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ORIGINAL RESEARCH

VSIG4 Silencing Inhibits Glioblastoma Growth by Regulating the JAK2/STAT3 Pathway

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Background: Glioblastoma (GBM) is the most common malignant primary brain tumour in adults. VSIG4 has been identified to be associated with GBM. We aimed to determine the downstream regulatory mechanisms of VSIG4 in GBM.

Methods: Differential expression of VSIG4 was analysed using GEPIA. The expression of VSIG4 was assessed by RT-qPCR and its downstream genes were screened by transcriptome sequencing. The expression of pyroptosis-related proteins and the JAK2/STAT3 pathway was measured by Western blotting. GBM cell viability, migration, and invasion were detected using CCK-8, scratch, and Transwell assays. The levels of pyroptosis-related factors were measured using ELISA. The effect of VSIG4 on GBM tumour growth in vivo was explored by constructing a xenograft tumour model.

Results: VSIG4 expression was upregulated in GBM. Functionally, silencing of VSIG4 inhibited proliferation, invasion, and migration of U251 and LN229 cells, and promoted pyroptosis. Mechanically, transcriptome sequencing revealed that the JAK2/STAT3 pathway might be a downstream regulator of VSIG4. Further studies proved that silencing of VSIG4 enhanced the expression of p-JAK2 and p-STAT3, and the JAK2/STAT3 pathway inhibitor relieved the suppression of VSIG4 silencing on GBM cell viability, invasion, and migration. Furthermore, in vivo experiments further validated that knockdown of VSIG4 inhibited the growth of GBM tumors.

Conclusion: In GBM, silencing VSIG4 promoted pyroptosis and inhibited tumor progression by regulating the JAK2/STAT3 signaling pathway.

Keywords: glioblastoma, VSIG4, JAK2/STAT3 pathway, pyroptosis

Introduction

As the most aggressive type of malignant brain tumour, glioblastoma has a high fatality rate and is one of the most difficult malignant solid tumours to treat. GBM is the most malignant tumour in adults, accounting for 48.6% of all malignant primary central nervous system tumors.[1] Although comprehensive treatment regimens, such as surgery, immunotherapy, and targeted therapy have been applied in clinical practice, the prognosis of patients with GBM is still poor.[2] Clinical studies have shown that the five-year survival rate is less than 10%, and the median survival time is less than 15 months.[3] More recently, with the rise and progress of precision medicine worldwide, many studies have attempted to explore the molecular mechanisms related to the generation and progression of GBM at the genetic level.

VSIG4 is a type I transmembrane protein of the B7 family of related proteins specifically expressed in macrophages, also known as complement receptor immunoglobulin superfamily molecules or immunoglobulin superfamily protein 39.[4] Many studies have shown that VSIG4 plays a crucial role in cancer. VSIG4 is reduced in hepatocellular carcinoma (HCC), and low expression of VSIG4 is associated with a poor prognosis in patients with hepatitis B virus-related HCC.[5] High expression of VSIG4 is also related to poor prognosis in patients with high-grade glioma.[6] GBM tissues express higher levels of VSIG4

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protein than adjacent normal tissues, illustrating that VSIG4 is an oncogene in GBM.[7] There are many studies on the upstream regulation of glioma by VSIG4,[6–8] but relatively few studies on its downstream regulatory mechanism are available.

Pyroptosis is an inflammatory form of cell death triggered by certain inflammasomes, leading to the cleavage of gasdermin D (GSDMD) and the activation of inactive cytokines such as IL-18 and IL-1β.[9] The development of tumours is influenced by pyroptosis. Lack of VSIG4 increased pyroptosis and IL-1β secretion in macrophages.[10] However, it remains unclear whether VSIG4 participates in GBM by regulating pyroptosis. Additionally, research on the downstream mechanisms of VSIG4 is limited. In this study, JAK2/STAT3 was identified as a possible downstream pathway using transcriptome analysis. JAK2/STAT3 is an important component of the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway and participates in cell proliferation and differentiation.[11] Abnormal activation of STAT3 in the JAK2/STAT3 signalling pathway is strongly associated with the development and progression of multiple malignant tumours.[12] Activation of the JAK2/STAT3 signalling pathway can also mediate pyroptosis in nephrology[13] and breast cancer.[14,15] However, whether the regulatory axis of VSIG4-JAK2/STAT3 is involved in pyroptosis in GBM is unclear.

In this study, we analysed the expression and function of VSIG4 in GBM cells and its role in pyroptosis. The downstream mechanisms of VSIG4 were explored using transcriptome analysis. We aimed to reveal a new regulatory mechanism of VSIG4 in JAK2/STAT3 pathway pyroptosis, providing a potential new target for the treatment of GBM.

Materials and Methods

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies.[16]

GEPIA Website and GlioVis Database

The GEPIA (Gene Expression Profiling Interactive Analysis) database (http://gepia.cancer-pku.cn/) integrates data from The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) projects. The GEPIA database allows for a variety of analyses, mainly including differential expression analysis, correlation analysis, and patient survival analysis, providing a valuable resource for cancer research.[17] In GEPIA database, we analysed the differential expression of VSIG4 in GBM. There were two groups, (T) GBM tissue (n=163) and (N) normal tissue (n=207). The GlioVis database (http://gliovis.bioinfo.cnio.es) collects approximately 6500 brain tumor samples (mainly gliomas) from 50 expression datasets, and allows analysis of genes for expression, copy number, mutations, and so on.[18] We used the Chinese Glioma Genome Atlas (CGGA) dataset in the GlioVis database to study the changes in VSIG4 expression in different GBM tumour grade. The GBM tumour grading system contained grades 2, 3, and 4.

Cell Culture and Transfection

U251 and LN229 cells (Pricella, Wuhan, China) were cultured in growth medium (Dulbecco's modified Eagle's medium and 10% fetal bovine serum) (Pricella). The design and synthesis of the siRNAs (si-NC and si-VSIG4) were performed by Gene Pharma (Shanghai, China). Following the manufacturer's instructions, Lipofectamine 3000 (Invitrogen, Carlsbad, CA, United States of America) was used to transfect siRNAs into cells.

