HLA Class I Antigen Down-Regulation in Primary Ovary Carcinoma Lesions: Association with Disease Stage

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ABSTRACT

Purpose: To investigate TAP1, TAP2, and HLA class I antigen expression in primary ovarian carcinoma lesions and to assess the clinical significance of defects in the expression of these molecules.

Experimental Design: Fifty-one formalin-fixed, paraffin-embedded primary ovarian carcinoma lesions were stained with affinity-purified rabbit anti-TAP1 and anti-TAP2 antibodies and with anti-HLA class I heavy chain monoclonal antibody (mAb) HC-10 using the immunoperoxidase reaction. The results of immunohistochemical staining were correlated with the histopathologic characteristics of the lesions and with patients’ survival.

Results: Ovarian surface epithelium, thecal cells of follicles, and stromal cells were stained by anti-TAP1, anti-TAP2, and anti-HLA class I antigen xenoantibodies with a homogeneous pattern. In contrast, no staining of lutein cells by these antibodies was detected. Forty-one and 32 out of 51 primary ovarian carcinoma lesions were stained by anti-TAP1 and anti-TAP2 xenoantibodies and by anti-HLA class I antigen mAb HC-10, respectively. The staining patterns by anti-TAP1 and anti-TAP2 xenoantibodies and by anti-HLA class I antigen mAb HC-10 were completely concordant, but did not correlate with that by anti-HLA class I heavy chain mAb HC-10. TAP1 and TAP2 expression was associated neither with the histopathologic characteristics of the lesions nor with clinical variables. On the other hand, HLA class I antigen down-regulation was associated with disease stage: the odds ratio of stage III for HLA class I antigen negative patients was 7.6 (95% confidence interval, 1.9-30.5; P = 0.007), whereas for TAP negative patients was 5.1 (95% confidence interval, 0.9-28.4; P = 0.07). Follow up was available for 39 out of the 51 patients. Multivariate analysis showed that both grading and staging were associated with a higher risk of death, whereas TAP and HLA class I antigen phenotypes were not.

Conclusions: The lack of association between TAP and HLA class I antigen expression is compatible with the possibility that multiple mechanisms underlie HLA class I antigen down-regulation in primary ovarian carcinoma lesions. The potential role of immunologic events in the clinical course of ovarian carcinoma suggests that the association between HLA class I antigen down-regulation and disease progression may reflect the escape of tumor cells from immune recognition and destruction.

INTRODUCTION

The limited efficacy of conventional chemotherapy in most malignant diseases has provided the impetus to develop and implement alternative strategies for the treatment of malignant diseases. Among them, immunotherapy has attracted much attention in recent years because of the revival of the role of immune surveillance in the control of tumor growth (1) and because of the significant progress in the identification of human tumor-associated antigens (TAA; ref. 2) and in the characterization of the molecular steps leading to an immune response (3). The emphasis has been on T cell–based immunotherapy, because T cells are generally believed to play the major, if not the only role in the control of tumor growth (4). The outcome of T cell–based immunotherapy of malignant diseases is influenced by many variables. Among them, an important role is played by HLA class I antigen–derived peptide complexes expressed on tumor cells, because they mediate the recognition of tumor cells by CTL. In humans, like in other animal species, HLA class I antigen–derived peptide complexes are generated, transported to the cell membrane, and presented to CTL through a series of sequential steps. The latter include (i) cleavage in the cytoplasm of proteins by proteasome, which modulates its activity by replacing the constitutive subunits β1, β2, and β3 with the immunosubunits LMP2, LMP10, and LMP7, respectively, upon exposure to IFN-γ (5); (ii) transport by the transporter associated with antigen processing TAP1-TAP2 complex of peptides to the endoplasmic reticulum (6); and (iii) loading of peptides on β2 microglobulin (β2m)-HLA class I heavy chain complex (7, 8). The peptide-β2m-HLA class I heavy chain complex then travels to the cell membrane and peptides are presented to CTL. Therefore, abnormalities in the expression and/or function of antigen processing machinery components and/or HLA class I antigens may lead to defects in the expression of HLA class I antigen-antipolate complexes and eventually in the recognition of targets by CTL. This mechanism
seems to be used by tumor cells to escape from immune recognition and destruction (9), because defects in the expression of TAP1 and/or HLA class I antigens in malignant cells are frequently associated with the histopathologic characteristics of the lesions and/or the clinical course of the disease in at least some malignancies (10–15).

