EWSR1 and *ATF1* rearrangements in clear cell odontogenic carcinoma: presentation of a case

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Clear cell odontogenic carcinoma (CCOC) is a rare odontogenic tumor of the jaws that is more common in the mandible than maxilla and has a female preponderance with a peak incidence in the sixth decade. It is characterized by locally aggressive behavior and has the potential to metastasize. This tumor was recently reported to have a rearrangement of the Ewing sarcoma breakpoint region 1 gene (EWS RNA-binding protein 1, *EWSR1*) in 5 of 8 cases tested and of the activating transcription factor 1 gene (*ATF1*) in 1 case tested. We report a case of CCOC in the premolar area of the mandible in a 59-year-old woman. This case demonstrated the presence of both *EWSR1* and *ATF1* gene rearrangements by fluorescence in situ hybridization. (Oral Surg Oral Med Oral Pathol Oral Radiol 2014;118:e115-e118)

In 1981, during an international oral pathology meeting held in Gothenburg, Sweden, Dr Gordon Rick presented a unique case of a mandibular tumor that exhibited aggressive clinical behavior. Histologically, it appeared to be of odontogenic origin and demonstrated a clear cell component. He designated this unusual tumor as clear cell odontogenic carcinoma (CCOC). It appears that this was the first description of the entity as a carcinoma.[1] Four years later, Hansen et al.[2] reported 3 cases of a locally aggressive tumor with a prominent clear cell component and coined the name clear cell odontogenic tumor. In 1989, Bang et al.[1] reported 3 further cases of a neoplasm with features identical to those described by Hansen et al. However, 1 of the 3 cases metastasized to a regional lymph node, and another of the cases metastasized to the lungs. These findings prompted the authors to propose a change in the nomenclature of the entity from clear cell odontogenic tumor to clear cell odontogenic carcinoma.[1]

CCOC is nearly twice as common in females compared with males (1.8:1 female-to-male ratio); has a mean age at diagnosis of about 55 years; is greater than 3 times more likely to arise in the mandible compared with the maxilla (3.67:1 mandible-to-maxilla ratio); and has the potential to metastasize to regional lymph nodes and distant sites. Histologically, CCOC consists of cords and nests composed of epithelial cells with round nuclei and eosinophilic to clear cytoplasm separated by fibrous septa.[1-7]

Tumors with clear cell morphology are a diagnostic challenge, because they share similar characteristics. Hyalinizing clear cell carcinoma (HCCC) of the salivary glands has a significant clear cell component with considerable histologic overlap with CCOC. Also, CCOC and HCCC share similar immunohistochemical profiles.[3] Antonescu et al.[8] identified the *EWSR1* (EWS RNA-binding protein 1, Ewing sarcoma breakpoint region 1) gene rearrangement in 18 of 22 cases of HCCC, in which 14 were confirmed to have the *ATF1* (activating transcription factor 1) rearrangement as well. Sequencing performed on 2 cases of HCCC confirmed *ATF1* to be the fusion partner of *EWSR1*. Subsequently, Bilodeau et al.[4] identified *EWSR1* rearrangement in 5 of 8 CCOC cases, with confirmation of *ATF1* involvement in 1 case. Here we present a case of CCOC that also had the *EWSR1* and *ATF1* rearrangements.

CASE REPORT

A 59-year-old woman presented to her general dentist complaining of numbness in her left lower jaw. She was referred to an oral and maxillofacial surgeon for further evaluation. A cone beam computed tomography scan was performed and revealed a 2.0 × 1.5cm well-circumscribed

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radiolucency with focal poorly defined borders in the left mandible, extending from the left mandibular second molar to the distal root surface of the left mandibular canine (Figure 1). An incisional biopsy of the area was performed. Microscopic examination revealed cords and nests of atypical epithelial cells, possessing a clear to eosinophilic cytoplasm supported by a fibrous stroma (Figure 2). Immunohistochemical stains demonstrated cytokeratin 7 positivity (Figure 3) and cytokeratin 20 negativity. A diagnosis of malignant epithelial neoplasm consistent with odontogenic carcinoma was rendered. Further work-up was recommended to rule out a metastatic deposit from an unknown primary tumor. A positron emission tomography scan was performed and was negative in all regions except for the left mandible. The patient underwent segmental resection of the left mandible. Modified neck dissection found 22 lymph nodes, all negative for metastatic disease. A vascularized free fibular flap and a reconstruction plate were placed. The gross specimen measured 5.5 × 4.0 × 1.4 cm. Microscopic review of the specimen showed areas of tumor with dense hyalinized connective tissue, a prominent clear cell component, and perineural invasion (Figure 4). Postoperative radiation was recommended, owing to the presence of perineural and intraneural invasion.

