Correlation between SATB1 and Bcl-2 expression in human glioblastoma multiforme

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Abstract. Special AT-rich sequence-binding protein-1 (SATB1) has been reported to be overexpressed in numerous human tumors. The aim of the present study was to determine the correlation and clinical significance between the expression of SATB1 and B-cell lymphoma 2 (Bcl-2) in human glioblastoma multiforme (GBM). Samples from 70 patients with GBMs were analyzed and 10 normal brain tissues were used as the control group. *In situ* hybridization was used to detect SATB1 mRNA expression and immunohistochemistry was used to detect Bcl-2 and proliferating cell nuclear antigen (PCNA) protein expression. Apoptosis was detected with flow cytometry. The SATB1 mRNA and Bcl-2 protein levels were found to be significantly higher in GBM tissues than in normal brain and their levels were associated with patient survival, but not associated with patient gender, age and tumor size and site. A positive correlation was observed between SATB1 mRNA and Bcl-2 protein and between SATB1 mRNA and PCNA. A negative correlation was observed between SATB1 mRNA and apoptosis and between Bcl-2 and apoptosis. A positive correlation existed between Bcl-2 and PCNA. Patients with GBM identified as SATB1 mRNA (+) and Bcl-2 (+) were associated with a poor prognosis. Therefore, assessment of SATB1 and Bcl-2 co-expression may provide important information for the diagnosis, therapy and prognosis of GBM.

Introduction

Glioblastoma multiforme (GBM) is the most common and lethal glial tumor of the adult brain, accounting for approximately 50% of all gliomas. GBM is characterized by an aggressive growth pattern, a marked degree of invasiveness and extremely poor prognosis. Even after multimodal treatment approaches, the median survival of patients with GBM following the primary diagnosis remains poor. An improved understanding of the genetic background and molecular pathogenic processes involved in the tumorigenesis of GBM is therefore critical for the development of rational, targeted therapies (1-5).

Special AT-rich sequence-binding protein 1 (SATB1) is a cell type-specific nuclear matrix attachment region-binding protein that links specific DNA elements to its cage-like network (6) and is predominantly expressed in thymocytes (7). SATB1 facilitates the formation of an open chromatin structure and is involved in the regulation of hundreds of genes. SATB1 has recently received considerable attention from the cancer research field due to high expression in various malignant tumor tissues (8-10), indicative of a tumor growth promoter. To explore the roles of SATB1 in the clinical progression of GBM, the present study examined the correlation between expression of SATB1 and Bcl-2 in GBM and tumor apoptosis, in order to shed new light on GBM therapy.

Materials and methods

Patients and tissue samples. Seventy cases of surgically resected GBMs were collected from the 2007-2010 pathology files of the Third People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine and Zhongnan Hospital of Wuhan University. Approval was obtained from the Ethics committee of the Third People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine and Zhongnan Hospital of Wuhan University. Specimens were handled and made anonymous according to ethical and legal standards. Written informed consent was obtained from all patients. There were 40 males and 30 females with a mean age of 46 years (range, 29-68). None of the patients had received chemical therapy or radiotherapy prior to surgery. Control brain tissues were obtained from 10 individuals, who had died in traffic accidents exhibiting no prior pathologically detectable condition. Based on the results of hematoxylin-eosin staining, histopathological diagnosis was performed by various neuropathologists.

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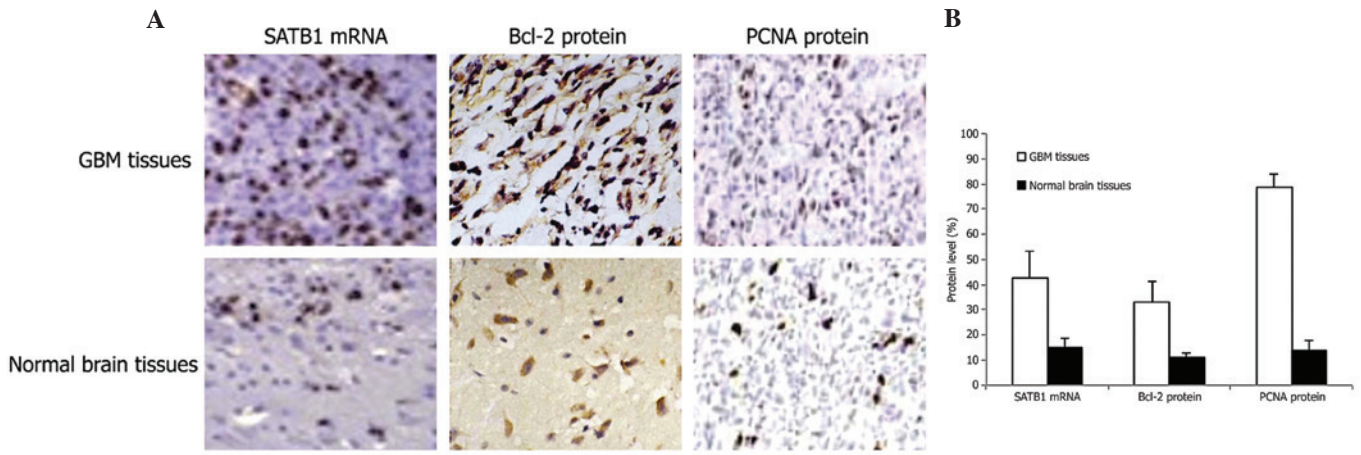

