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Immunohistochemical detection of retinoblastoma protein and E2 promoter-binding factor-1 in ameloblastomas

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BACKGROUND: To clarify the roles of cell cycle regulation in oncogenesis and cytodifferentiation of odontogenic tumors, expression of retinoblastoma protein (RB) and E2 promoter-binding factor-1 (E2F-1) 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 RB, E2F-1, and phosphorylated RB. Ki-67 antigen immunostaining was made as a marker of cell proliferation.

RESULTS: Immunohistochemical reactivity for RB, E2F-1, phosphorylated RB, and Ki-67 was detected in the nuclei of odontogenic epithelial cells near the basement membrane in tooth germs and benign and malignant ameloblastomas. The number of cells positive for phosphorylated RB was nearly equal to or slightly less than the number of cells positive for RB or E2F-1. The number of Ki-67-positive cells was slightly more than the numbers of cell positive for RB, E2F-1, or phosphorylated RB. The levels of immunoreactivity for RB, E2F-1, phosphorylated RB, and Ki-67 were slightly higher in benign and malignant ameloblastomas than in tooth germs. Plexiform ameloblastomas showed significantly higher expression of RB than follicular ameloblastomas. Ki-67 immunoreactivity was significantly higher in ameloblastic carcinomas than in metastasizing ameloblastomas.

CONCLUSION: Similar immunoreactivity for RB, E2F-1, phosphorylated RB, and Ki-67 in tooth germs and ameloblastomas indicated cellular expression of phosphorylated RB and active-free E2F-1 in both normal and neoplastic odontogenic tissues. Expression of RB, E2F-1, and phosphorylated RB was considered to be involved in cell proliferation and differentiation of odontogenic epithelium via control of the cell cycle.

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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 cytologic 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 cytologic 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.

Cell proliferation follows an orderly progression though the cell cycle, which is governed by various factors, including cyclins, cyclin-dependent kinases (CDKs), CDK inhibitors (CKIs), and other critical regulators (7, 8). Retinoblastoma protein (RB), the product of *retinoblastoma (RB)* tumor-suppressor gene, acts as a signal transducer connecting the cell cycle with the transcription machinery (9). During the time preceding G1-phase, underphosphorylated RB binds to transcriptional regulators termed E2 promoter-binding factors (E2Fs) and represses their transcriptional activation (9, 10). In late G1, RB is phosphorylated by several cyclin–CDK complexes and releases E2Fs,

enabling free E2Fs to transactivate genes whose products are important for S-phase entry (11, 12). Thus, RB and E2Fs control the cell cycle at the transition point from G1 to S, and uncontrolled cell cycles caused by aberration of these molecules have been identified in a variety of tumors (13–17).

Our previous study revealed cell cycle-related factors, such as cyclin D1, $p16^{INK4a}$, $p21^{WAF1/Cip1}$, and $p27^{Kip1}$, and cell cycle phase/cell proliferation markers, such as Ki-67, DNA topoisomerase IIα, and histone H3 mRNA, in ameloblastomas, suggesting that these molecules are associated with oncogenesis or cytodifferentiation of odontogenic epithelium (18, 19). In the present study, immunohistochemical expression of RB, E2 promoter-binding factor-1 (E2F-1), and phosphorylated RB was examined in benign and malignant ameloblastomas as well as in tooth germs to clarify the possible role of RB function in cell cycle regulation or alteration in epithelial odontogenic tumors. Ki-67 antigen immunoreactivity was concurrently studied to serve as a marker of cell proliferation.

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 1 to several days and were 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 40 ameloblastomas and five malignant ameloblastomas. Ameloblastomas were divided into 23 follicular and 17 plexiform types, including nine 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. After heating in 0.01 M citrate buffer (pH 6.0) for 10 min by autoclave (121°C, 2 atm), the sections were incubated with primary antibodies at 4°C overnight. The applied antibodies were mouse anti-RB monoclonal antibody (clone IF8; Santa Cruz Biotechnology, Santa Cruz, CA, USA; subclass IgG_1; diluted at 1:50), mouse anti-E2F-1 monoclonal antibody (clone KH95; Santa Cruz Biotechnology; subclass IgG_{2a}; diluted at 1:50), rabbit antiphosphorylated RB polyclonal antibody (#9308; Cell Signaling Technology, Beverly, MA, USA; diluted at 1:60), and mouse anti-Ki-67 monoclonal antibody (clone MIB-1; Dako, Glostrup, Denmark; subclass IgG_1; diluted at 1:50; 17, 20–22). The sections were allowed to react with peroxidase-conjugated antimouse IgG (for RB, E2F-1, and Ki-67) or antirabbit IgG (for phosphorylated RB) polyclonal antibody (Histofine Simple 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 methyl green. For control studies of the antibodies, the serial sections were treated with phosphate-buffered saline, mouse anti-OPD4 (CD45RO) monoclonal antibody (Dako; subclass IgG_1), anti-L26 (CD20) monoclonal antibody (Nichirei; subclass IgG_{2a}), and normal rabbit IgG instead of the primary antibodies and were confirmed to be unstained.

