Comparative immunohistochemical study of ameloblastoma and ameloblastic carcinoma

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Objective. Ameloblastic carcinoma combines the histologic features of ameloblastoma with cytologic atypia, regardless of whether it has metastasized. Because of its rarity, there are few immunoprofile studies of ameloblastic carcinoma and few comparative studies of ameloblastic carcinoma and ameloblastoma. In this study, we compared the expression levels of cytokeratins (CKs), matrix metalloproteinases (MMPs), and Ki-67 between ameloblastoma and ameloblastic carcinoma, and assessed the usefulness of these markers for differentiating the tumors.

Study design. We assessed CK7, CK14, CK18, CK19, MMP-2, MMP-9, and Ki-67 expression by immunohistochemistry in 10 cases of ameloblastoma and 7 cases of ameloblastic carcinoma and then compared expression patterns between the 2 groups.

Results. Immunostaining for CK14 and CK19 was diffuse and strongly positive in both tumor types, but staining for CK7 was focally positive in only 1 case of ameloblastoma and absent in all cases of ameloblastic carcinoma. However, there was a significant difference in CK18 expression between the 2 tumors (P = .000). Whereas 80% of ameloblastomas showed negative reactivity for CK18, most cases of ameloblastic carcinomas showed a moderate to strong intensity of immunostaining for CK18. Regarding the expression of MMPs, there were significant differences in parenchymal MMP-2 and stromal MMP-9 expression between the 2 tumors. Compared to ameloblastoma, ameloblastic carcinoma showed significantly strong expression of MMP-2 in parenchymal cells (P = .001) and MMP-9 in stromal cells (P = .013). However, there were no differences in MMP-2 expression of stromal cells and MMP-9 expression of parenchymal cells between ameloblastoma and ameloblastic carcinoma. The mean Ki-67 labeling index (LI) of ameloblastic carcinomas was 17.21%, which was significantly higher than that of ameloblastomas (3.57%; P = .002).


Ameloblastoma is the most common benign odontogenic tumor of the jaw and rarely exhibits malignant behavior. After much debate on the definition and classification of malignant versions of ameloblastoma,¹-³ the World Health Organization in 2005⁴ classified the malignant counterparts of ameloblastoma into malignant ameloblastoma and ameloblastic carcinoma. Malignant ameloblastoma gives rise to lung or regional lymph node metastases despite benign histologic features of the primary lesion. Ameloblastic carcinoma evidences cytologic atypia, even in the absence of the metastasis. Because of the obvious cytologic atypia in ameloblastic carcinoma, it is not difficult to differentiate ameloblastic carcinoma from ameloblastoma on routine histologic examination. However, it would be more reliable to differentiate the tumors based on biologic behavior, such as the growth fraction of tumors, the expression of invasiveness-related molecules, and...
the expression of distinct intermediate filaments, such as cytokeratin. Although there have been some case reports on ameloblastic carcinoma, there are few reports on the immunoprofile of ameloblastic carcinoma. Also, to our knowledge, there have not been any reports comparing cytokeratin (CK) expression of ameloblastoma and ameloblastic carcinoma.

Cytokeratins, a class of intermediate filaments, are essential intracellular components. CKs are expressed depending on epithelial cell type and degree of differentiation. Twenty different CK polypeptides have been identified in human epithelia. A limited number of reports have demonstrated that ameloblastomas express a variety of CKs, including CKs 5, 7, 8, 13, 14, 18, and 19. In particular, CK14 and CK19 seem to be mainly expressed in neoplastic epithelial cells of some odontogenic tumors, including ameloblastoma. However, in ameloblastoma, CK7 has been rarely detected and CK18 was either absent or weakly expressed in the focal area of tumor nests.

Matrix metalloproteinases (MMPs) are a family of zinc- and calcium-dependent proteolytic enzymes that are necessary to degrade the extracellular matrix (ECM). Degradation of ECM is an essential step in tumor invasion and metastasis. MMPs are divided into several subclasses according to their substrate specificity and structural characteristics: collagenases (MMPs 1, 8, and 13), gelatinases/type IV collagenases (MMP-2 and MMP-9), stromelysins (MMP-3 and MMP-10), matrilysins (MMP-7), elastase (MMP-12), MT-MMPs (MMPs 14, 15, 16, and 17), enameylsins (MMP-20), and other MMPs (MMP-11, MMP-19, and others). The type IV collagenases, MMP-2 and MMP-9, have been particularly emphasized because they are related to tumor invasion and metastasis. Some investigators have suggested that MMP-1,-2, and/or -9 might contribute to the invasive capacity of ameloblastoma.

