P21\textsuperscript{WAF1}, P27\textsuperscript{KIP1}, TP53 and C-MYC analysis in 204 ovarian carcinomas treated with platinum-based regimens

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Background: The prognostic and predictive value of cell cycle regulatory proteins in ovarian cancer has not been established. We evaluated the clinical and biological significance of P21\textsuperscript{WAF1}, P27\textsuperscript{KIP1}, C-MYC, TP53 and Ki67 expressions in ovarian cancer patients.

Materials and methods: Immunohistochemical analysis was performed on 204 ovarian carcinomas of International Federation of Gynecology and Obstetrics (FIGO) stage IIB to IV treated with platinum-based chemotherapy. Multivariate analysis with Cox and logistic regression models was performed in the whole group, and in the TP53-negative and TP53-positive subgroups.

Results: High P21\textsuperscript{WAF1} labeling index (LI) was an independent positive predictor of platinum-sensitive response (P = 0.02). Overall survival was positively influenced by P21\textsuperscript{WAF1} LI (P = 0.02) or by P21\textsuperscript{WAF1} plus P27\textsuperscript{KIP1} LI (P = 0.004) in the TP53-negative group only. Ki67 LI showed borderline association with disease-free survival (P = 0.05). Growth fraction was negatively associated with P21\textsuperscript{WAF1} and P27\textsuperscript{KIP1} indices in the TP53-negative group (P = 0.023 and 0.008, respectively), and these associations were borderline or lost in the TP53-positive group. Endometrioid and clear cell carcinomas differed from other carcinomas by having a low incidence of TP53 accumulation, a high incidence of C-MYC overexpression (70%) and a low median Ki67 LI (all with P < 0.001).

Conclusions: We have shown an independent predictive value of P21\textsuperscript{WAF1} LI in ovarian carcinoma patients. The prognostic value of P21\textsuperscript{WAF1} and P27\textsuperscript{KIP1} LI was determined by TP53 status. A high frequency of C-MYC overexpression in endometrioid and clear cell carcinomas may suggest its role in the development of these tumor types.

Key words: Ki67, C-MYC, ovarian cancer, P27\textsuperscript{KIP1}, P21\textsuperscript{WAF1}, TP53

Introduction

Tumor proliferation is an important parameter that potentially may influence both prognosis and response to chemotherapy in cancer patients. However, the clinical significance of cell cycle regulatory proteins in ovarian cancer has not been established and studies on large groups of uniformly treated patients are rare [1–4].

Proto-oncogene C-MYC and the tumor suppressor gene TP53 encode phosphoproteins that participate in the regulation of cellular proliferation, apoptosis and cell differentiation. C-MYC is a positive regulator of the cell cycle, while TP53 is a negative regulator. The main mechanism by which TP53 exerts its inhibitory effect on cell proliferation is induction of P21\textsuperscript{WAF1} expression, and this may be impaired by TP53 gene mutations [5]. On the other hand, some data suggest that C-MYC may antagonize P27\textsuperscript{KIP1} activity (reviewed in [6, 7]). P21\textsuperscript{WAF1} and P27\textsuperscript{KIP1} are inhibitors of cyclin-dependent kinases (cdk) and cause G\textsubscript{i} arrest by binding to cyclin–cdk complexes. P21\textsuperscript{WAF1} expression can also be regulated by epigenetic silencing [8] and TP53-independent pathways [9]. In some experimental models C-MYC inhibited P21\textsuperscript{WAF1} activity [10] and the TP53-dependent induction of P21\textsuperscript{WAF1} [11].

In ovarian carcinoma cell lines P21\textsuperscript{WAF1} gene transfer enhanced the cytotoxic effect of cisplatin [12]. However, its predictive role in ovarian carcinoma has not been demonstrated. Data on the prognostic importance of P21\textsuperscript{WAF1} in ovarian carcinoma are controversial. In the majority of studies P21\textsuperscript{WAF1} had no prognostic value [2, 4, 13–16], while in others, except in a study by Costa et al. [3], this has been demonstrated in univariate analyses only [1, 17].
As far as P27KIP1 is concerned, several groups have found it of prognostic significance in ovarian tumors [14, 18, 19], while others have not [2, 13]. Low levels of P27KIP1 expression were associated with chemoresistance in ovarian carcinoma patients in one study [20].

