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Carcinoma ex pleomorphic adenoma of minor salivary glands with major epithelial-myoepithelial component: clinicopathologic and immunohistochemical study of 3 cases☆

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ABSTRACT

In the present study, 3 cases of very rare intraoral carcinomas ex pleomorphic adenomas showing a striking differentiation of the malignant component towards epithelial-myoepithelial carcinoma were described. The tumors occurred in 2 men and 1 woman with median age of 56 years. Involved sites included palate and buccal mucosa. Two patients experienced local recurrences, of which one died of disease complications. In all cases, residual pleomorphic adenoma was present. The malignant component in all cases shared morphological aspects with epithelial-myoepithelial carcinoma. Those areas were characterized by eosinophilic duct-forming cells surrounded by layers of clear cells. The studied immunohistochemical markers highlighted a biphasic cell population. Duct-forming cells expressed pan-cytokeratin, cytokeratin 7, and focally cytokeratin 14, whereas the clear cell component strongly stained to cytokeratin 14, vimentin, and p63 but weakly stained to pan-cytokeratin and focally to α-smooth muscle actin, an immunophenotype compatible with both epithelial and myoepithelial differentiation. The Ki-67 proliferation index was up to 40% in malignant areas. Carcinoma ex pleomorphic adenomas of minor salivary glands with major epithelial-myoepithelial component are rare, locally aggressive, and potentially lethal tumors. The peculiar morphological and immunohistochemical aspects described may raise problems in diagnosis and classification of such cases, particularly in incisional biopsies.

1. Introduction

The term *epithelial-myoepithelial carcinoma* (EMC) lays emphasis on the characteristic biphasic cellular composition of a low-grade malignancy that can affect the salivary glands, initially described by Donath et al in 1972 [1–3]. Epithelial-myoepithelial carcinoma is a rare tumor, accounting for about 1% to 2% of all salivary gland tumors and 2% to 5% of all malignancies of the salivary glands, mainly in patients aged between the sixth and seventh decades of life with a slight female predilection [1,2]. Approximately 60% to 80% of the EMC cases occur in the parotid glands, with the remaining cases distributed almost equally among the submandibular and minor salivary glands, most commonly those of the palate. Rare cases were reported in sublingual glands [1,2,4,5].

Under histopathological examination, EMC is characterized by a dual cell population: intercalated duct-like luminal cells determine ductal structures and are surrounded by layers of large, polygonal, and clear neoplastic myoepithelial/abluminal differentiated cells [1,2]. Most cases are circumscribed masses and, rarely, encapsulated, often having a multilobular fashion [1,2]. Cytologic atypia is usually mild or absent [1,2,4].

Although they arise de novo, less than 2% of EMCs develop in a preexisting pleomorphic adenoma (PA), giving rise to an epithelial-myoepithelial carcinoma ex pleomorphic adenoma (CXPA) [4]. This event is exceptional and may represent a diagnostic dilemma, especially in small biopsy samples from the oral cavity, once EMC shares similar cellular composition and some morphological features with benign and malignant salivary gland tumors, including PA without carcinomatous transformation [1,2]. Therefore, we describe and discuss the clinical and histopathological aspects, as well as the immunohistochemical findings, in 3 cases of intraoral CXPA with major epithelial-myoepithelial component (CXPA-EMC).

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2. Material and methods

The local Committee of Ethics approved the present study. The archives and consultation practices of the Surgical Pathology Diagnostic Service at the School of Dentistry of the University of São Paulo and of the Department of Anatomic Pathology at the School of Medicine of the State University of Campinas were reviewed, and a total of 61 cases diagnosed as CXPA were found. Three of the 61 cases were identified as CXPA-EMC of intraoral minor salivary glands. Clinical and demographic data were collected from medical records.

Histopathological analysis was performed by 2 pathologists (AA and BTS) in a double-headed microscope using slides routinely stained in hematoxylin and eosin considering the following features: surgical margin status, pattern of growth (multilobular, solid, ductal, papillary, or cystic), presence of residual PA, tumor-associated dystrophic calcification, necrosis, stromal response (hyalinization, desmoplasia), and perineural and angiolymphatic invasion. Cases were also subclassified according to the degree of invasion through the capsule of the prior PA as intracapsular (without invasion), minimally invasive (≤1.5 mm of invasion), or frankly invasive (≥1.5 mm of invasion), according to World Health Organization (WHO) [6]. Cytologic characteristics considered for each case were nuclear atypia, mitotic rate, and cytoplasmic features. The degree of nuclear atypia was classified into mild, moderate, or severe based on grade of nuclear atypia for breast carcinomas [7]. Mitotic activity was expressed as the number of mitotic figures in 10 high-power fields (HPF) using a 40× objective. Cytoplasmic features were assessed in luminal and abluminal components by the cellular tinctorial quality (eosinophilic, amphophilic, or clear).

