P53 and Ki67 immunoexpression in mucinous malignant ovarian tumors

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Abstract
Ovarian cancer is the fourth cause of death by cancer among women and the first mortality cause in gynecological neoplasia. Our goal was to evaluate p53 and Ki67 immunoexpression and also the correlations with tumor stage and type. The study included 45 primary ovarian malignant mucinous tumors, diagnosed in patients in the IV and VI decade. From the standpoint of histopathology, there were 28 cases of borderline mucinous tumors and 17 mucinous carcinomas, predominantly stage I of the disease. The immunostaining for Ki67 was positive in all the cases, the highest levels being recorded in mucinous carcinomas (22.2% medium index) compared to the borderline tumors (9.5% medium index). Opposed to Ki67, the immunoreaction for p53 was present in 37.7% of all the tumors, predominantly in mucinous carcinomas where the stain has high values (52.3% medium index) in contrast with borderline lesions (15.5% medium index).

The study indicated significant differences in p53 and Ki67 immunostain in relation to the tumor stage and histological type, there being a direct correlation of the expression of both proteins, in the studied tumors. P53 and Ki67 are useful markers for evaluating aggressive tumoral behavior and differentiating between mucinous carcinomas and borderline mucinous tumors.

Keywords: malignant mucinous tumors, p53, Ki67.

Introduction
Ovarian cancer represents ca. 30% of all cancer cases of the female genital tract [1]. In approximately 70–75% of patients with ovarian cancer at the moment of diagnosis, the disease is extended beyond the structures of the pelvis [2].

Ovarian carcinomas are rarely the consequence of only one event so a singular biologic factor cannot offer concise information on the prognosis of these patients. The combination of two or more independent factors can generate an improved general prognosis index. Recent morphology, immunohistochemistry, and molecular genetics studies have led to the development of a new paradigm regarding the pathogenesis and the origin of these tumors, based on the dualist model of carcinogenesis which divides ovarian carcinoma into two categories named type I and II tumors [3, 4].

Mucinous carcinomas are included in type I tumors which rarely develop p53 mutations and are relatively genetically stable [4, 5].

We proposed to evaluate the p53 and Ki67 expression for malignant primary mucinous ovarian tumors and identifying the statistical significant correlations with tumor stage and type.

Materials and Methods
The study included 45 cases of primary mucinous malignant ovarian tumors, diagnosed in the Pathology Department of the Emergency County Hospital of Craiova, from which 28 of them were borderline tumors (BL) and 17 mucinous carcinomas (MC).

The biologic material was comprised of ovaries obtained after hysterectomy and salpingectomy surgery, which were processed by common histopathological technique using 10% formalin fixation, paraffin embedding and Hematoxylin–Eosin stain.

For the staging of the disease, we used the WHO 2004 classification [1].

The immunochemical processing was made on serial sections using the LSAB+ System-HRP (DAKO, code K0690) technique in the Pathology Laboratory of the University of Medicine and Pharmacy of Craiova.

The antibodies, clone, dilution, antigenic retrieval and external control are presented in Table 1.

Table 1 – Antibodies used panel

<table>
<thead>
<tr>
<th>Antibody</th>
<th>clone</th>
<th>Dilution</th>
<th>Antigenic recuperation</th>
<th>External positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>P53</td>
<td>DO-7</td>
<td>1:50</td>
<td>Citrate buffer, pH 6</td>
<td>Tonsil</td>
</tr>
<tr>
<td>Ki67</td>
<td>MIB1</td>
<td>1:100</td>
<td>Citrate buffer, pH 6</td>
<td>Mammary gland</td>
</tr>
</tbody>
</table>

For quantifying the immunoexpression of Ki67, we calculated the proliferation index (PI) for Ki67 and the positivity index (PoI) for p53, by referring the number of positive cells to the total number of cells counted with a 40× microscopic field [6].

In order to appreciate the immunostaining for p53 and Ki67 we used a quantitative score: score 0 – negative; score 1 – under 10%; score 2 – 10–50%; score 3 – over 50% marked cells.
The statistical analysis of the results was performed using the ANOVA test and the Pearson correlation coefficient in the SPSS 10 software.

The acquisition of the images was done with Nikon Eclipse E600 and software program Lucia 5.

Results

General clinico-pathological data

Our study included 45 cases of mucinous malignant ovarian tumors with primary localization out of which 28 borderline mucinous tumors and 17 mucinous carcinomas. The tumors were diagnosed in patients with ages between the IV and VI decade.

