Oral Diseases (2007) 13, 461–467. doi:10.1111/j.1601-0825.2006.01321.x

http://www.blackwellmunksgaard.com

ORIGINAL ARTICLE

Immunohistochemical detection of phosphorylated Akt, PI3K, and PTEN in ameloblastic tumors

Hiroyuki Kumamoto, Kiyoshi Ooya

Division of Oral Pathology, Department of Oral Medicine and Surgery, Tohoku University Graduate School of Dentistry, Sendai, Japan

OBJECTIVE: To evaluate roles of the Akt signaling pathway in oncogenesis and cytodifferentiation of odontogenic tumors, expression of phosphorylated Akt (pAkt), PI3K, and PTEN was analyzed in ameloblastic tumors as well as in tooth germs.

METHODS: 11 tooth germs, 40 ameloblastomas, and 5 malignant ameloblastic tumors were examined immunohistochemically with antibodies against pAkt, PI3K, and PTEN.

RESULTS: Immunoreactivity for pAkt, PI3K, and PTEN was detected predominantly in odontogenic epithelial cells near the basement membrane in tooth germs and ameloblastic tumors. The levels of immunoreactivity for pAkt and PI3K were slightly higher in ameloblastic tumors than in tooth germs. Plexiform ameloblastomas showed significantly higher expression of PI3K than follicular ameloblastomas, and PI3K immunoreactivity in ameloblastomas without cellular variation was significantly higher than that in acanthomatous ameloblastomas. The level of PTEN immunoreactivity was significantly lower in ameloblastomas than in tooth germs.

CONCLUSION: Expression of pAkt, PI3K, and PTEN in tooth germs and ameloblastic tumors suggests that these signaling molecules regulate cell survival and growth in normal and neoplastic odontogenic tissues by mediating growth factor signals. Increased expression of pAkt and PI3K and decreased expression of PTEN in ameloblastic tumors may participate in oncogenesis of odontogenic epithelium by activating the Akt signaling pathway.

Oral Diseases (2007) **13**, 461–467

Keywords: Akt; ameloblastoma; PI3K; PTEN; tooth germ

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: +81-22-717-8303, Fax: +81-22-717-8304, E-mail: kumamoto@mail.tains.tohoku.ac.jp
Received 22 May 2006; revised 12 July 2006; accepted 6 August 2006

Introduction

Tumors arising from the epithelium of the odontogenic apparatus or from its derivatives or remnants exhibit considerable histological variation and are classified into several benign and malignant entities (Sciubba *et al*, 2001; Philipsen *et al*, 2005). 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 (Sciubba *et al*, 2001; Philipsen *et al*, 2005). Histologically, the ameloblastoma shows considerable variation, including follicular, plexiform, acanthomatous, granular cell, basal cell, and desmoplastic variants (Philipsen *et al*, 2005). Malignant counterparts of ameloblastoma are classified into metastasizing ameloblastoma and ameloblastic carcinoma on the basis of metastatic spread and cytological malignant features (Philipsen *et al*, 2005). Recent studies have identified genetic and molecular alterations in these epithelial odontogenic tumors (Heikinheimo *et al*, 2002; Kumamoto, 2006); however, the detailed mechanisms of oncogenesis, cytodifferentiation, and tumor progression remain unknown.

A critical balance between the rate of cell survival and cell death is important during embryonic development and in maintenance of adult tissues, and perturbation of regulatory systems results in various pathologic conditions, such as neoplasms, autoimmune diseases, and degenerative disorders (Blume-Jensen and Hunter, 2001). The Akt signaling pathway functions downstream of many growth factor receptors, similar to the Ras/mitogen-activated protein kinase (MAPK) pathway, and provides survival signals that protect cells from apoptosis (Datta *et al*, 1999). Briefly, growth factor-activated receptor tyrosine kinases recruit phosphatidylinositol 3-kinase (PI3K) to the plasma membrane. Once localized to the plasma membrane, PI3K catalyzes the transfer of phosphate to membrane-localized phosphoinositides, thereby generating 3′-phosphorylated phosphoinositides, which recruit inactive Akt to the plasma membrane. Akt translocated to the plasma membrane is phosphorylated by regulatory kinases in proximity, and phosphorylated Akt (pAkt) regulates numerous molecules in the apoptotic

machinery, such as Bad, a Bcl-2 family member promoting cell death, and caspase-9, an initiator caspase, resulting in suppression of apoptosis and promotion of cell survival (Datta *et al*, 1999; Blume-Jensen and Hunter, 2001). In contrast to PI3K, phosphatase and tensin homolog deleted on chromosome 10 (PTEN) dephosphorylates PI3K-generated 3′-phosphorylated phosphoinositides, thereby negatively regulating the Akt signaling pathway (Stambolic *et al*, 1998). Until recently, alterations of Akt, PI3K, and PTEN involved in the Akt signaling pathway have been identified in a variety of human tumors (Staal, 1987; Li *et al*, 1997; Shayesteh *et al*, 1999).

