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# *p53* gene status and expression of p53, MDM2, and p14$^{ARF}$ proteins in ameloblastomas

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**BACKGROUND: To clarify the roles of the p53–MDM2–p14$^{ARF}$ cell cycle regulation system in oncogenesis and cytodifferentiation of odontogenic tumors, *p53* gene status and expression of p53, MDM2, and p14$^{ARF}$ proteins was analyzed in ameloblastomas as well as tooth germs.**

**METHODS: Paraffin sections of 16 tooth germs and 46 benign and 5 malignant ameloblastomas were examined immunohistochemically for the expression of p53, MDM2, and p14$^{ARF}$ proteins. Frozen tissue samples of 10 benign ameloblastomas and 1 malignant (metastasizing) ameloblastoma were analyzed by direct DNA sequencing to detect *p53* gene alteration.**

**RESULTS: Immunohistochemical reactivity for p53 was detected in 2 of 13 tooth germs, 13 of 29 ameloblastomas, and 5 of 5 malignant ameloblastomas, and the expression ratio of p53 in tooth germs was significantly lower than those in benign and malignant ameloblastomas. Direct DNA sequencing showed no alteration of *p53* gene exons 5–8 in any sample of 10 benign ameloblastomas and 1 metastasizing ameloblastoma. Expression of MDM2 and p14$^{ARF}$ was detected in all samples of normal and neoplastic odontogenic epithelium, and the expression ratios in tooth germs tended to be lower than those in benign and malignant ameloblastomas. In ameloblastomas, expression of p53, MDM2, and p14$^{ARF}$ was significantly higher in plexiform cases than in follicular cases. Markedly decreased reactivity for p53, MDM2, and p14$^{ARF}$ was detected in keratinizing and granular cells in ameloblastoma subtypes. Basal cell ameloblastoma showed slightly higher reactivity for p53, MDM2, and p14$^{ARF}$ as compared with other subtypes.**

**CONCLUSION: Elevated expression of p53, MDM2, and p14$^{ARF}$ in benign and malignant ameloblastomas suggests that alteration of the p53–MDM2–p14$^{ARF}$ cascade is involved in oncogenesis and/of malignant transformation of odontogenic epithelium. *p53* gene status implied that *p53* mutation might play a minor role in neoplastic changes of odontogenic epithelium. Immunoreactivity for p53, MDM2, and p14$^{ARF}$ in ameloblastoma variants suggests that these factors might be associated with tissue structuring and cytodifferentiation of ameloblastomas.**
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**Keywords:** ameloblastoma; MDM2; p14$^{ARF}$; p53

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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 (5–8); however, the detailed mechanisms of oncogenesis, cytodifferentiation, and tumor progression remain unknown.

A series of genetic alterations appears to promote the development of tumors via multiple steps (9, 10). *p53* gene is well recognized as a tumor suppressor gene situated on chromosome 17p13 and is one of the most frequently altered genes in tumors (11–13). Its gene product is a transcriptional factor that plays an important role in response to cellular DNA damage by inducing either G1/S cell cycle arrest to allow DNA repair or apoptosis if DNA has suffered irreversible damage (13, 14). Mutation and loss of heterozygosity

(LOH) of *p53* gene and/or accumulation of p53 product protein have been associated with increased cellular proliferation and malignant transformation (10, 12, 15–21). *MDM2* gene, mapped to chromosome 12q13-14, was originally identified as a highly amplified gene in a transformed tumorigenic fibroblast cell line (22). Its product protein forms a tight complex with both wild- and mutant-type p53 protein and inactivates wild-type p53 function (23). Amplification of *MDM2* gene and overexpression of its product protein have been reported to be involved in tumorigenesis or tumor development in several human malignancies (7, 17, 19, 24). $p14^{ARF}$ gene is located at the INK4a/ARF locus on chromosome 9p21, a region with a high rate of LOH in human tumors, and shared exons with $p16^{INK4a}$ gene, which encodes a cyclin-dependent kinase inhibitor, in an alternative reading frame (25, 26). $p14^{ARF}$ product directly interacts with MDM2 and neutralizes MDM2-mediated inhibition of p53 (27). Certain tumors have shown $p14^{ARF}$ gene alterations, including LOH, mutation, and hypermethylation (21, 28, 29).

