MOLECULAR PHENOTYPE OF TUMOR CELLS AS A POTENTIAL MARKER FOR RISK OF RELAPSE IN PATIENTS WITH SEROUS OVARIAN CANCER

Summary. Objective: the purpose of the study is to analyze the frequency of expression marker Ki-67 and CD44s in the primary tumor patients in serous ovarian cancer who develop relapses after cytoreductive surgery and adjuvant polychemotherapy. Object and methods: the study included 58 patients with ovarian cancer stage I–III with primary relapse after treatment. Methods: clinical, morphological and immunohistochemical, statistical. Results: relapses in patients with serous ovarian cancer stage I–III of the most frequently (62.0%) are associated with the expression CD44s in cells of the primary tumor (phenotype CD44s+Ki-67+ and CD44s+Ki-67−). The frequency of tumors with these phenotypes increases with the extension of primary tumor process in patients with a high degree of morphological malignancy (the coefficients of mutual conjugacy Chuprov indicate moderate interrelationship). Conclusion: phenotype CD44s+Ki-67+ and CD44s+Ki-67− of tumor cells in the primary focus of ovarian cancer can be used as potential markers of development of relapses.

Ovarian cancer (OC) is referred to the most mysterious tumors of the female reproductive system not only in the respect to its pathogenesis, but also peculiarities of its clinical course. Clinical experience of oncogynecologists shows that despite primary positive effect of treatment of patients with serous OC, after certain period in 70-90% of patients, metastases and relapses of tumor process within the abdominal cavity arise [1-3]. Therefore, in recent years OC is referred to as chronic disease of abdominal cavity or chronic tumor process. OC differs from tumors of other genesis by series of peculiarities of dissemination of tumor cells (TC). Isolated cells or their clusters, detaching from the primary tumor lesion and passively moving in peritoneal liquid, can be there free or attach to the omentum or mesothelium of abdominal cavity forming microlesions of tumor growth [4, 5]. In activity of metastatic process, essential role is played by high proliferation of TC – volume of tumor doubles each 2.5 months, and dissemination of serous OC cells starts at size of tumor that equals only 3 cm [6].

Significant involvement of abdominal cavity in neglected tumor process makes impossible to carry out radical cytoreductive surgeries, i.e. maximal removal of intraabdominal tumor lesions. Residues of tumor riddling on mesothelial lining of abdominal cavity after suboptimal or nonoptimal cytoreductive surgeries are the cause of development of further relapses, which arise in different periods after surgical and adjuvant treatment [3, 7].

Mentioned above shows that probable prognosis of relapses of OC is extremely relevant issue in oncogynecology. Studies conducted for many years have outlined range of unfavorable clinical, morphological and molecular-biological prognostic factors. To the unfavorable clinical factors are referred dissemination of tumor process (III-IV stages), extracapsular growth of tumor, disorder of integrity of its capsule, papillary growth on the surface of tumor, adhesion of tumor with adjacent tissues, ascites, presence of implantation metastases, resistance to cytostatics, as well as impossibility of carrying out optimal cytoreductive surgeries [1-3]. Morphological factors of unfavorable prognosis are low-differentiated variants of OC and high potential of malignancy, in which essential significance is given to the proliferation of TC. In the presence of prognostically unfavorable factors, survival of patients decreases regardless of the stage [7].
Today more publications arise, in which much emphasis is placed to the biological features of the tumors. According with the dualistic model of OC [8], biological features of tumor are connected with pathogenesis, aggressiveness and prognosis of tumor process. In particular, it has been showed that markers of proliferation, angiogenesis, intercellular adhesion may be prognostic factors; significance of the profile of gene expression of these factors, molecular phenotype of TC, their plasticity (especially of disseminated TC), as well as intercellular heterogeneity by specified characteristics [7, 9, 10], has been demonstrated. Adhering to the conception of clonal development and metastasis of tumors, some authors have showed that primary ovarian tumors are characterized by clonality of genetic changes, and metastases of OC have the same genetic changes as primary tumor. However, despite significant number of studies, criteria, which could be used as predictive markers of possible development of OC relapses, are still not finally determined.

