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Calcifying odontogenic cyst immunohistochemical detection of keratin and involucrin in cyst wall

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Summary. Calcifying odontogenic cysts (COC) were immunohistochemically described using different keratin proteins and involucrin as well as histopathology. The cystic lining epithelium was composed of calcifying, keratinizing, squamous, and columnar epithelial cells, and included calcified masses of irregular shape and various size as well as ghost cells. Calcifying epithelium gave negative or only trace staining for keratins detected with low molecular keratin (PKK1), but were regularly positive with high molecular keratin (KL1) and polyclonal antibody for keratin (TK). They were occasionally positive for involucrin. The cells located in the periphery of the calcified masses had a particular abundance of high molecular weight and total keratins (KL1 and TK). Calcified bodies and ghost cells were devoid of any immunoreactivity. Squamous epithelium was relatively similar to that of normal squamous cell epithelium in the oral mucosa. It were most commonly found in columnar cystic epithelial cells which displayed intense staining with all immunoreagents. It is postulated that such epithelial cells may have a strong potentiality to transform into ghost cells or to undergo metaplasia. They may develop altered synthesis of homogenous acellular materials and finally become transformed into calcifying epithelium containing dystrophic calcified masses.

Key words: Calcifying odontogenic cyst – Keratin – Involucrin – Immunoperoxidase technique

Introduction

Calcifying odontogenic cyst (COC), first reported by Gorlin et al. (1962; 1964) is composed of odontogenic epithelium containing "ghost cells" which is the most characteristic phenomenon of keratinization in epithelial cells derived from odontogenic epithelium (Gorlin et al. 1962, 1964; Gold 1963; Abrams and Howell 1968; Komiya et al. 1969; Fejerskov and Krogh 1972; Eda et al. 1971, 1974; Chen and Miller 1975; Regezi et al. 1975; Sapp and Gardner 1977; Farman et al. 1978; Vuletin et al. 1978; Donath and Kleinhans et al. 1979; Anneroth and Hansen 1982). Calcification occurs in ghost cells or independently in dystrophic materials. It is also seen to those ghost cells occasionally found in ameloblastoma, calcifying epithelial odontogenic tumour (CEOT), and odontoma (Pflüger 1956; Forest and Mercier 1967; Fejerskov and Krogh 1972; Levy 1973; Osborne et al. 1974; Sedano 1975; Sapp 1977; Farman et al. 1978; Vuletin et al. 1978; Praetorius et al. 1981; Fukushima 1983; Kerebel and Kerebel 1985). The clinical reviews and histological analysis in COC have been well described (Freedman et al. 1975; Lello and Makak 1986), and histologic features of COC have been described in detail, mainly in terms of their keratinizing or calcifying epithelium. While the COC which is a non-neoplastic cystic lesion is related to the category of benign odontogenic tumours by the classification of the World Health Organization (Pindborg et al. 1971). It has recently been pointed out and reevaluated that COC has two cystic entities, and a neoplasm termed "Dentinogenic ghost cell tumour" (Praetorius et al. 1981; Ellis et al. 1986; Tajima et al. 1986).

No description of the immunohistochemical properties or enzyme histochemistry of the particular epithelial cells of COC has been made except for SH protein histochemistry in calcified material (Eda et al. 1971, 1974). The purpose of the present study was to examine the immunohistochemical expression of keratin and involucrin, both of which

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are markers of keratinization. Their concentration indicates the degree of keratinization in cystic epithelial cells showing a wide variation in their histology. The observed patterns were compared with those of squamous cell epithelium and lesions in oral mucosa and other odontogenic epithelial lesions (Mori et al. 1985; 1988; Nakai and Mori 1986; Sumitomo et al. 1986).

Materials and methods

A 14-year-old male patient presented with radiopaque mass and impacted upper left canine by radiogram. The crown of an impacted canine was attached to the radiopaque focus. Under general anesthesia, the radiopaque mass and impacted canine was removed, providing material for examination (Case 1).

A 25-year-old female complained of pain in the lower left wisdom tooth area. Clinical examination showed that wisdom tooth was only partially developed, with half of its crown appearing above the gingiva which showed slight inflammation. By radiography, the areas normally occupied by wisdom teeth, displayed cystic radiolucency with a clearly defined margin containing a small amount of fine radiopaque materials (Case 2 – providing material from two cysts).

An 11-year-old female patient presented with a cystic lesion of lower right incisor. The X-ray picture revealed an odontoma-like radiopaque mass with a cystic lesion and also an impacted lower right canine. The mesial side of the impacted tooth was attached to the radiopaque mass. Under general anaesthesia, the cystic lesion including odontoma-like mass was removed as well as the impacted lower right canine. The pathological diagnosis was keratinizing and calcifying odontogenic cyst and a complex odontoma (Case 3).

