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PLASMA METALLOPROTEINASE ACTIVITY IS ENHANCED IN THE EUGLOBULIN FRACTION OF BREAST AND LUNG CANCER PATIENTS

Eduardo FARIAS[1], Stella RANUNCOLO[1], Carlos CRESTA[2], Sergio SPECTERMAN[3], Eduardo ARMANASCO[2], Mirta VARELA[3], José LASTIRI[3], María Guadalupe PALLOTTA[3], Elisa BAL DE KIER JOFFE[1] and Lydia PURICELLI[1]*

[1]*Research Area, "Angel H. Roffo," Buenos Aires, Argentina*
[2]*Breast Department of the Institute of Oncology, "Angel H. Roffo," Buenos Aires, Argentina*
[3]*Italian Hospital, Buenos Aires, Argentina*

Matrix metalloproteinases (MMP) have been implicated in tumor invasion and metastasis. We verified, by gelatin zymography, MMP activity in the euglobulin plasma fraction of 82 healthy controls, 66 patients with benign diseases and 149 patients with breast, lung, colon or brain cancer. The euglobulin fractions assayed showed 4 gelatinolytic bands of 62, 92, 120 and 200 kDa. The median (Md) value for 92 kDa-MMP activity was significantly increased in breast (Md 1.34 arbitrary units [AU]/ml plasma, range 0.0–7.2) and lung cancer patients (Md 1.43 AU/ml, range 0.0–3.6) compared with the controls (Md 0.48 AU/ml, range 0.0–1.8). Patients with colon cancer or gliomas presented values of MMP-9 similar to those of the healthy population. Multivariate analysis indicated that plasma MMP-9 activity was not predicted by the known clinicopathological parameters such as age, stage, tumor size, number of positive lymph nodes, histologic grade, histologic type, nuclear grade or mitotic index. Lung cancer patients also presented high values of MMP-9 (Md 1.43, range 0.0–3.6 [n = 26]), without association with tumor stage or histologic type. The levels of 92 kDa-MMP activity in the plasma euglobulin fraction could be a potentially useful tumor marker in breast and lung cancer. *Int. J. Cancer (Pred. Oncol.)* 89: 389–394, 2000.
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Many steps during invasion and metastasis require specific interactions between tumor cells and the extracellular matrix (ECM; Price *et al.*, 1997). Cellular invasiveness is also essential in some normal or other pathological conditions, such as embryonic development, inflammation, wound healing and rheumatoid arthritis (Price *et al.*, 1997).

The invasive phenotype depends on the ability of tumor cells to attach to the ECM, degrade matrix components and finally migrate through this partially degraded matrix. The repetitive cycling of these 3 processes enables tumor cells to invade host tissues (Nicolson, 1988). It is well known that tumor cells produce higher amounts of proteolytic enzymes than normal counterparts. A positive correlation between invasiveness properties and protease levels has been documented for all classes of proteinases both in animal tumor models and human tumors (MacDougall and Matrisian, 1995; DeClerck *et al.*, 1997; Boyer and Tannock, 1993).

The metalloproteinase family (MMPs) includes more than 20 members. Although MMPs have overlapping substrate specificities, they are divided in 5 groups with respect to their preferential degradation of the different matrix substrates: gelatinases, matrilysin, interstitial collagenases, stromelysins and membrane MMPs. The criteria used to define members of the MMP family include zinc metal-atom dependency, secretion as a zymogen, *in vitro* activation of the proenzyme by acidic treatment or organomercurial reagents, autoproteolytic removal of the N-terminus following activation and inhibition by tissue inhibitors of metalloproteinases (TIMPs) or chemical inhibitors like calcium chelators (EDTA; Gomez *et al.*, 1997; Nagase, 1997). Several MMPs, including type I collagenase (MMP-1), gelatinases (MMP-2 and MMP-9) and stromelysins (MMP-3 and MMP-10), have been implicated in cancer cell invasion and metastasis (Duffy and McCarthy, 1998).

MMP-2 and MMP-9 are normal components of human plasma released by different cells, like neutrophils and macrophages or other inflammatory cells, that need to digest the ECM to access organ parenchymas. Some studies have evaluated the diagnostic and/or prognostic utility of measuring these enzymes in plasma, serum or other body fluids from cancer patients (Zucker *et al.*, 1993, 1995; Garbisa *et al.*, 1992; Garzetti *et al.*, 1996; Moses *et al.*, 1998). Almost all these studies quantified the antigen concentration and only few works studied the activity of MMPs. The data are consistent with the conclusions that MMPs are important mediators of cancer progression and that their levels are frequently elevated in body fluids of cancer patients.

