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ORIGINAL RESEARCH

Serpin peptidase inhibitor, clade A member 3 (SERPINA3), is overexpressed in glioma and associated with poor prognosis in glioma patients

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Abstract: Glioma is the most common and aggressive human primary tumor in the central nervous system. Despite present clinical advancements, median survival time remains poor in this malignant tumor. Serpin peptidase inhibitor, clade A member 3 (SERPINA3), is a member of the serpin superfamily of protease inhibitors. Its aberrant expression has been observed in various tumors. However, its clinical significance and biological function in glioma remain unclear, especially for the prognosis of glioma patients. In this study, we investigated SERPINA3 expression in glioma tissue samples and its significance in predicting the prognosis of glioma patients. SERPINA3 protein expression was studied by immunohistochemistry, while real-time polymerase chain reaction was used to study SERPINA3 mRNA expression. We found that SERPINA3 was upregulated in glioma tissue at both mRNA and protein levels, compared with noncancerous brain tissues. We also found that high SERPINA3 expression in glioma tissues correlated significantly with advanced World Health Organization grade. Univariate and multivariate analyses revealed that high SERPINA3 expression was an independent prognostic factor for poor overall survival of glioma patients. Moreover, our findings were further validated by online Oncomine database. Taken together, our results suggest that SERPINA3 plays an oncogenic role in glioma progression and provide an insight into the application of SERPINA3 as a novel predictor of clinical outcomes and a potential biomarker of glioma.

Keywords: SERPINA3, immunohistochemistry, glioblastoma, prognosis, glioma

Introduction

Glioma is one of the most lethal kinds of cancer and the most common and aggressive human primary tumor in the central nervous system.[1,2] According to the World Health Organization (WHO) classification, glioma can be classified into four grades: pilocytic astrocytoma (grade I), diffuse astrocytoma (grade II), anaplastic astrocytoma (grade III) and glioblastoma multiform (grade IV).[3] Despite the great advancement in treatment modalities, especially the availability of radiotherapy and chemotherapy, the clinical outcome remains poor.[4,5] The high incidence and mortality prompt us to search for new therapeutic strategies. Identifying novel molecular biomarkers for glioma could be helpful for better understanding of the carcinogenesis mechanisms and for developing patient-specific treatments as well as improving the prognosis of glioma patients.

Serpin peptidase inhibitor, clade A member 3 (SERPINA3), is a member of the serpin superfamily of protease inhibitors and was previously known as α1-antichymotrypsin. SERPINA3 inhibits the activation of several serine proteases including chymotrypsin and cathepsin G. Also, SERPINA3 plays an important role in

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cytokinesis, proliferation, apoptosis and tumor metastasis.[6,7] Moreover, SERPINA3 overexpression is documented in several cancer types, including melanoma,[8] endometrial cancer,[9] lung cancer,[10] colon cancer,[11] breast cancer[12,13] and hepatocellular carcinoma.[6] Intriguingly, the overexpression of SERPINA3 has been demonstrated to have a significant positive relationship with poor prognosis in patients with gastric cancer,[14] ovarian cancer,[15] lung cancer[16] and acute leukemia.[17] Furthermore, SERPINA3 promoted endometrial carcinoma cells proliferation by regulating G2/M checkpoint in the cell cycle, and inhibited cell apoptosis and activation of MAPK/ERK1/2 and PI3K/AKT signaling.[9] The results suggested SERPINA3 as a novel predictor of clinical outcomes and a potential therapeutic target of endometrial carcinoma. Recent reports showed that high SERPINA3 expression was a marker of poor prognosis in ovarian cancer.[15] SERPINA3 expression was upregulated in recurrent ovarian cancer, which may play an important role in the progression and chemoresistance. In the study of brain diseases,[18] α1-antichymotrypsin has been suggested to be involved in the etiology of Alzheimer's disease, and the study showed that activation of NF-KB may be one possible cause of increased α1-antichymotrypsin levels in Alzheimer's disease. It is noteworthy that the overexpression of dominant-negative c-jun upregulated α1-antichymotrypsin expression in glioma cells.[19] However, few studies have investigated the expression and significance of SERPINA3 in glioma tissue samples, especially in the prognosis of glioma patients.

