Clin. Exp. Metastasis, 1996, **14**, 27–34

Expression and immunohistochemical localization of cathepsin L during the progression of human gliomas

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(*Received 20 May 1995; accepted in revised form 5 September 1995*)

Recent studies suggest that cysteine proteinase cathepsin L is involved in the process of tumor invasion and metastasis. We examined cathepsin L activity in brain tumor tissue samples by an enzymatic assay, and cathepsin L protein content by enzyme-linked immunoadsorbent assays and Western blotting to determine whether increased levels of cathepsin L correlate with the progression of human gliomas. Native and acid-activatable cathepsin L activities were highest in glioblastomas followed by anaplastic astrocytomas and were lowest in low-grade gliomas and normal brain tissues. Significantly higher amounts of an M_r 29 000 cathepsin L were present in glioblastomas and anaplastic astrocytomas than in normal brain tissues and low-grade glioma tissue extracts. Using specific antibodies to cathepsin L, we also studied its cellular distribution by immunohistochemical procedures. Higher diffuse cathepsin L immunoreactivity was found in glioblastomas than in low-grade gliomas and normal brain tissue samples. Finally, the addition of cathepsin L antibody inhibits the invasion of glioblastoma cell lines through Matrigel invasion assay. These results suggest the expression of cathepsin L is dramatically upregulated in malignant gliomas and correlates with the malignant progression of human gliomas *in vivo*.

Keywords: cathepsins, cysteine proteases, cysteine protease inhibitors, glioblastoma multiforme, invasiveness

Introduction

Transformed cells are associated with increased release of proteolytic enzymes that are thought to facilitate tumor cell growth and invasiveness by degrading components of basement membrane (BM). The identification of metalloendopeptidases [1], plasminogen activators [2], and lysosomal enzymes, such as cathepsin B [3], cathepsin L [4], cathepsin D [5], and cathepsin H [6] in human and rodent tumor tissues and cell lines and their association with tumor invasion and metastasis has been reported. Increased synthesis of these proteolytic enzymes has been found at different stages during neoplastic progression, and their role is thought to be in the ability of metastatic tumor cells to extravasate or invade through BMs. Dissolution of the BM during the extravasation of metastatic tumor cells may be dependent on the sequential action of several classes of endopeptidases.

Lysosomal enzymes are synthesized as high molecular weight precursor molecules that are subsequently post-translationally converted to the mature enzymes with increased proteolytic activities. This can be seen *in vitro* by incubating the fractions under acidic conditions [7] or by limited proteolysis that removes the proenzyme segment [8]. Proteolytic conversion of pro-cathepsin B [8], pro-cathepsin L [8], and pro-cathepsin H [9] to mature forms is strongly inhibited by pepstatin, suggesting that aspartic proteinase cathepsin D is involved in the proteolytic processing [8]. These lysosomal cysteine proteinases may undergo the similar post-translational processing

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and activation mechanisms within the lysosomes. Although both cathepsin B and L can degrade isolated components of the BM, cathepsin L has been reported to be more effective against intact BM [10].

Cathepsin L is a lysosomal cysteine proteinase that is important in intracellular protein catabolism [11] and shows the most potent collagenolytic and elastinolytic activity *in vitro* [12]. It has also been implicated in pathological processes, including myofibril necrosis [13], myopathies and myocardial ischemia [14] and in the renal tubular response of protein urea [15].

The secretion of cathepsin B by human glioma cell lines *in vitro* [16] and the expression of significantly greater levels of cathepsin B during the progression of human gliomas [17] have been reported recently. A possible role for cathepsin L in malignant transformation of murine and human tumors and cell lines has also been described [4]. Although cathepsin B, L, and D are lysosomal enzymes, the expression and activity of cathepsin B are relatively higher in melanomas, whereas the expression and activity of cathepsin L are relatively higher in hepatoma [18]. There are no reports on the expression and activity of cathepsin L in the central nervous system (CNS). Therefore we measured the cathepsin L expression and activity in human gliomas at different stages of tumor progression. We show here that there is a direct correlation between cathepsin L expression and the degree of malignancy of human gliomas.

Materials and methods

Materials

Cathepsin L and anti-rabbit cathepsin L antibody were purchased from Athens Research and Technology (Athens, GA, USA). Z-Phe-Arg-(4-methyl)coumaryl amide, 7-amino-4-methyl coumarin, and peroxidase-conjugated anti-rabbit IgG were purchased from Sigma (St Louis, MO, USA). Z-Phe-Phe-CHN_2 was purchased from Enzyme Systems Products (Livermore, CA, USA). Nitro-cellulose membranes were purchased from BioRad (Hercules, CA, USA). All other chemicals were of analytical grade.

