Original article

Folia Neuropathologica

Differential expression of MMP-9 and AQP4 in human glioma samples

Wei-Jiang Zhao[1,2], Wei Zhang[1], Gui-Lin Li[1], Yun Cui[1], Zhong-Fang Shi[1], Fang Yuan[1]

[1]Beijing Neurosurgical Institute, Capital Medical University, Beijing 100050, China, [2]Center for Neuroscience, Shantou University Medical College, Shantou, Guangdong Province 515041, China

Folia Neuropathol 2012; 50 (2): 176-186

Abstract

Background: *Metalloproteinase-9 (MMP-9) and aquaporin-4 (AQP4) have been individually reported in glioma development. Here, we co-analyzed their expression in multiple forms of human glioma tissues graded from II to IV.*
Material and methods: *Levels of MMP-9 and AQP4 were evaluated on 50 resected human glioma tissues using immunohistochemistry. Protein levels of both molecules were evaluated by a staining score system based on the percentage of positive cells/staining degree in each dot section. The transwell method was also used to discriminate fast migrating cells and slow migrating cells, in which expression of both MMP-9 and AQP4 was investigated by using immunofluorescence.*
Results: *The staining score of MMP-9 displayed a positively tumor grade dependent manner, whereas AQP4 expression showed a negatively tumor grade dependent manner. The nuclear translocation of both molecules was observed in astrocytomas with glioblastoma transition, or glioblastoma tissues. Fast migrating cells contain more AQP4, whereas more MMP-9 was localized in slow migrating cells.*
Conclusions: *Our findings suggest differential expression patterns of MMP-9 and AQP4 in different grades of gliomas. Nuclear translocation of MMP-9 and AQP4 may exert more functions in glioblastoma transition or deterioration. Co-analysis of MMP-9 and AQP4 may help to identify tumor type and their progression stages.*

Key words: *metalloproteinase-9 (MMP-9), aquaporin-4 (AQP4), human glioma.*

Introduction

Glioma is a kind of frequently occurring behaviorally malignant tumor in the nervous system (NS), accounting for 40-50% of all NS related tumors. It is characterized by invasive growth, high mortality, high recurrence, poor clinical prognosis and low survival time of less than 18 months [4]. Despite intense effort, we still fall back on surgery and radiation therapy for treatment options. A major reason for this status is that glioma development is molecularly a multiple-factor induced pathological condition. Recently, accumulated evidence has pointed to metalloproteinases and aquaporins (AQPs) as key molecules that play an important role in glioma development, especially in tumor related brain edema and metastasis [20].

Communicating author:
Weijiang Zhao, Center for Neuroscience, Shantou University Medical College, Shantou 515041, Guangdong Province, China, e-mail: neuromancn@yahoo.com.cn; Fang Yuan, Beijing Neurosurgical Institute, Capital Medical University, Beijing 100050, China, e-mail: florayuan@vip.sina.com

AQPs are a family composed of more than ten members, which function in water shuttling, glycerol and lipid metabolism [3]. Some specific members function in alleviating glioma-associated lactic acidosis by clearing glycerol and lactate from the extracellular space [31]. AQP4 is widely distributed in a variety of central nervous structures, including ependymal cells lining the lateral ventricle and aqueduct, choroid plexuses, pia mater, hypothalamus, supraoptic nucleus, dentate gyrus and cerebellar Purkinje cells [25]. AQP4 has also been reported to increase water permeability [15], and may be involved in astrocyte swelling and brain edema [1,16,24]. AQP4 deletion provides neuroprotection in a series of pathological settings, including a transient ischemia model of retinal injury [6]. In contrast, AQP4 expression in glioma has not been fully elucidated, and the correlation of AQP4 expression with glioma malignancy remains controversial.

Matrix metalloproteinases (MMPs) are a multigene family of Zn^{2+}-dependent enzymes, which upon activation can degrade the extracellular matrix [17], thus exerting key functions in wound healing, bone growth and remodeling, angiogenesis, macrophage infiltration, and axonal growth cone extension [20, 32]. MMP level correlates with tumor invasion and metastasis. Significantly increased MMP-9 levels in the euglobulin plasma serve as a potential tumor marker in breast and lung cancer [11]. MMP-9 expression in response to the signal cascade composed of TWEAK and NF-kappaB can lead to disruption of the neurovascular unit (NVU) structure and increase permeability of the blood-brain barrier (BBB) [23]. Exposure to hypoxia can cause increased activities of MMP-9 in endothelial cells [10]. Downregulation of MMP-9 can induce apoptosome-mediated apoptosis, a process related to inhibition of tumor invasion both in vitro and in vivo, as well as the inhibition of intracranial tumor growth [12].

MMP-9 expression correlates with glioma malignancy, whereas roles of AQP4 in glioma have not been well established. We here co-investigate the expression pattern of both molecules in different types of glioma tissues and gliomas of different grades.

Material and methods

Reagents

MMP-9 antibody was obtained from DAKO (catalogue no. A-0150, Dako, Denmark) and Bioss Biotechnology Company (catalogue no. bs-0397R, Beijing, China). AQP4 antibody was obtained from Chemicon (catalogue no. AB3086, Temecula, TX, USA) and Bioss Biotechnology Company (catalogue no. bs-0634R, Beijing, China). EnVision™ Detection Kit was purchased from DAKO (catalogue no. K4008, Dako, Denmark). Human U87-MG glioma cells were obtained from Chinese Type Culture Collection (CTCC, Shanghai, China).

