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Immunohistochemical detection of β-catenin and adenomatous polyposis coli in ameloblastomas

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BACKGROUND: To clarify the roles of the Wnt signaling pathway in oncogenesis and cytodifferentiation of odontogenic tumors, expression of β-catenin and adenomatous polyposis coli (APC) was analyzed in ameloblastomas as well as in tooth germs.

METHODS: Tissue specimens of 10 tooth germs, 40 benign ameloblastomas, and five malignant ameloblastomas were examined immunohistochemically with the use of antibodies against β-catenin and APC.

RESULTS: Immunohistochemical reactivity for β-catenin was detected in the cell membrane and cytoplasm of most odontogenic epithelial cells in tooth germs and ameloblastomas. Nuclear β-catenin expression was recognized in nine of 40 ameloblastomas and two of five malignant ameloblastomas, but not in tooth germs. APC was evidently expressed in odontogenic epithelial cells neighboring the basement membrane in tooth germs and ameloblastomas, and the reactivity was significantly lower in benign and malignant ameloblastomas than in tooth germs. Follicular ameloblastomas and acanthomatous ameloblastomas tended to show high nuclear β-catenin expression and low APC reactivity, as compared with other ameloblastoma variants.

CONCLUSION: Expression of β-catenin and APC in tooth germs and ameloblastomas suggests that aberration of the Wnt signaling pathway might play a role in oncogenesis and cytodifferentiation of odontogenic epithelium via deregulation of cell proliferation.

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Introduction

Tumors arising from epithelium of the odontogenic apparatus or from its derivatives or remnants exhibit considerable histological variation and are classified into several benign and malignant entities (1–4). Ameloblastoma is the most frequently encountered tumor arising from odontogenic epithelium and is characterized by a benign but locally invasive behavior with a high risk of recurrence (1, 2, 4). Histologically, ameloblastoma shows considerable variation, including follicular, plexiform, acanthomatous, granular cell, basal cell, and desmoplastic types (1). Malignant ameloblastoma is defined as a neoplasm in which the pattern of an ameloblastoma and cytological features of malignancy are shown by the primary growth in the jaws and/or by any metastatic growth (1). Recently, malignant ameloblastoma has been subclassified into metastasizing ameloblastoma and ameloblastic carcinoma on the basis of metastatic spread and cytological malignant features (3). Several recent studies have detected genetic and cytogenetic alterations in these epithelial odontogenic tumors (5, 6); however, the detailed mechanisms of oncogenesis, cytodifferentiation, and tumor progression remain unknown.

The Wnt signal transduction controls diverse developmental processes by regulating cell proliferation, morphology, motility, and fate in a variety of organisms ranging from nematode worms to mammals (7–9). Wnt signaling is regulated by the levels of an intracellular protein, β-catenin (7, 10). In the absence of Wnt signals, β-catenin is targeted for ubiquitination and proteasome-mediated degradation by phosphorylation through a multiprotein complex, including a serine/threonine kinase, glycogen synthase kinase 3β (GSK-3β), and a tumor suppressor gene product, adenomatous polyposis coli (APC) (10–12). When Wnt acts on its cell surface receptor, phosphorylation and degradation of β-catenin is suppressed, resulting in β-catenin accumulation in the cytoplasm and translocation into the nucleus. In the nucleus, β-catenin binds to transcription factors of the T-cell factor/lymphoid enhancing factor (TCF/LEF) family and stimulates the expression of target genes,

such as the cell cycle promoter cyclin D1 and the oncogene product c-myc (13–15). Recent data suggest that aberrant activation of the Wnt pathway by either mutations in *β-catenin* or by inactivation of *APC* leads to tumor formation (9, 16, 17).

Our previous study revealed expression of E-cadherin and α-catenin, with which β-catenin forms a complex required for cell adhesion, in epithelial odontogenic tumors, suggesting that these molecules are associated with cytodifferentiation or malignant potential of neoplastic odontogenic epithelium (18). Recently, mutations and nuclear expression of β-catenin have been seen in some epithelial odontogenic tumors (19). In the present study, immunohistochemical expression of β-catenin and APC in benign and malignant ameloblastomas as well as in tooth germs was examined to clarify the possible role of the Wnt signaling pathway in epithelial odontogenic tumors.

