Metachronous clear cell carcinoma of the tongue and kidney: a diagnostically challenging coincidence

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Clear cell carcinomas (CCCs) account for 1% of carcinomas of the salivary glands. A 63-year-old woman presented with a painless, nonulcerated, nodular mass on the right side of the tongue, without palpable neck nodes. After excision and cryotherapy of the mass, the histologic evaluation revealed CCC. At the age of 55, she had undergone radical nephrectomy for CCC of the kidney which extended into the renal vein (pT3aN0). Although she had remained metastasis-free during the follow-up, the clear cell morphology raised the possibility of late lingual metastasis of the renal CCC. A clinical search for metastases, and a series of immunostainings and analysis of the von Hippel–Lindau gene were therefore performed on paraffin-embedded blocks of both tumors: Primary metachronous CCC of the tongue was diagnosed. This case illustrates the diagnostic challenge posed by CCC of the tongue if there is a history of CCC of the kidney. (Oral Surg Oral Med Oral Pathol Oral Radiol 2012;114:e25-e30)

Not otherwise specified clear cell carcinoma of the salivary glands (synonyms: hyalinizing clear cell carcinoma [HCCC], glycogen-rich clear cell adenocarcinoma) is a rare low-grade tumor.[1-5] We report herein the case of a 63-year-old woman who presented with a painless lingual mass. The excised specimen submitted for histopathologic examination revealed an infiltrative tumor composed of clear cells. Seven years earlier, her left kidney had been removed because of clear cell carcinoma. The clear cell features of the lingual tumor raised the possibility of late metastatic renal cell carcinoma. The latter situation is unusual but well documented, and the prognosis for patients with lingual metastasis is poor. For a definitive diagnosis, the patient was examined clinically for the presence of occult metastases, and the histopathologic and immunohistochemical features of the renal and lingual tumor were carefully compared. Because the biallelic loss of the von Hippel–Lindau (VHL) gene frequently occurs in renal clear cell carcinoma,[6] and the alteration is maintained in metastatic deposits, the VHL gene status of both tumors was investigated by means of molecular pathologic methods. The results led to the diagnosis of primary metachronous HCCC of minor salivary glands in the tongue.

The first 2 authors contributed equally to this work.
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CASE REPORT

In 2008, a 63-year-old Caucasian woman was evaluated for a gradually enlarging painless mass of 2 months' duration on the right side of her tongue. Physical examination revealed a 2 × 2-cm ulceration-free elevated lesion on the right middle third of the tongue with no palpable neck nodes (Figure 1). The mass was firm and nontender on palpation. The patient stated that she did not consume alcohol or smoke. The hematologic and serum chemical laboratory indexes were within normal limits. Panoramic radiographs did not reveal any dental etiology or bony involvement. Because she had undergone radical nephrectomy and regional lymphadectomy for clear cell carcinoma of the kidney 7 years earlier, the differential diagnosis of the lingual mass extended not only to fibroma, neuroma, lipoma, adenoma, and granular cell tumor, but also to late metastasis of the kidney carcinoma. The clinical findings led to the decision to remove the tumor. Accordingly, a biopsy specimen was taken and cryosurgical therapy was applied.

After receiving the histopathologic report of clear cell carcinoma of the tongue, additional clinical tests and analyses were performed to explore whether the patient had distant occult metastases of the renal cancer. Ultrasonography did not reveal local recurrence, and there was no evidence of lymphadenopathy on either side of the neck. Significant enlargement of the neck nodes was not noted in the computerized tomographic images, and chest X-ray was negative for lung metastases. The laboratory findings, including complete blood count, blood biochemistry, and urine analysis, were within normal limits. The patient did