Evaluation of immunostaining and statistical analysis

Immunohistochemical reactivity for RB, E2F-1, phosphorylated RB, and Ki-67 was evaluated and classified into two groups: (+), $<5\%$ of epithelial or neoplastic cells positive and (++), more than 5% of epithelial or neoplastic cells 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. The *P*-values of <0.05 were considered to indicate statistical significance.

Results

Immunohistochemical reactivity for RB, E2F-1, phosphorylated RB, and Ki-67 in tooth germs and ameloblastomas is summarized in Table 1. Expression of RB, E2F-1, phosphorylated RB, and Ki-67 was detected in the nuclei of both normal and neoplastic odontogenic epithelium (Figs 1–4). Immunoreactivity for these molecules corresponded with each other in tooth germs and ameloblastomas, and the number of cells positive for phosphorylated RB was nearly equal to or slightly less than the numbers of cells positive for RB or E2F-1. The number of Ki-67-positive cells was slightly more than the number of cells positive for RB, E2F-1, or phosphorylated RB.

In tooth germs, RB, E2F-1, and phosphorylated RB were expressed scattered in inner enamel epithelium and stratum intermedium (Figs 1a and 3a). Immunohistochemical reactivity for these molecules was (+) in all tooth germs. Ameloblastomas showed reactivity for these molecules in some peripheral columnar or cuboidal neoplastic cells (Figs 1b, 2a and 3b), and a few central polyhedral cells were also positive in several cases of ameloblastoma. Six of 40 ameloblastomas showed (++) immunoreactivity for RB, and four ameloblastomas showed (++) immunoreactivity for E2F-1 and phosphorylated RB. The level of immunohistochemical reactivity for RB was significantly higher

Table 1 Immunohistochemical reactivity for RB, E2F-1, phosphorylated RB, and Ki-67 in tooth germs and ameloblastomas

		RB		*E2F-1*		*Phosphorylated RB*		*Ki-67*		
	n	+	++	+	++	+	++	+	++	
Tooth germ	10	10 (100)	0 (0)	10 (100)	0 (0)	10 (100)	0 (0)	10 (100)	0 (0)	
Ameloblastoma	40	34 (85)	6 (15)	36 (90)	4 (10)	36 (90)	4 (10)	32 (80)	8 (20)	
Follicular type	23	22 (96)	1 (4)	22 (96)	1 (4)	22 (96)	1 (4)	20 (87)	3 (13)	
Plexiform type	17	12 (71)	5 (29)	14 (82)	3 (18)	14 (82)	3 (18)	12 (71)	5 (29)	*
Acanthomatous subtype	9	9 (100)	0 (0)	9 (100)	0 (0)	9 (100)	0 (0)	9 (100)	0 (0)	
Granular subtype	6	6 (100)	0 (0)	6 (100)	0 (0)	6 (100)	0 (0)	6 (100)	0 (0)	
Basal cell subtype	3	2 (67)	1 (33)	2 (67)	1 (33)	2 (67)	1 (33)	2 (67)	1 (33)	
Desmoplastic subtype	4	4 (100)	0 (0)	4 (100)	0 (0)	4 (100)	0 (0)	4 (100)	0 (0)	
Non-cellular variation	18	13 (72)	5 (28)	15 (83)	3 (17)	15 (83)	3 (17)	11 (61)	7 (39)	
Malignant ameloblastoma	5	3 (60)	2 (40)	3 (60)	2 (40)	3 (60)	2 (40)	2 (40)	3 (60)	*
Metastasizing ameloblastoma	2	2 (100)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	2 (100)	0 (0)	
Ameloblastic carcinoma	3	1 (33)	2 (67)	1 (33)	2 (67)	1 (33)	2 (67)	0 (0)	3 (100)	*

Immunohistochemical reactivity: (+), < 5% of epithelial or neoplastic cells positive; (++), more than 5% of epithelial or neoplastic cells positive. Values in parentheses denote percentages.
*Statistical significance: $P < 0.05$.
RB, retinoblastoma protein; E2F-1, E2 promoter-binding factor-1.

