Original Article

A new prognostic model for glioblastoma multiforme based on coagulation-related genes

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Background: Glioblastoma multiforme (GBM) is the most aggressive, common, and lethal type of primary brain tumor. Multiple cancers have been associated with abnormalities in the coagulation system that facilitate tumor invasion and metastasis. In GBM, the prognostic value and underlying mechanism of coagulation-related genes (CRGs) have not been explored.

Methods: RNA sequencing (RNA-seq) and clinical information on GBM were obtained from The Cancer Genome Atlas (TCGA) and Chinese Glioma Genome Atlas (CGGA), respectively. Following the identification of differentially expressed CRGs (DECRGs) between GBM and control samples, the survival-related DECRGs were selected via univariate and multivariate Cox regression analyses to establish a prognostic signature. The prognostic performance and clinical utility of the prognostic signature were assessed by the Kaplan-Meier (KM) analysis and receiver operating characteristic (ROC) curve analysis, and a nomogram was constructed. The signature genes-related underlying mechanisms were analyzed according to gene set enrichment analysis (GSEA), Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and single-cell analysis. Finally, the difference in immune cell infiltration, stromal score, immune score, and Estimation of Stromal and Immune cells in Malignant Tumor tissues using Expression data (ESTIMATE) score were compared between different risk groups.

Results: A 5-gene prognostic signature (*PLAUR*, *GP6*, *C5AR1*, *SERPINA5*, *F2RL2*) was established for overall survival (OS) prediction of GBM patients. The predicted efficiency of the prognostic signature was confirmed in TGGA-GBM dataset and validated in the CGGA-GBM dataset, revealing that it could differentiate GBM patients from controls well, and high risk score was accompanied with poor prognosis. Moreover, biological process (BP) and signaling pathway analyses showed that signature genes were mainly enriched in the functions of blood coagulation and tumor invasion and metastasis. Moreover, high-risk patients exhibited higher levels of immune cell infiltration, stromal score, immune score, and ESTIMATE score than that of low-risk patients.

Conclusions: An analysis of coagulation-related prognostic signatures was conducted in this study, as well as how signature genes may affect GBM progress, providing information that might provide new ideas for the development of GBM-related molecular targeted therapies.

Keywords: Glioblastoma multiforme (GBM); coagulation-related genes (CRGs); prognosis; function enrichment analysis; tumor microenvironment (TME)

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Introduction

Gliomas are primary malignant tumors originating from glial cells in the central nervous system, comprising about 75% of brain tumors (1,2). In clinical terms, gliomas are divided into low-grade gliomas (LGGs) and glioblastoma multiformes (GBMs). GBMs grow rapidly, whereas LGGs grow slowly and can be resolved through surgical resection. Generally, patients with LGGs have a good prognosis (3). In contrast, GBM is a type of grade IV malignant glioma that is aggressive and resistant to treatment. GBM accounts for 50% of primary brain tumors in adults and results in over 15,000 fatalities annually in the United States (4). The development of a treatment plan for GBM patients requires a multidisciplinary approach (5). Although there have been multiple innovations in the treatment of GBM, it remains one of the most complex and difficult cancers to treat (6). Despite improvements in surgical resection rates, chemotherapy rates, and radiation rates, patients diagnosed with GBM continue to experience low survival rates, with a median overall survival (OS) of less than 2 years (7,8). In view of the limited treatment options available for GBM, new prognostic models are urgently needed to accurately and conveniently predict survival for patients with GBM.

One of the most important innate defense mechanisms is the coagulation system, especially tissue factor (TF) (9). The functions of these host-protected pathways are used by tumor cells for shaping the tumor microenvironment (TME), which they rely on for sustained metastasis. Research findings indicate that individuals with malignant tumors exhibit hypercoagulation and hyperfibrinolysis, denoting an augmented propensity for clot formation and heightened production of fibrin during the initial stages of the disease. This observation implies a correlation between coagulation impairment and the processes of tumor invasion, metastasis, as well as the overall prognosis of patients (10). Evidence has suggested that FGB of fibrinopeptide A increases when the clotting pathway is activated by tumors, resulting in fibrinolysis and the release of D-dimers. Coagulation, fibrinolysis, and platelet activation pathways can be blocked effectively to prevent tumor progression (11). In the meantime, it is possible to prevent metastatic tumor proliferation by inhibiting prothrombin activator, thrombin, and fibrin stages of clotting cascades and blocking selective platelet activation pathways (12). Invasive GBM is associated with hypercoagulable status, and the data support the hypothesis that plasma hypercoagulable status is associated with adverse outcomes in GBM patients (13). Currently, several biomarkers associated with coagulation disorders have shown significant prognostic associations in various cancers (14,15). The research conducted by Meli *et al.* demonstrated that the activation of thrombin and PAR-1 induces the production of pro-inflammatory factors, thereby promoting the proliferation of glioma (16). Thrombin, a protease, triggers the activation of a group of receptors known as protease-activated receptors (PARs), which include PAR-1, PAR-3, and PAR-4. Literature has reported a significant elevation in the incidence of venous thrombosis among glioma patients (17), which is attributed to the release of thrombin outside the blood vessels due to an imbalance in coagulation/fibrinolysis in patients with varying degrees of malignancy (18). Glioma stem cells (GSCs) activate platelets in a plasma-independent manner by utilizing both endogenous and extrinsic coagulation cascade factors. The production of thrombin by GSCs results in platelet activation, which in turn leads to the release of glioma platelets and subsequent promotion of vascular production of GBM endothelial cells. This process contributes to the complexity of the glioma vascular network (19). Within the glioma microenvironment, thrombin is generated through the activation of PARs by thrombin. Hence, the involvement of coagulation factors, such as thrombin and TFs, is pivotal in the onset and progression of glioma (20).

