Clin. Exp. Metastasis, 1996, **14**, 12–18

Expression and localization of 92 kDa type IV collagenase/gelatinase B (MMP-9) in human gliomas

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(Received 12 July 1995; accepted in revised form 17 August 1995)

Matrix metalloproteinases play an important regulatory role in tissue morphogenesis, cell differentiation and motility, and tumor cell invasiveness. We have recently demonstrated elevated activity of the 92 kDa type IV collagenase (MMP-9) in human glioblastoma and in the present study examine the relative amounts of MMP-9 protein and mRNA in human gliomas and as well as the distribution of MMP-9 in human glioma tumors *in vivo*. Using an enzyme-linked immunosorbent assay for the quantitative determination of MMP-9 protein, we found that levels were significantly higher in malignant astrocytomas, especially in glioblastoma multiforme, than in normal brain tissues and low-grade gliomas. In addition, the amount of MMP-9 mRNA, as determined by northern blot analysis was higher in anaplastic astrocytomas and glioblastoma multiforme than in normal brain tissue and low-grade gliomas. Immunocytochemical staining for MMP-9 showed strong cytoplasmic immunoreactivity in the tumor cells and the proliferating endothelial cells of glioblastoma multiforme and anaplastic astrocytomas. The staining intensity was lower in low-grade astrocytomas, and was undetectable or very low in normal brain astrocytes. The results indicate that expression of MMP-9 is dramatically upregulated in highly malignant gliomas and correlates with the highly malignant progression of human gliomas *in vivo*, and support a role for the MMP-9 in facilitating the invasiveness seen in malignant gliomas *in vivo*.

Keywords: 92 kDa type IV collagenase, astrocytoma, glioblastoma multiforme, invasiveness, matrix-metalloproteinase

Introduction

Local invasive growth is one of the key features of primary malignant brain tumors. Malignant brain tumor cells cause massive tissue destruction at the border zone between tumor and normal brain tissue [1]. The most common and malignant brain tumor, glioblastoma multiforme, is characterized by the presence of necrosis, vascular proliferation and aggressive invasion into surrounding normal brain tissue [2]. Glioblastoma multiforme spreads along nerve fiber tracts and frequently penetrates beyond the glial membrane limitans externa, leading to leptomeningeal dissemination of the tumor. This diffuse infiltrative nature of glioblastoma multiforme is one of the major obstacles to successful surgical control [3].

The specific mechanisms facilitating the invasive behaviors of brain cancers remain obscure; however, the interactions between cancer cells and the

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surrounding normal cells and extracellular matrix (ECM) are thought to be key aspects in tumor cell invasion [4]. To invade and spread through surrounding normal tissue, tumor cells must degrade multiple elements of the ECM, including fibronectin, laminin and type IV collagen [5]. A number of different proteases, including serine proteases, matrix metalloproteinases (MMPs) and cysteine proteases, have been proposed as potentiating the invasiveness and metastatic ability of a variety of malignant tumors [6].

MMPs are neutral proteinases encoded by a multigene family. These proteinases mediate, in part, ECM degradation and tissue turnover during physiological and pathological processes [7]. The MMPs are classified on the basis of their substrate specificities and include interstitial collagenase (type I collagenase/MMP-1); gelatinase A (72-kDa type IV collagenase/MMP-2); three stromelysins (MMP-3, -10 and -11); uterine metalloproteinase (PUMP-1/MMP-7); neutrophil collagenase (MMP-8); and gelatinase B (92-kDa type IV collagenase/MMP-9) [8]. All of these enzymes are secreted in latent forms and are activated by cleavage of amino-terminal propeptides at a conserved sequence. This cleavage can be mediated by proteases, such as plasmin, trypsin, kallikreins and cathepsins and by organomercurials [9]. The activities of the MMPs are regulated by gene expression [10], proenzyme activation, and inhibition of active enzymes by their specific tissue inhibitors of metalloproteinases (TIMPs) [8, 11].

