Research Article

Identification of an Amino Acid Metabolism Signature Participating in Immunosuppression in Ovarian Cancer

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1. Introduction

Ovarian (OV) cancer is one of the most lethal cancer types for female health, and it is the second fatal solid tumor of gynecologic cancers [1]; the routine treatment of OV is the combination of cytoreductive surgery and chemotherapy based on the platinum usage. Approximately 10% of OV cancers are familial syndromes, and 90% of them are sporadic [2]. The major risk factors for OV cancer are family history and the carrier of BRCA1/BRCA2 mutations; mean lifespan risk for OV cancer is 30% in BRCA1 mutation carriers and 27% in BRCA2 mutation carriers [2]. In recent years, the morbidity and mortality rates of OV cancer were slowly decreasing, but it still threatens the health of females, giving rise to the exploration of more effective cancer characteristic prediction and therapeutic strategies.

Metabolism reprogramming is a common feature in various cancer types that adapts cancer cells to the energy of substrates requirement of rapid proliferation or metastasis. Glycolysis has been noticed as the leading metabolic form of OV cancer [3], and many strategies were developed to target the glycolysis network to treat OV cancer. However, the effects vary due to the heterogeneity of the cancer histology and microenvironment. Amino acid metabolism is also a critical metabolic activity in cancers; it not only provides the substrate for protein production but also intersects the purine and one-carbon metabolism to fulfill the various biomass requirement of cancer cells [4]; the many amino
Amino acid metabolism can not only affect the proliferation of cancer cells themselves but also regulate the noncancer cells in the tumor microenvironment. Arginine is a critical substrate that functions during macrophages’ M1 and M2 polarization; M1 macrophages activate iNOS, a cancer-supporting factor [6, 7], to catalyze arginine, producing NO to attack cancer cells, whereas M2 macrophages express arginase 1, converting arginine to ornithine to further promote the cancer cells [5]. Tryptophan deprivation can inhibit the stabilization of T cells [8], and its catabolic product, kynurenine, can prevent the maturation of T17 cells and stimulate the regulatory T-cell proliferation [9]. However, the association between amino acid metabolism and cancer immunity remained unclear in OV cancer.

In this study, we aimed at constructing a risk score based on the amino acid metabolism-related gene sets to predict the OV cancer patient survival and clinical stage. We will also investigate the association between amino acid metabolism and the immune landscape in the tumor microenvironment using functional analyses and various algorithms. We believe that this study will provide a new perspective on the OV pathological mechanism, and benefit patients with better prognostic management and novel therapeutic target development.

2. Materials and Methods

2.1. Sequencing Data Collection. The sequencing data and the corresponding clinical information on ovarian cancers were obtained from The Cancer Genome Atlas (TCGA). The downloaded FPKM expression matrix was then transformed into the TPM matrix. The amino acid metabolism gene list was obtained from the GOBP_CELLULAR_-AMINO_ACID_METABOLIC_PROCESS of the Gene Ontology (GO). The samples without clinical survival information were removed. The gene sets for functional analyses were retrieved from the Molecular Signatures Database (MSigDB) of the Gene Set Enrichment Analysis (GSEA).

2.2. Principal Component Analysis and Risk Score Construction. The 286 amino acid metabolism-related genes were first tested by univariate cox regression to select the survival-associated candidates, and the genes that passed the test were used for principal component analysis (PCA) clustering to divide the samples into 2 clusters. After PCA clustering, we compared the prognostic diversity of the clusters by conducting a survival analysis. Furthermore, the least absolute shrinkage and selection operator (LASSO) regression [10] was applied to select the parameters from the genes that passed the univariate test and build a risk score for predicting the survival risks of the OV patients. The “lambda.min” was selected for obtaining the model with the lowest deviance, and the corresponding coefficients were also presented. The risk score was organized as follows:

\[ \text{Risk score} = \sum_{i=1}^{n} \beta_i \cdot g_i. \]  

The augment \( g_i \) refers to the expression of the gene \( i \) selected by LASSO and \( \beta_i \) means the coefficient of gene \( i \).

Besides, the expression differences of the survival-related amino acid metabolism genes between different risk groups were presented in a heatmap. And the expression differences of the amino-acid-metabolism-related pathways were analyzed by gene set enrichment analysis (GSEA) and gene set variation analysis (GSVA) [11].