Materials obtained from surgery were fixed in 10% formalin for 24 h. Serial 4 μm paraffin sections were made for histological examination by H & E, PAS, and immunohistochemical detection of keratins and involucrin. The details of methods have been shown in Table 1.

Results

A section of case 1 showed numerous irregular calcified masses and ghost cells in the cystic lining epitheium (Fig. 1A, 2A). The cystic wall was composed of 2 or 3 layers of keratinized squamous epithelium (Fig. 5A) and simple epithelium consisting of columnar and high columnar cells (Fig. 6A, E). The cystic lining in case 2 showed squamous cell epithelium with great atrophy and containing ghost cells. It was mainly composed of the intensely keratinized squamous epithelium (Fig. 3A, C) of two types: one strongly stained with haematoxylin and another refractory. The pathological features in bilateral wisdom teeth of the other cyst were almost the same. Some of the ghost cells revealed calcification. Inflammatory responses were dominant. Case 3 material revealed keratinizing and calcifying epithelium containing various

Table 1. Immunohistochemical staining method (Indirect Method)

1 Deparaffinization and protease digestion (PKK1 only): 0.01% trypsin/phosphate buffer saline (pH 7.6) solution, 37° C, 15 min

2 Inactivation of endogenous peroxidase activity: 0.3% H_2O_2 containing/methanol solution 30 min

3 Back ground blocking TK: normal goat serum, 1/20, 30 min
KL1 & PKK1: normal rabbit serum, 1/20, 30 min
Involucrin: normal goat serum, 1/20, 30 min

4 1st layer and **5** 2nd layer

Antibodies (1st layer)	Immunogen	Source	2nd layer	Source
Polyclonal anti-human keratin antiserum (TK: 41–65 kDa) 1/40, 1 h	stratum corneum of the sole of human foot	Dakopatts Denmark Copenhagen	HRP-labelled anti-rabbit IgG-Goat IgG F(ab)′ 1/20 30 h	Jimro Japan Takasaki
Monoclonal KL1 keratin (55–57 kDa) 1/50, 1 h	human keratinized squamous epithelium	Immunotech France Marseilles	HRP-labelled anti-mouse IgG-Rabbit IgG 1/20 30 h	Dakopatts Denmark Copenhagen
Monoclonal PKK1 keratin (44, 46, 52, 54 kDa) 1/80, 1 h	pig kidney epithelia cell line	Labsystem Finland Helsinki	HRP-labelled anti-mouse IgG-rabbit IgG 1/20 30 h	Dakopatts Denmark Copenhagen
Involucrin Immuno kit 1 h	the cross-linked envelope synthesized by mature cells of human stratified squamous epithelium	Biomedical Technology Cambridge USA	HRP-labelled anti-rabbit IgG-Goat IgG 30 h	Biomedical Technology Cambridge USA

6 Visualization of peroxidase activity: 0.02% diaminobenzidine hydrochloride (DAB)/0.05 M Tris buffer solution (pH 7.6) containing 0.005% H_2O_2

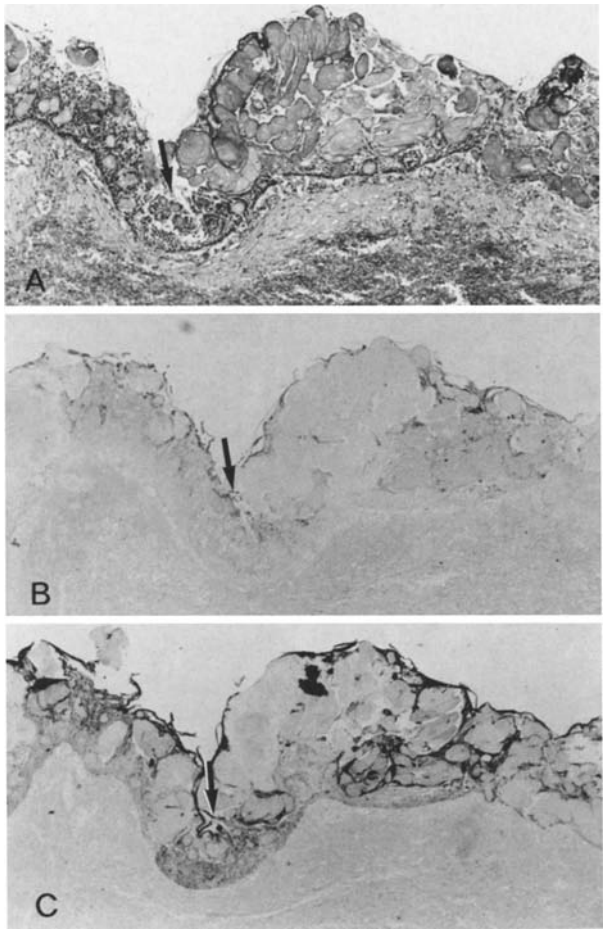

Fig. 1 A–C. Calcifying cystic epithelium in COC (Case 1). × 32. **A** H & E staining. Typical calcifying odontogenic cyst (COC). There are large numbers of ghost cells, irregular shaped calcified masses, and basal epithelial cells with dark staining. **B** PKK1 staining. The epithelial cells within cyst wall are almost negative but show some traces of staining. **c** KL1 staining. Reaction product is mainly shown to epithelial components in peripheral bands around calcified masses and ghost cells. Basal located cells are slight