Recent evidence suggests that T cell immunity may play a role in the clinical course of ovarian carcinoma (16). This possibility has stimulated interest in antigen processing machinery component and HLA class I antigen expression because defects in these molecules may provide ovarian carcinoma cells with an escape mechanism from T-cell recognition and destruction. To the best of our knowledge, no information is available about the expression of antigen processing machinery components in ovarian carcinoma lesions. Therefore, in the present study, we have analyzed the expression of TAP1 and TAP2 in surgically removed primary ovarian carcinoma lesions, because these molecules play a key role in the generation of HLA class I-peptide complexes and because defects in their expression seem to play a role in the clinical course of other malignant diseases (6). We have correlated TAP1 and TAP2 expression with HLA class I antigen expression, with the histopathologic characteristics of the lesions and with patients’ survival to assess the functional and clinical significance of TAP1 and TAP2 defects.

MATERIALS AND METHODS

Tissues. Primary ovarian carcinoma lesions were obtained from 51 untreated patients with an average age of 60.3 years (range, 32-77 years) who underwent surgery between 1988 and 1998 at the First Department of Obstetrics and Gynaecology, Medical School, University of Bologna, Bologna, Italy.

All patients were optimally (defined by residual individual tumor nodules measuring <1 cm in diameter) surgically debulked (laparoscopic omentectomy and omentectomy in case of peritoneal metastases, followed by pelvic and/or ilio-inguinal lymphadenectomy). The tumors analyzed included 4 clear cell adenocarcinomas, 9 endometrioid adenocarcinomas, 3 mucinous adenocarcinomas, and 35 serous adenocarcinomas. Lesions were staged following the International Federation of Gynaecology and Obstetrics criteria (17). All patients were treated with carboplatinum and taxol.

Tumors were fixed within 15 minutes following their surgical removal in 10% neutral buffered formaldehyde and routinely processed. Representative paraffin tissue blocks were selected from all tumor specimens and serially cut into 5-μm sections. Serial sections from each specimen were routinely stained with H&E for histologic examination and tumor grading.

Antibodies. Affinity-purified rabbit anti-TAP1 and anti-TAP2 antibodies and the monoclonal antibody (mAb) HC-10, which recognizes a determinant expressed on virtually all β2m-free HLA-B heavy chains and on β2m-free HLA-A10, HLA-A28, HLA-A29, HLA-A30, HLA-A31, HLA-A32, and HLA-A33 heavy chains were developed and characterized as described (18, 19). Biotinylated rabbit anti-mouse immunoglobulin and goat anti-rabbit immunoglobulin antibodies were purchased from Dako A/S (Gostrup, Denmark).

Immunohistochemical Staining. Tissue sections were dewaxed with xylene and rehydrated by passage through decreasing concentrations of ethanol (100-80%). Endogenous peroxidase activity was blocked by a 30-minute incubation at room temperature with methanol containing 3% H2O2. Tissue sections were then microwaved in a citrate solution (pH 6.0) at 750 W for 10 minutes. After rinsing in TBS (pH 7.4), tissue sections were preincubated for 30 minutes at room temperature with normal rabbit serum or normal goat serum diluted 1:5; prior to incubation in a humidified chamber for 90 minutes at room temperature with anti-HLA Class-I mAb HC-10 and overnight at 4°C with rabbit anti-TAP1 and anti-TAP2 antibodies. Tissue sections were then washed twice in TBS and incubated for 30 minutes at room temperature with either biotinylated rabbit anti-mouse immunoglobulin G or biotinylated goat anti-rabbit immunoglobulin G antibodies. Tissue sections were then washed again in TBS and incubated for 60 minutes at room temperature with avidin-biotin peroxidase complex (11). Peroxidase activity was detected by incubating tissue sections for 10 to 15 minutes with a solution of 3,3′-diaminobenzidine (Sigma Chemicals Co., St. Louis, MO; 1 mg/mL of 3,3′-diaminobenzidine in 5 mL of TBS) in the presence of 15 μL/mL of 3% H2O2. Tissue sections were counterstained with Mayer’s hemalum (Sigma Chemicals). Negative controls were done by omitting the primary antibodies.