In our study, we measured the activity of MMP in the euglobulin plasma fraction of 82 healthy donors and 215 patients with cancer or benign diseases in the breast, lung, colon and brain. It was demonstrated that the activity of plasma 92 kDa-MMP was significantly increased in patients with breast and lung cancer. The assay described herein could be useful to evaluate whether plasma MMP activity behaves as a useful tumor marker in these pathologies.

MATERIAL AND METHODS

Patients and control subjects

Plasma MMP activity was measured in patients with breast, lung, colon and brain cancer, in healthy subjects and in patients with benign diseases. Subjects did not receive any treatment before blood collection. The blood of cancer patients was obtained between 2–24 hr before surgery or before the beginning of any treatment. Information about patients was obtained by reviewing their medical charts.

The control group consisted of 82 healthy volunteers. The median (Md) age of this group was 41 years (range 19–83 years).

The breast disease group included 88 patients (Md age: 58, range 28–89 years) with breast tumors of different clinical stages and histopathology (56 ductal carcinomas,18 lobular carcinomas and 14 special tumors) and 49 women with benign breast disease (adenosis, typical hyperplasias, adenomas, fibroadenomas; Md age: 47, range 18–74 years).

The lung cancer group consisted of 26 patients (Md age: 60.5; range 41–80 years). Tumor histopathology showed 6 oat cell

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*Correspondence to: Lydia Puricelli, Area Investigación, Instituto de Oncología "Angel H. Roffo," Av. San Martín 5481, 1417 Buenos Aires, Argentina. Fax: (054-11) 4580-2811. E-mail: lydia@invrof.fmed.uba.ar

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carcinomas and 20 non–small-cell carcinomas (8 epidermoid cancers, 9 adenocarcinomas and 3 un-differentiated tumors).

The levels of plasma MMP activity were also studied in 21 patients with colon adenocarcinoma, 2 colonic villous adenocarcinomas, 1 rectal adenocarcinoma (n = 24) and 5 patients with colonic adenomas. The Md age of the colon disease group was 62 years (range 56–73).

The brain tumor group consisted of 12 patients with benign brain tumors (Md age: 54, range 27–60 years), 4 with anaplastic astrocytoma and 7 with glioblastoma. The Md age of the brain tumor group was 59 (range 47–62 years).

Our study was approved by the Ethical Committees of the Institute of Oncology, "Angel H. Roffo" and the Italian Hospital of Buenos Aires. All patients had knowledge about their participation in this protocol.

Preparation of euglobulins

Plasma was obtained from heparinized blood, aliquoted at 100 μl and frozen at -80°C until processing. There was no significant loss of activity after a year at this temperature.

Euglobulins were prepared by mixing 0.1 ml plasma with 0.9 ml cold deionized water and acidified to pH 5.5 with 40 ml 1% (v/v) acetic acid. This mixture was incubated for 30 min at 4°C and centrifuged 10 min at 2,500 rpm (Alonso *et al.*, 1993). Euglobulins were then dissolved in 1 ml PBS, pH 7.4, so that the final dilution was 1:10.

Zymography and densitometric analysis

Freshly prepared euglobulins were analyzed on 9% SDS-PAGE in the absence of reducing agents (Heussen and Dowle, 1980). Gels were co-polymerized with heat-denatured type I collagen (gelatin, GIBCO BRL, Gaithersburg, MD) as enzyme substrate (Pittman, 1985). Ten microliters of the final solution of euglobulin preparation was loaded onto each of the 15 lanes of the gelatin-polyacrylamide gels. The electrophoresis was performed with a Mini-Protean II (Bio-Rad, Richmond, CA) at 100 V constant voltage during approximately 120 min.

After electrophoresis, gels were washed twice in 2.5% Triton X-100 in aqueous solution for 30 min each time to remove SDS and incubated in 0.15 M NaCl, 10 mM $CaCl_2$ and 50 mM Tris-HCl buffer, pH 7.4, at 37°C for 20 hr. Previous studies, employing times of incubation between 6–72 hr demonstrated linearity in the MMP-9 activity up to 48 hr. When different aliquots of euglobulin preparation were analyzed by gelatin zymography, a linear correlation was observed between plasma volume (up to 20 μl) and MMP-9 activity.