in plexiform ameloblastomas than in follicular ameloblastomas ($P < 0.05$, Table 1). Keratinizing cells and granular cells in acanthomatous ameloblastomas and granular cell ameloblastomas were not reactive with RB, E2F-1, or phosphorylated RB. Expression of these molecules was recognized in some neoplastic cells of basal cell ameloblastomas and a few peripheral neoplastic cells of desmoplastic ameloblastomas (Fig. 2b). Metastasizing ameloblastomas showed RB, E2F-1, and phosphorylated RB expression patterns similar to those of benign ameloblastomas (Fig. 2c), whereas ameloblastic carcinomas exhibited reactivity for these molecules in some or many neoplastic cells (Figs 1c and 3c). Immunohistochemical reactivity for RB, E2F-1, and phosphorylated RB was (+) in all metastasizing ameloblastomas, and two of the three ameloblastic carcinomas showed (++) immunoreactivity for these molecules.

Ki-67 immunoreactivity showed similar distribution to that for RB, E2F-1, and phosphorylated RB in tooth germs, ameloblastomas, and malignant ameloblastomas (Fig. 4). Eight of 40 ameloblastomas and all ameloblastic carcinomas showed (++) immunoreactivity for Ki-67. Significant differences of the immunohistochemical reactivity levels for Ki-67 were found between tooth germs and malignant ameloblastomas and between metastasizing ameloblastomas and ameloblastic carcinomas ($P < 0.05$, respectively, Table 1).

Discussion

Regulation of the cell cycle is an essential process by which the cell monitors its growth and differentiation during embryonic development and morphogenesis (7, 9). Several cell cycle regulators, such as cyclins and CKIs, have been identified in tooth germs, suggesting that the development and fate of odontogenic cells are regulated by controlling the cell cycle (19, 23). RB is required for the developing lens, and homozygous inactivation of RB gene in the mouse leads to mid-gestational lethality with defects in erythropoiesis and neurogenesis (24, 25). Mice lacking E2F-1 are viable and fertile, yet have testicular atrophy, exocrine gland dysfunction, and defective thymocyte apoptosis (26, 27). In the present study, using human tooth germs at the initial stage of crown mineralization, expression of RB, E2F-1, phosphorylated RB, and Ki-67 was detected in some odontogenic epithelial cells of inner enamel epithelium and stratum intermedium, suggesting that proliferation and differentiation of normal odontogenic epithelium correlate with RB and E2F-1 expression during tooth development.

Retinoblastoma protein gene is a tumor-suppressor gene initially cloned because of its frequent mutation in retinoblastoma, and its product protein plays a fundamental role in cell cycle regulation (9, 28). Mutations or deletions affecting *RB* gene are encountered not only in retinoblastoma but also in other malignancies, such as osteosarcoma, lung small cell carcinoma, prostate carcinoma, and breast carcinoma (13–15, 29). Aberrant expression of RB, such as expression loss and increased expression, has been detected in various types of tumors, including lung, renal, colorectal, and oral carcinomas (20, 22, 30, 31). In the present study, immunohistochemical reactivity for RB and phosphorylated RB was identified mainly in neoplastic cells neighboring the basement membrane in benign and malignant ameloblastomas, and the number of phosphorylated RB-positive cells did not differ substantially from that of RB-positive neoplastic cells. These features suggest that most RB-positive neoplastic cells express phosphorylated protein in these epithelial odontogenic tumors. Immunohistochemical staining for Ki-67 detected proliferating cells near the basement membrane in ameloblastomas, as shown in previous studies (18, 32), and expression of RB was considered to correlate with cellular proliferation of neoplastic odontogenic epithelial cells. In this study, immunoreactivity for RB and phosphorylated RB in benign and malignant ameloblastomas was slightly higher than that in tooth germs,