In this study, a first attempt was made to identify the

Highlight box

Key findings

- There is a potential correlation between the expression of 5 prognostic biomarker genes (*PLAUR*, *GP6*, *C5AR1*, *SERPINA5*, *F2RL2*) and the advancement of glioma.

What is known and what is new?

- A correlation exists between coagulation impairment and the processes of glioblastoma multiforme (GBM) invasion, metastasis, as well as the overall prognosis of patients. Within the glioma microenvironment, thrombin is generated through the activation of protease-activated receptors by thrombin. The involvement of coagulation factors, such as thrombin and tissue factors, is pivotal in the onset and progression of GBM.
- A comprehensive screening was conducted to identify 5 biomarkers that exhibit a significant association with the progression of GBM.

What is the implication, and what should change now?

- In the future, we will persist in conducting further research on the role of these 5 biomarkers in the pathogenesis of GBM, with the potential to alter the incidence and progression of glioma through these mechanisms.

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prognostic effect and potential mechanism of coagulation-related genes (CRGs) in GBM based on the GBM-related public datasets, and a nomogram was constructed for clinical utilization of the CRGs-based prognostic signature. Besides, correlation of the signature and TME was systematically examined. The study aimed to provide reference for further treatment and prognostic prediction for GBM patients. We present this article in accordance with the TRIPOD reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-322/rc).

Methods

Data sources

Transcriptome sequencing data of TCGA-GBM dataset (including 5 healthy samples and 154 GBM samples) were downloaded from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/), wherein 153 GBM samples had complete clinical information. Moreover, RNA sequencing (RNA-seq) data of CGGA-GBM cohort were derived from the Chinese Glioma Genome Atlas (CGGA) database (http://www.cgga.org.cn/), wherein 657 case samples had associated follow-up information. CRGs within 2 coagulation pathways (hsa04610 and hsa04611) were collected from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.kegg.jp/kegg/pathway.html) (21). This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Identification of the differentially expressed CRGs (DECRGs)

Differentially expressed genes (DEGs) between GBM and control groups in TCGA-GBM dataset were identified using limma R package (Version 3.48.3; https://cran.r-project.org/) (22). The following thresholds were adopted: $|\log_2\text{fold change (FC)}| > 1$, $P<0.05$, and Ave Expr >1. Ggplot2 (Version 3.3.5) and pheatmap (Version 1.0.12) within R (23) were conducted to visualize gene expression of DEGs. Following overlapping the DEGs and CRGs, the DECRGs were selected for further analysis.

Construction and evaluation of the prognostic signature

Survival-related DECRGs were screened by the univariate and multivariate Cox proportional hazards regression analyses to construct the prognostic signature. Firstly, a total of 153 patients with complete clinical information in TCGA-GBM dataset were divided into a training set (n=108) and a testing set (n=45) with a ratio of 7:3. The risk score of each patient was calculated with the 'predict.coxph' function of survival R package (Version 3.2-13) (24), then the populations in the training set and testing set were stratified into high- and low-risk groups according to the median value of risk score. Kaplan-Meier (K-M) curves between two risk groups were plotted by survminer (Version 0.4.9) (25). Receiver operating characteristic (ROC) curve analysis was used to further evaluate the predictive accuracy of the prognostic signature. Similarly, the CGGA-GBM dataset (n=657) was chosen to independently validate the prognostic performance of the signature. Besides, clinical phenotype data in the training set, that is, treatment type, age, gender, and the prognostic signature, were analyzed using univariate and multivariate statistical analyses to investigate the independent prognostic factors of GBM patients. The nomogram model with the endpoints of OS was constructed by integrating the signature and clinical factors with a P value <0.05.

Function enrichment analyses of signature genes

To interpret the signature genes-related underlying mechanisms, gene set enrichment analysis (GSEA) was used to determine gene sets with significantly difference between high- and low-risk groups ($|\text{NES}| > 1$, NOM $P<0.05$, $q<0.25$). Signature genes were uploaded to the Metascape database (https://metascape.org/gp/index.html#/main/step1) (26) to excavate the enriched Gene Ontology (GO), KEGG pathways. Furthermore, single cell analysis of signature genes was performed through the cancer single-cell state atlas (CancerSEA) database (27).

Immune infiltration in TME of GBM

Considering the complex TME in GBM, the connections between 22 immune cells activity and the prognostic signature were investigated using the Cell-type Identification By Estimating Relative Subsets Of RNA Transcripts (CIBERSORT) analysis (28). Moreover, the immune score, Estimation of Stromal and Immune cells in Malignant Tumor tissues using Expression data (ESTIMATE) score, and stromal score were calculated by the ESTIMATE algorithm (29).

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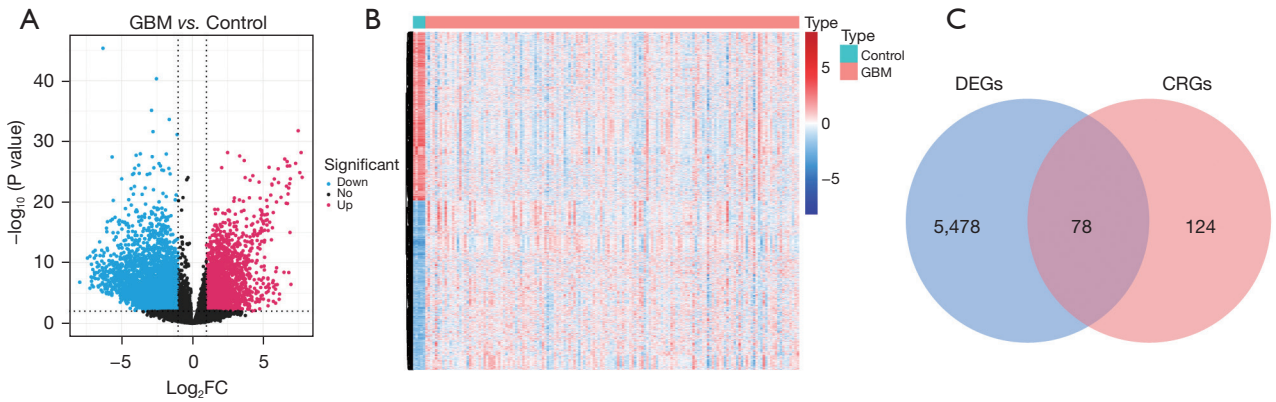