We estimated the prognostic value of the risk score by conducting a survival analysis for the patients separated by the median risk score, and receiver operating characteristics curves (ROC) were utilized to access the accuracy of the survival prediction of 1-year, 3-year, and 5-year by the risk score, and the prediction accuracy was compared among risk score, PCA cluster, and age.

2.3. Clinical Significance of the Risk Score and the Association between the Classifiers. Apart from the ability for predicting survival, we also evaluate the association between stages, age, and risk score. The risk level was compared between three stages and the age groups (separated by the median age of 59). Also, the ROC was applied to estimate the prediction accuracy of clinical stages by the risk score, PCA cluster, and age.

Subsequently, we performed unsupervised clustering of all the ovarian cancer samples by consensus clustering using the function “ConsensusClusterPlus” of the R package “ConsensusClusterPlus.” We selected the best \( k \) value by evaluating the cumulative distribution function (CDF) and the relative change in area under CDF curve. Survival analysis was used to access the prognostic significance of the consensus clusters, and a Sancy plot was drawn to explicit the distribution of the data flow among risk groups, PCA cluster, and the consensus clusters.

2.4. Functional Analysis of the Transcriptional Diversity between Risk Groups. To clarify the biological changes caused by the amino acid metabolism signature, we conducted functional enrichment analyses using the gene sets from GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) datasets. Gene set enrichment analysis (GSEA) was conducted to calculate the enrichment score of the biological processes from GO or the pathways from KGG. Meanwhile, gene set variation analysis (GSVA) [11] was performed to compare the gene sets’ variation between the high-risk and low-risk groups.

The stemness diversity of all samples was calculated from the signature (mRNAsi, EREGnRBAsi) calculated by innovative one-class logistic regression (OCLR) algorithm [12], and it was compared between the risk groups.

2.5. Immune Landscape and Immunocyte Infiltration Diversity between Risk Groups. Since the functional enrichment analyses had indicated the involvement of immune-related
biological processes and pathways, we first investigate the immune landscape of the two risk groups by Estimation of Stromal and Immune cells in MAALIGNant Tumor tissues using Expression data (ESTIMATE) analysis using the R package "estimate" [13], and the ESTIMATEScore, ImmuneScore, StromalScore, and TumorPurity were compared respectively between the high-risk and low-risk group. As for the immunocyte infiltration diversity, we utilized 4 different approaches to firmly evaluate the infiltrating levels of various immunocytes between the two groups by the function “deconvolute” of the R package "immunedeconv" [14]. A heatmap (scaled by rows) and box plots were presented to visualize the differences.

2.6. Stimulating and Inhibitory Immune Checkpoint Expression. The immune analyses suggested the immunosuppressive roles of high risk. We further explored whether immune checkpoints were employed to facilitate the depression of antitumor immunity. The expression of the 20 inhibitory and 35 stimulatory immune checkpoints [15] between the high-risk and the low-risk groups was compared and visualized by a heatmap and box plots. To further validate the correlation between immune checkpoints and amino acid metabolism, the enrichment levels of the aminoacid-metabolism-related gene sets in the samples ranged by immune checkpoint expression levels were analyzed by GSVA.

2.7. Antitumor Drug Development for Patients with High Risk. To develop a novel therapeutic strategy for high-risk patients, we searched the connective map (cMap) and PRISM compound databases to screen out high-sensitivity drugs. The top 50 compounds were presented in a heatmap showing the opposite similarity between the effects of the compounds and the transcriptional changes caused by the amino acid metabolism signature on 9 cancer cell lines. The mechanism of action (MoA) of these compounds, which showed the effective mechanisms of drugs, were presented in a scatter plot. The 50% inhibitory concentration (IC50) of drugs in the PRISM database for all ovarian cancer patients was predicted by the “callPhenotype” function of the R package "oncoPredict" [16], using the input of a training cell line expression matrix and a response matrix and the ridge regression algorithm. The top 4 significant drugs with IC50 lower than 30 were analyzed, and their IC50 was compared between the high-risk and the low-risk groups.