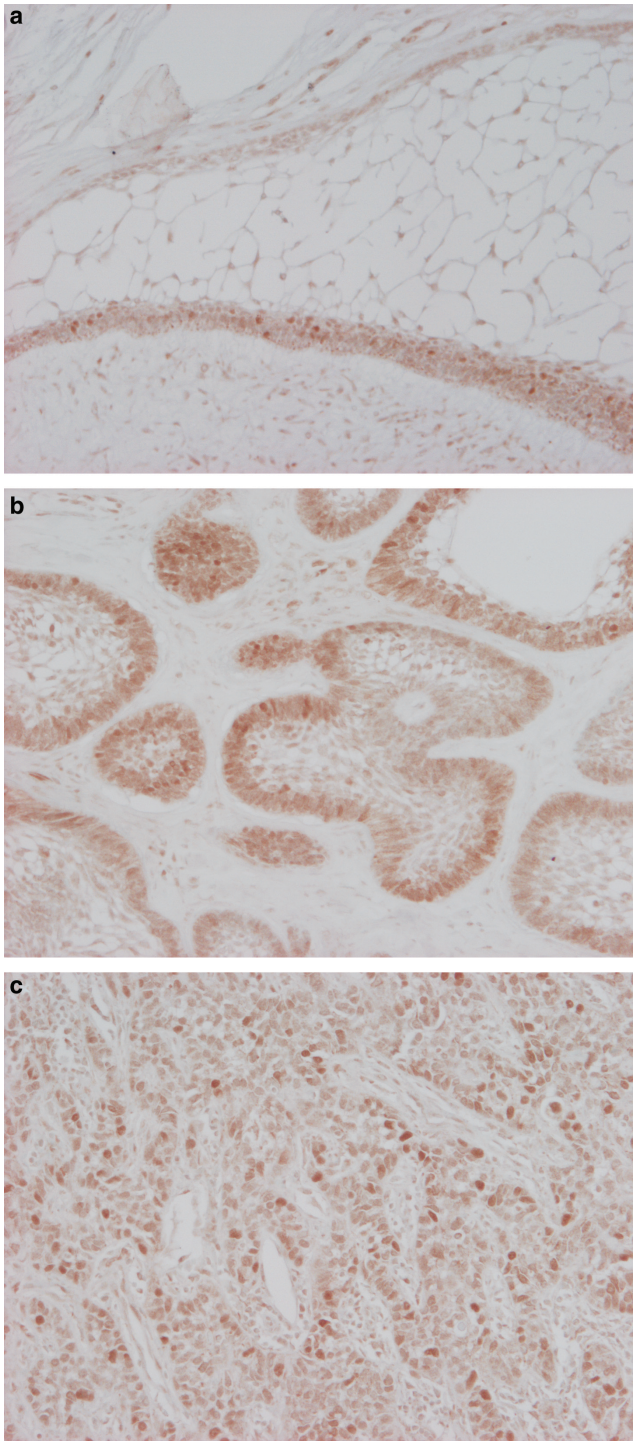

Figure 1 Immunohistochemical reactivity for retinoblastoma protein (RB). (a) Tooth germ showing scattered reactivity in inner enamel epithelium and stratum intermedium (×125). (b) Follicular ameloblastoma showing reactivity in some peripheral neoplastic cells (×120). (c) Ameloblastic carcinoma showing reactivity in many neoplastic cells (×115).

