HLA Class I Antigen Down-regulation in Primary Laryngeal Squamous Cell Carcinoma Lesions as a Poor Prognostic Marker

Takeshi Ogino, Hiroshi Shigyo, Hideyuki Ishii, Akihiro Katayama, Naoyuki Miyokawa, Yasuaki Harabuchi, and Soldano Ferrone

Abstract

We have investigated the role of antigen-processing machinery (APM) component defects in HLA class I antigen down-regulation in laryngeal squamous cell carcinoma (SCC) lesions and assessed the clinical significance of these defects. To this end, 63 formalin-fixed, paraffin-embedded tumor lesions were examined for APM component and HLA class I antigen expression by immunohistochemistry. Calnexin, calreticulin, and ERp57 were down-regulated in ~25% of the lesions tested, whereas LMP2, tapasin, and HLA class I antigens were down-regulated in at least 70% of the lesions tested. LMP2 and tapasin expression was significantly correlated with HLA class I antigen expression suggesting APM component defects as a mechanism underlying HLA class I antigen down-regulation in laryngeal SCC lesions. The expression of most APM components and HLA class I antigens was correlated with the extent of CD8+ T cell infiltration into tumor lesions. Furthermore, LMP2 and HLA class I antigen down-regulation and low CD8+ T cell infiltration were significantly associated with reduced patients’ survival. Multivariate analysis identified HLA class I antigen down-regulation as an independent unfavorable prognostic marker. This association is likely to reflect the reduction in the extent of CD8+ T cell infiltration in laryngeal SCC lesions. (Cancer Res 2006; 66(18): 9281-9)

Introduction

The revival of the role of immunosurveillance in the control of tumor growth (1) has stimulated interest in the characterization of the mechanisms used by malignant cells to avoid immune recognition and destruction. Many escape mechanisms have been identified and characterized. Among them, defects in HLA class I antigen expression and/or function in tumor cells have been extensively investigated (2) because of their potential role in the escape of tumor cells from T cell recognition and destruction (3, 4).

HLA class I antigen defects have been described in the large majority of head and neck squamous cell carcinoma (SCC) lesions, their frequency ranging between 6% and 78% (5–11). Laryngeal SCC is no exception to this finding. The frequency of HLA class I antigen defects in laryngeal SCC lesions reported in the literature ranges between 29% and 66% (6, 12–16). Because of the role of HLA class I antigens in the presentation of tumor antigen (TA)–derived peptides to the host’s immune system, these defects are likely to have a negative effect on the clinical course of the disease and on the outcome of T cell–based immunotherapy. Nevertheless, no information is available about the clinical relevance of these defects. Furthermore, the mechanisms underlying HLA class I antigen down-regulation and/or loss in laryngeal SCC lesions have been investigated only to a limited extent, although this information is required for the development of rational strategies to correct HLA class I antigen defects in laryngeal SCC cells. To the best of our knowledge, it has only been reported that HLA class I antigen loss in head and neck SCC lesions, including laryngeal SCC lesions, is not caused by defects in β2-microglobulin (β2m; ref. 10), which is required for the expression of the HLA class I antigen complex on the cell membrane. On the other hand, no information is available regarding the expression and function of antigen-processing machinery (APM) components in laryngeal SCC lesions, in spite of the major role of this machinery in the assembly and expression of stable HLA class I heavy chain-β2m-peptide complexes on the cell membrane.

As recently reviewed (17), peptides which are presented by HLA class I molecules to T cells are generated by the degradation of mostly intracellular proteins by immunoproteasome. Changes in the level of immunoproteasome subunits have recently been shown to impair the generation of antigenic peptides derived from several TAs. The peptides which have high affinity to HLA class I molecules are preferentially transported into the endoplasmic reticulum (ER) by the ATP binding transporter associated with antigen (TAP) presentation, which consists of the TAP1 and TAP2 subunits. In ER, the chaperones calnexin, calreticulin and ERp57 monitor the folding of newly synthesized HLA class I heavy chains. Tapasin bridges the HLA class I heavy chain-β2m-chaperone complex with TAP and selects high-affinity peptides. Finally, the stable trimeric HLA class I heavy chain-β2m-peptide complex is translocated to the cell surface. Defects in APM components may result in HLA class I antigen loss or down-regulation and/or in defects in binding of TA-derived peptides to HLA class I molecules.