Figure 1. SATB1 mRNA, Bcl-2 and PCNA protein expression in tissues. (A) Representative images of SATB1 mRNA, Bcl-2 and PCNA protein expression in GBM and normal brain tissues. (B) Quantitative analysis of SATB1 mRNA, Bcl-2 and PCNA protein expression in GBM tissues and the normal brain tissues. SATB1, special AT-rich sequence-binding protein-1; Bcl-2, B-cell lymphoma-2; PCNA, proliferating cell nuclear antigen; GBM, glioblastoma multiforme.

In situ hybridization. Frozen sections were immersed in a solution of 30% hydrogen dioxide and methanol for 30 min following brief warming at room temperature, then incubated at 37˚C with pepsin diluted by 3% citric acid. Sections were postfixed for 10 min in 1% paraformaldehyde and were incubated at 38-42˚C with DIG-labeled antisense cRNA probes overnight in a humidified chamber. Following multiple washes in 4X SSC at room temperature, the slides were incubated at 37˚C in a blocking reagent for 30 min, a biotinylated anti-digoxin antibody for 60 min, SABC for 20 min and the biotinylated peroxydase for 20 min, at 37˚C, followed by staining with DAB (Sigma, St. Louis, MO, USA). Sections were then covered with glycerol-gelatin and coverslips for microscopic examination (11,12). The SATB1 probe (sequence: 5'-TCTTTAATTTCTAATATATTTAGAA-3') was synthesized and labeled with biotin at the 5' end by Sangon Bioengineering Technology and Services Co., Ltd. (Shanghai, China). The probe was replaced with the dilution solution in control samples.

Immunohistochemical analysis. Antigen retrieval was performed in boiling citrate buffer for 15 min. Peroxide blocking was performed with 0.3% peroxide in absolute methanol. The slides were then incubated with anti-Bcl-2 polyclonal antibody (diluted 1:300; Sigma) or mouse anti-PCNA monoclonal antibody (diluted 1:100; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) at 4˚C overnight and washed twice with phosphate-buffered saline prior to incubation with a secondary antibody (Santa Cruz Biotechnology, Inc.) at room temperature for 30 min. After washing, the sections were incubated with immunoglobulins conjugated with horseradish peroxidase. The reaction was then developed with 3,3'-diaminobenzidine substrate (2,13). Tissue sections were counterstained with hematoxylin or methyl green.

Quantification of SATB1 mRNA, Bcl-2 and PCNA protein. The nucleoli of SATB1-positive cells, cytoplasm of Bcl-2-positive cells (14) and nuclei of PCNA-positive cells were stained brown-yellow. Images of the sections were obtained (magnification, x100) using the HPIAS-1000 High Resolution Color Pathological Image Analysis System (Tongji Medical College Qianping Imaging Engineering Co., Ltd., Shanghai, China) (15). Specimens with a positive cell ratio <30% were defined as negative.

Measurement of apoptosis by flow cytometry. Tissues collected in RPMI-1640 medium supplemented with 10% fetal bovine serum were processed routinely to generate single-cell suspensions (16,17). Suspensions were then fixed in 70% cold ethanol, treated with 10 g/l RNase and suspended and stained with 10 g/l propidium iodine (PI). After washing with PBS, the cells were stained directly with PI at a final concentration of 10 μg/ml and 2% Annexin-V Flous (Roche, Basel, Swizerland) in incubation buffer for 10 min. The cells were collected by FACSCalibur (BD Pharmingen, San Diego, CA, USA) following instrument set-up with controls (non-treated, stained cells) and two washes in PBS. In this experiment, cells with early apoptotic signals (stained with Annexin V) and cells with late death signals (stained with PI) were quantified and apoptotic cells were analyzed using the CellQuest software (18,19). Each assay was performed in triplicate.

