

Translational Insights into Cancer-Associated Fibroblast Infiltration-Related Biomarkers in Glioblastoma and Their Clinical Prognostic Value

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Purpose: Cancer-associated fibroblasts (CAFs) could promote the progression and migration of tumors. However, the roles of CAFs infiltration related genes in glioblastoma (GBM) were still unclear.

Methods: GBM-related transcriptome data and clinical information were downloaded from the UCSC Xena and CGGA databases. In this study, the abundance of fibroblasts were calculated by “MCPcounter”, and the CAFs infiltration related genes were identified by “WGCNA”. Then, the biomarkers of GBM were screened out, and based on it, the survival risk model (risk score) and the prognostic model (nomogram) were constructed to clinical predict the survival of GBM. Moreover, the function and mutation analyses were performed to further study the mechanisms of GBM. Furthermore, the competitive endogenous RNAs (ceRNA) regulatory network were used to reveal the potential regulation of biomarkers.

Results: Totals of 46 CAFs infiltration related genes were associated with focal adhesion. Four biomarkers, including STC1, BDKRB2, SOCS3 and FURIN were identified, and all of them were negative factors. Nomogram constructed based on risk scores and clinical indicators had good predictive performance (AUC > 0.68). Noticeable, COL5A1 might be the key gene, which were extremely positively associated with all these four biomarkers. Besides, the genes in high-risk groups were associated with the functions of epithelial mesenchymal transition (EMT) and angiogenesis. In addition, hsa-miR-107 could regulate STC1 through the TGF- β signaling pathway and further regulating the migration and invasion of tumour.

Conclusion: This study identified four CAFs infiltration related biomarkers associated with the prognosis of GBM. This finding might help to deepen the understanding the roles of CAFs in development of GBM.

Keywords: glioblastoma, cancer-associated fibroblasts, biomarkers, survival risk model, prognosis

Introduction

Glioblastoma (GBM) is the most prevalent primary malignant brain tumor, characterized by elevated recurrence and death rates.¹ Despite rigorous multimodal treatment, including surgical resection, chemotherapy, radiation, and targeted therapy, the overall median survival of GBM patients is still less than 2 years.^{2,3} GBM may expedite disease development by enhancing the release of pro-angiogenic factors, resulting in localized vascular growth, stasis, and thromboembolic blockage, hence worsening hypoxia inside the tumor.⁴ The processes of angiogenesis, cell migration, and infiltration not only facilitate the development of drug resistance but also make the efficient treatment of GBM very challenging.⁵ The tumor microenvironment of GBM is very complex and continuously evolving, where the overexpression of immune checkpoints serves as a critical mechanism for tumor cells to effectively avoid immune monitoring.⁶ Recent findings indicate that IGFBP3 may activate the JAK2/STAT3 signaling pathway, promoting death of Jurkat cells via the up-regulation of PD-L1 expression, resulting in immune evasion of GBM.⁷ The regular use of high-dose corticosteroids to mitigate cerebral edema may concurrently inhibit the anti-tumor immune response.⁴ Moreover, prolonged administration of anti-angiogenic agents like bevacizumab may exacerbate tumor hypoxia and promote the development of a more

infiltrative tumor phenotype, thus complicating the treatment landscape.⁸ Consequently, a thorough examination of the molecular pathogenesis of GBM, along with the identification of novel biomarkers and therapeutic targets, is critically significant for the advancement of more precise and effective treatment strategies aimed at enhancing the prognosis and quality of life for GBM patients.

Cancer-associated fibroblasts (CAFs) are activated fibroblasts, which are numerous and diverse stromal cells in the tumour microenvironment and are a prominent stromal component of many kinds of malignancies, including pancreatic, breast, and lung cancers.^{9,10} A increasing amount of data demonstrates that CAFs play a vital role in tumourigenesis and development.¹¹ CAFs facilitate extracellular matrix formation and remodeling, intimately interact with cancer cells to enhance their proliferation and migration and contribute to angiogenesis and inflammation via cytokine release.^{12,13} Ana Costa et al shown that cancer-associated fibroblasts (CAFs) facilitate cancer growth by circumventing immune monitoring and confer resistance to immunotherapy.¹⁴ Like other stroma-rich malignancies, GBM has a complex tumor microenvironment, characterized by a combination of tumor and non-tumor cells, including immune cells, vascular cells, and extracellular matrix components.¹⁵ Anne Clavreul et al conducted experimental tests revealing that markers linked to cancer-associated fibroblasts, including fibroblast surface proteins, α -smooth muscle actin, and platelet-derived growth factor receptor- β , were present in glioblastoma-associated stromal cells.¹⁶ Petr Busek et al indicate that the presence of stromal cells exhibiting a mesenchymal phenotype, which express markers of cancer-associated fibroblasts (CAFs) in the glioblastoma (GBM) tumor microenvironment, corresponds with a poor prognosis in GBM.¹⁷ Manuel Röhrich et al facilitated tumor proliferation and angiogenesis in human glioblastoma mice by the implantation of fibroblast activating protein.¹⁸ Moreover, interactions among diverse cell types in glioblastoma multiforme (GBM) influence the immunosuppressive tumor microenvironment, thereby diminishing the effectiveness of immunotherapy. This phenomenon appears to be associated with cancer-associated fibroblasts (CAFs) enhancing immunotherapy resistance by circumventing immune surveillance; however, the precise relationship between these factors remains ambiguous.²

This study aimed to identify biomarkers associated with CAFs infiltration that influence the prognosis and treatment of GBM patients. It sought to develop a novel CAFs infiltration-related risk model as a dependable tool for predicting clinical outcomes, thereby guiding clinical treatment strategies for GBM. Additionally, it aimed to investigate the potential mechanisms by which CAFs modulate GBM, enhancing the understanding of the disease.

Materials and Methods

Data Source

The RNA sequencing data, survival information, and clinical details of GBM were collected from two sources: the UCSC Xena database and the Chinese Glioma Genome Atlas (CGGA) database. Out of these, the UCSC Xena-GBM dataset was utilized as the training dataset, comprising 154 GBM samples with survival and clinical data. To ensure the availability of a risk survival model, the CGGA-GBM dataset (CGGA-mRNAseq-325), which contains 85 GBM samples with survival and clinical data, was utilized as the validation dataset.

Identification and Functional Enrichment Analyses of the CAF Infiltration Related Genes

In this study, the abundance of eight immune cells and two stromal cells (fibroblasts and endothelial cells) were calculated by “MCP-counter”.¹⁹ Then, the samples in UCSC Xena-GBM dataset were divided into high and low CAFs infiltration groups according the infiltration level of fibroblasts by “survminer” R package (version 3.3.5). On the other hand, the stromal score was calculated by “estimate” R package.²⁰ Based on it, the co-expression network was constructed by “WGCNA” R package (version 1.70–3), and the CAFs infiltration and stromal scores were used as the traits to screen the module genes which related to CAFs infiltration.²¹ Besides, the functional enrichment analysis of these CAFs infiltration related genes was conducted by “clusterProfiler” R package (adj.p. value < 0.05).^{22,23}

Construction of the Survival Risk Model and Prognostic Model of GBM

Univariate Cox regression analysis ($HR \neq 1$ and $p < 0.05$) was performed using the R package survival (version 3.2–13) based on the expression of CAFs infiltration related genes in the training set, which was screened to obtain prognosis related genes. Subsequently, least absolute shrinkage and selection operator (LASSO) regression analysis was performed on prognosis-related genes, and the optimal lambda value was determined by 10-fold cross validation, so as to select prognosis genes whose regression coefficients were not penalised to zero. The risk score was calculated by the algorithm: $risk\ scores = \sum_{i=1}^n COEfi \times Xi$ (COEfi is the LASSO risk coefficient of prognostic genes, and Xi is the gene expression of prognostic genes in the sample). Then, the tumour tissue samples of GBM patients with survival information in the training dataset were classified into two groups, high risk and low risk, based on the median risk score. To assess the reliability of the survival risk model, the Kaplan–Meier (K-M) survival curve and risk curve were plotted. In addition, the CGGA-GBM dataset, an external validation set, was used to confirm that this survival risk model was applicable. In addition, we used “Spearman” to evaluate the associations between biomarkers and the expressions of genes relevant to CAFs infiltration across various risk categories.

The “Wilcoxon” test was used to evaluate risk scores by clinical parameters (age, gender, tumor status, and radiation). A nomogram was created using cox analysis’ prognostic clinical variables. To test the prognostic model, the calibration curve and ROC curve of the nomogram were produced.

