Abstract. Aim: To investigate the role of CD44s in the biological behavior of surface epithelial ovarian tumors and its correlation with clinicopathological parameters, prognosis, p53, steroid receptor status and proliferative indices (PCNA, MIB1). Materials and Methods: We analyzed a total of 83 patients with ovarian surface epithelial tumors, for the immunohistochemical expression of CD44s and the possible correlation with clinicopathological factors and patients' outcome. Results: A statistically significant correlation was found between the expression of CD44s, which was higher in cancer cases than in benign cystadenomas (p<0.0001) and, between cancer cases, which was lower in borderline tumors, (p=0.05). No statistical correlation was found between CD44s expression and the examined markers. In overall survival analysis we did not detect a statistically significant correlation with the expression of CD44s. Conclusion: The current study demonstrates that CD44s may be functionally involved in the pathogenesis of epithelial ovarian lesions.

CD44 characterizes a polymorphic family of cell-surface glycoproteins, which are involved in cell-to-cell and cell to extracellular matrix adhesive interactions. They have been found to participate in several biological processes, such as cell trafficking, lymphocyte homing, haematopoiesis, wound healing and inflammation (1, 2). The biological aptitudes of these molecules have brought the CD44 family under intense investigation. Published data associate CD44 with cancer as a potential tumor marker (3, 4) and with chronic inflammation as a therapeutic intervention (5, 6).

CD44s, which represent the major type of CD44, found on leucocytes and fibroblasts but also in epithelial cells, is the most studied type in the ovary (17-19). This has been found expressed in normal ovarian epithelium but also in ovarian malignant cell lines and seems to play an important role in ovarian metastasis (20). In the metastatic process, CD44 is the primary receptor for hyaluronan and published data reported that the attachment of tumor ovarian cells to the peritoneal mesothelium is mediated through the interaction between CD44 expressed in malignant cells and hyaluronic acid expressed on the mesothelial surface (21, 22).

Correspondence to: N.J. Agrantis, Department of Pathology, Medical School, University of Ioannina, 45110 Ioannina, Greece. Tel: ++32651 9 99792, Fax: ++32651 0 99792.

Key Words: CD44s, ovarian cancer, ER, PR, p53, PCNA, MIB1.
The purpose of this study was to investigate the significance of the immunohistochemical expression of CD44s in the full spectrum of ovarian tumors that originate from the ovarian surface epithelium, from benign cystadenomas to cancer. We also investigated the correlations with p53, steroid receptor status, proliferative associated indices (MIB1, PCNA) and survival.

Materials and Methods

A total of 83 cases with diagnosis of ovarian surface epithelial tumors were retrieved from the archives of the Surgical Pathology Department of the University Hospital of Ioannina, Greece, for the period 1979 to 2003. All patients were surgically treated at the University Hospital of Ioannina. The surgical treatment for benign tumors was simple cystectomy, for borderline tumors conservative or radical surgery, but for malignant tumors radical surgery, which included hysterectomy with bilateral salpingo-oophorectomy and omentectomy. All patients with malignancy in this series received chemotherapy at the Medical Oncology Department of the same hospital according to running treatment protocols. Two pathologists reviewed the histological diagnosis and two representative blocks from each case were selected for immunohistochemistry. We used the method involving the avidin-biotin-peroxidase complex and developed the chromogen with immersion of the slides in a diamino-benzidine-H2O2 substrate for 5 min. The slides were counterstained in Harris’ haematoxylin, dehydrated and mounted. To assess the specificity of the reaction, negative controls were included, and tumor sections subjected to the procedure except for incubation without the primary antibody. The antibody sources and dilutions are shown in Table II.