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from cells or tissues using the TRIzol reagent (TaKaRa, Dalian, China). cDNA libraries of mRNA were reverse-transcribed using PrimeScript™ RT Master Mix (TaKaRa). SYBR® Green Pro Taq HS premix (Accurate Biology, Changsha, China) was used for qPCR analysis. The qPCR analysis was performed with the following procedure: 95°C for 30s, followed by 40 cycles of 95°C for 5 s and 60°C for 30s. We used $2^{-\Delta\Delta Ct}$ method to normalize the relative levels of mRNA expression to *GAPDH*. The primer sequences for VSIG4 were as follows: forward primer: TCCGTGTCCAGAAACACTCC (5'-3'), and reverse primer: CATGTCAGTGGTCCAGTCCC (5'-3').

Cell Counting Kit-8 (CCK-8) Assay

U251 and LN229 cells (10^4 cells/well) were seeded in 96-well plates and cultured for 0, 24, 48, 72, and 96 h. The cells in each well were treated with 10 μL of the CCK-8 reagent (Solarbio, Beijing, China). After 4 h of incubation, OD_{450} was measured at 450 nm by microplate reader (Thermo Fisher scientific, Cleveland, OH, United States of America).

Scratch Test

U251 and LN229 cells were seeded into 24-well plates at a density of 6×10^4 cells/well. After the cell density reached 85%, a scratch was made perpendicular to the marker line in the centre at the bottom of a 24-well plate. The medium was then replaced with serum-free medium after washing the dead cells. Each well was observed and photographed under a microscope (Leica, Wetzlar, Germany) at 0 and 24 h. ImageJ software was used to measure the scratch area.

Transwell

U251 and LN229 cells (6×10^4) were suspended in 100 μL serum-free medium. They were then seeded into the upper chamber, and 20% FBS was added to the lower chamber to induce cell invasion through the membrane. Matrigel (1:6 dilution; BD Biosciences) was then added to the upper chamber. After 24 h, cells that invaded across the Transwell membrane and were stained with crystal violet (Abcam, Cambridge, England) that invaded across the Transwell membrane were counted under an optical microscope (Leica, Wetzlar, Germany).

Western Blotting

Total cell protein was extracted using RIPA lysis buffer (Beyotime, Nanjing, China). Protein concentration was determined using a BCA Protein Assay kit (Beyotime, Nanjing, China). The protein sample (1.5 μg/well) was loaded into a 12% SDS-PAGE gel (Zoman, Beijing, China) and transferred to a PVDF membrane (Millipore, Billerica, MA, United States) by electroblotting. The transferred membranes were blocked with 1× TBST containing 5% bovine serum albumin (BSA) for 2 h at room temperature, followed by overnight incubation with the primary antibody (ZenBioScience, Chengdu, China) at 4°C. After washing the membranes three times with TBST, the appropriate antibody (1:2000) (Abcam, Cambridge, England) was used for incubation for 2 h at room temperature. Finally, the target proteins were detected using an ECL Chemiluminescence Detection Kit (Vazyme, Nanjing, China) with a Bio-Rad Chemi Doc XRS+ exposure system. The results of Western blotting were analysed using ImageJ Software.

RNA and Sequencing

The mRNA was enriched using oligo (dT) magnetic beads for ion displacement.[19] RNA was used as a template and cDNA was synthesized using 6-base random primers and reverse transcriptase. Library fragment enrichment was performed using PCR amplification. Library quality was assessed using an Agilent 2100 Bioanalyzer. Libraries containing different index sequences were then mixed proportionally based on the effective concentration of the library and the amount of data required. The pooled libraries were uniformly diluted to 2 nM, and single-stranded libraries were formed using alkalinity. After RNA extraction, purification, and library construction, Next-generation sequencing (NGS) technology was used to perform paired-end (PE) sequencing of the libraries using an Illumina sequencing platform.

RNA-Seq Data Analysis

The three RNA samples were submitted to the si-NC and si-VSIG4 groups for transcriptome sequencing. Differential expression analysis of the RNA was performed using the DESeq2 R package in the two groups (v1.32.0). The R software package ggplot2 was used to generate a volcano map of differentially expressed genes. We used the R software package Pheatmap to perform two-way cluster analysis of differential genes and samples from all comparison groups. We used topGO for gene ontology (GO) enrichment analysis and calculated the number of genes and gene list of each term using the differential gene annotation of the GO term. According to Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of differentially expressed genes (DEGs), pathway proteins with the smallest p-value (the most significant enrichment) were selected for further analysis.

Construction Xenograft Tumor Models in vivo

The Committee on Animal Research of Guangdong Provincial People's Hospital, Southern Medical University (Guangdong, China) granted approval for all animal experimentation procedures. A total of 12 BALB/c nude mice (weighed 18–20 g) obtained from the SPF (Beijing) Biotechnology Co., Ltd were randomly divided into two groups after one week of adaptive feeding. The PCR products of si-NC or si-VSIG4 were ligated into the GV115 vector using AgeI/EcoRI (Gikai, Shanghai, China). Subsequently, the plasmids were transfected into 293T cells using Lipofectamine 2000 (Thermo Fisher Scientific, New York, USA). The U251 cells were divided into two groups, including the LV-si-NC group (infected with si-NC interfering virus) and the LV-si-VSIG4 group (infected with si-VSIG4 interfering lentivirus). A total of 5×10^6 U87 cells of LV -si-NC or LV-si-VSIG4 were resuspended in PBS and injected subcutaneously into the right dorsum of nude mice. Tumour size was measured every 5 days following tumour formation using a vernier caliper. Tumour volume (V) was calculated using the formula: $V = (\text{width}^2 \times \text{length})/2$. After 5 weeks, the mice were euthanized, and the tumour tissues were observed, followed by the volume and weight of the tumour tissues were measured.