Mutation and Function Analyse in Different Risk Groups

The somatic mutation between different risk groups were compared by “TCGAbiolinks” R package) (version 0.3.0).^{24,25} Then, the differences in risk scores between different clinical indicators (age (≤ 60 and > 60), gender (male and female), tumor status, (tumor free and with tumor), radiotherapy (yes and no)) were analysed by Wilcoxon test and box plots were drawn. Next, the tumor mutation burden (TMB) were computed, and the correlation between TMB and risk score was examined by “Spearman” analysis.

Moreover, the gene set enrichment analysis (GSEA) were performed to study the function of genes in different risk groups by “clusterProfiler” R packages. On the other hand, the epithelial mesenchymal transition (EMT) score, TGF- β signaling score and angiogenesis score were calculated based on the 200 EMT-related genes, 54 TGF- β signaling-related genes and 36 angiogenesis-related genes by “ssGSEA” algorithm, respectively.²⁶ The correlations between risk score and these three scores was assessed by “Spearman”.

Construction of Competitive Endogenous RNAs (ceRNA) Regulatory Network

The miRNet, mirDIP and StarBase databases were used to estimate the matching miRNAs of model genes, and the targeted miRNAs were obtained by intersecting these three groups of predicted miRNAs. After that, the miRNet, mircode and StarBase databases were utilized to predict the lncRNA that corresponded to the targeted miRNA, similarly, the targeted lncRNAs were obtained by intersecting these three groups of predicted lncRNAs. Then, the ceRNA regulatory network was constructed by “Cytoscape” (version 3.8.2).²⁷

In vitro Validation of the BDKRB2 Gene

Cell Culture and Cell Transfection

Human glioblastoma U87 cells were acquired from iCell Bioscience located in Shanghai, China. U87 cells were grown in DMEM medium (Gibco, USA) supplemented with 10% FBS (Gibco, USA). Overexpressing lentivirus (oe-BDKRB2) of BDKRB2 and the empty control virus (oe-BDKRB2) were acquired from Shanghai Mega. U87 cells were infected with lentivirus, then screened in puromycin-containing media, and transfection efficiency was confirmed with PCR analysis. Table 1 lists the primers in the study.

Table 1 Primers for RT-PCR

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
BDKRB2	GTCTGTTTCGTGAGGACTCCG	AAAGGTCCCGTTAAGAGTGGG

Wound Healing Assay

Evaluation of cellular migratory potential with the Wound Healing Assay To evaluate the migratory potential of U87 cells, procedures were conducted as outlined in prior research after 0 hours and 24 hours of culture, and the migration extent of U87 was examined microscopically (OLYMPUS, Japan).

Transwell

Matrigel (Corning, USA) was thawed at 4°C. Matrigel was diluted with serum-free cell culture media at a 1:8 ratio on ice and gradually introduced into Transwell chambers, which were thereafter incubated at 37°C in a 5% CO₂ environment. 100 µL of serum-free media was dispensed into each well, and cells were injected at a density of 1000 cells per well. Five hundred microliters of medium with 10% FBS were introduced into the bottom chamber, while 100 to 200 microliters of cell suspension were placed in the top chamber and incubated in the incubator. Transwell staining was performed with crystal violet, and the cells were examined microscopically and enumerated post-staining.

Statistical Analysis

A minimum of three replications were used to assess the biological experiments in SPSS 25. The independent samples *t*-test was used to compare the two groups, with a *p*-value of less than 0.05 being deemed statistically significant.

Results

46 CAF Infiltration Related Genes

In this study, the K-M results indicated that there were significant differences between different CAFs infiltration and stromal score groups ($p < 0.05$) (Figure 1A and B). Sample clustering analysis was implemented and the results showed that there were six outlier samples need to cull (Figure 1C). When the optimal soft threshold value was identified as 8, the network was close to scale-free distribution (Figure 1D). Totals of 17 modules were identified, and the correlation analysis results showed that the MEcyan module had a significantly positive correlation with CAFs infiltration score ($\text{cor} = 0.76$, $p = 2e-29$) (Figure 1E and F). Then, a total of 46 CAFs infiltration related genes in the MEcyan module with $\text{MM} > 0.7$ and $\text{GS} > 0.4$ were screened for subsequent analysis (Figure 1G and Supplementary Table 1).

As the perspective of function, these 46 CAFs infiltration related genes were mainly enriched in endoplasmic reticulum lumen, focal adhesion, integrin binding and growth factor binding etc. 231 Gene Ontology (GO) functions. Besides, these genes were associated with focal adhesion, PI3K-Akt signaling pathway, ECM-receptor interaction etc. 16 Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways (Figure 1H, Supplementary Tables 2 and 3).

4 Biomarkers Were Used to Construct the Survival Risk Model

In this study, four biomarkers, including STC1, BDKRB2, SOCS3 and FURIN were identified, and all of them were negative factors (Hazard Ratio > 1) of GBM (Figure 2A and B). Then, the risk score was calculated and the high- and low-risk groups were divided. The risk curve and K-M curve showed substantial survival differences across risk groups ($p < 0.05$) (Figure 2C and D). The validation dataset risk curve and K-M curve matched the training dataset (Figure 2E and F). These findings showed that this survival risk model might be utilized to build a GBM prognostic model.

Construction of the Nomogram in GBM

Besides, we founded that most of the CAFs infiltration related genes were up-regulated in high-risk group (Figure 3A). Moreover, four biomarkers had positive correlations with COL5A1, ACTA2, COLIA2 etc. Genes, and had negative correlations with ZEB1, CDK2, SCRG1 etc. genes. Among them, COL5A1 might be the key gene, which was extremely positively associated with all these four biomarkers ($p < 0.0001$) (Figure 3B).

Furthermore, three factors including age, tumor status and risk score that associated with prognosis were screened, and all of them were negatively associated with patient survival (Figure 3C and D). The nomogram with these three prognostic factors was constructed (Figure 3E). The calibration curve of the nomogram showed that the slopes of 0.5-, 1.5-, and 2.5-year survival rate close to 1, and the area under ROC curve (AUC value) of 0.5-, 1.5-, 2.5-year were greater 0.6 (0.693, 0.686 and 0.714), all these results indicated that the nomogram could be used as an effective prognostic model of GBM (Figure 3F and G).

