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Analysis

Identified a novel prognostic model of HCC basing on virus signature for guiding immunotherapy

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Abstract

Oncolytic viral immunotherapy is a cancer treatment that uses native or genetically modified viruses that selectively replicate and destroy tumor cells. In this study, we aimed to construct a virus-based prognostic model for risk assessment and prognosis prediction in patients with hepatocellular carcinoma (HCC) and determine the most appropriate virus as a candidate vector for oncolytic virus immunotherapy. Microbiome and RNA sequencing data and clinical information were obtained from The Cancer Genome Atlas, and viruses with prognostic value were identified (Deltabaculovirus, Sicinivirus, and Cytomegalovirus) to construct the prognostic model. Correlation analyses were performed to evaluate the predictive function of the viral signature. Bioinformatics analyses were conducted to explore the functional enrichment of viral expression in HCC. The risk score generated by this model could distinguish patients with different survival outcomes, have excellent reliability and accuracy, and could be used as an independent prognostic indicator. The high-risk score group showed significantly lower overall survival, and this trend was also observed in subgroups with different clinicopathological features. Furthermore, *Deltabaculovirus* positively correlated with amino acid metabolism, energy metabolism signaling pathways, peroxisomes, and complement coagulation cascades. In addition, *Deltabaculovirus* was significantly related to immune cell infiltration; therefore, patients with high Delta-baculovirus expression might respond better to HCC immunotherapy. Our study identified a promising predictive viral signature for assessing clinical prognosis and guiding immunotherapy in HCC. *Deltabaculovirus* might be a suitable viral vector for oncolytic virus immunotherapy.

Keywords Hepatocellular carcinoma · Viruses · Prognosis · Metabolism · Immune infiltration

Abbreviations

HCC Hepatocellular carcinoma
HBV Hepatitis B virus

Shizhuan Huang and Dehai Wu contributed equally to this work.

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HCV Hepatitis C virus
ICI Immune checkpoint inhibitor
VV Vaccinia virus
VSV Vesicular stomatitis virus
MeV Measles vaccine
NDV Newcastle disease virus
AFP Alpha-fetoprotein
TIDE Tumor immune dysfunction and exclusion
TME Tumor microenvironment
TAM Tumor-associated macrophage
MDSC Myeloid-derived suppressor cell
WNV West Nile virus

1 Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths, and its incidence is increasing worldwide [1–4]. The major etiological factor of HCC is infection with the hepatitis B (HBV) or C virus (HCV), which is involved in approximately half of all HCC cases [5]. Other factors, including heavy alcohol consumption and nonalcoholic fatty liver disease, are also part of the complex etiology of HCC [6]. Hepatectomy and liver transplantation are the main treatments for patients with early-stage HCC, with a 5-year survival rate of approximately 70–80% [7–9]. However, tumor recurrence and metastasis tremendously limit the effectiveness of surgical treatment, and the specific mortality rate of the disease remains high [10]. Combination therapy with an immune checkpoint inhibitor (ICI) and targeted therapy (atezolizumab plus bevacizumab) showed a significant improvement in overall survival (OS) in patients with unresectable advanced HCC [11]. In addition, lenvatinib combined with ICIs showed promising efficacy and safety compared with lenvatinib alone in patients with unresectable HCC [12]. Oncolytic viruses are engineered viruses that can selectively lyse tumor cells. In other novel therapeutic approaches, virus-mediated oncolysis results in tumor regression and enhances antitumor immune responses by releasing soluble tumor antigens [13].

Viruses replicate in the intracellular milieu of host cells, and viral infection is associated with underlying pathogenicity and host inflammation induction [14]. Most viruses comprise three key structural elements: the genome, either single- or double-stranded RNA or DNA; the capsid, a protein coat surrounding the genetic material; and, in some viruses, the lipidic envelope (usually derived from the host plasma membrane), which surrounds the capsid and may facilitate viral attachment to host cell membranes, thus promoting viral entry [15]. The outcome of viral infection varies widely depending on the pathogenicity of the genes encoded by the virus, the interactions between the virus and the host immune system, and the ability of the virus to replicate and induce post-infection latency [16].

