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Adenomatoid odontogenic tumour: co-expression of keratin and vimentin

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Summary. Immunohistochemical observations of intermediate sized proteins in five cases of adenomatoid odontogenic tumour (AOT) are described. The immunohistochemical detections of keratins were made with polyclonal antiserum (TK, 41–65 kDa) and three monoclonal keratin antibodies (KL1: 55–57 kDa; PKK1: 40, 45, and 52.5 kDa and nos. 19, 18, 8; K8.12: nos. 16, 13) and vimentin and desmin monoclonal antibodies. Histologically, the tumour epithelia could be divided into two types: type A cells were a spindle or columnar shape and formed solid, ductal, tubular or whorled structures. Type B cells were small and compact cells at the periphery of the A cell-containing focus. Immunohistochemically, the type A cells showed very slight reaction with all antibodies to keratins, whereas the type B cells indicated slight-to-moderate expression of keratin and vimentin, and showed coexpression. Both types of cell showed a negative reaction for desmin. Only one case was associated with cystic lesions, and the cyst-lining was composed of thin squamous epithelium. Keratin expression in this epithelium was strong. In the histogenesis of AOT it was postulated that the tumour cells may have originated from undifferentiated odontogenic epithelium or stratum intermedium cells.

Key words: Adenomatoid odontogenic tumour – Keratin – Vimentin – Immunohistochemistry

Introduction

The adenomatoid odontogenic tumour (AOT) is an odontogenic epithelial neoplasm first recognized by Dreibladt (1907). As a variant of ameloblastoma it has been variously described (Ghosh 1934; Stafne and Minn 1948; Bernier and Tieche 1950; Miles 1951; Thoma 1955; Oehlers 1956; Lucas 1957; Gorlin and Chaudhry 1958; Golin et al. 1961; Ishikawa and Mori 1962; Shear 1962; Philipsen and Birn 1969). It is known that AOT generally has a marked female predilection, occurs most often in the second decade, shows a marked anterior maxillary preference, and in many cases is associated with impacted teeth (Courtney and Kerr 1975; Kinoshita et al. 1985).

The histological features of AOT have been well established and histochemical observations of tumour epithelium and homogenous amorphous materials have also been reported (Boyle and Kalnins 1960; Shear 1962; Spouge and Spruyt 1968; Lee 1974; Mori et al. 1980). Enzyme histochemistry of AOT has shown that alkaline phosphatase and hydrolytic enzymes as well as dehydrogenase are present in the tumour epithelial cells (Ishikawa and Mori 1962; Mori et al. 1970). Electron microscopic studies were also carried out in the epithelial components (Takagi 1967; Lee 1974; Khan et al. 1977; Hatakeyama and Suzuki 1978; Smith et al. 1979; Schlosnagle and Someren 1981; Yamamoto et al. 1981; Moro et al. 1982; Poulson and Greer 1983; Shimono et al. 1984; Kinoshita et al. 1985). However, no immunohistochemical study of intermediate filaments of AOT has been reported.

The present paper describes the immunohistochemical expression of intermediate filament proteins of AOT, compares those of other odontogenic and nonodontogenic epithelial tumours, and discusses histogenesis.

Materials and methods

Five cases of AOT were examined and an outline of the clinical and radiographic findings is shown in Table 1.

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Table 1. The cases of adenomatoid odontogenic tumour examined

Case	Sex	Age	Location	Clinical features	Clinical diagnosis
1	F	12	left mandible	bone expansion radiolucency (⌀ 1.5 cm)	follicular cyst
2	M	14	right maxillae	dislocation upper 2nd incisor left labial swelling radiolucency	adenomatoid odontogenic tumour
3	F	18	anterior mandible	lower 2nd incisor left impacted teeth cystic radiolucency	follicular cyst
4	F	48	left mandible	tumor mass with radiolucency in alveolar bone	benign tumours
5	F	10	left mandible	labial swelling radiolucency with well-demarcated border	follicular cyst

Table 2. Antibodies used in present study (indiect method)

