Immunohistochemical expression of matrilysins (MMP-7 and MMP-26) in ameloblastomas and adenomatoid odontogenic tumors

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Objective. The aim was to evaluate the expression of matrix metalloproteinases (MMPs) 7 and 26 in ameloblastomas and adenomatoid odontogenic tumors (AOTs).

Study design. Twenty intraosseous solid ameloblastomas and 10 intraosseous AOTs were evaluated regarding immunohistochemical expression of MMP-7 and -26 in the epithelium and stroma.

Results. There was no statistically significant difference in MMP-7 and -26 expression between the epithelium of ameloblastomas (P = .50) and AOTs (P = .90). Stromal staining for MMP-7 was evident in all cases. For MMP-26, stromal staining was observed in 65% of ameloblastomas and 50% of AOTs, and this difference was not statistically significant (P = .69).

Conclusion. The marked expression of these matrilysins suggests their role in the process of tissue remodeling and growth in the studied tumors, but it does not relate to their distinct patterns of aggressiveness. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:417-424)

Ameloblastoma is the most frequent tumor of epithelial odontogenic origin.1 Although benign, it is locally invasive, with a high recurrence rate if not adequately removed. The World Health Organization (WHO) has recently classified it into 4 variants: solid (multicystic ameloblastoma), extraosseous (peripheral ameloblastoma), desmoplastic, and unicystic.2

Adenomatoid odontogenic tumor (AOT) is a rather uncommon tumor of odontogenic origin.3,4 Its clinical and microscopic characteristics, biologic behavior, and prognosis are distinct from ameloblastoma. AOT presents an indolent behavior with rare recurrences, allowing a conservative treatment compared with that recommended for solid ameloblastomas.2,5

Matrix metalloproteinases (MMPs) are a family of zinc- and calcium-dependent proteolytic enzymes that degrade the extracellular matrix (ECM) into macromolecules, such as collagen, gelatin, fibronectin, tenascin, and laminin, at physiologic pH.6 More than 20 different members are currently known and classified according to the domain organization: collagenases (MMP-1, -8, -13, and -18), gelatinases (MMP-2 and -9), stromelysins (MMP-3 and -10), matrilysins (MMP-7, -26, and -11), membrane-type MMPs (MMP-14, -15, -16, -17, -24, and -25), and other MMPs (MMP-12, -19, -20, -21, -23, -27, and -28).7

In physiologic conditions, MMPs play a central role in the regulation of the ECM during embryonic development and tissue remodeling.2,8 However, the degradation of ECM has also been associated with diverse pathologic processes, including tumor growth, invasion, and metastasis.9,10

The matrilysins, also known as MMP-7 (matrilysin-1) and MMP-26 (matrilysin-2), are involved in cell proliferation, apoptosis, invasion, and metastasis. These matrilysins differ from other MMPs in their low molecular weight, their lack of a C-terminal hemopexin domain common to other MMPs, and their low expression in epithelial cells in physiologic conditions.11 Research has demonstrated their expression in malignant epithelial neoplasms12,13 and odontogenic keratocysts.14 However, no study has shown their expression in ameloblastoma and AOT.
In the present study, the expression of MMP-7 and MMP-26 in ameloblastoma and AOT was examined through immunohistochemistry, to clarify the possible role of these matrilysins in the biologic behavior of these tumors.

**MATERIALS AND METHODS**

Twenty cases of intraosseous solid ameloblastoma (14 of the follicular and 6 of the plexiform pattern) and 10 of intraosseous AOTs were obtained from the files of the Oral Pathology Department of the Federal University of Rio Grande do Norte (UFRN). Diagnoses were confirmed by the authors through the review of hematoxylin and eosin–stained sections, following the WHO classification.2 The specimens, embedded in paraffin blocks, were sliced in 3-μm-thick tissue sections and submitted to immunohistochemical examination. The study was approved by the Research Ethics Committee at UFRN.

