Development of A Vitamin-related Gene Signature to Predict the Immune Characteristics and Prognosis of Glioma

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Research

Keywords: Vitamins, Risk signature, Prognosis, Immunosuppressive, Glioma

Posted Date: September 22nd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-77380/v1>

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Abstract

Background: Vitamins not only play a pivotal role in maintaining homeostasis of the body, but also have complex impacts on the occurrence and progression of tumors. However, the effects of vitamins on glioma and the underlying mechanism have not been fully elucidated.

Methods: Vitamin -related genes were extracted from the Molecular Signature Database v7.1 (MSigDB). The overlapping overall survival (OS)-related genes in The Cancer Genome Atlas (TCGA), the Chinese Glioma Genome Atlas (CGGA), and GSE16011 cohorts screened out by univariate COX regression analysis were utilized to construct a risk model based on the TCGA cohort via random survival forest analysis and multivariate COX regression analysis. The powerful prognostic predictive potential of the vitamin-related risk signature was verified by Kaplan–Meier survival analysis and receiver operating characteristic (ROC) analysis in the three datasets. The ssGSEA method of the GSVA package was used for functional enrichment and immune cell component analyses. ESTIMATE score analysis was used for auxiliary analysis of glioma immune characteristics. A nomogram was constructed and assessed based on the TCGA dataset.

Results: The vitamin-related six-gene (POSTN, IRX5, EEF2, RAB27A, MDM2, and ENO1) risk signature constructed based on the TCGA dataset accurately predicted the outcomes of glioma patients and credibly distinguished between different levels and molecular subtypes of glioma in the TCGA, CGGA, and GSE16011 cohorts. Gliomas with high risk scores exhibited high immune scores, low tumor purity, and immunosuppressive features. The nomogram constructed by combining the vitamin-related risk signature and clinicopathological factors precisely predicted the 1-, 3-, and 5-year OS of glioma patients.

Conclusions: Our study revealed that the vitamin-related six-gene risk signature, as an independent prognostic factor, could accurately distinguish the grade, molecular subtype, and immune characteristics of glioma.

Background

Glioma, including low-grade glioma (LGG) and glioblastoma (GBM), is the most prevalent primary malignancy of the brain, representing 81% of intracranial malignancies (1). Glioma is classified to grade I – IV by the World Health Organization (WHO) in 2016 in the light of their aggressiveness (2). Despite surgical resection combined with postoperative radiotherapy and chemotherapy, OS improvements in glioma patients remain unfavorable (3). The unfavorable outcome of glioma is due to its strong invasion ability and the lack of effective therapeutic targets (4). Therefore, the discovery of efficient therapeutic targets and the development of new therapeutic drugs to improve the OS of glioma patients are critical.

Vitamins are organic compounds required in small amounts in cellular metabolism and are important for overall health and normal growth of the organism. Since vitamins cannot be generated in vivo and must be ingested through diet, deficiencies in these substances are known to cause clinical disease states. In addition to the well-known lack of vitamin D that can cause rickets, it has been shown that a lack of folic

acid, vitamin E, and vitamin B₃ (niacin) can increase the risk of coronary heart disease (5); lack of vitamin K can cause clotting dysfunction or hemorrhage (6). Vitamins also have an important impact on the occurrence and development of tumors. Vitamin C acts as a cofactor for many enzymes to regulate antioxidant defense, transcription and epigenetics in tumors (7). Some B vitamins, including folate, riboflavin, vitamin B₆, vitamin B₁₂, and choline, affect the transformation and progression of tumors by modulating their one-carbon metabolism (8, 9). Vitamin D can inhibit tumorigenesis and prolong the survival of tumor patients (10). Vitamins also have a regulatory effect on tumor immunity (11, 12). High-dose vitamin C regulates the infiltration of immune cells into the tumor microenvironment and suppresses cancer development in a T cell-dependent manner (13). Vitamin A can increase cytotoxic activity of macrophages in melanoma (14).

In glioma, vitamin A, vitamin C, and vitamin E have been proven to reduce the risk of glioma (15–17); vitamin D can not only attenuate the expression of stem cell markers in glioma stem cells, but also inhibit the self-renewal potential and mitochondrial respiration of glioma stem cells (18).