Statistical Analysis

SPSS software (21.0 version, IBM, United States) was used to perform the statistical analyses. Data are presented as mean ± standard deviation (SD) for all measurements. A Two-tailed Student's *t*-test was used to compare the average differences between the groups. One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used for multiple comparisons. Statistical significance was considered when $P < 0.05$. Three groups of parallel experiments were conducted.

Results

VSIG4 is Highly Expressed in GBM

To obtain the differential expression of VSIG4 in GBM, we used the GEPIA website for the analysis. The results showed that VSIG4 was highly expressed in GBM tissues compared to the adjacent non-tumor tissues ($P < 0.05$) (Figure 1A). We then used the Glio Vis database to investigate the relationship between VSIG4 expression and GBM tumour grades. Grade IV GBM exhibited significantly higher expression of VSIG4 compared to grade II and III ($P < 0.01$) (Figure 1B). qRT-PCR results showed that, compared with HEB cells, VSIG4 mRNA expression levels were increased in U251 and LN229 cells ($P < 0.01$), which are two GBM cell lines (Figure 1C).

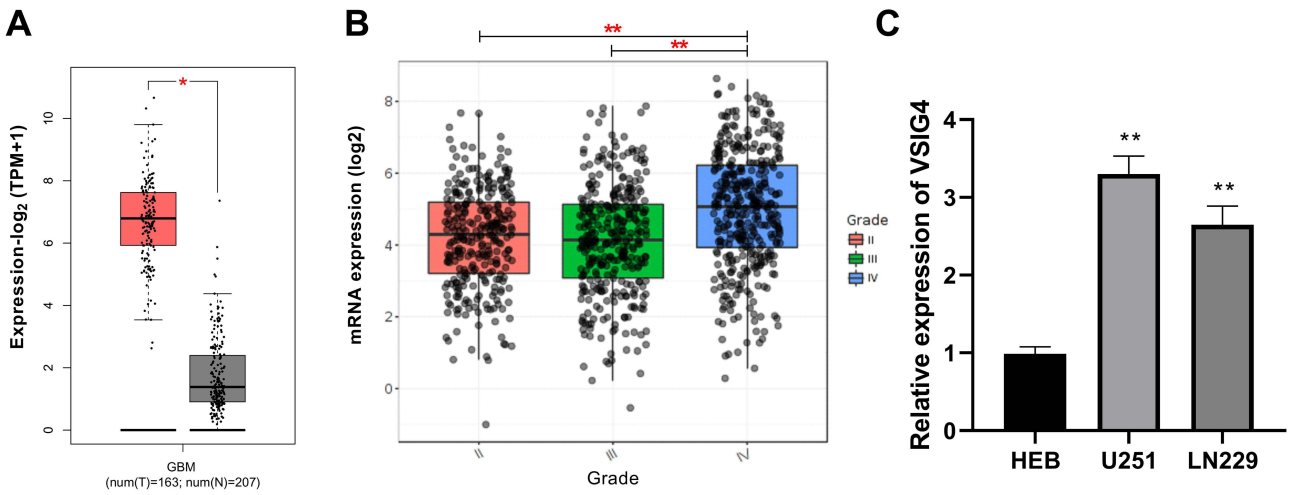

Figure 1 High expression of VSIG4 in GBM. (**A**) VSIG4 was overexpressed in GBM tumor tissues by analysis in the GEPIA database; (**B**) VSIG4 expression was significantly different in different grades of GBM by analysis in the GlioVis database; (**C**). RT-qPCR was used to detect the expression of VSIG4 in HEB, U251 and LN299 cells. RT-qPCR assay was repeated 5 times.

Notes: *Represents p-value less than 0.05; **Represents p-value less than 0.01.

Abbreviation: GEPIA, Gene Expression Profiling Interactive Analysis.

VSIG4 Silencing Inhibits the Viability, Migration and Invasion of GBM

To further explore the effect of VSIG4 on GBM, VSIG4 was silenced. Compared to the si-NC group, U251 and LN229 cells transfected with the si-VSIG4-1 and si-VSIG4-2 both reduced the mRNA expression level of VSIG4 ($P < 0.01$) (Figure 2A). We selected si-VSIG4-2 for subsequent experiments. To determine the cell viability, we performed a CCK-8 assay. The results showed that in U251 and LN229 cells, the viability of the si-VSIG4 group was reduced compared with that of the si-NC group ($P < 0.01$) (Figure 2B). We then determined whether VSIG4 affects cell migration and invasion. The scratch test showed that the cell migration rate in the si-VSIG4 group was lower than that in the si-NC group for both U251 and LN229 cells ($P < 0.01$) (Figure 2C). Transwall experiments showed that the cell invasion level in the si-VSIG4 group was reduced ($P < 0.01$) (Figure 2D). These findings showed that the absence of VSIG4 inhibited the viability, migration, and invasion of GBM cells.

Silencing VSIG4 Promotes Pyroptosis

We investigated the relationship between VSIG4 and pyroptosis by Western blotting. Our results indicated that pyroptosis-related proteins, including NLRP3, caspase-1, and GSDMD, were increased in cells transfected with si-VSIG4 ($P < 0.01$) (Figure 3A). We further determined the levels of several factors associated with pyroptosis. ELISA results (Figure 3B) showed that the levels of IL-18, IL-1β, and TNF-α in the si-VSIG4 group were increased ($P < 0.01$). These results demonstrated that VSIG4 silencing promoted pyroptosis.