In our study, we characterized SERPINA3 expression in clinical glioma tissue samples using immunohistochemistry (IHC) and real-time polymerase chain reaction (RT-PCR) and assessed the relationship of SERPINA3 expression with the clinicopathologic features of glioma patients. In addition, we investigated SERPINA3 expression in all 2007 WHO glioma grades. We found that the SERPINA3 expression level was positively correlated with the histologic staging. Also, we demonstrated through univariate and multivariate analyses that SERPINA3 overexpression in gliomas can indicate poor clinical prognosis. Our findings were further validated in online Oncomine database (https://www.oncomine.org/). To our knowledge, our study suggests that SERPINA3 overexpression plays an important role in glioma grade. Our data not only indicate that SERPINA3 is an independent prognostic factor of glioma patients, but also provide evidence for the potency of SERPINA3 for being a potential therapeutic target of glioma.

Materials and methods

Patients and tissue specimens

In our study, 180 paraffin-embedded glioma tissue specimens were retrieved from Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University from March 2005 to May 2011 for IHC. None of the patients had received radiotherapy or chemotherapy before surgery. The histologic tumor grade was determined according to the 2007 WHO classification system. Overall survival (OS) was calculated as the time from the date of diagnosis to the date of death or the date of the last follow-up (if death did not occur). In addition, 80 fresh glioma tissue specimens and 10 nontumorous brain tissue specimens were obtained from Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University from March 2013 to October 2016. Ten normal brain tissue specimens were taken from patients who underwent surgical operation for cerebral hemorrhage and cerebral trauma. All tissue samples were obtained with informed consent, and all procedures were performed in accordance with the Internal Review and the Ethics Board of the Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University. The ethics committee of Sun Yat-Sen Memorial Hospital also approved this research. The Karnofsky performance score (KPS) was evaluated as previously described.[20] Detailed clinicopathologic characteristics of all the patients are presented in Table 1.

Table 1 Relationship between SERPINA3 levels and various clinicopathologic characteristics in 180 glioma specimens

Variables	Number of cases (N=180)	SERPINA3 expression		χ^2	*P*-value[a]
		Low (n=73)	High (n=107)		
Age (years)				0.0014	0.9704
≤50	86	35	51		
>50	94	38	56		
Gender				3.804	0.0511
Female	78	38	40		
Male	102	35	67		
Tumor size (cm)				49.91	<0.0001
≤5	72	52	20		
>5	108	21	87		
Karnofsky score				36.98	<0.0001
≤90	112	26	86		
>90	68	47	21		
WHO grade				70.88	<0.0001
I + II	65	53	12		
III + IV	115	20	95		

Note: [a]Chi-square test.

Abbreviations: I, WHO grade I pilocytic astrocytoma; II, WHO grade II diffuse astrocytoma; III, WHO grade III anaplastic astrocytoma; IV, WHO grade IV glioblastoma multiforme; WHO, World Health Organization; SERPINA3, Serpin peptidase inhibitor, clade A member 3.

IHC staining of tissue specimens

SERPINA3 protein expression was analyzed by IHC as described in previously published papers.[21] Briefly, paraffin-embedded specimens were cut into 4 μm sections and baked at 65°C for 30 min. The sections were deparaffinized with xylene and rehydrated. The sections were submerged in ethylenediaminetetraacetic acid antigenic retrieval buffer and microwaved for antigenic retrieval. They were treated with 3% hydrogen peroxide in methanol to quench the endogenous peroxidase activity, followed by incubation with 1% bovine serum albumin to block the nonspecific binding. Mouse anti-SERPINA3 (1:200 dilution; Abcam) was incubated with the sections overnight at 4°C. For negative controls, the primary antibody was replaced by normal goat serum. After washing, the tissue sections were treated with biotinylated anti-mouse secondary antibody for 60 min at room temperature. After rinsing with phosphate-buffered saline (PBS), the slides were immersed for 10 min in 3,3′-diaminobenzidine (Sigma, St Louis, MO, USA) solution (0.4 mg/mL, with 0.003% hydrogen peroxide) and then monitored under the microscope. The reaction was terminated with distilled water. Slides were then counterstained with hematoxylin, dehydrated and coverslipped.

