Behavioral Comparison of Oral Squamous Cell Carcinoma between Original and Metastatic Lymph Node Lesions

Masataka Uehara, Tsugio Inokuchi, Hisazumi Ikeda, Joji Sekine, Takayoshi Tobita, Seigo Ohba, Yuji Mizoguchi, Mihoko Nonaka and Akihiko Fujisawa
Division of Oral and Maxillofacial Surgical Reconstruction and Functional Restoration, Department of Developmental and Reconstructive Medicine, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan

Immunoohistochemical expression of vascular endothelial growth factor (VEGF) and proliferating cell nuclear antigen (PCNA) in oral squamous cell carcinomas (OSCCs) was investigated comparatively between the original and the metastatic lymph node lesions of the same tumor.

Tissue samples of both the original and regional lymph node lesions were obtained from 11 patients who did not receive preoperative radiotherapy. The percentage of VEGF immunopositive area (PVIA) in the tumor was determined through computer image analysis. Proliferative activity of tumor cells was assessed in terms of the labeling index (LI) of PCNA. PCNA LI showed no correlation between the original and metastatic tumors, whereas the PVIA appeared to be similar between the original and regional lymph node lesions.

These results suggest that immunohistochemical expression of VEGF in the original tumor shows the characteristic behavior of a metastatic tumor, thus providing therapeutic information.

Key words: oral squamous cell carcinoma, original lesion, metastatic lymph node lesion, vascular endothelial growth factor, proliferating cell nuclear antigen

Introduction

Tumor growth and metastasis appear to be significantly associated with angiogenesis of the lesion (1). Vascular endothelial growth factor (VEGF) is a heparin-binding growth factor that affects the vascular endothelial cells and promotes cell proliferation and permeability. VEGF has been considered to be mainly responsible for the angiogenesis of tumors (2, 3), and it appears that expression of VEGF could be a prognostic marker for oral squamous cell carcinoma (OSCC) (4). Tanigawa et al. (5) reported that an increase of the microvessels around the tumor might worsen the prognosis, and that VEGF might mediate angiogenesis in squamous cell carcinoma. Thus, high expression of VEGF in the OSCC might induce angiogenesis of the tissue surrounding the tumor, resulting in a poor prognosis. In addition, the proliferative activity of OSCC indicates its malignant behavior and clinical outcome (6-8). However, it is not clear whether expression of VEGF correlates with proliferative activity of tumor cells in the OSCC.

The regional lymph node (RLN) metastasis is one of the poor prognostic factors in OSCC. It was reported that the invasion depth directly correlated with RLN metastasis (9). Furthermore, it appears that nuclear morphometry may also be a predictable marker for RLN metastasis (10). On the other hand, expression of VEGF or PCNA may not significantly indicate RLN metastasis in OSCC (4, 11). For the sake of treatment programming, it would be worthwhile to investigate the behavioral correlations between the original and metastatic tumors. The aim of this study, therefore, was to compare the characteristic behaviors of OSCC between the original and RLN metastatic lesion in terms of immunohistochemical VEGF and PCNA expression.
Materials and Methods

Patients and tissue samples

OSCC cases had metastatic lymph nodes and did not receive preoperative radiotherapy. Surgical specimens of the original and RLN metastatic tumors were obtained from 11 patients of OSCC with informed consent at the Division of Oral and Maxillofacial Surgical Reconstruction and Functional Restoration, Nagasaki University Hospital (Table 1).

PCNA immunohistochemistry and labeling indices

All specimens were fixed at 3.7% neutral phosphate-buffered formalin for 24 h, after which they were processed for routine paraffin embedding. Two 4-μm sections were prepared for each specimen, mounted on poly L-lysine-coated glass slides, and dried overnight on a hot plate at 37°C to promote adhesion. Immunohistochemical staining was performed using the avidin-biotin complex method, as described herein. In the first section, proliferating cell nuclear antigen (PCNA) immunohistochemical staining was performed. Sections were treated with 0.1% trypsin solution in 0.05 M Tris buffer (pH 7.6) at room temperature for 15 min, and washed in PBS three times. Endogenous peroxidase was inhibited by treatment with 0.3 H2O2 in methanol for 30 min. After three washes in PBS, the sections were incubated with diluted normal goat blocking serum for 20 min at room temperature to block a non-specific antibody reaction. Sections were incubated overnight at 4°C with anti-human PCNA polyclonal antibody (1:100 diluted, Santa Cruz FL-261) in PBS, followed by diluted biotinylated secondary antibody for 30 min, and ABC reagent for 30 min, using a Vectastain Elite ABC Kit (Vector Laboratories, Burlingame, CA, USA). Immunohistochemical reactions were developed with diaminobenzidine (DAB) solution (DAB; 20 mg in 100 ml 0.05 M Tris buffer containing 17 μl of 30% H2O2). After being washed in running water, the sections were counterstained lightly with Mayer’s haematoxylin. Negative controls, prepared by substituting normal goat serum for the primary antibody, resulted in no detectable staining.

The PCNA labeling index (LI) of tumor cells was determined as an assessment of tumor proliferative activity. The PCNA LI was calculated as the percentage of the PCNA-positive cells in 1000 tumor cells counted from four randomly selected fields at a magnification of 400×.