Samples

Normal brain tissue and various types of tumor tissues were collected in the operating room from patients undergoing craniotomy. The samples were flash-frozen in liquid nitrogen immediately after surgical removal and stored at −80°C. The samples included six meningiomas, five low-grade gliomas, seven anaplastic astrocytomas, 10 glioblastomas, and five normal brain tissues. The category of low-grade gliomas included only low-grade astrocytoma (fibrillary astrocytoma) and low-grade oligodendro. All tumor tissues examined in the present study were from primary resections; none of the patients had been subjected to chemotherapy or radiation therapy before resection.

Preparation of tissue

The frozen normal brain tissue and tumor tissues were thawed, homogenized in 50 mM acetate buffer (pH 5.5 with 0.1 M NaCl, 1 mM EDTA and 0.2% Triton X-100) on ice, and centrifuged at 10000 ***g*** at −10°C for 30 min. The pellet was discarded, and the supernatant was divided into aliquots that were used to determine total protein content [19].

Cathepsin L assay

Cathepsin L activity was determined in tissue extracts by Z-Phe-Arg-NHMec hydrolysis [20]. Enzyme assays were performed at pH 5.5. Acid-activatable cathepsin L was assayed after pretreatment at 37°C for 4 h at pH 3.8. Normal brain tissue and tumor tissue extracts (50 μg protein) were incubated with 50 μl of activation buffer (340 mM sodium acetate, 60 mM acetic acid, 4 mM disodium EDTA, pH 5.5, and freshly prepared 8 mM dithiothreitol) at 30°C for 1 min. The reaction was initiated by adding 50 μl of 20 μM substrate (Z-Phe-Arg-N H Mec) and incubation occurred at 37°C for 10 min. The enzymatic reaction was stopped by addition of 150 μl of 100 mM sodium monochloro acetate, 30 mM sodium acetate, and 70 mM acetic acid, pH 4.3 (made up to 2.0 ml with 1:1 stopping reagent and buffer-activator). Controls were performed by adding the enzyme after stopping reagent standards were prepared with 0.1–2.0 nM of 7-amino-4 methyl coumarin. The fluorescence was measured on a Shimadzu fluorescence spectrometer with excitation and emission wavelengths set at 370 and 460 nm, respectively. Cathepsin L activity was expressed as nmoles of methyl coumarin liberated per min per mg of protein. A specific and irreversible inhibitor, Z-Phe-Phe-CHN_2 [21] for cathepsin L was incorporated into the reaction mixture to confirm that the measured activities were indeed due to cysteine protease cathepsin L.

Western blotting

Normal brain tissue and brain tumor extracts (50 μg protein) were electrophoresed on a 12% SDS–polyacrylamide gel [22], followed by transfer of the proteins onto nitrocellulose paper according to the method of Towbin *et al.* [23]. The nitrocellulose paper was then incubated in blocking buffer for 2 h at room

temperature (1.5% bovine serum albumin, 0.15 M NaCl, 0.1 mM phenylmethyl sulfonyl fluoride, 20 mM Tris–HCl, pH 7.6) and washed with antibody buffer (0.3% bovine serum albumin, 0.15 M NaCl, 20 mM Tris–HCl, pH 7.6) three times at 10-min intervals. Stripes were then incubated with rabbit cathepsin L antibody (1:1000 dilution) at 4°C overnight or at room temperature for 2 h, washed as above, and incubated with a second antibody (anti-rabbit IgG peroxidase conjugate, 1:1000) for 2 h at room temperature. The strips were washed with Tris-HCl buffer as described above, and incubated with the substrate 2, 4-chloronaphthol in the dark for 15–30 min to develop color.

Enzyme-linked immunosorbent assay (ELISA)

Quantitative analysis of the content of cathepsin L in normal brain tissue and various grades of human glioma tissue extracts (50 μg) was performed by ELISA using cathepsin L specific antibodies. Tissue extracts and buffer containing cathepsin L were mixed with phosphate buffer and incubated overnight. The wells were washed with phosphate-buffered saline (PBS) and incubated with anti-cathepsin L antibody at 25°C for 3 h. The plates were washed and incubated with a second antibody, alkaline-phosphate conjugate, and the color was developed with p-nitrophenyl phosphate. The concentration of cathepsin L in the tissue extracts was determined based on a cathepsin L standard curve.

