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ORIGINAL ARTICLE

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Matrix metalloproteinase-2 and -9 expression in astrocytic tumors

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Abstract In this study, we investigated whether the expression of matrix metalloproteinase (MMP)-2 and MMP-9 correlated with invasiveness, proliferative potential, or prognosis in astrocytic tumors. Thirty-seven astrocytic tumors (8 diffuse astrocytomas, 15 anaplastic astrocytomas, and 14 glioblastomas) and three gliomatosis cerebri were investigated immunohistochemically. The invasive glioma group included three cases of gliomatosis cerebri and two of glioblastoma associated with cerebrospinal fluid dissemination. The expression of MMP-2 and MMP-9 was evaluated by assigning an immunohistochemical (IHC) score defined as the sum of expression frequency and intensity. mRNA expression patterns for the MMPs were also evaluated in a reverse transcription-polymerase chain reaction assay. Neither the MMP-2 nor MMP-9 IHC score was related to histological malignancy. The MMP-2 IHC score of the invasive glioma group was significantly higher than those of other kinds of astrocytic tumors. However, the MMP-9 IHC score did not correlate with dissemination among astrocytic tumors. An inverse correlation was observed between the MIB-1 labeling index and the IHC scores of MMP-2, but it was not significant. A Kaplan-Meyer survival analysis revealed no significant relationship between the survival rate and MMP-2 or MMP-9 expression. Our study showed that MMP-2 expression, but not MMP-9 expression, may be associated with invasion in astrocytic tumors.

Key words Glioma · Matrix metalloproteinase · Immunohistochemistry · Reverse transcription-polymerase chain reaction assay

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Introduction

Matrix metalloproteinases MMPs are a family of Zn^{2+}- and Ca^{2+}-dependent endopeptidases that catalyze degradation of diverse substrates in the matrix, attach to components of this matrix, and affect cellular motililty.[1] It is well known that tumor cells produce higher amounts of proteolytic enzymes than normal tissues, and that MMPs play an important role in the invasion and migration of tumor cells. Among MMPs, MMP-1, -2, -3, -9 and -10 have been implicated in cancer cell invasion and metastasis.[2] A major function of MMPs in metastasis is to facilitate the breakdown of the extracellular matrix, including the basement membrane, and they also play a substantial role in the maintenance of a microenvironment that facilitates the growth and angiogenesis of a tumor.[1] MMP-2 and MMP-9 are gelatinases that degrade various type of collagen, especially type IV collagen which is a chief constituent of the basement membrane of brain vessels. They are the two most abundant MMPs found in gliomas.[3,4]

Glioblastomas (GB) and anaplastic astrocytomas (AA) are characterized by highly diffuse infiltrative growth into the surrounding normal brain tissue. Glioma invasion involves cell adhesion and proteolytic degradation of the extracellular matrix.[5] Recent studies have indicated that the expression of MMPs, including that of MMP-2 and MMP-9, correlates with the degree of histological malignancy and invasiveness in gliomas.[5–7]

In the present study, we examined MMP-2 and MMP-9 expression immunohistochemically and with a reverse transcriptase-polymerase chain reaction (RT-PCR) assay, and analyzed the relationship between them and the histological grade, proliferative potential, and invasion or cerebrospinal fluid (CSF) dissemination of astrocytic tumors.

Subjects and methods

Patients

Thirty-seven astrocytic tumors and three gliomatosis cerebri were obtained from patients who had undergone surgery at our department between April 1990 and May 2001. Patients ranged in age from 1 to 74 years (median, 51.9 years); 21 were male and 19 were female. Tumor locations were as follows: 26 frontal lobe, 4 parietal lobe, 3 temporal lobe, 2 occipital lobe, 4 basal ganglia, and 1 cerebellum. Three patients were diagnosed clinically as having gliomatosis cerebri, and two patients with GB were found to have CSF dissemination on MRI at the initial diagnosis. We called these five cases the invasive glioma group. Tumors were classified according to the World Health Organization classification; 8 were diffuse astrocytomas (DA, Grade II), 15 were AA (Grade III), 14 GB (Grade IV), and 3 gliomatosis cerebri (Grade III). Clinical data, including age, sex, treatment, and survival after the initial operation, were obtained from the patients' records. All patients underwent surgery, and the extent of surgical resection was evaluated by computed tomography (CT) or magnetic resonance imaging (MRI). Although adjuvant therapy was not identical for patients with each grade of tumor, patients with supratentorial glioma were usually treated with a combination of intraoperative radiation therapy (IORT) followed by external beam irradiation (EXRT) and chemotherapy after surgical resection. We previously reported the procedures and results of IORT in the treatment of malignant gliomas.[8] For IORT, a dose of 20 or 25 Gy was given to the residual tumor during surgery, and adjuvant chemotherapies were performed using several agents, including ACNU, cisplatin, and carboplatin, administered intraarterially or intravenously after irradiation.