Scoring of staining

The degree of immunostaining of tissue specimens was scored separately by two independent investigators, who were blinded to the histopathologic features and the patient data of the samples, and the scores were determined by combining the proportion of positively stained tumor cells and the intensity of staining.[22] Scores given by the two independent investigators were averaged for further comparative evaluation of the SERPINA3 expression. The proportion of positive stained tumor cells was graded as follows: 0 (no positive tumor cells), 1 (<10% positive tumor cells), 2 (10%–50% positive tumor cells) and 3 (>50% positive tumor cells). The cells at each intensity of staining were recorded on a scale of 0 (no staining), 1 (weak staining, light yellow), 2 (moderate staining, yellowish brown) and 3 (strong staining, brown). The staining index (SI) was calculated as follows: SI = staining intensity × proportion of positively stained tumor cells. Using this method of assessment, we evaluated SERPINA3 expression in glioma tissue specimens by SI (scored as 0, 1, 2, 3, 4, 6 or 9). Cutoff values to define the high and low expression of SERPINA3 were chosen based on a measure of heterogeneity with the log-rank test statistics with respect to OS. SERPINA3 staining was quantified using a two-level grade system as follows: 0–3 indicates low expression and 4–9 indicates high expression.

IHC staining for SERPINA3 protein expression in the tumor and nontumorous brain tissue specimens was quantitatively analyzed with the AxioVision Rel. 4.6 computerized image analysis system assisted with the automatic measurement program (Carl Zeiss, Oberkochen, Germany). Briefly, to assess the mean optical density (MOD), the stained sections were evaluated at 200× magnification, and five representative staining fields of each section were analyzed to verify the mean absorbance, which represents the strength of staining signals as measured per positive pixel.

RNA preparation and quantitative RT-PCR

Total RNA was extracted from the tissues using Trizol reagent (Takara, Dalian, People's Republic of China) and reverse transcribed by PrimeScript RT reagent kit (Takara) according to the manufacturer's instruction. Quantitative RT-PCR (qRT-PCR) was subsequently performed with SYBR Premix Ex Taq (Takara) using a 7500 real-time PCR system (Applied Biosystems). Sequences of the primers are as follows: SERPINA3 forward primer 5′-CTTCACCAGCAAGGCTGACC-3′, SERPINA3 reverse primer 5′-GCACAGCCTTATGGACCACC-3′, GAPDH forward primer 5′-AAGATCATCAGCAATGCCTCC-3′ and GAPDH reverse primer 5′-TGGACTGTGGTCATGA GTCCTT-3′. PCR reactions were performed under the following conditions: pre-denaturation at 94°C for 7 min, denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, elongation at 72°C for 1 min and elongation at 72°C for 10 min. Expression data were normalized to the housekeeping gene GAPDH as a loading control.

Statistical analysis

All data were analyzed using SPSS17.0 statistical package (SPSS Inc., Chicago, IL, USA) or GraphPad Prism software 5.0 (GraphPad Software, San Diego, CA, USA). Associations between clinicopathologic parameters with SERPINA3 expression were identified using chi-square test. OS was evaluated by the Kaplan–Meier analysis and the difference between groups was assessed by log-rank test, while the prognostic significance of clinical and pathologic characteristics was determined using univariate Cox regression analysis. Continuous data were compared using Student's *t*-test. All statistical tests were two-tailed. Errors were standard

deviation (SD) of averaged results, and *P*-values <0.05 were accepted as significant differences.