2.8. Cell Culture, Small Interfering RNA Knockdown, and Western Blot Detection of the Protein Levels of OV Cells. The A2780 cells were cultured in 10% FBS containing the RPMI-1640 medium under 37°C and 5% CO2. The knockdown of *TPH1* was performed using small-interfering RNAs with transfection reagents. After transfection for 48 hours, the cells were harvested and lysised using RIPA lysis buffer. After ultrasound sonication and centrifugation, the proteins were heated with loading buffer in a mental bath. The electrophoresis and membrane transferring were conducted according to the standard protocol. The membranes were blocked with 5% skim milk powder and incubated with the primary antibodies overnight under 4°C. After secondary antibodies incubation, the membranes were washed, and the bands were detected using chemiluminescence. Each experiment was performed three times.

2.9. Clone Formation, Transwell Cell Migration, and Invasion Assay. After the transfection of 48 h, the cells were harvested and cultured at a low concentration (2000 cells per well) in a 6-well plate. After 14 days, the cells were washed with PBS and stained using crystal violet. We counted the effective clones to describe the clone formation ability of the cells. For transwell assay, the cells were resuspended in RPMI-1640 medium containing 1% FCS and added to the upper chamber with a concentration of 1 × 10⁴ cells per chamber. The lower room was filled with RPMI-1640 medium with 20% FBS. For invasion assay, each chamber was coated with Matrigel diluted in the medium before cells were planted. After incubation for 24 hours, the cells were stained with crystal violet for 1 hour, and the cells in the upper chamber were erased using a cotton swab. Each experiment was performed three times.

2.10. Statistical Analyses. All the statistical analyses were run on the R software. The mutual correlations of the genes were quantified by Pearson’s correlation coefficient. All the survival analyses were examined by log-rank test, and the ROC was used to calculate the AUC of the predictions. The risk, gene expression, and immunocyte infiltration level differences between different groups were tested by Student’s *t*-test or Wilcox test. Grouped comparison was tested by ANOVA (for normally distributed variables) or the Kruskal–Wallis test (for non-normally distributed variables), and Dunnett’s multiple comparisons test were applied as post hoc test for ANOVA. *P* < 0.05 was considered statistically significant. *, **, and *** referred to the *P* value less than 0.05, 0.01, and 0.001 respectively.

3. Results

3.1. PCA Clustering and Construction of an Amino Acid Metabolism-Related Risk Score. In order to identify a survival-related amino acid metabolism signature for OV patients, we downloaded a gene list of 286 amino acid metabolism from MsigDB of GSEA, and univariate Cox regression was applied to screen out survival-related genes. As a result, 19 genes were significantly associated with patient survival and their mutual correlations were presented in Figure 1(a); most of the genes shared low correlations. The genes that passed univariate analysis were then used to conduct PCA clustering, 2 clusters were identified, and the patients grouped in cluster 1 suffered a lower survival rate (Figures 1(b) and 1(c)).

Subsequently, the LASSO regression was used to select parameters to construct a risk score, and 17 genes were retained with the lambda.min value of 0.0144 (Figures 1(d) and 1(e)). The coefficients of the 17 genes are presented in
Figure 1: PCA clustering and risk score construction using LASSO. (a) The mutual correlations of the genes that passed the univariate cox analysis. (b) The PCA clustering of the patients based on the genes in Figure 1(a). (c) The Kaplan–Meier curves show the ability of the PCA clusters 1 and 2 in separating patient overall survival rate. (d) LASSO coefficients profiles. (e) LASSO deviance profile for selecting the best numbers of parameters. (f): Coefficients of the retained predictors. (g) Kaplan–Meier curve shows the survival prediction ability of the risk score. (h) ROC presents the predictive accuracy of 1-, 3-, 5-year overall survival of the risk score. (i) Comparison of the survival predictive accuracy between risk score, PCA cluster, and age. PCA, principal component analysis; LASSO, the least absolute shrinkage and selection operator; ROC, receiver operating characteristic curve.
3.3. Amino Acid Metabolism Was Involved in Extracellular Biological Activities in OV. To investigate the biological roles of amino acid metabolism in OV, we conducted GSEA and GSVA of the biological processes from the GO database and the pathways from the KEGG database. For the GO biological processes, we noticed that mesenchymal stem cell, fibroblast, and macrophage-related activities were highly enriched in the high-risk group, and the cell adhesion was negatively regulated (Figure 3(a)). Similarly, the GSVA results of GO showed mesenchymal cell-related pathways. Notably, the GSVA results also presented down-regulated immunity in the high-risk group (Figure 3(b)). As for the enrichment results from KEGG, we found the activated TGF-β signaling pathway and ECM-receptor interaction via both GSEA and GSVA. Moreover, the GSVA of KEGG discovered the active regulation of the cytoskeleton. These results suggested that the high amino acid metabolism status of OV may facilitate the interaction between the mesenchymal stem cell differentiation, fibroblasts, and cancer cells, and led to immunological changes and cell migration.

