Identification of Survival-Related Metabolic Genes and a Novel Gene Signature Predicting the Overall Survival for Patients with Uveal Melanoma

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\textbf{Keywords}
Uveal melanoma · Metabolic signature · Overall survival · Prognostic model · Immune-related features

\textbf{Abstract}

\textbf{Introduction:} Uveal melanoma (UM) is the most common primary intraocular malignancy among adults. Altered metabolism has been shown to contribute to the development of cancer closely, but the prognostic role of metabolism in UM remains to be explored. This study aimed to construct a metabolic-related signature for UM. \textbf{Method:} We collected the mRNA sequencing data and corresponding clinical information from The Cancer Genome Atlas and Gene Expression Omnibus databases. A univariate Cox regression analysis, the Lasso-penalized Cox regression analysis, and multivariate Cox regression analyses were used to construct a metabolic signature based on TCGA. The time-dependent ROC and Kaplan-Meier survival curves were calculated to validate the prognostic ability of the signature. The immune-related features and mutation profile were characterized by CIBERSORT and maftools between high- and low-risk groups. \textbf{Result:} A novel metabolic-related signature (risk score $= -0.246^{*}\text{SLC25A38} - 0.50186^{*}\text{ABCA12} + 0.032^{*}\text{CA12} + 0.086^{*}\text{SYNJ2}$) was constructed to predict the prognosis of UM patients. In TCGA and GSE22138, the signature had high sensitivity and specificity in predicting the prognosis of UM patients (survival probability; $p < 0.0001$, $p = 0.012$). Gene Ontology pathway enrichment analysis and GSEA were used to discriminate several significantly enriched metabolism-related pathways, including channel activity and passive transmembrane transporter activity, which may reveal the underlying mechanisms. The high-risk group had more immune cell infiltration and greater distribution of BAP1 mutations. \textbf{Conclusion:} Our study developed a robust metabolic-gene signature based on TCGA to predict the prognosis of UM patients. The signature indicates a dysregulated metabolic microenvironment and provides new metabolic biomarkers and therapeutic targets for UM patients.

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\textbf{Introduction}

Uveal melanoma (UM) is the most common primary malignancy of the adult eye with the incidence of 5.1 individuals per million per year worldwide [1–3]. Most UM patients suffer from visual loss and almost 50% of patients...
will eventually succumb to metastatic disease [4]. UM originates from melanocytes in the eye, including the iris, ciliary body, and choroid and differs from cutaneous melanoma at the genetic and molecular profiles [5]. As a consequence, many therapies that have proven effective in cutaneous melanoma have little or no success in UM. Considering the fact that the survival of UM has not improved over the past 30 years under the guidance of cutaneous melanoma therapies, elucidating the unique development of UM and obtaining a better understanding of the complex interaction between genetic factors, molecular features, and potential targets will be critical to develop innovative therapies specific for UM.

The abnormal alternations of metabolism in cancer cells make them well-distinguished from healthy cells, which are recognized as a crucial hallmark of cancer. The great significance of metabolic alternations on tumor was first revealed in 1924 by Otto Warburg [6]. It has been confirmed that metabolic reprogramming can support the biological processes (BPs) or promote the transformation to support the initiation and progression of tumor [7]. More importantly, the metabolic subtypes of cancer determine the heterogeneity of cancer in some way, suggesting that tumor relied on different metabolic energy metabolic pathways will develop different metastasis ability, immune-related features, sensitivity to treatment, and outcomes [8, 9]. For example, the cluster of glycolysis and pentose phosphate pathway (PPP) preferences had poor prognostic compared with the cluster of fatty acid oxidation (FAO), and glutaminolysis preferences in the study of breast cancers [10]. In the study of ovarian cancers, the dependence on glutamine and fatty acids determined highly sensitive to conventional chemotherapies and better prognostic compared with dependence on glycolysis [8]. Therefore, there is great importance in understanding the specificity of metabolism in UM to develop therapeutic strategies targeting the metabolism to help patients. However, the knowledge on the metabolic signatures of UM is limited currently.

