REVIEW ARTICLE

Treatment of Malignant Gliomas by Therapies Against Matrix Metalloproteinases

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INTRODUCTION

Malignant gliomas (glioblastoma multiforme and anaplastic astrocytomas) are the most common primary intracranial neoplasms and affect about 1500 Canadians annually (1,2). They are the second leading cause of cancer death in children. Despite intensive therapy (surgical resection followed by radiation or chemotherapy), they remain universally fatal with a median survival of only 12 months. Their prognosis has remained virtually unchanged for the past three decades. The remarkable advances in molecular biology, neurosurgical techniques, neuroimaging, radiation and chemotherapy have not yet contributed to improved patient outcomes (3,4).

Malignant gliomas are highly invasive and vascular tumors (5,6). They characteristically invade along basement membrane structures (blood vessels and the glial limitans externa) and myelinated (white) fibre tracts; microscopic spread into the contralateral hemisphere is not uncommon at the time of initial diagnosis (7,8). Their invasive behavior renders them surgically incurable. Even when successfully excised they tend to recur within a three centimeter margin of the resection cavity over 95% of the time (9,10). Neovascularization at the invading tumor edge recruits an increased blood supply to the metabolically active neoplastic cells. It may also facilitate invasion of glioma cells into the normal brain parenchyma. Interestingly, malignant gliomas only rarely metastasize outside the central nervous system (11). The explanation for this nonmetastatic phenotype is unknown but may be due to their inability to invade across basement membrane structures (for example, those surrounding blood vessels) despite their capacity to use them as permissive guiding structures for dissemination (5). This propensity for local invasion of the brain parenchyma is one of the greatest impediments to current locoregional therapies (surgical resection and radiation) (6).

Until recently, the emphasis in cancer research has been on mutations in critical growth control genes, i.e., oncogenes and tumor suppressor genes (6). Glioma research is not an exception, and a barrage of mutations has been identified in these tumors (12). A large proportion of these mutated genes encode growth factors, their receptors, or specific tumor suppressors. The resulting malignant phenotype is one of rapid and dysregulated proliferation. However, this is not the only hallmark of malignancy. Gliomas are also extremely vascular and invasive tumors. The genes mediating these processes in malignant gliomas are probably not mutated, but instead have altered regulation (13). Therefore, the dysregulation of a normal physiologic process rather than a tumor specific phenomenon seems to be responsible for the invasive and angiogenic phenotypes (14,15). The current authors believe that a better understanding of these processes will lead to targeted anti-invasive and anti-angiogenic therapies.

This review discusses matrix metalloproteinases (MMPs) in glioma biology. MMPs have been implicated in a wide range of physiologic and pathologic processes that involve the breakdown of the extracellular matrix (ECM) (16,17). In particular, the roles of gelatinase-A (MMP-2) and -B (MMP-9) in glioma invasion will be highlighted. New compounds with anti-MMP activity are already under development

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Table 1. The family of vertebrate matrix metalloproteinases (MMPs)[a].

Subgroups and MMP Family Members	MMP Number
Collagenases	
Interstitial Collagenase (Fibroblast type)	MMP-1
Neutrophil Collagenase	MMP-8
Collagenase-3	MMP-13
Collagenase-4 (Xenopus)	-
Stromelysins	
Stromelysin-1	MMP-3
Stromelysin-2	MMP-10
Stromelysin-3	MMP-11
Gelatinases	
Gelatinase-A (72 kDa Type IV collagenase)	MMP-2
Gelatinase-B (92 kDa Type IV collagenase)	MMP-9
Membrane-Type MMPs	
MT1-MMP	MMP-14
MT2-MMP	MMP-15
MT3-MMP	MMP-16
MT4-MMP	MMP-17
Others	
Matrilysin	MMP-7
Metalloelastase	MMP-12
Novel human MMP (unnamed)	-

[a] MMPs 4 to 6 were found to be identical to other members of the MMP family and these designations are no longer in use.

that target these enzymes. They will not only be useful in the treatment of brain cancers, but theoretically any disease in which MMPs have been shown to play important roles; for example, arthritis, multiple sclerosis and stroke.

THE FAMILY OF MMPs AND TISSUE INHIBITORS OF METALLOPROTEINASE

Integrity of the ECM is dependent on a balance between synthesis and degradation. This is governed by the interplay between matrix-degrading proteases (MMPs, serine proteases and cysteine proteases) and their inhibitors. Although members from all groups of proteases have been implicated in glioma biology, MMPs have been the most extensively studied (6,13,14,18).

MMPs are a large family of zinc-dependent endopeptidases (Table 1). At last count, 16 members have been defined that share extensive sequence homology. They are divided into subfamilies, based on substrate specificity, that include the collagenases, stromelysins and gelatinases (or type IV collagenases). A defining feature of these classical subfamilies is that they are secreted as proenzymes (6,19). Recently, a novel membrane-bound family of MMPs called membrane-type MMPs (MT-MMPs) has been characterized (20,21). Together, the MMPs are capable of degrading all protein constituents of the ECM and basement membrane structures. It is not surprising that these powerful degradative enzymes have been implicated in a wide range of physiologic and pathologic processes, including cancer invasion and metastasis (Table 2) (6,19).

MMPs are regulated at multiple levels including gene transcription, enzymatic cleavage and endogenous inhibition by tissue inhibitors of metalloproteinase (TIMP) (6,18,19). To date, the TIMP family consists of four members (TIMPs 1 to 4) (25,26). Although all members share the ability to inactivate MMPs in solution-based assays, there are several important differences between them. For example, TIMP-1 and -2 may have important and specific physiologic roles in the regulation of gelatinase-B and -A, respectively (23-25). More recently, TIMP-4 has also been implicated in the activation cascade of gelatinase-A (27).

Although both increases and decreases in TIMP expression or activity have been associated with the malignant phenotype, it is clear that an imbalance between MMP and TIMP activity favoring net proteolysis is critical in tumor growth, invasion, metastasis and angiogenesis (see below). Furthermore, it is now clear that the classical view of MMPs and TIMPs as only being involved in the regulation of ECM turnover is a narrow one. For example, a growing body of evidence suggests that the role of TIMPs in cellular biology is much more complex. They affect cellular proliferation and influence the invasive process independent of their MMP-inhibitory activity (28). TIMPs are clearly multifunctional molecules whose functional spectrum is yet to be understood (24,28).