Results

SERPINA3 expression is correlated with clinicopathologic features of patients with glioma

To determine the significance of the increased SERPINA3 expression in glioma, we analyzed the relationship between SERPINA3 expression levels and the clinicopathologic features of glioma tumor patients (Table 1). We confirmed SERPINA3 protein expression level in 180 glioma tissue specimens using IHC. In the 180 glioma patients, there were 78 women (43.33%) and 102 men (56.67%) with age ranging from 11 to 81 years (median 52.5 years), including 22 cases of grade I, 43 cases of grade II, 49 cases of grade III and 66 cases of grade IV astrocytomas according to the 2007 WHO classification. We observed that 59.44% (107/180) tissue samples showed high SERPINA3 expression and 40.56% (73/180) showed low expression. The diameter of most tumors was >5 cm, the proportion of which was 80.56% (87/108) in the high SERPINA3 expression group and 19.44% (21/108) in the low SERPINA3 expression group. We also evaluated the KPS for all of the patients before surgery. High SERPINA3 expression levels were found to be significantly associated with large tumor size (>5 cm; $P<0.0001$), low KPS score (<90; $P<0.0001$) and high WHO grade ($P<0.0001$). There were no significant correlations between SERPINA3 expression levels and age and gender.

Correlation between SERPINA3 expression and glioma grade

To determine whether SERPINA3 expression level was associated with glioma grade, all 180 glioma tissue specimens and 10 nontumorous brain tissues were analyzed by IHC experiments. Representative images of SERPINA3 staining in glioma patients and nontumorous brain tissues are shown in Figure 1A. Positive expression of SERPINA3 mainly existed in the cytoplasm of glioma cells. In contrast, SERPINA3 was hardly detectable in the normal brain tissue. Next, we summarized the MOD of SERPINA3 staining among ten nontumorous brain tissues and glioma tissue specimens of all grades. The MOD of SERPINA3 staining increased in a stepwise manner from lower grade to higher grade ($P<0.05$; Figure 1B). Furthermore, we measured the expression of SERPINA3 mRNA by qRT-PCR analysis in 80 glioma tissue specimens and 10 noncancerous brain tissue specimens. Compared with noncancerous brain tissues, the expression levels of SERPINA3 mRNA were markedly upregulated in all grades of glioma tissue specimens. Notably, the expression

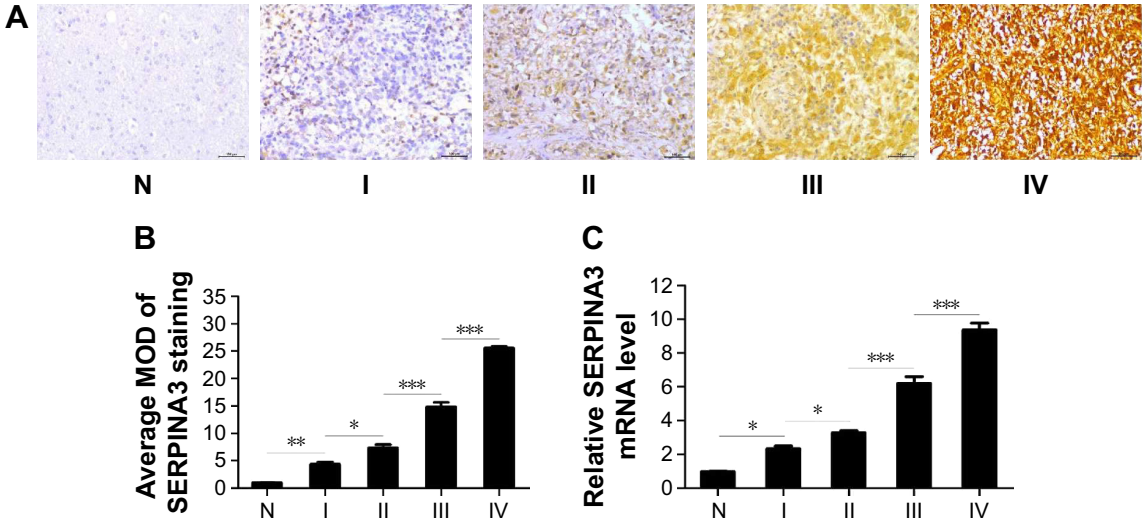

Figure 1 High expression of SERPINA3 in glioma tissues.