Materials and methods

Tissue preparation

Specimens were surgically removed from 45 patients with epithelial odontogenic tumors at the Department of Oral and Maxillofacial Surgery, Tohoku University Dental Hospital, and affiliated hospitals. The specimens were fixed in 10% buffered formalin for one to several days and were embedded in paraffin. The tissue blocks were sliced into 3-μm-thick sections for routine histological and subsequent immunohistochemical examinations. Tissue sections were stained with hematoxylin and eosin for histological diagnosis according to the WHO histological typing of odontogenic tumors (1). The tumors comprised 40 ameloblastomas and five malignant ameloblastomas. Ameloblastomas were divided into 23 follicular and 17 plexiform types, including 10 acanthomatous, six granular cell, three basal cell, and four desmoplastic subtypes. Malignant ameloblastomas were classified into two metastasizing ameloblastomas and three ameloblastic carcinomas according to the criteria of Eversole (3). Specimens of 10 tooth germs of the mandibular third molars, enucleated for orthodontic reasons at the initial stage of crown mineralization, were similarly prepared and compared with the epithelial odontogenic tumors.

Immunohistochemistry

The tissue sections were deparaffinized and immersed in methanol with 0.3% hydrogen peroxide. The sections for β-catenin immunostaining were heated in 0.01 M citrate buffer (pH 6.0) for 10 min by autoclave (121°C, 2 atm). After treatment with normal rabbit serum for 30 min, the sections were incubated with primary antibodies at 4°C overnight. The applied antibodies were mouse anti-β-catenin monoclonal antibody (Dako, Carpinteria, CA, USA; subclass IgG1; diluted at 1:200) and anti-APC monoclonal antibody (Novocastra, Newcastle, UK; subclass IgG2b; diluted at 1:30). The standard streptavidin-biotin-peroxidase complex method was performed to bind the primary antibodies with the use of Histofine SAB-PO Kits (Nichirei, Tokyo, Japan). Reaction products were visualized by immersing the sections in 0.03% diaminobenzidine solution containing 2 mM hydrogen peroxide for 1–3 min. Nuclei were lightly counterstained with methylgreen. For control studies of the antibodies, the serial sections were treated with phosphate-buffered saline, mouse anti-desmin monoclonal antibody (Nichirei; subclass IgG1), and anti-chromogranin A monoclonal antibody (Dako; subclass IgG2b) instead of the primary antibodies and were confirmed to be unstained.

Evaluation of immunostaining and statistical analysis

Immunohistochemical reactivity for β-catenin was evaluated for the localization: (M) membranous, (C) cytoplasmic reactivity, and (N) nuclear reactivity. Immunohistochemical reactivity for APC was evaluated and classified into three groups: (+) weak reactivity, (++) moderate reactivity, and (+++) strong reactivity.

The statistical significance of differences in the percentages of cases with different reactivity levels was analyzed by the Mann–Whitney *U*-test for differences between two groups or the Kruskal–Wallis test for differences among three or more groups. *P*-values < 0.05 were considered to indicate statistical significance.

Results

Immunohistochemical reactivity for β-catenin and APC in tooth germs and ameloblastomas is summarized in Table 1. Expression of β-catenin was detected in the cell membrane and cytoplasm of both normal and neoplastic odontogenic epithelial cells, and nuclear β-catenin reactivity was found only in neoplastic odontogenic epithelial cells (Fig. 1). In tooth germs, β-catenin was expressed in most epithelial cells of enamel organs and dental laminae (Fig. 1a). Ameloblastomas showed membranous and cytoplasmic reactivity for β-catenin in most neoplastic cells, and nuclear β-catenin expression was recognized in some peripheral columnar or cuboidal cells in six of 23 follicular ameloblastomas and three of 17 plexiform ameloblastomas (Fig. 1b,c). β-catenin reactivity was decreased in keratinizing cells of acanthomatous ameloblastomas and in granular cells of granular cell ameloblastomas, and nuclear expression was detected in four of 10 acanthomatous ameloblastomas and one of six granular cell ameloblastomas. Basal cell ameloblastomas and desmoplastic ameloblastomas did not show nuclear β-catenin reactivity. Metastasizing ameloblastomas and ameloblastic carcinomas showed membranous and cytoplasmic reactivity for β-catenin in most neoplastic cells, similar to benign ameloblastomas. Nuclear reactivity was recognized in some peripheral cells in one of two metastasizing ameloblastomas and in numerous neoplastic cells in one of three ameloblastic carcinomas (Fig. 1d).

