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Research Article

Ameloblastoma Containing Ghost Cells: Correlation with the Cystic Calcifying Odontogenic Tumor

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Abstract

Objective: The aim of this work was to assess the cytokeratins (Ks) 7, 10-13, 14, 19 and pan-K in two ameloblastomas containing clusters of ghost cells to check the ghost cells immune profile, and discuss the histopathogenesis within the odontogenic tumors group.

Material and methods: The keratins were immune labelled using the streptavidin-biotin immunoperoxidase system.

Results: The samples consisted of follicular and acanthomatous-cystic ameloblastomas showing eventual clusters of ghost cells. K14 was positive in the central stellate and peripheral columnar cells, despite the negative expression in some areas. K13 labeled cellular layers that lined cystic formations and squamous cells.

Discussion: Some of squamous cells were analogous to ghost cells in different stages of development, showing K13 restricted to the cytoplasmic periphery adjacent to the plasmatic membrane. Mature ghost cells were unreactive for all antibodies studied.

Conclusions: We concluded that ameloblastomas occasionally show typical ghost cells, representing microscopic similarity to the cystic calcifying odontogenic tumor(CCOTs), and the recurrence of some odontogenic tumors, as CCOT, can be linked to the ameloblastoma behavior.

ABBREVIATIONS

Ks: Keratins; A-Gcs: Ameloblastomas Containing Ghost Cells; Ccots: Cystic Calcifying Odontogenic Tumor; DGCT: Dentinogenic Ghost Cell Tumor; COC: Calcifying Odontogenic Cysts; WHO: World Health Organization

INTRODUCTION

The microscopic description of ameloblastomas usually refers to their several histopathologic patterns such as follicular, plexiform, acanthomatous, desmoplastic, basal cell, and granular cell types. In 80's and 90's scientific manuscripts, a number of them were reported containing ghost cells and dysplastic dentin, what were renamed using several descriptive terms. Later, these cases were grouped as "dentinogenic ghost cell tumor" (DGCT) what were considered a neoplastic category of calcifying odontogenic cysts (COC). Recently the revised World Health Organization (WHO) classification of odontogenic tumors separated the DGCT and COC in two distinct diseases, renaming COC as "calcifying cystic odontogenic tumor" (CCOT) [1-6].

In fact, the CCOT/DGCT epithelial cells resembles that of ameloblastoma, however, commonly it is referred as ameloblastomas to us proliferation because it not reveal the typical histologic alterations that characterize the true ameloblastoma epithelium: palisading basal layer cells with polarization ("piano-key" arrangement), hyperchromatism of basal cell nuclei and vacuolization [7,8]. Some of these tumors are locally aggressive, leading some authors to believe that they represent a form of ameloblastoma, or have malignant nature with potential for metastasis. [2,5,9,10]. Furthermore hybrid tumors composed by CCOT and ameloblastoma have been reported [11,12].

Crivelini et al. [13-15], assessed the cytokeratins (Ks) immunopattern in several odontogenic tumors. Ameloblastomas expressed K14 in most tumoral cells, except in part of the central stellate cells (follicular pattern) and peripheral columnar cells with vacuolated cytoplasm. Cytokeratins 13 and 19 labelled metaplastic squamous cells, some inner stellate cells and flattened cells lining cystic structures, indicating squamous differentiation. This immune profile was distinct of the human dental stellate reticulum, especially considering the follicular pattern. Regarding the CCOTs, the ghost cells did not express any cytokeratin, except some adjacent elements around them, showing K10-13 restricted to the cytoplasmic periphery adjacent to the plasmatic membrane. These cells were considered "transitory elements towards ghost cells", originated probably from the odontogenic epithelium with squamous maturation [14,15], or the "ghost cells in different stages of development" described by the WHO Classification of Tumors [1].

Thus, the present paper assessed cytokeratins in two obvious ameloblastomas containing ghost cells(A-GCs) to verify the ghost cells immunoprofile, and discussed the histopathogenesis within the odontogenic tumors group, with special attention to the CCOTs and DGCTs that have some A-GC's histopathological similarity.

