



Prognostic Value of SERPINA1 as a Biomarker for Poor Survival of Gliomas

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Background: Glioma is a primary brain tumor known for its aggressive behavior and poor prognosis. Alpha-1 antitrypsin (SERPINA1) is a protein with a crucial role in regulating inflammatory processes in the body. Prior research has shown that SERPINA1 enhances the invasive and metastatic capabilities of several cancer types, including lung, breast, and colorectal cancer. Notably, SERPINA1 has also been found to be overexpressed in human gliomas. Considering the poor prognosis and high mortality rate of glioma, there is an urgent demand for more reliable biomarkers to assess its outcome. **Aim:** This study aims to explore the potential role of SERPINA1 in glioma by leveraging the Gene Expression Profiling Interactive Analysis (GEPIA), Chinese Glioma Genome Atlas (CGGA), and Gene Expression Omnibus (GEO) databases to evaluate its viability as a prognostic biomarker for glioma. **Methods:** We analyzed GEPIA, CGGA, and GEO databases to assess SERPINA1 mRNA expression in glioma and its link to overall survival. The CGGA databases were utilized to investigate the interactions between SERPINA1 and proteins such as proteinase 3, elastase neutrophil expressed (ELANE), PRSS3P2, KLK3, and calnexin. **Results:** This study indicated that SERPINA1 mRNA is significantly overexpressed in glioma, with this overexpression strongly linked to poorer overall survival. In addition, analysis at the single-cell level revealed a significantly elevated expression of SERPINA1 in myeloid cells. Evaluation of the CGGA database further showed a strong correlation between SERPINA1 and ELANE expression in glioma. **Conclusion:** Our findings suggest that SERPINA1 is positively associated with poor survival in glioma and may serve as a valuable biomarker for predicting survival outcomes in glioma patients.

Key words: Glioma, SERPINA1, Chinese Glioma Genome Atlas, The Cancer Genome Atlas, Gene Expression Omnibus

INTRODUCTION

Glioma is the most prevalent primary brain tumor type and is characterized by its aggressive growth and resistance to conventional treatment modalities such as surgery, radiation therapy, and chemotherapy.¹ Worldwide, it causes nearly 80% of malignant brain cancers in the world with a high rate of relapse.²⁻⁴ The malignancy of glioma is graded based on its aggressiveness, with high-grade tumors having a poorer prognosis. Patients with glioma often experience more than ten symptoms simultaneously, including fatigue,

depression, and cognitive deficits, and these symptoms can also interact with each other, leading to a poor quality of life.^{2,5} Although epidemiological research has provided significant insights into sociodemographic factors, disease characteristics, and neurocognitive and psychological symptoms that are associated with glioma, there are currently limited diagnostic or prognostic biomarkers for this disease. Given these challenges, the identification of biomarkers associated with poor survival outcomes in glioma is urgently needed.

Received: October 21, 2024; Revised: December 23, 2024;
Accepted: December 29, 2024; Published: April 14, 2025
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How to cite this article: Cheng NJ, Hung HY, Chung TT, Song WS, Hong KT, Hueng DY. Prognostic value of SERPINA1 as a biomarker for poor survival of gliomas. J Med Sci 2025;45:77-85.

SERPINA1, also known as alpha-1 antitrypsin, was first discovered in cerebrospinal fluid of the brain and brain tumors in 1979,⁶⁻⁹ and subsequent research has highlighted its critical role in neuroinflammation, acute inflammatory responses, and neurodegeneration.¹⁰⁻¹³ Although primarily synthesized in the liver, SERPINA1 can also be produced by various other cells, including pulmonary alveolar cells and macrophages. Previous research has indicated that SERPINA1 can enhance invasive and metastatic potential in various cancers, such as lung, breast, thyroid, bladder, and colorectal carcinoma.¹⁴⁻¹⁷ In 2021, Zhang and his colleagues reported that SERPINA1 is a key gene detected in brain metastasis from lung adenocarcinoma.¹⁸ Another group also mentioned that SERPINA1 is a prognostic factor for distant metastasis in colon adenocarcinoma.¹⁹ In addition, overexpression of SERPINA1 has been observed in human gliomas, including pilocytic astrocytoma (a low-grade glioma) and glioblastoma multiforme (GBM) (a high-grade glioma).²⁰⁻²³