Immunohistochemistry

MMP-2 and MMP-9 protein expression were examined immunohistochemically using anti-MMP-2 mouse monoclonal antibody (Fuji Chemical Industry, Gohkakizawa, Japan) and anti-MMP-9 mouse monoclonal antibody (Fuji Chemical Industry). MIB-1 monoclonal antibody (Immunotech, Marseille, France) was also used to evaluate the proliferation potential. Formalin-fixed paraffin-embedded sections 6 μm thick were deparaffinized in xylene, rehydrated through graded alcohols, and immersed for 15 min in phosphate-buffered saline. For antigen retrieval, the sections were microwaved in 0.01 M citrate buffer (pH 6.0) for 20 min. After microwave pretreatment, the endogenous peroxidase activity was blocked with 3% hydrogen peroxidase in methanol for 10 min. The sections were incubated for 20 min with normal horse serum to block nonspecific staining. The sections were first incubated 6 h at 4°C with primary antibody in a humidity chamber. Then they were treated for 30 min with biotinylated secondary antibody and for 30 min more with avidin–biotin complex (ABC Elite, Vector, Burlingame, CA, USA), followed by 0.06% diaminobenzidine (Sigma, St. Louis, MO, USA) with 0.01% hydrogen peroxidase for 5 min. Finally, the sections were counterstained with hematoxylin.

As described by Strojnik et al.,[9,10] the expression of MMP-2 and MMP-9 was evaluated by assigning an immunohistochemical (IHC) score, defined as the sum of the frequency (0: none, 1: 0%–30%, 2: 30%–60%, 3: 60%–100%) and intensity (0: none, 1: weak, 2: moderate, 3: strong) of the expression. The MIB-1 labeling index (LI) was calculated as the percentage of 1000 nuclei that immunostained with MIB-1 monoclonal antibody.

Reverse transcription-polymerase chain reaction assay

Total RNA was isolated from the frozen tissue sample using ISOGEN reagent (Nippon Gene, Toyama, Japan). In brief, 100 mg of each tissue was homogenized in 1 ml of ISOGEN. Subsequently, 0.2 ml of chloroform was added, and the mix was centrifuged to separate the solution into an aqueous phase containing RNA, an interphase containing DNA, and an organic phase containing protein. The aqueous layer was aspirated and added to 0.5 ml of isopropanol for RNA precipitation. Following this, the solution (aqueous layer and isopropanol) was centrifuged, and then the pellet was washed with 75% ethanol and centrifuged again. Afterward, RNA was collected into 50 μl of diethylpyrocarbonate (DEPC)-treated water. Reverse transcription-polymerase chain reaction (RT-PCR) was performed using a First-Strand cDNA synthesis kit (Amersham Pharmacia Biotech, Woerden, The Netherlands). One microliter of total RNA (= 1 μg) was added to 14 l of RT mixture. After mixing, the samples were incubated at 37°C for 45 min, 95°C for 5 min, and 4°C for at least 5 min. We synthesized two oligodeoxynucleotide primers with the sequences for MMP-2 (5′ primer: GTGCTGAAGGACACACTAAA GAAGA; 3′ primer: TTGCCATCCTTCTCAAAGTTGT AGG, 605 bp), MMP-9 (5′ primer: CACTGTCCACCCCT CAGAGC; 3′ primer: GCCACTTGTCGGCGATAAGG, 263 bp), and β actin (5′ primer: ATCACCATTGGCAA TGAGCG, 3′ primer: TTGAAGGTAGAAACGTGGAT, 93 bp). Thirty-five microliters of a PCR mixture containing 10 nM of primers and *Taq* DNA polymerase (Amersham Pharmacia Biotech) was added to the RT products. Initial denaturation for 2 min at 94°C was followed by 30 cycles of 1 min at 94°C, 1 min at 55°C, 2 min at 72°C, and a final extension for 6 min at 72°C. The PCR products were separated on 2% agarose gels, and ethidium bromide-stained bands were recorded by Mupid-2R (Cosmo-Bio, Tokyo, Japan). The presence of the PCR product *MDR-1* was determined by the presence or absence of the appropriate bands.

Statistical analysis

We analyzed the relationship between mRNA expression, IHC score, of MMP-2 and MMP-9 and the MIB-1 LI. Student's *t*-test was used to evaluate significant differences. A Kaplan-Meyer survival curve was used to evaluate the

relationship between IHC scores and prognosis. Statview for Windows V. 5.0 was used to analyze each statistical parameter.