The zymograms were stained with Coomassie Brilliant Blue R-250 and de-stained with 30% methanol and 10% acetic. Bands of gelatin degradation can be seen as transparent areas against a blue background. Molecular weights were determined using standard pre-stained molecular weight markers (Bio-Rad). All reagents for electrophoresis were purchased from Sigma (St. Louis, MO).

Gelatinolytic bands were measured with an image analyzer (Bio-Rad densitometer, model GS-670) and referenced to a standard curve of bacterial collagenase (Sigma) at a range of concentrations from 0.025 to 0.25 U/ml and corrected by the plasma dilution. All data were expressed as arbitrary units (AU) per milliliter of plasma.

As a control, a purified MMP-9 isolated from human blood (Boehringer-Mannheim, Indianapolis, IN) was also electrophoresed and analyzed by zymography in some assays.

Control of specific metal-dependent protease activity was carried out using an incubation buffer containing 25 mM EDTA. The proteinase inhibitors aprotinin and phenylmethyl-sulphonylfluoride (PMSF) were employed to test the presence of serine protease activity.

The intra-assay coefficient of variation of the samples (tested in duplicate) was about 5% and the inter-assay coefficient of variation was about 10%.

An internal standard euglobulin fraction prepared from a pool of plasma from 20 healthy individuals was included on each gel for correction of inter-gel variation.

MMP identification by Western blot analysis

Western blot analysis for MMP-9 and MMP-2 was carried out using an SDS-PAGE that was performed as described by Laemmli (1970), using 10% separating and 4% stacking gels. It was employed as a sample buffer containing 25 mM Tris, 10% v/v glycerol, 12.5 mg/ml SDS, 0.125 mg/ml bromophenol blue and 1.25% 2β-mercaptoethanol (pH 6.8). After electrophoresis, gels were transferred to nitrocellulose membranes using an aquose electrical transfer (Bio-Rad) for 1 hr. To detect MMP-9 and MMP-2 expression, we used anti-human MMP-9 and MMP-2 monoclonal antibodies (MAb) from Calbiochem (La Jolla, CA; Oncogene Research Product). An anti-mouse biotinylated IgG (GIBCO) was used as the second antibody, the detection system was streptavidin-phosphatase alkaline conjugate (Vector, Burlingame, CA) and the BCIP/NBT solution was the substrate (Sigma).

Statistical analysis

Differences in the level of MMP-9 among the groups were compared using the Kruskall-Wallis and Mann-Whitney tests, appropriate Md tests for even skewed data. A receiver operator characteristic (ROC) curve (Fletcher *et al.*, 1988) was developed to determine the optimal reference value to differentiate patients from controls. Sensitivity, specificity, positive predictive value (PV+), negative predictive value (PV-) and positive likelihood ratio (LR+) were calculated employing the optimal cutoff point.

The chi-square test was used to assess statistical significance in bivariate comparisons. A difference of $p < 0.05$ was considered to be significant. We used unconditional logistic regression to summarize the joint effects of prognostic factors (such as age, stage, tumor size, number of positive lymph nodes [LN], histologic grade, histologic type, nuclear grade or mitotic index) on MMP positivity. SPSSPC+ statistical package was used for the aforementioned analyses.

RESULTS

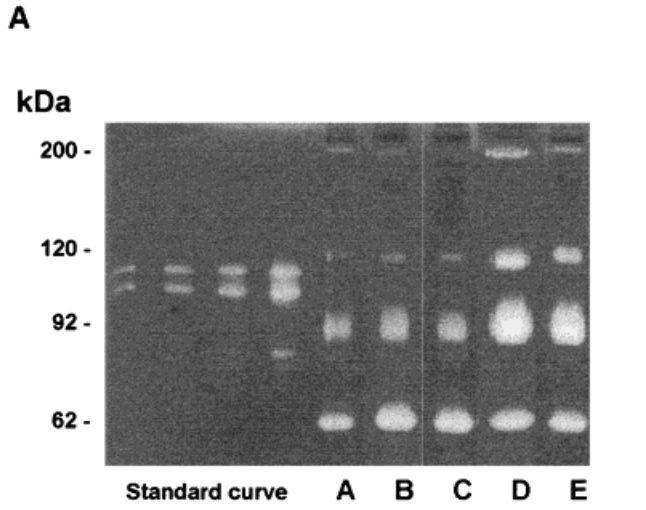
MMP activity in plasma euglobulin fraction

MMP activity was poorly detected by zymography in whole plasma samples either from healthy donors or from patients with benign diseases or malignant tumors (data not shown). The acetic precipitation used to prepare the euglobulin plasma fraction was highly effective to enhance MMP detection, allowing us to reveal several bands of circulating metal-dependent protease activity.