SERPINA1 is primarily synthesized in the liver and circulates in the bloodstream, where it inhibits the activity of various proteases, including calnexin (CANX), elastase neutrophil expressed (ELANE), and proteinase 3 (PRTN3). These proteases have been implicated in inflammatory processes and cancer progression, including brain tumors.²¹⁻²⁵ SERPINA1 also plays a role in regulating cytokines and chemokines, which are critical for tumor growth and invasion. Research has shown that SERPINA1 can increase the expression of interleukin-6 and vascular endothelial growth factor, both of which contribute to tumor angiogenesis and progression. However, the specific mechanism by which SERPINA1 regulates these cytokines and chemokines in glioma development requires further investigation.²⁶⁻²⁹ Recently, mounting studies suggest that SERPINA1 may also play a role in autophagy regulation in several types of cancer, including breast and colorectal cancer.^{19,30-35} Most noticeably, SERPINA1 was found to express in the spindle cells and pleomorphic cells from the sarcomatous area of GBM as early as 1990s.^{21,22} In addition, using high-grade glioma cell lines, the expression of SERPINA1 in glioma is observed.²³

To examine the possibility of SERPINA1 to serve as a novel prognostic biomarker for glioma, we have collected the bioinformatical databases to approach the role of SERPINA1 in glioma and reveal the potential proteins closely linked to SERPINA1 function.

MATERIALS AND METHODS

The study was conducted in accordance with the Declaration of Helsinki and was approved by TSGHIRB with E202416050; approval date: 01/01/2025. Informed written consent was waived by the IRB.

Online databases

Previous studies had defined the procedure for analyzing functional genomic databases.³⁶ We have accessed RNA-seq databases, as well as corresponding clinical characteristic data, for both lower grade glioma (LGG) and GBM samples from The Cancer Genome Atlas (TCGA), GTEx data from Gene Expression Profiling Interactive Analysis (GEPIA) (<http://gepia.cancer-pku.cn/index.html>), and Chinese Glioma Genome Atlas (CGGA) databases (<http://www.cgga.org.cn/index.jsp>). Furthermore, two single-cell sequencing datasets from the Gene Expression Omnibus (GEO) database were used: one for IDH wildtype gliomas (GSE131928, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE131928>) and another for IDH mutant (IDHmu) gliomas (GSE89567, <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE89567>).

SERPINA1-related protein function analysis

Protein–protein interaction (PPI) network was obtained using the methodology described by Huang *et al.*,³⁷ and the resulting network was analyzed using the STRING database version 11.5 (<https://string-db.org>) to identify signaling pathways involving SERPINA1-interactive proteins, as per the protocol by Szklarczyk *et al.*³⁸ The resulting PPI network was visualized using Cytoscape software.

Statistical analysis

To compare the expression levels of SERPINA1 between normal and mutated samples, analysis of variance was employed, whereas Kaplan–Meier curves were generated using the log–rank test. Specifically, a Kaplan–Meier survival curve was constructed for low- and high-SERPINA1 expression cohorts in glioma derived from GEPIA, with the median value as the cutoff point for SERPINA1 expression. GraphPad Prism was utilized for generating the figures, and statistical significance was determined at $P < 0.05$. The survival prognosis of SERPINA1 was analyzed using Kaplan–Meier analysis. Log2 (TPM + 1) was used to calculate the logarithm of the transcript count per million. Bioinformatics analyses were performed using R (version 4.1.0, www.r-project.org) along with associated R packages for the GEO database. For single-cell RNA sequencing analysis, we utilized the GSE131928 and GSE89567 datasets, processed, and analyzed through the BBrowser platform.³⁹

RESULTS

The SERPINA1 mRNA was higher in patients with low-grade glioma and glioblastoma multiforme than normal controls

The mRNA expression levels of SERPINA1 in normal

controls, LGG, and GBM samples were analyzed using the GEPIA databases and are shown in Figure 1. The results revealed significantly higher mRNA expression levels of SERPINA1 in both GBM ($n = 163$, $P < 0.0001$) and LGG ($n = 518$, $P < 0.0001$) groups compared to normal controls [Figure 1].