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 (26, 30–34). Several studies have examined alteration of *p53* gene and expression of p53 and MDM2 proteins in specific odontogenic tumors (7, 8, 18, 35). In the present study, the immunohistochemical expression of p53, MDM2, and $p14^{ARF}$ proteins and mutation of *p53* gene was examined in ameloblastomas as well as in tooth germs to clarify the possible role of p53 and its upstream regulators 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 51 patients with epithelial odontogenic tumor 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 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 5 malignant ameloblastomas. Ameloblastomas were divided into 29 follicular and 17 plexiform types, including 17 acanthomatous, 5 granular cell, 3 basal cell, and 4 desmoplastic subtypes. Malignant ameloblastomas were classified into two metastasizing ameloblastomas and three ameloblastic carcinomas according to the criteria provided by Eversole (3). For direct DNA sequencing, tumor tissues were immediately frozen on dry ice and stored at $-80^{\circ}$C. Specimens of 16 tooth germs of the mandibular third molars, enucleated for orthodontic reasons at the stage of crown mineralization, were similarly prepared and compared with the epithelial odontogenic tumors.

*Immunohistochemistry for p53, MDM2, and $p14^{ARF}$ expression*

The tissue sections were deparaffinized and immersed in methanol with 0.3% hydrogen peroxide. For antigen retrieval, the sections were 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-p53 monoclonal antibody (Dako, Glostrup, Denmark; subclass IgG2b; diluted at 1 : 50), mouse anti-MDM2 monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA; subclass IgG1; diluted at 1 : 200), and rabbit anti-$p14^{ARF}$ polyclonal antibody (Santa Cruz Biotechnology; diluted at 1 : 200). 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-chromogranin A monoclonal antibody (Dako; subclass IgG2b), mouse anti-desmin monoclonal antibody (Nichirei; subclass IgG1), and normal rabbit IgG instead of the primary antibodies and were confirmed to be unstained.

Immunohistochemical reactivity for p53, MDM2, and $p14^{ARF}$ was evaluated and classified into four groups: (−) negative, (±) weakly positive (less than 5% of epithelial or neoplastic cells), (+) moderately positive (5–25% of epithelial or neoplastic cells), and (++) strongly positive (more than 25% 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. *P*-values less than 0.05 were considered to indicate statistical significance.

*Direct DNA sequencing for p53 gene mutation*

Genomic DNA was extracted from frozen tissue samples of 10 benign ameloblastomas and 1 malignant ameloblastoma, which immunohistochemically showed moderately positive (+) reactions for p53 protein, using a QIAamp DNA Mini

**Table 1** Primers for *p53* sequencing

| *Exon* | *Codon* | *Sequence (5′–3′)* | *Product (bp)* |
|---|---|---|---|
| 5 | 146–186 | Forward: GCTGTGGGTTGATTCCACAC | 167 |
| | | Reverse: AACCAGCCCTGTCGTCTCTC | |
| 6 | 187–224 | Forward: GCCTCTGTTCCTCACTGATT | 175 |
| | | Reverse: TCCTCCCAGAGACCCCAGTT | |
| 7 | 225–261 | Forward: CCTCATCTTGGGCCTGTGTT | 171 |
| | | Reverse: CAGTGTGCAGGGTGGCAAGT | |
| 8 | 262–306 | Forward: TTCCTTACTGCCTCTTGCTT | 206 |
| | | Reverse: CACCGCTTCTTGTCCTGCTT | |

Kit (Qiagen, Hilden, Germany). *p53* exons 5–8, where most mutations of *p53* gene occur in human tumors, were separately amplified using a HotstarTaq Master Mix Kit (Qiagen) with specific primers (Table 1) in a DNA thermal cycler (Eppendorf, Hamburg, Germany). Polymerase chain reaction (PCR) was performed in a total volume of 50 µl, containing 0.5 µg of template DNA 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 *p53* exon were carried out with the PCR products purified using a 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 denaturing 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 Analyser (Amersham Biosciences).