In previous studies [11], using morphological and immunohistochemical analysis of surgical material of patients with serous OC, we have determined that markers of unfavorable course of tumor process are high grade of morphological malignancy of tumors in combination with high expression of markers of proliferation (Ki-67) and intercellular adhesion (CD44s). Basing on the stated above, we may presume that these very markers probably have significance for dissemination of TC in abdominal cavity and development of relapses in patients with OC.

Aim of the study was to analyze frequency of expression of markers Ki-67 and CD44s in primary tumor of patients with serous OC, in whom relapses after cytoreductive surgery and adjuvant polychemotherapy (PCT) developed.

**OBJECT AND METHODS**

Clinical data of 58 patients with OC I-III stages with relapses developed during 3 years after carried out surgical treatment have been included in the study (under informed consent of patients). Surgical treatment included different types of surgeries – optimal, suboptimal and nonoptimal; also adjuvant PCT by schemes CP and CAP (from 4 to 6 courses) was performed. Mean age of patients constituted 56.4 years with individual variations from 26 to 70. Optimal cytoreductive surgeries were carried out only in patients with OC I stage, in other patients – suboptimal and nonoptimal cytoreductive surgical interventions.

For evaluation of dissemination of tumor process at initial diagnostics, FIGO classification was used (International Federation of Gynecology and Obstetrics), for evaluation of histological structure of tumor – international classification of tumors of the World Health Organization, for evaluation of malignancy grade – complex of cytomeorphological criteria [11]. Phenotype of TC was characterized by expression (which was determined by immunohistochemical method) of adhesion molecule CD44s and marker of proliferation Ki-67 in histological sections (4-5 µm thick) of primary tumors. Primary McAB to CD44s (Clone DF 1485, «DakoCytomation», Denmark) and Ki-67 (Clone MIB-1, «DakoCytomation», Denmark) were used. Evaluation of the results was carried out using semi-quantitative method. Level of expression of CD44s was determined as percentage of positively stained TC among TC over the whole square of histological section. Such approach is stipulated by significant heterogeneity of this marker by location of staining of nuclei – necessity “to refer” its expression to the certain morphological structures. Cells were considered positive by CD44s expression, if staining of TC membranes developed, including isolated clusters or complexes of cells. According with the criteria represented in literature, expression of marker was considered high, if number of TC with moderate and strong staining of membranes exceeded 10%. Positive (CD44s+) lymphocytes in stromal component of tumors were taken as positive control of CD44s expression. Expression of Ki-67 was determined in percentage after analysis of 1000-2000 TC. In the presence of > 10% of Ki-67-positive (-Ki-67+) TC with strong and moderate intensity of staining of nuclei in histological section, we regarded tumor as having high proliferation, < 10% — low proliferation.

The following statistical methods were used: standard descriptive, parametric and nonparametric (Student’s t-criterion), coefficient of nonlinear correlation – Chuprov coefficient of mutual conjugacy, which varied within the limits from 0 to 1. Significant were considered differences at p ≤ 0.05.

**RESULTS AND DISCUSSION**

Analysis of clinical data of patients with relapses of OC has showed that dissemination of tumor process, which was specified at cytoreductive treatment, corresponded to the I stage in 9 (15.5%) patients, II stage – in 16 (27.6%), III stage – in 33 (56.9%) (Fig.1). It means that relapses were diagnosed in patients regardless of the stage of primary tumor, but with different frequency – number of patients with relapses significantly increased as the process disseminated. In the analyzed group, most patients had OC III stage (56.9%) that significantly exceeded number of patients with OC II or I stages (27.6 and 15.5%; p < 0.05 correspondingly).

Primary tumors of patients with relapses were different by morphological malignancy grades: high grade – 37 (63.8%), low — 21 (36.2%). Comparison by this criterion has showed that studied group contained 1.8 times higher number of patients with high malignancy grade of OC, than patients with low malignancy grade of OC (p < 0.05) (Fig. 2).

