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Expression of MMP-1 and MMP-11 in squamous cell carcinoma of the nasal cavity and paranasal sinuses

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RESUMEN

Antecedentes: el pronóstico en pacientes con carcinoma epidermoide de la cavidad nasal y de los senos paranasales es malo, sobre todo por invasión, recurrencia y metástasis locorregionales.
Objetivo: evaluar la expresión de MMP-1 y MMP-11 en las células escamosas de los carcinomas epidermoides de la cavidad nasal y los senos paranasales.
Pacientes y método: diecisiete pacientes con historia clínica completa, sin tratamiento previo al diagnóstico inicial con biopsia y material adecuado para el estudio inmunohistoquímico de MMP-1 y MMP-11. Se utilizaron la prueba de la χ^2 y la exacta de Fisher para evaluar la expresión de ambas endopeptidasas de los fibroblastos neoplásicos y peritumorales, y su relación con los parámetros clinicopatológicos.
Resultados: encontramos 10 mujeres y siete hombres con edad promedio de 58 años (37 a 85); dos en etapa clínica II, seis en III y nueve en IV. Trece y 12 casos fueron positivos en las células neoplásicas, y 13 y 10 en los fibroblastos peritumorales para MMP-1 y MMP-11, respectivamente.
Conclusiones: estas endopeptidasas son expresadas en carcinomas epidermoides de la cavidad nasal y de los senos paranasales por las células neoplásicas y por los fibroblastos peritumorales, lo que sugiere que podrían tener acciones adyuvantes en los mecanismos de progresión tumoral y que su baja expresión puede indicar un pronóstico más favorable.
Palabras clave: metaloproteasas, carcinoma de células escamosas, cavidad nasal y senos paranasales.

ABSTRACT

Background: Poor prognosis in patients with squamous cell carcinoma of the nasal cavity and paranasal sinuses is associated mainly with invasion, recurrence, and loco-regional metastases.
Objective: To evaluate the expression of MMP-1 and -11 in NCPS squamous cell carcinoma.
Patients and method: Seventeen patients with complete clinical record, no treatment before the initial diagnostic biopsy, and adequate material for the immunohistochemical study of MMP-1 and MMP-11. χ^2 and Fisher's exact test were used to assess MMP-1 and MMP-11 expression of neoplastic and peritumoral fibroblasts and its relation to clinicopathological parameters.
Results: We found 10 females and 7 males with 58 years old mean age (37 to 85); two were classified as clinical stage II, six as III, and nine as IV. For MMP-1, 13 cases (76%) were positive in neoplastic cells and 13 in peritumoral fibroblasts. For MMP-11, 12 (71%) were positive in neoplastic cells and 10 (59%) in peritumoral fibroblasts.
Conclusions: MMP-1 and MMP-11 are expressed in squamous cell carcinoma of the nasal cavity and paranasal sinuses by both neoplastic cells and peritumoral fibroblasts, suggesting that they might be exerting adjuvant actions in the mechanisms of tumor progression and that their low expression could be related to a favorable prognosis.
Key words: metalloproteases, squamous cell carcinoma, nasal cavity and paranasal sinuses.

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Malignant neoplasms of the nasal cavity and paranasal sinuses (NCPS) are infrequent tumors representing about 3% of all malignant neoplasms of head and neck.[1,2] The poor prognosis of patients with these neoplasms is related to the complex anatomy of the region, diagnosis in advanced stages, extension to adjacent structures, and frequent loco-regional recurrences.[1-3]

Sixty-three percent of the malignant NCPS neoplasms correspond to squamous cell carcinomas (SCC),[1-3] which are characterized by a high degree of invasion and local

recurrence. In this tumor, the degree of histological differentiation and the invasion pattern, either in small groups or individual neoplastic cells, lead to a more aggressive behavior.[4,5]

Tumor invasion and development of metastasis require degradation of the basal membrane (BM) and extracellular matrix (EM), a process in which several enzymes are involved. Among these enzymes are the matrix metalloproteases (MMPs) that are able to degrade completely all components of the BM and EM.[6] These zinc-dependent metalloproteases are classified by their specific substrate in: collagenases, stromelysins, gelatinases, matrilysins, and membranal-type MMPs.[6,7] Several MMPs have been described in different tumors and locations and may be considered as possible tumor markers and/or treatment targets. However, the large amount of available data has generated discrepancies in evaluating their precise involvement.[6-8]

Of the different MMPs, the interstitial collagenase (MMP-1) presents specific substrates for collagenases I, II, III, VII, VIII, X, and for proteoglicans.