Immunohistochemistry for cathepsin L

Cathepsin L immunoreactivity was analysed in 10% formalin-fixed and paraffin-embedded sections using a cathepsin L-specific polyclonal antibody (rabbit anti-human cathepsin L polyclonal antibody; Athens Research and Technology, Athens, GA, USA). An appropriate concentration of the primary antibody was determined by titration on positive control tissues. Sections of 4 μm thick were cut and mounted on aminoethoxysilane-coated glass slides. Cathepsin L expression was detected by using an indirect avidin–biotin complex immunohistochemical method. The slides were dewaxed and blocked with normal goat serum. The sections were then incubated with rabbit anti-human cathepsin L polyclonal antibody diluted 1:1000 in 1% bovine serum albumin in PBS (10.4 μg/ml) overnight at 4°C in a humidified chamber. After a brief washing in buffer, the tissue samples were incubated in biotinylated goat anti-rabbit secondary antibody (Vector Laboratories, Burlingame, CA, USA) and streptavidin-alkaline phosphatase (Dako, Carpinteria, CA, USA). Alkaline phosphatase activity was visualized by the addition of a substrate solution consisting of new fuchsin, levamisole, and naphthol AS-BI phosphate (Sigma) in *N*,*N*-dimethylformamide, which forms an intense pinkish color in the cell cytoplasm. Sections were counterstained in hematoxylin, and coverslips were mounted with Aquamount (Lerner Laboratories, Pittsburgh, PA, USA). A control study was performed by using a normal rabbit immunoglobulin fraction (Dako) as the primary antibody instead of rabbit anticathepsin L.

Invasion assay

Glioblastoma cell invasion was carried out using a method described previously [24, 25]. The glioblastoma cell lines (U251, UWR1 and UWR2) were incubated with various concentrations of cathepsin L antibody and the invasion assay was performed as described previously [24, 25]. We also stained the invaded cells through Matrigel in the presence and absence of cathepsin L antibody.

Results

Enzyme activity assay

Cathepsin L activity was determined in normal brain tissue and tumor tissue extracts at pH 5.5 (Figure 1). The activity of cathepsin L was 2- to 3-fold higher in anaplastic astrocytoma ($P<0.001$) and 6- to 7-fold higher in glioblastoma ($P<0.0001$) than in normal brain tissues and low-grade gliomas.

Western blotting

The molecular size of cathepsin L in normal brain tissue and glioma tissue extracts was determined by

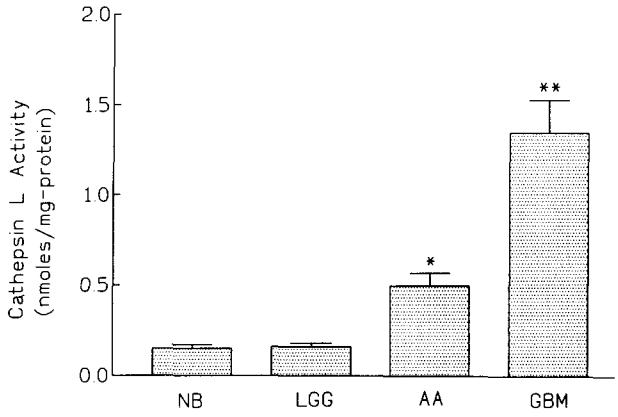

Figure 1. Activity of cathepsin L in normal brain and various types of human gliomas. The enzyme activity was expressed as nmoles of methyl coumarin liberated/min/mg of protein. Each value is the mean ±SD of five different tumors from each group. NB, normal brain tissue; LGG, low-grade glioma; AA, anaplastic astrocytoma; GBM, glioblastoma. $*P<0.001$; $**P<0.0001$.

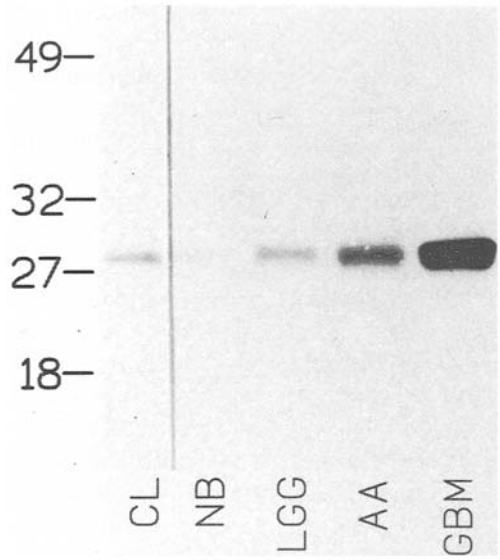

Figure 2. Western blots of normal brain tissue and glioma tissue tumor extracts. Tissue extracts (50 μg) and purified cathepsin L were subjected to SDS–PAGE polyacrylamide gel electrophoresis. The resulting proteins were transferred to nitrocellulose and detected as described in Materials and methods. CL, cathepsin L; NB, normal brain tissue; LGG, low-grade glioma; AA, anaplastic astrocytoma; GBM, glioblastoma.