Oncolytic viral immunotherapy is a cancer treatment that uses native or genetically modified viruses that selectively replicate and destroy tumor cells. Oncolytic viruses are thought to mediate antitumor activity through two distinct mechanisms of action: selective replication within neoplastic cells, resulting in a direct lytic effect on tumor cells, and induction of systemic antitumor immunity [16]. Adenoviruses and vaccinia viruses (VV) have been used in HCC treatment clinical trials [17, 18]. Most preclinical studies use adenoviruses and vesicular stomatitis virus (VSV) as vectors because of their natural ability to kill or target HCC cells [19, 20]. In addition, various studies have used other viral vectors to treat patients with HCC, including herpes simplex (HSV), measles vaccine (MeV), Newcastle disease virus (NDV), VSV, and VV [21].

This study aimed to construct a virus-based prognostic model using a viral signature derived from microbiome signature data obtained from TCGA-LIHC to predict the prognosis of patients with HCC via risk score calculation.

2 Materials and methods

2.1 Data collection

Microbiome signatures, virus expression, RNA sequencing (RNA-Seq), and clinical data of 335 TCGA-LIHC patients were downloaded from the cBioPortal for Cancer Genomics (https://www.cbioportal.org/).

2.2 Construction of Lasso regression model

The correlation between virus expression and the survival of the 335 patients with HCC was determined using Lasso-Cox analysis with "glmnet" and "survival" R software packages. In addition, we set up tenfold cross-validation to obtain the optimal model. Lambda was set to 0.05 to finally obtain 10 viruses. Univariate Cox regression analysis was performed to evaluate the relationship between viral expression levels and OS using the "survival" R package. Patients with HCC were divided into high- and low-expression groups based on the optimal viral expression cutoff value. The significance of the prognostic differences between the groups was evaluated using the log-rank test.

2.3 Prognostic signature development and HCC evaluation

Three viruses (*Deltabaculovirus*, *Sicinivirus*, and *Cytomegalovirus*) were screened because they were significantly associated with the OS of patients with HCC. We analyzed the risk score value, distribution, OS status, and heat map of the three-virus signature model. Subsequently, we used the aforementioned viruses to calculate the risk score for each patient with HCC using the following formula:

$$\begin{aligned} Riskscore =& expressionlevelofvirusa \times coefficienta + expressionlevelofvirusb \\ &\times coefficientb + expressionlevelofvirusc \\ &\times coefficientc + \cdots + expressionlevelofvirusn \times coefficientn. \end{aligned}$$

To evaluate model predictive performance, we set the optimal cutoff risk score as the cutoff value, and the patients were classified into high- and low-risk groups. Kaplan–Meier survival curves were applied for survival comparison between the two groups, and log-rank $P < 0.05$ was considered statistically significant (using R packages "survival" and "survminer"). Additionally, time-dependent receiver operating characteristic curves (ROC) (1-, 3-, and 5-year survival) were established (using the R package "survival ROC") to determine signature sensitivity and specificity.

2.4 Nomogram scoring system

Uni- and multivariate Cox regression analyses were performed to select independent factors ($p < 0.05$; Table 1). We used the independent factors (risk score, age, inflammation, and vascular invasion) to construct a nomogram to predict the survival of patients with HCC, which was assessed using the C-index value, time-dependent ROC curve, and calibration curves. Each variable contributed a point in the nomogram scoring system, adding to "Total Points" for each sample for predicting 1-, 3-, and 5-year survival.

Table 1 Univariate and multivariate Cox analysis of the prognostic value of viruses signature in term of OS in HCC

Characteristics	Univariate Cox analysis		Multivariate Cox analysis	
	HR (95%CI)	P-value	HR (95%CI)	P-value
Age	0.53 (0.28–1.03)	0.062	0.41 (0.2–0.82)	0.012
Gender	0.93 (0.48–1.8)	0.823	–	–
AFP	0.75 (0.33–1.72)	0.498	–	–
AJCC	2.17 (1.13–4.19)	0.02	1.68 (0.16–18.17)	0.669
T Stage	2.03 (1.04–3.96)	0.037	1 (0.08–12.07)	0.999
N Stage	2.51 (1.22–5.16)	0.012	1.14 (0.41–3.19)	0.798
M Stage	3.12 (1.5–6.49)	0.002	2.32 (0.83–6.48)	0.108
Fibrosis	0.68 (0.36–1.29)	0.24	–	–
Inflammation	1.75 (0.91–3.37)	0.094	2.31 (1.14–4.7)	0.020
Grade	0.83 (0.44–1.58)	0.576	–	–
Vascular	2.44 (1.26–4.73)	0.008	2.37 (1.16–4.87)	0.019
RiskScore	0.43 (0.22–0.83)	0.012	0.47 (0.23–0.99)	0.047