The assessment of proliferation has been applied in histopathology as a means to predict the behavior of tumors. Although Ki-67 and proliferating cell nuclear antigen (PCNA) are generally used to measure the proliferative activity of tumors, Ki-67 staining has been accepted as a more informative marker than PCNA staining because of many of the vagaries of PCNA in archival tissue sections. Although various studies of ameloblastoma have assessed cell proliferation using the expression of distinct intermediate filaments, such as cytokeratin. Although there have been some case reports on ameloblastic carcinoma, there are few reports on the immunoprofile of ameloblastic carcinoma. Also, to our knowledge, there have not been any reports comparing cytokeratin (CK) expression of ameloblastoma and ameloblastic carcinoma.

The aims of the present study were: 1) to compare the expression of CKs (CKs 7, 14, 18, and 19), MMPs (MMP-2 and -9), and Ki-67 between ameloblastoma and ameloblastic carcinoma; and 2) to find a useful marker for differentiating tumors.

**MATERIALS AND METHODS**

**Patients and tissue samples**

Ten cases of ameloblastoma and 7 cases of ameloblastic carcinoma were retrieved from the files of the Department of Oral Pathology, Seoul National University Dental Hospital. Slides were reviewed by 2 qualified and experienced oral pathologists.

**Immunohistochemistry**

Immunohistochemical staining was performed on the formalin-fixed paraffin-embedded sections (4 μm). The sections were deparaffinized through a series of xylene baths and then rehydrated in graded alcohols. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide for 15 minutes at room temperature. For antigen retrieval, the sections were treated with Target Retrieval Solution (Dako, Glostrup, Denmark) in the microwave for 15 minutes. Sections were then incubated with the primary antibodies, except for anti-MMP-2 antibody, for 1 hour at room temperature; the sections were incubated with monoclonal mouse antihuman MMP-2 antibody overnight at 4°C. Antibodies used in this study were monoclonal mouse antihuman CK7 (OV-TL 12/13, 1:50; Dako), CK14 (LL002, 1:50; DBS), CK18 (DC-10, 1:50; Dako), CK 19 (RCK 108, 1: 100; Dako), Ki-67 (MIB-1, 1:50; Dako), MMP-2 (4D3, 1:100; Santa Cruz Biotechnology), and polyclonal rabbit antihuman MMP-9 (1:50; DBS). The slides were stained using a Dako Real Envision/HRP kit. Immunohistochemical reactions were developed with diaminobenzidine as the chromogenic peroxidase substrate, and slides were counterstained with Meyer hematoxylin. Negative control samples were prepared by replacing the primary antibody with mouse or rabbit IgG isotype (Sigma, St. Louis, MO).

**Evaluation of staining**

Sections were evaluated by 2 blinded experienced investigators. The staining intensity of CKs was classified as − (negative), + (weak diffuse, focally moderate, or strong positive in <10% of tumor cells), ++ (moderate diffuse), and +++ (strong diffuse), with scoring as 0, 1, 2, and 3, respectively. The expression of MMPs was also assessed by semiquantitative analysis. The percentage of immunopositive cells was scored as 0 = 0%, 1 = <10%, 2 = 10%-50%, and 3 = >50%. The staining intensity was scored as 0 = negative, 1 = weak, 2 = moderate, and 3 = strong positive. The immunoscore for MMPs was calculated by multiplying the percentage score and intensity score, and then classified as follows: 0, negative (−); 1 and 2, weak posi-
Table I. Clinical information of patients with ameloblastoma