As shown in Figure 1*A*, all samples analyzed showed the gelatinolytic bands of 62 and 92 kDa. In many samples, bands of 120 and 200 kDa were also detected. These activities were completely suppressed by EDTA, but not by aprotinin or PMSF, confirming that the enzymes were metalloproteinases. The commercial MMP-9, isolated from human blood, also showed 3 bands of 92,120 and 200 kDa in the zymography (data not shown). Besides, Western blot analysis (Fig. 1*B*) using specific anti-human antibodies determined that the band of 62 kDa corresponded to MMP-2, whereas the 92 kDa band was MMP-9.

Plasma MMP activity in cancer patients

Densitometric analysis of the MMP-2 gelatinolytic band (Fig. 1*A*) showed that its activity did not differ between controls (n = 29) and cancer patients (n = 33): Md (range) 0.34 (0.03–0.84) and 0.54 (0.15–0.82), respectively. On the other side, zymograms revealed that 92, 120 and 200 kDa gelatinolytic bands were larger in the euglobulin fraction of cancer patients compared with healthy controls or individuals with benign disease (Fig. 1*A*). As 120 and 200 kDa bands were not constantly found in all samples, the



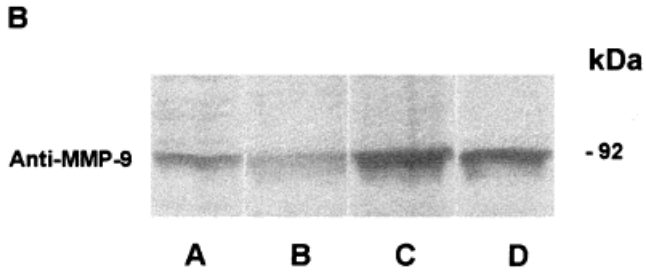


FIGURE 1 – (*a*) Gelatin zymogram of plasma euglobulin fraction. Internal standard preparation (lane A), healthy control subject (lane B), patient with benign breast tumor (lane C), breast cancer patient (lane D) and lung cancer patient (lane E). The first 4 lanes correspond to the standard curve of bacterial collagenase. (*b*) Western blot for MMP-9. Control subject (lane A), patients with benign breast disease (lane B), breast cancer (lane C) and lung cancer (lane D).

TABLE I – CIRCULATING 92 kDa-MMP ACTIVITY IN CANCER PATIENTS[1]

Groups		N	MMP-9 (AU/ml) Md (range)
Controls		82	0.48 (0.0–1.8)
Breast	B	49	0.60 (0.0–3.1)
	M	88	1.34 (0.0–7.2)[2]
Lung	M	26	1.43 (0.0–3.6)[3]
Colon	B	5	0.48 (0.0–2.4)
	M	24	0.31 (0.0–2.3)
Brain	B	12	0.19 (0.0–2.4)
	M	11	0.20 (0.0–2.2)

[1]Individual plasma euglobulin fractions were assayed for MMP activity by quantitative zymography as described in Material and Methods. B, patients with benign disease; M, patients with malignant tumors.–[2]$p < 0.05$ *vs.* the control and benign breast disease groups.–[3]$p < 0.05$ *vs.* the control group (Mann-Whitney test).

following quantitative analysis was only performed with the MMP of 92 kDa.

The control group showed an Md of 0.48 AU/ml (range 0.0–1.8) of 92 kDa-MMP activity in the plasma euglobulin fraction (Table I). A significant increase of this activity was observed in the plasma of patients with breast and lung cancer. No differences