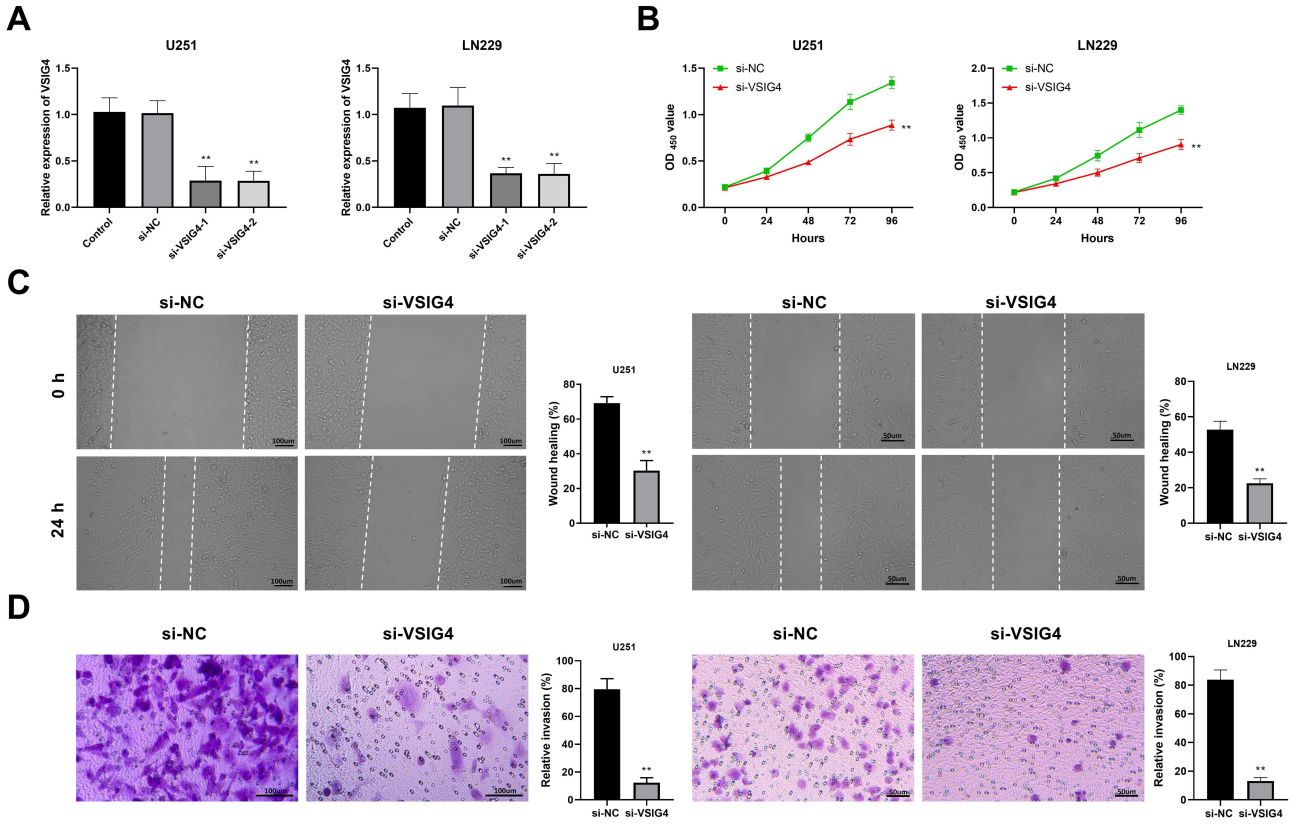

Figure 2 VSIG4 silencing inhibits the viability, migration and invasion of GBM. (**A**) Detection of VSIG4 silencing efficiency by RT-qPCR in U251 and LN229 cells, which were untreated and transfected with siNC, siVSIG41-1, and siVSIG41-2; (**B**) Detection of cell viability by CCK-8 assay in U251 and LN229 cells; (**C**) Detection of cell migration by scratch test in U251 and LN229 cells; (**D**) Detection of cell invasion by transwall assay in U251 and LN229 cells. Scale bar for U251 cells was 100μm, and scale bar for LN229 cells was 50μm. CCK-8 assay was repeated 4 times, and scratch test and transwall assays were repeated 3 times, respectively.

Notes: **Represents p-value less than 0.01.

Abbreviation: CCK-8 assay, Cell Counting Kit-8 assay.

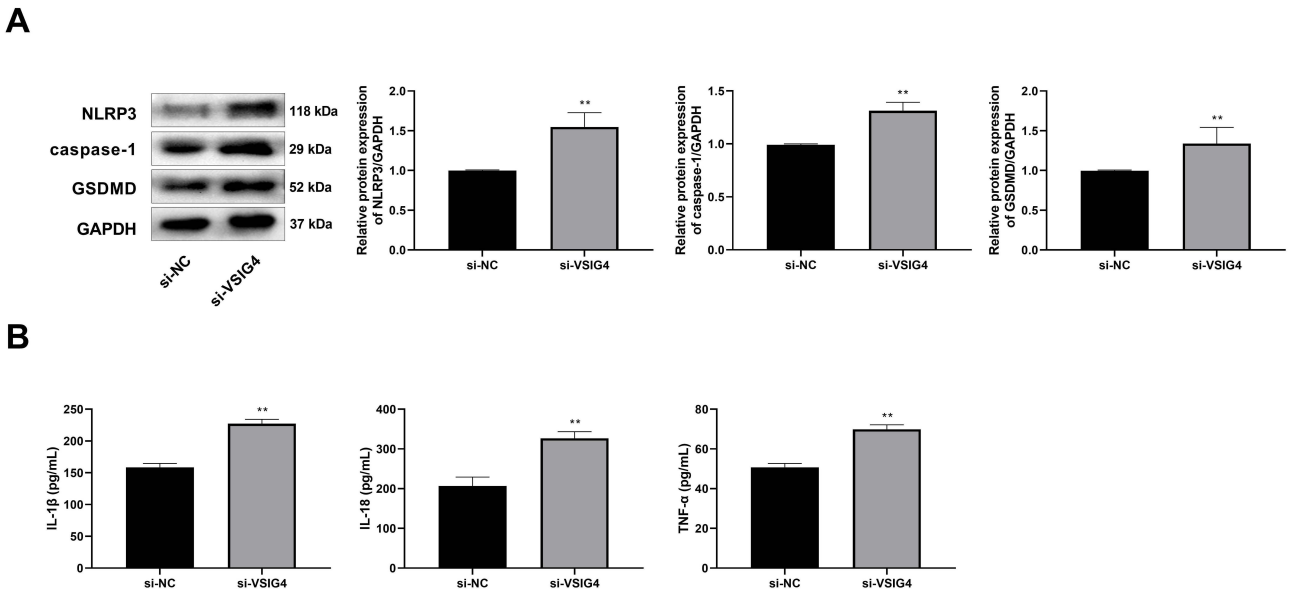

Figure 3 VSIG4 silencing promotes pyroptosis of GBM. (**A**) VSIG4 silencing influenced the protein expression levels of pyroptosis markers, including NLRP3, caspase-1, and GSDMD in the Western blot assay; (**B**) VSIG4 silencing influenced the concentration levels of pyroptosis-related inflammatory factors, including IL-18, IL-1β, and TNF-α in the ELISA assay. Western blot and ELISA assays were repeated 3 times, respectively.
Notes: **Represents p-value less than 0.01.