Figure 1 Differential gene screening for GBM and the intersection genes associated with coagulation. (A) The volcano plot illustrates the dissimilarly expressed genes linked to GBM, indicating a noteworthy distinction between the GBM and control cohorts. (B) Heat map of the DEGs associated with GBM. (C) A Venn diagram was constructed to illustrate the differential expression of genes in GBM and CRGs. The analysis revealed a total of 78 DECRGs between the GBM and control groups. GBM, glioblastoma multiforme; DEGs, differentially expressed genes; CRGs, coagulation-related genes; DECRGs, differentially expressed coagulation-related genes; FC, fold change.

Statistical analysis

All analyses were conducted using R language software. If not specified above, P<0.05 was regarded as statistically significant.

Results

Identification of DECRGs

Following the differential expressed analysis in TCGA-GBM dataset (*Figure 1A,1B*), a total of 78 DECRGs between GBM and control groups were identified by overlapping 5,556 DEGs and 202 CDGs (*Figure 1*, Table S1).

Development and validation of the prognostic signature of GBM

In order to explore the prognostic role of DECRGs, a total of five survival-related DECRGs (*PLAUR*, *GP6*, *C5AR1*, *SERPINA5*, *F2RL2*) (P<0.05) were identified to establish the prognostic signature (*Figure 2A,2B*; Tables S2,S3). Then, the high- and low-risk groups in the training set and testing set were classified according to the medium-risk score. As shown in *Figure 3A-3F*, patients with a high risk score had worse survival states and poor prognosis. The area under the ROC curve (AUC) values for survival prediction at 1–5 years were greater than 0.6, suggesting the prognostic signature had excellent efficiency. Meanwhile, it was further validated that the prognostic signature involved had a robust accuracy for survival prediction in CGGA-GBM dataset (*Figure 3G-3I*).

Results of the independent prognostic analyses suggested that only risk score and treatment type had independent prognostic value (*Figure 4A,4B*, Tables S4,S5). Therefore, a nomogram was drawn with the C-index of 0.6721 (*Figure 4C*). The calibration curve of the nomogram demonstrated a good agreement between predicted probabilities and the actual observed outcome of OS at 1- and 3-years (*Figure 4D*).

Biological process (BP) and pathway of signature genes

We conducted three enrichment analyses to explain the biological significance of signature genes, that is, GSEA analysis, function and pathway enrichment analysis, and single-cell analysis. The GSEA results indicated that a total of 6,385 GO and 174 KEGG pathways were significantly enriched in high- and low-risk groups, such as the BP of humoral immune response, the cellular components (CC) of external side of plasma membrane, and the molecular functions (MF) of cytokine activity (*Figure 5A*, available online: https://cdn.amegroups.cn/static/public/tcr-23-322-1.xlsx). According to the prediction results from Metascape database, these signature genes were markedly enriched in response to wounding, blood coagulation, and hemostasis (*Figure 5B*, Tables S6,S7). In addition, single-cell analysis of signature genes demonstrated that *F2RL2* and *SERPINA5* were significantly negatively correlated

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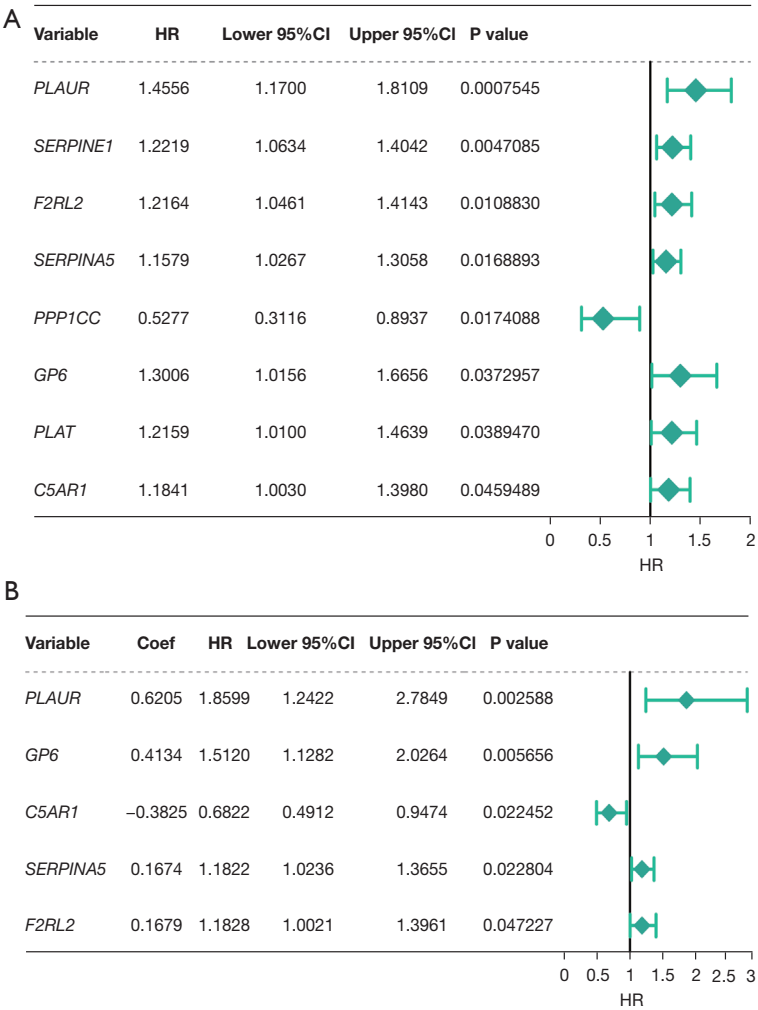