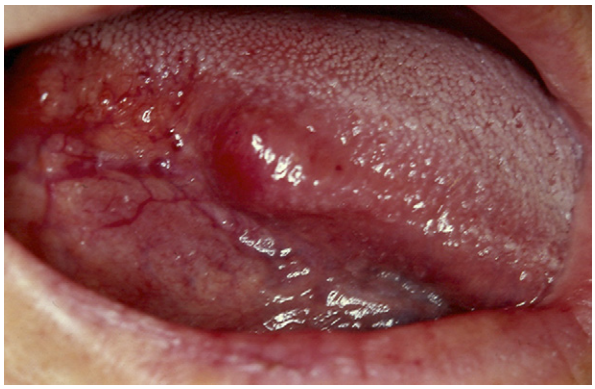

Fig. 1. The tumor on the right middle third of the tongue.

not receive further treatment, but returned at 2-month intervals for oncologic follow-up. She remained free of local or distant disease throughout the 3-year follow-up period.

HISTOPATHOLOGIC EVALUATION

The kidney and the lingual specimens were fixed in formalin and embedded in paraffin according to standard histopathology laboratory methods. Immunostainings with pancytokeratin (KL-1), epithelial membrane antigen (EMA), CD10, renal cell carcinoma (RCC) antigen, and vimentin were performed on paraffin blocks of both tumor samples. The lingual tumor was also investigated by means of periodic acid–Schiff (PAS) staining before and after diastase digestion, PAS–alcian blue (pH 2.5) staining, and myoepithelial markers, such as calponin, p63, smooth muscle-specific actin, S-100, and glial fibrillary acid protein.

MOLECULAR PATHOLOGY EVALUATION

DNA isolation

Areas rich in tumor cells and normal kidney tissue were selected by means of needle microdissection. The normal cell–tumor cell ratio was ≥60%. The QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) was used to acquire DNA according to the manufacturer's instructions. The DNA concentration of each sample was adjusted to ~10 ng/μL.

Loss of heterozygosity (LOH) analysis by microsatellite makers

Microsatellite (MS) marker (D3S2450, D3S1038, D3S 3651, D3S 1289, D3S 1582, D3S 3672, D3S 1613, and D3S 1300) sequences and locations were obtained from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/unists). Matched normal and tumor DNA samples were amplified in 10-μL reactions with 50 ng genomic DNA, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 200 μm each of deoxynucleoside triphosphate, 10 pmol Cy5-labeled forward primer, 5 pmol reverse primer, and 0.5 U Taq DNA polymerase (Fermentas). After 2 minutes of denaturation at 94°C, the polymerase chain reaction (PCR) mixes were subjected to 30 seconds at 94°C, 30 seconds at 61°C, and 40 seconds at 72°C for 28 cycles, followed by 10 minutes at 72°C in a CG1-96 thermal cycler (Corbet Research). Before loading, 20 μL stop solution containing 50 mM EDTA and 5 mg/mL dextran blue 2000 in 100% deionized formamide was added, and the samples were denatured at 95°C for 2 minutes. Analysis was carried out on an automated DNA sequencer (ALFexpress II; Amersham Pharmacia Biotech, Freiburg, Germany). The 6% denaturing polyacrylamide gels were run at 400 V, 55 mA, and 30 W in 1× Tris-borate EDTA buffer at a constant gel temperature of 55°C. The collected data were evaluated by using Fragment Manager (v. 1.2) software (Amersham, Pharmacia Biotech).