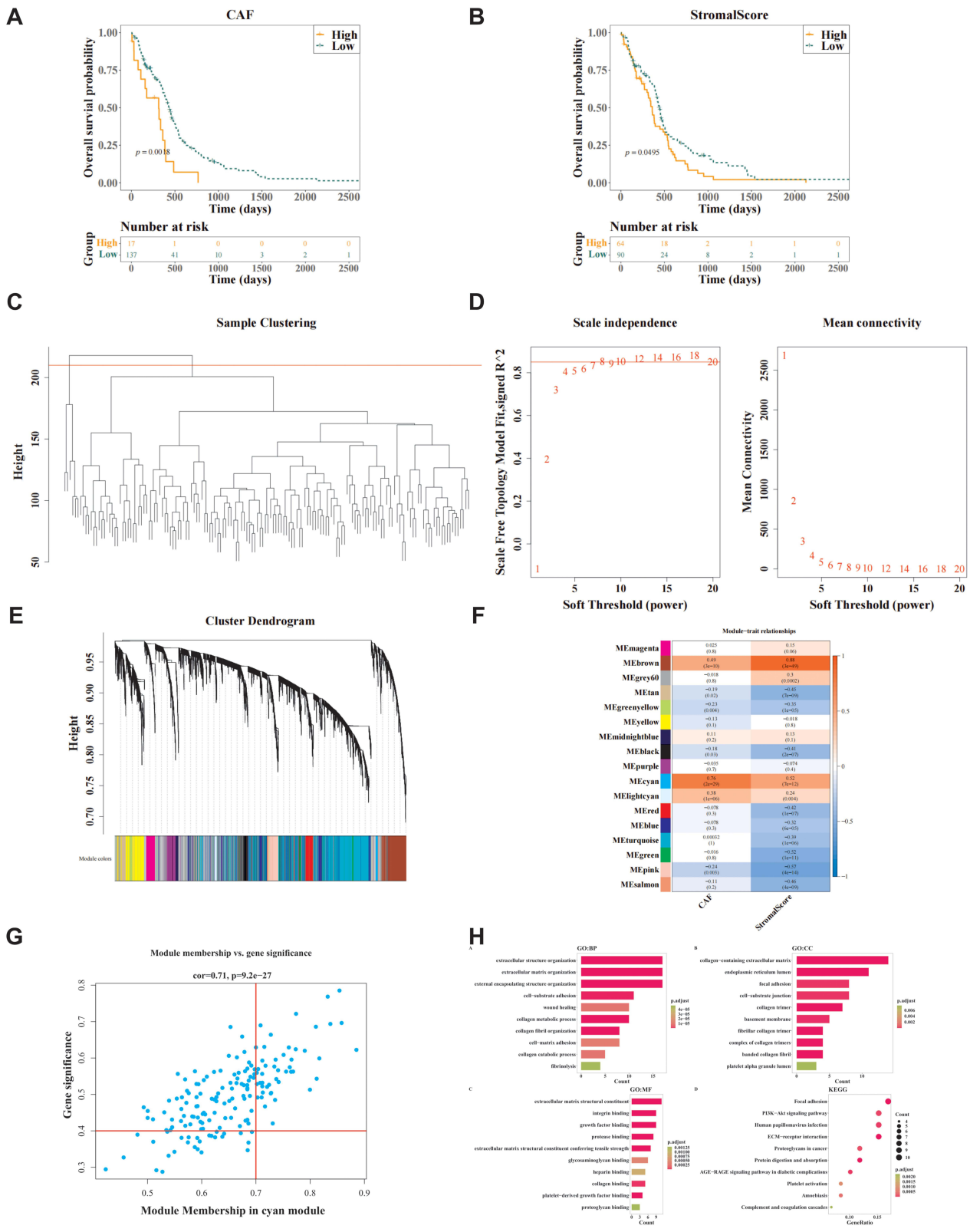


Figure 1 Identification and functional enrichment analyses of the CAF infiltration related genes. **(A)** High and low CAF infiltration groups; **(B)** High and low stromal score groups; **(C)** Screening of outlier samples. **(D)** The soft-thresholding power (β) was selected based on the scale-free topology criterion in TCGA-GBM. **(E)** Clustering dendrograms showing each gene with similar expression patterns were clustered into co-expression modules in TCGA-GBM **(F)** Module-trait relationships revealing the correlations between each gene module eigengene and phenotype in TCGA-GBM. **(G)** The horizontal axis is the correlation between the gene and co-expression module, and the vertical axis is the correlation between the gene and phenotype. **(H)** GO and KEGG analyses.

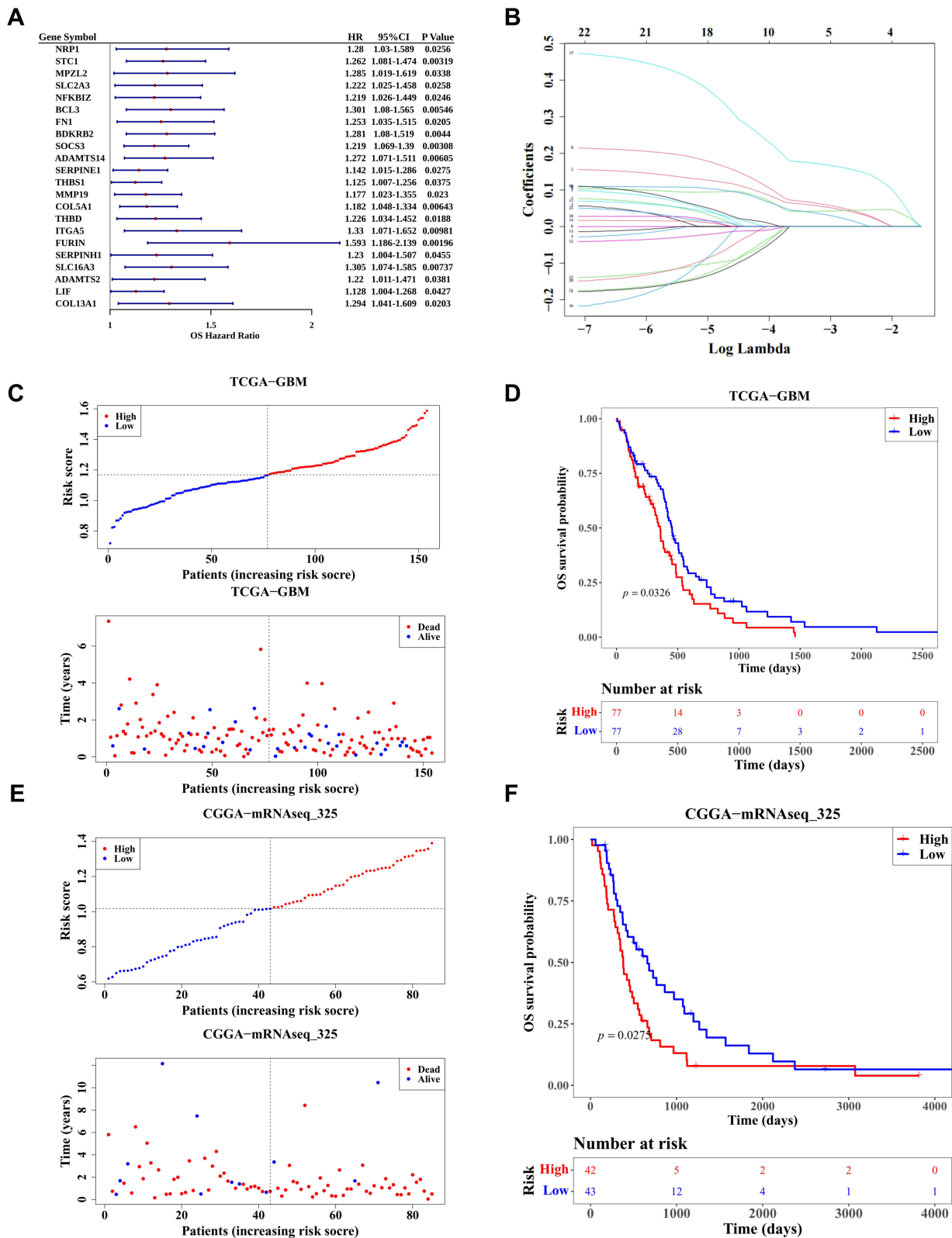


Figure 2 Survival risk model of 4 biomarkers. **(A)** Univariate cox analysis for the screening of overall survival-associated genes in TCGA-GBM; **(B)** Coefficient profiles of LASSO cox regression analysis, and the adjustment parameter (λ) was calculated based on the partial likelihood deviance with ten-fold cross validation. **(C)** The distribution of risk scores and survival states in TCGA-GBM cohort. **(D)** Kaplan-Meier analysis in TCGA-GBM cohort. **(E)** The distribution of risk scores and survival states in CGGA-mRNAseq-325 cohort. **(F)** Kaplan-Meier analysis in CGGA-mRNAseq-325 cohort.

