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THE ROLE OF MATRIX METALLOPROTEINASES IN GLIOMA INVASION

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1. ABSTRACT

The matrix metalloproteinase (MMP) family plays an important role in the degradation of extracellular matrix (ECM) in various physiological and pathological conditions. Accumulated evidence has suggested that MMPs contribute to cancer cell invasion of the surrounding normal tissues and metastasis through the cell-surface ECM degradation. Strong correlations have been reported between elevated MMP levels and tumor cell invasiveness in human gliomas. Among them, attention has been focused on gelatinases (MMP-2 and MMP-9) and membrane type MMPs (MT-MMPs). We discuss here the biological significance of these MMPs in the glioblastoma invasion processes. A better understanding of cell-ECM interactions will help in developing therapeutic strategies to decrease the invasion of gliomas.

2. INTRODUCTION

A characteristic pathological feature of malignant glioma cells is their ability to extensively invade surrounding brain parenchyma, particularly along white matter tracts, thus rendering focal therapies incapable of controlling tumor growth and resulting in inevitable recurrence. In this regard, identification of factors responsible for such invasion has become a central theme in glioma research. The underlying molecular mechanisms of brain tumor invasion are complex and involve a series of sequential steps. However, glioma cells are thought to penetrate the adjacent normal brain tissue through the disruption of extracellular matrix (ECM) components. Thus, ECM in the normal brain is a barrier to the glioma cell invasion. To overcome the ECM barrier, advancing cells express proteinases and/or proteinase activators at their leading edge, where complex proteolysis can direct cell migration. In fact, previous studies have reported the expression of many ECM-degrading proteinases in glioma tissues, which include matrix metalloproteinases (MMPs), serine proteinases (urokinase type plasminogen activator; uPA), cysteine proteinases (cathepsin B and S), and aspartic proteinases (cathepsin D) (1). Among them, MMPs are believed to play a major role in the tumor invasion since they can degrade almost all the ECM macromolecules in the brain (2-5). In this review, we present the data of our recent studies on glioma invasion and MMPs, and discuss the significance and possible role MMPs in the invasion of human glioblastomas.

3. MMP FAMILY

There are many reviews on MMPs in relation to tumors and other pathological processes (6-12). MMPs are a gene family of Zn^{2+}-dependent enzymes that are essential

Table 1. Matrix metalloproteinases MMP

	Name	Matrixin Designation	Activation Pathway	Localization In the brain	References
Collagenase					
	Interstitial collagenase	MMP-1	Extracelular	Glioma cell	46
	Neutrophil collagenase	MMP-8	Extracelular	Neutrophil	
	Collaganase-3	MMP-13	Pericellular	Unknown	
	Collaganase-4	MMP-18	Extracelular	Unknown	
Gelatinase					
	Gelatinase A	MMP-2	Pericellular	Glioma cell > astrocyte	27, 29, 30, 32, 34, 36, 41, 43, 45 , 48
	Gelatinase B	MMP-9	Extracelular	Glioma cell > astrocyte, Endothelial cell Neutrophil	27, 29, 30, 34, 36, 40, 43, 46
Stromelysin					
	Stromelysin 1	MMP-3	Extracelular	Glioma cell	33, 38, 46
	Stromelysin 2	MMP-10	Extracelular	Unknown	
	Stromelysin 3	MMP-11	Intracellular	Unknown	
Matrilysin					
	Matrilysin	MMP-7	Extracelular	Glioma cell > astrocyte	47
	Matrilysin-2	MMP-26	Extracelular	Unknown	
MT-MMP					
	MT1-MMP	MMP-14	Intracellular	Glioma cell, microglia	25, 26, 29, 32, 35, 42 , 44
	MT2-MMP	MMP-15	Intracellular	Glioma cell	32, 35
	MT3-MMP	MMP-16	Intracellular	Normal brain, microglia	32, 37
	MT4-MMP	MMP-17	Intracellular	Normal brain	55
	MT5-MMP	MMP-24	Intracellular	Glioma>normal brain	56
	MT6-MMP	MMP-25	Intracellular	Glioblastoma	28
Others					
	Metalloelastase	MMP-12	Extracelular	Glioblastoma	30
	RASI	MMP-19	Extracelular	Unknown	
	enamelysin	MMP-20	Extracelular	Unknown	
	XMMP	MMP-21	Extracelular	Unknown	
	CMMP	MMP-22	Extracelular	Unknown	
	Cystein array MMP	MMP-23	Intracellular	Unknown	
	No common name	MMP-27	Extracelular	Unknown	
	Epilysin	MMP-28	Intracellular	Unknown	