A common cysteine switch mechanism of MMP activation has been described (Figure 1) (22-24). According to this model, a critical cysteine-zinc bond between the enzyme's prodomain and catalytic domain, respectively, maintains proenzyme latency. Disruption of this bond (either by proteolytic cleavage of the prodomain or binding by organomercurials) is a prerequisite for enzyme activation. The gelatinase family (gelatinase-A and -B) will be the focus of discussion because of its major involvement in invasion and angiogenesis. This family exemplifies two fundamentally different strategies that are used to control MMP activation (summarized in Figure 2) (24).

Progelatinase-A Activation

Unlike progelatinase-B, progelatinase-A is constitutively expressed by a myriad of cell types. It lacks the required trans-activator sequences (AP-1 and

Table 2. The role of matrix metalloproteinases in physiologic and disease states that involve disruption of the extracellular matrix and basement membrane structures.

Physiologic Processes
Ovulation
Blastocyst implantation
Embryogenesis
Mammary development
Fetal membrane rupture
Bone growth and remodeling
Macrophage function
Neutrophil function
Cellular differentiation
Cellular migration
Apoptosis
Angiogenesis
Wound healing
Disease States
Cancer invasion
Tumor metastasis
Rheumatoid arthritis
Periodontitis
Fibrotic lung disease (70)
Pulmonary lymphangioleiomyomatosis (71)
Bone destruction in multiple myeloma
Abdominal Aortic Aneurysms (72)
Atherosclerosis / Atheromata rupture (73)
Chronic venous ulcers (74)
Gastric ulcers
Glomerulonephritis (75)
Pathologic (tumor-associated) angiogenesis

Modified from (6) with permission.

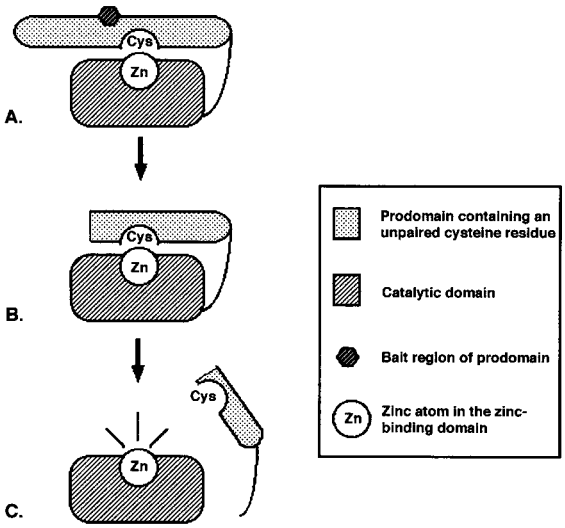

Figure 1. The cysteine switch mechanism of matrix metalloproteinase activation. **A:** A critical cysteine-zinc bond between the enzyme's prodomain and catalytic domain, respectively, maintains proenzyme latency. **B:** Proteolytic cleavage of the prodomain renders the molecule more susceptible to an autoproteolytic event. **C:** This results in disruption of the critical bond and enzyme activation.

PEA-3) in its promoter region that confer inducibility to various growth factors and cytokines. It is not surprising, therefore, that gelatinase-A production is regulated at the levels of proenzyme activation and inhibition by TIMPs and not primarily at the level of gene transcription (6,19,23).

MT1-MMP is a critical activator of progelatinase-A. Its recent discovery has led to the following model in which gelatinase-A activity might be concentrated at the cell surface (Figure 2A) (24,25). MT1-MMP is bound and inactivated by TIMP-2 by interaction with its catalytic domain. This complexed TIMP-2, however, is still free to bind progelatinase-A at a site distinct from its inhibitory domain. It thus recruits progelatinase-A to the cell surface (29,30). If TIMP-2 levels are low and unable to block all MT1-MMP activity, a second MT1-MMP will activate membrane-bound progelatinase-A. Gelatinase-A may function while bound to the cell surface in this fashion or be released to effect ECM turn-over. Eventually, gelatinase-A will be inactivated by free TIMP-1 to -4. On the other hand, if TIMP-2 concentrations are high and block all MT1-MMP activity, progelatinase-A will not be activated. Therefore, the availability of TIMP-2 both concentrates gelatinase-A activity at the cell surface and controls how long the enzyme will remain active.

Progelatinase-B Activation

In contrast to progelatinase-A, transcriptional regulation of progelatinase-B is critical (6,19,23,24). The promoter region of gelatinase-B possesses the AP-1 and PEA-3 trans-activator sequences. These promoter elements link the various mutations in growth factors and their receptors to the invasive phenotype (6,13). An active area of research is the development of strategies that interrupt second messenger pathways downstream of growth hormone receptors and decrease MMP expression.

Unlike the membrane-bound activation sequence described above, progelatinase-B activation is quite promiscuous (19,24). It is activated directly by serine proteases and some MMPs including stromelysin-1 (MMP-3) and gelatinase-A. It is also proposed that a member of the serine protease family, pro-urokinase-type plasminogen activator (pro-uPA), is secreted from cells and activated by a membrane-bound receptor designated uPAR on the surface of neoplastic cells (Figure 2B). Once membrane-bound activated "two-chain" uPA can cleave plasminogen to plasmin. Plasmin is capable of cleaving many constituents of the