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Age (y)</th>
<th>Gender</th>
<th>Location</th>
<th>Signs/symptoms</th>
<th>Perforation of the cortex</th>
<th>Treatment</th>
<th>Metastasis</th>
<th>DOF (mo)/Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 (F)</td>
<td>32</td>
<td>F</td>
<td>Mn</td>
<td>No</td>
<td>Lingual</td>
<td>Enucleation</td>
<td></td>
<td></td>
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<tr>
<td>A2 (F)</td>
<td>52</td>
<td>F</td>
<td>Mn</td>
<td>Swelling</td>
<td>Lingual</td>
<td>En bloc excision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3 (F)</td>
<td>25</td>
<td>M</td>
<td>Mn</td>
<td>Swelling</td>
<td>No</td>
<td>Mass excision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A4 (F)</td>
<td>73</td>
<td>F</td>
<td>Mn</td>
<td>Swelling, pain</td>
<td>Lingual</td>
<td>Mass excision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A5 (P)</td>
<td>27</td>
<td>M</td>
<td>Mn</td>
<td>No</td>
<td>No</td>
<td>Mass excision</td>
<td></td>
<td></td>
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<tr>
<td>A6 (P)</td>
<td>65</td>
<td>M</td>
<td>Mn</td>
<td>No</td>
<td>Lingual</td>
<td>Curettage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A7 (P)</td>
<td>36</td>
<td>M</td>
<td>Mn</td>
<td>Swelling</td>
<td>No</td>
<td>Mass excision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A8 (Ac)</td>
<td>29</td>
<td>F</td>
<td>Mn</td>
<td>Discomfort, swelling</td>
<td>No</td>
<td>Mass excision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A9 (Ac)</td>
<td>37</td>
<td>F</td>
<td>Mx</td>
<td>Swelling</td>
<td>No</td>
<td>En bloc excision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A10 (G)</td>
<td>42</td>
<td>F</td>
<td>Mn</td>
<td>Swelling</td>
<td>Lingual</td>
<td>Mass excision</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age</td>
<td>41.8</td>
<td>M:F 2:3</td>
<td>Mn:Mx 9:1</td>
<td>Pain in only 1 case</td>
<td>Perforation in 50% of cases</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ac, acanthomatous; A, ameloblastoma; F, follicular; G, granular cell; Mn, mandible; Mx, maxilla; P, plexiform.

Table II. Clinical information of patients with ameloblastic carcinoma

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Age (y)</th>
<th>Gender</th>
<th>Location</th>
<th>Signs/symptoms</th>
<th>Tumor size</th>
<th>Perforation of the cortex (pattern of bone invasion)</th>
<th>Treatment</th>
<th>Recur</th>
<th>Metastasis</th>
<th>DOF (mo)/Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC1</td>
<td>63</td>
<td>M</td>
<td>Mx</td>
<td>Swelling, bleeding</td>
<td>2.5 × 2</td>
<td>Buccal/palatal (pushing)</td>
<td>SR + RT</td>
<td>Yes</td>
<td>No</td>
<td>13/A</td>
</tr>
<tr>
<td>AC2</td>
<td>73</td>
<td>F</td>
<td>Mx</td>
<td>Pain, swelling</td>
<td>3 × 1</td>
<td>Buccal/palatal (infiltrative)</td>
<td>SR</td>
<td>No</td>
<td>No</td>
<td>31/A</td>
</tr>
<tr>
<td>AC3</td>
<td>61</td>
<td>M</td>
<td>Mx</td>
<td>Pain, ulcer, trismus</td>
<td>Diameter 5</td>
<td>Biopsy only</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AC4</td>
<td>46</td>
<td>M</td>
<td>Mn</td>
<td>Pain, swelling</td>
<td>Diameter 5</td>
<td>No (recurred lesion around the R-plate area)</td>
<td>SR + ND + RT</td>
<td>Yes</td>
<td>LN</td>
<td>18/A</td>
</tr>
<tr>
<td>AC5</td>
<td>58</td>
<td>M</td>
<td>Mx</td>
<td>Pain, ulcer</td>
<td>Diameter 3</td>
<td>Palatal (pushing)</td>
<td>SR + ND</td>
<td>No</td>
<td>No</td>
<td>12/A</td>
</tr>
<tr>
<td>AC6</td>
<td>65</td>
<td>M</td>
<td>Mn</td>
<td>Pain, ulcer, trismus</td>
<td>2.1 × 1.5</td>
<td>Lingual (pushing and infiltrative)</td>
<td>SR + ND + RT</td>
<td>No</td>
<td>LN</td>
<td>13/A</td>
</tr>
<tr>
<td>AC7</td>
<td>72</td>
<td>M</td>
<td>Mx</td>
<td>Pain</td>
<td>—</td>
<td>Buccal (−)</td>
<td>Biopsy only</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mean age</td>
<td>62.6</td>
<td>M:F 6:1</td>
<td>Mn:Mx 3:4</td>
<td>Pain in 85.7% of cases</td>
<td>1 cortex perforated in 85.7% of cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

—, Not applicable; A, alive; AC, ameloblastic carcinoma; DOF, duration of follow-up; LN, lymph node; ND, neck dissection; RT, radiation; SR, surgical resection.

ditive (+); 3 and 4, moderate positive (++) and 6 and 9, strong positive (+++). To evaluate proliferative activity, the Ki-67 labeling index (LI) was calculated. The Ki-67 LI was defined as the percentage of total tumor cells that were Ki-67 positive. Five fields from each case were randomly selected at a magnification of ×400.