The paucity of information about APM component expression in laryngeal SCC lesions, as well as in other types of tumors, reflects, at least in part, the limited availability of APM component–specific monoclonal antibodies (mAb) suitable for the immunohistochemical staining of lesions (18). To overcome this limitation, we have developed a panel of APM component–specific mAbs which stain formalin-fixed, paraffin-embedded lesions (19–21). Taking advantage of this unique panel of mAbs in this study, we have analyzed APM component expression in laryngeal SCC lesions, and we have assessed the role of defects in this machinery in HLA class I antigen down-regulation in laryngeal SCC lesions. Furthermore, we have determined the clinical significance of APM component and HLA class I antigen defects in laryngeal SCC lesions by correlating their expression with their histopathologic characteristics and with the clinical course of the disease.
Materials and Methods

Tissues. Tumor specimens from 63 patients included biopsies for diagnostic purposes and primary lesions surgically removed for therapeutic purposes. Tissue samples were fixed in 20% buffered formalin and embedded in paraffin following conventional procedures. All patients signed an informed consent form for clinical treatments and tissue studies, which received prior approval by the institutional review board.

Monoclonal antibodies. The mAb HC-10 which recognizes a determinant expressed on β2m-free HLA-A10, HLA-A28, HLA-A29, HLA-A30, HLA-A31, HLA-A32, and HLA-A33 heavy chains and on all β2m-free HLA-B and HLA-C heavy chains, the β2m-specific mAb I368, the LMP2-specific mAb SY-1, the TAP1-specific mAb TO-1, the calnexin-specific mAb TO-5, the calreticulin-specific mAb TO-11, the Erp57-specific mAb TO-2, and the tapasin-specific mAb TO-3 were developed and characterized as described (19–24). The human CD8-specific mouse mAb 144B was purchased from Dako Cytometrix, Glostrup, Denmark.

Immunohistochemical staining. Immunohistochemical staining of tissue sections using the EnVision+ system (Dako Cytomation, Carpinteria, CA) and evaluation of the staining were done as described elsewhere (11). The percentage of stained tumor cells in each lesion was enumerated independently by two investigators who had no knowledge of the patients’ characteristics and clinical outcome. Variations in the percentage of stained cells enumerated by two investigators were within a 10% range. In consideration of the error, we evaluated the percentage of stained tumor cells at 10% levels. Normal lymphocytes and vessel endothelia were used in each specimen as internal controls. Results were scored as positive, heterogeneous, or negative when the percentage of stained tumor cells in an entire lesion was >75%, 25% to 75%, and <25%, respectively, according to the criterion established by the HLA and cancer component of the 12th International Histocompatibility Workshop (23). Staining with CD8-specific mAb was done according to the manufacturer’s instructions. Results of staining with CD8-specific antibody were calculated by counting the number of stained infiltrating cells in 0.25 mm² of SCC lesion (11).

Statistical analysis. Correlation of APM component expression among themselves, with HLA class I antigen expression, and with the number of CD8+ T cells infiltrating into tumor lesions were analyzed by Spearman rank correlation coefficient. Differences in the expression levels of variables according to histopathologic and clinical characteristics were analyzed using the Mann-Whitney U test or the Kruskal-Wallis rank test (26). Regression analysis was applied to identify important molecules for HLA class I antigen expression and CD8+ T cell infiltration (27). Disease-free survival (DFS) and cause-specific survival (CSS) were calculated using the Kaplan-Meier method (28). Time was defined as the period starting from...
the date of diagnosis to the date of disease relapse (event) or that of last follow-up visit (censored) for DFS, and as the period starting from the date of diagnosis to the date of disease-specific death (event) or that of last follow-up visit (censored) for CSS. Deaths without a prior relapse in the DFS analysis and deaths not due to cancer in the CSS analysis were handled as censored.

A log-rank test was used for screening the possible prognostic factors in relation to the patients’ survival. A Cox proportional hazards model was applied for multivariate analysis to determine the independence of each prognostic factor (29). $P < 0.05$ was considered to be statistically significant.