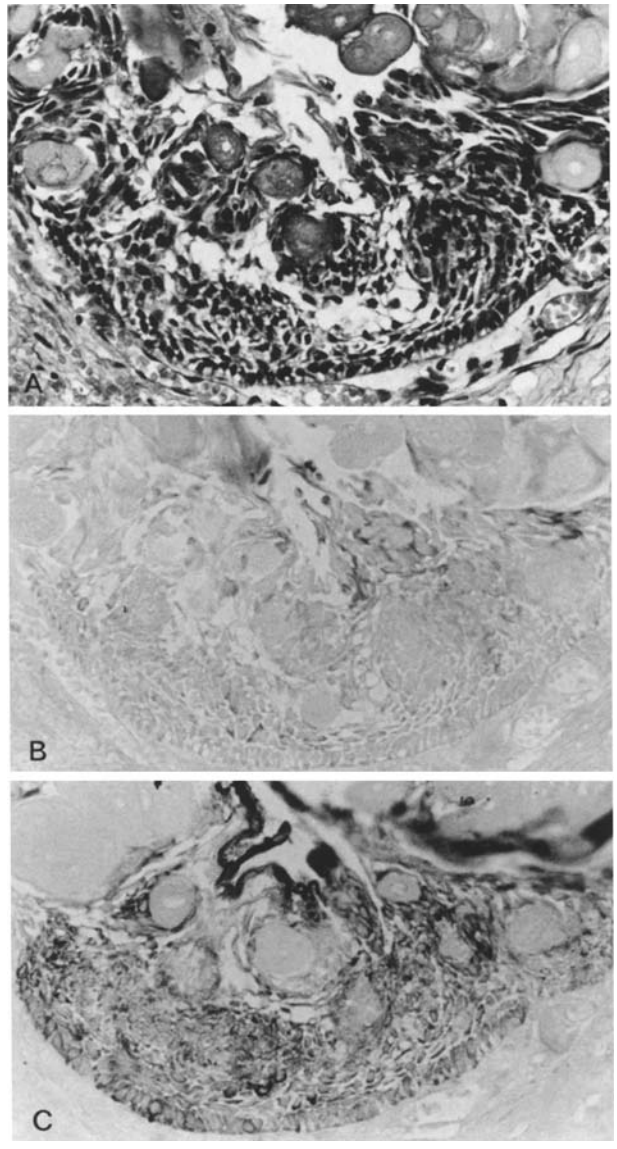

Fig. 2 A–C. Calcifying cystic epithelium. Higher magnification of Fig. 1 (*arrow*). × 140. **A** H & E staining. The lesion is composed of basal cells (cuboidal shape), ghost cells, and spindle-shaped (stellete reticum-like) cells. **B** PKK1 staining. There is no staining except a few upper epithelial cells with a trace. **C** KL1 staining. Calcified masses and ghost cells are negative, whereas basal and upper epithelial components are slight-to-moderate. Thin epithelial cells in upper peripheral area are markedly positive in reaction

amounts of dystrophic calcified materials and displaying severe inflammatory reactions (Fig. 4A).

In general, there were few epithelial cells and large amounts of irregularly shaped calcified masses. Epithelial cells were usually located at the basal aspects of the cystic wall (Fig. 1A). Basal epithelial cells were cuboidal in shape, which cells in upper strata were spindle-shape (Fig. 2A). In those epithelial structures, there was no reactivity with PKK1 monoclonal keratin antibody, whereas TK and KL1 keratin antibodies did bind (Figs. 1B, C, 2B, C). Basal and upper epithelial cells indicated similar staining intensities with TK and KL1, which were at a comparatively slight or moderate level. Calcified bodies were usually surrounded by thin epithelial cells which showing dark staining band or eosinophilic in nature (Fig. 4A). A dark staining band surrounding the calcified bodies was characterized by strong staining for KL1- and TK-detectable keratins (Fig. 4B, C). No staining with PKK1 was found in those materials and cells.