We have recently found that ovarian carcinomas with and without TP53 protein accumulation differ in clinical significance of apoptosis-regulating proteins [21]. The purpose of the present study was to examine the value of P21WAF1, P27KIP1, Ki67 and C-MYC proteins in predicting prognosis and response to chemotherapy in a large cohort of uniformly treated patients with advanced epithelial ovarian cancer, with respect to TP53 status.

Materials and methods

Patient population

The study was performed on archival material from 204 ovarian carcinoma patients treated in eight gynaecological oncology centers in Poland. The material was carefully selected from 548 cases submitted according to the following criteria: no chemotherapy before staging laparotomy, adequate staging procedure, International Federation of Gynecologists and Obstetricians (FIGO) stage IIB to IV, standard CP (cisplatin–cyclophosphamide or carboplatin–cyclophosphamide) or CAP (CP with addition of doxorubicin) chemotherapy, and tumor tissue from first laparotomy available. There was a central critical review of medical records by at least two clinicians. Tumors were staged according to the criteria of FIGO [22] (Table 1).

Follow-up time was based on patients’ date of death or the last information present in the medical records; it ranged from 3.1 to 157.5 (median 35.2) months and 160 patients (78%) died (Table 1).

Evaluation of clinical response to chemotherapy

Response to chemotherapy was evaluated retrospectively according to the World Health Organization (WHO) response evaluation criteria [23]. The evaluation was based on data from medical records describing patients’ clinical condition and CA 125 levels in 3- to 4-week intervals. Complete remission (CR) was defined as the disappearance of all clinical and biochemical symptoms of ovarian cancer evaluated after completion of first-line chemotherapy and confirmed at 4 weeks. Within the CR group we have distinguished a platinum-sensitive group (PS) according to criteria given by Christian and Trimble [24] (disease-free survival (DFS) longer than 6 months; 89 patients). Thus, the other tumors [partial remission (PR), progression (P), no change (NC)], as well as the CR group with DFS shorter than 6 months were described as resistant to cisplatin [24] (Table 1).

Histopathological data

All tumors came from the first laparotomy. They were uniformly reviewed histopathologically (J. Kupryjaczyk) and classified according to the criteria of WHO [25]: 159 (78%) tumors were of the serous type; 13 (6%) were of the endometrioid type, 10 (5%) were of the clear cell type, 12 (6%) were undifferentiated, and 10 were of other types. Histological grade was evaluated on a scale from 1 to 4: 1 (G1), 2 (G2), 3 (G3) and 4 (G4). The purpose of the present study was to examine the value of P21WAF1, P27KIP1, Ki67 and C-MYC proteins in predicting prognosis and response to chemotherapy in a large cohort of uniformly treated patients with advanced epithelial ovarian cancer, with respect to TP53 status.

Immunohistochemical analysis

Immunohistochemical stainings were performed on paraffin-embedded material. We used PAb1801 monoclonal antibody (1:500; Sigma-Genosys, Cambridge, UK) for the TP53 protein, sc-6246 monoclonal antibody (1:40; Santa Cruz Biotechnology Inc., Santa Cruz, USA) for P21WAF1, NCL-P27 monclonal antibody (1:50; Immunotech, Marseille, France) for P27KIP1, anti-C-MYC (both from Novocastra, UK), as well as MIB-1 monoclonal antibody (1:50; Immunotech, Marseille, France) for Ki67 antigen. We have recently found that ovarian carcinomas with and without TP53 protein accumulation differ in clinical significance of apoptosis-regulating proteins [21].

Table 1. Tumor characteristics (204 patients)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>NED 28 (14%)</th>
<th>AWD 16 (8%)</th>
<th>DOD 156 (76%)</th>
<th>DOC 4 (2%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotherapy</td>
<td>CP 146 (72%)</td>
<td>CAP 58 (28%)</td>
<td></td>
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</tr>
<tr>
<td>Response to chemotherapy</td>
<td>Complete remission 108 (53%)</td>
<td>Partial remission 32 (16%)</td>
<td>No change 7 (3%)</td>
<td>Progression 57 (28%)</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Age, years</th>
<th>Range</th>
<th>Mean (SD)</th>
</tr>
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<tr>
<td></td>
<td>24–76</td>
<td>53.5 (10.1)</td>
</tr>
</tbody>
</table>

