J Oral Pathol Med (2004) 33: 360–7

www.blackwellmunksgaard.com/jopm

K-*Ras* gene status and expression of Ras/mitogen-activated protein kinase (MAPK) signaling molecules in ameloblastomas

Hiroyuki Kumamoto[1], Nobuhiro Takahashi[2], Kiyoshi Ooya[1]

[1]*Division of Oral Pathology, Department of Oral Medicine and Surgery, and* [2]*Division of Oral Ecology and Biochemistry, Department of Oral Biology, Tohoku University Graduate School of Dentistry, Sendai, Japan*

BACKGROUND: To clarify the roles of rat sarcoma (Ras)/mitogen-activated protein kinase (MAPK) signaling pathway in oncogenesis and cytodifferentiation of odontogenic tumors, K-*Ras* gene status and expression of Ras, Raf1, MAPK/extracellular signal-regulated kinase (ERK) kinase (MEK)1, and ERK1/2 proteins were analyzed in ameloblastomas as well as in tooth germs.

METHODS: Paraffin sections of 10 tooth germs and 46 benign and 6 malignant ameloblastomas were examined immunohistochemically for the expression of K-Ras, Raf1, MEK1, and ERK1/2. Frozen tissue samples of 22 benign ameloblastomas and 1 malignant (metastasizing) ameloblastoma were analyzed by direct DNA sequencing to detect K-*Ras* gene alteration.

RESULTS: Immunohistochemical reactivity for K-Ras, Raf1, MEK1, and ERK1/2 was detected in both normal and neoplastic odontogenic epithelium, and these molecules were reactive chiefly with odontogenic epithelial cells neighboring the basement membrane. Plexiform ameloblastomas showed slightly stronger expression of these Ras/MAPK signaling molecules than follicular ameloblastomas. Keratinizing cells and granular cells showed decreased reactivity for the signaling molecules. Basal cell ameloblastomas showed slightly stronger reactivity for the signaling molecules than did the other subtypes. K-Ras immunoreactivity in malignant ameloblastomas was lower than that in dental lamina of tooth germs. Direct DNA sequencing showed a GGT to GCT point mutation at codon 12 of K-*Ras* gene in one ameloblastoma.

CONCLUSION: Expression of K-Ras, Raf1, MEK1, and ERK1/2 in tooth germs and ameloblastomas suggests that Ras/MAPK signaling pathway functions to regulate cell proliferation and differentiation in both normal and neoplastic odontogenic epithelium. K-*Ras* gene status implied that K-*Ras* mutations might play a minor role in oncogenesis of odontogenic epithelium.

J Oral Pathol Med (2004) **33**: 360–7

Keywords: ameloblastoma; ERK; K-Ras; MEK; Raf

Introduction

Tumors arising from epithelium of the odontogenic apparatus or from its derivatives or remnants exhibit considerable histologic 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 (5–7); however, the detailed mechanisms of oncogenesis, cytodifferentiation, and tumor progression remain unknown.

Ras proto-oncogenes were originally characterized on the basis of homology with the transforming genes of rat sarcoma viruses (v-*Ras*), and three *Ras* genes, H-*Ras*, K-*Ras*, and N-*Ras*, were identified in the mammalian genome (8–10). Activation of *Ras* genes by mutation contributes to malignant transformation, and K-*Ras* mutations have been detected in various human neoplasms (10–16). *Ras* genes encode highly similar

Correspondence: Hiroyuki Kumamoto, Division of Oral Pathology, Department of Oral Medicine and Surgery, Tohoku University Graduate School of Dentistry, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan. Tel.: +81 227178303. Fax: +81 22 7178304. E-mail: kumamoto@mail.tains.tohoku.ac.jp
Accepted for publication October 21, 2003

guanine nucleotide-binding proteins of approximately 21 kDa ($p21^{Ras}$), and $p21^{Ras}$ is involved in the transduction of external stimuli most likely induced by growth factors (10, 17, 18). These stimuli activate $p21^{Ras}$ by inducing the exchange of GDP to GTP, and GTP-bound $p21^{Ras}$ contributes the activation of three closely related Raf serine/threonine kinases: Raf1, B-Raf, and A-Raf (10, 16, 18). In downstream, activated Raf phosphorylates and activates mitogen-activated protein kinase (MAPK) kinases, MAPK/extracellular signal-regulated kinase (ERK) kinase (MEK)1 and MEK2 (16,18). Phosphorylated MEK functions as dual-specificity kinases and phosphorylates tandem threonine and tyrosine residues in MAPK, ERK1, and ERK12 to activate them (16, 18). Once activated, ERK translocates to the nucleus and activates a variety of substrates, including nuclear transcription factors (16–18). Thus, Ras/MAPK signaling pathway functions as a key regulator of cell proliferation and differentiation, and aberration of involved signaling components has been identified in a various of human tumors (16, 18–23).

Our previous studies confirmed cellular kinetics, including proliferation and cell death modulators, in tooth germs and ameloblastomas, suggesting that these factors are associated with oncogenesis or cytodifferentiation of odontogenic epithelium (24–29). Several studies have examined the expression of specific Ras/MAPK signaling molecules in tooth germs or odontogenic cysts and tumors (30–32). In the present study, the immunohistochemical expression of K-Ras, Raf1, MEK1, and ERK1/2 proteins and mutation of K-*Ras* gene were examined in ameloblastomas as well as in tooth germs to clarify the possible role of Ras/MAPK signaling pathway in epithelial odontogenic tumors.

