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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 lesions. 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-neoplastic. These lesions are characterized by a well-delineated cystic proliferation of the odonto-
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 dystro-
phic. 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 a-1 (cbfa-1).

MATERIALS AND METHODS

A 26-year-old woman was admitted to a pri-
ivate dental office on November 3, 2001, com-
plaining of a painful swelling in her right
mandibular molar region. A general exami-
nation 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 man-
dible. It was clinically diagnosed as a para-
dental cyst. On November 5, 2001, an extrac-
tion 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 rou-
tine methods. For light microscopic obser-
vations, paraffin sections were stained with
hematoxylin and eosin (HE). For immuno-
histochemical 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 (H₂O₂) in sodium
azide for 4 minutes at 37°C. Non-specific bind-
ing was blocked by treatment with 10% nor-
mal 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 biding factor a-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) tech-
nique 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, odonto-
genic 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 squa-
mous 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 odonto-
genic 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 superfi-
cial 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

<table>
<thead>
<tr>
<th>Area</th>
<th>Lining epithelium</th>
<th>Odontogenic epithelial island</th>
<th>Periodontal ligament</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calcification</td>
<td>Non calcification</td>
<td>Calcification</td>
</tr>
<tr>
<td>CK13</td>
<td>#</td>
<td>+</td>
<td>#</td>
</tr>
<tr>
<td>CK19</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>cbfa-1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

+: 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 restiform 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”. 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 cyst. In 1981, Praetorius et al. classified COC into cystic and neoplastic variants. In 1986, Ellis and Shmookler used the term “epithelial odontogenic ghost cell tumor” for the neoplastic variant of COC. More recently, the WHO classified COC as an odontogenic tumor.

The mechanisms of calcification are classified into three calcareous categories: 1. degeneration, 2. hypercalcemia, and 3. products of cellularity forming calcifications. 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. Recently, the expression of cbfa-1 has been confirmed in the tooth germ, but the mechanism involved remains unclear. 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 strongly. 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. On the other hand, Gordeff and Clergeau-Guerithault reported that CK did not stain ghost cells in COC.

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.

REFERENCES


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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) (×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) (×600).

Fig. 3 Microphotograph of cbfa-l staining
The ghost cell in the lining epithelium is negative for cbfa-l (arrow) (×1,200).

Fig. 4 Microphotograph of CK13 staining
Malassez’s epithelial rest is negative for CK13 (arrow) (×600).

Fig. 5 Microphotograph of CK19 staining
Malassez’s epithelial rest is positive for CK19 (arrow) (×600).

Fig. 6 Microphotograph of cbfa-l staining
Cbfa-l positive reactions were uniformly observed in nuclei of spindle cells of the periodontal ligament (arrow) (×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) (×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) (×600).