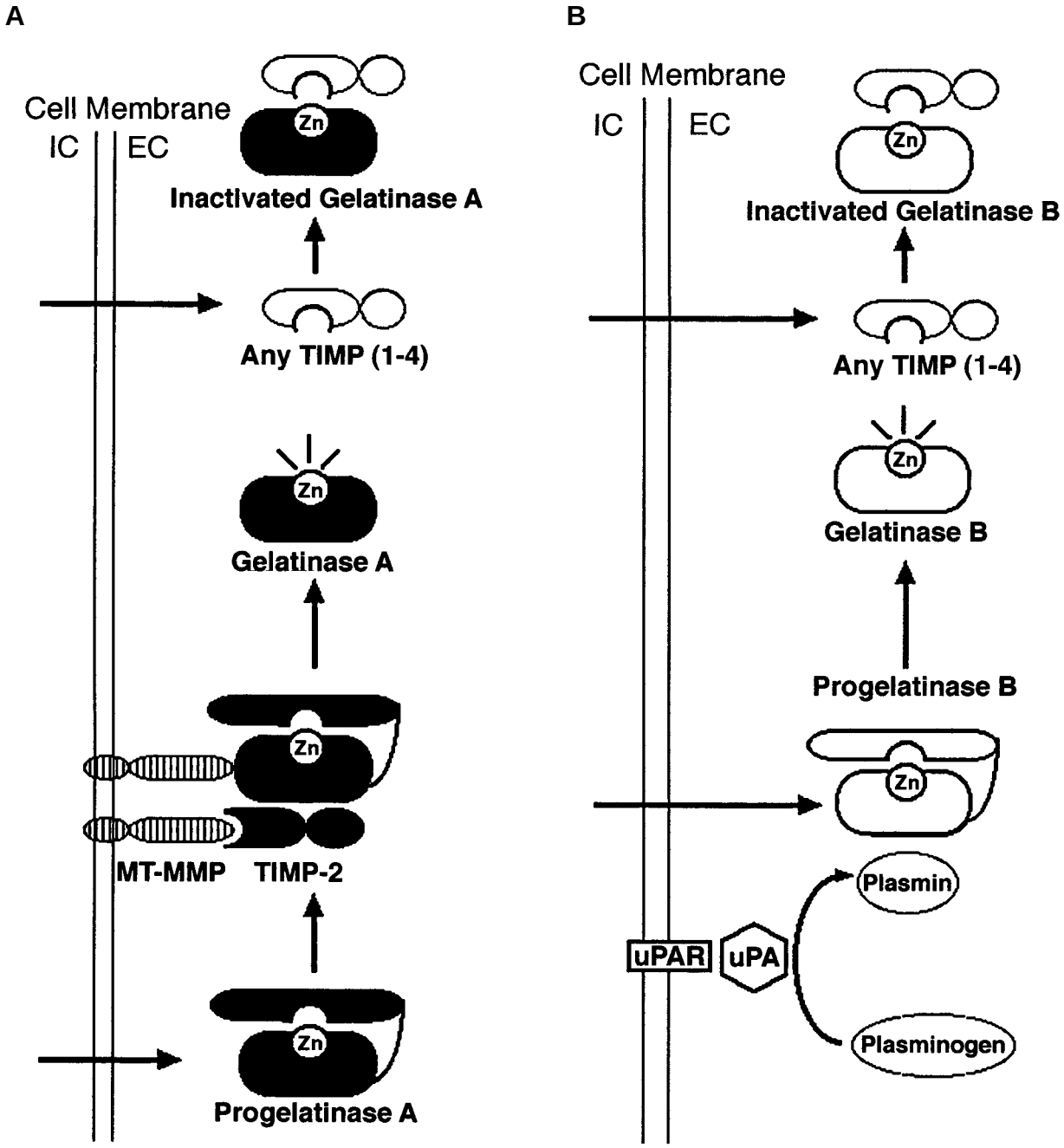

Figure 2. Different activation cascades for progelatinase-A and -B. **A:** MT-MMPs are the critical activators of progelatinase-A. In order to recruit free progelatinase-A to the cell surface, this membrane-bound protein binds TIMP-2 through its catalytic domain and is inactivated. This complexed TIMP-2 retains its ability to bind progelatinase A at a site distinct from its catalytic domain and thus can recruit it into an MT-MMP/TIMP-2/progelatinase-A complex. This complex functions to bring progelatinase-A into close association with other membrane-bound MT-MMPs which activate progelatinase-A. **B:** In contrast, progelatinase-B activation is quite promiscuous and is mediated by both serine proteases and other MMPs. The membrane-localized pro-urokinase-type plasminogen activator system directly activates progelatinase-B and indirectly activates it by first activating stromelysin-1 (MMP-3) (not shown). Similar to the role of TIMP-2 in progelatinase-A activation, TIMP-1 has been implicated in the activation of progelatinase-B (not shown). **EC**: extracellular; **IC**: intracellular; **MT-MMP**: membrane-type matrix metalloproteinase; **TIMP**: tissue inhibitor of metalloproteinases; **uPA**: urokinase-type plasminogen activator; **uPAR**: urokinase-type plasminogen activator receptor.

ECM and several MMPs including stromelysin-1, a known activator of progelatinase-B. The pro-uPA system can also activate progelatinase-B directly. Similar to the dual role of TIMP-2 in gelatinase-A activation, recent evidence suggests that TIMP-1 interacts with progelatinase-B at a site distinct from its catalytic domain (19,25). This may have special significance in its activation.

MMPs, TIMPs AND GLIOMA BIOLOGY

In the early 1980s, Liotta and colleagues demonstrated that increased MMP activity was associated with the malignant potential of B16 melanoma cells (31). Since then, the expression of MMPs has been directly correlated with the invasive phenotype and metastatic potential of an increasing number of solid tumors including those of the lung (32), breast (33,34), ovary (35), prostate (36), colon (37,38), stomach (39), thyroid (40), and head and neck (41). Their involvement has even been implicated in the pathogenesis of multiple myeloma (42), lymphomas (33,43-45) and the leukemias (46). It is only more recently that interest has focused on the role of MMPs in the invasive potential of malignancies of the central nervous system (6,47).