Statistical analysis. Quantitative values were expressed as the mean ± SD. Statistical analysis was performed using the Pearson method and a Student's or Chi-squared test using SPSS 12.0 (for Windows; SPSS, Chicago, IL, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

SATB1 expression by in situ hybridization. The majority of brown-positive staining for *in situ* hybridization of SATB1 mRNA was homogeneously distributed within the nucleolus (Fig. 1A). SATB1 mRNA expression levels were found to be significantly higher in GBM than in the normal brain tissues (Fig. 1B; $P<0.01$).

Immunohistochemical analysis of Bcl-2 and PCNA protein expression. Immunohistochemical staining revealed Bcl-2 protein expression in both the cytoplasm and cell membrane

Table I. Correlation of SATB1 and Bcl-2 expression with clinical variables of GBM.

Variables	n	SATB1 (%)	P-value	Bcl-2 (%)	P-value
Gender					
Male	40	43.63±7.23	0.332	33.24±5.75	0.558
Female	30	41.83±8.14		32.35±6.88	
Age (years)					
≥46	34	41.92±6.96	0.302	32.23±6.21	0.388
<46	36	43.74±7.65		33.45±5.54	
Tumor size (cm^3)					
≥4	41	44.12±7.45	0.109	34.02±7.12	0.103
<4	29	41.07±8.12		31.21±6.85	
Tumor site					
Supratentorial	45	42.79±7.52	0.919	32.80±5.23	0.906
Infratentorial	25	42.98±7.44		32.96±5.78	
Survival rate (years)					
≥1	29	37.18±7.68	0.000	28.34±6.23	0.000
<1	41	46.87±8.92		36.05±7.48	

SATB1, special AT-rich sequence-binding protein-1; Bcl-2, B-cell lymphoma-2; GBM, glioblastoma multiforme.

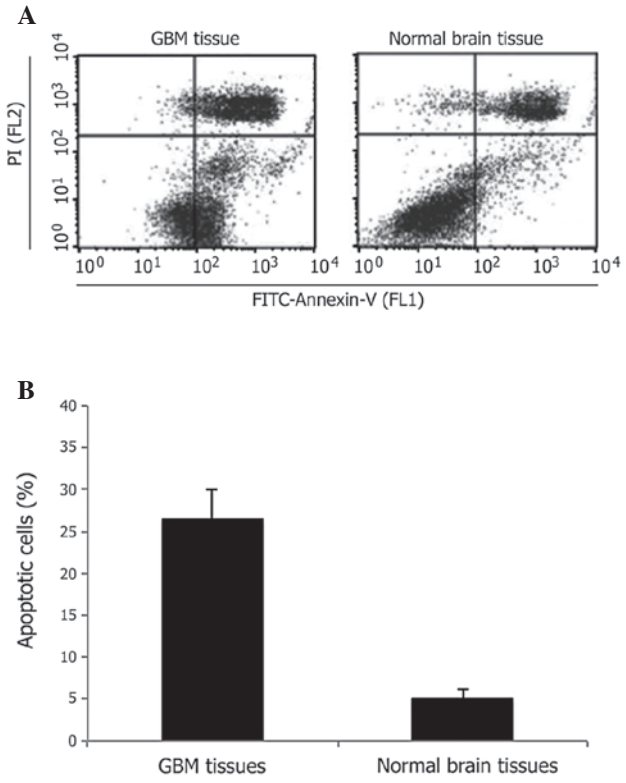

Figure 2. FACS analysis of Annexin-V staining of cells in GBM tissues and the normal brain tissues. (A) Representative FACS scatter plots of cells indicating apoptotic cell death. (B) Percentages of apoptotic cells in tissues. GBM, glioblastoma multiforme.

(Fig. 1A). PCNA protein expression was primarily detected in the nucleolus (Fig. 1A). Expression of Bcl-2 and PCNA protein was noted at significantly higher levels in GBM tissues than in normal brain tissues (Fig. 1B, P<0.01).