Subsequently, we proceeded to validate our novel findings in an additional database. On analysis of the CGGA mRNAseq693 dataset, we observed a trend indicating that

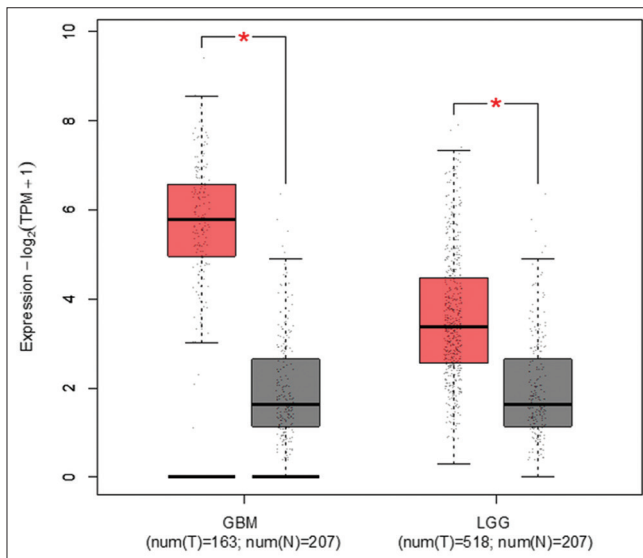


Figure 1: Comparison of mRNA expression levels of SERPINA1 between normal controls and individuals with low-grade glioma and glioblastoma multiforme (GBM). Based on the Cancer Genome Atlas datasets, the mRNA expression levels of SERPINA1 were compared between normal brain controls ($n = 207$) with low-grade gliomas ($n = 518$) and GBM ($n = 163$) patients, respectively. * $P < 0.0001$, statistically significant

higher grades of glioma may correspond to increased levels of SERPINA1 mRNA expression ($P = 6.1e-24$) [Figure 2].

Overexpression of SERPINA1 mRNA was associated with poor survival outcomes

The impact of differential SERPINA1 mRNA expression on survival was evaluated using TCGA and GTEx datasets obtained from GEPIA databases. The analysis revealed that LGG patients with higher SERPINA1 expression had a shorter survival time ($P = 2.7e-6$) [Figure 3a]. In contrast, GBM patients with higher SERPINA1 expression showed a similar survival time ($P = 0.23$) [Figure 3b]. When the data from LGG and GBM patients were combined, those with higher SERPINA1 expression had significantly shorter survival times than those with lower expression ($P < 0.0001$) [Figure 3c].

The impact of SERPINA1 mRNA expression on survival time was further validated among patients with primary glioma, including those with WHO Grade II, III, and IV [Figure 4]. In the primary glioma group, comprising 404 cases, individuals with higher SERPINA1 mRNA expression levels exhibited significantly shorter survival times compared to those with lower SERPINA1 mRNA expression levels ($P < 0.0001$) [Figure 4a]. Similarly, in the WHO grade II group, comprising 130 cases, individuals with higher SERPINA1 mRNA expression levels had significantly shorter survival times compared to those with lower SERPINA1 mRNA expression levels ($P = 0.0093$) [Figure 4b]. In the WHO Grade III group, comprising 141 cases, individuals with higher SERPINA1 mRNA expression levels had significantly shorter survival times compared to those with lower SERPINA1 mRNA expression levels ($P = 0.0470$) [Figure 4c]. Finally, in the WHO Grade IV group, comprising 133 cases, there was

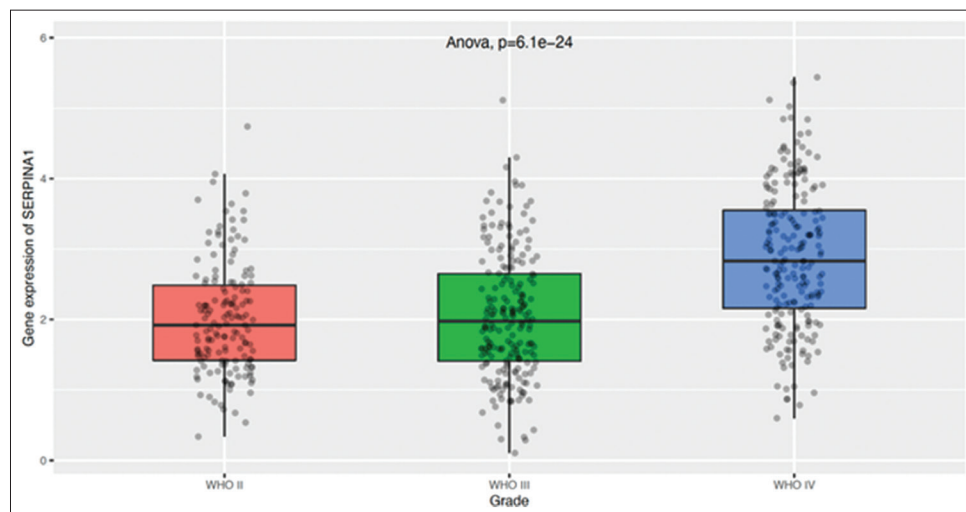


Figure 2: Comparison of mRNA expression levels of SERPINA1 among individuals with Grade II, III, and IV. Based on the Chinese Glioma Genome Atlas datasets, the mRNA expression levels of SERPINA1 were compared among Grade II, Grade III, and Grade IV patients, respectively. * $P < 0.0001$, statistically significant

