ORIGINAL ARTICLE

Immunoeexpression of MMPs-1, -2, and -9 in ameloblastoma and odontogenic adenomatoid tumor

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OBJECTIVE: The aim of this study was to evaluate and compare the expression of metalloproteinases-1, -2, and -9 in solid ameloblastoma and adenomatoid odontogenic tumor.

METHODS: A total of 20 cases of solid ameloblastoma and 10 cases of adenomatoid odontogenic tumors were selected and immunohistochemically assessed. Metalloproteinases-1, -2, and -9 immunoexpression and their distribution pattern were noted and semiquantitatively scored. The scores obtained were statistically analyzed.

RESULTS: Matrix metalloproteinase (MMP)-1 showed a predominant expression in both tumors and was found in stroma and parenchyma. For MMP-2, there was a varied expression, with 80% and 60% of immunoreactive tumor cells in ameloblastoma and adenomatoid odontogenic tumor respectively. Regarding stromal cells, 65% of ameloblastomas and 80% of adenomatoid odontogenic tumors showed positivity. There was immunoeexpression of the MMP-9 in parenchymal and stromal cells in all cases of both tumors analyzed. A statistically significant difference in the expression of MMP-1 in relation to the expression of MMP-2 and -9 in ameloblastomas (P < 0.001) was observed.

CONCLUSION: The results suggest that these metalloproteinases are related to growth and progression of tumors analyzed, and particularly in ameloblastoma, its highest aggressiveness may be, in part, a result of the active participation of the stromal cells and their products, such as the MMPs studied.

Keywords: ameloblastoma; odontogenic adenomatoid tumor; matrix metalloproteinases; extracellular matrix

Introduction

The ameloblastoma and adenomatoid odontogenic tumor are two representatives of benign tumors derived from odontogenic epithelium, without the participation of the ectomesenchyma (Barnes et al., 2005).

The solid ameloblastoma is an odontogenic neoplasm that, although it has a benign nature, also has an aggressive and invasive behavior and high rate of recurrence (Neville et al., 2004; Barnes et al., 2005; Kumamoto and Ooya, 2006). The adenomatoid odontogenic tumor is a slow-growing, but progressive, lesion (Silva et al., 2004; Leon et al., 2005).

The matrix metalloproteinases (MMPs) comprise a family of zinc-dependent endopeptidases that have the ability to degrade the components of the extracellular matrix (ECM), participating in physiologic events and pathologic processes (Mott and Werb, 2004). Several studies have emphasized the action and the importance of MMPs in the growth of pathologic processes of all kinds. These proteases have the ability to act on the components of the ECM, favoring the invasion and proliferation of neoplastic cells (Souza and Line, 2002).

To better understand the interaction between tumor cells and the ECM in ameloblastoma and adenomatoid odontogenic tumor, this study aimed to evaluate and compare the immunohistochemical expression of MMPs-1, -2, and -9 in odontogenic tumors cited by a semi-quantitative descriptive and comparative analysis of the pattern of the expression of these metalloproteinases.

Materials and methods

The research received approval from the Research Ethics Committee of the Federal University of Rio Grande do Norte for its implementation.

The selected sample at the Laboratory of Pathologic Anatomy of the Discipline of Oral Pathology at Federal University of Rio Grande do Norte consisted of 20 cases
of solid ameloblastomas and 10 cases of adenomatoid odontogenic tumors diagnosed according to the classification of the World Health Organization (Barnes et al., 2005).