Materials and methods

All immunohistochemistry was performed using the Autostainer Link 48 system (Dako, Glostrup, Denmark); 4-μm-thick sections of routinely processed formalin-fixed, paraffin-embedded (FFPE) tissue were stained with monoclonal mouse antihuman antibodies for cytokeratin 7 (Clone OV-TL 12/30; Dako) and cytokeratin 20 (Clone K_S20.8; Dako). Manufacturer-suggested protocols were followed.

Vysis Locus Specific Identifier (LSI) break-apart *EWSR1* DNA Probe from Abbott Molecular (Des Plaines, IL, USA) was used for fluorescence in situ hybridization (FISH) analysis of the *EWSR1* gene rearrangement. FISH analysis was performed on 5-μm-thick sections of FFPE tissue according to the manufacturer's suggested protocol with minor modifications including increase of the Protease I (Abbott Molecular) step from 10 to 40 minutes and omission of the final formalin fixation step. These modifications were elected for optimization. Slides were counterstained with DAPI II (4′,6-diamidino-2-phenylindole) and viewed with fluorescence microscopy.

FISH for the *ATF1* rearrangement was performed using bacterial artificial chromosomes (BACs) from BACPAC sources of Children's Hospital of Oakland Research Institute (CHORI; Oakland, CA, USA; http://bacpac.chori.org), as previously described.[9] In brief, DNA from individual BACs was isolated according the manufacturer's instructions, labeled with different fluorochromes in a nick translation reaction, denatured, and hybridized to pretreated slides. Slides were incubated, washed, and mounted with DAPI in an antifade solution as previously described. Slides were viewed with fluorescence microscopy.

At least 200 nonoverlapping tumor epithelial nuclei with full-complement signal were analyzed for each probe.

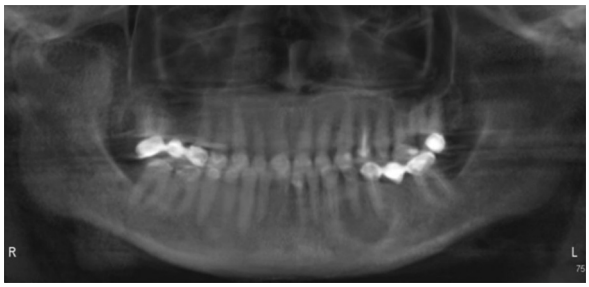

Fig. 1. Cone beam computed tomography image converted to a panoramic view showing a unilocular radiolucency with focal poorly defined borders in the left mandible.

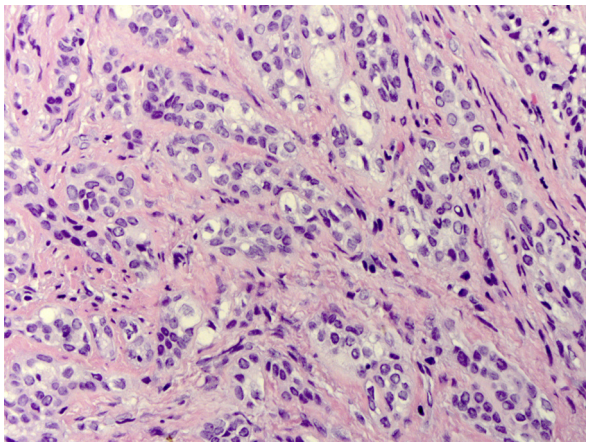

Fig. 2. Incisional biopsy specimen showing cords and nests of epithelial cells exhibiting a clear to eosinophilic cytoplasm with a fibrous connective tissue stroma (hematoxylin-eosin, original magnification ×400).

RESULTS

EWSR1 and *ATF1* probes were successfully hybridized to the tumor sections and exhibited break-apart and fusion signals. Of the 200 tumor nuclei counted for each probe, more than 20% were found to exhibit rearrangement by showing separation of the 5′ and 3′ probes for both *EWSR1* and *ATF1* probes (Figures 5 and 6, respectively).

DISCUSSION

CCOC is a rare tumor of the jaws that occurs more commonly in females than males, typically in adulthood, and has a predilection for the mandible over the maxilla. It is characterized by aggressive clinical behavior and has been reported to metastasize to regional lymph nodes as well as the lung. Histologically, the tumor is composed of nests and cords of round to polygonal epithelial cells exhibiting an eosinophilic to clear cytoplasm and round dark nuclei. The background consists of a fibrous connective tissue