By utilizing the genome sequencing and bioinformatics, identifying multiple metabolic biomarkers with high sensitivities and specificities is particularly crucial to predict the prognosis and potential therapeutic targets of UM. In this study, we used metabolic-related genes expression profiles and clinicopathological data obtained from TCGA to establish a metabolic-gene signature to predict the outcome of UM patients. What’s more, we evaluated the prognosis value of this signature based on mRNA expression profiles and clinical information obtained from TCGA and GEO database. Finally, underlying mechanism, metabolic pathways, immune-related features, and mutation data related to this signature was explored thoroughly.

Materials and Methods

Collection of Data

We collected the mRNA sequencing data and corresponding clinical information of 80 UM patients from The Cancer Genome Atlas (TCGA, http://cancergenome.nih.gov/) [1]. The GSE22138 data served as testing data with 63 tumor samples were downloaded from Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) datasets using R package "GEO query" [11].

Construction and Validation of the Prognostic Metabolic-Related Gene Signature

After excluding patients without survival information, clinical information from the TCGA database was used to assess the prognostic associations of the metabolic genes with clinical outcomes. Univariate Cox proportional hazards regression analysis was used to identify differentially expressed overall survival (OS)-related metabolic genes, and adjusted p values <0.001 were considered statistically significant. Lasso-penalized Cox regression and multivariate Cox regression analyses were used to construct a new prognostic gene signature, which was calculated by the following formula: risk score = (coefficient mRNA1*expression of mRNA1) + (coefficient mRNA2*expression of mRNA2) + (coefficient mRNA3*expression of mRNA3). The cutoff point of the risk score was accessed using the R package “survminer,” which divided the patients into high- and low-risk groups. Kaplan-Meier survival curves were drawn using the R package “survival” to analyze the OS of the models. Time-dependent receiver-operating characteristic (ROC) analysis was used to evaluate the predictive performance of the gene expression signature.

External Evaluation of the Prognostic Metabolic Gene Signature in the GEO Validation Cohorts

To verify the prognostic value of the signature in the GEO dataset, patients in the GSE22138 were divided into high- and low-risk groups using the same cutoff values of the prognostic signature constructed from the TCGA cohorts. The time-dependent ROC and Kaplan-Meier survival curves were constructed identically to the analyses of the TCGA dataset.

Differentially Expressed Gene Identification

The TCGA cohort was divided into two groups based on the cutoff point of estimated score. The limma R package was used to identify differentially expressed genes (DEGs) between the high and low metabolic score groups. An adjusted p value <0.05 and a |log2-fold change (FC)| > 1 were set as the selection criteria to determine the significant DEGs.

Gene Functional Enrichment Analysis

Gene Ontology (GO) terms and pathway enrichment analysis were performed to classify genes into DEG sets based on their functions. An adjusted p value <0.05 was considered statistically significant for BP, cellular components, and molecular functions. GSEA was implemented to identify the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and molecular mecha-
nisms of the signature genes in the TCGA cohort. Gene sets with a $p$ value <0.05 were considered statistically significant.

**Difference of Energy-Related Metabolic Genes Identification**

To identify differences in optimal energy metabolic pathways between two groups, we analyzed the expression levels of genes involved in four central metabolic pathways: glycolysis, PPP, FAO, and glutaminolysis, according to the papers [10].

**Evaluating the Immune-Related Features between High- and Low-Risk Groups**

We used CIBERSORT to dissect the immune infiltration data in each sample downloaded from the TCGA, which is a newly developed deconvolution algorithm for obtaining the abundance of 22 immune cell types from complex tissues. The differential abundance of 22 immune cells between two groups was compared using a two-sample t test. We compared the expression of immune checkpoint points between the two groups. The immune score and the stromal score of each sample were calculated using the ESTIMATE algorithm in both cohorts.