Immunohistochemistry. On two selected paraffin blocks, from each case, we performed immunohistochemistry on 4-μm tissue sections placed on poly-L-lysine-coated glass slides. Consequently, the sections were deparaffinised in xylene and dehydrated. All sections were treated for 30 min with 0.3% hydrogen peroxide (in methanol) to quench endogenous peroxidase activity and then were incubated with primary antibodies. We used the method involving the avidin-biotin-peroxidase complex and developed the chromogen with immersion of the slides in a diamino-benzidine-H2O2 substrate for 5 min. The slides were counterstained in Harris’ haematoxylin, dehydrated and mounted. To assess the specificity of the reaction, negative controls were included, and tumor sections subjected to the procedure except for incubation without the primary antibody. The antibody sources and dilutions are shown in Table II.

Immunohistochemical evaluation. Anti-CD44s reactivity was evaluated as positive when cytoplasmic membrane staining was observed and was reported as percentage of positive epithelial cells in relation to the total number of cells. 

Anti-ER and anti-PR reactivity was evaluated by the observation of the positive epithelial cell nuclei. The scoring system was as follows: 0=negative, (1+) for positive cells below 10%, (2+) 11–50% and (3+) for more than 51% stained cells. Only cases with score 2+ and 3+ were considered as positive.

Anti-p53 reactivity was evaluated only when brown nuclear staining was detected, and was scored as follows: 0 when less than 10% reactive cells, (1+) when the reactivity was between 10% and 25%, (2+) for 26% to 50% and (3+) when more than 51% cells were positive. Any case scored at least as (1+) was considered positive. The selection of this scoring system was based on the observation that, when more than 10% of the tumor nuclei are stained with anti-p53, the highest correlation with the presence of structural mutations in the p53 gene is observed (27).

Anti-Mib1 and anti-PCNA reactivity was evaluated as positive, only when epithelial nuclear staining was observed. For the statistical analysis the cases were divided for MiB1 into two groups, (<10% and >10%) and for PCNA into three groups (<10%, 11-50% and >50%).

Statistical analysis. All data were statistically analyzed by SPSS ver10 statistical programme. Non-parametric test, Mann-Whitney U-test type, was used for the association of continuous variables. For relapse-free and overall survival analyzed by expression of CD44 we used Kaplan-Meier plots and the log-rank test, while the Graph pad Prism 4 programme analyzed the results. p values less than 0.05 were considered statistically significant.
Results

Patients' age at the time of the diagnosis ranged from 17 to 85 years old (median = 58, mean = 53) and was clearly related to the presence of malignancy: age was lower for patients diagnosed with benign and higher for those with malignant tumors (Figure 1). Regarding the 48 carcinomas, 10 were well-differentiated (20.8%), 14 moderately-differentiated (29.2%) and 24 poorly-differentiated (50%); by histology, 38/48 (79.2%) were serous cystadenocarcinomas, 4/48 (8.3%) mucinous cystadenocarcinomas, 3/48 (6.3%) mixed carcinomas and 3/48 (6.3%) clear cell carcinomas.

All the positive tumor cells were characterized by intense membrane staining. In the positive tumor cases small foci, single or multiple, of intense membrane staining were noticed, but the rest of the tumor cells were negative (Figures 4, 5). The mean value of CD44s expression in cancer cases was 17.4%. Fifty percent of the cases were negative (24/48), in 29.2% (14/48) of the cases the expression was between 0 to 50% and in 10 out of 48 cases (20.8%) the expression was higher than 50%. A statistically significant correlation was found between the expression of CD44s, which was higher in cancer cases than in benign cystadenomas ($p < 0.0001$) and between cancer cases, which was lower than in borderline tumors, ($p = 0.05$) (Figure 2).

The mean value of CD44s expression was 12 in grade I (well-differentiated), 18.2 in grade II (moderately-differentiated) and 19.1 in grade III (poorly-differentiated), but no statistical correlation was reached between grade or histological type and CD44s expression. ER expression was in 33/48 (68.8%) cases lower than 10% and in 15 (31.3%) higher than 10%. PR expression was in 39/48 (81.3%) lower than 10% and in 9/48 (18.8%) higher than 10%. No statistical correlation was found between CD44s expression and the examined markers (Table III).

In borderline tumors the mean value of CD44s expression was 20.3. The expression of CD44s in 42.9% (6/14) of the cases was between 10 and 50%. No statistical correlation was found between CD44s expression and the examined markers (Table III).