Measurement of apoptosis by flow cytometry. To quantify apoptotic cell death in tissues, ~1x10^6 cells were double stained with Annexin V-FITC and PI at various times post-transfection. Apoptotic cell death was detected in GBM and normal brain tissues (Fig. 2A). FACS analysis identified a significantly higher number of apoptotic cells in GBM tissues than normal brain tissues (Fig. 2B; P<0.01).

Correlation between SATB1 and Bcl-2 expression and clinical characteristics of GBM. SATB1 mRNA and Bcl-2 protein expression levels were associated with the survival rate of patients (P<0.01), but were not associated with patient gender, age and tumor size and site (Table I).

Correlation between SATB1 and Bcl-2 expression, PCNA and apoptosis. Statistical analysis revealed a positive correlation between SATB1 mRNA and Bcl-2 protein levels (P<0.05) and between SATB1 mRNA and PCNA protein levels (P<0.01). A negative correlation was identified between SATB1 and apoptosis (P<0.01) and between Bcl-2 and apoptosis (P<0.01). However, a positive correlation was observed between Bcl-2 and PCNA (P<0.01), whereas a negative correlation was found between PCNA and apoptosis (P<0.01; Table II).

Co-expression of SATB1 and Bcl-2. SATB1-positive cases (+) were divided into two groups consisting of a survival rate <1 and ≥1 year. Differences between the groups were evaluated according to the Bcl-2 protein expression and identified as χ^2=20.95 (P<0.001; Table III).

Discussion

SATB1 is a tissue-specific nuclear matrix-attachment DNA-binding protein, which is located on chromosome 3p23.

Table II. Correlation between SATB1, Bcl-2, PCNA expression and apoptosis in GBM.

Variables	SATB1	Bcl-2	PCNA	Apoptosis
SATB1	-	0.542[a]	0.615[b]	-0.534[a]
Bcl-2	0.542[a]	-	-0.536[a]	-0.586[b]
PCNA	0.615[b]	-0.536[a]	-	-0.532[a]
Apoptosis	-0.534[a]	-0.586[b]	-0.532[a]	-

[a]P<0.05, [b]P<0.01. Values indicate the correlation coefficient r. SATB1, special AT-rich sequence-binding protein-1; Bcl-2, B-cell lymphoma-2; PCNA, proliferating cell nuclear antigen; GBM, glioblastoma multiforme.

Table III. Correlation between SATB1, Bcl-2 and survival time.

	Survival time	
Variables	≥1 year	<1 year
S^+B^+	6	29
S^+B^-	16	4

SATB1, special AT-rich sequence-binding protein-1; Bcl-2, B-cell lymphoma 2; S, SATB1; B, Bcl-2.

SATB1 has previously attracted considerable attention in the cancer research field due to its high expression in tumor tissues of a variety of malignancies (8-10), indicative of a crucial role in the promotion of tumor growth and prediction of tumor prognosis. It was previously demonstrated that overexpression of SATB1 correlates with the metastatic potential of human gastric cancer and may be suitable for use as a novel independent prognostic marker for the prediction of gastric cancer outcome (20). Bcl-2 is expressed in various tissues under normal conditions, with the physiological function of the modulation of apoptotis and cell number balance (21). Two important factors of cell number control are rate of apoptosis and proliferation (22,23). Overexpression of SATB1 and/or Bcl-2 disturbs this balance and contributes to the proliferation and anti-apoptotic functions of the abnormal cell.

Results of the present study have demonstrated that expression of SATB1 mRNA and Bcl-2 protein is significantly higher in GBM tissues than in the normal brain tissues. With regard to clinical features, expression of SATB1 and Bcl-2 was correlated with patient survival, but was not associated with patient gender, age and tumor size and site. Overexpression of SATB1 mRNA and Bcl-2 protein was higher in the survival <1 year group than the ≥1 year and a significant positive correlation between SATB1 and Bcl-2 was observed. We analyzed the correlation between SATB1, Bcl-2, PCNA and apoptosis. A positive correlation between SATB1 mRNA and PCNA was observed. A negative correlation between SATB1 mRNA and apoptosis and between Bcl-2 and apoptosis was observed and a positive correlation was found between Bcl-2 and PCNA. These data suggest that SATB1 functions in the promotion of cell proliferation and inhibition of apoptosis. Function of Bcl-2 is restricted to inhibiting apoptosis. Consistent with this hypothesis, cases positive for SATB1 and Bcl-2 were associated with poor prognosis, thus, assessment of SATB1 and Bcl-2 co-expression may provide useful information for the diagnosis, therapy and prognosis of GBM.

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