Materials and methods

The study protocol was reviewed and approved by the Research Ethics Committee of Tohoku University Graduate School of Dentistry.

Tissue preparation

Specimens were surgically removed from 52 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 embedded in paraffin. The tissue blocks were sliced into 3-μm-thick sections for routine histologic and subsequent immunohistochemical examinations. Tissue sections were stained with hematoxylin and eosin for histologic diagnosis according to the WHO histologic typing of odontogenic tumors (1). The tumors comprised 46 ameloblastomas and 6 malignant ameloblastomas. Ameloblastomas were divided into 30 follicular and 16 plexiform types, including 15 acanthomatous, 5 granular cell, 3 basal cell and 4 desmoplastic subtypes. Malignant ameloblastomas were classified into two metastasizing ameloblastomas and four ameloblastic carcinomas according to the criteria of Eversole (3). For direct DNA sequencing, tumor tissues were immediately frozen on dry ice and stored at −80°C. 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 for K-Ras, Raf1, MEK1, and ERK1/2 expression

The tissue sections were deparaffinized, immersed in methanol with 0.3% hydrogen peroxide, and heated in 0.01 M citrate buffer (pH 6.0) for 10 min by autoclave (121°C, 2 atm). After treatment with normal serum for 30 min, the sections were incubated with primary antibodies at 4°C overnight. The applied antibodies were mouse anti-K-Ras monoclonal antibody (Oncogene, Boston, MA, USA; subclass IgG2a; diluted at 1 : 20), mouse anti-Raf1 monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA; subclass IgG1; diluted at 1 : 100), mouse anti-MEK1 monoclonal antibody (Santa Cruz Biotechnology; subclass IgG2b; diluted at 1 : 100), and rabbit anti-ERK1/2 polyclonal antibody (Cell Signaling Technology, Beverly, MA, USA; diluted at 1 : 20). 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-L26 (CD20) monoclonal antibody (Nichirei; subclass IgG2a), mouse anti-OPD4 (CD45RO) monoclonal antibody (Dako, Glostrup, Denmark; subclass IgG1), mouse antichromogranin A monoclonal antibody (Dako; subclass IgG2b), and normal rabbit IgG instead of the primary antibodies and were confirmed to be unstained.

Immunohistochemical reactivity for K-Ras, Raf1, MEK1, and ERK1/2 was evaluated and classified into three groups: (+) focally weak to moderate reactivity; (++) focally strong reactivity or diffusely weak to moderate reactivity; and (+++) diffusely 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 less than 0.05 were considered to indicate statistical significance.

*Direct DNA sequencing for K-*Ras *gene mutations*

Genomic DNA was extracted from frozen tissue samples of 22 benign ameloblastomas and 1 malignant ameloblastoma using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). K-*Ras* exons 1 and 2, including hotspot codons 12, 13, and 61, were separately amplified using a HotstarTaq Master Mix Kit (Qiagen) with specific primers in a DNA thermal cycler (Eppendorf, Hamburg, Germany). Primers used in this study were as follows: 5′-GACTGAATATAAACTTGTGG-3′

(forward) and 5′-CTATTGTTGGATCATATTCG-3′ (reverse) for exon 1, yielding a 107-bp product, and 5′-GATTCCTACAGGAAGCAAGT-3′ (forward) and 5′-CTATAATGGTGAATATCTTTC-3′ (reverse) for exon 2, yielding a 185-bp product. Polymerase chain reaction (PCR) was performed in a total volume of 50 μl, containing 0.5 μg of template DNA, 1.5 mM (for exon 1) or 3 mM (for exon 2) of $MgCl_2$, and 0.5 mM of each specific primer set. The procedure for amplification included 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and elongation at 72°C for 60 s, with heat starting at 95°C for 15 min, and final elongation at 72°C for 10 min.

Sequencing reactions of each K-*Ras* exon were carried out with the PCR products purified using an GFR PCR DNA and Gel Band Purification Kit (Amersham Biosciences, Little Chalfont, UK), the above-mentioned PCR primers, and a Thermo Sequenase Cy5 Dye Terminator Sequencing Kit (Amersham Biosciences). The sequencing products were separated on denatured 8% polyacrylamide gel on an automated laser fluorescence sequencer (ALFexpress II DNA Sequencer; Amersham Biosciences), and the sequencing data were analyzed with the use of an ALFwin Sequence Analyzer (Amersham Biosciences).

Results

Immunohistochemical reactivity for K-Ras, Raf1, MEK1, and ERK1/2

Immunohistochemical reactivity for K-Ras was detected in the cytoplasm and cell membrane of normal and neoplastic odontogenic epithelial cells (Fig. 1). In tooth germs, K-Ras reactivity in inner and outer enamel epithelium and dental lamina was more evident than that in stratum intermedium and stellate reticulum (Fig. 1A). Ameloblastomas showed K-Ras reactivity in many peripheral columnar or cuboidal cells and some central polyhedral cells (Fig. 1B). K-Ras expression in keratinizing cells in acanthomatous ameloblastomas and granular cells in granular cell ameloblastomas was low. Basal cell ameloblastomas and desmoplastic ameloblastomas showed K-Ras reactivity in most neoplastic cells. Metastasizing ameloblastomas showed a K-Ras expression pattern similar to that of follicular ameloblastomas, while ameloblastic carcinomas demonstrated weak to moderate K-Ras reactivity in neoplastic cells (Fig. 1C). Reactivity for K-Ras in malignant ameloblastomas was significantly lower than that in dental lamina ($P < 0.05$) (Table 1).