Notes: (**A**) Paraffin-embedded specimens of a total of 10 nontumorous brain tissues and 180 glioma specimens including WHO grade I to IV glioma samples were stained by immunohistochemistry using an anti-SERPINA3 antibody. (magnification ×200; scale bars, 100 μm). (**B**) Comparative quantification of the MOD of SERPINA3 staining among nontumorous brain tissues and glioma specimens of different stages. The expression levels of SERPINA3 in grades I, II, III and IV glioma increased in a stepwise manner. (**C**) qRT-PCR analysis of SERPINA3 mRNA levels in a total of 90 tissue samples (10 nontumorous brain tissues, 10 GI, 22 GII, 18 GIII and 30 GIV glioma tissue samples). Increased levels of transcripts in higher grade glioma tissue samples were significant when compared with lower grade and nontumorous brain tissue samples. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Abbreviations: N, nontumorous brain tissues; MOD, mean optical density; I, WHO grade I pilocytic astrocytoma; II, WHO grade II diffuse astrocytoma; III, WHO grade III anaplastic astrocytomas; IV, WHO grade IV glioblastoma multiforme; qRT-PCR, quantitative real-time polymerase chain reaction; SERPINA3, Serpin peptidase inhibitor, clade A member 3.

levels of SERPINA3 mRNA also increased from lower grade to higher grade in all gliomas ($P<0.05$; Figure 1C). This result indicates that SERPINA3 could play a critical role in glioma initiation and progression process.

Positive expression of SERPINA3 is associated with poor prognosis of glioma patients

To evaluate the association of SERPINA3 protein expression with glioma patients' prognosis, the expression pattern was categorized into two groups: low SERPINA3 expression and high SERPINA3 expression. This categorization was based on positively stained tumor cells and the intensity of staining. The prognostic significance of SERPINA3 levels in patients with glioma was investigated using a Kaplan–Meier survival analysis, which showed that patients with high SERPINA3 levels had a significantly shorter OS than those with low SERPINA3 levels ($P<0.0001$; Figure 2A). In addition, the median survival time of patients whose tumors showed high SERPINA3 expression levels was only 21 months (hazard ratio [HR]=0.3687; 95% confidence interval [CI]: 0.2637–0.5154), whereas the median survival time of those with low SERPINA3 expression levels was 44 months (HR=2.095; 95% CI: 1.383–2.808). Also, the cumulative 5-year survival rate was 31.51% (23/73) in the low SERPINA3 expression group, whereas it was only 5.61% (6/107) in the high SERPINA3 expression group. Next, we further examined the prognostic value of SERPINA3 expression in different subgroups of glioma patients. We found that high SERPINA3 expression correlated significantly with shorter OS time in glioma WHO grade subgroups. Significant differences were observed wherein patients with high SERPINA3 expression had significantly shorter OS than the ones with low SERPINA3 expression in either grade I+II subgroup (n=65, $\chi^2=7.071$, $P=0.0078$) or grade III+IV subgroup (n=115, $\chi^2=16.47$, $P<0.0001$; Figure 2B and C). In addition, univariate and multivariate analyses were performed for 180 cases of glioma patients to determine the relative risk of prognostic parameters (Table 2). The results indicated that the SERPINA3 expression level, tumor size and WHO grade were independent prognostic factors for poor OS in glioma patients. Taken together, SERPINA3 protein overexpression is associated with poor prognosis in glioma clinical cases and appears to be a potential therapeutic target.

To further validate these findings, we searched the Oncomine database for SERPINA3 expression in human glioma patients (Table 3). Eight datasets show that SERPINA3 protein expression is higher in glioma patients tissues compared with that in normal noncancerous tissue. Two datasets show a significant association between SERPINA3 protein expression and glioma WHO grade. Also, there is an important difference between SERPINA3 overexpression and patient treatment response in one dataset. Similarly, SERPINA3 overexpression is significantly associated with EGFR gene amplification and loss of heterozygosity Chromosome. Not surprisingly, high SERPINA3 expression is significantly associated with recurrence and short survival time. In conclusion, the results show that SERPINA3 overexpression plays an important role in glioma growth and progression, indicating a potential therapeutic strategy for personalized medicine by targeting SERPINA3 in glioma patients.

