Bioinformatics analysis of SH2D4A in Glioblastoma Multiforme to evaluate immune features and predict prognosis

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Abstract

**Background:** Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor in adults. The predictive role of SH2D4A has been shown to be closely related to various cancers progression, but there is no comprehensive analysis of the clinical significance in glioblastoma. Hence, this study aimed to explore the relationship between the prognosis of GBM and SH2D4A expression.

**Methods:** The expression of SH2D4A in GBM was analyzed using TIMER2.0 and GEPIA, and validated by qRT-PCR experiments. The CGGA database analyzed the differential expression of SH2D4A in glioma and evaluated the impact of SH2D4A on the survival of glioma patients. LinkedOmics database and GeneMANIA database were studied for SH2D4A co-expression network. A lasso regression model and nomogram were constructed to assess the prognosis of GBM. TCGA database was used to do a GSEA to find functional differences. The relationship between SH2D4A expression and tumor-infiltrating immune cells was analyzed using xCELL, the CIBERSORT algorithm and the TIMER database.

**Results:** In GBM patients, we found that the expression of SH2D4A was upregulated, and the elevated expression of SH2D4A was strongly associated with the grade of the tumor. High SH2D4A expression was found to be a significant independent predictor of poor overall survival (OS) in GBM patients by survival curve analysis and multivariate cox regression analysis. GSEA revealed that SH2D4A was mainly enriched in extracellular matrix tissues, and the expression level of SH2D4A was inversely correlated with the level of infiltration of CD8+ T cells, CD4+ T cells, B cells, neutrophils and macrophages in GBM, but was positively correlated with the level of dendritic cell infiltration. Immunoassays suggest that altered SH2D4A expression may affect the immune infiltration of GBM tissues and thus affect the survival outcome of GBM.

**Conclusion:** In addition to being a possible prognostic marker and therapeutic target for GBM, SH2D4A may also accelerate the progression of GBM.

Introduction

The most prevalent and aggressive type of primary brain cancer is glioblastoma, which has a median survival time of 16 to 20 months and a 5-year survival rate of under 5% [1, 2]. With poor survival outcomes as compared to standard therapy, high-grade gliomas like GBM continue to pose considerable therapeutic challenges. Therefore, clinicians and scientists are working to identify molecular markers associated with tumors and to personalize treatment for patients in combination with pathological classification [3, 4]. Therefore, finding the key signaling molecules that contribute to GBM tumorigenesis is crucial to improve clinical treatment strategies and patient prognosis.

SH(2)A is encoded by the SH2 structural domain 4A (SH2D4A) at site 8p21.3. This protein has a SH2 structural domain that is very similar to the T cell-specific adapter protein and the adapter protein of unknown function and has tyrosine residues with potential sites for phosphorylation of LCK protein tyrosine kinase [5]. It has been demonstrated that these proteins control the transduction of T cell
receptor (TCR) signaling [5]. In the HEK cell line of the human embryonic kidney, SH2D4A has been shown to inhibit cell proliferation [6]. SH2D4A also plays different roles in different human cancers, and previous studies have reported the validation of the impact of SH2D4A as an oncogene. Hepatocellular carcinoma usually results in the deletion and downregulation of six gene clusters on chromosome 8p, including SH2D4A, and low expression of these genes is connected to a worse prognosis for HCC patients [7, 8]. According to a recent study on the effects of SH2D4A downregulation in colon cancer, the downregulation of several genes on chromosome 8p, including SH2D4A, promotes carcinogenesis and creates an immunocompromised cold tumor microenvironment and a poor prognosis [9].

TIICs (Tumor infiltrating immune cells) can promote or regulate tumor progression and growth through cell types and their interactions, which is a development in molecular studies. There has been some advancement in the understanding of immune cell infiltration in CNS malignancies. However, little is known about its role in the origin of tumors and patient prognosis. CD8+ T-cell enrichment is related to glioma hypermutation at diagnosis or recurrence. Notably, following radiation therapy, glioma short-term recurrence is similarly linked to M2 macrophages [10]. It has been demonstrated that glioma-associated macrophages/monocytes (GAMPs) promote glioma growth and invasion by acting as tumor-supporting cells that can infiltrate gliomas from the circulation [11].

