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Detection of cytokeratins in ghost cells of calcifying cystic odontogenic tumor indicates an altered keratinization and hair follicle differentiation for their development

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ABSTRACT

Calcifying cystic odontogenic tumors (CCOTs) are benign cystic lesions of odontogenic origin characterized by an ameloblastoma-like epithelium and the presence of a group of cells named *ghost cells*. The pattern of cytokeratin (Ck) expression on these lesions remains unclear and needs to be clarified. To this end, the expression of Ck6, Ck13, Ck14, Ck18, and Ck19 in the epithelium lining of 7 cases of CCOTs was evaluated by immunohistochemistry. For this, the epithelium lining was divided into 3 distinct regions: basal layer, suprabasal layer, and the compartment composed of ghost cells. In this study, 6 cases (85.7%) were classified as type 1 and 1 (14.3%) as type 4. All cases were negative for Ck13 and Ck18, despite the epithelial layer, as well as in the ghost cells. Ck6 was only positive in the ghost cells. Positivity for Ck14 and Ck19 was found in the basal and suprabasal layers, including the ghost cells. The results showing positivity for Ck14 and Ck19 in all of the analyzed cases reinforce CCOT as being of odontogenic origin, and the restricted expression of Ck6 in the ghost cells may be indicative that these cells suffer an altered differentiation into hair follicles in CCOTs.

1. Introduction

The calcifying cystic odontogenic is an uncommon benign lesion of odontogenic origin that is characterized by an ameloblastoma-like epithelium and a number of ghost cells that underlie a fibrous capsule [1]. Since its first description by Gorlin et al [2], there has been great controversy regarding the nature of this lesion because some authors classify it as a cystic lesion and others as a neoplasm [3]. Recently, the World Health Organization renamed this tumor the calcifying cystic odontogenic tumor (CCOT), and in 2008, an international collaborative study distinguished 4 variants of CCOT after reviewing 122 cases as follows: type 1 (simple cystic CCOT), type 2 (odontomas-associated CCOT), type 3 (ameloblastomatous proliferation), and type 4 (associated with benign tumors, except for odontoma) [4].

Cytokeratins (Cks) are proteins that belong to the group of intermediate filaments present in the cytoplasm of epithelial cells [5]. Although the main function of these proteins is the promotion of mechanical support, some Cks have also been implicated in other functions inside cells, such as apoptosis, cell growth, tissue polarity, wound response, and tissue remodeling [6]. Each epithelial cell expresses a profile of Cks that varies according to the epithelium type, the degree of differentiation, and the pathological process [6,7]. Some reports have studied a set of Cks, including Ck7, Ck8, Ck14, Ck18, Ck19, and Ck13, in CCOT, primarily attempting to identify the nature of the ghost cells and also to characterize the degree of maturation of its epithelium [8]. Despite this, no consensus has been reached, and the results found in the literature are largely diverse [3,4]. Notwithstanding this, a recent study showed immunoreactivity for hair keratin only in ghost cells and suggested a possible differentiation of them into hair follicles in CCOT [9]. Confirming such findings, another study identified hard keratin in the cytoplasm of ghost cells during their development [10]. However, there are no studies evaluating the expression of a specific hair keratin in CCOT to confirm this hypothesis.

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Considering the aforementioned, the aim of this study was to evaluate the expression of a set of Cks, including Ck6, a keratin that is detected in hair follicles, in an attempt to show a possible mechanism for ghost cell development in CCOT.

2. Materials and methods

The current study was approved by the Human Research Ethics Board at the Federal University of Uberlândia (protocol number 305/07).

Our sample corresponded to 7 cases of CCOT that were referred to and diagnosed at the Department of Oral Pathology of the Federal University of Piauí and Federal University of Uberlândia. Clinical data of the selected cases were gathered from the patients' pathological files and included the location of the lesion and the age and sex of each patient. Slides of the retrieved cases from both departments were reviewed according to the novel classification criteria proposed by Ledesma-Montes et al [4]. Only biopsied tissue samples were used to evaluate the expression of Cks.

Immunohistochemistry was performed on tumoral tissue sections to assess the expression of Ck6, Ck13, Ck14, Ck18, and Ck19 using a streptavidin-biotin-peroxidase technique. The primary antibody specifications, dilutions, and times of incubation used for each antibody are shown in Table 1. Briefly, formalin-fixed, paraffin-embedded tissues were cut into 3-μm tissue sections. Next, the samples were deparaffinized and then treated with 10% ammonium hydroxide diluted in 95% ethanol for 10 minutes to remove the formalin pigment. Antigen retrieval was performed in 10 mM, pH 6.0, citric acid in a steam cooker for 5 minutes. Endogenous peroxidase was quenched in 10 V H_2O_2, twice, for 15 minutes. After incubation with the primary antibodies, the sections were treated with a secondary biotinylated antibody (kit LSAB-HRP; Dako, Carpinteria, California) for 30 minutes at room temperature. The immunoreactivity was observed with the chromogen 3,3-diaminobenzidine tetrahydrochloride (Sigma, St Louis, Missouri), and the sections were counterstained with hematoxylin. Samples of normal skin were used as positive controls. For the negative control, the primary antibodies were omitted and a diluted solution was used in the reactions.