Discussion

Malignant glioma is one of the most aggressive cancers, and the overall prognosis for glioma patients remains poor.[4,5,23–25]

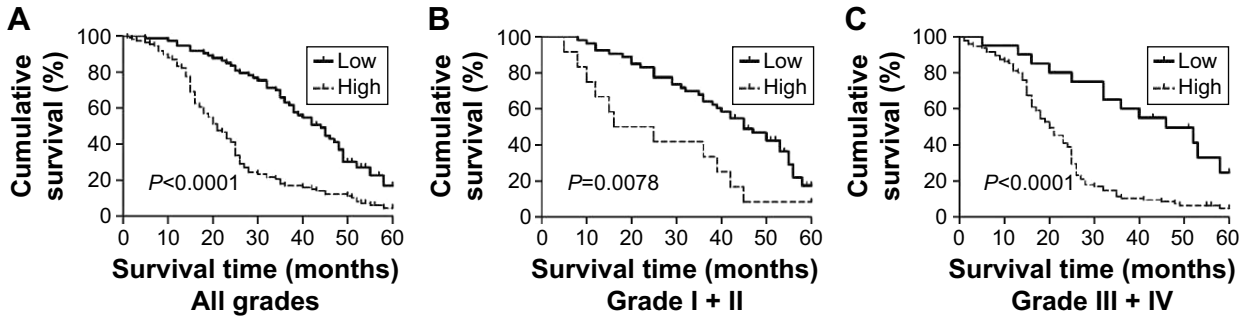

Figure 2 Kaplan–Meier curves with log-rank test for patients with low SERPINA3 expression (bold line) versus high SERPINA3 expression tumors (dotted line).
Notes: (**A**) High SERPINA3 expression was markedly associated with a reduced overall survival in all grades glioma patients ($P<0.0001$). (**B, C**) The overall survival of glioma patients with high SERPINA3 expression was significantly decreased than those with low SERPINA3 expression in either grade I + II subgroup ($P=0.0078$) or grade III + IV subgroup ($P<0.0001$).
Abbreviations: I, WHO grade I pilocytic astrocytoma; II, WHO grade II diffuse astrocytoma; III, WHO grade III anaplastic astrocytomas; IV, WHO grade IV glioblastoma multiforme; SERPINA3, Serpin peptidase inhibitor, clade A member 3.

Table 2 Univariate and multivariate analyses of prognostic parameters for survival in patients with glioma

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value[a]
Age (years)						
≤50 vs >50	0.839	0.512–3.368	0.446			
Gender						
Female vs male	0.985	0.637–3.521	0.535			
Tumor size (cm)						
≤5 vs >5	0.512	0.338–0.798	<0.001*	0.538	0.349–0.845	<0.001*
SERPINA3 level						
Low vs high	2.158	1.381–3.224	<0.0001*	2.269	1.487–3.478	<0.0001*
WHO grade						
I + II vs III + IV	1.858	1.349–3.378	<0.0001*	2.289	1.456–3.527	<0.0001*

Notes: [a]Log-rank test. *$P<0.05$

Abbreviations: I, WHO grade I pilocytic astrocytoma; II, WHO grade II diffuse astrocytoma; III, WHO grade III anaplastic astrocytoma; IV, WHO grade IV glioblastoma multiforme; CI, confidence interval; HR, hazard ratio; WHO, World Health Organization.

Therefore, identifying the mechanism that is responsible for glioma progression may provide insights into the design of effective and therapeutic strategies for glioma.[26,27] The main finding in our study is that the progression of human glioma is associated with upregulation of SERPINA3 expression. At first, we found that high SERPINA3 expression levels were significantly associated with large tumor size (>5 cm; $P<0.0001$), low KPS score (<90; $P<0.0001$) and high WHO grade ($P<0.0001$). Moreover, we demonstrated the expression format and prognostic importance of SERPINA3 expression in all grades of human glioma tissue samples and nontumorous brain tissues using IHC and RT-PCR. IHC analysis suggested SERPINA3 protein overexpression in glioma tissue samples as compared with nontumorous brain tissues. The score of SERPINA3 staining increased in a stepwise manner from lower to higher grade glioma tissues. Furthermore, the expression levels of SERPINA3 mRNA also increased from lower grade to higher grades of glioma ($P<0.05$). Next, using Kaplan–Meier survival analysis, we investigated the prognostic significance of SERPINA3 levels in glioma patients. Our results showed that patients with high SERPINA3 levels had a significantly shorter OS than those with low SERPINA3 levels ($P<0.0001$). In addition, the cumulative 5-year survival rate was 31.51% (23/73) in the low SERPINA3 expression group, whereas it was only 5.61% (6/107) in the high SERPINA3 expression group. Our results obtained using univariate and multivariate analyses indicated that SERPINA3 may act as an independent prognostic factor for the OS of glioma patients.

