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Immunohistochemical expression of amelogenins in odontogenic epithelial tumours and cysts

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Summary. Amelogenins, enamel proteins in odontogenic tumours, were detected immunohistochemically using a monoclonal antibody. They were strongly expressed in amyloid-like material, ghost cells, and the cells surrounding ghost cells of calcifying epithelial odontogenic tumours and cysts, whereas calcified bodies within the tumours and cysts showed negative staining. The expression of amelogenins was also positive in tumour cells of ameloblastoma, adenomatoid odontogenic tumour, squamous odontogenic tumour and ameloblastic fibroma. Peripheral tumour cells of the follicular ameloblastoma were positive with relatively intense staining. Undifferentiated or flattened tumour cells of adenomatoid odontogenic tumour and non-keratinized tumour cells of the squamous odontogenic tumour showed marked staining. Reduced ameloblasts in the odontoma displayed the strongest staining for amelogenins. The study suggests that biosynthesis of amelogenins may occur in the homogeneous materials of calcifying epithelial odontogenic tumours and cysts.

Key words: Amelogenin – Odontogenic tumour – Immunohistochemistry

Introduction

The epithelial components in calcifying epithelial odontogenic tumours (CEOT) and calcifying odontogenic cysts (COC) consist histologically of homogeneous acellular materials that include ghost cells and show evidence of calcification (Pindborg 1958; Gorlin et al. 1962). It has been suggested that both the homogeneous material and ghost cells contain amyloid, identified by Congo red staining or fluorescence techniques (Vickers et al. 1965; Ranly and Pindborg 1966; Meenaghan et al. 1972), and that they contain enamel matrix proteins or enamel-like proteins, identified by classic histochemical methods (Solomon et al. 1975; Mori and Makino 1977; Mori et al. 1980). Epithelial tumour cells of the CEOT and the COC originate from enamel organ cells, and the homogeneous acellular material and ghost cells in these tumours may be synthesized or secreted from tumour cells. These materials possibly coincide with amelogenins.

Enamel proteins have been reported to be essential for the formation of the enamel matrix during enamel formation using classical techniques (Deguchi 1966; Nikiforuk and Gruca 1969). Enamel proteins are divided into two classes: amelogenins, which are soluble in neutral salts, and enamelins, which are strictly bound to apatite crystals (Herold et al. 1987). In young or immature enamel, amelogenin antibodies stain 90–95% of the

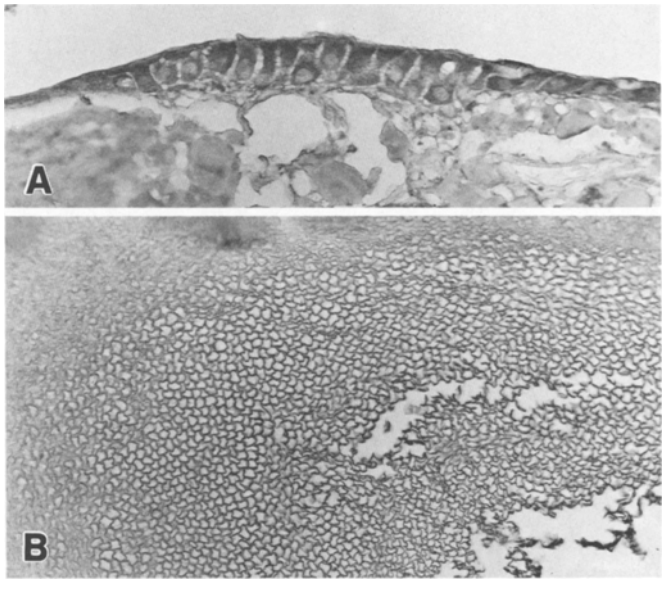

Fig. 1. A Reduced ameloblastic layer in the odontoma. The apical site of reduced ameloblasts strongly stains with amelogenins. ×85. **B** Enamel in the odontoma (decalcifying specimens). Amelogenin reacts strongly in the enamel structure. ×85