However, the role of vitamins in tumors and the underlying mechanisms are still unclear. Some studies have confirmed that vitamin-related receptors play an important role in signal transduction. For instance, the nuclear vitamin D receptor binds to vitamin D through genomic pathways and further forms a heterodimeric complex by combining with a retinoid-X-receptor to regulate the transcription of vitamin D downstream genes such as CDKN1A, C-MYC, CDH1, and CYP24A1 (19). Other studies have found that vitamins play a role in tumors through their dependent factors. A typical example is the protein S, a vitamin K-dependent factor, which can not only promote the migration of brain neural stem cells to glioma cells, but also enhance the phagocytic ability of brain neural stem cells on apoptotic glioma cells (20). Although these studies do not fully explain the mechanism of vitamins in tumors, they at least show that the expression of vitamin-related genes is crucial in tumors and these genes may serve as potential therapeutic targets.

In our study, we comprehensively investigated the vitamin-related genes in glioma. We collected vitamin-related genes and identified six genes related to patients' prognosis. The vitamin-related six-gene risk signature was constructed and acted as an independent prognosis factor for glioma patients that can distinguish the immune characteristics in glioma microenvironment. Additionally, we established a nomogram that integrates the vitamin-related risk scores with clinicopathological features and assessed its capability in estimating 1-, 3-, and 5-year OS rates of glioma patients.

Methods

Human vitamin-related gene set

Vitamin-related genes were extracted from MSigDB (21). All the vitamin-related gene sets are shown in Table S1. After removing the overlapped genes, 622 vitamin-related genes were obtained (Table S2).

Patients and datasets

The RNA-sequencing transcriptome data and relevant clinicopathological data of 558 glioma patients were obtained from TCGA (<http://cancergenome.nih.gov/>) as a training set. Similarly, data of 416 glioma patients from CGGA (www.cgga.org.cn) were downloaded as a validation set. GSE16011 gene expression and clinicopathological data were obtained through the Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo/>) as another validation set. Raw data of GSE16011 were processed utilizing the *affy* R package, and robust multi-array analysis (RMA) was used for background correction and normalization. Patient characteristics are shown in Table S3.

Construction of a vitamin-related risk model

Univariate and multivariate Cox regression analyses, and the random survival forests-variable hunting (RSFVH) algorithm were adopted to examine the prognostic significances of vitamins-related genes in estimating glioma patients' OS. Genes were considered as potential prognostic factors at $p < 0.05$. RSFVH algorithm and multivariate Cox regression analyses with the Akaike information criterion (AIC) method were performed to further screen and narrow of the prognostic related genes. Hazard ratios (HRs) and regression coefficients were counted for each gene, and six reasonable genes were eventually filtered out. Afterwards, the vitamin-related risk model was constructed via a linear integration of the regression coefficient extracted from the multivariate Cox regression analysis and expression level of the six genes. The formula for calculating the risk scores was as follows: $Risk\ score\ (patient) = Coefficient_{mRNA1} \times Expression_{mRNA1} + Coefficient_{mRNA2} \times Expression_{mRNA2} + Coefficient_{mRNA3} \times Expression_{mRNA3} + \dots + Coefficient_{mRNA_n} \times Expression_{mRNA_n}$.

Assessing of the risk signature

All samples in the training and validation cohorts were divided into low- and high-risk groups according to the median value of risk scores. Kaplan–Meier (KM) survival curves of the two groups and the time-dependent receiver operating characteristic (ROC) curves for OS evaluation were implemented to evaluating the accuracy of the vitamin-related risk model.

Expression and prognostic characteristics of the six genes

The correlation analysis between the six genes was performed using the *corrplot* package in R. The expression level of the six genes in normal brain and glioma tissues was detected via Gene Expression Profiling Interactive Analysis (GEPIA) (22). The Human Protein Atlas (HPA) (<http://www.proteinatlas.org>) was used to analyze the expression of proteins encoded by the six genes in glioma. The genetic alteration features of the six genes were extracted from the cBioPortal (23).