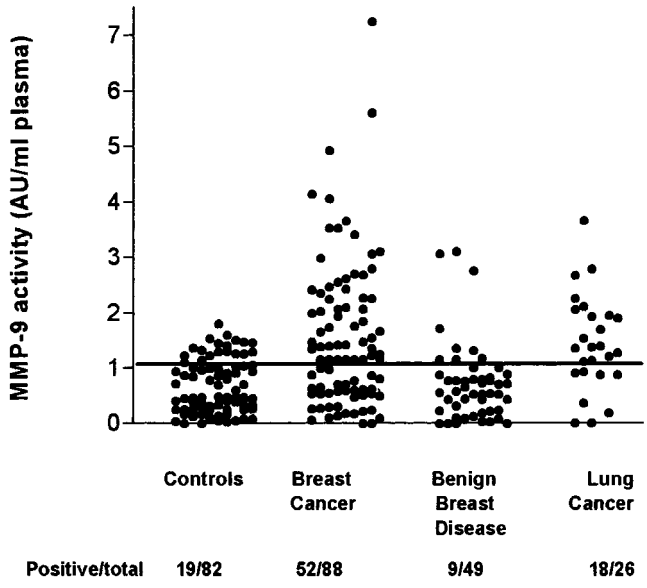


FIGURE 2 – 92 kDa-MMP activity in individual euglobulin fraction samples of cancer patients. The line indicates the optimal cutoff value (1.15 AU/ml plasma). A higher number of patients with elevated MMP-9 values was observed in the breast cancer group, compared with controls or patients with benign diseases (chi-square test, $p < 0.001$). Similar results were found in lung cancer patients with respect to the control population (chi-square test, $p < 0.001$).

were observed in the level of circulating 92 kDa-MMP activity in patients with malignant colon cancer and gliomas, with respect to controls or to patients with benign pathologies at the same localization (Table I). No correlation was observed between 92 kDa-MMP activity and age or sex (data not shown).

92 kDa-MMP activity in breast cancer patients

As mentioned, the breast cancer patient group exhibited significantly higher 92 kDa-MMP activity than that observed in the control or benign breast disease groups (Table I). Figure 2 shows the distribution of the 92 kDa-MMP activity in the individual euglobulin fraction of these subjects.

The reference value of 1.15 AU/ml plasma was close to the inflection point on the ROC curve (data not shown), thereby maximizing sensitivity and specificity. This point corresponded to the 75 percentile value in normal control subjects. Values above this concentration of 92 kDa-MMP were defined as elevated. On the basis of the optimal cutoff point, 59.1% (52 of 88) of patients with breast cancer had elevated values, whereas only 23.2% (19 of 82) of controls and 18.4% (9 of 49) of patients with benign breast disease had high values. The difference between the number of elevated values in the breast cancer group *vs.* the control or benign groups was highly significant.

The assay of 92 kDa-MMP activity in the studied breast cancer population exhibited a sensitivity of 59.1% and a specificity of 76.8%. The PV+ was 73.2%, the PV- was 63.6% and the LR+ was 2.6.

We analyzed whether already established variables were associated with plasma 92 kDa-MMP activity levels. No association was observed between MMP activity enhancement and the known clinicopathological prognosis predictors (Table II). Logistic regression analysis indicated that MMP positivity was not predicted by the prognostic values studied (data not shown).

Lung cancer patients

The lung cancer group also exhibited a significantly higher Md of 92 kDa-MMP activity with respect to the control population

TABLE II – CLINICOPATHOLOGICAL FEATURES OF THE BREAST CANCER POPULATION IN RELATION TO THE PLASMA 92 kDa-MMP ACTIVITY

Features	Positive/ negative	Percentage of positives	Chi-square	*p*
Age (years)				
<50	18/11	62.1		
51–60	14/10	58.3		
61–70	8/9	47.1		
>70	12/5	70.6	2.05	0.56
Stage				
I	7/5	58.3		
II	21/20	51.2		
III	18/8	69.2	2.12	0.35
Tumor size (cm)				
<1.0	2/2	50.0		
1.1–2.0	14/10	58.3		
2.1–3.0	13/9	59.1		
3.1–5.0	5/5	50.0		
>5.cm	12/8	60.0	0.40	0.98
Number of LN with metastasis				
0	18/14	56.3		
1–3	8/8	50.0		
4–10	9/5	64.3		
>10	5/1	83.3	2.26	0.52
Histologic grade				
I	11/3	78.6		
II	12/11	52.2		
III	12/6	66.7	2.73	0.26
Nuclear grade				
I	4/1	80.0		
II	21/18	53.8		
III	17/6	73.9	3.18	0.20
Mitotic index				
I	12/11	52.2		
II	8/12	40.0		
III	3/2	60.0	0.96	0.62
Histologic type				
Lobular	9/9	50.0		
Ductal	34/22	60.7		
Special	9/5	64.3	0.83	0.66