Several laboratories have found an association between MMPs and the invasive properties of malignant gliomas. In 1993, Rao and colleagues demonstrated an eight- to 10-fold increase in gelatinase-B activity in human glioblastomas when compared with lower-grade brain tumors and normal brain tissue (48). Subsequently, gelatinase-A mRNA, protein and gelatinolytic activity were found to be significantly higher in malignant gliomas than in low-grade and non-neoplastic brain tissue (11,49-51). This suggested that gelatinases may be important in glioma biology. In a comprehensive study of 41 human brain tumors that included 11 malignant gliomas, six metastatic tumors and 24 low-grade brain tumors, Nakagawa and colleagues confirmed that the 17 high-grade tumors demonstrated significantly higher gelatinolytic activity and positive immunostaining for MMP-1, -2, -3 and -9 compared to low-grade astrocytomas and normal brain tissue (52). In addition, TIMP-1 immunohistochemistry showed that it was not expressed in some malignant tumors. The non-invasive meningiomas and neurinomas expressed only moderate gelatinase activity but showed intense immunoreactivity for TIMP-1. Taken as a whole, this study suggested that the balance of MMPs and their inhibitors might determine the invasiveness of malignant gliomas. This was further supported by the observation that the most highly invasive of seven human astrocytoma cell lines was the only one to demonstrate a relative abundance of gelatinase-A and -B over TIMP-1 and -2 transcript levels. The least invasive astrocytoma cell line demonstrated a relative abundance of TIMP-1 and -2 transcripts over gelatinase-A and -B transcripts (53). The immunohistochemical localization of gelatinase-B and TIMP-1 to proliferating endothelial cells of glioblastomas suggests a central role for these proteins in tumor-related angiogenesis (52). The current authors' lab has recently confirmed this pattern of gelatinase-B expression by *in situ* hybridization and immunohistochemistry (54). However, gelatinase-A has a more universal distribution. This implies a more selective role for gelatinase-B in glioma angiogenesis.

An association, however, does not imply causality. Important experiments were conducted that showed that overexpression of gelatinase-A in non-transformed astrocytes resulted in increased invasive potential *in vitro*. Pharmacologic inhibition of MMPs resulted in an over 90% reduction in glioma invasiveness (55). These results suggest a causal role for gelatinase-A in glioma invasion.

Inhibition of the activation cascades of gelatinase-A and -B may represent an alternative strategy to effectively decrease gelatinolytic activity. It has been shown that MT1-MMP mRNA levels correlate with the expression and activation of gelatinase-A in the progression to malignancy of human gliomas *in vivo* (56). Furthermore, it has been demonstrated by immunohistochemistry that MT1-MMP expression is restricted to microglial cells of white matter (57). This suggests the possibility that the pattern of glioma invasion along white matter tracts *in vivo* may be due to the activation of gelatinase-A by microglial-associated MT1-MMP in the surrounding brain (5,6). In addition, the current authors' laboratory has recently demonstrated high levels of activated gelatinase-A in two rare patients with extraneural metastases from a malignant glioma (11). Taken together, these data suggest progelatinase-A activation is a critical event in the development of the invasive phenotype in human gliomas.

This membrane-localized model of progelatinase-A activation has two important implications for the therapy of malignant gliomas. First, the membrane-bound activation cascade of progelatinase-A effectively concentrates proteolytic activity at the invading edge of the tumor (6,13). This is consistent with immunohistochemical and *in situ* data from the current authors' laboratory (54) and immunohistochemical data from others that localize gelatinase-A expression to the tumor margin (52). Effective therapies must therefore be delivered in high enough concentrations that local inhibitory activity can counter the concentrated gelatinolytic activity at the tumor's invading front.

Second, pharmacologic manipulation of MT1-MMP expression and/or activity is an attractive target for control of progelatinase-A activation (13). Inhibition of protein kinase C (PKC), a second messenger upstream of MT1-MMP induction, can decrease gelatinase-A activity and the invasiveness of gliomas in an *in vitro* model (6,55). This highlights the therapeutic potential of targeting pathways involving MT1-MMP and gelatinase-A to reduce glioma invasion. It has also recently been shown that ablation of uPAR by antisense targeting markedly reduces the invasiveness of a glioblastoma cell line *in vitro* (58). This supports the hypothesis that therapeutic manipulation of the progelatinase-B activation cascade might be effective in the treatment of malignant gliomas.

Recently, the current authors' laboratory has investigated the pattern of expression of TIMPs 1 to 4 in 46 primary brain tumors including 26 malignant gliomas (54). It was demonstrated that TIMP-1 was strongly increased at the RNA level in high grade tumors, while TIMP-4 expression was highest in low and middle grade tumors. These results suggest a different role for these molecules in malignant gliomas. One possibility is that the expression of TIMP-1 is compensatory and increases in parallel to increases in expression of gelatinase-A and -B. This compensatory pattern of TIMP-1 gene expression has been observed in other cancers (28). Alternatively, TIMP-1 could be functioning as a growth factor and thereby contribute to the malignant phenotype. In contrast, downregulation of TIMP-4 may allow these tumors to invade widely. These results emphasize the complicated relationships of MMPs and their inhibitors in the biology of gliomas and the need to know the spatial and temporal distribution of their expression within the tumor. If the MMP/TIMP axis is to become an important target for therapeutic manipulation, these complex interactions and the distinct biologic roles of each protein must be more clearly defined (23-26).

INHIBITION OF MMPs IN THE TREATMENT OF MALIGNANT GLIOMAS

The evidence implicating MMPs in the development of malignant gliomas is growing. It has been demonstrated *in vitro* that pharmacologic manipulation of the activation cascades of gelatinase-A and -B results in the decreased invasive potential of gliomas (55,58). The balance between MMPs and TIMPs has also been implicated in the processes of angiogenesis and tumor cell growth (26,59). This is thought to be due at least in part to the release of angiogenic and growth factors from a partially degraded ECM. Consequently, anti-MMP strategies can potentially counter the three essential aspects of the malignant phenotype of human