The selected material was fixed in formalin at 10% and embedded in paraffin. There were histologic sections of 3 μm thick which were extended on glass slides containing adhesive based on 3-amino-silane-propiltrie-toxi (Signa Chemical CO, St. Louis, MO, USA). The histologic sections were submitted to dewaxing in xylene through two baths, the first being at 60°C for 30 min and the second at room temperature for 20 min. The sections were re-hydrated in string of alcohol in the water and washed up in two passages of distilled water for 5 min each. The blocking of endogenous peroxidase activity was performed using hydrogen peroxide of 10 volumes. Subsequently, the sections were washed in water and immersed twice in buffer solution of Tris (hydroxymethyl) aminomethane (TRIS-HCL), pH 7.4, for 5 min each. There was the incubation of the sections with antibodies diluted in TRIS-HCL buffer solution (Table 1) with the streptavidin–biotin complex (Dako-Cytomation LSAB+ System-HRP, Dakocytomation, A/S, Glostrup, Denmark) during 30 min at room temperature. It was applied the chromogenic agent diaminobenzidine diluted in TRIS-HCL added with hydrogen peroxide at 10 volumes in a dark room for 3 min to the revelation. The samples were counterstained with Mayer hematoxylin for 10 min, rinsing with water after each step. Finally, dehydration in ethanol and xylene clearing were carried out for assembling the glass slides with Erv-mount.

The immunohistochemical analysis, the presence or absence of immunohistochemical expression of MMP-1, -2, and -9 and the pattern of distribution (focal and diffuse), was verified by two examiners at different times. The pattern of distribution was performed using the following scores (adapted from Nagel et al., 2004): 0 (<10% of positive tumor cells), 1 (10–50% of positive tumor cells), 2 (>50% of positive tumor cells). After obtaining the data, a descriptive analysis of the results was carried out. In the statistical analysis, the non-parametric Pearson’s chi-square test, with a significance level of 5%, was used to evaluate the difference between the scores in each tumor. The scores were dichotomized to reduce the dispersion of data.

Results

There was immunohistochemical expression of MMPs-1, -2, and -9 in the parenchyma and the stroma of solid ameloblastomas and adenomatoid odontogenic tumors, and the MMP-1 had a predominant immunostaining in the two tumors analyzed. The neoplastic cells in ameloblastoma showed immunoreactivity in the cell cytoplasm independent of being located on the periphery or on the central portion of nests for all MMPs analyzed.

In solid ameloblastomas, the MMP-1 was observed in all examined cases in both parenchyma and stroma (Figure 1). For the MMP-2, the expression was varied. There was positivity in 80% of the specimens (Figure 2) and 20% had no immunostaining in tumor cells. Considering the stroma, 65% of cases of ameloblastoma were positive. The immunoreactivity for MMP-9 was evident in parenchymal and stromal cells (Figure 3) of all specimens of ameloblastoma analyzed. The distribution of scores in ameloblastomas is presented in Table 2. Regarding the pattern of distribution, there was a predominance of the diffuse pattern for MMP-1 and -9, while for the MMP-2, there was no predominance in the pattern of distribution of the cells, with 50% being focal and 50% diffuse. The expression of MMP-1 in tumor cells of ameloblastomas was statistically significant (P < 0.001) when compared with the expression of the MMP-2 and -9 (Table 3).

The adenomatoid odontogenic tumors showed positivity in tumor cells, regardless of their location, and in stromal cells in all cases examined for MMP-1 (Figure 4) and -9 (Figure 5). In areas where the cell was

Table 1 Applied monoclonal antibodies and stained conditions

<table>
<thead>
<tr>
<th>Clone</th>
<th>Specification</th>
<th>Source</th>
<th>Dilution</th>
<th>Incubation time</th>
<th>Antigen retrieval</th>
</tr>
</thead>
<tbody>
<tr>
<td>41-1E5</td>
<td>MMP-1</td>
<td>Calbiochem</td>
<td>1:100</td>
<td>Overnight (18 h)</td>
<td>Citrate pH 6.0 Pa</td>
</tr>
<tr>
<td>17B11</td>
<td>MMP-2</td>
<td>Novocastra</td>
<td>1:50</td>
<td>60 min</td>
<td>EDTA pH 8.0 Pa</td>
</tr>
<tr>
<td>2C3</td>
<td>MMP-9</td>
<td>Novocastra</td>
<td>1:20</td>
<td>Overnight (18 h)</td>
<td>Citrate pH 6.0 Pa</td>
</tr>
</tbody>
</table>
organized in a predominantly cordonal arrangement, there has been less exuberant immunoexpression. Considering the MMP-2, there was a positive expression in 60% of cases in tumor cells and 40% were negative. The positivity in the stroma cells was varied, with eight cases (80%) showing positivity and two showing negativity (20%). The percentage of immunopositive cells is shown in Table 4. Regarding the pattern of distribution, diffuse pattern was predominant for MMP-1 and -9, while for the MMP-2, the focal pattern was predominant.