Tissue microarrays

Glioma tissue microarray was prepared using resected glioma tissues from patients diagnosed with glioma before an operation in Beijing Tiantan Hospital from January to September, 2006. 50 glioma samples graded from II to IV were collected from 35 male and 15 female patients. Patient age ranged from 14 to 66 years, with the average age 41.1 ± 12.5 years. Glioma types were further confirmed through hematoxylin and eosin staining based microscopic investigations after the operation. Each sample was classified and scored based on the WHO standard for tumor classification. An experienced neuropathologist delicately selected 2 tumor areas with a diameter of 1 mm sampled in each case to ensure they were accurately in coincidence with the donor cases. Microarrayed tissues were sectioned at 4 μm for immunohistochemical observations.

Immunohistochemistry of gliomas

After deparaffinization, sections were rehydrated through a graded series of ethanol to phosphate buffer saline (PBS). Antigen retrieval was performed using 10 mM citrate buffer (pH 6.0) at 98° for 30 min followed by cooling down at room temperature for at least 60 min. Then sections were subjected to 3% H_2O_2 incubation for endogenous peroxidase clearance. Afterwards, these sections were individually incubated with anti MMP-9 and AQP4 antibodies diluted in 10% normal goat serum at 1 : 200 and 1 : 50 respectively (Chemicon, USA) at 4°C overnight. The antigen-antibody complexes were visualized using the EnVision™ Detection Kit (catalogue no. K4008, DAKO, Denmark). Counterstaining was undertaken with Meyer's hemotoxylin. MMP-9 and AQP4 immunostains were evaluated using 10× and 20× lenses by light microscopy. MMP-9 and AQP4 expressions were quantified as percentage of immunopositive neoplastic cells per section. In the representative areas, positive tumor cells were determined and scored in a semiquantitative fashion of 0 up to 3+: 0 (–) = less than 10% weak positive tumor cells, 1+ = = 11-25% intermediate positive tumor cells, 2+ = 26-50%

strong positive tumor cells, 3+ = 51-100% very strong positive cells.

Cell culture

Human glioma U87-MG cells were routinely cultured in the 75 cm^2 plate in DMEM low glucose culture medium supplemented with 10% fetal bovine serum (FBS) and 1% each of penicillin and streptomycin mixture at 37°C in a 5% CO_2 environment. Transwell methods were used to discriminate fast migrating cells and low migrating cells. For screening, 1×10^5 cells were loaded onto the upper well of the transwell, whose bottom surface was contacting the medium surface in the lower well. After cell seeding for 24 hours, the upper well was transferred to another well containing the normal cell culture medium. Cells were permitted to migrate for another 24 hours. Migrated cells from both lower wells were individually transferred to the 75 cm^2 plate for routine culture and further cultured for 2 passages until being used for the immunofluorescence experiment.

Immunofluorescence

Fast migrating and slow migrating cells (1×10^4 cells/well) were individually seeded on a cover slip in regular culture medium in a 24-well cell culture plate. 48 hours after seeding, regular culture medium was aspirated and cells were washed briefly with PBS and were fixed with 4% PBS buffered paraformaldehyde (PFA) for 10 min and then washed briefly with PBS. Samples were blocked with 10% normal donkey serum (NDS) for 30 min and incubated individually overnight at 4°C with the following primary antibodies: polyclonal rabbit anti-MMP-9 (1 : 200; Biosynthesis Biotechnology Co., LTD, Beijing, China) or polyclonal rabbit anti-AQP4 (1 : 200; Biosynthesis Biotechnology Co., LTD, Beijing, China) antibody. After being washed, samples were incubated at room temperature with secondary donkey anti-rabbit IgG/Dylight™ 594 antibody (1 : 1000; Jackson ImmunoResearch Laboratories Inc., PA, USA) for 120 min. Nuclei were counterstained with Dapi. Confocal images of U87-MG cells were acquired using an Olympus FluoView FV1000 confocal laser scanning microscope (Olympus, Japan). Dapi was excited at 405 nm, and Dylight™ 594 at 568 nm.

Statistical analysis

Data were expressed as mean ± SEM. Independent Student's *t*-test was used for the comparison between 2 groups, with $p < 0.05$ as the significance level.

Results

Localization of MMP-9

In normal brain tissues, MMP-9 was selectively and weakly detected in cytoplasm and in brain parenchyma in a dotted pattern (Fig. 1A). In astrocytomas, MMP-9 was localized mainly in the tumor cytoplasm, with enhanced dotted staining in the brain parenchyma (Fig. 1B). By contrast, MMP-9 was selectively expressed in the cytoplasm in the oligodendroastrocytoma tissues (Figs. 1C, D). In astrocytoma tissues displaying anaplastic presentation, cytoplasmic staining of MMP-9 was enhanced (Fig. 1E), and some samples displayed a perinuclear staining pattern (Fig. 1F). In glioblastoma tissues and anaplastic astrocytoma and oligodendroastrocytoma tissues, MMP-9 were expressed in most tumor cells, where they were mostly localized in the cytoplasm and nuclei (Figs. 1G-I). Such a localization pattern was obvious in gliosarcoma samples (Fig. 1J).

Localization of AQP4

AQP4 was accumulatively and intensively localized in the end-feet surrounding cerebrovascular walls, with sporadic and dotted localization in the prominences of the astrocytic cells (Fig. 2A). AQP4 was diffusely and extensively accumulated in astrocytoma specimens, with more intensive localization surrounding the vasculature and processes in the astrocytomic processes (Fig. 2B). In oligodendroastroglioma tissues, more intense localization of AQP4 surrounding the vascular walls was observed, with its selective distribution in tumor parenchyma (Figs. 2C, D). By contrast, AQP4 was mainly located on cellular membrane in oligodendrocytomas (Fig. 2E).