Figure 2 This forest plot displays the results of a univariate and multivariate Cox regression analysis of DECRGs with GBM. (A) The results of a univariate Cox regression; the P values for *PLAUR*, *SERPINA5*, *GP6*, *F2RL2*, and *C5AR1* are less than 0.05; (B) The results of a multivariate Cox regression; the P values for *PLAUR*, *SERPINA5*, *GP6*, *F2RL2*, and *C5AR1* are less than 0.05. DECRGs, differentially expressed coagulation-related genes; GBM, glioblastoma multiforme; HR, hazard ratio; CI, confidence interval.

with invasion process, whereas *PLAUR* was closely related to the functions of metastasis, inflammation, and hypoxia (*Figure 5C*, Table S8).

Exploration of immune microenvironment and generation of TME scores

From the perspective of immune infiltration analysis, the infiltration levels of 22 immune cells are displayed in *Figure 6A*, in which 5 innate immune cells demonstrated a distinct difference between high- and low-risk groups, namely, M2 macrophages, resting mast cells, neutrophils, activated natural killer (NK) cells, and resting memory $CD4^+$ T cells. Simultaneously, the patients in high-risk group presented higher stromal score, immune score, and ESTIMATE score than those in the low-risk group (*Figure 6B*).

Discussion

The development of imaging and directional biopsy techniques has enabled rapid diagnosis of GBM. However, the current findings on molecular genetic characteristics, including *IDH* mutation, *PTEN* mutation, *EGFR* amplification, and *TP53* mutation, have not changed the prognosis of GBM; patients with GBM relapse quickly after surgery and have a very short survival. As molecular

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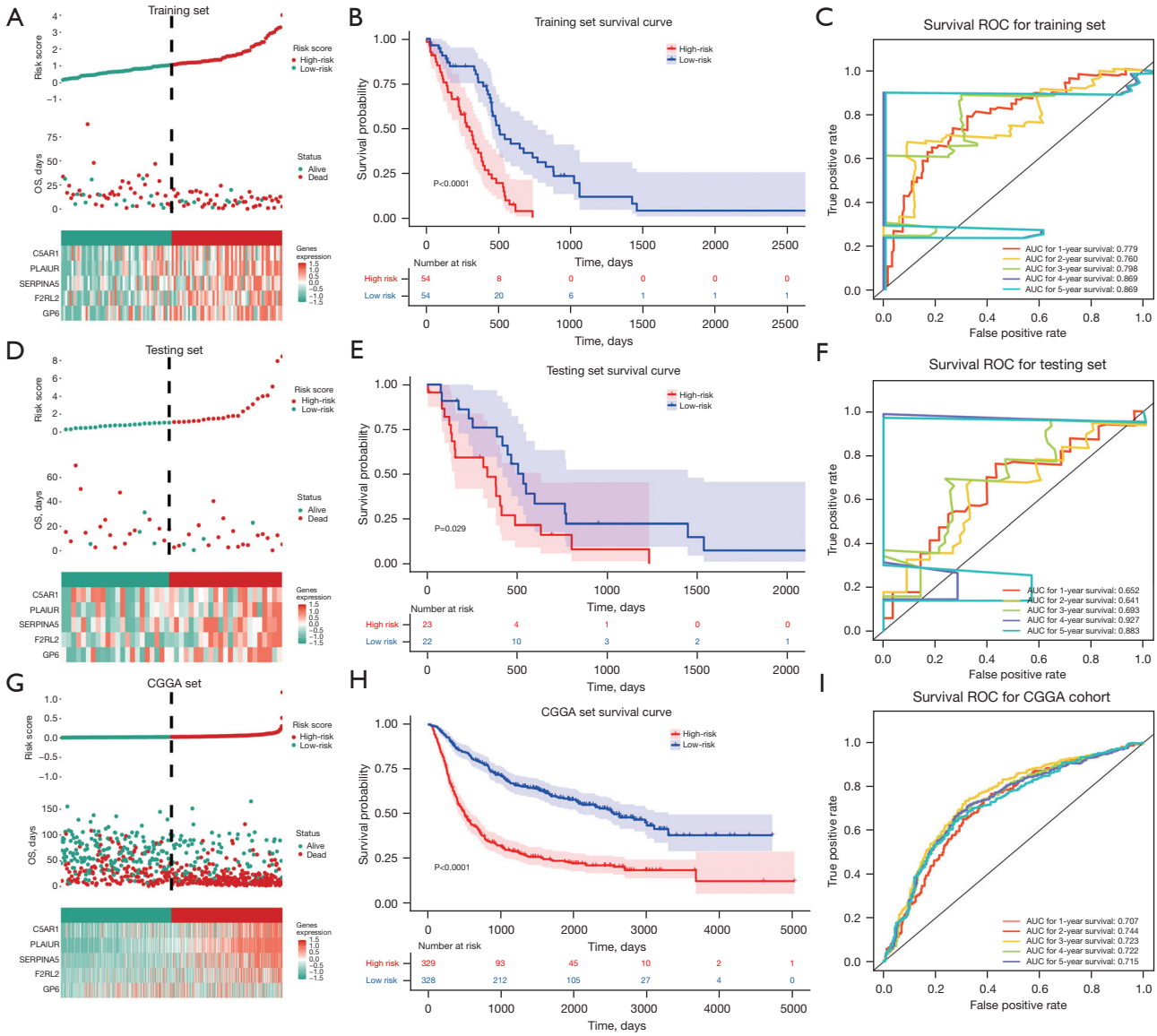