3.4. The High Risk of OV Was Related to High Cancer- Associated Fibroblasts and Low CD8+ T-Cell Infiltration. To seek the diversity of the immune status caused by the amino acid metabolism signature, we first calculated the ESTIMATE of the samples. As presented in Figure 4(a), the high-risk group showed higher ESTIMATE Score, StromalScore, and lower TumorPurity. We employed 4 different approaches to calculate the immune infiltration to go further with the immune infiltration. The heatmap of the immune cytotype results exhibited that cancer-associated fibroblast infiltration, identified by both the Xcell and Epic algorithms, increased as the risk grew (Figure 4(b)). We then conducted statistical comparisons of immune levels between the high-risk and low-risk groups. The cancer-associated fibroblasts were significantly enriched in the high-risk group according to the Epic and Xcell approaches (Figures 4(c) and 4(d)). Besides, we noticed that the enrichment of CD8+ T cells was lower (Figures 4(c)–4(e)) and macrophages were higher in the high-risk group (Figures 4(d)–4(f)). The immune landscape of the OV indicated that the immunity was affected.

3.5. High Risk Was Associated with a Higher Level of CD276 and TGFB1. Immune checkpoints have been discovered as critical factors contributing to immunity depression. Hence, we compared the expression of the immune checkpoint between the risk groups. As the heatmap showed, many stimulatory immune checkpoints seemed to decrease as the risk grew, including CXCL10, BTN3A1, BTN3A2, CD40LG, GZMA, PRF1, CD27, CXCL9, IFNG, CD80, and ICOS, while the inhibitory immune checkpoints TGFB1 and CD276 seemed to increase (Figure 5(a)). The expression of those molecules was then compared between the risk groups. As exhibited in Figure 5(b), the expression of BTN3A1, BTN3A2, CD27, CD40LG, CD80, ICOS, IFNG, IL2, IL2RA, and PRF1 was decreased in the high-risk group. As for inhibitory immune checkpoints, we noticed that CD276 and TGFB1 were upregulated in the high-risk group, suggesting their roles in mediating immune suppression. To further confirm the association between amino acid metabolism and CD276 and TGFB1, we analyzed the amino-acid-metabolism-related gene set enrichment levels in samples ranged by CD276 and TGFB1, respectively. As presented in Figure 5(d) and Figure 5(e), the enrichment levels of these gene sets increased as the expression of CD276 and TGFB1 was elevated.
**Figure 2:** Clinical relevance of the risk score. (a) and (b) The semi-violin plots exhibit the risk differences between stages (a) and age groups (b). (c) ROC estimates the predictive accuracy of the stage by risk score, PCA cluster, age. (d)–(f) The consensus clustering results were presented by the consensus matrix (d), consensus CDF of different $k$ value (e), relative change in area under CDF (f). (g) Kaplan–Meier curve testing the survival predictive ability of the consensus clusters. (h) The sankey plot exhibited the data distribution between PCA cluster, consensus cluster, and risk group. ROC, receiver operating characteristic curve; PCA, principal component analysis; CDF, cumulative distribution function.
Running Enrichment Score

**Go**

1. fibroblast migration
2. mesenchymal stem cell differentiation
3. negative regulation of cell-substrate junction organization
4. negative regulation of focal adhesion assembly
5. positive regulation of extracellular matrix assembly
6. positive regulation of fibroblast proliferation
7. regulation of macrophage migration
8. somatic stem cell division

**KEGG**

1. Cytokine–cytokine receptor interaction
2. ECM–receptor interaction
3. EGFR tyrosine kinase inhibitor resistance
4. Leukocyte transendothelial migration
5. Neutrophil extracellular trap formation
6. PI3K–Akt signaling pathway
7. TGF–beta signaling pathway

**Figure 3: Continued.**
3.6. Development of Novel Drugs Targeting the High-Risk OV.
For the high-risk OV patients, we sought for more sensitive compounds to treat them. The cMap online tool was employed to analyze the transcriptional changes that arose from the median risk score, the top 50 compounds with opposite transcriptional disturbance to those that arose by median risk score were presented in a heatmap, and their MoA was also shown (Figures 6(a) and 6(b)). The most associated compound was chaetocin, a histone lysine methyltransferase inhibitor, and three of the compounds were adrenergic receptor agonists.