TABLE III – CLINICOPATHOLOGICAL FEATURES OF THE LUNG CANCER POPULATION IN RELATION TO THE LEVEL OF PLASMA 92 kDa-MMP ACTIVITY

Features	Positive/ negative	Percentage of positives	Chi-square	*p*
Age (years)				
<50	6/1	85.7		
51–60	4/2	66.7		
61–70	6/3	66.7		
>70	2/2	50.0	1.63	0.65
Stage				
II	1/0			
III	4/1	80.0		
IV	8/6	57.1	1.41	0.49
Histologic type				
Epidermoid	7/1	87.5		
Adenocarcinoma	4/5	44.4		
Undifferentiated	2/1	66.7		
Oat cell	5/1	83.3	4.42	0.22

(Table I). The cutoff point calculated for the control and lung cancer groups was identical to that obtained for breast cancer patients. On this basis, 69.2% (18 of 26) of patients exhibited elevated 92 kDa-MMP activity (Fig. 2).

The specificity of the test for detecting lung cancer was 77.1%, the sensitivity was 69.2%, the PV+ 48.6%, the PV- 90.0% and the LR+ 3.00. No association was found between levels of 92 kDa-MMP activity and age, tumor stage or histologic type (Table III).

DISCUSSION

In murine and human models, tumor metastatic potential correlates with the degradation of basement membrane type IV collagen and the levels of metalloproteinases able to degrade the ECM (MacDougall and Matrisian, 1995). Over-production of MMP by a tumor might result in increased levels of MMP activity in body fluids and its levels could have a potential utility as tumor marker.

Several authors have studied the expression of MMP in human tumor tissues employing several techniques (Sier *et al.*, 1996; Duffy *et al.*, 1995; Davies *et al.*, 1993*a*,*b*; Nomura *et al.*, 1995; Stearns and Wang, 1993; Urbansky *et al.*, 1992). Other studies have analyzed circulating MMPs, at the protein level, in cancer patients. In this way, high levels of MMP-9 were detected in plasma of patients with both breast and colorectal cancer (Zucker *et al.*, 1993) and increased MMP-2 was measured in the serum of patients with lung (Garbisa *et al.*, 1992) and ovarian (Garzetti *et al.*, 1996) tumors. Besides, MMP-9/TIMP complexes were also increased in the plasma of gastrointestinal and gynecologic cancer patients (Zucker *et al.*, 1995).

MMP gelatinolytic activity in serum or plasma was studied in several animal tumor models (Isidoro *et al.*, 1995; Nakajima *et al.*, 1993), whereas only a few works have studied the enzymatic activity of each circulating MMP in cancer patients. Hashimoto *et al.* (1998) reported elevated serum levels of type IV collagenolytic activity in hepatocellular cancer patients, but the substrate degradation assay employed did not distinguish between MMP-2 and MMP-9. In our study, we analyzed the activity of each circulating MMP after the precipitation of plasma proteins at pH 5.5 (regular euglobulin fraction) previous to electrophoresis in SDS-gels copolymerized with gelatin. In this way, 4 bands of gelatinolytic activity were detected. Other authors (Zucker *et al.*, 1994; Vartio *et al.*, 1989) obtained the same pattern of bands employing a more complex methodology, *e.g.*, Sepharose chromatography followed by zymography (Zucker *et al.*, 1994). When whole plasma or serum was employed, we could only detect slight MMP activity in comparison with euglobulins. (Garbisa et al., 1992) reported a high variability when plasma MMP levels were measured. The method developed in our laboratory was highly specific and reproducible.

Zucker *et al.* (1994) demonstrated that the vast majority of plasma gelatinases circulate as latent enzymes. We do not know for certain why the acetic precipitation of plasma proteins is highly effective to reveal the activity of MMPs. As reported by several authors (Kato *et al.*, 1992), both the acid treatment and the addition of SDS to the samples to run in a zymogram convert latent MMP to catalytically active forms, without proteolytic cleavage of the N-terminal inhibitory sequence. However, SDS-PAGE of whole plasma samples exhibited a very low ability to activate MMP. Perhaps, the acetic acid precipitation of the euglobulin may be increasing the activity through the concentration of the samples.

We showed that the size and intensity of MMP bands of 92, 120 and 200 kDa were markedly increased in the euglobulin fraction of cancer patients with respect to the control population. As the striking differences between controls and cancer patients were mainly observed with the band of 92 kDa, further analysis was performed on this MMP. The 92 kDa band corresponded to MMP-9, according to its molecular weight and Western blot analysis.

