Overexpression of matrix metalloproteinases and their inhibitors in mononuclear inflammatory cells in breast cancer correlates with metastasis-relapse

An immunohistochemical study was performed using tissue microarrays and specific antibodies against matrix metalloproteinase (MMP)-1, -2, -7, -9, -11, -13 and -14, tissular inhibitors of metalloproteinase (TIMP)-1, -2 and -3. More than 2600 determinations on cancer specimens from 131 patients with primary ductal invasive tumours of the breast were performed. To identify specific groups of tumours with distinct expression profiles the data were analysed by unsupervised hierarchical cluster analysis by each cellular type. We did not find well-defined cluster of cases for tumour cells or fibroblastic cells. However, for mononuclear inflammatory cells the dendogram shows a first-order division of the tumours into two distinct MMP/TIMP molecular profiles, designated group 1 (n = 89) and group 2 (n = 42). Matrix metalloproteinase-7, -9, -11, -13 and -14, and TIMP-1 and -2, were identified as showing significant high expression in group 2 compared with group 1. Multivariate analysis demonstrated that clustering for mononuclear inflammatory cells was the most potent independent factor associated with distant relapse-free survival (group 2: 5.6 (3.5–9.6), P < 0.001). We identify a phenotype of mononuclear inflammatory cells infiltrating tumours, which is associated with the development of distant metastasis. Therefore, this finding suggests that these host inflammatory cells could be a possible target for inhibition of metastasis.

Keywords: breast cancer; prognosis; MMP; TIMP; tumoural invasion; metastasis

Degradation of the stromal connective tissue and basement membrane components are key elements in tumour invasion and metastasis. Some components of the extracellular matrix, particularly the interstitial collagens, are very resistant to proteolytic attacks, being degraded only by matrix metalloproteinases (MMPs) (Nelson et al, 2000). Matrix metalloproteinases are also able to impact on tumour cell behaviour in vivo as a consequence of their ability to stimulate the tumoural growth, antiapoptotic factors, motility cellular or angiogenesis (Manes et al, 1999; Rifkin et al, 1999; Fingleton et al, 2001; Noe et al, 2001; Sternlicht and Werb, 2001; Egeblad and Werb, 2002; Turk et al, 2004).

Several MMPs, in particular the gelatinases MMP-2 (Jones et al, 1999; Duffy et al, 2000; Talvensaari-Mattila et al, 2001, 2003; Baker et al, 2002; Grieu et al, 2004; Li et al, 2004; Sivula et al, 2005) and MMP-9 (Chantrain et al, 2004; Li et al, 2004; Pellikainen et al, 2004), have been recently studied as prognostic factors in breast cancer, being associated with a poor outcome in various subsets of patients. Discordant data have also been published on the prognostic value of the above-mentioned MMPs (Jones et al, 1999; Hirvonen et al, 2003; Talvensaari-Mattila et al, 2003; Grieu et al, 2004; Sivula et al, 2005; Talvensaari-Mattila and Turpeenniemi-Hujanen, 2005). Nevertheless, it has been suggested that the coexpression of various MMPs and/or tissular inhibitors of metalloproteinases (TIMPs) might provide additional prognostic information in breast cancer. Accordingly, it has been reported that several other MMPs and TIMPs may be overexpressed and/or related to clinical outcome in breast cancer, such as MMP-11 (Duffy et al, 2000) MT1-MMP (MMP-14) (Jones et al, 1999; Mimori et al, 2001) MMP-13 (Nielsen et al, 2001), TIMP-1 (Ree et al, 1997; McCarthy et al, 1999; Nakopoulou et al, 2002a; Schroth et al, 2003, 2004) or TIMP-2 (Visscher et al, 1994; Ree et al, 1997; Remacle et al, 2000). In addition, there are at least two elements adding more complexity to the role of MMPs and TIMPs in cancer. On the one hand, it is now assumed that TIMPs are multifactorial proteins also involved in the induction of proliferation and the inhibition of apoptosis (Baker et al, 1999; Jiang et al, 2002) TIMP-3 (Guedez et al, 2001) and TIMP-1. On the other hand, we should consider that the cellular type (tumoural cell/stromal cell) expressing these factors might be of biological importance in breast cancer (see...
Decock et al., 2005, for review). Thus, for example, it has been demonstrated that positive stromal MMP-9 expression is related to HER-2 overexpression in oestrogen receptor-positive tumours and predicts a shorter relapse-free and overall survival, whereas MMP-9 expression in carcinomatous cells predicts a longer relapse-free survival (Pellikainen et al., 2004).