Tissue sections were read independently by two investigators. A lesion was classified as negative when <25% of tumor cells were stained; positive, when >75% of cells were stained, independently from the intensity of staining. In case of heterogeneous stained tumors (25-75% of stained cells), corresponding tumor cell clusters were observed in seriated sections. If the cluster resulted positive in repetitive subsequent sections, the lesion was scored as positive. Normal ovarian epithelium, stromal cells, endothelial cells, and/or infiltrating lymphocytes were examined in each tissue section as internal controls. The staining intensity was scored as (−) when no staining was detected, (+) when the intensity was lower than that of the normal cells in the tissue section, and (++) when the intensity was similar to that of the normal cells in the tissue section.

Statistical Analysis. The association of HLA class I antigen and TAP and expression (alone or in combination) with the grade and stage of the ovary carcinoma lesions tested was investigated using log-linear models as described by Agresti (20) and by the Kruskal-Wallis test (21). In this analysis, the only lesion from a patient with disease stage IV was excluded.

Moreover, in a further analysis, the disease stage was dichotomized in an early stage (which combined stages I and II) and an advanced stage (stage III). The odds ratios for advanced and early disease stage were calculated according to TAP and HLA class I antigen expression of the lesions (20). The same approach was followed to calculate the odds ratios for tumor grading.

Finally, patients’ survival rates were calculated using the Kaplan-Meyer method; differences between groups were tested using the log-rank test; association between each variable under study and survival was done using the Cox regression models (21). All the statistical tests were done at P = 0.05, although P’s between 0.05 and 0.1 are also shown. All the analyses were done using the BMDP/Dynamic computer programs (22).
RESULTS

Preliminary assays tested the staining of formalin-fixed, paraffin-embedded normal ovary tissues with the affinity-purified rabbit anti-TAP1 and anti-TAP2 antibodies and with the anti-HLA class I mAb HC-10. The three antibodies stained homogeneously the ovarian surface epithelium as well as the thecal cells of the follicles and stromal cells. The staining by anti-HLA class I mAb had a high intensity on cell membrane and was associated with some cytoplasmic staining. On the other hand, the staining by anti-TAP1 and anti-TAP2 antibodies was restricted to cytoplasm and had an intermediate to high intensity. TAP1 and TAP2 expression, as determined by staining with antibodies, was always associated with that of HLA class I antigens in normal tissues. No staining of luteinized cells by the three antibodies was detected. Representative results are shown in Fig. 1.

Fifty-one primary ovary carcinoma lesions were stained with rabbit anti-TAP1 and anti-TAP2 antibodies and with anti-HLA class I mAb HC-10 (Figs. 2 and 3). Forty-one out of the 51 lesions tested were stained by anti-TAP1 and anti-TAP2 antibodies. The staining pattern of malignant cells was similar to that of their normal counterparts. A complete concordance was found in the expression of TAP1 and TAP2 in the malignant lesions analyzed. Therefore, the results are presented in terms of TAP expression. Thirty-two lesions were stained by anti-HLA class I mAb HC-10. No correlation was found between TAP and HLA class I antigen expression.

As shown in Table 1, only 27 lesions stained positive for both TAP and HLA class I antigens. Of the remaining lesions, 14 were positive for TAP but negative for HLA class I antigens, 5 lesions were negative for both TAP and HLA class I antigens, and 5 were negative for TAP, but positive for HLA class I antigens.