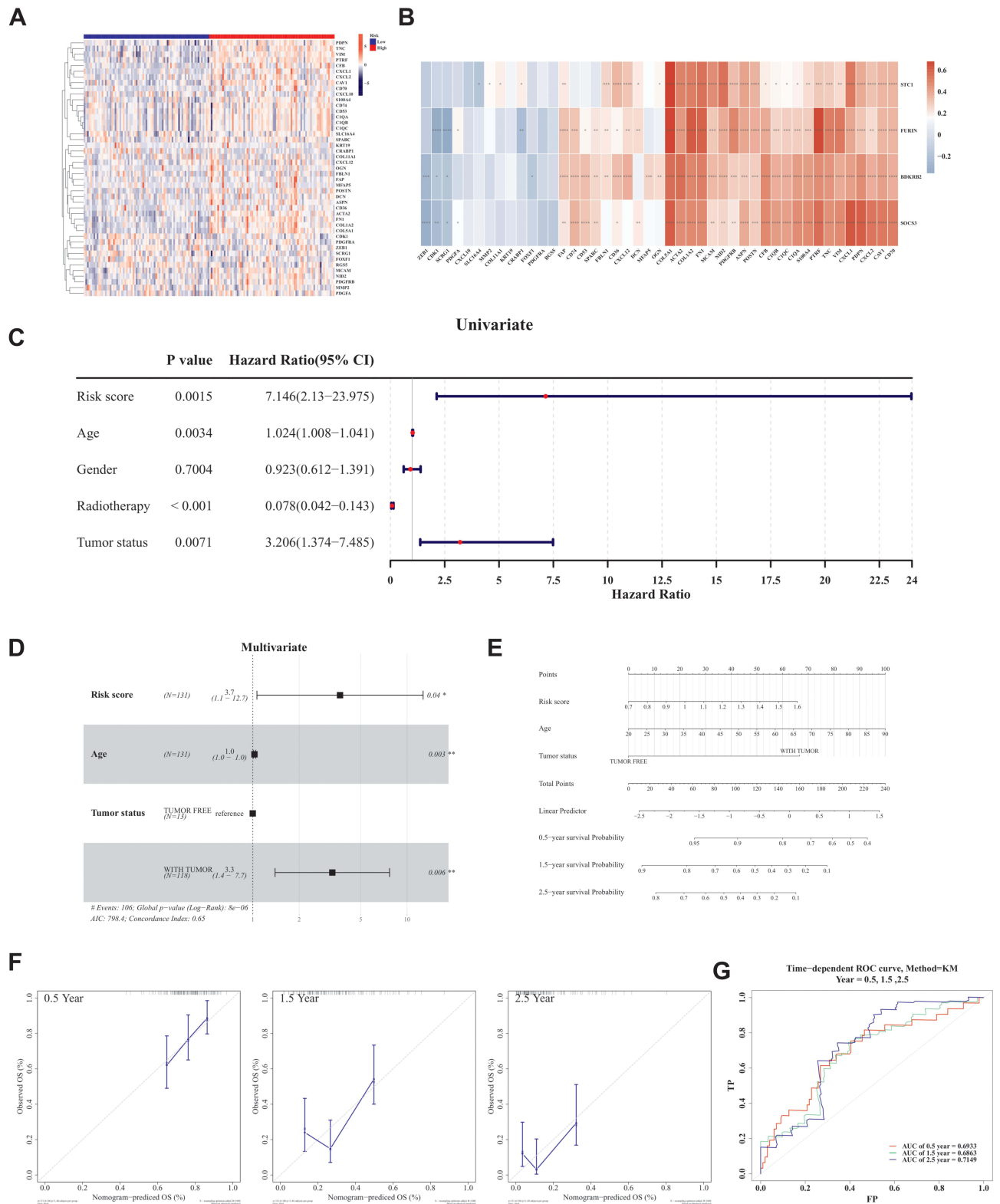


Figure 3 Construction of the nomogram in GBM. **(A)** The heatmap revealing the expression patterns of CAF markers in TCGA-GBM cohorts. **(B)** The correlations between 4 signature genes and CAF markers in TCGA-GBM cohort. **(C)** Univariate Cox regression analysis of the risk scores and clinical parameters. **(D)** Multivariate Cox regression analysis of the risk scores and clinical parameters. **(E)** The nomogram was constructed based on three independent prognostic factors. **(F)** The calibration plots of the nomogram predicting 0.5-year, 1.5-year and 2.5-year OS. **(G)** ROC curve of the Nomogram in TCGA-GBM.

The EMT and Angiogenesis Functions Played a Vital Role in the Progress of GBM

The GSEA results showed that the chemokine signaling pathway, cytokine-cytokine receptor interaction, ECM receptor interaction etc. pathways were significantly highly enriched in high-risk group, and the oxidative phosphorylation etc. pathways were significantly highly enriched in low-risk group (Figure 4A and B, Supplementary Table 4). Furthermore, risk scores did not differ significantly in clinical traits such as age, gender, radiotherapy and tumour status (Supplementary Figure 1). However, the risk score was significantly positively correlated with EMT, TGF- β , and angiogenesis scores (Figure 4C–E).

In addition, it was worth noting that there was a significant difference in mutation of PTEN in high-risk group (mutation rate = 38%), and a significant difference in mutation of TP53 in low-risk group (mutation rate = 42%) (Figure 5A and B). Besides, the risk score was significantly negatively correlated with the TMB ($Cor = -0.18, p = 0.028$) (Figure 5C and D).

CeRNA Network Construction

In this study, we identified 61 targeted miRNAs and 83 targeted lncRNAs (Figure 6A and B). Then, the ceRNA regulatory network was constructed with 3 mRNAs, 10 miRNAs and 32 lncRNAs (Figure 6C). In this network, hsa-miR-107 and hsa-miR-17-5p were the key targeted miRNAs, which could regulate *FURIN* and *STC1* at the same time. Notably, the *KCNQ1OT1* could competitively conjugated with 11 targeted miRNAs, *NEAT1* could competitively conjugated with eight targeted miRNAs, *XIST* could competitively conjugated with eight targeted miRNAs, *MALAT1* could competitively conjugated with four targeted miRNAs, *PVT1* could competitively conjugated with four targeted miRNAs, *MCM3AP-AS1* could competitively conjugated with three targeted miRNAs, and further regulating *FURIN*, *STC1* and *SOCS3*.

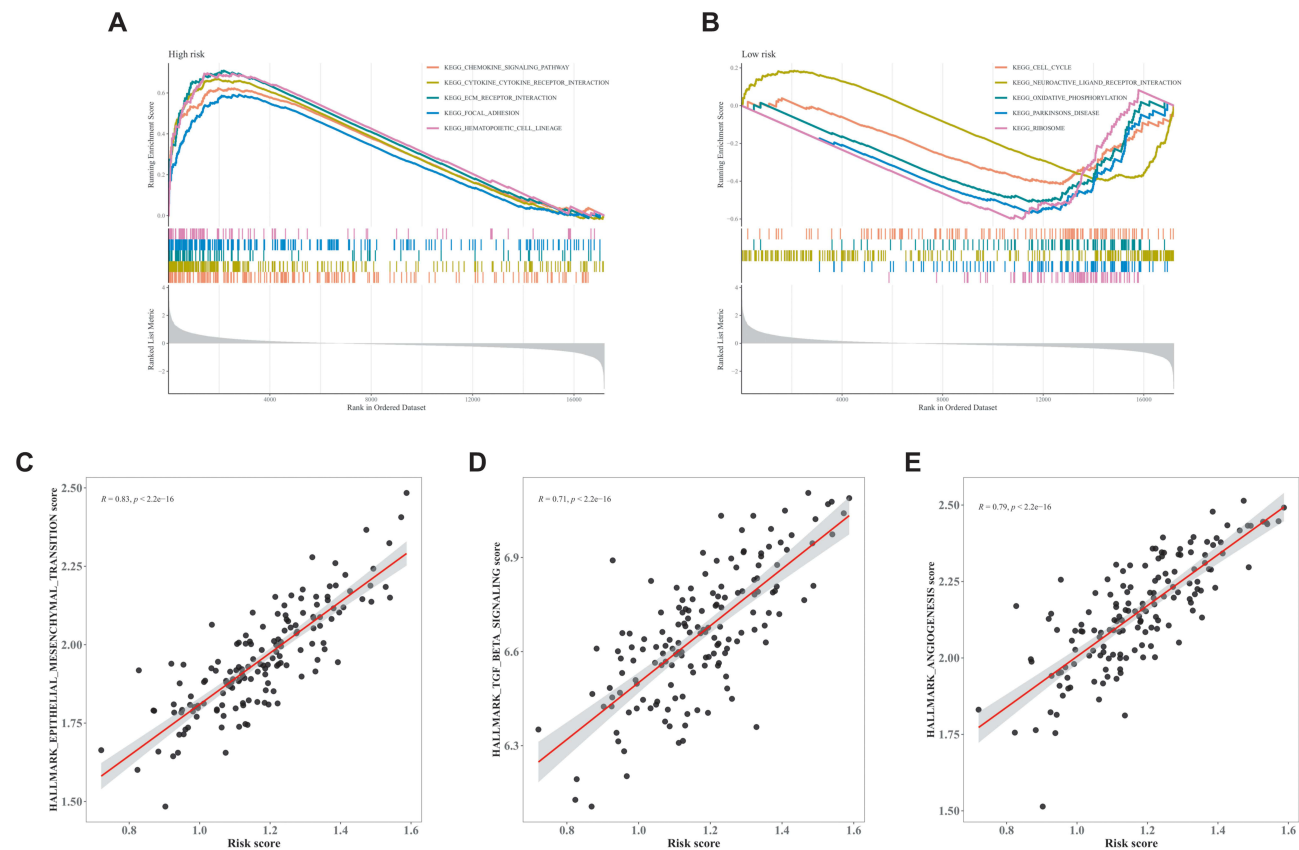


Figure 4 The GSEA analyse. (A and B) Gene set enrichment analysis (GSEA) of KEGG gene sets between high-and low-risk groups. (C–E) the correlation analyses between risk score and EMT, TGF- β and angiogenesis enrichment scores in TCGA-GBM.

