Case Report

A CASE OF CALCIFYING ODONTOGENIC CYST WITH NUMEROUS CALCIFICATIONS: IMMUNOHISTOCHEMICAL ANALYSIS

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Abstract

The purpose of this study was to investigate a case of calcifying odontogenic cyst (COC) in which numerous calcifications were observed not only in the lining epithelium, but also in the cyst wall, using cytokeratins 13 (CK13), 19 (CK19), and core binding factor a-1 (cbfa-1) as primary antibodies. Cells of Malassez's epithelial rest were stained as controls.

Cells of the epithelial nests in the cyst wall were reactive for CK13, but their CK19 staining was similar to that observed in the lining epithelial cells. Calcifying nodules were reactive only for CK13. Cells of Malassez's epithelial rest were reactive for CK19 but not for CK13. Cbfa-1 positive reactivity was observed only in nuclei of spindle cells in the periodontal ligament. CK13 was positive superficial to the prickle cells. CK19 was positive in the basal cells of the oral mucosa. In the lining epithelium of the cyst, the expressions of CK13 and CK19 were similar to their immunoreactions in the oral mucosa. These results suggest that the odontogenic epithelium differentiated into squamous epithelial cells, which began as ghost cells in the COC, and that this process depended on the dystrophic calcification of differentiated odontogenic epithelial cells, not of osteogenic cells.

Key words: Calcifying odontogenic cyst—Ghost cell—Calcification—
Immunohistochemistry

INTRODUCTION

Calcifying odontogenic cyst (COC) was first described as a distinct entity by Gorlin and associates in 1962, and COC are now known as rare developmental odontogenic lesions⁹⁾.

The World Health Organization (WHO) has classified COC as a neoplasm rather than as an epithelial cyst, but they state that most COCs are non-neoplastic¹¹⁾.

These lesions are characterized by a well-delineated cystic proliferation of the onto-

genic epithelium with ghost cells, which are degenerated epithelial cells. They are usually calcified. It is known that this calcification of odontogenic epithelium in COC is dystrophic. However, odontoid or bone-like hard tissue has sometimes been reported to be formed in the cyst wall. In these cases, the hard tissues in cyst wall were produced by mesenchymal cells.

In the present study, we investigated a COC containing numerous calcifications and odontogenic epithelial islands in the fibrous capsule by using immunohistochemical stains to detect cytokeratins (CK) 13 and 19 and core binding factor α -1 (cbfa-1).

MATERIALS AND METHODS

A 26-year-old woman was admitted to a private dental office on November 3, 2001, complaining of a painful swelling in her right mandibular molar region. A general examination revealed a well-nourished female who did not appear ill or in any distress.

A dental X-ray revealed a cystic, completely radiolucent zone about the size of a thumb caput in an area adjacent to the distal root of the impacted third molar of her right mandible. It was clinically diagnosed as a paradental cyst. On November 5, 2001, an extraction of the impacted tooth with the cystic lesion was performed intraorally. The cystic lesion was in contact with the distal root of the impacted third molar. Surgical specimens were fixed in 10% formalin, embedded in paraffin, and cut in serial sections using routine methods. For light microscopic observations, paraffin sections were stained with hematoxylin and eosin (HE). For immunohistochemical staining, the paraffin sections were deparaffinized with xylol, and the slides were microwaved for 5 minutes at 60°C for antigen retrieval. Endogenous peroxidase activity was then eliminated by treatment with 3% hydrogen peroxide (H_2O_2) in sodium azide for 4 minutes at 37°C. Non-specific binding was blocked by treatment with 10% normal goat serum (Meneki Seibutu Kenkyujo,

Gunma, Japan) for 10 minutes at 37°C. Anti-CK13 (PROGEN BIOTECHNIK GMBH, Heidelberg, Germany, diluted at 1:50), anti-CK19 (PROGEN BIOTECHNIK GMBH, Heidelberg, Germany, diluted at 1:50), and anti-core binding factor α -1 (cbfa-1; supplied by Dr. Sasaguri, Department of Orthodontics, Kanagawa Dental College, Japan, diluted at 1:1,000) were used as primary antibodies. The sections were incubated in those antibodies at 37°C for 32 minutes. The immunoperoxidase (avidin biotin peroxidase complex) technique was performed using an automatic immunostaining device (Ventana NX System, Tucson, Arizona, U.S.A./Tokyo, Japan) and Ventana kits. Finally Gill's hematoxylin was used for counterstaining (37°C, 2 minutes).

