

Presence of ghost cells and the Wnt signaling pathway in odontomas

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BACKGROUND: Although it has been reported that ghost cells are present in odontomas, the generation mechanism of these cells is unclear. To evaluate the presence of ghost cells and involvement of the Wnt signaling pathway, we examined the expression of hard keratins, β -catenin and Lef-1 in odontomas.

METHODS: Sixty-nine cases of odontoma were examined immunohistochemically with the use of antibodies against human hair proteins, β -catenin and Lef-1.

RESULTS: Expression of hard keratins was found only in the cytoplasm of ghost cells in 46 (66.7%) of the 69 odontomas. Compound odontomas (78.8%) showed a higher incidence of ghost cells than complex odontomas (29.4%). Histopathologically, ghost cells were found within odontogenic epithelium adjacent to immature enamel and in the centre of Liesegang-ring-like calcified materials. Expression of β -catenin and Lef-1 was observed in the cytoplasm and nucleus of odontogenic epithelial cells adjacent to the ghost cells in immature odontomas.

CONCLUSION: These findings suggest that odontoma is a hard keratin-expressing tumor-like lesion, and that the Wnt signaling pathway may be involved in the formation of ghost cells in odontomas.

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Keywords: odontogenic tumors; compound odontoma; complex odontoma; ghost cells

Introduction

Odontoma, one of the most common odontogenic tumors, is a tumor-like malformation (hamartoma) in which enamel, dentin or cementum are present, and is generally divided into two types: complex and compound odontomas.

Although calcifying cystic odontogenic tumor (CCOT or calcifying odontogenic cyst) is characterized by proliferation of ameloblastoma-like epithelial components with ghost cells, it is well known that ghost cells are also present in odontomas. Several reports have indicated that the incidence of ghost cells in odontomas is not constant (1–6). These ghost cells have been recognized as enlarged, eosinophilic epithelial cells with aberrant keratinization. Other than CCOT and odontoma, two tumor types display ghost cells or their homologue, shadow cells: adamantinomatous cranio-pharyngioma of the pituitary gland and pilomatrixoma of the skin. Recently, Kusama et al. (7) reported that newly developed antibodies against human hair proteins reacted with ghost, shadow or transitional cells of CCOT, adamantinomatous cranio-pharyngioma and pilomatrixoma. Human hair proteins are composed of hard keratins and matrix proteins, and it is thought that the Wnt- β -catenin-Tcf/Lef (T cell factor/lymphoid enhancer factor) pathway is involved in the expression of hard keratins (8, 9). This signaling pathway also has an important role in tumorigenesis, and β -catenin mutation and accumulation in these hard keratin-expressing tumors has been reported (10–17).

The present study investigated the presence of ghost cells and the Wnt signaling pathway in odontomas using immunohistochemistry.

Materials and methods

Cases diagnosed histopathologically as odontoma at the Division of Pathology, Department of Diagnostic and Therapeutic Sciences, Meikai University School of Dentistry, between August 1982 and September 2006 were used in this study. A total of 69 cases of odontoma were analysed. The specimens were fixed in 10% (v/v) neutral buffered formalin, demineralized in Plank and Rychlo solution, and embedded in paraffin wax. Each section was prepared for hematoxylin-eosin staining and immunohistochemical analysis.

Data on patient age, gender and lesion site were obtained from information submitted with the biopsy request forms and also reviews of the dental charts

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Table 1 Age, gender and histological variants of odontomas gender

Age	Male	Female	Compound	Complex
0–9	9 (5)	5 (3)	14 (8)	0 (0)
10–19	20 (15)	11 (7)	24 (21)	7 (1)
20–29	9 (7)	2 (2)	7 (7)	4 (2)
30–39	2 (2)	3 (2)	3 (3)	2 (1)
40–49	1 (0)	1 (1)	2 (1)	0 (0)
50–59	2 (0)	2 (1)	0 (0)	4 (1)
60–69	0 (0)	1 (1)	1 (1)	0 (0)
70–79	1 (0)	0 (0)	1 (0)	0 (0)
Total	44 (29)	25 (17)	52 (41)	17 (5)

Number of cases of ghost cells expression are given in parentheses.