MMP-9 can degrade elastin, casein, gelatin and collagen types IV, VII and X by cleavage of their helical domains [12]. Degradation of ECM proteins is thought to be required for tumor cell invasion and metastasis [13]. For example, the invasiveness of murine and human tumor cells can be inhibited by TIMPs [14] and by an antibody to MMP-9 [15]. This experimental evidence implies a link between the production of MMP-9 by tumor cells and their invasive behavior. Disrupting the balance between MMP and its inhibitors in favor of increased degradative activity could be important in tumor cell invasion and metastasis formation. A similar role for a membrane-associated MMP in C6-glioma-cell motility and invasiveness was reported previously [16, 17].

MMP-9 is synthesized by a variety of cells, including tumor cell lines of glial or neuroectodermal origin [18]. We previously showed that MMP-9 activity was dramatically higher in human malignant brain tumors than in less malignant tumors or normal brain tissue *in vivo* [19] and we have also shown that MMP-9 facilitates the invasiveness of a glioblastoma cell line in an *in vitro* invasion model [20]. To determine whether upregulation of MMP-9 is associated with the malignant progression of astrocytoma we quantitated the levels of MMP-9 mRNA and determined the distribution of MMP-9 protein in various types of human gliomas and normal brain tissue. Our results support the notion that MMP-9 is abnormally regulated in malignant gliomas and that its increased expression probably facilitates the invasiveness of these tumor cells.

Materials and methods

Surgical specimens

Various types of fresh human glioma tissues and normal brain tissue samples were obtained in the operating room from patients undergoing craniotomy for removal of a brain tumor. Samples used for quantitative enzyme protein and northern blot analysis of MMP-9 were flash-frozen in liquid nitrogen immediately after surgical removal and stored at −80°C. Tissue samples for immunohistochemical analysis of MMP-9 were fixed in 10% formalin and embedded in paraffin. The histological diagnosis was confirmed for each tissue block by standard light-microscopic evaluation of sections stained with hematoxylin and eosin. The classification of human brain tumors used in this study was based on the World Health Organisation criteria [21]. The samples included seven glioblastoma multiforme, six anaplastic astrocytomas, six low-grade gliomas and six normal brain tissue samples. None of the patients had been subjected to chemotherapy or radiation therapy before resection.

ELISA

Quantitative protein analysis of MMP-9 in tumor tissue and normal brain tissue extracts (75 μg) was performed by ELISA using antibody specific for MMP-9 [22]. Tissue extracts and buffer containing MMP-9 were mixed with phosphate buffer and incubated overnight. The wells were washed with PBS and incubated with anti-MMP-9 antibody at 25°C for 3 h. The plates were then washed with PBS, incubated with a secondary antibody (alkaline phosphate conjugate) and the color developed with *p*-nitrophenol phosphate. The concentration of MMP-9 in the tissue extracts was determined using the standard curve for MMP-9.

RNA extraction and northern blot analysis

Quantitation steady-state MMP-9 mRNA was determined by northern blot analysis as described previously [23]. Frozen tumor tissue and normal brain

tissue were ground to powder in liquid nitrogen and dissolved in 4 M guanidinium isothiocyanate, and total RNA was isolated by the guanidinium thiocyanate–phenol–chloroform extraction method [24]. Total RNA (20 μg) was electrophoresed in formaldehyde containing 1.5% agarose gel, transferred to Hybond (Amersham, Arlington Heights, IL, USA) membranes by capillary blotting overnight in 10 × SSC buffer (1 × SSC; 150 mM NaCl, 15 mM sodium citrate) and hybridized with DNA probe randomly labeled with [α^{32}P]dCTP (6000 Ci/mmol; NEN Research Products, Boston, MA, USA). Hybridization was carried out overnight at 42°C with the prehybridization solution containing the radiolabeled and denatured cDNA specific for MMP-9 mRNA (provided by W.G. S.-S.). The filters were then washed in 0.5 × SSC, 1.0% sodium dodecyl sulfate at 65°C and dried and then autoradiographed for 1–3 days at −80°C using Hyperfilm (Amersham) and intensifying screens. Subsequently, the blots were reprobed with β-actin cDNA probe to confirm RNA loading equalities. Relative hybridization signal intensities were calculated by assigning an arbitrary value of 1 to the least intense signal seen by northern blot analysis. In each group, the band of MMP-9 mRNA was scanned in three positions at different exposures, and the peak areas were averaged.