The activity of 92 kDa-MMP was significantly enhanced in the euglobulin fraction of breast and lung cancer patients compared with the healthy population or with patients with benign disease. This finding seems to be confined to some tumor pathologies because patients bearing colon and brain tumors presented values of plasma 92 kDa-MMP activity that were not significantly elevated compared with those observed in the control group. Surprisingly, the Md value of MMP-9 activity in both benign and malignant brain diseases was even lower than the Md of control individuals. This result could be probably attributed to the small size of the evaluated group, which should be increased to confirm

the observation. Employing a cutoff point of 1.15 AU/ml of plasma, about 59% of breast cancer patients and 69% of lung cancer patients exhibited high values, whereas only about 24% of healthy controls did. The sensitivity of the test to detect breast cancer was higher than that observed with the conventional markers, although we must take into account that our estimations may have a little bias for being obtained from the same data used to pick up the value of the cutoff point.

Our results suggest that plasma MMP activity is a good marker for detecting the presence of primary breast and lung tumors. Further studies are necessary to establish whether high MMP values could be associated with local recurrence or metastatic dissemination. In this sense, the dosage of MMPs, added to the currently available circulating tumor markers, such as CA15-3 or carcinoembryonic antigen (CEA), could improve the follow-up of breast cancer patients. Our preliminary results (unpublished data) with 60 breast cancer patients followed during 3 years suggest that the levels of plasma 92 kDa-MMP, measured every 3 months, may be useful to determine the effectiveness of a primary treatment.

No association was found between the main clinicopathological prognostic parameters and the levels of 92 kDa-MMP activity in the plasma of breast cancer patients, indicating that MMP-9 could be an independent tumor marker. We did not find that the quantities of the circulating enzyme were reflective of tumor burden, as currently available breast tumor markers do. Probably, high amounts of MMP-9 identify some other biological characteristics of the cancer cells such as the invasive behavior (Stearns *et al.*, 1993) or the presence of metastatic cells (Nakajima *et al.*, 1990). Variable results were obtained by other authors when MMP-9 expression in tumor biopsies of breast cancer and known prognosis markers were compared (Duffy *et al.*, 1995; Davies *et al.*, 1993*a*; Monteagudo *et al.*, 1990). We are now expanding our studies to evaluate whether plasma MMP-9 activity could be considered a prognosis tumor marker.

Plasma 92 kDa-MMP activity is also elevated in lung cancer patients. We did not find any association between levels of this enzyme and tumor stage or histologic type. Almost all these patients were classified as stage III or IV. To determine whether circulating MMP levels have a possible value as a tumor marker, it is necessary to complete our study including patients of lower stages. Zucker *et al.* (1993) reported no increase in the circulating 92 kDa-MMP, at protein level, in patients with this pathology.

Although other authors reported the finding of high levels of MMP-9 antigen in the plasma of patients with gastrointestinal tract cancer (Zucker *et al.*, 1993) and in colorectal biopsies (Monteagudo *et al.*, 1990), we found that colon cancer patients have similar values of 92 kDa-MMP activity than controls or patients bearing benign colon tumors. It would be interesting to compare both concentration and activity of plasma MMP-9 in the same patient sample to determine its role in this pathology.

The origin of MMP in the plasma of cancer patients is not known. It could derive directly from the tumor cells or from stromal fibroblasts or infiltrating cells, such as neutrophils, eosinophils or macrophages. There is strong evidence indicating that 92 kDa-MMP is not expressed by the tumor cells but by cells in the surrounding stroma (Davies *et al.*, 1993*a*). It is believed that, after local secretion, MMP can reach the blood stream. It has been demonstrated recently that MMPs are also found in urine samples and that their analysis could be useful to determine disease status in a variety of cancers, both within and outside the urinary tract (Moses *et al.*, 1998).

In conclusion, we developed a specific and reproducible test to quantify the activity of individual metalloproteinases in plasma. We believe that the dosage of 92 kDa-MMP (MMP-9) in the plasma of breast and lung cancer patients could be a useful tool for the oncologist in managing these patients. A high plasma level of this enzyme seems to be an independent marker not associated with known clinicopathological parameters. More studies are necessary to determine whether MMP activity levels are useful to prognosticate the evolution of the disease and/or the effectiveness of treatments.

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