Few studies have examined SH2D4A in GBM, and there is little data available. We systematically investigated the relationship between SH2D4A and GBM prognosis and validated it using resources from different databases. The unique and complex immune microenvironment of gliomas is an important obstacle to immunotherapy [12]. Therefore, in order to increase the effectiveness of immunotherapy, we will look at the relationship between SH2D4A and immune cells that infiltrate GBM. TCGA and CGGA were used to gather our data. We employed the CIBERSORT, xCELL, and TIMER/TIMER2.0 to measure the density of large tumor-infiltrating immune cells in various tumor microenvironments.

## Results

### Relationship between the prognosis of GBM and SH2D4A expression

Based on the TCGA database, we first analyzed the differentially expressed genes of mRNAs in GBM. The analysis showed that there were 6765 differentially expressed genes, including 3413 up-regulated genes and 3352 down-regulated genes, as shown in the generated volcano plot (Fig.1d). Using the GEPIA database, we looked at the SH2D4A expression pattern in GBM tissues. We found that the expression level of SH2D4A was significantly greater in GBM tissues than in normal peritumor tissues (Fig.1b), and interestingly, upregulation of SH2D4A was observed in highly aggressive GBM but not in low-grade glioma compared with normal brain tissues (Fig.1b). We examined the connection between SH2D4A expression and pan-cancer in the TIMER2.0 database to verify the outcomes of the GEPIA database study and further investigate the link between SH2D4A expression and GBM. We confirmed that SH2D4A expression was upregulated in GBM, renal small pigmented cell carcinoma, cervical adenocarcinoma and cervical squamous cell carcinoma, thyroid cancer, and gastric cancer. In contrast, SH2D4A expression
pattern was decreased in head and neck squamous cell carcinoma, renal clear cell carcinoma, and lung squamous carcinoma (Fig.1a).

Thus, we found that SH2D4A is significantly upregulated in GBM and has prognostic value for GBM. We verified the differential expression between GBM (U87, T98G) and normal human astrocytes HA1800 cells by qRT-PCR analysis (Fig.1c). Relative to HA1800 cells, SH2D4A was upregulated in GBM cells, especially in the U87 cell line. In conclusion, these findings imply that SH2D4A promotes malignancy in GBM.

**The clinical and prognostic significance of SH2D4A expression according to the CGGA database**

We conducted an online study of the CGGA database's mRNA expression data (mRNAseq 325array) and the accompanying clinicopathological characteristics to further evaluate the predictive value for GBM. We discovered that SH2D4A was considerably up-regulated in GBM (Fig.2a-e). Interestingly, consistent with the GEPIA and TCGA results, GBM patients with high SH2D4A expression had poorer survival outcomes (Fig.3a-d), with shorter survival times corresponding to patients with upregulated SH2D4A expression in WHO grades III and IV (p < 0.05). Additionally, there was a substantial correlation between SH2D4A differential expression and age, 1p19q deletion status, and IDH mutation (Fig.2a-e). The above results suggest that SH2D4A is differentially expressed in gliomas and may be a potential biomarker for GBM progression.

**SH2D4A Co-expression network**

The GeneMANIA website was used to identify functionally similar genes and build a PPI network by entering the specific gene we needed, SH2D4A, with 19 functionally similar genes located in the outer loop and hub genes in the inner loop (Fig.4a). Based on the STRING database, the PPI network of these crossover genes was constructed, and the 10 hub genes identified included SH2D4A, SH3GL1, LYN, PPP1CB, PPP1R7, PPP1CA, DTD1, GRB2, DBNL, and HCLS1 (Fig.4b). The co-expression pattern of SH2D4A in the TCGA cohort was studied utilizing the functional module of LinkedOmics in order to comprehend the biological significance of SH2D4A in TCGA. Based on RNAseq, we screened 19660 genes associated with SH2D4A (false discovery rate (FDR) <0.01) (Fig.4c). Two heatmaps displaying the top 50 significant genes related with SH2D4A both negatively and positively are provided (Fig.4d-e).