In locally anaplastic astrocytoma specimens, cell density was apparently low with enhanced AQP4 localization in some tangled astrocytomic processes (Fig. 2F) or accumulated in processes proximal to the cell body (Fig. 2G). By contrast, in local anaplastic oligodendroastrocytoma specimens, AQP4 was exclusively expressed in vasculature surrounding structures (Fig. 2H). Of note, in grade III oligodendroastrocytoma with an anaplastic character of the glioblastoma, cells displayed significant localization of AQP4 in the nucleus (Fig. 2I), which was also observed in the nucleus or in the cytoplasm in the glioblastoma specimens (Fig. 2J).

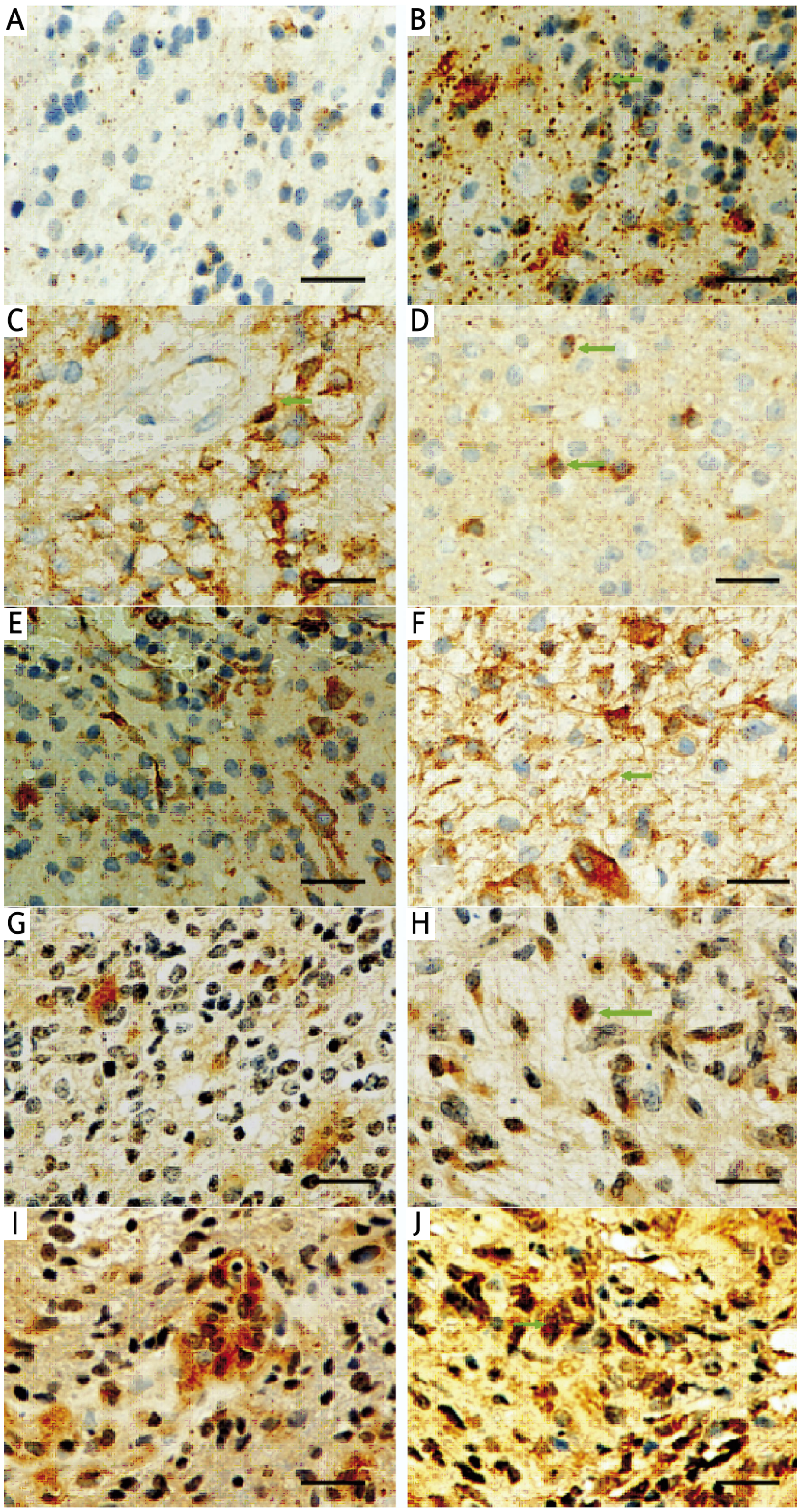


Fig. 1. Immunohistochemical staining of MMP-9 in human glioma tissues. Staining of MMP-9 was observed in **A)** normal brain tissue, **B)** astrocytoma, **C**, **D)** oligodendroastrocytoma, **E**, **F)** anaplastic astrocytoma, **G)** glioblastoma, **H)** anaplastic astrocytoma, **I)** anaplastic oligodendroastrocytoma, **J)** gliosarcoma. Scale bars = 30 µm.