**Mutation Analysis**

The mutation data of UM patients were obtained from the TCGA dataset. Mutation annotation format files were analyzed and mapped using maftools [12].

**Results**

**Construction of the Prognostic Signature in TCGA Training Cohort**

First, univariate COX regression using the OS and mRNA sequencing data of TCGA UM patients was applied to evaluate the prognostic value of the metabolic-related genes (Table 1). Next, we performed the LASSO Cox selection method (Fig. 1) to identify 10 survival-related genes, including IDH2, SLC25A38, SLC45A2, ABCA12, ATP8B3, CA12, MANEAL, PAPSS2, PLCD1, and SYNJ2. After multivariate Cox regression to define the independent predictive power, a prognostic signature consisting of 4 genes (SLC25A38, ABCA12, CA12, SYNJ2) was established. The risk score = mRNA expression level of $-0.246 \ast SLC25A38 - 0.50186 \ast ABCA12 + 0.032 \ast CA12 + 0.086 \ast SYNJ2$. The upregulation of SLC25A38 and ABCA12 is related to low risk, while the upregulation of CA12 and SYNJ2 is associated with high risk.

**Validation of the Prognostic Risk Signature in TCGA and GEO**

According to the OS model, we calculated the risk scores of UM patients in TCGA cohorts and GEO cohorts, respectively. Based on the median risk score, patients were divided into high- and low-risk groups. The distribution of risk score, survival data, and the expression of 4 OS-related genes in TCGA cohorts and GEO cohorts are illustrated in Figure 2a and Figure 3a, respectively. As shown in Figures 2a and 3a, patients who died were more clearly distributed on the high-risk groups than the low-risk groups. Subsequently, the area under the ROC curve for 1 year and 3 years was 0.94 and 0.92, respectively, in TCGA cohorts (Fig. 2b). The area under the ROC curve for 1 year, 3 years, and 5 years was

| Gene     | p value     | HR  | Low 95% CI | High 95% CI |
|----------|-------------|-----|------------|-------------|
| CA12     | 0.00000061  | 1.000271 | 1.000164   | 1.000377    |
| SLC45A2  | 0.00000080  | 1.000442 | 1.000266   | 1.000617    |
| IDH2     | 0.00000083  | 1.000648 | 1.000390   | 1.000906    |
| ATP8B3   | 0.00000106  | 1.004195 | 1.002508   | 1.005885    |
| TRPV2    | 0.00000344  | 1.000212 | 1.000122   | 1.000301    |
| HSD11B2  | 0.00000404  | 1.020887 | 1.01952    | 1.029900    |
| G6PC3    | 0.00000491  | 1.000238 | 1.000136   | 1.000340    |
| GUSB     | 0.00000588  | 1.000783 | 1.000444   | 1.001123    |
| NDUFB8   | 0.00000617  | 1.001346 | 1.000762   | 1.001930    |
| KCNN3    | 0.00000648  | 1.019906 | 1.011207   | 1.028679    |
| NUDT14   | 0.00000750  | 1.000946 | 1.000525   | 1.001367    |
| CYC1     | 0.00001220  | 1.000115 | 1.000063   | 1.000166    |
| SLC39A4  | 0.00001230  | 1.000492 | 1.000272   | 1.000713    |
| PAPSS2   | 0.00001400  | 1.003801 | 1.002084   | 1.005520    |
| MGST2    | 0.00001440  | 1.000522 | 1.000286   | 1.000757    |
| NQO1     | 0.00001510  | 1.000158 | 1.000087   | 1.000230    |
| ABCC4    | 0.00001530  | 1.002514 | 1.001374   | 1.003565    |
| CHST9    | 0.00001560  | 1.002376 | 1.001297   | 1.003456    |
| TTYH3    | 0.00001900  | 1.000069 | 1.000037   | 1.000100    |
| ASS1     | 0.00001940  | 1.000908 | 1.000491   | 1.001325    |