Expression of Raf1 and MEK1 was found in the cytoplasm of normal and neoplastic odontogenic epithelial cells (Figs. 2 and 3). Tooth germs showed Raf reactivity in inner enamel epithelium and dental lamina (Fig. 2A). In ameloblastomas, Raf1 reactivity in peripheral columnar or cuboidal cells was more evident than that in central polyhedral cells. Plexiform ameloblastomas exhibited statistically higher Raf1 expression than follicular ameloblastomas ($P < 0.05$) (Table 1). Keratinizing cells in acanthomatous ameloblastomas and granular cells in granular cell ameloblastomas showed markedly decreased reactivity for Raf1. Basal cell ameloblastomas and desmoplastic ameloblastomas showed diffuse Raf1 expression in neoplastic cells, and staining intensity in basal cell ameloblastomas was

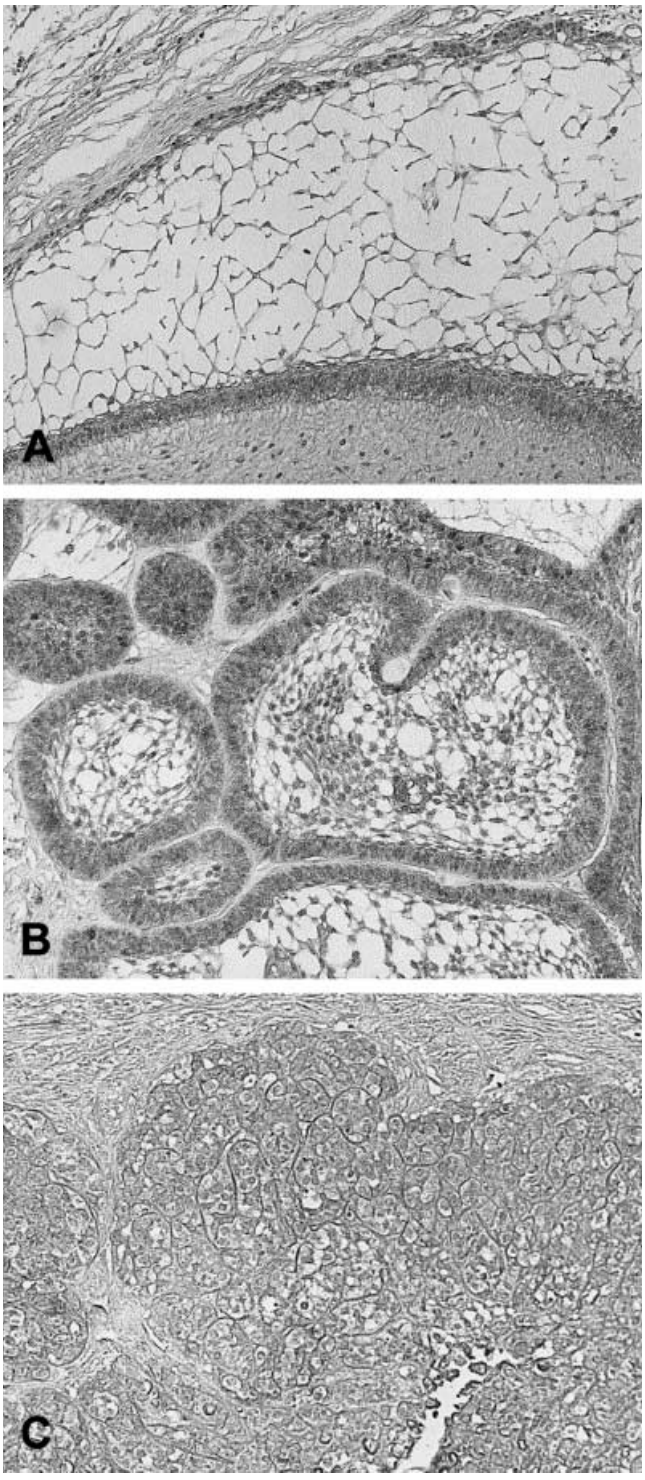

Figure 1 Immunohistochemical reactivity for K-Ras. (A) Tooth germ showing moderate to strong reactivity in inner and outer enamel epithelium and weak reactivity in stratum intermedium and stellate reticulum (×100). (B) Follicular ameloblastoma showing strong reactivity in peripheral columnar cells and weak to moderate reactivity in central polyhedral cells (×125). (C) Ameloblastic carcinoma showing weak reactivity in most neoplastic cells (×120).

Table 1 Immunohistochemical reactivity for K-Ras, Raf1, MEK1, and ERK1/2 in tooth germs and ameloblastomas