**SH2D4A acted as an independent risk factor of poor prognosis in GBM patients**

With lasso regression and the “glmnet” R package, we ran a screening on the 107 samples of TCGA mRNA sequencing data. The change in trajectory for each variable is plotted in (Fig.5a), and (Fig. 5c) displays the confidence range for each. SH2D4A expression was identified as a risk factor affecting the prognosis of GBM patients using correlation analysis utilizing one-way cox regression Table1. In a multifactorial Cox analysis, SH2D4A expression and radiation status were independent predictor variables (Fig.6b,Table1). Based on the median risk score for each cohort, samples from the TCGA cohort were split into low-risk and high-risk categories. According to KM analysis, patients in the low-risk category had better outcomes than those in the high-risk group (Fig.5b).
In the TCGA dataset, we developed a nomogram based on age, sex, radiation status, and risk score to predict 1-year, 2-year, and 3-year OS. Each factor's score in the nomogram was determined by how much risk it posed to OS (Fig. 6a). For the 1-year OS rate in the TCGA cohort, calibration curves revealed a significant agreement between predicted survival time and actual survival time (Fig. 6c). An AUC area of 0.72 is found for the anticipated time-dependent 1-year survival nomograms (Fig. 6d), and additional pertinent clinical investigations are required to confirm the validity of this nomogram.

**SH2D4A-related signaling pathways in glioblastoma**

To further investigate the biological role of SH2D4A in GBM, GSEA was performed (Fig. 7c). The results showed a range of functions of the highly expressed region of SH2D4A, including extracellular matrix organization, cytokine-mediated signaling pathways. The 2 KEGG items, neuroactive ligand-receptor interactions, protein digestion and uptake, showed significant enrichment differences in the SH2D4A high expression phenotype (Fig. 7b). We performed GO analysis including BP, MF and CC (Fig. 7a). GO analysis showed BP was significantly enriched in epidermal growth and extracellular matrix tissue, CC analysis showed enrichment of collagen containing extra cellular matrix, MF enrichment to DNA binding transcription repressor activity, RNA polymerase II-specific and extracellular matrix structural constituent.

**Relationship between immune cells that invade tumors and SH2D4A expression**

We were interested in determining if immune infiltration in GBM is correlated with SH2D4A expression. Using the CIBERSORT method, we first looked at the immune infiltration of 22 immune cell subpopulations in GBM tissue. The fraction of immune cells in each GBM sample is depicted in Fig. 8a using various colors, and the immune cell population is represented by the length of the bars in the bar graph. According to the graphs, GBM tissues had comparatively large concentrations of M0, M1 and M2 macrophages and monocytes. Then, using the R package, we displayed the correlation heat map to identify the correlation between the above 8 hub genes and 21 immune cells (Fig. 8b). It shown a positive correlation between SH2D4A and the expression of T cells with activated CD4 memories and a negative correlation between SH2D4A and activated NK cells. In order to determine the association between SH2D4A and other stromal cells, we calculated the levels of 64 immune cells using the xCELL algorithm (Fig. 8c). The findings revealed that SH2D4A expression correlated with th1 cells, neurons, plasma cells, megakaryocytes, and eosinophils.

We examined the relationship between SH2D4A expression and the degree of immunological infiltration using TIMER as well. Our findings demonstrated that higher levels of SH2D4A expression were linked to a worse prognosis and compromised immune response in GBM. Additionally, there was a negative correlation between the levels of SH2D4A expression and B-cell infiltration \((r = -0.094, p=5.50e-02)\), CD4\(^+\) T cells \((r = -0.116, p=1.79e-02)\), CD8\(^+\) T cells \((r = -0.046, p=3.52e-01)\), Macrophage \((r = -0.081, p=1.00e-01)\), Neutrophil \((r = -0.192, p=7.87e-05)\) and DCs \((r = 0.314, p=5.01e-11)\) (Fig. 9a). Following that, the univariate Cox survival analysis were performed using the TIMER data. According to the findings of the univariate analysis, dendritic cells and SH2D4A had an impact on the prognosis for GBM patients' survival (Fig. 9b).
Furthermore, the degree of immune infiltration of GBM did not seem to be correlated with variations in SH2D4A copy number (Fig.9c). The significance of SH2D4A in the immunological infiltration of Dendritic Cells is clearly supported by our findings.

## Discussion

The median survival of GBM patients is 15 months, with a 5-year survival rate of less than 5%, and current drugs do not significantly improve the prognosis of patients \[13\]. Therefore, to increase the overall survival of GBM patients, successful identification of critical chemicals for GBM growth and tumor resistance is crucial.