Functional enrichment analysis

The ssGSEA method of *GSVA* package in R was employed to calculating the GSVA scores of each gene set from the Gene Ontology (GO) biological processes and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways for each sample. The *limma* package in R was applied to determine differentially expressed genes and differential gene sets scores in the low- and high-risk groups. GO and KEGG analyses were conducted with the differentially expressed genes between low- and high-risk groups via

the *clusterProfiler* package in R. GSEA analysis was performed via the GSEA v4.0.3 desktop program (<http://software.broadinstitute.org/gsea/datasets.jsp>) to further verify the GO analysis results.

Estimation of the glioma immune microenvironment

The *ESTIMATE* R package was employed to calculate the ESTIMATE score of each sample (24). The ssGSEA method of the GSVA package was used to calculate the infiltration levels of different immune cells in glioma microenvironment (25–27). The *immuneSubtypeClassifier* package was adopted to identify different immune subtypes of glioma samples (28).

Independence of the risk signature in predicting prognosis

Univariate and multivariate Cox regression analyses were adopted to assess the independent prognostic prediction potential of the vitamin-related risk model for glioma patients. To better evaluation the prognostic significance of the risk signature and clinicopathological features, the *ggplot2* package in R was adopted to draw the forest plots.

Establishment and evaluation of the nomogram

To improve the quality with a quantitative tool, we developed a nomogram using the *rms* package to predict the 1-, 3-, and 5-year OS of glioma patients. Calibration plots were employed to assess the accuracy of the nomogram. Decision curve analysis (DCA) has been recommended to evaluate the latent clinical effectiveness of predictive nomograms. In this study, DCA was employed to assess the clinical utility of the nomogram by calculating the net benefits for a range of threshold possibilities. DCA was performed as a statistical method that integrated the results of a decision to determine the clinical utility of a model. Therefore, the DCA can examine the net benefit to evaluate the clinical utility of a nomogram (29, 30). A net reduction curve was used as a supplementary verification for DCA (31).

Statistical analysis

Statistical analysis of this exploratory research was implemented utilizing R software (v3.6.3) or GraphPad Prism v7.00 (GraphPad Software Inc., USA). Quantitative data are presented as the mean $\pm$ standard deviation (SD). The Wilcoxon test was employed to weigh the statistical differences between two groups; the Kruskal – Wallis H test was adopted to compare multiple groups. $p < 0.05$ was believed statistically significance. The Venn diagram was plotted by *jvenn* (32).

Results

Six vitamin-related genes were screened out to develop a risk signature

To identify vitamin-related genes that were significantly associated with glioma OS, we first performed univariate COX regression analysis based on the TCGA, CGGA, and GSE16011 datasets. There were 482, 354, and 290 vitamin-related genes, respectively, in the TCGA, CGGA, and GSE16011 datasets that were

obviously linked with OS of glioma patients (Fig. 1A, Tables S4 – 6). Eleven vitamins-related genes closest linked with the prognosis of glioma patients were selected out from the 193 overlapped genes (Fig. 1A, Table S7) in the three databases according to the permutation importance score by the RSFVH algorithm based on TCGA data (Fig. 1B). To identify an optimal prognostic model and avoid overfitting of the risk signature, the above 11 genes were subjected to multivariate Cox regression analysis with a stepwise model selection to establish an optimal gene combination for constructing the risk signature based on TCGA data. Finally, six genes (POSTN, IRX5, EEF2, RAB27A, MDM2 and ENO1) were filtered out (Fig. 1C). Among the six genes, POSTN, IRX5, RAB27A, MDM2 and ENO1 were unfavorable factors for glioma survival with HR > 1, and EEF2 was a conservatory factor with HR < 1.