## Results

### *Immunohistochemical reactivity for p53, MDM2, and p14ARF*

The results of immunohistochemical studies of p53, MDM2, and p14$^{ARF}$ are summarized in Table 2. Immunohistochemical reactivity for p53 was detected in the nuclei of normal and neoplastic odontogenic epithelial cells (Fig. 1). In tooth germs, p53 expression was found in limited epithelial cells in 2 of 13 dental laminae. Ameloblastomas showed p53 reactivity scatteredly in peripheral columnar or cuboidal cells in 13 of 29 follicular cases and 16 of 17 plexiform cases (Fig. 1A,B). p53 expression in ameloblastomas was significantly higher than that in enamel organs ($P < 0.05$) and dental laminae ($P < 0.01$) of tooth germs. Plexiform ameloblastomas exhibited statistically higher p53 expression than follicular ameloblastomas ($P < 0.001$). Keratinizing cells in acanthomatous ameloblastomas and granular cells in granular cell ameloblastomas were not reactive with anti-p53 antibody. Basal cell ameloblastomas showed p53 reactivity in scattered neoplastic cells, whereas p53 expression in desmoplastic ameloblastomas was found in a few neoplastic cells. Expression of p53 in malignant ameloblastomas was detected in all five cases and was significantly higher than that in enamel organs and dental laminae ($P < 0.01$). Metastasizing ameloblastomas showed a p53 expression pattern similar to that of follicular ameloblastomas, while ameloblastic carcinomas demonstrated increased p53 expression in neoplastic cells (Fig. 1C).

Immunohistochemical reactivity for MDM2 and p14$^{ARF}$ was detected in the nuclei of normal and neoplastic odontogenic epithelial cells: mesenchymal cells in tooth germs and stromal cells in benign and malignant ameloblastomas were faintly reactive with anti-MDM2 and anti-p14$^{ARF}$ antibodies (Figs. 2 and 3). In all tooth germs, MDM2 expression was found in scattered epithelial cells of outer enamel epithelium and dental laminae. Ameloblastomas showed MDM2 reactivity in many peripheral columnar or

**Table 2** Immunohistochemical reactivity for p53, MDM2, and p14$^{ARF}$ proteins in tooth germs and ameloblastomas

| | *p53* | | | | | *MDM2* | | | | | *p14ARF* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (–) | (±) | (+) | (++) | | (–) | (±) | (+) | (++) | | (–) | (±) | (+) | (++) | |
| Tooth germ ($n = 16$) | | | | | | | | | | | | | | | |
| Enamel organ ($n = 8$) | 8 (100) | 0 (0) | 0 (0) | 0 (0) | | 0 (0) | 2 (25) | 6 (75) | 0 (0) | | 2 (25) | 5 (62) | 1 (13) | 0 (0) | |
| Dental lamina ($n = 13$) | 11 (85) | 2 (15) | 0 (0) | 0 (0) | * | 0 (0) | 2 (15) | 6 (47) | 5 (38) | * | 0 (0) | 5 (38) | 4 (31) | 4 (31) | ** |
| Ameloblastoma ($n = 46$) | 17 (37) | 9 (20) | 20 (43) | 0 (0) | ** | 0 (0) | 3 (7) | 17 (37) | 26 (56) | | 0 (0) | 5 (11) | 30 (65) | 11 (24) | |
| Follicular type ($n = 29$) | 16 (55) | 6 (21) | 7 (24) | 0 (0) | *** | 0 (0) | 3 (10) | 14 (48) | 12 (42) | ** | 0 (0) | 4 (14) | 23 (79) | 2 (7) | ** |
| Plexiform type ($n = 17$) | 1 (6) | 3 (18) | 13 (76) | 0 (0) | | 0 (0) | 0 (0) | 3 (18) | 14 (82) | | 0 (0) | 1 (6) | 7 (41) | 9 (53) | |
| Acanthomatous subtype ($n = 17$) | 6 (35) | 2 (12) | 9 (53) | 0 (0) | ** | 0 (0) | 2 (12) | 7 (41) | 8 (47) | | 0 (0) | 2 (12) | 13 (76) | 2 (12) | ** |
| Granular subtype ($n = 5$) | 2 (40) | 3 (60) | 0 (0) | 0 (0) | | 0 (0) | 1 (20) | 3 (60) | 1 (20) | | 0 (0) | 1 (20) | 4 (80) | 0 (0) | * |
| Basal cell subtype ($n = 3$) | 0 (0) | 0 (0) | 3 (100) | 0 (0) | | 0 (0) | 0 (0) | 1 (33) | 2 (67) | | 0 (0) | 0 (0) | 0 (0) | 3 (100) | ** |
| Desmoplastic subtype ($n = 4$) | 2 (50) | 2 (50) | 0 (0) | 0 (0) | | 0 (0) | 0 (0) | 0 (0) | 4 (100) | | 0 (0) | 0 (0) | 4 (100) | 0 (0) | |
| Malignant ameloblastoma ($n = 5$) | 0 (0) | 2 (40) | 2 (40) | 1 (20) | | 0 (0) | 1 (20) | 0 (0) | 4 (80) | | 0 (0) | 0 (0) | 2 (40) | 3 (60) | |
| Metastasizing ameloblastoma ($n = 2$) | 0 (0) | 1 (50) | 1 (50) | 0 (0) | | 0 (0) | 1 (50) | 0 (0) | 1 (50) | | 0 (0) | 0 (0) | 2 (100) | 0 (0) | * |
| Ameloblastoma carcinoma ($n = 3$) | 0 (0) | 1 (33) | 1 (33) | 1 (33) | | 0 (0) | 0 (0) | 0 (0) | 3 (100) | | 0 (0) | 0 (0) | 0 (0) | 3 (0) | |

