Neurosci Bull February 1, 2013, 29(1): 83–93. http://www.neurosci.cn
DOI: 10.1007/s12264-012-1296-5

·Original Article·

Antisense MMP-9 RNA inhibits malignant glioma cell growth *in vitro* and *in vivo*

Cuiyun Sun[1], Qian Wang[1], Hongxu Zhou[1], Shizhu Yu[1], Alain R. Simard[2], Chunsheng Kang[1], Yanyan Li[1], Yanling Kong[1], Tongling An[1], Yanjun Wen[1], Fudong Shi[3], Junwei Hao[3]

[1]*Department of Neuropathology, Tianjin Neurological Institute, Tianjin Medical University General Hospital; Key Laboratory of Post-trauma Neuro-repair and Regeneration in the Central Nervous System, Ministry of Education; Tianjin Key Laboratory of Injuries, Variations and Regeneration of the Nervous System, Tianjin 300052, China*

[2]*Département de Chimie et Biochimie, Université de Moncton, Moncton, New Brunswick E1A 3E9, Canada*

[3]*Department of Neurology, Tianjin Neurological Institute, Tianjin Medical University General Hospital, Tianjin 300052, China*

Corresponding authors: Shizhu Yu, Junwei Hao. E-mail: tjyushizhu@yahoo.com; hjw@tmu.edu.cn

ABSTRACT

The matrix-degrading metalloproteinases (MMPs), particularly MMP-9, play important roles in the pathogenesis and development of malignant gliomas. In the present study, the oncogenic role of MMP-9 in malignant glioma cells was investigated *via* antisense RNA blockade *in vitro* and *in vivo*. TJ905 malignant glioma cells were transfected with pcDNA3.0 vector expressing antisense MMP-9 RNA (pcDNA-AS-MMP9), which significantly decreased MMP-9 expression, and cell proliferation was assessed. For *in vivo* studies, U251 cells, a human malignant glioma cell line, were implanted subcutaneously into 4- to 6-week-old BALB/c nude mice. The mice bearing well-established U251 gliomas were treated with intratumoral pcDNA-AS-MMP9-Lipofectamine complex (AS-MMP-9-treated group), subcutaneous injection of endostatin (endostatin-treated group), or both (combined therapy group). Mice treated with pcDNA (empty vector)-Lipofectamine served as the control group. Four or eight weeks later, the volume and weight of tumor, MMP-9 expression, microvessel density and proliferative activity were assayed. We demonstrate that pcDNA-AS-MMP9 significantly decreased MMP-9 expression and inhibited glioma cell proliferation. Volume and weight of tumor, MMP-9 expression, microvessel density and proliferative activity in the antisense-MMP-9-treated and therapeutic alliance groups were significantly lower than those in the control group. The results suggest that MMP-9 not only promotes malignant glioma cell invasiveness, but also affects tumor cell proliferation. Blocking the expression of MMP-9 with antisense RNA substantially suppresses the malignant phenotype of glioma cells, and thus can be used as an effective therapeutic strategy for malignant gliomas.

Keywords: matrix-degrading metalloproteinase 9; antisense MMP-9 RNA; cell proliferation; malignant glioma cells

INTRODUCTION

Glioblastoma is an aggressive tumor characterized by extensive brain invasion. This infiltrative nature makes curative surgical resection almost impossible and contributes to the short median survival of glioblastoma patients. Meanwhile, the importance of angiogenesis in glioblastoma growth was recognized many decades ago. Angiogenesis and glioblastoma invasion share common features, at least in their initial stages, as they both require controlled degradation of extracellular matrix (ECM) components in order to allow tumor cell and vascular endothelial cell migration, as

well as facilitate neovascularization and tumor infiltration[1-8].

The matrix-degrading metalloproteinases (MMPs) play fundamental roles in these processes. Among the human MMPs reported to date, MMP-9 plays a vital role in the degradation of the ECM because of its substrate specificity for type IV collagen, the major component of the basement membrane. High expression levels of MMP-2 and MMP-9 have been frequently correlated with increased tumor invasiveness and poor prognosis in various types of human cancer[5,9-13]. It is widely accepted that degradation of the ECM, and the consequently increased invasive capacity and metastatic potential of tumor cells, result from an imbalance between the activities of these proteases and their inhibitors[14]. These observations have created novel opportunities for developing therapeutics based on differentiation or targeting the proliferative features of malignant gliomas.

The present study was designed to investigate the effects of antisense human MMP-9 on glioma cell growth, by constructing an expression vector consisting of MMP-9 antisense cDNA against the template of MMP-9.

