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Case Report

Calcifying epithelial odontogenic tumor: report of three cases with immunohistochemical study

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Abstract: Calcifying epithelial odontogenic tumor (CEOT) is a rare benign odontogenic tumor. Here we report 3 cases of CEOT. All patients were women and the age of the patients ranged from 39-48 years. All cases were located in the maxilla. Histologically, one case was epithelial-predominant and consisted of large irregular cribriform sheets of polygonal epithelial cells, dense eosinophilic amyloid-like globules, and frequent concentric calcifications. The other two cases were amyloid-rich and consisted of scattered small nests of polygonal epithelial cells, abundant stroma with amyloid-like globules, and lacked calcifications. Immunohistochemically, tumor cells in all cases were diffusely strongly positive for AE1/AE3, cytokeratin (CK)5, Cam5.2, CK19, 34βE12, and p63. Tumor cells in two cases were positive for CK7 and in one case, they were positive for CD10. Vimentin was strongly positive in one case, whereas weakly positive in two cases. A variable number of CD1a-positive Langerhans cells were observed among nests of tumor cells in all cases. In summary, CEOT exhibits distinct but various histological and immunohistochemical features.

Keywords: Calcifying epithelial odontogenic tumor, amyloid, calcification, immunohistochemistry

Introduction

Calcifying epithelial odontogenic tumor (CEOT) is a rare odontogenic tumor that accounts for approximately 1% of all odontogenic tumors [1]. It is a benign, slow-growing, locally invasive odontogenic tumor. It generally occurs in patients between 20-60 years of age, with a mean age of diagnosis of 40 [1, 2]. It affects men and women equally. Histologically, CEOT consists of three distinct histological components: sheets of polyhedral epithelial cells, amyloid deposits, and calcifications [1, 2]. Here we present 3 cases of CEOT with unique immunohistochemical findings.

Case reports

Case 1

A 48-year-old woman complained of a painful swelling in her right cheek that had been present for 2 months. A panoramic radiograph revealed a mixed radiolucent-radiopaque mass in the right maxillary bone. A facial computed tomography (CT) revealed a large osteolytic mass (4.8×3.3×3.9 cm) with amorphous and stippled calcifications (**Figure 1A**). The mass was associated with an unerupted tooth. Incisional biopsy was performed and the diagnosis of CEOT was made. Subsequently, partial hemimaxillectomy was performed. Macroscopically, the mass appeared as a nonencapsulated, circumscribed, pale gray, firm solid. Microscopically, the tumor had an epithelial-predominant pattern. The tumor consisted of large irregular cribriform sheets of polygonal epithelial cells, abundant extracellular dense eosinophilic amyloid-like globules, and concentric lamellar calcifications called Lisengang rings (**Figure 1B**). The epithelial cells had abundant eosinophilic cytoplasm, well-developed intercellular bridges, moderate nuclear pleomorphism, occasional binucleation, smudged or vesicular chromatin, inconspicuous or small nucleoli, and no mitotic figures (**Figure 1C**). The amyloid-like material was stained intensely with Congo red and showed apple-green birefringence when subjected to polarized light (**Figure 1D**). Tumor

