ORIGINAL ARTICLE

SPP1 promoter polymorphisms and glioma risk in a Chinese Han population

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SPP1 was found to be significantly upregulated in many kinds of malignant tumors, including gliomas. Considering that gene polymorphisms have been implicated in the development of gliomas, we performed an association study between *SPP1* functional promoter region polymorphisms and glioma risk in a Chinese population. We found significant evidence of an association between *SPP1* promoter polymorphisms and glioma risk. For the −155_156insG variant, the −155_156GG allele was found to be significantly associated with an increased risk of glioma (P=0.020, odds ratio (OR)=1.202, 95% confidence interval (CI): 1.028–1.408). Individuals with the genotype containing the GG allele had a 1.372-fold increased risk (P=0.006, OR=1.372, 95% CI: 1.095–1.719). Further stratified analyses suggested that a significant association existed in adult glioma patients, male subjects and in cases without a family history of cancer. Alternatively, the study of single-nucleotide polymorphism −443C/T in a recessive model revealed that the genotype CC + CT significantly decreased the risk of glioma when compared with TT (P=0.023, OR=0.774, 95% CI: 0.621–0.966). After the analysis of haplotypes, the haplotype −155_156GG/−443T was represented at a significantly higher frequency in cases (P=0.029, OR=1.192, 95% CI: 1.018–1.395). Cellular assay indicated that the transcriptional activity of the *SPP1* promoter containing the −155_156GG allele significantly increased in glioma cells. Thus, variants of the *SPP1* promoter might influence the risk of glioma by regulating promoter activity. Further analyses are necessary to validate our observation in larger samples or in other ethnic groups.
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INTRODUCTION

Osteopontin (OPN) is encoded by *SPP1* and is a glycophosphocytokine with multiple functions.[1] It is expressed and secreted by various cells, and has a role in cell adhesion, chemotaxis, prevention of apoptosis, invasion, migration and anchorage-independent growth of tumor cells.[2–6] Extensive research has demonstrated the pivotal role of OPN in the regulation of signals that control neoplastic and malignant transformation. Consistent with these tumorigenic functions, elevated expression of *SPP1* has been observed in a variety of cancers. Moreover, OPN has been linked with tumor metastasis and signifies a poor prognosis for the patient.[1,7]

Although it has not been extensively studied as a mediator of glioma pathobiology to date, *SPP1* is known to be overexpressed in human high-grade gliomas;[8] in a serial analysis of gene expression analysis comparing C6 rat glioma cells with normal rodent astrocytes, *SPP1* was identified as one of the most overexpressed genes.[9] In addition, on the basis of results from a study by Jang *et al.*,[7] which used microarray analysis to compare the gene expression patterns of induced tumors in rat brains, *SPP1* was the most upregulated gene in gliomas. Therefore, the process of upregulation of *SPP1* during glioma genesis is important.

It is well known that *SPP1* is predominantly a transcriptionally regulated gene with a highly conserved promoter.[10] Its transcription can be activated by various stimuli through AP1[11–13] and RUNX2,[14] and both of these factors could interact with SP1 in a cooperative manner.

Several polymorphisms in the *SPP1* gene noticeably affect its expression and have been associated with various diseases.[15–18] Reporter gene expression experiments with the *SPP1* promoter polymorphisms −443C/T, −155_156insG and −66T/G revealed that the sequence variants resulted in a significantly increased reporter gene expression. The strongest expression was conferred by G-insertion at position −155, in combination with the −66T allele.[15] Further analysis of the *SPP1* promoter sequence revealed putative transcription factor binding sites for SP1 around −66, for CBFA1/RUNX2 around −155 and for MYT1 zinc-finger factor at −443.[15] These results suggest that different haplotypes of the *SPP1* promoter might alter *SPP1* transcription and finally affect tumor risk.