SDS–PAGE, followed by Western blotting using a specific antibody for cathepsin L (Figure 2). From the Western blot the prominent cathepsin L at M_r 29 000 band was present in low-grade gliomas, anaplastic astrocytomas and glioblastomas. The M_r 29 000 cathepsin L band was faintly detectable in normal brain tissue extracts. Based on the intensity of the M_r 29 000 band, it is apparent that there is an increase in cathepsin L protein with progression and histological grade of gliomas (glioblastomas > anaplastic astrocytomas > low-grade gliomas).

ELISA

Using specific antibodies for cathepsin L, we determined the levels of cathepsin L in normal brain tissue and tumor extracts by ELISA. Figure 3 shows that cathepsin L was increased in anaplastic astrocytomas 3-fold ($P < 0.001$) and glioblastomas 6-fold ($P < 0.0001$) compared to normal brain tissue and low-grade glioma.

Immunohistochemical localization of cathepsin L in human brain tumors and normal brain tissues

Using paraffin-embedded sections by immunohistochemical analysis we determined the relative levels of expression and the distribution of cathepsin L in the tumor and normal brain tissue. Antibodies against cathepsin L showed diffuse, strong immunoreactivity in glioblastomas. Immunoreactivity was localized to tumor cells (Figure 4a, b). Adjacent normal brain exhibited little detectable immunoreactivity (Figure 4b). The endothelial cells within capillaries and larger vessels in the tumor tissues were devoid of cathepsin L immunoreactivity. Anaplastic astrocytomas displayed weak, focal immunoreactivity in scattered tumor cells (Figure 4c). Low-grade astrocytomas showed almost no immunoreactivity, except for two samples out of five that showed a few areas with weak immunostaining (Figure 4d), whereas normal CNS white matter exhibited almost no immunoreactivity (Figure 4e). In benign meningiomas, only one sample showed focal weak immunoreactivity in tumor cells, whereas the remaining four cases were negative (Figure 4f). There was no detectable staining of the tumor cells in the presence of a nonspecific rabbit IgG instead of primary antibody (data not shown). These results correlate well with the ELISA and Western blot analyses and demonstrate that there are higher levels of cathepsin L protein in glioblastomas and anaplastic astrocytomas and low levels in low-grade gliomas, normal brain tissues, and benign meningiomas.

Inhibition of glioblastoma cell invasion by cathepsin L antibody

Cathepsin L antibody at a concentration of 20 μg/ml inhibited 90–95% of glioblastoma cell invasion through Matrigel (Figure 5) and the number of cells stained in the lower filter at a higher concentration of cathepsin L antibody was less compared to controls without antibody (Figure 6).

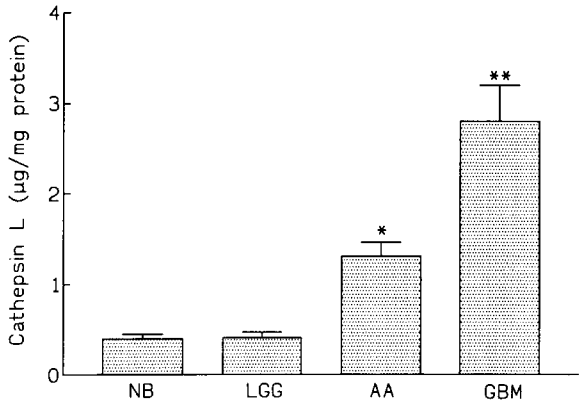

Figure 3. Cathepsin L content in normal brain tissues and in tumor extracts was determined by ELISA using cathepsin L-specific antibodies. The data are shown as mean ± SD of five different samples from each group. NB, normal brain tissue; LGG, low-grade glioma; AA, anaplastic astrocytoma; GBM, glioblastoma. *$P < 0.001$; **$P < 0.0001$.