Transcriptome Sequencing Analysis

The sequencing results showed that 208 genes were significantly differentially expressed in the si-NC and si-VSIG4 groups. Compared with the si-NC group, 129 genes were significantly upregulated and 79 genes were downregulated in the si-VSIG4 group (Figure 4A and B). GO enrichment analysis showed many significantly different categories, including mainly positive regulation of peptide-tyrosine phosphorylation, cellular response to molecules of bacterial origin, cellular response to biotic stimulus, and cellular response to lipopolysaccharide (Figure 5A and B). KEGG enrichment analysis was performed

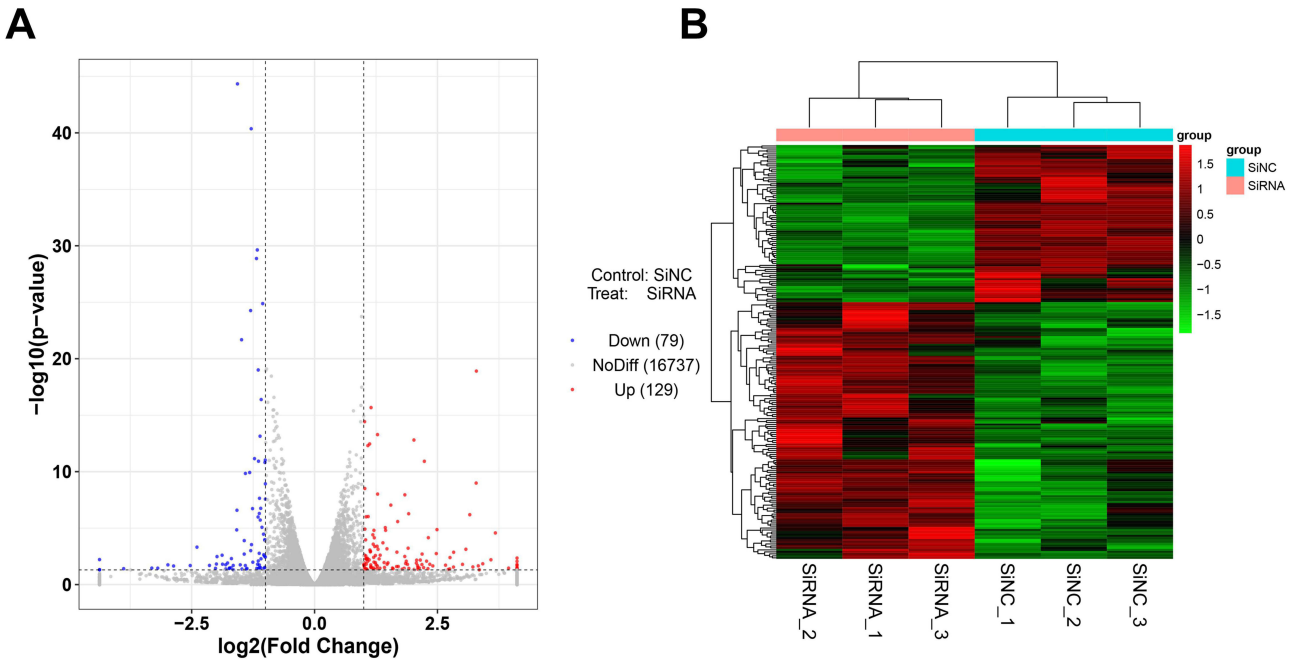

Figure 4 The exhibition of DEGs of transcriptome sequencing. (**A**) Volcano plot of the DEGs regulated by VSIG4. Red dots represent genes with high expression, blue dots represent genes with low expression, and black dots represent genes with no difference in expression level; (**B**) The heatmap displayed the DEGs variation modulated by VSIG4.
Abbreviation: DEGs, differentially expressed genes.

