Immunohistochemical expression of matrix metalloproteinases in squamous cell carcinoma of the tongue and lower lip

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Article info

Article history:
Accepted 8 November 2010

Keywords:
Matrix metalloproteinases
Immunohistochemistry
Carcinoma

Abstract

Objective: To evaluate the immunohistochemical expression of MMP-1, -2, -7, -9 and -26 in oral squamous cell carcinomas (SCCs) according to tumour site and histological grade of malignancy.

Study design: Fifteen cases of SCC of the lower lip and 15 cases of tongue SCC were selected and divided into low grade malignancy (n = 17) and high grade malignancy (n = 13).

Results: Higher immunohistochemical expression of MMPs by neoplastic cells was observed in tongue SCCs, with a statistically significant difference for MMP-9 (P < 0.05). High-grade SCCs showed a higher expression of MMPs, except for MMP-2, with a statistically significant difference for MMP-7 (P < 0.05) and MMP-26 (P < 0.05). In addition, a direct association was observed between morphological scores of malignancy and MMP immunoreactivity, with the association being significant for MMP-7 and MMP-26.

Conclusion: The present results demonstrate the important role of MMPs in the development of SCCs of the lower lip and tongue.

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1. Introduction

Squamous cells carcinomas (SCCs) account for approximately 95% of all oral malignant neoplasms and for about 38% of all malignant head and neck tumours, affecting especially the tongue and lip. The aggressiveness of these tumours depends on a series of factors; however, tongue carcinomas generally exhibit a much more aggressive biological and clinical behaviour, whereas lip tumours tend to have a good prognosis and low metastatic potential.6 The prognosis of SCC is related to the proliferative activity of the tumour, degree of differentiation, and invasion and metastatic potential.6,7 The last two processes involve multiple steps, such as degradation of the basement membrane and extracellular matrix (ECM), alterations in cell adhesiveness, tumour cell motility, and angiogenesis.7

At present, therapeutic decisions in SCCs are based on clinical and pathological parameters, including age, metastasis stage and histological grade of the tumour. However, these factors frequently fail to distinguish between more or less
aggressive tumours. Therefore, specific markers need to be identified that are related to tumour progression and thus permit the establishment of therapeutic strategies against specific antigens.6

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that are able to degrade all ECM proteins.9,10 MMPs are associated with tumour progression and a poor prognosis due to their capacity to rupture these physical barriers and to regulate angiogenesis and cell proliferation.11–13 These enzymes are abundantly expressed in various malignant neoplasms and are implicated in all stages of tumour progression.11,14–17 Interestingly, in a significant number of cases MMPs are expressed by stromal cells, a finding indicating that, in addition to endogenous production of these enzymes, tumour cells utilize proteinases produced by stromal cells.18,19 Several studies have reported an increased immunoeexpression of MMPs in oral and head and neck carcinomas,20,21,9 with the observation of more intense staining at the tumour invasion front22–24 and of an association with the development of metastases.21,25–27 The exact determination of the MMPs involved in the processes of tumour invasion and metastasis in different tumours would permit the development of drugs designed to work against specific MMPs or of inhibitors of these proteins.7,28

The objective of the present study was to evaluate the immunohistochemical expression of MMP-1, -2, -7, -9 and -26 in SCCs of the lower lip and tongue in order to determine the presence or absence of a correlation between the expression of these enzymes and the anatomic location of the tumour and histological grade of malignancy. The results are expected to contribute to a better understanding of the biological behaviour of oral SCCs and of the role of MMPs in the invasiveness of these tumours.

2. Materials and methods

Fifteen cases of tongue squamous cell carcinoma and fifteen of lower lip squamous cell carcinoma were obtained from the files of the São Marcos Hospital in Teresina-PF. Patients were surgically treated without radiotherapy or chemotherapy prior. Samples were excluded from the study in case of incision biopsy, specimens with inadequate material or extensive areas of necrosis. The study was approved by the Research Ethics Committee at UFRN.