These enzymes are produced in small amounts by a variety of normal cells. Nevertheless, it has been reported that some tumors express high levels of this enzyme, mainly the invasive neoplastic cells,[9] and this has been related with poor prognosis in patients with breast, skin,[10] gallbladder,[11] esophageal,[7] stomach, colorectal and oral cavity[7-11] cancers.

Stromelysin-3 (MMP-11) specific substrates are casein, laminin, fibronectin, gelatinin, collagenase IV, and carboxymethylated transferrin. Its participation in malignant transformation of cells is currently being considered, and it has been correlated with an aggressive biological behavior and poor prognosis in patients with breast, lung, ovarian, laryngeal, and oral cavity cancers.[12-15]

There are few studies on the participation of these enzymes in NCPS squamous cell carcinoma. Considering that these regions have epidemiological, histological, and clinical characteristics that differ from the rest of head and neck structures, we decided to evaluate the expression of MMP-1 and -11.

MATERIAL AND METHODS

From the pathological records of the National Cancer Institute in Mexico, 55 cases with histological diagnosis by biopsy of primary SCC of NCPS were identified between 1998 and 2003. Of these, 17 fulfilled the following inclusion criteria: complete clinical record, no treatment before the initial diagnostic biopsy, and adequate material for the immunohistochemical study of MMP-1 and MMP-11. Of the 17 cases, ten were females and seven were males, with an average age of 58 years (range 37 to 85). Two patients were classified as clinical stage II, six to stage III, and nine to stage IV. Eight patients presented extension to adjacent critical structures. Twelve cases (70%) were moderately differentiated SCC, four (24%) were poorly differentiated, and one (6%) was a well-differentiated carcinoma. Eleven cases (65%) presented an invasion pattern in layers, cords, and solid bands; six (35%) presented invasion in small groups and individual neoplastic cells. Regarding nuclear polymorphism, 50% to 70% of mature malignant cells (considered as moderate nuclear polymorphism) were found in ten cases (59%). The remnant seven cases (41%) presented less than 25% of mature neoplastic cells (high nuclear polymorphism). Most patients received combined treatment based on radiotherapy and surgery (eight patients), four patients received chemotherapy, and two patients were subjected to chemo- and radiotherapy. Ten patients presented local recurrences, three developed lymphatic node metastasis, and one of the latter metastasized to the brain. Tumor persistence was observed in four patients (23.5%), six developed lymphatic node metastasis. The 14 patients with persistence and/or recurrence with tumor activity died (82%). Of the remainder three patients (18%) two were alive without tumor activity and one died without tumor. The postoperative follow-up of patients was 40 months, with an average 6 to 24 months (table 1).

IMMUNOHISTOCHEMISTRY

Paraffin blocks were cut into 2μ sections containing representative areas. They were deparaffinized in xylene and rehydrated in ethanol. Retrieval of epitopes was performed in 0.1 M sodium citrate (pH 6.0) for 10 min. Samples were blocked for endogenous peroxidase with 3% H_2O_2 for 5 min and incubated for 1 h at room temperature with the MMP-1 rabbit polyclonal antibody (collagenase-1, Ab-7 NeoMarkers, USA, 1:100 dilution) and the MMP-11 mouse monoclonal antibody (stromelysin-3 Ab-5 clone SL3.05 NeoMarkers, USA, 1:50 dilution). The second-

Table 1. Clinical and histological characteristics and MMP-1 and MMP-11 expression in squamous cell carcinoma of the nasal cavity and paranasal sinuses

