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...
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).

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 (Table 2) (24). TIMPs are clearly multifunctional molecules whose functional spectrum is yet to be understood (24,28).

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**Table 1.** The family of vertebrate matrix metalloproteinases (MMPs)

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

* MMPs 4 to 6 were found to be identical to other members of the MMP family and these designations are no longer in use.
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 extracellular matrix.
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 a more universal distribution. This implies a more selective role for gelatinase-B in glioma angiogenesis.

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

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

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 hemangiomia 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
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.

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