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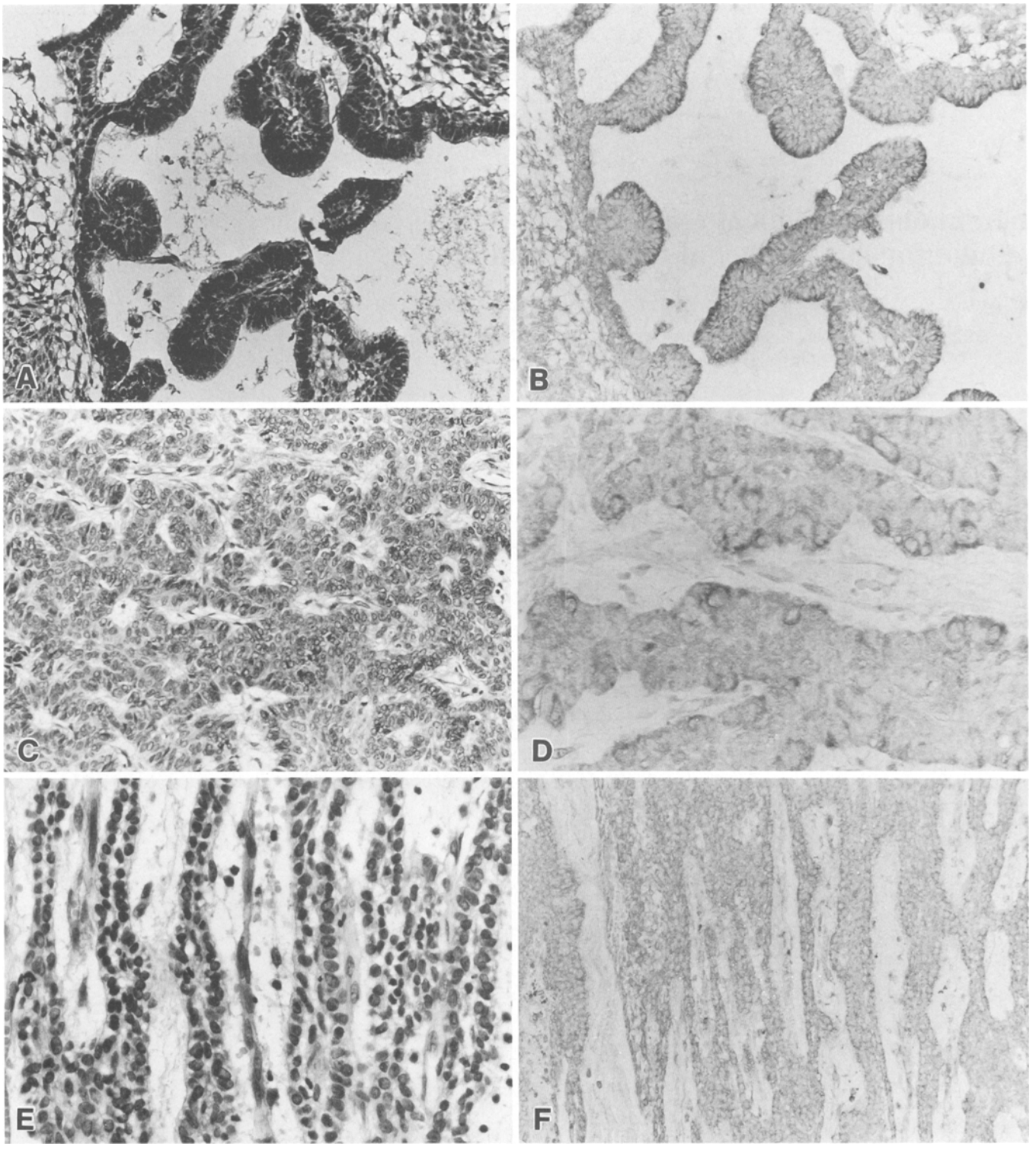

Fig. 2. A, B Follicular ameloblastoma. Amelogenin staining is confined to the periphery of the ameloblastoma foci. ×100. **C, D** Follicular ameloblastoma. Scattered tumour cells in the follicular ameloblastoma show marked amelogenin staining. ×200. **E, F** Ameloblastoma. Small tumour cell strands stain slightly for amelogenins. ×100

enamel protein and enamelins the remaining 5–10%. Both proteins, amelogenins and enamelins, were biosynthesized in young ameloblasts and secreted into the extracellular enamel matrix (Graver et al. 1978; Zeichner-David et al. 1983; Nanci et al. 1985; Rosenbloom et al. 1986; Herold et al. 1987). Immunohistochemical observations of amelogenins have been described using specific antiserum or monoclonal antibodies during tooth development of the human (Christner et al. 1983; Rosenbloom et al. 1986; Inage et al. 1989; Uchida et al. 1989).