2.5 Kaplan–Meier curves survival analysis and correlation between risk score and viruses and different clinicopathological features

We used the R package "maxstat" version: 0.7–25 to calculate the optimal cut-off value. The minimum and maximum sample sizes were set to be >25% and <75%, respectively. The patients were divided into high and low groups based on the optimal cutoff value. The "survfit function" of the "survival" R software package was used to analyze the prognostic difference between the two groups. Significant prognostic differences between the groups were determined using the log-rank test. In addition, we implemented a Mann–Whitney U test to analyze the correlation between risk score, viruses, and different clinicopathological features, including age, AJCC cancer staging, alpha-fetoprotein (AFP) levels, sex, inflammation, fibrosis, vascular invasion, and grade. Survival analysis was performed for different clinical factors.

2.6 Gene set enrichment analysis

For gene set enrichment analysis (GSEA) [22], we used the GSEA website (http://software.broadinstitute. org/gsea/index.jsp). We divided the sample into high- and low-expression groups according to the risk score and viral expression levels and downloaded the c2.cp kegg.v7.4.symbols.gmt sub aggregate from the Molecular Signatures Database [23] (http://www.gsea-msigdb.org/gsea/downloads.jsp) to evaluate related pathways and molecular mechanisms. Based on the gene expression profile and phenotypic grouping, the minimum and maximum gene sets were set to 5 and 5000, with 1000 times of resampling. $p < 0.05$ and FDR < 0.25 were considered statistically significant.

2.7 Correlation between viruses and KEGG signaling pathways

The Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway scores were calculated using single-sample GSEA analysis. Forty-one KEGG signaling pathways selected by univariate Cox regression analysis were significantly associated with OS in patients with HCC (Supplementary Fig. 1). Pearson's correlation analysis was used to determine the relationship between the viruses and KEGG signaling pathways.

2.8 GO and KEGG enrichment analyses

For functional enrichment analysis of gene sets, Gene Ontology (GO) [24] annotations of genes in the "org.Hs.eg.db" R software package were used as the background to mapping genes into the background set. Subsequently, enrichment analysis was performed using the "clusterProfiler" R software package. The minimum and maximum gene sets were set as 5 and 5000, respectively, and $p < 0.05$ and FDR < 0.25 were considered statistically significant.

For gene set functional enrichment analysis, we used KEGG [25] REST API (https://www.kegg.jp/kegg/rest/keggapi.html) to obtain the latest KEGG pathway gene annotations to map genes into the background set. Subsequently, enrichment analysis was performed using the "clusterProfiler" R software package. The minimum and maximum gene sets were set as 5 and 5000, respectively, and $p < 0.05$ and FDR < 0.25 were considered statistically significant.

2.9 Immune cell infiltration levels and immune-related functional scores related to the viruses

Tumor immune dysfunction and exclusion (TIDE) scores were calculated using the TIDE database (http://tide.dfci.harvard.edu/faq/) [26, 27]. A total of 115 complement cascade-related genes were identified and downloaded from the MSigDB database (https://www.gsea-msigdb.org/gsea/msigdb/). Pearson's correlation analysis was used to determine the linear relationship between the viruses and complement cascade-related genes.

2.10 Statistical analysis

Sangerbox 3.0 (http://www.sangerbox.com/tool) [28], GraphPad Prism 7.00 (GraphPad Software, La Jolla, CA, USA), and R software v4.2.3 (R Foundation for Statistical Computing, Vienna, Austria) were used for the statistical analysis. Student's *t*-test was used for comparisons between two groups. Comparisons among multiple groups were performed using the Kruskal–Wallis test. Pearson's correlation analysis was used to determine linear relationships between two groups. Multivariate analysis was performed using a Cox regression model. Kaplan–Meier curves were used to compare survival

and the log-rank test was used to compare survival between different groups. Results were considered significant when $p < 0.05$ (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$).