Figure 3 CRGs-based establishment and validation of a prognostic model for GBM. (A) Upon analyzing the curves and model genes heat maps of high- and low-risk groups in TCGA training, notable disparities in the expression of 5 biomarkers were detected between cohorts classified as high- and low-risk. (B) The KM curves depicting the high-risk and low-risk groups in the TCGA training set exhibit statistically significant differences (P<0.0001). (C) The AUC values of the ROC curve for the training set indicated that the prognostic signature exhibited exceptional efficacy for predicting survival at 1–5 years, with values exceeding 0.7. (D) In the TCGA test set, the heat map depicting the curves and model genes of the high- versus low-risk groups revealed notable variations in the expression of 5 biomarkers between the two groups. (E) The KM curves depicting the high- and low-risk groups in the TCGA test set exhibit statistically significant differences (P=0.029). (F) The AUC values of the ROC curve for the test set revealed that the prognostic signature demonstrated remarkable effectiveness in forecasting survival within the 1–5 year timeframe, with values surpassing 0.6. (G) In the CGGA test set, the heat map depicting the curves and model genes of the high-risk versus low-risk group revealed notable variations in the expression of 5 biomarkers between the two groups. (H) The KM curves depicting the high- and low-risk groups in the CCGA test set exhibit statistically significant differences (P<0.0001). (I) The AUC values of the ROC curve for the CGGA test set revealed that the prognostic signature demonstrated remarkable effectiveness in forecasting survival within the 1–5-year timeframe, with values surpassing 0.7. CRG, coagulation-related gene; GBM, glioblastoma multiforme; TCGA, The Cancer Genome Atlas; KM, Kaplan-Meier; AUC, area under the curve; ROC, receiver operating characteristic; CGGA, Chinese Glioma Genome Atlas; OS, overall survival.

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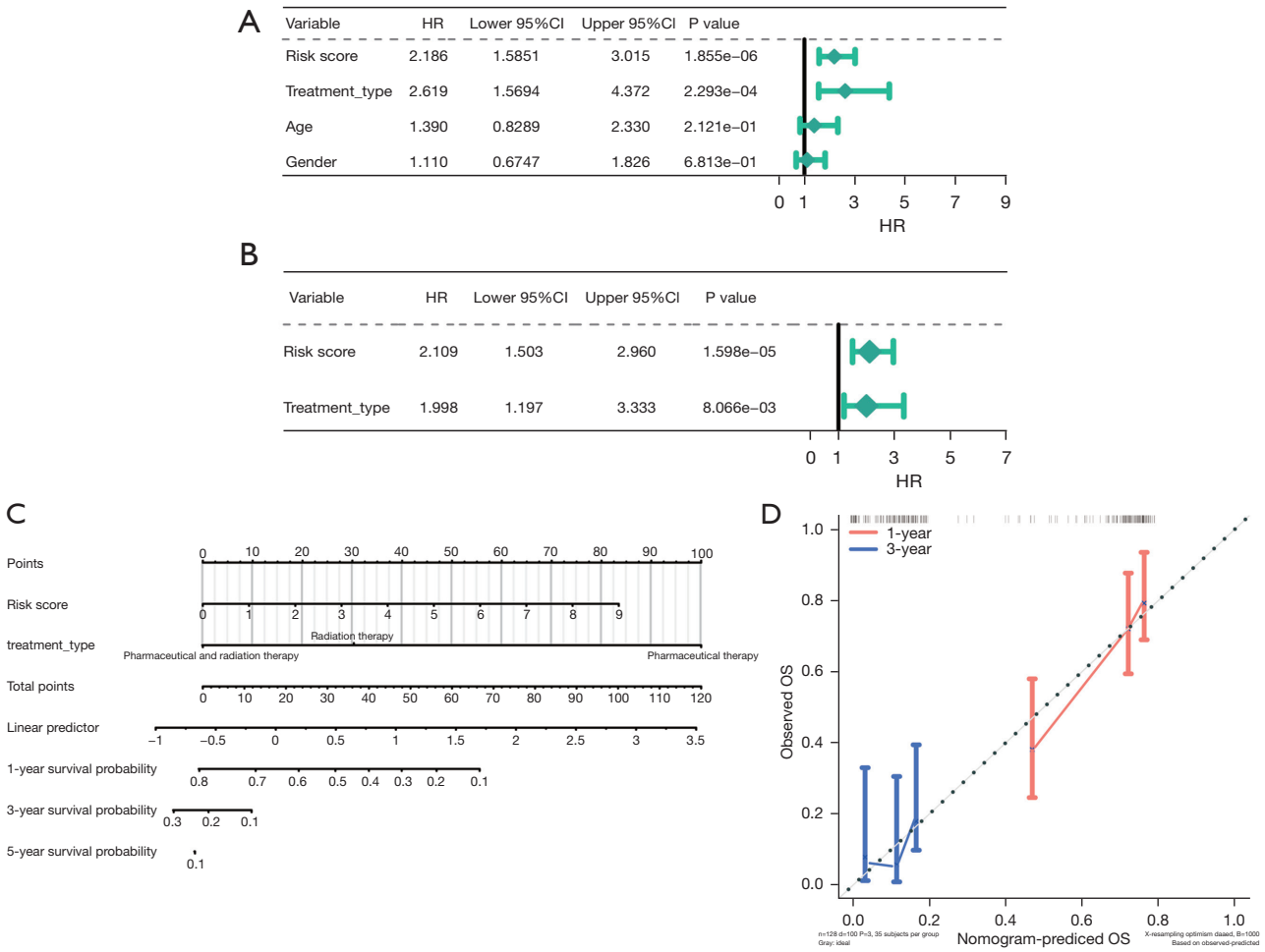