Immunocytochemical staining for MMP-9

Immunoreactivity of the MMP-9 was analyzed in 10%-formalin-fixed, paraffin-embedded sections by using a MMP-9-specific rabbit anti-human polyclonal antibody raised against a synthetic peptide, LGRFQTFEGDLKWHH, corresponding to a sequence in the pro-MMP-9 molecule described previously [23]. The appropriate concentration of the primary antibody was determined by testing on control tissues. Sections (4 μm) were cut and mounted on silane-coated slides. MMP-9 was detected by using an indirect avidin–biotin complex immunohistochemical method. The slides were dewaxed and blocked with normal goat serum. The sections were incubated with rabbit anti-human MMP-9 polyclonal antibody diluted 1:100 in PBS for 1 h at room temperature in a humidified chamber. After a brief washing in buffer, the tissue samples were incubated in biotinylated goat anti-rabbit secondary antibody and streptavidin–alkaline phosphatase (Biogenese Laboratories, San Ramon, CA, USA). Alkaline phosphatase activity was visualized by the addition of a substrate solution consisting of naphthol AS-BI phosphate, levamisole and fast-red TR, which forms an intense red color. Sections were counterstained in hematoxylin. A control study was performed using a nonspecific IgG or antiserum that had been pre-absorbed with 10 μg/ml of the peptide antigen.

Results

ELISA

Using specific antibody for MMP-9 analysis of normal brain tissue and various grades of glioma tissue extracts by ELISA showed that the 92-kDa type IV collagenase content was approximately 12 times higher in glioblastomas ($P < 0.0001$) and four times higher in anaplastic astrocytoma ($P < 0.001$) than in low-grade gliomas or normal brain tissue samples (Figure 1).

Northern blot analysis

Total RNA was extracted from various types of human gliomas and normal brain tissue, and the steady-state levels of MMP-9 mRNA were compared by northern analysis. MMP-9 mRNA was present in all tissue samples, but at lower levels in normal brain tissue and low-grade gliomas than in anaplastic astrocytoma and glioblastoma multiforme (Figure 2a). Quantitative evaluation of hybridization signals by scanning autoradiograms with laser densitometry indicated that MMP-9 mRNA levels were three- to fourfold higher in anaplastic astrocytomas ($P < 0.001$) and 12- to 14-fold higher in glioblastomas ($P < 0.0001$) than in normal brain tissues and low-grade gliomas (Figure 2b). Anaplastic astrocytomas and glioblastomas with high-levels of MMP-9 protein had proportionately higher levels of MMP-9 mRNA than did normal brain

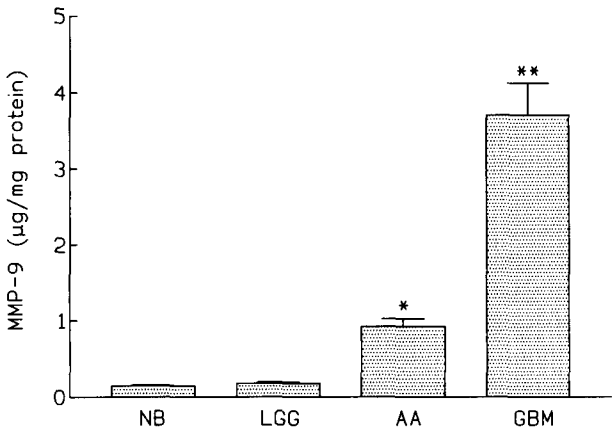

Figure 1. MMP-9 content in tumor tissue and normal brain tissue extracts determined by ELISA using MMP-9-specific antibodies. Data are mean values ± SD for six different samples from each group. NB, normal brain; LGG, low-grade glioma; AA, anaplastic astrocytoma; GBM, glioblastoma. *$P < 0.001$. **$P < 0.0001$.