VHL gene sequencing

The PCR volume of 15 μL contained 1× buffer (75 mmol/L Tris-HCl (pH 8.8), 20 mmol/L $(NH_4)_2SO_4$, 0.01% (v/v) Tween 20), 3.75 nmol/L $MgSO_4$, and 10 pmol forward and 10 pmol reverse primers for the amplification of VHL exons. The primer sequence was: Exon 1a F: M13(−20)-AgCgCgTTCCATCCTCTAC; exon 1a R: CTgCgATTgCAgAAgATgAC; exon 1b F: M13(−20)-TACggCCCTgAAgAAgACgg; exon1b R: gggCTTCA-gACCgTgCTATC; exon 2 F: M13(−20)-AggACggTCTT-gATCTC; exon 2 R: gATTggATAACgTgCCTgAC; exon 3 F: M13(−20)-gTTggCAAAgCCTCTTgTTC; and exon 3R: gAAggAACCAgTCCTgTATC. The amplification steps were denaturation at 95°C for 20 seconds, annealing at 53°C for 20 seconds, and extension at 70°C for 30 seconds. The PCR products were sequenced in 1 direction on Megabace 1000 (Amersham Biosciences, Uppsala, Sweden) and evaluated with Sequence Analyzer 4.0.

RESULTS

Pathologic features of the renal tumor

Grossly, a spherical predominantly bright-yellow tumor measuring 95 × 60 × 45 mm with focal hemorrhage and cystic change was identified. The neoplasm extended into the renal sinus, the renal pelvis, and the renal vein. Histologically, the tumor cells were polygonal in shape, with a clear cytoplasm and nuclear atypia of Fuhrman grades I to II, and displayed a mixture of solid, acinar, and microcystic growth patterns (Figure 2). The tumor cells were diffusely positive with pancytokeratin and EMA and focally positive with vimentin, CD10 (Figure 3), and RCC antigen. The scanty

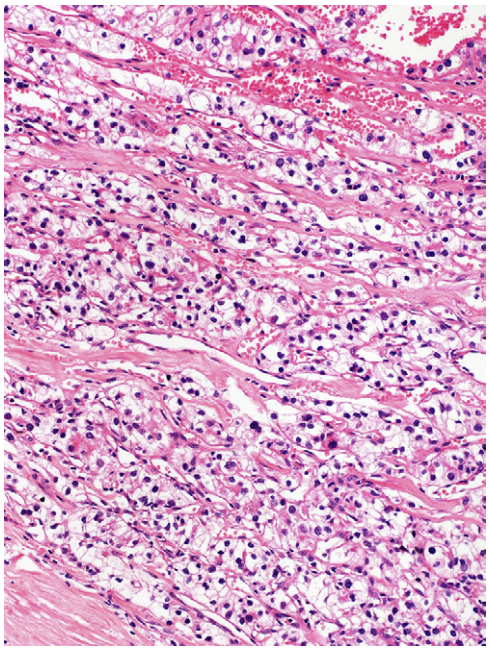

Fig. 2. Renal cell carcinoma. Solid and microcystic nests of clear cells with a fine capillary vascular background and Fuhrman grade II nuclear pleiomorphism. Hematoxylin-eosin, ×20.

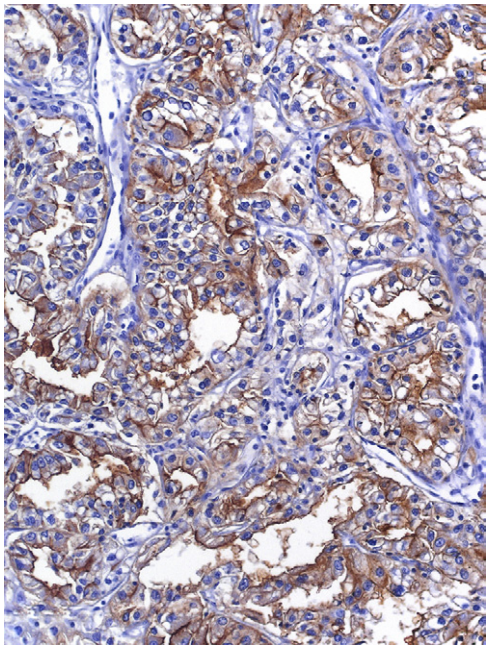

Fig. 3. Renal cell carcinoma. CD10 positivity of tumor cells. The architecture was tubular in this visual field. ×20.

stroma had a rich capillary network. The surgical margins were negative. The adrenal gland and 3 lymph nodes were devoid of metastasis. Low-grade clear cell carcinoma of the kidney (pT3aN0) was diagnosed.