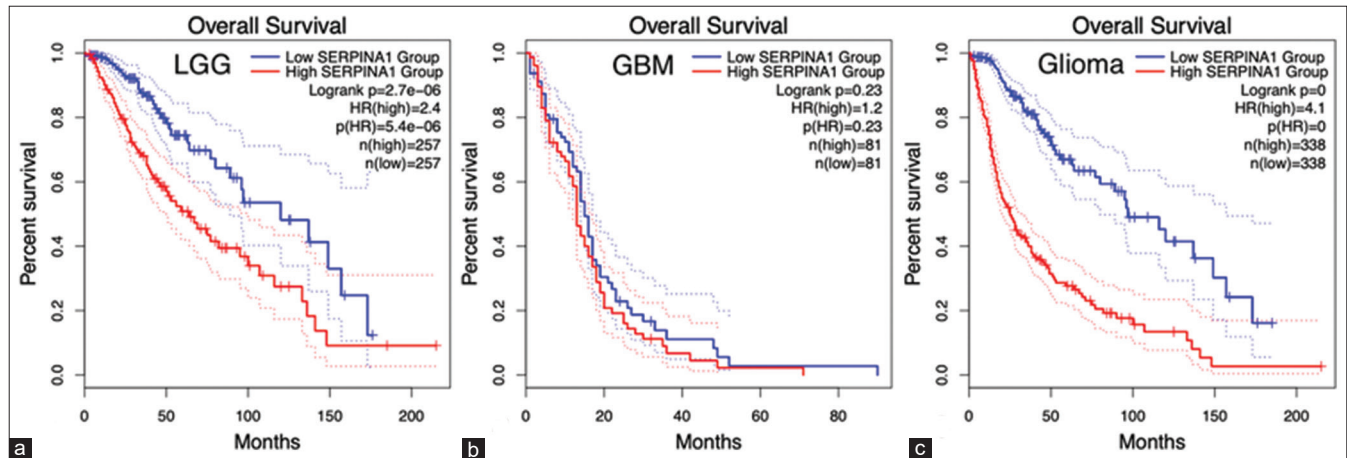


Figure 3: The relationship of the expression of SERPINA1 mRNA with overall survival (OS) time among patients of various glioma stages. Kaplan–Meier curves for comparison of the OS of subgroups in The Cancer Genome Atlas (TCGA) datasets. Kaplan–Meier survival curves were generated to compare OS in the TCGA dataset (lower grade glioma group, $n = 514$; glioblastoma multiforme group, $n = 162$; total glioma group, $n = 676$)

no significant difference in survival time between individuals with higher and lower SERPINA1 mRNA expression levels ($P = 0.49$) [Figure 4d].

Single-cell RNA analysis of SERPINA1 shows significantly higher SERPINA1 expression in myeloid cells than other cell types in IDH wild-type and IDH mutant gliomas

Using single-cell RNA sequencing, we examined SERPINA1 expression patterns in gliomas stratified by IDH mutation status: IDH wild-type (IDHwt) and IDHmu. In IDHwt gliomas [Figure 5a-c], SERPINA1 expression was predominantly localized to the myeloid cell population, indicating its active involvement in tumor-associated myeloid cell dynamics. Similarly, in IDHmu gliomas [Figure 5d-f], SERPINA1 was also expressed in myeloid cells, though at a slightly reduced intensity compared to IDHwt gliomas. Violin plots demonstrated significant enrichment of SERPINA1 expression in myeloid cells compared to other cell types, including T cells, glial cells, and tumor cells ($***P < 0.001$). These findings align with the role of myeloid cells, such as tumor-associated macrophages and microglia, in shaping the tumor microenvironment and driving glioma aggressiveness.