MMP-4, 5, 6 were found to be identical to other MMP genes on sequencing.

for ECM turnover in normal and pathological conditions. They can be classified into six different classes based on their substrate specificities and structural differences: collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs and others (Table 1). All members of the MMP family are synthesized as inactive proenzymes (proMMPs) that require enzymatic cleavage of the propeptide domain for activation. The activities of the MMPs are regulated by gene expression, proMMP activation, and inhibition of active MMPs by a family of endogenous tissue inhibitors, i.e. tissue inhibitors of metalloproteinases (TIMPs) (13) and a general inhibitor, i.e. a2-macroglobulins (14-17). Balance between the levels of activated MMPs and free inhibitors determines the overall MMP activity (7). Maintenance of this critical equilibrium is essential, because a disturbed balance or ratio of MMPs and TIMPs affects the invasive process (13). Decreased TIMP gene expression results in increased tumor invasiveness, whereas its overexpression leads to reduce invasive growth *in vivo* (13-14).

3.1. Activation pathway

Activation of proMMPs is essential to degrade ECM components by MMPs. There are three pathways for the activation (Figure1, Table 1).

3.1.1. Extracellular activation

Most MMPs are secreted in latent precursor forms, which are activated in the extracellular spaces. MMP latency is maintained by a 'cysteine switch' formed through the interaction between the sulfhydryl group of a conserved cysteine residue within the propeptide and a catalytic zinc (18). ProMMPs-1, 3, 7, 8, 9, 10, 12 13, 18, 19, 20, 21, 22, 26 and 27 are activated in this manner (7). This activation can be mediated either by proteinases such as plasmin, trypsin, kallikreins and cathepsins or by organomercury compounds (19). Among them, plasmin may be the most important for the activation of most MMPs.

3.1.2. Pericellular activation

ProMMP activation on the cell surface is very important for the invasion of cancer cells. Discovery of MT1-MMP gave a new insight into the cell surface activation of proMMP-2, which is resistant to the serine proteinases-mediated activation that is common in other proMMPs (20). Because MMP-2 is an important enzyme for basement membrane breakdown due to its ability to degrade type IV collagen, proMMP-2 activation mediated by MT-MMPs on the tumor cell surface is thought to be critical for the tumor cell invasion. MMP-13 may be also activated by this manner (7). MMP-2 activation by MT-MMPs is carried out in a two-step mechanism, analogous to the extracellular activation in other MMPs: the initial cleavage is mediated by direct action of MT-MMPs in a region of the proMMP-2 propeptide domain that is exposed

