TEACHING CASE

Oncocytic lipoadenoma of the parotid gland: Immunohistochemical and cytogenetic analysis

Marius Ilie, Véronique Hofman, Florence Pedentour, Rita Attias, Joseph Santini, Paul Hofman

Abstract

Salivary gland oncocytic lipoadenoma is an exceptional benign tumor composed of mature adipose tissue associated with a mixture of oncocyes. We report a case of oncocytic lipoadenoma showing sebaceous differentiation, and provide a cytogenetic analysis, which has not yet been described. A 64-year-old male developed a left parotid gland, well-encapsulated tumor measuring 3.5×3 cm², showing mature fat cells associated with oncocytic changes of epithelial components. Immunohistochemistry showed a dual epithelial population with ductal (positivity for AE1/AE3, CK19, CK7 antibodies) and basal-cell (positivity for p63, CK14, CK5,6 antibodies) differentiation in oncocytic areas. Moreover, oncocytic cells were stained with anti-alpha-1 antic- hymotrypsin antibody and phosphotungstic acid–hematoxylin staining. Molecular cytogenetic analysis showed a translocation t(12;14), resulting in structural rearrangement of the region framing the HMGA2 gene at 12q14.3. Such alterations in HMGA2 have been described in both lipomas and pleomorphic adenomas of the salivary glands.

Keywords: Oncocytic lipoadenoma; Parotid gland; Immunohistochemistry; Cytogenetic

Introduction

Oncocytic lipoadenoma of the salivary glands is an exceptional benign tumor arising in parotid and submandibular glands [1,9,13,14]. This tumor is well-encapsulated and composed of large areas of mature adipose tissue and areas of oncocytic cells [1,9,13,14]. These tumors belong to the group of salivary gland tumors with both adipose and epithelial tissues, including sialolipoma and lipoadenoma tumors [10,18,20,22]. The first case of oncocytic lipoadenoma of the salivary gland was described by Hirokawa et al. [9] in 1998. Since then, three supplementary cases have been published [1,13,14]. The purpose of the present study is to report a new case of oncocytic lipoadenoma of the parotid gland.
with immunohistochemical study. Moreover, we provide the first cytogenetic analysis of such tumor.

**Case report**

A previously healthy 64-year-old male was seen in the Department of ORL (Pasteur Hospital, Nice, France) for evaluation of a painless swelling in the left preauricular area of 2-year duration. The CT scan revealed a deep lobe and an ill-defined lesion of low density with a heterogeneous aspect that led to the diagnosis of pleomorphic adenoma. A partial parotidectomy was performed with preservation of the facial nerve. A partial parotidectomy was performed with preservation of the facial nerve. The patient was followed up for 2 years without any sign of recurrence.

**Methods**

The surgical specimen measured $5 \times 4.5 \times 3$ cm$^3$ and weighed 15 g. The tumor had a fatty consistency, measured 3.5 cm in its largest diameter, and was completely encapsulated. Examination of crossing sections showed a yellowish tumor with small micronodules of light gray tan measuring 0.2–0.4 cm. A diagnosis of a benign adipose tumor with oncocytic areas was made on the basis of frozen sections. The surgical specimen was fixed in formalin, embedded in paraffin, and deparaffinized sections were stained with hematoxylin, eosin, and saffron (HES) and phosphotungstic acid–hematoxylin (PTAH). Immunohistochemical staining was performed using an automated Ventana BenchMark® instrument (Tucson, AZ, USA). A standard avidin–biotin–peroxidase complex staining technique was performed using the indicated primary antibodies (Table 1). Normal salivary gland tissue adjacent to the tumor was used as a control. After surgical excision, a fresh fragment from the tumor was prepared for cytogenetic and fluorescence in situ hybridization (FISH) analyses. Mechanical and collagenase dissociation of the tumor sample was performed according to Limon et al. [16]. Bacterial artificial chromosome (BAC) clones RP11-30I11 and RP11-118B13 located proximal to the 5' and distal to the 3' region of the HMGA2 gene (http://genome.ucsc.edu) were used as a two-color break-apart probe to detect rearrangements in the HMGA2 region, as described previously [11]. The BAC clones from the Roswell Park Cancer Institute Library (Buffalo, NY) were obtained from the Children's Hospital Oakland Research Institute (http://www.bacpac.chori.org). They were hybridized to metaphase cells according to standard procedures.