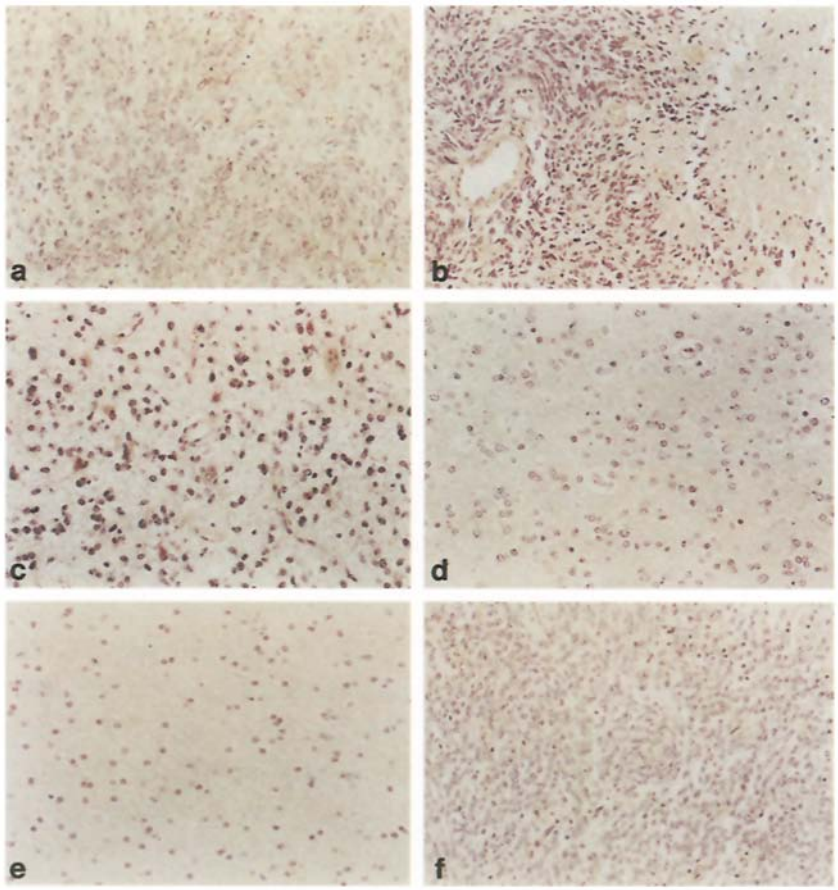

Figure 4. Immunohistochemical localization of cathepsin L in various types of human astrocytomas, benign meningiomas and normal brain tissue, using cathepsin L-specific antibody, performed as described in Materials and methods. (a) Cathepsin L immunoreactivities in a glioblastoma. (b) Glioblastoma and adjacent normal brain tissue. (c) Anaplastic astrocytoma. (d, e, f) Low-grade astrocytoma, normal white matter, and meningioma, respectively. × 260.

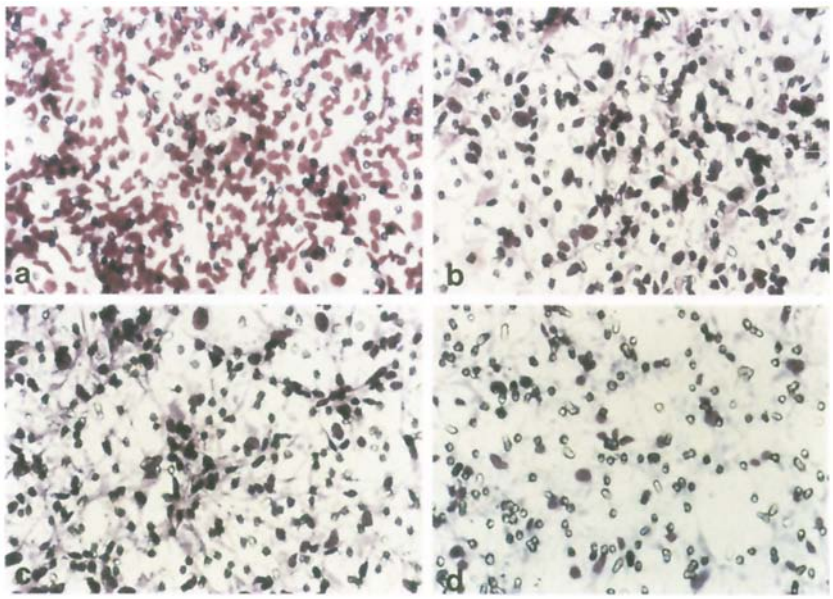

Figure 6. The invaded cells were stained without cathepsin L antibody (a), and with 5, 10 and 20 μg/ml of cathepsin L antibody (b, c and d, respectively).