3 Results

3.1 Establishment of prognostic performance of viruses

Virus signature data were obtained from the TCGA-LIHC dataset. Based on the Lasso-Cox regression analysis results, five viruses (L5-like virus, *Deltabaculovirus*, *Aparavirus*, *Sicinivirus*, and *Cytomegalovirus*) were filtered and found to be significantly associated with the OS of the 335 patients with HCC (Fig. 1A–C). Kaplan–Meier curves were used to analyze the correlation between viral expression levels and OS and disease-free survival (DFS) of patients with HCC. The results showed that L5-like virus and *Aparavirus* expression had no significant relationship with the OS of patients with HCC (Fig. 1D, E). However, the expressions of *Deltabaculovirus*, *Sicinivirus*, and *Cytomegalovirus* were significantly associated with the OS of patients with HCC (Fig. 1F–H). Patients with higher *Deltabaculovirus* and *Sicinivirus* levels were predicted to have a better prognosis, whereas patients with higher *Cytomegalovirus* levels were predicted to have a poorer prognosis (Fig. 1F–H). The Kaplan–Meier curves also showed that L5-like virus, *Aparavirus*, *Deltabaculovirus*, and *Sicinivirus* expression positively correlated with the DFS of patients with HCC (Fig. 1I–M). We used different methods to further verify the correlation between five viruses and the OS of patients with HCC and take the intersection to further narrow down the target virus, making our screening results more accurate. In summary, we hypothesize that these viruses can predict the prognosis of patients with HCC.

3.2 Construction and verification of virus signature and nomogram scoring system

We selected *Deltabaculovirus*, *Sicinivirus*, and *Cytomegalovirus* to construct a viral signature prognostic model and calculate risk scores owing to their significant association with OS in patients with HCC. Using the risk–survival status plots of patients, we revealed that the risk scores of patients negatively correlated with the survival of HCC patients. Furthermore, heatmaps showed that *Cytomegalovirus* was positively associated with the risk signature, whereas *Deltabaculovirus* and *Sicinivirus* were negatively associated (Fig. 2A). Kaplan–Meier analysis demonstrated that the high-risk score group correlated with worse survival ($p = 9.1e{-}5$). The 1-, 3-, and 5-year area-under-the-curve (AUC) values of the ROC curve were 0.62, 0.67, and 0.70, respectively, indicating that the viral signature prognostic model performed well in predicting the survival rate of patients with HCC (Fig. 2B, C).

To further verify the predictability and effectiveness of the virus signature prognostic model, uni- and multivariate Cox regression analyses demonstrated that risk score, age, inflammation, and vascular status were independent factors predicting the prognosis of patients with HCC (Table 1). Based on these independent factors, we constructed a nomogram scoring system to predict survival time in patients with HCC. Each variable contributed a point in the nomogram scoring system, adding to "Total Points" for each sample to predict 1-, 3-, and 5-year survival (Fig. 2D). The C-index value was 0.731 (95%CI: 0.687–0.775). The time-dependent ROC curve showed AUC values of 0.66 (1-year), 0.67 (3-year), and 0.62 (5-year). The 1-, 3-, and 5-year calibration curves showed stable and accurate performance (Fig. 2E–H). The viral signature exhibited good predictability and effectiveness in predicting the survival rate of patients with HCC. Therefore, the viral signature may be used to clinically manage patients with HCC.

3.3 Correlation between risk score and clinical features

To further validate the viral signature as a significant clinicopathology-related indicator, we analyzed the correlation between risk scores and clinical data. The risk score was significantly lower in the groups of patients with tumors at AJCC stages I–II stage and fibrosis 1–4. However, the risk score was not significantly associated with age, AFP levels, sex, inflammation, vascular invasion, or tumor grade (Fig. 3A–H).

To further explore viral signature prognostic value in patients with HCC, patients were categorized into subgroups according to clinical variables (age, AJCC stage, AFP, sex, inflammation, fibrosis, vascular invasion, and clinical grade). The survival of patients in the high-risk score group was worse than that in the low-risk score group in most subgroups (Fig. 3I–S), suggesting that the viral signature prognostic model has good predictive and discriminative abilities. The