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In this study, we hypothesized that *SPP1* promoter single-nucleotide polymorphisms (SNPs) might account for the higher *SPP1* mRNA level during glioma genesis and therefore promote tumor risk and outcome. We present data from a case–control association study on Chinese glioma patients with the three functional sequence variations spanning the whole *SPP1* promoter region.

MATERIALS AND METHODS

Study population
The characteristics of subjects in the study have previously been described elsewhere.[19] Briefly, this was a hospital-based case–control study that included 670 glioma cases and 680 cancer-free controls. All subjects were of Han Chinese origin. Newly diagnosed patients with histopathologically confirmed glioma were recruited between October 2004 and May 2006 from the Huashan Hospital of Fudan University and from the Changzheng Hospital of the Second Military Medical University without any restrictions on age, sex and histology. The exclusion criteria for the control subjects included known central nervous system-related diseases, a self-reported history of another type of cancer, and chemotherapy for unknown disease conditions. All control subjects were frequency matched to cases on the basis of age, sex and area of residence (urban or rural). Among all eligible glioma patients and eligible control subjects, a total of 670 case patients and 680 control subjects participated in this study. Each participant was scheduled for a face-to-face interview to answer a structured questionnaire that elicited information about demographic factors and health characteristics. After the interview, a one-time sample of approximately 3–5 ml of venous blood was collected from each of the study participants.

DNA extraction and genotyping
Blood samples were collected in EDTA-containing tubes. Genomic DNA was extracted from white blood cell fractions using the Qiagen Blood Kit (Qiagen, Chatsworth, CA, USA). This study was approved by the Fudan University Ethics Committee for Human Subject Research.

Three functional SNPs, −66T/G (rs28357094), −155_156insG (rs17524488) and −443C/T (rs11730582), were genotyped using the PCR–ligation detection reaction (PCR–LDR) method. The amplification primers used were the following: forward, 5′-CTGAAGCAGCCCTCTCAAGCA-3′;and reverse, 5′-ACAAC-CAAGCCCTCCCAGAAT-3′. PCRs were carried out on ABI 9600 (Applied Biosystems, Foster City, CA, USA) in a total volume of 20 μl, which included 20 ng of genomic DNA, 1× PCR buffer, 2.5 mM MgCl$_2$, 0.2 mM dNTPs, 0.5 μM of each primer and 1 U of hot-start Taq DNA polymerase (Qiagen). Cycling parameters were as follows: 95 °C for 15 min; 35 cycles of 94 °C for 30 s, 60 °C for 2 min, 72 °C for 60 s; and a final extension step at 72 °C for 10 min. The probes for LDR were −66T/G common probe, 5′-P-CCGCCTCCCTGTG TTGGTGGAGGAT-FAM-3′; −66T/G T-specific probe, 5′-tttCAGAAAACCT-CATGACACATTCTCT-3′; −66T/G G-specific probe, 5′-CAGAAAACCTCAT-GACACATTCTCG-3′; −155_156insG common probe, 5′-P-TTTTTTTTTGTT TTAACCACAAAACttt-FAM-3′; −155_156insG G-specific probe, 5′-tttttGTA-GATTGTGTGTGTGCGATTTTG-3′; −155_156insG GG-specific probe, 5′-ttttttttGTAGATTGTGTGTGTGCGATTTTGG-3′; −443C/T common probe, 5′-P-TCTGAACTCCTTGCAGGCTTGAACAtttttt-FAM-3′; −443C/T C-specific probe, 5′-tttttttttAGTAGTAAAGGACAGAGGCTAGTTC-3′; −443C/T T-specific probe, 5′-ttttttttttttAGTAGTAAAGGACAGAGGCTAGTTT-3′. The common probe was labeled at the 3′-end with 6-carboxyXuorescein (FAM) and was phosphorylated at the 5′-end. The ligation reaction for each PCR product was carried out in a final volume of 20 μl, which contained 2 μl of 10× ligation buffer, 2 μl of PCR product, 1 pmol of each discriminating probe and 20 U of Taq DNA ligase (New England Biolabs, Ipswich, MA, USA). LDR parameters were 94 °C for 2 min, followed by 40 cycles of 94 °C for 30 s and 60 °C for 2 min. Following the LDR reaction, 1 μl of LDR reaction product was mixed with 1 μl of ROX and 1 μl of loading buffer. The mixture was then analyzed by the ABI Prism 373 DNA Sequencer (Applied Biosystems).