Biochemical analysis of enamel matrix proteins has also been described in five mammalian species (Fincham et al. 1982) and human fetuses (Fincham et al. 1983).

It is the purpose of the present paper to explore the immunohistochemical expression of amelogenins in the homogeneous material of the CEOT and the COC, the epithelial tumour cells in odontogenic epithelial tumours, and the reduced ameloblastic layer of odontomas.

Materials and methods

The following odontogenic tumours were studied: ameloblastoma ($n=22$), granular cell ameloblastomas ($n=3$), adenomatoid odontogenic tumours ($n=5$), ameloblastic fibromas ($n=4$), squamous odontogenic tumours ($n=2$), CEOT ($n=2$), COC ($n=8$). Reduced odontogenic epithelium (ameloblastic layer) in odontomas ($n=2$) was also used to detect amelogenin staining. All tissue was embedded in paraffin following 10% formalin fixation, and 4-μm serial sections were employed to detect amelogenins immunohistochemically.

A monoclonal antibody (mAb) to bovine amelogenins (IgM) was used at a 1:1000 dilution. Deparaffinized sections were incubated with 0.05% pronase for 30 min at 37° C, immersed in methanol containing 0.05% H_2O_2 for 30 min, and treated with normal rabbit serum (1:20) for 30 min. Sections were then reacted with the mAb for 1 h at room temperature. The ABC method was used for detecting amelogenins. Details of the biochemical properties of the mAb have been described previously (Shimokawa et al. 1984; Takagi et al. 1984).

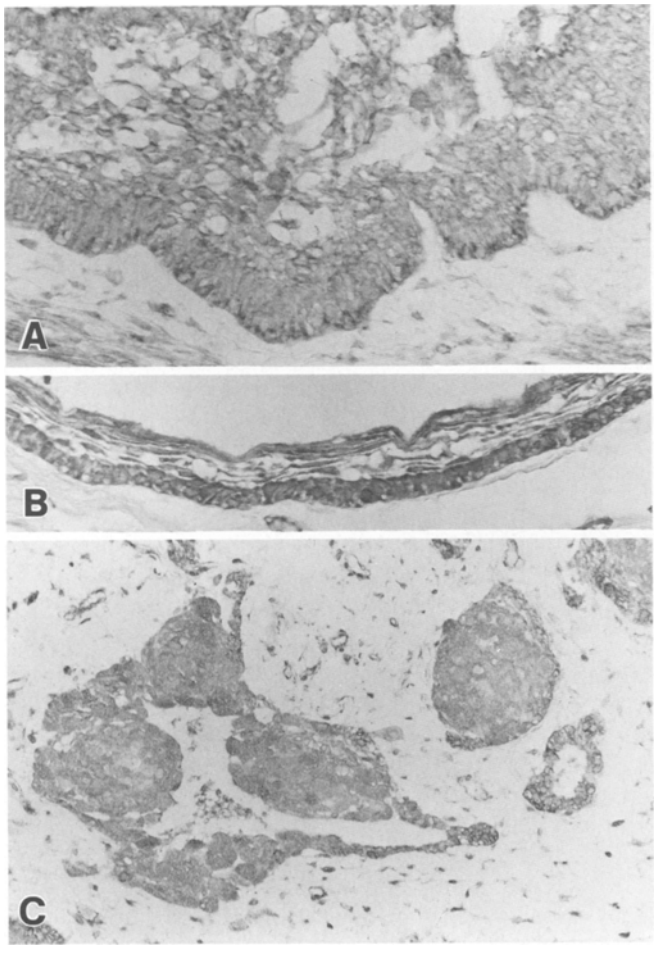

Fig. 3. **A** The follicular ameloblastoma shows abundant staining to amelogenins in the periphery of columnar cells. ×85. **B** The cystic ameloblastoma shows positive amelogenin staining in cystic epithelial cells. ×85. **C** Granular cells of the granular ameloblastoma indicate slight positive staining to amelogenins. ×85