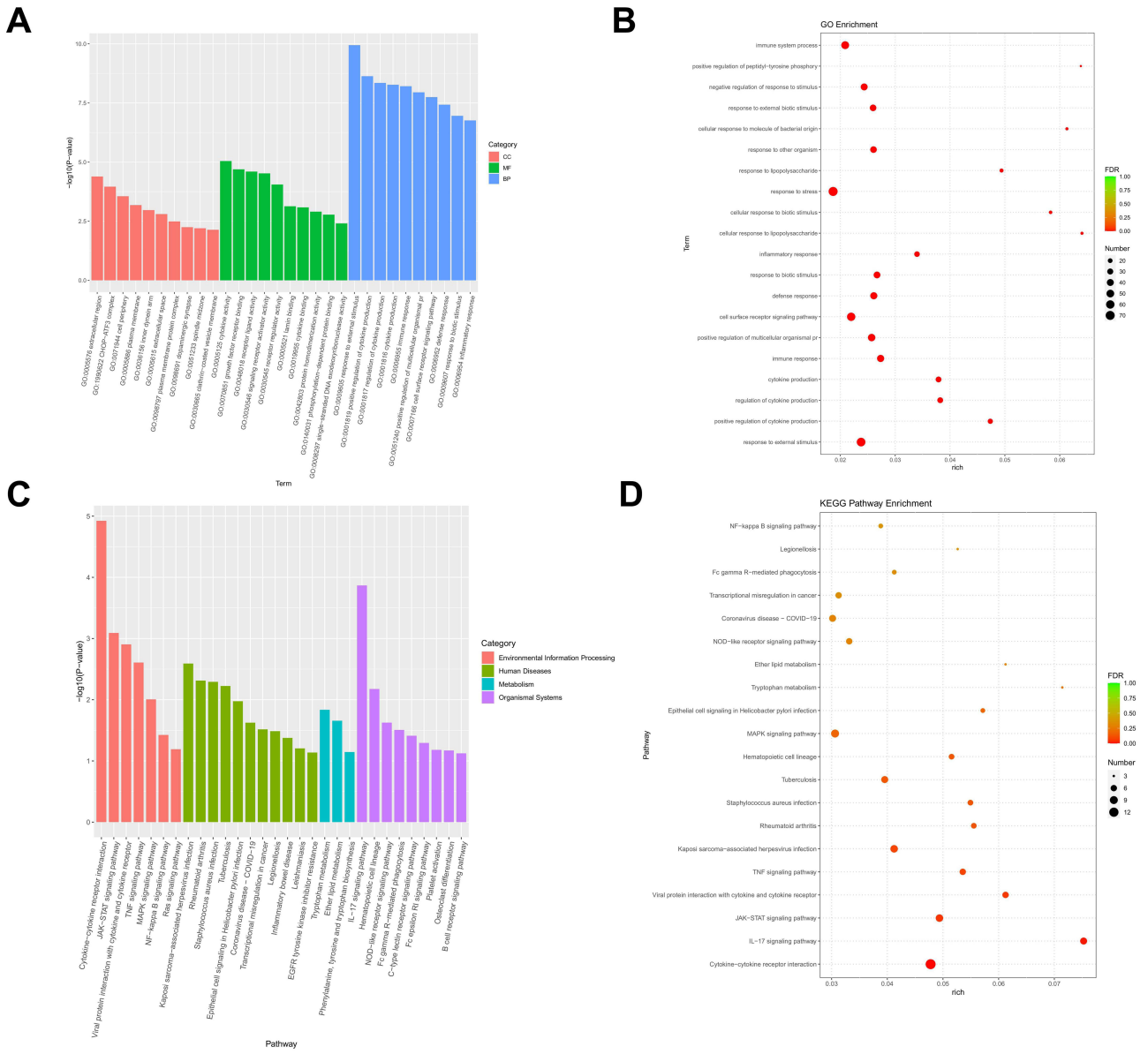

Figure 5 GO and KEGG analysis of DEGs regulated by VSIG4. (**A**) A bar chart of GO analysis for DEGs; (**B**) A bubble plot of GO analysis for DEGs; (**C**) A bar diagram of KEGG analysis for DEGs; (**D**) A bubble diagram of KEGG analysis for DEGs.
Abbreviations: GO, Gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes.

according to genes that were differentially expressed, revealing the JAK-STAT signalling pathway, MAPK signalling pathway, and NF-kappa B signalling pathway (Figure 5C and D). We chose the JAK-STAT signalling pathway because of its correlation with pyroptosis. Furthermore, we performed Western blotting to determine whether VSIG4 regulates the JAK-STAT pathway. We found that the expression of p-JAK2 ($P < 0.01$), and p-STAT3 ($P < 0.01$) in the si-VSIG4 group was significantly higher than that in the si-NC group (Figure 6), which was consistent with the sequencing results.

Silencing VSIG4 Inhibits the Viability, Migration and Invasion of GBM Cells by Regulating JAK2/STAT3 Pathway

Silencing VSIG4 could activate the JAK2/STAT3 pathway; therefore, we added AG490, an inhibitor of the JAK2/STAT3 pathway, to perform feedback validation. We found that the cell viability in the si-VSIG4+AG490 group was significantly higher than that in the si-VSIG4 group (Figure 7A) ($P < 0.01$). Next, we assessed cell migration and invasion. The results

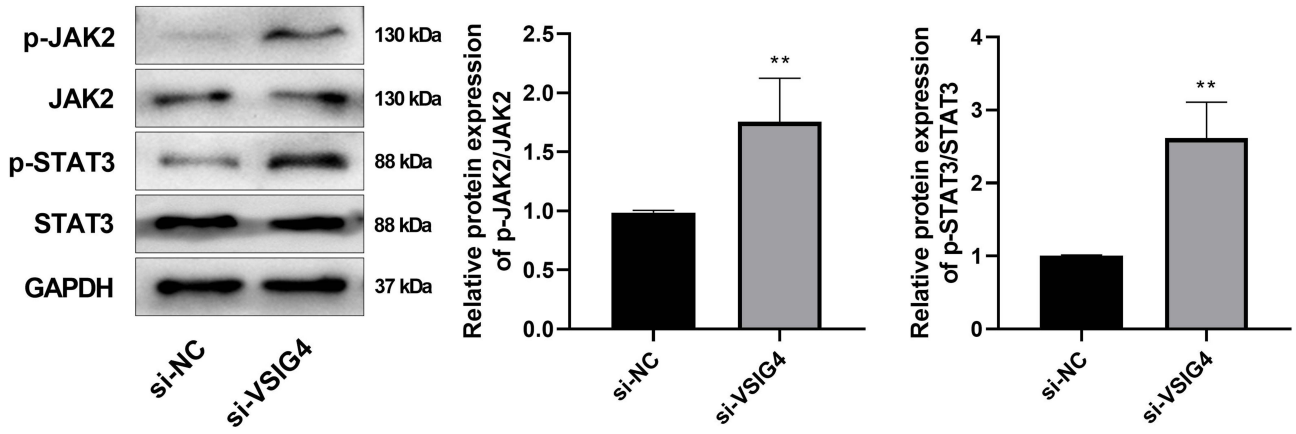

Figure 6 WB validation of JAK2/STAT3 pathway-related proteins, including p-JAK2, JAK2, p-STAT3, and STAT3. Western blot assay was repeated 3 times.
Notes: **Represents p-value less than 0.01.
Abbreviation: WB, Western Blotting.

