Oncocytoma and Oncocytic Carcinoma of the Salivary Glands, Single Institute Experience

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Background: Oncocytic neoplasms of the salivary glands are rare and the differential diagnosis between oncocytic carcinomas (OCs) and oncocytomas is difficult. We present 5 cases of oncocytoma and 3 cases of OC of the salivary glands with clinicopathological and immunohistochemical comparisons.

Methods: Eight cases of oncocytic neoplasms diagnosed at Asan Medical Center between 1998 and 2009 were reviewed for clinical data and histological features. Immunohistochemical staining for epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (Her-2), c-kit, p53, and Ki-67 was done.

Results: Cytological differences between oncocytomas and OCs were not obvious, but unequivocal infiltrative growths were identified in 3 cases, rendering the diagnosis of oncocytic carcinoma. When the remaining cases were classified as oncocytomas, there was no difference in age, size, and clinical symptoms between oncocytomas and OCs. Two of 3 OCs showed strong membranous expression of c-kit, but all oncocytomas were negative. The proportion of p53-positive cells was larger in OCs than oncocytomas. Her-2 or EGFR expression was absent, and Ki-67 labeling indices were less than 1% in all cases.

Conclusions: An infiltrative growth pattern, strong membranous expression of c-kit, and an increased proportion of p53-positive cells are features that can differentiate OCs from oncocytomas of the salivary glands.

Key Words: Adenoma, oxyphilic; Adenocarcinoma; Oxyphil cells; Salivary glands; Proto-oncogene protein c-kit
ly incubated with primary antibodies, biotinylated antimouse immunoglobulin, and peroxidase-labeled streptavidin in that order. 3,3′-diaminobenzidine was used as a chromogen and the sections were counterstained with Harris hematoxylin.

Her-2 results were scored according to the College of American Pathologists (CAP) guideline recommendations for Her-2 testing in breast cancer. EGFR results were analyzed using the same criteria as for Her-2. The result of c-kit immunostaining was interpreted by the intensity of cytoplasmic or membranous staining. The nuclear staining of p53 and Ki-67 was interpreted by the percentage of positive cells.

Clinical history and follow up data were obtained by review of the medical records of each patient. All patients were evaluated for the following parameters: age, sex, additional relevant history, previous biopsy or cytology, length of follow-up, recurrence and metastasis.

**RESULTS**

The clinical features of 8 cases are summarized in Table 1. All cases occurred at old ages (median, 56.5 years) and the male to female ratio was 1 : 1. In most cases the tumor presented as a slow-growing mass, except for case 4 which exhibited a bloody skin discharge. Seven tumors developed in the parotid glands, and one case (case 3) was presumed to be of submandibular gland origin, considering the patient’s history of a submandibular gland resection and radiation therapy about 30 years prior. On computed tomography scans, 6 cases were interpreted as benign masses. Case 3 was suspected to be a recurrent malignancy, and in case 4, a lobulating parotid gland mass extended to the surrounding soft tissue and skin, suggesting a malignant nature. The tumor sizes varied from 1 cm to 4.2 cm in greatest dimensions (mean, 2.2 cm). Preoperative fine needle aspiration cytology had been performed on 7 cases, but none had been diagnosed as oncocytoma or OC. All patients received complete resection of their tumors. Adjuvant therapy was not given. Follow up varied from 7 days (case 5), who was immediately lost to the study after the first visit following the operation to 36 months (case 3). No case showed evidence of recurrence or metastasis at the time of writing of this paper.

All tumors were microscopically composed of large, round to polygonal cells with fine, granular, eosinophilic cytoplasm (Fig. 1A). The cells had central or eccentric, round and vesicular nuclei and small but distinct nucleoli (Fig. 1B). While 5 tumors were well-circumscribed masses within fibrous capsules (Fig. 1A), cases 3, 4 and 8 showed extracapsular and/or extraparenchymal extension (Fig. 2). Although nuclear membrane irregularity and wrinkling were commonly observed in focal areas, nuclear atypia or pleomorphism was not noted in most cases. Mitotic figures were very rarely found; there were fewer than 1 per 50 high power fields. Only the tumor cells of case 3 showed mild cellular pleomorphism, nuclear atypia and prominent nucleoli (Fig. 3A). Lymphovascular invasion was not observed in any cases, and perineural invasion was present only in case 3 (Fig. 3B).