Immunohistochemical reactivity: (–) negative; (±) weakly (less than 5% of epithelial or neoplastic cells) positive; (+) moderately (5–25% of epithelial or neoplastic cells) positive; (++) strongly (more than 25% of epithelial or neoplastic cells) positive.
Values in parentheses denote percentage values.
Statistical significance: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

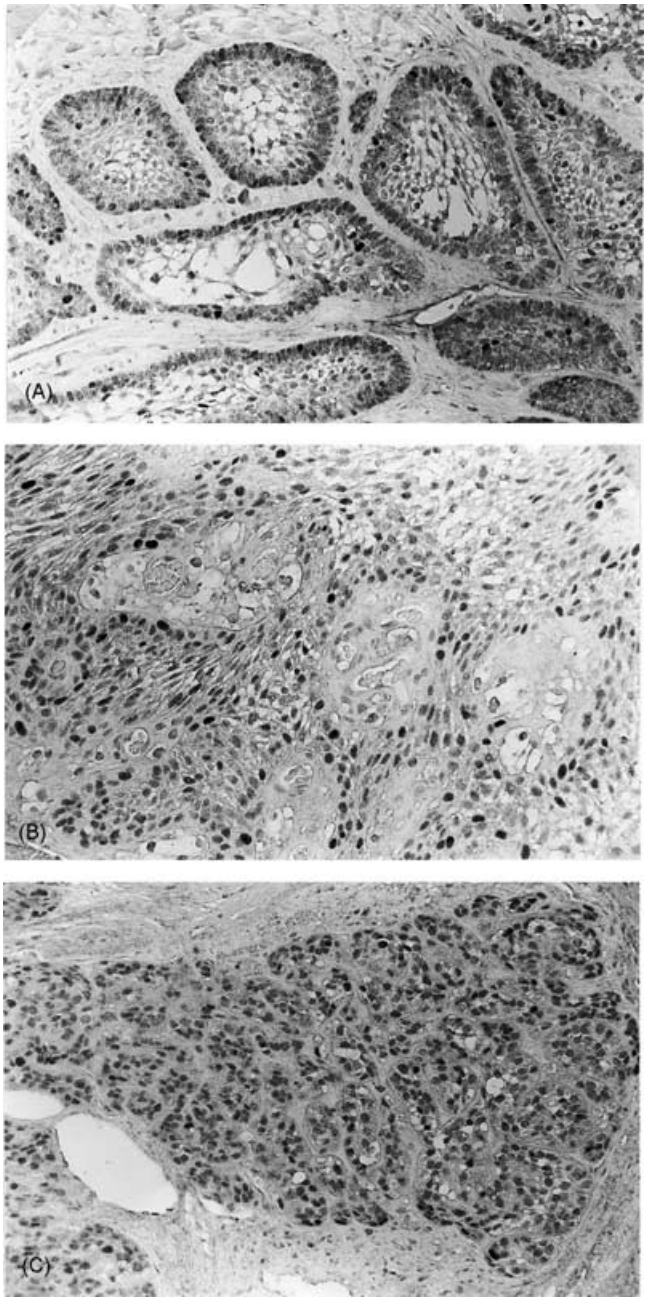


**Figure 1** Immunohistochemical reactivity for p53 in ameloblastoma (A,B) and malignant ameloblastoma (C). Follicular ameloblastoma (A) and plexiform ameloblastoma (B) showing reactivity scatteredly in peripheral columnar or cuboidal cells (A: ×160; B: ×160). Ameloblastic carcinoma (C) showing reactivity in most neoplastic cells (×160).