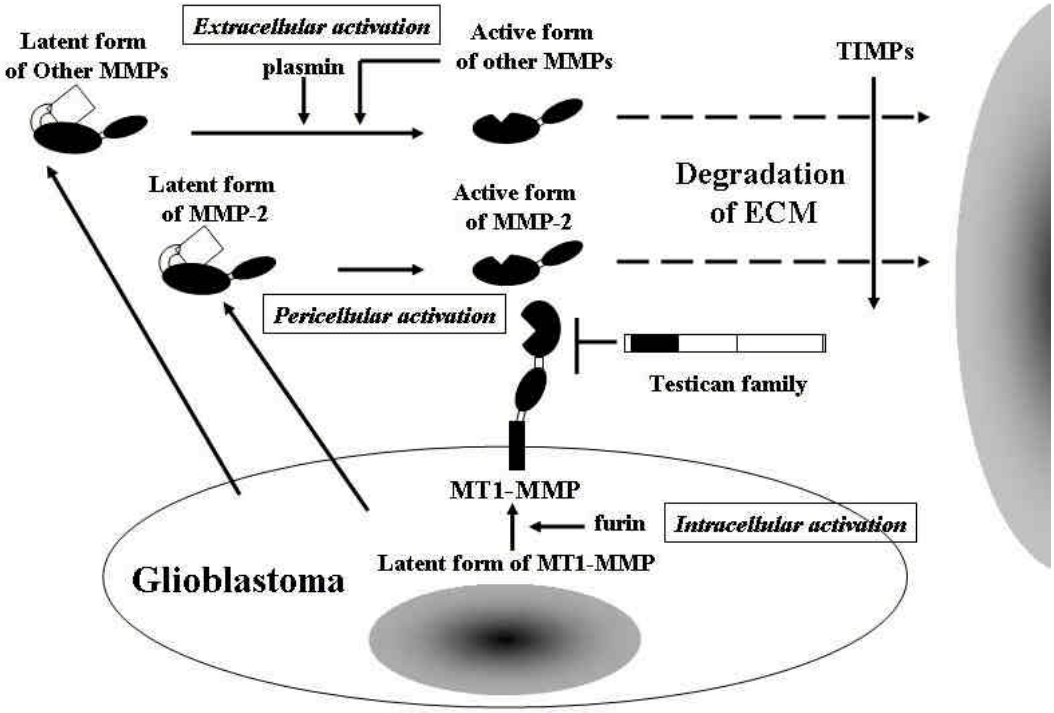

Figure 1. Proposed model for MMP mediated proteolysis during glioma invasion. MT1-MMP on the glioma cell surface activates proMMP-2 which is produced by glioma cells. Other MMPs are activated by plasmin or other active form of MMPs in the exrtracellular space. All MMPs are inhibited by TIMPs. MT-MMPs are inhibited by the component of brain ECM, testican family. The imbalance between MMPs and their inhibitors contributes to the invasion of glioma.

to solvent, whereas the secondary cleavage is autoproteolytic. This activation process seems to involve the trimolecular complex formation composed of proMMP-2, TIMP-2 and MT1-MMP that is crucial for the efficiency of activation on cell surfaces (21). TIMP-2 is essential for MMP-2 activation, however, excessive TIMP-2 inhibit MMP-2 activation (13).

3.1.3. Intracellular activation

MT-MMPs contain a furin-sensitive motif between the pro-peptide and catalytic domains, suggesting that the proenzymes are activated intracellularly in the trans-Golgi network by the proprotein convertase, furin or related enzymes (22-23). Actually, experimental studies have demonstrated the intracellular activation of the MT-MMPs (22). MMP-11, 23 and 28, albeit secretary MMPs, are also activated by furin and secreted into ECM (7,24).

4. GLIOMA INVASION AND MMPs

MMPs are overexpressed in various human cancers including malignant brain tumors (Figure 2). Many authors have demonstrated the expression of MMPs in gliomas and much attention has been drawn to gelatinases (MMP-2 and MMP-9) and MT-MMPs (Table 1, 25-47). The actual substrate which is a target of MMP is not elucidated on the occasion when the glioma cells invade the surrounding normal brain. The brain ECM consists mainly of hyaluronic acid and various proteoglycans which are localized especially within the basal lamina of blood vessels. MMPs might degrade these ECM and permit glioma cells to migrate along the structure, which is well known as typical route of the glioma invasion.

4.1. MMP-2

Although MMP-2 is constitutively expressed in many cells, this MMP is believed to be particularly important for invasion and metastasis by the following three reasons: (1) MMP-2 degrades type IV collagen and other ECM, (2) it is activated rather specifically in tumor tissues and (3) its activation correlates with tumor spread and poor prognosis. In our studies, we have demonstrated that the production level of proMMP-2 in human glioblastomas is significantly higher than those in the low grade astrocytomas, metastatic brain tumors, or normal brains (Figure 2) as is also shown by other papers (32,41). Similar findings are observed by Northern blotting or quantitative RT-PCR (29,33,43). Furthermore, the level of MMP-2 activity correlated with the degree of invasion in vitro (32,45,48). In situ hybridization and immunohistochemistry indicated the expression of MMP-2 mainly in the neoplastic cells in contrast to virtual absence of labeling in the brain tissue surrounding the tumor mass. (29,32,41,46,49). In addition, the neural stem cells which show extensive migration within the brain express MMP-2 but not MMP-9, suggesting that MMP-2 was closely related to the invasive character (50). Taken together, it is generally accepted that MMP-2 activity is important for the human glioma invasion. On the other hand, the fragment derived from the proteolysis of MMP-2 in the human glioblastoma cells and culture medium was recently reported to have an ability to inhibit angiogenesis, cell proliferation and migration contrary to active form of MMP-2 (51).

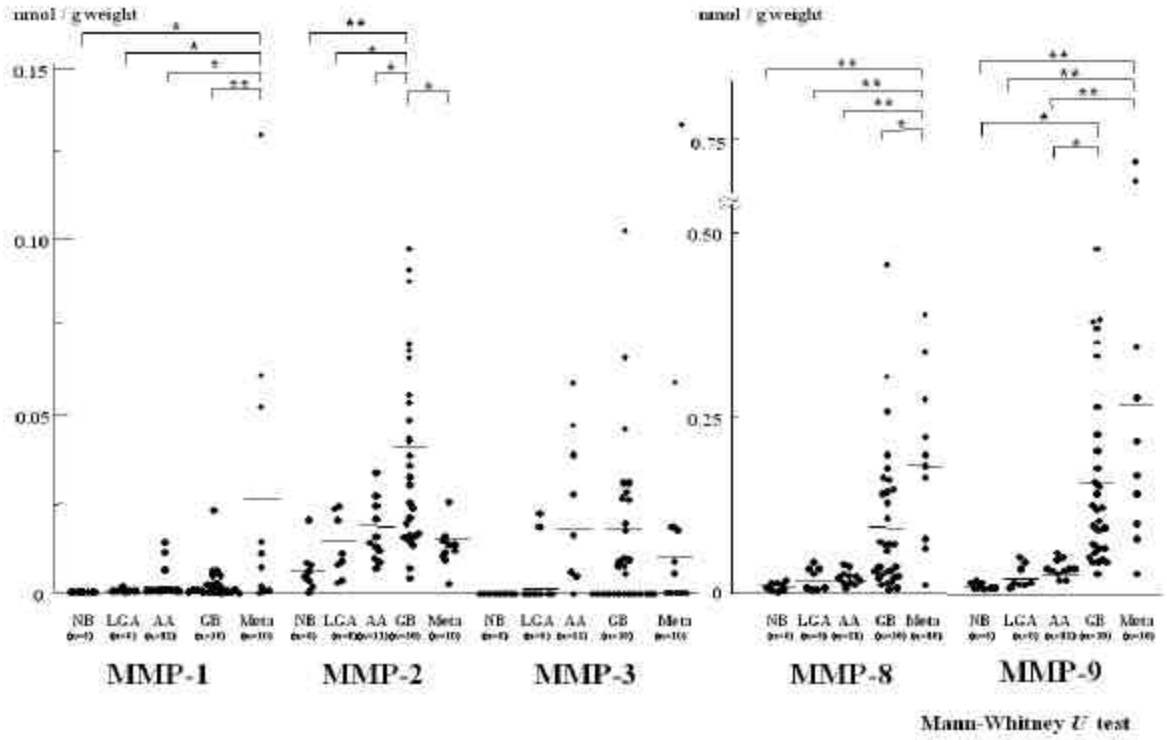

Figure 2. Amounts of MMPs in the tissue homogenates of astrocytic tumors, metastatic brain tumors and normal brains. The supernatants from normal brain (NB), low-grade astrocytoma (LGA), anaplastic astrocytoma (AA), glioblastoma (GB) and metastatic brain tumor (Meta) tissues were prepared, and MMPs was measured by enzyme immunoassay (EIA). The EIA systems for MMP-1, -3, and -8 measure both the precursor and active forms of the MMPs, but those for MMP-2, and -9 detect only their latent forms. Bars indicate the mean value. *, $p < 0.05$; **, $p < 0.01$.