MATERIALS AND METHODS

Plasmid Constructs

The 528-bp fragment of the 5'-end of MMP-9 cDNA was obtained by RT-PCR and inserted both forward and reverse into the eukaryotic expression vector pcDNA3.0 to construct sense and antisense RNA expression plasmids. TJ905 cells were designated randomly into control, vector, sense and antisense groups. The cells in each of the latter three groups were transfected with the expression vector, sense or antisense RNA expression plasmid. The primer sets (5'–3') were as follows: MMP-9 cloning, AGA CACCTCTGCCCTCACCATGAG and AACTGGATGAC GATGTCTGCGTCC. The plasmids used for transfection, pcDNA3.0-AS-MMP9 and pcDNA3.0-S-MMP9, were isolated from *E. coli* strain DH5α and purified using the Wizard Plus SV Minipreps kit (Promega, Madison, WI). The DNA concentration and purity were determined using an ultraviolet scanning spectrophotometer. The isolated MMP-9 antisense and sense cDNAs were confirmed by 1% agarose gel electrophoresis after digestion with the restriction enzymes Xho I and Hind III (Fermentas) and by DNA sequencing.

Cell Culture and Transfection

Human malignant glioma TJ905-MG cells were obtained from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum, 4 mmol/L glutamine, 50 U/mL penicillin and 50 μg/mL streptomycin. For transfection, cells were plated onto 6-well plates at 2×10^5 cells per well and grown overnight until 50–80% confluent. Empty plasmid pcDNA3.0, pcDNA-S-MMP9 or pcDNA-AS-MMP9 was transfected into TJ905 cells by Lipofectamine according to the manufacturer's instructions. Cells were subcultured at 1:5 dilution in 300 μg/mL G418-containing medium. Positive stable transfectants were selected and expanded for further study.

Semiquantitative RT-PCR

Total RNA was extracted using TRIzol reagents (Life Technologies, Carlsbad, CA), according to the manufacturer's instructions. Isolated RNA was electrophoresed in 1.0% agarose-formaldehyde gels to verify its quality. First-strand cDNA was generated from 1 μg of total RNA in a final volume of 20 μL using SuperScript II (Life Technologies) and oligo (dT) primers. PCR amplification was performed using a Perkin Elmer DNA thermal cycler (PTC-200). The sequences of PCR primers were as follows: MMP-9, forward 5'-CGGAGCACGGAGACGGGTAT-3', reverse 5'-TGAAGGGGAAGACGCACAGC-3'; β-actin, forward 5'-TCCCTGGAGAAGAGCTACGA-3', reverse 5'-GATC CACACGGAGTACTTGC-3'. The synthesized cDNA was amplified in a total volume of 50 μL containing 200 μmol/L of each of the four dNTPs, 2 μmol/L of each MMP-9 primer and 1 U Taq polymerase (Takara Bio). PCR conditions were as follows: 94°C for 30 s; 56°C for 30s; 72°C for 90 s; 30 cycles, final extension at 72°C for 5 min. β-Actin was used as a loading control. PCR products were analyzed by electrophoresis on 1% agarose gel containing 0.1 mg/mL ethidium bromide.

Western Blotting Analysis

After G418 selection, individual clones were washed three times with ice-cold PBS. Then the cells were solubilized in 1% Nonidet P-40 lysis buffer (containing, in mmol/L: 20 Tris, pH 8.0, 137 NaCl, 1% Nonidet P-40, 10% glycerol, 1 $CaCl_2$, 1 $MgCl_2$, 1 phenylmethylsulfonyl fluoride, 1 sodium fluoride,

1 sodium orthovanadate, and a protease inhibitor mixture). Homogenates were centrifuged at 20 000 g for 15 min at 4°C and protein concentrations were determined using a bicinchoninic acid protein assay kit (Pierce, Rockford, IL). Samples were adjusted to equal content and volume, and subjected to SDS-PAGE on 8% SDS-acrylamide gel. Proteins were then transferred to PVDF membranes (Millipore, Billerica, MA) followed by blocking. The membranes were incubated with primary antibody against MMP-9 (1:500 dilution, Santa Cruz Biotechnology, Santa Cruz, CA), followed by incubation with HRP-conjugated secondary antibody (1:1 000 dilution, Zymed, Carlsbad, CA). The specific protein was detected using a SuperSignal protein detection kit (Pierce). After washing with stripping buffer, the membrane was reprobed with antibody against β-actin (1:500 dilution, Santa Cruz Biotechnology) using the same procedures as above.

Immunocytochemistry and Immunohistochemistry

The expression of MMP-9 and Ki-67 was assessed by immunostaining using the ABC-peroxidase method. Briefly, cells cultured on coverslips were fixed in acetone and incubated with primary MMP-9 or Ki-67 antibody (1:200 dilution) overnight at 4°C, then incubated with a biotinylated secondary antibody (1:200 dilution) at room temperature for 1 h, followed by incubation with ABC-peroxidase reagent (1:200 dilution, Vector, Burlingame, CA) for an additional 1 h. After washes with PBS, cells were incubated with DAB (3,3' diaminobenzidine, 30 mg dissolved in 100 mL Tris-buffer containing 0.03% H_2O_2) for 5 min, rinsed in water and counterstained with hematoxylin. The percentage of positive-staining cells in a total number of 500–1 000 cells was determined under a light microscope at 400× magnification. Immunohistochemical staining was analyzed as follows. First, the most intensively stained area was chosen using 10× objective magnification. Four staining categories were used: "0", negative staining, only blue background seen on nuclei; "1", positive brown staining seen with difficulty and background color easily seen; "2", moderate positivity, brown staining seen better than blue background; and "3", strong positivity, background color hardly seen.