**Results**

Microscopic examination showed a well-circumscribed proliferation, consisting primarily of mature adipose tissues (70% of the tumor area) surrounded by a thin fibrous capsule with incomplete septae extending into the tumor mass. Islands or less delimited zones consisting of oncocyes were found in the lesion (Figs. 1A and B). These cells exhibited abundant eosinophilic fine granular cytoplasm and a single small rounded nucleus, and were arranged in microglandular or solid patterns or were isolated, and admixed with adipocytes (Fig. 1C). A few oncocytic populations were composed of small, densely eosinophilic cells with pyknotic nuclei (Fig. 1D). Mature sebaceous glands were present in some rare areas, admixed with oncocytic cells, and more rarely with mature adipocytes (Figs. 1E and F). Oncocytic cells and adipocytes showed no mitotic figures and atypia, and invasion through the fibrous capsule was not seen. Some acini and ductal structures were present in the adipose tissue and in oncocytic nodules, surrounded by lymphoid and plasmatic inflammatory infiltrates (Fig. 1G).

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Manufacturers</th>
<th>Clones</th>
<th>Dilution</th>
<th>Intensity</th>
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<tr>
<td>Cytokeratin 19</td>
<td>Dako</td>
<td>RCK108 monoclonal</td>
<td>1:30</td>
<td>+ to +++</td>
<td>LC</td>
</tr>
<tr>
<td>Cytokeratin 14</td>
<td>Novostra</td>
<td>LL002 monoclonal</td>
<td>1:20</td>
<td>+ + +</td>
<td>BC and MEC</td>
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<tr>
<td>Cytokeratin 5/6</td>
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<td>BC, MEC, and LC</td>
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<td>Dako</td>
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<td>+ + +</td>
<td>LC</td>
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<td>AE1/AE3</td>
<td>Ventana</td>
<td>AE1/AE3/PCK26 monoclonal</td>
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<td>BC and LC</td>
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<tr>
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<td>Biogenex</td>
<td>A1A88</td>
<td>1:200</td>
<td>+ + +</td>
<td>BC and LC</td>
</tr>
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LC: luminal cells; BC: basal cells; MEC: myoepithelial cells. SMA: smooth muscle actin.
Fig. 1. (A) Oncocytic nodule and mature adipose tissue (HES × 100). (B) Oncocytic cells. (HES × 200). (C) Oncocytes arranged in a solid and microglandular (arrow) pattern (HES × 400). (D) Dark cells (arrows) (HES × 400). (E, F) Metaplastic sebaceous gland (E: HES × 200 and F: HES × 400, inset × 800). (G) Lymphocytic infiltration around the ducts (HES × 200). (H) PTAH staining ( × 200, inset × 800).
The surrounding normal salivary gland showed atrophy of epithelial cells and chronic inflammatory cell infiltration, as well as some scattered adipocytes without associated oncocytic cells. Histochemically, PTAH staining revealed diffuse cytoplasmic granularity in oncocytic cells (Fig. 1H).

Immunohistochemistry showed that oncocytic areas were strongly and diffusely positive for AE1/AE3, CK7, and alpha-1-antichymotrypsin, whereas CK19, CK14, CK5,6, and p63-positive cells were present mainly at the periphery of neoplastic acini (Figs. 2A–D). Immunostaining with an anti-EMA antibody stained the luminal surface of the oncocytes (Fig. 2E), and differentiated sebaceous cells were positive for CK14 and EMA. Scattered myoepithelial cells stained with anti-alpha-smooth muscle actin and S100 protein antibodies in residual acini, but not in tumor areas (Fig. 2F). Data concerning the immunostaining are summarized in Table 1. Cytogenetic analysis showed a translocation involving the long arms of chromosomes 12 and 14 (Fig. 3A). The breakpoints were located at 12q14 and 14q24, respectively. FISH analysis confirmed and assigned more precisely the breakpoint at 12q14.3, and showed that it was located in the region of the HMGA2 gene framed by the RP11-30I11 and RP11-118B13 BAC probes (Fig. 3B). The karyotype was 46, XY, t(12;14) (q14.3;q24).

**Discussion**

Benign soft-tissue tumors represent 2–5% of all salivary glands neoplasms [3,5]. Oncocytic lipoadenomas of
Salivary glands are benign, exceptional tumors recently described in the literature, but currently not included in the WHO classification of the salivary gland tumors [3]. To our knowledge, four cases of oncocytic lipoadenomas have previously been published in the literature [1,9,13,14]. In those cases, the tumors were located in major salivary glands (parotid gland: 3 cases; submandibular gland: 1 case) of an adult man (1 case) or women (3 cases), respectively [1,9,13,14]. As in the present case, those tumors were always encapsulated, associating mature adipose tumor tissue and micronodules or islands of oncocytic areas with "light" and "dark" oncocytes without atypia and mitotic features. Residual normal acini and ducts can be present in the mesenchymal and epithelial tumor components. Polycystic changes in the tumor can be observed, with associated non-specific inflammatory cell infiltrates. As for the case described by Klieb and Perez-Ordonez [14], we noted sebaceous metaplasia of a couple of oncocytes and adipocytes. Interestingly, sebaceous metaplasia can be noted in different lesions of the salivary glands, such as pleomorphic adenoma, oncocytoma, Warthin's tumor, sialolipoma, and in the non-tumor salivary gland parenchyma [14,5]. The oncocytic origin of the cells described in the oncocytic lipoadenoma of the salivary glands is easily confirmed by strong staining of these cells with PTAH stain or with antibodies against mitochondria and by electron microscopy [13,14].