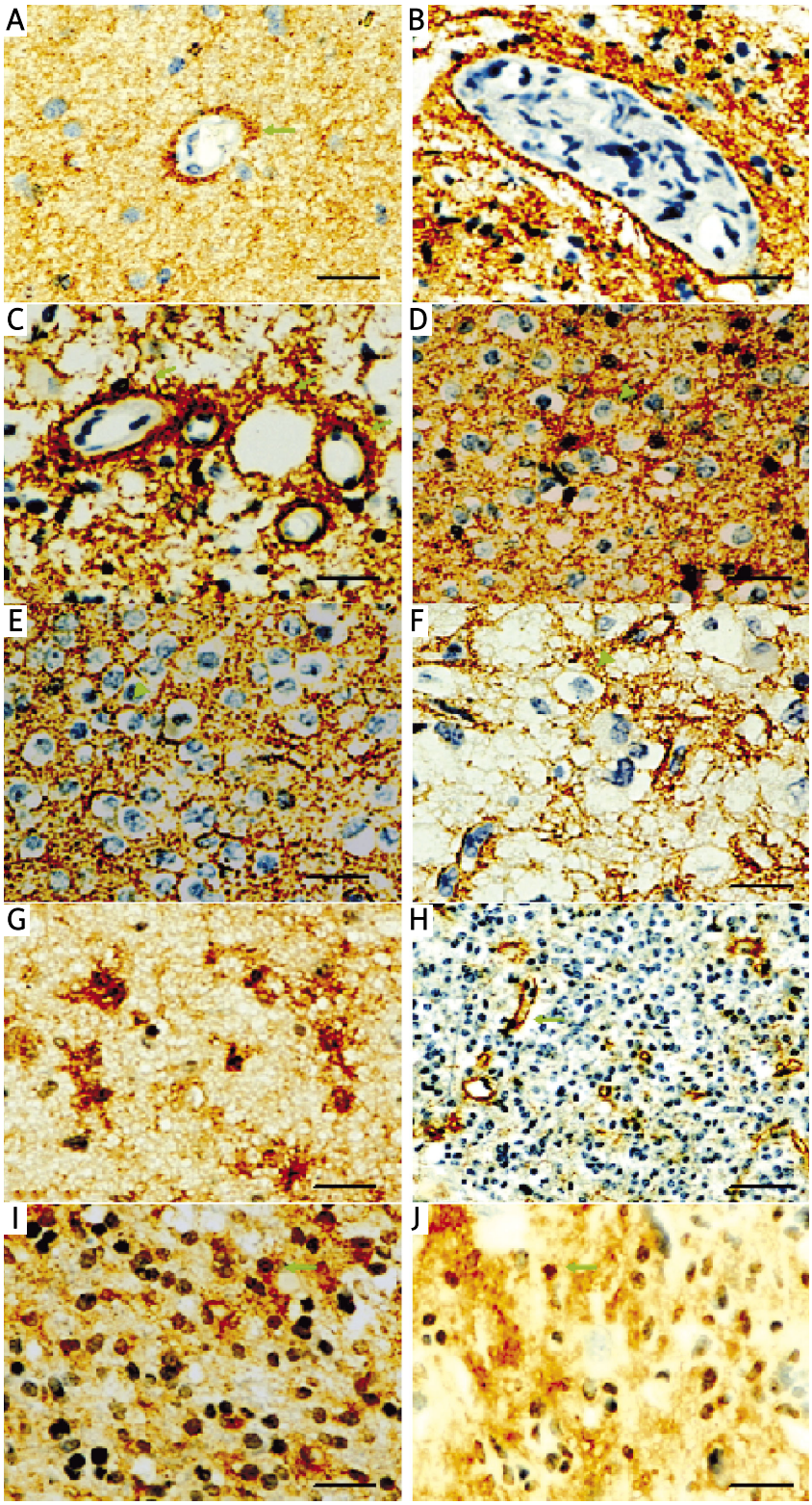


Fig. 2. Immunohistochemical staining of AQP4 in human glioma tissues. Staining of AQP4 was observed in **A)** normal brain tissue, **B)** astrocytoma, **C**, **D)** oligodendroastrocytoma, **E)** oligodendrocytoma, **F**, **G)** anaplastic astrocytoma, **H**, **I)** anaplastic oligodendroastrocytoma, **J)** glioblastoma. Scale bars = 30 μm.

Immunohistochemical staining of MMP-9 and AQP4 in human glioma tissues

In grade II glioma specimens, 13 astrocytomas were identified, among which the numbers of tumor tissues for MMP-9 staining score graded from 1 to 3 were 3, 5, and 5 respectively, and for AQP4 were 1, 7, and 5 respectively. The proportion for MMP-9 staining score in 4 oligodendroglioma tissues was grade 0 (1/4), grade 1 (1/4), grade 2 (1/4), and grade 3 (1/4), and for AQP4 it was grade 3 (4/4). Among 7 oligodendroastrocytoma tumor tissues, the distribution of staining score for MMP-9 is 0 (2/7), 1 (2/7), 2 (2/7), 3 (1/7), and for AQP4 the number is 0 (0/7), 1 (0/7), 2 (1/7), and 3 (6/7) (Tables I and II).

In grade III glioma tissues, 8 anaplastic astrocytomas were identified, in which the proportion for MMP-9 staining score is 0 (1/8), 1 (2/8), 2 (1/8) and 3 (4/8), respectively, and for AQP4 it is 0 (1/8), 1 (2/8), 2 (2/8) and 3 (3/8). One anaplastic oligodendroglioma was identified, showing grade 3 for MMP-9 staining, but 0 for AQP4 in the staining score. Two anaplastic oligodendroastrocytomas showed grade 2 for MMP-9 staining, and showed grade 1 and grade 3 for AQP4 in the staining score. One glioblastoma was included, whose staining score for MMP-9 is 3, but is 1 for AQP4 (Tables I and II).