### Results

**Patients.** Between 1990 and 2001, 63 Japanese patients (58 males and 5 females) with a median age of 67 years (range, 42-85 years) were newly diagnosed and treated for laryngeal SCC in the Department of Otolaryngology-Head and Neck Surgery, Asahikawa Medical College, Asahikawa, Japan. Table 1 summarizes the patients’ characteristics. The anatomic subsite of primary lesions was the supraglottis in 30 (48%) patients, the glottis in 26 (41%), and the subglottis in 7 (11%). Tumors were well differentiated in 23 (37%) patients, moderately differentiated in 33 (52%), and poorly differentiated in 7 (11%). According to the Tumor-Node-Metastasis Classification of Malignant Tumors (6th edition), the T classification of the disease was T1 in 20 (32%) patients, T2 in 29 (46%), T3 in 6 (9%), and T4 in 8 (13%). Sixteen (25%) patients had lymph node metastasis; the N classification of the disease at diagnosis was N0 in 47 (75%), N1 in 1 (1%), N2 in 12 (19%), and N3 in 3 (5%) patients.

Of the 63 patients investigated, 38 (60%) were treated with surgery. Eleven (17%) underwent total laryngectomy, 19 (30%) underwent total laryngectomy and neck dissection, and 8 (13%) underwent laser surgery. The remaining 25 (40%) patients were treated with radiotherapy. Disease relapsed in 10 (26%) of the 38 patients treated with surgery. Five of them died from this disease.

Disease relapsed in 7 (24%) of the 25 patients treated with radiotherapy. One of them died from this disease. The follow-up period ranged from 1 to 136 months with a median of 37 months for all patients.

**HLA class I antigen expression in primary laryngeal SCC lesions.** Sixty-three primary laryngeal SCC lesions were stained with HLA class I heavy chain–specific mAb HC-10 using the immunoperoxidase reaction. Only 62 lesions were stained with $\beta_2 m$-specific mAb L368 because one lesion was not available for staining with this mAb. Figure 1 shows representative examples of the staining patterns of SCC lesions with the mAb HC-10 and L368. The majority of tumor cells stained by the two mAbs in the lesions were scored as positive (Fig. 1A and C). Using these two mAbs, staining of the tumor cells was not detected in the lesions scored as negative (Fig. 1B and D).

Staining of lymphocytes and vessel endothelium was used as a quality control of the immunohistochemical staining in each tissue section. As summarized in Table 2, HLA class I heavy chain–specific mAb HC-10 and $\beta_2 m$-specific mAb L368 stained 12 (19%) and 8 (13%) SCC lesions with a positive score, 38 (60%) and 40 (64%) with a heterogeneous score, and 13 (21%) and 14 (23%) with a negative score, respectively. HLA class I heavy chain expression was significantly correlated with that of $\beta_2 m$, the Spearman rank correlation coefficient being $r = 0.85$ and $P < 0.001$ (Fig. 2). This finding suggests that $\beta_2 m$ is expressed only in association with HLA class I heavy chains in laryngeal SCC cells.

**APM component expression in primary laryngeal SCC lesions.** Representative examples of the staining patterns of primary laryngeal SCC lesions with APM component–specific mAb are shown in Fig. 1. In lesions scored as positive, the majority of tumor cells are stained by LMP2-specific mAb SY-1, TAP1-specific mAb TO-1, tapasin-specific mAb TO-3, calnexin-specific mAb TO-5, Erp57-specific mAb TO-2, and calreticulin-specific mAb TO-11 (Fig. 1E, G, I, K, L, and M, respectively). In lesions scored as

