasms were consequently negative for both p21 WAF1 and p53. The lack of p53 reactivity was observed with all three anti-p53 antibodies used. PCNA expression was assessed using monoclonal anti-PCNA antibody, clone PC10; (NCL-PCNA Novocastra Lab. Ltd., UK) at 1:50 dilution.

Immunohistochemical staining. The direct peroxidase-antiperoxidase test was performed on cryostat acetone fixed tissue sections as previously described (23). After inhibition of endogenous peroxidase and 30-min incubation with 1% normal swine serum the sections were treated with primary antibodies against p53 protein (DO-7, PAb240, PAb1620) and against p21 WAF1 protein and against PCNA. The appropriate dilutions of all antibodies were determined in preliminary immunohistochemical staining. Replacement of the primary antibody by Tris-buffer served as a negative control. After 60-min incubation with the primary antibodies peroxidase conjugated rabbit anti-mouse IgG (Dako, Denmark) was applied for 30 min. After washing with Tris-buffer, the sections were treated with peroxidase-conjugated swine anti-rabbit IgG (Dako, Denmark) followed by 3′3′-diaminobenzidine (Sigma St. Louis, MO) as a chromogen.

The preparations were counterstained with hematoxylin and mounted. Immunohistochemical staining was evaluated by two independent observers using a double-headed BHS Olympus microscope. Because of intratumoral heterogeneity of staining the average percentage of reactivity of at least 20 microscopic fields were evaluated in each sample. According to recently published data (17) and our earlier report (24) the tumors were judged as p53 positive if immunostaining was observed in more than 5% of cells. Any clearly visible nuclear staining for p21 WAF1 and PCNA was considered positive.

Statistical analysis. The relation between p53, p21 WAF1 and PCNA expression and clinicopathological parameters of the disease were analysed by Chi-square test. The level of statistical significance was p<0.05. In addition, the relationship between p21 WAF1 and PCNA reactivity was evaluated by Spearman correlations.

Results

p53, p21 WAF1 and PCNA expression in normal ovaries. All specimens of normal ovarian surface epithelium were negative for both p21 WAF1 and p53. The lack of p53 reactivity was observed with all three anti-p53 antibodies used. PCNA expression was observed in 9 of 10 normal ovaries and the mean percentage of stained cells was 10% (range 5-20%).

differences in the percentage of reactive cases and p53 protein expression detected by all three antibodies was shown in 4 (8%) carcinomas however in 18 (36%) cases only reactivity with DO-7, and in 4 of all cases (8%) only staining with PAb1620 was detected. Positive correlation between PAb240 and PAb1620 reactivity was shown in 4 (8%) carcinomas and in 8 (16%) cases only PAb240 and in 11 (22%) carcinomas only PAb1620 staining was observed. In no case the coexistence of PAb240 and PAb1620 reactivity without accompanied DO-7 positivity was found. A comparison of p53 expression detectable by three different monoclonal antibodies to clinicopathological features of ovarian carcinomas showed that independently of the antibody used no statistically confirmed correlation between p53 reactivity and histology, stage of disease, and grade of differentiation was found (p>0.05). However, p53 protein expression detected by all three antibodies was observed more often in FIGO stages III/IV than in I/II and p53 reactivity with DO-7 and PAb240 tended to be more frequently associated with serous than non-serous histology of ovarian carcinomas. PAb1620 reacted more often with poorly differentiated carcinomas. Independently of the antibody used benign ovarian neoplasms were consequently negative for p53 staining.

p21 WAF1 immunoreactivity. p21 WAF1 immunostaining was confined to tumor cell nuclei. The mean percentage of p21 WAF1 positive nuclei was 4% (range 0-30%). p21 WAF1 expression did not correlate with histology, tumor grade and FIGO stage (p>0.05). In three serous cystadenomas (33%) a weak p21 WAF1 positivity limited to 5-10% of cells was observed.

PCNA immunoreactivity. Fig. 1 presents the comparison between PCNA expression in ovarian carcinomas and benign ovarian neoplasms.