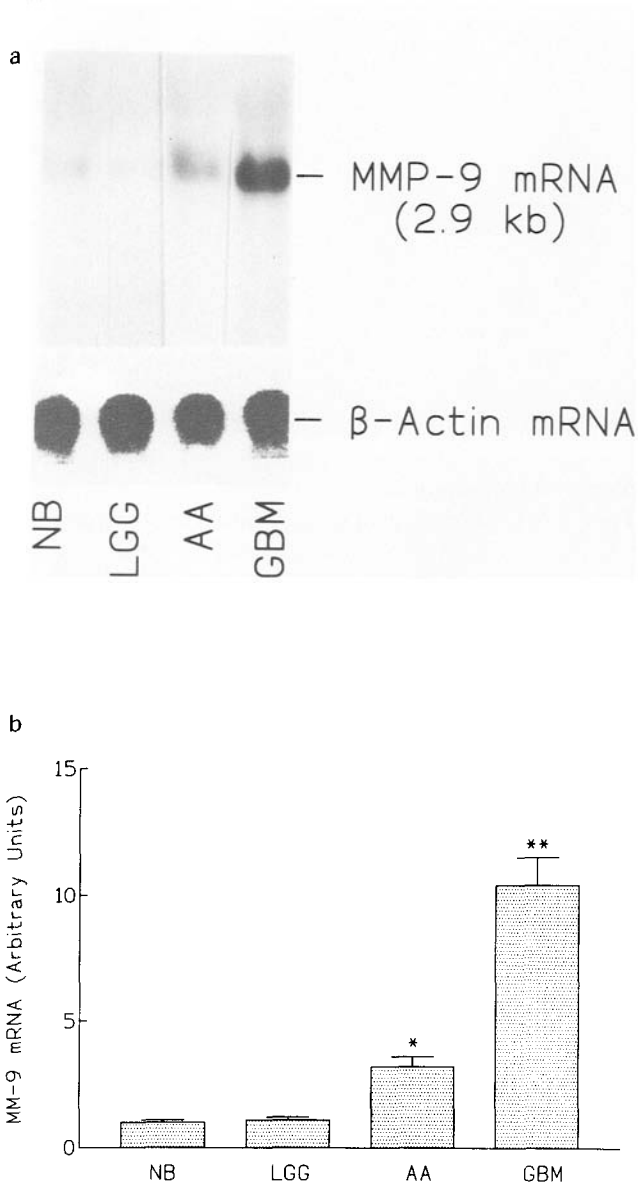

Figure 2. Northern blot analysis: MMP-9 mRNA in normal brain (NB), low-grade glioma (LGG), anaplastic astrocytoma (AA) and glioblastoma (GBM). **a** total RNA (15 μg) was electrophoresed in a 1.5% agarose gel and transferred to nytran-modified nylon filters by capillary action. The membrane was then hybridized with radio-labeled cDNA probe specific for MMP-9 mRNA. After stripping the blots were rehybridized with a β-actin probe to check loading equalities. **b** MMP-9 mRNA levels measured by scanning autoradiograms with a laser densitometer: relative hybridization signals were calculated by assigning an arbitrary value of 1 to the least intense signal seen by northern blot analysis after mRNA loading equalities based on β-actin probe reactivity. In each group, MMP-9 mRNA bands were scanned in three positions at different exposures by laser densitometry and the peak areas were averaged to give the values presented. Columns represent the mean $\pm$ SD (bars) for samples from six different patients from each group. $*P < 0.001$; $**P < 0.0001$.

tissues and low-grade gliomas. Thus a correlation between mRNA quantities and MMP-9 protein content was found in these tissues.