Histopathologically, the 28 cases of borderline mucinous tumors were intestinal type in 23 cases and endocervical in five cases. The association of borderline mucinous tumors with areas of mucinous cystadenoma was fairly common, in 14 (50%) cases out of the total of 28 borderline tumors analyzed. Mucinous carcinomas were present in 17 cases out of which 11 cases were low grade and six of them high-grade. In 14 of the cases with mucinous carcinomas, there were areas of borderline tumor associated and in 11 cases out of the 14, aspects of benign mucinous tumor were present (Figure 1).

TNM staging revealed that most of the cases were diagnosed in stage I with 43 cases (95.6%) out of which 28 borderline mucinous tumors (62.2%) and 17 malignant tumors (37.8%). Only two cases (4.4%) were diagnosed in stage II, both of them being mucinous carcinomas (Table 2).

The immunochemical study followed the immunoexpression of Ki67 and p53 and also the relation with tumor stage (Table 3).

The immunoexpression study indicated nuclear positivity in 17 cases representing 37.7% of the analyzed cases. Negative cases belonged to both tumor categories but predominantly in the borderline tumor compared to the mucinous carcinomas. We observed positivity for eight (28.5%) of the borderline mucinous tumors and for nine (52.9%) of the mucinous carcinomas.

In borderline tumors, the p53 stain had a mean PoI of 15.5% with values between 5 and 30%, presenting a score of 1 respectively score 2 in each four cases (50%). For studied mucinous carcinomas, PoI had a mean value of 52.3% with limits between 35 and 78%. In these cases, p53 reactions presented a score of 2 in three cases (33.3%) and a score of three in six cases (66.6%). The p53 score analysis indicated a maximum value (score 3) only in mucinous carcinomas, this score being present in 75% of the carcinomas in stages IC and IIC. In addition, in five of the mucinous carcinomas investigated we observed positivity not only in the malignant component of the tumor but in the borderline or benign areas of the tumor as well, with a much lower score conferring the tumors heterogeneity as a whole (Figure 2).

The immunostain for Ki67 was identified in all cases, present at the nuclear level. Borderline tumors presented a mean PI Ki67 of 9.5%, with values between...
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3–18%, the reactions indicating a score of 1 in 21 cases (75%) and a score of 2 in seven cases (25%). The mucinous carcinomas had a mean PI Ki67 of 22.2% with values between 8 and 45%, a score of 1 being found in two cases (11.8%) and a score of 2 in 15 cases (88.2%) (Figure 3).

In reference to the tumor stage, the immunoreactions presented a maximum score in 45.9% of the analyzed stages IA–IB tumors and 83.3% in stages IC–IIC.

The ANOVA test indicated significant differences of p53 immunoexpression in relation to the tumor type \[ F(1.43)=16.27, p=0.000 \] and as well for the tumor stage \[ F(3.41)=5.72, p=0.002 \], a high expression being associated with carcinomas in an advanced stage. Also, the ANOVA test showed significant differences in the values of the proliferation index of Ki67 in relation to the tumor type \[ F(3.41)=10.82, p=0.000 \] and also for tumor stage \[ F(1.43)=39.67, p=0.000 \]. The immunostain has high values in advanced stages carcinomas. The Pearson test indicated a positive direct correlation between the expression of p53 and Ki67 in malignant mucinous tumors of the ovary \( r(43)=0.396, p=0.007 \) (Figure 4).

Discussion

In mucinous ovarian tumors, there were many biologic markers evaluated in order to better understand the molecular events that lead to the disease’s progression and to study the correlation of those events with tumor stage or behavior. Uncontrolled cellular proliferation is one of the most important biological mechanisms involved in oncogenesis [7], p53 and Ki67 being two of the most researched markers in this matter.

In our study, we observed the positivity of p53 and Ki67 in both malignant mucinous tumors categories that we investigated (borderline and carcinomas) but in different ratios. P53 immunoexpression was identified...
TP53 is a tumor-suppressing gene situated on the short arm of chromosome 17. This acts by suppressing cell growth control in the beginning of the S-phase of the cellular cycle [8]. The mutation and/or overexpression of the p53 are the most frequent anomalies described in human cancers [9]. Studies have shown a correlation between the mutation/overexpression of p53 and the patient prognosis with different types of tumors like breast cancer [10], rectal and intestinal cancer [11], lung cancer [12] and also ovarian cancer [13].