Results

The clinical characteristics of the 40 patients with astrocytic tumors or gliomatosis cerebri in this study are shown in Table 1. Out of 29 patients with AA or GB, 11 received IORT followed by postoperative EXRT or only IORT after tumor removal to various extents. The remaining 18 patients received EXRT without IORT. The total radiation dose for EXRT ranged from 40 to 50Gy. Twenty-six patients received adjuvant chemotherapy using ACNU, cisplatin, or carboplatin. Of the 40 patients, 25 had died by the time of data analysis. The survival of patients with DA was significantly longer than survival of those with either AA or GB. Among the patients with AA or GB, survival of patients receiving IORT was not significantly different from that of those who did not receive IORT. There was no evidence that different chemotherapy agents affected survival time in this series, partly because of the small number of patients undergoing each type of treatment.

Immunohistochemical examination revealed that astrocytic tumor cells were stained in a diffuse cytoplasmic pattern for both MMP-2 and MMP-9 (Fig. 1). The IHC score of MMP-2 for GB (2.00 ± 1.35) may be higher than that for DA (1.75 ± 1.67) or AA (1.87 ± 1.77); however the differences were not significant (Fig. 2). The IHC score for MMP-9 for GB (2.92 ± 1.93) was higher than that for DA (2.25 ±

Table 1. Summary of clinical characteristics of 40 patients with astrocytic tumors

Case no.	Age/ sex	Histology	Site	Extent of surgery	Adjuvant therapy	MIB-1 LI (%)	MMP-2 IHC score	MMP-9 IHC score	Prognosis (months)
1	62/M	DA	F	Sub	n.d.	0	0	2	Alive (21)
2	31/M	DA	F	Sub	IORT, EXRT, Chemo	2.2	2	3	Dead (36)
3	43/M	DA	F	Sub	n.d.	0.2	4	0	Alive (20)
4	16/M	DA	F	Sub	n.d.	1.5	0	0	Alive (56)
5	46/F	DA	T	Sub	EXRT	3.0	0	2	Alive (2)
6	56/F	DA	F	Sub	n.d.	0.1	2	4	Alive (21)
7	1/F	DA	Opt	Sub	n.d.	10.8	2	5	Dead (4)
8	51/M	DA	F	Sub	EXRT, Chemo	0	4	2	Dead (29)
9	68/M	AA	T	Sub	EXRT, Chemo	6.8	2	4	Alive (10)
10	42/M	AA	P	Sub	IORT, EXRT, Chemo	18.2	0	0	Dead (29)
11	67/M	AA	F	Total	IORT, Chemo	3.9	0	3	Alive (20)
12	56/M	AA	F	Sub	EXRT, Chemo	7.7	2	3	Alive (18)
13	62/M	AA	F	Partial	IORT, EXRT	0	0	4	Dead (18)
14	44/F	AA	F	Sub	EXRT, Chemo	0	4	2	Dead (26)
15	66/F	AA	P	Sub	IORT, Chemo	15.0	0	3	Dead (13)
16	61/M	AA	BG	Partial	EXRT, Chemo	20.0	3	3	Dead (10)
17	51/M	AA	BG	Partial	EXRT, Chemo	19.7	0	2	Dead (13)
18	58/M	AA	F	Sub	EXRT, Chemo	26.9	3	3	Alive (38)
19	60/M	AA	F	Sub	IORT, EXRT, Chemo	2.1	2	0	Dead (31)
20	62/F	AA	F	Partial	IORT, EXRT, Chemo	10.3	4	5	Dead (6)
21	65/M	AA	F	Sub	IORT, EXRT, Chemo	3.3	5	2	Dead (11)
22	65/F	AA	F	Sub	EXRT, Chemo	13.6	0	0	Dead (12)
23	40/F	AA	F	Sub	IORT, EXRT, Chemo	3.5	3	2	Dead (90)
24	45/M	GB	F	Partial	EXRT, Chemo	3.6	3	2	Dead (12)
25	67/M	GB	F	Sub	EXRT, Chemo	9.7	4	3	Dead (3)
26	50/F	GB	P	Sub	IORT, EXRT, Chemo	14.3	2	0	Dead (22)
27	62/F	GB	P	Sub	IORT, EXRT, Chemo	4.7	3	4	Dead (12)
28	13/F	GB	BG	Sub	EXRT, Chemo	35.8	2	2	Dead (6)
29	74/F	GB	O	Sub	EXRT	0.1	2	6	Alive (19)
30	58/F	GB	F	Sub	EXRT, Chemo	6.6	3	2	Dead (10)
31	65/M	GB	C	Partial	EXRT, Chemo	18.2	0	0	Alive (12)
32	54/F	GB	F	Sub	IORT, EXRT, Chemo	10.0	3	3	Dead (8)
33	36/F	GB	F	Partial	EXRT, Chemo	13.5	0	6	Alive (36)
34	61/F	GB	F	Sub	EXRT, Chemo	8.3	2	3	Alive (4)
35	57/F	GB	T	Sub	EXRT	14.1	0	4	Dead (11)
36	59/M	GB*	F	Partial	EXRT, Chemo	13.2	4	5	Dead (12)
37	56/F	GB*	F	Partial	EXRT, Chemo	13.1	3	3	Dead (10)
38	55/M	GC	F&O	Partial	EXRT, Chemo	2.5	4	0	Dead (12)
39	28/M	GC	BG	Partial	EXRT, Chemo	n.d.	4	3	Alive (15)
40	43/F	GC	F	Partial	EXRT, Chemo	4.8	5	5	Alive (15)