In addition, the representative PCR products were subjected to direct DNA sequencing in an ABI Prism 310 Sequencer (Applied Biosystems) to confirm the accuracy of this method.

Construction of luciferase reporter plasmids
A 644/643 bp fragment from −645 to −2 bp of the *SPP1* promoter was prepared by PCR amplification from genomic DNA of homozygous individuals for the four most common haplotypes (forward primer: 5′-ggggtaccTGAAGCA GCCCTCTCAAGCA-3′; reverse primer: 5′-gaagatctACAACCAAGCCCTCCCA GAAT-3′). DNA fragments containing different alleles of the human *SPP1* gene from −645 to −2 (−155_156G/−443T, −155_156G/−443C, −155_156GG/ −443T and −155_156GG/−443C) were cloned into the pGL3 Basic Vector (Promega, Madison, WI, USA) between KpnI and BglII restriction sites, which were designated as A, B, C and D plasmids, respectively.

Cell culture, transfection and luciferase assay
Human embryonic kidney (HEK293) cells and human glioma cells (U373) were obtained from Fudan University (Shanghai, China). The cells were cultured in Dulbecco's modified Eagle's medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum, 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin and 2 mmol l^{-1} L-glutamine in a humidified incubator with 5% CO$_2$ at 37 °C. One day before transfection, the cells were cultured in a 96-well plate in 100 μl culture medium without antibiotics to 90–95% confluence at the time of transfection. The cells were transfected using Lipofectamine 2000 Reagent according to the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). Briefly, 0.2 μg of the pGL3 vector containing an *SPP1* promoter fragment was used for each well. pGL3-Basic was used as negative control. In each transfection, 10 ng pRL-TK (Promega) was used to normalize the transfection efficiency. The culture medium was replaced 6 h after transfection and the cells were maintained in culture for an additional 42 h before luciferase assays.

The transfectants were lysed in Passive Lysis buffer 48 h after transfection, and 20 μl aliquots of supernatant were analyzed for luciferase activity by using the Dual-Luciferase Reporter Assay System in a GloMax 96 Microplate luminometer (Promega). Promoter activities were expressed as the ratio between *Firefly* luciferase and *Renilla* luciferase activities.

Statistical analysis
Pearson's χ^2-test without Yates' continuity correction was used to evaluate differences between cases and controls in the frequency of selected demographic variables, including smoking status, family history of cancer, histology and genotype distributions of *SPP1* promoter region polymorphisms. Hardy–Weinberg equilibrium was tested by χ^2-test for goodness of fit using a web-based program (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). The associations between these polymorphisms and glioma risk were estimated by odds ratios (ORs) and their 95% confident intervals (95% CI), which were computed using the logistic regression model. Stepwise forward selection/backward elimination procedure of multivariate analysis was also performed. Student's *t*-test was used to examine the differences in luciferase reporter gene expression. Haploview (http://www.broadinstitute.org/haploview/haploview) software and PHASE 2.1 (http://www.stat.washington.edu/stephens/software.html) were used to calculate haplotype frequencies, linkage disequilibrium and index *D′*. We used an age of 18 years as the cutoff point and divided the subjects into two groups of children ($\leqslant 18$) and adults (> 18). In addition, we stratified patients into four subgroups of WHOI, WHOII, WHOIII and WHOIV according to WHO. All statistical analyses were performed with the computer software SPSS12.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was used as the criterion of statistical significance and the statistical tests were two-sided.

