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ORIGINAL ARTICLE

Matrilysins may not predict the metastatic potential in squamous cell carcinoma of the tongue

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Abstract

Objective. To examine immunoexpression of matrix metalloproteinase (MMP)-7 and -26 in squamous cell carcinoma (SCC) of the tongue and its relation with cervical metastasis. ***Material and methods.*** Twenty-four cases were selected and divided into two groups: a metastatic group ($n = 12$) and a non-metastatic group ($n = 12$). Cases were graded as either negative (score 0), positive (score +) or strongly positive (score ++). ***Results.*** MMP-7 expression was identical in both groups, with 17% of the cases graded as score 0, 50% as score + and 33% as score ++. MMP-26 expression was 25% score 0, 8% score + and 67% score ++ in the metastatic group, and 8% score 0, 50% score + and 42% score ++ in the non-metastatic group. Statistical analysis showed no differences between the studied groups and no correlations between proteins. ***Conclusions.*** MMP-7 and -26 immunostaining is not a useful indicator of the metastatic potential of SCCs of the tongue. However, the role of these proteins in the process of invasion and metastasis cannot be ruled out since their more marked presence along the tumor invasion front compared to more central areas of the tumors indicates higher secretion of these proteases in this region, facilitating the invasion process.

Key Words: *Immunohistochemistry, matrilysins, metastasis, squamous cell carcinoma, tongue*

Introduction

Squamous cell carcinoma (SCC) is the most important of all oral malignancies because of its high incidence in the mouth. It accounts for 90–95% of all malignant tumors diagnosed in this region and for 38% of head and neck tumors. SCCs can occur in any region of the mouth but SCCs of the tongue show some peculiarities because of their more infiltrative character and their high potential for developing metastases even when the tumor diameter is small. Generally, SCCs exhibit an aggressive clinical course and an unfavorable prognosis [1]; however, in their biologic behavior, oral SCCs are characterized by significant heterogeneity and tumors of the same clinical stage often show differences in clinical course and treatment response [2]. Thus, identification of factors affecting invasion and metastasis and establishment of biomarkers to predict malignant potential and identify different risk groups are of paramount importance.

Both tumor invasion and metastasis are phenomena that involve a series of steps, including the proteolytic degradation of the basement membrane and interstitial extracellular matrix (ECM). In this respect, the establishment of the relationship between tumor cells and tissue stroma has been considered to be the most critical event in the development of metastases, with the stroma promoting the survival of tumor cells and favoring the formation of new neoplastic colonies [3].

One essential component required in order for tumor cells to adapt to different tissues is the capacity of these cells to remodel the host's ECM. This remodeling capacity is attributed to the secretion of a series of proteases, including metalloproteinases (MMPs) [4,5].

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MMPs can be classified into five groups according to their functional and structural properties: collagenases, gelatinases, stromelysins, membrane-type MMPs and other types. The last group includes matrilysins, which are proteins with unique characteristics because of their minimal organization in terms of the number of domains necessary for their activation, secretion and latency. MMP-7 and -26 belong to this group and are expressed in normal and neoplastic epithelial tissues [6]. In view of the importance of matrilysins in the carcinogenesis of some human tumors and the scarcity of studies regarding their role in oral SCCs, the objective of the present investigation was to analyze the immunoexpression of matrilysins (MMP-7 and -26) in SCC of the tongue in order to identify a possible relationship with the presence or absence of metastases.

Material and methods

The study was approved by the Ethics Committee of the Federal University of Rio Grande do Norte (UFRN), Natal-RN, Brazil.

A case study investigating a possible association between the expression of MMP-7 and -26 and the presence of cervical metastases of SCC of the tongue was conducted. Twenty-four cases of SCC of the tongue were selected and divided into two groups: those without metastases (n = 12) and those with (n = 12). There were 16 men and eight women, ranging in age from 35 to 80 years (mean 60.4 years). The follow-up period ranged from 2 to 10 years.

The criterion for inclusion in the respective groups was the presence or absence of cervical metastases at the time of diagnosis and before institution of the therapeutic protocol. Metastases were demonstrated by imaging examinations such as CT or MRI. Moreover, the following aspects were taken into consideration: appropriate surgical treatment performed according to standard procedures with resection of the primary tumor; complete clinicopathologic data; and availability of sufficient paraffin-embedded tumor material.

For immunohistochemical analysis, 3-μm thick sections were obtained from paraffin-embedded tumors and submitted to immunohistochemistry by the streptavidin–biotin method as follows: deparaffinization; hydration in a decreasing ethanol series; removal of formalin pigment with 10% ammonium hydroxide in 95% ethanol; blockade of endogenous peroxidase with 10 volumes of hydrogen peroxide solution (twice for 10 min); and incubation with the monoclonal antibody according to the specifications shown in Table I. The material was immersed in Tris buffer, pH 7.4, between reaction steps. Next, the slides were incubated with the secondary antibody and the streptavidin–biotin complex for 30 min at room temperature, and the reaction was developed with diaminobenzidine. The slides were counterstained with Mayer's hematoxylin and cover-slipped with Permount.