4.2. MMP-9

Like MMP-2, the expression of MMP-9 has been shown to correlate with increasing malignancy in glial tumors (27,29-30,35,43), and the data in our sandwich enzyme immunoassay also demonstrated that the MMP-9 production level in the glioblastomas is significantly higher than that in the normal brains (Figure 2). In addition, recent study indicates that high grade glioblastoma cells which were stably transfected with an antisense vector capable of expressing an antisense transcript complementary to MMP-9 are unable to form tumors in the brain of nude mice, unlike parental cells or vector-transfected or sense-transfected clones, indicating that MMP-9 is necessary to form glioblastoma in the brain (52). However, these data could be findings closely linked with abundant angiogenesis in the glioblastoma, since MMP-9 is expressed within and around the vasculature, especially endothelial cells (27,29,33,36,46). Most importantly, activated form of proMMP-9 is not commonly observed in these studies, and thus there is no definite proof that MMP-9 is really acting in the invasive glioma tissues. Further studies are necessary to prove that MMP-9 activity is involved in the invasive process of gliomas.

4.3. MT-MMPs

Among the six members of the MT-MMP subfamily, MT1-, MT2-, MT3- and MT5-MMP are anchored to the plasma membrane via a transmembrane domain (20,53-56), but MT4- and MT6-MMP are glycosylphosphatidyl inositol-anchored proteinases (57-60). Among the MT-MMPs, MT3- and MT4-MMPs are expressed in normal brain, suggesting the physiological functions of these MT-MMPs in brain (32,37,55,59). On the other hand, MT1-, 2-, 5- and MT6-MMPs are reported to be highly expressed in glioblastoma tissues (26,29,32,35,42,56,59). Accumulated lines of data have indicated that MT-MMPs, especially MT1-MMP, are directly involved in tumor cell invasion and metastasis (20-21,42,61-64). In human gliomas, MT1-MMP overexpression is strongly associated with malignant progression and invasive behavior of tumor cells(26,29,32,35,42). Expression of MT1-MMP is predominantly observed in glioblastoma tissues and its expression levels correlate with activation ratio of proMMP-2 and tumor grades (26,29,32,35,42). In addition, immunohistochemical and *in situ* hybridization indicated that MT1-MMP and MMP-2 are colocalized (29,32). Experimental studies by Deryugina *et al* have also shown that glioma cells transfected with cDNA encoding MT1-MMP resulted in increased collagen degradation, and cell migration in tumor spheroid outgrowth assay through the cell surface activation of proMMP-2 (65-67). All these data strongly suggest the possibility that MT1-MMP contributes to the glioma cell invasion through the activation of proMMP-2. In our previous studies, we further demonstrated the possible involvement of MT2-MMP as well as MT1-MMP in the activation of proMMP-2 in human gliomas (32). Using a novel method, i.e. *in situ*