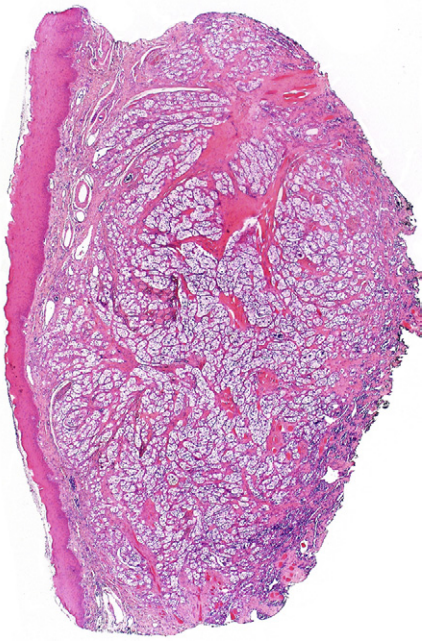

Fig. 4. Hyalinizing clear cell carcinoma of the tongue. Low power showing fascicles of clear cells and stromal bands of hyalinized collagen beneath the mucosal squamous epithelial layer (left). The tumor is uncapsulated and infiltrative. Hematoxylin-eosin, ×5.

Pathologic features of the lingual tumor

Grossly, the sample measured 10 × 15 × 10 mm. The mucosal surface was intact. Microscopically, the sample was covered by nondysplastic squamous stratified epithelium, under which a relatively circumscribed, noncapsulated, invasive tumor was identified (Figure 4). The tumor cells displayed a clear cytoplasm, distinct cell borders, small nuclei, and, at low magnification, barely visible nucleoli. The cells were arranged into small solid nests (Figure 5). The amount of stroma varied, and thin bands of hyalinized collagen separated the nests (Figure 4) in a few visual fields. Lymphovascular invasion was not encountered. Special stains demonstrated a moderate amount of glycogen in 20% of the tumor cells. The lesion was negative for mucin Immunohistochemically, the tumor cells were diffusely positive for pancytokeratin and EMA and negative for CD10, RCC antigen, vimentin, and markers of myoepithelial differentiation.

Molecular pathology results

In the course of the LOH examination, an allelic imbalance at the D3S2450 MS locus upstream of the VHL gene was found in the tumor of the kidney but not in that of the tongue. Downstream of this gene, all informative loci showed allele retention (Figure 6). An additional 3 markers (D3S 1289, D3S 1582, and D3S

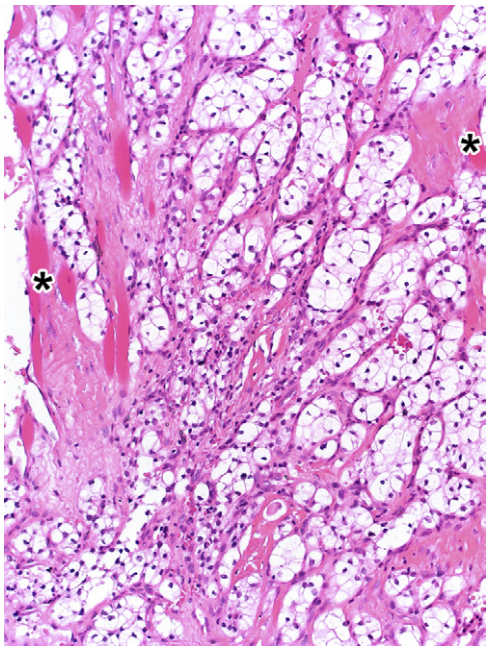

Fig. 5. Hyalinizing clear cell carcinoma of the tongue. Main features: a monomorphous population of tumor cells with clear cytoplasm and very mild nuclear atypia, arrangement into solid nests, and invasion of the lingual striated muscle (*asterisks*). Hematoxylin-eosin, ×20.