RESULTS

Characteristics of the study population
Our final analysis included 670 glioma cases and 680 cancer-free controls. The characteristics of the patients and control subjects are summarized in Table 1. No statistically significant differences were found between patients and controls in term of smoking status. More notably, in this study population, a family history of cancer seemed to account for an increased glioma risk of about 44% ($P=0.024$, OR=1.44, 95% CI: 1.05–1.97), which points to inherited genes being responsible for glioma susceptibility in the Chinese population. Among the 670 patients, 67 (9.9%) had a WHOI histology type, 230 (34.2%) a WHOII, 118 (17.6%) a WHOIII and 255 (38.5%) a WHOIV (Table 1).

Table 1 Characteristics of selected patients with glioma and controls

Variable	Patients (n=670), no. (%)	Controls (n=680), no. (%)	P-value[a]
Gender			0.082
Male	397 (59.7)	371 (54.6)	
Female	273 (40.3)	309 (45.4)	
Age (years)			0.640
Children (⩽18)	62 (9.4)	58 (8.7)	
Adults (>18)	608 (90.6)	622 (91.3)	
Smoking status			
Never	469 (70.2)	493 (72.3)	
Ever	65 (9.7)	70 (10.5)	0.895
Still	136 (20.1)	117 (17.2)	0.157
Still+ever	201 (29.8)	187 (27.7)	0.310
Family history of cancer			
No	566 (84.8)	603 (88.9)	0.024
Yes	104 (15.2)	77 (11.1)	
Histology			
WHOI	67 (9.9)		
WHOII	230 (34.2)		
WHOIII	118 (17.4)		
WHOIV	255 (38.5)		

[a]Two-sided χ^2-test.

Association between an individual SNP and risk of glioma

After genotyping by LDR methods, we did not find the G allele of the −66T/G variant, indicating that the −66T/G variant was not present in the Chinese population. SNP −155_156insG was successfully genotyped in 664 cases and 667 controls, resulting in a 99% genotyping rate. SNP −443C/T was successfully genotyped in 663 cases and 667 controls, yielding a genotyping rate of 98%. Allele frequencies and genotype distributions of *SPP1* in patients and controls are shown in Table 2. The −155_156GG allele frequencies of the −155_156insG SNP were 0.363 in controls and 0.409 in patients. The genotype frequencies of this site for −155_156GG/GG, −155_156GG/G and −155_156G/G in cases and controls were 0.149, 0.52, 0.331 and 0.135, 0.457, 0.408, respectively. The allele and genotype frequencies differed from those previously reported in Caucasians.[20] The allele frequencies of the −443C/T SNP for *SPP1*T were 0.654 in controls and 0.672 in patients. The genotype frequencies of this site for CC, CT and TT in cases and controls were 0.103, 0.448, 0.448 and 0.115, 0.463, 0.423, respectively (Table 2). The frequencies of both controls and patients fit the Hardy–Weinberg equilibrium (data not shown).

A logistic regression model was used to estimate associations between the alleles or genotypes of *SPP1* and the risk of glioma (Table 2). For the −155_156insG variant, the −155_156GG allele was found to be significantly associated with an increased risk of glioma (P=0.020, OR=1.202, 95% CI: 1.028–1.408). Furthermore, individuals who carried the genotype −155_156GG/G and −155_156GG/GG had a 1.372-fold increased risk (P=0.006, OR=1.372, 95% CI: 1.095–1.719). Results of the stepwise forward selection/backward elimination procedure also indicated that the genotype of the −155_156insG polymorphism was the most important variable for glioma risk, as well as familial history of cancer. When considering the differences in the pathogenesis of glioma between childhood and adult populations, we age stratified glioma into childhood glioma (age ⩽ 18 years) and adult glioma (age > 18 years) and found that the −155_156GG allele was associated with an increased risk in both populations. However, the observation was only significant in the case of adult glioma (P=0.006, OR=1.372, 95% CI: 1.095–1.719) (Table 3). In addition, we examined the association between *SPP1* alleles and glioma risk in subgroups of participants stratified by smoking status, family history of cancer and histology type. No significant association was observed between the −155_156insG polymorphism and glioma risk in the subgroups of never smoking, ever smoking, still smoking, with family history of cancer and type WHOI, II, III, IV glioma patients. Moreover, as shown in Table 2, the −155_156GG/G genotype of the −155_156insG polymorphism was associated with an increased risk of glioma among subjects without a family history of cancer with an OR of 1.224 (P=0.018, OR=1.224, 95% CI: 1.035–1.448). A similar result was also seen in male patients (P=0.016, OR=1.286, 95% CI: 1.048–1.578) (Table 3).