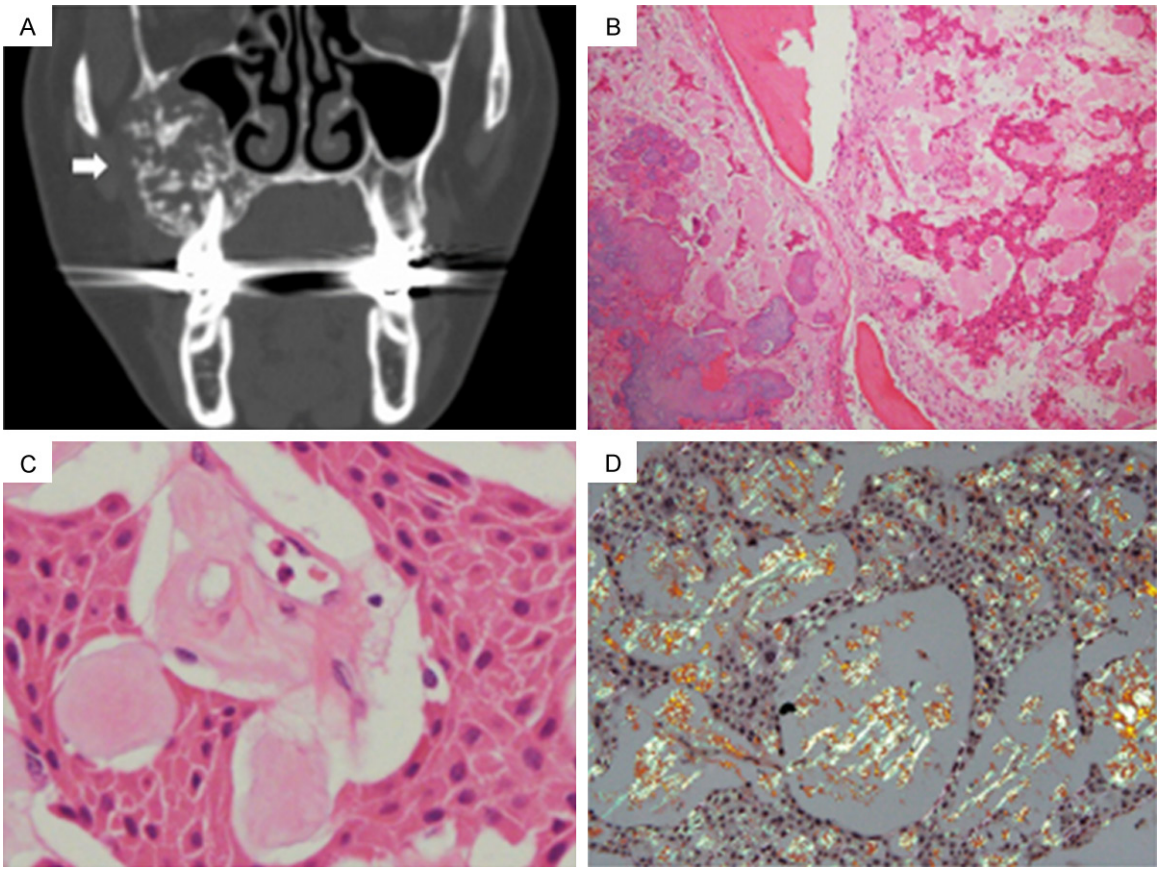

Figure 1. Case 1: A. The coronal view of facial CT reveals a large osteolytic mass (arrow) with amorphous and stippled calcifications. B. The tumor consists of large irregular cribriform sheets of epithelial cells surrounded by eosinophilic amyloid-like material and concentric calcification. C. Higher magnification of polyhedral epithelial cells showing abundant eosinophilic cytoplasm, well-developed cell borders and distinct intercellular bridges. D. Apple-green birefringence under polarized light after staining with Congo red.

cells had infiltrated between bone trabeculae extensively. The patient was disease-free 7 years later.

Case 2

A 39-year-old woman had a painless maxillary mass that was discovered incidentally. A panoramic radiograph revealed a radiolucent defect in the anterior maxilla (**Figure 2A**). A facial CT revealed an expansile lesion (2×1.5×2 cm) with mild bulging of the bony cortex and scalloped marginal sclerosis (**Figure 2B**). Excision of the mass was performed. Microscopic examination revealed an amyloid-rich pattern. The tumor consisted of scattered small nests or strands of polygonal epithelial cells dispersed within fibromyxoid stroma with abundant globular amyloid-like material (**Figure 2C**). No calcification was detected. Tumor cells had abundant granular cytoplasm, indistinct cytoplasmic borders, anisonucleosis, irregular nuclear membrane, frequent intranuclear cytoplasmic inclusions, smudged or vesicular chromatin and inconspicuous nucleoli (**Figure 2D**). No mitotic figures were observed. The amyloid-like material was stained with Congo red and exhibited apple-green birefringence under polarizing microscopy. No recurrence was detected after one month.