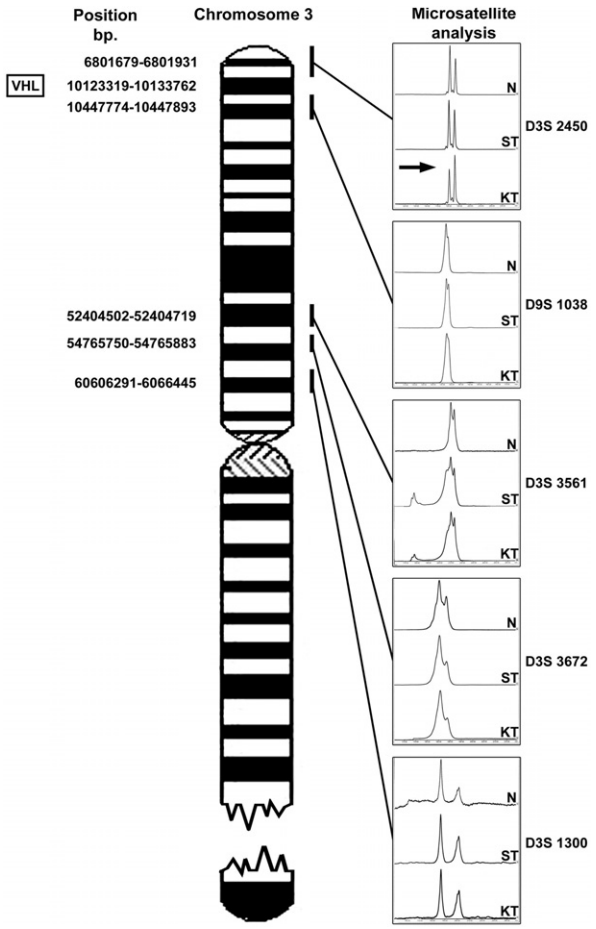

Fig. 6. Microsatellite markers and their approximate location on chromosome 3p. The D3S2450 marker shows an allelic imbalance in the renal tumor (*arrow*). *N*, Normal kidney; *ST*, salivary tumor; *KT*, kidney tumor.

1613) were not informative, because of the equal lengths of the repeat numbers of both loci. The sequence analysis did not reveal any alteration in the VHL gene in either the renal or the lingual carcinoma (data not shown).

DISCUSSION

From 1970 to 2008, 203,389 patients were treated in our Department of Oral Surgery. 172 patients (0.08%) had a malignant salivary gland tumor, including 2 (1%) diagnosed as HCCC. In one of those patients, the neoplasm was located in the parotid gland. The other patient is the subject of the present report.

In 2001, the patient had a large, renal vein–invasive, low-grade clear cell RCC. Local recurrence and distant metastasis did not develop during the follow-up. Seven years later, an insidiously developing lingual tumor was noted, and the low-grade clear cell morphology of the excised sample raised the possibility of late metastatic RCC. The careful comparison of the histologic and immunohistochemical features of the renal and the lingual tumor revealed distinct differences, however. The clear cell RCC displayed a solid, acinar, and microcystic architecture and had a rich capillary network, and the nuclei frequently exhibited Fuhrman grade II atypia. A significant proportion of the tumor cells were positive for vimentin, CD10, and RCC antigen, the standard markers of clear cell RCC. In contrast, the lingual neoplasm lacked a microcystic architecture; there were stromal hyaline bands, the capillary network was not prominent, and nuclear atypia was minimal. The tumor cells were focally positive for glycogen and negative for mucin, vimentin, CD10, RCC antigen, and markers of myoepithelial differentiation. These features were consistent with the pathology of HCCC, and the possibility of metastatic RCC or other primary clear cell neoplasms in minor salivary glands, such as clear cell mucoepidermoid carcinoma, adenoid cystic carcinoma, polymorphous low-grade adenocarcinoma, epithelial-myoepithelial carcinoma, and myoepithelial carcinoma, could be excluded.