The study of SNP −443C/T revealed that the distribution of alleles was not significantly different between cases and controls (P=0.420, OR=1.069, 95% CI: 0.909–1.256) (Table 2). However, in a recessive genetic model, CC+CT genotypes significantly decreased the risk of glioma when compared with TT (P=0.023, OR=0.774, 95% CI: 0.621–0.966). The evaluation of different populations in the stratified analysis showed that the T allele of this variant was associated with an increased risk in the male population (P=0.045, OR=1.243, 95% CI: 1.004–1.539) (Table 3).

Association between haplotypes and the risk of gliomas

Finally, we analyzed the haplotypes of these two SNPs. The estimated haplotype frequencies suggested that these two loci had high linkage disequilibrium (D′=0.9915). All four possible haplotypes were identified and the rarest haplotype, −155_156GG/−443C, was present only in patients, and haplotype −155_156GG/−443T, which combined two increasing risk alleles, displayed a significantly higher representation in cases (P=0.029, OR=1.192, 95% CI: 1.018–1.395) (Table 2). Because the rarest haplotype included an increasing risk allele, −155_156G, and a decreasing risk allele, −443C/T, the influence on the risk of glioma should be neutral, except if an unknown interaction between these two polymorphisms exists.

Promoter activity assay

The location of these two glioma-associated polymorphisms in the immediate 500 bp at the 5′ end of the *SPP1* gene prompted us to investigate their effect in promoter activity. DNA fragments from −645 to −2 of the *SPP1* promoter corresponding to the four haplotypes were inserted in pGL3 expression plasmids upstream of the luciferase reporter gene. Transient transfection experiments were conducted in glioma cell lines U373 and HEK293.

Figure 1 shows that in both cell lines haplotypes −155_156GG/−443T and −155_156GG/−443C showed higher promoter activity compared with haplotypes −155_156G/−443T and −155_156G/−443C, which indicated that *SPP1* promoter activation might depend on −155_156insG polymorphism. The transcriptional activity of the *SPP1* promoter containing the −155_156GG allele significantly increased by 1.3-fold compared with other haplotypes in glioma cells, whereas it slightly increased by 0.3-fold in HEK293 cells.

DISCUSSION

In this study, we conducted a case–control association analysis in a Chinese population and the results revealed that both the −155_156insG and -443C/T variants influenced susceptibility to

Table 2 Analysis on association between *SPP1* polymorphisms or haplotypes and risk of glioma in overall population

