Granular Cell Tumor of the Tongue: An Electron Microscopical and Immunohistochemical Study

Hiroyuki OKADA, Hirotsugu YAMAMOTO
Tomonori KAWANA, Takao KATOH
Yukishige KOZAWA and Hirotsugu IZUMI

(Received 9 January and accepted 12 January 1990)

Key words: granular cell tumor, electron microscopy, immunohistochemistry

Abstract

Granular cell tumor of the tongue in a 29-year-old woman is reported including details of studies using electron microscopy and immunohistochemistry. Immunohistochemically, the tumor cells showed moderate positivity for S-100 protein. Electron microscopically, basal lamina, angulate bodies, myelin-like figures and axon-like structures were observed in the tumor. These findings may indicate that this tumor is a lesion of Schwann cell origin. The Japanese literature on oral granular cell tumor is also reviewed and discussed.

Introduction

Granular cell tumor is a relatively rare neoplasm, most frequently found in superficial soft tissue. There are some reports on ultrastructural and immunohistochemical studies of this tumor[1-13]. However, its histogenesis and nature are still controversial.

Recently, we encountered a case of granular cell tumor of the tongue and studied it using light and electron microscopy, and immunohistochemistry. The findings are reported here, together with a review and discussion of the literature on oral granular cell tumor.

Case report

A 29-year-old woman visited the Nihon University Dental Hospital at Matsudo on November 14, 1986 with a chief complaint of swelling of the right side of the tongue. She had noticed swelling in the posterior portion of the tongue at the end of the previous year, but had not sought therapy because the swelling was painless. However, the swelling subsequently enlarged, and an abnormal sensation was also present upon chewing. She was introduced to our hospital through her...
private dentist. On her first visit, laboratory tests and examination of her general condition showed no abnormality.

Oral examination revealed a small soybean-sized, yellowish, elastic slightly stiff mass in the right posterior portion on the tongue (Fig. 1). The suspected clinical diagnosis was benign tumor. Under local anesthesia using 2% xylocaine, the tumor was removed. Up to the time of writing, there has been no sign of recurrence.

![Fig. 1. The nodule present in the right posterior portion of the tongue.](image)

![Fig. 2. Macroscopical view of the cut surface of the removed specimen.](image)

**Materials and Methods**

Macroscopically, the removed specimen was a nodular, oval-shaped yellow-gray tumor measuring $6 \times 5 \times 4$ mm. The cut surface was glossy and grayish-yellow (Fig. 2).

Light microscopy: Following fixation with 10% neutral formalin, the mass was
cut into small pieces and routine paraffin sections were made. The sections were stained with hematoxylin and eosin, and various staining techniques, including periodic acid-Schiff reaction (PAS) with and without diastase digestion, combined PAS-alcian blue at pH 2.6, toluidine blue at pH 2.4 and 4.1, Masson's trichrome, Best's carmine, phosphotungstic acid-hematoxylin (PTAH), silver impregnation, Klüver-Barrera and Grimelius stainings.

Electron microscopy: Parts of the material fixed with 10% neutral formalin were washed and refixed with 2% glutaraldehyde and 1% osmium tetroxide. The tissue was then embedded in Quetol 812 by the usual method. Ultrathin sections were post-stained with uranyl acetate-lead citrate and observed with a Hitachi HS-9 electron microscope.

Immunohistochemistry: Primary antibodies against S-100 protein, neuron-specific enolase, neurofilament, glial fibrillary acidic protein, myelin basic protein, carcinoembryonic antigen, epithelial membrane antigen, keratin, vimentin, myosin, lysozyme, fibronectin and α2-antichymotrypsin were purchased from Dakopatts, Copenhagen, and commercially available antibodies against actin, myosin and desmin were obtained from Tanner Japan, Kobe. For detection of the antigens, the peroxidase-anti-peroxidase (PAP) method of STERNBERGER[14] was used. Deparaffinized sections were treated with 0.1% trypsin to enhance specific tissue staining. Endogenous peroxidase activity was blocked by incubating the sections with methanol containing 0.3% H2O2 for 20 min. The primary antibodies were generally used at a dilution of 1 : 200 and the incubation time was 1 h at room temperature, according to the instructions of the supplier. Peroxidase activity was developed using 3,3′ diaminobenzidine tetrahydrochloride substrate containing 0.05% H2O2.

