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Mitochondrial serine hydroxymethyltransferase 2 is a potential diagnostic and prognostic biomarker for human glioma

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ABSTRACT

Objective: Scholars have gradually come to appreciate the relevance of serine and glycine metabolism. Recently, researchers have discovered that mitochondrial serine hydroxymethyltransferase 2 (SHMT2) is overexpressed in various types of cancer. However, the function of SHMT2 in glioma is not clear. In this study, we sought to examine the expression of SHMT2 in glioma, the association between SHMT2 expression and clinicopathological characteristics, and the association of SHMT2 expression with prognosis in glioma patients.
Methods: We evaluated the expression of SHMT2, Ki67, O-6-methylguanine-DNA methyltransferase (MGMT), and Glutathione *S* Transferase pi (GST-pi) in 150 glioma patients using immunohistochemistry assays. The associations among the expression of SHMT2, clinicopathological parameters, and outcome of glioma patients were statistically analysed.
Results: The expression of SHMT2 was increased in gliomas compared to normal brain tissue and gradually increased with increasing WHO grade. The SHMT2 expression was positively correlated with Ki67 expression and WHO degree ($p < 0.01$) but was not correlated with other clinicopathological parameters, including sex, age, Karnofsky Performance Status (KPS), tumour diameter, MGMT, and GST-pi ($p > 0.05$). Kaplan–Meier survival curves and Cox regression analyses showed that SHMT2 expression and the WHO grade were independent prognostic indicators for glioma patients.
Conclusion: The expression of SHMT2 in glioma was significantly increased compared to normal brain tissue. SHMT2 promoted tumour proliferation, and there was no association between SHMT2 and drug resistance mechanisms of glioma. SHMT2 may be a novel and valuable biomarker for the diagnosis of glioma and an independent prognostic parameter of glioma.

1. Introduction

In the 1920s, Otto Warburg, a German physiologist, discovered that cancer cells, even under aerobic conditions, exhibit active glucose uptake and glycolysis, which has become widely known as the Warburg Effect [1]. Since then, energy metabolism in tumour tissue has become an important research direction for scholars. Abnormal metabolic change is an important feature of malignant tumours, and it also plays an important role in the occurrence and development of the tumour [2–4]. The study of tumour metabolism may provide new indicators and intervention targets for the diagnosis and treatment of tumours.

In recent years, scholars have recently come to appreciate amino acid metabolism for its role in tumourigenesis. Serine and glycine metabolism are important in the occurrence and devel-

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opment of the tumour [5–7]. Serine metabolism is not only an important branch of glycolysis but also an important source of one-carbon metabolism [8,9]. One-carbon units, which constitute the raw material for the synthesis of purines and pyrimidines, are the hub of amino acid metabolism and nucleotide metabolism, while also providing the methyl source for the methylation of various compounds in vivo [10]. Mitochondrial serine hydroxymethyltransferase 2 (SHMT2) is a crucial enzyme in the process of serine metabolism, which catalyses the conversion of serine to glycine [11–13]. Recently, researchers have discovered that SHMT2 is overexpressed in liver cancer, lung cancer, and breast cancer and is associated with a poor prognosis [14–16]. However, the expression of SHMT2 in the human glioma and its relationship with prognosis has not been reported to our knowledge.

We hypothesize that SHMT2 may facilitate glioma growth. SHMT2 may be a novel and valuable biomarker for the diagnosis and prognosis of glioma. In this study, we evaluated the expression of SHMT2 in 150 glioma patients using immunohistochemistry assays. In doing so, we demonstrated a correlation between the expression of SHMT2 and clinicopathological parameters from glioma patients. Furthermore, the prognostic value of SHMT2 expression was determined.

2. Materials and methods

2.1. Patients and tissue specimens

This study was approved by the Tianjin Huanhu Hospital ethics committee. Informed consent for this study was obtained from each patient. Clinical data were collected during the period from January 2012 to January 2013, with a total amount of 150 patients being randomly chosen as samples from the Department of Neurosurgery of Tianjin Huanhu Hospital, China. Clinical information included age, sex, Karnofsky Performance Status (KPS) and tumour diameter.