Tumor Growth *in vivo*

Female immune-deficient nude mice (BALB/c-nu; 6 weeks old) were purchased from the Animal Center of the Cancer Institute of the Chinese Academy of Medical Sciences, bred at the laboratory animal facility, Tianjin University, and housed individually in microisolator ventilated cages with water and food *ad libitum*. All experimental procedures were carried out according to the regulations and internal biosafety and bioethics guidelines of Tianjin Medical University General Hospital and the Tianjin Municipal Science and Technology Commission, and were approved by the Animal Care Committee of Tianjin Medical University.

Four mice were injected subcutaneously with 1×10^8 U251 cells, in 50 μL PBS premixed with an equal volume of Matrigel (Becton Dickinson, Franklin Lakes, NJ). Mice were monitored daily and three of the four formed subcutaneous tumors. When the tumor size reached ~5 mm in length, it was surgically removed, cut into 1–2 mm^3 pieces, and injected into another 40 mice. Again, when the tumor size reached ~5 mm, the mice were randomly divided into eight groups: (1) untreated for 4 weeks (U251-4W), (2) treated with pcDNA3.0 empty vector for 4 weeks (pcDNA-4W), (3) injected i.p. with endostatin for 4 weeks (E-4W), (4) treated with pcDNA-AS-MMP9 for 4 weeks (AS-4W), (5) treated with pcDNA-AS-MMP9 and injected i.p. with endostatin for 4 weeks (AS+E-4W), (6) treated with pcDNA3.0 empty vector for 8 weeks (pcDNA-8W), (7) treated with pcDNA-AS-MMP9 for 8 weeks (AS-8W), and (8) treated with pcDNA-AS-MMP9 and injected i.p. with endostatin for 8 weeks (AS+E-8W). Each group consisted of five mice. Five micrograms of plasmid DNA in 20 μL of Lipofectamine (once per day for 4 days) and/or 2.5 mg/kg endostatin in 100 μL PBS (once a day for 14 days) were injected into subcutaneous tumors using a multi-site injection method. Mice in control groups received 10 μL PBS alone. Tumor size was measured with a digital vernier caliper three times weekly (volume = length×$width^2$/2). After four or eight weeks of treatment, mice were sacrificed by CO_2 euthanasia, and tumors were weighed. Routine hematoxylin-eosin staining was performed to assess histopathological changes in the tumors. The expression of GFAP, MMP-9 and Ki-67 was examined on paraffin-embedded sections of homograft tumor tissues by immunohistochemical staining with the ABC-peroxidase method. All antibodies were from Santa Cruz Biotechnology. The Perdrau staining method for reticulum was carried out on sections to count the microvessel density of tumors.

Statistical Analysis

SPSS 11.0 was used for statistical analysis. One-way

analysis of variance and the *q*-test were used to analyze the significance of differences between groups. Statistical significance was set at $P < 0.05$.

RESULTS

Plasmid Constructs

The fidelity and orientation of the heterogeneous fragments in the recombinant plasmids were confirmed by restriction endonuclease digestion and DNA sequencing (Fig. 1). Results showed that digestion of pcDNA-AS-MMP9 and pcDNA-S-MMP9 by Hind III + Xho I released a 500-bp fragment, which is consistent with the theoretical length from MMP-9 RT-PCR. Since there is a BamH I site at position 391 of the MMP-9 cDNA, we then proceeded to digest the construct with BamH I + Hind III, or BamH I + Xho I, and obtained fragments of ~400 bp and 140 bp, respectively (Fig. 1). This demonstrates that the cDNA fragments were correctly inserted into the vectors. In addition, DNA sequencing confirmed that the S- and AS-MMP-9 insertions in the plasmids were correct in fidelity and orientation.

MMP-9 Expression in TJ905 Glioma Cells Transfected with pcDNA-AS-MMP9

As shown by semi-quantitative RT-PCR analysis, MMP-9 expression was hardly changed in TJ905 cells transfected with control vector or pcDNA-S-MMP9, compared with parental TJ905 cells. However, MMP-9 expression was dramatically decreased by ~90% in cells transfected with pcDNA-AS-MMP9 ($P < 0.001$; Fig. 2A). Meanwhile, Western blotting and immunocytochemistry demonstrated similar downregulation of MMP-9 expression that was consistent with the results from semi-quantitative RT-PCR ($P < 0.001$; Fig. 2B, C). These results showed that the MMP-9 expression was significantly inhibited by AS-MMP9 RNA in TJ905 glioblastoma cells.