Previous studies have confirmed the expression of growth factor receptors with tyrosine kinase activity, including epidermal growth factor receptor, fibroblast growth factor receptors, and c-Met, and Ras/MAPK signaling molecules in odontogenic tumors, suggesting that growth factor signaling contributes to cell proliferation and differentiation of odontogenic tissues (Heikinheimo *et al*, 1993; So *et al*, 2001; Kumamoto *et al*, 2002, 2004b). In the present study, immunohistochemical expression of pAkt, PI3K, and PTEN was examined in benign and malignant ameloblastic tumors as well as in tooth germs to evaluate the role of the Akt signaling pathway in oncogenesis and cytodifferentiation of 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 classification of odontogenic tumors (Philipsen *et al*, 2005). The tumors comprised 40 ameloblastomas and five malignant ameloblastic tumors. Ameloblastomas were divided into 22 follicular and 18 plexiform types, including nine acanthomatous, six granular cell, three basal cell, and four desmoplastic subtypes. Malignant ameloblastic tumors were classified into two metastasizing ameloblastomas and three ameloblastic carcinomas. Specimens of 11 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 serial sections were deparaffinized and immersed in methanol with 0.3% hydrogen peroxide. The sections were heated in 1 mM ethylenediamine tetraacetic acid (EDTA) buffer (pH 8.0; for pAkt and PI3K) or 0.01 M citrate buffer (pH 6.0; for PTEN) for 10 min by autoclave (121°C, 2 atm). The sections were then incubated with primary antibodies at 4°C overnight. The applied antibodies were rabbit anti-pAkt polyclonal antibody (Cell Signaling Technology, Beverly, MA, USA; diluted at 1:15), mouse anti-PI3K monoclonal

Table 1 Immunohistochemical reactivity for pAkt, PI3K, and PTEN in tooth germs and ameloblastic tumors

		pAkt		*PI3K*		*PTEN*	
	n	+	++	+	++	+	++
Tooth germ	11	9 (82)	2 (18)	8 (73)	3 (27)	1 (9)	10[*3] (91)
Ameloblastoma	40	19 (48)	21 (52)	17 (43)	23 (57)	21 (52)	19[*3] (48)
Follicular type	22	12 (55)	10 (45)	12 (55)	10[*1] (45)	12 (55)	10 (45)
Plexiform type	18	7 (39)	11 (61)	5 (28)	13[*1] (72)	9 (50)	9 (50)
Acanthomatous subtype	9	6 (67)	3 (33)	7 (78)	2[*2] (22)	6 (67)	3 (33)
Granular subtype	6	4 (67)	2 (33)	3 (50)	3 (50)	4 (67)	2 (33)
Basal cell subtype	3	2 (67)	1 (33)	2 (67)	1 (33)	2 (67)	1 (33)
Desmoplastic subtype	4	3 (75)	1 (25)	3 (75)	1 (25)	2 (50)	2 (50)
Non-cellular variation	18	4 (22)	14 (78)	2 (11)	16[*2] (89)	7 (39)	11 (61)
Malignant ameloblastic tumors	5	3 (60)	2 (40)	2 (40)	3 (60)	2 (40)	3 (60)
Metastasizing ameloblastoma	2	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)
Ameloblastic carcinoma	3	2 (67)	1 (33)	1 (33)	2 (67)	1 (33)	2 (67)

Immunohistochemical reactivity: (+) weakly to moderately positive and (++) strongly positive.
Values in parentheses denote percentage values.
Statistical significance: $^{*1-3}P < 0.05$.