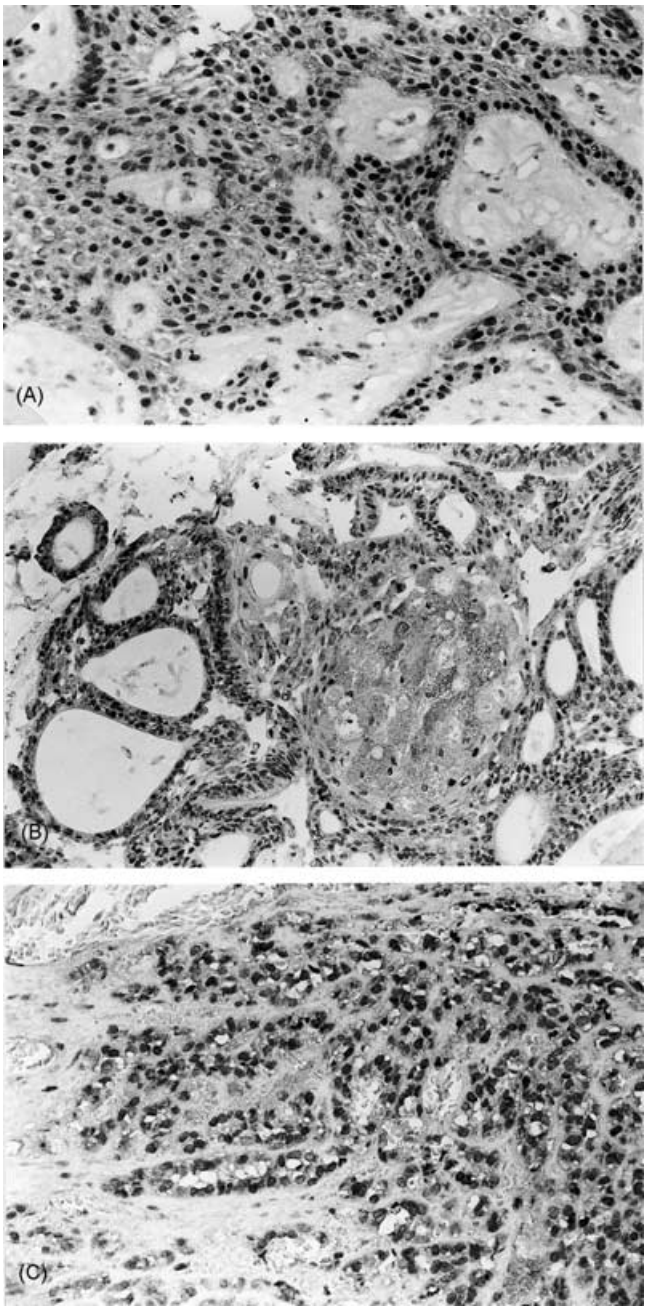


**Figure 2** Immunohistochemical reactivity for MDM2 in ameloblastoma (A,B) and malignant ameloblastoma (C). (A) Plexiform ameloblastoma showing reactivity in many peripheral cuboidal cells and some central polyhedral cells (×175). (B) Granular cell ameloblastoma showing a little reactivity in granular cells (×140). (C) Ameloblastic carcinoma showing reactivity in most neoplastic cells (×180).

cuboidal cells and some central polyhedral cells in all cases (Fig. 2A). MDM2 expression in ameloblastomas was significantly higher than that in enamel organs of tooth germs ($P < 0.05$). Plexiform ameloblastomas exhibited statistically higher MDM2 expression than follicular ameloblastomas ($P < 0.01$). MDM2 reactivity was markedly decreased in keratinizing cells in acanthomatous ameloblastomas and granular cells in granular cell ameloblastomas (Fig. 2B). Basal cell ameloblastomas and desmoplastic ameloblastomas showed diffuse MDM2 expression in neoplastic cells, and staining intensity in desmoplastic ameloblastomas was low. Expression of MDM2 in malignant ameloblastomas was detected in all cases. Metastasizing ameloblastomas showed a MDM2 expression pattern similar to that of follicular ameloblastomas, while ameloblastic carcinomas were diffusely positive for MDM2 in most neoplastic cells (Fig. 2C).

