

Original Article

The expression of MMP19 and its clinical significance in glioma

Qisheng Luo^{1,2*}, Hongcheng Luo^{3*}, Xiaoping Chen^{5,6*}, Peng Yan^{2*}, Huangde Fu², Haineng Huang², Huadong Huang², Chuanyu Li², Chengjian Qin², Chuanhua Zheng², Lan Chuanliu², Qianli Tang^{1,4}

¹College of Integrated Chinese and Western Medicine, Hunan University of Chinese Medicine, Changsha, Hunan, China; ²Departments of ²Neurosurgery, ³Laboratory Medicine, ⁴Surgery, Affiliated Hospital of Youjiang Medical University for Nationalities, Baise, Guangxi, China; ⁵Department of Neurology, Guangxi Zhuang Autonomous Region People's Hospital, Nanning, Guangxi, China; ⁶Guangxi Medical University Graduate School, Nanning, Guangxi, China. *Equal contributors.

Received September 16, 2018; Accepted September 25, 2018; Epub November 1, 2018; Published November 15, 2018

Abstract: Aims: The expression of phosphoglycerate kinase 1 (MMP19) is elevated in some cancers. However, the clinical features and prognostic value of glioma patients with MMP19 expression are unclear. In this study, the expression level of MMP19 and the correlation between the level of MMP19 expression and the clinicopathologic data in glioma patients including survival were examined. Methods and results: Using real-time PCR, the mRNA expression of MMP19 was examined in 61 fresh glioma tissues and 32 brain samples. The result indicated that MMP19 mRNA was obviously elevated in glioma tissues compared to brain tissues. Further, we observed that MMP19 mRNA was much higher in stage III patients than it was in stage I-II patients. The expression of the MMP19 protein was determined by immunohistochemical analysis in 156 paraffin-embedded glioma samples and 35 normal paraffin-embedded brain samples. The MMP19 protein level was significantly increased in glioma tissues compared to brain tissues ($P = 0.008$). Furthermore, we observed that a high expression of MMP19 protein was positively associated with clinical stage ($P = 0.008$) but did not correlate with age, gender, or histological type. An increased MMP19 protein expression was associated with poor overall survival rates ($P = 0.001$). A stratified analysis showed that patients with high MMP19 protein expression indicated a worse prognosis occurring in WHO III-IV stages ($P = 0.001$). A Multivariate analysis indicated that a high expression of the MMP19 protein was an independent prognostic indicator of patient survival ($P = 0.009$). Conclusions: MMP19 is overexpressed and plays a significant role in disease progression and poor outcome in glioma patients.

Keywords: MMP19, overexpression, glioma, disease progression, outcome

Introduction

Glioma is a basic type of brain tumor that comprises about one-third of all brain tumors. It may arise from different types of glial cells, which include astrocytes, oligodendrocytes, and ependymal cells. As with many tumors, the cause of glioma is still unknown. It affects people from all ages, although it is more common in adults than children and is more likely to occur in men than in women [1].

Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of the extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis [2, 3]

and tumor metastasis [4-8]. Matrix metalloproteinase-19 (MMP-19), also known as matrix metalloproteinase RAS1, is an enzyme that in humans is encoded by the MMP19 gene. In a previous study, MMP19 was reported to have a higher expression in glioma tissues than in brain tissues [9]. However, the correlation of MMP19 expression with clinical features and the prognosis of glioma has never been reported.

MMP19 is the first ATP-generating enzyme of the glycolytic pathway

In this study, we examined the expression of MMP19 and explored the correlation of MMP19 expression with clinical features and patient prognosis in glioma. Our data demonstrate that

MMP19 overexpression in glioma

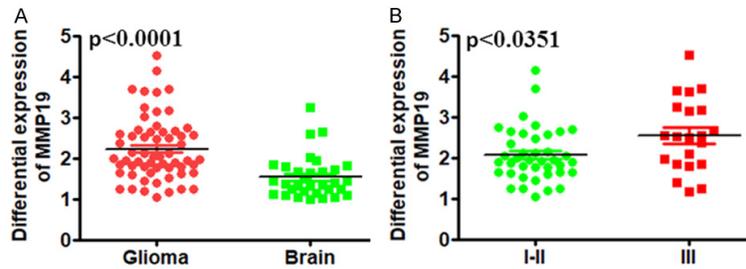


Figure 1. Increased MMP19 mRNA expression was positively associated with clinical progression in Glioma. A. Increased MMP19 mRNA expression in glioma compared to brain tissues. B. Elevated MMP19 mRNA expression was positively associated with clinical progression in Glioma (I-II vs. III-IV).

an elevated expression of MMP19 promotes the pathogenesis of glioma and results in a poor outcome.