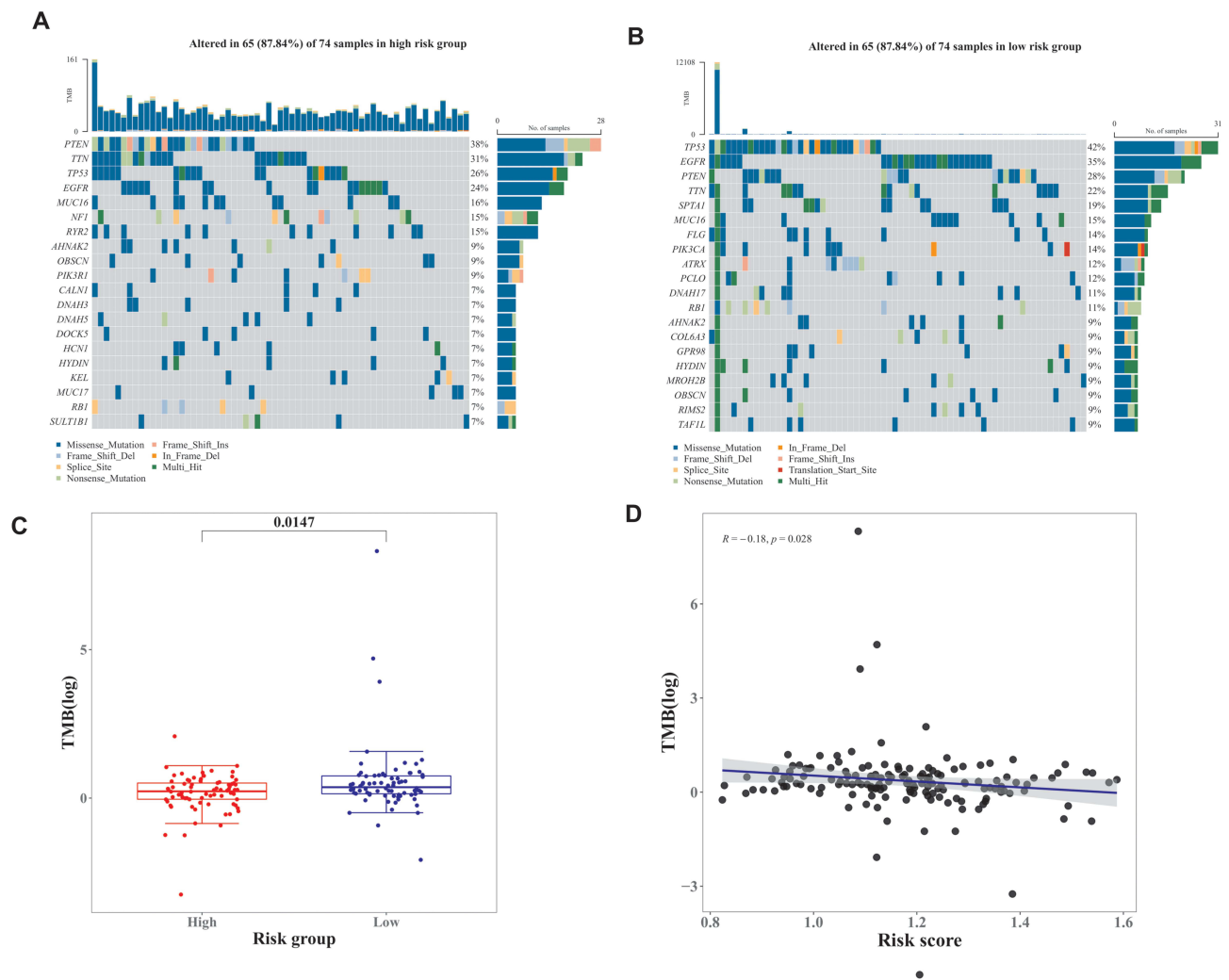


Figure 5 Gene mutation analysis. **(A and B)** Oncoplots depicting the top 20 mutational genes in high- **(A)** and low- **(B)** risk groups of TCGA-GBM. **(C)** The boxplot of TMB values in low- and high- risk group. **(D)** Spearman correlation analyses of risk score and TMB value.

BDKRB2 Gene Function Experiment

As shown in **Figure 7A**, qRT-PCR confirmed successful transfection of U87 human glioma cells. **Figure 7B and C** illustrate that the cell scratch experiment and the transwell assay, respectively, demonstrated that overexpressing the BDKRB2 gene promoted cell healing and cell invasion, respectively.

Discussion

CAFs facilitate the creation and remodeling of the extracellular matrix, engage intimately with cancer cells, and enhance their proliferation and migration.¹² Mesenchymal phenotype stromal cells expressing CAF markers in GBM are associated with tumor development and angiogenesis in GBM.¹⁷ This demonstrates a significant connection between GBM and CAFs. In this study, we initially assessed the negative prognostic significance of cancer-associated fibroblasts (CAFs) in glioblastoma (GBM) by analyzing CAF infiltration levels within the GBM tumor microenvironment. Our findings indicated that patients with high CAF infiltration exhibited a markedly reduced survival probability compared to those with low CAF infiltration, corroborating previous research.²⁸

This research revealed 46 infiltration-associated genes related to CAFs, mostly implicated in pathways including focal adhesion, the PI3K-Akt signaling pathway, and ECM receptor interaction. A substantial body of research demonstrates that focal adhesion has a role in all phases of cancer development, is linked to cell invasion and migration and is crucial

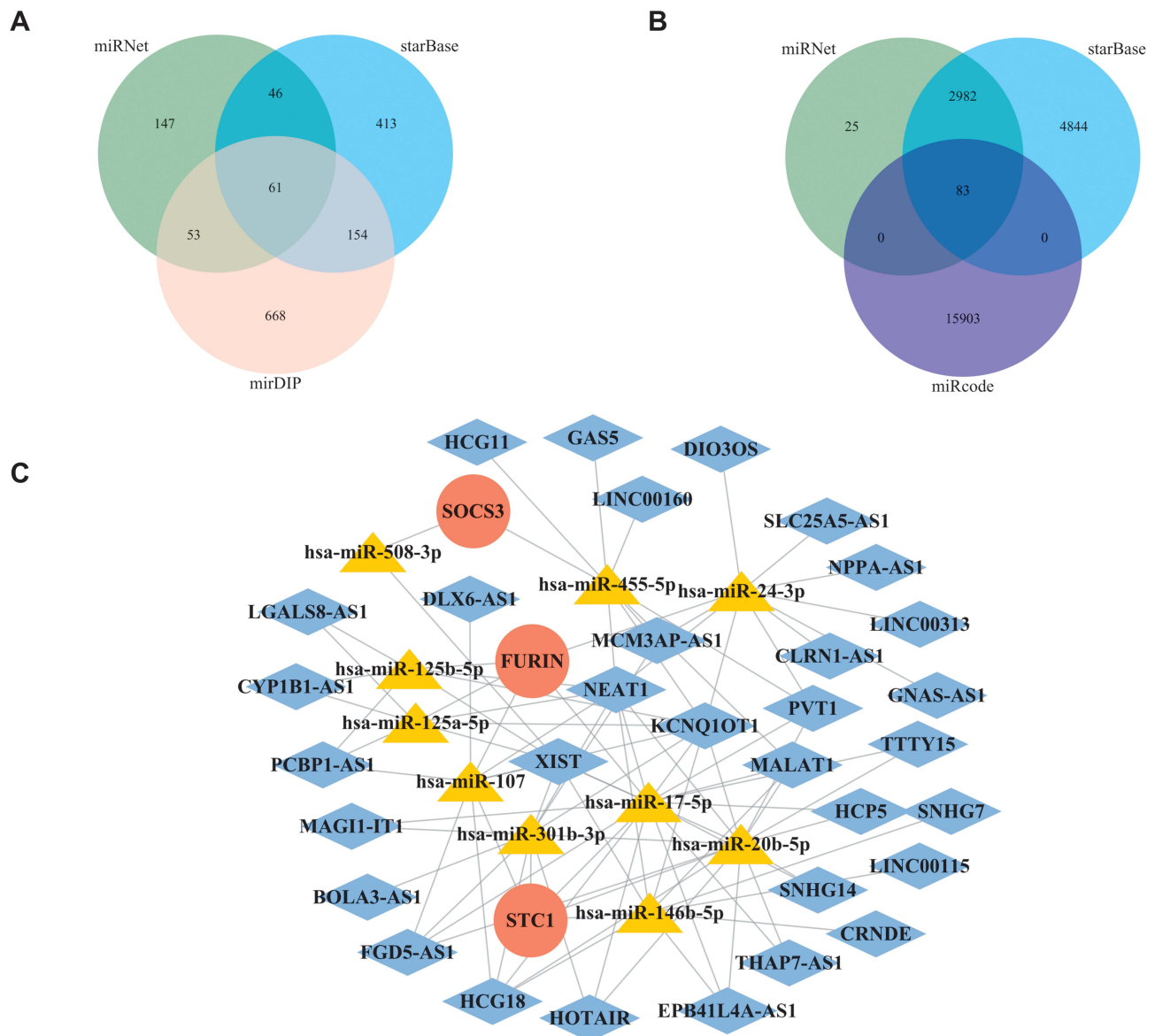


Figure 6 CeRNA network. (A and B) The prediction of miRNA and lncRNA. (C) CeRNA network construction.

for cancer cells to sustain their survival and modify their local microenvironment. Interactions between the extracellular matrix, cell adhesion, proliferation, and migration within the tumor microenvironment are fundamentally connected to cancer pathways.^{29,30} Gabler L has asserted that the primary function of fibroblast growth factor in facilitating glioblastoma is cell invasion.³¹ The hyperactivation of the PI3K-Akt signaling pathway is a crucial mechanism in the survival, growth, and proliferation of several tumors, including glioblastoma multiforme (GBM).³² This route has been shown to be linked to processes of GBM-specific radioresistance.³³ We propose that the genes associated with CAF infiltration facilitate the progression of GBM by influencing the tumor microenvironment and augmenting cellular proliferation, migration, and invasion primarily via the activation of signaling pathways such as PI3K-Akt.