The APC immunoreactivity was detected in the cytoplasm of both normal and neoplastic odontogenic epithelial cells (Fig. 2). In tooth germs, APC was expressed in epithelial cells of enamel organs and dental laminae, and reactivity in inner and outer enamel epithelium was stronger than that in other epithelial

Table 1 Immunohistochemical reactivity for β-catenin and APC in tooth germs and ameloblastomas

		β-catenin		*APC*			
	n	*M+C*	*N*	*+*	*++*	*+++*	
Tooth germ	10	10 (100)	0 (0)	0 (10)	5 (50)	5 (50)	**
Ameloblastoma	40	40 (100)	9 (22)	10 (25)	28 (70)	2 (5)	*
Follicular type	23	23 (100)	6 (26)	7 (30)	15 (65)	1 (5)	
Plexiform type	17	17 (100)	3 (18)	3 (18)	13 (76)	1 (6)	
Acanthomatous subtype	10	10 (100)	4 (40)	5 (50)	5 (50)	0 (0)	
Granular subtype	6	6 (100)	1 (17)	1 (17)	5 (88)	0 (0)	
Basal cell subtype	3	3 (100)	0 (0)	0 (0)	3 (100)	0 (0)	
Desmoplastic subtype	4	4 (100)	0 (0)	1 (25)	3 (75)	0 (0)	
Non-cellular variation	17	17 (100)	4 (24)	3 (18)	12 (70)	2 (12)	
Malignant ameloblastoma	5	5 (100)	2 (40)	2 (40)	3 (60)	0 (0)	
Metastasizing ameloblastoma	2	2 (100)	1 (50)	0 (0)	2 (100)	0 (0)	
Ameloblastic carcinoma	3	3 (100)	1 (33)	2 (67)	1 (33)	0 (0)	

β-catenin reactivity: M, membranous; C, cytoplasmic; N, nuclear.
APC reactivity: +, weak; ++, moderate; +++, strong.
Values in parentheses denote percentage values.
Statistical significance: *$P < 0.05$; **$P < 0.01$.