2.1. Histopathology

Sections (5 µm) were stained with haematoxylin–eosin (HE) for evaluation of the tumour invasion front. Next, the carcinomas were classified according to the histological grading system proposed by Bryne,29 which considers four parameters for the determination of the grade of malignancy: degree of keratinization, nuclear pleomorphism, invasion pattern, and inflammatory infiltrate. This system was adapted by the author from his first classification system,30 which included five morphological parameters. In view of the revision in the number of parameters evaluated by the method of Bryne,29 a classification of cases based on the sum of scores was adapted for this study, with cases presenting 4–8 points being classified as low grade and those with more than 8 points being classified as high grade of malignancy.31

2.2. Immunohistochemical methods

For immunohistochemistry, 3-µm thick sections were mounted on glass slides previously prepared with organosilane adhesive (3-aminopropyltrithoxy-silane, Sigma Chemical Co., USA) and submitted to the streptavidin–biotin method. The histological sections were deparaffinized in xylene and rehydrated in a decreasing alcohol series. The sections were then submitted to antigen retrieval (Table 1) and blockade of endogenous peroxidase with 10 volumes of hydrogen peroxide, washed in water, and incubated with Tris–HCl, pH 7.4, for 10 min. The sections were then incubated with the primary antibodies (Table 1) diluted in 1% BSA/Tris–HCl, pH 7.4. The reactions were developed with 0.03% diaminobenzidine as chromogen and the slides were counterstained with Mayer’s haematoxylin for 10 min. Finally, the sections were dehydrated in alcohol and cleared in xylene for mounting in Permount resin (Fisher Scientific, USA) under a coverslip. The positive control for MMP-1 was section of breast carcinoma tissue, for MMP-2 was section of inflamed large bowel, for MMP-9 was section of liver and 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.

Immunostaining at the invasion front was evaluated in triplicate by the same examiner at different times under a light microscope. Staining for each MMP was evaluated in neoplastic cells and expression was analysed semiquantitatively11 and classified as follows: negative (−); low expression, less than 10% positive cells (+); moderate expression, 10–50% positive cells (++); intense expression, more than 50% positive cells (+++). In addition, the expression of MMPs by stromal cells adjacent to neoplastic cells of the invasion front was analysed. The specimens were classified as follows according to an adaptation of the method of Franchi et al.11: negative or weak staining, 0–10% positive cells; expressive staining, more than 10% positive cells.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clones</th>
<th>Dilution</th>
<th>Manufacturer</th>
<th>Antigen retrieval</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-MMP-1</td>
<td>41-1E5</td>
<td>1:100</td>
<td>Cabiochen (Oncogene)</td>
<td>Citrato, pH 6.0, 30 min, Steamer</td>
<td>Overnight</td>
</tr>
<tr>
<td>Anti-MMP-2</td>
<td>17B11</td>
<td>1:50</td>
<td>Novocastra</td>
<td>EDTA, pH 8.0, 30 min, Steamer</td>
<td>60 min</td>
</tr>
<tr>
<td>Anti-MMP-7</td>
<td>Ab-1/ID2</td>
<td>1:250</td>
<td>Labvision/Neomarkers</td>
<td>Pepsina, pH 1.8, 1%, 37° C, 60 min</td>
<td>Overnight</td>
</tr>
<tr>
<td>Anti-MMP-9</td>
<td>2C3</td>
<td>1:20</td>
<td>Novocastra</td>
<td>Citrato, pH 6.0, 30 min, Steamer</td>
<td>Overnight</td>
</tr>
<tr>
<td>Anti-MMP-26</td>
<td>AHP756</td>
<td>1:250</td>
<td>Serotec</td>
<td>Pepsina, pH 1.8, 1%, 37° C, 60 min</td>
<td>Overnight</td>
</tr>
</tbody>
</table>
Fig. 1 – Immunohistochemical expression of MMPs in SCC of the tongue (400×). (a) High grade malignancy showing moderate expression for MMP-1. (b) Low grade malignancy showing moderate expression for MMP-1. (c) High grade malignancy showing weak expression for MMP-2. (d) Low grade malignancy showing no expression for MMP-2. (e) High
3. Results

3.1. Morphological study

Seventeen (56.67%) of the 30 cases studied exhibited a low degree of malignancy (8 lower lip SCCs and 9 tongue SCCs), whereas 13 (43.33%) were classified as high degree of malignancy (7 lower lip SCCs and 6 tongue SCCs). No significant association was observed between the anatomical location of the tumour and histological degree of malignancy (P > 0.05, chi-square and Fisher’s exact tests).

