

THE ROLE OF SELECTED MATRIX METALLOPROTEINASES IN THE PATHOGENESIS OF GLIOMAS

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A b s t r a c t

The progression of tumor formation is a multifactorial process that involves changes at different levels. Within this intricate molecular and cellular landscape of tumorigenesis, specific enzymes, namely matrix metalloproteinases (MMPs), emerge as pivotal contributors. They may influence this process at the level of tissue remodeling, angiogenesis, changes in cell signalling, invasion and metastasis, but may also serve as prognostic markers or therapeutic targets. Although the function of MMPs has been known for a long time, their specific role in the pathogenesis of brain tumors has only begun to be investigated in the last three decades. Deregulation of MMPs expression, frequently observed in brain tumor tissue, is associated with malignant phenotype, dependent on the grade of malignancy and associated with a worse prognosis. They participate in tissue remodelling under physiological and pathological conditions, which predetermines their action especially in the process of invasion and migration of tumor cells into the surrounding tissue. Related to their potential in tumor progression, they are also being investigated as possible targets in anticancer therapy. It would be very difficult to characterize in detail the role of all known MMPs in the context of brain tumor pathogenesis, so we have selected those that have an essential effect in this issue.

Key words: gliomagenesis, metalloproteinases, extracellular matrix

INTRODUCTION

Gliomas represent the prototypical and prevalent tumors of brain tissue, stemming from the transformation of glial cells, constituting approximately 90% of all cells within the nervous system. In 2021, the fifth edition of the WHO classification for central nervous system (CNS) tumors was published, which is the sixth updated version of the international standard for the classification of brain and spinal cord tumors. In addition to histological and immunohistochemical characterization, the latest classification is based on the irreplaceable role of molecular diagnostics in these tumors (1).

Glioblastoma multiforme (GBM) belongs to WHO grade IV glioma group and represents 53.8% of all gliomas (2). Glioblastoma is one of the most common and aggressive brain

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neoplasms with a relatively unfavourable prognosis and a median survival of only 12–15 months after the diagnosis. Surgical resection followed by radiation therapy and chemotherapy has been a conventional therapy for almost three decades. Due to the rapid growth with a high degree of infiltration into the surrounding tissues, a perfect surgical resection of GBM is still a major issue and the disease is in many cases recurrent (3). Although Temozolomid (TMZ) has been a standard chemotherapeutic in the treatment of glioblastoma, since its approval in 2005, the response to its use among brain tumor cells varies (4). The development of TMZ resistance in a significant number of patients is one of the main reasons for the failure of GBM treatment. The origin and progression of glioblastoma is multifactorial, so it involves a combination of genetic and environmental factors. In this process, specific enzymes called matrix metalloproteinases (MMPs) undoubtedly play a multifaceted role, contributing to tumor invasion, angiogenesis, and disturbances in cell signalling or immune modulation.

MMPS IN CENTRAL NERVOUS SYSTEM

A total of 23 human matrix metalloproteinases (MMPs), known so far as zinc-dependent endopeptidases, are key in the degradation of the extracellular matrix (ECM) (table 1) (5). Of these, more than 10 different types have been detected in the mammalian central nervous system at both transcriptomic and proteomic levels (6). Studies have demonstrated that MMPs exhibit a varied expression profile depending on the developmental program of neuronal tissue. Several MMPs, including MMP-2, -9, -11, -12, -13, -14, -15, and -24, show a developmental regulation, while others such as MMP-3, -7, and -10 remain unchanged during this process (7). Based on their sub-cellular distribution and specificity for components of the ECM, the MMPs are divided into membrane-type matrix metalloproteinases (MT-MMPs), collagenases, gelatinases, stromelysins, and matrilysins. The ECM, which comprises various proteins like fibronectin, thrombospondin-1, laminin, and osteopontin, holds significance in cancer progression. These ECM proteins impact the tumor behaviour by affecting cell movement and the formation of new blood vessels (angiogenesis). The interplay between cancer cells and ECM elements is crucial for various stages of cancer development, including cell transformation and carcinogenesis not only in CNS tissue (8,9).

MMPS AND BRAIN CARCINOGENESIS

Despite cancer cells produce MMPs in small quantities, they leverage their influence by stimulating neighbouring host cells to produce MMPs through the secretion of interleukins, interferons, growth factors, and other extracellular MMP inducers in a paracrine manner (10). Normal cells surrounding the cancer cells can also secrete MMPs that attach to the surface of cancer cells and can be utilized by them (11).