DA, diffuse astrocytoma (grade II); AA, anaplastic astrocytoma Grade III); GB, glioblastoma (Grade IV); GC, gliomatosis cerebri; GB*, glioblastoma with cerebrospinal fluid dissemination; LI, labeling index; HIC score, immunohistochemical score; F, forntal lobe; T, temporal lobe; P, parietal lobe; O, occipital lobe; BG, basal ganglia; Opt, optic nerve; C, cerebellum; Total, total removal; Sub, subtotal removal; Partial, partial removal; EXRT, external beam irradiation; IORT, intraoperative radiotherapy; Chemo, chemotherapy; n.d., not done

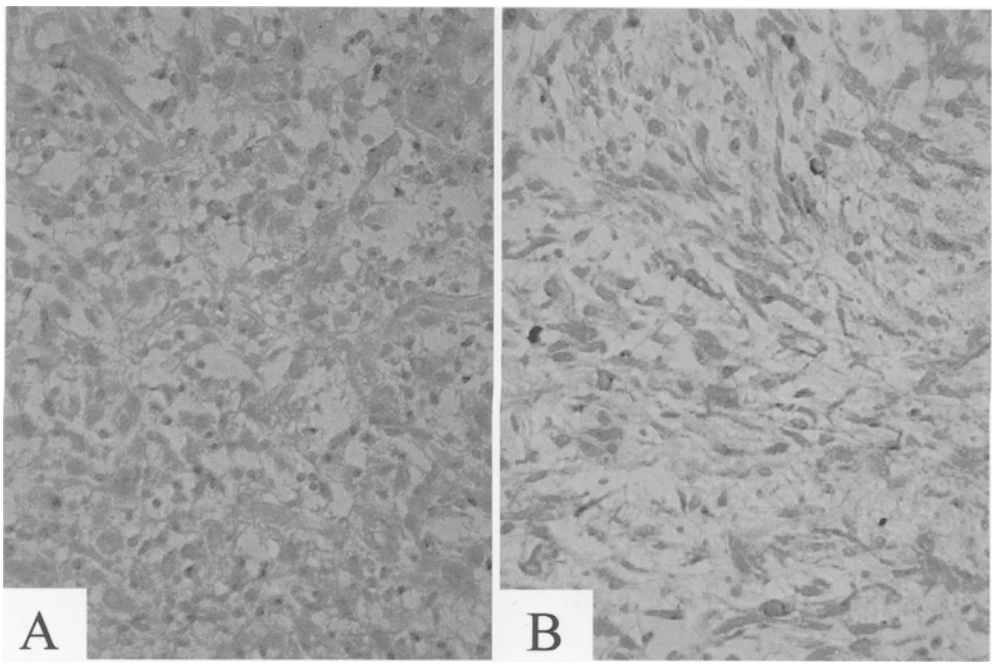

Fig. 1A,B. Photomicrographs showing immunohistochemical staining of matrix metalloproteinase (*MMP*)-2 and MMP-9. **A**, anaplastic astrocytoma. Diffuse cytoplasmic staining for MMP-2 was observed in almost all tumor cells. The immunohistochemical score was 4 in this specimen (×400). **B**, anaplastic astrocytoma. Anti-MMP-9 antibody revealed many strongly stained tumor cells. The immunohistochemical score was 5 (×400)

1.75) or AA (2.40 ± 1.50), but the differences were not significant (Fig. 2). Therefore, neither the MMP-2 IHC score nor that of MMP-9 was associated with histological malignancy in astrocytic tumors.

The MMP-2 IHC score of the invasive glioma group (4.00 ± 0.71) was significantly higher than those of the other astrocytic tumor types, including DA (1.75 ± 1.67, $P = 0.0166$), AA (1.87 ± 1.77, $P = 0.0184$), and GB (2.00 ± 1.35, $P = 0.0073$) (Fig. 2). The MMP-9 IHC score of the invasive glioma group (3.20 ± 2.05) was possibly higher than that of DA (2.25 ± 1.75), AA (2.26 ± 1.50), or GB (2.92 ± 1.93), although the differences were not statistically significant (Fig. 2).