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The immunohistochemical procedure has been described previously [27, 28]. Briefly, deparaffinized sections were boiled in a citrate buffer (pH 6.0) for 5 min for P27KIP1; for P21WAF1 detection, the sections were boiled in the same buffer for 5 min at 120 °C and 3% H2O2, respectively. Biotinylated goat anti-mouse immunoglobulin G peroxidase reactivities were blocked with 10% bovine serum albumin (BSA) and 3% H2O2, respectively. Biotinylated goat anti-mouse immunoglobulin G (IgG; 1:1500, cat. no. 816), peroxidase-conjugated streptavidin (1:500, cat. no. 309) (both from Immunotech, Marseille, France), and diaminobenzoilene (DAB) were used as a detection system. Tissue sections were incubated with primary antibodies for 1 h at room temperature (anti-TP53, anti-Ki67) or overnight at 4°C (anti-P27KIP1, anti-P21WAF1, anti-C-MYC). Ovarian carcinomas with and without a TP53 gene missense mutation were controls for TP53 while a human tonsil was a control for proliferation antigens. Ovary with corpus luteum and
plasma cells were positive controls for C-MYC. An isotype-matched antibody from the same company directed against a cytoplasmic antigen [anti-MEK1 (clone H-8), Santa Cruz Biotechnology Inc.], was used as a negative control for anti-P21WAF1. Anti-P27KIP1 antibody was a negative control for anti-C-MYC (both of the same isotype). Normal mouse IgG of the same subclasses and concentrations as the primary antibodies served as negative controls also (all from Dako, Glostrup, Denmark).

**Evaluation of immunohistochemical stainings**

TP53 protein accumulation was described as present (>10% of positive cells) or absent. MIB1-positive cells were counted in the three different foci most rich in proliferating cells: 500 cells were counted in each focus (total 1500). P21WAF1 and P27KIP1 expressions were evaluated in four different randomly chosen places and 200 cells were counted in each focus (total 800). Ki67, P21WAF1 and P27KIP1 LIs were defined as a proportion of positive cells to total cells counted. We also created a combined variable, which was a sum of P21WAF1 and P27KIP1 LIs, i.e. P21WAF1 plus P27KIP1. For C-MYC expression the following staining categories were created: weak, moderate and strong.

**Statistical analysis**

Probability of survival and DFS were estimated using the Kaplan–Meier method. Overall survival and DFS time analyses were performed with multivariate Cox’s proportional hazards models [29]; factors that may determine tumor response to chemotherapy were evaluated in the multivariate logistic regression model. Important factors were selected using backward selection technique, where factors not significant at 0.1 were drawn one by one out of the model. The analysis was performed in all ovarian carcinomas, and separately in the TP53(−) and TP53(+) subgroups.

Associations between protein expressions and clinico-pathological parameters were studied by chi-square or Fisher’s exact test. All tests were two-sided and the level of significance was set at 5%. Associations between numerical indices and protein expressions or clinical and immunohistochemical parameters were performed using the Student’s t-test or F-test. All calculations were performed using the STATA 7.0 program.

In all multivariate models the cut-off points for numerical indices were determined at median value. The combined P21WAF1 plus P27KIP1 variable has been evaluated in statistical analyses alternatively to separate P27KIP1 and P21WAF1 variables.

**Results**

**TP53, Ki67, P21WAF1, P27KIP1 and C-MYC expressions**

TP53, Ki67, P21WAF1 and P27KIP1 antigens showed nuclear localization (P27KIP1 showed weak cytoplasmic staining also), while C-MYC protein was distributed in the cytoplasm (inconstant perinuclear staining was observed also) and showed homogeneous staining. TP53 protein accumulation was observed in malignant cells only (Figure 1A), while the other proteins were expressed by normal cells or tissues also. P21WAF1 and P27KIP1 were strongly expressed by mesothelium, endothelium, ovarian stromal cells, ~50% of tumor stroma fibroblasts (Figure 1B and C), smooth muscle cells and moderately in oviductal mucosa. In addition, strong P27KIP1 expression was seen in lymphocytes and plasma cells (Figure 1C). C-MYC was expressed by mesothelium, oviductal mucosa, plasma cells and inconsistently by fibroblasts and endothelium.