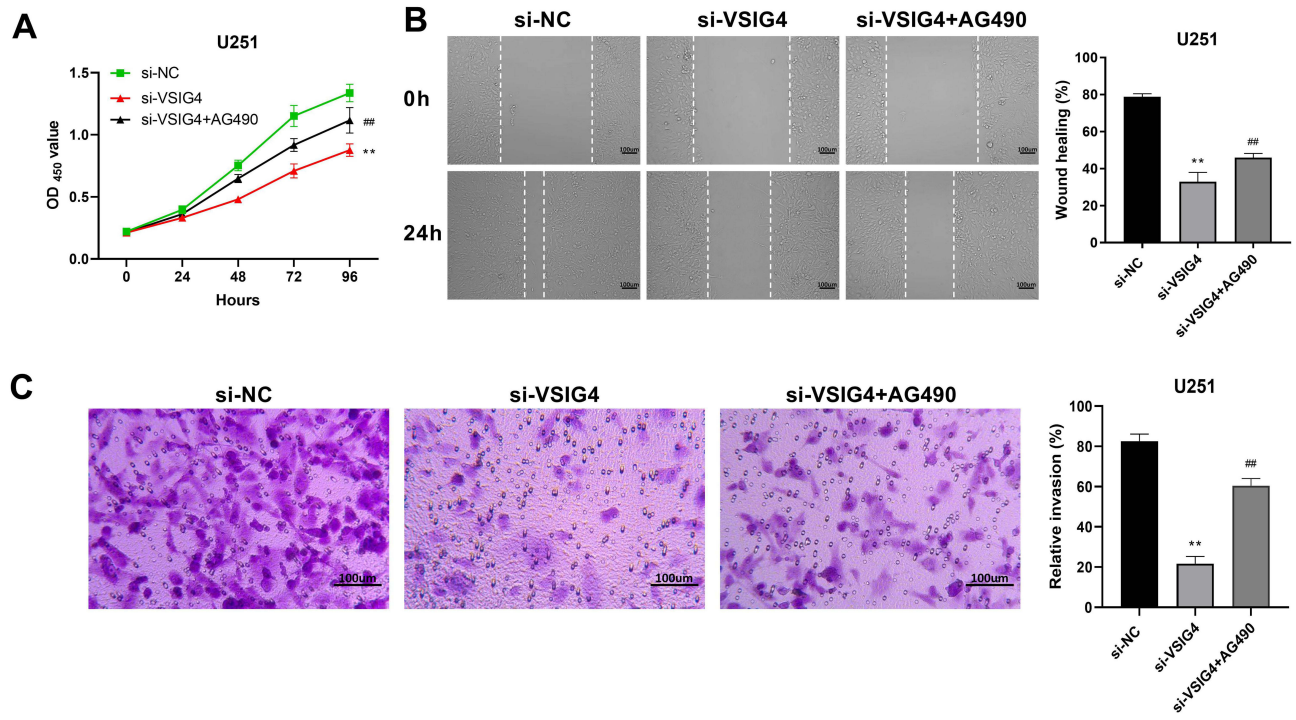

Figure 7 VSIG4 could regulate JAK2/STAT3 pathway to promote GBM viability, migration and invasion. (**A**) Detection of cell viability by CCK-8 in si-NC, si-VSIG4, and si-VSIG4+AG490 groups; (**B**) Detection of cell migration in si-NC, si-VSIG4, and si-VSIG4+AG490 groups by scratch assay; (**C**) Detection of cell invasion in si-NC, si-VSIG4, and si-VSIG4+AG490 groups by transwall assay. The scale bar was 100μm. CCK-8 assay was repeated 4 times, and scratch test and transwall assay were repeated 3 times, respectively.
Notes: **And ##Represent p-value less than 0.01.
Abbreviation: CCK-8, Cell Counting Kit-8 assay.

showed that the rate of cell migration was significantly higher in the si-VSIG4+AG490 group than in the si-VSIG4 group ($P < 0.01$) (Figure 7B). The results of the transwell experiment showed that AG490 partially restored the cell invasion inhibited by si-VSIG4 ($P < 0.01$) (Figure 7C). These results indicate that silencing VSIG4 inhibited GBM cell viability, migration, and invasion through the JAK2/STAT3 pathway.

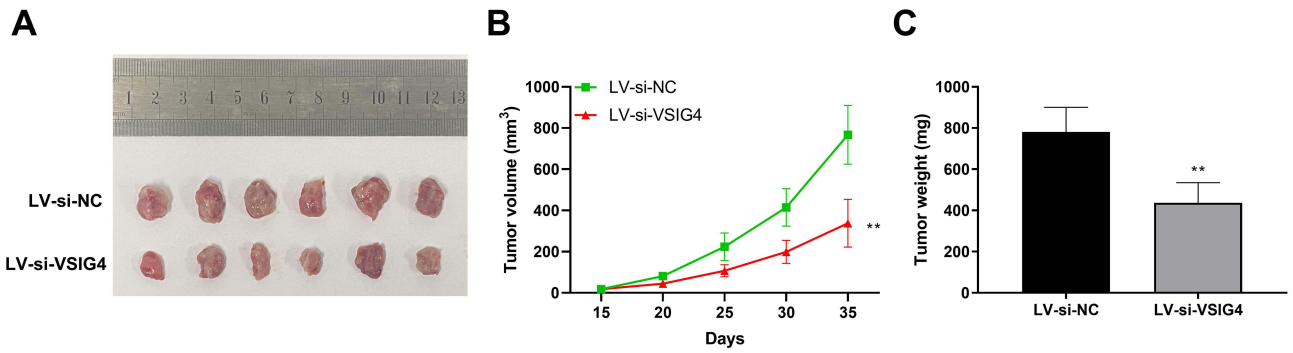

Figure 8 Knockdown of VSIG4 inhibits GBM tumor growth in vivo. (**A**) Representative images of tumors in the LV-si-NC and LV-si-VSIG4 groups; (**B**) Tumor volume growth curve in the LV-si-NC and LV-si-VSIG4 groups; (**C**) Tumor weight histogram in the LV-si-NC and LV-si-VSIG4 groups. Tumor volume and weight measurements were repeated 6 times, respectively.
Notes: **Represents p-value less than 0.01.