Table 2 Location and histological variants of odontomas

Location	Maxilla	Mandibule	Compound	Complex
Incisor	28 (20)	12 (11)	39 (30)	1 (1)
Incisor-premolar	3 (3)	6 (5)	7 (6)	2 (2)
Premolar	3 (3)	4 (3)	6 (5)	1 (1)
Molar	7 (0)	6 (1)	0 (0)	13 (1)
Total	41 (26)	28 (20)	52 (41)	17 (5)

Number of cases of ghost cells expression are given in parentheses.

(Tables 1, 2). Odontomas in which ghost cells were observed were also studied clinicopathologically.

Immunohistochemical examination was conducted using the streptavidin peroxidase biotin complex method. Deparaffinized sections were immersed in absolute methanol containing 0.3% (v/v) hydrogen peroxide for 20 min at room temperature to block endogenous peroxidase activity. After washing with running water and phosphate-buffered saline (PBS, pH 7.4), the sections were immersed in a 0.01-M concentration of citrate buffer (pH 6.0) and heated in a microwave oven for 15 min (for β -catenin), as described by Shi et al. (18). The sections were incubated in 2% (w/v) bovine serum albumin in PBS for 15 min at room temperature to block non-specific reactions. Appropriately diluted polyclonal antibody against hair proteins 1 and 2 (PA-HP1 and PA-HP2) (10 μ g/ml), monoclonal antibody against hair protein 1 (MA-HP1) (supernatant, 1:50), anti- β -catenin antibody (1:200; BD Transduction Laboratories, KY, USA) and anti-Lef-1 antibody (1:20; Santa Cruz Biotechnology Inc., CA, USA) were applied to each section for 1 h at room temperature. The tissue sections were washed with PBS and then incubated with biotinylated goat anti-mouse IgG (heavy and light chains) antibody (dilution 1:200; Vector Laboratories Inc., Burlingame, CA, USA) or biotinylated horse anti-mouse IgG (heavy and light chains) antibody (dilution 1:200; Vector Laboratories) for 30 min at room temperature. An appropriately diluted streptavidin-peroxidase antibody (GIBCO-BRI, Grand Island, NY, USA) was applied to the tissue sections for 30 min. The sections were washed with PBS, immersed for 10 min in 0.05% (w/v) 3, 3'-diaminobenzidine tetrahydrochloride in a 0.05 M concentration of Tris-hydrochloride buffer (pH 7.6)

containing 0.01% (v/v) hydrogen peroxide, and then counterstained with Mayer's hematoxylin.

Results

All three antibodies, PA-HP1, PA-HP2 and MA-HP1, produced similar staining patterns. The antibodies reacted only with the cytoplasm of ghost cells in 46 (66.7%) of the 69 cases (Table 1).

Ghost cells positive for hair proteins were often observed in the odontogenic epithelium adjacent to immature enamel (Fig. 1A). They were frequently found within the odontogenic epithelium, in the calcified materials in the connective tissue, or in the interstitial portion of dental hard tissues. The ghost cells usually formed small foci and sometimes large masses (Fig. 1B). In immature odontomas, small numbers of ghost cells were found in the epithelial components resembling the enamel organ (Fig. 1C). In odontomas with broad calcification, few odontogenic epithelia were observed in comparison with immature odontomas. The ghost cells became mineralized to form calcified materials resembling Liesegang rings. Immunoreactivity for hair proteins was often detected in the centre of the Liesegang-ring-like structures (Fig. 1D).

Although positivity for β -catenin was usually found on the membrane of odontogenic epithelial components in odontomas, the epithelial cells adjacent to the ghost cells showed strong immunoreactivity in the nucleus as well as the cytoplasm, especially in immature odontomas (Fig. 2A). Weak positivity was also found in some ghost cells (Fig. 2B). Positivity for Lef-1 was also found in the nucleus of odontogenic epithelial cells adjacent to the ghost cells (Fig. 2C) and in the cytoplasm of the ghost cells themselves (Fig. 2D).