Twenty-five of the 229 initially stained cases (13%) were totally negative for P21WAF1 expression and were rejected from the study as false negative. After HIER in the autoclave 28 of 204 tumors (14%) showed focal P21WAF1 labeling. TP53 protein nuclear accumulation was present in 124 tumors (61%) and the percentage of positive nuclei ranged from 20 to 98 (median 80). Ki67 LI ranged from 7 to 93% (mean 60.4%, median 64), P21WAF1 LI ranged from 22 to 98% (mean 74.9%, median 78) and P27KIP1 LI ranged from 19 to 88% (mean 61.8%, median 64). C-MYC expression was weak in 23 (12%) cases and moderate in 96 (47%) cases; strong C-MYC expression was seen in 84 cases (41%) and it was qualified as overexpression (Figure 1D).

**Associations between Ki67, P21WAF1 and P27KIP1**

Ki67 LI showed negative association with P21WAF1 LI (P = 0.0004), P27KIP1 LI (P = 0.0021) and with combined P21WAF1 plus P27KIP1 LI value (P < 0.0001). P21WAF1 LI was positively correlated with P27KIP1 LI (P < 0.0001).

Analysis in the TP53(−) and TP53(+) subgroups revealed that associations between Ki67 LI and P21WAF1, P27KIP1 and P21WAF1 plus P27KIP1 expressions are much stronger in the TP53(−) group (P = 0.023, 0.008 and 0.002, respectively) than in the TP53(+) group (P = 0.05, 0.24 and 0.05, respectively), despite its larger size. In TP53(+) and TP53(−) subgroups, P21WAF1 was positively associated with P27KIP1 at the same level of statistical significance (P = 0.004).

**TP53 associations with Ki67, P21WAF1, P27KIP1 and C-MYC**

TP53(+) carcinomas had higher Ki67 LIs (range 27–93, mean 65.7) than TP53(−) carcinomas (range 7–88, mean 52.0) (P < 0.0001). P21WAF1 LI was lower in the TP53(+) group than in the TP53(−) group; however, the difference was not statistically significant (P = 0.08). Neither P27KIP1 LI nor C-MYC expression showed differences in relation to the TP53 status.

**C-MYC associations with Ki67, P21WAF1 and P27KIP1**

C-MYC overexpression was associated with lower Ki67 LI (P = 0.02). In accordance, P27KIP1 LI was higher in tumors with C-MYC overexpression; however, the difference was not statistically significant (P = 0.09). C-MYC overexpression did not show an association with P21WAF1 LI.

**TP53, Ki67, P21WAF1, P27KIP1, C-MYC and clinicopathological parameters**

Endometrioid and clear cell carcinomas differed from serous, undifferentiated and other carcinomas in having a low incidence of TP53 accumulation, a low median Ki67 LI and a high incidence of C-MYC overexpression (P = 0.0004) (Table 2). The differences between histological types were not due to differences in tumor differentiation degree, because there was no association between type and differentiation. Serous carcinomas differed from undifferentiated and other carcinomas in having a slightly lower Ki67 LI only (P = 0.027) (Table 2).

Loss of tumor differentiation (G2 versus G3 versus G4) was accompanied by an increase in Ki67 LI (P < 0.0001) and in inci-
dence of TP53 accumulation ($P = 0.004$). It was also accompanied by a decrease of mean values of $P21^{WAF1}$ LI ($P = 0.07$) and $P27^{KIP1}$ LI, and a lower incidence of C-MYC overexpression (56% versus 42% versus 33%); however, the differences were not statistically significant.

The proteins studied did not correlate with FIGO stage. $P27^{KIP1}$ LI was the only LI associated (negatively) with residual tumor size ($P = 0.05$).

**Overall survival, disease-free survival and tumor response to chemotherapy**

Overall survival (OS) in the whole group was positively influenced by lower patients’ age, lower FIGO stage, lower residual tumor size, and not by any immunohistochemical parameter studied. Analysis of the TP53(−) group revealed an independent prognostic value of $P21^{WAF1}$ LI, and alternatively included $P21^{WAF1}$ plus $P27^{KIP1}$ LI (Figure 2, Kaplan–Meier). These associations have not been confirmed in the TP53(+) group despite its larger size (Table 3).

Disease-free survival (DFS) was longer with lower FIGO stage ($P = 0.07$ or $0.001$ or $<0.001$, depending on stages compared) and with lower Ki67 LI [$P = 0.051$, relative risk (RR) = 1.6, 95% confidence interval (CI) for RR 1.0, 2.5].