Involucrin was usually absent in this epithelium and in calcified bodies of COC but some limited

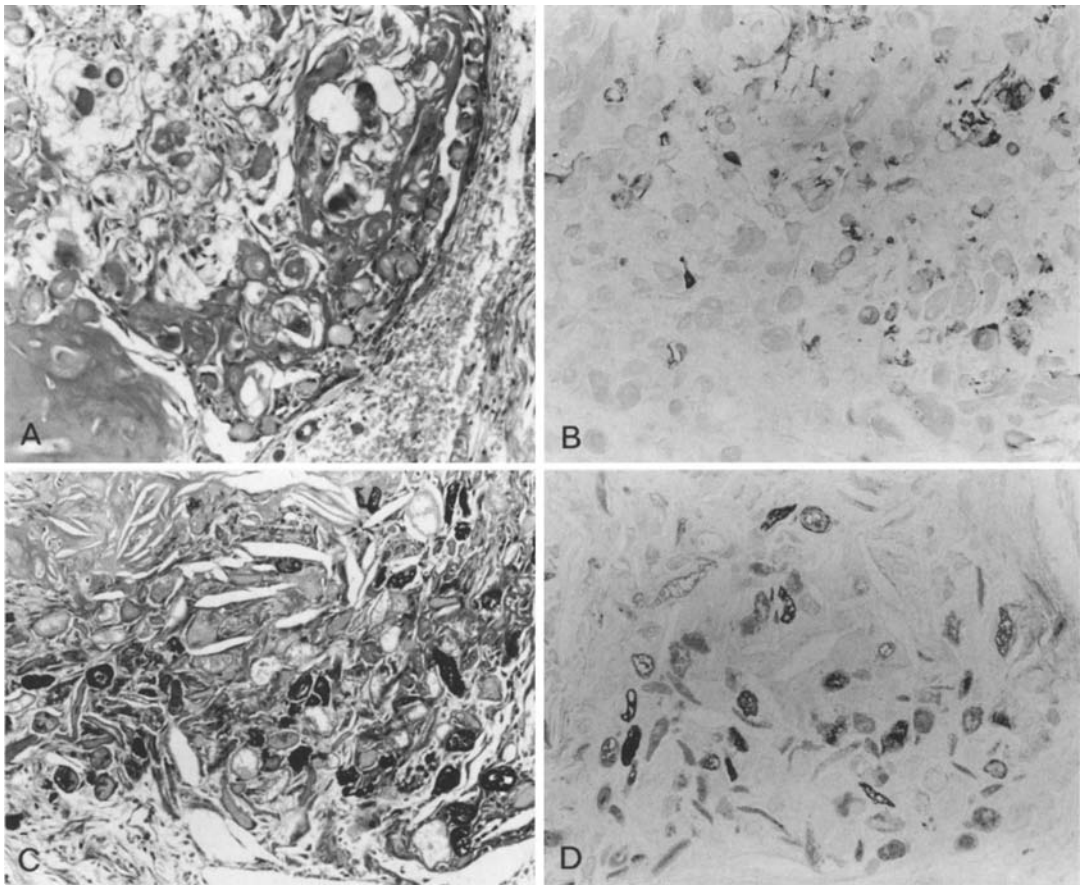

Fig. 3A–D. Keratinizing epithelium in COC (Case 2). ×80. **A, C** H & E staining. Many ghost cells and dystrophic calcification intermingle with keratinized cell residues or bodies. The lesions show chronic inflammatory response. **B, D.** KL1 staining. Keratinized bodies show an intense positive reaction

numbers of cells or bodies present in calcified foci showed conspicuous staining (Fig. 4D).

In the case 2 (Fig. 3A, C), the epithelial cells gave a negative response to PKK1, KL1, and TK, while keratinized bodies sometimes showed a strongly positive reaction with KL1 and TK (Fig. 3B, D). Involucrin staining was obtained in the limited epithelial cells.

In case 1, the epithelium was composed of thin keratinized cell layer and two or three layers of epithelial cells (Fig. 5A). No basal cell proliferation or epithelial hyperplasia were noted. The epithelium showed a reaction negative to PKK1, but positive staining with KL1, TK, and involucrin in superficial keratinized cells (Fig. 5B).

The sections from case 1 showed a simple columnar epithelium. However, higher magnification of the epithelium revealed that it was composed of small round or cuboidal cells at the basal aspect and tall columnar or spindle cells in the upper layer (Fig. 6A, E). This epithelium contained an abundance of keratins and involucrin, as evidence by the intense staining which all the immunoreagents (Fig. 6B–D, F–H).

These results are summarized in Table 2.