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
Monclonal KL1 keratin (55–57 kDa) 1/50 1 h	human keratinized squamous epithelium	Immunotech France Marseilles		
Monoclonal PKK1 keratin (40, 45 and 52.5 kDa) 1/80 1 h	pig kidney epithelium cell line	Labsystem Finland Helsinki	HRP-labelled anti-mouse IgG-Rabbit IgG 1/20 30 h	Dakopatts Denmark Copenhagen
Monoclonal K8.12 keratin (48 and 54 kDa) 1/20 1 h	bovine epidermal keratins	Bio-yeda Islael		
Monoclonal Vimentin (57 kD) 1/10 1 h	porcine eye lene	Dakopatts Denmark Copenhagen		
Monoclonal Desmin (53 kD) 1/50 1 h	porcine stomach	Dakopatts Denmark Copenhagen		

1) Deparaffinization; 2) Protease digestion (only PKK1 keratins): 0.01% trypsin/PBS solution (pH 7.6) 15 min, 37° C; 3) Inactivation of endogenous peroxidase: 0.3% H_2O_2; 4) Background blocking: Normal swine serum (TK) 1/20, 30 min, Normal rabbit serum (KL1 & PKK1) 1/20, 30 min; 5) 1st layer; 6) 2nd layer; 7) Visalization of peroxidase activity: 0.02% 3-3′diaminobenzidine hydrochloride (DAB)/0.05 M Tris buffer solution (pH 7.6) containing 0.005% H_2O_2 for 5 min

Materials obtained by surgical removal were fixed in 10% formalin. No decalcification procedures were carried out, if the specimens were only slightly calcified. Paraffin sections at 4 μm were made for routine histological examination with the H&E stain and for immunohistochemical staining.

In the present study, the indirect immunohistochemical method was applied. For detection of keratins, polyclonal anti-keratin antiserum and 3 monoclonal antibodies to keratins (KL1, PKK1 and K8.12) were used, and monoclonal antibodies for vimentin and desmin were also employed. The details of the staining protocol are shown in Table 2.

Double staining for monoclonal keratin PKK1 of K8.12 and vimentin was performed. The 1st step was demonstrated with the indirect method using DAB and the 2nd step was done for vimentin detection with the indirect method using 4-cl-l-naphthol (20 mg/100 ml) as chromogen. Sections were mounted in glycerol.

Results

The tumours were composed of sheets, cords, and nests of epithelial cells that had proliferated to form solid masses, duct-like structures, and occasional cribriform structures. Epithelial tumour cells could be classified into distinct two types in the present study.

The type A cell was spindle-shaped with oval or elongated nuclei in the solid foci (Fig. 1A), or numerous small whorled structures gathered together. Duct-like or tubule-like structures were occasionally found in the solid masses. Those structures showed pseudorosette formation with or