The MMP-2 IHC score and MIB-1 LI, seemed to be inversely correlated because when cases with the MMP-2 IHC score was more than 4, the MIB-1 LI (4.89 ± 4.99) was lower, and when the MMP-2 IHC score was low, the MIB-1 LI was high (9.91 ± 8.70), but the differences were not significant ($P = 0.1090$) (Fig. 3). Similarly, there were no significant differences between the MIB-1 LI of the group with high MMP-9 IHC score (7.13 ± 5.57) and that of the group with low MMP-9 IHC score (9.21 ± 9.18) ($P = 0.4900$) (Fig. 3).

The results of the RT-PCR analysis for MMP-2 and MMP-9 mRNA are shown in Fig. 4. Bands for MMP-2 mRNA were observed in three of eight cases, and bands for MMP-9 mRNA were present in three of eight cases. mRNA expression appeared to be correlated with the IHC scores of the MMPs, but the sample sizes were too small for statistical analyses to be performed.

The Kaplan-Meyer analysis showed no significant relationship between the survival rate and MMP-2 or MMP-9 expression, but the group with lower MIB-1 LI lived significantly longer than that with higher LI ($P < 0.05$) (Fig. 5).

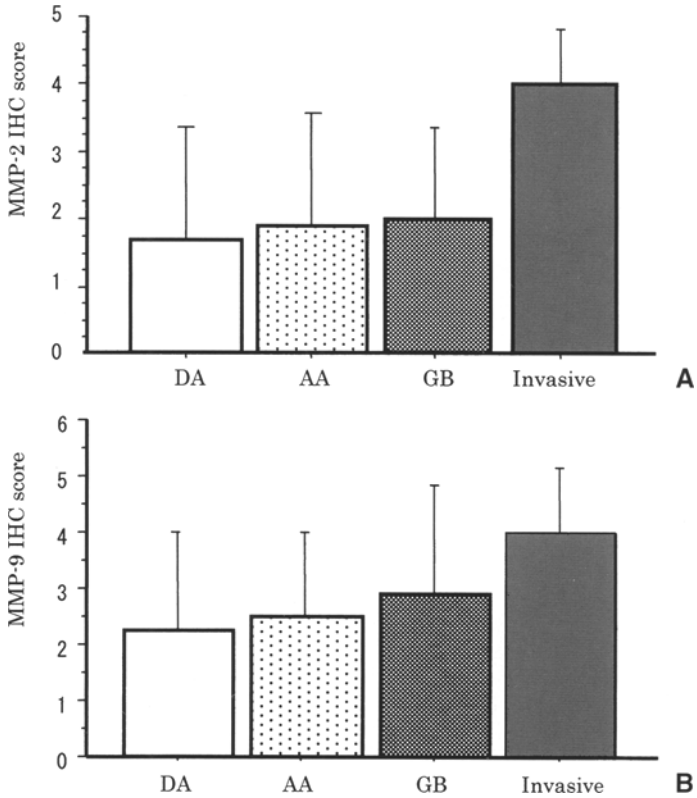

Fig. 2. The immunohistochemical (*IHC*) score of matrix metalloproteinase (*MMP*)-2 of the invasive glioma group (*invasive*, 4.00 ± 0.71) was significantly higher than that of diffuse astrocytoma (*DA*, 1.75 ± 1.67; $n = 8$), anaplastic astrocytoma (*AA*, 1.87 ± 1.77; $n = 15$), or glioblastoma (*GB*, 2.00 ± 1.35; $n = 12$) (**A**).The IHC score of MMP-9 of the invasive glioma group (3.20 ± 2.05) appeared to be higher than those of DA (2.25 ± 1.75), AA (2.26 ± 1.50), and GB (2.92 ± 1.93), although there were no significant differences between them (**B**). The invasive glioma group included three cases with gliomatosis cerebri and two glioblastomas with cerebrospinal fluid dissemination

Discussion

MMP-2 and MMP-9 expression were not correlated with histological malignancy because neither AA nor GB showed higher expression of MMP-2 or MMP-9 than DA. MMP-2 expression tended to be inversely related to the MIB-1 LI, but the relationship was not statistically significant. MMP-9 expression was not related to the MIB-1 LI. The MMP-2 IHC score of the invasive glioma group was significantly higher than those of other malignant or benign astrocytic tumors not associated with dissemination; therefore MMP-2 is related to dissemination or invasiveness.