Results

Microscopically, the tumor was made up of strands and fascicles of large polyhedral cells, was not tunicated, and covered by stratified squamous epithelium showing pseudoepitheliomatosus hyperplasia (Fig. 3). The tumor cells had small

Fig. 3. Microscopically, the tumor is made up of strands and fascicles of large polyhedral cells (H. E., original magnification ×40).
nuclei containing one or two distinct nucleoli and extremely granular eosinophilic cytoplasm which revealed a PAS-positive reaction with resistance to diastase digestion (Fig. 4). The cell borders were often clear and syncytial formations were occasionally found. Some multinucleated giant cells were sometimes seen. Collagen fibers were observed in the stroma of the tumor. Striated muscle fibers were occasionally interspersed among the tumor cells, but did not transmigrate to the tumor cells.

![Figure 4](image4.png)

**Fig. 4.** The tumor cells have small nuclei and extremely granular eosinophilic cytoplasm (PAS, original magnification ×400).

![Figure 5](image5.png)

**Fig. 5.** Many granules (G), nucleus (N) and axon-like structures with cytoplasmic filaments and microtubules (arrows) are evident in the cytoplasm of a tumor cell (×9,000).

Electron microscopically, the tumor cells were generally ovoid or polyhedral, containing numerous granules. Groups of the tumor cells were surrounded by
basal lamina. The nuclei were mostly polygonal and rather small. Their chromatin was dispersed and a few distinct nucleoli were frequently observed. Some mitochondria, free ribosomes and rough endoplasmic reticulum were evident in the cytoplasm, but the Golgi apparatus was not distinct. Many granules showing marked variation in size, shape and density were also identified in the cytoplasm (Fig. 5). Some myelin-like figures with concentric laminae and angulate bodies with many fibrils were observed in the cytoplasm (Fig. 6,A,B). Furthermore, some axon-like structures containing cytoplasmic filaments and microtubules resembling Schwann cells could be identified in the tumor (Fig. 5).

Immunohistochemically, S-100 protein was moderately positive in the cytoplasm and nucleus of almost all tumor cells (Fig. 7). There was no immunoreactivity for myelin basic protein, neuron-specific enolase, glial fibrillary acidic protein, neurofilament, actin, myosin, desmin, vimentin, fibronectin, lysozyme, α1-antichymotrypsin, epithelial membrane antigen, keratin or carcinoembryonic antigen in the tumor cells.

![Image](image-url)

Fig. 6. Myelin-like figures with centric laminae (arrows, A) and angulate bodies with many fibrils (double arrows, B) are evident in the cytoplasm ((A) ×60,000,(B) ×13,000).
Discussion

Granular cell tumor was first described in 1926\(^{[15]}\). Since then, many authors have discussed the histogenesis and nature of the tumor\(^{[1-8,11-13,15-27]}\).

In Japan, 53 cases\(^{[3,6,13,27-33]}\) of oral granular cell tumor, including the present case, have been reported, and 55 tumors have been removed, since two cases were multiple. Seventy three percent, or about three fourths, of the tumors were located in the tongue, and women were found to be affected almost three times as often as men (Table 1). The ages of the patients ranged from newborn to 60 years, with a majority in the 10- to 30-year age group (Table 2). The average patient age was 27.8