Immunohistochemical localization of MMP-9 in various types of human gliomas and normal brain tissues

Using paraffin-embedded tissues we determined the distribution of MMP-9 in the tumor and normal brain tissue by immunohistochemical analysis. Antibodies against MMP-9 showed intense immunoreactivity in the tumor cells and proliferating endothelial cells of glioblastomas and anaplastic astrocytomas (Figures 3a & b). Low-grade astrocytoma exhibited weak immunoreactivity (Figure 3c). In normal brain tissues, MMP-9 was almost undetectable (Figure 3d). No staining was seen when a nonspecific IgG was substituted for the anti-MMP-9 antibody (data not shown).

The intensity of immunohistochemical staining of MMP-9 was assessed in a semiquantitative manner for each tissue group (Table 1). All but one of the samples of glioblastoma showed strong anti-MMP-9 immunoreactivity. Of the anaplastic astrocytoma samples, one showed strong immunoreactivity, three showed positive immunoreactivity and two showed weak positive immunoreactivity. Staining intensities were negative or only weakly positive for the normal brain tissue and low-grade glioma samples. These results are consistent with the ELISA and northern blot analyses demonstrating abundant levels of MMP-9 protein and mRNA in glioblastoma and anaplastic astrocytoma but low levels in low-grade gliomas and normal brain tissue samples.

Discussion

MMPs mediate degradation of the ECM thereby altering morphogenic tissue interactions [25]. These enzymes are secreted from various normal cell types, including monocytes and macrophages, trophoblasts and fibroblasts treated with phorbol esters [26], as well as tumor cells [27]. It has been proposed that increased expression of MMPs might be a transient phenomenon correlating with tissue remodeling in development, cellular growth, tissue repair or cellular invasion [28]. MMP-9 is an important member of the MMP family and has recently been investigated as an important factor that facilitates the invasiveness and angiogenesis of malignant tumors outside the central nervous system. In many malignant tumors, including carcinoma of the lung [29], breast [30], squamous cell [23] and colon [31], and in metastatic cell lines [22]