	K-Ras				*Raf1*				*MEK1*				*ERK1/2*			
	(+)	(++)	(+++)		(+)	(++)	(+++)		(+)	(++)	(+++)		(+)	(++)	(+++)	
Tooth germ ($n = 10$)																
Enamel organ ($n = 10$)	0 (0%)	7 (70%)	3 (30%)		0 (0%)	10 (100%)	0 (0%)		0 (0%)	6 (60%)	4 (40%)		0 (0%)	5 (50%)	5 (50%)	
Dental lamina ($n = 5$)	0 (0%)	3 (60%)	2 (40%)		0 (0%)	5 (100%)	0 (0%)		0 (0%)	2 (40%)	3 (60%)		0 (0%)	2 (40%)	3 (60%)	
Ameloblastoma ($n = 46$)	4 (9%)	38 (82%)	4 (9%)		5 (11%)	30 (65%)	11 (24%)		1 (2%)	32 (70%)	13 (28%)		4 (9%)	29 (63%)	13 (28%)	
Follicular type ($n = 30$)	4 (13%)	24 (80%)	2 (7%)		4 (13%)	22 (74%)	4 (13%)	*	1 (3%)	20 (67%)	9 (30%)		4 (13%)	19 (64%)	7 (23%)	
Plexiform type ($n = 16$)	0 (0%)	14 (87%)	2 (13%)		1 (6%)	8 (50%)	7 (44%)		0 (0%)	12 (75%)	4 (25%)		0 (0%)	10 (62%)	6 (38%)	
Acanthomatous subtype ($n = 15$)	2 (13%)	11 (74%)	2 (13%)	*	3 (20%)	10 (67%)	2 (13%)		0 (0%)	12 (80%)	3 (20%)		1 (9%)	10 (66%)	4 (27%)	
Granular subtype ($n = 5$)	1 (20%)	4 (80%)	0 (0%)		1 (20%)	3 (60%)	1 (20%)		0 (0%)	4 (80%)	1 (20%)		1 (20%)	4 (80%)	0 (0%)	
Basal cell subtype ($n = 3$)	0 (0%)	2 (67%)	1 (33%)		0 (0%)	1 (33%)	2 (67%)		0 (0%)	2 (67%)	1 (33%)		0 (0%)	1 (33%)	2 (67%)	
Desmoplastic subtype ($n = 4$)	0 (0%)	4 (100%)	0 (0%)		0 (0 %)	4 (100%)	0 (0%)		0 (0%)	3 (75%)	1 (25%)		0 (0%)	1 (25%)	3 (75%)	
Malignant ameloblastoma ($n = 6$)	1 (17%)	5 (83%)	0 (0%)		1 (17%)	4 (66%)	1 (17%)		0 (0%)	4 (67%)	2 (33%)		0 (0%)	4 (67%)	2 (33%)	
Metastasizing ameloblastoma ($n = 2$)	0 (0%)	2 (100%)	0 (0%)		0 (0%)	2 (100%)	0 (0%)		0 (0%)	2 (100%)	0 (0%)		0 (0%)	2 (100%)	0 (0%)	
Ameloblastic carcinoma ($n = 4$)	1 (25%)	3 (75%)	0 (0%)		1 (25%)	2 (50%)	1 (25%)		0 (0%)	2 (50%)	2 (50%)		0 (0%)	2 (50%)	2 (50%)	

Immunohistochemical reactivity: (+) focally weak to moderate reactivity; (++) focally strong reactivity or diffusely weak to moderate reactivity; (+++) diffusely strong reactivity.
Statistical significance: $^*P < 0.05$.

Figure 2 Immunohistochemical reactivity for Raf1. (A) Tooth germ showing reactivity in inner enamel epithelium (×105). (B) Basal cell ameloblastoma showing reactivity in most neoplastic cells (×115). (C) Ameloblastic carcinoma showing reactivity in most neoplastic cells (×115).

remarkable (Fig. 2B). Metastasizing ameloblastomas showed a Raf1 expression pattern similar to that of follicular ameloblastomas, while ameloblastic carcinomas were positive for Raf1 in most neoplastic cells (Fig. 2C).