Many cancerous tissues, including the thyroid, stomach, kidney, and brain, can express SH2D4A. Our knowledge of the literature on SH2D4A's impact on GBM's poor prognosis is limited. So, in order to investigate the potential function of SH2D4A in GBM, we first examined SH2D4A expression in GBM patients. We observed that SH2D4A was substantially expressed in GBM tissues in our investigation using information from the GEPIA database, indicating that SH2D4A may be a reliable biomarker for determining the prognosis of GBM patients.

According to multifactorial studies, the expression of SH2D4A in GBM patients represents a distinct prognostic factor. As a prospective cancer biomarker, SH2D4A expression patterns were observed to be linked with survival outcomes in the current investigation. We were able to reaffirm SH2D4A's prognostic significance in GBM and the correlation between tumor grade and SH2D4A mRNA expression by using data from the CGGA database.

Additionally, our study demonstrated a relationship between SH2D4A expression and various degrees of immune infiltration in GBM. So, we propose that SH2D4A may potentially affect tumor immunology. T cells, some of which have been shown to respond to immune checkpoint inhibition and their survival, have traditionally been the focus of analysis of the involvement of TIIC in human malignancies \[14, 15\]. It's interesting that SH2D4A was connected to the control and invasion of cytotoxic T cells \[16\]. Therefore, T-cell density may be connected to the negative effects of SH2D4A on GBM.

We draw the conclusion that SH2D4A expression may be a molecular indicator of poor prognosis in GBM patients. Furthermore, the extracellular matrix's structure and interactions with neuroactive ligand receptors may be a crucial mechanism for GBM that is controlled by SH2D4A. The findings also imply that high SH2D4A mRNA expression may be used as an independent prognostic factor to help GBM patients have a better clinical outcome. Bioinformatic techniques were used exclusively on public databases (TCGA and CGGA) to analyze and confirm the association between SH2D4A expression and prognosis, and our study will carry out additional in vivo and in vitro experiments to gradually strengthen the evidence for the biological impact of SH2D4A \[17\].

## Materials And Methods
Data sets and data collection

Clinical data and TCGA RNA-seq transcriptome data were retrieved from The Cancer Genome Atlas (TCGA) database [18] (http://cancergenome.nih.gov/). The Chinese Glioma Genome Atlas (CGGA) database [19] (http://www.CGGA.org.cn) was used to collect the mRNA expression data (mRNAseq 325) and associated clinicopathological characteristics. In order to formalize the pooled gene numbers into gene symbols for analysis, we utilized Perl [20] scripts to acquire the GTEx data from UCSC Xena (http://Xena.UCSC.edu/). The R software was used to process the raw data, and robust multi-array analysis was used to normalize and compensate for the backdrop.

Expression analysis by GEPIA

An online database called Gene Expression Profile Interaction Analysis (GEPIA) [21] (http://GEPIA.cancer-pku.cn/index.html) is utilized to confirm the relationship between clinicopathological data and SH2D4A expression in GBM. For the purpose of determining the differential expression of SH2D4A, we created box plots using the disease status (tumor or normal) as a variable.

Cell Culture

T98G, U87 and HA1800 cell lines were purchased from the Shanghai Cell Bank, Type Culture Collection Committee, Chinese Academy of Sciences. Cells were cultured using DMEM (WISENT, Canada) supplemented with 10% fetal bovine serum (WISENT, Canada). All cell lines were stored in a humidified atmosphere containing 5% CO2 at 37°C.

PPI network analysis

GeneMANIA [22] (http://genemania.org/) was used to predict functionally comparable genes of hub genes and build PPI networks once SH2D4A was added to its database. Additionally, it makes predictions about the relationships, pathways, physiological and biochemical responses, co-expression, and co-localization between functionally related genes and the main gene. In order to examine the interaction network of hub gene-encoded proteins, the resulting hub genes were added to the STRING database (version 11.5, https://string-db.org/). The necessary minimal interaction score was set to 0.4 (medium confidence level).

LinkedOmics analysis

For the analysis of multivariate histology data for 32 different cancer types, visit LinkedOmics (http://www.linkedomics.org/) [23]. The co-expression of the SH2D4A gene was examined using Pearson correlations, and the results were displayed as a volcano and a heat map.