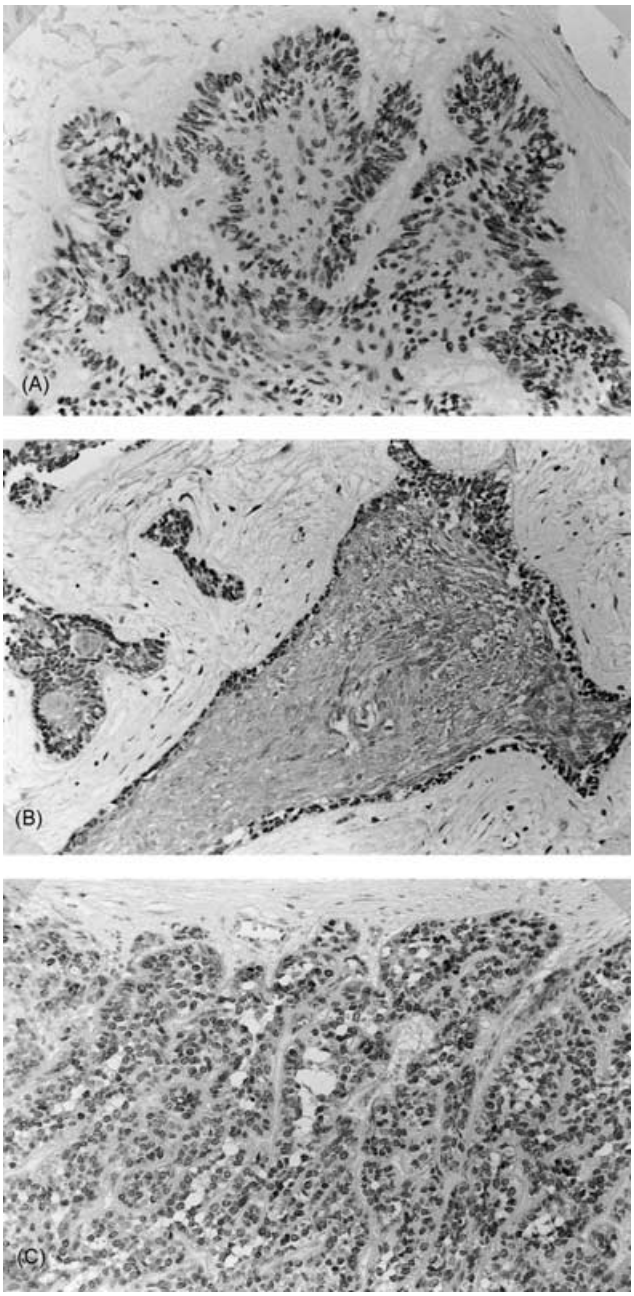


**Figure 3** Immunohistochemical reactivity for $p14^{ARF}$ in ameloblastoma (A,B) and malignant ameloblastoma (C). (A) Follicular ameloblastoma showing reactivity in many peripheral columnar cells and some central polyhedral cells (×160). (B) Desmoplastic ameloblastoma showing reactivity in neoplastic cells neighboring the basement membrane (×130). (C) Ameloblastic carcinoma showing reactivity in most neoplastic cells (×140).

$p14^{ARF}$ expression was found in scattered epithelial cells of outer enamel epithelium and dental laminae in all but two tooth germs, and dental laminae showed significantly higher $p14^{ARF}$ expression than enamel organs did ($P < 0.05$). Ameloblastomas showed $p14^{ARF}$ reactivity in many peripheral columnar or cuboidal cells and some central polyhedral cells in all cases (Fig. 3A). $p14^{ARF}$ expression in ameloblastomas was significantly higher than that in enamel organs of tooth germs ($P < 0.01$). Plexiform ameloblastomas exhibited statistically higher $p14^{ARF}$ expression than follicular ameloblastomas ($P < 0.01$). Keratinizing cells in acanthomatous ameloblastomas and granular cells in granular cell ameloblastomas demonstrated little or no reactivity for $p14^{ARF}$. Basal cell ameloblastomas showed diffused $p14^{ARF}$ expression in neoplastic cells, whereas $p14^{ARF}$ reactivity in desmoplastic ameloblastomas was localized in neoplastic cells neighboring the basement membrane (Fig. 3B). $p14^{ARF}$ expression in basal cell ameloblastomas was significantly higher than that in acanthomatous ameloblastomas ($P < 0.05$) and granular cell ameloblastomas ($P < 0.01$). Expression of $p14^{ARF}$ in malignant ameloblastomas was detected in all cases and was significantly higher than that in enamel organs of tooth germs ($P < 0.01$). Metastasizing ameloblastomas showed a $p14^{ARF}$ expression pattern similar to that of follicular ameloblastomas, while ameloblastic carcinomas were diffusely positive for $p14^{ARF}$ in most neoplastic cells (Fig. 3C). Ameloblastic carcinomas exhibited statistically higher $p14^{ARF}$ expression than metastasizing ameloblastomas ($P < 0.05$).