Ghost cells positive for hair proteins were observed in 41 (78.8%) of 52 compound odontomas and 5 (29.4%) of 17 complex odontomas (Table 1). The age of the patients ranged from 2 to 63 years, with a mean of 19.1 years. The highest incidence was 22 cases in the second decade of life (Table 1). By gender, 29 (65.9%) out of 44 males and 17 (68.5%) out of 25 females showed ghost cells in their odontomas (Table 1). By location, 26 (63.4%) of 41 maxillary cases and 20 (71.4%) of 28 mandibular cases showed ghost cells (Table 2). The most common location was the maxillary incisor region in 20 cases, followed by the mandibular incisor region in 11 (Table 2). Thirteen cases of compound odontoma contained ghost cells in the incisor region, but there was not much difference between locations in cases of complex odontoma (Table 2).

Discussion

Ghost cells, which are the most characteristic feature of CCOT, are also found in odontomas. The reported incidence of ghost cells in odontomas has ranged from 11% to 37.1% (1–4). However, Chang et al. reported a higher incidence of 83% in odontomas among Taiwanese (5). However, these studies were performed by using H-E-stained sections, which might have made it difficult

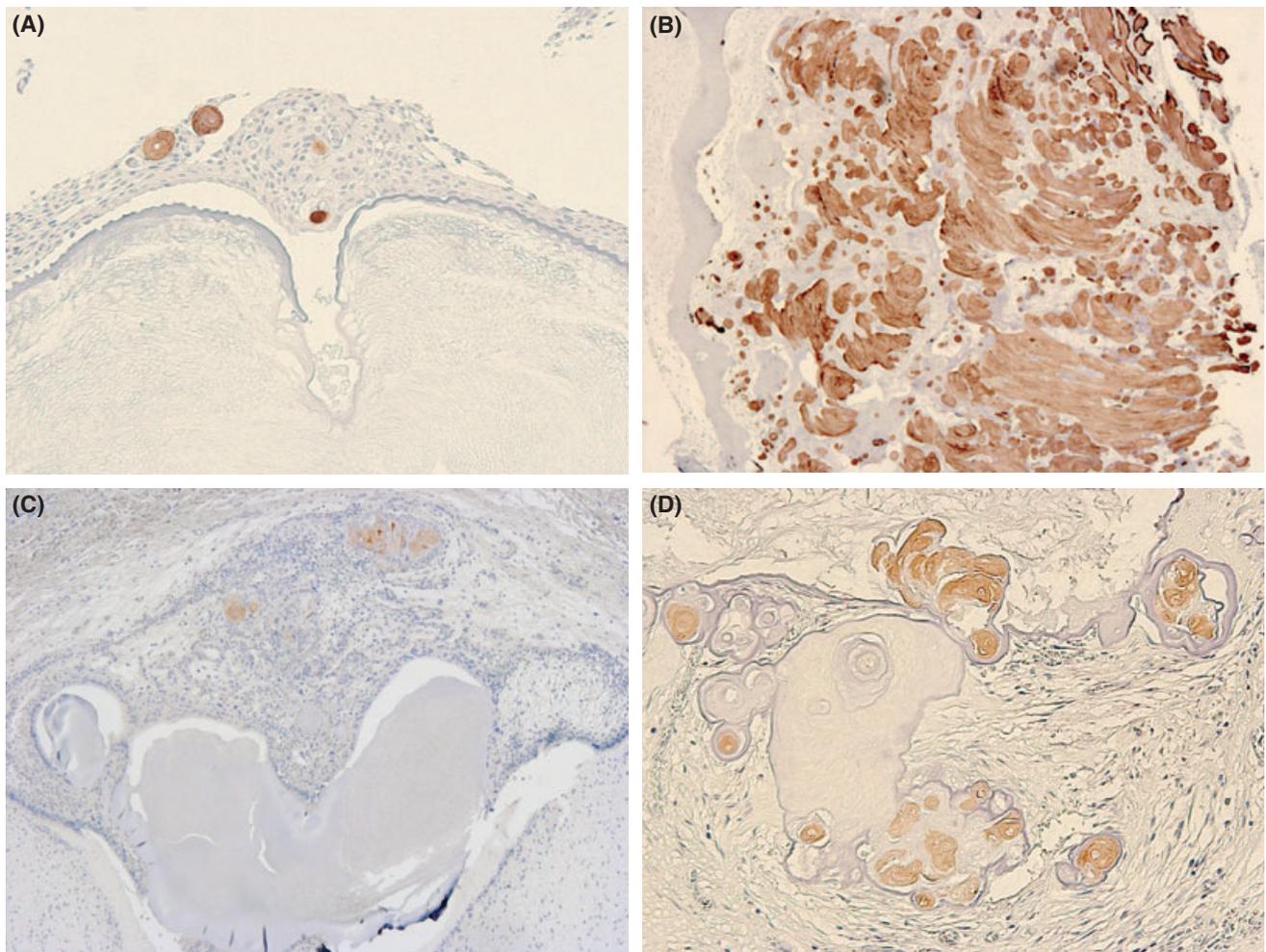


Figure 1 (A) Ghost cells show a positive reaction with antibody against hard keratins and are well recognized near the immature enamel matrix. (B) Many ghost cells form large clusters. (C) Immature odontoma in a young person. There is abundant odontogenic epithelium resembling the enamel organ and forming hard tissues. Several ghost cells are present in the odontogenic epithelium, but not in the calcified substances. (D) Ghost cells are often evident in Liesegang-ring-like structures consisting of dystrophic calcification.

to distinguish ghost cells among the other components of odontoma microscopically. In the present study, antibodies against hair proteins were applied to detect ghost cells in odontomas, and the cells were accurately observed in 66.7% of the 69 cases, which was a relatively high incidence.