The protein–protein interaction network of SERPINA1 and its close correlation with elastase neutrophil expressed (ELANE) expression

To identify potential interacting candidates with SERPINA1, we constructed a PPI network using the STRING database. The results revealed that SERPINA1 may have close associations with PRTN3, ELANE, PRSS3P2, KLK3, and CANX [Figure 6]. To further analyze the correlation between SERPINA1 and its interacting proteins, we obtained data from

the CGGA databases and presented the results in Figure 5. We found that the correlation between SERPINA1 and CANX and PRTN3 was not statistically significant ($P = 5.4e-01$ and $1.73e-01$, respectively) [Figure 7a and b]. Interestingly, a strong correlation was observed between SERPINA1 and ELANE ($P = 1.26e-11$) [Figure 7c]. However, we observed weak correlations between SERPINA1, PRSS3P2, and KLK3 (data not shown). These results suggest that SERPINA1 may have a specific regulatory relationship with ELANE in glioma, while its association with other interacting proteins may be weaker or absent.

DISCUSSION

Glioma is a primary brain tumor that originates from the supportive cells of the central nervous system, namely the glial cells. This type of tumor is the most commonly occurring brain tumor and is often highly aggressive, leading to significant morbidity and mortality.⁴⁰ The malignancy level of glioma is classified based on its grade, with high-grade tumors having a poorer prognosis and being more aggressive. Despite ongoing research, the etiology of glioma is not yet fully understood, and identifying a practical biomarker could greatly aid in the diagnosis and/or prognosis of this disease.

To overcome the challenge of limited glioma samples, we utilized worldwide-accessible databases to ensure the credibility and representativeness of our study. Specifically, we consulted the GEPIA, CGGA, and GEO databases. First, in the GEPIA database, we observed higher levels of SERPINA1 mRNA expression in both GBM and LGG cases [Figure 1]. Subsequently, we validated this finding using the CGGA dataset and found a significant correlation between glioma grade and SERPINA1 mRNA expression levels [Figure 2].