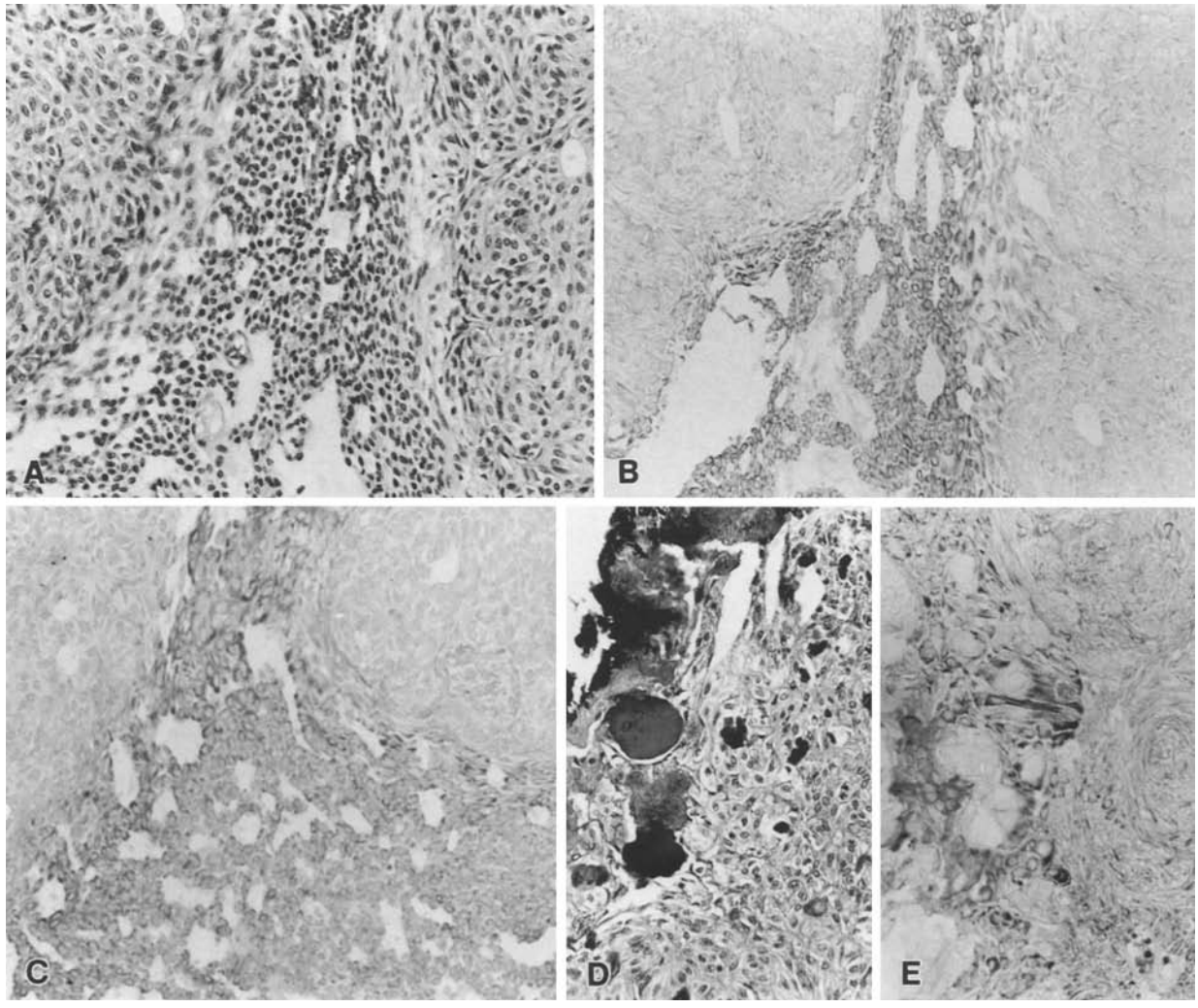

Fig. 1. Typical structures of adenomatoid odontogenic tumour (AOT), (× 100). (**A**) H&E staining. Tumour epithelial focus consists of spindle-shaped cells (type A) and small compacted cells (type B), (case 1). (**B**) PKK1 staining. Serial section of Fig. 1A. The type A cells show very slight, whereas the type B cells slight-to-moderate (**C**) Vimentin staining. Serial section of Fig. 1A and 1B. The type B cells show positive staining. The type B cells coexpress with keratin and vimentin. (**D**) H&E staining. Highly calcified foci exist in solid masses of the type A cells. (**E**) TK staining. Serial section of Fig. 1D. The type A cells focus indicate slight-to-moderate, peripheral tumour cells of highly keratinized focus shows moderate

without luminal cavities and walls consisting of tall columnar cells or cuboidal cells. Eosinophilic amorphous material was observed in the lumen (Fig. 2A).

The type B cell was small and compact with a round, polyhedral, or flat in shapes. These cells were located around adenomatoid foci consisting of type A cells (Fig. 1A). The small round cells were distributed irregularly (Fig. 2D). Duct-like structures and large rosette formations contained flattened cells in their outer zone (Fig. 2A). The type B cells were arranged as ductal structures with single raw in the connective tissue stroma, or were scattered sparsely and irregularly at the periphery.

Calcification was evident in the type A cell focus, but rarely in the type B, and was occasionally seen in the stromal tissue (Fig. 1D). Calcified foci stained intensely with haematoxylin and homogeneous staining material was also observed.

The cystic lining epithelium in one case (case 5) was composed of thin squamous epithelium with two or three cellular layers without distinct typical stratification or keratinization (Fig. 3).