For determination of the effectiveness of the technique, fragments of the minor salivary gland situated deep inside the tumors analyzed were used as a positive internal control. As a negative control, the incubation step with the primary antibody was omitted. All slides were scored by two investigators without knowledge of the metastases information. Occasional disagreements were discussed in order to reach a consensus.

The expression of MMP-7 and -26 in neoplastic cells was analyzed semiquantitatively by light microscopy, identifying the tumor invasion front which corresponded to the site used for the analysis of immunostaining. Staining was classified in each case as either negative or inexpressive, positive or strongly positive. For this purpose, scores of 0, + and ++, respectively were attributed at a magnification of ×400, with 0 corresponding to the total absence of expression or up to 5% of immunostained neoplastic cells (inexpressive staining), + corresponding to cellular staining of >5% and <50% and ++ corresponding to expression of >50%.

The data were analyzed statistically by the Mann–Whitney U-test in order to detect possible differences in the expression of MMP-7 and -26. Statistical analysis was performed with Statistica 6.0 software (Tulsa, Oklahoma, USA).

Results

The MMP-7 and -26 immunostaining results are shown in Tables II and III, respectively. As can be seen in Table II, MMP-7 immunostaining was similar in the groups with and without metastases, with no significant difference ($P > 0.05$). Most cases were classified as score +. MMP-7 immunostaining was also detected in glandular epithelium, muscle fibers and blood vessels amidst tumor specimens. MMP-26 immunostaining was more marked in the group with metastases, with score ++ being observed in eight of the 12 cases analyzed versus only five of 12 cases in the group without metastases, but this difference was not significant ($P > 0.05$).

Table I. Description of the antibodies used.

Clone	Specificity	Source	Dilution	Incubation	Antigen retrieval
Ab-1/ID2	MMP-7	Labvision/Neo Markers (Fremont, CA)	1:250	Overnight (18 h)	No treatment
AHP756	MMP-26	Serotec (Kidlington, UK)	1:250	Overnight (18 h)	1% Pepsin, pH 1.8, 60 min in an oven

Table II. Scores attributed to the immunoexpression of MMP-7 in neoplastic cells of cases of SCC of the tongue with and without metastases.

Group with metastases		Group without metastases	
Case	Score	Case	Score
1	+	1	+
2	+	2	++
3	++	3	0
4	0	4	+
5	++	5	+
6	+	6	+
7	++	7	++
8	+	8	+
9	+	9	0
10	+	10	++
11	+	11	+
12	++	12	++

Table III. Scores attributed to the immunoexpression of MMP-26 in neoplastic cells of cases of SCC of the tongue with and without metastases.

Group with metastases		Group without metastases	
Case	Score	Case	Score
1	++	1	+
2	++	2	++
3	++	3	++
4	++	4	+
5	++	5	+
6	0	6	+
7	0	7	+
8	0	8	++
9	+	9	+
10	++	10	0
11	++	11	++
12	++	12	++

Furthermore, MMP-7 (Figure 1) and -26 (Figure 2) immunostaining was more marked along the tumor invasion front, especially in small cell clusters, whereas extensive negative areas were observed in central regions of the tumors studied.

Discussion

Matrix metalloproteinases (MMPs) have been implicated in tumor invasiveness and several of them have shown prognostic significance in several human cancers, because are capable of cleaving ECM and basement membranes. The activity of MMPs on chemokines, growth factors and theirs receptors, adhesion molecules and apoptotic mediators is also required for tumor progression thought to be a key event in both the local invasion and metastasis. In this study, we analyzed the immunoprofile of matrilysins (MMP-7 and -26) in SCCs of the tongue. According to De Vicente et al. [2], MMP-7 seems to play a central role in tumor invasion and metastasis but another important characteristic of this protein is that, unlike the other MMPs which are also synthesized by stromal cells, matrilysin is produced exclusively by cancer cells.

Curiously, in the present study the intensity of MMP-7 immunostaining was identical in the metastatic and non-metastatic SCC of the tongue groups, with the observation of significant expression of this protein in both groups. This finding suggests a possible role of this protease in the development and progression of SCCs of the tongue, in agreement with the findings of Birkedal-Hansen et al. [7], who

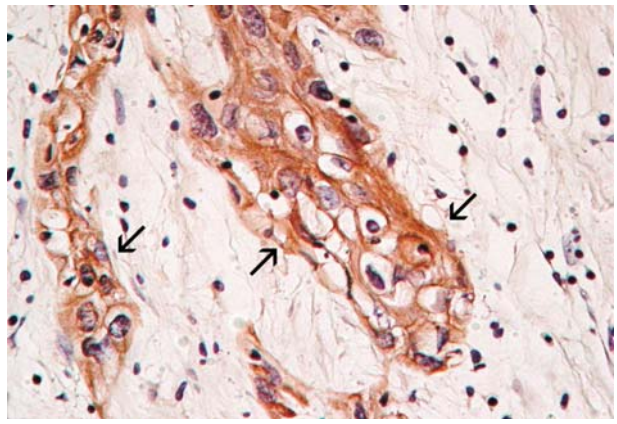

Figure 1. MMP-7. Intense immunoexpresssion in a metastatic group case (*arrows*). Score ++ (streptavidin–biotin staining; original magnification ×400).