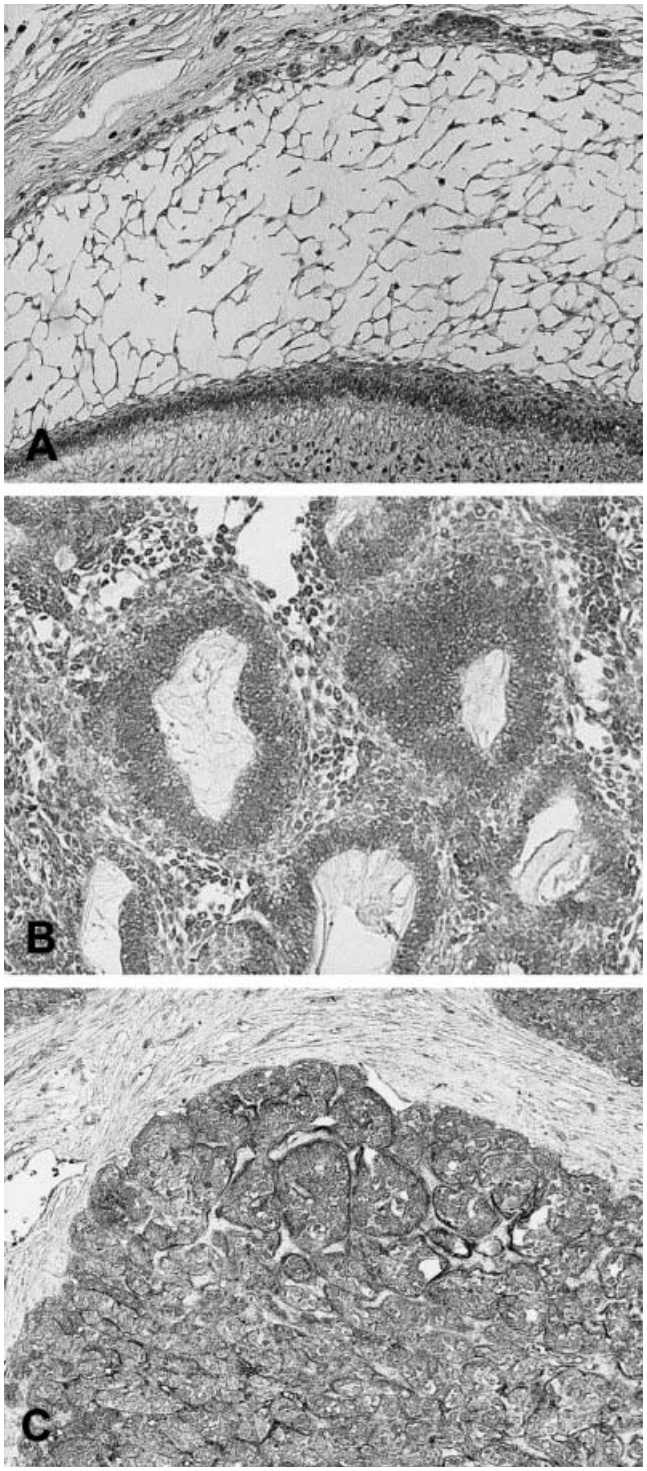

Figure 3 Immunohistochemical reactivity for MEK1. (A) Tooth germ showing strong reactivity in inner enamel epithelium and weak to moderate reactivity in outer enamel epithelium, stratum intermedium and stellate reticulum (×100). (B) Plexiform ameloblastoma showing strong reactivity in peripheral columnar or cuboidal cells and weak to moderate reactivity in central polyhedral cells (×125). (C) Ameloblastic carcinoma showing strong reactivity in most neoplastic cells (×100).

MEK1 expression was detected in most epithelial cells in tooth germs, and reactivity in inner enamel epithelium was stronger than that in other epithelial components (Fig. 3A). Ameloblastomas showed MEK1 expression in most neoplastic cells, and reactivity in peripheral columnar or cuboidal calls was stronger than that in central polyhedral cells (Fig. 3B). Keratinizing cells in acanthomatous ameloblastomas and granular cells in granular cell ameloblastomas demonstrated low reactivity for MEK1. Basal cell ameloblastomas and desmoplastic ameloblastomas showed diffuse MEK1 expression in neoplastic cells. Metastasizing ameloblastomas showed a MEK1 expression pattern similar to that of follicular ameloblastomas, while ameloblastic carcinomas were moderately to strongly positive for MEK1 in most neoplastic cells (Fig. 3C).

Immunohistochemical reactivity for ERK1/2 was detected usually in the cytoplasm and often in the nuclei of normal and neoplastic odontogenic epithelial cells (Fig. 4). In tooth germs, ERK1/2 expression was found in most epithelial cells, and reactivity in inner and outer enamel epithelium was stronger than that in other epithelial components (Fig. 4A). Ameloblastomas showed ERK1/2 expression in most neoplastic cells, and reactivity in peripheral columnar or cuboidal cells was stronger than that in central polyhedral cells. Keratinizing cells in acanthomatous ameloblastomas and granular cells in granular cell ameloblastomas demonstrated markedly decreased reactivity for ERK1/2 (Fig. 4B). Basal cell ameloblastomas and desmoplastic ameloblastomas showed diffuse ERK1/2 expression in neoplastic cells. Metastasizing ameloblastomas showed a ERK1/2 expression pattern similar to that of follicular ameloblastomas, while ameloblastic carcinomas were moderately to strongly positive for ERK1/2 in most neoplastic cells (Fig. 4C).

*Mutation analysis of K-*Ras *gene*

Direct DNA sequencing for K-*Ras* gene mutations was carried out in 22 ameloblastomas (13 follicular and 9 plexiform cases, including 7 acanthomatous, 3 granular cell, 1 basal cell and 1 desmoplastic subtypes) and 1 malignant ameloblastoma (1 metastasizing ameloblastoma). A GGT to GCT (glycine to alanine) point mutation was detected at codon 12 in exon 1 of K-*Ras* gene in one follicular ameloblastoma without cellular subtype (Fig. 5). Mutational alteration was not detected at codon 13 in exon 1 or codon 61 in exon 2 of K-*Ras* gene in any of the 23 cases.

Discussion

RAS/MAPK signaling pathway is a primordial signaling system that controls such fundamental cellular processes as cell proliferation and differentiation (16, 18). Mouse embryos homozygous for K-*Ras* mutation die *in utero*, suggesting that K-*Ras* is essential for embryogenesis (33). Expression of Raf1 is recognized in various mouse fetal tissues (34). MEK1 and ERK are known to be necessary for PC12 cell neuronal differentiation (35, 36). These features suggest that Ras/MAPK signaling pathway plays a role in cellular regulation during developmental processes (34–36). Raf-1 expression has been detected at different stages of mouse tooth