**Discussion**

The ameloblastoma and adenomatoid odontogenic tumor are benign odontogenic tumors arising from odontogenic epithelium and have different behaviors (Kumamoto et al., 2003; Sempere et al., 2006). The epithelial–mesenchymal interaction is necessary for the control and regulation of cell functions, such as proliferation, differentiation, apoptosis, and migration (Medeiros, 2001; Raitz et al., 2003; Mott and Werb, 2004).

The matrix metalloproteinases are important proteases that act on the structural and functional modifications of ECM components. In physiologic conditions, the MMPs

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**Table 2** Immunoreactive score for MMPs-1, -2, and -9 in ameloblastoma. Natal/RN, 2008

<table>
<thead>
<tr>
<th>Ameloblastoma</th>
<th>MMP-1</th>
<th>MMP-2</th>
<th>MMP-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 2</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Score 1</td>
<td>1</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Score 0</td>
<td>1</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>100</td>
<td>20</td>
</tr>
</tbody>
</table>

**Table 3** Immunoreexpression of MMPs-1, -2, and -9 in ameloblastoma. Natal/RN, 2008

<table>
<thead>
<tr>
<th>Metalloproteinases</th>
<th>MMP-1</th>
<th>MMP-2</th>
<th>MMP-9</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Absence or until 50.0%</td>
<td>2</td>
<td>10.0</td>
<td>17</td>
<td>85.0</td>
</tr>
<tr>
<td>More than 50.0%</td>
<td>18</td>
<td>90.0</td>
<td>3</td>
<td>15.0</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>100.0</td>
<td>20</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Pearson chi-square test $P < 0.001$. 

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**Figure 2** Immunohistochemical staining for MMP-2 in tumor and stromal cells of ameloblastoma. Cells from the nests and cords arrangement of the ameloblastoma showed brown immunostaining in the cytoplasm. In the stroma, endothelial and inflammatory cells also showed immunopositivity (SABC, streptavidin–biotin complex, DAB chromogen, Mayer’s hematoxylin counterstain, 400x)

**Figure 3** MMP-9 immunoexpression in parenchymal and stromal cells of ameloblastoma. Cells from periphery and in the central portion of nests and cords of ameloblastoma showed brown immunostaining in the cytoplasm (SABC, streptavidin–biotin complex, DAB chromogen, Mayer’s hematoxylin counterstain, 400x)

**Figure 4** Immunoreexpression of MMP-1 in adenomatoid odontogenic tumor. Whorl-shaped tumor cells nests and duct-like structures were stained in brown in the cytoplasm (SABC, streptavidin–biotin complex, DAB chromogen, Mayer’s hematoxylin counterstain, 200x)
are poorly expressed by tissues, while in disease processes there is superexpression of these due to the imbalance in the metalloproteinases activities and their inhibitors, such as the tissue inhibitors of matrix metalloproteinases (TIMPs) (Pereira et al, 2005; Nagase et al, 2006; Verma and Hansch, 2007).

Considering the odontogenic tumors, few studies have been conducted to evaluate the expression of metalloproteinases in these lesions. In this study, in general, the MMPs were expressed both in tumor cells and in the two stromal tumors analyzed, the expression of MMP-1 being more evident.

All cases of ameloblastoma showed positivity for MMP-1 in tumor and stromal cells. This result is comparable with that obtained by Pinheiro et al (2004), in which the MMP-1 was observed in tumor cells, in the stroma, and in the interface of bone-growth ameloblastomas. However, these authors found the presence of MMP-1 only in columnar cells on the periphery of nests of ameloblastoma, unlike that in this study, which found tagging enzyme in cells of the periphery and the central portion of the nests of epithelium. This result can be explained by the presence of tenascin, which is a substrate of MMP-1, detected in the central portion of nests that resemble the stellate reticulum of the enamel organ observed by Medeiros (2001).