3.2. Immunohistochemical study

All cases investigated expressed at least two of the MMPs studied. Some positive staining for MMP-1 in tumour cells at the invasion front was observed in 93.33% of cases. This rate was 60% for MMP-2, 63.33% for MMP-9, 93.33% for MMP-7, and 100% for MMP-26 (Table 2).

In general, tongue SCCs exhibited more intense MMP staining in the parenchyma than lower lip SCCs (Figs. 1a-j and 2a–j), although this difference was statistically significant only for MMP-9 (P < 0.05, Mann–Whitney test).

With respect to histological grade of malignancy, higher expression of MMPs by neoplastic cells at the invasion front was observed in high-grade tumours (Figs. 1a, c, e, g, i and 2j), except for MMP-2 which showed the opposite tendency (Fig. 2c and d). A significant difference in expression between high-grade and low-grade tumours was observed for MMP-7 (P < 0.05) and MMP-26 (P < 0.05, Mann–Whitney test for both).

Stromal expression varied widely between the MMPs investigated. All cases studied exhibited expressive staining for MMP-1, whereas MMP-26 was expressed in only 3.33%. No significant difference in the expression of MMP-1, - 7, -9 or -26 was observed between lower lip and tongue SCCs (Table 3). However, the stromal expression of MMP-2 was significantly higher in lower lip SCCs (P < 0.05).

With respect to morphological grade of malignancy, no significant difference was observed in the stromal expression of MMP-1, -2, -9 or -26. However, high-grade tumours exhibited significantly more intense stromal staining for MMP-7 (P < 0.05).

After analysis of the sample as a whole, the data were analysed statistically within each subgroup divided according to tumour site: lower lip and tongue. For lower lip SCCs, higher expression of MMP-1 (P < 0.05), MMP-7 (P < 0.05) and MMP-26 (P < 0.05) by neoplastic cells was observed in high-grade tumours when compared to low-grade tumours. For tongue SCCs, no significant differences in MMP-1, -2, -9 or -26 staining were observed between high-grade and low-grade tumours. Expression of MMP-7 was significantly higher in parenchyma of high-grade tongue SCCs (P < 0.05). Immunostaining in stromal cells did not differ significantly between high-grade and low-grade tumours in either subgroup. There was no significant difference in the immunohistochemical expression of MMP-1, -2, -7, -9 or -26 between tongue carcinomas with and without metastases.

Table 2 – Immunohistochemical reactivity for metalloproteinases (MMPs -1, 2, 7, 9 and 26) in the invasive front of SCC of the tongue and lower lip. Natal/RN-2006.

<table>
<thead>
<tr>
<th>MMP</th>
<th>% of positive cells</th>
<th>Low grade (n = 17)</th>
<th>High grade (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>MMP-1</td>
<td>0</td>
<td>2</td>
<td>11.76</td>
</tr>
<tr>
<td></td>
<td>&lt;10%</td>
<td>2</td>
<td>11.76</td>
</tr>
<tr>
<td></td>
<td>10–50%</td>
<td>12</td>
<td>70.58</td>
</tr>
<tr>
<td></td>
<td>&gt;50%</td>
<td>1</td>
<td>5.88</td>
</tr>
<tr>
<td>MMP-2</td>
<td>0</td>
<td>7</td>
<td>41.17</td>
</tr>
<tr>
<td></td>
<td>&lt;10%</td>
<td>4</td>
<td>23.52</td>
</tr>
<tr>
<td></td>
<td>10–50%</td>
<td>6</td>
<td>35.29</td>
</tr>
<tr>
<td></td>
<td>&gt;50%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MMP-7</td>
<td>0</td>
<td>2</td>
<td>11.76</td>
</tr>
<tr>
<td></td>
<td>&lt;10%</td>
<td>5</td>
<td>29.41</td>
</tr>
<tr>
<td></td>
<td>10–50%</td>
<td>8</td>
<td>47.05</td>
</tr>
<tr>
<td></td>
<td>&gt;50%</td>
<td>2</td>
<td>11.76</td>
</tr>
<tr>
<td>MMP-9</td>
<td>0</td>
<td>8</td>
<td>47.05</td>
</tr>
<tr>
<td></td>
<td>&lt;10%</td>
<td>5</td>
<td>29.41</td>
</tr>
<tr>
<td></td>
<td>10–50%</td>
<td>4</td>
<td>23.52</td>
</tr>
<tr>
<td></td>
<td>&gt;50%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MMP-26</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&lt;10%</td>
<td>5</td>
<td>29.41</td>
</tr>
<tr>
<td></td>
<td>10–50%</td>
<td>8</td>
<td>47.05</td>
</tr>
<tr>
<td></td>
<td>&gt;50%</td>
<td>4</td>
<td>23.52</td>
</tr>
</tbody>
</table>