MATERIALS AND METHODS

The materials consisted of 02 acanthomatous ameloblastomas retrieved from the files of the Department of Pathology and Clinical Propaedeutic of the São Paulo State University, School of Dentistry, Araçatuba-SP, Brazil. Paraffin sections of formalin fixed tissue were used for both immunohistochemical and H.E. histological evaluation.

For the immunohistochemical technique, 3 µm tissue sections were deparaffinized and treated with citric acid (10 mM, pH 6.0), three times for 5 minutes, at high power 700W microwave oven. Two incubations (05 min. each) with 0.6% hydrogen peroxide/methanol blocked endogenous peroxidase activity, followed by washing with Tris pH 7.4. Primary antibodies were then incubated. Their sources, concentrations and times of incubation are listed in Table (1). After this procedure, sections were thoroughly washed with Tris pH 7.4 and incubated for 20 minutes using the Universal Large Volume DAKO LSAB_+ Kit (Dako) Corporation, Carpinteria, CA, USA), a biotinylated link antibody that labels primary antibodies produced in rabbit, mouse or goat. The color reaction was developed using a freshly prepared solution of 3.3-diaminobenzidine tetra hydrochloride for 3 min, followed by a solution of 0.5% copper sulfate for 5 min. Finally Mayer's hematoxylin was used for counterstaining.

Data were scored by observing the presence of a brown end-product at the site of the target antigen under a light microscope. Positive controls for each K and vimentin were used as indicated by the supplier, labeling in normal tissues the following: K7, K8, K19 the glandular epithelia, K10/13 the suprabasal cells of epidermis, K14 the basal cells of epidermis. Negative controls were performed by Tris replacing the primary antibody.

RESULTS

The predominant histopathologic patterns of these ameloblastomas were acanthomatous and follicular, showing palisading basal layer cells with polarization, hyperchromatism nuclei and vacuolization. Cysts formations with several sizes commonly were present within the epithelial islands of one case. The "lining" cystic cells always showed squamous metaplasia arranged in few layers, some of them with more abundant eosinophilic cytoplasm or vacuolar degeneration. Among them, some microcystic structures apparently arose from the fusion of vacuolated cells, containing intracytoplasmic eosinophilic or amphophilic homogeneous materials. Cells similar to ghost cells were found in rare small foci, especially close to the lining cysts formations. Their morphological features included loss of nuclei, eosinophilic cytoplasm and preservation of the cell outline (Figures 1A and 1B). Tumoral stroma consisted of mature fibrous connective tissue.

Table 1: Monoclonal antibodies used.

Antibodies	Clone	Concentration	Incubation time (minutes)	Temperature
Multi-Ka	AE1-AE3	1:50	60	Room
K7b	OV-TL 12/30	1:60	60	Room
K10/13b	DE-K13	1:80	120	Room
K14b	LL002	1:50	30	Room
K18b	DC10	1:600	60	Room

Abbreviation: K: ketarin.

The immunohistochemical labelling was consistent to K7, K13, K14, AE1-AE3, but not to K18. A number of squamous cells were able to express K7 and K13, unless those with abundant cytoplasm. The K13 labelled especially the layers bordering cyst spaces, including those with vacuolated cells. However eventual sparse squamous cells strongly expressed K13 in the cytoplasmic periphery whereas the portion near the nucleus was weakly positive (Figure 1C). The central stellate cells of follicular pattern and the peripheral columnar cells were K14 positive, despite a negative expression had occurred in some of them (Figures 1D and 1E). Overall the K7 and K13-positive cells were un reactive to the K14. None cytokeratin could be identified in the "ghost" similar cells (Figure 1F).