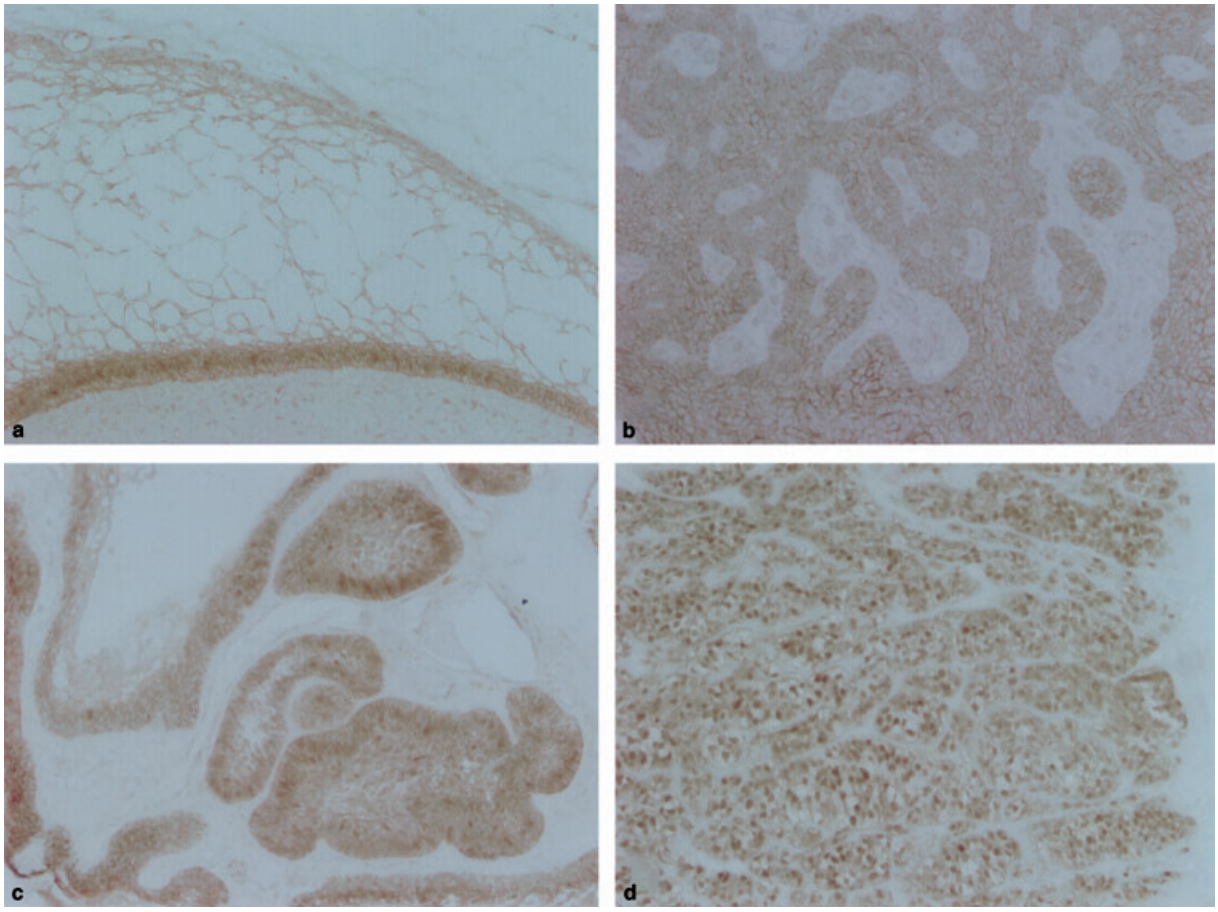

Figure 1 Immunohistochemical reactivity for β-catenin. (a) Tooth germ showing membranous and cytoplasmic reactivity in enamel organ (×105). (b) Plexiform ameloblastoma showing membranous and cytoplasmic reactivity in neoplastic cells (×105). (c) Follicular ameloblastoma showing membranous and cytoplasmic reactivity in neoplastic cells. Nuclear expression is also found in scattered peripheral neoplastic cells (×105). (d) Ameloblastic carcinoma showing nuclear reactivity in many neoplastic cells. Membranous and cytoplasmic expression is weak (×105).

components (Fig. 2a). APC reactivity levels in tooth germs were significantly higher than those in ameloblastomas ($P < 0.01$) and malignant ameloblastomas ($P < 0.05$), as analyzed by the Kruskal–Wallis test. Ameloblastomas showed APC reactivity in many peripheral columnar or cuboidal cells and some central