Discussion

Histologically, COC lesions are composed of various types of cystic lining epithelia and their neoplastic variants. Previous investigations have focussed on the calcifying components including ghost cells and have not shown specific interest in other types of lining epithelium. In the present study, the following three immunohistochemical features of calcifying cystic epithelial cells are noteworthy: (1) all the cells were negative with to PKK1 keratin antibodies, which detected lower molecular weight keratin and usually bind only to basal cells of squamous epithelium; (2) peripheral cells or peripheral bands (which stain strongly with haematoxlin) of calcified bodies showed striking

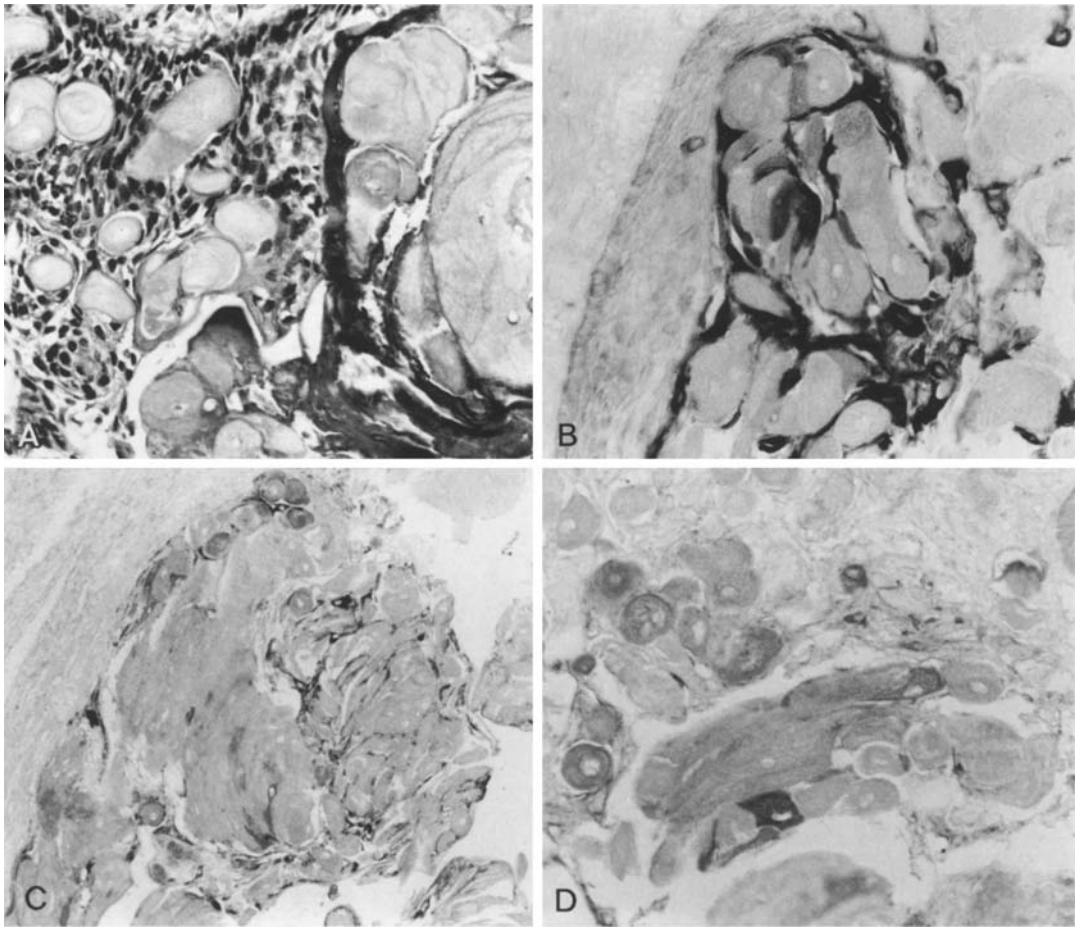

Fig. 4A–D. Calcifying cystic epithelium with complex odontoma (Case 4). Fig. 4A. H & E staining. Thin epithelium with dark staining is adjacent to calcified masses. ×160. **B** KL1 staining. Same area of Fig. 3A. Thin epithelium among calcified body shows intensely strong reaction, whereas other cystic epithelial staining is trace-to-slight. ×160. **C** TK staining. Marked staining is observed in thin epithelium included in the massive calcified foci. ×80. **D** Involucrin staining. A few epithelial cells are positive to involucrin. ×160