![Figure 1. PCNA expression in individual patients with malignant and benign ovarian neoplasms.](image)
tumors. Forty-eight out of 50 (96%) ovarian carcinomas showed heterogeneous PCNA staining which was independent of histology and clinical advancement of the disease (p>0.05) (Table I) however, it was higher in poorer differentiated carcinomas (p=0.03). Two cases with undetectable PCNA activity were well differentiated mucinous carcinomas in FIGO stage I of the disease. All p53 reactive ovarian carcinomas were highly positive for PCNA staining. In benign tumors PCNA expression was observed in 8 out of 10 cases (80%) however, the intensity of staining and mean percentage of reactive cells was lower than in ovarian carcinomas (52.4% in carcinomas vs. 22% in benign neoplasms) (Fig. 1).

Relation between p53 and p21WAF1 proteins expression. In Table III the relations between p53 and p21WAF1 proteins expression, considering p53 reactivity with three anti-p53 antibodies (DO-7, PAb240, PAb1620) are shown. The detectable association between p21WAF1 and p53 immunoreactivity seems to be dependent on the antibody used for the detection of p53. DO-7 antibody and the anti-p21WAF1 antibody (4D10) gave negative results in 12 (24%) and in 15 cases (30%) DO-7 as well as 4D10 gave a positive staining. Fourteen p53 positive cases were p21WAF1 negative (28%) and 9 p53 negative cases were p21WAF1 positive (18%). The positive results for both proteins with PAb240 were found in 9 cases (18%), negative results in 22 carcinomas (44%), p53+/p21WAF1- phenotype was detected only in 3 (6%) and p53/p21WAF1+ in 16 (32%) cases. Similarly heterogeneous results were obtained with the PAb1620 antibody. Eight cases (16%) were positive for p53 and p21WAF1 proteins, whereas 19 (38%) were negative for both. Seven p53 positive cases remained p21WAF1 negative (14%) and 16 p53 negative carcinomas were p21WAF1 positive (32%). The differences between detectable p53/p21WAF1 phenotypes were significantly related to applied antibody, which recognizes distinct conformationally dependent p53 epitopes (p=0.524 for DO-7; p=0.00006 for PAb240; p=0.001 for PAb1620).

Relation between p21WAF1 and PCNA. Mutual relation between p21WAF1 and PCNA immunostaining in individual patients with ovarian carcinoma is presented in Fig. 2. High cell proliferation was usually accompanied by p21WAF1 negative or weak positive ovarian carcinomas however the inverse correlation between these two variables was not statistically confirmed (Spearman r=0.098, p=0.499).

table III. Relations between p53 immunological status and p53/p21WAF1 phenotypes in ovarian carcinomas.

<table>
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<tbody>
<tr>
<td></td>
<td>positive/total (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DO-7</td>
<td>14/50 (28.0)</td>
<td>15/50 (30.0)</td>
<td>9/50 (18.0)</td>
<td>12/50 (24.0)</td>
<td>0.524</td>
</tr>
<tr>
<td>PAb240</td>
<td>3/50 (6.0)</td>
<td>9/50 (18.0)</td>
<td>16/50 (32.0)</td>
<td>22/50 (44.0)</td>
<td>0.00006</td>
</tr>
<tr>
<td>PAb1620</td>
<td>7/50 (14.0)</td>
<td>8/50 (16.0)</td>
<td>16/50 (32.0)</td>
<td>19/50 (38.0)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Discussion