Case 3

A 41-year-old woman was referred by an orthodontist because of an incidentally discovered maxillary mass. A panoramic radiograph revealed a radiolucent defect in the right maxilla. A dental cone beam CT revealed a well-defined radiolucent lesion with cortical thinning and perforation of palate (**Figure 3A**). Excision of the mass was performed. Microscopically, the tumor had an amyloid-rich pattern. The

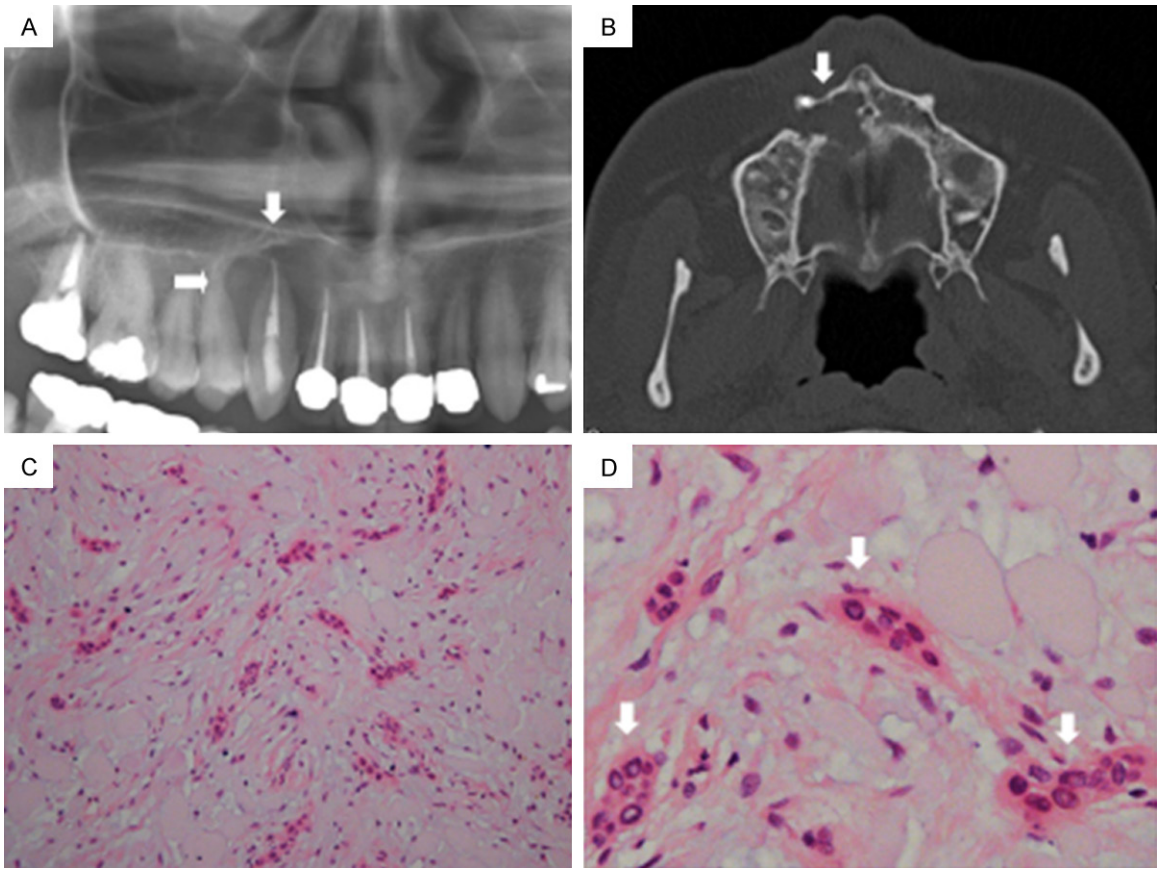

Figure 2. Case 2: A. Panoramic radiograph showing a radiolucent defect (arrows) in the anterior maxilla. B. The axial view of facial CT scan reveals a small expansile mass (arrow) with mild bulging of the bony cortex and scalloped marginal sclerosis. C. The tumor consists of scattered small nests of epithelial cells and fibrous stroma with abundant eosinophilic globular amyloid-like substance and lack of calcification. D. Higher magnification of epithelial cells showing indistinct intercellular bridges, anisonucleosis, highly irregular nuclear membrane, and frequent intranuclear cytoplasmic inclusions (arrows).