Materials and methods

Sample collection

Sixty-one fresh glioma tissues, 32 fresh brain tissues, 156 paraffin-embedded glioma samples, and 35 normal paraffin-embedded brain samples were collected from the Affiliated Hospital of Youjiang Medical University for Nationalities between 2010 and 2014. The paraffin-embedded glioma cases included 106 males and 50 females ranging in age from 15 to 78 years (median, 43.8 years). All specimens had a confirmed pathological diagnosis and were classified according to the World Health Organization (WHO) criteria. For the use of these clinical materials for research purposes, prior consent from the patients and approval from the Ethics Committees of this hospital were obtained.

RNA extraction and Real-time PCR

RNA was extracted from the glioma and brain tissues using Trizol (Takara, Shiga, Japan). The RNA was transcribed into cDNA and amplified with a specific MMP19 forward primer: 5'-GGATGGTCTGGCAACATGGA-3', The reverse primer: 5'-AGTCCCATGTCACTCCCAT-3'. GAPDH gene was used as an internal control, using the sense primer 5'-CGGAGTCAACGGATTTGGTCGTAT-3' and the antisense primer 5'-AGCCTTCTCCATGGTGGTGAAGAC-3'. The assays were performed in accordance with the manufacturer's instructions (Takara, Shiga, Japan). Cycling conditions were 95°C for 10 min to activate

DNA polymerase, followed by 45 cycles of 95°C for 13 s, 56°C for 13 s, and 72°C for 13 s. PCR reactions for each gene were repeated three times.

Immunohistochemistry

Following standard protocols, the glioma paraffin tissue sections (3 μ m thick) were deparaffinized in 100% xylene and rehydrated in a descending

ethanol series (100%, 90%, 80%, and 70% ethanol). Heat-induced antigen retrieval was carried out in 10 mM citrate buffer for 2 min at 100°C. A peroxidase blocking reagent containing 3% hydrogen peroxide and serum was used to block endogenous peroxidase activity and non-specific antigens. Samples were then incubated with mouse anti-human MMP19 monoclonal antibody (1:100 dilution) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) at 4°C overnight. The sections were visualized with 3,3'-diaminobenzidine, counterstained with hematoxylin, mounted in neutral gum, and analyzed using a bright field microscope.

Evaluation of staining

The stained tissue sections were reviewed separately by two pathologists blinded to the clinical parameters and evaluated for the presence of cytoplasm staining. The staining intensity was scored from 1-4 (negative expression: 1; weak expression: 2; positive expression: 3; strong expression: 4). The percentage of positive staining areas of the cells was defined on a scale of 0-3 (0: < 10%, 1: 10-25%, 2: 26-75%, and 3: > 76%). For the statistical analysis, a final staining score of 0-5 and 6-7 in the cytoplasm was considered to be low or high expression, respectively.

Statistical analyses

Statistical analyses were performed using GraphPad Prism 5 (<http://www.graphpad.com/company/>) and SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA). A two-tailed Student's t-test was employed for the comparisons between two groups. A chi-square test was used to examine the correlation between the

MMP19 overexpression in glioma

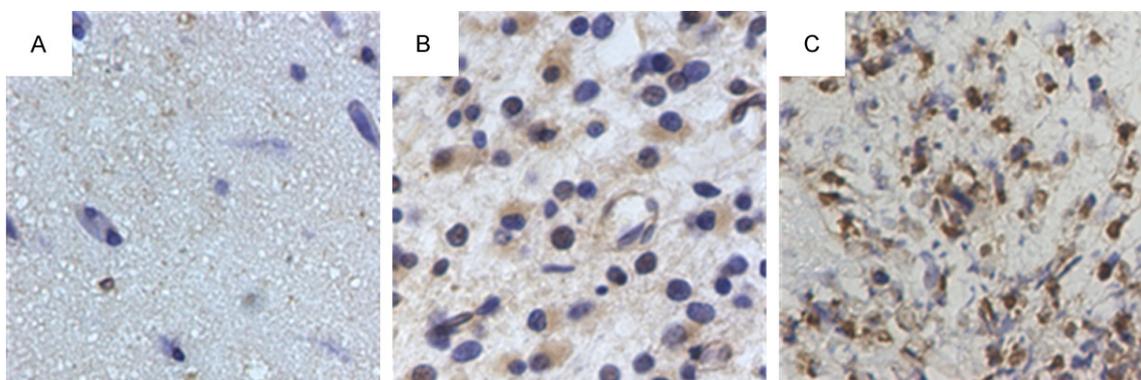


Figure 2. MMP19 protein expression in glioma (original magnification: $\times 400$). A. Negative expression of MMP19 protein in brain tissues. B. Low expression of MMP19 protein in glioma tissues of I-II stage. C. High expression of MMP19 protein in glioma tissues of III stage.