The staining reaction in the type A cells, irrespective of the histological variants solid focus, ductal, and tubular elements) was very slight with TK, KL1 and K8.12 and only trace activity was seen with PKK1 (Fig. 1B, Fig. 2B, C, and Fig. 2E).

The type B tumour cells stained more markedly

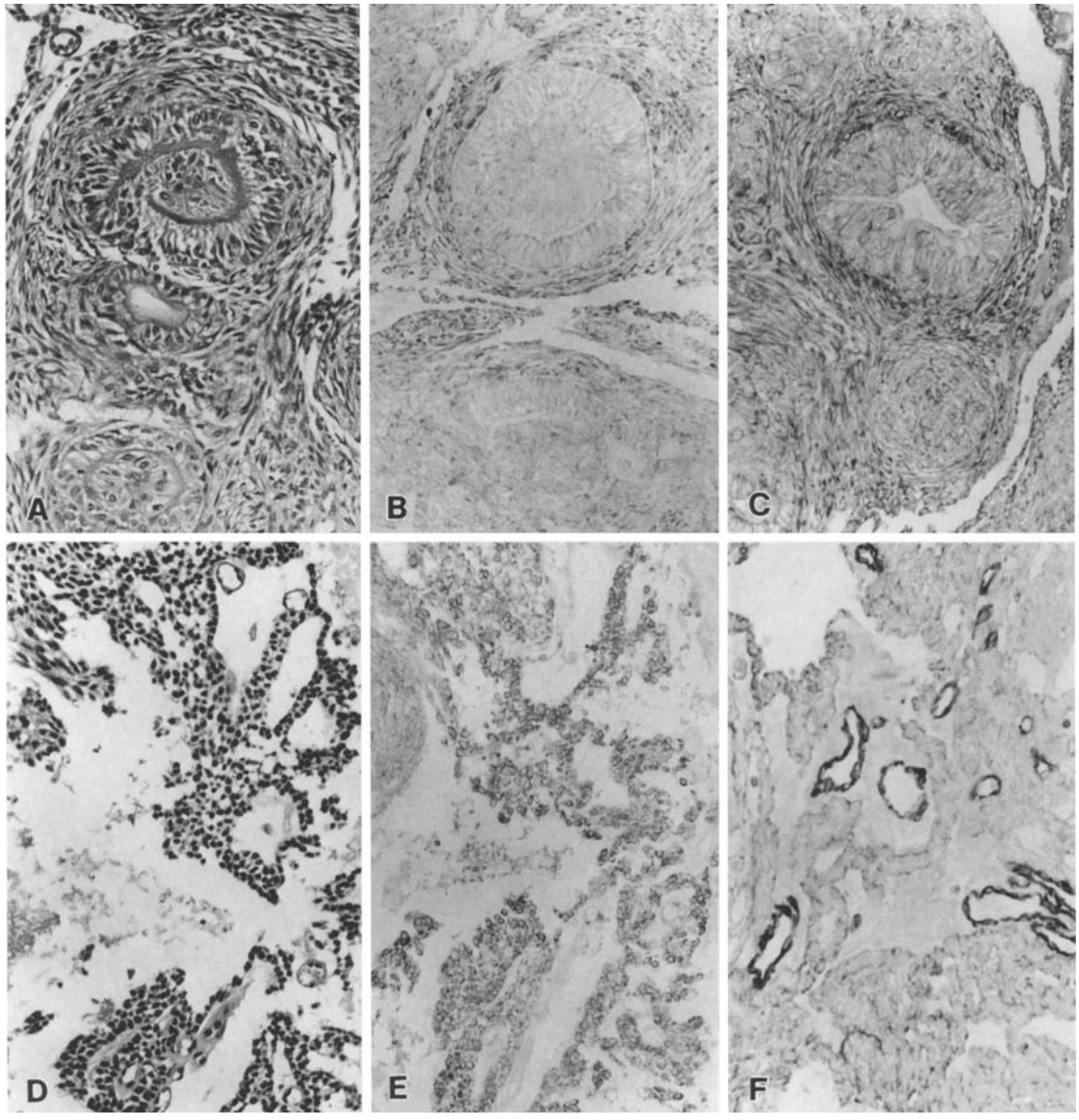

Fig. 2. AOT lesion with duct-like or rosett structures (the type A cells), (×100). (**A**) H&E staining. Pseudotuble structure (the type A) and surrouding flattend epithelial cells (the type B), (case 3). (**B**) KL1 staining. Serial section of Fig. 2A. Reaction product of the type B cells is a little stronger than that in the type A (rosett forming cells). (**C**) TK staining. Serial section of Fig. 2A and 2B. Surrouding tumour cells showing flattend shape (the type B cells) of tubular structures indicate moderate deposition. (**D**) H&E staining. Small compact tumour cells (the type B) infiltrate into non-collagenous stromal tissue, (Case 5). (**E**) KL1 staining. Serial section of Fig. 2D. Moderate reaction for KL1 is distributed to the type B cells. (**F**) Vimentin staining. Serial section of Fig. 2D and 2E. The type B cells show slightly positive staining for vimentin, and blood vessels display strongly deposition