Discussion

The possible involvement of metallo, serine and cysteine proteinases and their inhibitors in degradation of the BM and regulation of fibrinolysis during physiological and pathological conditions *in vivo* and *in vitro* have been reported previously [1]. In particular, there is increasing evidence correlating the invasive or meta-static abilities of a number of cell lines and tumors with increased activities of the cysteine proteases cathepsin B and cathepsin L [3, 4, 26, 27]. In normal cells these enzymes are targeted to lysosomes, but in malignant cells where they are overexpressed, they are also secreted or associated with the plasma membrane where they can interact with the extracellular environ-ment. Extracellular cell-

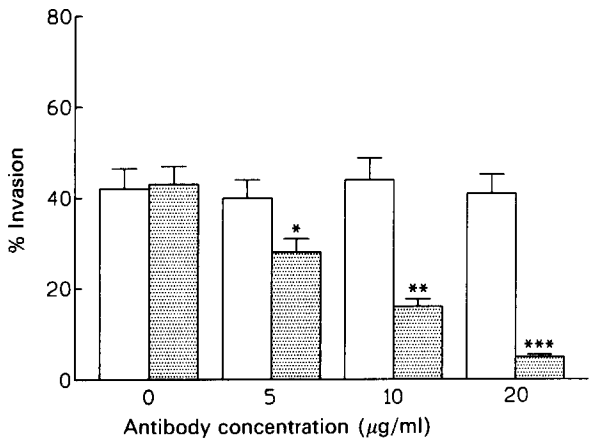

Figure 5. Invasion assays with glioblastoma cells in the presence of cathepsin L antibody. Glioblastoma cells were first plated on Matrigel-coated transwell clusters with different concentrations of cathepsin L antibody and irrelevant antibody. Columns (bars) represent the mean values (±SD) from five experiments. $*P < 0.05$; $**P < 0.001$; $***P < 0.0001$. □, U251 cells + irrelevant antibody; ▨, U251 cells + cathepsin L antibody.

associated cathepsin B can degrade BM components either directly or indirectly by activating pro-uPA and pro-type IV collagenase [10, 28]. Our previous studies on various types of human intracranial tumors showed that there were higher cathepsin B activities in glioblastomas that in normal brain tissues and low-grade gliomas and moderate levels in anaplastic astrocytomas [17]. Immunohistochemical localization of cathepsin B showed intense immunoreactivity in tumor cells and endothelial cells of glioblastomas and anaplastic astrocytomas but only weak expression of cathepsin B in low-grade gliomas and normal brain tissues [17].

Overexpression of cathepsins has been reported in a number of transformation and metastatic systems. For example, in *ras*-transformed metastatic 10T 1/2 cells, cathepsin L levels are higher than in nontransformed cells [29], and inhibitors of cathepsin L reduced the invasive abilities of a number of cell lines [30]. Chambers *et al.* [31] reported that in *ras*-transformed NIH 3T3 cells, the levels of both cathepsin B and cathepsin L mRNA were increased in metastatic cells. They found that the association of metastatic properties with cathepsin L levels was stronger than the association with cathepsin B, suggesting that cathepsin L may be a good marker for tumor progression. Cathepsin L purified from human liver has been compared with that isolated from rabbit and rat liver [32], and it was observed that cathepsin L from the three different species have similar properties. However, the presence of cathepsin L in normal human brain and brain tumor tissues has not been reported. Rozhin *et al.* [18] have found an association of native and acid-activatable cathepsin L with metastasis of human and murine melanomas. The finding of plasma membrane fraction with cathepsin L activity suggests that this enzyme may participate in a focal pericellular proteolysis of the BM during the metastatic process [18].