*Mutation analysis of p53 gene*

Direct DNA sequencing for *p53* gene mutation was carried out in 10 ameloblastomas (five follicular and five plexiform cases) and 1 malignant ameloblastoma (one metastasizing ameloblastoma), which were moderately positive for p53 protein. Mutational alteration was not detected in *p53* gene exons 5–8, including hotspot codons 175, 245, 248, 249, 273, and 282, in any of the 11 cases (Fig. 4).

## Discussion

Mutation of *p53* gene results in accumulation of a conformationally altered and functionally defective protein, and overexpression of p53 protein has been detected in various types of tumors (17–21). The present study was performed, employing a monoclonal antibody reactive with wild- and mutant-type p53 protein. Tooth germ tissue showed no or little p53 expression, whereas nuclear accumulation was recognized in benign and malignant ameloblastomas. These features suggest that p53 expression is associated with oncogenesis of odontogenic epithelium. In ameloblastomas, reactivity for p53 was significantly higher in plexiform-type than in follicular-type, suggesting that tissue structuring of ameloblastomas might be affected by p53 expression. In our previous study, keratinizing cells in acanthomatous ameloblastomas and granular cells in granular cell ameloblastomas showed increased apoptotic cell death as compared with other neoplastic cells (30, 32). The present study found no p53 expression in keratinizing or granular cells in ameloblastomas. Apoptosis of these cells was thus apparently not induced by a p53-dependent pathway.

In a wide variety of human tumors, *p53* gene mutations have been detected mainly in exons 5–8, including several hotspot codons (10, 12, 13, 15, 16, 19, 21). Ameloblastomas have shown infrequent *p53* mutations in limited number of neoplastic cells on ELISA and yeast functional assay (7, 8). In the present study using direct *p53* sequencing, alteration of *p53* exons 5–8 was not detected in 10 benign ameloblastomas or 1 metastasizing ameloblastoma, although these