TAP expression in lesions was not associated with tumor grading and disease stage, considered as linear variables. HLA class I antigen expression was not associated with tumor grading but was significantly \( P = 0.003 \) associated with disease stage (Table 2). Tumor grading and staging were then divided into high (\( \rightarrow \) advanced disease: G3 or stage III) and low categories (\( \rightarrow \) early disease: G1 and G2, stage I and stage II). Also in this analysis, no association was found between TAP/HLA class I antigen down-regulation and tumor grading. Disease stage showed an association with both TAP and HLA class I antigen down-regulation (Table 3). However, the association reached the level of statistical significance only for HLA class I antigens. The odds ratio of lesions from patients with disease stage III for TAP and HLA class I antigen down-regulation were 5.1 (95% confidence interval, 0.9-28.4; \( P = 0.07 \)) and 7.6 (95% confidence interval, 1.9-30.5; \( P = 0.007 \)), respectively.

Additional analyses tested whether TAP and HLA class I antigen down-regulation in primary carcinoma lesions was associated with disease-free interval and survival. The clinical information was available only for 39 patients (Table 4).
Survival was calculated using the time of surgical removal of the lesions as a starting point and death or time of the last examination for patients who had no evidence of disease and were still alive. Twelve patients (30.8%) were still alive in March 2003, with a median of 26.03 months (range, 2.8-177.9 months). Survival rates at 1, 3, and 5 years were 87.2%, 46.2%, and 30.8%, respectively. None of the patients with tumor grade G1 or disease stage I died during the study period. Multivariate analysis by Cox models confirmed that both tumor grade and disease stage are associated with a higher risk of death and showed that TAP and HLA class I antigen down-regulation are not. In fact, although HLA class I antigen down-regulation was significantly associated with advanced tumor staging (stage III), TAP and HLA class I antigen expression in carcinoma lesions was not associated with survival. However, this finding might be caused by the small number of patients for whom a continuous follow-up in a 5- to 15-year period was available.

DISCUSSION

Immunohistochemical staining of 51 formalin-fixed, paraffin-embedded primary ovarian carcinoma lesions has shown TAP and HLA class I antigen down-regulation in 19.6% and 37.2% of the lesions tested, respectively. In view of the role immunoselection seems to play in the generation of malignant lesions with HLA class I defects, the high frequency of malignant lesions with HLA class I antigen defects we have found in our study is compatible with the development of tumor antigen–specific T-cell immunity in a high proportion of the patients from whom the ovarian carcinoma lesions analyzed had been obtained. This possibility is supported by the detection of tumor antigen–specific CTL in the peripheral blood of many patients with ovarian carcinoma. The tumor antigen–specific T-cell immunity developed in patients is expected to favor the expansion and overgrowth of ovarian carcinoma cell subpopulations which escape T-cell recognition and killing because of HLA class I antigen defects. If this interpretation is correct, one might wonder whether the correlation of the presence of intratumoral T cells with disease recurrence and survival described in a group of patients with ovarian carcinoma (16) may improve by taking into account HLA class I antigen expression in malignant lesions in the analysis of the results.

No correlation was found between TAP and HLA class I antigen down-regulation in primary ovarian carcinoma lesions. This finding is not unique of ovarian carcinoma, because an association between TAP and HLA class I antigen down-regulation has been found in colorectal cancer (9, 14) but not in cervical carcinoma (13) and breast cancer (11). The lack of correlation between TAP and HLA class I antigen expression
Table 1  Distribution of ovary cancer lesions according to grading, staging, and phenotype

<table>
<thead>
<tr>
<th>Lesion no.</th>
<th>HLA class I antigens</th>
<th>TAP+</th>
<th>TAP−</th>
<th>TAP+</th>
<th>TAP−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>9</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>G2</td>
<td>18</td>
<td>12</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>G3</td>
<td>24</td>
<td>10</td>
<td>3</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>25</td>
<td>19</td>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>III</td>
<td>20</td>
<td>4</td>
<td>3</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>27</td>
<td>5</td>
<td>14</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2  Association of HLA class I antigen downregulation in primary ovarian carcinoma lesions with disease stage