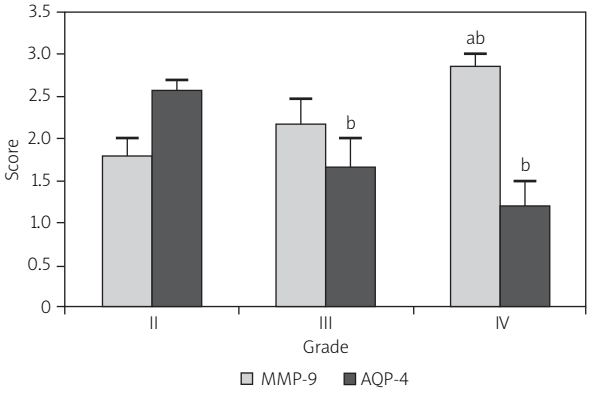
Seven glioblastomas were included in grade IV glioma tissues, among which one (1/7) showed grade 1 score for MMP-9 staining, with the others showing grade 3 (6/7). In contrast, the proportion of AQP4 staining score in glioma tissues was 0 (2/7), 1 (3/7), and 2 (2/7). Two astrocytomas and 4 anaplastic oligodendroastrocytomas, all with transition for glioblastoma, and 1 glioma sarcomatosum were also included, all showing 3+ in the staining score for MMP-9. Two astrocytomas displaying glioblastoma transition showed 1+ and 3+ for AQP4 in the staining score. In 4 anaplastic oligodendroastrocytoma tissues with glioblastoma

Table I. Staining intensity analysis of MMP-9 in the human glioma tissue microarray

Grade	MMP-9 expression				Total	Positive incidence
	–	+	++	+++		
II						
astrocytoma		3	5	5	**13**	
oligodendroglioma	1	1	1	1	**4**	
oligodendroastrocytoma	2	2	2	1	**7**	
						21/24
III						
astrocytoma (anaplasia)	1	2	1	4	**8**	
oligodendroglioma (anaplasia)				1	**1**	
oligodendroastrocytoma (anaplasia)			2		**2**	
glioblastoma				1	**1**	
						11/12
IV						
glioblastoma		1		6	**7**	
astrocytoma with transition to glioblastoma				**2**	**2**	
anaplastic oligodendroastrocytoma, with transition to glioblastoma				4	**4**	
glioma sarcomatosum				1	**1**	
						14/14

Table II. Staining intensity analysis of AQP4 in the human glioma tissue microarray

Grade	AQP-4 expression				Total	Positive incidence
	–	+	++	+++		
II						
astrocytoma		1	7	5	13	
oligodendroglioma				4	4	
oligodendroastrocytoma			1	6	7	
						24/24
III						
astrocytoma (anaplasia)	1	2	2	3	8	
oligodendroglioma (anaplasia)	1				1	
oligodendroastrocytoma (anaplasia)		1		1	2	
glioblastoma		1			1	
						12/12
IV						
glioblastoma	2	3	2		7	
astrocytoma with transition to glioblastoma		1		1	2	
anaplastic oligodendroastrocytoma, with transition to glioblastoma	2		1	1	4	
glioma sarcomatosum		1			1	
						10/14



[a]p < 0.05 compared to values in the grade II gliomas, and [b]p < 0.01 compared to values in the grade III gliomas

Fig. 3. Immunohistochemical staining score analysis of MMP-9 and AQP4 levels in the human glioma tissue microarray.

transition, the proportion for AQP4 staining score is 0 (2/4), 2 (1/4), and 3 (1/4) respectively. One glioma sarcomatosum showed 1+ for AQP4 (Tables I and II).

Staining score analysis

To clarify whether expression of both molecules is related to tumor severity, we compared the staining scores of both MMP-9 and AQP4 between glioma tissues of different grades.

Staining scores of MMP-9 in glioma tissues graded from II to IV showed a malignancy dependent manner, and were 1.79 ± 1.02, 2.17 ± 1.03 and 2.86 ± 0.53 respectively. Compared to grade II and III, the staining score in grade IV tumor samples was significantly higher ($p < 0.01$ and $p < 0.05$ respectively). No significance was revealed when staining scores in grade II and III tumor tissues were compared to each other (Fig. 3).