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**Figure 1.** Representative staining patterns of formalin-fixed, paraffin-embedded primary laryngeal SCC lesions with HLA class I antigen, APM component, and CD8-specific mAb. The staining with HLA class I heavy chain–specific mAb HC-10 (A and B), with $\beta_2 m$-specific mAb L368 (C and D), with LMP2-specific mAb SY-1 (E and F), with TAP1-specific mAb TO-1 (G and H), and with tapasin-specific mAb TO-3 (I and J) was scored as positive (A, C, E, G, and I) and as negative (B, D, F, H, and J), respectively. The staining with calnexin-specific mAb TO-5 (K), with Erp57-specific mAb TO-2 (L) and with calreticulin-specific mAb TO-11 (M) was scored as positive. The staining with CD8-specific mAb 144B was less than 20 (N) and at least 20 (O). Magnification, x200.
negative, the majority of tumor cells are not stained (Fig. 1F, H, and I, respectively). Only 51 lesions were tested for LMP2 and TAP1 expression, and only 59, 58, 57, and 61 lesions for calnexin, ERp57 calreticulin, and tapasin expression, respectively, because some lesions were not available for the staining with the indicated mAb. As summarized in Table 2, LMP2-specific mAb SY-1, TAP1-specific mAb TO-1, calnexin-specific mAb TO-5, calreticulin-specific mAb TO-11, ERp57-specific mAb TO-2, and tapasin-specific mAb TO-3 stained 11 (22%), 15 (29%), 39 (66%), 46 (81%), 43 (74%), and 13 (21%) SCC lesions with a positive score; 23 (45%), 23 (45%), 20 (34%), 11 (19%), 15 (26%), and 29 (48%) lesions with a heterogeneous score; and 17 (33%), 13 (26%), 0 (0%), 0 (0%), 0 (0%), and 19 (31%) lesions with a negative score, respectively. Analysis by the Spearman rank correlation coefficient of the correlation of APM component expression with HLA class I antigen expression showed that only LMP2 and tapasin expression was significantly correlated with HLA class I antigen and all APM component expressions except calreticulin (Fig. 3). The correlation was especially high for LMP2 (P = 0.0021), tapasin (P < 0.001), and HLA class I antigens (P < 0.001). Simple regression analysis showed that LMP2, TAP1, calnexin, calreticulin, ERp57, tapasin, and HLA class I antigens were likely to contribute to the increase of CD8+ T cell infiltration into tumor lesions (P < 0.05). In addition, multiple regression analysis identified HLA class I antigen expression as the most significant variable for the extent of CD8+ T cell infiltration (P = 0.020; Table 4).

The level of HLA class I antigen and APM component expression was not significantly correlated with the histopathologic characteristics of the lesions and with the patients’ clinical characteristics such as gender, age, primary site of the tumor, tumor differentiation, T classification, N classification, and clinical stage of the disease (Table 1). However, the level of HLA class I antigen expression was significantly associated with disease recurrence (P = 0.016) and disease-specific death (P = 0.011). Furthermore, the extent of CD8+ T cell infiltration into tumor lesions was significantly (P = 0.001) associated with tumor differentiation (Table 1).

Association of APM component expression, HLA class I antigen expression, and CD8+ T cell infiltration into tumor lesions with patients' survival. Among the APM components analyzed, only LMP2 expression was significantly correlated with patients' DFS (Fig. 4). The DFS of the 11 patients whose lesions were stained by LMP2-specific mAb SY-1 with a positive score was because they are the only ones likely to affect tumor cells directly. The number of infiltrating CD8+ T cells which ranged from 0 to 158 in 0.25 mm² was significantly correlated with HLA class I antigen and all APM component expressions except calreticulin (Fig. 3).

Table 2. HLA class I antigen and APM component expression in laryngeal SCC lesions

<table>
<thead>
<tr>
<th>Stained tumor cells (%)</th>
<th>HLA class I</th>
<th>β₂m</th>
<th>LMP2</th>
<th>TAP1</th>
<th>Tapasin</th>
<th>Calnexin</th>
<th>ERp57</th>
<th>Calreticulin</th>
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<tbody>
<tr>
<td>&gt;75% (positive)</td>
<td>12% (19%)</td>
<td>8</td>
<td>11</td>
<td>15</td>
<td>13</td>
<td>39</td>
<td>43</td>
<td>46</td>
</tr>
<tr>
<td>25-75% (heterogeneous)</td>
<td>38 (60%)</td>
<td>40</td>
<td>23</td>
<td>23</td>
<td>29</td>
<td>20</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>&lt;25% (negative)</td>
<td>13 (21%)</td>
<td>14</td>
<td>17</td>
<td>13</td>
<td>19</td>
<td>0</td>
<td>0</td>
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*Number of lesions with the indicated staining score.
†Percentage of lesions with the indicated staining score.
patients whose number of CD8+ T cells infiltrating into the tumor and DFS.

Furthermore, HLA class I antigen expression in the lesions was significantly associated with patients' survival (Fig. 5). The DFS of the 13 patients whose lesions were stained by HLA class I heavy chain-specific mAb HC-10 with a negative score was significantly shorter than those of the 12 and 38 patients whose lesions were stained with a positive (P = 0.0044) and a heterogeneous (P = 0.036) score, respectively. The CSS of the patients whose lesions were stained with a negative score was significantly shorter than those of the patients whose lesions were stained with a positive (P = 0.020) and a heterogeneous (P = 0.010) score, respectively. Similar results were obtained when β2m expression in laryngeal SCC lesions was correlated with DFS and CSS (Fig. 5).