Figure 4 The independent prognostic analyses of prognostic model for GBM based on CRGs. (A) The univariate Cox forest map indicates that the P values for risk score, treatment type, age, and gender are all less than 0.05, signifying their independent prognostic significance. (B) In the multivariate Cox forest map of independent prognostic analyses, only the P value of risk score and treatment type exhibit statistical significance with a value less than 0.05, indicating independent prognostic analyses. (C) In conjunction with the outcomes of univariate and multivariate Cox analyses, it was determined that solely risk score and treatment category exhibited autonomous prognostic significance. Consequently, a nomogram was developed to formulate a prognostic framework, the C-index is 0.6721. (D) The calibration curve of the nomogram demonstrated a good agreement between predicted probabilities and the actual observed outcome of OS at 1 and 3 years. GBM, glioblastoma multiforme; CRGs, coagulation-related genes; OS, overall survival; HR, hazard ratio.

genomics develops rapidly, conventional diagnostics and treatments may be improved with the help of specific diagnostic and prognostic biomarkers. As a result, this study investigated the impact of CRGs on the prognosis of GBM patients, and various bioinformatics analyses were used to identify key CRGs with excellent prognostic value in GBM. Subsequently, prognostic risk models for GBM patients were constructed using *PLAUR*, *GP6*, *C5AR1*, *SERPINA5*, and *F2RL2*. Besides, the characteristics of TME infiltration in different risk populations were explored, thus providing new ideas for the treatment of patients with GBM.

Malignant tumors can provoke changes in the blood vessels, stroma, and microenvironment when they are genetically altered. All changes in inflammation, angiogenesis, and tissue repair procedures can contribute to the local and systemic activation of the clotting system. BPs of tumor growth, initiation, dormancy, invasion, angiogenesis, metastasis, and therapeutic response are affected by abnormal coagulation function in malignant tumors. A driver mutation in key oncogenes that is

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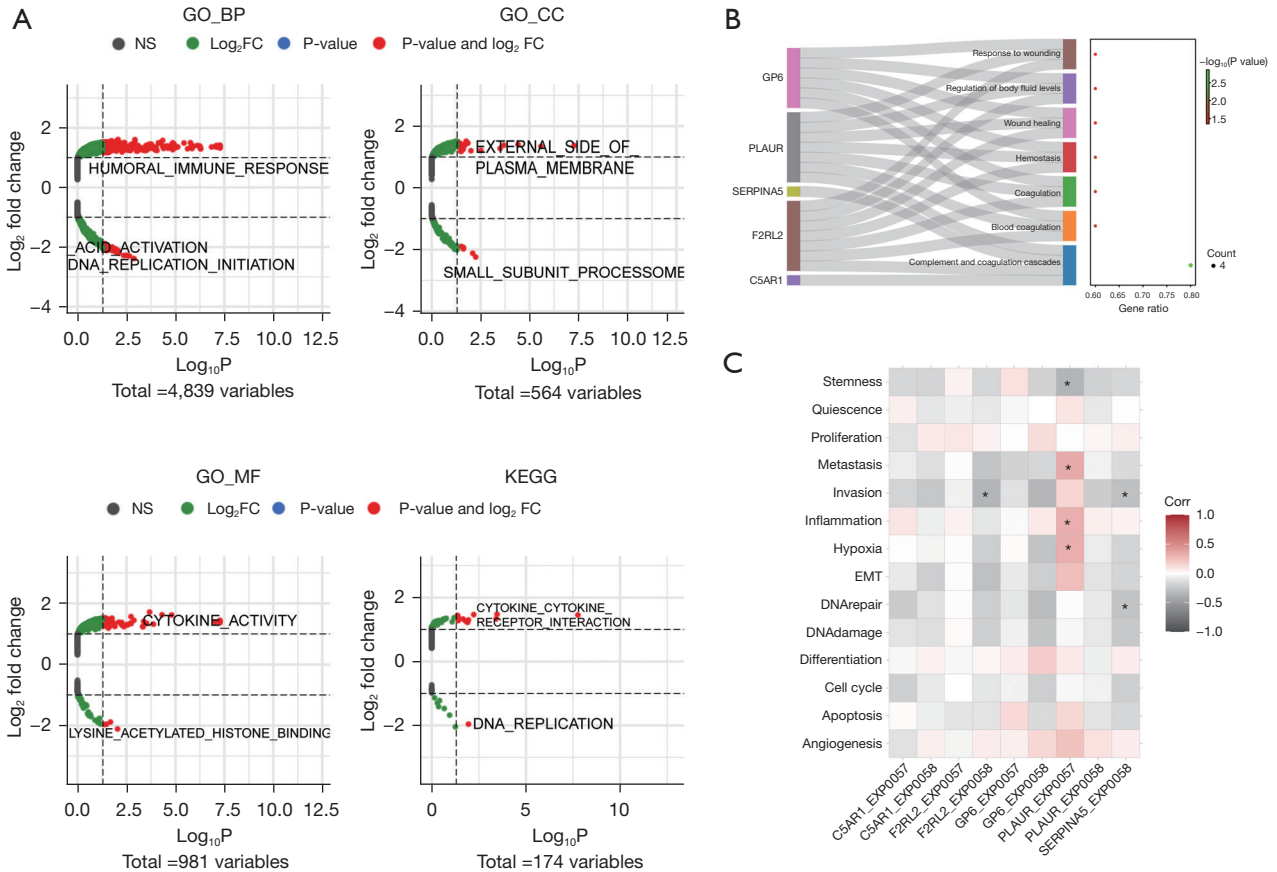