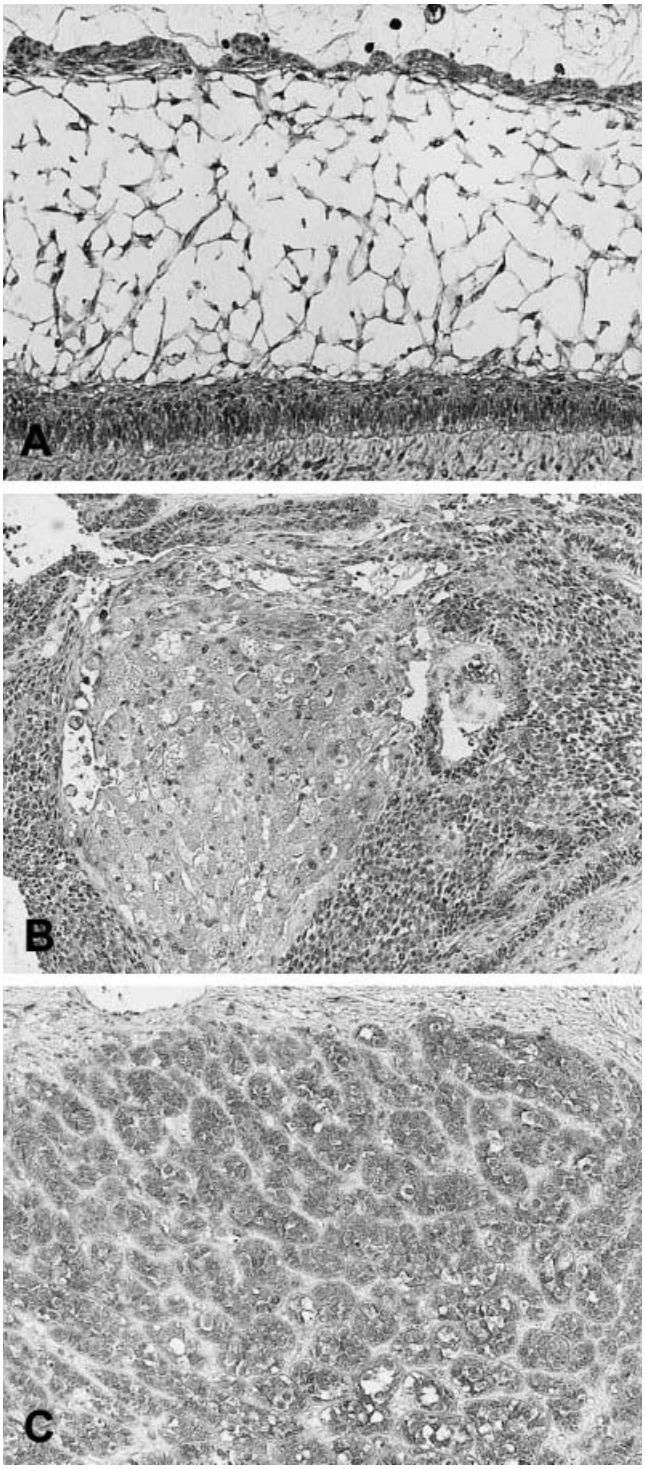

Figure 4 Immunohistochemical reactivity for ERK1/2. (A) Tooth germ showing strong reactivity in inner and outer enamel epithelium and weak to moderate reactivity in stratum intermedium and stellate reticulum (×125). (B) Granular cell ameloblastoma showing marked reactivity in peripheral cuboidal cells and central polyhedral cells and decreased reactivity in granular cells (×95). (C) Ameloblastic carcinoma showing strong reactivity in most neoplastic cells (×115).

germ development (31), and ERK reactivity has been studied in odontogenic epithelial rests neighboring human odontogenic cysts (32). In the present study,

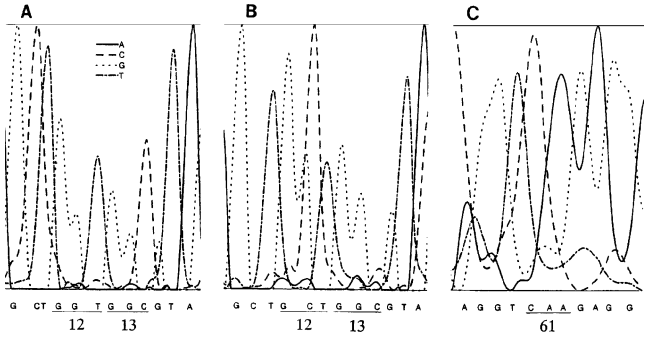

Figure 5 Direct DNA sequencing of K-*Ras* gene in ameloblastoma. (A) No mutation at codons 12 and 13 in exon 1. (B) A GGT to GCT point mutation at codon 12 in exon 1. (C) No mutation at codon 61 in exon 2.

Ras/MAPK signaling molecules were detected in epithelial components of tooth germs at the initial stage of crown mineralization. These features suggest that Ras signaling plays a role in cell proliferation and differentiation during tooth development.

Ras signaling functions as a relay switch in downstream of cell surface receptor tyrosine kinases, including receptors for many growth factors, such as epidermal (EGF), hepatocyte (HGF), platelet-derived (PDGF), insulin-like (IGF), fibroblast (FGF), vascular endothelial (VEGF), and nerve (NGF) growth factors (17, 18). Receptors for EGF, FGF, and HGF have been investigated in odontogenic tumors, suggesting that these receptor tyrosine kinases affect cell proliferation in oncogenesis or malignant transformation of odontogenic epithelium (29, 37–39). Aberrant expression and/or activation of signal transducing proteins are linked with neoplastic change (16, 20, 22). Alterations in Ras/MAPK signaling pathway, such as overexpression or constitutive activation of signaling molecules, have been detected in various malignancies, including lung, renal, prostate, breast, ovarian, and oral carcinomas (19, 21, 23, 40–42). Overexpression of $p21^{Ras}$ in ameloblastomas and activation of ERK in odontogenic cysts are related to the biological behavior of the odontogenic lesions (30, 32). In the present study, ameloblastomas expressed Ras/MAPK signaling molecules evidently in peripheral neoplastic cells, and basal cell ameloblastomas tended to show stronger reactivity for the signaling molecules than did the other subtypes. These features suggest that Ras/MAPK signaling pathway plays a role in promoting the proliferation of ameloblastoma cells. However, expression of Ras/MAPK signaling molecules in ameloblastomas did not clearly differ from that in tooth germs or malignant ameloblastomas. K-Ras immunoreactivity in malignant ameloblastomas was lower than that in dental lamina of tooth germs, whereas no apparent difference was found in reactivity for Raf1, MEK1, or ERK1/2 between tooth germs and malignant ameloblastomas. These results did not clearly show that these molecules have a specific role in oncogenesis or malignant transformation of odontogenic epithelium. In this study, expression of Ras/MAPK signaling molecules in plexiform ameloblastomas was slightly stronger than that in

follicular ameloblastomas. Keratinizing cells and granular cells showed decreased reactivity for the signaling molecules in acanthomatous and granular cell ameloblastomas. These features suggest that Ras/MAPK signaling might play a role in tissue structuring and/or cytodifferentiation of ameloblastomas.