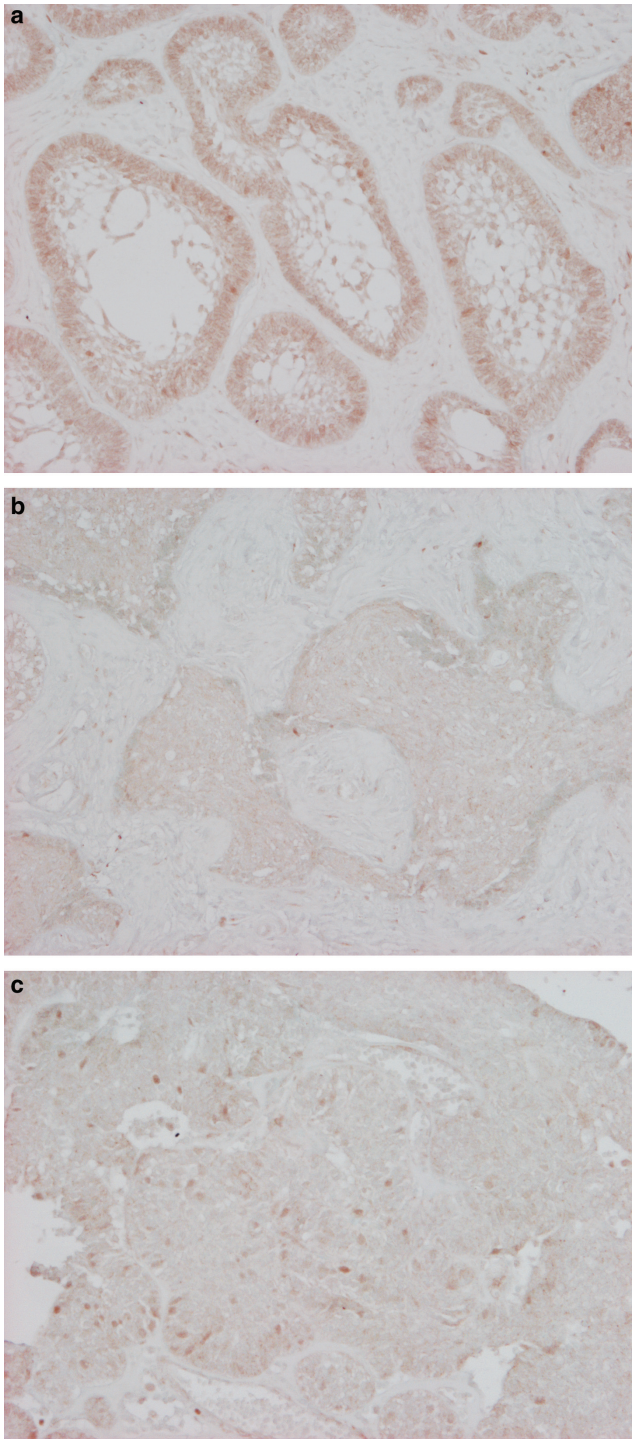

Figure 2 Immunohistochemical reactivity for E2 promoter-binding factor-1 (E2F-1). (a) Follicular ameloblastoma showing reactivity in some peripheral neoplastic cells (×100). (b) Desmoplastic ameloblastoma showing reactivity in a few peripheral neoplastic cells (×105). (c) Metastasizing ameloblastoma showing reactivity in some peripheral and a few central neoplastic cells (×125).

and plexiform ameloblastomas tended to show higher expression of RB and phosphorylated RB than follicular ameloblastomas. These results suggest that RB expression might be involved in oncogenesis or tissue structuring of these epithelial odontogenic tumors. RB function is controlled by a negative feedback loop associated with cyclin D1 phosphorylating RB and $p16^{INK4a}$ evicting cyclin D1, termed $p16^{INK4a}$-cyclin D1-RB pathway or RB pathway (33, 34). Derailments of this pathway, caused by inactivation of $p16^{INK4a}$ or RB