We identified four CAFs infiltration-related markers linked to the prognosis of GBM patients: BDKRB2, FURIN, SOCS3, and STC1, all of which serve as poor prognostic indicators for GBM. We subsequently developed a risk model utilizing these four biomarkers, categorizing GBM patients into high- and low-risk groups. Functional analyses revealed a significant positive correlation between the risk scores and the epithelial-mesenchymal transition (EMT) signaling pathway, the TGF- β signaling pathway, and the angiogenic pathway. BDKRB2 has been extensively documented in

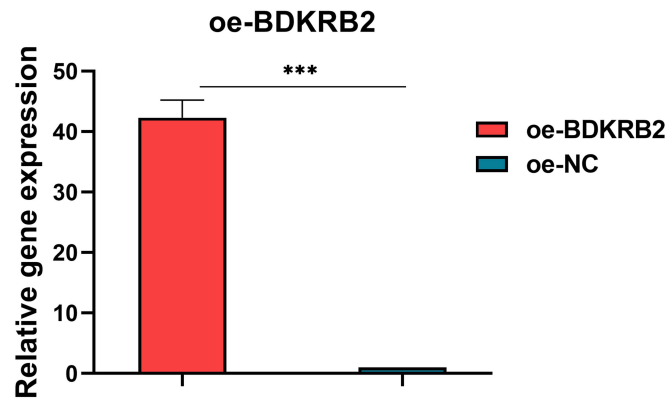
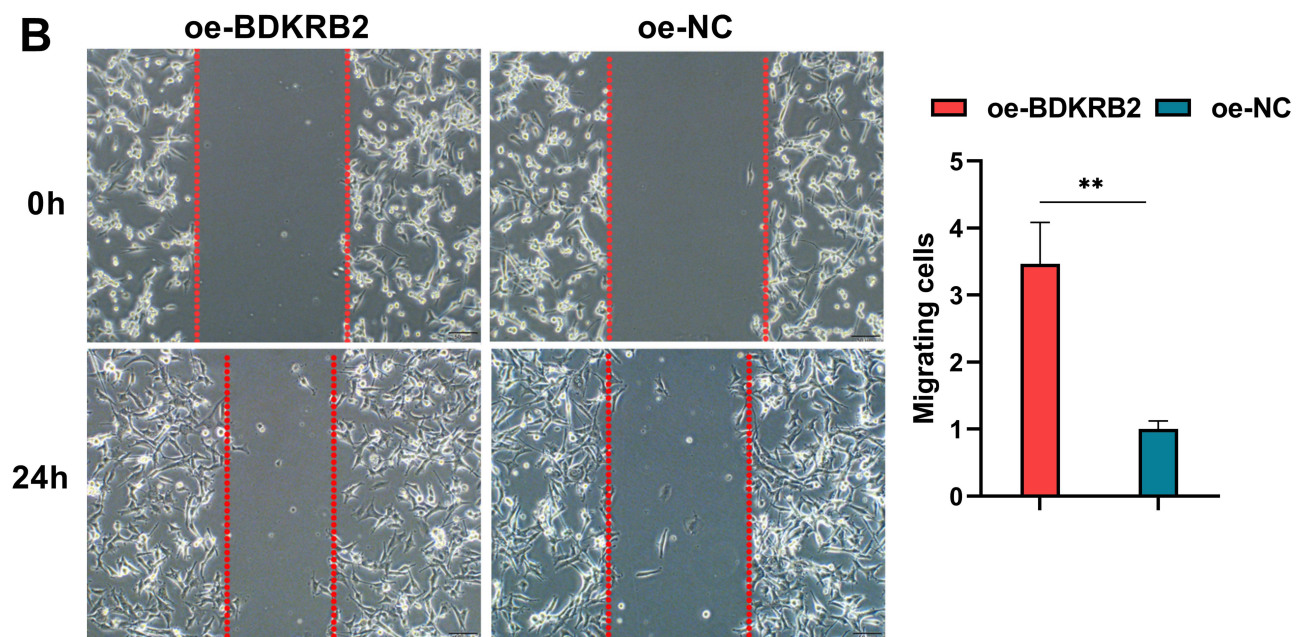
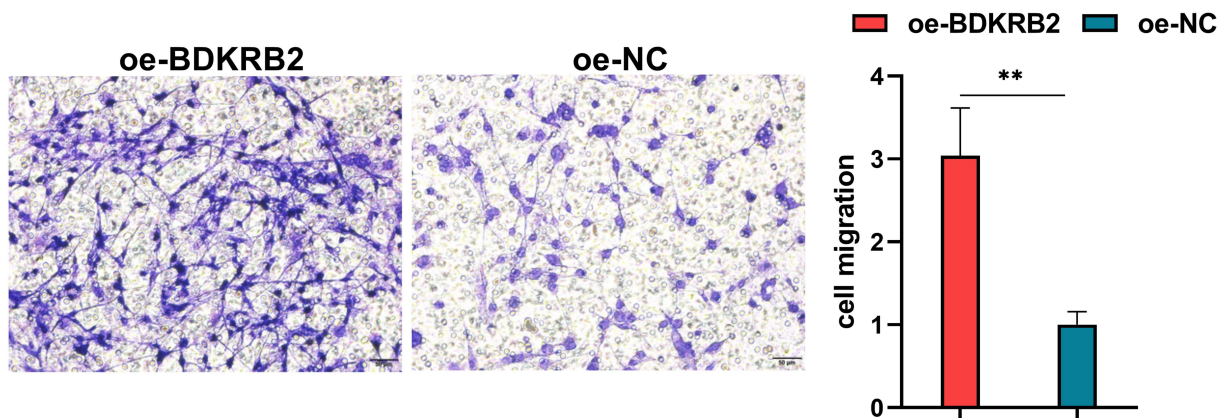
A**B****C**

Figure 7 BDKRB2 Gene Function Experiment. (A) PCR experiments show high staining efficiency of BDKRB2. (B) Wound Healing Assay and (C) transwell showed that decreased BDKRB2 overexpression contributed to increased migration and invasiveness of U87 cells. ** $p < 0.01$, *** $p < 0.001$.