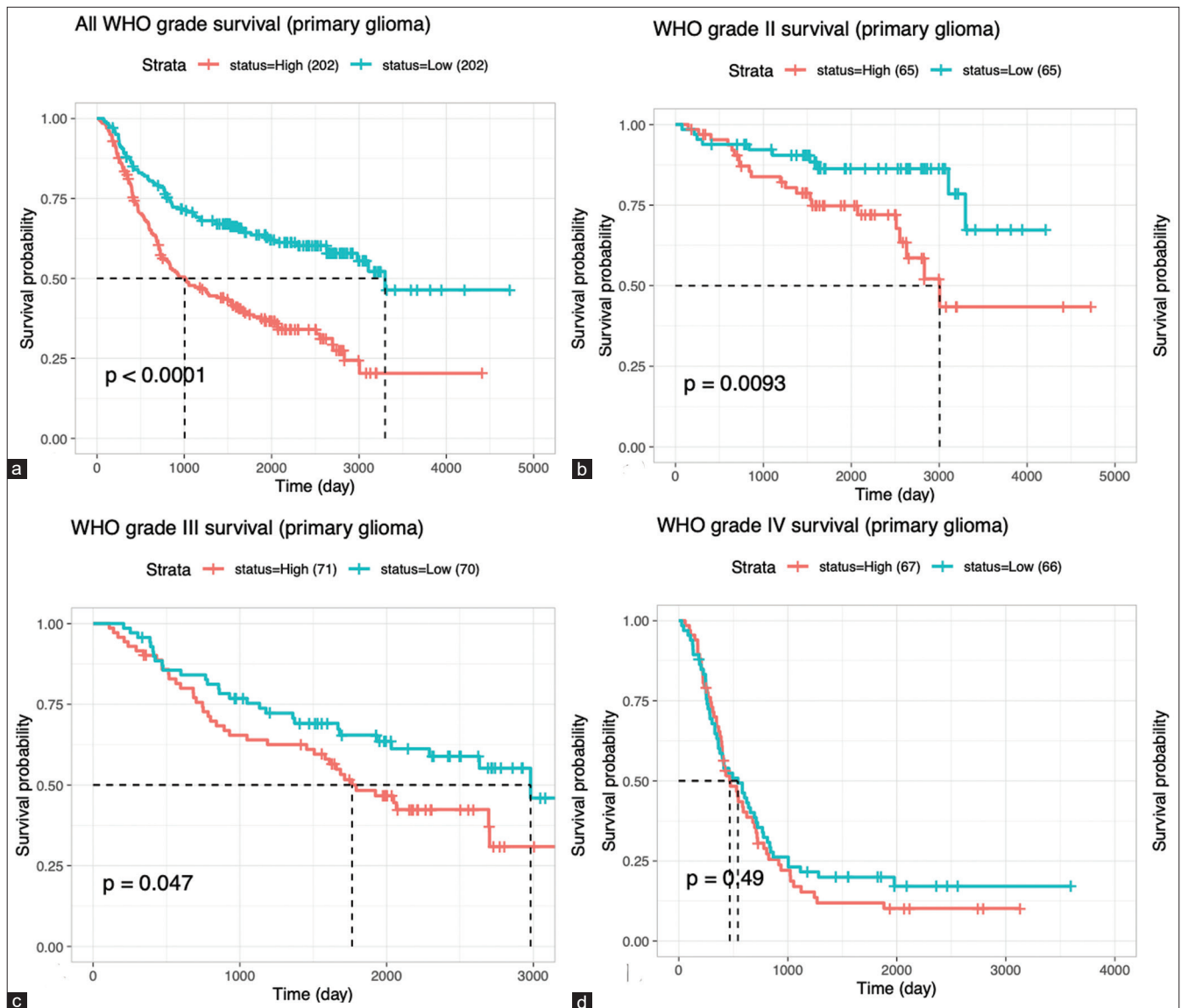


Figure 4: The relationship of the expression of SERPINA1 mRNA with overall survival (OS) time. Kaplan–Meier curves for comparison of the OS of subgroups in Chinese Glioma Genome Atlas datasets (total primary glioma group, $n = 404$; WHO Grade II group, $n = 130$; WHO Grade III group, $n = 141$; WHO Grade IV group, $n = 133$)

Although the exact sample size for each subgroup was not specified, nor were normal brain controls mentioned, the overall finding of elevated SERPINA1 mRNA expression in glioma patients was confirmed.

The initial investigation into the effects of differential SERPINA1 mRNA expression on survival time was conducted by Ookawa *et al.*,²³ who focused on high-grade glioma patients. Despite the limited sample size and the analysis being restricted to high-grade glioma patients, the study provided valuable insights into the potential use of SERPINA1 as a novel prognostic marker for glioma. In the current study, a bioinformatics approach was utilized to examine

the hypothesis that SERPINA1 could be a novel prognostic marker for glioma [Figure 1]. With the analysis of hundreds of glioma samples, the hypothesis was further validated for both low-grade [Figure 3a] and high-grade [Figure 4] gliomas. Although there is some inconsistency between different databases regarding GBM glioma patients [Figures 3b and 4d], the overall trend suggests that high-grade gliomas exhibit higher levels of SERPINA1 mRNA expression remains consistent.

The evidence presented in this study suggests that SERPINA1 has the potential to serve as a biomarker for predicting survival outcomes in patients with glioma.