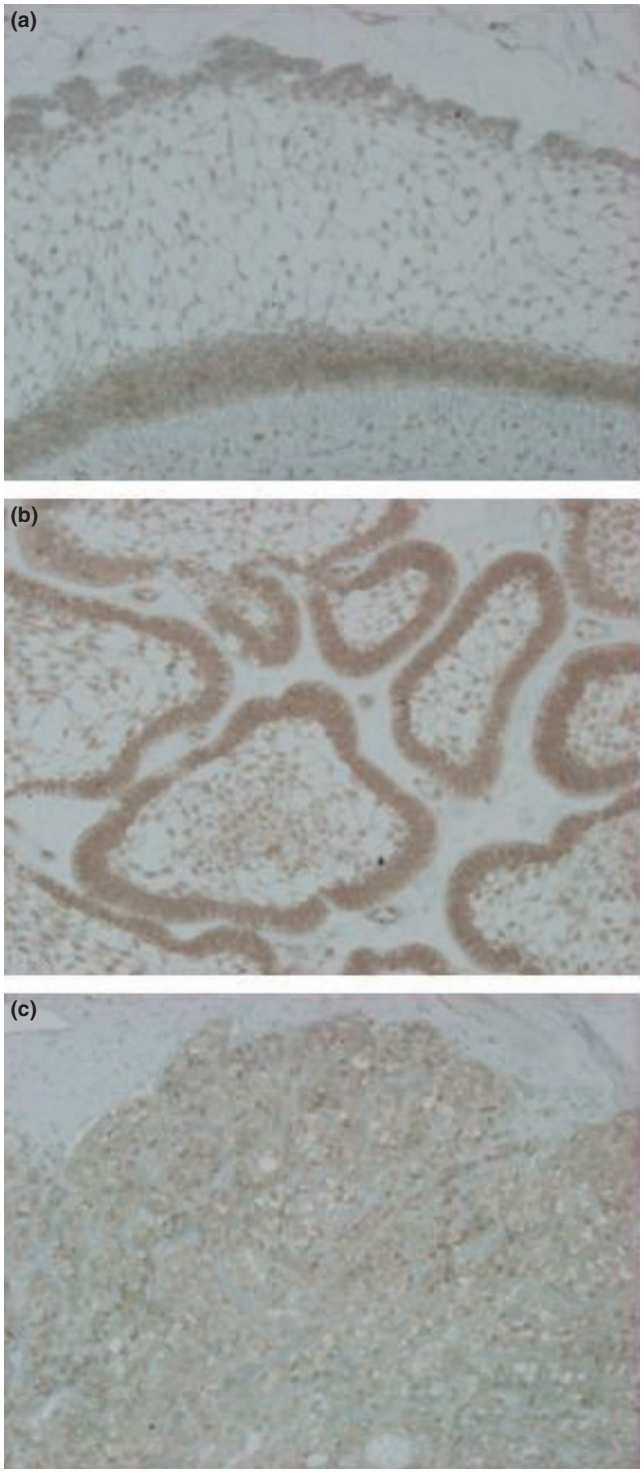

Figure 1 Immunohistochemical reactivity for pAkt. (**a**) Tooth germ showing strong reactivity in inner and outer enamel epithelium and weak reactivity in stratum intermedium and stellate reticulum. (x115) (**b**) Follicular ameloblastoma showing strong reactivity in peripheral columnar cells and weak reactivity in central polyhedral cells. (x120) (**c**) Ameloblastic carcinoma showing weak to moderate reactivity in most neoplastic cells. (x115)