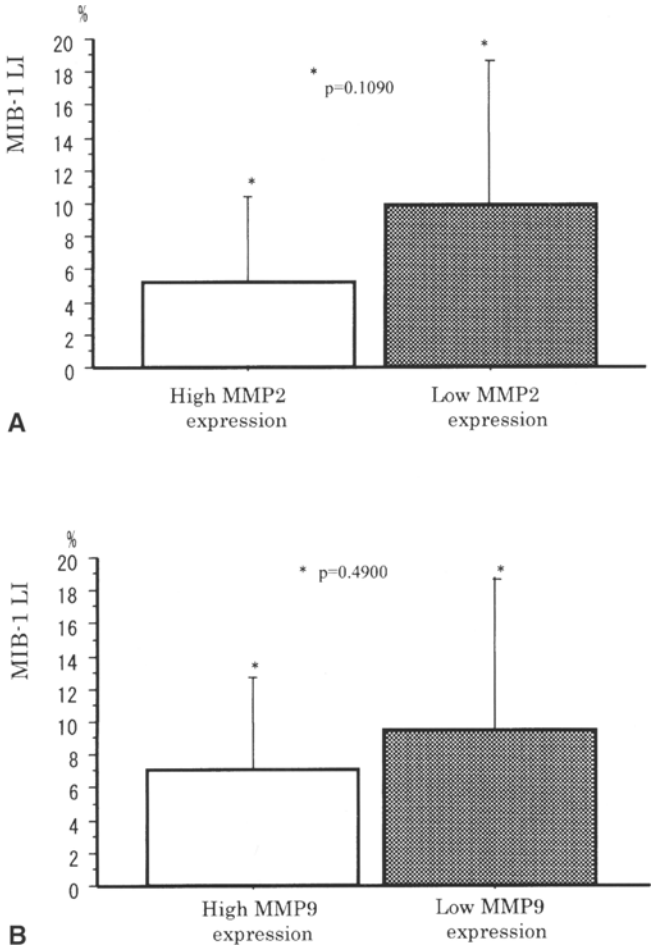

Fig. 3. The MIB-1 labeling index (LI) of the cases with a matrix metalloproteinase (MMP)-2 immunohistochemical (IHC) score of more than 4 (4.89 ± 4.99) appeared to be lower than that of those with a low MMP-2 IHC score (9.91 ± 8.70), although the difference was not significant ($P = 0.1090$) (**A**). The difference between the MIB-1 LI of the group with a high MMP-9 IHC score (7.13 ± 5.57) and that of the group with a low MMP-9 IHC score (9.21 ± 9.18) was not significant ($P = 0.4900$) (**B**)

Several studies[3,4,9,10,11] have shown by immunohistochemistry or gelatin zymography that the expression of MMPs, including that of MMP-2 and MMP-9, is higher in malignant gliomas than in low-grade gliomas. In astrocytes of the normal adult brain, MMP-2 is undetectable by immunohistochemical methods.[10] A few studies[12] also found a correlation between MMP expression and survival of patients with malignant gliomas. The results of the present study contrast with those of the previous reports because MMP expression did not correlate with histological malignancy. One reason for this discrepancy may be differences in the immunohistochemical methods used because we pretreated the sections in a microwave before incubation with the primary antibodies. Also, we used monoclonal antibodies, whereas several other studies used polyclonal antibodies. Furthermore, we used a different system to evaluate immunohistochemical staining (the IHC scoring system).

On the other hand, Thier et al.[13] found an inverse correlation between proliferation potential and MMP-2 expression in glial and neural cell lines. They also suggested that differentiated nonproliferating cells produced significantly more MMP-2 than proliferating cells. Our results were similar, in that MMP-2, but not MMP-9, expression in gliomas inversely correlated with proliferative potential as measured by the MIB-1 LI in this in vivo study. Other in vitro studies using glioma cell lines showed correlations between MMP-2 and MMP-9 expression and invasion, and suggested that MMPs enhance the capability of glioma cells to degrade extracellular matrix and spread into the extracellular space.[4,9,14] This observation leads us to surmise that the higher expression of MMPs in gliomatosis cerebri and glioblastoma with CSF dissemination might be responsible for the invasive nature of the tumor cells.

Many factors affect the degradation of extracellular matrix. Tissue inhibitors of matrix metalloproteinases (TIMPs) inhibit MMPs by forming noncovalent associations with their active sites. Several studies reported less production of TIMPs in brain tumors.[9,15,16] Generally, an imbalance between the production of MMPs and that of TIMPs is considered to be responsible for histological

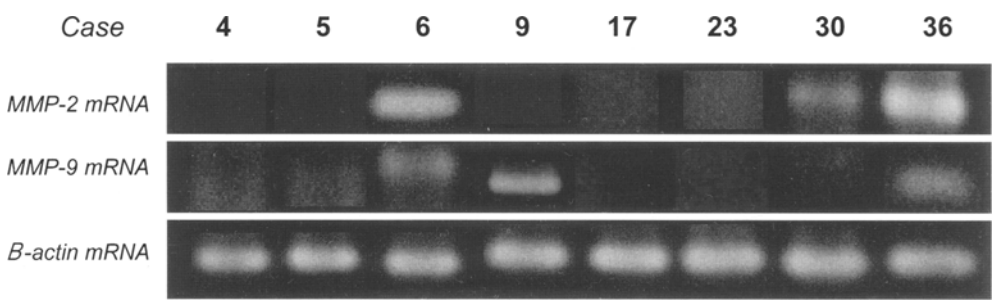

Fig. 4. The reverse transcription-polymerase chain reaction results show the mRNA expression of matrix metalloproteinase (MMP)-2 (605 bp) and MMP-9 (263 bp). Bands were observed in three of eight cases for both MMP-2 and MMP-9