Table 3 – Immunohistochemical reactivity for metalloproteinases (MMPs -1, 2, 7, 9 and 26) in the stromal cells adjacent to the invasive front of SCC of the tongue and lower lip. Natal/RN-2006.

<table>
<thead>
<tr>
<th>MMP</th>
<th>% of positive cells</th>
<th>Low grade (n = 17)</th>
<th>High grade (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>MMP-1</td>
<td>&lt;10%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>&gt;10%</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td>MMP-2</td>
<td>&lt;10%</td>
<td>9</td>
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<td></td>
<td>&gt;10%</td>
<td>8</td>
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<tr>
<td></td>
<td>&gt;10%</td>
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<td>64.70</td>
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<tr>
<td>MMP-9</td>
<td>&lt;10%</td>
<td>10</td>
<td>58.82</td>
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<td></td>
<td>&gt;10%</td>
<td>7</td>
<td>41.17</td>
</tr>
<tr>
<td>MMP-26</td>
<td>&lt;10%</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>&gt;10%</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

grade malignancy showing strong expression for MMP-7. (f) Low grade malignancy showing moderate expression for MMP-7. (g) High grade malignancy showing moderate expression for MMP-9. (h) Low grade malignancy showing weak expression for MMP-9. (i) High grade malignancy showing strong expression for MMP-26. (j) Low grade malignancy showing strong expression for MMP-26.
Fig. 2 – Immunohistochemical expression of MMPs in SCC of the lower lip (400×). (a) High grade malignancy showing moderate expression for MMP-1. (b) Low grade malignancy showing moderate expression for MMP-1. (c) High grade malignancy showing no expression for MMP-2. (d) Low grade malignancy showing weak expression for MMP-2. (e) High
Spearman’s correlation between the morphological scores of SCCs and immunohistochemical expression of MMPs showed that, in general, the higher the score of malignancy, the higher the expression of MMPs. This correlation was statistically significant for MMP-7 and MMP-26. MMP-7 showed a direct and significant correlation with almost all partial scores and MMP-26 was correlated with the parameter of tumour invasion pattern. Only the degree of keratinization showed an inverse correlation with MMP-1 and MMP-9, but this correlation was not significant.

4. Discussion

The biological behaviour of SCCs depends on a series of factors, including the anatomical location of the tumour. It has been well established in the literature that malignant tumours located in the tongue are more aggressive, showing high rates of recurrence and metastasis.32–35 On the other hand, lower lip carcinomas are poorly aggressive and rarely produce metastases.36 These factors, together with the fact that an early diagnosis is easier to be established, seem to contribute to the good prognosis of these carcinomas.4 On the basis of this evidence, SCCs of the tongue and lower lip were selected to compare tumours that commonly exhibit a distinct biological behaviour.

Semiquantitative analysis of the expression of MMPs at the tumour invasion front of the carcinomas studied here demonstrated an overall higher expression of these enzymes in cases of tongue SCCs. However, this difference was only significant for MMP-9 (P < 0.05, Mann–Whitney test). MMP-1 showed moderate to strong expression in most cases of lip (80%) and tongue (80%) carcinomas. On the other hand, gelatinases were less expressed, with MMP-2 mainly showing weak to moderate expression in both lip and tongue carcinomas (60%). Slightly higher expression of MMP-9 was observed in cases of tongue SCCs, with weak to moderate expression in 67% of cases. In contrast, weak or no expression of MMP-9 was observed in 94% of lip SCC cases. Matrixins showed a similar pattern of immunoreactivity, with moderate to strong expression of MMP-7 and MMP-26 in both lip (73% of cases) and tongue (80% of cases) carcinomas.

The abundant staining for MMP-1 clearly reflects the important role of this enzyme in the invasion of adjacent tissues by the tumour through the destruction of ECM components, especially collagen I. Cao et al.37 emphasized the importance of MMP-1 for invasion and metastasis of tongue carcinomas.