<table>
<thead>
<tr>
<th>Location</th>
<th>No.</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tongue</td>
<td>40</td>
<td>(73)</td>
</tr>
<tr>
<td>Gingiva</td>
<td>4</td>
<td>(7)</td>
</tr>
<tr>
<td>Buccal mucosa</td>
<td>4</td>
<td>(7)</td>
</tr>
<tr>
<td>Palate</td>
<td>2</td>
<td>(4)</td>
</tr>
<tr>
<td>Floor of mouth</td>
<td>2</td>
<td>(4)</td>
</tr>
<tr>
<td>Others</td>
<td>3</td>
<td>(5)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>55</td>
<td>(100)</td>
</tr>
</tbody>
</table>

Table 1 Locations of granular cell tumor in the Japanese literature

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>0~9</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>10~19</td>
<td>1</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>20~29</td>
<td>2</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>30~39</td>
<td>5</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>40~49</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>50~59</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>60~69</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>14</td>
<td>36</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 2 Age & sex of affected patients reported in the Japanese literature

Fig. 7. PAP immunostaining for S-100 protein reveals a moderately positive reaction in almost all tumor cells (original magnification ×200).
years. Five cases were so-called congenital epulis, which occurs in infancy. Most of the tumors were within 9 mm in diameter. The data on location, age, and sex are basically in agreement with some reports in Europe and America.[8,34–36]

There are many theories concerning the origin of this tumor, such as myoblasts[15,21], fibroblasts[22], histocytes[23], epithelial cells[24], undifferentiated mesenchymal cells[11,26], neural cells[1,2,13,16,25], and various other cell types[8,12]. Although the tumor was classically thought to arise from myoblasts, ultrastructural investigations have suggested a neural origin, especially Schwann cells[3–7,17–20]. The concept of a Schwann cell origin is based on light and electron microscopic studies showing that the tumor cells lie within peripheral nerve bundles. Some investigators[2,8,10,18,19,27] have recognized axon-like structures in the cytoplasm of some tumor cells. We were able to observe axon-like structures containing cytoplasmic filaments and microtubules in the tumor cells, and the cells including axon-like structures resembled Schwann cells, even though our material for electron microscopy was fixed with 10% neutral formalin. The basal lamina and many myelin-like figures[10,11,27] showing concentric laminae and many angulate bodies[2,12,18,26] with many fibrils were also recognized in the present study, as described previously.

Moreover, immunohistochemical studies on S-100 protein, which is one of the specific neural proteins isolated from bovine brain, have been reported for this tumor. Some investigators reported that S-100 protein was positive in the tumor cells and suggested a Schwann cell origin[4–7,17]. S-100 protein is seen in the satellite Schwann cells among peripheral nerve tissues and tumor cells of nerve sheath origin. In the present immunohistochemical study, S-100 protein was moderately positive in the tumor cells, but myelin basic protein was not. Recently, some reporters have suggested that myelin basic protein could be a specific marker for neoplastic Schwann cells[37–39], and therefore its antibody has come to be used in studies of granular cell tumor[39,40,41]. However, immunoreactivity for myelin basic protein was negative in the present case, perhaps due to a technical error, heterogeneity or disappearance of staining intensity due to its degeneration. Neuron-specific enolase, neurofilament and glial fibrillary acidic protein were also negative in the tumor cells. Furthermore, actin, myosin, desmin, vimentin, fibronectin, lysozyme, α1-antichymotrypsin, carcinoembryonic antigen, epithelial membrane antigen and keratin were absent. These findings confirmed that this tumor did not originate from myoblasts, undifferentiated mesenchymal cells, fibroblasts, histocytes or epithelial cells.

The precise histogenesis of granular cell tumor is not known. However, a neural cell (Schwann cell) origin was confirmed in the present study by electron microscopy and immunohistochemistry.

References


[26] **SOBEL, H. J., SCHWARZ, R. and MARQUET, E.:** Light- and electron-microscope study of the
origin of granular-cell myoblastoma, *J. Pathol.*, 109, 101-111, 1973


[37] **Mogollon, R., Penneys, N. S., Albores-Saavedra, J. and Nadil, M.** Malignant schwannoma presenting as a skin mass: Confirmation by the demonstration of myelin basic protein within tumor cells, *Cancer*, 53, 1190-1193, 1984