**Table 1.** Univariate Cox proportional regression analysis of the top 20 DEGs
Identification of DEGs in the High- and Low-Risk Groups and Functional Annotations

A total of 1,458 upregulated genes and 731 downregulated genes were identified between the high- and low-risk groups. The volcano plot and heatmap of DEGs were present in Figure 4. GO-BP analysis demonstrated that the DEGs were significantly enriched in cell morphogenesis involved in neuron differentiation, axon development, and axon genesis (Fig. 5a). It was shown in the GO-cellular component analysis that the DEGs were enriched in extracellular matrix, collagen-containing extracellular matrix, and plasma membrane protein complex (Fig. 5b). Moreover, GO-molecular function analysis showed that these genes were significantly associated with channel activity, passive transmembrane transporter activity, and substrate-specific channel activity (Fig. 5c). Furthermore, the GSEA analysis was employed to stratify the transcript message of UM patients. The most significantly enriched metabolism-related pathways of the low-risk groups were the circadian rhythm mammal, the ribosome, histidine metabolism, primary bile acid biosynthesis, limonene and pinene degradation, thyroid cancer, tyrosine metabolism, and tight junction pathways (Fig. 5d). Moreover, the enrichment of calcium signaling, Wnt signaling, apoptosis, JAK-STAT signaling, and MAPK signaling pathways was significantly different between two groups (Fig. 5e).

Different Expressions of Genes Associated with Optimal Energy Metabolic Pathways

We analyzed the expressions of genes involved in four central metabolic pathways: glycolysis, PPP, FAO, and glutaminolysis between high- and low-risk groups. The expression of TPI1 and PKM2 was significantly higher in the high-risk group, which encodes enzymes participating in glycolysis (Fig. 6a). Moreover, the expression of CPT1B, an FAO-related gene, was significantly lower in the high-risk group, while ACADVL showed higher mRNA levels in the high-risk group (Fig. 6b).

Difference of Immune-Related Features among Two Groups

Given the inextricable interaction between tumor metabolism and tumor immune microenvironment, we analyzed the difference of immune characteristics among two groups. As shown in Figure 7a, the abundance of T cells CD8, T cells regulatory, macrophages M0, and macrophages M1 was significantly more enriched in the high-risk group. Additionally, plasma cells, monocytes, macrophages M2, neutrophils were enriched more in the low-risk group. Meanwhile, we thoroughly explored the expression of PD-1, PD-L1, PD-L2, and CTLA-4. The ex-
Expression of PD-1 and CTLA-4 was higher in the high-risk group (Fig. 7b). Finally, the immune score and stromal score were significantly higher in the high-risk group. Overall, the results showed that the high-risk group tend to have more immune cell infiltration compared with the low-risk group.

Fig. 2. Identification of the prognostic model in the training group. a Risk score, survival data, and heatmap of mRNA expression in the TCGA. b Time-dependent ROC curves of the signature in the TCGA dataset. 1 year, AUC = 0.94; 3 years, AUC = 0.92. c Kaplan-Meier curve of the signature in the TCGA dataset, p < 0.0001.
Fig. 3. Identification of the prognostic model in the testing group. a Risk score, survival data, and heatmap of mRNA expression in the GEO dataset. b Time-dependent ROC curves of the signature in the TCGA dataset. 1 years, AUC = 0.67; 3 years, AUC = 0.75; 5 years, AUC = 0.73. c Kaplan-Meier curve of the signature in the GEO dataset, $p = 0.012$. 

A Metabolic Signature Predicting the Overall Survival of Uveal Melanoma
The Mutation Profile of the Prognostic Risk Signature

We thoroughly explored the mutation characteristics of all UM samples in TCGA dataset. As shown in Figure 8a, gene GNA11 mutated most frequently approximately accounting for 52% in the high-risk group, followed by BAP1 (48%) and GNAQ (40%). As for the low-risk group (Fig. 8b), the most frequently mutated gene was GNAQ (59%), followed by GNA11 (34%) and SF3B1 (32%). Among the alterations, missense mutation was the most common variant classification.