Figure 5 Functional analysis of biomarkers in GBM prognostic model. (A) The results of the GSEA revealed that the high- and low-risk groups exhibited significant enrichment in a total of 6,385 GO and 174 KEGG pathways. Notably, the enriched pathways included the BP of humoral immune response, the CC of external side of plasma membrane, and the MF of cytokine activity. (B) The results of the prediction analysis from the Metascape database indicate a significant enrichment of signature genes associated with the BPs of wound healing, blood coagulation, and hemostasis. (C) The results of the single-cell analysis of signature genes indicate a significant negative correlation between *F2RL2* and *SERPINA5* and the invasion process, whereas PLAUR exhibited a close association with the functions of metastasis, inflammation, and hypoxia. *, a significance level of P<0.05. GBM, glioblastoma multiforme; GSEA, gene set enrichment analysis; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; MF, molecular function; CC, cellular components; NS, no significant; FC, fold change.

synergistic with hypoxia, differentiation, and other factors, alters the expression of TFs and a number of hemostat-associated molecules, including transmembrane receptors such as protease-activating receptors (PAR-1, PAR-2) and clotting factors (FII and FVII) (30).

Evidence suggests that subpopulations of specific malignancies, including GBM, differ in their expression profiles of CRGs (20,21,31). In GBM, the coagulation cascade interacts with endothelial cells and angiogenic activity to drive growth and invasion. A clotting cascade alone cannot explain glioma progression. Throughout the course of GBM, a variety of complex interactions trigger the coagulation cascade, including tumor hypoxia, upregulation of VEGR expression, and increases in both tumor cell-specific TF expression and inducible TF expression in numerous intrinsic regulatory pathways (32). It remains unclear whether the coagulation cascade leads to GBM progression or whether GBM progression leads to hypercoagulability. This study showed that all five prognostic biomarker genes (*PLAUR*, *GP6*, *C5AR1*, *SERPINA5*, *F2RL2*) were highly expressed among patients at high risk, which might be involved in the development of GBM through the coagulation pathways. Several studies have reported an association between *PLAUR*

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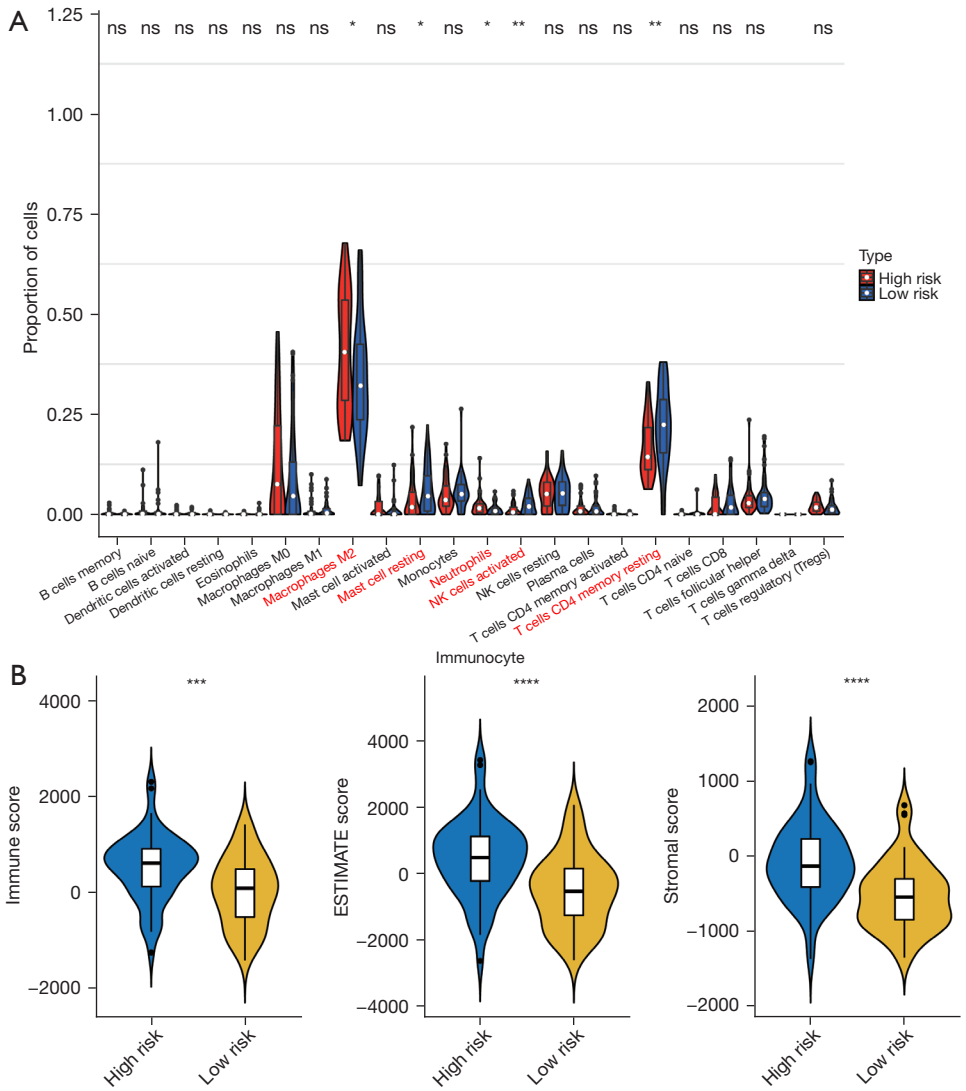