tumor consisted of nests or strands of polyhedral epithelial cells embedded within dense fibrous stroma with a large amount of globular eosinophilic amyloid-like material (**Figure 3B**). No calcification was identified. The epithelial cells had abundant eosinophilic cytoplasm, indistinct cytoplasmic borders, mild nuclear pleomorphism, occasional binucleation, smudged or vesicular chromatin, occasional intranuclear inclusions, inconspicuous nucleoli and no mitotic figures (**Figure 3C**). The amyloid-like material exhibited apple-green birefringence under polarizing microscopy after Congo red staining (**Figure 3D**). No recurrence was detected after 29 months.

Immunohistochemical findings

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded sections using a BOND-MAX automated immunostainer (Leica Biosystems, Bannockburn, IL, USA). The primary antibodies used are summarized in **Table 1**. Tumor cells in all cases were diffuse, strongly positive for AE1/AE3, cytokeratin (CK)5, Cam5.2, CK19 (**Figure 4A**), 34βE12, and p63, but negative for epithelial membrane antigen (EMA), smooth muscle actin (SMA), calponin, and cytokeratin 20.

Tumor cells in all cases were stained with β-catenin in a normal membranous pattern. CK7 was non-homogeneously positive with strong or moderate intensity in cases 1 and 3 (**Figure 4B**), but negative in cases 2. CD 10 was non-homogeneously positive with strong intensity in case 1, but negative in cases 2 and 3. Vimentin was strongly but non-homogeneously positive in case 1 (**Figure 4C**), and very weakly positive in cases 2 and 3. Vimentin was not