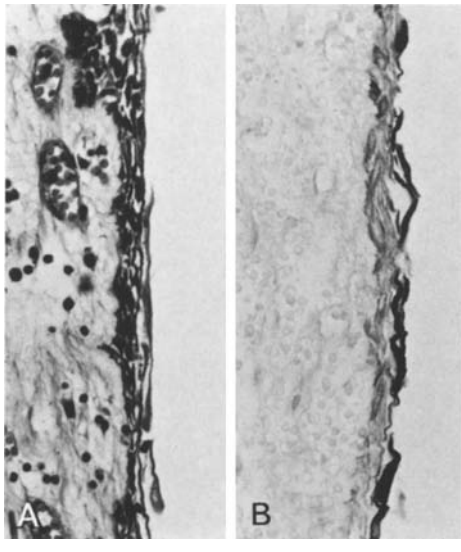

Fig. 5A, B. Squamous epithelium in COC (Case 1). ×80. **A** H & E staining; **B** KL1 staining. Positive reaction is limited to superficial layers

reactivity with KL1 and TK immunoreagents which detected high molecular weight keratin and wide spetrum keratins respectively; and (3) epithelial cells containing calcified bodies which showed a tendency toward keratinization, as indicated by their reaction with KL1 and TK keratins, whereas both the calcified bodies, irrespective of size or shape, and ghost cells were negative for keratin and involucrin, although ultrastructurally, ghost cells contain abundant of tonofilaments similar to keratinocytes (Fejerskov and Krogh 1972; Eda et al. 1974; Chen and Miller 1975; Regezi et al. 1975; Sapp and Gardner 1977; Vuletin et al. 1978; Donath et al. 1979; Kerebel and Kerebel 1985).

In the normal process of terminal kertinization, upper spinous cells in squamous epithelium contain abundant amounts of KL1- and TK-detectable keratins, as well as involucrin (Mori et al. 1985; Nakai an Mori 1986; Sumitomo 1986). From this description, these epithelial cells did not

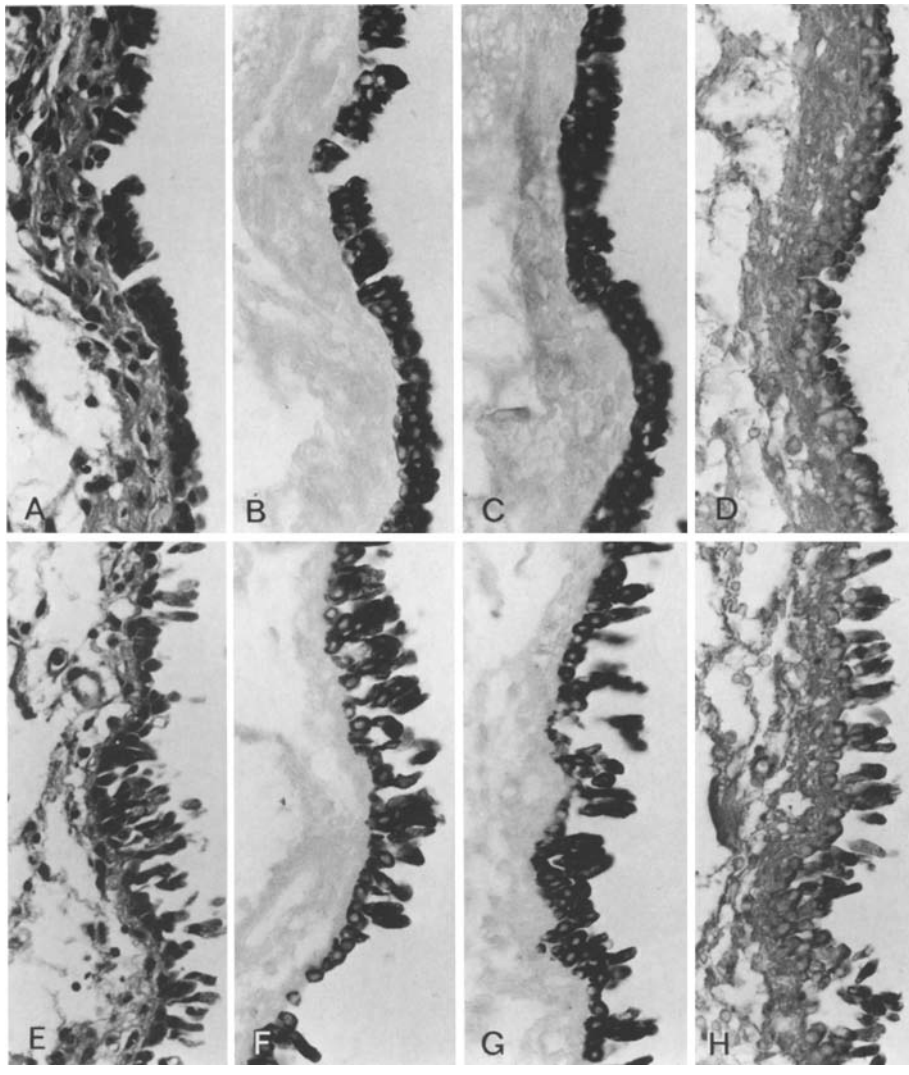

Fig. 6A–H. Columnar Epithelium on COC (Case 1) ×80. **A, E** H & E staining; **B, F** PKK1 staining; **C, G** KL1 staining. Columnar epithelium in cystic wall strongly reacts to these immunoreagents. **D, H** Involucrin staining. Reaction product is intensely strong to upper luminal aspect in cystic cavity

show the regular or normal level of involucrin staining and it is suggested that the mechanism of keratinization was not the same as that seen in normal conditions or other lesions of squamous epithelium origin. In particular, the antigenecity of keratin polypeptides in ghost cells may product other different subclasses of keratins because ghost cells strongly indicate the tendency of the degenerative changes or abnormal squamous metaplstic feature in lesions.