Independent prognostic role of the risk signature

Univariate and multivariate Cox regression analyses were carried out, the outcomes of independent predictor analyses were presented as forest plots, and patients were divided into high- and low-risk groups based on median risk scores in order to ascertain whether the risk profile associated with high
SH2D4A expression was dependent on other clinicopathological factors (such as age, gender, and radiotherapy) that predicted patient OS. This was followed by a log-rank tests and then a Kaplan-Meier overall survival (OS) analysis. Utilizing the R program, the aforementioned analyses were all completed. When p < 0.05, differences were statistically significant.

**Development and assessment of the nomogram**

The nomogram generate custom predictive models that show the likelihood of clinical events using simple graphs of statistical predictive models. The nomogram was drawn using R-pack “survival” and “rms”, and it included scores for age, sex, radiation status, and risk associated with SH2D4A. Using calibration curves to assess the accuracy of nomogram in predicting one-year, two-year, and three-year survival in patients with glioma. The more closely the calibration curves' projected and actual curves coincide, the greater their predictive value for the nomogram. Analysis of the subject operating characteristic (ROC) curve was done to evaluate the impact of the prediction model.

**Functional enrichment analysis**

Gene Set Enrichment Analysis (GSEA) is a computational technique for evaluating whether there are consistently different biological states between the two states and the statistical significance of a collection of genes selected a priori [24]. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and gene ontology (GO) biological processes related to risk factors were evaluated using the “GSVA” package in R. We identified the various biological processes that were enriched in the high- and low-risk groups by comparing the differences in scores across risk groups after the GSVA software assessed GO biological processes and KEGG pathways in each sample. To find differentially expressed genes and gene sets in the various groups, the "limma" package in R was utilized. The "clusterProfiler" package in R was used to conduct GO and KEGG analysis of differentially expressed genes in order to further validate the feature-related KEGG pathways and GO processes.

**Assessing the number of tumour-infiltrating immune cells using TIMER/TIMER2.0**

We employ the Tumor Immunity Estimation Resource (TIMER/TIMER2.0) [25, 26] as a complete resource on the investigation of the immune infiltration system in various cancer types (https://cistrome.shinyapps.io/timer/). TIMER2.0 utilized a previously announced statistical technique known as deconvolution, which estimates the amount of immune cells that have infiltrated tumors(TIICs) using gene expression profiling [27]. The abundance of 6 tumor-infiltrating immune cell subtypes (B cells, CD4+ T cells, CD8+ T cells, macrophages, neutrophils, and DCs) and the expression of SH2D4A were analyzed and visualized.

**xCORE Analysis and Cell Type Identification by Estimating Relative Subsets of RNA Transcripts (CIBERSORT) Analysis**
An R package called "xCell" calculates integrated levels for 64 different cell types, including 14 stromal cells. Using CIBERSORT, each sample's abundance scores for 21 different immune cell types could be precisely quantified (T cells gamma delt information was missing in 22 immune cell types in this study). To determine the ratio of stromal cell and immune cell abundance in GBM samples separately, we used xCELL and CIBERSORT.

**Real-Time qRT-PCR**

Using qRT-PCR, the relative mRNA expression of SH2D4A was evaluated. TRIzol (Invitrogen) was used to extract total RNA from GBM cell lines. RNA was reverse transcribed into cDNA using a reverse transcription kit (Vazyme, China). PCR reactions were performed using ChamQ Universal SYBR qPCR Master Mix (Vazyme, China) and ABI 7500 qPCR instrument (Applied Biosystems, Carlsbad, CA, USA) were performed. Quantitative PCR was performed on an using SYBR Green. The relative expression of genes was calculated by the 2-ΔΔCt method. The primers used for the experiments were SH2D4A, forward: 5′-AGAAAGGAAGGCGGTGAGAGG-3′, reverse: 5′-ACTGAGGCTTGGGTGGAAGGG-3′, with ACTIN as the internal reference, forward: 5′-CCTGGCACCCAGCACAAT-3′, reverse: 5′ GGGCCGGACTCGTCATAC-3′.

**Statistical analysis by R-4.2.1**

The TCGA statistics were combined and run through R 4.2.1. The R package "ggplot2" was used to create the DEG volcano plot. Cox regression was used to examine correlations between clinical data and SH2D4A expression. In order to determine the impact of SH2D4A expression and other clinicopathological variables (age, sex, radiation) on survival, multifactorial and univariate Cox regression analyses were performed. The cut-off value was set at p < 0.05.

**Declarations**

**Acknowledgements**

None.