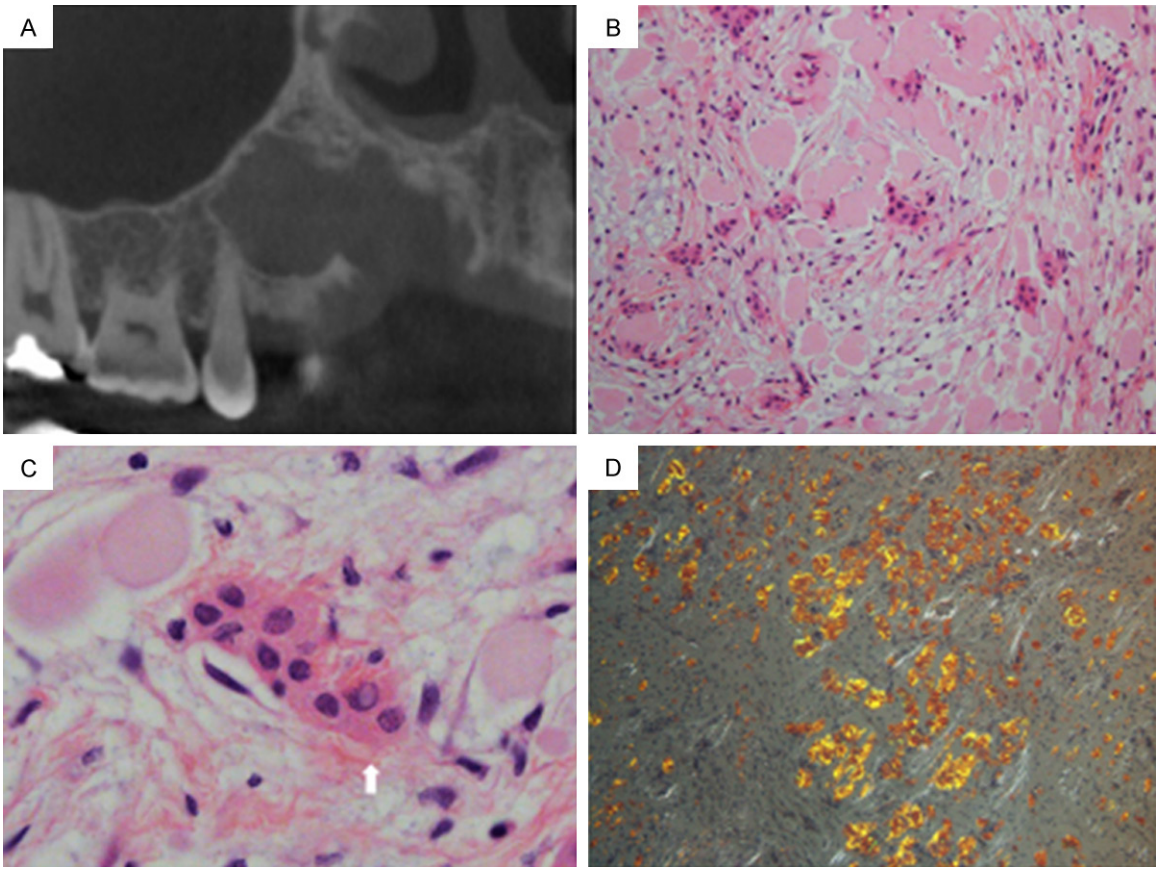

Figure 3. Case 3: A. Dental cone beam CT reveals a well-defined radiolucent lesion with cortical thinning and perforation of palate in the right maxilla. B. The tumor consists of small nests of polygonal epithelial cells, abundant eosinophilic globular material, and lack of calcification. C. Higher magnification of epithelial cells showing abundant eosinophilic cytoplasm, indistinct intercellular bridges, and intranuclear cytoplasmic inclusion (arrow). D. The amyloid exhibits apple-green birefringence under polarizing microscopy after staining with Congo red.

stained throughout the cytoplasm of the tumor cells, but stained a part of the cytoplasm of the tumor cells in all cases. In cases 2 and 3, many Langerhans cells among nests of tumor cells were observed, which were consistently positive for CD1a (**Figure 4D**), whereas inconsistently positive for S100 proteins. In case 1, only a few CD1a-positive Langerhans cells were observed. The Ki67 index was less than 2% in all 3 cases.

Discussion

CEOT was described and defined by Pindborg in 1955 [3]; it is histologically characterized by three components, polyhedral epithelial cells, amyloid deposits, and calcification. The amount of calcification varies, and some tumors reveal no calcification at all [2, 4]. However, the other two components are necessary for a diagnosis of noncalcifying CEOT [5]. Cases 2 and 3 reported herein correspond to the noncalcifying variant. Some tumors are epithelial-predominant, like case 1, while others are amyloid-rich, like cases 2 and 3. Most CEOTs are intraosseous but approximately 6% of CEOT are extraosseous [6]. Intraosseous tumors occur more often in the mandible than in the maxilla [2, 7]. Extraosseous cases have a predilection for the anterior gingiva and commonly lack calcification [1, 2, 6]. Given the locally invasive nature of CEOT, small tumors may be enucleated, but the definite therapy should include resection of the entire mass, with a tumor-free surgical margin [1].

Odontogenic epithelial tumors are heterogeneous lesions that are classified according to the histological features of the odontogenic epithelium and stroma, which are applicable to