In overall survival analysis we did not detect a statistical difference in this small number of patients when categorized by expression of CD44s (Figure 3).

Discussion

Ovarian epithelial cancer is a leading cause of death among gynecological malignancies due to advanced stage at presentation and the development of resistance to
chemotherapy. The majority of women, after an initial response to first-line chemotherapy, will eventually relapse to a more resistant status (28). It is noticeable that, despite improvements in the treatment of ovarian cancer, incidence and mortality rate steadily remain high (29, 30). The five-year survival for women diagnosed with ovarian cancer is only 50% (31). These clinical data strongly support the need for establishing tumor markers, which could offer predictive information with regard to biological behavior of a tumor and expected response to chemotherapy and thus facilitate the decision about the initial clinical management.

Surface epithelial tumors are categorized by histopathological criteria for grading, as benign, borderline or low malignant potential (LMP) and malignant tumors. It is unknown if this classification denotes a sequence to malignant transformation or whether it simply represents a spectrum of diseases. In this respect great interest has been raised in studying LMP tumors. In a large study of Kurman and Trimble, which included 953 serous LMP tumors, only less than 1% demonstrated a malignant transformation. This was the reason that this pathological entity was renamed as proliferative tumor (32).

Surface epithelial tumors originate from the celomic epithelium that forms epithelial glands and cysts (33). The epithelium is continuous with the mesothelium that covers the peritoneal cavity. This anatomical consideration explains the metastatic behavior of these types of tumors, which is actually unique. The dissemination occurs principally within the abdominal cavity, by intraperitoneal adhesion of the tumor cells to the mesothelium and penetration to the

---

**Table III. Immunohistochemical expression of ER, PR, p53, MIB1, PCNA and correlation with CD44s in cancer cases.**

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>%</th>
<th>CD44s +ves</th>
<th>CD44s -ves</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>Less than 10%</td>
<td>31</td>
<td>64.6%</td>
<td>15 (31.3%)</td>
</tr>
<tr>
<td></td>
<td>Greater than 10%</td>
<td>17</td>
<td>35.4%</td>
<td>7 (14.6%)</td>
</tr>
<tr>
<td>PR</td>
<td>Less than 10%</td>
<td>39</td>
<td>81.3%</td>
<td>18 (37.5%)</td>
</tr>
<tr>
<td></td>
<td>Greater than 10%</td>
<td>9</td>
<td>18.8%</td>
<td>6 (12.5%)</td>
</tr>
<tr>
<td>p53</td>
<td>Less than 10%</td>
<td>25</td>
<td>52.1%</td>
<td>16 (33.3%)</td>
</tr>
<tr>
<td></td>
<td>Greater than 10%</td>
<td>23</td>
<td>47.9%</td>
<td>8 (16.7%)</td>
</tr>
<tr>
<td>MIB1</td>
<td>Less than 10%</td>
<td>5</td>
<td>10.4%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Greater than 10%</td>
<td>43</td>
<td>89.6%</td>
<td>24 (50%)</td>
</tr>
<tr>
<td>PCNA</td>
<td>Less than 10%</td>
<td>3</td>
<td>6.3%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>10-50%</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Greater than 50%</td>
<td>11</td>
<td>78.6%</td>
<td>11 (78.6%)</td>
</tr>
</tbody>
</table>