Platinum-sensitive response (CR with DFS longer than 6 months) was associated with small residual tumor size, lower FIGO stage and high $P21^{WAF1}$ LI. Predictive value of $P21^{WAF1}$ LI has been confirmed in the TP53(+) group only, at a lower level of significance (Table 4). CR status was associated with small residual tumor size only ($P <0.001$). However, when residual tumor size was divided into five categories (0 versus <1 versus ≤2 versus 2–5 versus >5 cm) instead of two (≤2 versus >2 cm), we observed a positive impact of high $P21^{WAF1}$ LI on both CR and platinum sensitivity.

In determining relative risk, clinical parameters such as patient age, residual tumor size and clinical stage were more important than immunohistochemical markers.
Discussion

We have shown an independent predictive value of P21\textsuperscript{WAF1} expression in ovarian carcinoma patients, as well as a prognostic value of P21\textsuperscript{WAF1} and P21\textsuperscript{WAF1} plus P27\textsuperscript{KIP1} in a subgroup without TP53 protein accumulation. Experimental studies have previously shown a role for P21\textsuperscript{WAF1} in ovarian cancer response to cisplatin treatment; however, it has not been confirmed by clinical studies. Poulain et al. [30] suggested that loss of both wild type TP53 and P21\textsuperscript{WAF1} induction resulted in cisplatin resistance due to defective G1/S checkpoint. In a study by Lincet et al. [12], transfection of P21\textsuperscript{WAF1} cDNA led to enhanced sensitivity to cisplatin and prevented repopulation of cancer cells. Specifically, the latter findings may be clinically reflected by CR with a longer DFS (platinum-sensitive group) observed in high P21\textsuperscript{WAF1} expressors in our study.

P21\textsuperscript{WAF1} predictive value has been demonstrated in all patients and confirmed in the TP53(+) group only at the lower level of significance, which might have been caused by the smaller size of the subgroup; this could also explain why this association has not been found in the TP53(−) group. On the other hand, TP53 status might determine P21\textsuperscript{WAF1} and P21\textsuperscript{WAF1} plus P27\textsuperscript{KIP1} prognostic value, since it has been observed in the TP53(+) group only, and not in all patients nor in the large TP53(+) group. We have previously observed the differential clinical importance of BCL-2 and BAX proteins depending on TP53 status [21]. TP53 is a pleiotropic protein with complex interactions at gene and protein levels, including regulation of transcriptional activation of P21\textsuperscript{WAF1}, BCL-2 and BAX genes [5, 31]. These functions are largely lost by mutant TP53, which results in profound alterations of cellular processes, such as signaling of DNA damage and its repair, cell cycle arrest and apoptosis. Possibly, decreased P21\textsuperscript{WAF1} expression may demonstrate its clinical significance in tumors with wild-type TP53, but as a biological change may be too subtle to show an influence in the TP53(+) group. Our results confirm our previous observations that separate evaluation of TP53(+) and TP53(−) subgroups may help to identify molecular markers of clinical importance in ovarian cancer.

Some studies suggested that P21\textsuperscript{WAF1} has an influence on clinical end points in ovarian cancer, but generally it has not been confirmed by multivariate analyses [1, 3, 17]. Costa et al. [3] reported P21\textsuperscript{WAF1} as a factor of overall survival in a group stratified for clinical stage and nuclear grade. Some authors reported that a variable of combined P21\textsuperscript{WAF1} and TP53 may be a marker of prognosis [1, 32] and such findings may be somehow related to our result obtained for the TP53(−) group. Our results confirm our previous observations that separate evaluation of TP53(+) and TP53(−) subgroups may help to identify molecular markers of clinical importance in ovarian cancer.