Polymorphisms or haplotypes	Allele or genotype	Case (%)	Control (%)	P-value[a]	Adjusted OR[b]	95% CI[b] Lower	Upper
−155_156insG	G	785 (59.1)	852 (63.7)		1		
	GG	543 (40.9)	486 (36.3)	0.020	1.202	1.028	1.408
	G/G	220 (33.1)	273 (40.8)		1.000		
	G/GG	345 (52.0)	306 (45.7)	0.005	1.399	1.106	1.770
	GG/GG	99 (14.9)	90 (13.5)	0.070	1.365	0.975	1.910
	G/GG + GG/GG vs G/G			0.006	1.372	1.095	1.719
	GG/GG vs G/G + G/GG			0.503	1.112	0.815	1.516
−443C/T	C	437 (32.8)	465 (34.6)		1.000		
	T	897 (67.2)	879 (65.4)	0.420	1.069	0.909	1.256
	CC	69 (10.3)	77 (11.5)		1.000		
	CT	299 (44.8)	311 (46.3)	0.814	1.045	0.726	1.503
	TT	299 (44.8)	284 (42.3)	0.508	1.131	0.785	1.631
	CT + TT vs CC			0.640	1.086	0.768	1.536
	CC + CT vs TT			0.023	0.774	0.621	0.966
Haplotypes	−155_156G/-443C	297 (22.5)	311 (23.3)	0.615	0.954	0.796	1.145
	−155_156G/-443T	483 (36.5)	535 (40.2)	0.066	0.863	0.737	1.010
	−155_156GG/-443C	2 (0.1)	0 (0)	0.999	NA	NA	NA
	−155_156GG/-443T	540 (40.8)	486 (36.5)	0.029	1.192	1.018	1.395

Abbreviations: 95% CI, 95% confident intervals; NA, not applicable; OR, odds ratios.
[a]P-values from unconditional logistic regression analyses.
[b]Adjusted for age and gender.

Table 3 Analysis on association between *SPP1* polymorphisms and risk of glioma in stratified population

Variable	Polymorphisms	Comparisons of alleles	P-value[a]	Adjusted OR[b]	95% CI[b] Lower	Upper
Male	−155_156insG	GG vs G	0.016	1.286	1.048	1.578
	−443C/T	T vs C	0.045	1.243	1.005	1.539
Female	−155_156insG	GG vs G	0.501	1.088	0.850	1.393
	−443C/T	T vs C	0.296	0.877	0.685	1.122
Childhood glioma	−155_156insG	GG vs G	0.400	1.248	0.745	2.089
	−443C/T	T vs C	0.510	0.838	0.497	1.416
Adult glioma	−155_156insG	GG vs G	0.032	1.197	1.016	1.411
	−443C/T	T vs C	0.262	1.102	0.930	1.305
Never smoking	−155_156insG	GG vs G	0.166	1.140	0.947	1.372
	−443C/T	T vs C	0.702	0.964	0.797	1.165
Ever smoking	−155_156insG	GG vs G	0.067	1.324	0.980	1.789
	−443C/T	T vs C	0.060	1.348	0.988	1.839
With family history of cancer	−155_156insG	GG vs G	0.997	1.001	0.627	1.599
	−443C/T	T vs C	0.883	1.036	0.646	1.661
Without family history of cancer	−155_156insG	GG vs G	0.018	1.224	1.035	1.448
	−443C/T	T vs C	0.445	1.070	0.900	1.271
WHOI	−155_156insG	GG vs G	0.117	1.335	0.930	1.916
	−443C/T	T vs C	0.596	1.108	0.759	1.617
WHOII	−155_156insG	GG vs G	0.110	1.194	0.960	1.656
	−443C/T	T vs C	0.191	1.165	0.927	1.463
WHOIII	−155_156insG	GG vs G	0.132	1.245	0.936	1.599
	−443C/T	T vs C	0.790	0.961	0.718	1.287
WHOIV	−155_156insG	GG vs G	0.016	1.286	1.048	1.578
	−443C/T	T vs C	0.760	1.035	0.828	1.294