Construction and evaluation of the vitamin-related risk signature

After extracting the coefficient values obtained from the multivariate Cox regression analysis on the basis of TCGA data, we calculated individualized risk scores with coefficient-weighted expression levels of six vitamin-related genes with the following formula: risk score = $(0.1363 \times \text{expression level of POSTN}) + (0.2367 \times \text{expression level of IRX5}) + (-0.4342 \times \text{expression level of EEF2}) + (0.2201 \times \text{expression level of RAB27A}) + (0.2183 \times \text{expression level of MDM2}) + (0.4314 \times \text{expression level of ENO1})$. Individuals were classified as low- or high-risk based on the median value of the risk scores; consequently, a vitamin-related six-gene risk signature was established. The ROC curve analysis displayed excellent discrimination with the areas under the curve (AUC) of 0.900, 0.936, and 0.874 at 1-, 3-, and 5-year OS, respectively, in the TCGA dataset (Fig. 2A). ROC curve analysis based on the CGGA and GSE16011 datasets also indicated that the vitamin-related risk signature had high sensitivity and specificity in prognostic prediction (Fig. 2B, C). To further examine the prognostic prediction ability of the vitamin-related risk model in glioma, KM analysis was employed to determine the difference of OS between the low- and high-risk groups, which revealed that the low-risk group had a considerably increased OS compared with the high-risk group in the TCGA, CGGA, and GSE16011 datasets (Fig. 2D – F). The distribution of risk scores and survival status displayed in Fig. 2G and H and Figure S1 further confirmed the excellent prognostic prediction potential of the risk model. These results confirmed that the vitamin-related risk signature could act as a reliable prognostic predictor for glioma patients.

Genetic and expression characteristics of the six genes

To better understand the six vitamin-related genes, we analyzed the correlations of the six genes in the TCGA, CGGA, and GSE16011 datasets. We found that POSTN and RAB27A had a significantly positive correlation while EEF2 was negatively correlated with RAB27A in the three datasets (Fig. 3A – C). We next explored the expression of the six genes in glioma and normal brain tissue. GEPIA, the online tool with data sourced from TCGA and GTEx, was adopted to detect the mRNA expression of these genes (22). As show in Fig. 3D, except for EEF2, the expression of the remaining five genes in GBM was higher than that in LGG, and the expression of the six genes in glioma was higher than that in normal brain tissue. We

further explored the protein expression of POSTN, EEF2, MDM2, and ENO1 in the HPA dataset; characteristic images are shown in Fig. 3E. However, we did not find IRX5 and RAB27A proteins expression in the HPA database. Consistent with the multivariate Cox regression analysis results, KM survival curves presented that samples with higher expression levels of POSTN, IRX5, RAB27A, MDM2, or ENO1 had worse outcomes and samples with higher expression levels of EEF2 had a more favorable outcome in the TCGA cohort (Fig. 3F – K). Then, we examined the genetic alteration of these risk-associated genes in glioma; among the 794 patients included in the cBioportal for Cancer Genomics database, 66 (8.3%) patients showed genetic alterations. Amplification was the most prevalent genetic alteration (Figure S2).

The correlation of risk scores and clinicopathologic features

We further detected the risk scores in glioma patients classified by WHO grades, IDH status, 1p19q status, MGMT promoter, and subtype. The risk score was significantly increased along with WHO grades in the TCGA, CGGA, and GSE16011 cohorts (Fig. 4A – C), and the remarkably increased risk scores were also observed in IDH wild type (Fig. 4D – F), 1p19q non-codeleted (Fig. 4G, H), MGMT promoter unmethylated (Fig. 4I, J), and mesenchymal subtype (Fig. 4K, L) glioma patients. These data suggested a notable association between the vitamin-related risk signature and clinical molecular features of glioma patients.

Prognostic significance of the risk signature in stratified patients

To comprehensively examine the risk model in glioma, we detected the correlation between risk scores and OS of glioma patients classified by WHO grades, IDH status, 1p19q status, and MGMT promoter. KM analysis showed samples in the low-risk group had an obviously longer OS than those in the high-risk group for LGG patients in the TCGA, CGGA, and GSE16011 datasets (Fig. 5A – C). Consistent results were also found in most stratified samples except in 1p19q codeleted patients (Fig. 5D – T). These findings demonstrated the powerful potential of the vitamin-related risk model in predicting the outcome of glioma patients.

Functional enrichment analysis

To unearth the vitamin-related risk signature associated biological processes, the GSVA score of KEGG pathways and GO biological processes (c5.bp.v7.1.symbols.gmt) was investigated between the low- and high-risk groups in the TCGA, CGGA, and GSE16011 datasets. The top 30 KEGG pathways and top 30 GO biological processes significantly enriched in the high-risk group ($p < 0.05$) were screened out. We found that most of the processes were linked with immune and inflammatory responses in the TCGA (Fig. 6A – B), CGGA (Figure S3A – B), and GSE16011 (Figure S4A – B) datasets. To confirm this result, we performed GO and KEGG analyses of the differentially expressed genes between the two groups. The biological

processes and pathways enriched in the high-risk group was similar to the results of GSVA in the three datasets (Fig. 6C, D, Figure S3C, D, Figure S4C, D). Additionally, the GSEA analysis of GO biological processes in the three databases was also consistent with the GO analysis (Fig. 7A – C). These data denoted that the vitamin-related risk model may be able to distinguish the immune characteristics of gliomas.