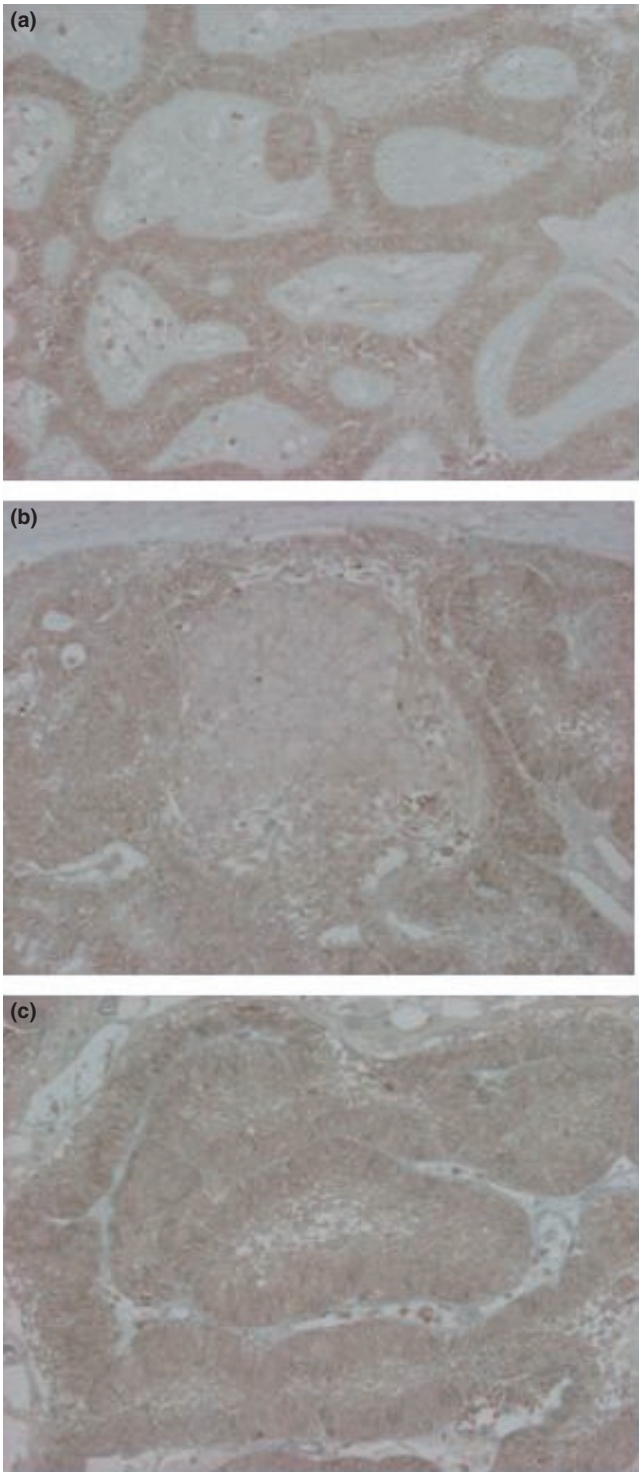

Figure 2 Immunohistochemical reactivity for P13K. (**a**) Plexiform ameloblastoma showing strong reactivity in peripheral cuboidal cells and weak reactivity in central polyhedral cells. (x105) (**b**) Granular cell ameloblastoma showing no reactivity in granular cells. (x95) (**c**) Metastasizing ameloblastoma showing strong reactivity in peripheral columnar cells and weak reactivity in central polyhedral cells. (x105)

antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA; subclass IgG1; diluted at 1:15), and mouse anti-PTEN monoclonal antibody (Lab Vision Corporation, Fremont, CA, USA; subclass IgM; diluted at 1:25). The sections were allowed to react with peroxidase-conjugated anti-rabbit IgG (for pAkt) or anti-mouse IgG (for PI3K and PTEN) polyclonal antibody (Histofine Simple

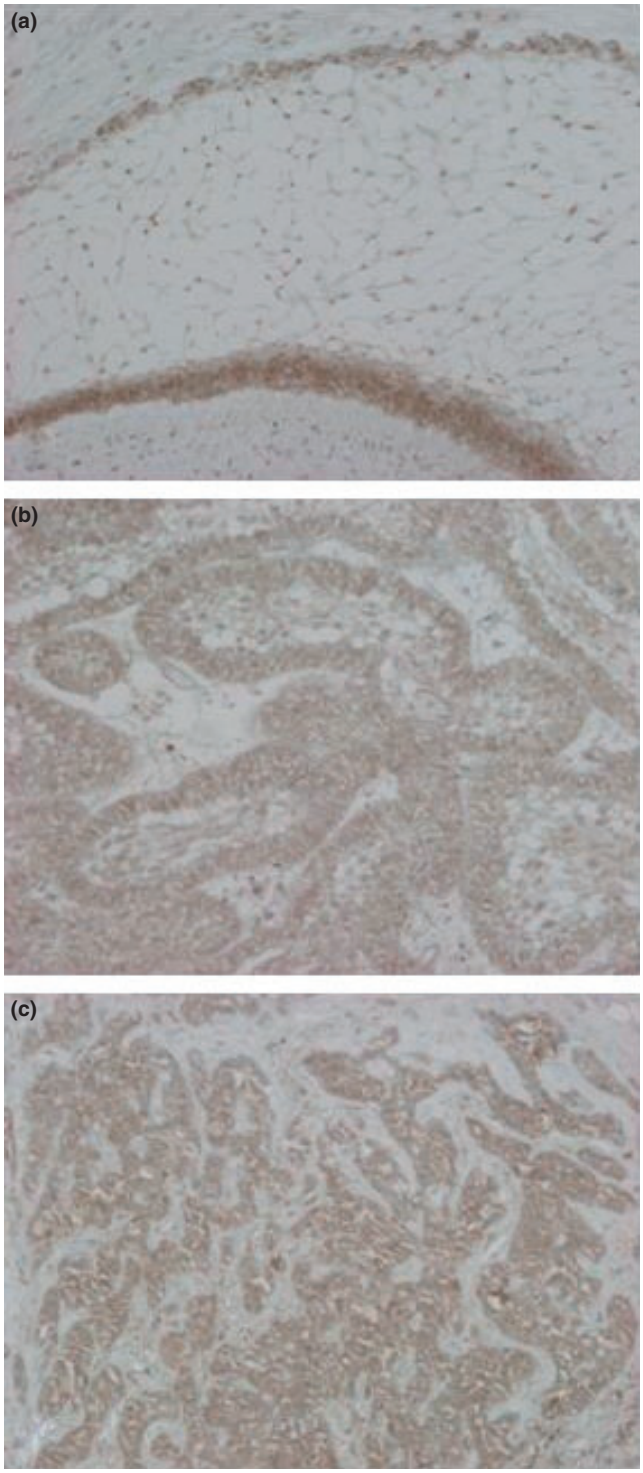

Figure 3 Immunohistochemical reactivity for PTEN. (**a**) Tooth germ showing strong reactivity in inner enamel epithelium and weak reactivity in other epithelial components. (x95) (**b**) Follicular ameloblastoma showing reactivity in many peripheral columnar cells and some central polyhedral cells. (x120) (**c**) Ameloblastic carcinoma showing reactivity in most neoplastic cells. (x115)