As far as the association of CD8+ T cell infiltration into malignant lesions with patients' survival is concerned, the CSS of the 32 patients whose number of CD8+ T cells infiltrating into the tumor (at least 20) was significantly (P = 0.020) longer than that of the 27 patients whose number of CD8+ T cells infiltrating into the tumor was less than 20 (Fig. 6). However, there was no statistically significant association between the extent of CD8+ T cell infiltration into the tumor and DFS.

To determine whether any of the variables analyzed was an independent prognostic factor, data were analyzed by multivariate Cox proportional hazards model. The variables analyzed included age (≥61), tumor differentiation (poor differentiation), tumor location (supraglottis and subglottis), T classification (T3 and T4), N classification (N1, N2, and N3), and HLA class I antigen expression (negative; Table 5). β2m, LMP2, tapasin, and CD8+ T cell infiltration were excluded from this analysis because each of these variables was correlated with HLA class I antigen expression. HLA class I antigen down-regulation was the only variable found to be a statistically significant independent prognostic factor for DFS [hazard ratio (HR), 3.29; P = 0.028] and CSS (HR, 12.4; P = 0.017).

**Discussion**

Immunostaining of 63 formalin-fixed, paraffin-embedded primary laryngeal SCC lesions with a panel of mAbs has identified defects in APM component expression in the majority of the lesions analyzed. The frequency of defects varied markedly among the components analyzed, as LMP2, TAP1, and tapasin were down-regulated or not detectable in ~70% of the lesions analyzed, whereas calnexin, calreticulin, and ERp57 were down-regulated in only ~20% to 30% of the lesions. The reason(s) for these differences is (are) not known because the molecular mechanisms underlying the defects we have identified have not been characterized. In view of the available information that was characterized in almost all the malignant lesions, APM component defects are caused by functional and not structural abnormalities (30–32), the different frequencies of the APM component defects in the laryngeal SCC lesions we have analyzed is likely to reflect the differential susceptibility to dysregulation of the distinct mechanisms which control their expression.

Comparison of the frequency of APM component expression in laryngeal SCC lesions with that in other types of malignant lesions is limited by the scanty available information about APM component expression in malignant lesions because only some have been tested in a few malignant lesions and cell lines of different histopathologic type. The only exception is represented by TAP1, which has been investigated in a number of malignancies, although still in a relatively small number of lesions within each type of malignancy. Mutations in the TAP1 gene have been found to severely affect HLA class I antigen cell surface expression in a melanoma cell line (33) and in a lung cancer cell line (34). Although abnormal TAP1 mRNA and protein were detected in these two cell lines, the mutant proteins did not function as a transporter, resulting in HLA class I antigen down-regulation in both cell lines.

Calnexin, calreticulin, and ERp57 expression has been investigated only in maxillary sinus SCC lesions (11). The frequency of calnexin and ERp57 down-regulation in the latter malignancy is

![Figure 3. Correlation of the number of infiltrating CD8+ T cells into tumor lesions with HLA class I antigen and APM component expression in primary laryngeal SCC lesions.](image)

Table 3. Regression analysis to identify variables which influence HLA class I antigen expression

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Significantly longer than that of the 23 and 17 patients whose lesions were stained with a heterogeneous (P = 0.012) and a negative (P = 0.036) score, respectively.

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lower than that we have found in laryngeal SCC lesions, whereas that of calreticulin is higher. In addition, the frequency of LMP2 down-regulation in laryngeal SCC lesions is similar to that described in colorectal carcinoma lesions (35), but is markedly lower than that found in renal cell carcinoma lesions (36), and is slightly higher than that found in melanoma lesions (37). In interpreting the data about the expression of LMP2, it is noteworthy that this subunit of the immunoproteasome, like the other ones, is assumed not to be expressed under basal conditions but is induced following exposure of cells to IFN-γ. Furthermore, no data are available regarding the expression of immunoproteasome subunits in normal tissues. Therefore, at present, it is not possible to determine whether the expression of an immunoproteasome subunit in malignant cells is a normal phenotype and its lack of expression is a down-regulation or loss, or whether the lack of expression of an immunoproteasome subunit is a normal phenotype and its expression reflects regulatory defects.