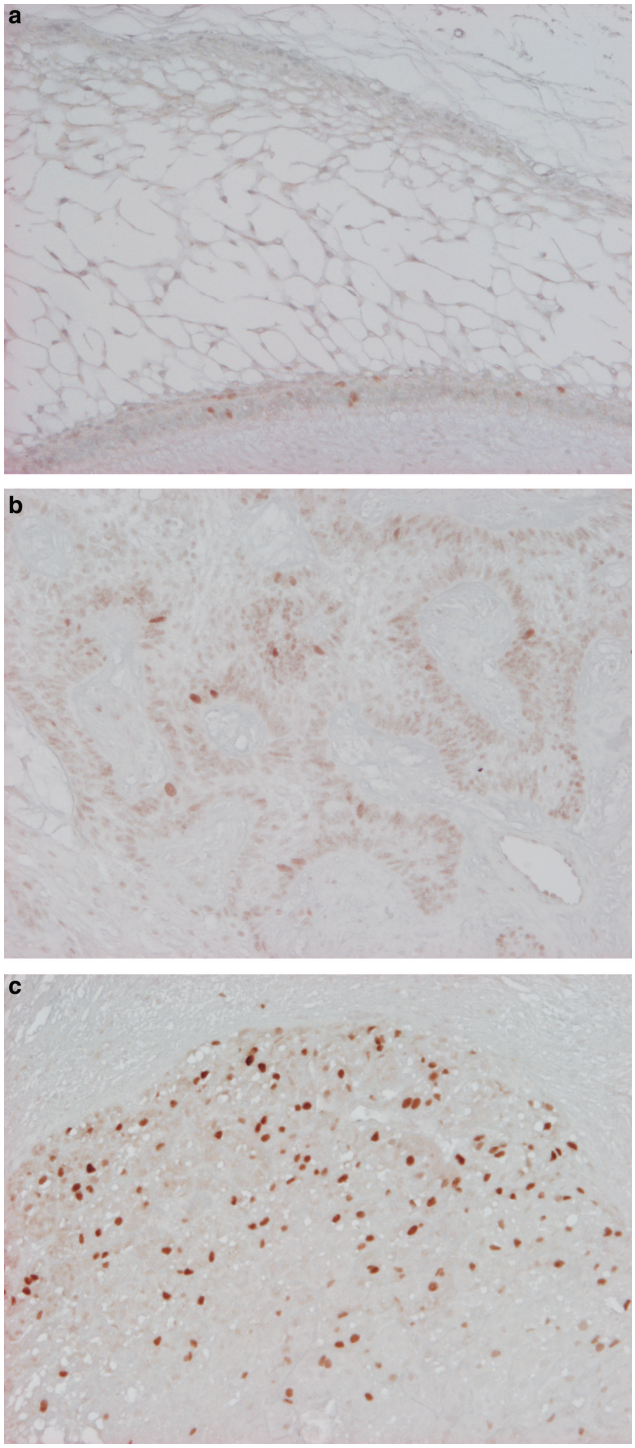

Figure 3 Immunohistochemical reactivity for phosphorylated retinoblastoma protein (RB). (a) Tooth germ showing scattered reactivity in inner enamel epithelium and stratum intermedium (×125). (b) Plexiform ameloblastoma showing reactivity in some peripheral neoplastic cells (×125). (c) Ameloblastic carcinoma showing reactivity in many neoplastic cells (×120).

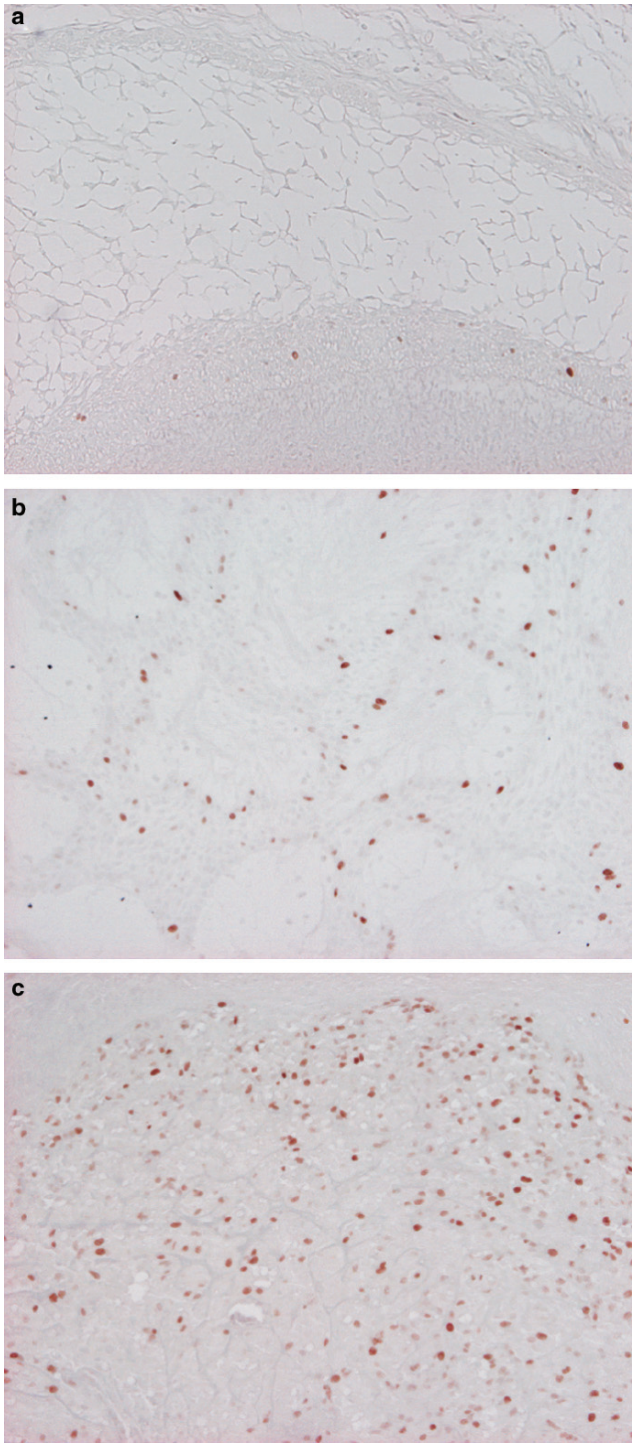

Figure 4 Immunohistochemical reactivity for Ki-67. (a) Tooth germ showing scattered reactivity in inner enamel epithelium and stratum intermedium (×110). (b) Plexiform ameloblastoma showing reactivity in peripheral neoplastic cells (×110). (c) Ameloblastic carcinoma showing reactivity in many neoplastic cells (×110).

or overexpression of cyclin D, are implicated in dysregulation of the cell cycle machinery, and components of this pathway are frequently altered in many types of human tumors (13–15, 34–36). Our previous study revealed that expression of cyclin D1 and $p16^{INK4a}$ did not largely differ between normal and neoplastic odontogenic epithelium (19). In the present study, immunoreactivity for RB was slightly higher in

benign and malignant ameloblastomas than in tooth germs.