All these data suggest the importance of the consideration of new techniques that would allow evaluation of the expression of several MMPs and TIMPs by each cellular type of the tumoural scenario, to contribute to the understanding of the molecular complexity of breast cancer and to a more precise prognostic evaluation. Immunohistochemistry on tissue microarrays (TMAs) provide an efficient platform to observe protein expression in a large number of cases with a limited amount of reagents and with a short analysis time (Gulmann et al., 2003). Recently, we reported the clinical value of this technology to evaluate the expression of MMP-1, -2, -7, -9, -11, -13 and -14, and TIMP-1, -2 and 3, in breast cancer (Vizoso et al., 2007). In this study, we validated this method against the determination of the expression of these parameters in the whole-tissue sections of the tumours, and we also found significant associations between the expression of several MMPs and the occurrence of distant metastasis. Following with our investigations, the objectives of the present work were (i) to investigate the relationship between these several MMPs and TIMPs expressions by the cellular type (tumoural/stromal cells) and the clinicopathological and biological characteristics of the tumours; and (ii) to attempt the identification of the different phenotypes of tumour or stromal cells associated with the development of distant metastases.

MATERIALS AND METHODS

Patients' selection

This study comprised 131 women with a histologically confirmed diagnosis of early invasive breast cancer of ductal-type treated between 1990 and 2001, which were previously included in our preliminary study on the expression of MMPs and TIMPs in breast cancer (Vizoso et al., 2007). This study population include a significant number of patients with relapse in both node-negative and node-positive patient's subgroups (half of cases with distant metastasis during the follow-up period in each one of these subgroups) for securing the statistical power of the survival analysis. Data about treatment and following of the patients were described elsewhere (Vizoso et al., 2007). The study adhered to national regulations and was approved by our Institution's Ethics and Investigation Committee.

Tissue microarrays and immunohistochemistry

Routinely fixed (overnight in 10% buffered formalin), paraffin-embedded tumour samples stored in our pathology laboratory files were used in this study. Details about TMAs technique were described elsewhere (Vizoso et al., 2007). From our 131 tumour samples available, four tissue array blocks were prepared, each containing 33 tumour samples, as well as internal controls including four normal breast tissue samples from two healthy women that underwent reductive mammary surgery. Immunohistochemistry was carried out on sections of 5 μm of TMA fixed in 10% buffered formalin and embedded in paraffin using a TechMate TM50 autostainer (Dako, Glostrup, Denmark). Antibodies for MMPs and TIMPs were obtained from Neomarker (Lab Vision Corporation, Fremont, CA, USA). The dilution for each antibody was established based on negative and positive controls (1 out of 50 for MMP-2, -7 and -14, TIMP-2 and -3; 1 out of 100 for MMP-1, -9 and -13 and TIMP-1; and 1 out of 200 for MMP-11).

The immunohistochemistry technique was also described for us in a recent report (Vizoso et al., 2007).

Tissue microarray analysis

For each antibody preparation studied, the location of immunoreactivity in each cellular type was determined. In each case, immunoreactivities were classified into two categories in each cell type (tumour, fibroblastic and inflammatory mononuclear cells), depending upon the percentage of cells stained (negative: 0–10% positive cells; positive: >10% positive cells). We studied both cores that were carried out for each patient and averaged the results. If there was no tumour in a particular core, then the results of the other was given. Staining was studied by two pathologists (LOG and AMM) blinded to the clinical outcome of the patients.