After excision, resected tissue samples were fixed immediately in 4% neutral-buffered formalin and then processed for paraffin sections. The WHO classification system was used by two senior neuropathologists to evaluate tumour pathology. None of the selected patients received radiotherapy, adjuvant chemotherapy or other therapies that may impact the study before surgeries. In comparison, a control group consisting of tissue from 10 patients with serious brain trauma and malignant swelling. In these 10 patients, 6 cases were frontal lobe contusion patients and 4 cases were temporal lobe contusion patients. During operation, contusive brain tissue was excised. The relatively normal brain tissues were selected for use as a control group.

All patients received follow-up at regular intervals for up to 3 years. Survival time was calculated from the date of initial surgery to the date of death or the last date of regular follow-up. Meanwhile, patients' records of radiotherapy and chemotherapy had been collected. According to the clinical results, patients who died from a disease not directly related to glioma or other unexpected causes were excluded from this study.

2.2. Immunohistochemistry assay

Paraffin-embedded tissue sections (5-μm thick), were deparaffinized and rehydrated. Endogenous peroxidase was neutralized with 3% H_2O_2 in methanol (10 min) after antigen retrieval in 0.1 M citrate buffer (pH 5.8) at 95° C for 5 min and cooled at 25° C for 1 h. Sections were blocked with normal goat serum (10 min) and then incubated with the following primary antibodies overnight at 4° C: Anti-SHMT2 antibody, Anti-Ki67 antibody, Anti-*O*-6-methylguanine-DNA methyltransferase (MGMT) antibody, Anti-Glutathione *S* Transferase pi (GST-pi) antibody (1:200, Abcam, Cambridge, UK). After incubation with biotinylated secondary antibody, the immunoreaction was visualized with diaminobenzene (DAB) and the nuclei were counterstained with Mayer's haematoxylin. Sections were dehydrated in an ascending alcohol series, cleared in dimethylbenzene, and coverslipped with neutral balsam. Negative controls were incubated in the same solutions without primary antibodies. SHMT2- and Ki67-positive cells were defined as having light to deep yellow nuclear staining. We randomly selected five areas at 400 magnification and counted 200 cells in each area. Then, we calculated the percentage of positive cells. MGMT- and GST-pi-positive stained cells were identified in a similar manner. Cells with no positive expression or weak staining were categorized as negative cells. Under this identification method, tumour cells with obvious staining were categorized as positive.

2.3. Statistical analysis

Data are presented as the mean ± SD. A p value of less than 0.05 ($p < 0.05$) was considered statistically significant. The following statistical analyses were conducted using the SPSS package version 16.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA with post hoc LSD test was conducted to compare the gliomas with different grades. The correlations between SHMT2 and other indexes were assessed by Spearman's rank tests. Survival curves were plotted with the Kaplan-Meier method and differences between the survival curves were tested using the log-rank test. Cox's proportional hazards model was used to identify factors with an independent influence on survival.

3. Results

3.1. Clinical characteristics of patients

Of the 150 patients, 76 (50.7%) were males and 74 (49.3%) were females with ages ranging from 17 to 70 (mean 48 ± 11 years). The Karnofsky Performance Scale (KPS) score ranged from 40 to 100 (mean 72 ± 11). Among the samples, 23 cases were WHO grade I glioma, 33 cases were WHO grade II glioma, 45 cases were WHO grade III glioma, and 49 cases were WHO grade IV glioma. The diameter of glioma ranged from 1.3 cm to 13 cm (mean 5.3 ± 1.9 cm). None of the patients had received chemotherapy or radiotherapy before surgery. After surgery, 45 patients did not receive radiotherapy or chemotherapy and 105 patients received radiotherapy or chemotherapy (Table 1).

Table 1
Clinicopathological characteristics of the study samples.

WHO Grade	n	Sex (M/F)	Median Age (years)	Median KPS	Tumour Diameter	Adjuvant Therapies (YES/NO)
1	23	11/12	40(16–56)	80(60–90)	4.9(3.7–13.0)	0/23
2	33	13/20	43(30–56)	70(60–90)	5.6(3.1–6.7)	10/23
3	45	24/21	47(24–66)	70(50–90)	6.5(1.6–12.0)	38/7
4	49	28/21	48(28–68)	70(30–90)	6.4(1.5–9.1)	33/16

n Case number.