Discussion

Cancer is a metabolic disease, and metabolic reprogramming is a key event in cancer development and progression [13, 14]. The reliable predictive value of metabolic-related prognostic signature has been shown in various types of cancers, including lung adenocarcinoma [15], stomach adenocarcinoma [16], hepatocellular carcinoma [17], breast cancer [18], and so on. Accumulating evidence suggests that metabolic reprogramming is a dominant determinant of tumor growth and metastasis in UM [19]. For example, the expression of mitochondrial metabolism-related genes is higher in metastatic UM compared to nonmetastatic UM [20]. High glycolytic activity is another characteristic of UM tumors, and higher total glycolytic activity was associated with poor OS of patients [21]. Therefore, characterization of the leading metabolites and pathways of UM should provide detectable biomarkers and therapeutic targets for patients. To the best of our knowledge, this is the first study to construct a metabolic-related prognostic signature of UM based on public datasets. The present study developed a prognostic signature with four metabolic genes based on the TCGA dataset and verified its value in the GSE22138 dataset [1, 11]. The results showed that patients in the high-risk group showed poorer overall survival outcomes, which indicated that the signature efficiently stratified the OS values of UM patients.

Four genes in our prognostic signature were involved in the development of cancer. The upregulation of SYNJ2 and CA12 was related to high risk, and the upregulation of ABCA12 and SLC25A38 was associated with low risk.
SYNJ2 is a member of the inositol-polyphosphate 5-phosphatase family, and it encodes an inositol polyphosphate phosphatase that participates in membrane trafficking and signal transduction pathways. Recent studies showed that the overexpression of SYNJ2 in breast cancer patients correlated with poor survival [22, 23]. The oncogenic activity of SYNJ2 in tumor growth and metastasis was thoroughly confirmed in a mouse model [22]. Mutations in SYNJ2 are associated with an increased risk for colon cancer [24]. CA12 is a membrane-associated enzyme that plays an important role in the regulation of tumor pH and the malignant phenotype, and it is overexpressed in various human cancers [25]. An inhibitor of CA12 reduced cell proliferation and migration in breast cancer and glioblastomas [26, 27]. ABCA12 encodes a member of the ATP-binding cassette family of transporters, and carriers of ABCA12 mutations tend to respond well to chemotherapy [28]. SLC25A38 is a proapoptotic protein that plays an important role in the pathological progression of Alzheimer’s disease [29]. A decreased expression of SLC25A38 was found in hepatocellular carcinoma tissues compared to normal tissues [30]. Although the roles of these four genes were reported in other cancers, the confirmed mechanisms of the roles of these genes in the development of UM must be thoroughly examined.

According to the GO pathway enrichment analysis, DEGs were most significantly enriched in cell morphogenesis involved in neuron differentiation, BPs, and channel activity. Consistent with the GO analysis results, GSEA showed that the value of the calcium signaling pathways between two groups by GSEA.
pathway in the high-risk group was significantly higher than the low-risk group. Calcium signaling is a central pathway in various cellular phenotypic transitions, which makes it relevant in the development and maintenance of cancers. Excessive activation of calcium channels promoted the metastasis of breast cancer [31]. Deregulation of the calcium signaling pathway exerted an enormous effect on pancreatic ductal carcinoma [32]. Taken together, we speculated that abnormal activation of the calcium signaling pathway was relevant to the poor prognosis of...
UM patients. We found that the expression of TPI1 and PKM2 was significantly higher in the high-risk group. TPI1 encodes an enzyme that plays a key role in glycolysis. The upregulation of TPI1 is a novel biomarker for poor prognosis of intrahepatic cholangiocarcinoma and pancreatic cancer [33, 34]. PKM2 is a key metabolic component of glycolysis and a catalytic driver of the Warburg effect and oncogenesis [35]. These results revealed metabolic heterogeneity between the two groups. The overexpression of TPI1 and PKM2 in the high-risk group highlights the role of glycolysis in the development of UM and indicates that malignant cells tend to gain energy via glycolysis, which would facilitate UM progression.