<table>
<thead>
<tr>
<th>HLA class I antigens</th>
<th>TAP+</th>
<th>TAP−</th>
<th>TAP+</th>
<th>TAP−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>6 (66.6%)</td>
<td>3 (33.3%)</td>
<td>7 (77.7%)</td>
<td>2 (22.2%)</td>
</tr>
<tr>
<td>G2</td>
<td>13 (72.2%)</td>
<td>5 (27.7%)</td>
<td>16 (88.9%)</td>
<td>2 (11.1%)</td>
</tr>
<tr>
<td>G3</td>
<td>13 (54.2%)</td>
<td>11 (45.1%)</td>
<td>18 (75.0%)</td>
<td>6 (25.0%)</td>
</tr>
<tr>
<td>K-W</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Log-Lin</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>4 (80.0%)</td>
<td>1 (20.0%)</td>
<td>4 (80.0%)</td>
<td>1 (20.0%)</td>
</tr>
<tr>
<td>II</td>
<td>20 (80.0%)</td>
<td>5 (20.0%)</td>
<td>23 (92.0%)</td>
<td>2 (8.0%)</td>
</tr>
<tr>
<td>III</td>
<td>7 (35.3%)</td>
<td>13 (65.0%)*</td>
<td>13 (65.0%)</td>
<td>7 (35.0%)</td>
</tr>
<tr>
<td>IV</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>*K-W</td>
<td>P &lt; 0.005</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Log-Lin</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NOTE: All percentages were calculated on the total number of patients with each phenotype. The case classified as stage IV was excluded from the statistical analysis. K-W: The Kruskal-Wallis test was used to determine the association between ordinal and nominal variables (grade and stage were considered as ordinal variables). Log-Linear models. For each variable, a test of partial association is shown.

*The interactions between TAP and HLA class I antigen phenotypes were not significantly associated at the 0.05 level.

Table 3  Association of TAP and HLA class I antigen expression with tumor grade and disease stage grouped as low and advanced

<table>
<thead>
<tr>
<th>HLA class I antigens</th>
<th>TAP +</th>
<th>TAP −</th>
<th>Odds ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (G1-G2)</td>
<td>19</td>
<td>8</td>
<td>23</td>
</tr>
<tr>
<td>Adv. (G1)</td>
<td>13</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (I-II)</td>
<td>24</td>
<td>6</td>
<td>27</td>
</tr>
<tr>
<td>Adv. (III)</td>
<td>7</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7</td>
</tr>
</tbody>
</table>

*Odds ratio of G3 or stage III lesions for negative versus positive TAP and HLA class I antigen phenotypes was calculated by logistic regression analysis with both TAP and HLA class I antigen phenotypes included in the models as independent variables. P corresponds to the likelihood ratio test on each variable.

Table 4  Survival of patients with ovarian carcinoma according to tumor grade, disease stage, and TAP and HLA class I antigen phenotype

<table>
<thead>
<tr>
<th>Lesion no.</th>
<th>Alive (n, %)</th>
<th>P (log-rank test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>39</td>
<td>12 (30.8)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>&lt;50</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>50-59</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>&gt;59</td>
<td>13</td>
</tr>
<tr>
<td>Grade</td>
<td>G1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td>22</td>
</tr>
<tr>
<td>Stage</td>
<td>I</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>19</td>
</tr>
<tr>
<td>TAP</td>
<td>+</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>30</td>
</tr>
<tr>
<td>HLA class I antigen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>22</td>
<td>6 (35.3)</td>
</tr>
<tr>
<td>−</td>
<td>17</td>
<td>6 (27.3)</td>
</tr>
</tbody>
</table>
ovarian carcinoma, it seems of the utmost relevance to unravel the defects in the antigen presentation machinery that are likely to generate cancer cells able to escape the host’s T-cell control.

The likely negative impact of HLA class I defects in the interactions of ovarian carcinoma cells with host’s immune system emphasizes the need to characterize the molecular defects underlying HLA class I antigen down-regulation or loss in ovarian carcinoma cells. This information may suggest strategies to correct HLA class I antigen defects in ovary as well as to select patients to be treated with T cell–based immunotherapy. These approaches are likely to improve the efficacy of T cell–based immunotherapy.

REFERENCES


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