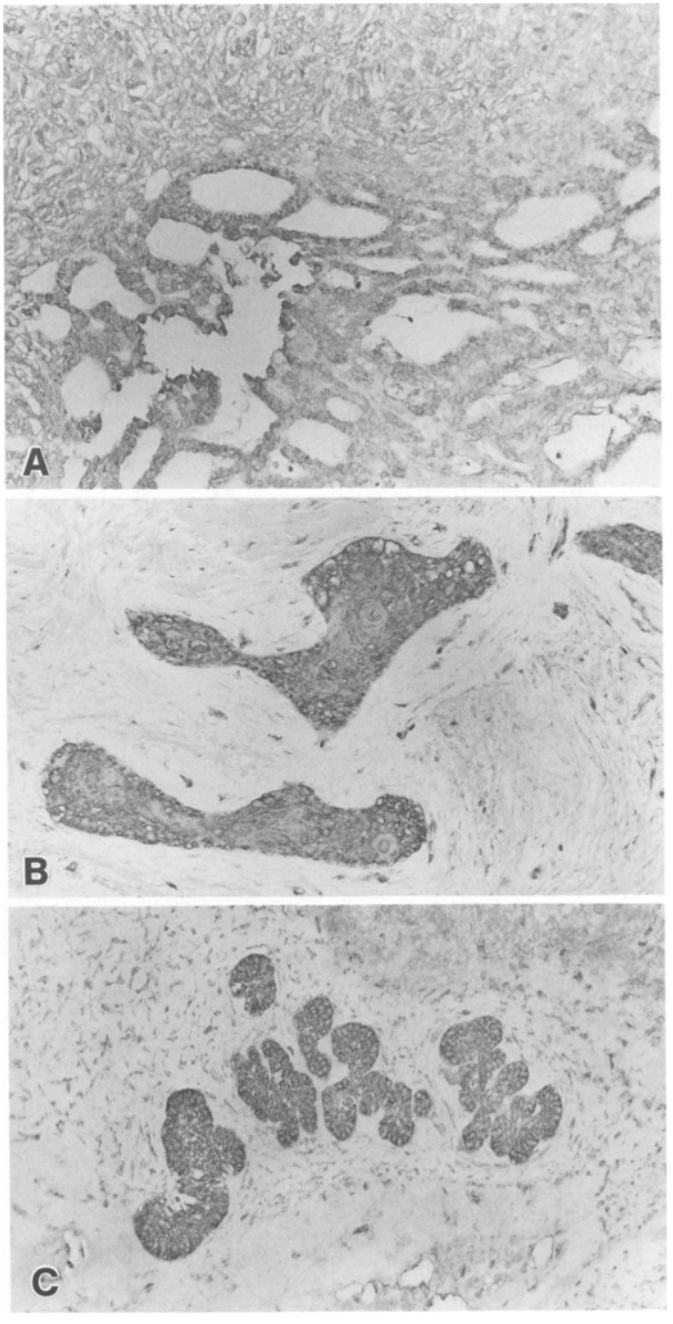

Fig. 4. **A** The adenomatoid odontogenic tumour shows positive amelogenin staining in undifferentiated odontogenic epithelial cells surrounding pseudoductal structures. ×85. **B** The squamous odontogenic tumour reveals moderate amelogenin staining in odontogenic tumour cells and weak staining in keratinized tumour cells. ×85. **C** The ameloblastic fibroma shows positive amelogenin staining in epithelial cells. ×85