One of the most important metalloproteinases in the context of brain tumors are gelatinases MMP-2 and MMP-9. Gelatinases are primarily responsible for the degradation of gelatin and collagens facilitating tumor cell invasion into the surrounding healthy brain tissue. They are often overexpressed in high-grade gliomas, correlating with the increased invasive potential of these malignancies (12). Among other processes, they are also involved in the neovascularization of the tumor mass and can participate in the disruption of the blood-brain barrier (13). Dobra et al. examined the MMP-9 content of small extracellular vesicles (sEVs) from patients with tumors with a different invasion capacity. They found a relation between low MMP-9 level in sEVs and improved survival of glioblastoma patients, and MMP-9 levels showed a positive correlation with aggressiveness. These findings suggest that vesicular MMP-9 level might be a promising prognostic marker for brain tumors (14). Experiments with glioma cells U251 and nude mice showed that the expression of MMP-2

and MMP-9 in recurrent gliomas was significantly higher than those in primary gliomas and radiotherapy increased the expression of MMP-9 proving a poor prognosis in glioma recurrence. This suggests that MMP-9 may be an important target also in radiosensitization of gliomas (15). MMP-2 and MMP-9 have been also shown to present an increased activity in cortex neuronal nuclei after focal cerebral ischemia. Their increased gelatinolytic activity in nucleus occurs to be linked with MMP-dependent cell death triggering neuroinflammatory reactions (16).

Collagenase MMP-13 plays a crucial role in initiating the invasive progression of glioma due to its proteolytic activity. Its expression is notably higher in glioma compared to the surrounding normal brain tissue, especially in advanced grades of glioma. Some researchers propose MMP-13 as a potential biomarker for tracking the progression of glioblastoma (GBM). A stimulation of a highly invasive glioma cell line U251 in vivo with endothelin resulted in an increased expression of MMP-13, MMP-9, and enhanced cell migration. The addition of MMP-13 and MMP-9 inhibitors successfully mitigated this heightened cell migration (17,18).

ECM-degrading enzymes play a significant role in influencing the survival of metastatic cells by modulating the process of apoptosis. In particular, MMP-7 contributes to the survival of tumor cells by cleaving the Fas ligand. This cleavage action removes the ligand from the cell surface, thereby preventing it from stimulating the Fas death receptor. The Fas death receptor is a powerful mediator of innate apoptotic pathways (19). By evading apoptosis through this mechanism, malignant cells not only escape cell death but also potentially develop a resistance to chemotherapeutic treatments.

Matrix metalloproteinase-14 (MMP-14), also recognized as membrane-type matrix metalloproteinase 1 (MT1-MMP), is attached to the cell membrane and possesses the ability to activate other MMPs. It specifically activates proMMP-2 directly and indirectly influences MMP-2 and MMP-9. MMP-14 can enzymatically break down potent inhibitors of central nervous myelin, including BN-220. Moreover, MMP-14 is capable of digesting proteins with adhesion functions. Interestingly, MMP-14 is not limited to extracellular processes; it is also involved in intracellular activities. It passes along the tubulin cytoskeleton and plays a role in intracellular recycling pathways. Abnormalities in MMP-14 expression are associated with mitotic spindle aberrations and chromosomal instability, ultimately leading to a malignant transformation of neoplastic cells (20).

An important key element of tumor cell growth and proliferation is angiogenesis. This process can be stimulated by various signalling molecules produced by tumor cells or by surrounding tissue. MMPs play a significant role in the formation of new blood vessels to supply nutrients and oxygen to the growing tumor. Malignant cells and endothelial cells release MMPs to remodel the ECM, creating a path for new blood vessels to grow. They contribute to the release and activation of various angiogenic factors, such as vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF), both potent inducers of angiogenesis often bound to the ECM (21). One of the extensively studied MMPs involved in angiogenesis is the aforementioned MMP-14. MMP-14 is a key effector in the generation of pro-angiogenic factor VEGF. It interacts with cell surface molecules such as CD44 and sphingosine 1-phosphate receptor 1 (S1P1), promoting endothelial cell migration. Furthermore, MMP-14 is crucial in the proteolytic degradation of anti-angiogenic factors like decorin. Moreover, there is an evidence suggesting that MMP-14 can degrade pro-transforming growth factor-beta (pro-TGF- β) and endoglin (TGF- β receptor), indicating its pivotal role in vessel maturation and angiogenesis, respectively (22). Additionally, MMP-14 seems to be indispensable in determining ECM adhesion and the formation of tubes by human endothelial cells through the modulation of MMP-2 expression. This underscores its significant involvement in regulating angiogenesis-related functions in human endothelial cells (23).