Statistical analysis
Statistical analysis was accomplished using SPSS (version 11.5). The mean score of the expression of CKs and MMPs and the mean Ki-67 LIs were compared between the groups using the t test. P < .05 was considered to be statistically significant.

RESULTS
The clinical information for all patients is summarized in Tables I and II. Representative histopathologic features of ameloblastoma and ameloblastic carcinoma are shown in Fig. 1. The results of immunostaining for CKs, MMPs, and Ki-67 are presented in Table III.

Cytokeratin expression
Both ameloblastoma and ameloblastic carcinoma showed uniformly strong expression of CK 14 in most tumor cells (Fig. 2, a and b). For the expression of CK19, both tumors exhibited diffuse immunoreactivity in most tumor cells (Fig. 2, c and d). There was no significant difference in the staining intensity of CK14 and CK19 between the 2 tumors. CK7 reactivity was focally positive in some tumor nests in 1 case of ameloblastoma, but it was absent in the remaining cases of ameloblastoma and all cases of ameloblastic carcinoma (Fig. 2, e and f). For CK18, most cases of ameloblastoma showed negative reactivity, except for 2 cases showing weak or focally moderate expression in the
central area of the tumor nests (Fig. 2, g), whereas ameloblastic carcinoma showed diffusely moderate to strong expression in almost all cells of the tumor nests in 5 out of 7 cases (Fig. 2, h). Therefore, there was a statistically significant difference in the expression level of CK18 between the 2 groups \( (P = .000) \).

**MMP expression**

MMP-2 was expressed in the cytoplasm of both peripheral columnar cells and stellate reticulum–like cells of ameloblastoma, except for 1 case with negative reactivity. MMP-2 was also present in the surrounding stromal cells. Tumor nests or strands of ameloblastoma showed negative or weak reaction for MMP-2 in 70% of cases, whereas tumor cells in almost all cases of ameloblastic carcinoma showed strong reactivity (Fig. 3, a and b). There was a statistically significant difference in MMP-2 expression in parenchymal cells between the 2 tumors \( (P = .001) \). However, in the stromal cells, all cases except for only 1 case of ameloblastoma showed weak to moderate expression of MMP-2 without significant difference between ameloblastoma and ameloblastic carcinoma.

MMP-9 was also detected in both tumor cells and stromal cells of all examined cases. In contrast to MMP-2, tumor cells in both tumors showed moderate to strong expression of MMP-9 without significant difference between the 2 tumors. However, there was a statistically significant difference in MMP-9 expression in the stromal cells between ameloblastoma and ameloblastic carcinoma \( (P = .013) \). Stromal cells in ameloblastoma showed weak to moderate expression of MMP-9 in most cases, whereas ameloblastic carcinoma showed moderate to strong expression in all cases (Fig. 3, c and d).

**Ki-67 expression and labeling index**

The mean Ki-67 LI of ameloblastomas was 3.57%, whereas that of ameloblastic carcinomas was 17.21%. There was a statistically significant difference in the mean Ki-67 LI between the 2 groups \( (P = .002) \).

**DISCUSSION**

Ameloblastic carcinoma is a rare malignant odontogenic tumor that arises de novo or from a preexisting ameloblastoma. Sometimes it may be difficult to diagnose the malignant transformation of ameloblastoma, because a single definitive microscopic criterion for ameloblastic carcinoma is elusive and the microscopic distinction from ameloblastoma may be subjective. Slater designated the term “atypical ameloblastoma” or “proliferative ameloblastoma” for some ameloblastomas exhibiting basilar hyperplasia and an increased mitotic index, because these features are insufficient to permit a malignant designation in the absence of nuclear pleomorphism, perineural invasion, or other histologic evidence of malignancy.25 The microscopic fea-
Table III. Immunoprofiles of ameloblastoma and ameloblastic carcinoma