Consistent with the observations of our study, accumulating evidence has indicated that elevated SERPINA3 protein level is correlated with cancer progression and decreased survival. Previous studies[8] showed overexpression of SERPINA3 in invasive and metastatic melanomas, compared to normal nevi and melanoma-in-situ. Also, knockdown of SERPINA3 was sufficient to decrease melanoma migration and invasion abilities. In melanoma patients, high SERPINA3 expression was strongly associated with worse overall survival and disease-specific survival at 5 years. Multivariate Cox regression analysis showed that SERPINA3 expression was an independent prognostic factor in predicting melanoma patients' clinical outcome. A study also indicated that SERPINA3 expression was significantly upregulated in endometrial cancer samples and was closely correlated with lower differentiation, higher stage, positive lymph node or vascular thrombosis and negative estrogen receptor, indicating a poor prognosis.[9] A previous study suggested that staining for α1-antichymotrypsin and α1-antitrypsin is of little value in the differential diagnosis of neuroepithelial or mesenchymal lesions in the brain.[28] Some studies also found that α1-antichymotrypsin was expressed in human glioblastoma multiforme cells.[29] However, there is no report on whether SERPINA3 expression level is related to glioma patients' prognosis. In our study, using IHC and RT-PCR, we found that SERPINA3 protein level and mRNA level in glioma were higher than in nontumorous brain tissues. Overexpression of SERPINA3 increased from lower grade to higher grade in all glioma cases. High SERPINA3 expression levels were found to be significantly associated with large tumor size (>5 cm), low KPS score (<90) and high WHO grade. The survival time of patients with high SERPINA3 expression level was shorter than that of those with low expression.

Multiple steps are involved in the invasion and metastasis of malignant cells to distant tissues, including cancer cell attachment to ECM, degradation of ECM components and

Table 3 Correlation of SERPINA3 expression and clinical features in glioma in Oncomine online database