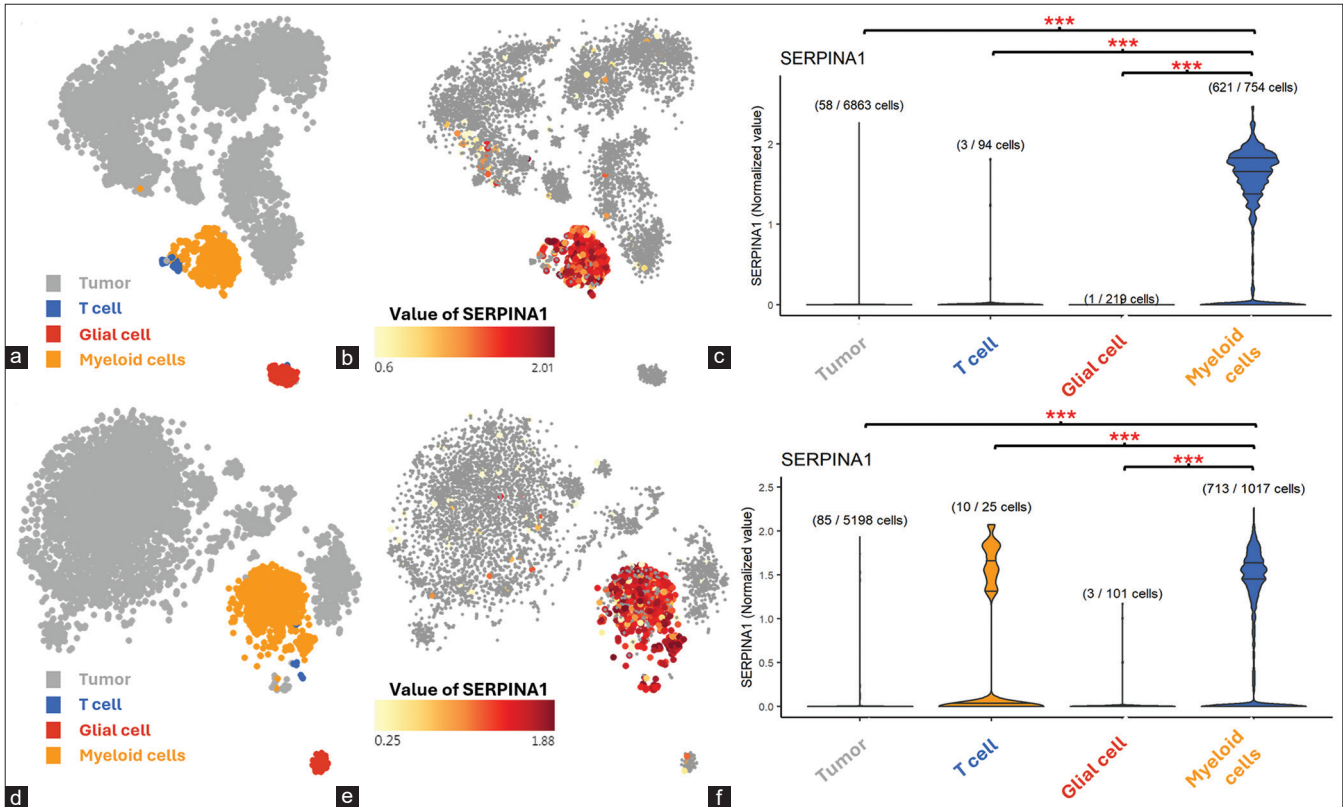


Figure 5: Single-cell analysis of SERPINA1 expression in gliomas. (a-c) IDH wild-type (IDHwt) gliomas and (d-f) IDH-mutant (IDHmu) gliomas. UMAP plots illustrate cell-type clusters, with SERPINA1 expression concentrated in myeloid cells. Violin plots show significantly higher SERPINA1 expression in myeloid cells than other cell types in IDHwt and IDHmu gliomas (*** $P < 0.001$)

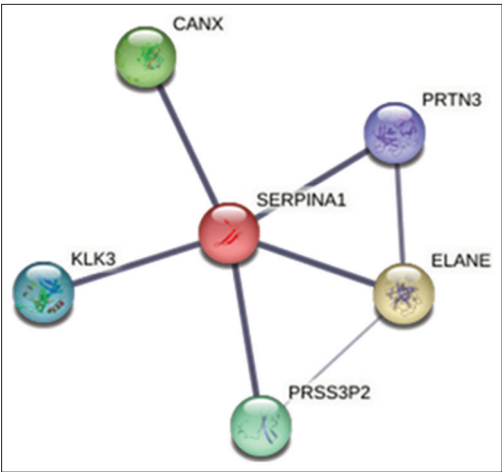


Figure 6: The SERPINA1 protein-protein interaction (PPI) network. SERPINA1 and its regulated proteins were generated by STRING datasets. Each node with different colors in the network diagram represents a specific protein, and each line indicates an interaction between the proteins. The thickness of the lines reflects the strength of the PPIs

However, to fully comprehend its role in tumor development and progression, further research is urgently needed. Previous studies have reported that SERPINA1 modulates the invasive

and metastatic capacity in lung, gastric, and colorectal cancer.⁴¹⁻⁴³ Therefore, it is highly possible that SERPINA1 could also enhance the invasive and metastatic capacity of glioma, leading to a shorter survival period for patients. Nonetheless, the exact mechanism underlying the role of SERPINA1 in glioma development and progression remains unclear and requires further investigation.

Based on GEO databases, we observed significantly higher SERPINA1 expression in myeloid cells compared to other cell types in both IDHwt and IDHmu gliomas using single-cell RNA sequencing [Figure 5c and 5f]. These findings suggest the potential of SERPINA1 as a biomarker for characterizing myeloid cell activation and its prognostic implications. Our results indicate that SERPINA1 serves as a marker of myeloid-driven immunosuppression and tumor progression, establishing a mechanistic link between myeloid cell activity and glioma prognosis. Further research into the functional role of SERPINA1 in myeloid cell biology may uncover novel therapeutic opportunities targeting the tumor microenvironment.