The lining epithelium of the cyst, odontogenic epithelial islands in the fibrous capsule of the cyst wall, ghost cells and multiform calcifications were observed. As a control, Malassez's epithelial rests, which are located in the periodontal ligament of the impacted tooth, were also observed.

RESULTS

Histopathological findings

The cyst was lined with a stratified squamous epithelium of varying thickness, which included many ghost cells and calcifications. The cyst wall consisted of a cell-rich fibrous connective tissue in which many odontogenic epithelial nests and calcifications were observed. The calcifications were more or less granular and presented as basophilic in the peripheral area but as slightly eosinophilic in the central area.

Immunohistochemical findings

The results of the immunohistochemical staining are shown in Table 1. Strong CK13 positive reactions were observed in the lining epithelium except in the basal cells; in other words, CK13 was positive in the layers superficial to the first and second layers and in the ghost cells. In the adjoining basal layer, the positive reactions were attenuated (Fig. 1). In

Table 1 Results of immunohistochemical staining

Area	Lining epithelium		Odontogenic epithelial island		Periodontal ligament		
	Calcification	Non calcification	Calcification	Non calcification	Cementicle	Malassez's epithelial rest	Fibroblast
CK13	‡	+	‡	±	‡	—	—
CK19	—	+	—	±	—	+	—
cbfa-1	—	—	—	—	—	—	+

‡: strongly positive; +: positive; ±: weakly positive; —: negative

In calcification, CK19 is negative while CK13 is strongly positive. Only CK19 is positive in the Malassez's epithelial rest. Only cbfa-1 is positive in the fibroblasts of the periodontal ligament.

the restiformal growth areas and subjacent odontogenic islands, weak reactions could be seen in the cytoplasm. In the calcification areas, CK13 positive reactions were seen in the comparatively large granules as nuclei, in micro-granules, and in the odontogenic epithelium in the fibrous capsule. Slight immunoreactivity for CK13 was observed in cytoplasm (Fig. 7). However, CK13 was negative in the Malassez's epithelial rests in the periodontal ligament used as a control (Fig. 4).

CK19 positive reactions were observed in cells of the entire layer of the lining epithelium (Fig. 2) and in restiformal growth areas of the cyst. Positive reactions for CK19 were observed both in the cytoplasm of cells in the odontogenic epithelial islands (Fig. 8, and in Malassez's epithelial rest in the periodontal ligament (Fig. 5). However, the ghost cells in the lining epithelium were negative for CK19 (Fig. 2).

Cbfa-1 positive reactions were uniformly observed in the nuclei of spindle cells of the periodontal ligament (Fig. 6). However, there was no reactivity in the ghost cells in the lining epithelium (Fig. 3), the odontogenic epithelial island, Malassez epithelial rest, or the lining epithelium near the cells of the calcifications.

DISCUSSION

COC is characterized by a well-delineated cystic proliferation of the odontogenic epithelium with ghost cells, which may calcify,

and by a fibrous connective tissue capsule. Sometimes, the dentinoid may contact the lining epithelium. Occasionally, COC is associated with dental hard tissue formation and resembles complex or compound odontoma. However, there is much discussion about the diverse histology of COC. In 1972, Fejerskov and Krogh used the term "*calcifying ghost cell odontogenic tumor*"⁶⁾. Sauk reported that the osteoid or dentinoid in the COC is formed by true derivation development from the epithelium, and that COC should be considered a tumor-like disease rather than a cyst¹⁸⁾. In 1981, Praetorius *et al.* classified COC into cystic and neoplastic variants¹⁶⁾. In 1986, Ellis and Shmookler used the term "*epithelial odontogenic ghost cell tumor*" for the neoplastic variant of COC⁵⁾. More recently, the WHO classified COC as an odontogenic tumor¹¹⁾.