Western blot analysis

Western blotting of metalloproteinases (MMP-1, -11, -13 and -14) from human breast cancer cytosolic extracts or from human placenta (used as positive control) was performed as follows: samples were subjected to 12% SDS–PAGE and transferred onto a nitrocellulose membrane for 1 h at room temperature. Nitrocellulose membrane was blocked with 2% non-fat dry milk in TBS (50 mM Tris–HCl, 100 mM NaCl, pH 8.0) with 0.1% Tween 20 for 1 h at room temperature. Blots were then immunolabelled overnight at 4°C with a monoclonal anti-MMP-11 (1:200, Lab Vision Corporation). After three washes for 5 min each in TBS, membranes were incubated with goat anti-mouse IgG (1:2500) (or 1:5000), anti-rabbit IgG for MMP-1 and -14, see below) peroxidase-conjugated secondary antibody, using the ECL Western blotting analysis system (Amersham Biosciences, CE Healthcare, Buckinghamshire, UK), and visualised by placing the blot in contact with standard X-ray film according to the manufacturer’s instructions. Membranes were stripped by incubation in 0.2 M glycine, pH 2.2, containing 0.1% SDS and 1% Tween 20 at room temperature for 1 h, and then re-probed with an anti-MMP-1 (1:200, Lab Vision Corporation), anti-MMP-13 (1:100, Calbiochem, Barcelona, Spain), and anti-MMP-14 antiserum (1:200, Lab Vision Corporation), or an anti-β-actin monoclonal antibody (1:2500; Sigma-Aldrich, St Louis, MO, USA) to confirm that equivalent amounts of total protein were added to each well.

Data analysis and statistical methods

Differences in percentages were calculated with the χ2 test. For metastasis-free survival analysis we used the Cox’s univariate method. Cox’s regression model was used to examine interactions of different prognostic factors in a multivariate analysis. Expression profiles were analysed by unsupervised hierarchical clustering method that organises proteins in a tree structure, based on their similarity. Data were reformatted as follows: –3 designated negative staining, 3 positive staining, missing data were left blank. The score values were reformatted (positive–negative) choosing the median as cutoff value. We used the Cluster 3.0 programme (average linkage, Pearson’s correlation). Results were displayed with Treeview (Eisen et al., 1998). The SPSS 11.5 programme was used for all calculations.

RESULTS

Figure 1 shows examples of tissue with immunostaining for proteins evaluated. The percentage of positive cells for each protein was always higher than 50% in positive case for each cellular type.

Matrix metalloproteinase-1 was immunohistochemically detected in 88.3% of breast carcinomas, MMP-2 in 42%, MMP-7 in
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To identify specific groups of tumours with distinct MMP/TIMP immunohistochemical expression profiles the data were analysed by unsupervised hierarchical cluster analysis by each cellular type. The algorithm orders proteins on the horizontal axis and samples on the vertical axis based on similarity of their expression profiles. This did not produce a dendrogram with well-defined cluster of cases for tumour cells or fibroblastic cells (Figure 2A and B). However, for mononuclear inflammatory cells the dendogram shows a first-order division of the tumours into two distinct MMP/ TIMP molecular profiles, designated group 1 (n = 89) and group 2 (n = 42) (Figure 2C). Matrix metalloproteinase-7, -9, -11, -13 and -14, and TIMP-1 and -2, were identified as showing significant high expression in group 2 compared with group 1 (Table 1). The presence of the proteins was confirmed by Western blot analysis of breast tumour cytosols samples (Figure 3). Western blots clearly showed immunoreactive bands corresponding to MMP-1, -11, -13 and -14 in seven samples from breast carcinomas (Figure 3A, upper panel). Two major immunoreactive bands were readily visible in samples from MMP-1 (glycosylated and unglycosylated form) and MMP-14 (latent and active form). As positive control, we also performed a Western blot for MMP-11 protein detection in placenta. As shown in Figure 3B, the anti-MMP-11 antibody recognised a band of approximately 47 kDa corresponding to the expected size in both, breast carcinomas (lanes 1 and 2), and placenta (lane 3).