than the type A cells, indicating a distinct difference in keratin distribution between the type A cells and the type B (Fig. 1B, 2C). In duct-like structures and rosette-like configrations, the type B cells at outer or peripheral zones revealed a slight deposition of TK, KL1 and K8.12 keratins (Fig. 2C), but not of PKK1 (Fig. 2B). The focus composed of the type B cells only, in spite of cellular proliferation and arrangement, showed slight to moderate staining for TK, KL1 and K8.12 (Fig. 2E, 2F). Tumour cells in large areas of calcification expressed a positive reaction with TK, KL1 and K8.12; however, small or micro foci with irregular calcification in a solid focus (containing type A cells) was devoid of any keratin staining (Fig. 1E).

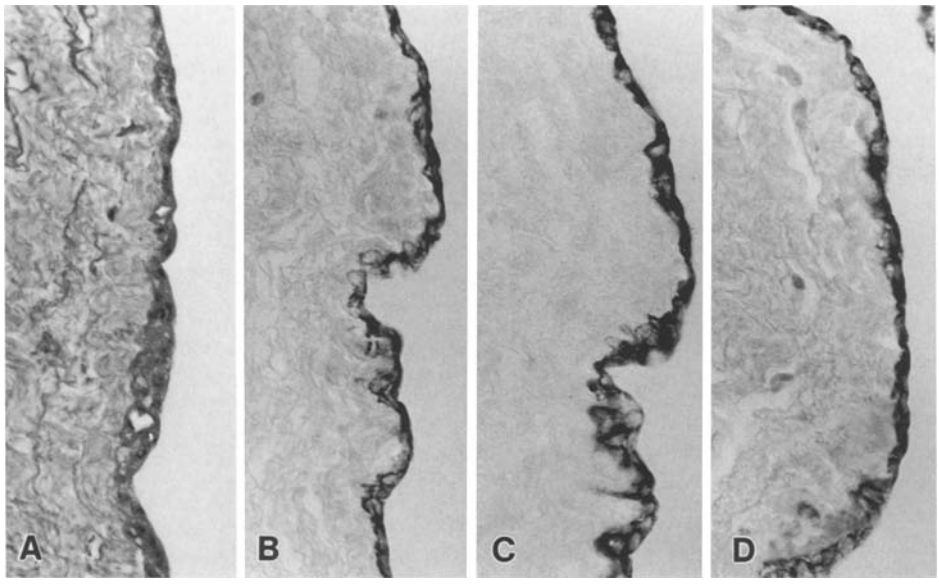

Fig. 3. Thin squamous epithelium in cystic lining accompanying with AOT (Case 5), (×200). (**A**) H&E staining, Fig. 3B, PKK1 staining, Fig. 3C, KL1 staining, and Fig. 3D, TK staining. These sections are serial sections. Epithelial cells show higher reactions for all the keratins

Squamous epithelium in the cystic lining displayed the strongest staining of keratin antibodies in all of the cellular layers (Fig. 3B–D).

The type B tumour cells showed positive staining to vimentin, but negative to desmin. Blood capillaries in the focus indicated the strongest vimentin staining (Fig. 1C, 2F).

Double staining for keratin (PKK1 or K8.12) and vimentin showed co-expression in the type B cells only.