Comparing the results of keratin immunohistochemistry in calcifying epithelial odontogenic tumour (CEOT) with those of COC, in CEOT, PKK1 staining in tumour cells was slightly positive, while that in COC was almost negative or very weak; and KL1- and TK-detectable keratins were nearly the same in the two lesions (Mori et al. 1988). The pattern of keratin distribution in lining epithelial cells of COC was not strongly different from that seen in CEOT.

Keratin distribution in squamous epithelium of COC was relatively similar to that of squamous cell epithelium in oral mucosa except for the finding of PKK1 keratins. Normal squamous epithelium reveals a regular zonal distribution: basal layer cells react with PKK1 antibodies; spinous and granular epithelial cells, with KL1 antibody and the completely hornified layer, showing orthokeratinization, is devoid of reactivity keratins (Mori et al. 1985; Nakai and Mori 1986). Squamous epithelium in cystic lining of COC may be a result of metaplasia of odontogenic epithelium.

Table 2. Immunohistochemical findings in epithelial component of COC

	TK	KL1	PKK1	Involucrin
Calcifying epithelium				
Basal and upper epithelial cells	+1–+2	+1–+2	0	0 (or +3)
Thin epithelial cells	+2–+3	+3–+4	0–±	0 (or +3)
Calcified bodies and ghost cells	0	0	0	0
Keratinizing epithelium				
Epithelial cells	0–±	±	±	0–±
Keratinized bodies	0 or +3	0 or +3	0 or +3	0–±
Squamous epithelium				
Upper cells	0	+3–+4	+3–+4	+3
Basal cells	0	+1	+1	0
Columnar epithelium				
Upper columnar cells	+4	+4	+4	+4
Basal cuboidal cells	+4	+4	+4	+3–+4

0: negative; ±: trace; +1: slight; +2: moderate; +3: strong, +4: strongest

The columnar epithelium in the COC lesion was of particular interest, although cuboidal and columnar epithelial cells have been noted occasionally in other odontogenic cysts. Immunohistochemically detectable keratins and involucrin were most specifically expressed in these epithelial cells which were reactive with all the antibodies used. Such strong staining for all keratin types and involucrin in the same epithelial cells has not been reported previously. However, these columnar epithelial cells did not show any signs of keratinization from routine histology. The characteristics of these epithelial cells may suggest that keratinizing and calcifying odontogenic epithelia seem to arise from this type of simple odontogenic epithelium via several types of transformation such as metaplasia, leading to focal intense keratinization, and dystrophic calcification in such epithelial cells including neoplastic variants.

Toto et al. (1967) pointed out that the constitutions of amino acid of the keratin-like substances in odontogenic epithelium apparently differented from those of keratinized epithelium, however, immunohistochemical detection of keratin polypeptides in odentogenic epithelium and lesions have not been carreid out in detail. In conclusion, it may be thought that the keratin distribution in odontogenic epithelium and lesions are more characteristic features in comparison with squamous epithelium in oral mucosa. Furthermore it is necessary to investigate the immunohistochemistry of these cystic lining epithelial components of COC containing irregular calcified bodies and ghost cells, in order to clarify the histogenesis of COC.

References

Abrams AM, Howell FV (1968) The calcifying odontogenic cyst. Report of four cases. Oral Surg 25:594–606

Anneroth G, Hansen LS (1968) Variations in keratinizing odontogenic cysts and tumors. Oral Surg 54:530–546

Chen SY, Miller AS (1975) Ultastructure of the keratinizing and calcifying odontogenic cyst. Oral Surg 39:769–780

Donath K, Kleinhans V, Gundlach KKH (1979) Zur Pathogenese der calcifizierenden odontogenen Cyste (Gorlin-Cyste). Virchows Arch A (Pathol Anat) 384:307–324

Eda S, Kawahara H, Yamamura T, Imaizumi I, Ohi M, Ichikawa T (1971) A case of calcifying odontogenic cyst associated with odontoma. Bull Tokyo Dent Vol 12:1–7