These two groups showed significant differences with regard to PgR status and peritumoural inflammation. Group 1 showed a higher percentage of PgR-positive tumours, whereas group 2 showed a higher percentage of tumours with peritumoural inflammation. In addition, group 2 showed a significantly higher percentage of tumours positive for TIMP-1, MMP-9 and -11 in the tumour cells; as well as a significantly higher percentage of tumours positive for TIMP-2, TIMP-3, MMP-9, -11, -13 and -14, in fibroblastic cells (Table 2). However, our result did not show significant association between the cluster group of tumours with other clinicopathological characteristics, such as age, menopausal stage, tumour size, nodal status, histological grade, Nottingham Prognostic Index, oestrogen receptors, desmoplastic reaction, tumour advancing edge or vascular invasion (data not shown). On the other hand, during the study period, distant metastasis-relapse was confirmed in 41 of 42 (97.61%) patients of group 2 and only in 24 of 89 (26.96%) patients of group 1 tumours. Kaplan–Meier analysis shows that the difference in relapse-free survival between these two groups is highly and statistically significant (P < 0.0001; Figure 4). Multivariate analysis according to Cox model demonstrated that tumoural stage (II: relative risk (RR) (confidence interval (CI)) = 1.6 (0.8–3.1); III: 3.5 (1.7–7.1); P < 0.001) and ER status (positive: 0.5 (0.3–0.8), P < 0.001) were significantly and independently associated with relapse-free survival. However, this same analysis also demonstrated that clustering for mononuclear inflammatory cells was the most potent independent factor associated with relapse-free survival (group 2: 5.6 (3.5–9.6), P < 0.001).

DISCUSSION

The results of the present study led us to several considerations, on the cellular type expressing each MMPs or TIMPs, the cellular origin of its production, the importance of stromal–tumour cell interactions, and the clinical value of these proteins not only in predicting outcome in breast cancer but also as a potential biological therapeutic target.

Breast cancer, as a solid tumour, consists of a variable mixture of neoplastic cells and non-neoplastic tumour stroma, comprising endothelial cells, pericytes, fibroblast cells and variable representation of inflammatory cells. Over the past few years, evidence shows...
that both changes in stromal behaviour and the interaction between tumour cells and stromal cells are intimately linked to the processes of tumorigenesis, tumour invasion and metastasis (Liotta and Kohn, 2001; Bhowmick et al., 2004). In fact, it is now known that in addition to their production by epithelial tumour cells, MMP and/or TIMP gene expression may be induced in stromal fibroblasts and/or in vascular and inflammatory cells that infiltrate tumours around them (Visscher et al., 1994; Nielsen et al., 2001; Nakopoulos et al., 2002b; De Wever and Mareel, 2003).

Table 1  Expression of MMPs and TIMPs in two cluster groups of breast carcinomas with distinct MMP/TIMP molecular profile in the stromal mononuclear inflammatory cells

|          | Group 1 n = 89 | Group 2 n = 42 | P-value |
|----------|----------------|----------------|---------|
| MMP-1    | 53 (61.6)      | 32 (76.2)      | NS      |
| MMP-2    | 1 (1.1)        | 1 (2.4)        | NS      |
| MMP-7    | 33 (38.8)      | 30 (71.4)      | 0.001   |
| MMP-9    | 0              | 14 (33.3)      | 0.0001  |
| MMP-11   | 4 (4.6)        | 36 (85.7)      | 0.0001  |
| MMP-13   | 21 (23.6)      | 23 (54.8)      | 0.001   |
| MMP-14   | 29 (32.6)      | 38 (90.5)      | 0.0001  |
| TIMP-1   | 9 (10.1)       | 24 (57.1)      | 0.0001  |
| TIMP-2   | 15 (16.9)      | 35 (83.3)      | 0.0001  |
| TIMP-3   | 43 (48.3)      | 26 (61.9)      | NS      |

Data are expressed as number of cases (%). Note: samples on tissue sections were either insufficient or lost for analysis in three cases for MMP1, two cases for MMP-2, four cases for MMP-7, one case for MMP-9, two cases for MMP-11, one case for TIMP-1 and one case for TIMP-2. The values shown corresponding to the total number of cases were analysed for each protein.

Figure 2  Hierarchical clustering analysis of global matrix metalloproteinases (MMPs)/tissular inhibitors of metalloproteinases (TIMPs) expressions in the different cell types of breast cancer as measured by immunohistochemistry on tissue microarray (TMA). Graphical representation of hierarchical clustering results in tumour cells (A), fibroblasts (B) and mononuclear inflammatory cells (C). Rows: tumoural samples; columns, MMPs/TIMPs. Protein expressions are depicted according to a color scale: red, positive staining; green, negative staining; grey, missing data. No major clusters of tumours are shown in tumour cells and fibroblasts. Two major clusters of tumours (1 and 2) are shown in mononuclear inflammatory cells.