However, Kumamoto et al (2003) found MMP-1 immunoreexpression only in stromal cells of the ameloblastomas and not in the tumors cells. The divergent results can be explained by the use of different clones or, possibly, by the temporal expression of this MMP depending on the needs of the development of neoplasm.

The statistically significant higher expression of MMP-1 in relation to the MMP-2 and -9 in ameloblastoma, detected by Chi-square test of Pearson ($P < 0.001$), shows that this metalloproteinase is one of the major enzymes that act in the degradation of the ECM, being essential for tumor growth, which is easily verified and explained in ameloblastoma through its major growth potential. The high expression of MMP-1 in ameloblastoma may be related to the largest existing cell proliferation in this neoplasia, as has been verified by Meer et al (2003), Santana et al (2004), and Barboza et al (2005).

The adenomatoid odontogenic tumor, characterized as a less aggressive lesion and with a lower proliferative potential than ameloblastoma (Barboza et al, 2005), also had immunostaining for the MMP-1 in parenchymal and stromal cells, revealing the extreme importance of this enzyme in the degradation of the ECM, regardless of the aggressiveness of the tumor.

The presence of tenascin and the collagen I, substrates of MMP-1, in adenomatoid odontogenic tumor verified by Medeiros (2001) may explain the positivity for MMP-1 found in this work, both in parenchymal cells and in stroma, confirming the role of this enzyme in tumor growth and expansion.

The expression of metalloproteinases-2 and -9 in ameloblastomas and adenomatoid odontogenic tumors was varied, with the MMP-9 showing higher immunoreactivity when compared with MMP-2. The same was evident in works performed by Teronen et al (1995) and Silveira et al (2007) in odontogenic cyst. These findings may suggest a secondary role of gelatinases in the lesions evaluated; as the MMP-1 is the main protease responsible for degrading collagen I, it has a crucial role in the degradation of ECM and tumor growth. The MMP-2 and -9 act in a combined form with the MMP-1 as they are essential for degradation of gelatin from the breakdown of collagen type I, initiated by the MMP-1.

Poor expression of MMP-2 in parenchymal cells in most cases examined in the two tumors was already expected. Similar results were obtained by Pinheiro et al (2004) that found immunostaining for the MMP-2 in tumor cells, but only those located on the periphery of the nests of odontogenic epithelium of ameloblastoma. Kumamoto et al (2003) and this work showed weak reactivity for MMP-2 in both cells of the periphery and in cells of the central portion of the nests of odontogenic epithelium of ameloblastomas.

The MMP-2 mainly degrades the collagen type IV, present in the basement membrane, and other components of ECM. We believe that the low expression of MMP-2 is due to the need to preserve at least some of

### Table 4 Immunoreactive score for MMPs-1, -2, and -9 in AOT.

<table>
<thead>
<tr>
<th></th>
<th>MMP-1</th>
<th>MMP-2</th>
<th>MMP-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 2</td>
<td>10</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Score 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Score 0</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>100</td>
<td>10</td>
</tr>
</tbody>
</table>

OAT, adenomatoid odontogenic tumor.

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**Figure 5** Immunoreexpression of MMP-9 in tumor cells of adenomatoid odontogenic tumor. Tumor cells, rosette-like and duct-like structures, stained in brown in the cytoplasm. Endothelial and inflammatory cells were also immunostained in the cytoplasm (SABC, streptavidin–biotin complex, DAB chromogen, Mayer’s hematoxylin counterstain, 200x).
the constituents of the basement membrane, crucial to the process of differentiation of neoplastic cells as well as for maintaining the structural architecture in the tumor nests, characteristic in ameloblastoma.