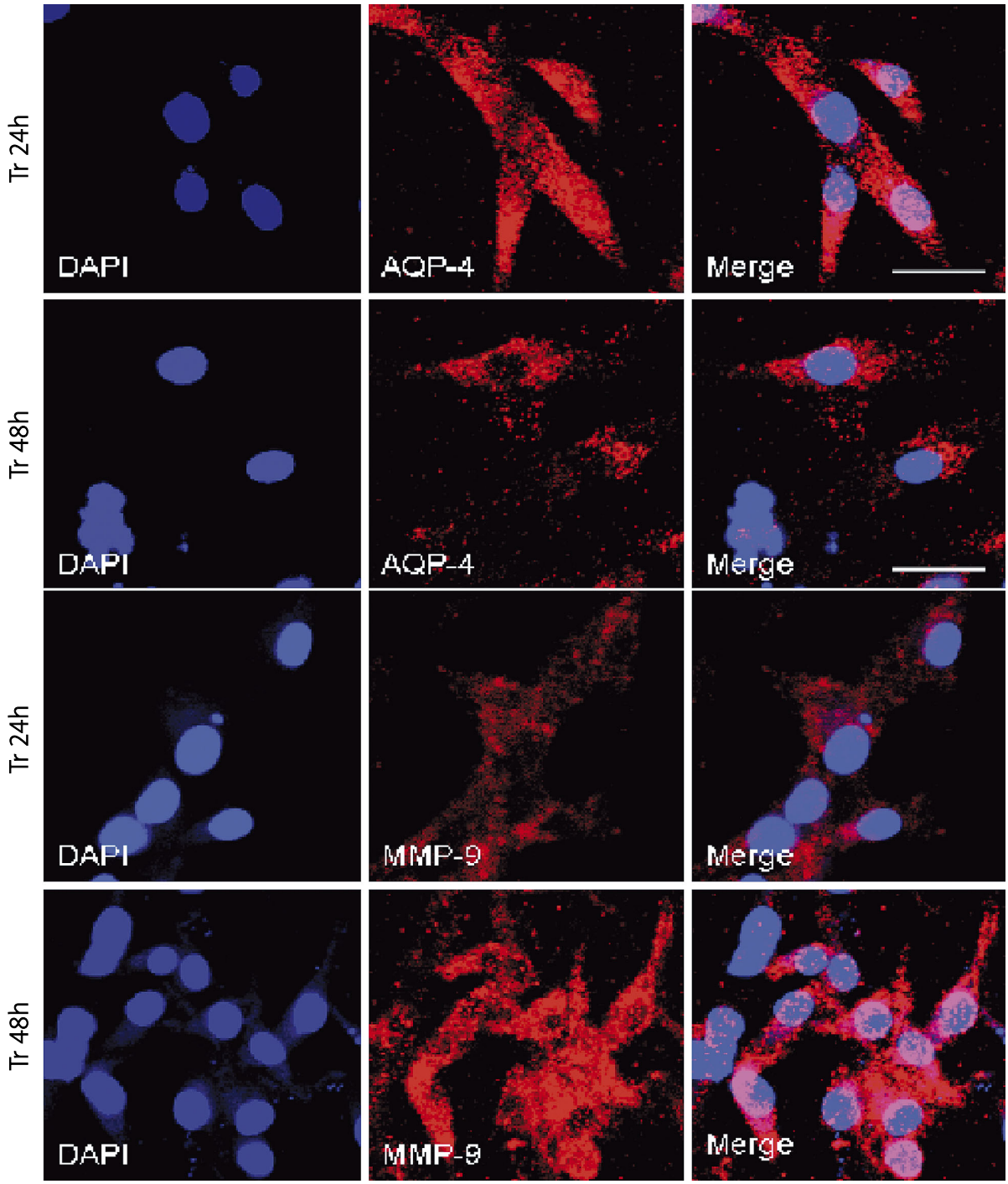


Fig. 4. Immunofluorescence staining of MMP-9 and AQP4 in fast migrating and slow migrating human glioblastoma U87-MG cells. Scale bars = 20 µm.

In contrast to MMP-9, staining scores of AQP4 in glioma tissues graded from II to IV demonstrated a malignancy dependent reduction manner, and were 2.58 ± 0.58, 1.67 ± 1.15 and 1.21 ± 1.05 respectively. Staining scores in both grade III and IV tumor samples were significantly lower than that in grade II tumor samples ($p < 0.01$ for both). No significance was revealed when staining scores in grade II and III glioma tissues were compared (Fig. 3).

Immunofluorescence staining

We employed immunofluorescence staining to compare the levels of MMP-9 and AQP4 in cells with more migration potential and less migration potential. The results demonstrated that cells displaying more migration potential expressed less MMP-9, in contrast to those with slow migration potential. However, more AQP4 was expressed in cells with more migration potential, suggesting the possible involvement of AQP4 in glioma cell migration (Fig. 4).

Discussion

The high invasiveness and infiltrability of malignant gliomas makes chemotherapy and gene therapy of gliomas largely unsuccessful. In the present study, we investigated the expression and localization of MMP-9 and AQP4 in microarrayed human glioma tissues. Overall, MMP-9 expression showed a malignancy dependent pattern, whereas AQP4 expression showed a negatively malignancy dependent manner.

We primarily found that expression of AQP4 is not absolutely dependent on the glioma malignancy, but shows a high correlation with tumor type. This suggests that AQP4 may selectively function in the development and pathophysiological behaviors of human gliomas. However, most of our knowledge about AQPs is from the previous data about AQP1, which has been detected in cultured glioma cell lines [14]. AQP1 mRNA and protein are present at very low levels in primary rat brain microvessel endothelial cells [8]. In the central nervous system, AQP1 is selectively localized in the choroid plexus and is thought to participate in cerebrospinal fluid production [21]. Upregulation of AQP1 was induced by DES in the female rat anterior pituitary [34,35]. In cultured 9L gliosarcoma cells, AQP1 expression was induced by multiple conditions, functioning as a base for potential invasion of glioma cells in the perivascular space [14]. In astrocytomas and other pathological conditions, AQP1 was expressed in microvessel endothelia and neoplastic astrocytes and may participate in the formation of brain tumor edema [9,26,33].

In contrast to AQP1, the role of AQP4 remains to be understood. Through stable reintroduction of either AQP1 or AQP4 into glioma cell lines, which lost all AQP proteins once contained in their derived cell line, McCoy *et al.* [18] found that each AQP is sufficient to restore water permeability. However, only AQP1 could enhance cell growth and migration. And AQP4 enhanced cell adhesion. In contrast, we found that fast migrating glioma cells contain more AQP4 than slow migrating cells, suggesting that this molecule may directly or indirectly participate in the process of cell migration. This suggested that AQP4 may play roles different from those of AQP1 in glioma cell biology.

AQPs are involved in the dynamics of brain edema formation or resolution during subarachnoid hemorrhage (SAH) [2]. It has been reported that upregulation of AQP-4, within a certain range, may facilitate the clearance of water content accumulated in the extracellular space, whereas extremely increased AQP4 expression can cause accumulation of water in the glia cells, thus leading to cell death [28]. AQP4 expression significantly correlates with the degree of cerebral edema, possibly involved in edema clearance. The restriction of AQP4 to the endfoot membrane has been reported to be necessary for maintaining the integrity of the blood-brain barrier (BBB) [29]. Since electron microscopy revealed tight junction opening in high-grade astrocytoma microvessels, the reduced expression of AQP4 around these regions in high-grade glioma tissues may further lead to increased edema. AQP4 was found to be redistributed in high-grade astrocytomas, rather than in low-grade astrocytomas. As a result of the breakdown of BBB, the redistribution of AQP4 in glioblastoma cells may function as a vasogenic edema executor to facilitate reabsorption of excess fluid [30]. This over-expression on the astrocytic processes and disappearance of polarization on astrocytic endfeet reinforce the hypothesis that AQPs may be involved in the dynamics of brain edema formation or resolution [2]. Controversial results demonstrated that addition of glioma cells in cultured BBB content induced a significantly decreased level of expression of AQP4 in the astrocytes [5]. There was a significant correlation between blood-brain barrier opening and upregulated AQP4 expression. Increased AQP4 expression in high grade astrocytomas and adenocarcinomas

has also been postulated to facilitate the flow of edema fluid [26].