<table>
<thead>
<tr>
<th></th>
<th>CK7</th>
<th>CK14</th>
<th>CK18</th>
<th>CK19</th>
<th>MMP-2 Tumor cells</th>
<th>MMP-2 Stromal cells</th>
<th>MMP-9 Tumor cells</th>
<th>MMP-9 Stromal cells</th>
<th>Ki-67 LI</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>−</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3.00</td>
</tr>
<tr>
<td>A2</td>
<td>−</td>
<td>+++</td>
<td>−</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3.45</td>
</tr>
<tr>
<td>A3</td>
<td>+</td>
<td>+++</td>
<td>−</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.75</td>
</tr>
<tr>
<td>A4</td>
<td>−</td>
<td>+++</td>
<td>−</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>A5</td>
<td>−</td>
<td>+++</td>
<td>−</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>1.38</td>
</tr>
<tr>
<td>A9</td>
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<td>+++</td>
<td>−</td>
<td>+++</td>
<td>+</td>
<td>+</td>
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<td>+++</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
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<td>+</td>
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<tr>
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<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>18.80</td>
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<tr>
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<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>38.08</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>P = .000*</td>
<td>NS</td>
<td>P = .001*</td>
<td>NS</td>
<td>P = .013*</td>
<td>P = .002*</td>
<td></td>
</tr>
</tbody>
</table>

CK, Cytokeratin; LI, labeling index; MMP, matrix metalloproteinase; NS, not significant.
*Significant difference in expression between ameloblastoma and ameloblastic carcinoma.

Tissues favoring the malignant transformation include the presence of sheets, islands, or trabeculae of epithelium, the absence of stellate reticulum–like structures, and round-to-spindled epithelial cells with little or no differentiation toward the columnar cell morphology of ameloblastoma.26 Other features of malignancy include hyperchromatism, large or atypical nuclei, increased mitotic index, necrosis and calcification, and particularly neural and vascular invasion. In addition to the histologic changes, demographic features and biologic behavior of the tumor may be helpful to differentiate between the 2 tumors. In the present study, ameloblastic carcinoma showed a much higher mean age, a higher rate of occurrence in men, and a relatively higher proportion of maxillary lesions compared with ameloblastoma. Regarding the differences of clinical and biologic features, Hall et al. demonstrated that ameloblastic carcinoma exhibited 4 characteristics, namely, rapid growth, propensity to perforate the cortex, pain, and paresthesia, that are distinct from ameloblastoma.26 Our study also found that pain was a characteristic symptom in almost all cases of ameloblastic carcinoma and that bone destruction was more aggressive accompanying the perforation of both cortices in ameloblastic carcinoma. Therefore, the integration of histologic changes with the demographic and biologic features seems to be of importance differentiating between ameloblastoma and ameloblastic carcinoma.

Earlier studies had been interested in the expression pattern of CKs in developing tooth germ to understand the histogenesis of either odontogenic cyst or tumor.6,27,28 Analysis of the CK expression of tooth germ has demonstrated that CKs 5, 7, 8, 14, and 19 are expressed in the enamel organ, CK13 is locally distributed in dental lamina, and CK18 is absent.6,9,27,28 Several studies have described the expression pattern of various CKs in ameloblastoma.6-10 Although there were some differences in CK types expressed between the reported cases of ameloblastoma, strong expression of CK14 and CK19 was common in most cases. Immunoreactivity for CK7 was absent in almost all reported cases, except for a case reported by Ong’uti et al. demonstrating a weakly positive reaction in the suprabasal and stellate reticulum–like cells of tumor nests.7,9,10,12 However, CK7 positivity has been detected in other odontogenic tumors, such as odontoma, calcifying epithelial odontogenic tumor, and ameloblastic fibroma.9,12 CK18 has been absent or focally positive in the majority of cases of ameloblastoma according to earlier reports.6,7,9,10 In the present study, ameloblastoma showed uniformly strong positivity for CK14 and CK19 but mostly negative reactivity for CK7 and CK18. These results were in agreement with those from earlier studies.6-11 To our knowledge, no investigation has explored the expression of CKs in ameloblastic carcinoma. In our previous report, we demonstrated that CKs 5, 14, and 18 were strongly positive and CK7 was negative in ameloblastic carcinoma.29 In the present study, comparison of CK expression between ameloblastoma and ameloblastic carcinoma showed that there were no differences in the expression
of CKs 7, 14, and 19. However, there was a significant difference in CK18 expression between the 2 tumors. Five (71.4%) of the 7 ameloblastic carcinomas showed moderate to strong positivity, unlike ameloblastoma, which had negative reactivity for CK18 in 80% of cases. Therefore, expression of CK18 is a distinctive feature of ameloblastic carcinoma compared with ameloblastoma.
CKs are expressed depending on epithelial cell type and degree of differentiation. CK18 is the major intermediate filament of simple epithelial tissue. CK18 and its coexpressed complementary subunit, CK8, are commonly expressed in normal glandular epithelium, transitional epithelium, and hepatocytes, but not in stratified squamous epithelium. Nevertheless, aberrant expression of simple epithelial CK18 has been found in squamous cell carcinomas derived from stratified epithelia. Although CK18 is not expressed in normal oral mucosa cells, there have been some reports demonstrating that CK8/18 expression is increased in squamous cell carcinoma in the head and neck area. This up-regulation has been shown to be related to poor prognosis in squamous cell carcinoma of the oral cavity and esophagus. Therefore, apart from the reflection of organ type and degree of differentiation, CK18 may have some regulatory role in malignant transformation. In an in vitro study, it was reported that transfection of CK8 and CK18 into mouse L-cell fibroblasts and a