Several studies have investigated the role of MMP-2 and MMP-9 in oral SCCs and demonstrated a relationship between the expression of these gelatinases and tumour aggressiveness,20,9,38,39 a fact permitting the use of these MMPs as prognostic markers or for therapeutic purposes. However, other studies were unable to confirm these results.11,40–43 The expression of gelatinases observed in the present study suggests that these enzymes also play an effective role in the development of oral SCCs, although staining was generally less intense than that observed for MMP-1. A distinct presentation might be observed at earlier stages of development of these carcinomas since rupture of the basement membrane is necessary for tumour invasion. The invasion process is initiated by the cleavage of basement membrane proteins that are the preferential substrates of MMP-2 and MMP-9, such as collagen IV. Thus, immunoeexpression of these MMPs in oral SCCs may reflect the ability of these enzymes to degrade collagen IV and gelatin.

The strong staining for matrixins (MMP-7 and MMP-26) observed in the oral SCC cases studied seems to indicate a high potential of invasion and metastasis since these enzymes are able to degrade basement membrane proteins such as laminin and collagen IV.

The cooperation or synergism between neoplastic cells and stromal fibroblasts in the production of MMPs has been demonstrated in numerous experiments,11,9,41,44,45 a finding emphasizing the fundamental role of the microenvironment in tumour progression. The stromal area adjacent to the invasion front was considered for the analysis of MMP immunoreactivity in tumour strata and only weak or negative staining (0–10% positive cells) and expressive staining (>10% positive cells) were evaluated. Thus, predominantly expressive staining for MMP-1 and MMP-7 was observed in lip (100% and 87%, respectively) and tongue carcinomas (100% and 73%). MMP-2 staining was expressive in most cases of lip SCC (73%) and weak in tongue carcinomas (73%). In contrast, immunoeexpression of MMP-9 and MMP-26 was weak in most cases of lip (67% and 100%, respectively) and tongue carcinomas (80% and 93%). Statistical analysis of these results only revealed significance for MMP-2 which, curiously, was more expressed in lower lip tumours (P < 0.05).

Taken together, the results of this study showed that stromal cells of the oral SCC cases studied were able to produce MMPs and that this production was more or less efficient depending on the MMP investigated. It is believed that these stromal enzymes potentiate the action of MMPs produced by the parenchyma. This fact supports the view of a marked interaction between neoplastic cells and the adjacent stroma. This strategic interaction permits tumours to induce stromal cells to produce proteolytic enzymes that act in synergism with tumour enzymes and thus facilitate the processes of invasion, migration and metastasis.

Studies in the literature have investigated the association between an increased expression of MMPs in oral SCCs and the primary tumour site. In this respect, Vicente et al.9 found no significant association between the immunohisto-

-grade malignancy showing moderate expression for MMP-7. (f) Low grade malignancy showing moderate expression for MMP-7. (g) High grade malignancy showing no expression for MMP-9. (h) Low grade malignancy showing weak expression for MMP-9. (i) High grade malignancy showing strong expression for MMP-26. (j) Low grade malignancy showing moderate expression for MMP-26.
chemical expression of MMP-2 or MMP-9 in oral SCCs and tumour site.

Analysis of the expression of MMPs in parenchyma and stroma showed an overall higher expression in tongue carcinomas compared to lower lip carcinomas, a finding demonstrating the higher aggressiveness of the former as documented in the literature. However, this difference was only significant for MMP-9.

Another important parameter is the association between the expression of MMPs and the histological grade of malignancy of carcinomas. Tunuguntla et al. and Fang et al. studied endometrial and colorectal carcinomas, respectively, observed a correlation between the higher expression of MMPs and histological grades II and III, the presence of lymph node metastases and invasion of other tissues, consequently contributing to a poorer prognosis. In contrast, Pilka et al. and Ahokas et al. found expressive staining for MMP-26 in grades I and II of endometrial and skin carcinomas, respectively, but not in less differentiated carcinomas classified as grade III. Vicente et al. demonstrated a significant association between the histological grade of malignancy of oral SCCs and the immunohistochemical expression of MMP-9 but not MMP-2. In contrast, Liu et al. observed no significant association between MMP-2 or MMP-9 immunostaining and the degree of cell differentiation in laryngeal carcinomas.