Immunohistochemical staining was semiquantitatively assessed considering the intensity of expression in 3 distinct regions of the tumoral epithelium: basal, suprabasal, and the compartment composed of ghost cells. For this, the immunoreactivity for each Ck was evaluated using the following intensity categories: (0) negative, (1+) weak, (2+) moderate, and (3+) strong staining.

3. Results

The clinicopathologic data of 7 cases are summarized in Table 2. Six cases were histologically classified as type 1 (85.7%) and 1 case as type 4 (14.3%). Men were slightly more affected than women, and the mean age was 37.4 years. Five of the 7 cases involved the mandible, with most of the lesions arising in the premolar region (5 cases). Only 5 patients developed tumors in the incisive region, with 1 case affecting the mandible and another in the maxilla.

With respect to the immunohistochemical staining, Table 3 depicts the pattern of Ck expression for each case. Positivity for Ck14 and Ck19 was found in 100% of the cases in the 3 regions analyzed from the tumoral epithelium, and the intensity of expression was always strong for both proteins. On the other hand, all of the cases were negative for Ck13 and Ck18 independent of the epithelial region. One interesting aspect found here was that only the ghost cells were positive for Ck6 in all cases investigated, and the staining was seen peripherally surrounding these cells, as for Ck14 and Ck19 (Fig.). Regarding Ck6 immureactivity, in all cases studied, the ghost cells exhibited a moderate intensity of expression, although some cells showing a weak and even a strong expression could be seen, even in the same area.

Table 1
List of primary antibodies

Cks	Dilutions	Clone	Supplier
6	1:75	LHK6B	Bio System, Dako, Carpinteria, CA
13	1:50	KS-1A3	Bio System
14	1:75	LL002	Bio System
18	1:100	DC-10	Santa Cruz, Santa Cruz, CA
19	1:100	A53-B/A2	Santa Cruz

Table 2
Clinical and histopathologic findings of CCOTs

Case	Age (y)	Sex	Location	Type[a]
1	36	M	Maxilla (premolar)	1
2	45	M	Mandible (incisive)	1
3	15	M	Mandible (premolar)	1
4	65	M	Maxilla (premolar)	1
5	45	F	Mandible (premolar)	4
6	20	F	Mandible (canine-premolar)	1
7	36	F	Maxilla (incisive)	1

Abbreviations: F, female; M, male.
[a] According to Ledesma-Montes et al [4].

4. Discussion

Being considered a controversial lesion since its first publication by Gorlin et al [2], primarily because of its nature, the calcifying odontogenic cyst has recently been reviewed and renamed and is now known as the CCOT [1]. More recently, an international collaborative study described a useful classification for CCOT after analyzing more than 120 cases [4]. In this study, it was proposed that CCOT may be categorized into 4 variants in respect to its histomorphologic features: type 1 (simple cystic), type 2 (with associated odontomas), type 3 (with presence of ameloblastomatous proliferation), and type 4 (associated with benign tumors different from odontomas). Regardless of subtypes, CCOT characteristically behaves as a painless, slow-growing lesion and microscopically shows a stratified epithelial lining composed by 3 distinct regions: a basal layer, which is formed of cuboidal cells; a suprabasal layer, which is similar to the stellate reticulum of the enamel organ; and a compartment constituted of cells known as ghost cells [1,4]. In the present study, CCOT type 1 was more prevalent, representing 85.7% of the cases studied. One case was classified as type 4 because it was associated with an adenomatoid odontogenic tumor. A similar frequency of occurrence has been observed in the literature [4]. In the current cohort, the mandible was more affected than maxilla, and in both locations, the premolar region was the most frequently involved site. This finding is in disagreement with a previous report that observed a high incidence of the CCOT in the anterior maxillary region [4].

In this study, the expression of some Cks in the tumoral epithelium of 7 cases of CCOT was assessed. Cytokeratin expression has been frequently seen in cells of the epithelium lining, but its detection in

Table 3
The intensity of expression for each Ck in 3 distinct regions of the tumoral epithelium investigated

Cks	Basal layer	Suprabasal layer and stellate reticulum	Ghost cells
6	0 (0%)	0 (0%)	2+ (100%)
13	0 (0%)	0 (0%)	0 (0%)
14	3+ (100%)	3+ (100%)	3+ (100%)
18	0 (0%)	0 (0%)	0 (0%)
19	3+ (100%)	3+ (100%)	3+ (100%)

Positiveness rate: number of positive case/total cases.