Table 1. Primary antibodies used for immunohistochemistry

Antibody	Clone	Source	Dilution
AE1/AE3	5D3/LP34	Novocastra, Newcastle upon Tyne, UK	1:200
CK5	XM26	Novocastra	1:200
CK7	OV-TL 12/30	Dako, Glostrup, Denmark	1:400
Cam5.2	CAM5.2	Becton Dickinson Biosciences, San Jose, CA	Ready to use
CK19	b170	Novocastra	1:800
CK20	Ks 20.8	Dako	1:400
34βE12	34βE12	Dako	1:200
p63	4A4	Thermo Fisher Scientific, Fremont, CA, USA	1:8000
CD10	56C6	Novocastra	1:100
Vimentin	V-9	BioGenex, Fremont, CA USA	1:3200
EMA	GP1.4	Novocastra	1:400
SMA	αsm-1	Novocastra	1:200
Calponin	CALP	Dako	1:1600
CD1a	O10	NeoMarkers, Fremont, CA, USA	1:400
S100	Polyclonal	Novocastra	1:800
β-catenin	17C2	Novocastra	1:1600
Ki67	SP6	CELL-MARQUE, Rocklin, CA, USA	1:200

CK, cytokeratin; EMA, epithelial membrane antigen; SMA, smooth muscle actin.

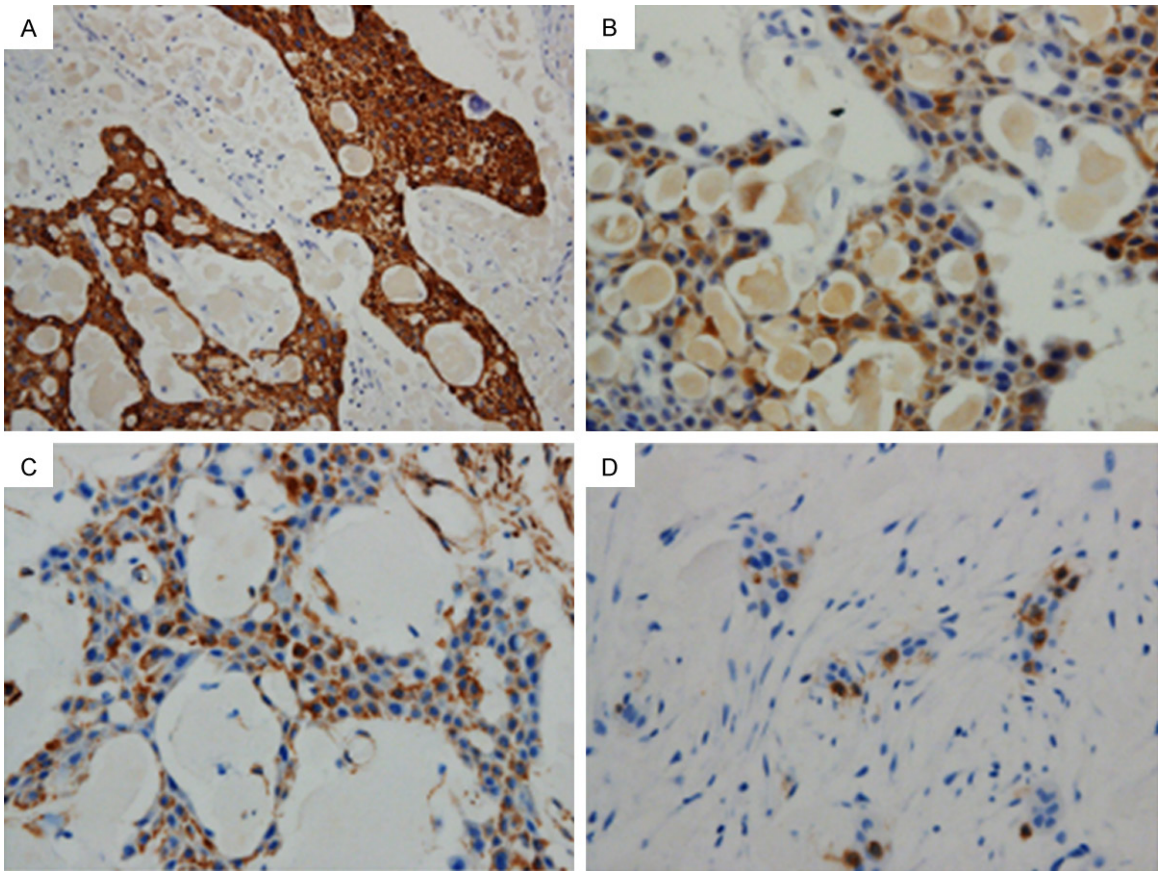

Figure 4. Immunohistochemical findings. Tumor cells are diffusely strongly positive for CK19 (A), moderately positive for CK7 (B) and vimentin (C) in case 1. Many Langerhans cells intermingled in the epithelial nests are positive for CD1a (D) in case 3.