Regarding the VHL gene status, we found an allelic imbalance at the most distal MS locus (D3S2450) upstream of the VHL gene and retention of all informative alleles downstream from it in the RCC, but not in the carcinoma of the tongue. To explain the possible background of the allelic imbalance, the mosaicism of the

tumor cells or normal cell "contamination" from parenchyma should be considered. By sequence analysis, wild-type VHL gene was confirmed at both sites. The results imply that the evolution of the RCC was associated with deletion of the VHL gene, and this genetic feature was not present in the carcinoma of the tongue, indicating that the latter was a primary tumor. The clinical data, the histopathologic features of the renal and the lingual tumors, and the results of VHL gene analysis collectively indicated that the patient had recovered from her renal cancer and that the later disease was primary metachronous HCCC of the tongue. She was free of her lingual disease at 3-year follow-up.

Clear cell salivary gland tumors were first described in the German-language literature by Kleinasser et al. in 1968.[7] In 1977, Batsakis et al. proposed a unified concept of "clear cell" tumors with the exclusion of acinic cell carcinoma and mucoepidermoid carcinoma of the salivary glands,[8] and they concluded that all clear cell tumors of the salivary glands were low-grade malignancies. In 1983, Chen[9] divided clear cell carcinomas into the dimorphic variant (an outer layer of clear cells and an inner layer of eosinophilic cells) and the monomorphic variant (composed only of clear cells). The dimorphic variant corresponds to epithelial-myoepithelial carcinoma. In 1994, Milchgrub et al. reported a series of 11 cases that they called HCCC.[10] The latest WHO classification of salivary gland tumors applies the term clear cell carcinoma not otherwise specified for HCCC, because not all cases display a stroma composed of thick bands of hyalinized collagen.[11]

HCCC is an infrequent salivary gland neoplasm, with a reported incidence of 1%. The clinical presentation, the behavior, and the histopathologic features of the present case corresponded with those in recent reviews.[12,13] HCCC usually occurs in middle-aged patients; women are affected more commonly than men. The predominant site is the palate, followed by the tongue. The classic initial symptom is a slowly growing painless submucosal mass without surface ulceration. Twenty-five percent of patients may have regional lymph node metastases at presentation. Hematogenous metastasis to the lung occurs infrequently.[12] The origin of HCCC is still uncertain. It may arise from intercalated ducts, because the myoepithelial markers are negative. The EWSR1-ATF1 fusion gene was recently demonstrated in a series of 23 HCCC cases, but not in 3 cases of mucoepidermoid carcinoma or 5 cases of epithelial-myoepithelial carcinoma, although the alteration was recommended as a molecular marker of HCCC.[14] We do not have the ability to test this novel finding, because the archived lingual tumor sample was entirely used for the analysis of the VHL gene status.

Among malignant epithelial tumors, lung carcinoma, breast carcinoma, and RCC may metastasize to the head and neck region, but the condition is rare. RCC metastases to the tongue are exceptional. From 1973 to 2011, 33 cases of a lingual metastasis of a primary kidney tumor were published in the English-language literature.[15-19] Five of those cases presented initially with lingual metastases before the diagnosis of primary RCC. The routes of metastasis to the tongue are not obvious. Most metastases are located on the base of the tongue. The prognosis is poor: <6 months. Although the clinical appearance of the lingual mass in the present case did not raise the suspicion of lingual metastasis, the history of RCC necessitated the approach presented.

In summary, a case has been reported in which clear cell carcinoma appeared in 2 different locations at a 7-year interval, both as primary lesions. The history of clear cell carcinoma of the kidney caused a challenge in the diagnostic work-up of HCCC of the tongue. To our best knowledge, a similar case has not been published in the English-language literature to date.

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