The expression of matrix metalloproteinase-2 and -9 in human gliomas of different pathological grades

Received: August 18, 2003 / Accepted: September 9, 2003

Abstract Matrix metalloproteinases (MMPs) have been implicated to play a critical role in glioma invasiveness. In this study, we aimed to investigate the expression of MMP-2 and MMP-9 in human gliomas of different degrees of malignancy, and evaluated the correlation between MMP-2 and MMP-9 expression in gliomas. The samples from 65 cases of glioma were divided into four groups according to the WHO classification: there were 16 cases of grade I, 17 cases of grade II, 20 cases of grade III, and 12 cases of grade IV. Normal brain samples served as the control group, and biopsy specimens were obtained from 8 glioma patients with a needle placed into the adjacent brain 1 cm from the margin after tumor resection. All the samples were stained with hematoxylin and eosin and immunohistochemistry. A computer-aided image-analysis system was employed to measure the integral optical density (IOD) of positive slides. No positive staining was found in the control group. The positive staining was localized in the cytoplasm of glioma cells, the extracellular matrix (ECM), the basement membrane (BM), and the endothelial cells of blood vessels. Positive staining rates increased significantly when the degree of malignancy of gliomas was elevated. The IOD value of MMP-2 and MMP-9 also indicated that the intensity of MMP-2 and MMP-9 expression was elevated significantly with the degree of malignancy of the gliomas. There was a positive correlation between MMP-2 and MMP-9 expression in gliomas. Glioma invasion and angiogenesis were particularly seen in the biopsied tissues, and MMP-9 immunostaining seemed to be much more intense and extensive than MMP-2 immunostaining in these samples. These results suggest that MMP-2 and MMP-9 staining in gliomas is localized in the cytoplasm of tumor cells, BM, and endothelial cells, and that MMP-2 and MMP-9 together play an important role in the invasiveness of gliomas, mediating the degradation of the ECM and angiogenesis. MMP-2 and MMP-9 could be molecular targets in the treatment of malignant glioma.

Key words MMP-2 · MMP-9 · Gliomas · Invasion

Introduction

Gliomas are the most common primary malignant brain tumors, and they display extensive infiltrative growth behavior but seldom metastasize to distant organs. This invasiveness into the surrounding normal brain tissue makes glioma a major challenge for aggressive therapeutic interventions. Total surgical resection of gliomas is impossible, and recurrence of tumor growth is common. The mean survival time is 8–12 months. Recent studies focusing on the mechanisms of glioma invasion suggest that matrix metalloproteinases (MMPs) play a critical role in this process. MMPs, a large family of zinc-dependent natural endopeptidases, enhance tumor cell invasion by degrading extracellular matrix proteins, such as collagen, fibronectin, and proteoglycans.

Among the MMP family, MMP-2 and MMP-9 have received considerable attention because their expression correlates with the progression of gliomas. Although substantial progress has been made toward understanding the roles of MMP-2 and MMP-9 in glioma invasion and in angiogenesis, the correlation between the levels of MMP-2 and MMP-9 expression in the border of gliomas relating to the histopathological grade of malignancy, and MMP-2 and MMP-9 expression in the surrounding tissue of gliomas, are still poorly documented.

Therefore, in the present study, we aimed to investigate the expression of MMP-2 and MMP-9 in the margin of
glioma tissue and to discuss their biological significance, with evaluation of the correlation between MMP-2 and MMP-9 expression.

**Materials and methods**

Tissue specimens and classification

From January 2000 to October 2002, in the First Hospital of XJTU, 65 patients with glioma underwent operation. There were 33 male patients and 32 females, with ages ranging from 15 to 72 years (average, 42.3 years). During the operation, specimens were incised from the margin of the tumors, and the margin of the tumor mass was identified by the chief operator macroscopically. Additionally, 8 cases (6 males and 2 females) underwent biopsy with a needle from the “non-functional area” of the brain 1 cm outside of the tumor margin under the microscope following tumor resection. None of these patients received any treatment before the operation.

Subsequently all the samples were fixed in 10% formalin within 1 h after operation. The sections were processed for histopathologic examination, stained with hematoxylin and eosin (H&E), and examined by a neuropathologist.

According to the glioma classification of WHO and Kernohan’s grading, we divided these patients into four groups: grade I, 16 cases (13 cases of pilocytic astrocytoma and 3 cases of ependymoma); grade II, 17 cases (7 cases of fibrillary astrocytoma, 7 cases of protoplasmic astrocytoma, and 3 cases of oligodendrogloma); grade III, 20 cases (11 cases of anaplastic astrocytoma, 7 cases of anaplastic oligodendrogloma, and 2 cases of anaplastic ependymoma); and grade IV, 12 cases (all glioblastoma multiforme). In addition, 12 control samples of normal brain tissue were taken from patients with head injuries who underwent cerebral decompression (7 males and 5 females; age, 23–58 years; average age, 38.3 years).

Immunohistochemistry

Staining was carried out by using an immunoperoxidase staining kit for mouse monoclonal antibodies with goat immunoglobulin as the second antibody. The primary mouse antihuman monoclonal antibodies and corresponding concentrations used were anti-MMP-2 10 μg·ml⁻¹ and anti-MMP-9 10 μg·ml⁻¹ (both mouse monoclonal, Calbiochem, Bad Soden, Germany).

Formalin-fixed, paraffin-embedded blocks with samples of tumor and control samples were stained for MMP-2 and MMP-9 in every case. Paraffin-embedded specimens were sectioned at 4 μm. The sections were baked at 60°C and deparaffinized with xylene, dehydrated in an ethanol gradient for 15 min, and washed with distilled water. The slides were then treated with 3% hydrogen peroxide for 30 min to block endogenous peroxidase. After three washes with phosphate-buffered saline (PBS), the sections were incubated with citrate buffer for 15 min and washed again with PBS three times; then the sections were incubated for 5 min with 2% normal serum to block nonspecific binding, followed by incubation with the primary mouse antihuman monoclonal antibody MMP-2 or MMP-9 in a humid chamber at 4°C overnight. After being washed in PBS three times, the sections were incubated with goat antimouse secondary antibody of biotinylated anti-immunoglobulin vector of appropriate species specificity for 1 h at room temperature and washed three times with PBS. Finally, incubation with avidin-biotin-horseradish peroxidase complex for 1 h at room temperature, immunoperoxidase reaction was visualized with 3,3′-diaminobenzidine tetrahydrochloride used as the enzyme substrate. The slides were briefly counterstained with hematoxylin and washed three times with PBS, covered with glass coverslips, and mounted and examined under an Olympus BH-2 microscope. Negative control slides received normal mouse serum as the primary antibody. Immunopositivity in more than 20% of tumor cells was considered to be positive staining. The expression site and distribution of the positive cells were observed.

**Results**

**Integral optical density (IOD) of positive slides**

In each group, each positively stained slide was measured by a computer-aimed microscope chromatic pathological slice image-analysis system (Beijing Aerospace University), at the same brightness and the same magnification (10 × 40), and five views were randomly selected for a value of IOD. This value of IOD is the positive staining degree of slide and it was staining quantification of MMP-2 and MMP-9.

**Statistical analysis**

Statistical analysis was performed with the SPSS 8.0 for Windows software program. MMP-2 and MMP-9 expression rates were compared in different groups by Spearman correlative analysis. Correlation analysis between MMP-2 and MMP-9 expression in the same group was performed by Fisher’s exact test. The IOD values for MMP-2 and MMP-9 in different groups were compared by Student’s t-test. Statistical significance was taken as P < 0.05.