**Table IV. Immunohistochemical expression of ER, PR, p53, MIB1, PCNA and correlation with CD44s in LMP tumor.**

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>%</th>
<th>CD44s +ves</th>
<th>CD44s -ves</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>Less than 10%</td>
<td>13</td>
<td>92.9%</td>
<td>12 (85.7%)</td>
</tr>
<tr>
<td></td>
<td>Greater than 10%</td>
<td>1</td>
<td>7.1%</td>
<td>1 (7.1%)</td>
</tr>
<tr>
<td>PR</td>
<td>Less than 10%</td>
<td>14</td>
<td>100.0%</td>
<td>13 (92.9%)</td>
</tr>
<tr>
<td></td>
<td>Greater than 10%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>p53</td>
<td>Less than 10%</td>
<td>14</td>
<td>100.0%</td>
<td>13 (92.9%)</td>
</tr>
<tr>
<td></td>
<td>Greater than 10%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MIB1</td>
<td>Less than 10%</td>
<td>3</td>
<td>21.4%</td>
<td>2 (14.3%)</td>
</tr>
<tr>
<td></td>
<td>Greater than 10%</td>
<td>11</td>
<td>78.6%</td>
<td>11 (78.6%)</td>
</tr>
<tr>
<td>PCNA</td>
<td>Less than 10%</td>
<td>3</td>
<td>21.4%</td>
<td>2 (14.3%)</td>
</tr>
<tr>
<td></td>
<td>10-50%</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Greater than 50%</td>
<td>8</td>
<td>57.1%</td>
<td>8 (57.1%)</td>
</tr>
</tbody>
</table>
submesothelial matrix. Haematogenous dissemination is rare and lymphatic spread also occurs at low frequency (34).

The significance of the immunohistochemical expression of the CD44 family in ovarian cancer has been investigated during recent years. Cannistra and collaborators (35) investigated CD44s and CD44v9 in 31 cases of ovarian cancer and found no prognostic significance. In contrast Uhl-Steidl et al. (36), also in 1995, published a study, which included 44 patients and reported that CD44-positive cancer cases were associated with shortened overall survival. In 1999 three studies were published, which associated CD44s and variants with poor prognosis. Schroder et al., examined CD44s and CD44v5-v8 and v10 in 50 cases and reported that CD44v5 and v6 were associated with intraperitoneal implantation. Kayastha et al. (37) investigated the immunohistochemical expression of CD44s in a series of 56 cases, and reported that CD44s was associated with poorer overall survival. Saegusa et al., (38) in 115 cases, using immunohistochemistry and RT-PCR, examined CD44s, v3 and v6, and reported that CD44v3 loss of expression is associated with poor prognosis. In 2001, Ross et al. (19) in a study, which included 101 cases, investigated CD44s by immunohistochemistry and reported that decreased expression of CD44s was associated with shortened survival. In the present study, with a small number of patients with complete follow-up, we did not reveal any statistically significant correlation between the expression of CD44s and the examined markers, the clinicopathological parameters or survival. The benign lesions, as a group, showed minimal or even no reactivity for CD44s. On the other hand the expression of CD44s in the LMP group was higher when compared to malignant lesions.

The expression of ER, PR in cancer cases was lower than 10% in 64.6% and 81.3%, respectively, and we did not reveal any statistically significant correlation between these two markers and CD44s. It remains to be found if there is a relationship between them.

Despite that, the immunohistochemical expression of PCNA and MIB1, which are markers of proliferation, was higher in cancer cases than in the two other examined groups (LMP, benign tumors) (Tables III, IV) and could be used as a method of distinguishing them. We are in agreement with Anreder et al., that light microscopy remains the method of choice to evaluate proliferative from malignant lesions (39). p53 has been correlated with CD44 in renal carcinoma (23). In ovarian cancer no association was found between CD44v6 and p53 (40). In the current study we did not reveal any statistically significant correlation between p53 and CD44s. So, it remains to be discovered whether these two markers are independent or correlated in epithelial ovarian cancer. In LMP tumors the expression of p53 was in all the examined cases lower than 10% or even absent. We agree with the results published by Wertheim et al. (41), who investigated the p53 gene mutation in borderline tumors and concluded that the gene mutation is a rare event and is not important in the pathogenesis of these lesions, which represent a distinct biological entity. No correlation was found between p53 and CD44s. The existing published data do not concur. This could be related to differences in technical methods or in the interpretation of the immunohistochemical results.

In conclusion, the current study demonstrates that CD44s may be functionally involved in the pathogenesis of epithelial ovarian lesions.

Acknowledgements

We thank Mrs Antigoni Christodoulou for her technical assistance.

References