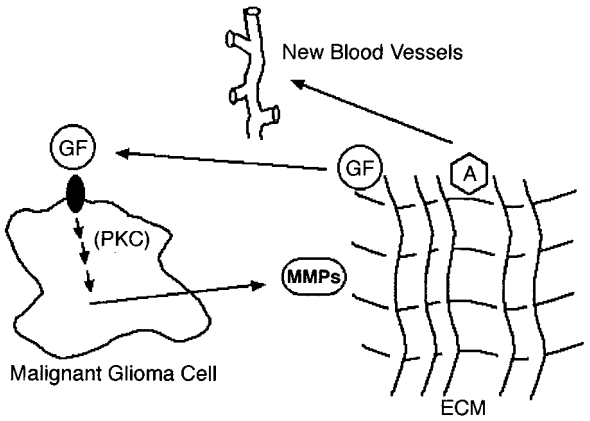

Figure 3. A model for the varied biologic effects of MMPs in glioma malignancy. MMP activity is inducible by PKC which is a second messenger downstream of growth factor receptors. Once activated MMPs can degrade the surrounding extracellular matrix and release growth and angiogenic factors. These growth factors can stimulate the production of more MMPs to establish a vicious cycle of increased cell growth, angiogenesis and invasion. Abbreviations: **A**: angiogenic factor; **ECM**: extracellular matrix; **GF**: growth factor; **MMP**: matrix metalloproteinase; **PKC**: protein kinase C

gliomas; namely, proliferation, angiogenesis and invasion (Figure 3).

Although TIMPs effectively neutralize the degradative activity of MMPs, their use as pharmacologic agents is unlikely because of their poor pharmacokinetics. Their use in gene therapy protocols where TIMP genes can be directly delivered to the tumor may prove more valuable. The current authors' lab is investigating this hypothesis for a number of TIMPs in murine glioma models. As an alternative, more than a dozen synthetic inhibitors have been developed by the pharmaceutical industry. The best characterized of these is Batimastat (BB-94) (60,61). The critical hydroxamate residue of this molecule binds to the zinc atom in the active site and results in potent, but reversible, inhibition of MMPs. Batimastat has shown early promise in inhibiting tumor growth and metastasis in xenograft models of human ovarian (62) and colorectal (63,64) carcinomas. In addition, Batimastat inhibited murine hemangioma growth suggesting it may reduce angiogenesis *in vivo* (65). The current authors' lab has recently shown that the administration of a synthetic hydroxamate MMP inhibitor significantly reduced the growth and invasion of human glioma cells implanted subcutaneously in SCID/NOD mice (Figure 4). These results underline the promise of this class of agents in controlling glioma growth and spread.

Although these drugs were initially hypothesized to act primarily by inhibiting tumor invasion, it is now clear that they also have important growth inhibitory activity (28). This may be due to the decreased

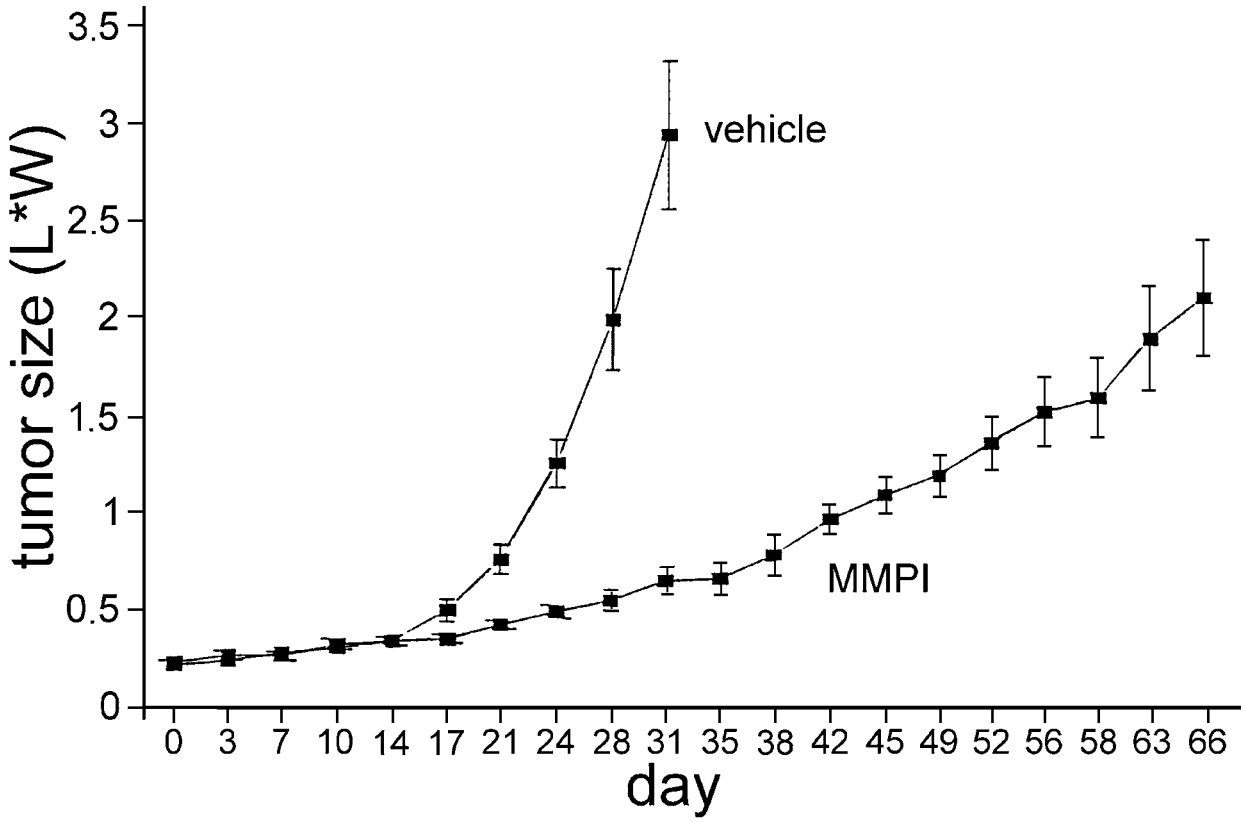

Figure 4. Inhibition of tumor growth in a murine orthostatic model by a synthetic hydroxamate MMP inhibitor. U87 glioma cells were grown in SCID/NOD mice. One group of eight mice received the MMP inhibitor and the other received a vehicle control. **MMPI**: matrix metalloproteinase inhibitor. Data are from Price et al. (76).

recruitment of blood supply by the tumor and decreased release of growth factors in the ECM during matrix turnover, or a direct anti-proliferative effect on the tumor cells. The mechanism that these anti-invasive drugs use to markedly reduce tumor growth in animal models is unknown. Clinical trials of these synthetic inhibitors in cancer patients began in 1990 and a large number are underway including a single phase III, randomized placebo-controlled trial in glioma patients (66,67).