**Immunohistochemical methods**

The tissue sections were deparaffinized and immersed in methanol with 0.3% hydrogen peroxide to block endogenous peroxidase activity. Tissue sections were then washed in phosphate-buffered saline (PBS). The antigen retrieval, antibody dilution, and clone type for MMP-7 and -26 are shown in Table I. After treatment with normal serum, the sections were incubated in a moist chamber with primary antibodies. The sections were then washed twice in PBS and treated with streptavidin-biotin-peroxidase complex (Dako, Glostrup, Denmark) at room temperature to bind the primary antibodies. Peroxidase activity was visualized by immersing tissue sections in diaminobenzidine (D5637; Sigma Chemical, St. Louis, MO), resulting in a brown reaction product. Finally, tissue sections were counterstained with Mayer hematoxylin and coverslipped. Positive controls for MMP-7 and -26 were sections of ordinary human placenta. As negative controls, samples were treated as above, except that the primary antibody was replaced by a solution of bovine serum albumin in PBS.

**Immunohistochemical expression evaluation and statistical analysis**

Immunohistochemical staining of MMP-7 and -26 was evaluated using descriptive and semiquantitative analysis. In the latter analysis, scores from 1 to 3 were used for the stained cells in the epithelium (Table II). Staining in the stroma was evaluated and classified as positive (+) and negative (−). Parameters were independently analyzed through light microscopy by 3 of the authors. Then they analyzed the cases as a group to reach a consensus.

Statistical analysis was performed using SPSS for Windows version 15.0 (SPSS, Chicago, IL). The Mann-Whitney test was performed in the epithelium immunoscores, and the chi-squared test in the stroma staining. P values of <.05 were considered to indicate statistical significance.

**RESULTS**

The majority of both the ameloblastomas (60%) and the AOTs (70%) showed score 3 for MMP-7 in the epithelium. In the ameloblastoma, staining was especially evident in central cells of epithelial nests (Fig. 1, A and B). Stromal staining was observed in all studied cases in fibroblasts and inflammatory and endothelial cells (Fig. 1, E). In AOT, staining for this matrilysin was evident in cuboidal and columnar epithelial cells present in nests and duct-like structures (Fig. 1, C and D). Stromal staining in AOT was shown in some fibroblasts and endothelial cells (Fig. 1, F).

The expression analysis of MMP-26 in the ameloblastoma epithelium showed that 55% of the ameloblastomas exhibited score 3. A variation in the location pattern of the stained cells was observed. In the plexiform pattern, expression was more homogeneously distributed (Fig. 2, A), whereas in the follicular pattern immunoreactivity was especially observed in central cells of nests (Fig. 2, B). In the AOT epithelium, 50% of the cases presented score 3. Staining was shown in nests and duct-like and rosette-like structures (Fig. 2, C and D). In the stroma, it was observed that 65% of the

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<tr>
<th>Antibody clones</th>
<th>Specificity</th>
<th>Dilution</th>
<th>Antigen retrieval</th>
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<tr>
<td>Ab-1/DD2*</td>
<td>MMP-7</td>
<td>1:250</td>
<td>Pepsin pH 1.8, oven 37°C, 60 min</td>
<td>Overnight (18 h)</td>
</tr>
<tr>
<td>AHP756†</td>
<td>MMP-26</td>
<td>1:250</td>
<td>Pepsin pH 1.8, oven 37°C, 60 min</td>
<td>Overnight (18 h)</td>
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*Labvision/Neomarkers, Fremont, CA.
†Serotec, Kidlington, Oxford, U.K.

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<th>Score</th>
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<tr>
<td>1</td>
<td>&lt;10%</td>
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<tr>
<td>2</td>
<td>10%-50%</td>
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<td>3</td>
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amebblastomas and 50% of the AOTs exhibited staining for MMP-26. Stromal staining for this matrilysin was similar for both tumors, being observed in fibroblasts as well as in endothelial cells (Fig. 2, E and F).