The presence of fibronectin, substrate of MMP-2, observed by Medeiros (2001) in the stroma of ameloblastomas and adenomatoid odontogenic tumor, in the epithelial-mesenchymal interface, as well as in the parenchymal cells of both tumors, can justify the results obtained in this study, in which the MMP-2 was expressed, even if weakly, in the parenchyma and stroma of the two tumors analyzed.

The positivity for MMP-9 observed in tumor cells of ameloblastoma and adenomatoid odontogenic tumor in this study highlights the involvement of metalloproteinase degradation of the ECM. The MMP-9 is important, as well as MMP-2, for the complete degradation of collagen initiated by the MMP-1 (Teronen et al, 1995; Cotrim et al, 2002) and consequent promotion of tumor growth. The results found by Kumamoto et al (2003) and Pinheiro et al (2004) in ameloblastoma are similar in part to the results presented in this study; the expression of MMP-9 was found only in the cells of the periphery in the earlier studies, while in this work, both the central portion and the periphery of nests and cords of odontogenic epithelium showed immunoreactivity for this metalloproteinase.

The low expression of MMP-2 reported during the odontogenesis in the organ of enamel, which has as one of its constituents the inner epithelium of the organ of enamel composed of cells that undergo differentiation into ameloblasts during odontogenesis, may be associated with the lowest positivity of this enzyme in ameloblastoma, as during odontogenesis it acts in the degradation of the basement membrane, before the start of the formation of the enamel and dentin matrix. As this process of differentiation in ameloblastoma is not complete, a structural modification of the basement membrane through its degradation is not necessary; therefore, large amounts of MMP-2 and -9 to degrade the collagen IV are also not necessary.

The presence of MMP-2 and -9 in ameloblastomas and in adenomatoid odontogenic tumor is possibly related to cell differentiation that occurs in tumor cells. According to Murata et al (2000), cells that form the duct-like structures in adenomatoid odontogenic tumor cells are differentiated in cells similar to ameloblasts, but do not undergo the process of maturation and have the capacity to produce molecules of ECM. Similarly, the ameloblastomas exhibit high columnar or cubic cells of the periphery of the nests of odontogenic epithelium, recalling the ameloblasts, but also did not reach the necessary maturity to the formation of enamel (Tsujigiwa et al, 2005).

The positivity displayed by the three metalloproteinases studied in stromal cells of the two tumors shows that these enzymes are produced by fibroblasts and endothelial and inflammatory cells, such as lymphocytes, plasma cells, macrophages, and neutrophils that are also involved in the degradation of ECM, contributing to the growing tumor. Similarly, the results of Kumamoto et al (2003) and Pinheiro et al (2004) showed positivity for the MMPs evaluated in stromal cells of ameloblastomas, showing that these enzymes are probably synthesized by tumor induction and in response to cytokines, growth factors, and hormones, and are linked to tumor progression.

Although not statistically significant difference was found between the expression of MMPs in the parenchymal cells of ameloblastomas and of adenomatoid odontogenic tumors, the greater aggressiveness of ameloblastomas may possibly be associated with the highest amount of stromal cells in this tumor; the presence of the metalloproteinases detected in mesenchymal cells, confirms the participation of the enzymes in the degradation of the constituents of ECM, thereby promoting tumor growth.

It should be emphasized that through immunohistochemical technique, in this study as well as in the one from Kumamoto et al (2003), it is possible to detect the presence or absence of MMP-1, -2, and -9 in the tumors tested, though it is not possible to determine the enzymatic activity of these metalloproteinases. But in the study developed by Ikebe et al (1999), it was observed that there is correlation between the results of the zimography technique performed to verify the activity of metalloproteinases and the degree of expression displayed by immunohistochemical tests.

From the obtained results, which characterized the presence of MMP-1, -2, and -9 in the cells of the stroma and the parenchyma of ameloblastomas and adenomatoïd odontogenic tumors and its active participation in the growth of those tumors, it is believed that studies using more sensitive techniques must be carried out in an attempt to better understand the role and influence of these enzymes in the local behavior of the tumors studied.

References


Kumamoto H, Yamauchi K, Yoshida M, Ooya K (2003). Immunohistochemical detection of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases...