Expression and prognostic significance of matrix metalloproteinases and their tissue inhibitors in primary neuroendocrine carcinoma of

Expression and Prognostic Significance of Matrix Metalloproteinases and Their Tissue Inhibitors in Primary Neuroendocrine Carcinoma of the Skin

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Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) have been implicated in the development and progression of many tumors, but data for primary neuroendocrine carcinoma (PNC) of the skin are lacking. The aim of the study was to assess the expression of MMPs and TIMPs in PNC and to evaluate their prognostic significance. Expression of MMP-1, MMP-2, MMP-3, MMP-9, MMP-11, MMP-13, and MMP-14 and TIMP-1, TIMP-2, and TIMP-3 was evaluated by immunohistochemistry on 23 samples of PNC of the skin. The results were matched with clinical features and patient survival. In the 23 specimens of PNC, high (>20% of positive neoplastic cells) MMP-1 expression was found in 13 (56.5%) cases. MMP-2 was evidenced in 12 (52.1%) cases, 8 (34.7%) of which showed high expression in neoplastic cells. MMP-3 was detected in 11 cases (47.8%), with high expression in 9 (39.1%) of them. High MMP-9 expression was observed in 3 (13%) cases, whereas high MMP-14 expression was detected in 11 (47.8%) specimens. Expression of TIMP-1 by neoplastic cells was found in 8 (34.7%) cases, with high expression in 3 cases, whereas high TIMP-3 expression was detected in 21 (91.3%) cases. No immunoreactivity for MMP-11, MMP-13, or TIMP-2 was found. Statistical analysis failed to identify a significant correlation between MMP/TIMP expression and clinical parameters. By univariate analysis, stage >I ($P = 0.01$), high expression of MMP-1 ($P = 0.04$) and MMP-3 ($P = 0.01$) resulted significant negative prognostic factors, whereas by multivariate analysis, stage was the only factor that affected survival ($P = 0.02$). Our results suggest that MMP-1 and MMP-3 may influence the invasive and metastatic potential of PNCs. It is conceivable that future attempts to specifically block MMP-1 and MMP-3 activity may provide a novel means to inhibit invasiveness and distant spread in selected patients with PNC. Hum Pathol 34:80-88.

Key words: **matrix metalloproteinase, tissue inhibitor of metalloproteinase, neuroendocrine carcinoma, immunohistochemistry, prognosis.**

Abbreviations: **MMP, matrix metalloproteinase; PNC, primary neuroendocrine carcinoma; TIMP, tissue inhibitor of matrix metalloproteinase.**

Primary neuroendocrine carcinoma (PNC) of the skin is the currently favored designation for the tumor originally described by Toker in 1972 as *trabecular carcinoma*,[1] and subsequently termed *Merkel cell carcinoma* on the basis of the electron microscopic findings of neurosecretory granules.[2] PNC is a rare, highly malignant neuroendocrine tumor that occurs most commonly in elderly Caucasian patients. Clinically, it usually appears as a rapidly growing nodule on sun-damaged skin of the head and neck region and on the extremities, often being indistinguishable from other cutaneous neoplasms on clinical grounds alone. Affected patients have a high risk of disease progression: early local recurrences occur in about 33% of cases, and approximately 50% of patients will develop regional lymph node metastases with eventual death due to systemic dissemination in more than 33% of cases.[3-4] Because of its rarity, prognostic factors have not been fully established. However, cumulative data from small series have suggested that stage, size of the primary tumor, and gender may have an influence on survival, whereas the prognostic impact of anatomic location and age are more controversial.[5-8]

Although various factors can determine the aggressiveness of PNCs, the capacity for invasion and metastasis of tumor cells, involving the degradation of components of basement membranes and the extracellular matrix, is certainly important. The matrix metalloproteinases (MMPs) are a large family of zinc-dependent proteolytic enzymes[9,10] involved in the degradation of different components of the extracellular matrix. At present, there is considerable evidence indicating that MMPs play important roles in local invasion and tumor spread.[10-12] The family of human MMPs comprises several members classified into 4 main classes according to their structure and in vitro substrate specificity: collagenases, gelatinases, stromelysins, and membrane-type MMPs[11-12] (Table 1).

Tissue inhibitors of matrix metalloproteinases (TIMPs) are the major natural inhibitors of MMPs. To date, 4 types of TIMPs (TIMP-1, TIMP-2, TIMP-3, and TIMP-4) have been recognized. The TIMPs are secreted proteins that complex MMPs and are involved in the inhibition of individual MMPs and regulation of their activity.[13] Indeed, it is thought that the balance between activated MMPs and TIMPs determines the

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TABLE 1. Nomenclature and Substrate Specificity of Individual MMPs

MMP	Synonym	Substrates
MMP-1	Interstitial collagenase	Fibrillary collagens type I, II, III, VI, IX; proteoglycans
MMP-2	Gelatinase A, 72-kD gelatinase, collagenase type IV	Gelatin type I, II, III; collagen type IV, V, VII, X; fibronectin; elastin
MMP-3	Stromelysin-I, procollagenase, transin	Cartilage proteoglycans; fibronectin; laminin; gelatin type I, III, IV, V; collagen type III, IV, I, IX; procollagenase
MMP-7	Matrylisin, PUMP-I	Gelatin type I, III, IV, V; cartilage proteoglycans; fibronectin; procollagenase; TNF-α precursor; collagen type IV
MMP-8	Neutrophil collagenase	Collagens type I, II, III
MMP-9	Gelatinase B, 92 kD gelatinase	Gelatin type I, V; collagen type IV, V
MMP-10	Stromelysin-2, transin-2	Gelatin type I, III, IV, V; collagen type III, IV, V; procollagenase; fibronectin
MMP-11	Stromelysin-3	Laminin, fibronectin, casein
MMP-12	Metalloelastase	Elastin
MMP-13	Collagenase-3	Collagen type I, II, III
MMP-14	MTI-MMP, membrane type I MMP	Activated progelatinase-A
MMP-15	MT2-MMP, membrane type I MMP	Activated progelatinase-A
MMP-16	MT3-MMP, membrane type I MMP	Activated progelatinase-A
MMP-17	MT4-MMP, membrane type I MMP	ND
MMP-18	Putative MMP only, partial cDNA identified	ND
MMP-19	Rheumatoid arthritis-associated	ND
MMP-20	Enamelysin	Amelogenenin

Abbreviation: ND, not determined.
Modified from Curran and Murray.[12]

overall MMP activity and proteolysis in vivo. However, recent experimental data suggest that TIMPs not only have MMPs inhibitory functions, but also are multifunctional molecules, with apparent paradoxical effects on tumor progression. Indeed, although early studies reported that TIMPs had antitumor or antimetastatic effects, recent studies suggest a positive correlation between TIMP level and poor outcome in individual types of tumors.[12]

Some investigators have hypothesized that the disruption of the MMP/TIMP balance may be a factor in the progression of tumors to a more malignant phenotype and that certain MMPs may predict poor prognosis when expressed at high levels.[14] Indeed, the level of expression of some of these proteins has been correlated with tumor aggressiveness, as implied by increasing histologic grade;[15-16] advanced clinical stage;[17-19] poor patient survival[17,20-21] in gastric, pancreatic, and lung cancer; and increased relapse rate in colorectal cancer.[22]

Recently, the crucial role of MMPs in cancers has also generated considerable interest in the use of broad-spectrum synthetic MMP inhibitors as potential therapeutic agents for clinical use.[23-24] Because there may be an organ- or cell-specific expression of certain members of the MMP family in each malignant tumor, future efforts should be focused on selecting the most appropriate inhibitor for each tumor type to determine which MMP to target to obtain the best effect with the fewest side effects.

We undertook an extensive immunohistochemical study of the expression of MMP-1, MMP-2, MMP-3, MMP-9, MMP-11, MMP-13, and MMP-14 and TIMP-1, TIMP-2, and TIMP-3 on a series of 23 PNCs of the skin to determine whether the expression profile of these enzymes reflects differences in biological aggressiveness of tumor cells. Although the literature on this subject has grown in recent years, expression patterns of MMPs and TIMPs in PNCs have not yet been investigated. The results described here will provide valuable data on the role of proteinases in the biological behavior of PNCs and the potential use of proteinases in clinical practice.

MATERIALS AND METHODS

Patients

A total of 33 patients treated for PNC at the Department of Dermatological Sciences, University of Florence were enrolled in the study. Patients with histologic material not available, those with an unconfirmed diagnosis, and those lost to follow-up were dropped from the study. Thus, the study group comprised a total of 23 patients with PNC of the skin with adequate histologic material, complete clinical data, and a minimum follow-up of 6 months (for living patients). The following baseline data were obtained from patients' records: age, sex, anatomic site and size of the tumor, number of lesions (single versus multiple), stage, and date of diagnosis. Further clinical information on management and outcome in each patient, including type of therapy (ie, surgical excision versus surgical excision plus postoperative local/regional radiotherapy), clinical response, development of recurrences and/or metastases, current status, and survival time was collected. Patients were staged according to the absence (stage I) or presence (stage II) of positive regional lymph nodes and by the presence of systemic dissemination (stage III).[25] Stage I was further subdivided based on tumor size into stage Ia (tumors $\leq$2 cm in diameter) and stage Ib (>2 cm), according to a recently published modified staging system.[7] Staging workup included detailed history and physical examination, complete blood count, routine serum chemistries, chest X-ray, and abdominal ultrasound and other tests as deemed necessary. In each case, the tumor was studied by routine

TABLE 2. Antibodies Used in the Immunohistochemical Study

Antibody	Clone	Source	Dilution	Incubation Time	Pretreatment
MMP-1	41-1E5	Oncogene, Boston, MA	1:400	1 hour	None
MMP-2	42-5D11	Oncogene	1:10	2 hour	10 mM citrate buffer, MW (Micromed T/T Mega-Mileston Srl., Sorisole (BG), Italy) 35 minutes
MMP-3	55-2A4	Oncogene	1:20	1 hour	0.5% proteasi XIV, r.t. 15 minutes
MMP-9	56-2A4	Oncogene	1:10	1 hour	10 mM citrate buffer, MW 35 minutes
MMP-11	SL3.05	Neomarkers, Fremont, CA	1:200	1 hour	None
MMP-13	VIIIA2	Neomarkers	1:10	2 hour	10 mM citrate buffer, MW 35 minutes
MMP-14	113-5B7	Chemicon International, Temecula, CA	1:40	2 hour	10 mM citrate buffer, MW 35 minutes
TIMP-1	102D1	Oncogene	1:10	2 hour	10 mM citrate buffer, MW 35 minutes
TIMP-2	T2-N IC3	Oncogene	1:10	2 hour	10 mM citrate buffer, MW 35 minutes
TIMP-3	136-13H4	Chemicon International	1:50	2 hour	10 mM citrate buffer, MW 35 minutes

hematoxylin and eosin–stained sections and immunohistochemical stains. The diagnosis was confirmed by electron microscopy in 5 cases. On histopathologic examination, features noted included cell size (small versus large); architectural pattern (organoid versus diffuse) and mitotic rate (0 to 5 mitoses versus 6 to 10 mitoses versus >10 mitoses per high-power field; ×40), according to previously published criteria.[4] On revision, in all cases the diagnosis was confirmed by immunohistochemical stains, including cytokeratins (clone AE1/AE3; Biogenex, San Ramon, CA), cytokeratin 20 (Biogenex), neuron-specific enolase (Biogenex), and chromogranin A (Biogenex).

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue specimens from PNCs used for all immunohistochemistry studies were retrieved from the Department of Human Pathology and Oncology, University of Florence. Tissue sections were incubated with primary antibodies to MMP-1, MMP-2, MMP-3, MMP-9, MMP-11, MMP-13, and MMP-14 and TIMP-1, TIMP-2, and TIMP-3 (Table 2). The specific MMPs were selected because of previous reports showing their increased expression, and even a prognostic impact for some of them, in several solid tumors, including small-cell neuroendocrine lung cancer. Immunohistochemistry was performed by the standard avidin-biotin-peroxidase complex method (Dako SpA, Milan, Italy) with diaminobenzidine as a chromogen and hematoxylin as a counterstain. The final dilution of each antibody was determined after preliminary serial dilution studies. Human colon adenocarcinoma was used as a positive control. Procedures used as negative controls included omission of the primary antibody from the staining protocol and replacement of the primary antibody with normal rabbit or mouse IgG in appropriate concentrations.

Analysis of immunohistochemical staining was independently performed by 2 investigators (D.M. and A.F.) without knowledge of the clinical and survival data. Immunoreactivities were semiquantitatively evaluated as "negative" (−) when no positive cells were found, "focal" (+) when positive tumor cells accounted for <20% of the total number, "moderate" (++) when 21% to 50% of tumor cells were positive, and "diffuse" (+++) when >50% tumor cells were positive. For statistical analysis, negative/focal stainings were classified as low expression and moderate/diffuse stainings as high expression of the respective antigens. Stromal staining of TIMPs was also assessed in fibroblasts and collagen stroma.

Statistical Analysis

Statistical analysis was performed using the SPSS statistical software, release 8.0 (SPSS, Chicago, IL). The relationship between categorical variables was assayed by the 2-tailed Fisher exact test. The Cox proportional hazards model was used to assess the prognostic values of the clinical and immunohistochemistry variables. Survival was measured from the date of death or last follow-up time before study closure. The Kaplan-Meier product-limit method was used to estimate the overall survival for the group and to illustrate the effect of each variable on survival.[26] The log-rank test was used to evaluate the differences between survival curves. A multivariate analysis (Cox proportional hazards model) was used to determine which variable had an independent effect on clinical outcome.[27] A P value <0.05 was considered statistically significant.

RESULTS

Patient Population

Clinical details for the study group are given in Table 3. The study included 7 (17.4%) males and 16 (69.6%) females with an age range of 54 to 91 years (mean, 77 years; median, 81 years). The tumors were located mainly on the lower extremities (n = 11) and the head and neck region (n = 10), with 1 case arising on the upper extremities and 1 case on the back. Fifteen (65.2%) tumors exceeded 2 cm in greatest dimension at the time of diagnosis. Follow-up of patients ranged from 4 to 291 months (mean, 42.2 months; median, 16 months). Disease progression was observed in 13 (56.5%) cases. All patients had been treated with surgery plus adjuvant radiotherapy. Nine patients were alive without evidence of disease, 2 were alive with tumor, 2 had died of other causes, and 10 had died of disseminated PNC. The 2-year survival rate was 54.2%. On histopathologic examination, only 2 cases exhibited surface ulceration. Cytologically, the small-cell type was observed in most cases (n = 20), whereas the tumor cell population comprised an admixture of small and intermediate to large cells in 3 cases. In most cases (n = 21) the architectural pattern was diffuse, whereas in the other 2 cases a mixture of organoid, trabecular, and

TABLE 3. Clinical Data in 23 Patients with PNC of the Skin

Case	Age (year)	Sex	Site	Number of Lesions	Stage	Type of Progression	Status	Follow-Up (months)
1	57	M	Right leg	Multiple	Ib	Skin rec; systemic dis	DOD	47
2	54	F	Back	Single	Ia	—	NED	291
3	91	M	Neck	Single	II	Skin rec; systemic dis	DOD	5
4	84	F	Right leg	Multiple	Ib	Skin rec; systemic dis	DOD	13
5	65	F	Right leg	Single	Ib	—	NED	132
6	84	F	Left cheek	Single	Ib	N+; systemic dis	DOD	11
7	83	F	Forehead	Single	Ia	Skin rec; systemic dis	DOD	4
8	84	M	Right leg	Single	Ib	Skin rec; skin met	DOD	16
9	91	F	Right cheek	Single	II	Systemic dis	DOD	22
10	85	F	Lower eyelid	Single	Ib	—	DOC	76
11	81	M	Right leg	Multiple	Ib	—	NED	83
12	68	M	Forehead	Multiple	Ib	Skin rec; N+; systemic dis	DOD	10
13	81	M	Right leg	Single	Ia	—	NED	55
14	57	F	Left thigh	Multiple	III	—	DOD	4
15	85	F	Right thigh	Single	Ib	Systemic dis	DOD	24
16	67	F	Right thigh	Single	Ia	—	NED	42
17	80	F	Right cheek	Single	Ia	—	NED	37
18	78	F	Left leg	Single	Ib	Skin rec	AWD	42
19	89	F	Left knee	Single	Ib	Skin rec	DOC	9
20	73	F	Left cheek	Single	Ia	Skin rec	AWD	16
21	78	F	Left cheek	Single	Ia	—	NED	9
22	89	M	Forehead	Single	Ia	—	NED	10
23	69	F	Left arm	Multiple	Ib	—	NED	14

Abbreviations: N, lymph node metastasis; skin rec, local recurrence in the skin; skin met, metastasis in the skin or subcutaneous tissue beyond the regional lymph nodes; systemic dis, systemic dissemination; NED, no evidence of disease; AWD, alive with disease; DOD, dead of disease; DOC, dead of other causes.

diffuse patterns was seen. In terms of mitotic activity, 6 cases showed 0 to 5 mitoses per high-power field, 10 cases displayed 6 to 10 mitoses, and 7 cases showed ≥10 mitoses. As expected, the immunohistochemical profile showed diffuse positivity for cytokeratins AE1/AE3 and neuron-specific enolase in all cases. CK20 positivity (paranuclear dot pattern) was detected in 21 (91.3%) cases. Chromogranin A was positive in 18 (78.2%) cases.

Distribution of MMP and TIMP Expression by Immunohistochemistry

In the 23 PNC specimens, tumoral immunoreactivity for MMPs was quite variable and heterogeneous within cases (Fig 1). Immunoreactivity was present in the cytoplasm of neoplastic cells. Although positive staining for some MMPs was noted in scattered fibroblasts, vascular endothelial cells, and inflammatory cells, such expression was not specifically evaluated and quantified. Stromal staining was poorly defined and gave the impression of being present predominantly in the extracellular matrix. MMP expression was also seen, at varying frequencies and intensities, within the epidermis and adnexal structures, with a particularly intense and constant immunoreactivity observed in eccrine glands. High (>20%) MMP-1 expression was found in 13 (56.5%) cases. MMP-2 was evidenced in 12 (52.1%) cases, 8 (34.7%) of which showed moderate or diffuse distribution of the immunostaining in neoplastic cells (high expression). MMP-3 was detected in 11 cases (47.8%), with high expression in 9 (39.1%) cases. MMP-9 expression was observed in 3 (13%) cases and MMP-14 expression in 11 (47.8%) cases, all with a moderate to diffuse staining. No immunoreactivity for MMP-11 and MMP-13 was found. Tumoral expression of TIMP-1 was found in 8 (34.7%) cases, with high expression in 3 cases, whereas high tumoral TIMP-3 expression was detected in 21 (91.3%) cases (Fig 2). TIMP-2 expression was observed neither in neoplastic cells nor in stromal cells. Conversely, stromal cell expression of TIMP-1 and TIMP-3 was found in >90% of cases. Table 4 summarizes MMP and TIMP expression for each case. Statistical analysis failed to identify any statistically significant correlation between MMP/TIMP expression and clinical parameters.

Prognostic Factor Analysis

On univariate analysis, the only significant clinical factor that adversely affected survival was status greater than stage I ($P = 0.01$). High expression of MMP-1 ($P = 0.04$) and MMP-3 ($P = 0.01$) were also significant negative prognostic factors (Table 5). Conversely, none of the other MMPs and TIMPs analyzed in this study demonstrated prognostic significance. Patients whose tumors showed high MMP-1 expression had a significantly shorter overall survival time than those with tumors with low expression ($P = 0.02$, log-rank test; Fig 3). Similarly, patients whose tumors showed high MMP-3 expression had a significantly shorter overall survival time than those with tumors with low expression ($P = 0.006$, log-rank test; Fig 4).

Clinical and immunohistochemistry parameters

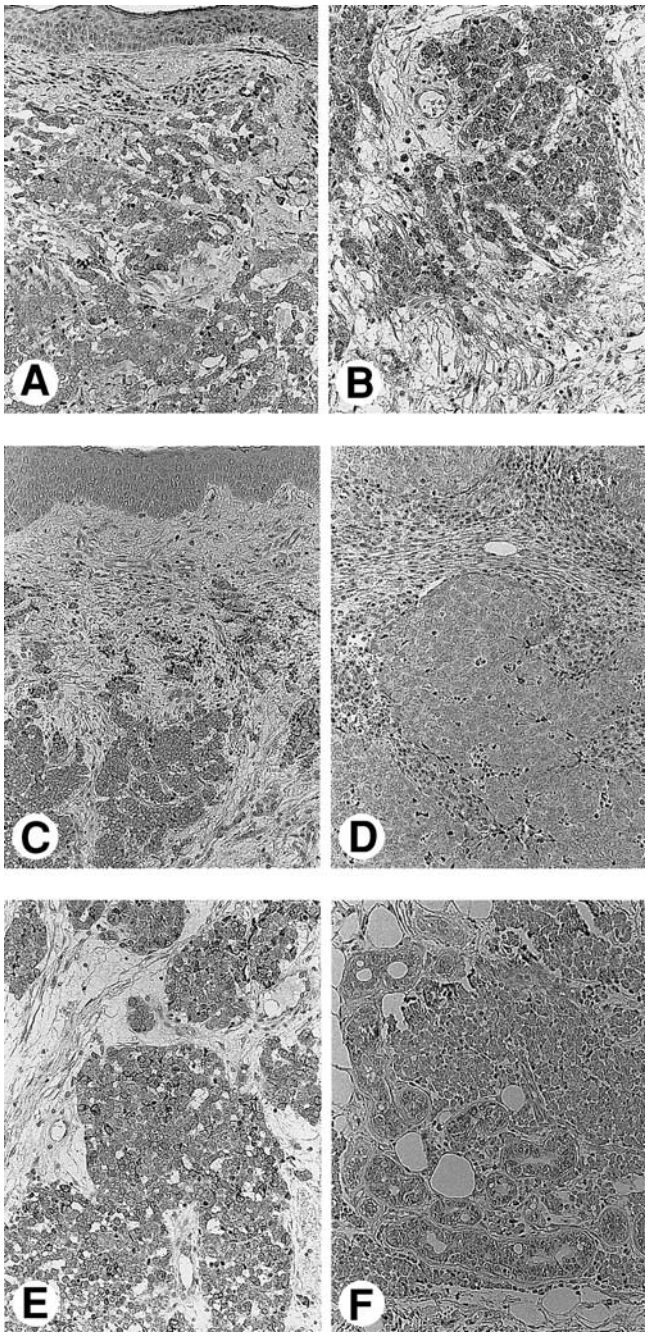

FIGURE 1. Representative immunohistochemistry of MMPs in PNCs of the skin. (A) MMP-1 immunoreactivity is present in the cytoplasm of neoplastic cells, as well as in the deeper epidermal layers. (B) Positive staining for MMP-2 is demonstrated in neoplastic cells and fibroblasts, as well as in endothelial cells around the tumor. (C) Diffuse MMP-3 expression in tumor cells, stromal cells, and the overlying epidermis is present. (D) Neoplastic cells do not express MMP-13, whereas scattered inflammatory cells and stromal cells around the tumor are positive. (E) Immunostaining for MMP-14. (F) Neoplastic cells do not express MMP-9, whereas eccrine glands are strongly positive.

were also assessed for their prognostic value on survival by multivariate analysis. Only stage ($P = 0.02$; hazard ratio, 4.4; 95% confidence interval, 1.2 to 15.8) persisted as a significant independent prognostic factor.

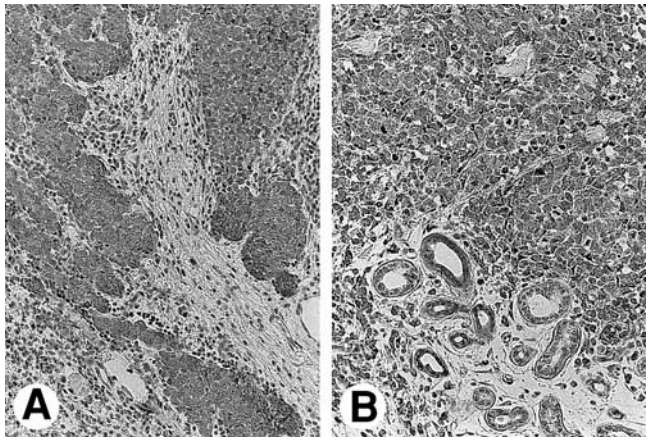

FIGURE 2. Representative immunohistochemistry of TIMP-1 (A) and TIMP-3 (B) in PNCs of the skin. Note that TIMP-1 expression is observed predominately within neoplastic cells, whereas TIMP-3 immunostaining is located within tumor cells, scattered stromal cells, and the epithelium of eccrine glands.

DISCUSSION

The present study is the first report on the immunohistochemical expression of MMPs and TIMPs in PNC of the skin. We focused our evaluation on the expression of these proteins and their inhibitors by tumor cells, and we demonstrated that immunoreactivity for MMPs and TIMPs was quite variable and heterogeneous. Among the different MMPs evaluated, MMP-1, MMP-2, MMP-3, and MMP-14 were expressed in a high proportion of cases, with the frequency of

TABLE 4. MMPs and TIMPs Expression by Neoplastic Cells in 23 PNCs of the Skin

Case	MMP-1	MMP-2	MMP-3	MMP-9	MMP-14	TIMP-1	TIMP-3
1	−	−	−	−	−	+	−
2	−	+	−	+	−	+	−
3	+++	−	+	−	+++	−	+++
4	+++	+++	+++	−	−	−	+++
5	+++	+	++	++	+++	++	+++
6	+++	−	+++	−	+++	−	+++
7	+++	−	+++	++	+++	−	+++
8	+++	+++	+++	−	++	−	+++
9	+++	+	++	−	++	+	+++
10	−	−	−	−	−	−	+++
11	−	+++	−	−	+++	++	+++
12	+++	−	+	−	−	−	+++
13	+++	−	−	−	−	−	+++
14	−	+	++	−	+++	++	+++
15	+++	++	++	−	+++	−	+++
16	−	+++	−	−	−	+	+++
17	+	−	−	−	−	−	++
18	−	+++	−	−	−	−	+++
19	++	+++	−	−	−	+	+++
20	++	+++	−	−	+++	−	+++
21	+++	−	++	−	+++	−	+++
22	−	−	−	−	−	−	++
23	−	−	−	−	−	−	+++

−, Negative staining; +, focal staining when positive tumor cells accounted for <20% of the total number; ++, moderate staining when 21% to 50% of tumor cells were positive; and +++, diffuse staining when >50% tumor cells were positive.

TABLE 5. Univariate Analysis (Cox Proportional Hazards Method) Assessing the Influence of Clinical and IHC Variables on Survival

Variable	Cases (%)	Hazard Ratio	95% Confidence Interval	*P* Value
Age				0.78
≤65 years	4 (17.4%)	1.0		
>65 years	19 (82.6%)	1.2	0.2-6.1	
Sex				0.57
Male	7 (30.4%)	1.0		
Female	16 (69.6%)	0.6	0.1-2.4	
Site				0.22
Head/neck	10 (43.5%)	1.0		
Other sites	13 (56.5%)	0.4	0.1-1.6	
Number of lesions				0.25
Single	17 (73.9%)	1.0		
Multiple	6 (26.1%)	2.1	0.5-7.5	
Stage				0.01
I	20 (87.0%)	1.0		
>I	3 (13.0%)	6.3	1.4-27.1	
MMP-1				0.04
Low expression	10 (43.5%)	1.0		
High expression	13 (56.5%)	4.9	1.0-23.5	
MMP-2				0.61
Low expression	15 (65.2%)	1.0		
High expression	8 (34.8%)	0.7	0.1-2.7	
MMP-3				0.01
Low expression	14 (60.9%)	1.0		
High expression	9 (39.1%)	5.4	1.3-21.6	
MMP-9				0.55
Low expression	22 (95.7%)	1.0		
High expression	1 (4.3%)	0.04	0.001-1438.0	
MMP-14				0.07
Low expression	12 (52.2%)	1.0		
High expression	11 (47.8%)	3.4	0.8-13.5	
TIMP-1				0.60
Low expression	20 (87.0%)	1.0		
High expression	3 (13.0%)	0.5	0.07-4.6	
TIMP-3				0.65
Low expression	2 (8.7%)	1.0		
High expression	21 (91.3%)	1.6	0.1-13.2	

NOTE. Low expression, ≤20% of positive cells; high expression, >20% of positive cells.

expression ranging from 47.8% to 56.5%. Expression of TIMP-1 and TIMP-3 was found in neoplastic cells in 34.7% and 91.3% of cases, respectively. In stromal cells, staining for both TIMP-1 and TIMP-3 was seen in more than 90% of PNC sections. Our results support the hypothesis that certain members of the MMP family may play a crucial role in PNC development and progression.

A major aim of this study was to assess the prognostic importance of MMP and TIMP expression on survival, while accounting for generally accepted significant clinical factors. In this regard, univariate analysis indicated that stage greater than I ($P = 0.01$) and high expression of MMP-1 ($P = 0.04$) and MMP-3 ($P = 0.01$) resulted in statistically significant factors that adversely affected survival, although multivariate analysis indicted that only stage was an independent prognostic indicator. Thus our results suggest that MMP-1 and MMP-3 may contribute to the aggressive behavior of and influence the invasive and metastatic potential of PNCs by allowing breakdown or proteolytic degradation of components of basement membrane and extracellular matrix. In particular, MMP-1 (also known as interstitial collagenase, mainly produced by fibroblasts and macrophages[28-29]) preferentially cleaves fibrillar collagen. In line with our observations in PNCs, MMP-1 expression has been associated with an invasive process in the stromal tissues of breast, esophageal, gastric, colorectal, pancreatic, and lung cancers.[30-37] Furthermore, its expression has been significantly associated to shortened survival in esophageal and colorectal carcinoma.[33,35] MMP-3 (also known as stromelysin-I) degrades a wide spectrum of extracellular matrix components, including laminin, fibronectin, proteoglycans, gelatins, and nonhelical regions of type IV collagens.[38] In line with our observations, MMP-3 overexpression has been correlated with poor outcome in patients with esophageal and lung cancer.[21,39]

The MMP/TIMP profile has been evaluated in other types of neuroendocrine cells and tumors, including thyroid C cells and medullary thyroid carcinomas,[40] normal pancreatic islet cells and islet cell tumors,[41] anterior pituitary cells and pituitary adenomas,[41] and carcinoids and small-cell lung cancers.[21,42] Interestingly, normal thyroid C cells were found strongly positive for MMP-2, MMP-9, TIMP-1, and TIMP-2, whereas medullary thyroid carcinomas were relatively more weakly stained.[40] Similarly, pancreatic islet cells and islet cell tumors were found to be positive for more MMP family members (MMP-1, MMP-2, MMP-3, and MMP-9) and TIMPs (TIMP-1 and TIMP-2), although in general islet cell tumors were weakly stained in comparison with normal islets in the adjacent pancreatic tissue.[41] On the basis of these observations, it has been suggested that MMPs/TIMPs can be considered possible markers of neuroendocrine cells and that MMP activity exists in endocrine tumors, although the MMP/TIMP balance may not be the major determinant in endocrine tumor invasion and metastasis.[40] At variance with this hypothesis, however, Michael et al[21] demonstrated that in small-cell neuroendorine carcinomas of the lung (which share morphologic similarities with PNCs), MMPs, and TIMPs were widely expressed and that increased tumoral expression of MMP-3, MMP-11, and MMP-14 were independent negative prognostic factors for survival, supporting their role in small-cell cancer progression.[21]

To better clarify the significance of the MMP/TIMP profile in the development of PNCs of the skin, it would be appropriate to evaluate their expression in the normal counterpart of PNCs. However, the histogenesis of PNC is controversial. Originally thought to be of eccrine origin,[1] PNC was subsequently believed to originate from Merkel cells, the normal neuroendocrine cell population of the skin, on the basis of the ultrastructural recognition of neuroendocrine granules within the cytoplasm of tumor cells.[2] A more recent hypothesis is that pure PNCs and mixed neuroendocrine-epithelial cutaneous carcinomas are of epithelial (epidermal or adnexal) origin, having undergone complete or partial neuroendocrine differentiation.[43] In

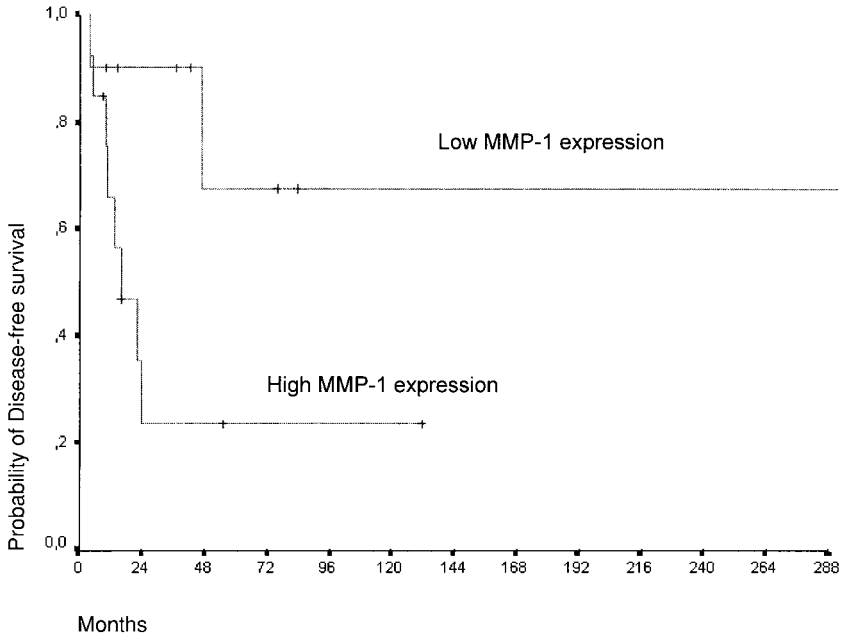

FIGURE 3. Probability of overall survival by MMP-1 expression (low expression versus high expression).

this view, it is important to emphasize that although MMP expression was seen at varying frequencies and intensities within the epidermis and adnexal structures, a particularly strong and constant immunoreactivity was observed in normal eccrine glands.

Among the different techniques that can be used to investigate the MMP/TIMP profile, immunohistochemistry has the advantage of allowing direct correlation with morphology. Furthermore, in immunohistochemical studies mRNA may not be translated, whereas protein expression is functionally more relevant. Another practical advantage is that immunohistochemistry can be performed on paraffin-embedded specimens, and thus it is practical for routine assessment of MMPs and TIMPs in diagnostic practice. However, it has been suggested that immunohistochemistry cannot differentiate between latent MMPs and activated forms.[12] Other techniques, including zymography and quenched fluorescent substrate hydrolysis, separate latent and active forms of MMPs, although they do not allow correlation with morphology.[37]

It is fair to comment that interpreting the results of

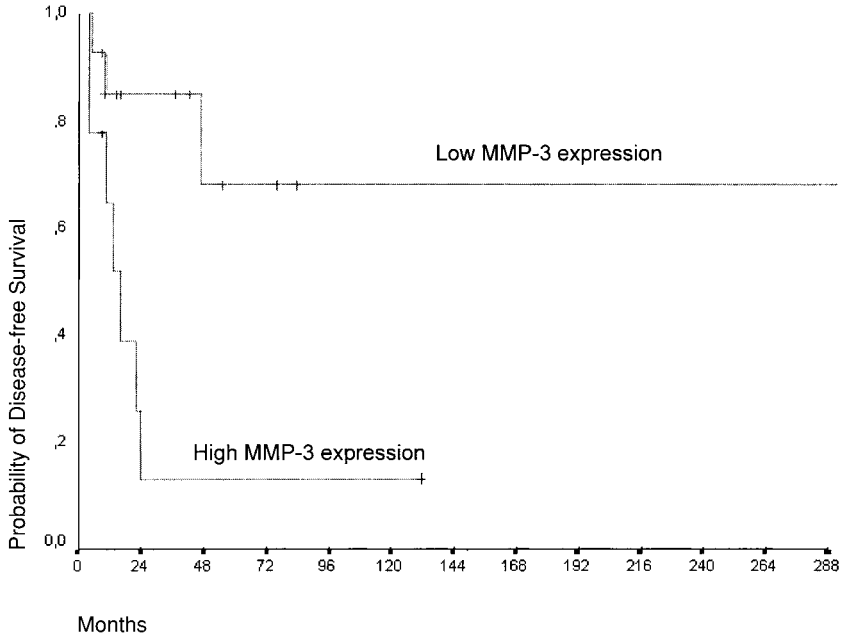

FIGURE 4. Probability of overall survival by MMP-3 expression (low expression versus high expression).

MMP and TIMP immunohistochemical expression in human tumors is rather complicated. Indeed, tissue localization of specific MMPs and TIMPs in and around the tumor can be heterogeneous, with variable expression within the tumor and adjacent stromal cells. It is not yet clear whether the overexpression of a particular enzyme or inhibitor reflects its functional role in the malignant process or rather is a sign of the host response to the tumor itself.[11] In our study, immunolocalization of MMPs and TIMPs was found largely in tumor cells, as well as in fibroblasts and endotelial cells in the adjacent stroma. Our study confirms previous findings and provides further evidence that tumor cells and stromal cells are the major sources of various members of MMP and TIMP families in tumor tissues. Indeed, it is conceivable that dynamic host–tumor interactions modulate MMP/TIMP activity in the progression of human tumors, including PNCs.

In conclusion, the present study has for the first time comprehensively evaluated by immunohistochemistry the MMP/TIMP profile in specimens of human PNC. By univariate analysis, high MMP-1 and MMP-3 expression was shown to be a negative prognostic factor for survival, suggesting that MMP-1 and MMP-3 may contribute to the aggressive behavior of and influence the invasive and metastatic potential of PNCs. There is no doubt that the pathophysiologic roles of specific MMPs and TIMPs in the biology of PNC requires further study; however, if the current results are confirmed in independent datasets, then it is conceivable that future attempts to specifically block MMP-1 and MMP-3 activity may provide a novel means to inhibit invasiveness and distant spread in selected patients with PNC.

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