Figure 3  (A) Representative Western blots of immunoreactive matrix metalloproteinases (MMPs). (A) Eighty micrograms of cytosol extract from seven human breast carcinomas (lanes 2–8) were subject to 12% SDS–PAGE and transferred onto a nitrocellulose membrane and then immunolabelled with MMP-1, -11, -13, -14 and β-actin (used as loading control) antisera. Lane 1, molecular weight marker (50 kDa). (B) Western blots of immunoreactive MMP-11 and β-actin (used as loading control) from two human breast carcinomas (lanes 1 and 2) and from human placenta (used as positive control, lane 3).
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different MMPs and TIMPs expressions, in tumour cells or fibroblastic cells,

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Table 2

Number and percentage of positive cases of tumours for the
different MMPs and TIMPs expressions, in tumour cells or fibroblastic cells,
in the different cluster groups

|     | Group 1 n = 89 | Group 2 n = 42 | P-value |
|-----|---------------|---------------|--------|
| MMP-7 |               |               |        |
| TC   | 72 (84.7)     | 32 (92.9)     | NS     |
| FC   | 57 (67.1)     | 33 (78.6)     | NS     |
| MIC  | 33 (38.8)     | 30 (71.4)     | 0.001  |
| MMP-9 |               |               |        |
| TC   | 57 (64.8)     | 38 (90.5)     | 0.004  |
| FC   | 6 (6.8)       | 14 (33.3)     | <0.001 |
| MIC  | 0             | 14 (33.3)     | <0.001 |
| MMP-11 |              |               |        |
| TC   | 73 (83.9)     | 41 (97.6)     | 0.04   |
| FC   | 48 (55.2)     | 40 (95.2)     | <0.001 |
| MIC  | 4 (4.6)       | 36 (85.7)     | <0.001 |
| MMP-13 |              |               |        |
| TC   | 65 (73)       | 32 (76.2)     | NS     |
| FC   | 36 (40.4)     | 28 (66.7)     | 0.009  |
| MIC  | 21 (23.6)     | 23 (54.8)     | 0.001  |
| MMP-14 |              |               |        |
| TC   | 77 (86.5)     | 41 (97.6)     | NS     |
| FC   | 67 (75.3)     | 39 (92.9)     | 0.03   |
| MIC  | 29 (32.6)     | 38 (90.5)     | <0.001 |
| TIMP-1 |             |               |        |
| TC   | 82 (92.1)     | 41 (97.6)     | NS     |
| FC   | 41 (46.1)     | 23 (54.8)     | NS     |
| MIC  | 9 (10.1)      | 24 (57.1)     | <0.001 |
| TIMP-2 |             |               |        |
| TC   | 69 (77.5)     | 41 (97.6)     | 0.008  |
| FC   | 21 (23.6)     | 34 (81)       | <0.001 |
| MIC  | 15 (16.9)     | 35 (83.3)     | <0.001 |
| TIMP-3 |             |               |        |
| TC   | 75 (84.3)     | 38 (90.5)     | NS     |
| FC   | 46 (51.7)     | 34 (81)       | 0.003  |
| MIC  | 43 (48.3)     | 26 (61.9)     | NS     |

F = fibroblasts; MIC = mononuclear inflammatory cells; MMP = matrix metallopro-
teinase; TC = tumoural cells; TIMP = tissular inhibitors of metalloproteinase. Data are
expressed as number of cases (%). Notes: samples on tissue sections were either
insufficient or lost for analysis in four cases for MMP-7, one case for MMP-9, two
cases for MMP-11, one case for TIMP-1 and one case for TIMP-2. The values shown
_corresponding to the total number of cases were analysed for each protein.

Rather, it may in fact actively participate in the process of cancer
invasion.