In ovarian tumors, the mutation and/or overexpression of p53 were reported variably. In 62 studies that included 75 to 9448 patients, the mean percentage of p53 positive tumors was 50% (13.7–82% interval) [13]. Literature data regarding p53 positivity in mucinous ovarian tumors varies from 22% [14] to 6.3% [15]. Other studies indicate mutations of the p53 gene in 52% of carcinomas but also their absence in borderline tumors or cystadenoma [16]. Studies that have investigated comparative immunexpression of p53 in mucinous benign tumors, borderline and malignant ones report that the accumulation of p53 protein is associated with a similar level in mucinous cystadenoma and borderline tumors [17]. This fact suggested that p53 mutations can play an important role in the debut of malignancy in ovarian mucinous tumors. Similar to the aspects observed in cases, other studies reported that immunostaining for borderline tumors was also present in the benign morphology areas indicating that despite their aspect they have made an important step forward in the evolution of malignancy [17].

Our study reported that the immunoexpression analysis for p53 indicated positivity for 28.5% of borderline tumors and 52.9% of the investigated carcinomas regardless of their degree of differentiation. Advanced stage mucinous carcinomas have presented the highest scores, the immunostain being heterogenous in different areas of the same tumor (benign, borderline, malignant).

The prognostic significance of p53 expression is controversial [15, 18]. A significant association of p53 expression with general survival in a univariated analysis was reported in 38.6% of cases [13]. While some studies report that the mutation/overexpression of p53 is an important prognosis factor [19–21], others have not confirmed such results [22, 23]. The importance of accumulating p53 as a negative prognosis marker in ovarian cancer has been demonstrated by many studies [18–21]. The expression of p53 was associated with other inauspicious prognostic factors like FIGO advanced stage, suboptimal cytoreduction, serous histological subtype and tumor grade [18–21].

Ki67 is a nuclear protein expressed in cells during the phases of the cellular cycle (G1, S, G2 and M) and is absent during the G0 phase. The expression of Ki67 was associated with tumor aggression, reserved prognosis, vascular invasion and tumor metastasis [24]. Ki67 is overexpressed in malignant tumors compared to benign or borderline tumors [24]. In a study of Kübel M et al. (2008), it has been indicated that the Ki67 index has a greater value in high-grade serous carcinomas while the mucinous type has an intermediate proliferative capacity with a mean Ki67 index of 12.9% [25]. A study focused on evaluating the Ki67 index in mucinous ovarian tumors reported a value of 12.2±10.9% while well differentiated mucinous carcinomas had a value of 16.8±10.2% [26].

In our study, the mean proliferation index for Ki6 was 9.5% for borderline tumors and 22.2% for mucinous carcinomas, the highest values being recorded in patients with advanced stages of the disease.

Studies involving Ki67 index and its prognostic value in ovarian cancer have posted controversial results [24, 27, 28]. Some studies consider that the Ki67 index is an independent prediction tool for stage III ovarian cancer prognosis [28], while others have suggested that the Ki67 index cannot be used a sensitive indicator of proliferation in mucinous and serous ovarian carcinomas [29]. Other studies appreciate that MIB1 can be an additional instrument for the adjuvant treatment in early stage carcinoma patients but which should be tested in prospective studies [27].

Min KW et al., in 2007 [30], were analyzing Ki67 and p53 expression and they reported that the expression of Ki67 and p53 (>50%) correlates with the type and grade of the tumor in ovarian cancer, the overexpression being absent in borderline tumors and present in ovarian carcinomas.

Conclusions

Our study demonstrated a correlation between the expression of p53 and Ki67 markers in ovarian mucinous carcinomas and borderline tumors. While the expression of p53 was present in some of the tumors, the expression of Ki67 was observed in all the studied tumors. The immunochemistry study of the mucinous malignant ovarian tumors indicated significant differences of the Ki67 and p53 expression in relation to the type and stage of the tumor which demonstrates that these markers can be useful in evaluating aggressive tumor behavior but also for differentiating between carcinomas and borderline tumors.

Acknowledgments

Constantin Kamal Kamal acknowledges the support received through the project entitled “Doctorate an Attractive Research Career”, contract number POSDRU/ID/88/1.5/S/52826 co-financed by European Social Fund through Sectoral Operational Programme for Human Resources Development 2007–2013.

References
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Received: June 5th, 2012
Accepted: October 26th, 2012