In the present research, we found weak positivity of AQP4 surrounding the capillary vessel in low-graded human glioma tissues, while in higher-grade samples, distribution of AQP4 was not confined to a position contacting the basal laminae, which is involved in the construction of the blood-brain barrier. This further confirms previous reports about redistribution of AQP4 with the increase of glioma grade. Thus, AQP4 and its redistribution may be grade dependent and reflect the grade of gliomas. Ding *et al.* [7] reported enhanced cell-cell adhesion ability in AQP4-downregulated LN229 cells. Increased MMP-9 expression led to loss of agrin and dystroglycan, and is thus involved in the disruption of the regular assembly and expression of AQP4 [21]. It is therefore postulated that migration and cell adhesion may represent two independent but key steps in the development of glioblastoma. High expression of AQP4 may facilitate cell polarity formation and shape change, thus leading to fast cell migration. In contrast, more MMP-9 may allow longer local cell anchoring by enhancing cell adhesion.

MMP-9 upregulation is generally indicative of the infiltrative phenotype of glioma. More histologically malignant astrocytomas display significantly higher expression levels of MMP-9 compared to tumors of lower grades. MMP-9 was also subjected to the modulation of multiple molecules, including HMGB1, which triggers MMP-9 upregulation in neurons and astrocytes predominantly via TLR4. Immunostaining with different monoclonal antibodies against MMP-9 revealed dynamic localization of MMP-9 at the different stages of cell division. Intracellular MMP-9 is involved in the process of cell division in neuroblastoma cells [27]. Roles of MMP-9 have been strongly indicated in nuclear content reorganization and the involvement of chromatid segmentation. Reduced MMP-9 expression also reduced cell growth. This was further confirmed by our observation showing nuclear localization of MMP-9 in high graded glioblastoma and anaplastic astrocytomas. Based on our histochemical observations here, we propose that MMP-9 translocation may exert similar functions to those described in neuroblastoma cells.

In brief, our findings suggest differential expression patterns of MMP-9 and AQP4 in different grades of gliomas. Nuclear translocation of MMP-9 and AQP4 may exert more functions in glioblastoma transition or deterioration. Co-analysis of MMP-9 and AQP4 may help to identify tumor type and their progression stages.

Acknowledgements

The authors are grateful to the National Natural Science Foundation of China (grant no. 81171138 to WJ. Z. and grant no. 30840083 to F. Y.) for support. The work was also supported by Beijing Natural Science Foundation (grant no. 7102028 to F. Y.).

References

1. Alexander JJ, Jacob A, Cunningham P, Hensley L, Quigg RJ. TNF is a key mediator of septic encephalopathy acting through its receptor, TNF receptor-1. Neurochem Int 2008; 52: 447-456.
2. Badaut J, Brunet JF, Grollimund L, Hamou MF, Magistretti PJ, Villemure JG, Regli L. Aquaporin 1 and aquaporin 4 expression in human brain after subarachnoid hemorrhage and in peritumoral tissue. Acta Neurochir Suppl 2003; 86: 495-498.
3. BeitzE, Schultz JE. Themammalian aquaporin water channel family: a promising drug target. CurrMed Chem 1999; 6: 457-467.
4. Boon K, Edwards JB, Eberhart CG, Riggins GJ. Identification of astrocytoma associated genes including cell surface markers. BMC Cancer 2004; 4: 39.
5. Chen YZ, Xu RX, Xu ZJ, Yang ZL, Jiang XD, Cai YQ. Relationship between aquaporin-4 expression in astrocytes and brain edema caused by glioma. Di Yi Jun Yi Da Xue Xue Bao 2003; 23: 566-568, 571.
6. Da T, Verkman AS. Aquaporin-4 gene disruption in mice protects against impaired retinal function and cell death after ischemia. Invest Ophthalmol Vis Sci 2004; 45: 4477-4483.
7. Ding T, Ma Y, Li W, Liu X, Ying G, Fu L, Gu F. Role of aquaporin-4 in the regulation of migration and invasion of human glioma cells. Int J Oncol 2011; 38: 1521-1531.
8. Dolman D, Drndarski S, Abbott NJ, Rattray M. Induction of aquaporin 1 but not aquaporin 4 messenger RNA in rat primary brain microvessel endothelial cells in culture. J Neurochem 2005; 93: 825-833.
9. Endo M, Jain RK, Witwer B, Brown D. Water channel (aquaporin 1) expression and distribution in mammary carcinomas and glioblastomas. Microvasc Res 1999; 58: 89-98.
10. Ezhilarasan R, Mohanam I, Govindarajan K, Mohanam S. Glioma cells suppress hypoxia-induced endothelial cell apoptosis and promote the angiogenic process. Int J Oncol 2007; 30: 701-707.
11. Farias E, Ranuncolo S, Cresta C, Specterman S, Armanasco E, Varela M, Lastiri J, Pallotta MG, Bal de Kier Joffe E, Puricelli L.Plasma metalloproteinase activity is enhanced in the euglobulin fraction of breast and lung cancer patients. Int J Cancer 2000; 89: 389-394.
12. Gondi CS, Dinh DH, Gujrati M, Rao JS. Simultaneous downregulation of uPAR and MMP-9 induces overexpression of the FADD-associated protein RIP and activates caspase 9-mediated apoptosis in gliomas. Int J Oncol 2008; 33: 783-790.
13. Gondi CS, Talluri L, Dinh DH, Gujrati M, Rao JS. RNAi-mediated downregulation of MMP-2 activates the extrinsic apoptotic pathway in human glioma xenograft cells. Int J Oncol 2009; 35: 851-859.