When analyzing clinical data, it also has been determined that primary relapses of OC arose in different periods after treatment of patients. By the period of their occurrence, we have singled out early relapses (arise within the 6 months after primary treatment) and late relapses (arise after 6 months). General characteristic and frequency of primary relapses
depending on stage of OC are represented in Table 1. Data in this table show that in patients with OC I and II stages, early relapses were not observed, and number of late relapses in this dissemination of tumor process was not equal. Part of patients, who at the time of cytoreductive intervention had OC I stage, constituted 15.5%, II stage – was 1.8 times higher and constituted 27.6% (p < 0.05). In patients with OC III stage, both early (17.2%) and late (39.7%) relapses were detected. As it was previously stated, total number of the latter was 33, out of them 10 (30.3%) were early, 23 (69.7%) – late. It means that in patients with disseminated forms of tumor process that corresponds to the OC III stage, late relapses also prevailed – their part was 2.3 times greater, than part of the early relapses (p < 0.01).

![Fig. 1. Number of patients with relapses (n = 58) depending on stage of serous OC](image1)

![Fig. 2. Number of patients with relapses (n=58) depending on morphological malignancy grade of serous OC](image2)

**Table 1**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Total number of patients with relapses</th>
<th>Number of patients with early relapses</th>
<th>Number of patients with late relapses</th>
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<tr>
<td></td>
<td>n</td>
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<tr>
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<td>0</td>
</tr>
<tr>
<td>III</td>
<td>33</td>
<td>56.9</td>
<td>10</td>
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<tr>
<td>Total</td>
<td>58</td>
<td>100.0</td>
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According with the set aim, we have analyzed frequency of relapses depending on characteristic of phenotype of cells of primary tumor. Basing on the results of immunohistochemical evaluation of expression of adhesion and proliferation markers in histological sections of primary serous OC, following TC phenotypes were singled out: CD44s"Ki-67", CD44s"Ki-67", -CD44s"Ki-67", CD44s Ki-67" (Fig. 3). In studied group, part of patients with TC expressing marker CD44s (phenotype CD44s"Ki-67" and CD44s"Ki-67") was the largest — 36 (62.0%). The smallest part of patients (15.5%) had tumors without expression of studied markers (phenotype CD44s Ki-67").
Fig. 3. Frequency of phenotype of primary tumor cells in patients with relapses of OC: 1 — phenotype CD44s’Ki-67+; 2 — phenotype CD44s’Ki-67−; 3 — phenotype CD44s Ki-67+; 4 — phenotype CD44s Ki-67−

Analysis of frequency of TC with various phenotypes has showed their variability depending on stage of OC (Table 2). As data show, frequency of tumors with phenotype of TC CD44s’Ki-67+ (19/32.7%) and CD44s’Ki-67− (17/29.3%) was the highest. Phenotype CD44s Ki-67− also was determined frequently (13/22.4%), contrary to the phenotype CD44s Ki-67+. Frequency of OC phenotype without expression of studied markers decreased and frequency of phenotype CD44s + Ki-67+ increased as tumor process disseminated (see Table 2). Frequency of phenotype CD44 Ki-67+ almost did not depend on stage of OC. Statistical processing of obtained data with determination of Chuprov coefficient of mutual conjugacy has showed moderate positive correlation between frequency of cells with various phenotype and stage of OC (K= 0.40).

Taking into account that development of relapses depends not only on stage of OC, analysis of frequency of cells with different phenotype depending on morphological malignancy grade of OC has been carried out. As data in Fig.4 show, phenotype CD44s’Ki-67+ is mostly (73.7%) associated with high malignancy grade of primary tumor; upon the condition of low malignancy grade, frequency of such phenotype is significantly lower (26.3%; p < 0.05). Concerning phenotypes CD44s’Ki-67− and CD44s’Ki-67+, their frequency is also higher in patients with high malignancy grade of primary tumor. Statistical processing of data on frequency of cells with different phenotypes in patients with relapses depending on grade of morphological malignancy of OC has showed moderate correlation between these parameters, Chuprov coefficient of mutual conjugacy constitutes 0.65.