CS	*ECA*	*PI*	*NP*	*MMP-1TC*	*MMP-1 PF*	*MMP-11 TC*	*MMP-11 PF*	*Status*	*Metastasis*	*Survival*
II	Fs	II	II	+++	+++	+++	+++	R-17 months	Lymph node	DWT (17 months)
II	-	II	II	+++	+++	++	++	R-12 months	-	DWT (12 months)
III	-	II	II	-	++	-	-	R-10 months	-	DWT (13 months)
III	Of	II	II	+++	+++	+++	+++	P	Lymph node	DWT (6 months)
III	-	II	IV	+++	-	+++	+++	P	Lymph node	DWT (16 months)
III	Of	II	II	-	-	-	-	WTA	-	DWOT (63 months)
III	-	II	IV	++	++	-	-	R-12 months	-	DWT (15 months)
III	-	IV	II	-	-	-	-	WTA	-	AWOT (50 months)
IV	-	II	II	+++	+++	+++	+++	R-12 months	-	DWT (12 months)
IV	-	II	II	++	++	++	++	R-10 months	Lymph node	DWT (10 months)
IV	Of	II	II	+++	+++	+++	+++	R-35 and 39 months	brain	DWT (74 months)
IV	-	IV	IV	++	++	++	++	R-24 and 10 months	-	DWT (35 months)
IV	Of	II	IV	+++	+++	+++	-	R-8 months	Lymph node	DWT (10 months)
IV	Of	IV	IV	++	++	++	-	R-6 months	-	DWT (7 months)
IV	Of	IV	IV	+++	+++	+++	+++	P	-	DWT (8 months)
IV	N	IV	IV	+++	+++	+++	+++	P	Lymph node	DWT (10 months)
IV	-	IV	II	-	-	-	-	WTA	-	AWOT (46 months)

CS: clinical stage, ECA: extension to adjacent areas, Fs: frontal sinus, OF: orbital floor, N: nasopharynx, PI: pattern of invasion, NP: nuclear polymorphism, TC: tumoral cells, PF: peritumoral fibroblasts, R: recurrence, P: persistence, WTA: without tumor activity, DWT: death with tumor, DWOT: death without activity, AWOT: alive without tumor.

ary antibody labeled with biotin was added for 15 min at room temperature. Afterwards, streptavidin conjugated to peroxidase (LSAB + System HRP Universal, Dako Cytomation, USA), was added to react with diaminobenzidine (Liquid DAB, Dako Cytomation, USA) and counterstained with Mayer's hematoxylin. As positive control, ovary and placental tissue samples, treated under the same conditions, were included.

MMP-1 and MMP-11 expression was assessed by two independent observers (AMG and LSR) in the area corresponding to the invasive front in both neoplastic and peritumoral fibroblasts, in five high-resolution (40x) fields. Cases considered positive were those exhibiting more than 20% of moderate to intense expression in the cytoplasm of the studied cells. The surface epithelium and the ducts were used as internal basal control.[8]

STATISTICAL ANALYSIS

Statistical analysis was based on χ^2 and Fisher's exact test to assess MMP-1 and MMP-11 expression of neoplastic and peritumoral fibroblasts and its relation to clinico-

pathological parameters, using the Sigma Stat Ver. 3.00 software (SPSS, USA). A p value < 0.05 was considered statistically significant.

RESULTS

Expression varied from moderate to intense in the positive cases for both MMP-1 and MMP-11. For MMP-1, 13 cases (76%) were positive in neoplastic cells and 13 in peritumoral fibroblasts. For MMP-11, 12 (71%) positive cases were found in neoplastic cells and in ten (59%) positivity was expressed in peritumoral fibroblasts (figure 1). Table 2 shows the clinical characteristics of each patient and the assessment of MMP-1 and MMP-11 expression.

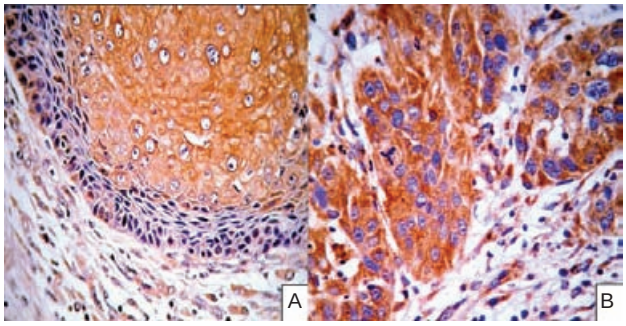

Figure 1. A) Microphotograph of MMP-1 immunoexpression in SCC of NCPS showing neoplastic and peritumoral stromal cells with an invasion pattern in pushing borders (40x). B) Microphotograph of MMP-11 immunoexpression in SCC of NCPS showing nests of neoplastic and peritumoral stromal cells (40x).

Dependence of MMP-1 expression in tumor and peritumoral fibroblasts on clinicopathological parameters

MMP-1 expression in tumor cells was found to be associated with patient status (p = 0.015) but not with metastasis.

Dependence of MMP-11 expression in tumor and peritumoral fibroblasts on clinicopathological parameters

MMP-11 expression in tumor cells was found to be associated with all clinicopathological parameters assessed (metastasis p = 0.044 and status p = 0.003)

The literature on different malignant neoplasms of NCPS, revision period among 4 to 20 years with major populations of 80 cases, where the percentages of SCC are between 20 to 30%. In this study of 52 cases, patients had neoplasms in advanced stages when the diagnosis was made, with non specific, poor prognosis and a maximal survival of five years.[11,16,17]

In this series of SCC cases of NCPS, clinical (advanced clinical stage and invasion to adjacent structures) and histological (pleomorphism and invasion pattern) characteristics considered as predictors of the status and survival of patients did not correlate with data referred in the literature.[1-3] These discrepant findings indicate the need to understand the diverse mechanisms involved in the biological behavior of neoplasms.

Different enzymes mediate tumoral invasion, with MMPs been associated with tumor progression. However,

Table 2. Positive (+) and negative (-) cases of MMP-1 and MMP-11 expression and their association with the patient status, survival, and metastasis

Clinicopathological parameters	*MMP-1*				*MMP-11*			
	Tumor cell		*Peritumoral fibroblasts*		*Tumor cell*		*Peritumoral fibroblasts*	
	+	-	+	-	+	-	+	-
Metastasis								
With	7	0	6	1	7	0	6	1
Without	6	4	7	3	5	5	4	6
p value	ns		ns		0.044		ns	
Status								
Recurrence	8	1	9	0	8	1	6	3
Persistence	4	0	3	1	4	0	4	0
Without tumor activity	1	3	1	3	0	4	0	4
p value	0.015		ns		0.003		0.005	
Survival								
Death with tumor	12	1	12	1	12	1	10	3
Alive without tumor	1	3	1	3	0	4	0	4
p value	0.022		0.022		0.002		0.015	

NS: not significant.

up to now the role played by the diverse MMPs has not been clearly established.

The main group of MMPs widely studied in SCC of head and neck are the gelatinases (MMP-2 and MMP-9), which have been associated with invasion processes and metastases (the advanced stages in tumoral progression). Those enzymes have specific substrates for collagen types I, II, III, IV, V, VII, X, XI, and XIV; gelatin, elastin, fibronectin, laminin-1, laminin-5 galectin-3, and proteoglicans, among others. Nonetheless, contradictory results are reported in the literature regarding their participation, suggesting that they are not the only MMPs involved in these processes.[6-11,17]

In our series, we detected the presence of MMP-1 and MMP-11 in neoplastic and peritumoral stromal cells by means of immunohistochemistry.

This technique allows the direct assessment of expression intensity in the cells and allows the correlation with invasion at the biological level.[17]

MMP-1 initiates degradation of collagen I, which is abundant in the extracellular matrix[16] and is essential for keratinocyte migration; several authors consider that these mechanisms facilitate tumoral invasion.[6,7,9,11] MMP-1 expression has been described in both neoplastic and peritumoral stromal cells;[16,17] however, its presence is considered more important in the zone of greatest activity corresponding to the tumoral front.[9,17] In a study of SCC of the larynx, in which MMP-1 was evaluated, no significant tendency was found in its expression in invasion processes and metastasis.[11] On the other hand, Kurahara et al.,[8] in a study of the oral cavity, suggested that participation of MMP-1 together with other MMPs is essential for the invasion process. In our series, the presence of MMP-1 in both types of cells at the level of the tumoral front suggests its participation in the processes of tumor persistence and recurrence, showing a more aggressive behavior and a poor prognosis in patients with SCC of the NCPS.