We have examined whether expression of cathepsin L is associated with malignant progression in astrocytomas *in vivo* and whether overexpression of cathepsin L is associated with the invasive properties of malignant primary CNS tumors. We found significantly higher levels of cathepsin L activity in glioblastomas than in low-grade gliomas and normal brain tissues. An attempt to prove that the increased activity of an enzyme is not a simple reflection of increased cellularity secondary to increased malignancy, prompted several studies, such as normalizing cellular enzymatic content by comparing enzymatic activity with total protein content and DNA *in vitro*, because comparing enzymatic activity with DNA might be problematic *in vivo*. Normalization using an aneuploid state of tumor DNA is not accurate in an *in vivo* system. Immunohistochemical studies clearly showed that cathepsin L activity was upregulated in tumor cells in malignant astrocytomas. We also found inhibition of glioblastoma cell invasion in the presence of cathepsin L antibody. The rate of synthesis and secretion of cathepsin L is increased following malignant transformation by several different oncogenes [33]. A number of different growth factors like PDGF and EGF [34] and tumor promoters such as phorbol myristate acetate (PMA or TPA) have been shown to increase cathepsin L expression in mouse fibroblast 3T3 cells [35]. In addition, increased synthesis or secretion of cathepsin L has been reported during activation of macrophages [36]. Cathepsin L may also control growth regulation in cells by modulating the levels of certain proteins involved in signal transduction [37], and its overexpression by transformed cells might be related to their abnormal growth properties. Cathepsin L secreted by cancer cells has also been shown to interfere with antigen processing [38]. Thus increased amounts of cathepsin L and its increased localization in human brain tumor samples during glioma progression may indicate that it is intimately related to the malignant transformation of progression and malignancy of CNS tumors.

Acknowledgements

We thank Alan Rayford for technical help, Norma Adams for preparing the manuscript, and Leslie

Wildrick for reviewing the manuscript. Support for these studies was provided, in part, by NCI grant CA 56792 and ACS grant EDT-91 (to J. S. R.).

References

1. Stetler-Stevenson WG, 1990, Type IV collagenases in tumor invasion and metastasis. *Cancer Metastasis Rev*, **9**, 289–303.
2. Danø K, Andreasen PA, Grøndahl-Hansen J, Kristensen P, Nielsen LS and Skriver L, 1985, Plasminogen activators, tissue degradation and cancer. *Adv Cancer Res*, **44**, 139–66.
3. Sloane BF, Moin K, Krepela E and Rozhin J, 1990, Cathepsin B and its endogenous inhibitors: the role in tumor malignancy. *Cancer Metastasis Rev*, **9**, 333–52.
4. Chauhan SS, Goldstein LJ and Gottesman MM, 1991, Expression of cathepsin L in human tumors. *Cancer Res*, **51**, 1478–81.
5. Rochefort H, 1990, Biological and clinical significance of cathepsin D in breast cancer. *Semin Cancer Biol*, **1**, 153–60.
6. Nicolson GL, Nakajima M, Herrmann JL, *et al.* 1994, Malignant melanoma metastasis to brain: role of degradative enzymes and responses to paracrine growth factors. *J Neuro-Oncol*, **18**, 139–49.
7. Briozzo P, Morisset M, Capony F, Rougeot C and Rochefort H, 1988, *In vitro* degradation of extracellular matrix with M_r 52000 cathepsin D secreted by breast cancer cells. *Cancer Res*, **48**, 3688–92.
8. Nishimura Y, Kawabata T and Kato K, 1988, Identification of latent procathepsins B and L in microsomal lumen: characterization of enzymatic activation and proteolytic processing *in vitro*. *Arch Biochem Biophys*, **261**, 64–71.
9. Nishimura Y and Kato K, 1988, Identification of latent procathepsin H in microsomal lumen: characterization of proteolytic processing and enzyme activation. *Arch Biochem Biophys*, **260**, 712–18.
10. Baricos WH, Zhou Y, Mason RW and Barrett AJ, 1988, Human kidney cathepsins B and L: characterization and potential role in degradation of glomerular basement membrane. *Biochem J*, **252**, 301–4.
11. Bohley P, Kirschke H, Schaper S and Wiederanders B, 1984, Principle of the regulation of intracellular proteolysis. *Symposia Biologocia Hungarica*, **25**, 101–18.
12. Mason RW, Johnson DA, Barrett AJ and Chapman HA, 1986, Elastinolytic activity of human cathepsin L. *Biochem J*, **233**, 925–7.
13. Kominami E, Il K and Katunuma N, 1987, Activation of the intramyofibral autophagic lysosomal system in muscular dystrophy. *Am J Pathol*, **127**, 461–6.
14. Tsuchida K, Aihara H, Isogai K, Hanada K and Shibata N, 1986, Degradation of myocardial structural proteins in myocardial infarcted dogs is reduced by Ep459, a cysteine proteinase inhibitor. *Biol Chem Hoppe-Seyler*, **367**, 39–45.
15. Olbricht CJ, Cannon JK, Garg LC and Tisher CC, 1986, Activities of cathepsins B and L in isolated nephron segments from proteinuric and nonproteinuric rats. *Am J Physiol*, **250**, F1055–F1062.
16. McCormick D, 1993, Secretion of cathepsin B by human gliomas *in vitro*. *Neuropathol Appl Neurobiol*, **19**, 146–51.
17. Sivaparvathi M, Sawaya R, Wang SW, *et al.* 1995, Overexpression and localization of cathepsin B during the progression of human gliomas. *Clin Exp Metastasis*, **13**, 49–56.
18. Rozhin J, Wade RL, Honn KV and Sloane BF, 1989, Membrane associated cathepsin L: a role in metastasis of melanomas. *Biochem Biophys Res Commun*, **164**, 556–61.
19. Bradford MM, 1976, A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt Biochem*, **72**, 248–54.
20. Barrett AJ and Kirschke H, 1981, Cathepsin B, cathepsin H, and cathepsin L. *Method Enzymol*, **80**, 535–61.
21. Kirschke H and Shaw E, 1981, Rapid inactivation of cathepsin L by Z-Phe-Phe-CHN_2 and Z-Phe-Ala-CHN_2. *Biochem Biophys Res Commun*, **101**, 454–8.
22. Laemmli UK, 1970, Cleavage of structural proteins during the assembly of the head of bacteriophage T-4. *Nature*, **227**, 680–5.
23. Towbin H, Staehelin T and Gordon J, 1979, Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci, USA*, **76**, 4350–4.
24. 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.
25. Mohanam S, Sawaya R, McCutcheon I, Ali-Osman F, Boyd D and Rao JS, 1993, Modulation of *in vitro* invasion of human glioblastoma cells by urokinase-type plasminogen activator receptor antibody. *Cancer Res*, **53**, 4143–47.
26. Sloane BF, Rozhin J, Hatfield JS, Crissman JD and Honn KV, 1987, Plasma membrane-associated cysteine proteinases in human and animal tumors. *Exp Cell Biol*, **55**, 209–24.
27. Rozhin J, Robinson D, Stevens MA, *et al.* 1987, Properties of a plasma membrane associated cathepsin B-like cysteine proteinase in metastatic B16 melanoma variants. *Cancer Res*, **47**, 6620–8.
28. Kobayashi H, Schmitt M, Goretzki L, *et al.* 1991, Cathepsin B efficiently activates the soluble and the tumor cell receptor bound form of the proenzyme urokinase-type plasminogen activator (Pro uPA). *J Biol Chem*, **266**, 5147–52.
29. Denhardt DT, Greenberg AH, Egan SE, Hamilton RT and Wright JA, 1987, Cysteine proteinase cathepsin L expression correlates closely with the metastatic potential of H-*ras*-transformed murine fibroblasts. *Oncogene*, **2**, 55–9.
30. Yagel S, Warner AH, Nellans HN, Lala PK, Waghorne C and Denhardt DT, 1989, Suppression by cathepsin L