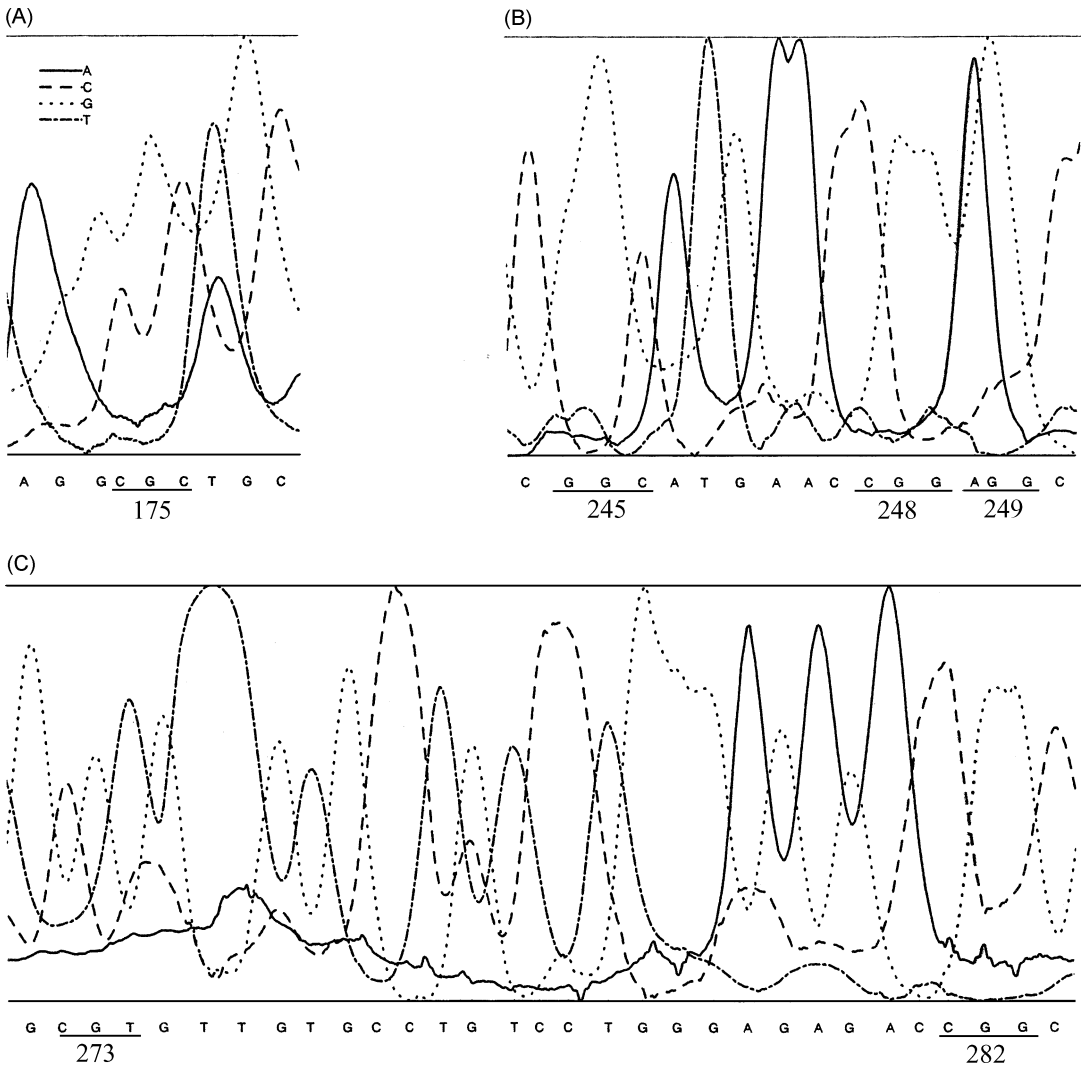


**Figure 4** Direct DNA sequencing of *p53* gene in ameloblastoma. No mutation is detected at hotspot codons: codon 175 in exon 5 (A), codons 245, 248, and 249 in exon 7 (B) and codons 273 and 282 in exon 8 (C).

cases were immunohistochemically positive for p53. These findings suggest that mutation of *p53* gene might play a minor role in, and not be essential for, neoplastic changes of odontogenic epithelium. Wild-type p53 protein transcriptionally activates genes involved in cell cycle arrest, such as $p21^{WAF1/Cip1}$, or genes modulating apoptosis, such as bax (13, 36). Our previous study demonstrated low reactivity for bax protein and obvious $p21^{WAF1/Cip1}$ expression in ameloblastomas (26, 31). These features indicate that wild-type p53 protein regulates the cell cycle via $p21^{WAF1/Cip1}$ protein in ameloblastomas. In the present study, several ameloblastic carcinomas immunohistochemically showed increased p53 reactivity, and p53 was possibly associated with malignant transformation of odontogenic epithelium. Mutation analysis of *p53* gene in ameloblastic carcinomas could not be investigated because of the rarity of the malignancy, and further studies should be carried out to determine the association between p53 and malignant changes of odontogenic epithelium. Kropveld et al. (37) has revealed that 33% of all mutations are located outside the core domain of *p53* gene in head and neck squamous cell carcinomas. In our study, mutation analysis was performed only in the core domain of *p53* gene, and sequencing analysis of all 11 exons might be needed to guarantee absence of *p53* mutations.

The $p14^{ARF}$–MDM2–p53 cascade, called the p53 pathway, is an important cell cycle regulatory system in G1 arrest (25, 38), and aberration of this system strongly correlates with neoplastic transformation (7, 17, 19, 21, 24, 28, 29). The ability to generate mice lacking *p53* implies that *p53* is dispensable for embryonic development, while expression of MDM2 and $p14^{ARF}$ during development suggests that these molecules have a primary role in developmental processes (13,39). In the present study, tooth germs showed higher immunohistochemical reactivity for MDM2 and $p14^{ARF}$ as compared with that for p53, indicating that MDM2 and $p14^{ARF}$ play certain roles in tooth development. MDM2 expression in ameloblastomas has been reported to be associated with proliferative activity (7). Our previous study revealed that expression of $p16^{INK4a}$ protein did not

differ distinctly between tooth germs and ameloblastomas (26). In the present study, expression of MDM2 and p14$^{ARF}$ was higher in ameloblastomas and malignant ameloblastomas than in tooth germs, suggesting that these upstream regulators of p53 are involved in oncogenesis and/or malignant transformation of odontogenic epithelium. Plexiform ameloblastomas showed higher expression of MDM2 and p14$^{ARF}$ than follicular ameloblastomas, and markedly decreased reactivity for MDM2 and p14$^{ARF}$ was found in keratinizing and granular cells in ameloblastomas, similar to p53 expression in ameloblastomas. In addition, basal cell ameloblastomas demonstrated high reactivity for p53, MDM2, and p14$^{ARF}$ as compared with other subtypes of ameloblastomas, and p14$^{ARF}$ expression in desmoplastic ameloblastomas was localized in neoplastic cells of basal areas. These features suggest that the p53–MDM2–p14$^{ARF}$ cell cycle regulation system might be related to tissue structuring and cytodifferentiation of ameloblastomas.

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