The increased expression of various MMPs observed in brain tumors is the result of deregulation of several intracellular signalling pathways that have long been a subject of increased

interest. MMP-2 has been found to have intracellular activity and play a role in processes occurring in the cell nucleus. MMP-2 directly interacts with p21 activated kinase 4 (PAK4) which aberrant expression was found to be associated with an enhanced tumor progression in various carcinomas. Complex PAK4/MMP-2 is supposed to regulate integrin mediated pathways in gliomas and earlier study revealed that MMP-2 knock down glioma cells entered on apoptosis pathway (24,25).

MMPS AS TARGETS FOR ANTICANCER THERAPY

Many MMPs have become interesting candidates for diagnostic tools and therapeutic interventions in the context of cancer due to their key involvement at virtually every critical stage of tumor development. MMPs modulation can be approached through three primary strategies: transcriptional regulation, activation control, or direct inhibition. At the transcriptional level, the interference with extracellular factors, such as interferon, and the blockade of signal transduction pathways like MAPK or ERK can effectively hinder MMPs synthesis. Critical to MMPs inhibition is the modulation of nuclear transcription factors, including NF- κ B or AP-1 (26). Another key aspect of MMPs regulation is their activation process, given that they are initially secreted as inactive zymogens. Monoclonal antibodies targeting MMPs are considered an effective means of inhibiting their activation. Such antibodies have been successfully developed, for example, against gelatinase B (MMP-9) as well as for MT1-MMP (MMP-14) (27,28). In healthy organisms, MMP activity is regulated by endogenous TIMPs that are natural inhibitors of MMPs. High TIMPs levels lead to ECM accumulation due to inhibition of the degradation processes, whereas low TIMPs activity results in elevated proteolysis (29). TIMPs can also inhibit the growth, invasion and metastasis of malignant tumors. On the other hand, there are non-specific inhibitors such as α 1-proteinase inhibitor and α 2-macroglobulin that can affect the regulation of MMPs activity (30).

Another strategy to exploit the potential of MMPs is their ability to recognize and cleave peptides with specific sequences, making it a hot spot for targeted drug release studies (31). In a recent study dealing with the treatment of GBM, MMP-2 was used for this purpose, precisely because of its high expression in glioma tissue (32).

Despite encouraging results from preclinical studies targeting MMP inhibition as a potential cancer treatment, the results of phase III trials were ultimately disappointing. The lack of success can be attributed to the inadequate design of the clinical trials, the specific properties of the MMP inhibitors used, the limited understanding of the complex nature of MMPs, and the differences between the results observed in mouse models and the patient populations participating in the clinical trials (33). It is therefore extremely important to show interest in extending the current knowledge of these multifactorial enzymes.

CONCLUSION

In recent years, matrix metalloproteinases (MMPs) have revealed novel biochemical properties that exhibit both extracellular and intracellular activities, including intranuclear functions. These activities have been implicated in the invasiveness of brain tumors, particularly gliomas. Understanding the integral connection between MMP function and essential cellular processes, such as apoptosis, cell migration, and angiogenesis – frequently implicated in glioma pathogenesis – presents MMPs as potential tumor markers critical for developing targeted therapies.

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Table 1 Classification of matrix metalloproteinases (MMPs)

Subgroup	MMP number	Nomenclature
collagenases	MMP-1 MMP-8 MMP-13	Collagenase-1 Collagenase-2 Collagenase-3
gelatinases	MMP-2 MMP-9	Gelatinase-A Gelatinase-B
stromelysins	MMP-3 MMP-10 MMP-11 MMP-12	Stromelysin-1 Stromelysin-2 Stromelysin-3 Metalloelastase
matrilysins	MMP-7 MMP-26	Matrilysin-1 Matrilysin-2, Endometase
membrane type MMP	MMP-14 MMP-15 MMP-16 MMP-17 MMP-24 MMP-25 MMP-23	MT1-MMP MT2-MMP MT3-MMP MT4-MMP MT5-MMP MT6-MMP CA-MMP
others	MMP-19 MMP-20 MMP-18 MMP-22 MMP-27 MMP-28	RASI-I Enamelysin Epilysin