Stain MAX-PO; Nichirei, Tokyo, Japan) for 45 min, and reaction products were visualized by immersing the sections in 0.03% diaminobenzidine solution containing 2 mM hydrogen peroxide for 2–3 min. Nuclei were lightly stained with methylgreen. For control studies of the antibodies, the serial sections were treated with phosphate-buffered saline, normal rabbit IgG, mouse anti-OPD4 (CD45RO) monoclonal antibody (Dako, Glostrup, Denmark; subclass IgG1), and mouse anti-LeuM1 (CD15) monoclonal antibody (Becton-Dickinson, San Jose, CA, USA; subclass IgM) instead of the primary antibodies and were confirmed to be unstained.

Immunohistochemical reactivity for pAkt, PI3K, and PTEN was evaluated and classified into two groups: (+) weakly to moderately positive and (++) strongly positive. 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. $P < 0.05$ was considered to indicate statistical significance.

Results

Immunohistochemical reactivity for pAkt, PI3K, and PTEN in tooth germs and ameloblastic tumors is summarized in Table 1. Expression of pAkt and PI3K was detected in the cell membrane and cytoplasm of cellular components in normal and neoplastic odontogenic tissues (Figures 1 and 2). Immunohistochemical reactivity for these molecules were similar in tooth germs and ameloblastic tumors. In tooth germs, pAkt and PI3K expression was strong in the inner and outer enamel epithelium and dental lamina and weak in the stratum intermedium and stellate reticulum (Figure 1a). Some endothelial cells in dental papillae and dental follicles were also weakly reactive. Ameloblastomas and metastasizing ameloblastomas showed strong reactivity for pAkt and PI3K in peripheral columnar or cuboidal cells and weak reactivity in central polyhedral cells (Figures 1b and 2a,c). The level of immunohistochemical reactivity for PI3K was significantly higher in plexiform ameloblastomas than in follicular ameloblastomas ($P < 0.05$, Table 1). Keratinizing cells in acanthomatous ameloblastomas or granular cells in granular cell ameloblastomas exhibited no expression of pAkt or PI3K (Figure 2b). Basal and desmoplastic ameloblastomas and ameloblastic carcinomas showed pAkt and PI3K expression in most neoplastic cells (Figure 1c). The level of immunohistochemical reactivity for PI3K was significantly lower in acanthomatous ameloblastomas than in ameloblastomas without cellular variation ($P < 0.05$, Table 1). In these ameloblastic tumors, some stromal endothelial cells showed weak reactivity for pAkt and PI3K.

Immunoreactivity for PTEN was detected in the cytoplasm of cellular components in normal and neoplastic odontogenic tissues (Figure 3). In tooth germs, PTEN was expressed strongly in the inner enamel epithelium and weakly in other epithelial components (Figure 3a). Some endothelial cells in dental papillae and dental follicles were also weakly reactive. Ameloblastomas and metastasizing ameloblastomas showed PTEN reactivity in many peripheral columnar or

cuboidal cells and some central polyhedral cells (Figure 3b). The level of immunohistochemical reactivity for PTEN was significantly lower in ameloblastomas than in tooth germs ($P < 0.05$, Table 1). Keratinizing cells in acanthomatous ameloblastomas or granular cells in granular cell ameloblastomas exhibited no PTEN expression. Basal cell ameloblastomas showed PTEN expression in many neoplastic cells, and some neoplastic cells neighboring the basement membrane in desmoplastic ameloblastomas were reactive with PTEN. Ameloblastic carcinomas showed PTEN expression in most neoplastic cells (Figure 3c). In these ameloblastic tumors, some stromal endothelial cells showed weak PTEN reactivity.