The mechanisms of calcification are classified into three calcareous categories: 1. degeneration, 2. hypercalcemia, and 3. products of cellularity forming calcifications^{2,12,19)}. In the conventional calcification of calcareous degeneration, calcareous depositions are observed with abnormal calcifications representing dystrophic calcareous degeneration, usually even without elevation of serum calcium in necrobiosis organization or a foreign body in the organization. In contrast, the deposition of calcium salts in normal tissue is known as metastatic calcification. It almost always reflects some derangement in calcium metabolism and leads to hypercalcemia. Calcium deposits made by cells are produced by

mesenchymal cells such as osteoblasts, odontoblasts; or ameloblasts. Cbfa-1 is a genetic transcription factor expressed in osteoblasts, and it is related to the expression of osteocalcin and osteopontin, which are known to be involved in the differentiation of osteoblasts with bone formation^{3,17}. Recently, the expression of cbfa-1 has been confirmed in the tooth germ, but the mechanism involved remains unclear^{4,7,10,13,20}. In this study, positive reactions for cbfa-1 were observed only in mesenchymal cells in the periodontal ligament, but not in cells of the lining epithelium or in odontogenic epithelial islands. Taken together, this suggests that the calcification in this COC was due to calcareous degeneration. In COC, Lukinmaa *et al.* reported that low-molecular-weight cytokeratin 19 stained the epithelial intrusions strongly¹⁴. Elsewhere in the covering epithelium, the staining reaction, where present, was barely detectable. Ghost cells failed to stain, and the connective tissue was also negative. In osteofibrous dysplasia, the epithelial-like component, CK19, was reported to be strongly expressed, but the antibody to CK13 showed a negative response^{1,15}. On the other hand, Gordeff and Clergeau-Guerithault reported that CK did not stain ghost cells in COC⁸.

In this present report, the odontogenic epithelial cells were positive for CK1, and CK19 and had the same appearance as the lining epithelial cells and odontogenic epithelial cells in the cyst wall. No expression of CK13 was seen in the control cells of Malassez's epithelial rest. Generally, odontogenic epithelial cells are positive for cytokeratins of comparatively high molecular weights, which are related to either the framework of differentiation or the maturation of cells.

In conclusion, the mechanism of calcification of the odontogenic epithelium, which begins as the formation of ghost cells in the COC, depends on the dystrophic calcification of less differentiated odontogenic epithelial cells; these are positive for CK19 expression. It is clear that the fibroblasts, which existed in lining epithelial circumference of the COC, were not the bone forming cells.

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Legend of figures

- Fig. 1 Microphotograph of cytokeratin 13 staining
Strong CK13 positive reactions were observed in the lining epithelium except for the basal cells and the ghost cell (arrow) ($\times 600$).
- Fig. 2 Microphotograph of cytokeratin 19 staining
In the lining epithelium, positive reactions were observed in the cytoplasm from the upper layer to the basal side, but the staining is negative in the ghost cell (arrow) ($\times 600$).
- Fig. 3 Microphotograph of cbfa-1 staining
The ghost cell in the lining epithelium is negative for cbfa-1 (arrow) ($\times 1,200$).
- Fig. 4 Microphotograph of CK13 staining
Malassez's epithelial rest is negative for CK13 (arrow) ($\times 600$).
- Fig. 5 Microphotograph of CK19 staining
Malassez's epithelial rest is positive for CK19 (arrow) ($\times 600$).
- Fig. 6 Microphotograph of cbfa-1 staining
Cbfa-1 positive reactions were uniformly observed in nuclei of spindle cells of the periodontal ligament (arrow) ($\times 600$).
- Fig. 7 Microphotograph of CK13 staining
In areas of calcification, CK13 positive reactions were observed, as uniformly deep staining in the cytoplasm, comparatively big granules as nuclei, minute granules, and mixed type (arrowhead). Weak positive reactions were also seen in the cytoplasm of odontogenic epithelial islands (arrow) ($\times 600$).
- Fig. 8 Microphotograph of CK19 staining
Weak immunoreactivity for CK19 is found in the cytoplasm of odontogenic epithelial island cells (arrow), but the staining is negative in calcium depositions (arrowhead) ($\times 600$).