One representative paraffin block from each case was chosen for immunohistochemical studies. The following primary antibodies were used: anti–pan-cytokeratin, anti-cytokeratin (CK) 7, anti-CK14, anti-vimentin, anti–α-smooth muscle actin (α-SMA), anti-p63, and anti-Ki-67. Briefly, the immunohistochemical staining was performed as follows: the 4-μm sections were deparaffinized and hydrated, and endogenous peroxidase activity was quenched by immersion of the slides in 10% hydrogen peroxide. The antigen retrieval was achieved by boiling, in a steamer, in citrate buffer (pH 6.0) or Tris-EDTA buffer (pH 9.0) according to the primary antibody used (Table 1). After cooling, the sections were incubated at 4°C with the primary antibody and then with the EnVision polymer (DakoCytomation, Carpinteria, CA) for 1 hour at 37°C. Subsequently, sections were stained for 5 minutes at 37°C with 3,3 -diaminobenzidine tetrahydrochloride and counterstained with Mayer hematoxylin. Appropriate positive controls were used in all immunohistochemical reactions. Negative control was obtained by omission of the primary antibodies.

Immunoreactivity of the studied markers was assessed separately in both residual PA and CXPA-EMC. The labeled sections were qualitatively evaluated, and the Ki-67 expression was estimated as the percentage of positive cells in relation to all tumor cells in 3 "hotspot" chosen areas.

Table 1
Index of immunohistochemical stains

Antibody	Clone	Immunohistochemical dilution	Antigenic retrieval	Incubation
Pan-cytokeratin	AE1/AE3	1:50	Citrate	1 h
CK7	OV-TL12130	1:50	Citrate	1 h
CK14	LL02/1	1:500	Citrate	1 h
Vimentin	Vim 3B4	1:800	Citrate	1 h
α-SMA	1A4	1:200	Citrate	1 h
p63	4A4	1:400	Tris-EDTA	1 h
Ki-67	MIB1	1:75	Tris-EDTA	1 h

3. Results

3.1. Clinical findings

The incidence of CXPA-EMC was 4.9% of all CXPA cases. Two patients were men and one was a woman, and their ages ranged from 42 to 70 years (median, 56 years). The involved sites were palate/maxilla and buccal mucosa. Follow-up information was available in all cases that were T3/T4 tumors treated by wide surgical resection without neck dissection. Two patients underwent adjuvant radiotherapy. The surgical margins were focally positive in all 3 cases, 2 patients experienced multiple local recurrences, and 1 patient died of disease complications 48 months after tumor resection. The clinical findings of CXPA-EMC are summarized in Table 2.

3.2. Histopathological findings

The histopathological findings of CXPA-EMC cases are summarized in Table 3. Recognizable areas of PA were identified in all cases. These areas were composed of occasional double-layered ductiform structures, cords, and islands of polygonal to plasmacytoid myoepithelial cells in an extensive hyalinized stroma with focal myxoid areas (Fig. 1A). In 1 case, the transition from adenoma to carcinoma was abrupt (Fig. 1B).

The CXPA-EMC component displayed an infiltrative growth and was predominant in relation to the benign areas in all cases. A multinodular pattern of growth was present in 2 tumors (Fig. 1C); and in 1, it was solid with focal papillary structures. Two distinct cellular populations were evident under microscopic examination: eosinophilic duct-forming cells and clear cells. The former were polygonal in shape with eosinophilic cytoplasm and determined subtle ducts (predominant) and occasional well-defined duct-like spaces. These structures were surrounded by layers of clear cells (Fig. 1D). Additional but nonspecific findings included coagulative necrosis (2 cases), dystrophic calcification (2 cases), and squamoid (1 case) and apocrine change (1 case) (Fig. 2A and B). No area of perineural or angiolymphatic invasion was detected. All cases were subclassified as frankly invasive CXPAs (Fig. 2C). Two cases had mild nuclear atypia and one case moderate. The mitotic rate ranged from 1 to 3 per 10 HPF.

3.3. Immunohistochemical findings

Luminal/ductal cells of the PA areas showed strong and diffuse positivity to pan-cytokeratin and CK7. The myoepithelial component was positive to pan-cytokeratin, CK14, vimentin, and p63 but focal to α-SMA. Similar to EMC, a biphasic cellular component was demonstrated in malignant areas by immunohistochemistry. The eosinophilic duct-forming cells expressed pan-cytokeratin, CK7, and focally CK14, whereas the clear cell population demonstrated myoepithelial-like immunophenotype diffusely staining to CK14, vimentin, and p63 but focally to α-SMA and weakly to pan-cytokeratin. The Ki-67 proliferation index in all cases was up to 40% of positive neoplastic cells in the carcinomatous areas (ranging from 25% to 40%), whereas that in the residual PA areas was little significant (less than 5% of positive cells) (Fig. 3A-F). The morphological findings associated with immunohistochemical profile of the 3 cases were consistent with the diagnosis of CXPA of minor salivary glands with major epithelial-myoepithelial component.

4. Discussion

In the current study, we have demonstrated the clinical, pathological, and immunohistochemical features in 3 CXPA cases involving intraoral minor salivary glands with a major epithelial-myoepithelial carcinoma component. Among the 34 epithelial neoplasms of the salivary glands recognized by WHO, PA is considered as the most common benign tumor; and its malignant transformation as a CXPA is a rare