On the other hand, the frequency of TAP1 down-regulation in laryngeal SCC lesions is lower than that in colorectal carcinoma (35) and renal cell carcinoma (36) lesions, and is markedly higher than that in melanoma lesions (37). Lastly, the frequency of tapasin down-regulation in laryngeal SCC lesions is markedly higher than that in maxillary sinus SCC (11) and tonsillar carcinoma lesions (38), and is markedly lower than that in colorectal carcinoma (35) and renal cell carcinoma (36) lesions. Whether the differences we have indicated reflect differences in the extent of genetic instability and selective pressure on tumor cell populations in various types of malignancies, in the clinical characteristics of the patients investigated, in the histopathologic characteristics of the lesions analyzed, and/or in the sensitivity of the immunohistochemical technique used remains to be determined.

As far as the association of APM component abnormalities with HLA class I antigen down-regulation is concerned, close association of TAP with MHC class I antigen expression has been well documented (39–41). TAP abnormality may cause diminution in the translocation of peptides into the ER, resulting in decreased formation of stable HLA class I molecule-peptide complexes. In addition, TAP heterodimer can preferentially translocate high-affinity peptides into the ER lumen (42). However, in the present study, TAP1 did not seem to be associated with HLA class I antigen expression in laryngeal carcinoma lesions. On the other hand, LMP2 and tapasin were associated with HLA class I antigen expression in the laryngeal carcinoma lesions analyzed. It has been reported that tapasin increases the efficiency of peptide translocation by increasing the level of TAP and facilitates direct loading and assembly of MHC class I molecules by association with MHC class I molecules (43). Therefore, tapasin can greatly contribute to HLA class I antigen cell surface expression. In addition, MHC class I antigen expression is not down-regulated in LMP2-deficient mice; however, the level of CD8+ T cells is lower and the capability of generating influenza nucleoprotein–specific CTL precursors is much lower than in the nondeficient mice (44). Moreover, proteasome activity has been reported to limit MHC class I antigen assembly after IFN-γ stimulation (45), and LMP2 and TAP1 have been reported to affect HLA class I antigen cell surface expression on lung carcinoma cells (46).

HLA class I antigens were down-regulated in 81% of the laryngeal SCC lesions tested. This frequency is similar to that found in 70 maxillary sinus SCC lesions (11), but is higher than that previously described in laryngeal SCC lesions, which range between 29% and 66% (6, 12–16). Our study differs from the other published

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**Table 4. Regression analysis to identify variables which influence CD8⁺ T cell infiltration**

<table>
<thead>
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<th>Variable</th>
<th>Simple regression analysis</th>
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<tbody>
<tr>
<td></td>
<td>Regression coefficient P</td>
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</tr>
<tr>
<td>HLA class I antigen</td>
<td>0.83 &lt;0.0001</td>
<td>0.58 0.020</td>
</tr>
<tr>
<td>Tapasin</td>
<td>0.81 &lt;0.0001</td>
<td>0.19 0.59</td>
</tr>
<tr>
<td>LMP2</td>
<td>0.70 0.0001</td>
<td>0.12 0.64</td>
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<tr>
<td>TAP1</td>
<td>0.52 0.0029</td>
<td>0.17 0.40</td>
</tr>
<tr>
<td>Calnexin</td>
<td>0.78 0.0036</td>
<td>0.28 0.44</td>
</tr>
<tr>
<td>ERP57</td>
<td>0.74 0.019</td>
<td>0.076 0.84</td>
</tr>
</tbody>
</table>