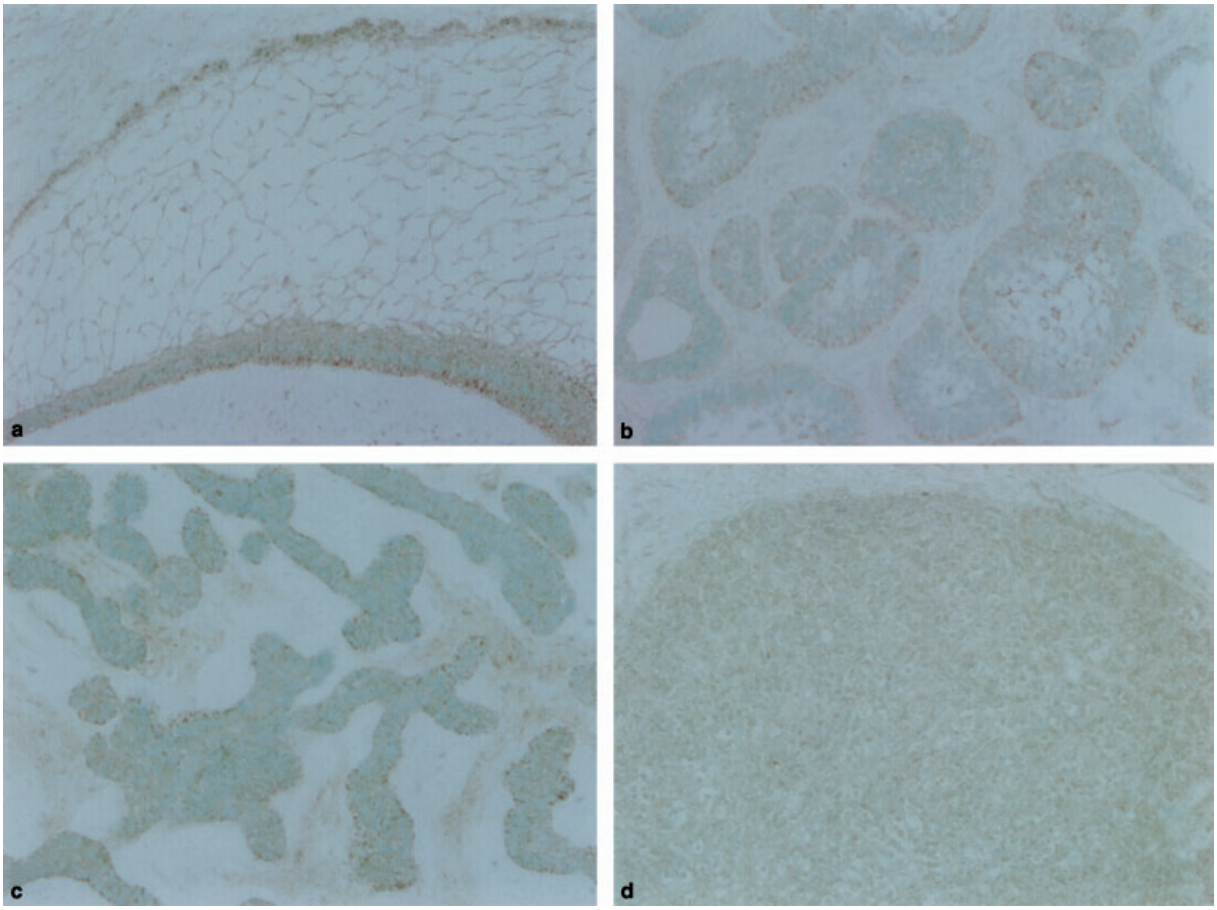

Figure 2 Immunohistochemical reactivity for APC. (a) Tooth germ showing moderate to strong reactivity in inner and outer enamel epithelium and weak reactivity in stratum intermedium and stellate reticulum (×95). (b) Follicular ameloblastoma showing reactivity in many peripheral columnar cells and some central polyhedral cells (×125). (c) Basal cell ameloblastoma showing reactivity in most neoplastic cells (×115). (d) Ameloblastic carcinoma showing weak reactivity in most neoplastic cells (×105).

polyhedral cells (Fig. 2b). Keratinizing cells in acanthomatous ameloblastomas and granular cells in granular cell ameloblastomas exhibited no APC reactivity. Basal cell ameloblastomas and desmoplastic ameloblastomas showed APC reactivity in most neoplastic cells (Fig. 2c). Metastasizing ameloblastomas showed an APC expression pattern similar to that of benign ameloblastomas, whereas ameloblastic carcinomas exhibited weak to moderate reactivity in most neoplastic cells (Fig. 2d).