Table 1. Elevated expression of the MMP19 protein in lung adenocarcinoma

| | N | MMP19 protein expression | | P value |
|--------|-----|--------------------------|-----|---------|
| | | High | Low | |
| Cancer | 156 | 75 | 81 | 0.008* |
| Normal | 35 | 8 | 27 | |

*, Statistically significant.

Table 2. Correlation between the clinicopathologic characteristics and expression of MMP19 in glioma

| Characteristics | N | MMP19 Protein expression | | |
|-------------------|-----|--------------------------|-----|---------|
| | | High | low | P value |
| Age (y) | | | | |
| < 50 | 74 | 38 | 36 | 0.521 |
| ≥ 50 | 82 | 37 | 45 | |
| Gender | | | | |
| Male | 50 | 26 | 24 | 0.607 |
| Female | 106 | 49 | 57 | |
| Histological type | | | | |
| AT | 112 | 59 | 53 | 0.109 |
| OT | 20 | 11 | 9 | |
| Other | 24 | 17 | 7 | |
| WHO | | | | |
| I-II | 75 | 19 | 56 | 0.008* |
| III-IV | 81 | 25 | 56 | |

*, Statistically significant.

expression of MMP19 and the clinicopathologic features of breast cancer. The correlation between the protein expression of MMP19 and survival was performed using a Kaplan-Meier analysis with the log-rank test. A P value < 0.05 was considered statistically significant.

Results

Elevated MMP19 mRNA in glioma

In order to assess the role of MMP19 in glioma, qRT-PCR was explored to measure MMP19 mRNA transcripts in 61 freshly collected MMP19 tissues and brain tissues. Compared with the brain tissues, the glioma tissues exhibited higher expression levels of MMP19 mRNA ($P = 0.003$) (Figure 1A). Further, we observed that MMP19 expression was obviously increased in WHO stages III-IV compared to stages I-II (Figure 1B).

Immunohistochemistry of MMP19

Specific MMP19 protein staining was detected in the cytoplasm of the brain and tumor tissues (Figure 2A-C). MMP19 expression was significantly elevated ($P = 0.008$) in the glioma tissues (75/156) compared to the brain tissues (8/35) (Table 1).

The correlation between MMP19 expression and clinicopathologic parameters

A significant relationship between MMP19 protein expression with patient age, gender, and histological type in the 156 glioma cases was not observed, but high MMP19 protein expression was positively correlated with the clinical stage of the disease (Table 2; $P = 0.008$).

High expression of MMP19 is correlated with poor overall survival outcome

A Kaplan-Meier analysis with a log-rank test was used to analyze the correlation between

MMP19 overexpression in glioma

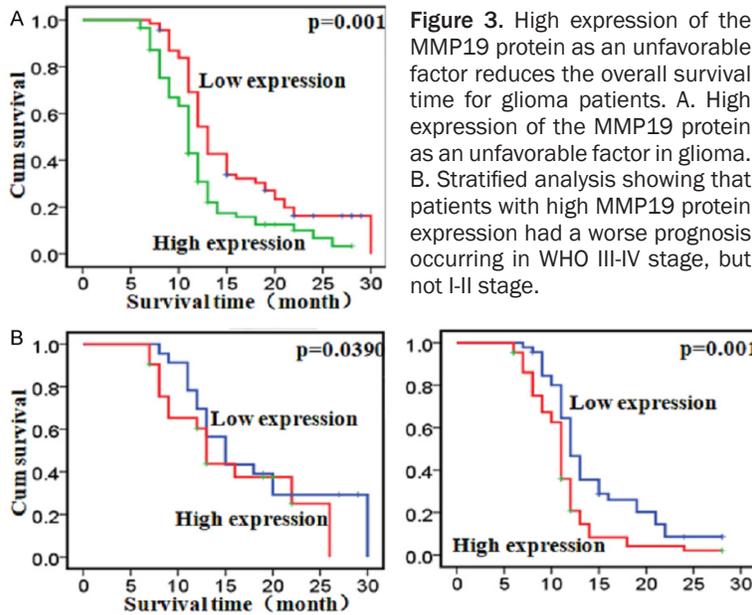


Figure 3. High expression of the MMP19 protein as an unfavorable factor reduces the overall survival time for glioma patients. A. High expression of the MMP19 protein as an unfavorable factor in glioma. B. Stratified analysis showing that patients with high MMP19 protein expression had a worse prognosis occurring in WHO III-IV stage, but not I-II stage.

the level of MMP19 protein expression in glioma tissue and patient survival. The level of MMP19 protein expression was negatively correlated with the overall survival time of the glioma patients. Patients with high MMP19 protein expression had a significantly worse prognosis ($P = 0.001$) compared to patients with low MMP19 protein expression (**Figure 3A**). Further, a stratified analysis showed that patients with high MMP19 protein expression indicated a worse prognosis occurring in WHO stages III-IV ($P = 0.001$), but not in stages I-II ($P = 0.039$).