Knockdown of VSIG4 Inhibits GBM Tumor Growth in vivo

Next, we investigated the role of VSIG4 on tumor growth in vivo. The results showed that the tumor volume and tumor weight of mice in the LV-si-VSIG4 group were significantly decreased than those in the LV-si-NC group (Figure 8A–C), indicating that knockdown of VSIG4 inhibited tumor progression in vivo.

Discussion

GBM is a devastating primary brain malignancy.[20,21] GBM is one of the toughest tumours to cure owing to its high heterogeneity, rapid growth rate, strong invasion ability, and poor prognosis.[22,23] There is an urgent need to determine the mechanism underlying the generation and progression of GBM and identify therapeutic targets. We found that VSIG4 is highly expressed in GBM tissues. The expression levels in U251 and LN229 cells also support this hypothesis. This is consistent with the findings of a previous study.[6] Meanwhile, VSIG4 silencing inhibited GBM cell viability, migration, and invasion and promoted pyroptosis. We selected the downstream mechanisms of VSIG4 via transcriptome sequencing. The absence of VSIG4 upregulated JAK2 and STAT3 phosphorylation Therefore, we demonstrated that VSIG4 induced pyroptosis, and promoted GBM progression through the JAK2/STAT3 pathway.

VSIG4 expression correlates with the occurrence of cancer. For example, VSIG4 may lead to increased C3b deposition, thereby promoting the formation of new blood vessels and progression of ovarian cancer.[24] VSIG4 facilitates lung tumour growth by inhibiting T-cell proliferation and cytokine production.[25] VSIG4 plays a regulatory role not only in the above cancers but also in gliomas. VSIG4 can predict the effect of immunotherapy in glioma by performing a protein-protein interaction network.[26] Overexpression of VSIG4 can induce EMT in GBM U-87MG cells and significantly promote their invasion and migration, suggesting that VSIG4 may be an oncogene in GBM.[7] In our study, we found that VSIG4 was overexpressed in GBM, and silencing VSIG4 inhibited the viability, migration, and invasion of GBM cells. Furthermore, knockdown of VSIG4 inhibited GBM tumour growth in vivo. Recently, there have been many studies on inhibitors that target various key proteins in GBM, such as lercanidipine and ivacaftor. Lercanidipine is mainly used for the treatment of hypertension.[27] It has been shown to inhibit Wnt/β-catenin signaling-dependent tumourigenesis in GBM by inhibiting GLT8D1 expression.[28] Ivacaftor was approved by the FDA in 2019 for the treatment of cystic fibrosis. It inhibits stem cell maintenance in GBM by decreasing the expression of CD133, CD44 and Sox2.[29] In U-87MG cells, VSIG4 upregulates proteins that enhance temozolomide resistance in human GBM cells, including P4HB, VAMP8 and CX43.[7] Therefore, inhibitors that target the expression of VSIG4 in GBM cells are likely to exert their antitumor effects. Our study provided a theoretical basis for clinical treatment of GBM.

Pyroptosis is an inflammatory cell death pathway mediated by caspase.[30] Pyroptosis plays a significant part in the generation and progression of some tumours. Inhibition of pyroptosis can promote the development of digestive, cervical, and breast cancer.[9] A recent study has constructed a new prognostic model for predicting the prognosis of patients with GBM based on three pyroptosis-related genes. Our data showed that silencing VSIG4 promoted pyroptosis and increased the levels of inflammatory

factors such as IL18. There have been several previous studies on the relationship between VSIG4 and pyroptosis. For example, VSIG4/NLRP3 pathway induces pyroptosis during sepsis by regulating Lipopolysaccharide-induced extracellular histone H3.[31] In our study, silencing VSIG4 was found to induce pyroptosis in GBM, which has not been widely reported before. In addition, it has been shown that VSIG4-encoded proteins of the V-set and immunoglobulin domains play a control point role in immunity, inflammation, and tumors.[32] Based on the above studies, we hypothesized that VSIG4 played an important role in pyroptosis of tumors.

We explored the VSIG4 downstream mechanism of in pyroptosis using transcriptome analysis. We found the most interesting pathway in the KEGG enrichment analysis results to be the JAK2/STAT3 pathway. We verified that the expression of p-JAK2 and p-STAT3 increased when VSIG4 was silenced, which was consistent with the sequencing results. The JAK2/STAT3 pathway is involved in the development of many tumours. Activating JAK2/STAT3 affects prognosis and reduces survival time in patients with NPC.[33] Moreover, the JAK2/STAT3 pathway mediates the inhibition of pioglitazone on breast cancer proliferation and migration.[34,35] VSIG4 occupancy can activate JAK2-STAT3-A20 cascades, triggering Ser232 and Ser235 phosphorylation in MS4A6D.[10] Similarly, in our study, the JAK2/STAT3 pathway participated in the regulation of VSIG4 in GBM, verifying the existence of the VSIG4-JAK2/STAT3 pathway and providing more targets for the treatment of GBM.

Conclusion

In summary, we demonstrated that VSIG4 expression was higher in GBM. The knockdown of VSIG4 increased their pyroptosis and inhibited the viability, migration, and invasion of GBM cells by regulating the JAK2/STAT3 pathway. Our research provided new molecular mechanisms and possible therapeutic targets for the clinical treatment of GBM.

Data Sharing Statement

The data that support the findings of this study are available from [Guangdong Provincial People's Hospital (Guangdong Academy of Medical Sciences), Southern Medical University] but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [Guangdong Provincial People's Hospital (Guangdong Academy of Medical Sciences), Southern Medical University].

Hang Shu should be contacted if someone wants to request the data from this study.

Author Contributions

Congying Zheng and Chengliang Mao are co-first authors. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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