In the present case, immunohistochemical examination showed that oncocytic cells had both the phenotype of typical normal ductal cells (positivity of staining with AE1/AE3, CK19, and CK7) and of normal basal cells (positivity of the staining with p63, CK14, and CK5/6). As previously described, oncocytic cells showed positivity for staining with α1-antichymotrypsin and with PTAH [14,19]. A few cells were stained with anti-smooth muscle actin antibodies, confirming that a few myoepithelial cells were present. These results confirmed a previous case report showing a double subpopulation of oncocytic cells in oncocytic lipoadenoma of the parotid gland [14]. For some authors, these latter tumors had basal–myoepithelial differentiation [14,6,4]. Some oncocytic cells demonstrated the ultrastructural characteristics of normal striated duct cells [13]. Finally, various oncocytic lesions of the salivary glands, including benign oncocytoma, oncocytic carcinoma, and other lesions, can be detected using an anti-mitochondria antibody [21].

As an exceptional tumor, oncocytic lipoadenoma of salivary glands can have differential diagnoses, mainly other tumors with a large adipose tissue component. Sialolipomas are very rare tumors, which represent 0.5% of salivary tumors, and were recently described to be distinct from lipomas [20]. Lipoadenomas are very rare tumors arising in salivary glands [10,22]. Glandular structures are numerous, showing features of striated ducts, without associated myoepithelial cells. No acini are identified after immunohistochemical examination [10]. Oncocytic metaplasia can be observed focally. Associated sclerotic and polycystic changes can be described due to a chronic evolution [10]. This entity is under discussion. Some authors regard these tumors as adenolipoma as described for the breast, thyroid, or skin [20,4], whereas others consider them as different tumors [9]. It seems that lipoadenoma is rather a neoplastic glandular tumor than a hamartoma [4]. Pleomorphic adenoma can show a large adipose tissue component corresponding to a lipomatous pleomorphic adenoma or a pleomorphic adenoma with extensive adipose metaplasia [15]. A myoepithelial component, as well as chondroid or myxoid stroma, must be demonstrated [8]. Finally, salivary gland oncocytoma can be easily eliminated by the presence of associated adipose cells in oncocytic lipoadenoma [5,2].
We provide here the first cytogenetic analysis of an oncocytic lipoadenoma. We observed a reciprocal translocation involving chromosomes 12 and 14. The breakpoint at 12q14.3 was located within or very close to the HMGA2 gene, as demonstrated by FISH analysis. The potential biological implication of HMGA2 alteration in the pathogenesis of this tumor could be linked to a fusion of HMGA2 with a gene located on the long arm of chromosome 14, and this fusion gene onset might play a direct role in cell proliferation in this case. It was not possible to establish with certainty which of the tumor cell components – adipose, oncocytic, and/or basal cells – were the origin of the karyotypic anomaly, since a whole fresh specimen of the tumor was prepared for cell culture. However, cultured cells showed fibroblastic features, suggesting at least that the adipose component contained the t(12;14) translocation. Rearrangements of HMGA2 are frequent in lipomas and other benign tumors, such as uterine leiomyomas and salivary gland pleomorphic adenomas [7]. The alteration most often consists of a fusion of the first three exons of HMGA2 with the 3′ part of another gene. The fusion partner genes of HMGA2 are highly variable, the most frequent being LPP (for lipoma-preferred partner) at 3q28. Strikingly, lipomas and pleomorphic adenomas of the salivary glands share some cytogenetic and molecular similarities [5]. While a majority of pleomorphic adenomas of the salivary gland are characterized by a rearrangement in the PLAG1 gene at 8q11, approximately 8% of these tumors show a rearrangement in HMGA2 [3]. The most frequent aberration is fusion of HMGA2 with the NFIB gene at 9p22. This fusion HMGA2-NFIB has also been described in lipomas [12]. Of note, HMGA2 structural rearrangements have also been described in benign mesenchymal tumors, e.g. uterine leiomyomas and hamartomas. However, it is currently not known whether particular chromosomal patterns are specifically associated with oncocytic type tumors and, if so, whether they relate to a malignant potential of these tumors [17].

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References