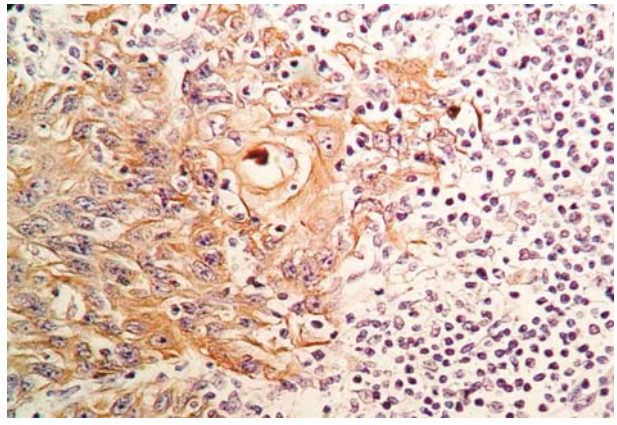

Figure 2. MMP-26. Intense immunoexpression in the tumor invasion front in a metastatic group case. Score ++ (streptavidin–biotin staining; original magnification ×400).

concluded that the expression of MMP-3, -7, -10 and -14 observed in carcinomas provides important information about ECM degradation and tumor progression. We also believe that MMP-7 detected in neoplastic cells of the cases analyzed might be acting on the selection of cell clones that are less likely to be eliminated by the immune system.

Impola et al. [8] demonstrated a difference in MMP-7 immunostaining between SCCs and verrucous carcinomas, with MMP-7 expression being higher in the former, and suggested that the absence of staining for this protein in neoplastic cells might be a good prognostic indicator. This fact might explain the presence of MMP-7 in the two tumor groups in the present study, suggesting that all tongue carcinomas possess a marked invasive potential. In addition, the involvement of this protein as a determining factor in the development of metastatic lesions might be elucidated by studying the interaction of this MMP with other proteins.

One result of this study that should be emphasized is the more marked presence of MMP-7 along the tumor invasion front compared to other more central areas of the tumor. This is in agreement with the findings of Yasmashita et al. [9], who observed a significant correlation between increased expression of MMP-7 at the invasion front and a poorer prognosis in esophageal carcinomas.

MMP-26 degrades collagen IV, vitronectin, insulin-like growth factor, fibronectin, fibrinogen and gelatin [10], and is widely expressed in neoplasms of epithelial origin, such as those arising in the lung, prostate gland and breast. According to Park et al. [10], one of the factors associated with the involvement of MMP-26 in the development and progression of cancer is the ability of this enzyme to activate gelatinase-B (MMP-9), which degrades fibronectin and collagen IV, an event essential for tumor invasion. Although MMP-7 and -26 belong to the same metalloproteinase group, Park et al. [10] suggested that these proteases may have different substrates and, therefore, distinct activation mechanisms and roles in carcinogenesis. MMP-26 was included in the present study because of the scarcity of reports on the role of this protein in oral carcinogenesis.

Yamamoto et al. [11] reported that MMP-26 may play a role in normal turnover and-or repair. In your research the expression of this MMP was detected in all 10 lymph node metastases in esophageal squamous cell carcinoma (ESCC), that showed correlation with depth of invasion, lymph node and distant metastasis as advanced tumor stage. They suggested that overexpression of MMP-26 (matrilysin-2) plays an essential role in the development of lymph node metastasis in this neoplasia. Additionally, they suggested that analysis of expression of matrilysin-2 and MMP-9 could be an important routine part of the management of patients with ESCC. Use of the diagnostic strategy examined in this study and advances in therapeutic approaches, including the use of MMP inhibitors, should improve the prognosis of patients with ESCC. In conclusion, although no significant correlation was observed between the immunohistochemical expression of MMP-7 and -26 and the metastatic potential of the SCCs of the tongue studied, the role of these proteins in the process of invasion and metastasis cannot be ruled out since their more marked presence along the tumor invasion front compared to more central areas of the tumors indicates higher secretion of these proteases in this region, facilitating the invasion process. In addition, this study demonstrated that MMP-7 and -26 immunostaining is not a useful indicator of the metastatic potential of SCCs of the tongue, suggesting that these tumors possess a marked invasion potential.

Declaration of interest: The authors declare that they have no conflicts of interest.

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