Considering the specificity of the eye as a privileged site of immunity, the infiltration of lymphocytes in UM was associated with poor prognosis, unlike most cancers.

Fig. 7. The difference of immune-related features among two groups. a The abundance of tumor-infiltrating immune cells between risk groups: more immune cells infiltrating in the high-risk group. b The expressions of immune checkpoint point between two groups: PD-1 and CTLA-4 highly expressed in high-risk group. c The immune score and stromal score of two groups.
Consistent with previous studies, we found that the abundance of tumor-infiltrating cells was significantly higher in high-risk group, including CD8 T cells and M1 macrophages. Unexpectedly, the abundance of M2 macrophages was higher in the low-risk group, and M1 macrophages were upregulated in the high-risk group, which contradicts previous research, showing that infiltration of M2 macrophages was related to worse prognosis [39]. One hypothesis is that macrophages are extremely plastic cells that are able to adjust metabolic preference by stimulating the tumor microenvironment [40, 41]. The microenvironment of the high-risk group encouraged macrophages to rely primarily on glycolysis metabolism and polarize into the M1 subtype. Because of the complexity of the tumor microenvironment, further studies are needed to confirm the hypothesis, including single-cell sequencing and experimental research. Although the high-risk group showed a higher expression of PD-1 and CTLA-4, future studies should identify unique hallmarks of UM because agents targeting the typical immune checkpoint have a limited impact on the survival of UM patients. The mutation analysis showed that mutation of BAP1 was visibly more distributed in the high-risk group and the occurrence of SF3B1 mutation was more distributed in the low-risk group. Robertson et al. [1] and Smit et al. [42] reported that patients with SF3B1 mutations had longer disease-free survival and later metastatic occurrence than patients carrying BAP1 mutations. Our mutation analysis is consistent with previous studies.

Our study had several limitations. First, basic experiments should be performed to validate our findings. Second, the size of our study was limited, and studies with larger samples and broader scopes on the metabolic-gene signature are needed in the future.

In this study, we developed a robust metabolic-gene signature based on TCGA to predict the prognosis of UM patients. Our signature indicates a dysregulated metabolic microenvironment of UM, which would provide new metabolic biomarkers and therapeutic targets for UM patients.

**Acknowledgments**

We thank TCGA and GEO databases for providing their platforms and uploading their meaningful datasets. We thank AJE for its linguistic assistance during the preparation of this manuscript.

**Statement of Ethics**

The local ethical committee (Clinical Research Ethics Committee of Renmin Hospital of Wuhan University) approved that written informed consent from participants was not required for the study (20210459). Data presented in this study were provided by TCGA and GEO databases. The patients involved in the database have obtained ethical approval. Users can download relevant data for free for research and publish relevant articles. The study adhered fully to the Declaration of Helsinki.

**Conflict of Interest Statement**

The authors have no conflicts of interest to declare.
Funding Sources

This study was supported by the project of Health Commission of Hubei Province (WJ2017Z004 and WJ2021156). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions

G.X. and Y.X. contributed equally to this work. G.X. and Y.X. performed the data acquisition and analysis; G.X., Y.X., and L.F. interpreted the results; X.Q. and R.H. prepared the figures and tables; G.X. and Y.X. wrote the main manuscript text; C.Z. and X.Y. contributed to conception, design the study, and critically revised the manuscript; All authors read and approved the final manuscript.

Data Availability Statement

The data that support the findings of this study are available anonymously from the corresponding author.

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