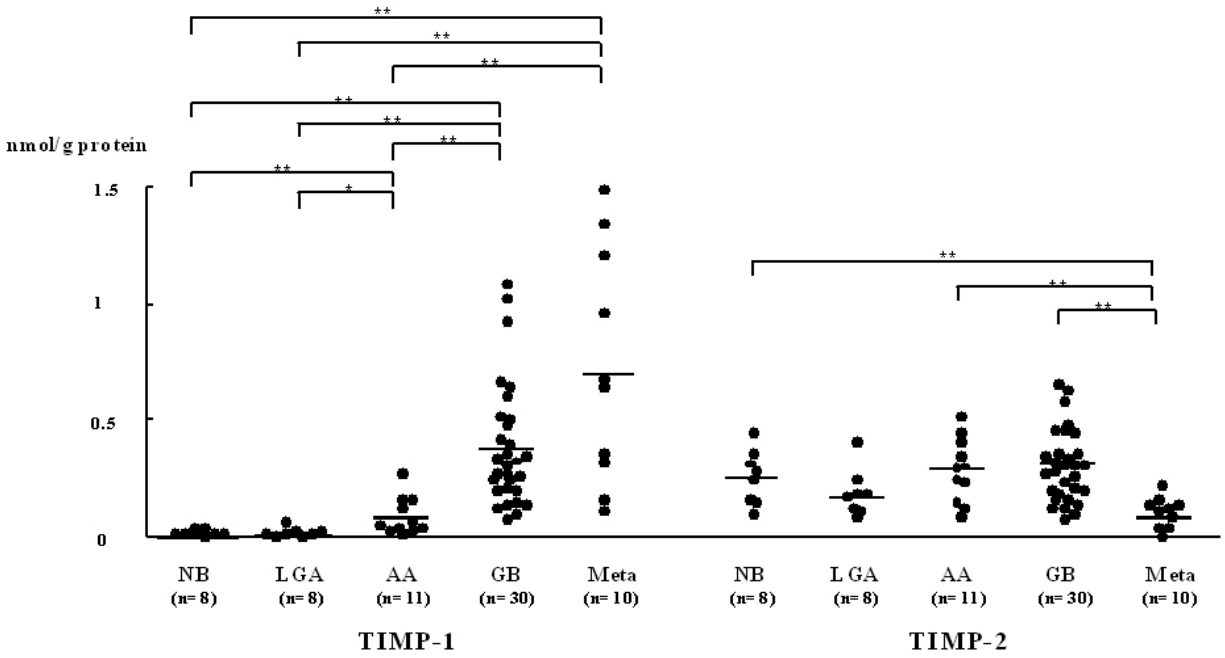

Figure 3. Amounts of TIMP-1 and TIMP-2 in the tissue homogenates of astrocytic tumors, metastatic brain tumors and normal brains. TIMP-1 and TIMP-2 were measured by corresponding EIA systems. Bars indicate the mean value. *, $p < 0.05$; **, $p < 0.01$ (Reproduced permission. Reprinting from (25)).

zymography, we could demonstrate gelatinolytic activity in glioblastoma tissue but not in normal brain tissue (32). Since the activity was completely blocked with a synthetic MMP inhibitor, BB94, and its localization was consistent with immunohistochemical data of MMP-2, MT1-MMP and MT2-MMP, the activity is considered to be derived from MMP-2 activation by MT1-MMP and MT2-MMP.

MT-MMPs may contribute to glioma invasion by acting not only as an activator of proMMP-2 but also as ECM degradation enzymes. MT1-MMP has the ability by itself to digest ECM components such as fibrillar collagens, vitronectin, fibronectin, and aggrecan (68-69). Belien *et al* have shown that MT1-MMP enables invasive migration of glioma cells due to its ability to digest central nervous system myelin-inhibitory proteins (70).

Inhibitor profile of MT-MMPs is different from other MMPs, since the activities of MT-MMPs are inhibited by TIMP-2 but not by TIMP-1 (22). In addition, we have recently demonstrated that MT1- and MT3-MMP are inhibited selectively by N-Tes, a newly identified ECM molecule in the brain (71). N-Tes can form stable complexes with either MT1- or MT3-MMP resulting their inhibition. Transfection of N-Tes genes into U251 glioma cells suppressed their invasive growth in collagen gel (71).

4.4. Other MMPs

The expression of various MMPs including MMP-2, 9 and MT-MMPs has been reported in human gliomas (Table 1). Although most of them are expressed by glioma cells, MMP-8 is produced by neutrophils (72). MMP-1, 3, 7 and 12 were reported to be localized in the glioma cells (30,33,38,46-47), but further studies are necessary to clarify their roles in glioma invasion.

5. TIMP FAMILY

The TIMP family is comprised of four different members which possess 12 conserved cysteine residues (Table 2, 13,73). TIMPs inhibit MMP activities by forming non-covalent complexes with active MMPs. However, recent studies have demonstrated that TIMP-1 does not inhibit the activity of MT-MMPs, although other TIMPs do (74). TIMPs bind to the highly conserved active zinc-binding site of the MMPs. However, unlike other proMMPs, proMMP-2 and proMMP-9 can make complexes with TIMP-2 and TIMP-1, respectively (75). The activation of proMMP-9 is inhibited in the proMMP-9/TIMP-1 complex form. On the other hand, proMMP-/TIMP-2 complex formation is implicated in the efficient activation of proMMP-2 by MT1-MMP on the cell surfaces (21).