Discussion

The clinicopathological features of AOT have been well documented (Giansanti et al. 1970; Kinoshita et al. 1985). The neoplastic cells in AOT were classified into two types according to previous investigations in the present study. The type A tumour cells were characterized by their occurrence in whorled and tubule and duct-like structures with columnar or spindle-shaped cells. Smith et al. (1979) stated that AOT cells could be classified into two distinct types of epithelium – type I and II – by light and electron microscopy, and Schlosnagle and Someren (1981) also noted similar finding. The type I epithelial cells correspond without the type A cells, whereas the type II epithelial cells may coincide with our description of the type B cells. Hatakeyama and Suzuki (1978) described 4 types of tumour cells: the compactly proliferating cell layer, flat epithelial cell layer, star-shaped epithelial cell layer, and cuboidal epithelial layer. The flat epithelial cells of these four categories seem to be included in our type B cells, whereas the other three types were in type A. These type B cells were also identified in previous reports by other names. These include the cribriform area showing the remains of stroma and cystic change (Lee 1974); Fig. 5 of Spouge (1967) were no detailed explanation is given; small cells in basal and parabasal layers of the cystic portion (Dunlap and Fritzlen 1972); and the cells having a condensed uniform eosinophilic cytoplasm with small round to oval-shaped hyperchromatic nuclei (Courtney and Kerr 1975).

Enzyme histochemically, Ishikawa and Mori (1962) stated that alkaline phosphatase activity was confined to flattened tumour cells (type B cells) and Mori et al. (1970) compared phosphatases and dehydrogenase distribution between tumour cells of AOT and enamel organ of tooth germs. Their examination indicated alkaline phosphatase activity in flattened tumour cells (the type B) but not in the type A cells. In the developing rat tooth, keratin profiles in the enamel organ were dependent on the degree of differentiation during amelogenesis. Keratins were invariably localized in stratum intermedium cells, but were very weakly detected or lacking in the ameloblasts (Nakai et al. 1986). It is suggested that the type B cells of AOT possible arise from stratum intermedium cells, whereas the type A cell may correspond to young or immature ameloblasts which have not fully differentiated. These findings seem to be in agreement with the many previous studies in the interrelation ship of AOT cells and enamel organ. Co-expression of keratin and vimentin in the type B tumour cells was a novel finding among odontogenic epith-

erial tumours. It has been reported that tumour cells in calcifying epithelial odontogenic tumours (CEOT) showed coexpression of keratin and vimentin (Mori et al. 1988).

The histochemical constitution of calcified matrix or homogeneous materials in AOT have been reported (Boyle and Kalnins 1960; Shear 1962; Spouge and Spruyt 1968; Lee 1974; Mori et al. 1980). It is known that the biological properties of the calcification site indicate that they contain proteins not much different from those found in human enamel. However, the details of biological significance in the calcified areas are unknown.

AOT associated with cystic lining epithelium is occasionally reported as evidence of derivation from dentigerous cyst, globulomaxillary cyst or residual cyst (Stafne and Minn 1948; Bhaskar 1964; Khan et al. 1977; Glickman et al. 1983; Saito et al. 1983; Kuntz and Reichart 1986). Bhasker (1964) described that AOTs arise from the epithelial lining of follicular cyst. In calcifying odontogenic cysts (COC), a preceding paper reported that cystic epithelium composed of thin keratinizing, squamous, and columnar epithelium and especially columnar epithelium, indicated positive staining for keratins (Yamamoto et al. 1988). The cystic lining composed of thin squamous epithelium associated with AOT in the present case also indicated positive keratin staining. These cystic epithelial cells of odonotogenic origin have unique keratin expressions, whereas it is known that squamous cell epithelium in oral mucosa and skin shows a regular zonal distribution for keratins (Nakai and Mori 1986). We suggest that this particular thin cystic epithelium probably originates from undifferentiated odontogenic epithelium. A previous paper has noted that cystic lining cells accompanying with COC can be classified into 4 types and that immunohistochemical characteristics of keratin proteins were found in columnar epithelial cells (Yamamoto et al. 1988). This cystic lining epithelium which may have the potential for tumour development, could possibly arise via squamous differentiation (metaplasia) or by alternative expression of cellular properties (as AOT).

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