Despite the promise of this approach, MMP inhibition may produce some unwanted effects. Since MMPs are critical in normal physiologic remodeling of the ECM, gross inhibition of MMP activity might lead to dysregulated cell homeostasis. It may also interfere with the migration and activity of inflammatory cells which play important roles in host defense against tumor cells (68). However, one of these synthetic inhibitors, Marismastat (BB-2516), is well-tolerated in healthy volunteers and produces few side effects (69).

The anti-angiogenic properties of MMP inhibitors might not only restrict an important nutrient supply for tumor cells, but might also diminish the number of permissive pathways into normal parenchyma for tumor cell dissemination. This may indirectly reduce glioma invasion. Also, the MMP inhibitors have dramatic growth inhibitory activity in animal cancer models. Consequently, it may be possible to maintain tumor foci, primary and secondary, in a state of pseudo-dormancy (14,15). Although the patient would not be cured of their cancer, they could continue to live with it for many years. Anti-MMP strategies might halt continued tumor invasion and save critical brain structures (for example, motor and speech areas) from involvement and thereby dramatically improve the patient's quality of life.

Anti-invasive therapies define a new paradigm for the treatment of malignant gliomas and MMP-related strategies are but one example (14,15). Traditional therapies have focused on decreased tumor burden as an end result of therapy. This includes surgery, radiation therapy and cytotoxic chemotherapeutics. Patient survival was dependent on the complete destruction of the tumor cell population, a very rare event in the treatment of these lethal malignancies. However, anti-invasive therapies offer the hope of disease stabilization and improved patient survival. Used in combination with more traditional cytoreductive

strategies, it may be possible to dramatically decrease tumor burden while at the same time preventing recurrence, growth and invasion of neoplastic cells that have not been destroyed. MMP inhibitors offer renewed hope in the fight against malignant gliomas.

REFERENCES

1. Forsyth PA, Cairncross JG. Treatment of malignant glioma in adults. Current Opinion in Neurology 8: 414-418; 1995.
2. Canadian Cancer Statistics 1994. National Cancer Institute of Canada. Toronto, Canada; 1994.
3. Halperin EC. Malignant gliomas in older adults with poor prognostic signs. Oncology 9: 229-234; 1995.
4. Forsyth PA, Cairncross JG. Chemotherapy for malignant gliomas. Baillière's Clinical Neurology 5: 371-393; 1996.
5. Giese A, Westphal M. Glioma invasion in the central nervous system. Neurosurgery 39: 235-250; 1996
6. Uhm JH, Dooley NP, Villemure JG, et al. Mechanisms of glioma invasion: role of matrix-metalloproteinases. Canadian Journal of Neurological Sciences 24: 3 -15; 1997.
7. Giese A, Kluwe L, Laube B, et al. Migration of human glioma cells on myelin. Neurosurgery 38: 755-764; 1996.
8. Scherer HJ. The forms of growth in gliomas and their practical significance. Brain 63: 1-35; 1940.
9. Gaspar LE, Fischer BJ, MacDonald DR, et al. Supratentorial malignant glioma: patterns of recurrence and implications for external beam local treatment. International Journal of Radiation Oncology, Biology, Physics 24: 55-57; 1992.
10. Burger PC, Dubois PJ, Schold EC, et al. Computerized tomographic and pathologic studies of the untreated, quiescent, and recurrent glioblastoma multiforme. Journal of Neurosurgery 58: 159-169; 1983.
11. Forsyth PA, Laing TD, Gibson AW, et al. High levels of gelatinase-B and active gelatinase-A in metastatic glioblastoma. Journal of Neuro-oncology 36: 21-29; 1998.
12. Barker FG, Israel MA. The molecular biology of brain tumors. Neurologic Clinics 13: 701-722; 1995.
13. Coussens LM, Werb Z. Matrix metalloproteinases and the development of cancer. Chemistry and Biology 3: 895-904; 1996.
14. Kohn EC, Liotta LA. Molecular insights into cancer invasion: strategies for prevention and intervention. Cancer Research 55: 1856-1862; 1995.
15. Stetler-Stevenson WG, Hewitt R, Corcoran M. Matrix metalloproteinases and tumor invasion: from correlation and causality to the clinic. Seminars in Cancer Biology 7: 147-154; 1996.
16. Liotta LA. Tumor invasion and metastasis: role of the extracellular matrix. Rhoades Memorial Award Lecture. Cancer Research 46: 1-7; 1986.
17. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 86: 353-364; 1996.
18. Corcoran ML, Kleiner DE, Stetler-Stevenson WG. Regulation of matrix metalloproteinases during extracellular matrix turnover. Advances in Experimental Medicine and Biology 385: 151-159; 1995.
19. Murphy G, Knauper V. Relating matrix metalloproteinase structure to function: why the "hemopexin" domain? Matrix Biology 15: 511-518; 1997.
20. Sato H, Takino T, Okada Y, et al. A matrix metalloproteinase expressed on the surface of invasive tumor cells. Nature 370: 61-65; 1994.
21. Sato H, Okada Y, Seiki M. Membrane-type matrix metalloproteinases (MT-MMPs) in cell invasion. Thrombosis and Haemostasis 78: 497-500; 1997.
22. Springman EB, Angleton EL, Birkedal-Hansen H, et al. Multiple modes of activation of latent human fibroblast collagenase: evidence for the role of a Cys73 active-site zinc complex in latency and a "cysteine switch" mechanism for activation. Proceedings of the National Academy of Sciences (USA) 87: 364-368; 1990.
23. Yu AE, Hewitt RE, Kleiner DE, et al. Molecular regulation of cellular invasion — role of gelatinase A and TIMP-2. Biochemistry and Cell Biology 74: 823-31; 1996.
24. Nagase H. Activation mechanisms of matrix metalloproteinases. Biological Chemistry 378: 151-160; 1997.
25. Nagase H, Suzuki K, Itoh Y, et al. Involvement of tissue inhibitors of metalloproteinases (TIMPs) during matrix metalloproteinase activation. Advances in Experimental Medicine and Biology 389: 23-31; 1996.
26. Edwards DR, Beaudry PP, Laing TD, et al. The roles of tissue inhibitors of metalloproteinases in tissue remodeling and cell growth. International Journal of Obesity 20 (Supplement 3): S9-S15; 1996.
27. Bigg HF, Shi YE, Liu YE, et al. Specific, high affinity binding of tissue inhibitor of metalloproteinase-4 (TIMP-4) to the COOH-terminal hemopexin-like domain of human gelatinase A. Journal of Biological Chemistry 272: 15496-15500; 1997.
28. Chambers AF, Matrisian LM. Changing views of the role of matrix metalloproteinases in metastasis. Journal of the National Cancer Institute 89: 1260-1270; 1997.
29. Butler GS, Butler MJ, Atkinson SJ, et al. The TIMP2 membrane type 1 metalloproteinase "receptor" regulates the concentration and efficient activation of progelatinase A. Journal of Biological Chemistry 273: 871-880; 1998.
30. Zucker S, Drews M, Conner C, et al. Tissue inhibitor of metalloproteinase-2 (TIMP-2) binds to the catalytic domain of the cell surface receptor, membrane type 1-matrix metalloproteinase 1 (MT1-MMP). Journal of Biological Chemistry 273: 1216-1222; 1998.
31. Liotta LA, Tryggvason K, Garbisa S, et al. Metastatic potential correlates with enzymatic degradation of basement membrane collagenase. Nature 284: 67-68; 1980.
32. Urbanski SJ, Edwards DR, Maitland A, et al. Expression of metalloproteinases and their inhibitors in primary pulmonary carcinomas. British Journal of Cancer 66: 1188-1194; 1992.
33. Kossakowska AE, Huchcroft SA, Urbanski SJ, et al. Comparative analysis of the expression patterns of metalloproteinases and their inhibitors in breast neoplasia, sporadic colorectal neoplasia, pulmonary carcinomas and malignant, non-Hodgkin's lymphomas in humans. British Journal of Cancer 73: 1401-1408; 1996.
34. Lee KS, Rha SY, Kim SJ, et al. Sequential activation and production of matrix metalloproteinase-2 during breast cancer progression. Clinical and Experimental Metastasis 14: 512-519; 1996.
35. Takemura M, Azuma C, Kimura T, et al. Type-IV collagenase and tissue inhibitor of metalloproteinase in ovarian cancer tissues. International Journal of Gynaecology and Obstetrics 46: 303-309; 1994.
36. Stearns M, Stearns ME. Evidence for increased activated metalloproteinase 2 (MMP-2a) expression associated with human prostate cancer progression. Oncology Research 8: 69-75; 1996.
37. Urbanski SJ, Edwards DR, Hershfield N, et al. Expression pattern of metalloproteinases and their inhibitors changes with the progression of human sporadic colorectal neoplasia.