Inflammatory cells can account for as much as 50% of the total
tumour mass in invasive breast carcinomas. Mononuclear
inflammatory cells infiltrate in breast carcinomas include a
variable representation of macrophages, plasma cells, mast cells
and B and T lymphocytes (CousSENS and Werb, 2002; Lin
and Pollard, 2004). Historically, tumour-infiltrating leukocytes have
been considered to be manifestations of an intrinsic defence
mechanism against developing tumours (Johnson et al, 1989; Lin
and Pollard, 2004). However, our data are in accordance with the
increasing evidence indicating that leukocytes infiltration can
promote tumour phenotypes, such as angiogenesis, growth and
invasion (Adams et al, 1987; CousSENS and Werb, 2002; Daniel
et al, 2005). This may be due to inflammatory cells probably
influencing cancer promotion by secreting cytokines, growth
factors, chemokines and proteases, which stimulate proliferation
invasiveness of cancer cells. Nevertheless, the prognostic signifi-
cance of the lymphoid infiltrate at the tumour site remains
controversial, perhaps because the evaluation criteria for tumour
infiltrates are not sufficiently standardised to yield reliable and
reproducible results in different institutions. Therefore, our results
may contribute to characterise a phenotype of infiltrating mono-
nuclear inflammatory cells associated with a poorer prognosis
in breast cancer. These mononuclear inflammatory cells were
characterised by the expression of MMP-7, -9, -11, -13, -14,
TIMP-1 and -2.

Matrix metalloproteinase-7 (matrilysin 1) is a stromelysin,
which degrades type IV collagen, fibronectin and laminin. It was
showed that MMP-7 is aberrantly expressed in human breast
tumours, and that elimination of MMP-7 is associated with low
invasiveness and slow tumour growth (Jiang et al, 2005). Matrix
metalloproteinase-9 (gelatinase B) is related to tumour invasion
and metastasis by their special capacity to degrade the type IV
collagen found in basement membranes (Jones and Walker, 1997),
and to induce angiogenesis (Egeblad and Werb, 2002). High MMP-
9 expression correlates significantly with tumoural aggressiveness
and poor prognosis (Chantry et al, 2004; Li et al, 2004; Pellikainen
et al, 2004). Similarly to other studies, our data showed that MMP-11 (Stromaly
isin-3) was preferentially expressed by peritumoral stromal cells (Basset et al, 1990, 1997) and that
high levels of MMP-11 were associated with tumour progression
and poor prognosis (Muller et al, 1993; Anderson et al, 1995;
Chenard et al, 1996; Ahmad et al, 1998; Tetu et al, 1998; Lochter
Figure 4 Kaplan–Meier survival curves as a function of the two major
clusters of tumours (Groups 1 and 2) shown in mononuclear inflammatory
cells.
et al; Nakopoulos et al, 2002b). Matrix metalloproteinase-13 (collagenase-3) has been found to have an exceptionally wide substrate specificity when compared with other MMPs (Freije et al, 1994; Knauper et al, 1997). Moreover, it is thought to play a central role in the MMP activation cascade, both activating and being activated by several other MMPs (MMP-14, -2 or -3). Nielsen et al (2001) have reported that MMP-13 expression by myofibroblasts was often associated with microinvasive events, and they have proposed that this MMP may play an essential role during the transition of ductal carcinoma in situ lesions to invasive ductal carcinoma of the breast. Matrix metalloproteinase-14 (membrane type 1 MMP or MT1-MMP) is a key metalloproteinase involved in the degradation of extracellular matrix, activates pro-MMP-13 (Knauper et al, 1996) and pro-MMP-2 (Stroopgin et al, 1985), and is expressed on cell surface, and plays crucial roles in molecular carcinogenesis, tumour cell growth, invasion and angiogenesis. On the other hand, the positive relationship between TIMP expression by inflammatory mononuclear cells and cancer progression could look paradoxical, because both TIMP-1 and -2 are well-known inhibitors of MMP activity. If TIMPs inhibit MMPs in vivo, it should be expected that high levels of inhibitors would prevent tumour progression and thus relate to good outcome in patients with cancer. However, there is an increasing body of evidence suggesting that TIMPs are multifunctional proteins that, in addition to its MMP-inhibitory effect, also promote the prolifera-

**REFERENCES**

Adams TE, Alpert S, Hanahan D (1987) Non-tolerance and autoantibodies to a transgenic self antigen expressed in pancreatic beta cells. Nature 325: 223 – 228

Ahmad A, Hanby A, Dublin E, Poulsom R, Smith P, Barnes D, Rubens R, Aglard P, Hart I (1998) Stromelysin-3: an independent prognostic factor for relapse-free survival in node-positive breast cancer and demonstration of novel breast carcinoma cell expression. Am J Pathol 152: 721 – 728