Odontomas are generally classified into compound and complex types. The incidence of ghost cells in complex odontomas is reportedly higher than that in compound odontomas (2, 3, 5). However, in the present series, immunohistochemistry revealed a higher incidence of these cells in compound odontomas (78.8%) than in complex odontomas (29.4%). This result suggests that the presence of ghost cells might be correlated with the degree of differentiation of odontomas. Furthermore, the incidence of ghost cells in odontomas was not considered to be associated with age, gender, or location.

It has been reported that ghost cells in odontomas originate through metaplasia of the odontogenic epithelium with abnormal keratinization (1, 2, 6).

However ghost cells are not revealed by immunostaining with common cytokeratin antibodies (6). Recent studies have identified the expression of hard keratins in the cytoplasm of ghost cells in CCOT, adamantinomatous craniopharyngioma and pilomatrixoma by immunohistochemistry (7, 19, 20). In the present study, strong expression of hard keratins was also detected in the ghost cells of odontomas.

Activated alteration of the β -catenin gene and accumulation of β -catenin have been demonstrated in CCOT, adamantinomatous craniopharyngioma and pilomatrixoma, suggesting that the Wnt- β -catenin-Tcf/Lef activated pathway is closely related to tumorigenesis of these lesions (13–23). It has been reported that most hair keratin genes possess a Lef-1 binding site in the promoter domain (10–12). Interestingly, the Wnt signaling pathway is involved in the development of teeth (24), and the amelogenin and enamelin genes also possess a Lef-1 binding site in the promoter domain (17). In this study, immunoreactivity for β -catenin and Lef-1 was found in the cytoplasm and nucleus of