Fig. 3. Matrix metalloproteinase (MMP) immunoreactivity. a and b, MMP-2 is significantly expressed in tumor cells of ameloblastic carcinoma in comparison with those of ameloblastoma (×200). c and d, In contrast to MMP-2, MMP-9 is significantly expressed in stromal cells of ameloblastic carcinoma compared to those of ameloblastoma (×100).

Fig. 4. Ki-67 immunoreactivity (×200). Ameloblastic carcinoma (a) showed a significantly high Ki-67 labeling index compared with ameloblastoma (b).
human melanoma cell line resulted in higher migratory and invasive ability compared to the control. Raul et al. reported that the transfection of fetal buccal mucosa cell with CK8 resulted in an increase in CK8/18 filament formation, producing significantly altered cellular morphology and increased cell motility, which are characteristic of invasive tumor behavior. However, CK8/18 expression may not directly correlate with malignant transformation. Oshima et al. demonstrated that oncogenes activating Ras signal-transduction pathways stimulate the expression of CK8 and CK18 genes through transcriptional factors, such as AP-1 and ETS families. Up-regulation of CK18 may merely reflect integrated transcriptional activation of such transcription factors. Taken together, a significant increase of CK18 expression in ameloblastic carcinoma could be interpreted as a change in differentiation (dedifferentiation) of tumor cells or as evidence of malignant transformation, whether or not the up-regulation of CK18 is directly related with the induction of transformed phenotype.

Increased production of MMPs has been found in a number of different malignancies and has been associated with tumor aggressiveness and patient prognosis. The expression of MMP-2 and -9, which can degrade type IV collagen, the major component of basement membrane, has been investigated frequently and has been shown to play a role in the invasion and metastasis of oral carcinoma. Although a number of studies of MMP expression have been reported for various tumors, there have been only a few studies of its expression in odontogenic tumors characterized by local invasion. Increased expression of MMPs 1, 2, or 9, or MT1-MMP in the tumor cells and/or surrounding stromal cells has been associated with regulation of tumor progression in ameloblastic tumors. Recently, Zhang et al. demonstrated that inhibition of MMP-2 activity could suppress the local invasiveness of ameloblastoma in vitro and in vivo. These results indicate that MMP-2 activity is related to the invasive capacity of ameloblastoma. These MMPs are expressed in both tumor cells and stromal cells of ameloblastoma. Kumamoto et al. demonstrated that the expression of MMP-2 and -9 was prominently detected in the stromal cells surrounding tumor nests in most cases of ameloblastoma. In addition, MMP-9 expression in stromal cells of ameloblastoma was significantly stronger than that in the mesenchymal component of tooth germ, suggesting that increased MMP-9 might be related to neoplastic changes in odontogenic tissue. Unlike stromal cells, some tumor cells of ameloblastoma showed weak reactivity for MMP-2 in most cases and for MMP-9 in some cases, resulting in higher expression of MMP-2 than MMP-9 in tumor cells. Ribeiro et al. demonstrated MMP-2 and -9 expression in both the parenchyma and the stroma of ameloblastoma, with MMP-9 showing higher immunoreactivity than MMP-2. This finding contrasts with the results of Kumamoto et al. In the present study, MMP-2 and -9 were detected in both tumor cells and stromal cells of ameloblastoma. Although there was no difference between the immunoreactivity of both MMPs in stromal cells, our results showed stronger expression of MMP-9 than MMP-2 in tumor cells of ameloblastoma, which is consistent with the results of Ribeiro et al. The higher expression of MMP-9 compared with MMP-2 in ameloblastoma was also found in a recent report by Shen et al. Such heterogenous results for the expression of MMP-2 and -9 might be explained by the different antibodies used, tissue processing differences in different laboratories, or less objective evaluation for the immunoreactivity using a semiquantitative analysis.