DISCUSSION

According to the recent literature, ghost cells rarely appear in the ameloblastoma histopathology. Rather tumors with these peculiar cells are diagnosed as DGCTs or CCOTs. Our reported case consisted of acanthomatous and follicular ameloblastoma, in which tumoral epithelial islands showed hyperchromatism and basal cell with palisading, polarization and vacuolization of nuclei, the typical ameloblastoma features [7,8]. However, some small clusters of ghost cells were found in eventual foci. These cells could be safely identified due to their peculiar morphology and typical negative keratin immunoexpression in our results, as reported by Crivelini et al [14,15]. Furthermore eventual cells showed K10/13 expression restricted to the cytoplasmic periphery, adjacent to the plasmatic membrane, similar to ghost cells transitory elements [15]. We preferred the ameloblastoma diagnostic instead of CCOT, because the Vickers; Gorlin [7] delineative histopathology for ameloblastomas was present,

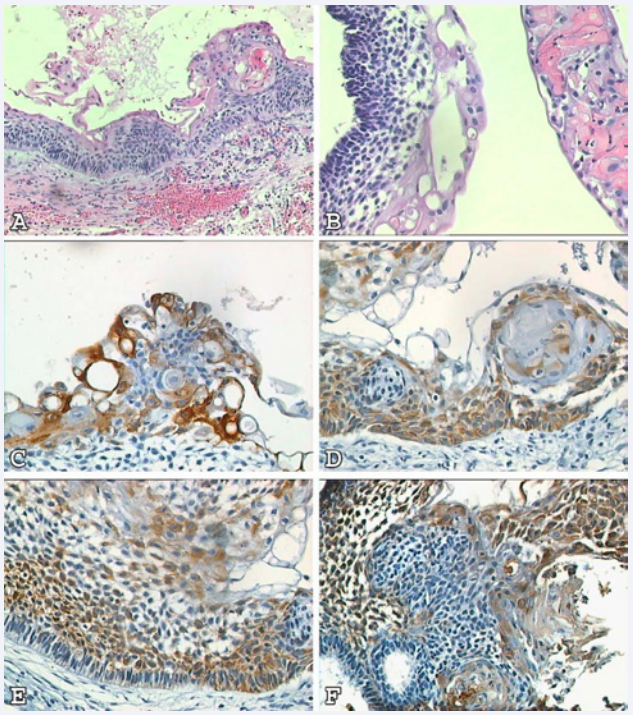

Figure 1 (A, B) - Acanthomatous-cystic ameloblastoma showing clusters of ghost cells inner the tumoral tissue (arrows). HE X200-A; HE X400-B. (C) - Some K13-labelled cells with weak positivity near the nucleus (arrow).Streptavidin-biotin X400. (D) - K14 expression in several cells except ghost cells.Streptavidin-biotin X400.(E) - Variable expression of K14 in the typical ameloblastoma epithelium.Streptavidin-biotin X400. (F) - Ghost cells negative to pan-K (arrow). Streptavidin-biotin X400.

andthe K14 and K10/13 distribution followed the ameloblastoma immunopattern [13]. CCOT's K14 and K10/13 expression are similar to the squamous stratified epithelium, with K14 in the basal layer and K10/13 in the upper cells [14].

In this study the ameloblastoma showed predominance of acanthomatous and cystic pattern, with layers of squamous cells lining the cystic structures. Investigators reported that the squamous differentiated ameloblastoma cells immunoexpress proteins closed to ketarinization process, i.e. K10, K11, K13, K16, KL1 and involucrin. [13,16,17,18]. Ultrastructural studies described desmosomes and to no filaments similar to basal and suprabasal keratinocytes, what was in favour of metaplastic transformation of stellate and columnar cells, a possible regressive phenomenon towards the oral surface epithelium phenotype [19,20]. Our results showed some squamous cells strongly eosinophilic resembling the skin stratum corneum. Close to them were clusters of ghost cells, corroborating to the opinion that ghost cells arise from odontogenic epithelium with squamous maturation [15]. In addition some of cystic squamous lining cells showed more abundant eosinophilic cytoplasm, resembling those above-mentioned squamous transitory elements towards ghost cells, or the ghost cells in different stages of development.

CONCLUSION

Therefore we concluded that ameloblastomas occasionally show typical ghost cells, evidencing some microscopic similarity to the CCOTs, that also show ameloblastomatous epithelium. Recurrence of some odontogenic tumors, as CCOTs, can be linked to the ameloblastoma behavior, what is corroborated by reported cases of hybrid tumors involving closely both diseases. [11,12].

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