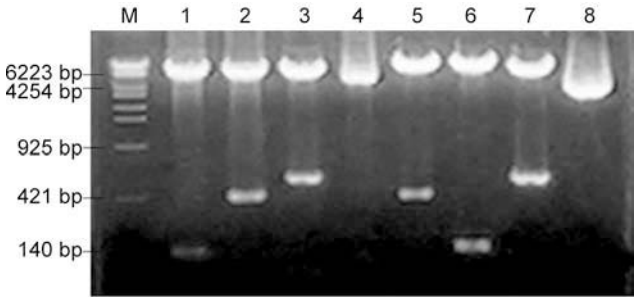

Fig. 1. DNA electrophoresis of vectors digested by restriction endonuclease digestion. Lane 1, sense MMP-9 vector digested by BamH I+Xho I; lane 2, sense MMP-9 vector digested by BamH I+Hind III; lane 3, sense MMP-9 vector digested by Hind III+Xho I; lane 4, sense MMP-9 vector; lane 5, antisense MMP-9 vector digested by BamH I+Xho I; lane 6, antisense MMP-9 vector digested by BamH I+Hind III; lane 7, antisense MMP-9 vector digested by Hind III+Xho I; lane 8, antisense MMP-9 vector. M, Marker.

Proliferation of Transfected TJ905 Malignant Glioma Cells

Immunocytochemical analysis showed that MMP-9 expression was substantially lower in TJ905 cells transfected with pcDNA-AS-MMP9 (Fig. 2C). This finding correlated with the decrease in Ki-67 expression (a cellular marker for proliferation) (Fig. 2D), therefore suggesting that the proliferation of malignant glioma cells was inhibited by AS-MMP9 RNA.

Antitumor Effect of MMP-9 Knockdown on U251 Glioma Xenograft Model

Our *in vitro* experiments demonstrated that the antisense approach efficiently inhibited cell proliferation, as shown by lower Ki-67 expression. Therefore, we further investigated the antitumor effect of AS-MMP9 *in vivo* using a U251 glioma xenograft model and Lipofectamine-mediated gene therapy. Mice were treated with empty vector alone, pcDNA-AS-MMP9 *in situ* by Lipofectamine-mediated gene therapy, i.p. injection of endostatin, both pcDNA-AS-MMP9 and endostatin, or PBS only as a control. All the mice were monitored every 2 days for 4 or 8 weeks, and the tumor volumes were measured and compared.

Three mice in the 8-week vector-alone group died in 6–7 weeks, while mice from the other groups lived until sacrification. The rates of tumor growth during 4- and 8-week-monitoring in different groups are presented in Fig. 3A. The mean volume and weight of tumors were higher in control and empty-vector groups than in the other therapy groups ($P < 0.001$; Fig. 3B). In comparison with those injected i.p. with endostatin alone and those transfected with pcDNA-AS-MMP9, the volume and weight of tumors with combined pcDNA-AS-MMP9 + endostatin were lower ($P < 0.05$). These results suggested that AS-MMP9 and endostatin treatments individually inhibit tumor growth, while