VEGF immunohistochemistry and quantification of VEGF expression

For the second sections, VEGF immunohistochemical staining was carried out. Sections were treated with 0.1% trypsin solution in 0.05 M Tris buffer (pH 7.6) at room temperature for 15 min, and washed in PBS three times. Endogenous peroxidase was inhibited by treatment with 0.3 H2O2 in methanol for 30 min. After three washes in PBS, the sections were incubated with diluted normal goat blocking serum for 20 min at room temperature to block a non-specific antibody reaction. Sections were incubated overnight at 4°C with anti-human VEGF polyclonal antibody (1:200 diluted, Santa Cruz A-20) in PBS, followed by diluted biotinylated secondary antibody for 30 min, and ABC reagent for 30 min, using a Vectastain Elite ABC Kit (Vector Laboratories, Burlingame, CA, USA). Immunohistochemical reactions were developed with DAB solution. After being washed in running water, the sections were counterstained lightly with Mayer’s haematoxylin. Negative controls, prepared by substituting normal goat serum for the primary antibody, resulted in no detectable staining.

The VEGF expression was assessed in terms of

<table>
<thead>
<tr>
<th>Tumor site</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tongue</td>
<td>5</td>
</tr>
<tr>
<td>Palate</td>
<td>1</td>
</tr>
<tr>
<td>Lower alveolus and gingiva</td>
<td>2</td>
</tr>
<tr>
<td>Buccal mucosa</td>
<td>1</td>
</tr>
<tr>
<td>Floor of mouth</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 1: Site of the oral squamous cell carcinoma

<table>
<thead>
<tr>
<th>Tumor site</th>
<th>Original tumor</th>
<th>Metastatic tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVIA</td>
<td>37.1 ± 17.1%</td>
<td>32.1 ± 20.3%</td>
</tr>
<tr>
<td>PCNA LIs</td>
<td>43.6 ± 13.0%</td>
<td>40.6 ± 11.1%</td>
</tr>
</tbody>
</table>

Table 2: Percentage of VEGF immunopositive area (PVIA) and PCNA LIs
percentage of VEGF immunopositive area (PVIA) (4) in the tumor through computer-assisted image analysis (Macintosh Image 1.62 program, Apple Computer Power Book G4, USA). The sections were photographed using a Nikon digital camera, COOLPIX 4500 (Nikon Co., Tokyo, Japan), at a 50 × magnification. After saving the captured image in a personal computer, the image was cropped to a 256 × 256 pixel image. After noise reduction treatment and edge enhancement, image analysis was performed according to the method of Wu et al. (12). Statistical analysis was performed using Pearson’s correlation coefficient.

Results

PCNA immunohistochemical staining and labeling index

PCNA positive cells were distinguished by the brown-stained nuclei in immunohistochemical staining. The mean labeling indices of original and RLN metastatic tumors were 43.6 ± 13.0% and 40.6 ± 11.1%, respectively (Table 2).

VEGF expression and percentage of VEGF immunopositive area (PVIA)

VEGF was found in all specimens as shown by the presence of brown stain (Fig. 1). VEGF expression was shown to be mainly located in the cytoplasm of the tumor cells. The mean PVIAs of primary and RLN metastatic tumors were 37.1 ± 17.1% and 32.1 ± 20.3%, respectively (Table 2).

Relationship between PVIA and PCNA LI

The correlations of PVIA or PCNA LI between the original tumor and the RLN metastatic tumor are shown in Figs. 2 and 3, respectively. VEGF expression tended to correlate between the original tumor and the RLN metastatic tumor, although statistical significance was not
observed (r=0.596, Y=5.81+0.707X, P=0.0523; Fig. 2). No significant correlation for PCNA LI was indicated between the original tumor and the RLN metastatic tumor (r=-0.250, P=0.4704; Fig. 3).

There were no significant correlations between PVIA and PCNA LI in either the original tumor or the RLN metastatic tumor (original tumor; r=0.473, P=0.1457, RLN metastatic tumor; r=-0.150, P=0.6697).

Discussion

The proliferative activity of the tumor is an important prognostic indicator for head and neck cancer (13, 14). Although Mattern et al. (15) have found a close correlation between VEGF expression and tumor cell proliferation in epidermoid lung carcinoma, the present study demonstrated that there was no correlation between PCNA LI and PVIA in either the original tumor or the RLN metastatic tumor. The mitogenic activity of VEGF is specific for vascular endothelial cells (16, 17), and our results suggested that VEGF did not associate with proliferating activity of OSCC tumor cells. Furthermore, there was no association for PCNA LI between these two lesions, indicating that the proliferative behavior of the RLN metastatic tumor does not depend on that of the original tumor. On the other hand, VEGF expression was comparable between the original and the metastatic lesions. These results imply that the RLN metastatic tumor may behave like the original lesion in angiogenesis. Accordingly, the expression of VEGF in the original tumor could predict the proliferative behavior of the metastatic tumor, and high expression of VEGF in the original tumor could be suggestive of the necessity for postoperative radiotherapy and/or chemotherapy to the neck after neck dissection.

In conclusion, VEGF immunohistochemistry of OSCC at the original site appears to provide therapeutic strategies for metastatic lesions of the same tumor.

References


(Accepted for publication March 25, 2005)