On immunohistochemical study, cases 4 and 8 showed strong membranous c-kit expression, while the others showed no or weak cytoplasmic expression (Fig. 4A, B). The percentages of p53-positive cells were generally lower than 3%, but those of cases 3, 4, and 8 were higher (10-20%). However, immunohistochemical results of Her-2 and EGFR were negative in all cases according to the CAP guidelines. Ki-67 labeling indices were less than 1% in all cases. The results of immunohistochemical staining are summarized in Table 2.

We reclassified the oncocytic neoplasms according to the reassessed findings regardless of the original diagnosis; only cases 3, 4, and 8, which manifested unequivocal invasive growths, were considered to be oncocytic carcinomas, and the others were

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>Sex</th>
<th>Chief complaint</th>
<th>Treatment</th>
<th>Recurrence</th>
<th>Size (cm)</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58</td>
<td>M</td>
<td>Slow-growing mass</td>
<td>Total P</td>
<td>-</td>
<td>2</td>
<td>Parotid gland</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>M</td>
<td>Slow-growing mass</td>
<td>Total P</td>
<td>-</td>
<td>1.7</td>
<td>Parotid gland</td>
</tr>
<tr>
<td>3</td>
<td>49</td>
<td>M</td>
<td>Slow-growing mass</td>
<td>Wide excision</td>
<td>Possible</td>
<td>1</td>
<td>Submandibular gland</td>
</tr>
<tr>
<td>4</td>
<td>66</td>
<td>M</td>
<td>Bloody skin discharge</td>
<td>Total P</td>
<td>-</td>
<td>4.2</td>
<td>Parotid gland</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>F</td>
<td>Slow-growing mass</td>
<td>Superficial P</td>
<td>-</td>
<td>1.7</td>
<td>Parotid gland</td>
</tr>
<tr>
<td>6</td>
<td>53</td>
<td>F</td>
<td>Slow-growing mass</td>
<td>Total P</td>
<td>-</td>
<td>3.5</td>
<td>Parotid gland</td>
</tr>
<tr>
<td>7</td>
<td>56</td>
<td>F</td>
<td>Slow-growing mass</td>
<td>Total P</td>
<td>-</td>
<td>2</td>
<td>Parotid gland</td>
</tr>
<tr>
<td>8</td>
<td>53</td>
<td>F</td>
<td>Slow-growing mass</td>
<td>Superficial P</td>
<td>-</td>
<td>1.4</td>
<td>Parotid gland</td>
</tr>
</tbody>
</table>

M, male; F, female; P, parotidectomy.
classified as oncocytomas. When we compared these two groups, patients with OC were slightly older (mean, 57.5 years) than oncocytoma patients (mean, 52.5 years), with tumors slightly larger (mean, 2.6 cm) than oncocytomas (mean, 2.2 cm), but...
these differences were not statistically significant. Interestingly, 2 of 3 OCs showed strong membranous expression of c-kit, while none of the oncocytomas did. The percentage of p53-positive cells was higher in OCs (10-20%) than in oncocytomas (less than 3%). Her-2 or EGFR expression was absent in both groups, and Ki-67 labeling indices were less than 1% in all cases.

**DISCUSSION**

The term “oncocyte” was first used by Hamperl in 1931 to describe cells with abundant, finely granular, eosinophilic cytoplasm. The terms “oncocytoma” and “oncocytic carcinoma” are commonly used to designate tumors – benign and malignant – consisting of oncocytic cells. With the exception of the thyroid and kidney, neoplasms composed of oncocytic cells are generally rare, but have been reported in various organs. OCs are malignant oncocytic neoplasms, which may have general malignant features such as cellular atypia, pleomorphism, large irregular nuclei, invasive growth, and perineural invasion.

