Prognostic Value of Ki67 and VEGF Expression in Laryngeal Squamous Cell Carcinoma

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OBJECTIVE To investigate the prognostic value of measuring Ki67 and VEGF expression in laryngeal squamous cell carcinoma (LSCC).

METHODS The expression of Ki67 and VEGF in 32 LSCC tissues was studied by immunohistochemical staining. Of these cases, 5 recurred (recurrent group), 3 cases metastasized (metastatic group), 8 cases died (deceased group) and 24 cases survived (survival group) over a 3 year period of follow-up after their operation.

RESULTS The expression of Ki67 and VEGF in the deceased group was higher compared to that in the survival group (P<0.01). Overexpression of Ki67 was found in the recurrent group and in the metastatic group (P<0.05). VEGF expression was higher in the recurrent group than in the non-recurrent group (P<0.05). With Cox-regression of multivariate analysis, Ki67 (RR: 3.236; P=0.001), the clinical T stage (RR: 1.382; P=0.029) and metastasis in lymph nodes (RR: 0.316; P=0.033) were shown to be independent prognostic factors for survival of LSCC patients.

CONCLUSION Ki67 and VEGF expression is related to the prognosis of LSCC. Overexpression of the 2 markers indicated poor prognosis of the disease, a finding which might be helpful for the treatment of laryngocarcinoma.

KEYWORDS: laryngeal neoplasms, carcinoma, squamous cell, Ki67, endothelial growth factors, prognosis.

Ki67 is a protein which reflects the status of cellular proliferation and its expression is related to the development, metastasis, and prognosis of many types of malignant tumors. Vascular endothelial growth factor (VEGF) is the most powerful angiogenic factor known at present. It can enhance tumor growth and metastasis. Expression of these 2 proteins can closely reflect the biological behavior of malignant tumors.

We have previously investigated the expression of both Ki67 and VEGF in laryngeal squamous cell carcinomas (LSCC), and found high expression of the 2 markers in LSCC. The levels of expression related to the metastasis in lymph nodes and the clinical T stage of the carcinoma, suggesting that Ki67 and VEGF expression might correlate with the prognosis of laryngocarcinomas. At present, only a few reports have referred to the relationship of the 2 markers with the prognosis of laryngocarcinoma, and at present there is still some debate about the correlation. We studied the expression of Ki67 and VEGF in LSCC by an immunohistochemical method, examined the correlation of the 2 markers with the prognosis, and investigated the significance of Ki67 and VEGF expression for the treatment of laryngocarcinoma.
MATERIALS AND METHODS

Patients
We studied 38 patients, 35 men and 3 women, aged 49 to 66 (mean 58.1) years, who were operated on for LSCC from January 1997 to October 2002 at the Department of Otolaryngology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology. Tumor staging was performed according to the UICC 1992 TNM classification. T1 stage was recorded in 7 cases, T2 stage in 13 cases, T3 stage in 11 cases, and T4 stage in 7 cases. Lymphoid node metastases were found in 11 patients. No patients had been treated by radiotherapy or chemotherapy before operations. Thirty-two cases were followed-up from 10 to 95 (mean 42.5) months. Of the 32 cases, 5 recurred (recurrent group), 3 cases metastasized (metastatic group), 8 cases died (deceased group), and 24 cases survived (survival group) for 3 years after their operation.

Chief agents
A SP kit was purchased from Beijing Zhongshan Biotechnology Ltd. Ki67 immunohistochemical antibody I was obtained from NeoMarkers Ltd, and VEGF immunohistochemical antibody I was a product from Wuhan Boster Biological Technology Ltd.

Methods

Ki67 immunohistochemical staining method
Paraffin sections were immersed in 3% H2O2 at room temperature for 5–10 min to inhibit endogenous peroxidase activity, washed with distilled water, and then with PBS for 5 min. For antigen retrieval, the sections were heated in a microwave oven. The sections were blocked with 5–10% normal goat serum (PBS dilution), incubated at room temperature for 10 min, covered with 1:100 Ki67 antibody I Ki67 Ab-2, and incubated at 37°C for 1–2 h. After washing with PBS, the slides were treated with a general type mono-antibody IgG (1% BAS-PAS dilution), and incubated at 37°C for 10–30 min. Then the slides were treated with horseradish enzyme labelling chain antibiotin (PBS dilution), incubated at 37°C for 10–30 min, and stained with developer (DAB). Finally they were thoroughly washed, counterstained, and mounted.

VEGF immunohistochemical staining method
With the exception that antibody I was changed to 1:100 rabbit anti-VEGF, the VEGF immunohistochemical staining method was fundamentally identical with that for Ki67.

Section evaluation
The positive results for Ki67 staining are brown-stained tumor nuclei, and brown-stained tumor cytoplasm is a positive result for VEGF staining. The HPIAS-1000 high-definition multicolor-pathologic artwork writing analytical system of the Pathology Department, Tongji Medical College, Huazhong University of Science and Technology was utilized to quantitatively process the staining results. A representative slice from each specimen, with clear positive grains and no non-specific background coloration, was selected to reflect the degree of staining of the specimen. Relative optical density (ROD) of each positive cell was surveyed in the selected microscopic field at ×200, and the mean value of the positive cells was considered to be the assessment of the specimen.

Statistical analysis
Statistical processing was conducted by a SPSS12.0 statistical package. Group comparisons were analyzed by the t-test, and correlative study of Ki67 and VEGF with prognosis of LSCC was performed by Cox multivariate regression analysis. P<0.05 was considered significant.

RESULTS

Positive results of Ki67 and VEGF are shown in Figs.1 and 2, and the correlation between optical density of Ki67 and VEGF with the prognosis of LSCC is shown in Table 1. From Table 1 it can be seen that the expression of Ki67 and VEGF in the deceased group was higher compared to that in the survival group (P<0.01). Overexpression of Ki67 was found in both the recurrent and metastatic groups (P<0.05). VEGF expression was higher in the recurrent group compared to the non recurrent group (P<0.05).

Table 1. Correlation between optical density of Ki67 and VEGF with prognosis of LSCC (x±s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Ki67</th>
<th>VEGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival group</td>
<td>24</td>
<td>0.1742 ± 0.0436</td>
<td>0.091 ± 0.0392</td>
</tr>
<tr>
<td>Deceased group</td>
<td>8</td>
<td>0.2853 ± 0.0176</td>
<td>0.1783 ± 0.0471</td>
</tr>
<tr>
<td>Recurrent group</td>
<td>5</td>
<td>0.2815 ± 0.0191</td>
<td>0.1757 ± 0.0146</td>
</tr>
<tr>
<td>Non recurrent group</td>
<td>27</td>
<td>0.1873 ± 0.0558</td>
<td>0.1173 ± 0.0496</td>
</tr>
<tr>
<td>Metastatic group</td>
<td>3</td>
<td>0.2916 ± 0.0163</td>
<td>0.1758 ± 0.0862</td>
</tr>
<tr>
<td>Non metastatic group</td>
<td>29</td>
<td>0.1927 ± 0.0576</td>
<td>0.1213 ± 0.0450</td>
</tr>
</tbody>
</table>