Immune features of the low- and high-risk groups

Considering the role of vitamins in the immune system (11, 12) and the results of the above-mentioned functional enrichment analysis, we speculated that the vitamin-related risk signature may be linked with the tumor immune characteristics of glioma. To verify this conjecture, we first compared the ESTIMATE scores between the low- and high-risk groups. ESTIMATE scores in the high-risk group were significantly higher than that in the low-risk group in the TCGA (Fig. 8A), CGGA (Fig. 8E), and GSE16011 (Figure S5A) datasets. Similarly, the immune and stromal scores were obviously higher in the high-risk group than in the low-risk group in the three datasets; while tumor purity showed the opposite result (Fig. 8B – D, F – H, Figure S5B – D). Correlation analysis presented that the ESTIMATE, immune and stromal scores were all significantly positively correlated with the risk score, but tumor purity was negatively correlated with the risk score in the TCGA, CGGA, and GSE16011 datasets (Fig. 8I – P, Figure S5E – H). These results indicated that the higher the risk score, the lower the purity of the glioma, indicating that the higher the risk score, the more complex the microenvironment of the glioma. We previously confirmed that, in glioma, lower tumor purity indicated a worse prognosis (33). This was accordant with the prognosis prediction of the risk signature of this study, and further confirmed that the vitamin-related risk model was accurate in predicting the tumor purity of glioma and the prognosis of glioma patients.

Immune cells, as an important component in the glioma microenvironment, affect tumor purity of glioma (34, 35). As glioma purity is reduced, the local immune status can be enhanced (33) and the vitamin-related risk score is significantly negatively correlated with tumor purity. We speculated that the risk score may be linked with the distribution of immune cells in the glioma microenvironment. To investigate their relationship, we employed GSVA to estimate signatures of multiple immune cells and compared the distribution of immune cells in low- and high-risk groups. We found that most immune cells, whether innate or adaptive immune cells, were notably more enriched in the high-risk group in the TCGA (Fig. 9A, B), CGGA (Fig. 9C, D), and GSE16011 (Figure S6A, B) datasets. This indicated that the higher the risk score, the more immune cells were enriched in the glioma microenvironment. At the same time, it also suggested that the vitamin-related risk signature can directly predict the local immune status of glioma, highlighting its strong predictive ability. It has been shown that higher immune cells infiltration indicates a worse outcome in both LGG and GBM (36). This further confirmed the accuracy and reliability of the vitamin-related risk signature in predicting the local immune response of the glioma microenvironment and the prognosis of glioma patients.

To further characterize intratumoral immune states, we analyzed the components of immune subtypes in the TCGA, CGGA, and GSE16011 datasets using the *immuneSubtypeClassifier* package. In the three

datasets, the C4 subtype was the main component of the high-risk group, while the C5 subtype was mainly concentrated in the low-risk group (Fig. 9E). The OS of tumor patients classified as C4 subtype is worse than that of tumor patients classified as C5 subtype (28), which is consistent with the prognosis of glioma in the low- and high-risk groups differentiated by the vitamin-related risk model. These results indicated that the vitamin-related risk model could not only accurately predict the immune status, but also could exactly reveal the prognosis of glioma patients.