Although clinical evidence of malignancy such as metastasis...
or death did not occur in our patients, the metastatic potential of salivary gland OCs has been well documented.\textsuperscript{6} And, as with other forms of salivary gland carcinomas,\textsuperscript{11,12} the submandibular gland location of OCs has been associated with aggressive tumor behavior.\textsuperscript{13,14} Tumor size was also reported to be a prognostic factor.\textsuperscript{13,14} Our case 3, another OC of the submandibular gland, was presumed to be a recurrent tumor, but its aggressiveness seemed to be much less than that of the other reported cases, considering the time interval to the recurrence being almost 30 years. Anyhow, this case showed the most obvious cytologic atypia including high nucleocytoplasmic ratio, nuclear hyperchromasia and pleomorphism, and prominent nucleoli.

Cytologic atypia was not evident in the other oncocytic carcinomas. Our case 4 could easily be underdiagnosed as an oncocytoma by cellular morphology only, but showed definite invasion of the adjacent tissue, causing the symptom of bloody skin discharge. The most reliable histologic criterion of malignancy in salivary gland oncocytic neoplasms is considered to be the growth pattern and invasiveness. This classification was further supported by immunohistochemical characteristics of carcinoma cells.

Immunohistochemical staining of oncocytic neoplasms in the past was mainly designed to prove the presence of mitochondria in the cytoplasm,\textsuperscript{7,15-18} a substitute method for electron microscopy. Commonly used antibodies were alpha-1-antitrypsin, alpha-1-antichymotrypsin, and antibodies against mitochondrial antigen. There were only a few comparative studies that differentiated oncocytic neoplasms. Ozolek \textit{et al.}\textsuperscript{17} suggested that CD10 and cytokeratin 20 are useful for the differentiation between oncocytomas of the salivary glands and renal oncocytomas. Mai \textit{et al.}\textsuperscript{10} used CD117 (c-kit) and progesterone receptor (PR) to distinguish oncocytic papillary renal cell carcinomas from renal oncocytomas. The renal oncocytoma showed positivity for c-kit and PR while oncocytic papillary renal cell carcinomas were negative for both. For salivary gland oncocytic neoplasms, the index of Ki-67 immunostaining was recommended by Ito \textit{et al.}\textsuperscript{16} to distinguish benign from malignant oncocytomas. However, Ki-67 labeling indices were not different between oncocytomas and oncocytic carcinomas in our study. This might be due to a slow growth rate of the tumors; the infra-auricular mass of case 4 persisted for over 10 years.

c-kit, a tyrosine kinase receptor, is involved in the growth and development of normal tissues and some types of neoplasms, and is expressed in both neoplastic and non-neoplastic salivary gland diseases.\textsuperscript{20} c-kit expression of salivary oncocytic carcinomas has not been described previously. Although the imatinib therapy in salivary gland OCs were rarely chosen, since the tumors rarely metastasize, a full validation of c-kit gene abnormality would have to be completed in more cases of salivary gland OCs. It was interesting that p53 overexpression was unexpectedly present in OCs but not in oncocytomas. Matzonkas-Antonio \textit{et al.}\textsuperscript{15} suggested that mutations in the p53 gene were related to salivary gland neoplasm pathogenesis. A possibility of p53 involvement in oncocytic carcinogenesis was raised, but needs to be confirmed by more studies. c-kit and p53 are expected to be useful ancillary markers in salivary gland OCs, especially when encountered at early stages. EGFR and Her-2 were candidates for targeted therapy of salivary duct carcinomas in recent studies\textsuperscript{22,23} but OCs were not included in those series because of their rareness. OCs did not express these surface receptors in our study.

In summary, the most reliable histological criterion for the diagnosis of OC was not the cytologic atypia of oncocytes but the invasive growth pattern. c-kit and/or p53 overexpression might be helpful ancillary markers and may represent possible involvement of these genes in oncocytic carcinogenesis.

REFERENCES