We further explored the PRISM database for potential sensitive drugs, the top 4 drugs with the most remarkable fold change and IC50 less than 30 were selected, and their IC50 was compared between the high-and low-risk group, where the drug BRD-K4700838-001-01-6 showed the lowest IC50 in the high-risk group (Figures 6(c)–6(f)).

3.7. Knockdown of TPH1 Expression Depressed the Clone Formation, Migration, and Invasion Ability of OV Cancer Cells.
We performed the western blot detection of the protein levels in control, TPH1-si-RNA-#1, and TPH1-si-RNA-#2 groups. As exhibited in Figures 7(a) and 7(b), the protein levels in TPH1-si-RNA-#1 and TPH1-si-RNA-#2 groups were decreased. The clone formation assay results showed that the clone assay of OV cancer cells in the TPH1-si-RNA-#1 and TPH1-si-RNA-#2 groups was depressed (Figure 7(c)). Similarly, the transwell results also presented that TPH1 knockdown inhibited the OV cancer cell migration and invasion (Figure 7(d)). The quantification results of these experiments are shown in Figures 7(d)–7(g). These results implied the oncogenic roles of TPH1 in OV cancers.

4. Discussion
Amino acid metabolism controls the protein synthesis of cells, which are the most critical components in cellular activities. Here, we built a risk score for OV patients based on the amino acid metabolism signature using LASSO. Previously, many metabolism-related cancer risk scores have been reported to predict the overall survival of OV patients, including total metabolism, energy metabolism, and lipid metabolism signature. However, the roles of amino acid metabolism in affecting OV patients’ survival have not been explored so far. Herein, we first established an amino acid metabolism-based risk score in OV cancers and revealed its roles in patient survival. This risk score can predict patient survival, especially long-term survival, with high accuracy, outperforming the previous metabolism-related models [17, 18]. And similarly, it can predict the clinical stage of OV cancers with high performance as well. The diversity of the expression pattern of the amino-acid-metabolism-related genes and gene sets validated the association between the risk score and amino acid metabolism.

Amino acid metabolism plays an important role in regulating anticancer immunity in many cancers. As the previous studies found, the metabolites of tryptophan...
Figure 4: Continued.
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metabolism supported tumor-associated macrophages to facilitate immunosuppression in pancreatic cancer [19]. High levels of arginase that catalyze the L-arginine can inhibit the proliferation of antigen-specific T cells in lung cancer [20]. Besides, serine and glycine metabolism can destroy the anticancer function of macrophages and neutrophils [21]. In OV cancers, only glutamine was found as a key molecule in modulating myeloid-derived suppressor cells (MDSCs) activities, and targeting glutamine can ease the immunosuppressive effects led by MDSCs [22], and the evidence of the association between amino acid metabolism and cancer immunosuppression is still lacking. In this study, we discovered the potential association between highly infiltrated cancer-associated fibroblasts, decreased CD8+ T cells, and the amino acid signature, and to our knowledge, this is the first study revealing this association.

For the potential underlying mechanism, fibroblasts were found to utilize the extracellular lactate, which promoted their amino acid biosynthesis and inhibited the tricarboxylic acid (TCA) cycle [23]. The acidification of the microenvironment was attributed mainly to the glycolysis of cancer [24], which transferred the cancer cells from TCA to macromolecule metabolism and lactate production. This may also be one of the factors explaining the association between amino acid metabolism and fibroblasts in the tumor microenvironment. But importantly, amino acid biosynthesis, such as glycine and proline, directly provided the substrates for extracellular collagen production and assembly, and this may result from the glycolysis in fibroblasts themselves [25]. Apart from collagen production, fibroblasts were also found to provide necessary fuels (such as lactate, amino acids, and fatty acids) to cancer cells, and this process was facilitated by cancer cell-derived paracrine oxidative stress [26]. Shortly, the amino acid metabolism triggered by the glycolysis inside the fibroblasts or the lactate stimulation from cancer cell glycolysis promoted the extracellular matrix production and fuel provision for cancer cells, leading to immunity exclusion and cancer growth support. A study has reported that the inhibition of a metabolism enzyme of the tryptophan of fibroblast restored the T-cell response in vivo [27], and this was in accordance with our finding that CD8+ T cells were decreased in the high-risk group, indicating that T cells were the main immunosuppressive target of fibroblasts driven by abnormal amino acid metabolism.