Diagnostic Molecular Pathology 2: 81-89; 1993.
38. Liabakk NB, Talbot I, Smith RA, et al. Matrix metalloproteinase 2 (MMP-2) and matrix metalloproteinase 9 (MMP-9) type IV collagenases in colorectal cancer. Cancer Research 56: 190-196; 1996.
39. Nomura H, Fujimoto N, Seiki M, et al. Enhanced production of matrix metalloproteinases and activation of matrix metalloproteinase 2 (gelatinase A) in human gastric carcinomas. International Journal of Cancer 69: 9-16; 1996.
40. Kameyama K. Expression of MMP-1 in the capsule of thyroid cancer – relationship with invasiveness. Pathology, Research and Practice 192: 20-26; 1996.
41. Boyd DD, Nicolson GL. Mechanisms of invasion by head and neck cancers. Cancer Treatment and Research 74: 117-130; 1995.
42. Barille S, Akhoundi C, Collette M, et al. Metalloproteinases in multiple myeloma: production of matrix metalloproteinase-9 (MMP-9), activation of proMMP-2, and induction of MMP-1 by myeloma cells. Blood 90: 1649-1655; 1997.
43. Kossakowska AE, Urbanski SJ, Watson A, et al. Patterns of expression of metalloproteinases and their inhibitors in human malignant lymphomas. Oncology Research 5: 19-28; 1993.
44. Kossakowska AE, Urbanski SJ, Huchcroft SA, et al. Relationship between the clinical aggressiveness of large cell immunoblastic lymphomas and expression of 92 kDa gelatinase (type IV collagenase) and tissue inhibitor of metalloproteinases-1 (TIMP-1) RNAs. Oncology Research. 4: 233-240; 1992.
45. Kossakowska AE, Urbanski SJ, Edwards DR. Tissue inhibitor of metalloproteinases-1 (TIMP-1) RNA is expressed at elevated levels in malignant non-Hodgkin's lymphomas. Blood 77: 2475-2481; 1991.
46. Ismair MG, Ries C, Petrides PE. Matrix metalloproteinases and their inhibitors in acute myeloid leukemia. Leukemia 11 (Supplement): 527-529; 1997.
47. Yong VW, Krekoski CA, Forsyth PA, et al. Matrix metalloproteinases and diseases of the CNS. Trends in Neurosciences 21: 75-80; 1997.
48. Rao JS, Steck PA, Mohanam S, et al. Elevated levels of Mr 92,000 type IV collagenase in human brain tumors. Cancer Research 53: 2208-2211; 1993.
49. Saxena A, Robertson JT, Kufta C, et al. Increased expression of gelatinase A and TIMP-2 in primary human glioblastomas. International Journal of Oncology 7: 469-473; 1995.
50. Sawaya RE, Yamamoto M, Gokaslan ZL, et al. Expression and localization of 72 kDa type IV collagenase (MMP-2) in human malignant gliomas *in vivo*. Clinical and Experimental Metastasis 14: 35-42; 1996.
51. Costello PC, Del Maestro RF, Stetler-Stevenson WG. Gelatinase A expression in human malignant gliomas. Annals of the New York Academy of Sciences 732: 450-452; 1994.
52. Nakagawa, T, Kubota T, Kabuto M, et al. Production of matrix metalloproteinases and tissue inhibitor of metalloproteinases-1 by human brain tumors. Journal of Neurosurgery 81: 69-77; 1994.
53. Rutka JT, Matsuzawa K, Hubbard SL, et al. Expression of TIMP-1, TIMP-2, 72- and 92-kDa type IV collagenase transcripts in human astrocytoma cell lines: correlation with astrocytoma cell invasiveness. International Journal of Oncology 6: 877-844; 1995.
54. Forsyth PA, Wong H, Laing TD, et al. Survey of gelatinase-A and -B and MT1-MMP in gliomas. Submitted.
55. Uhm JH, Dooley NP, Villemure J-G, et al. Glioma invasion in vitro: regulation of matrix metalloprotease-2 and protein kinase C. Clinical and Experimental Metastasis 14: 421-433; 1996.
56. Yamamoto M, Mohanam S, Sawaya R, et al. Differential expression of membrane-type matrix metalloproteinase and its correlation with gelatinase A activation in human malignant brain tumors in vivo and in vitro. Cancer Research 56: 384-392; 1996.
57. Yamada T, Yoshiyama Y, Sato H, et al. White matter microglia produce membrane-type matrix metalloprotease, an activator of gelatinase A, in human brain tissues. Acta Neuropathologica 90: 421-424; 1995.