The E2F-1, one of the E2F family of transcription factors originally identified as a cellular transcriptional regulator that could bind to and activate the adenovirus E2 promoter, transactivates genes that encode cell cycle regulators, such as *c-myc*, *cdc2*, and *cyclin A*, as well as genes encoding proteins for cell cycle-regulated biochemical processes, such as DNA polymerase α, thymidine synthetase, and dihydrofolate reductase, resulting in cell cycle progression (12, 37). *E2F-1* gene amplification and its overexpression have been detected in several human tumors, such as lung, bladder, gastrointestinal, and head and neck carcinomas, suggesting that its product protein acts as oncoprotein (16, 17, 21, 38). Transgenic mice expressing *E2F-1* gene under the control of a keratin 5 promoter develop skin tumors and ameloblastomas (39). In the present study, immunohistochemical reactivity for E2F-1 closely correlated with that for RB and phosphorylated RB in neoplastic odontogenic epithelium. E2F-1 expression in benign and malignant ameloblastomas was slightly higher than that in tooth germs, and plexiform ameloblastomas tended to show higher E2F-1 expression than follicular ameloblastomas, similar to RB, phosphorylated RB, and Ki-67 expression. These features suggest that most E2F-1-positive neoplastic cells possess free and active E2F-1 in these epithelial odontogenic tumors. Such E2F-1 expression was considered to affect cellular proliferation and differentiation of neoplastic odontogenic epithelial cells. Recent studies have shown that increased E2F-1 expression induces $p14^{ARF}$, a p53 upstream regulator, and p73, a p53 homolog, leading to cell cycle arrest or apoptosis (40, 41). In our previous studies, expression of $p14^{ARF}$ and p73 was confirmed in many neoplastic cells of benign and malignant ameloblastomas (42, 43), when compared with expression of E2F-1. These features suggest that the apoptotic effect of E2F-1 is low in these epithelial odontogenic tumors.

References

1. Kramer IRH, Pindborg JJ, Shear M. *WHO histological typing of odontogenic tumours*. Berlin, Germany: 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, USA: 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. Beijersbergen RL, Bernards R. Cell cycle regulation by the retinoblastoma family of growth inhibitory proteins. *Biochim Biophys Acta* 1996; **287**: 103–20.
8. Sherr CJ, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev* 1999; **13**: 1501–12.
9. Weinberg RA. The retinoblastoma protein and cell cycle control. *Cell* 1995; **81**: 323–30.
10. Chellappan SP, Hiebert S, Mudryj M, Horowitz JM, Nevins JR. The E2F transcription factor is a cellular target for the RB protein. *Cell* 1991; **65**: 1053–61.
11. Mihara K, Cao XR, Yen A, et al. Cell cycle-dependent regulation of phosphorylation of the human retinoblastoma gene product. *Science* 1989; **246**: 1300–3.
12. Lavia P, Jansen-Durr P. E2F target genes and cell-cycle checkpoint control. *Bioessays* 1999; **21**: 221–30.
13. Toguchida J, Ishizaki K, Sasaki MS, et al. Chromosomal reorganization for the expression of recessive mutation of retinoblastoma susceptibility gene in the development of osteosarcoma. *Cancer Res* 1988; **48**: 3939–43.
14. Lee EY, To H, Shew JY, Bookstein R, Scully P, Lee WH. Inactivation of the retinoblastoma susceptibility gene in human breast cancers. *Science* 1988; **241**: 218–21.
15. Bookstein R, Shew JY, Chen PL, Scully P, Lee WH. Suppression of tumorigenicity of human prostate carcinoma cells by replacing a mutated *RB* gene. *Science* 1990; **247**: 712–5.
16. Suzuki T, Yasui W, Yokozaki H, Naka K, Ishikawa T, Tahara E. Expression of the E2F family in human gastrointestinal carcinomas. *Int J Cancer* 1999; **81**: 535–8.
17. Gorgoulis VG, Zacharatos P, Mariatos G, et al. Transcription factor E2F-1 acts as a growth-promoting factor and is associated with adverse prognosis in non-small cell lung carcinomas. *J Pathol* 2002; **198**: 142–56.
18. 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.
19. Kumamoto H, Kimi K, Ooya K. Detection of cell cycle-related factors in ameloblastomas. *J Oral Pathol Med* 2001; **30**: 309–15.
20. Pande P, Mathur M, Shukla NK, Ralhan R. pRb and p16 protein alterations in human oral tumorigenesis. *Oral Oncol* 1998; **34**: 396–403.
21. Rabbani F, Richon VM, Orlow I, et al. Prognostic significance of transcription factor E2F-1 in bladder cancer: genotypic and phenotypic characterization. *J Natl Cancer Inst* 1999; **91**: 874–81.
22. Hedberg Y, Ljungberg B, Roos G, Landberg G. Retinoblastoma protein in human renal cell carcinoma in relation to alterations in G1/S regulatory proteins. *Int J Cancer* 2004; **109**: 189–93.
23. Jernvall J, Aberg T, Kettunen P, Keranen S, Thesleff I. The life history of an embryonic signaling center: BMP-4 induces *p21* and is associated with apoptosis in the mouse tooth enamel knot. *Development* 1998; **125**: 161–9.
24. Lee EY, Chang CY, Hu N, et al. Mice deficient for Rb are nonviable and show defects in neurogenesis and haematopoiesis. *Nature* 1992; **359**: 288–94.
25. Morgenbesser SD, Williams BO, Jacks T, DePinho RA. *p53*-dependent apoptosis produced by Rb-deficiency in the developing mouse lens. *Nature* 1994; **371**: 72–4.
26. Yamasaki L, Jacks T, Bronson R, Goillot E, Harlow E, Dyson NJ. Tumor induction and tissue atrophy in mice lacking E2F-1. *Cell* 1996; **85**: 537–48.
27. Field SJ, Tsai FY, Kuo F, et al. E2F-1 functions in mice to promote apoptosis and suppress proliferation. *Cell* 1996; **85**: 549–61.