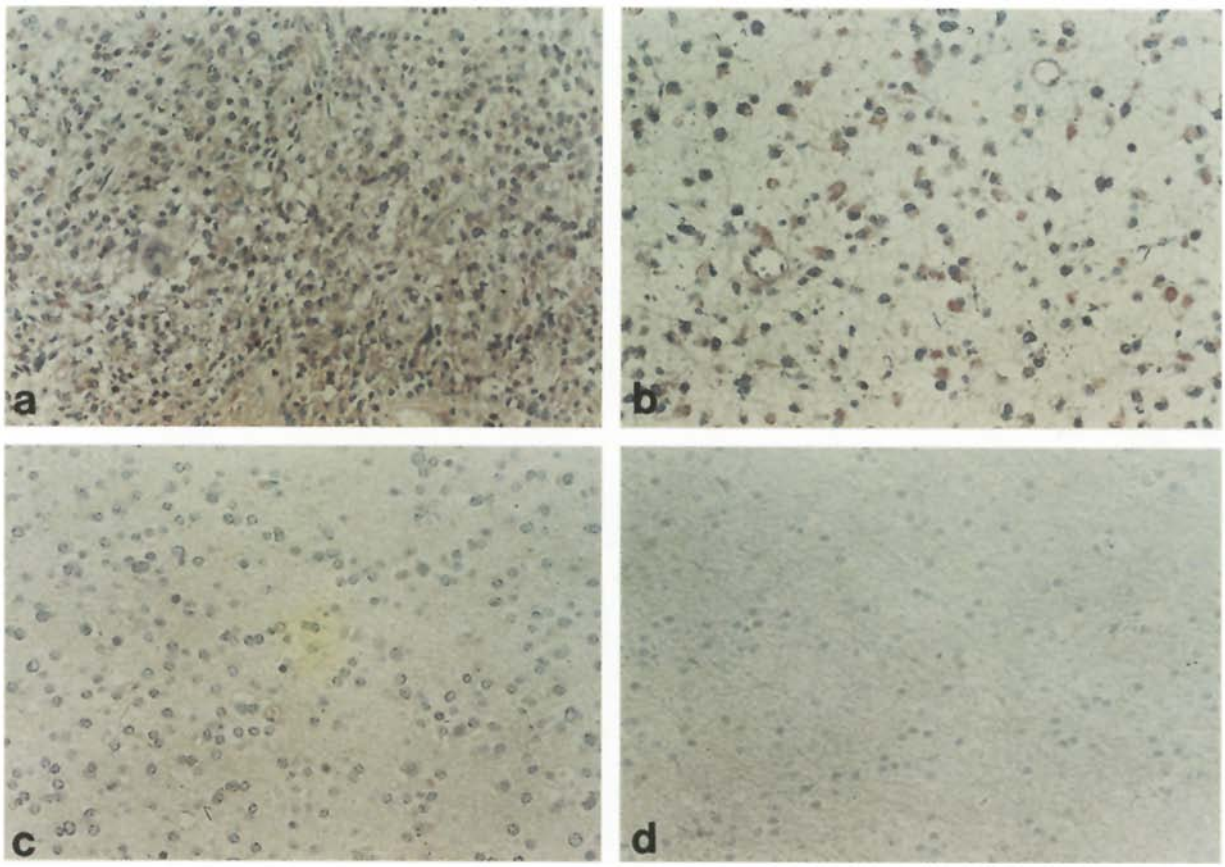

Figure 3. Immunohistolocalization of MMP-9 in **a** glioblastoma, **b** anaplastic astrocytoma, **c** low-grade astrocytoma and **d** normal brain tissue.

Table 1. MMP-9 staining intensities in normal tissues and various types of human gliomas

	No. of samples	MMP-9 staining			
		Negative (−)	Weakly positive (±)	Positive (+)	Strongly positive (++)
Normal	6	5	1	0	0
Low-grade	6	4	2	0	0
Anaplastic	6	0	2	3	1
Glioblastoma	7	0	0	1	6

there is a correlation between the production of MMP-9 and the invasive or metastatic phenotype.

Although the expression of MMP-9 in CNS tumors has been determined in several *in vitro* studies [16–18], little is known about the expression and distribution of MMPs in CNS tumor tissue *in vivo*, particularly with respect to the invasive behaviors of malignant gliomas [19]. The only previous comprehensive study of immunohistochemical localization of MMP-9 in CNS tumors is that of Nakagawa *et al.* [32], who investigated the expression and distribution of MMP-9 and TIMP-1 in various brain tumors and found that MMP-9 was highly expressed in malignant astrocytoma and localized within tumor cells and endothelial cells. In the present study, we found higher amounts of MMP-9 mRNA and protein in malignant astrocytomas

than in normal CNS tissue and low-grade glioma. We also noted a strong correlation between the histological grade of CNS tumor and MMP-9 content. Increased levels of MMP-9 mRNA correlated with elevated levels of MMP-9 protein in tumor tissue. Immunohistochemical analysis indicated that MMP-9 protein is localized within astrocytoma cells and proliferating endothelial cells and is heterogeneously distributed within glioblastomas.

Local invasive growth is one of the key features of malignant astrocytomas [1]. Our results, together with those of a previous report [20], suggest that the specific mechanisms that enhance the invasive behavior of malignant astrocytomas, especially glioblastomas, might depend in part on the increased expression of MMP-9. Changes in the expression of MMPs might be important in some types of human malignant tumors for sustaining growth and invasiveness.

The mechanism underlying the upregulation of MMP-9 in malignant astrocytomas is unknown. Soluble factors such as growth factors and cytokines that directly stimulate cell proliferation and invasiveness have been shown to regulate the secretion of MMPs. For example, interleukin-1β, epidermal growth factor, transforming growth factor-β and tumor necrosis factor-α can stimulate the secretion of MMP-9 [33, 34]. We have demonstrated increased levels of MMP-9 protein and mRNA in malignant gliomas, and an intense cytoplasmic immunoreactivity in tumor cells and proliferating endothelial cells in these tumors. The data suggest that this gene is activated or upregulated in malignant gliomas and that upregulation of MMP-9 is closely correlated with the malignant progression of gliomas. A further understanding of the mechanisms of increased expression of MMP-9 in malignant gliomas may provide important information about the regulatory pathways controlling cellular invasion and tumor progression.