However, it has been shown that MMP-11 is expressed in peritumoral fibroblasts of the area corresponding to the tumoral front, indicating its stromal origin, based on its paracrine stimulation by tumoral cells. It is considered that this stromelysin can also be related with progression of phenotypic alterations acquired during malignant transformation.[6,7] In some studies on colorectal cancer in mice, MMP-11 deficiency coincides with a decrease in tumor genesis. The mechanisms implicating in MMP-11 in tumor progression include inhibition of neoplastic cells death, thereby fostering survival in the stromal environment.[6]

Studies on MMP-11 in head and neck tumors are scarce; Arora et al. performed a study in patients with SCC of the oral cavity in normal tissue, pre-malignant and invasive lesions also in a cell line.

In our study, we observed a relation between MMP-11 expression and persistence and recurrence of tumors, which could suggest participation of this enzyme in a more aggressive behavior of the disease as seen in other tumors.[12,13]

For tumoral cells, blood circulation is considered a hostile environment, as they can be affected by mechanical deformation, immunologic attack, serum toxicity, or high shear stress. Neoplastic cells acquire a variety of mechanisms in which MMPs are involved. Based on experiments, it has been suggested that MMP-11 generates bioactive fragments of the α-1-proteinase inhibitor, which could reduce the action of natural killer cells and, thereby, increase tumor growth and invasion *in vivo*.[6]

Immunopositivity for MMP-11 was found in both neoplastic and peritumoral stromal cells in biopsies of patients with metastases. This is relevant because in patients without treatment, expression of this enzyme could be an important predictor of metastasis.

In conclusion, our results show that these enzymes are expressed in SCC of the NCPS in neoplastic and peritumoral stromal cells, suggesting that they might coparticipate in tumor progression processes, and that a lower expression of these enzymes might indicate a favorable prognosis. In addition, it could be considered that MMP-11 might play a relevant role in fostering metastasis, but it is necessary to increase the number of cases as well as to perform other types of study to understand the diverse mechanisms in which these enzymes participate in the pathogenesis of tumors and their interaction with the extracellular matrix.

The objective of this study was to explore MMPs -1 and -11 expression in SCC of the NCPS, because these MMPs were found in other similar tumors as well as in oral cavity squamous cell carcinoma. This research could be the start for other studies regarding MMPs expression and activity in these aggressive tumors. It would be important to increase the number of cases as well as patients follow-up to assess its prognostic significance.

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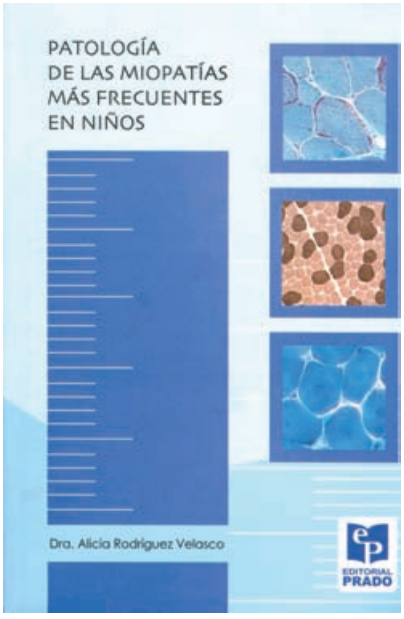

Patología de las miopatías más frecuentes

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Este libro de la doctora Alicia Rodríguez es un regalo para cualquiera que esté interesado en las enfermedades musculares de los niños. En unas cuantas páginas se podrán revisar los hallazgos clínicos, paraclínicos histopatológicos, de histoquímica y ultraestructura característicos de las miopatías más frecuentes. Es muy útil para pediatras, neurólogos, neuropediatras, ortopedistas, fisioterapeutas, genetistas, patólogos y estudiantes de medicina.