many malignant neoplasms, including cervical cancer, breast cancer, hepatocellular carcinoma, and squamous cell carcinoma of the head and neck.^{34–36} In several malignancies, BDKRB2 functions as an oncogene, facilitating tumor growth by enhancing cell proliferation, migration, and angiogenesis.^{37,38} Ying Yang et al identified BDKRB2 as a new biomarker linked with epithelial-mesenchymal transition (EMT) that predicts poor survival in gliomas.³⁹ We hypothesize that BDKRB2 may facilitate GBM growth by stimulating the EMT signaling pathway. Andrea Shergalis et al found 20 genes, including FURIN, that are overexpressed in glioblastoma patients by bioinformatics analysis and correlate with worse survival outcomes.⁴⁰ FURIN has a tumorigenic effect in glioma starting cells by facilitating the activation of TGF β 1 and TGF β 2. Furthermore,⁴¹ FURIN has been linked to various tumor progression phenomena; it has been documented as overexpressed in thyroid cancer patients, correlating significantly with aggressive clinicopathological features and unfavorable outcomes. Additionally, FURIN is implicated in the activation of the Notch signaling pathway, which facilitates hepatic metastasis in breast cancer.⁴² Mercapide et al also noted that in astrocytoma cells, inhibition of FURIN reduced cell proliferation and invasiveness.⁴³ The worse prognosis of FURIN in GBM may be linked to invasion. SOCS3 was downregulated in the majority of TCGA cancer datasets but had high expression in brain tumors, breast cancer, esophageal cancer, colorectal cancer, and lymphomas. Yeuni Yu et al identified SOCS3 as a significant biomarker for GBM, linked to the proliferation of neural tissue.⁴⁴ Lirui Dai et al confirmed its elevated expression in LGG and GBM by immunohistochemistry and discovered that SOCS3 contributes to cancer progression primarily via the JAK/STAT signaling pathway and cytokine receptor activation.⁴⁵ New research by Shuhua Zheng et al demonstrates that the expression level of SOCS3 correlates with immunotherapy or chemotherapy across various cancer types, identifying SOCS3 as a possible predictive biomarker for anti-angiogenic treatment.⁴⁶ Our work indicated a substantial correlation between the risk score model and the angiogenic pathway, with SOCS3 potentially playing a crucial role in this relationship. Sakata J et al indicated that the life duration of GBM patients exhibiting elevated STC1 levels was markedly reduced compared to those with diminished STC1 levels, as determined using survival analysis.⁴⁷ Yan Xiong et al assessed the mRNA expression level of STC1 in GBM via quantitative PCR and discovered that its expression was elevated and significantly associated with poor outcomes in GBM patients.⁴⁸ They also demonstrated that STC1 modulates the migration and invasion of GBM through the TGF- β signaling pathway, as evidenced by transfection experiments. Our research indicated that the risk score model correlates with the TGF- β signaling pathway. In our study, we found significant positive correlations between COL5A1 and four biomarkers among 46 infiltration-associated genes associated with CAFs. COL5A1 is a regulatory fibre-forming collagen that has been associated with breast, gastric and other cancerous diseases.⁴⁹ It was found that down-regulation of COL5A1 in vitro inhibited the proliferation and migration of glioma cells and enhanced their sensitivity to temozolomide,⁵⁰ and overexpression of COL5A1 in the LN-229 cell line enhanced the migration and invasive ability of the cells, suggesting that COL5A1 may be associated with the migration of GBM.⁵¹ Our study indicated that overexpression of the BDKRB2 gene promoted cell healing and cell invasion. Notably, there was a significant positive correlation between BDKRB2 and COL5A1, and we speculate that there may be some synergistic effect between the two in regulating glioma cell behaviour. However, the specific mechanism of action between them still requires further in-depth study.

Finally, we established a CeRNA network including 3 biomarkers, 10 miRNAs, and 32 lncRNAs.⁵² Has-miR-107, situated at the core of the network, can modulate both FURIN and STC1. It has been documented to participate in the TGF- β signaling pathway and influence prostate cancer progression, indicating that has-miR-107 may regulate STC1 and affect GBM migration and invasion via the TGF- β signaling pathway.

This work found four CAFs-related biomarkers that influence cell adhesion and invasion, impacting GBM advancement primarily via the activation of the EMT signaling route, the TGF- β signaling pathway, and the angiogenic pathway. Nonetheless, this research has several drawbacks. Initially, confounding variables (eg, age, treatment method, etc) were not accounted for in the investigation, thereby compromising the accuracy of the findings. Future stratified analyses might be conducted to mitigate the impact of these confounding factors and validate the reliability of the results. Secondly, although our work investigated the function of the BDKRB2 gene in human glioblastoma cells by cell scratch and transwell assays, we regrettably did not pursue further investigation and confirmation of other potentially relevant biomarkers. Moreover, some key discoveries in this work are based on predictions derived from bioinformatics analysis, which provide significant insights but need definitive experimental validation. Consequently, further comprehensive and

stringent experimental investigations, including gene knockdown, overexpression assays, and animal model studies, are required to further corroborate and enhance the existing results.

Conclusion

In this research, four biomarkers associated with CAF infiltration were screened and a prognostic model of GBM was established, which not only provides a novel perspective for clinical diagnosis but also reveals the potential targets for patient prognosis and points out the direction for the development of novel drugs. More importantly, this model is expected to assist physicians in clinical practice to more accurately assess the disease progression and prognosis of patients and thus enable the personalisation of treatment plans. In addition, this study also predicted the upstream miRNA regulation of these biomarkers, thus deepening the understanding of the molecular mechanism of GBM. In summary, this study not only enriches the molecular mechanism of GBM but also provides new perspectives for its clinical diagnosis and treatment. We expect that future studies will further validate these findings and promote their translation to clinical applications.

Data Sharing Statement

The TCGA count (<https://portal.gdc.cancer.gov>) and the CGGA database (<http://www.cgga.org.cn/>) both provide online access to the datasets that were used in this research experiment.

Ethics Statement

According to the Measures for Ethical Review of Life Science and Medical Research Involving Human Beings (February 2023 Edition), jointly issued by many departments of the People's Republic of China. Article 32: Human information data or biological samples used to conduct life sciences and medical research involving humans may be exempt from ethical review if no harm is caused, sensitive personal information is not involved, or commercial interests are not involved. This reduces unnecessary burdens on scientific researchers and promotes human research. (1) Research using legally obtained public data or observational data; (4) Research using human cell lines from the biological sample bank, within the provider's authorization, and avoiding the use of human germ cells, embryos, reproductive cloning, chimerism, etc. The study complied with local legal requirements and ethical exemptions were available.

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Disclosure

The authors declare no conflicts of interest in this work.

References

1. Bai RY, Staedtke V, Riggins GJ. Molecular targeting of glioblastoma: drug discovery and therapies. *Trends Mol Med.* 2011;17(6):301–312. doi:10.1016/j.molmed.2011.01.011
2. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005;352(10):987–996.
3. Li D, Hu J, Li S, et al. LINC01393, a novel long non-coding RNA, promotes the cell proliferation, migration and invasion through MiR-128-3p/NUSAP1 axis in glioblastoma. *Int J mol Sci.* 2023;24(6):5878. doi:10.3390/ijms24065878
4. Oronsky B, Reid TR, Oronsky A, Sandhu N, Knox SJ. A review of newly diagnosed glioblastoma. *Front Oncol.* 2020;10:574012. doi:10.3389/fonc.2020.574012
5. Nazzaro JM, Neuwelt EA. The role of surgery in the management of supratentorial intermediate and high-grade astrocytomas in adults. *J Neurosurg.* 1990;73(3):331–344. doi:10.3171/jns.1990.73.3.0331
6. Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. *Cell.* 2015;161(2):205–214. doi:10.1016/j.cell.2015.03.030
7. Zhao L, Wang Y, Mu P, et al. IGFBP3 induces PD-L1 expression to promote glioblastoma immune evasion. *Cancer Cell Int.* 2024;24(1):60. doi:10.1186/s12935-024-03234-3
8. Iwamoto FM, Abrey LE, Beal K, et al. Patterns of relapse and prognosis after bevacizumab failure in recurrent glioblastoma. *Neurology.* 2009;73(15):1200–1206. doi:10.1212/WNL.0b013e3181bc0184
9. Ligorio M, Sil S, Malagon-Lopez J, et al. Stromal microenvironment shapes the intratumoral architecture of pancreatic cancer. *Cell.* 2019;178(1):160–175.e27. doi:10.1016/j.cell.2019.05.012