Acknowledgments

This work was supported in part by the Physicians Referral Service funds of The University of Texas M. D. Anderson Cancer Center, Houston, Texas; by NCI grant CA 56792 and ACS grand EDT-91 (J. S. R.) and National Foundation for Cancer Research, Inc. (G. L. N.). We thank Pamela P. Pyle, Janet E. Quinones, Norva Steward and Alan Rayford for their technical help, Norma Adams for preparation of the manuscript, and Stephanie Deming for manuscript review.

References

1. Bjerkvig R, Laerum OD and Mella O, 1986, Glioma cell interactions with fetal rat brain aggregates *in vitro* and with brain tissue *in vivo*. *Cancer Res*, **46**, 4071–9.
2. Russel DC and Rubinstain LJ, 1989, *Pathology of Tumors of the Nervous System*, 5th ed. London: Edward Arnold.
3. Gleave JR, 1986, Surgery for primary brain tumors. In: Blehen NM, ed. *Tumors of the Brain*. Berlin: Springer-Verlag, pp. 101–120.
4. Liotta LA, 1984, Tumor invasion and metastases: role of the basement membrane. *Am J Pathol*, **117**, 339–48.
5. Kwaan HC, 1992, The plasminogen–plasmin system in malignancy. *Cancer Metastasis Rev*, **11**, 291–311.
6. Goldfarb RH and Liotta LA, 1986, Proteolytic enzymes in cancer invasion and metastasis. *Semin Thromb Hemostasis*, **12**, 294–307.
7. Liotta LA, Steeg PS and Stetler-Stevenson WG, 1991, Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. *Cell*, **64**, 327–36.
8. Woessner JF, 1991, Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB J*, **5**, 2145–54.
9. Nagase H, Ogata Y, Suzuki K, Enghild JJ and Salvensen G, 1991, Substrate specificities and activation mechanisms of matrix metalloproteinases. *Biochem Soc Trans*, **19**, 715–8.
10. Sato H and Seiki M, 1993, Regulatory mechanism of 92-kDa type IV collagenase gene expression which is associated with invasiveness of tumor cells. *Oncogene*, **8**, 395–405.
11. Stetler-Stevenson WG, Krutzsch HC and Liotta LA, 1989, Tissue inhibition of metalloproteinase (TIMP-2). A new member of the metallproteinase inhibitor family. *J Biol Chem*, **264**, 17374–8.
12. Hibbs MS, Hoidal JR and Kang AH, 1987, Expression of a metalloproteinase that degrades native type V collagen and denatured collagens by cultured human alveolar macrophages. *J Clin Invest* **80**, 1644–50.
13. Liotta LA and Stetler-Stevenson WG, 1990, Metalloproteinases and cancer invasion. *Semin Cancer Biology*, **1**, 99–106.
14. Schultz RM, Silberman S, Persky B, Bahkoski AS and Carmichael DF, 1988, Inhibition by human recombinant tissue inhibitor of metallproteinases of human amnion invasion and lung colonization by murine B16-F10 melanoma cells. *Cancer Res*, **48**, 5539–45.
15. Wang M and Stearns ME, 1988, Blocking of collagenase secretion by estramustine during *in vitro* tumor cell invasion. *Cancer Res*, **48**, 6262–71.
16. Paganetti PA, Caroni P and Schwab ME, 1988, Glioblastoma infiltration into central nervous system tissue *in vitro*: involvement of a metalloproteinase. *J Cell Biol*, **107**, 2281–91.
17. Vaithilingam IS, McDonald W, Stroude EC, Cook RA and Del Maestro RF, 1992, Proteolytic activity during the growth of C6 astrocytoma in the murine spheroid implantation model. *Can J Neurol Sci*, **19**, 17–22.
18. Apodaca G, Rutka JT, Bouhana K, *et al.* 1990, Expression of metalloproteinases and metalloproteinase inhibitors by fetal astrocytes and glioma cells. *Cancer Res*, **50**, 2322–9.
19. Rao JS, Steck PA, Mohanam S, Stetler-Stevenson WG,