Anderson IG, Sugarbaker DJ, Ganju RK, Tsarswah DG, Richards WG, Sunday M, Kobzik L, Shipp MA (1995) Stromelysin-3 is overexpressed by stromal elements in primary non-small cell lung cancers and regulated by retinoic acid in pulmonary fibroblasts. Cancer Res 55: 4120 – 4126

Baker AH, George SJ, Zaltaus AB, Murphy G, Newby AC (1999) Inhibition of invasion and induction of apoptotic cell death of cancer cell lines by overexpression of TIMP-3. Br J Cancer 79: 1347 – 1355

Baker EA, Stephenson TJ, Reed MW, Brown NJ (2002) Expression of proteases and inhibitors in human breast cancer progression and survival. Mol Pathol 55: 300 – 304

Basset P, Bellocq JP, Lefebvre O, Noel A, Chenard MP, Wolf C, Anglard P, Rio MC, Chambon P (1990) A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. Nature 348: 699 – 704

Bhowmick NA, Neilson EG, Moses HL (2004) Stromal fibroblasts in cancer. Nature 432: 432 – 447

Decock J, Paridaens R, Cufer T (2005) Proteases and metastasis: clinical relevance nowadays? Curr Opin Oncol 17: 545 – 550

Duffy MJ, Maguire TM, Hill A, McDermott E, O’Higgins N (2000) Metalloproteinases: role in breast carcinogenesis, invasion and metastasis. Breast Cancer Res 2: 252 – 257

Egeblad M, Werb Z (2002) New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2: 161 – 174

Eisen MB, Spellman PT, Brown PO, Botstein D (1998) Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci USA 95: 14863 – 14868

Fingleton B, Vargo-Gogola T, Crawford HC, Matrisian LM (2001) Matrilysin [MMP-7] expression selects for cells with reduced sensitivity to apoptosis. Neoplasia 3: 459 – 468

Freije JM, Diez-Irta I, Balbin M, Sanchez LM, Blasco R, Tolivia J, Lopez-Otin C (1994) Molecular cloning and expression of collagenase-3, a novel human matrix metalloproteinase produced by breast carcinomas. J Biol Chem 269: 16766 – 16773

Grieu F, Li WQ, Iacopetta B (2004) Genetic polymorphisms in the MMP-2 and MMP-9 genes and breast cancer phenotype. Breast Cancer Res Treat 88: 197 – 204

Guede L, Courtemanch L, Stetler-Stevenson M (1998) Tissue inhibitor of metalloproteinase (TIMP)-1 induces differentiation and an antiapoptotic phenotype in germinal center B cells. Blood 92: 1342 – 1349

Guede L, McMarlin AJ, Kingma DW, Bennett TA, Stetler-Stevenson M, Stetler-Stevenosn WG (2001) Tissue inhibitor of metalloproteinase-1 alters the tumorigenicity of Burkit’s lymphoma via divergent effects on tumor growth and angiogenesis. Am J Pathol 158: 1207 – 1215

Gulmann C, Butler D, Kay E, Grace A, Leader M (2003) Biopsy of a biopsy: validation of immunoprofiling in gastric cancer biopsy tissue micro-arrays. Histopathology 42: 70 – 76

Heppner KJ, Matrisian LM, Jensen RA, Rodgers WH (1996) Expression of most matrix metalloproteinase family members in breast cancer represents a tumor-induced host response. Am J Pathol 149: 273 – 282

Hirvonen R, Talvensaari-Mattila A, Paakko P, Turpeenniemi-Hujanen T (2003) Matrix metalloproteinase-2 (MMP-2) in T(1–2)NO breast carcinoma. Breast Cancer Res Treat 77: 85 – 91

Jiang WG, Davies G, Martin TA, Parr C, Watkins G, Mason MD, Mokbel K, Mansel RE (2005) Targeting matrilysin and its impact on tumor growth in vivo: the potential implications in breast cancer therapy. Clin Cancer Res 11: 6012 – 6019

We thank Professor Román Pérez from Departamento de Fisiología, Facultad de Medicina, Universidad de Santiago de Compostela for support and for helpful comments. This work was supported by grants from Fondo de Inversión Sanitaria del Instituto Carlos III (FIS-PI040137) (FIS-Spain), Red de Centros de Cáncer RTICCC (C03/10) and Obra Social Cajastur.