6. GLIOMA INVASION AND TIMPs

It has been emphasized that the balance between MMPs and TIMPs is critical for control of invasion and dissemination in glioma cells (25,30,76). Our analyses by sandwich enzyme immunoassay systems indicated that the production levels of TIMP-1, but not TIMP-2, are significantly higher in glioblastomas than in other grades of astrocytic tumors and normal brain tissues (Figure 3, 25). Actually this is a common finding for human cancers including stomach, thyroid, endometrial and oral carcinomas (62,63,77-78). Several authors demonstrated that MMP-2 gelatinolytic activity is detected by *in situ* zymography in the cancer tissues even in the

Table 2. The inhibitor of MMPs

Inhibitor		Substrates	Localization in the brain	Reference
TIMP family				
	TIMP-1	active form of MMPs except for MT-MMPs, proform of MMP-9	Glioma>normal brain, Glioma cell	25, 26, 30, 35, 43, 46, 79, 80
	TIMP-2	active form of MMPs, proform of MMP-2	Glioma ≦ normal brain	25, 26, 30, 35, 43, 79, 80
	TIMP-3	active form of MMPs	Glioma=normal brain	80
	TIMP-4	active form of MMPs	Normal brain>glioma, Glioma cell	80
Testican family				
	Testican-1, 3, N-Tes	MT1, MT3-MMP	Normal brain>glioma, Neuron	71
a2-macrogloblin		Protease including active form of MMPs	Glioma>normal brain, Glioma cell	16, 17

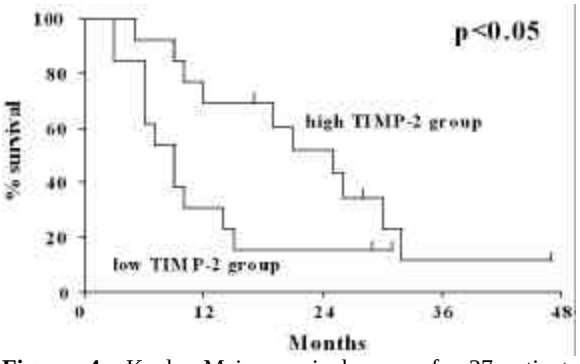

Figure 4. Kaplan-Meier survival curves for 27 patients with glioblastoma according to the production level of TIMP-2. The survival curves were tested by using the generalized Wilcoxon test.

presence of abundant TIMP-1 (32,63.64), suggesting that overproduced TIMP-1 can not function as inhibitor in the cancer tissues. On the other hand, conflicting results are found regarding the expression of TIMP-2 in glioma. Some authors demonstrated the low expression levels in glioblastomas (26,30,79), but others showed no correlation between TIMP-2 level and tumor grade (25,35,43,80). However, the data that the virus vector-driven overexpression of TIMP-2 decreases invasiveness of glioma cells (81) suggest that TIMP-2 may be a key regulator for glioma cell invasion. Our study showed that low production level of TIMP-2 correlates with short survival in the patients with glioblastoma (Figure 4).

7. PERSPECTIVES

Although significant technical advances in surgical and radiation treatment for gliomas have emerged in recent years, their impact on clinical outcome for patients has been disappointing. A fundamental source of the management challenge presented by glioma patients is the insidious propensity of the malignant cells to invade adjacent normal brain. Recent advances in understanding the biological mechanisms and molecular determinants of glioma cell invasion provide valuable insight to the biology of gliomas as well as illuminating possible new therapeutic targets such as anti-invasion therapy. The importance of MMPs in invasion is underlined by the data that synthetic MMP inhibitors limit tumor growth in animal models. MMP inhibitors, Batimastat and Marimastat, effectively reduced glioma invasion in Matrigel-coated transwell assays and cocultures of tumor spheroids with fetal rat brain aggregates, although higher concentrations were required in the latter systems (82-83). AG3340, SI-27 and BE16627B, other synthetic MMP inhibitors, inhibited the growth of the glioma cell line implanted in SCID mice (84-85). Furthermore, as mentioned above the fragment derived from the proteolysis of MMP-2 or N-Tes may be a candidate for new anti-invasion therapy. The investigation into MMPs biology is very attractive and exciting area and will continue to provide insight into glioma invasion. It is hoped that more information concerning the regulation of the invasion process of glioma cells in brain will provide clinically important strategies for targeting invading cells for anti-cancer therapy.

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