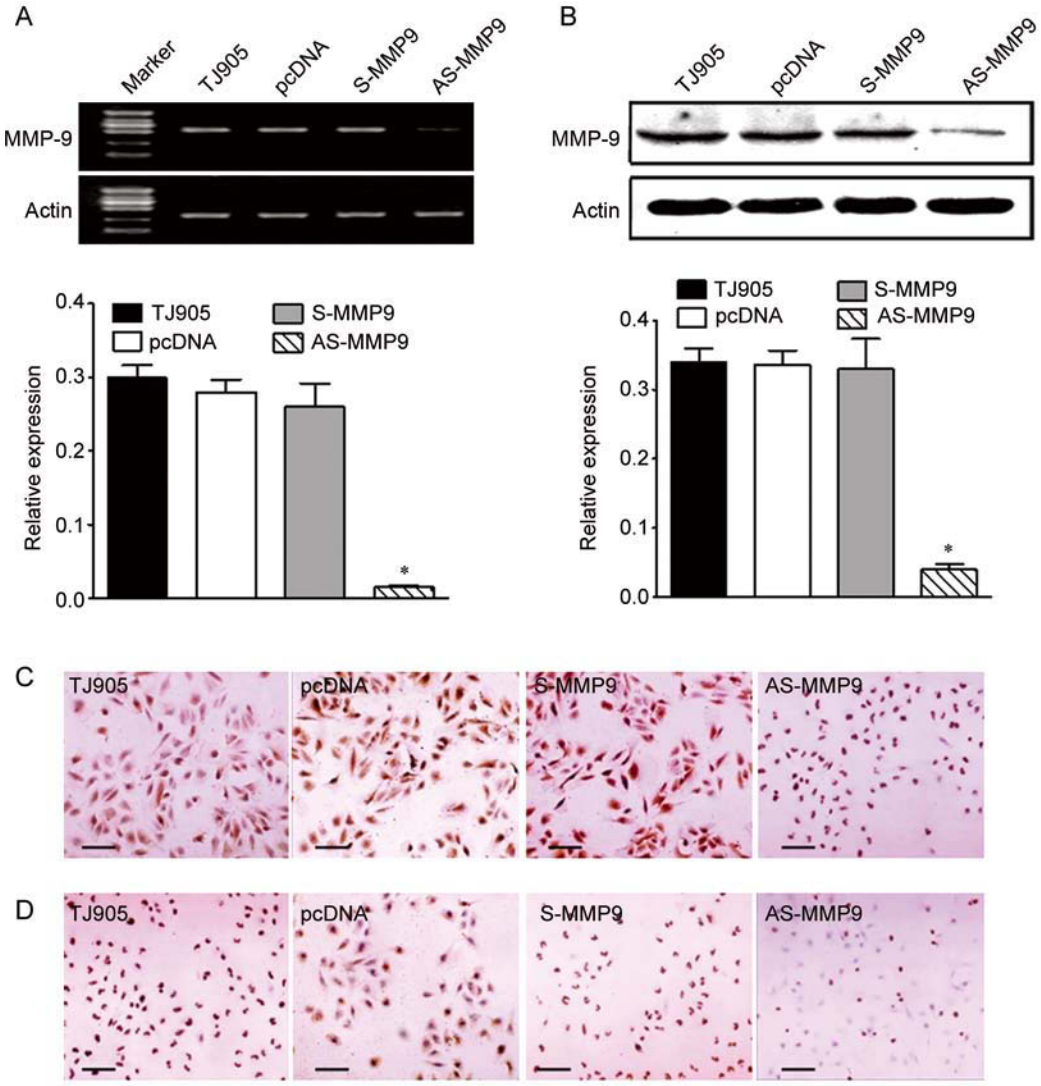

Fig. 2. MMP-9 expression was decreased by antisense MMP-9 RNA in cultured TJ905 glioma cells. A: RT-PCR analysis of MMP-9 expression using total RNA isolated from the cells. The assays were performed in triplicate. ****P* **<0.001 compared with the other three groups. B: Western blot analysis of MMP-9 protein expression using total protein extracted from the cells. ****P* **<0.001 compared with the other three groups. C and D: Immunostaining of MMP-9 (C) and Ki-67 (D) expression in cultured TJ905 cells. TJ905, parental TJ905 cells; pcDNA, TJ905 cells transfected with empty pcDNA vector; S-MMP9, TJ905 cells transfected with sense MMP-9 construct; AS-MMP9, TJ905 cells transfected with antisense MMP-9 construct. Scale bars, 100 μm.

combined therapy may be synergistic.

At the end of the 4- and 8-week treatments, the tumors were isolated and embedded in paraffin. Sections were prepared and immunohistopathological staining was performed using anti-GFAP, MMP-9 and Ki-67 antibodies. GFAP, an intermediate filament protein predominantly expressed in cells of astroglial origin, is also used as a marker for glioma diagnosis. Histopathological analysis and positive expression of GFAP showed that the tumors were in fact glioblastomas (Fig. 3C). Immunohistopathological analysis showed that MMP-9 expression in the tumor group transfected with pcDNA-AS-MMP9 and combined treatment group was lower (P <0.001) than that in control and the endostatin groups. However, MMP-9 expression in the endostatin group was not different from control group. Expression of MMP-9 in tumor cells did not significantly differ between the