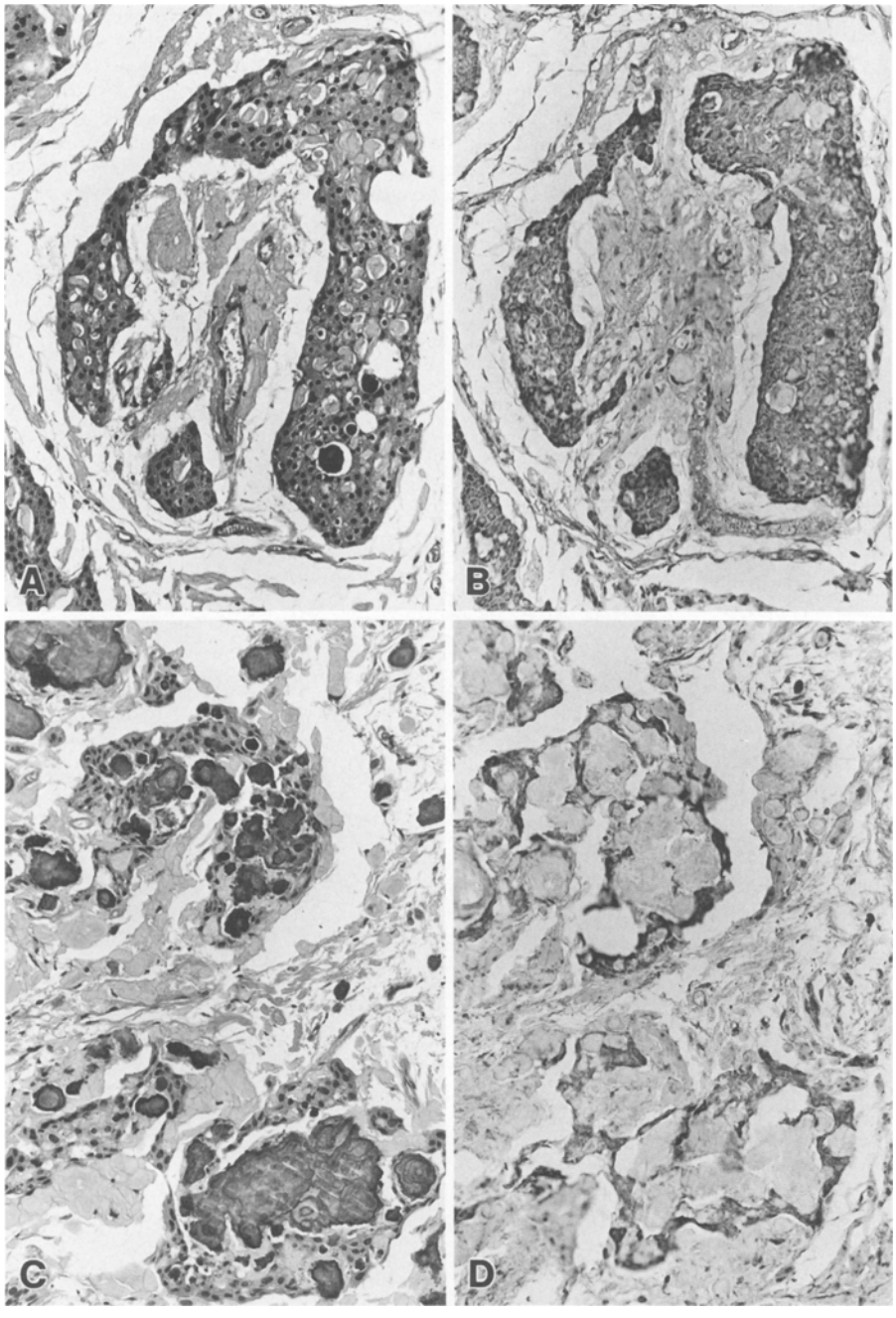

Fig. 5A–D. The calcifying epithelial odontogenic tumour (CEOT) × 85. **A, B** The CEOT focus contains much amorphous material (**A**), and amelogenin staining is marked both in CEOT cells and amorphous material. (**B**). **C, D.** Highly calcified CEOT foci indicate marked amelogenin staining in tumour cells located in the periphery of highly calcified masses. The calcified masses were negative for amelogenin staining (**D**)

Results

Positive staining of the mAb for amelogenins was more intense in pronase pre-treated sections as compared to non-treated sections. Trypsin-treated sections tended to strip away from the slides, particularly in peripheral tissue of calcified masses; therefore, pronase pre-treated sections gave the best staining results.

In the reduced enamel epithelium and enamel matrix in odontoma, the most intense reaction for amelogenins was found distributed in reduced ameloblasts and the enamel matrix (Figs. 1A, B).