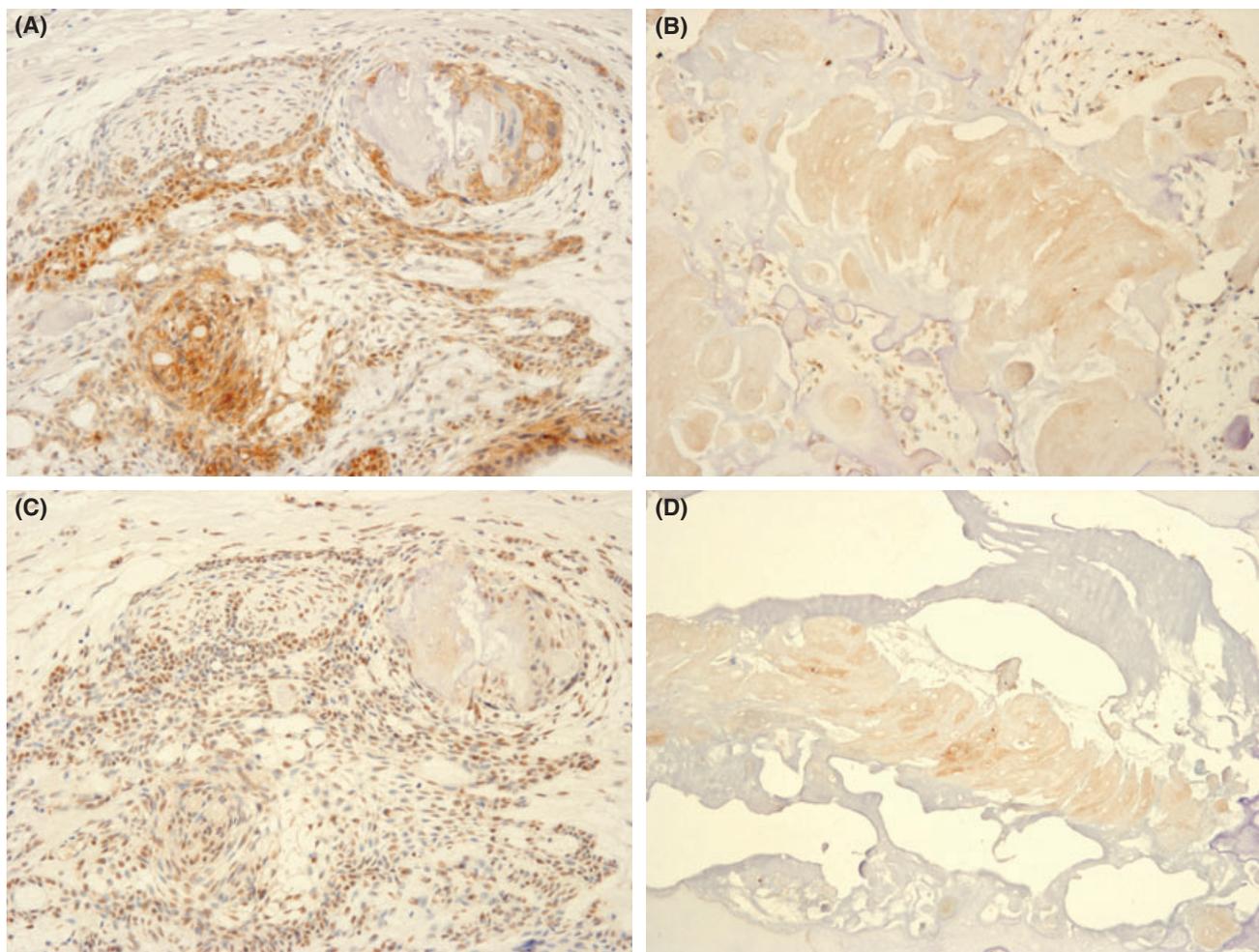


Figure 2 (A) Immature odontoma showing a positive reaction for β -catenin in the nucleus, cytoplasm and membrane of odontogenic epithelial cells around the ghost cells. (B) Some of the ghost cells are weakly positive for β -catenin. (C) Positive reactivity for Lef-1 is seen in the nucleus of odontogenic epithelial cells around the ghost cells. (D) Some of the ghost cells are weakly positive for Lef-1.

odontogenic epithelial cells adjacent to ghost cells in odontomas.

In conclusion, odontoma is a hard keratin-expressing tumor-like lesion, in which the Wnt signaling pathway may be involved in the formation of ghost cells. In addition, dystrophic calcification associated with ghost cells might be an important element in odontomas.

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