Considering the 30 cases evaluated in this study, higher expression of MMPs, except for MMP-2, was observed in high-grade tumours, with this difference being significant for MMP-7 and MMP-26 (P < 0.05). This finding indicates a greater proteolytic potential of tumours with a high grade of malignancy in the cases of oral SCCs investigated. These findings explain the greater potential of invasion and metastasis shown by high-grade malignant tumours.

The present study also evaluated the expression of MMP-1, -2, -7, -9 and -26 within each subgroup (lower lip and tongue carcinomas), analysing staining in stroma and parenchyma of low-grade and high-grade tumours, since the two neoplastic components are known to play a determinant role in tumour progression. For SCCs of the lower lip, neoplastic cells at the invasion front of high-grade tumours expressed significantly more MMP-1, -7 and -26 than those of low-grade malignant tumours (P < 0.05). Expressive staining for MMP-1 and absent or weak staining for MMP-26 were observed in stromal cells adjacent to the invasion front in all cases of tongue carcinomas. No significant differences in stromal staining were observed, with a predominance of expressive staining for MMP-1 and weak staining for MMP-26.

Analysis of tumour cells at the invasion front in tongue carcinomas showed a tendency towards increased expression of MMPs in high-grade tumours compared to low-grade tumours. This difference was significant for MMP-7 (P < 0.05).

One important factor for the determination of the prognosis of patients with malignant neoplasms is the presence of metastases, which has been used as a parameter to test the efficiency of possible markers of tumour aggressiveness. Hong et al. observed a stronger expression of MMP-2 and MMP-9 in metastatic oral SCCs. In head and neck carcinomas, Katayama et al. and Franchi et al. reported a significant correlation between the expression of MMP-9 and the occurrence of nodal or distant metastases. In contrast, other investigators did not find a significant correlation between the presence of metastases and the expression of MMP-2 or MMP-9. In the study of Huang et al. on tongue carcinomas, the expression of EMMPRIN, a glycoprotein that stimulates the production of MMPs by stromal fibroblasts, was not associated with nodal metastasis. Sasaki et al. studying MMP-7 in lung carcinomas, and Yamamoto et al. investigating MMP-26 in esophageal SCCs, reported a significant association between metastasis and the expression of these proteases. In the present study, although MMP reactivity tended to be higher in the parenchyma of metastatic carcinomas (6 cases of tongue carcinoma), no significant difference was observed.

The correlation between morphological and immunohistochemical findings observed in the present study reaffirms and supplements one of the statements discussed earlier, i.e., that the expression of MMP-7 and MMP-26 is significantly increased in high-grade oral SCCs. This may explain the higher metastatic potential exhibited by tumours with greater histological malignancy.

Kumagai et al. demonstrated that the invasion pattern of SCCs is related to the loss of basement membrane continuity. In agreement with these authors, the present study showed that the more dispersed the invasion pattern of the tumours studied, the higher the expression of MMP-26, a finding indicating the degradation of basement membrane components.

Analysis of the present results as a whole showed that neoplastic cells at the invasion front of the tumours studied were able to synthesize MMPs and to induce the production of these enzymes by stromal cells. The high rates of positive staining for MMPs, especially MMP-1 and matrilysins, in lower lip and tongue carcinomas indicate the importance of these enzymes for tumour progression.

A significant correlation between histological grade of malignancy and expression of MMP-7 was observed for both types of carcinomas. Since the basement membrane is important for guidance of the process of differentiation of membrane-bound cells, it is perfectly reasonable to believe that degradation of this structure by MMP-7 is related to a greater histological severity of oral SCCs.

Significant differences between groups were more frequently observed for the expression of MMPs by tumour cells, whereas in most cases stromal expression of MMPs did not differ according to tumour site or histological grade of the carcinomas studied.

The present results clearly demonstrate the marked expression of MMPs in SCCs of the lip and tongue, particularly in tumours with a high histological grade of malignancy. Although this expression was more prominent in parenchymatous cells, it is believed that the tumour stroma is also a determinant factor for tumour progression. Despite a lack of statistical significance, the small differences observed may indicate distinct stages of tumour progression, with different events being involved in the initial establishment of the primary tumour and in metastatic invasion.