Vitamin-related signature indicates an immunosuppressive microenvironment

In the process of the immune system's elimination of tumor cells, a series of anti-tumor immune response processes are initiated and expanded iteratively; these processes are defined as the cancer-immunity cycle (37). Immune cells play a vital role in tumor clearance in this cycle. However, in glioma, the high-risk group had significantly higher immune cell enrichment than the low-risk group, but the prognosis was worse than that of the low-risk group, indicating that there may be other immunosuppressive mechanisms in the high-risk group. To explore this, we first detected the expression of cancer-immunity cycle inhibitors in the two groups (38). The expression levels of most cancer-immunity cycle inhibitors in the high-risk group were obviously higher than those in the low-risk group in the TCGA, CGGA, and GSE16011 (Fig. 10A, B, Figure S7A) datasets. TGFB1, VEGFA, IL10, ARG1, CSF1, and FGL2 are immunosuppressive factors secreted by glioma cells, while CD70 and FASLG are glioma cell surface immunosuppressive factors (39). We further compared the expression levels of these genes in the two groups in the TCGA, CGGA, and GSE16011 datasets. As presented in Fig. 10C – E, the expression levels of these immunosuppressive factors in the high-risk group were evidently higher than those in the low-risk group in the three datasets.

In addition to cancer-immunity cycle inhibitors, immune checkpoints also play a vital role in tumor immune tolerance. Some immune checkpoint blockers have emerged as powerful weapons in the oncological armamentarium (40). Similar to cancer-immunity cycle inhibitors, the expression of most immune checkpoints in the high-risk group was notably higher than that in the low-risk group in the TCGA (Fig. 11A), CGGA (Fig. 11B), and GSE16011 (Figure S7B) datasets. Since CTLA4, PD1 and PD-L1 blockers have shown clinical benefit in tumor treatment (41, 42), we separately explored their expression in the two groups and their relationship with risk scores in the TCGA and CGGA datasets. As shown in Fig. 11C – N, the expression levels of CTLA4, PD1 and PD-L1 in the high-risk group were significantly higher than those in the low-risk group and significantly positively correlated with the risk scores.

These findings indicated that glioma with high vitamin-related risk scores tended to form an immunosuppressive microenvironment via overexpression of cancer-immunity cycle inhibitors and immune checkpoints. This also explained why the high-risk group had more immune cells infiltration than the low-risk group but their prognosis was worse.

Building and validating a nomogram

To examine whether the prognostic significance of vitamin-related risk model did not depend on other clinicopathological factors, univariate and multivariate Cox regression analyses were conducted in the TCGA dataset. We detected that the risk signature was obviously linked with OS even when adjusted by other clinical factors (Fig. 12A, B). The same results were also obtained in the CGGA and GSE16011 datasets (Figure S8A – D).

To develop a clinically utilizable method for predicting the 1-, 3-, and 5-year OS of glioma patients, we constructed a nomogram based on the TCGA cohort. The factors of the nomogram included clinicopathological variables (age, gender, WHO grade, IDH status, 1p19q status, and MGMT promoter status) and the vitamin-related risk score (Fig. 12C). Calibration curves were employed to evaluate the accuracy of the nomograms in predicting the OS of glioma patients. The 45-degree line denoted the most accurate prediction. Calibration curves displayed that the nomogram performed excellently in both the TCGA and CGGA cohorts (Fig. 12D, E). The DCA based on the TCGA dataset suggested that the risk score-based nomogram could amplify net benefits and display a broad range of threshold possibility in the prediction of 1-, 3-, and 5-year OS (Fig. 12F – H). Consistent results were also found in the CGGA dataset (Figure S8E – G). The net reduction analyses based on the TCGA and CGGA cohorts further confirmed the results of the DCA (Fig. 12I – K, Figure S8H – J).

Discussion

Vitamins play a multi-faceted role in tumors. In addition to affecting their occurrence and progression, vitamins can also influence the effects of radiotherapy and chemotherapy in tumor patients, and have an effect on the treatment of cachexia in tumor patients (43, 44). However, different vitamins can have completely different effects. The specific mechanisms of the different effects of vitamins in tumors are complex and diverse and involve a variety of signaling and biological pathways (19, 20).

In this study, we first selected all vitamin-related genes from MSigDB. From these, genes related to the OS of glioma patients in the TCGA, CGGA, and GSE16011 datasets were screened out. Based on the TCGA database, vitamin-related six-gene risk model was constructed using survival-related genes. Among these six genes (POSTN, IRX5, EEF2, RAB27A, MDM2, and ENO1), vitamin D can suppress the metastasis potential of breast cancer cells by inhibiting the expression of POSTN through its receptor (45). IRX5 is also regulated by vitamin D in prostate cancer (46). RAB27 is related to the level of 25(OH)D in serum (47) and can regulate the invasion ability of melanoma cells (48). The expression of MDM2 is affected by folic acid and can inhibit the effects of ADR and p53 (49–51). ENO1 can regulate the metabolism of vitamin D3 and indole (52). EEF2 plays a vital role in the tumorigenesis and progression of some tumors (53–55).