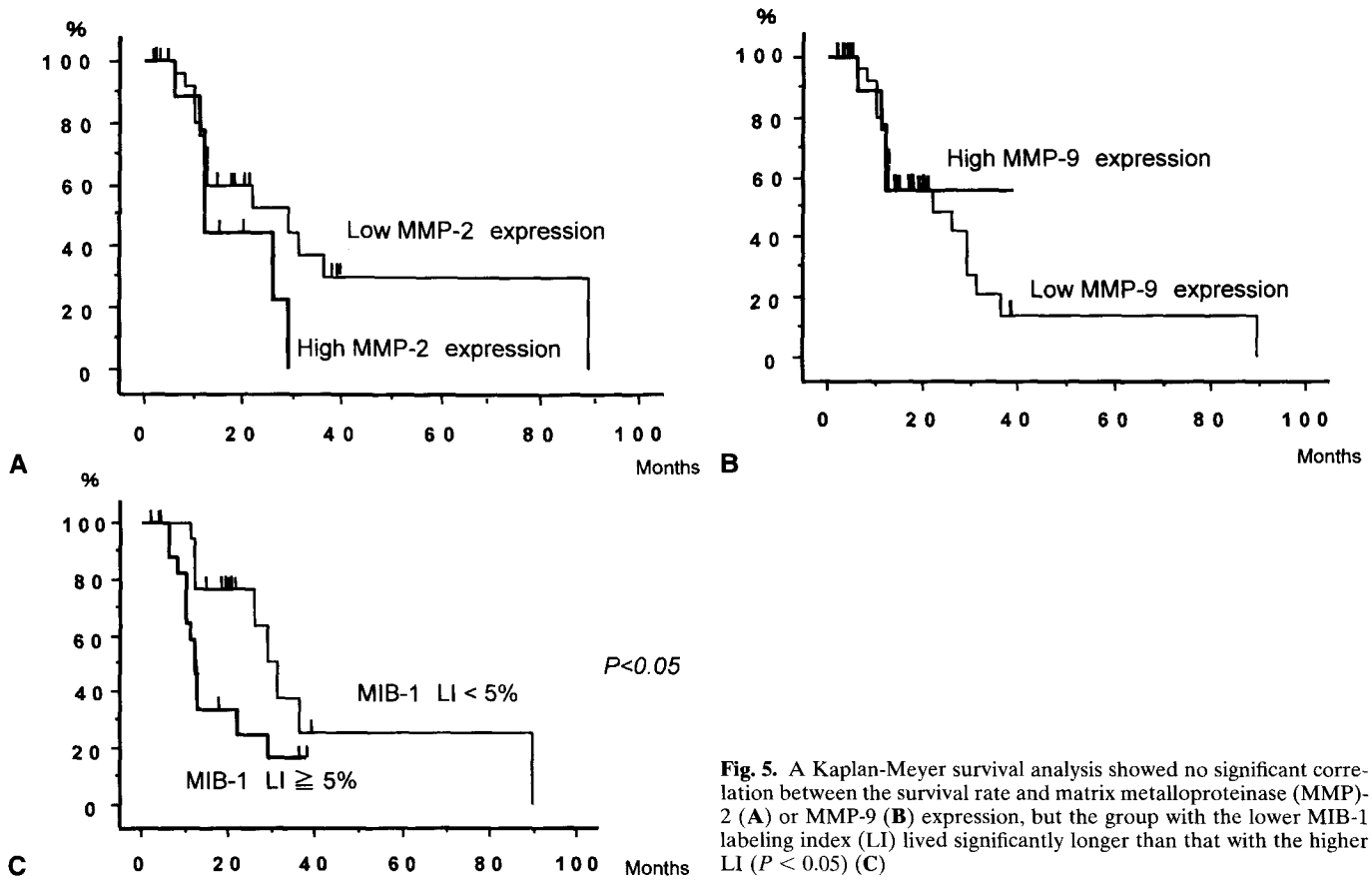

Fig. 5. A Kaplan-Meyer survival analysis showed no significant correlation between the survival rate and matrix metalloproteinase (MMP)-2 (**A**) or MMP-9 (**B**) expression, but the group with the lower MIB-1 labeling index (LI) lived significantly longer than that with the higher LI ($P < 0.05$) (**C**)

Table 2. Immunohistochemical and reverse transcription-polymerase chain reaction assay data

Case no.	Age/sex	Histology	MMP-2 IHC score	MMP-9 IHC score	MMP-2 mRNA	MMP-9 mRNA
4	16/M	DA	0	0	−	−
5	46/F	DA	0	2	−	−
6	56/F	DA	2	4	+	+
9	68/M	AA	2	4	−	+
17	51/M	AA	0	2	−	−
23	40/F	AA	3	2	−	−
30	58/F	GB	3	2	+	−
36	59/M	GB	4	5	+	+

malignancy and tumor recurrence. These data suggest that enhanced levels of MMPs might be predictive of invasion, tumor histological grade, or prognosis.