Table 2. Associations of protein expressions with histological tumor type\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>Serous (159 tumors)</th>
<th>Endometrioid, clear cell (23 tumors)</th>
<th>Undifferentiated, other (22 tumors)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53(−)</td>
<td>54 (34%)</td>
<td>19 (83%)</td>
<td>7 (32%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TP53(+)</td>
<td>105 (66%)</td>
<td>4 (17%)</td>
<td>15 (68%)</td>
<td></td>
</tr>
<tr>
<td>Pairwise comparisons</td>
<td>1 versus 2</td>
<td>2 versus 3</td>
<td>3 versus 1</td>
<td>P &lt;0.001</td>
</tr>
<tr>
<td></td>
<td>P &lt;0.001</td>
<td>P = 0.001</td>
<td>P = 0.84</td>
<td></td>
</tr>
<tr>
<td>Ki67 (min, max)</td>
<td>(7, 93)</td>
<td>(20, 85)</td>
<td>(36, 91)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>60.6 (18.3)</td>
<td>47.1 (19.7)</td>
<td>71.5 (15.4)</td>
<td></td>
</tr>
<tr>
<td>Pairwise comparisons</td>
<td>1 versus 2</td>
<td>2 versus 3</td>
<td>3 versus 1</td>
<td>P = 0.004</td>
</tr>
<tr>
<td></td>
<td>P = 0.004</td>
<td>P &lt; 0.001</td>
<td>P = 0.027</td>
<td></td>
</tr>
<tr>
<td>P27\textsuperscript{KIP1} (min, max)</td>
<td>(19, 88)</td>
<td>(46, 83)</td>
<td>(30, 85)</td>
<td>0.05</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>61.2 (15.5)</td>
<td>68.7 (10.1)</td>
<td>58.9 (14.5)</td>
<td></td>
</tr>
<tr>
<td>Pairwise comparisons</td>
<td>1 versus 2</td>
<td>2 versus 3</td>
<td>3 versus 1</td>
<td>P = 0.07</td>
</tr>
<tr>
<td></td>
<td>P = 0.07</td>
<td>P = 0.08</td>
<td>P = 1.0</td>
<td></td>
</tr>
<tr>
<td>C-MYC 1, 2</td>
<td>98 (62%)</td>
<td>14 (30%)</td>
<td>14 (64%)</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td>61 (38%)</td>
<td>33 (70%)</td>
<td>8 (36%)</td>
<td></td>
</tr>
<tr>
<td>Pairwise comparisons</td>
<td>1 versus 2</td>
<td>2 versus 3</td>
<td>3 versus 1</td>
<td>P = 0.001</td>
</tr>
<tr>
<td></td>
<td>P = 0.008</td>
<td>P = 0.86</td>
<td>P = 0.86</td>
<td></td>
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</tbody>
</table>

\textsuperscript{a}P21\textsuperscript{WAF1} did not show an association.
\textsuperscript{b}Additional 24 tumors included.
SD, standard deviation.
studied prostatic carcinomas with the use of the same antibody, and they found similarly high P21\textsubscript{WAF1} indices (mean 85\%) [33].

In our analysis P21\textsubscript{WAF1} and P27\textsubscript{KIP1} expressions showed stronger associations with growth fraction in the TP53(–) than in the TP53(+) ovarian carcinomas. This finding is unexpected, particularly in relation to P27\textsubscript{KIP1}, and may suggest some cooperation between P27\textsubscript{KIP1} and TP53 or P21\textsubscript{WAF1} in cell cycle regulation. Furthermore, in our study P21\textsubscript{WAF1} and P27\textsubscript{KIP1} expressions were positively associated (reported also by Baekelandt et al. [2]) and their cumulative value (defined by a sum of P21\textsubscript{WAF1} and P27\textsubscript{KIP1} indices) showed stronger association with overall survival than either protein alone. We have found one study showing that P21\textsubscript{WAF1} may upregulate P27\textsubscript{KIP1} protein by inhibiting its phosphorylation, which prevents P27\textsubscript{KIP1} ubiquitination and destruction [34].

Data on C-MYC expression and/or amplification in ovarian carcinomas are scarce and its clinical significance has not been proven [35–38]. We have found that C-MYC was associated with better tumor differentiation, higher P27\textsubscript{KIP1} expression and lower growth fraction, which seems paradoxical in the context of its biological function. However, some authors have also reported higher levels of C-MYC in better differentiated breast and gastric cancers [39, 40]. In our study, there was a striking association of C-MYC overexpression with endometrioid and clear cell carcinomas, which suggests a role of C-MYC in the development of these carcinoma types and should be the subject of further analysis.

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\[\text{Figure 2. Kaplan–Meier curves for overall survival in the TP53(–) group in relation to P21\textsubscript{WAF1} LI and P21\textsubscript{WAF1} plus P27\textsubscript{KIP1} LI.}\]
References