We checked and verified the prognostic prediction potential of the risk signature in the above three databases via ROC curve, KM curve and risk score distribution plots. We also revealed correlations, expression characteristics, prognostic effects, and mutation characteristics of the six genes in glioma. These six genes were significantly linked with the outcomes of glioma patients, and the vitamin-related

six-gene risk signature could accurately predict the outcome of glioma patients. Additionally, the risk signature can accurately distinguish different grades and molecular subtypes of glioma, and can predict the outcomes of glioma patients with different grades and molecular subtypes.

Functional enrichment analysis found that there was an accumulation of immune response-related pathways in the high-risk group. The ESTIMATE score displayed that the high-risk group had higher immune scores and lower tumor purity than the low-risk group, indicating that the risk model might be linked with the characteristics of the immune microenvironment of glioma. Immune cell component analysis confirmed this conclusion. The expression characteristics of cancer-immunity cycle suppression molecules and immune checkpoints verified that high vitamin-related risk scores were related to glioma immunosuppression.

To make full use of the vitamin-related risk signature, we combined it with clinicopathological factors to construct a nomogram. This nomogram has strong potential in predicting the outcome of glioma patients.

Although the vitamin-related risk signature has great potential in predicting the outcome of glioma patients, it still has some limitations. First, the construction of this model is based on the mRNA expression of the six genes, which needs to be verified at the protein expression level. In addition, due to the various types and functions of vitamins, we cannot enumerate the specific role of every vitamin-related gene set in glioma.

Conclusion

Overall, our study screened out six vitamin-related genes, which were used to construct an independent prognostic risk signature, that can accurately distinguish different WHO grades and molecular subtypes of glioma, and accurately predict the immune characteristics of glioma.

Abbreviations

MSigDB

molecular signature database;

TCGA

the cancer genome atlas;

CGGA

Chinese glioma genome atlas;

ROC

receiver operating characteristic;

KM

Kaplan–Meier survival analysis

GBM

glioblastoma;
LGG
low-grade glioma;
GEO
gene expression omnibus;
RMA
robust multi-array analysis;
RSFVH
random survival forests-variable hunting algorithm;
AIC
Akaike information criterion method;
HRs
hazard ratios;
GEPIA
gene expression profiling interactive analysis;
HPA
human protein atlas;
GO
gene ontology;
KEGG
Kyoto encyclopedia of genes and genomes;
DCA
decision curve analysis;
AUC
areas under the curve;

Declarations

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

Not applicable.

Availability of data and materials:

Publicly available datasets used in this study can be acquired at: [http:// www.cgga.org.cn/](http://www.cgga.org.cn/) (CGGA), <https://portal.gdc.cancer.gov/> (TCGA), <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE16011> (GSE16011).

Competing interests:

The authors declare that they have no competing interests.

Funding:

This work was supported by grants from the National Natural Science Foundation of China (grant numbers: 81172409, 81472360, and 81872054), and Liaoning Science and Technology Plan Projects (grant number: 2011225034).

Authors' contributions:

Qing Zhang and Anhua Wu: project conception and design. Qing Zhang: data interpretation, statistical analysis, and preparation of the manuscript. Anhua Wu: manuscript review and revision. All authors approved the final manuscript.

Acknowledgments:

The authors thank the Department of Pathology, China Medical University, Shenyang, China for technical support.

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Figures

Figure 1

Identification of prognostic-related genes to establish a risk model. (A) Overlapping OS-related genes in the TCGA, CGGA, and GSE16011 datasets. (B) RSFVH analysis revealed the chosen genes based on

important score. (C) Six vitamin-related genes (POSTN, IRX5, EEF2, RAB27A, MDM2 and ENO1) were established by multivariate Cox regression analysis to develop the risk model.

