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Original contribution

Expression of high-mobility group AT-hook protein 2 and its prognostic significance in malignant gliomas☆,☆☆

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Summary High-mobility group AT-hook protein 2 (HMGA2) is an architectural transcription factor associated with malignancy, invasiveness, and poor prognosis in a variety of human neoplasms. This study investigated HMGA2 expression and prognostic value in human gliomas. We also correlated HMGA2 expression with Ki-67 labeling index and matrix metalloproteinase-2. Expression of HMGA2 in 78 human gliomas and 7 human normal brain samples was studied using immunohistochemistry, and 29 gliomas were randomly selected and studied along with the normal brain by real-time quantitative polymerase chain reaction and Western blot analysis. Expression of HMGA2 protein was significantly higher in glioblastoma multiforme (World Health Organization [WHO] grade IV; $P = .007$) and anaplastic astrocytoma (WHO grade III; $P = .037$) than in diffuse astrocytoma (WHO grade II). Expression of HMGA2 correlated significantly with expression of Ki-67 ($r = 0.415$, $P < .01$) and matrix metalloproteinase-2 ($r = 0.363$, $P < .01$), but not with patient sex and age. The real-time quantitative polymerase chain reaction and Western blot analysis revealed similar results. Patients with tumors expressing HMGA2 at a higher level had a significantly shorter progression-free survival time (11.2 months versus 18.8 months; $P = .021$). Expression of HMGA2 significantly correlates with tumor cell proliferation, invasion, and survival in gliomas. The results suggest that HMGA2 has an important role in the treatment and prognosis of these cancers.

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1. Introduction

Malignant gliomas, including astrocytoma (World Health Organization [WHO] grade II), anaplastic astrocytoma (AA; WHO grade III), and glioblastoma multiforme (GBM; WHO grade IV), are the most common and lethal primary central nervous system tumors [1]. Gliomas show an aggressive growth pattern and scatter along the white matter. The growth pattern makes total resection almost impossible, and gliomas can recur quickly after gross total resection. Despite aggressive treatment with chemotherapy, radiotherapy, or both, the median survival time is only 12 to 15 months for patients with GBM, 2 to 5 years for patients with AA, and 5 to 10 years for patients with diffuse astrocytoma (DA; WHO grade II) [2]. Increasingly, evidence shows that the pathogenesis of malignant gliomas is related to tumor proliferation and invasion; therefore, gene therapy targeting cell proliferation and invasion is becoming a more effective strategy [3–5]. However, effective therapeutic targets have always been sought in order to inhibit tumor growth.

High-mobility group AT-hook protein 2 (HMGA2) belongs to a family of nonhistone chromosomal proteins expressed mainly in the early embryo and suppressed in differentiated cells [6]. With its 3 AT hooks, HMGA2 can be bound to the narrow minor groove of AT-rich regions of DNA, affecting the transcription of target genes by altering chromatin architecture through protein-protein and protein-DNA interactions [6,7]. The protein is not expressed in normal human adult tissues but can be detected in many human neoplasms such as colorectal, lung, gastric, and ovarian cancers and is associated with the extent of malignancy and invasiveness and poor prognosis [8–11]. Silencing of the *HMGA2* gene leads to growth inhibition and increased apoptosis in ovarian cancer and liposarcoma cells [9,12].

Increasing evidence shows that tumor proliferation and invasiveness are related to the recurrence interval and patient survival in malignant gliomas [13]. However, there is no research focusing on the pathologic role of HMGA2 in human gliomas. The Ki-67/MIB-1 labeling index is a common marker for evaluating the proliferation of tumors, and matrix metalloproteinases (MMPs), especially MMP-2, are important cytokines in tumor invasiveness [14]. The aim of this study was to investigate HMGA2 expression in human gliomas. To assess the role of HMGA2, we detected Ki-67 and MMP-2 and correlated HMGA2 expression with tumor WHO class, Ki-67, MMP-2, and progression-free survival (PFS) time to further demonstrate the role of HMGA2 in the proliferation and invasion of gliomas and its possible role as a prognostic biomarker and therapeutic target.

2. Materials and methods

2.1. Patient selection and sample collection

We retrospectively selected 78 consecutive patients with histologically confirmed gliomas who underwent gross total resection at Provincial Hospital Affiliated to Shandong University between October 2010 and May 2013. Normal brain tissue samples were obtained from 7 patients with spontaneous intracerebral hemorrhage. Clinicopathological information was retrieved from the medical records. All hematoxylin and eosin–stained sections were reevaluated and classified by 2 pathologists according to the criteria of the WHO histologic classification [15]. Of the 78 glioma specimens, 27 were classified as DA, and 25 and 26 tumor specimens were classified as AA and GBM, respectively. Demographic data and tumor characteristics are summarized in the Table. The mean age of the patients (female 37, male 41) was 43 years. Among these, 32 patients who had undergone primary surgical resection between October 2010 and December 2011, including 11 with DA, 9 with AA, and 12 with GBM, received follow-up for 24 to 38 months. None of the patients received chemotherapy or radiotherapy prior to surgery, but all AA and GBM patients received postoperative radiotherapy or chemotherapy. None of the DA patients received postoperative therapy. Written consent to use stored specimens was obtained from all living patients. The study was approved by the Research Ethics Committee of Provincial Hospital Affiliated to Shandong University.

The surgical specimens were either kept in 4% para formaldehyde or preserved at −80 °C for later RNA and protein extraction.

The PFS time was defined as the period between the initial craniotomy and the day of the second operation for the first instance of tumor recurrence. Recurrence was defined as any new tumor visible on surveillance MRI, a ≥25% increase in known residual volume, and/or neurologic deterioration despite increased steroid use.

2.2. Immunohistochemistry staining

Representative formalin-fixed and paraffin-embedded blocks were selected and cut 5 μm thick for staining. The primary antibodies used in the study were as follows: HMGA2 (rabbit polyclonal, 1:50 dilution; Santa Cruz Biotechnology, Dallas, TX), MMP-2 (rabbit monoclonal antibody, 1:100 dilution; Boster Immunoleader, Pleasanton, CA), and Ki-67 (mouse monoclonal, 1:100 dilution; Dako, Carpenteria, CA).

Immunohistochemical staining was performed using the standard avidin-biotin method. Briefly, slides were deparaffinized and treated in citrate buffer (pH 6.0) using the microwave-induced epitope retrieval protocol. Endogenous peroxidase activity was quenched with 0.3% hydrogen peroxide for 15 minutes at 37°C, and then slides were incubated with primary antibodies in working solution overnight at 4°C. After several washes, the sections were incubated with biotinylated secondary antibodies (Beijing Zhongshan Goldenbridge Bio, Beijing, China) for 30 minutes at 37°C. Subsequently, the slides were incubated with peroxidase-conjugated streptavidin complex reagent and developed with 3,3′-