A series of genetic alterations appear to promote the development of tumors via multiple steps (13, 43). Point mutations at codons 12, 13, and 61 of K-*Ras* gene are found in approximately 30% of solid tumors, and the incidences of these mutations are high in pancreatic, colorectal, lung, ovarian, and endometrial carcinomas (13, 14, 44–46). Mutated Ras product constitutively transduces signals and promotes cellular proliferation (10, 16). In the present study using direct DNA sequencing, a point mutation of K-*Ras* was detected at codon 12 in only 1 of 22 ameloblastomas and 1 metastasizing ameloblastoma, suggesting that K-*Ras* mutation might play a minor role in neoplastic change of odontogenic epithelium. Our immunohistochemical examination revealed relatively low reactivity for K-Ras product in ameloblastic carcinomas; however, mutation analysis of K-*Ras* gene in ameloblastic carcinomas could not be investigated because of the rarity of this malignancy. Further studies should be carried out to determine the association between K-*Ras* and the malignant potential of odontogenic epithelium.

References

1. Kramer IRH, Pindborg JJ, Shear M. *WHO Histological Typing of Odontogenic Tumours*. Berlin: Springer-Verlag, 1992; 11–27.
2. Melrose RJ. Benign epithelial odontogenic tumors. *Semin Diagn Pathol* 1999; **16**: 271–87.
3. Eversole LR. Malignant epithelial odontogenic tumors. *Semin Diagn Pathol* 1999; **16**: 317–24.
4. Sciubba JJ, Fantasia JE, Kahn LB. *Tumors and Cysts of the Jaw*. Washington, DC: Armed Forces institute of Pathology, 2001: 71–99.
5. Heikinheimo K, Jee KJ, Niini T, et al. Gene expression profiling of ameloblastoma and human tooth germ by means of a cDNA microarray. *J Dent Res* 2002; **81**: 525–30.
6. Jaakelainen K, Jee KJ, Leivo I, Saloniemi I, Knuutila S, Heikinheimo K. Cell proliferation and chromosomal changes in human ameloblastoma. *Cancer Genet Cytogenet* 2002; **136**: 31–7.
7. Shibata T, Nakata D, Chiba I, et al. Detection of TP53 mutation in ameloblastoma by the use of a yeast functional assay. *J Oral Pathol Med* 2002; **31**: 534–8.
8. Der CJ, Krontiris TG, Cooper GM. Transforming genes of human bladder and lung carcinoma cell lines are homologous to the *ras* genes of Harvey and Kirsten sarcoma viruses. *Proc Natl Acad Sci USA* 1982; **79**: 3637–40.
9. Shimizu K, Goldfarb M, Suard Y, et al. Three human transforming genes are related to the viral *ras* oncogenes. *Proc Natl Acad Sci USA* 1983; **80**: 2112–6.
10. Barbacid M. *ras* genes. *Ann Rev Biochem* 1987; **56**: 779–827.
11. Capon DJ, Seeburg PH, McGrath JP, et al. Activation of Ki-ras2 gene in human colon and lung carcinomas by two different point mutations. *Nature* 1983; **304**: 507–13.
12. Almoguera C, Shibata D, Forrester K, Martin J, Arnheim N, Perucho M. Most human carcinomas of the exocrine pancreas contain mutant c-K-*ras* genes. *Cell* 1988; **53**:549–54.
13. Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. *N Engl J Med* 1988; **319**: 525–32.
14. Bos JL. *ras* oncogenes in human cancer: a review. *Cancer Res* 1989; **49**: 4682–9.
15. White MA, Nicolette C, Minden A, et al. *Cell* 1995; **80**: 533–41.
16. Seger R, Krebs EG. The MAPK signaling cascade. *FASEB J* 1995; **9**: 726–35.
17. Marshall CJ. Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. *Cell* 1995; **80**: 179–85.
18. Kolch W. Meaningful relationships. the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions. *Biochem J* 2000; **351**: 289–305.
19. Hajj C, Akoum R, Bradley E, Paquin F, Ayoub J. DNA alterations at proto-oncogene loci and their clinical significance in operable non-small cell lung cancer. *Cancer* 1990; **66**: 733–9.
20. Gulbis B, Galand P. Immunodetection of the p21-*ras* products in human normal and preneoplastic tissues and solid tumors: a review. *Hum Pathol* 1993; **24**: 1271–85.
21. Oka H, Chatani Y, Hoshino R, et al. Constitutive activation of mitogen-activated protein (MAP) kinases in human renal cell carcinoma. *Cancer Res* 1995; **55**: 4182–7.
22. Hoshino R, Chatani Y, Tamori T, et al. Constitutive activation of the 41-/43-kDa mitogen-activated protein kinase signaling pathway in human tumors. *Oncogene* 1999; **18**: 813–22.
23. Albanell J, Codony-Sevat J, Rojo F, et al. Activated extracellular signal–regulated kinases: association with epidermal growth factor receptor/transforming growth factor α expression in head and heck squamous carcinoma and inhibition by anti-epidermal growth factor receptor treatments. *Cancer Res* 2001; **61**: 6500–10.
24. Kumamoto H. Detection of apoptosis-related factors and apoptotic cells in ameloblastomas. analysis by immunohistochemistry and an in situ DNA nick end-labelling method. *J Oral Pathol Med* 1997; **26**: 419–25.
25. Kumamoto H, Ooya K. Immunohistochemical analysis of bcl-2 family proteins in benign and malignant ameloblastomas. *J Oral Pathol Med* 1999; **28**: 343–9.
26. Kumamoto H, Kimi K, Ooya K. Immunohistochemical analysis of apoptosis-related factors (Fas, Fas ligand, caspase-3 and single-stranded DNA) in ameloblastomas. *J Oral Pathol Med* 2001; **30**: 596–602.
27. Kumamoto H, Kinouchi Y, Ooya K. Telomerase activity and telomerase reverse transcriptase (TERT) expression in ameloblastomas. *J Oral Pathol Med* 2001; **30**: 231–6.
28. Kumamoto H, Kimi K, Ooya K. Detection of cell cycle-related factors in ameloblastomas. *J Oral Pathol Med* 2001; **30**: 309–15.
29. Kumamoto H, Yoshida M, Ooya K. Immunohistochemical detection of hepatocyte growth factor, transforming growth factor-β and their receptors in epithelial odontogenic tumors. *J Oral Pathol Med* 2002; **31**: 539–48.
30. Sandros J, Heikinheimo K, Happonen R-P, Stenman G. Expression of p21RAS in odontogenic tumors. *APMIS* 1991; **99**: 15–20.
31. Sunohara M, Tanzawa H, Kaneko Y, Fuse A, Sato K. Expression patterns of Raf-1 suggest multiple roles in tooth development. *Calcif Tissue Int* 1996; **58**: 60–4.
32. Nickolaychuk B, McNicol A, Gilchrist J, Birek C. Evidence for a role of mitogen-activated protein kinases in proliferating and differentiating odontogenic epithelia