mature, fibrous stroma or odontogenic ectomesenchyme [2]. CEOT falls into the category of odontogenic epithelial tumors with mature, fibrous stroma without odontogenic ectomesenchyme [2]. The epithelium of CEOT is characterized by sheets or nests of polygonal epithelial cells with intercellular bridges. The stroma of CEOT contains rounded amyloid-like material which undergoes concentric calcification [3].

Until now, immunohistochemical studies of CEOT have been limited. According to the literature, the epithelial cells of CEOT are positive for various cytokeratins (CKs), such as AE1/AE3, CK5/6, Cam5.2, CK14, CK19, 34βE12 and p63 [8-12]. The strong immunoreactivity for AE1/AE3, CK5, Cam5.2, CK19 and 34βE12 was the same in all three of our cases, even though the histologic patterns differed. However, the immunoreactivities of CK7, CD10 and vimentin were variable in our cases. Gratzinger *et al.* [10] reported that two out of three CEOTs were positive for CK7. Tumor cells in our cases 1 and 2 were positive for CK7. We consider CK7 expression in CEOT very noticeable because CK7 expression is not common in other odontogenic epithelial tumors [12-16].

Some previous articles [8, 11, 15, 17] have reported positive reaction to vimentin in CEOT. Crivelini *et al.* [15] reported that vimentin stained consistently but non-homogeneously in all of 5 cases of CEOT. They suggested that CEOT origin is the Hertwing root sheeth, based on the presence of vimentin and CK7. All three of our cases were positive for vimentin strongly or at least weakly. Vimentin expression may be a valuable finding of CEOT in distinguishing CEOT from other odontogenic epithelial tumors.

Gratzinger *et al.* [10] reported that odontogenic epithelial tumors such as CEOTs, ameloblastomas, and calcifying cystic odontogenic tumors (CCOTs) show distinctive immunohistochemical and ultrastructural features which overlap with those of myoepithelial-derived salivary gland neoplasms. However, those authors failed to provide definitive support for myoepithelial differentiation because other, more definitive, markers of myoepithelial differentiation, including S-100 protein and SMA, were not present [10]. In our three cases, the expression of AE1/AE3, CK5, CK7, Cam5.2, CK19, 34βE12, p63, CD10 and vimentin partly supports their suggestion. However, S100 protein, calponin, and SMA were not expressed in our cases either.

The presence of Langerhans cells in CEOT has been reported, especially in the non-calcifying variant of CEOT and in Asian individuals [4, 18, 19]. Significantly increased numbers of Langerhans cells in CEOT suggest that the cases are the Langerhans cell-rich variant of CEOT [4, 18]. Cases 2 and 3 reported herein correspond to the Langerhans cell-rich variant. In our cases, Langerhans cells were not identified by hematoxylin-eosin staining; however, they were revealed by distinct immunoreactivity of CD1a. The significance of Langerhans cells in CEOT remains to be clarified.

The diagnosis of CEOT is based on its distinct histology. The main differential diagnosis for CEOT includes CCOT and dentinogenic ghost cell tumor (DGCT). CCOT and DGCT are similar to CEOT in regards to calcification [2, 7, 14, 16, 17]; however, they are characterized by ghost cells and ameloblastoma-like epithelium, which are not observed in CEOT. Furthermore, dentinoid material is prominent in cases of DGCT [2, 17].

In summary, CEOT exhibits distinct but various histological and immunohistochemical features. Further large-scale comprehensive studies are required to clarify that.

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Disclosure of conflict of interest

None.

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