There have been a few cases of research on MMP expression in ameloblastic carcinoma. Kumamoto et al. reported detection of MT1-MMP expression in ameloblastic tumors and demonstrated that all 5 malignant cases, including 3 cases of ameloblastic carcinoma, showed strongly positive expression of MT1-MMP. Also, Zhang et al. demonstrated that most cases of ameloblastic carcinoma showed strong expression of MMP-2. However, neither of these reports compared the expression of MT1-MMP or MMP-2 between ameloblastoma and ameloblastic carcinoma. In the present study, MMP-2 was significantly expressed in tumor cells and MMP-9 in stromal cells of ameloblastic carcinoma. In contrast to the weak expression of MMP-2 in tumor cells of ameloblastoma, most cases of ameloblastic carcinoma showed significantly strong expression of MMP-2 in tumor cells, which was consistent with the results of Zhang et al. Although there have been no studies on the expression of MMP-9 in ameloblastic carcinoma, a significant increase in stromal MMP-9 expression in malignant odontogenic tumor was detected in another report, demonstrating that the positive staining of MMP-9 in ghost cell odontogenic carcinoma is significantly stronger than staining in its benign counterparts, calcifying cystic odontogenic tumor and dentinogenic ghost cell tumor. The present report suggests that significant expression of MMP-9 in the stroma is associated with the invasive ability of these tumors. This finding is consistent with our results showing a significant increase in MMP-9 expression in stromal cells of the malignant counterpart. Although more studies are necessary to better understand the role and influence of these enzymes in the local behavior of ameloblastic tumors, a significant increase in MMP-2 or MMP-9 expression in their ma-
lignant counterpart can be interpreted as more aggressive progression of disease.

The Ki-67 LI has been frequently used in the differential diagnosis of benign and malignant tumors, as an adjunct in the grading of tumors, and as a prognostic marker. Various values of Ki-67 LI for ameloblastoma have been reported (including 5.08%, 2.8%, and 6.4%). However, ameloblastic carcinoma has shown a much higher proliferative index than ameloblastoma. According to the comparison with ameloblastoma by Akrish et al., ameloblastic carcinoma had approximately 20% Ki-67–positive cells, whereas solid ameloblastoma showed a relatively low proliferative index of ~5%-10%. According to a recent study of ameloblastic carcinoma, the mean Ki-67 LI was 18.2% (range 13.4%-21.4%), which is significantly higher than that of ameloblastoma (6.4%). Also, in the present study, the 7 cases of ameloblastic carcinoma had a mean Ki-67 LI of 17.21%, which was ~5 times higher than that for ameloblastoma (3.57%). In a review of the literature, the highest mean Ki-67 LI of ameloblastic carcinoma was 41.2%, which is higher than our highest Ki-67 LI of 38.08%. Based on these findings, ameloblastic carcinoma appears to be a highly proliferative and aggressive tumor.

Ameloblastic carcinoma is demographically, clinically, and biologically, distinctive from ameloblastoma, as previously mentioned. Therefore, when differentiating the 2 tumors, it may be necessary to combine the subtle histologic changes with the demographic and clinical features. Ameloblastic carcinoma shows a significant expression of CK18, parenchymal MMP-2, stromal MMP-9, and Ki-67 compared with ameloblastoma, suggesting that differential expression of these markers could be used to support the diagnosis of ameloblastic carcinoma. However, in a small series of ameloblastic carcinoma, there seems to be a lack of relationship between the expression patterns of these markers and the clinical behavior of tumors or patient outcome. Therefore, further study in a larger sample of ameloblastic carcinomas is needed to identify the prognostic and therapeutic value as well as to validate the utility of these markers for differential diagnosis.

REFERENCES


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