Discussion

The Akt signaling pathway mediates growth factor-dependent cell survival in a variety of cell types (Datta *et al*, 1999; Blume-Jensen and Hunter, 2001). *akt*-deficient mice are small with increased neonatal mortality and show increased apoptosis in the thymus and testis (Chen *et al*, 2001). Mice lacking *PI3K* show increased insulin sensitivity and impaired B cell development and proliferation (Fruman *et al*, 1999; Terauchi *et al*, 1999). Homozygous *PTEN*-mutant mice undergo embryonic death due to defective chorio-allantoic development (Di Cristofano *et al*, 1998). Thus, the Akt signaling pathway is involved in various developmental processes. Expression of growth factors that bind receptor tyrosine kinases, including epidermal, fibroblast, hepatocyte, and insulin-like growth factors, has been detected temporally and spatially during tooth development (Snead *et al*, 1989; Ayer-le Lievre *et al*, 1991; Cam *et al*, 1992; Tabata *et al*, 1996). In the present study, immunoreactivity for pAkt, PI3K, and PTEN was found in tooth germ tissues, suggesting that the Akt signaling pathway regulates cell survival and growth during tooth development by mediating growth factor signals.

Akt, also referred to as *protein kinase B* (*PKB*), was identified as the cellular homolog of the transforming viral oncogene v-*akt* (Staal, 1987). Its product protein is a serine/threonine kinase activated by various growth factors and survival stimuli, and activated Akt (pAkt) phosphorylates many substrates, including Bad, caspase-9, cyclic AMP-response element-binding protein (CREB), Forkhead family members, glycogen synthase-3 (GSK-3), IκB kinase (IKK), and mammalian target of rapamycin (mTOR), thereby blocking apoptosis and promoting cell survival (Datta *et al*, 1999; Blume-Jensen and Hunter, 2001). In addition, pAkt controls cyclin-dependent kinase inhibitors, $p21^{WAF1/Cip1}$ and $p27^{Kip1}$, and a p53 upstream regulator, MDM2, affecting the RB and p53 pathways (Blume-Jensen and Hunter, 2001; Mayo and Donner, 2002). *Akt* gene amplification and/or overexpression and constitutive activation of its product protein have been detected in various human malignancies (Staal, 1987; Altomare and Testa, 2005). Recent studies have revealed that treatments with tumor necrosis factor-α and RANKL induce upregulation of Akt phosphorylation in cultured ameloblastoma cells (Hendarmin *et al*, 2005; Sandra *et al*, 2005). In the present study, immunoreactivity for pAkt was detected predominantly in neoplastic cells near the basement membrane in ameloblastomas and malignant ameloblastic tumors, suggesting that Akt contributes to neoplastic cell survival in these epithelial odontogenic tumors. Our previous studies demonstrated that expression of caspase-9 was obvious in ameloblastic tumors as compared with that of caspase-8, suggesting that the mitochondrial pathway has an important role in apoptotic cell death in odontogenic tumors, rather than the death receptor-mediated pathway (Kumamoto and Ooya, 2005a,b). These features suggest that pAkt suppresses caspase-9 function and blocks apoptosis in the ameloblastic tumors. Previous studies have revealed expression of $p21^{WAF1/Cip1}$, $p27^{Kip1}$, and MDM2 in odontogenic tumors (Kumamoto *et al*, 2001, 2004a; Sandra *et al*, 2002), suggesting that pAkt might be associated with regulation of the cell cycle and p53-dependent cell death in epithelial odontogenic tumors. In this study, the expression levels of pAkt in benign and malignant ameloblastic tumors were slightly higher than that in tooth germs, suggesting that upregulation of this signaling molecule might play a role in oncogenesis of odontogenic epithelium.

PI3K was first identified in association with activated platelet-derived growth factor receptor (Whitman *et al*, 1988). Subsequently, PI3K activity was found to be required for the growth factor-dependent survival of a wide variety of cultured cell types (Yao and Cooper, 1995). This lipid kinase generates specific inositol lipids that are implicated in recruitment to the plasma membrane and subsequent activation of Akt (Datta *et al*, 1999; Blume-Jensen and Hunter, 2001). Oncogenic effects of *PI3K* have been proved in chicken angiosarcoma cells and mouse T-cell lymphoma cells (Chang *et al*, 1997; Jimenez *et al*, 1998). In human neoplasms, *PI3K* amplification was originally reported in some carcinomas, whereas mutations have been detected frequently in many malignancies (Shayesteh *et al*, 1999; Samuels *et al*, 2004). In the present study, immunoreactivity for PI3K in ameloblastic tumors showed a similar localization pattern to that for pAkt and was slightly higher than the level in tooth germs, similar to pAkt. These features suggest that PI3K expression contributes to Akt activation in ameloblastic tumors and is possibly involved in neoplastic changes of the odontogenic epithelium. In this study, the expression level of PI3K in plexiform ameloblastomas was significantly higher than that in follicular ameloblastomas, and ameloblastomas without cellular variation showed significantly higher PI3K expression than acanthomatous ameloblastomas. These trends in PI3K reactivity were similar to those in pAkt reactivity among ameloblastoma types and subtypes. These features suggest that the Akt signaling pathway might be involved in tissue structuring and cell differentiation of ameloblastomas.