Figure 6 Immune microenvironment analysis of biomarkers in GBM prognostic model. (A) A discernible distinction between high- and low-risk groups was observed in terms of immune infiltration analysis, specifically with regard to M2 macrophages, resting mast cells, neutrophils, activated NK cells, and resting memory CD4+ T cells. (B) The difference of immunization scores between high and low risk groups was P<0.001, and the difference of ESTIMATE and stromal scores between high- and low-risk groups was P<0.0001. Those of the high-risk group are higher than the low-risk group. *, a significance level of P<0.05; **, a significance level of P<0.01; ***, a significance level of P<0.001; ****, a significance level of P<0.0001; ns, no significant difference. GBM, glioblastoma multiforme; NK, natural killer; ESTIMATE, Estimation of Stromal and Immune cells in Malignant Tumor tissues using Expression data.

and tumor progression. Using immunohistochemistry and real-time quantitative polymerase chain reaction (qRT-PCR) (33,34), *PLAUR* expression was very high in GBM, but virtually undetectable in LGG, indicating that *PLAUR* may be one of the characteristic genes of GBM. A study showed that *PLAU* and *PLAUR* expression was positively correlated with invasion, angiogenesis, epithelial-mesenchymal transformation, tumor stem cell-like characteristics, and metastasis in GBM (35). *PLAUR* activation promotes extracellular protease cascades involved in tumor matrix remodeling and cell migration. It has not been reported whether *GP6* is differentially expressed in gliomas. Breast cancer tissue has high levels of *GP6*, which are significantly higher than those in

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adjacent tissues (36). A genetic signature associated with clots has been studied in breast cancer, which suggests that *GP6* may also influence GBM processes through angiogenesis. *CA5R1* is a receptor for complement CA5, and cancer survivors with high expression have a poorer prognosis (37). Based on immunohistochemistry and transcription-level expression validation by RT-PCR, *C5AR1* was highly expressed in GBM (38). Complement and coagulation play an Important role in bodily defense. Each plays a different role in physiology and pathophysiology of the systemic circulation, but their functions are interconnected (39). The regulatory role of *SERPINA5* in hemostasis and thrombosis has been observed in various organs. However, the expression relationship of *SERPINA5* in GBM remains unreported, despite its implication in numerous diseases such as osteoarthritis (40), gastric cancer (41), and thyroid cancer (42). Researchers found that LGG patients with high *SERPINA5* expression had a poor prognosis (43). In other words, *SERPINA5* is highly expressed in LGG patients, and LGG may progress to GBM. Although it is rare to find a report on coagulation *F2RL2* in studies on gliomas, *F2RL2* was revealed to be a promising biomarker for glioma in a recent analysis. *PAR3* plays a key role in inflammatory reactions and immune responses, as well as tumorigenesis and metastasis in many types of cancer, including gliomas. *PAR3* is encoded by *F2RL2*, which is a G-protein-coupled receptor (GPCR). As a result, we hypothesized that *F2RL2* is related to *PAR3* through inflammatory processes in primary gliomas (44). In conclusion, there may be a close relation between all five prognostic biomarker genes (*PLAUR*, *GP6*, *C5AR1*, *SERPINA5*, *F2RL2*) and the progression of glioma.

The potential value in predicting the prognosis of GBM for these characteristic genes was explored. With ROC analysis, it is suggested that AUCs at 1, 3 and 5 years were all greater than 0.6. Randomly, patients were grouped into high- and low-risk groups according to their median risk scores. Low-risk survivors had longer survival times than high-risk survivors, supporting the validity of the risk model. Moreover, risk models as well as treatment modalities were considered independent predictors of GBM survival. A nomogram for clinical use was also validated.

Based on the results of the GO and KEEG functional enrichment analysis of the 5 prognostic biomarkers, almost all of them were related to blood coagulation. It appears that coagulation plays an important role in GBM progression. In single-cell functional analysis, biomarkers *F2RL2*, *PLAUR*, and *SERPINA5* were significantly correlated with cell invasion, metastasis, inflammation, repair, and other functions. According to the tumor immune microenvironment analysis, high-risk patients had significantly higher matrix scores, immune scores, as well as ESTIMATE composite scores. As compared to the low-risk group, there were significant differences between the five types of immune cells: macrophages M2, mast cells resting, neutrophils, NK cells activated, and T cells CD4 memory resting. According to these results, the immune microenvironment appears to play a significant role in GBM development. The interaction between the TME, coagulation, inflammation, and immunization plays an important role throughout the tumor life cycle (45).

In the study for GBM, CRGs were independently identified for the first time as having a prognostic effect through the bioinformatic analysis. The relationship between the prognostic signature and immune cell infiltration in GBM was investigated as well. It is essential to observe the characteristics of GBM from an entirely new perspective in order to understand its potential pathogenesis. It has been demonstrated that *PLAUR* and *C5AR1* are involved in glioma cell growth (35,36). The *GP6*, *SERPINA5*, and *F2RL2* genes need to be studied in more clinical experiments, as well as further mechanistic studies to verify the prognostic utility of the CRGs-based risk model. In the future, we will continue to investigate the prognostic role of these genes, which are essential steps in predicting GBM disease progression and managing treatment. In the future, we will persist in conducting further research on the role of these 5 biomarkers in the pathogenesis of GBM, with the potential to alter the incidence and progression of glioma through these mechanisms.

Conclusions

This study aimed to conduct an analysis of coagulation-related prognostic signatures, resulting in the identification of 5 biomarkers (*PLAUR*, *GP6*, *C5AR1*, *SERPINA5*, and *F2RL2*) through the screening process. Additionally, the study explored the potential impact of these signature genes on GBM progression, offering valuable insights that could potentially contribute to the development of molecular targeted therapies for GBM.

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Footnote

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Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-322/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-322/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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