10. Scherz-Shouval R, Santagata S, Mendillo ML, et al. The reprogramming of tumor stroma by HSF1 is a potent enabler of malignancy. *Cell*. 2014;158(3):564–578. doi:10.1016/j.cell.2014.05.045
11. Su S, Chen J, Yao H, et al. CD10(+)/GPR77(+) cancer-associated fibroblasts promote cancer formation and chemoresistance by sustaining cancer stemness. *Cell*. 2018;172(4):841–856.e16. doi:10.1016/j.cell.2018.01.009
12. Sahai E, Astsaturov I, Cukierman E, et al. A framework for advancing our understanding of cancer-associated fibroblasts. *Nat Rev Cancer*. 2020;20(3):174–186. doi:10.1038/s41568-019-0238-1
13. Turley SJ, Cremasco V, Astarita JL. Immunological hallmarks of stromal cells in the tumour microenvironment. *Nat Rev Immunol*. 2015;15(11):669–682. doi:10.1038/nri3902
14. Costa A, Kieffer Y, Scholer-Dahirel A, et al. Fibroblast heterogeneity and immunosuppressive environment in human breast cancer. *Cancer Cell*. 2018;33(3):463–479.e10. doi:10.1016/j.ccell.2018.01.011
15. Lukas RV, Wainwright DA, Ladomersky E, Sachdev S, Sonabend AM, Stupp R. Newly diagnosed glioblastoma: a review on clinical management. *Oncology*. 2019;33(3):91–100.
16. Clavreul A, Etcheverry A, Chassevent A, et al. Isolation of a new cell population in the glioblastoma microenvironment. *J Neurooncol*. 2012;106(3):493–504. doi:10.1007/s11060-011-0701-7
17. Busek P, Balaziová E, Matrasova I, et al. Fibroblast activation protein alpha is expressed by transformed and stromal cells and is associated with mesenchymal features in glioblastoma. *Tumour Biol*. 2016;37(10):13961–13971. doi:10.1007/s13277-016-5274-9
18. Röhrich M, Loktev A, Wefers AK, et al. IDH-wildtype glioblastomas and grade III/IV IDH-mutant gliomas show elevated tracer uptake in fibroblast activation protein-specific PET/CT. *Eur J Nucl Med Mol Imaging*. 2019;46(12):2569–2580. doi:10.1007/s00259-019-04444-y
19. Aran D, Hu Z, Butte AJ. xCell: digitally portraying the tissue cellular heterogeneity landscape. *Genome Biol*. 2017;18(1):220. doi:10.1186/s13059-017-1349-1
20. Yoshihara K, Shahmoradgoli M, Martínez E, et al. Inferring tumour purity and stromal and immune cell admixture from expression data. *Nat Commun*. 2013;4(1):2612. doi:10.1038/ncomms3612
21. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinf*. 2008;9:559.
22. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS*. 2012;16(5):284–287. doi:10.1089/omi.2011.0118
23. Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*. 2000;28(1):27–30. doi:10.1093/nar/28.1.27
24. Colaprico A, Silva TC, Olsen C, et al. TCGAAbiolinks: an R/Bioconductor package for integrative analysis of TCGA data. *Nucleic Acids Res*. 2016;44(8):e71. doi:10.1093/nar/gkv1507
25. Mayakonda A, Lin DC, Assenov Y, Plass C, Koeffler HP. Maftools: efficient and comprehensive analysis of somatic variants in cancer. *Genome Res*. 2018;28(11):1747–1756. doi:10.1101/gr.239244.118
26. Hänzelmann S, Castelo R, Guinney J. GSEA: gene set variation analysis for microarray and RNA-seq data. *BMC Bioinf*. 2013;14(1):7. doi:10.1186/1471-2105-14-7
27. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;13(11):2498–2504. doi:10.1101/gr.1239303
28. Zhang Y, Wu D, Yu T, Liu Y, Zhao C, Xue R. Prognostic value of TMTC1 in pan-cancer analysis. *Heliyon*. 2024;10(19):e38308. doi:10.1016/j.heliyon.2024.e38308
29. Makrilia N, Kollias A, Manolopoulos L, Syrigos K. Cell adhesion molecules: role and clinical significance in cancer. *Cancer Invest*. 2009;27(10):1023–1037. doi:10.3109/07357900902769749
30. Li Q, Jiang S, Feng T, Zhu T, Qian B. Identification of the EMT-related genes signature for predicting occurrence and progression in thyroid cancer. *Onco Targets Ther*. 2021;14:3119–3131. doi:10.2147/OTT.S301127
31. Gabler L, Jaunecker CN, Katz S, et al. Fibroblast growth factor receptor 4 promotes glioblastoma progression: a central role of integrin-mediated cell invasiveness. *Acta Neuropathol Commun*. 2022;10(1):65. doi:10.1186/s40478-022-01363-2
32. Seol MY, Choi SH, Lee JJ, et al. Selective inhibition of PI3K isoforms in brain tumors suppresses tumor growth by increasing radiosensitivity. *Yonsei Med J*. 2023;64(2):139–147. doi:10.3349/ymj.2022.0414
33. Rivera-Caraballo KA, Nair M, Lee TJ, Kaur B, Yoo JY. The complex relationship between integrins and oncolytic herpes simplex virus 1 in high-grade glioma therapeutics. *mol Ther Oncolytics*. 2022;26:63–75. doi:10.1016/j.omto.2022.05.013
34. Zhou Y, Wang W, Wei R, et al. Serum bradykinin levels as a diagnostic marker in cervical cancer with a potential mechanism to promote VEGF expression via BDKRB2. *Int J Oncol*. 2019;55(1):131–141. doi:10.3892/ijo.2019.4792
35. Chen Y, Yu Y, Sun S, et al. Bradykinin promotes migration and invasion of hepatocellular carcinoma cells through TRPM7 and MMP2. *Exp Cell Res*. 2016;349(1):68–76. doi:10.1016/j.yexcr.2016.09.022
36. Zhang W, Bhola N, Kalyankrishna S, et al. Kinin b2 receptor mediates induction of cyclooxygenase-2 and is overexpressed in head and neck squamous cell carcinomas. *mol Cancer Res*. 2008;6(12):1946–1956. doi:10.1158/1541-7786.MCR-07-2197
37. da Costa PL, Sirois P, Tannock IF, Chammas R. The role of kinin receptors in cancer and therapeutic opportunities. *Cancer Lett*. 2014;345(1):27–38. doi:10.1016/j.canlet.2013.12.009
38. Yang WH, Chang JT, Hsu SF, et al. Bradykinin enhances cell migration in human chondrosarcoma cells through BK receptor signaling pathways. *J Cell Biochem*. 2010;109(1):82–92. doi:10.1002/jcb.22383
39. Yang Y, Wang J, Shi F, Shan A, Xu S, Lv W. BDKRB2 is a novel EMT-related biomarker and predicts poor survival in glioma. *Aging*. 2021;13(5):7499–7516. doi:10.18632/aging.202614
40. Shergalis A, Bankhead A, Luesakul U, Muangsin N, Neamati N. Current challenges and opportunities in treating glioblastoma. *Pharmacol Rev*. 2018;70(3):412–445. doi:10.1124/pr.117.014944
41. Poyil PK, Siraj AK, Padmaja D, et al. Overexpression of the pro-protein convertase furin predicts prognosis and promotes papillary thyroid carcinoma progression and metastasis through RAF/MEK signaling. *Mol Oncol*. 2023;17(7):1324–1342. doi:10.1002/1878-0261.13396
42. Chen M, Pan Y, Liu H, et al. Ezrin accelerates breast cancer liver metastasis through promoting furin-like convertase-mediated cleavage of Notch1. *Cell Oncol Dor*. 2023;46(3):571–587. doi:10.1007/s13402-022-00761-x
43. Mercapide J, Lopez De Cicco R, Bassi DE, Castresana JS, Thomas G, Klein-Szanto A. Inhibition of furin-mediated processing results in suppression of astrocytoma cell growth and invasiveness. *Clin Cancer Res*. 2002;8(6):1740–1746.

44. Yu Y, Sung SK, Lee CH, et al. SOCS3 is related to cell proliferation in neuronal tissue: an integrated analysis of bioinformatics and experiments. *Front Genet.* 2021;12:743786. doi:10.3389/fgene.2021.743786
45. Yamamoto T. Engineering of *Bacillus thuringiensis* insecticidal proteins. *J Pestic Sci.* 2022;47(2):47–58. doi:10.1584/jpestics.D22-016
46. Zheng S, Tao W. Identification of novel transcriptome signature as a potential prognostic biomarker for anti-angiogenic therapy in glioblastoma multiforme. *Cancers.* 2021;13(5):1013. doi:10.3390/cancers13051013
47. Sakata J, Sasayama T, Tanaka K, et al. MicroRNA regulating stanniocalcin-1 is a metastasis and dissemination promoting factor in glioblastoma. *J Neurooncol.* 2019;142(2):241–251. doi:10.1007/s11060-019-03113-2
48. Xiong Y, Wang Q. STC1 regulates glioblastoma migration and invasion via the TGF- β /SMAD4 signaling pathway. *Mol Med Rep.* 2019;20(4):3055–3064. doi:10.3892/mmr.2019.10579
49. Yang M, Lu Z, Yu B, et al. COL5A1 promotes the progression of gastric cancer by acting as a ceRNA of miR-137-3p to upregulate FSTL1 expression. *Cancers.* 2022;14(13):3244. doi:10.3390/cancers14133244
50. Gu S, Peng Z, Wu Y, et al. COL5A1 serves as a biomarker of tumor progression and poor prognosis and may be a potential therapeutic target in gliomas. *Front Oncol.* 2021;11:752694. doi:10.3389/fonc.2021.752694
51. Tsai H-F, Chang Y-C, Li C-H, et al. Type V collagen alpha 1 chain promotes the malignancy of glioblastoma through PPRC1-ESM1 axis activation and extracellular matrix remodeling. *Cell Death Discov.* 2021;7(1):313. doi:10.1038/s41420-021-00661-3
52. Wu J, Li X, Luo F, Yan J, Yang K. Screening key miRNAs and genes in prostate cancer by microarray analysis. *Transl Cancer Res.* 2020;9(2):856–868. doi:10.21037/tcr.2019.12.30

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