In ameloblastoma amelogenin reactivity was positive in follicular ameloblastomas and was particularly abundant in peripheral tumour cells (Figs. 2A, B, 3A) and cystic areas of ameloblastoma (Fig. 3B). Centrally located tumour cells of the follicular type also indicated positive staining. Some cells located in the tumour epithelium showed relatively marked staining (Fig. 2C, D). Granular cells of ameloblastoma showed slight to moderate staining (Fig. 3C).

In adenomatoid odontogenic tumours there was slight positive staining for amelogenins in tumour cells showing pseudoglandular and ductal structures, and relatively marked staining in undifferentiated odontogenic cells located in the periphery of pseudoglandular structures (Fig. 4A). Homogeneous acellular material stained for amelogenins.

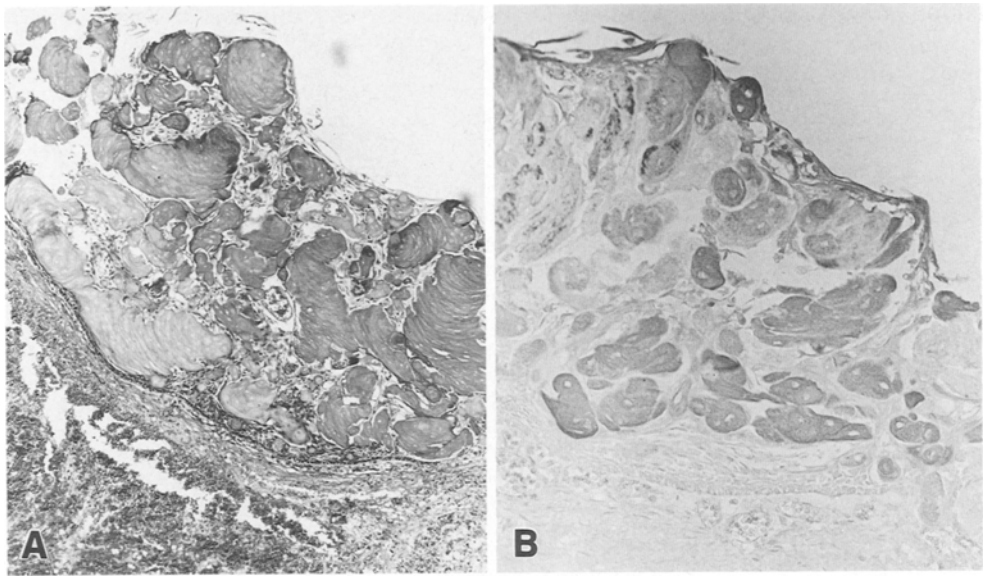

Fig. 6A, B. Calcifying epithelial cyst (COC). ×85. There are variously shaped ghost cells in the COC lining (**A**), and these ghost cells stain positive for amelogenins (**B**)

In squamous odontogenic tumours, amelogenin staining was localized in odontogenic epithelial islands, and the staining was less intense in keratinized tumour cells than in non-keratinized tumour cells (Fig. 4B).

Ameloblastic fibroma showed epithelial cells positively stained for amelogenins, while fibroma cells were not (Fig. 4C).

Immunostaining of the mAb for amelogenins was strongly evident in the eosinophilic amorphous material of CEOT and in tumour cells present at the periphery of the well-calcified material. No reaction product was found in calcified material. Tumour cells of the CEOT showed a moderate reaction (Fig. 5A–D).

COC showed positive staining in ghost cells in cystic epithelium at varying intensity, from trace amounts to intermediate grades. No calcified cells stained immunohistochemically for amelogenins. The epithelial cells of the COC were almost devoid of amelogenin staining (Fig. 6A, B).