Liotta LA and Sawaya R, 1993, Elevated levels of M_r 92,000 type IV collagenase in human brain tumors. *Cancer Res*, **53**, 2208–11.
20. Rao JS, Steck PA, Tofilon P, *et al.* 1994, Role of plasminogen activator and of 92-kDa type IV collagenase in glioblastoma invasion using an *in vitro* matrigel model. *J Neuro-Oncol*, **18**, 129–38.
21. Kleihus P, Burger PC and Schneithauer BW, 1993, The new WHO classification of brain tumours. *Brain Pathol*, **3**, 255–68.
22. Nakajima M, Welch DR, Belloni PN and Nicolson GL, 1987, Degradation of basement membrane type IV collagen and lung subendothelial matrix by rat mammary adenocarcinoma cell and clones of differing metastatic potentials. *Cancer Res*, **47**, 4869–76.
23. Juarez J, Clayman G, Nakajima M, *et al.* 1993, Role and regulation of expression of 92-kDa type-IV collagenase (MMP-9) in two invasive squamous-cell-carcinoma cell lines of the oral cavity. *Int J Cancer*, **55**, 10–18.
24. Chomczynski P and Sacchi N, 1987, Single-step method of RNA isolation by acid quanidinium thiocyanate–phenol–chloroform extraction. *Anal Biochem*, **162**, 156–9.
25. Matrisian LM, 1992, The matrix-degrading metallo-proteinases. *Bioessays*, **14**, 455–63.
26. Salo T, Turpeenniemi-Hujanen T and Tryggvason K, 1985, Tumor-promoting phorbol esters and cell proliferation stimulate secretion of basement membrane (type IV) collagen-degrading metalloprotease by human fibroblasts. *J Biol Chem*, **260**, 8526–31.
27. Salo T, Liotta LA, Keski J, Turpeenniemi-Hujanen T and Tryggvason K, 1982, Secretion of basement membrane collagen degradation enzyme and plasminogen activator by transformed cells—role in metastasis. *Int J Cancer*, **30**, 669–73.
28. Bernhard EJ, Gruber SB and Muschel RJ, 1994, Direct evidence linking expression of matrix metalloproteinase (92-kDa gelatinase/collagenase) to the metastatic phenotype in transformed rat embryo cells. *Proc Natl Acad Sci USA*, **91**, 4293–7.
29. Muller D, Breathnach R, Eagelmann A, *et al.* 1991, Expression of collagenase-related metalloproteinase genes in human lung or head and neck tumours. *Int J Cancer*, **48**, 550–56.
30. Monteagudo C, Merino MJ, San-Juan J, Liotta LA and Stetler-Stevenson WG, 1990, Immunohistochemical distribution of type IV collagenase in normal, benign and malignant breast tissue. *Am J Pathol*, **136**, 585–92.
31. Hewitt RE, Leach IH, Powe DG, *et al.* 1991, Distribution of collagenase and tissue inhibitor of metalloproteases (TIMP) in colorectal tumors. *Int J Cancer*, **49**, 666–72.
32. Nakagawa T, Kubota T, Kubota M, *et al.* 1994, Production of matrix metalloproteinases and tissue inhibitor of metalloproteinases-1 by human brain tumors. *J Neurosurg*, **81**, 69–77.
33. Okada Y, Tsuchiya H, Shimizu H, *et al.* 1990, Induction and stimulation of 92-kDa gelatinase/type IV collagenase production in osteosarcoma and fibrosarcoma cell lines by tumor necrosis factor α. *Biochem Biophys Res Commun*, **171**, 610–17.
34. Shima I, Sasaguri Y, Kusukawa J, *et al.* 1993, Production of matrix metalloproteinase 9 (92-kDa gelatinase) by human oesophageal squamous cell carcinoma in response to epidermal growth factor. *Br J Cancer*, **67**, 721–7.