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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%; $P = .002$).

Conclusions. The significant expression of CK18, parenchymal MMP-2, stromal MMP-9, and Ki-67 could provide useful markers for differentiating ameloblastic carcinoma from ameloblastoma. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:767-776)**

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 2005[4] 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

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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.[5] A limited number of reports have demonstrated that ameloblastomas express a variety of CKs, including CKs 5, 7, 8, 13, 14, 18, and 19.[6-10] In particular, CK14 and CK19 seem to be mainly expressed in neoplastic epithelial cells of some odontogenic tumors, including ameloblastoma.[6,7,9,11] However, in ameloblastoma, CK7 has been rarely detected[7,12] and CK18 was either absent or weakly expressed in the focal area of tumor nests.[6,9]

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), matrilysin (MMP-7), elastase (MMP-12), MT-MMPs (MMPs 14, 15, 16, and 17), enamelysin (MMP-20), and other MMPs (MMP-11, MMP-19, and others).[13] The type IV collagenases, MMP-2 and MMP-9, have been particularly emphasized because they are related to tumor invasion and metastasis.[14] Some investigators have suggested that MMP-1, -2, and/or -9 might contribute to the invasive capacity of ameloblastoma.[15-19]

The assessment of proliferation has been applied in histopathology as a means to predict the behavior of tumors.[20] 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.[21] Although various studies of ameloblastoma have assessed cell proliferation using Ki-67,[22-24] there have been a limited number of studies examining the expression of Ki-67 in 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 semiquantitive 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-