The relationship between two important cell-cycle regulators p53 and p21WAF1 has been investigated by immunohistochemical analysis by many authors in various types of human cancers, but the results are still controversial. In ovarian carcinomas the mutual relations between these two proteins have rarely been studied, seem to be complex and remain ambiguous. The majority of observations showed that in ovarian carcinomas p21WAF1 induction is mostly p53 independent (7,10,17,25). On the other hand some reports indicate the necessity of the presence of wild-type p53 for p21WAF1 activation (3,12). In our study for the first time the associations between p53 and p21WAF1 expression were examined analysing the reactivity of three anti-p53 monoclonal antibodies detecting different p53 epitopes. According to our earlier results (24) it was shown that the frequency of p53 accumulation in ovarian carcinomas is related to the
antibody applied. Similarly, Würl et al (26) described the significant association between the antibody used, p53 expression and survival time in patients with primary soft-tissue sarcomas. The differences in immunoreactivity of antibodies detecting conformationally/immunologically distinct p53 forms were also indicated by others (22,27-29). In our earlier (24) and in the present study using PAb1620 a cytoplasmic, diffuse staining was frequently observed. Mutation or mutation independent partial denaturation of the p53 protein destroy the PAb1620 epitope (28) which is not well defined and this antibody should be considered unreliable for the immunohistochemical detection of p53 status in human cancer. Monoclonal DO-7 antibody detected more positive cases than PAb240 however all PAb240 reactive carcinomas were also positive with DO-7. The coincidence of positivity between PAb240 and PAb1620 was shown only in 4 carcinomas. These results are consistent with earlier data indicating that exposure of the PAb240 epitope causes the loss of PAb 1620 reactivity (29). These observation could be explained by the binding of antibodies to different epitopes of p53 protein indicating that the level of detection of the p53 conformation and immunohistochemical analysis of p53 protein in tumor tissue is clearly dependent on the monoclonal antibodies directed against the p53 protein (28,30). For immunohistochemical detection of p53 alterations, DO-7 antibody which recognizes a specific linear epitope located in the amino-terminal region of p53 seems to be much better than both PAb240 and PAb1620 (24,26). According to recently published data (31) on the effect of mutation and conformation on the p53 function in colorectal carcinoma seem to be probable that the detection of different p53 conformations may be important to predict more precisely the biological behavior of carcinomas. It clearly confirms the complex relationship between immunohistochemical detection of p53 protein, p53 mutation and p53 function (28,32) and indicates that the presence, of a p53 mutation is not always associated with a complete protein inactivation (31). In addition to molecular-genetic events, p53 function can also be changed by its binding to other proteins, altered subcellular localization and by post-transcriptional modifications (27,30-33). Immunohistochemical detection of p53 accumulation might indicate either the presence of an underlying gene mutation in most carcinomas or p53 inactivation by other mechanisms. Thus, the knowledge of the p53 status based on protein conformation may be really helpful in understanding the functional p53 pathways and in the determination of the p53 dysfunction as an indicator of clinical course and response for therapy in carcinoma patients (31).

In the present study p21WAF1 expression was detected in 48% of ovarian carcinomas but the positive signal was usually observed in a small percentage of cells (5-10%) and only in three cases p21WAF1 reactivity was visible in 20-30% of tumor cell nuclei. The low level of p21WAF1 reactivity in ovarian carcinomas was also described by others (18,34). According to recently published data (7) we found neither a positive nor a negative correlation between p21WAF1 and p53 proteins expression, indicating that in ovarian carcinomas the level of p21WAF1 is regulated mainly by p53-independent mechanisms (7,17,25,34). Moreover, our results showed that the detection of an association between p21WAF1 and p53 immunoreactivity is at least partially dependent on the anti-p53 antibody used. The intratumoral heterogeneity of p21WAF1 and p53 staining was clearly revealed by the application of antibodies detecting different p53 epitopes, indicating a highly complex relationship between these variables in ovarian carcinomas (12). It also confirmed that p21WAF1 induction is multifactorial, both p53 dependent and p53-independent (6,35). However, independent of a possible role of many other genes and factors interacting in the complex system of cell-cycle regulation, the relative ratio of p53 and p21WAF1 proteins could also be important (2). In our study, both in p53 positive and p53 negative tumors, usually low p21WAF1 expression was detected. The strong p21WAF1 staining seems to be an indicator for an intact cell cycle regulation and relative level of this protein may be critical in determining the threshold kinase activity of various cyclin/cdk complexes (6,36-38).

Similarly to the results of Geisler et al (18) analysing the altered pattern of p53/p21WAF1 expression in ovarian carcinomas different p53/p21WAF1 phenotypes were identified, indicating the existence of a mixture of cell subpopulations with different genetic ‘make up’. Recently Ceccarelli et al (2) using image cytometry have been also reported that the ratio analysis of p53 and p21WAF1 expression in primary breast carcinomas allowed defining three subgroups of tumors with distinct biological and pathological properties. All these data clearly indicate that it is reasonable to accept that p21WAF1 activation in human carcinomas is very complex (4,6,7).