Table III shows that there was no significant difference between the metalloproteinase staining medians in the ameloblastoma epithelium and the AOTs. The same was observed when comparing MMP-7 (P = .50) and
MMP-26 ($P = .90$) staining between the 2 lesions. The chi-squared test (Table IV) also showed no significant difference in stromal MMP-26 staining between the 2 lesions. Regarding MMP-7, no statistical tests were performed, because its presence was verified in 100% of the samples of both lesions.

Fig. 2. Immunohistochemical reactivity for matrilysin-2 (metalloproteinase 26) in ameloblastomas and adenomatoid odontogenic tumors (AOTs). **A**, Plexiform ameloblastoma showing expression in epithelium ($\times$200). **B**, Follicular ameloblastoma showing expression in central cells of epithelial nests ($\times$400). **C**, AOT showing expression in epithelium ($\times$200). **D**, AOT showing expression in epithelial cells in nests and duct-like and rosette-like structures ($\times$400). **E**, Ameloblastoma showing expression in fibroblasts and endothelial cells ($\times$400). **F**, AOT showing expression in fibroblasts and endothelial cells ($\times$400).
DISCUSSION

Although ameloblastoma and AOT are classified by WHO in the same group, formed by epithelial odontogenic tumors with mature fibrous stroma without odontogenic ectomesenchyme, these lesions present distinct biologic behavior. Ameloblastoma is a potentially destructive tumor, whereas AOT presents slow, progressive, and circumscribed growth.  

Various factors have been associated with the aggressive behavior of ameloblastomas, such as the increase in the proliferative potential, changes in the expression of tumor suppressor genes and their protein products, and modifications in cell cycle mechanisms. Besides these, other factors have also been investigated in an attempt of better understanding the behavior of these tumors, such as growth factors, adhesion molecules, and changes in stromal components and the expression of metalloproteinases and their inhibitors, tissue inhibitor of metalloproteinases (TIMPs).

Previous research has confirmed MMP-1, -2, and -9 expression in ameloblastomas and odontogenic myxomas, suggesting that these proteinases are involved in the process of ECM degradation and contribute to the local invasiveness of these tumors. The inhibition of MMP-2 activity was also related to the decrease in local invasiveness in ameloblastomas. In addition to the high expression of MMP-2 and -9, the aggressive behavior of these tumors has also been associated with changes in the expression of vascular endothelial growth factor and E-cadherin. Silveira et al. also verified greater expression of MMP-1, -2 and -9 in the mesenchymal cells of nonsyndrome odontogenic keratocysts than in radicular, residual, and dentigerous cysts, confirming the participation of these enzymes in this lesion growth, as well as justifying that the first lesions are more aggressive than the latter three. Kumamoto et al. detected strong expression of MMP-1, -2, and -9 and TIMP-1 and -2 in mesenchymal components of dental follicles and dental papillae and in stromal cells of ameloblastomas. Those findings suggest that these molecules might play a role in regulating tumor progression in ameloblastomas as well as in regulating developmental processes in tooth germs.

Studies involving matrilysins and odontogenic tumors are even rarer. Cavalcante et al. evaluated the expression of MMP-7 and -26 in syndrome and nonsyndrome odontogenic keratocysts and observed stronger epithelial expression in cases associated with Gorlin syndrome than in nonsyndrome cases, which may explain the more aggressive behavior of the keratocysts associated with this syndrome.

In the present study, most cases of ameloblastoma and AOT showed strong expression of MMP-7 and -26 in the epithelium as well as the stroma, which suggests the participation of these MMPs in tissue remodeling.