For the molecular discoveries of the high-risk disturbance in OV cancer, we noticed the elevated expression of TGFβ1. This discovery further confirmed the involvement of fibroblasts in high-risk OV cancer, since TGFβ1 is the driver of collagen accumulation produced by fibroblasts and immune suppression, and the inhibition of TGF-β signaling pathway and expression of LOXL2 depressed the fibroblast activities and pathological collagen accumulation [28]. Moreover, the inhibition of TGF-β1 improved the function of CD8+ T cells [29]. These suggested that abnormal amino acids employed TGF-β signaling pathway to control the microenvironment alteration. Interestingly, we also noticed the elevated CD276 expression in the high-risk group. CD276 has been reported to increase HIF-1α expression and promote the glycolysis of cancer cells [30]. This provided a hypothesis that the high-risk OV cancers highly expressed CD276 to enhance glycolysis, which directly stimulated the amino acid metabolism of fibroblast or acidated the microenvironment to prompt fibroblast metabolism. At the same time, they expressed TGFβ1 to promote fibroblast activities, and as a result, both the two pathways may support tumor growth and depress normal immunocytes, like T cells. For the association between the two immune checkpoints and amino acid metabolism, only a recent study has demonstrated that glutamine metabolism inhibition decreased CD276 expression and enhanced granzyme B produced from CD8+ T cell via ROS [7]. And there is no evidence of the correlation between amino acid metabolism and TGFβ1. Hence, our study presents a novel mechanism of immune checkpoints-mediated effects on OV cancers.
In this study, we finally confirmed the oncogenetic role of TPH1 in OV cancer. TPH1 catalyzed the reaction of enhanced degradation of tryptophan to serotonin, and its oncogenetic has been discovered in breast cancer, bladder cell carcinoma, and colon cancer [31–33]. Similarly, we found that the knockdown of TPH1 inhibited OV cancer.

Figure 5: Immune checkpoints expression level comparison. (a) A heatmap shows the 55 immune checkpoints expression level of each sample. (b) and (c) Comparison of the 35 stimulatory (b) and 20 inhibitory (c) immune checkpoints expression differences between risk groups. (d) and (e) The expression pattern between CD276 (d), TGFB1 (e), and the amino-acid-metabolism-related pathways presented by heatmaps.
Figure 6: Development of risk-sensitive drugs to OV patients. (a) and (b) A heatmap presents the top 50 cMap compounds causing opposite transcriptional disturbance to that caused by the median risk (a) and the scatter plot shows their corresponding MoA (b). (c) Comparison of the IC50 levels between the risk groups of 4 drugs from PRISM database. cMap, connective map; MoA, mechanism of action; PRISM, profiling relative inhibition simultaneously in mixtures.
Figure 7: Continued.
clone formation and migration ability, which is consistent with previous findings. Interestingly, TPH1 participated in mast cell-mediated immunosuppression, indicating its potential role in tumor-supporting and immunity depression [33]. However, this requires further investigation.

5. Conclusion
Comprehensively, we identified a novel risk score based on OV cancer amino acid metabolism, the risk score predicted survival and tumor stages with high accuracy, and the oncogenic role of the risky gene TPH1 was experimentally validated. We also discovered the immunological roles of amino acid metabolism signature. The high-risk group was associated with increased cancer-associated fibroblast infiltration and decreased CD8+ T cells. Besides, the immune checkpoints CD276 and TGFB1 were highly expressed. We believe this study sheds light on the understanding of the association between amino acid metabolism and immunosuppression in OV cancer. It will benefit patients with better prognostic management and provide novel targets for developing more effective therapeutic strategies.

Data Availability

Conflicts of Interest
The authors declare that there are no conflicts of interest regarding the publication of this article.

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Supplementary Materials
Supplementary Figure S1. The association between the risk groups and the amino-acid-metabolism-related gene set expression. A: The heatmap shows the expression diversity of the amino-acid-metabolism-related gene that passed the univariate analysis between the risk groups. B, C: enrichment and variation differences of the amino-acid-metabolism-related gene sets between the risk groups by GSEA (B) and GSVA (C). (Supplementary Materials)

References
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