It is known that alterations in p53 gene are associated with enhanced proliferative activity of cells contributing to the evolution of carcinomas (12,39) however the mutual relations between cell proliferation and p21WAF1 expression are unclear. Some authors found an inverse correlation between Ki-67 or PCNA labelling and p21WAF1 expression (11,12) while the others were unable to show any significant association between these variables (10,13,14).There are also reports describing the positive, not inverse correlation between p21WAF1 and Ki-67 or PCNA expression (15,16). In ovarian cancer the results regarding the relationship between proliferation activity and p21WAF1 expression are also equivocal (10,12). Anttila et al (12) have demonstrated an inverse correlation between p21WAF1 and Ki-67 proliferation marker, contrary to the observation of Werness et al (10). In the current study, cell proliferation estimated by PCNA staining was high, detectable in 96% of carcinomas and usually accompanied by negative or weak p21WAF1 expression, suggesting that low p21WAF1 levels are unable to negatively regulate cell proliferation in ovarian cancer. It is possible that more p21WAF1 may be required to bind to and to inhibit PCNA activity (15). A p21WAF1 overexpression reported by some authors in various carcinomas (13,16,37) seems surprising because high p21WAF1 expression is expected to lead to cell growth arrest (37). Groeger et al (13) indicated that the control of DNA repair by PCNA is a more important activity than its contribution to cell proliferation, at least during lung carcinogenesis.

According to our earlier results (24,39) no statistically significant differences between p53 expression and histological subtypes and grade of differentiation were found. Regardless of the anti-p53 antibody the visible trend for higher p53 accumulation in III/IV than in I/II FIGO stages was observed but this difference was not statistically confirmed (24). The
relations between p21\textsuperscript{WAF1} and clinicopathological variables in ovarian carcinomas remain unclear. Our studies did not show any correlation between p21\textsuperscript{WAF1} expression or its combination with p53 reactivity and histology, stage and grade of ovarian carcinomas. These results are in agreement with some earlier published data (3,17). On the contrary, Anttila \textit{et al} (12) described that low p21\textsuperscript{WAF1} expression was significantly associated with high grade of the tumor, advanced FIGO stage and serous or miscellaneous histological type. On the other hands some reports (7,18,34) described that p21\textsuperscript{WAF1} overexpression correlated with lower FIGO stage. Recently, Geisler \textit{et al} (18) showed that the combination of p21\textsuperscript{*} and p53\textsuperscript{*} phenotype is a better independent indicator for prognosis and survival in patients with ovarian carcinomas than p21\textsuperscript{WAF1} or p53 estimated separately. However, the clinical impact of various p21\textsuperscript{WAF1}/p53 immunophenotypes can only be evaluated in the large studies including high number of one histological tumor type and at least 5-year follow-up of the patients.

High PCNA expression in our study was observed in almost all ovarian carcinomas and two PCNA negative tumors were classified as well differentiated mucinous carcinomas in stage I of disease. The comparison of PCNA activity in malignant and benign neoplasms clearly showed that strongly enhanced cell proliferation is a characteristic feature of ovarian carcinomas.

Our results showed that in ovarian carcinomas significant inter- and intratumoral heterogeneity of the p53 and p21\textsuperscript{WAF1} expression exists and different p53/p21\textsuperscript{WAF1} phenotypes were identified. The further clinical observation of patients considering various immunophenotypes should allow defining their possible impact on tumor behavior and prognosis. Independent of the anti-p53 antibody used, no correlation between p53 and p21\textsuperscript{WAF1} expression was found, which indicates that p21\textsuperscript{WAF1} activation in ovarian carcinomas could be induced at least partially by a p53-independent pathway. Immunohistochemical determination of p21\textsuperscript{WAF1} and p53 proteins expression did not correlate with conventional clinicopathological variables of the disease. Ovarian epithelial carcinomas showed high level of PCNA expression usually accompanied by undetectable or low p21\textsuperscript{WAF1} expression.

\section*{Acknowledgements}

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