Abbreviations: 95% CI, 95% confident intervals; OR, odds ratios.
[a]P-values from unconditional logistic regression analyses.
[b]Adjusted for age and gender.
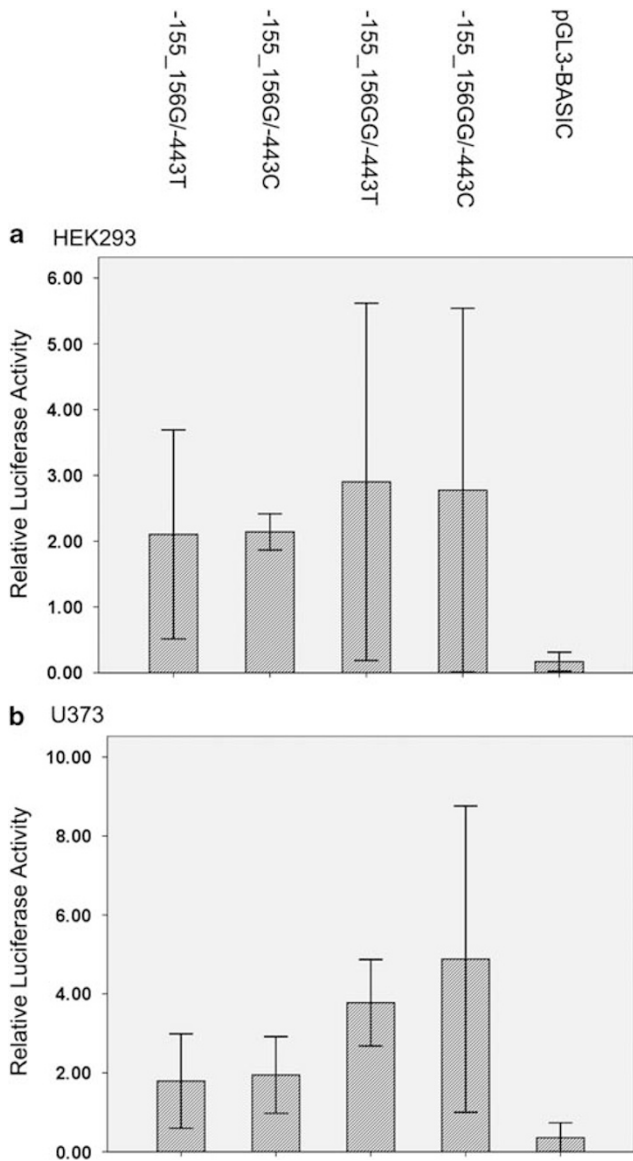
Figure 1 Transcriptional activity of four SNP haplotypes in HEK293 cells (**a**) or in U373 cells (**b**). *SPP1* promoter fragments representing the haplotypes of SNPs −443C/T and −155_156insG were cloned into the pGL3-basic vector. The relative *Firefly* luciferase activities, standardized to *Renilla* luciferase, were reported as the mean of triplicate samples (results are means ± s.e.m.). **P<0.01, Student's *t*-test.

glioma. As the coding gene of OPN, it was reported that *SPP1* was highly expressed in almost all kinds of tumors examined, including gliomas.[7,8,21] In normal tissues, OPN is produced mainly by epithelial cells,[22] but is not found in other types of cells, including astrocytes. The fact that OPN is expressed in glioma cells and negligibly expressed in normal astrocytes suggested that its expression in tumor cells might alter the malignant properties.[21] Therefore, upregulation of *SPP1* transcription was important during glioma genesis. In our study population, the familial clustering of gliomas revealed that this disease is inherited to some extent. Considering that genetic variants might be implicated in the development of gliomas,[23] the polymorphisms in the *SPP1* promoter region were reasonable candidates to be regarded as potential markers of glioma susceptibility.