PTEN, also known as *mutated in multiple advanced cancers 1* (*MMAC1*) and *transforming growth factor β-regulated and epithelial cell-enriched phosphatase 1* (*TEP1*), is a tumor suppressor gene first identified as a

locus mutated in many types of human tumors (Li *et al*, 1997). Its product protein is a lipid phosphatase that functions as a negative regulator of the Akt signaling pathway, thereby promoting cell cycle arrest and apoptosis (Stambolic *et al*, 1998). Germline mutations in *PTEN* cause rare autosomal dominant inherited hamartoma-cancer syndromes, Cowden disease (CD) and Bannayan-Zonana syndrome (BZS), that share specific developmental defects as well as multiple hamartomas in the skin, intestine, breast, and thyroid, and increased susceptibility to breast and thyroid malignancies (Liaw *et al*, 1997; Marsh *et al*, 1997). Heterozygous *PTEN* mice show hyperplastic-dysplastic changes in the prostate, skin, and colon and neoplastic development in the thyroid and colon, which are characteristic of CD and BZS (Di Cristofano *et al*, 1998). Various types of human sporadic neoplasms show somatic deletions or mutations of *PTEN*, often in association with decreased levels or loss of its gene products (Li *et al*, 1997; Altomare and Testa, 2005). In the present study, immunoreactivity for PTEN was detected in neoplastic cells of ameloblastomas and malignant ameloblastic tumors, suggesting that this molecule suppresses the Akt signaling pathway in these epithelial odontogenic tumors. However, the expression level of PTEN in ameloblastomas was significantly lower than that in tooth germs. A previous study detected frequent allelic losses of chromosome 10q in ameloblastic tumors (Nodit *et al*, 2004). These features suggest that decreased PTEN expression might participate in oncogenesis of odontogenic epithelium by activating the Akt signaling pathway.

References

Altomare DA, Testa JR (2005). Perturbations of the AKT signaling pathway in human cancer. *Oncogene* **24:** 7455–7464.

Ayer-le Lievre C, Stahlbom PA, Sara VR (1991). Expression of IGF-I and -II mRNA in the brain and craniofacial region of the rat fetus. *Development* **111:** 105–115.

Blume-Jensen P, Hunter T (2001). Oncogenic kinase signalling. *Nature* **411:** 355–365.

Cam Y, Neumann MR, Oliver L *et al*. (1992). Immunolocalization of acidic and basic fibroblast growth factors during mouse odontogenesis. *Int J Dev Biol* **36:** 381–389.

Chang HW, Aoki M, Fruman D *et al*. (1997). Transformation of chicken cells by the gene encoding the catalytic subunit of PI 3-kinase. *Science* **276:** 1848–1850.

Chen WS, Xu PZ, Gottlob K *et al*. (2001). Growth retardation and increased apoptosis in mice with homozygous disruption of the akt1 gene. *Genes Dev* **15:** 2203–2208.

Datta SR, Brunet A, Greenberg ME (1999). Cellular survival: a play in three Akts. *Genes Dev* **13:** 2905–2927.

Di Cristofano A, Pesce B, Cordon-Cardo C *et al*. (1998). *Pten* is essential for embryonic development and tumour suppression. *Nat Genet* **19:** 348–355.

Fruman DA, Snapper SB, Yballe CM *et al*. (1999). Impaired B cell development and proliferation in absence of phosphoinositide 3-kinase p85α. *Science* **283:** 393–397.

Heikinheimo K, Voutilainen R, Happonen RP *et al*. (1993). EGF receptor and its ligands, EGF and TGF-alpha, in developing and neoplastic human odontogenic tissues. *Int J Dev Biol* **37:** 387–396.

Heikinheimo K, Jee KJ, Niini T *et al*. (2002). Gene expression profiling of ameloblastoma and human tooth germ by means of a cDNA microarray. *J Dent Res* **81:** 525–530.

Hendarmin L, Sandra F, Nakao Y *et al*. (2005). TNFα played a role in induction of Akt and MAPK signals in ameloblastoma. *Oral Oncol* **41:** 375–382.

Jimenez C, Jones DR, Rodriguez-Viciana P *et al*. (1998). Identification and characterization of a new oncogene derived from the regulatory subunit of phosphoinositide 3-kinase. *EMBO J* **17:** 743–753.

Kumamoto H (2006). Molecular pathology of odontogenic tumors. *J Oral Pathol Med* **35:** 65–74.