Discussion

The Wnt signaling pathway controls many developmental decisions, including embryonic axis formation, axial specification in limb development, and organogenesis of the nervous system, heart, kidney, mammary gland, hair follicles, and teeth (7, 8, 20). Homozygous inactivation of *β-catenin* or *APC* causes embryonic lethality (21, 22). Several Wnt signaling molecules, including β-catenin and APC, are expressed during tooth development (20, 23–25). Mice targeted for the Wnt signaling molecule *LEF-1* show inhibition of tooth morphogenesis (26). Familial adenomatous polyposis (FAP) caused by germline mutations in *APC* gene is an autosomal dominant inherited disease characterized by multiple adenomatous polyps in the colon and rectum, leading to the development of colorectal cancer, and is often associated with dental anomalies, such as odontomas and supernumerary teeth (27, 28). In the present study, immunoreactivity for β-catenin and APC was recognized in epithelial components of tooth germ tissues, similar to murine odontogenic tissues, suggesting that Wnt signal transduction is required for tooth formation, especially amelogenesis by enamel organs (23–25).

β-catenin, originally isolated by its association with the cytoplasmic domain of the cell adhesion molecule cadherin, has a signaling role in the Wnt pathway to control cellular proliferation or cell death (7, 29). Mutations in *β-catenin* gene have been reported in a wide variety of human tumors, including colorectal carcinoma, gastric carcinoma, hepatocellular carcinoma, malignant melanoma, desmoid tumor, craniopharyngioma, and pilomatricoma, and are considered to result in nuclear accumulation of β-catenin and constitutive activation of TCF/LEF-dependent transcription, which are implicated in tumor formation (9, 16, 30–32). Our previous study detected expression of E-cadherin and α-catenin, which form a cell adhesion complex with

β-catenin, in epithelial odontogenic tumors (18). In the present study, β-catenin reactivity was recognized in the cell membrane and cytoplasm of most neoplastic cells in ameloblastomas. These features suggest that β-catenin plays dual roles in cell-cell adhesion and signal transduction in neoplastic odontogenic epithelium. Ameloblastomas have shown nuclear β-catenin accumulation, although *β-catenin* mutations are rare (19). In our study, nuclear β-catenin expression was detected in some ameloblastomas but not in tooth germs. Neoplastic odontogenic epithelial cells with nuclear β-catenin expression were distributed near the basement membrane, and the localization of such expression was similar to the expression of cyclin D or c-myc, targets of Wnt signaling, in ameloblastomas (33, 34). These features suggest that activation of the Wnt signal pathway is associated with oncogenesis of odontogenic epithelium. In addition, nuclear β-catenin expression was slightly higher in follicular ameloblastomas than in plexiform ameloblastomas, and acanthomatous ameloblastomas tended to show higher nuclear β-catenin expression than the other ameloblastoma subtypes, suggesting that the Wnt signaling pathway might affect tissue structuring or cytodifferentiation of ameloblastomas.

The tumor suppressor protein APC down-regulates the Wnt signaling pathway by inducing β-catenin degradation, inhibiting cell cycle progression (7, 10, 35). Defects of *APC* gene cause the loss of β-catenin regulation and facilitate activation of the Wnt signaling pathway, similar to *β-catenin* mutations (9, 16, 17). Germline mutations in *APC* gene are responsible for FAP, while somatic *APC* mutations occur in 60–80% of sporadic colorectal tumors (17, 27, 36). Mice heterozygous for *APC* develop multiple intestinal neoplasia, and these neoplastic lesions show decreased APC expression (37, 38). In the present study, APC expression was evident in neoplastic cells neighboring the basement membrane in ameloblastomas. Our previous studies detected proliferating cells neighboring the basement membrane and apoptotic cells detached from the basement membrane in ameloblastomas (33, 34, 39). These features suggest that APC might control cell proliferation of neoplastic odontogenic epithelium. In our previous study, E-cadherin and α-catenin were expressed strongly in central polyhedral cells and weakly in peripheral columnar or cuboidal cells in benign ameloblastomas (18). These results provide evidence that binding of APC and cadherin to β-catenin is mutually exclusive (25, 40). In this study, immunoreactivity for APC in benign and malignant ameloblastomas was significantly lower than that in tooth germs. Follicular ameloblastomas and acanthomatous ameloblastomas tended to show low APC reactivity, as compared with other ameloblastoma variants. This decreased APC expression is considered to be linked to nuclear β-catenin translocation in ameloblastomas, and the dysregulated Wnt signaling pathway is possibly involved in oncogenesis or cytodifferentiation of odontogenic epithelium.

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