Eda S, Yanagisawa Y, Koike H, Yamamura T, Kato T, Noma H, Inagami K, Kawashima Y (1974) Two cases of calcifying odontogenic cyst associated with odontoma, with an electronmicroscopic observation. Bull Tokyo Dent Coll 15:77–90

Ellis GL, Shmookler BM (1986) Aggressive (malignant?) epithelial odontogenic ghost cell tumor. Oral Surg 61:471–478

Farman AG, Smith SN, Nortje CJ, Grotepass FW (1978) Calcifying odontogenic cyst with ameloblastic fibro-odontome: one lesion or two? J Oral Pathol 7:19–27

Fejerskov O, Krogh J (1972) The calcifying ghost cell odontogenic tumor or the calcifying odontogenic cyst. J Oral Pathol 1:273–287

Forest D, Mercier P (1967) Compound composite odontome associated with keratinizing masses. J Can Dent Assoc 33:487–493

Freedman PD, Lumerman H, Gee JK (1975) Calcifying odontogenic cyst. A review and analysis of seventy cases. Oral Surg 40:93–106

Fukushima M (1983) Simple ameloblastoma with ghost cell and granular cell components. Acta Pathol Jpn 33:1215–1221

Gold L (1963) The keratinizing and calcifying odontogenic cyst. Oral Surg 16:1414–1424

Gorlin RJ, Pindborg JJ, Clausen FP, Vickers RA (1962) The calcifying odontogenic cyst-A possible analogue of the cutaneous calcifying epithelioma of malherbe. An anolysis of fifteen cases. Oral Surg 15:1235–1243

Gorlin RJ, Pindborg JJ, Redman RS, Williamson JJ, Hansen LS (1964) The calcifying odontogenic cyst. A new entity and possible analogue of the cutaneous calcifying epithelioma of Malherbe. Cancer 17:723–729

Kerebel B, Kerebel LM (1985) Ghost cells in complex odontoma: A light microscopic and SEM study. Oral Surg 59:371–378

Komiya Y, Susa A, Kawachi H, Yamamura T, Eda S, Kawachi T (1969) Calcifying odontogenic cyst. Report of a case. Oral Surg 27:90–94

Lello GE, Makek M (1986) Calcifying odontogenic cyst. Int J Oral Maxilofac Surg 15:637–644

Levy BA (1973) Ghost cells and odontomas. Oral Surg 36:851–855

Mori M, Nakai M, Hyun K-H, Noda Y, Kawamura K (1985) Distribution of keratin porteins in neoplastic and tumor like lesions of squamous epithelium. Oral Surg 59:63–69

Mori M, Tatemoto Y, Yamamoto N, Okada Y (1988) Immunohistochemical expression of keratin proteins detected with the use of polyclonal and monoclonal antibodies on calcifying epithelial odontogenic tumors. J Oral Pathol (in press)

Nakai M, Mori M (1986) Immunohistochemical distribution of monoclonal antibodies against keratin in papillomas and carcinomas from oral and nasopharyngeal regions. Oral Surg 62:292–302

Osborne TP, Park LK, Levy BA, Tewews WE (1974) Odontoma containing ghost cells in the maxillary sinus. Oral Surg 38:819–823

Pflüger H (1956) Über die von Zahnbildenden Gewebe ausgehenden Geschwülste Adamantinom and Odontom. Deutsch Zahn Mund- und Kieferheilk 25:97–121

Pindborg JJ, Kramer IRH, Torloni H (1971) Histological typing of odontogenic tumors, jaws cysts, and allied lesions. World Health Organization, Geneva

Praetorius F, Hjorting-Hansen E, Gorlin RJ, Vickers RA (1981) Calcifying odontogenic cyst. Range, variations and neoplastic potential. Acta Odontol Scand 39:227–240

Regezi JA, Countney RM, Kerr DA (1975) Keratinization in odontogenic tumors. Oral Surg 39:447–455

Sapp JP, Gardner DG (1977) An ultratructural study of the calcifications in calcifying odontogenic cysts and odontoma. Oral Surg 44:754–766

Sedano HO, Pindborg JJ (1975) Ghost cell epithelium in odontomas. J Oral Pathol 4:27–30

Sumitomo S, Kumasa S, Iwai Y, Mori M (1986) Involucrin expression in epithelial tumors of oral and pharyngeal mucosa and skin. Oral Surg 62:155–163

Tajima Y, Ohno J, Ustumi N (1986) The dentinogenic ghost cell tumor. J Oral Pathol 15:359–362

Toto PD, O'Malley JJ, Grandel EP (1967) Similarities of keratinization and amelogenesis. J Dent Res 46:602–607

Vuletin JC, Solomon MP, Pertschuk LP (1978) Peripheral odontogenic tumor with ghost-cell keratinization. A histologic, fuorescent microscopic, and ultrastructural study. Oral Surg 45:406–415

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