Some studies have evaluated MMPs expression in mostly distinct pathologies. Nonetheless, studies involving cysts and odontogenic tumors are still scarce. Among the MMPs, collagenase-1 (MMP-1) and gelatinases (MMP-2 and -9) seem to play an important role in the development of these lesions, actively participating in the interactions between epithelial cells and mesenchymal components.
gen, and in noncomponents of this matrix, such as fibrinogen and inactive serpin. Because matrilysins degrade ECM proteins, their primary function has been presumed to be tissue remodeling. However, their action on other substrates, such as growth factors, growth factor protein bindings, and adhesion molecules, may modulate cell behavior, which might make matrilysins to play a central role in tumor invasion and metastasis. Additionally, MMP-7 activates latent forms of other MMPs (proMMP-1, -2, and -9) and MMP-26 activates MMP-9. Because gelatinases are involved in tumor invasion processes and metastasis, the present findings indirectly suggest the participation of MMP-7 and -26 in invasive processes in ameloblastomas, because their strong immunoreactivity can be correlated with the activation of MMPs -2 and -9.

Some MMPs are expressed by tumor and host cells. Expression of MMP-7 has been described in epithelial cells, myofibroblasts, macrophages, and endothelial cells. Cloned MMP-26 was expressed by macrophages and fibroblasts in vivo and in culture, suggesting that this MMP may contribute to both physiologic processes of tissue remodeling and various benign skin disorders. MMP-26 expression was shown in normal tissues of endometrium, placenta, and kidney. Furthermore, up-regulation of these matrilysins was associated with tumors of epithelial origin.

In the present study, expression of these matrilysins was shown in both tumor and stromal cells. MMP-7 was evident in epithelial cells, fibroblasts, and endothelial cells of both tumors and in inflammatory cells of the ameloblastomas. And MMP-26 was observed in epithelial cells, fibroblasts, and endothelial cells in both ameloblastomas and AOTs.

Even though there was neither association between MMP staining and the studied lesions nor significant difference between medians of MMPs in the epithelium, our results, when analyzed as a whole, suggest that stromal cells from both studied tumors are capable of producing MMPs with more or less efficiency depending to the studied MMP. That suggests that these enzymes can potentialize the action of epithelium-produced MMPs.

The literature agrees with the role of the stromal microenvironment in tumoral progression, showing evidence of cooperation or synergy between neoplastic cells and stromal fibroblasts in MMP production. According to Lynch and Matrisian, MMPs of either tumoral or stromal origin may process cell surface molecules, proteins, or growth factors and cytokines stored in the EMC, causing changes in the microenvironment and thereby favoring tumor growth, migration, invasion, angiogenesis, and selection of cell clones resistant to apoptosis. Accordingly, those authors believe that MMPs can be used as a means of communication between tumor and stromal cells.

In addition, changes in stromal components may occur due to an imbalance in cytokines, resulting in changes that may ease the transdifferentiation of fibroblasts into myofibroblasts (MFs). MFs are specialized stromal cells that display a hybrid phenotype between fibroblasts and smooth muscle cells. Earlier studies have identified the presence of these cells in neoplasms, including odontogenic tumors, relating them to the biologic behavior of these lesions. MFs were identified in ameloblastomas, and a high rate of these cells was observed in more aggressive tumors, such as solid ameloblastomas and odontogenic keratocysts. MFs may produce high levels of MMP-2, and the intensive presence of these cells and gelatinases in solid ameloblastomas has been associated with disruption of cortical bone and increased aggressiveness of these tumours.

From our findings, it is plausible to conclude that the expression of these matrilysins suggests their participation in the process of tissue remodeling and ameloblastoma and AOT growth, but this expression does not relate to the their distinct patterns of aggressiveness. However, these matrilysins may indirectly act through the activation of other MMPs associated with changes in the stromal components, which could contribute to the greater aggressiveness of ameloblastomas. We therefore believe that further studies investigating the expression of MMPs and their relationship to stromal components, as well as the use of other techniques, such as zymography, should be undertaken in an attempt to better understand the role and influence of these enzymes in the behavior of the studied tumors.

REFERENCES


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