28. Friend SH, Bernards R, Rogelj S, et al. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. *Nature* 1986; **323**: 643–6.
29. Harbour JW, Lai SL, Whang-Peng J, Gazdar AF, Minna JD, Kaye FJ. Abnormalities in structure and expression of the human retinoblastoma gene in SCLC. *Science* 1988; **241**: 353–7.
30. Gorgoulis VG, Zacharatos P, Kotsinas A, et al. Alterations of the p16-pRb pathway and the chromosome locus 9p21–22 in non-small-cell lung carcinomas: relationship with p53 and MDM2 protein expression. *Am J Pathol* 1998; **153**: 1749–65.
31. Yamamoto H, Soh JW, Monden T, et al. Paradoxical increase in retinoblastoma protein in colorectal carcinomas may protect cells from apoptosis. *Clin Cancer Res* 1999; **5**: 1805–15.
32. Slootweg PJ. p53 protein and Ki-67 reactivity in epithelial odontogenic lesions. An immunohistochemical study. *J Oral Pathol Med* 1995; **24**: 393–7.
33. Sherr CJ. Cancer cell cycles. *Science* 1996; **274**: 1672–7.
34. Tsuda H, Yamamoto K, Inoue T, Uchiyama I, Umesaki N. The role of p16-cyclin D/CDK-pRb pathway in the tumorigenesis of endometrioid-type endometrial carcinoma. *Br J Cancer* 2000; **82**: 675–82.
35. Kamb A, Gruis NA, Weaver-Feldhaus J, et al. A cell cycle regulator potentially involved in genesis of many tumor types. *Science* 1994; **264**: 436–40.
36. Donnellan R, Chetty R. Cyclin D1 and human neoplasia. *Mol Pathol* 1998; **51**: 1–7.
37. Kovesdi I, Reichel R, Nevins JR. Identification of a cellular transcription factor involved in E1A *trans*-activation. *Cell* 1986; **45**: 219–28.
38. Zhang SY, Liu SC, Johnson DG, Klein-Szanto AJ. *E2F-1* gene transfer enhances invasiveness of human head and neck carcinoma cell lines. *Cancer Res* 2000; **60**: 5972–6.
39. Pierce AM, Schneider-Broussard R, Gimenez-Conti IB, Russell JL, Conti CJ, Johnson DG. E2F1 has both oncogenic and tumor-suppressive properties in a transgenic model. *Mol Cell Biol* 1999; **19**: 6408–14.
40. Bates S, Phillips AC, Clark PA, et al. $p14^{ARF}$ links the tumour suppressors RB and p53. *Nature* 1998; **395**: 124–5.
41. Lissy NA, Davis PK, Irwin M, Kaelin WG, Dowdy SF. A common E2F-1 and p73 pathway mediates cell death induced by TCR activation. *Nature* 2000; **407**: 642–5.
42. Kumamoto H, Izutsu T, Ohki K, Takahashi N, Ooya K. *p53* gene status and expression of p53, MDM2, and $p14^{ARF}$ proteins in ameloblastomas. *J Oral Pathol Med* 2004; **33**: 292–9.
43. Kumamoto H, Ohki K, Ooya K. Expression of p63 and p73 in ameloblastomas. *J Oral Pathol Med* 2005; **34**: 220–6.