inhibitors of the invasion of amnion membranes by murine cancer cells. *Cancer Res*, **49**, 3553–7.
31. Chambers AF, Colella R, Denhardt DT and Wilson SM, 1992, Increased expression of cathepsins L and B and decreased activity of their inhibitors in metastatis *ras*-transformed NIH 3T3 cells. *Molec Carcino*, **5**, 238–45.
32. Mason RW, Green GDJ and Barrett AJ, 1985, Human liver cathepsin L. *Biochem J*, **226**, 233–41.
33. Gottesman MM, 1978, Transformation-dependent secretion of a low molecular weight protein by murine fibroblasts. *Proc Natl Acad Sci, USA*, **75**, 2767–71.
34. Scher CD, Dick RL, Whipple AP and Locatell KL, 1983, Identification of a BALB/c-3T3 cell protein modulated by platelet-derived growth factor. *Molec Cell Biol*, **3**, 70–81.
35. Gottesman MM and Sobel ME, 1980, Tumor promoters and Kirsten sarcoma virus increase synthesis of a secreted glycoprotein by regulating levels of translatable mRNA. *Cell*, **19**, 449–55.
36. Portnoy DA, Erickson AH, Kochan J, Ravetch JV and Unkeless JC, 1986, Cloning and characterization of a mouse cysteine proteinase. *J Biol Chem*, **261**, 14697–703.
37. Laumas S, Abdel-Ghany M, Leister K, Resnick R, Kandrach A and Racker E, 1989, Decreased susceptibility of a 70-kDa protein to cathepsin L after phosphorylation by protein kinase C. *Proc Natl Acad Sci, USA*, **86**, 3021–5.
38. McCoy K, Gal S, Schwartz RH and Gottesman MM, 1988, An acid protease secreted by transformed cells interferes with antigen processing. *J Cell Biol*, **106**, 1879–84.