14. Hayashi Y, Edwards NA, Proescholdt MA, Oldfield EH, Merrill MJ. Regulation and function of aquaporin-1 in glioma cells. Neoplasia 2007; 9: 777-787.
15. Huang CG, Lamitina T, Agre P, Strange K. Functional analysis of the aquaporin gene family in Caenorhabditis elegans. Am J Physiol Cell Physiol 2007; 292: C1867-1873.
16. Ito H, Yamamoto N, Arima H, Hirate H, Morishima T, Umenishi F, Tada T, Asai K, Katsuya H, Sobue K.Interleukin-1beta induces the expression of aquaporin-4 through a nuclear factor-kappaB pathway in rat astrocytes. J Neurochem 2006; 99: 107-118.
17. Matrisian LM. The matrix-degrading metalloproteinases. BioEssays 1992; 14: 455-463.
18. McCoy E, Sontheimer H. Expression and function of water channels (aquaporins) in migrating malignant astrocytes. Glia 2007; 55: 1034-1043.
19. Monard D. Cell-derived proteases and protease inhibitors as regulators of neurite outgrowth. Trends Neurosci 1988; 11: 541-544.
20. Mou K, Chen M, Mao Q, Wang P, Ni R, Xia X, Liu Y. AQP-4 in peritumoral edematous tissue is correlated with the degree of glioma and with expression of VEGF and HIF-alpha. J Neurooncol 2010; 100: 375-383.
21. Noell S, Wolburg-Buchholz K, Mack AF, Ritz R, Tatagiba M, Beschorner R, Wolburg H, Fallier-Becker P. Dynamics of expression patterns of AQP4, dystroglycan, agrin and matrix metalloproteinases in human glioblastoma.Cell Tissue Res 2012; 347: 429-441.
22. Oshio K, Binder DK, Liang Y, Bollen A, Feuerstein B, Berger MS, Manley GT. Expression of the aquaporin-1 water channel in human glial tumors. Neurosurgery 2005; 56: 375-381; discussion 375-381.
23. Polavarapu R, Gongora MC, Winkles JA, Yepes M. Tumor necrosis factor-like weak inducer of apoptosis increases the permeability of the neurovascular unit through nuclear factor-kappa B pathway activation. J Neurosci 2005; 25: 10094-10100.
24. Rama Rao KV, Chen M, Simard JM, Norenberg MD. Increased aquaporin-4 expression in ammonia-treated cultured astrocytes. Neuroreport 2003; 14: 2379-2382.
25. Rash JE, Yasumura T, Hudson CS, Agre P, Nielsen S. Direct immunogold Labeling of aquaporin-4 in square arrays of astrocyte and ependymocyte plasma membranes in ratbrain and spinal cord. Proc Natl Acad Sci USA 1998; 95: 11981-11985.
26. Saadoun S, Papadopoulos MC, Davies DC, Bell BA, Krishna S. Increased aquaporin 1 water channel expression in human brain tumours. Br J Cancer 2002; 87: 621-623.
27. Sans-Fons MG, Sole S, Sanfeliu C, Planas AM. Matrix metalloproteinase-9 and cell division in neuroblastoma cells and bone marrow macrophages. Am J Pathol 2010; 177: 2870-2885.
28. Vizuete ML, Venero JL, Vargas C, Ilundáin AA, Echevarría M, Machado A, Cano J. Differential upregulation of aquaporin-4 mRNA expression in reactive astrocytes after brain injury: potential role in brain edema. Neurobiol Dis 1996; 6: 245-258.
29. Warth A, Kröger S, Wolburg H. Redistribution of aquaporin-4 in human glioblastoma correlates with loss of agrin immunoreactivity from brain capillary basal laminae. Acta Neuropathol (Berl) 2004; 107: 311-318.
30. Warth A, Mittelbronn M, Wolburg H. Redistribution of the water channel protein aquaporin-4 and the K+ channel protein Kir4.1 differs in low- and high-grade human brain tumors. Acta Neuropathol (Berl) 2005; 109: 418-426.
31. Warth A, Mittelbronn M, Hülper P, Erdlenbruch B, Wolburg H. Expression of the water channel protein aquaporin-9 in malignant brain tumors. Appl Immunohistochem Mol Morphol 2007; 15: 193-198.
32. Woessner JF. The family of matrix metalloproteinases. Ann NY Acad Sci 1994; 732: 11-21.
33. Yatsushige H, Ostrowski RP, Tsubokawa T, Colohan A, Zhang JH. Role of c-Jun N-terminal kinase in early brain injury after subarachnoid hemorrhage. J Neurosci Res 2007; 85: 1436-1448.
34. Zhao W, Shen H, Yuan F, Li G, Sun Y, Shi Z, Zhang Y, Wang Z. Induction stage-dependent expression of vascular endothelial growth factor and aquaporin-1 in diethylstilbestrol-treated rat pituitary. Eur J Histochem 2009; 53: 53-60.
35. Zhao W, Shi Z, Yuan F, Li G, Sun Y, Zhang Y, Wang Z. Melatonin modulates effects of DES on the pitutiary of the female wistar rat. Folia Histochem Cytobiol 2010; 48: 278-283.