Elevated expression of MMP19 is an independent prognosis factor

Univariate and multivariate analyses both suggested that MMP19 protein expression was significantly associated with patient survival ($P = 0.008$ and 0.009 , respectively). A high expression of the MMP19 protein was an independent prognostic marker for glioma patients (**Table 3**).

Discussion

The MMPs are the most prominent family of proteases involved in tumor invasion (40). MMP19, as a member of the MMPs family, had been reported to promote tumor growth, invasion, metastasis and chemoresistance including non-small cell lung cancer [10-12], a demonstration of its significance in tumor pathogenesis. Furthermore, its overexpression

was also observed in melanoma and glioma [13]. However, the correlation of MMP19 expression with the clinical features of glioma is still determined.

Glioma is the predominant type of tumor in the brain. The present study indicated that MMP19 mRNA expression was significantly elevated compared to brain tissues. These data were consistent with Lettau's et al. report [9] on glioma. Further, we found that MMP19 expression was much higher in the glioma tissues of WHO stage III compared to WHO grade I-II. This result hinted that increased MMP19

expression was involved in the progression of glioma, which had never been reported. Further, we examined the MMP19 protein expression by immunohistochemistry. The result showed the cytoplasm expression of MMP19 in glioma and normal brain cells. Further, the MMP19 protein was significantly elevated compared to a normal brain, which was also consistent with the finding of Lettau's et al. [9]. This result indicates that MMP19 may act as an oncogene and that it may participate in the pathogenesis of glioma. However, the correlation of MMP19 protein expression with clinical features remains to be definitively determined in glioma patients.

In a previous study, in contrast to the early stages, MMP19 was upregulated during the vertical growth phase of melanoma and in metastases [13]. Presently, the overexpression of MMP19 is not correlated with age and gender but is clearly associated with the clinical stage of glioma patients, consistent with prior findings. Thus, the overexpression MMP19 is related to the promotion of glioma pathogenesis.

In the past few years, overexpressed MMP19 has never been identified as a prognostic factor in tumors. The present data provide evidence that MMP19 protein expression in glioma is inversely correlated with overall survival. Patients with higher expression of MMP19 protein had shorter overall survival times. Inversely, patients with lower expression of the MMP19

MMP19 overexpression in glioma

Table 3. Summary of univariate and multivariate Cox regression analyses of overall survival

| Parameter | Univariate analysis | | | Multivariate analysis | | |
|--|---------------------|-------|-------------|-----------------------|-------|-------------|
| | P | HR | 95% CI | P | HR | 95% CI |
| Age ≥ 60 versus < 60 years | 0.007* | 0.615 | 0.431-0.876 | 0.010* | 0.625 | 0.436-0.895 |
| Gender Male versus female | 0.442 | 0.864 | 0.595-1.255 | | | |
| Histological type High versus Medium versus Low | 0.268 | 0.875 | 0.691-1.108 | | | |
| WHO classification I-II versus III-IV | 0.001* | 2.000 | 1.319-3.033 | 0.002* | 1.933 | 1.268-2.947 |
| Expression of MMP19 High versus low expression | 0.008* | 1.607 | 1.130-2.285 | 0.009* | 1.606 | 1.126-2.289 |

*, Statistically significant.

protein had longer overall survival times. Interestingly, a stratified analysis indicated that higher MMP19 caused a poor prognosis occurring WHO stages III-IV, and this data suggested that MMP19 functions an oncogene in advanced glioma.

MMP19 overexpression has never been reported as an independent prognostic factor in tumors. In this study, we demonstrated by univariate and multivariate analyses that increased protein expression of MMP19 is an independent prognostic factor predicting a poor prognosis for glioma patients. The data reinforce the malignant role of MMP19 in glioma pathogenesis.

Conclusions

An increased protein expression level of MMP19 is clearly correlated with the clinical progression and poor prognosis of glioma. In addition, increased MMP19 is an important independent prognostic factor of glioma.

Acknowledgements

This work was supported by the National Natural Science Foundation of China, Grant/Award Number: 81760450; Guangxi Natural Science Foundation of China, Grant/Award Number: 2017GXNSFAA198322.

Disclosure of conflict of interest

None.

Address correspondence to: Qianli Tang, College of Integrated Chinese and Western Medicine, Hunan University of Chinese Medicine, Changsha 410208, Hunan, China. E-mail: 2283782346@qq.com

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