Although the actual role and meaning of the expression of MMPs in the astrocytic tumors remain to be validated, the present results suggest a contribution of MMP-2 to the invasion or dissemination of astrocytic tumors. Thus, the expression of MMP-2 may be useful as an indicator of invasiveness. Immunohistochemistry does not provide enough information on the functional state of these enzymes, so gelatin zymography must be performed to know their gelatinolytic activities. Wild-Bode et al.[14] demonstrated a good correlation between MMP-2 and MMP-9 protein expression and gelatinolytic activity as shown on zymograms, and also suggested that the activities of MMP-2 and MMP-9 were the best predictors of glioma cell invasion. Takano et al.[17] evaluated the gelatinase activities in glioma tissue using film in situ zymography and demonstrated localization of gelatinase acticities in situ.

In this study, we showed that the immunohistochemical expression of MMP-2 may be associated with tumor invasion, although no significant correlation was observed between MMP expression and histological malignancy or survival.

References

1. Chintala SK, Tonn JC, Rao JS (1999) Matrix metalloproteinases and their biological function in human gliomas. Int J Dev Neurosci 17:495–502

2. Duffy MJ, McCarthy K (1998) Matrix metalloproteinases in cancer: prognostic markers and targets for therapy. Int J Oncol 12: 1343–1348
3. Forsyth PA, Wong H, Laing Td, et al. (1999) Gelatinase-A (MMP-2), gelatinase-B (MMP-9) and membrane type matrix metalloproteinase-1 (MT1-MMP) are involved in different aspects of the pathophysiology of malignant gliomas. Br J Cancer 79:1828–1835
4. Ulm JH, Dooley NP, Villemure JG, et al. (1997) Glioma invasion in vitro: regulation by matrix metalloproteinase-2 and protein kinase C. Clin Exp Metastasis 14:421–433
5. Giese M, Westphal M (1996) Glioma invasion in the central nervous system. Neurosurgery 39:235–250
6. Nakano A, Tani E, Miyazaki K, et al. (1995) Matrix metalloproteinases and tissue inhibitors of metalloproteinases in human gliomas. J Neurosurg 83:298–307
7. Pilkington GJ (1994) Tumor cell migration in the central nervous system. Brain Pathol 4:157–166
8. Kunishio K, Morisaki K, Kawai N, et al. (1998) Analysis of intraoperative radiotherapy for malignant gliomas. Neuro-Oncology 8:21–27
9. Nakagawa T, Kubota T, Kabuto M, et al. (1994) Production of matrix metalloproteinases and tissue inhibitor of metalloproteinases-1 by human brain tumors. J Neurosurg 81:69–77
10. Sawaya RE, Yamamoto M, Gokaslan ZL, et al. (1996) Expression and localization of 72-kDa type IV collagenase (MMP-2) in human malignant gliomas in vivo. Clin Exp Metastasis 14:35–42
11. Nakada M, Nakamura H, Ikeda E, et al. (1999) Expression and tissue localization of membrane-type 1, 2, and 3 matrix metalloproteinases in human astrocytic tumors. Am J Pathol 154:417–428
12. Jaalinoja J, Herva R, Korpela M, et al. (2000) Matrix metalloproteinase 2 (MMP-2) immunoreactive protein is associated with poor grade and survival in brain neoplasms. J Neurooncol 46:8190
13. Thier M, Roeb E, Breuer B, et al. (2000) Expression of matrix metalloproteinase-2 in glial and neural tumor cell lines: inverse correlation with proliferation rate. Cancer Lett 149:163–170
14. Wild-Bode C, Weller M, Wick W (2001) Molecular determinants of glioma cell migration and invasion. J Neurosurg 94:978–984
15. Beliveau R, Delbecchi L, Beaulieu E, et al. (1999) Expression of matrix metalloproteinases and their inhibitors in human brain tumors. Ann N Y Acad Sci 886:236–239
16. Nakada M, Kita D, Futami K, et al. (2001) Roles of membrane type 1 matrix metalloproteinase and tissue inhibitor of metalloproteinase 2 in invasion and dissemination of human malignant glioma. J Neurosurg 94:464–473
17. Takano S, Tsuboi K, Matsumura A, et al. (2001) Localization of gelatinase activities in glioma tissues by film in situ zymography. Brain Tumor Pathol 18:145–150