Clinical features	*P*-value[a]	Database (case)
Upregulation of SERPINA3 in glioblastoma		
Glioblastoma vs normal	6.28E-20	Bredel Brain 2 (54)
Glioblastoma vs normal	1.03E-5	TCGA Brain (557)
Glioblastoma vs normal	1.71E-9	Sun Brain (180)
Glioblastoma vs normal	0.020	TCGA Brain 2 (1531)
Glioblastoma vs normal	0.003	Shai Brain (42)
Glioblastoma vs normal	0.003	Lee Brain (101)
Upregulation of SERPINA3 in anaplastic astrocytoma		
Anaplastic astrocytoma vs normal	1.70E-4	Sun Brain (180)
Upregulation of SERPINA3 in anaplastic oligoastrocytoma		
Anaplastic oligoastrocytoma vs normal	1.35E-6	Bredel Brain 2 (54)
Anaplastic oligoastrocytoma vs normal	0.033	French Brain (33)
Upregulation of SERPINA3 in anaplastic oligodendroglioma		
Anaplastic oligodendroglioma vs normal	0.028	French Brain (33)
Upregulation of SERPINA3 in astrocytoma		
Astrocytoma vs normal	0.007	Rickman Brain (51)
Upregulation of SERPINA3 in brain glioblastoma		
Brain glioblastoma vs normal	3.53E-5	TCGA Brain (557)
Upregulation of SERPINA3 in diffuse astrocytoma		
Diffuse astrocytoma vs normal	0.002	Sun Brain (180)
Upregulation of SERPINA3 in oligodendroglioma		
Oligodendroglioma vs normal	0.004	Bredel Brain 2 (54)
Oligodendroglioma vs normal	0.019	Sun Brain (180)
Upregulation of SERPINA3 in grade type		
Grade I vs normal	0.007	Rickman Brain (51)
Grade II vs normal	0.019	Sun Brain (180)
Grade III vs normal	1.70E-4	Sun Brain (180)
Grade IV vs normal	1.71E-9	Sun Brain (180)
Correlation of SERPINA3 overexpression and patient treatment response		
PCV response vs no response	<0.05	French Brain (33)
PCV complete response vs partial response	<0.05	French Brain (33)
Temozolomide response vs no response	<0.05	French Brain (33)
Temozolomide complete response vs partial response	<0.05	French Brain (33)
Correlation of SERPINA3 overexpression and molecular subtype		
EGFR amplification vs no loss	<0.05	French Brain (33)
Loss of heterozygosity chromosome 10q vs no	<0.05	French Brain (33)
Loss of heterozygosity chromosome 19q vs no	<0.05	French Brain (33)
Loss of heterozygosity chromosome 1p vs no	<0.05	French Brain (33)
LDHA mutation vs no	<0.05	French Brain (33)
Correlation of SERPINA3 overexpression and recurrence		
Primary occurrence vs no	<0.05	TCGA Brain 2 (1531)
Recurrence vs no	<0.05	TCGA Brain 2 (1531)
SERPINA3 and poor prognosis of glioma		
Dead at 1 year vs alive at 1 year	<0.05	TCGA Brain 2 (1531)
Dead at 1 year vs alive at 1 year	<0.01	Shai Brain (42)
Dead at 1 year vs alive at 1 year	<0.00001	TCGA Brain (557)
Dead at 3 years vs alive at 3 years	<0.01	Shai Brain (42)
Dead at 3 years vs alive at 3 years	<0.00001	TCGA Brain (557)
Dead at 3 years vs alive at 3 years	<0.05	French Brain (33)
Dead at 3 years vs alive at 3 years	<0.05	French Brain (33)
Dead at 5 years vs alive at 5 years	<0.01	Shai Brain (42)
Dead at 5 years vs alive at 5 years	<0.00001	TCGA Brain (557)
Dead at 5 years vs alive at 5 years	<0.05	French Brain (33)
Dead at 5 years vs alive at 5 years	<0.05	French Brain (33)

Note: [a]Statistically significant.

Abbreviations: I, WHO grade I pilocytic astrocytoma; II, WHO grade II diffuse astrocytoma; III, WHO grade III anaplastic astrocytoma; IV, WHO grade IV glioblastoma multiforme; WHO, World Health Organization; PCV, procarbazine, lomustine, and vincristine; SERPINA3, Serpin peptidase inhibitor, clade A member 3; TCGA, The Cancer Genome Atlas.

subsequent infiltration into adjacent normal tissue.[30] The proteases, such as matrix metalloproteinases, are, therefore, considered as a key factor in this process.[31,32] Moreover, protease inhibitors are generally expected to have an anti-malignant role.[33] However, some serine protease inhibitors have been reported to be regulated in many tumors, indicating a potential role in tumor progression.[34] As one of the most abundant protease inhibitors in human plasma, SERPINA3 has shown overexpression in many types of tumor.[8,9,11,12,35,36] In this study, our findings suggested that SERPINA3 was associated with the progression of glioma. Further testing using Kaplan–Meier survival analysis confirmed that significant difference was obtained wherein patients with high SERPINA3 expression had significantly shorter OS than the ones with low SERPINA3 expression in either grade I + II subgroup or grade III + IV subgroup. Cox regression confirmed that the SERPINA3 expression level, tumor size and WHO grade were independent prognostic factors for poor OS in glioma patients.

In Oncomine online database, SERPINA3 overexpression in glioma has already been proven. There was a significant association between SERPINA3 protein expression and glioma WHO grade. Significant difference was observed that SERPINA3 overexpression is significantly associated with patient treatment response in dataset. Consistent with this, SERPINA3 overexpression was significantly associated with EGFR amplification and loss of heterozygosity chromosome. Not surprisingly, high SERPINA3 expression was significantly associated with recurrence and short survival time.

Conclusion

On the basis of these observations, we confirmed that SERPINA3 can serve as a potential predictive biomarker for the prognosis of gliomas and may be a potential therapy target.

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Disclosure

The authors report no conflicts of interest in this work.

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