Discussion

Immunohistochemically detectable amelogenins and enamelins, including enamel proteins, have been described in developing teeth. Secretory ameloblasts and enamel matrix were positive for amelogenins with specific antibody techniques (Graver et al. 1978; Christner et al. 1983, 1985; Nanci et al. 1985; Rosenbloom et al. 1986; Herold et al. 1987; Inage et al. 1989; Uchida et al. 1989) and autoradiography (Zeichner-David et al. 1983). Classic histochemical studies of developing tooth germs have been reported in human fetuses by Deguchi (1966). Odontogenic epithelial tumours have been reported to develop from the enamel organ which is composed of ameloblasts, stellate reticulum and external enamel epithelium. The present immunohistochemical study aimed to identify amelogenins in the tumour cells of odontogenic epithelial tumours. Tumour epithelial cells of ameloblastoma, adenomatoid odontogenic tumour, and ameloblastic fibroma were positive for amelogenin staining. Granular cells in ameloblastoma were also positively stained for amelogenins. These cells are characterized not only by the existence of lysozomal enzymes, acid phosphatase and glucuronidase (Mori 1970), but also by a strong lectin binding affinity (Mori et al. 1985). The homogeneous material in the adenomatoid odontogenic tumour showed amelogenin deposition, suggesting that the material was similar to that of ghost cells in CEOT and COC. In the present study some, but not all, of the ghost cells in COC and the cells surrounding the amyloid material in CEOT displayed varying degrees of positive staining for amelogenins. Tumour cells in CEOT usually displayed a strong expression for amelogenins, whereas calcified bodies within the CEOT were devoid of staining. Tumour cells in CEOT and epithelial cells in the COC may biosynthesize or a product with similar amelogenin immunoreactivity. The lack of a reaction for amelogenins in well-calcified material suggests only trace amounts or the absence of amelogenins, as seen in well-calcified enamel. In the present study, the enamel matrix or low calcifying enamel of the odontoma showed the strongest reaction to amelogenins (Fig. 1A). It has been suggested that amorphous material in the COC and the CEOT might be dystrophic and atrophic substances, and they contain amelogenins synthesized from tumour epithelial cells.

The biological significance of homogeneous material in the CEOT, and ghost cells in the COC suggesting amyloid or amyloid-like substances (Vickers et al. 1965; Ranly and Pindborg 1966; Gardner et al. 1968; Meenaghan et al. 1972) and enamel protein-like material (Solomon et al. 1975; Mori and Makino 1977; Mori et al. 1980) is controversial. Our previous reports have stated that the amorphous material in the CEOT contained proteins similar to enamel matrix or pre-enamel proteins as identified by the specific staining technique of the DNFBH-acid reaction. This identifies tyrosine, histidine

and amino groups (Mori and Makino 1977; Mori et al. 1980). We also suggested that protein material may be a synthetic product of tumour cells of the CEOT or from cystic epithelial cells of the COC (Yamamoto et al. 1988; Kakudo et al. 1989) which may originate from enamel organs.

Keratin immunohistochemistry in ameloblastoma (Lesot et al. 1982; Thesleff and Ekblom 1984; Mori et al. 1985), adenomatoid odontogenic tumour (Tatemoto et al. 1988), squamous odontogenic tumour (Tatemoto et al. 1989), COC (Kakudo et al. 1989; Yamada et al. 1988) and CEOT (Mori et al. 1988) has been described previously. Keratin staining of follicular ameloblastoma was comparatively intense in central stellate tumour cells compared with peripheral cells. The keratin distribution was different from the distribution of amelogenins. In the adenomatoid odontogenic tumour, many mAb to keratin were strongly expressed in flattened tumour cells or peripherally located tumour cells of pseudoglandular structures, and tubular-ductal or pseudoglandular tumour cells were slight or very weakly positive with mAb to keratin (Tatemoto et al. 1988). These keratin findings resemble the staining pattern of amelogenins. The squamous odontogenic tumour showed positive keratin staining in keratinized cells in the tumour foci (Tatemoto et al. 1989), whereas keratinized cells were almost devoid of amelogenin expression. Calcified bodies, ghost cells, and acellular material in the CEOT and the COC were usually devoid of keratin staining and showed no evidence of involucrin and filaggrin staining (Kakudo et al. 1989; Yamamoto et al. 1988). In contrast, cells surrounding calcified cells or ghost cells usually showed strong positive staining for amelogenins. It is a particular feature that ghost cells and homogeneous material without calcification were characterized by a strong expression of amelogenin, and the most consistent finding among odontogenic epithelial tumours. We conclude that ghost cells in COC and homogeneous acellular material in CEOT (rarely in the adenomatoid odontogenic tumour) contain the amelogenins found in ameloblasts of developing teeth.

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Note added in proof

Following paper entitled, "The presence of amelogenin in calcifying epithelial odontogenic tumor" by Komiyama K, Oda Y, Tanaka T, Moro I, J Oral Pathol Med, will be published soon.