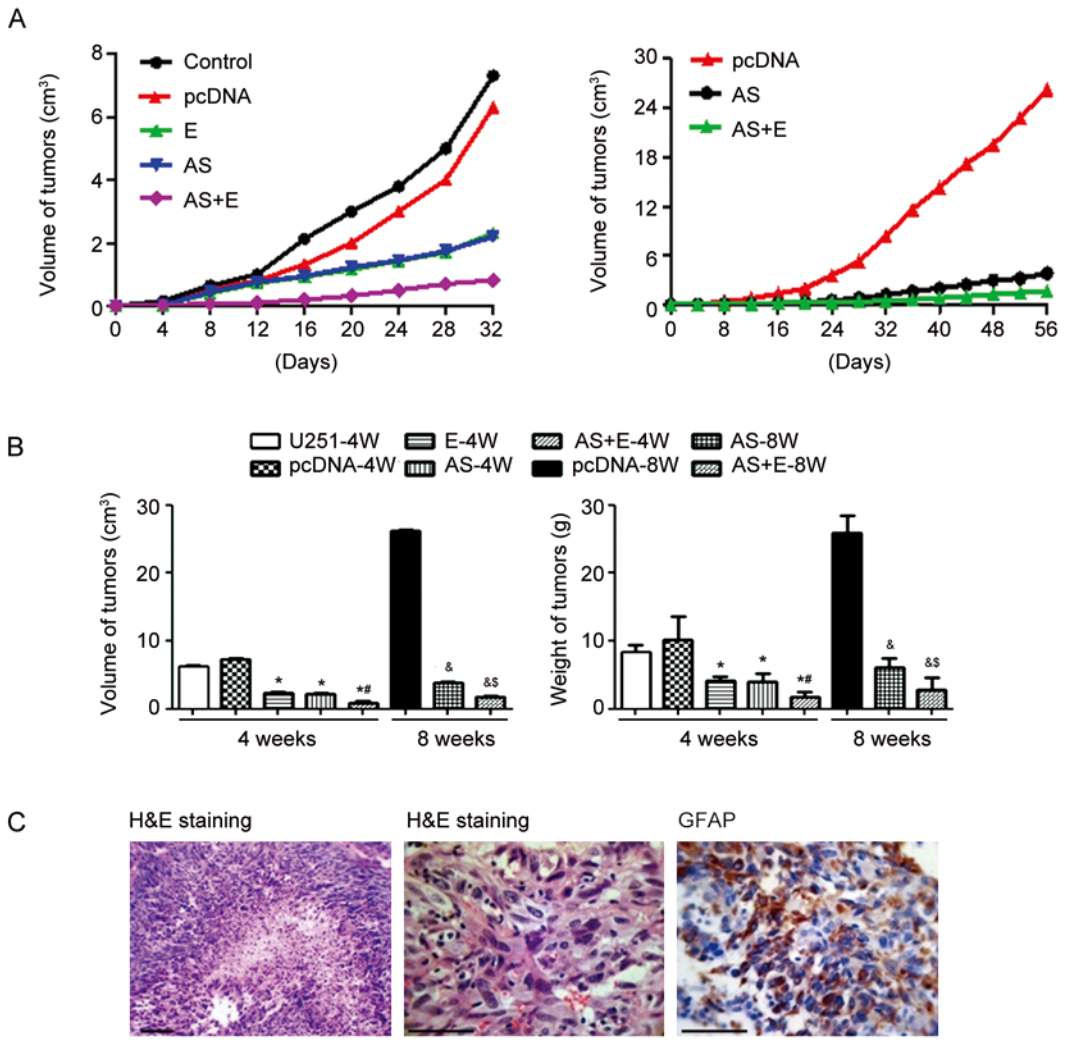

Fig. 3. Homograft tumor growth and character. A: Rate of tumor growth over 4 (left) and 8 (right) weeks in different groups. B: Tumor volume and weight in different groups. *P <0.001 compared with U251-4W and pcDNA-4W groups, $^{\#}P$ <0.05 compared with AS-4W and E-4W groups, $^{\&}P$ <0.001 compared with pcDNA-8W group, $^{\$}P$ <0.05 compared with AS-8W group. C: Morphology of homograft stained with hematoxylin and eosin (H&E) and GFAP staining confirmed the glioblastoma. Scale bars, 100 μm.

pcDNA-AS-MMP9 and combined therapy groups (Fig. 4A).

Tumors in the control and empty-vector groups showed much higher proliferation (Ki-67) (Fig. 4B) and microvessel density (Fig. 4C) than those of the other therapy groups. The proliferation activity and microvessel density were lower in the combined therapy group than in either the endostatin or the pcDNA-AS-MMP9 group (Table 1). There were no differences between the 4- and 8-week treatments in animals receiving either AS-MMP9 alone or combined therapy, implying that antisense MMP-9 therapy continuously inhibited the proliferation of tumors for at least 8 weeks.

DISCUSSION

MMP-2 and -9 belong to the matrix metalloproteinase family. Previous reports have suggested that MMP-9 is highly expressed in malignant gliomas and its expression rises with the degree of malignancy. The expression level of MMP-9 is far higher than that of MMP-2, which suggests that MMP-9 plays a role in the development and the invasive phenotype of malignant gliomas[8, 15, 16]. In the present