Many transcription factors have been shown to be directly implicated in *SPP1* transcription, including progesterone, glucocorticoids, 1a25-dihydroxyvitamin D3 and basic helix-loop-helix proteins.[10,24] According to previous reports, three functional SNPs, −66T/G (rs28357094), −155_156insG (rs17524488) and −443C/T (rs11730582), might influence the binding affinity of transcription factors. The sequences around these polymorphisms constitute the binding motifs of SP1, CBFA1/RUNX2 and MYT1, respectively.[15] SP1 is a human transcription factor necessary for basal transcription, especially in the early development of an organism.[25,26] Both RUNX2 and MYT1 are tumor-related transcription factors. RUNX2 is essential for skeletal development and has oncogenic potential.[27] In addition to the binding site around the −155_156insG SNP in the *SPP1* promoter region, another highly conserved RUNX2-binding site was found 14 bp downstream of the former polymorphism, which indicated an important role of RUNX2 in *SPP1* transcription. Myt1 is a key regulator of the G(2) cell cycle checkpoint and the localization of MYT1 in brain tumor regions suggested an association of MYT1 with cell proliferation.[28]

According to the previous report, RUNX2 factor was shown to bind better to the −155_156GG allele than to the −155_156G allele.[15] In addition, the T to C substitution in the −443C/T SNP results in a decreased binding affinity of the MYT1 transcription factor.[15] The results of our epidemiological study were consistent with those from the above-mentioned research. The haplotype −155_156GG/−443T displayed significantly greater representation in cases. In addition, our cellular experiments revealed that haplotypes with the −155_156GG allele yielded higher *SPP1* transcription activity in glioma cells. We postulated that the possible pattern of *SPP1* promoter polymorphisms influencing glioma risk was as follows: In the beginning of glioma genesis, several transcription factors, especially RUNX2, were overexpressed or activated. These proteins bound to the promoter region of *SPP1* and enhanced its expression level, finally altering tumorigenesis. Individual genotypes of the *SPP1* promoter displayed different regulatory efficiencies by transcription factors including RUNX2, resulting in diverse susceptibilities for glioma.

Another finding from our results was that all distributions of the three SNPs we investigated differed from those of former reports focusing on a Caucasian population. For the −66T/G SNP, we did not find the G allele in our population. The GG allele of the −155_156insG variant and the T allele of the −443C/T variant were represented at a much higher frequency in Chinese individuals. The difference in genotype distributions might account for the different morbidity of glioma observed in different populations.

OPN encoded by *SPP1* belongs to the small integrin-binding ligand N-linked glycoproteins (SIBLINGs) family and was reported to be an important factor in many stages of cancer progression.[21] Because of its detection in various human cancers and the demonstration of its key functional roles during malignant transformation, invasion and metastasis, OPN was a protein that could potentially serve as a diagnostic and prognostic tool, and also as a new therapeutic target. Moreover, our investigation suggested that the genetic variants of the *SPP1* promoter also had the potential to be clinical biomarkers for distinguishing unique subsets of persons at higher risk of developing gliomas. Furthermore, the relationship between prognosis of glioma and *SPP1* polymorphisms needed further research. However, our results did not identify obvious differences in the influence of the variants between study groups stratified by WHO grades.

Our study has several limitations. First, the sample size of our study may not be large enough to draw a final conclusion. Second, all samples were collected from a Chinese population. There may be

different results in different ethnic groups. Finally, although we postulated as to why *SPP1* promoter SNPs influenced glioma risk, it should be confirmed by further experiments in the future.

In conclusion, we investigated the association between functional polymorphisms in the promoter of *SPP1* and glioma risk. Data analysis provided support for an association between *SPP1* promoter polymorphisms and glioma risk in a Chinese population. For the −155_156insG variant, the −155_156GG allele was found to be significantly associated with an increased risk of glioma. Alternatively, the study of SNP −443C/T in a recessive genetic model revealed that CC+CT significantly decreased the risk of glioma compared with TT. In addition, during the analysis of haplotypes, −155_156GG/−443T was represented at a higher frequency in cases. All these results indicated that variants of the *SPP1* promoter might influence the risk of glioma by regulating promoter activity. The transcriptional activity of *SPP1* promoter containing the −155_156GG allele significantly increased in glioma cells. However, on the basis of the limitations of our investigation, our results should be viewed with caution. Further analyses are necessary to confirm our observation about *SPP1* polymorphisms and glioma risk in larger samples or in other ethnic groups.

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