Antisense MMP-9 RNA inhibits malignant glioma cell growth \textit{in vitro} and \textit{in vivo}

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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 \textit{in vitro} and \textit{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 \textit{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\textsuperscript{[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\textsuperscript{[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\textsuperscript{[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 AACTGGATGACGATGTCTGCGTCC. The plasmids used for transfection, 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.

**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\textsuperscript{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'-GATCCACGGGAGTACTTGC-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\textsubscript{2}, 1 MgCl\textsubscript{2}, 1 phenylmethylsulfonyl fluoride, 1 sodium fluoride,