Table 2

<table>
<thead>
<tr>
<th>Stage</th>
<th>Phenotype CD44s' Ki-67+</th>
<th>Phenotype CD44s' Ki-67−</th>
<th>Phenotype CD44s Ki-67+</th>
<th>Phenotype CD44s Ki-67−</th>
<th>Total</th>
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<td>I</td>
<td>2 22.2</td>
<td>2 22.2</td>
<td>3 33.3</td>
<td>2 22.2</td>
<td>9</td>
</tr>
<tr>
<td>II</td>
<td>6 37.5</td>
<td>4 25.0</td>
<td>4 25.0</td>
<td>2 12.5</td>
<td>16</td>
</tr>
<tr>
<td>III</td>
<td>11 33.3</td>
<td>7 21.2</td>
<td>10 30.3</td>
<td>5 15.1</td>
<td>33</td>
</tr>
<tr>
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<td>13 22.4</td>
<td>17 29.3</td>
<td>9 15.5</td>
<td>58</td>
</tr>
</tbody>
</table>
patients with relapses of OC depending on phenotype of TC and grade of morphological malignancy of primary tumor: 1 — CD44s*Ki-67*; 2 — CD44s*Ki-67*; 3 — CD44s*Ki-67*; 4 — CD44 Ki-67*

Thus, conducted study shows that frequency of TC phenotypes CD44s*Ki-67* and CD44s*Ki-67* reliably increases as tumor process disseminates and morphological malignancy grade of OC increases that allows to evaluate this phenotype with expression of CD44s as marker of potential relapses. Significance of such phenotype as prognostic marker is substantiated by data of literature concerning the role of molecule of intercellular adhesion CD44s in growth of tumor and dissemination of TC. It is known that CD44s is multifunctional molecule. Firstly, it can stimulate proliferation of cells, their mobility and invasiveness. Secondly, it can function as co-receptor for activation of tyrosine kinases of other receptors. Thirdly, some isoforms of CD44s are capable to act as cellular-surface ligands for interaction with endothelium. They also can delay and fix TC in different tissues and organs, regulate function of endothelial cells and tumor angiogenesis [12–14]. All described processes are components of complex mechanism of metastasis of TC not only of OC, but also tumors of other genesis. Moreover, presence of expression of CD44s, which is marker of stem cancer cells, can be associated with higher growth potential increasing tumorigenic potential by this way. It has been showed that TC with CD44s expression are characterized by higher clonogenicity compared with cells having no such expression [1]. However, there are data that not only expression of CD44*, which is connected with the stage of OC, histological structure of tumor, but also other markers of stem TC (CD24*, CD133*, EpCAM*) are important molecular markers of aggressive potential, tumorigenicity, chemoresistance and unfavorable prognosis of serous OC [15, 16]. Perhaps, molecules of intercellular adhesion and proliferation play key role in development of relapses in patients with OC, though the role of other factors cannot be excluded, in particular, microenvironment of TC, matrix metalloproteinases, hypoxia, state of mesothelium of abdominal cavity, growth factor of endothelium vessels VEGF, as well as pathogenesis of OC [17]. All these issues require further detailed study, results of which will contribute to the determination of subtypes of serous OC with different metastatic potential that represents relevant problem of oncogynecology [18].

CONCLUSIONS

1. Relapses in patients with serous OC I-II stages are associated with dissemination of tumor process and molecular phenotype of cells of primary tumor: the largest part (62.0%) is formed by tumors with expression of CD44s (phenotypes CD44s*Ki-67* and CD44s*Ki-67*), which were determined in patients with different stages of tumor process, mostly – III stage of OC.

2. In patients with relapse of serous OC, moderate direct correlation between expression of CD44s in cells of primary tumor and its high grade of morphological malignancy has been determined (Chuprov coefficient of mutual conjugacy 0.65).

3. Expression of molecul of intercellular adhesion CD44s and marker of proliferation Ki-67 in cells of serous OC is the sign of aggressiveness of tumor process and may be used for the predictive evaluation of individual prognosis of the disease as potential marker of relapses.

REFERENCES