of inflammatory and developmental cysts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002; **93**: 720–9.
33. Johnson L, Greenbaum D, Cichowski K, et al. K-*ras* is an essential gene in the mouse with partial functional overlap with N-*ras*. *Genes Dev* 1997; **11**: 2468–81.
34. Storm SM, Cleveland JL, Rapp UR. Expression of *raf* family proto-oncogenes in normal mouse tissues. *Oncogene* 1990; **5**: 345–51.
35. Qui M-S, Green SH. PC12 cell neuronal differentiation is associated with prolonged $p21^{ras}$ activity and consequent prolonged ERK activity. *Neuron* 1992; **9**: 705–17.
36. Cowley S, Paterson H, Kemp P, Marchall C. Activation of MAP kinase kinase is necessary and sufficient for PC12 differentiation and for transformation of NIH 3T3 cells. *Cell* 1994; **77**: 841–52.
37. Shrestha P, Yamada K, Higashiyama H, Takagi H, Mori M. Epidermal growth factor receptor in odontogenic cysts and tumors. *J Oral Pathol Med* 1992; **21**: 314–7.
38. Heikinheimo K, Voutilainen R, Happonen R-P, Miettinen PJ. EGF receptor and its ligands, EGF and TGF-α, in developing and neoplastic human odontogenic tissues. *Int J Dev Biol* 1993; **37**: 387–96.
39. So F, Daley TD, Jackson L, Wysocki GP. Immunohistochemical localization of fibroblast growth factors FGF-1 and FGF-2, and receptors FGFR2 and FGFR3 in the epithelium of human odontogenic cysts and tumors. *J Oral Pathol Med* 2001; **30**: 428–33.
40. Sivaraman VS, Wang H-Y, Nuovo GJ, Malbon CC. Hyperexpression of mitogen-activated protein kinase in human breast cancer. *J Clin Invest* 1997; **99**: 1478–83.
41. Magi-Galluzzi C, Mishra R, Fiorentino M, et al. Mitogen-activated protein kinase phosphatase 1 is overexpressed in prostate cancers and is inversely relate to apoptosis. *Laboratory Invest* 1997; **76**: 37–51.
42. Kupryjanczyk J, Szymanska T, Madry R, et al. Evaluation of clinical significance of TP53, BCL-2, BAX and MEK1 expression in 229 ovarian carcinomas treated with platinum-based regimen. *Br J Cancer* 2003; **88**: 848–54.
43. Fearon ER, Vogelstein. A genetic model for colorectal tumorigenesis. *Cell* 1990; **61**: 759–67.
44. Forrester K, Almoguera C, Han K, Grizzle WE, Peruch M. Detection of high incidence of K-*ras* oncogenes during human colon tumorigenesis. *Nature* 1987; **327**: 298–303.
45. Enomoto T, Weghorst CM, Inoue M, Tanizawa O, Rice JM. K-*ras* activation occurs frequently in mucinous adenocarcinomas and rarely in other common epithelial tumors of the human ovary. *Am J Pathol* 1991; **139**: 777–85.
46. Imamura T, Arima T, Kato H, Miyamoto S, Sasazuki T, Wake N. Chromosomal deletions and K-*ras* gene mutations in human endometrial carcinomas. *Int J Cancer* 1992; **51**: 47–52.