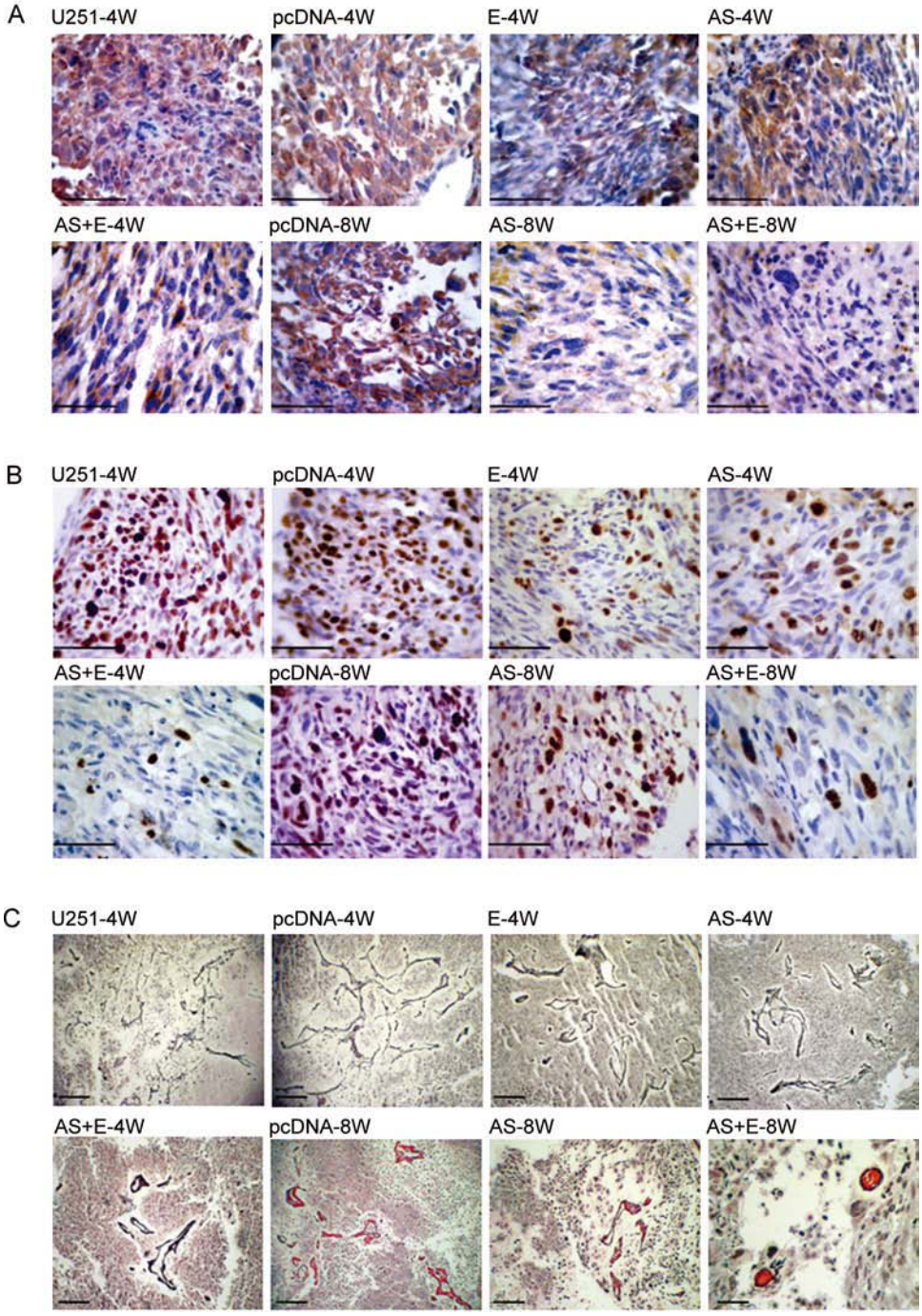

Fig. 4. Immunohistochemical staining of MMP-9 (stained brown) (A) and Ki-67 (brown) (B), and silver staining of microvessel density (C) in tumor tissues treated with PBS (U261), empty vector (pcDNA), endostatin (E), or antisense MMP-9 alone (AS), and those treated with antisense MMP-9 + endostatin (AS + E) for 4 (4W) or 8 weeks (8W). Scale bars, 100 μm.

study, we characterized the expression levels of MMP-9 using RT-PCR, Western blot and immunohistochemistry and found that both its mRNA and protein were highly expressed in TJ905 glioma cells, confirming that overexpression of MMP-9 is a stable characteristic of malignant glioma[17]. Then, we used the antisense approach to investigate

Table 1. Ki-67 expression rate and microvessel density (MVD) in homograft tumor of all the groups

Groups	*n*	Ki-67LI (%)	MVD
U251-4W	5	46.56 ± 7.17	9.64 ± 0.54
pcDNA-4W	5	46.67 ± 9.72	9.82 ± 0.67
E-4W	5	32.62 ± 5.63*	4.80 ± 0.73*
AS-4W	5	29.44 ± 3.91*	4.70 ± 0.92*
AS+E-4W	5	21.46 ± 2.84$^{*\#}$	3.54 ± 0.45$^{*\#}$
pcDNA-8W	2	47.72 ± 8.92	11.20 ± 0.50
AS-8W	5	28.74 ± 2.04$^{\&}$	5.58 ± 1.30$^{\&}$
AS+E-8W	5	21.07 ± 2.15$^{\&\$}$	4.26 ± 0.63$^{\&\$}$

Ki-67LI, Ki-67 labelling rate (%); MVD, microvessel count per field of vision at high magnification. Shown are mean values of duplicate experiments ± SD. $^{*}P$ <0.001 compared with U251-4W and pcDNA-4W groups; $^{\#}P$ <0.05 compared with AS-4W and E-4W groups; $^{\&}P$ <0.001 compared with pcDNA-8W group; $^{\$}P$ <0.05 compared with AS-8W group.

the oncogenic role of MMP-9 in the development of malignant gliomas. We demonstrated that antisense MMP-9 RNA was effective in inhibiting the elevated MMP-9 expression in glioma cells both *in vitro* and *in vivo*. Following transfection with antisense MMP-9 RNA, the growth rate and proliferation of glioma cells were significantly inhibited. In addition, Ki-67 expression in glioma cells was downregulated *in vitro* and *in vivo*. These results indicated that antisense MMP-9 RNA is able to reverse the malignant phenotype of glioma cells.

We used 528-bp MMP-9 mRNA as the target sequence of antisense RNA, which included MMP-9 initiation coding and did not overlap with the following RT-PCR-amplified sequence to avoid the influence of RNA transcription mediated by the expression plasmid. Meanwhile, we set the empty plasmid and sense RNA groups to exclude the influence of the expression plasmid vector and Lipofectamine, respectively, on glioma cells. Our data showed that MMP-9 mRNA and protein as well as the Ki-67 proliferation index dramatically decreased in the antisense RNA therapy group, which demonstrated that blocking MMP-9 by antisense RNA can inhibit tumor cell proliferation *in vitro*.