**Author contributions**

†Tian Yang and Zhiyou Sun contributed equally to this work. Tian Yang participated in the design of this study, Tian Yang, Zhiyou Sun and Chujun Li drafted the manuscript text. Kexin Cheng, Hongwei Ma, Yanhong Ren prepared all figures and tables. Zhengkui Zhang and Rutong Yu contributed to the critical revision of the manuscript. All authors reviewed the manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors provided consent for publication.

Competing interests

The authors declare that they have no conflicts of interest.

References


Table 1

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<th>Clinical characteristics</th>
<th>HR(95%CI)</th>
<th>p-Value</th>
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<td>1.59 (1.03,2.47)</td>
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<tr>
<td>gender</td>
<td>1.06 (0.67,1.69)</td>
<td>0.804</td>
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<tr>
<td>radiation therapy</td>
<td>0.21 (0.11,0.42)</td>
<td>&lt; 0.001</td>
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<td>age</td>
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<td>0.022</td>
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<tr>
<td>SH2D4A</td>
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<td>0.004</td>
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<tr>
<td>b.</td>
<td></td>
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<tr>
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<tr>
<td>SH2D4A</td>
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a. Utilizing Cox regression, associations with overall survival and clinicopathologic characteristics were found in TCGA patients. b. Cox regression for multivariate survival.

Figures
Figure 1

Levels of SH2D4A expression in GBM. Using TIMER2.0, (a) compares the expression of SH2D4A mRNA in pan-cancer malignant and paracancerous tissues. (b) In GBM, SH2D4A expression levels were noticeably greater than those of normal controls (p<0.05). (c) Verification of SH2D4A expression in GBM cell lines using qRT-PCR. The P values are labeled using asterisks (ns, no significance, *p< 0.05, ***p< 0.001). (d) Valcano plot of the TCGA dataset’s variously expressed RNA (Blue: downregulated expression; Red: upregulated expression).
Figure 2

Correlation study between clinical characteristics and SH2D4A expression. Analysis of the relationships between SH2D4A expression and several clinical characteristics. (a) WHO grade, (b) IDH mutation status, (c) 1p19q codeletion status, (d) gender, (e) age.
Figure 3

The relationship between the expression level of SH2D4A and the survival rate in the CGGA dataset. (a) ALL WHO grade, (b) WHO grade , c WHO grade  d WHO grade
Figure 4

The genes that SH2D4A co-expresses with in GBM. (a) Examination of 21 hub genes. (b) 10 hub genes form the PPI network. In the TCGA cohort based on LinkedOmics, (c) SH2D4A mRNA was significantly linked with genes identified by the pearson test. (d-e) Heatmaps of the top 50 genes in TCGA based on LinkedOmics that are negatively and positively linked with SH2D4A.
Figure 5

The prognostic risk model’s construction. (a) Profiles of Lasso coefficients from the TCGA dataset. (b) Risk scores, survival time, and survival status in the TCGA dataset. From low to high risk scores are represented in the scatterplot at the top. At the bottom are the survival times and survival statuses that correlate to the various risk scores of the samples. (c) Selection of the optimal parameter (Lambda) in the Lasso model.
Figure 6

Creating and approving a nomogram survival model. (a) Based on factors such as age, gender, risk, and radiation therapy, the nomogram plot was created. (b) Forest plot of the TCGA cohort’s multivariate Cox regression analyses. (c) Nomogram calibration plot based on TCGA data. (d) Time dependent ROC curves for the prediction of the 1-, 3-, and 5-year survival rate.
Figure 7

Bioinformatic analysis of TCGA database. (a) GO and (b) KEGG analysis of DEGs. (c) GSEA analysis. (c) The GSEA analysis showed that genes with high expression in the SH2D4A phenotype were differentially enriched in the extracellular matrix tissue and cytokine mediated signaling pathways.
Figure 8

Immune infiltration analysis in relation to SH2D4A. (a) The lengths of the bars in the bar chart show the levels of the immune cell populations, and the percentage of immune cells in each GBM sample are denoted by various colors. (b) Hub gene expression and immune cell expression are correlated. (c) A correlation matrix for the proportions of 64 immune cells.
**Figure 9**

In GBM, SH2D4A is connected to immune cell infiltration. (a) The association between immune infiltration and SH2D4A. (b) Survival curve for immune-cell invasion. (c) The association between SH2D4A copy number variation and the degree of immune cell infiltration.