58. Mohanam S, Chintala SK, Go Y, et al. In vitro inhibition of human glioblastoma cell line invasiveness by antisense uPA receptor. Oncogene 14: 1351-1359; 1997.
59. Folkman J. What is the evidence that tumors are angiogenesis dependent? Journal of the National Cancer Institute 82: 4-6; 1990.
60. Brown PD, Giavazzi R. Matrix metalloproteinase inhibition: a review of anti-tumor activity. Annals of Oncology 6: 967-974; 1995.
61. Wojtowicz-Praga SM, Dickson RB, Hawkins MJ. Matrix metalloproteinase inhibitors. Investigational New Drugs 15: 61-75; 1997.
62. Davies B, Brown PD, East N, et al. A synthetic matrix metalloproteinase inhibitor decreases tumor burden and prolongs survival of mice bearing human ovarian carcinoma xenografts. Cancer Research. 53: 2087-2091; 1993.
63. Watson SA, Morris TM, Crosbee DM, et al. Inhibition of organ invasion by the matrix metalloproteinase inhibitor, batimastat (BB-94), in two human colorectal carcinoma metastasis models. Cancer Research 55: 3629-3633; 1995.
64. Wang X, Fu X, Brown PD, et al. Matrix metalloproteinase inhibitor BB-94 (batimastat) inhibits human colon tumor growth and spread in a patient-like orthotopic model in nude mice. Cancer Research 54: 4726-4728; 1994.
65. Taraboletti G, Garofalo A, Belotti D, et al. Inhibition of angiogenesis and murine hemangioma growth by batimastat, a synthetic inhibitor of matrix metalloproteinases. Journal of the National Cancer Institute 87: 293-298; 1995.
66. Beattie GJ, Young HA, Smyth JF. Phase I study of intraperitoneal metalloproteinase inhibitor BB-94 in patients with malignant ascites. Abstract presented at the 8th NCI-EORTC Symposium of New Drug Development, Amsterdam; March 1994.
67. Macaulay VM, O'Byrne KJ, Saunders MP, et al. Phase I study of matrix metalloproteinase (MMP) inhibitor batimastat (BB-94) in patients with malignant pleural effusions. British Journal of Cancer 71(Supplement XXIV): 11; 1995.
68. Leppert D, Waubant E, Galardy R, et al. T cell gelatinases mediate basement membrane transmigration in vitro. Journal of Immunology 154: 4379-4389; 1995.
69. Brown PD. Synthetic matrix metalloproteinase inhibitors: from cancer models to cancer patients. Proceedings of the American Association of Cancer Research 37: 633-334; 1996.
70. Hayashi T, Stetler-Stevenson WG, Fleming MV, et al. Immunohistochemical study of metalloproteinases and their tissue inhibitors in the lungs of patients with diffuse alveolar damage and idiopathic pulmonary fibrosis. American Journal of Pathology 149: 1241-1256; 1996.
71. Hayashi T, Fleming MV, Stetler-Stevenson WG, et al. Immunohistochemical study of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) in pulmonary lymphangioleiomyomatosis (LAM). Human Pathology 28: 1071-1078; 1997.
72. Tamarina NA, McMillan WD, Shively VP, et al. Expression of matrix metalloproteinases and their inhibitors in aneurysms and normal aorta. Surgery 122: 264-271; 1997.
73. Galis ZS, Sukhova GK, Lark MW, et al. Increased expression of

matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. Journal of Clinical Investigation 94: 2493-2503; 1994.

74. Vaalamo M, Matilla L, Johansson N, et al. Distinct populations of stromal cells express collagenase-3 (MMP-13) and collagenase-1 (MMP-1) in chronic ulcers but not in normally healing wounds. Journal of Investigative Dermatology 109: 96-101; 1997.
75. Akiyama K, Shikata K, Sugimoto H, et al. Changes in serum concentrations of matrix metalloproteinases, tissue inhibitors of metalloproteinases and type IV collagen in patients with various types of glomerulonephritis. Research Communications in Molecular Pathology and Pharmacology. 95: 115-128; 1997.
76. Price A, Raja JB, Rewcastle NB, et al. Marked inhibiion of tumour growth in a malignant glioma tumor model by the novel synthetic matrix metalloproteinase inhibitor AG3340. Proceedings of the 89th Annual Meeting of the American Association for Cancer Research 39: 301-302; 1998.

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