The current study utilized PPI analysis to predict potential links between SERPINA1 and candidate proteins, including

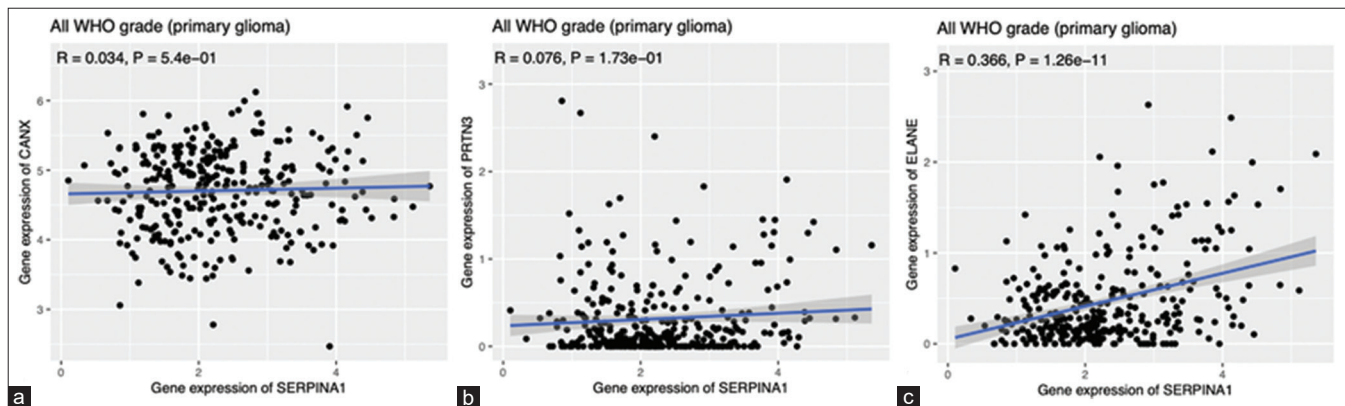


Figure 7: Analysis of expression levels between SERPINA1 with calnexin, proteinase 3, and elastase neutrophil expressed. Based on Chinese Glioma Genome Atlas databases, the expression levels were dot-blotted and the correlation between SERPINA1 and its regulated protein genes was evaluated. $P < 0.0500$ was identified as significantly correlated with each other

PRTN3, ELANE, PRSS3P2, KLK3, and CANX [Figure 6], which is consistent with the findings reported by Chao *et al.*²⁴ However, detailed investigations regarding the mechanisms are still limited. Of particular interest is the strong correlation observed between SERPINA1 and ELANE [Figure 7], which may have novel implications. Proteases are crucial in regulating inflammatory processes, highlighting the significance of SERPINA1 as a biomarker for assessing myeloid cell activation and its potential prognostic value. It is essential to uncover how these proteins work together and the specific molecules involved. Jülicher *et al.* reported in a mouse model that the SERPINA1-paralog DOM-7 functionally inhibits ELANE, and further studies are needed to explore the interactions between SERPINA1 and ELANE.⁴⁴

The study is subject to several limitations that need to be considered. First, due to the limited availability of clinical data, it was not possible to perform further analysis on factors related to the quality of life of glioma patients. Second, the small number and diverse subtypes of gliomas present challenges in the analysis of potential markers for each subtype. Third, it is important to note that there may be differential patterns of gene expression between different ethnic groups and populations, and the GEPIA and CGGA databases used in this study represent different geographic and genetic backgrounds. Nonetheless, the findings suggest that SERPINA1 may be a promising biomarker for predicting prognostic outcomes in glioma patients of different ethnicities and genetic backgrounds. Further investigation is needed to determine the most effective strategies for targeting SERPINA1 in the treatment of glioma.

CONCLUSION

The study revealed that glioma is correlated with the upregulation of the SERPINA1 gene and that higher expression

levels of SERPINA1 are associated with worse survival outcomes, indicating that SERPINA1 could be a promising biomarker for predicting poor survival. Further research is necessary to fully comprehend the underlying mechanisms of SERPINA1 in glioma progression and to establish effective approaches for targeting SERPINA1 in glioma treatment.

Acknowledgments

This research was supported by the Tri-Service General Hospital and the Tri-Service General Hospital Penghu Branch. We thank our team for providing valuable insights and expertise that greatly assisted this research.

Data availability statement

The genotyping results and clinical data supporting the findings of this study are available from the corresponding authors upon reasonable requests via email at syndrome1028@gmail.com

Financial support and sponsorship

This study was supported by grant from Tri-Service General Hospital (TSGH-D-111090) and Tri-Service General Hospital Penghu Branch (TSGH-PH-D-112004).

Conflicts of interest

Dr. Dueng-Yuan Hueng, an editorial board member at Journal of Medical Sciences (Taiwan), had no role in the peer review process of or decision to publish this article. The other authors declared no conflicts of interest in writing this paper.

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