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**Figure 4.** Association of LMP2, TAP1, and tapasin down-regulation in primary laryngeal SCC lesions with DFS and CSS in patients with laryngeal SCC. The DFS and CSS of patients with lesions stained with positive (solid line), heterogeneous (broken line), and negative (dotted line) scores were compared using the Kaplan-Meier method. Differences in patients’ survival were analyzed using a log-rank test.
studies in at least two aspects, i.e., the substrate used in the immunohistochemical reactions and the specificity of the mAb used to stain the lesions. All the studies in the literature (6, 12–16) have used frozen tissue sections, whereas we have used formalin-fixed, paraffin-embedded tissue sections. However, this difference is not likely to account for the higher frequency of HLA class I antigen down-regulation we found because a previous study (47) did not find marked differences in the sensitivity of immunohistochemical staining of frozen and formalin-fixed tissue sections with HLA antigen–specific mAbs. On the other hand, the specificity of the mAb used to detect HLA class I antigens may play a role in the difference between our results and the information in the literature. Most studies have used mAb W6/32 or mAb sl.34/28 to detect framework determinants expressed on all gene products of HLA-A, HLA-B, and HLA-C loci. In contrast, we have used the mAb HC-10 which recognizes a determinant expressed on all HLA-B and HLA-C allospecificities, but only some HLA-A allospecificities (24). Therefore, we cannot exclude that the restricted reactivity pattern of mAb HC-10 reduces the sensitivity of the immunohistochemical reaction. This possibility cannot be tested at present, because to the best of our knowledge, neither mAb W6/32 nor mAb sl.34/28 stain formalin-fixed, paraffin-embedded tissues, and none of the few available mAbs which stain formalin-fixed, paraffin-embedded tissues recognizes a framework determinant expressed by HLA-A, HLA-B, and HLA-C antigens. In spite of these limitations, the HLA class I antigen-specific mAb which stain formalin-fixed, paraffin-embedded tissues facilitate the implementation of retrospective studies to characterize the expression of these molecules in malignant lesions and to assess the clinical significance of defects in their expression.

The association between HLA class I antigen down-regulation and poor prognosis has already been described in maxillary sinus

Figure 5. Association of HLA class I antigen and MHC class I down-regulation in primary laryngeal SCC lesions with DFS and CSS in patients with laryngeal SCC. The DFS and CSS of patients with lesions stained with positive (solid line), heterogeneous (broken line), and negative (dotted line) scores were compared using the Kaplan-Meier method. Differences in patients’ survival were analyzed using a log-rank test.

Figure 6. Association of CD8+ T cell infiltration into primary tumor lesions with DFS and CSS in patients with laryngeal SCC. The DFS and CSS of patients with CD8+ T cell infiltration with score >20 (solid line) were compared with those of patients with scores < 20 (broken line) using the Kaplan-Meier method. Differences in patients’ survival were analyzed using a log-rank test.
SCC (11), esophageal carcinoma (48), and melanoma (37). In the present study, we have shown for the first time that HLA class I antigen down-regulation is significantly correlated with an unfavorable clinical course of the disease in patients with laryngeal SCC. Furthermore, using multivariate analysis, we have shown that HLA class I antigen down-regulation is an independent prognostic factor for disease recurrence and disease-specific death. These findings may reflect the lack of recognition by HLA class I antigen–restricted, TA-specific CTL of laryngeal SCC cells which have lost or down-regulated HLA class I antigen expression. This possibility is supported by the positive correlation between HLA class I antigen expression levels and CD8+ T cell infiltration into the laryngeal SCC lesions, which we have found in the present study.

As far as the association of APM component expression with clinical and histopathologic features and patients’ survival is concerned, only LMP2 expression was found to be significantly associated with DFS of the patients. However, TAP1 and tapasin expression levels and CD8+ T cell infiltration into tumor lesions supported by the positive correlation between HLA class I antigen down-regulation and CD8+ T cell infiltration into the laryngeal SCC lesions, which we have found in the present study. However, TAP1 and tapasin expression levels and CD8+ T cell infiltration into tumor lesions. Clinical and histopathologic features and patients’ survival is concerned.

Table 5. Multivariate analysis for independent prognostic factors in patients with laryngeal SCC

<table>
<thead>
<tr>
<th></th>
<th>DFS</th>
<th></th>
<th>CSS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% confidence interval)</td>
<td>P</td>
<td>HR (95% confidence interval)</td>
<td>P</td>
</tr>
<tr>
<td>T classification</td>
<td>0.47 (0.10-2.12)</td>
<td>0.33</td>
<td>0.86 (0.09-8.10)</td>
<td>0.89</td>
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<tr>
<td>T classification</td>
<td>1.31 (0.44-3.89)</td>
<td>0.63</td>
<td>2.71 (0.52-14.09)</td>
<td>0.24</td>
</tr>
<tr>
<td>N classification</td>
<td>2.61 (0.95-7.22)</td>
<td>0.063</td>
<td>7.00 (1.18-41.52)</td>
<td>0.032</td>
</tr>
</tbody>
</table>

Acknowledgments

References


HLA Class I Antigen Down-regulation in Primary Laryngeal Squamous Cell Carcinoma Lesions as a Poor Prognostic Marker

Takeshi Ogino, Hiroshi Shigyo, Hideyuki Ishii, et al.


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