Figure 2

Construction and evaluation of the prognostic prediction potential of the vitamin-related risk model. (A–C) The accuracy of the 1-, 3-, and 5-year OS prediction of the risk model was assessed by ROC curves in the TCGA (A), CGGA (B), and GSE16011 (C) datasets. (D–F) K-M curves presented the OS differences

between low- and high-risk groups in the three datasets. (G, H) Risk score and survival status distribution of glioma patients in the TCGA (G) and CGGA (H) datasets.

Figure 3

Expression characteristics and prognostic significance of the six genes. (A-C) Correlation between the six genes in the TCGA (A), CGGA (B), and GSE16011 (C) cohorts. (D) Expression of the six genes in normal brain, LGG and GBM tissues acquired from GEPIA database. (E) Protein expression levels of POSTN,

EEF2, MDM2, and ENO1 were obtained from the HPA database via immunohistochemistry staining. (F-K) Expression level of the six genes were significantly linked with the prognosis of glioma patients in TCGA.

Figure 4

The vitamin-related risk score could distinguish the grades and molecular subtypes of glioma. (A-C) The risk score increased with the grade of glioma in the TCGA (A), CGGA (B), and GSE16011 (C) cohorts. (D-F) Vitamin-related risk scores in IDH wild-type glioma were notably higher than those in IDH mutant glioma in the three datasets. (G, H) Vitamin-related risk scores in 1p19q non-codeleted glioma were significantly higher than those in 1p19q codeleted glioma in the TCGA (G) and CGGA (H) datasets. (I, J) MGMT promoter methylated glioma had relatively low risk scores in the TCGA (I) and CGGA (J) datasets.

(K, L) Mesenchymal subtype had the highest risk scores among the four subtypes in the TCGA (K) and GSE16011 (L) datasets.

Figure 5

Prognosis prediction of the vitamin-related risk signature in stratified glioma patients. (A-T) High risk score was linked with unfavorable outcome in glioma patients stratified by grade, IDH status, MGMT promoter, and 1p19q codeletion status in the TCGA, CGGA, and GSE16011 datasets.

Figure 6

Functional enrichment analysis of the vitamin-related risk model. (A, B) Top 30 GO processes (A) and KEGG pathways (B) enriched in the high-risk group in the TCGA dataset. (C, D) GO (C) and KEGG (D) analyses based on genes overexpressed in the high-risk group.

Figure 7

GSEA analysis of the vitamin-related risk signature. (A–C) GSEA analysis of GO biological processes based on the median value of the risk score in the TCGA (A), CGGA (B), and GSE16011 (C) datasets.

Figure 8

ESTIMATE score analysis of the risk signature. (A-H) ESTIMATE, immune, and stromal scores, and tumor purity in low- and high-risk groups in the TCGA (A-D) and CGGA (E-H) cohorts. (I-P) Correlation of ESTIMATE, immune, and stromal scores, tumor purity and risk scores in the TCGA (I-L) and CGGA (M-P) datasets.

Figure 9

Composition of immune cells. (A–D) Distribution of innate and adaptive immune cells in the high- and low-risk groups in the TCGA (A, B) and CGGA (C, D) cohorts. (E) The immune subtype distribution in the two groups in the TCGA, CGGA and GSE16011 datasets.

Figure 11

Expression profile of immune checkpoints. (A, B) Heatmap of immune checkpoints in the TCGA (A) and CGGA (B) datasets. (C-H) Expression of CTLA4, PD-L1, and PD1 in the two groups in the TCGA (C-E) and CGGA (F-H) cohorts. (I-N) Correlation of the expression levels of CTLA4, PD-L1, PD1, and risk scores in the TCGA (I-K) and CGGA (L-N) datasets.

Figure 12

Establishment and evaluation of the nomogram. (A, B) Univariate (A) and multivariate (B) COX regression analyses assessed the effect of the risk signature and clinicopathological factors on the OS of glioma patients in the TCGA cohort. (C) Nomogram developed based on TCGA dataset. (D, E) Calibration curves established in the TCGA (D) and CGGA (E) datasets to evaluate the sensitivity and specificity of the nomogram in predicting 1-, 3-, and 5-year OS of glioma patients. (F–K) Net benefit (F–H) and net

reduction (I-K) curves evaluating the nomogram in predicting the 1-, 3-, and 5-year OS of glioma patients in the TCGA cohort.

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