Kumamoto H, Ooya K (2005a). Expression of tumor necrosis factor α, TNF-related apoptosis-inducing ligand, and their associated molecules in ameloblastomas. *J Oral Pathol Med* **34:** 287–294.

Kumamoto H, Ooya K (2005b). Detection of mitochondria-mediated apoptosis signaling molecules in ameloblastomas. *J Oral Pathol Med* **34:** 565–572.

Kumamoto H, Kimi K, Ooya K (2001). Detection of cell cycle-related factors in ameloblastomas. *J Oral Pathol Med* **30:** 309–315.

Kumamoto H, Yoshida M, Ooya K (2002). Immunohistochemical detection of hepatocyte growth factor, transforming growth factor-β and their receptors in epithelial odontogenic tumors. *J Oral Pathol Med* **31:** 539–548.

Kumamoto H, Izutsu T, Ohki K *et al*. (2004a). *p53* gene status and expression of p53, MDM2, and $p14^{ARF}$ proteins in ameloblastomas. *J Oral Pathol Med* **33:** 292–299.

Kumamoto H, Takahashi N, Ooya K (2004b). K-*Ras* gene status and expression of Ras/mitogen-activated protein kinase (MAPK) signaling molecules in ameloblastomas. *J Oral Pathol Med* **33:** 360–367.

Li J, Yen C, Liaw D *et al*. (1997). *PTEN*, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* **275:** 1943–1947.

Liaw D, Marsh DJ, Li J *et al*. (1997). Germline mutations of the *PTEN* gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet* **16:** 64–67.

Marsh DJ, Dahia PL, Zheng Z *et al*. (1997). Germline mutations in *PTEN* are present in Bannayan-Zonana syndrome. *Nat Genet* **16:** 333–334.

Mayo LD, Donner DB (2002). The PTEN, Mdm2, p53 tumor suppressor-oncoprotein network. *Trends Biochem Sci* **27:** 462–677.

Nodit L, Barnes L, Childers E *et al*. (2004). Allelic loss of tumor suppressor genes in ameloblastic tumors. *Mod Pathol* **17:** 1062–1067.

Philipsen HP, Reichart PA, Slootweg PJ *et al*. (2005). Odontogenic tumours. In: Barnes L, Eveson JW, Reichart PA, Sidransky D, eds. *WHO classification of head and neck tumours*. Pathology & Genetics. Lyon: IARC Press, pp. 283–327.

Samuels Y, Wang Z, Bardelli A *et al*. (2004). High frequency of mutations of the *PIK3CA* gene in human cancers. *Science* **304:** 554.

Sandra F, Nakamura N, Kanematsu T *et al*. (2002). The role of MDM2 in the proliferative activity of ameloblastoma. *Oral Oncol* **38:** 153–157.

Sandra F, Hendarmin L, Kukita T *et al*. (2005). Ameloblastoma induces osteoclastogenesis: a possible role of ameloblastoma in expanding in the bone. *Oral Oncol* **41:** 637–644.

Sciubba JJ, Fantasia JE, Kahn LB (2001). *Tumors and cysts of the jaw*. Washington, DC: Armed Forces institute of Pathology, pp. 71–99.

Shayesteh L, Lu Y, Kuo WL *et al*. (1999). *PIK3CA* is implicated as an oncogene in ovarian cancer. *Nat Genet* **21:** 99–102.

Snead ML, Luo W, Oliver P *et al*. (1989). Localization of epidermal growth factor precursor in tooth and lung during embryonic mouse development. *Dev Biol* **134:** 420–429.

So F, Daley TD, Jackson L, Wysocki GP (2001). 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* **30:** 428–433.

Staal SP (1987). Molecular cloning of the *akt* oncogene and its human homologues *AKT1* and *AKT2*: amplification of *AKT1* in a primary human gastric adenocarcinoma. *Proc Natl Acad Sci U S A* **84:** 5034–5037.

Stambolic V, Suzuki A, de la Pompa JL *et al*. (1998). Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell* **95:** 29–39.

Tabata MJ, Kim K, Liu JG *et al*. (1996). Hepatocyte growth factor is involved in the morphogenesis of tooth germ in murine molars. *Development* **122:** 1243–1251.

Terauchi Y, Tsuji Y, Satoh S *et al*. (1999). Increased insulin sensitivity and hypoglycaemia in mice lacking the p85α subunit of phosphoinositide 3-kinase. *Nat Genet* **21:** 230–235.

Whitman M, Downes CP, Keeler M *et al*. (1988). Type I phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate. *Nature* **332:** 644–646.

Yao R, Cooper GM (1995). Requirement for phosphatidylinositol-3 kinase in the prevention of apoptosis by nerve growth factor. *Science* **267:** 2003–2006.