In view of the fact that cultured glioma cell lines lack a tumor matrix and the positive correlation between the Ki-67 proliferation index and MMP-9 expression found in this study, we speculate that MMP-9 promotes proliferation by means of another more direct mechanism rather than remodeling cellular adhesion and the tumor matrix. Active MMP-9 is able to mobilize VEGF from its ECM reservoir. Therefore, MMPs could promote VEGF-mediated angiogenesis in glioblastomas by both transcriptional (MT1-MMP) and post translational (MMP-9) mechanisms[2]. A recent study reported that MMP-9 releases activated insulin-like growth factor II (IGFII) after hydrolyzing the complex composed of IGFII and IGF-conjugated protein 2, which may be the mechanism resulting in the proliferation of malignant gliomas[2]. In addition, decreased pre-growth factors on the cell membrane and a weakened anti-apoptotic role of MMP-9 due to lower expression levels of the metalloproteinase also may be important causes of the inhibition of tumor cell proliferation[18-20].

In the subcutaneous tumor model, we found that MMP-9 was overexpressed at the tumor border, where growth is fastest and the invasive potential is strongest. MMP-9 is also expressed on endothelial cells and the basement membrane of blood vessels, where proliferation and angiogenesis are active[21-24]. Therefore, we speculated that MMP-9 plays multiple roles in the invasion process. On one hand, overexpression of MMP-9 may facilitate the degradation of collagen type IV and support glioma cells breaking through the basement barrier to invade the surrounding environment. On the other hand, overexpression of MMP-9 in endothelial cells would promote degradation of the vessel basement membrane[25], encouraging endothelial cells to proliferate and migrate to form tumor microvessels[26,27]. We found that both a high density of microvessels and high expression of MMP-9 occurred at the edge of tumors, especially at the site of tumor invasion. We thus speculate that expression of MMP-9 may be closely connected with vascular endothelial growth[28-30].

Expression of MMP-9 was significantly lower in the antisense MMP-9 construct and combined therapy groups than in the control and empty vector groups. However, MMP-9 expression in the endostatin group did not differ from those in control and empty vector groups. All these data suggest that antisense MMP-9 inhibited the expression of MMP-9. We used Ki-67LI to measure the proliferative activity as an estimate of the biological behavior of malignant glioma cells. We found that Ki-67LI in the AS-MMP9 construct, endostatin, and combined therapy groups was

significantly lower than that in the control and empty vector groups. These data show that both AS-MMP9 and endostatin inhibit the proliferation of malignant glioma cells[31,32]. In addition, Ki-67LI in the combined therapy group was lower than those in the AS-MMP9 construct and endostatin groups, demonstrating that the combination has synergistic effects on proliferation. There was no difference between the 4- and 8-week groups, meaning that the treatments continuously inhibited tumor proliferation for at least 8 weeks. The possible reasons for these observations are as follows: (1) endostatin, an angiogenesis inhibitor, inhibits tumor progression by directly affecting tumor cells and endothelial cells[32-34]; (2) antisense MMP-9 inhibits the transcription and translation of MMP-9 to depress the expression and impair the biological function of tumor cells, followed by a decrease in proliferation-promoting growth factors, thereby reducing tumor proliferation; and (3) low expression of MMP-9 around the microvessels inhibits remodeling of the ECM[35,36]. Collagen type IV represents ~50% of all basement membrane proteins, and has the capacity to self-assemble into organized networks; it is crucial for basement membrane stability and assembly. Therefore, since collagen type IV is a key MMP-9 substrate, it is not surprising that suppression of MMP-9 gene expression resulted in a significant decrease in collagen type IV expression[15,37].

As noted above, MMP-9 plays a critical role in the malignant progression of glioma. Inhibition of MMP-9 expression with antisense MMP-9 RNA might contribute to the inhibition of malignant glioma growth and angiogenesis, as well as inhibiting tumor cell proliferation. The effect was strengthened when combined with endostatin therapy. In conclusion, this study supports the premise that MMP-9 not only promotes the invasive growth of malignant glioma cells, but also directly affects tumor cell proliferation[38-40]. Antisense RNA technology aimed at the 528-bp sequence of MMP-9 mRNA 5' may be a valid treatment strategy for the treatment of malignant gliomas. The current study, therefore, describes a general method for the knockdown of MMP-9 gene expression that could have therapeutic applications.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (30770827, 31170864 and 81100887), National Basic Research Development Program of China (973 Program, 2010CB529405), Key Laboratory Project of Tianjin Municipality for Science and Technology (10SYSYJC28800), Major Program of Research on Applied Fundamentals and Frontier Technologies (10JCZDJC19400), Key Program of Higher Education of Tianjin Municipality for Science and Technology (2004ZD06, 20060202), Program for New Century Excellent Talents in University of China (NCET-11-1067), Key Project of Natural Science Foundation of Tianjin Municipality, China (12JCZDJC24200) and Key Project for Science and Technology of Ministry of Education, China (212005).

Received date: 2012-04-12; Accepted date: 2012-07-07

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