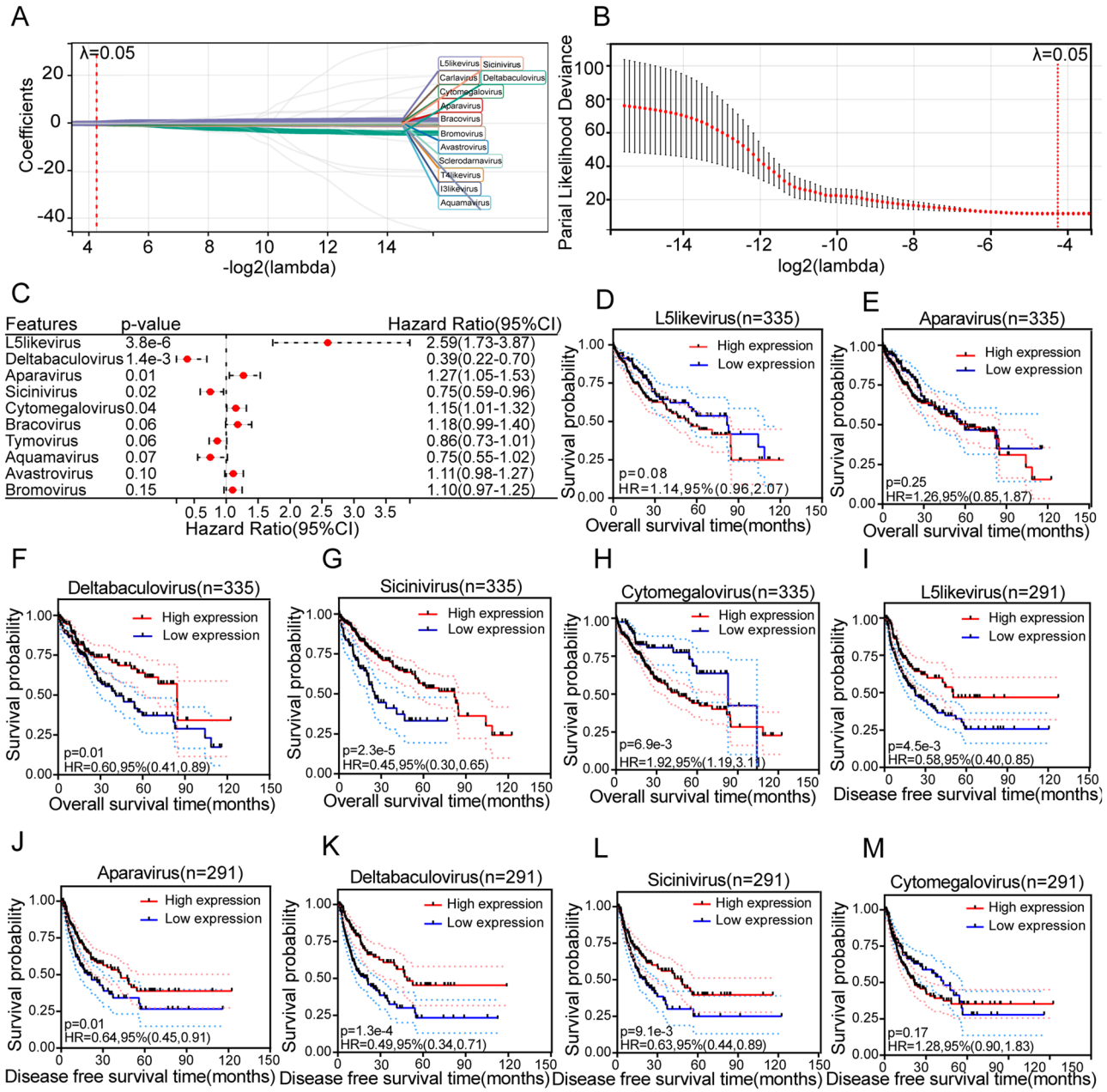

Fig. 1 Construction of prognostic performance of viruses **A**–**C** LASSO Cox regression was performed to identify 5 viruses significantly related to the prognosis of the patients with HCC. **D**–**M** Kaplan–Meier survival curves of patients in the high- and low-expression groups within 5 viruses, including L5likevirus, Aparavirus, Deltabaculovirus, Sicinivirus, and Cytomegalovirus, respectively

survival of patients in the high-risk score group and low-risk score group had no significant difference in AJCC stages I–II stage, female, fibrosis 0, no vascular invasion, and tumor grade III–IV subgroups (Supplementary Fig. 2A–E).

3.4 Gene set enrichment and immune cell infiltration level analyses

GSEA revealed the KEGG signaling pathway terms related to risk scores in patients with HCC; the cell cycle, nucleotide excision repair, homologous recombination, RNA degradation, and mismatch repair signaling pathways were enriched in patients with high-risk scores. In addition, risk score positively correlated with the cell cycle, DNA replication, spliceosome, base excision repair, pentose phosphate pathway, mismatch repair, RNA degradation, and nucleotide excision repair

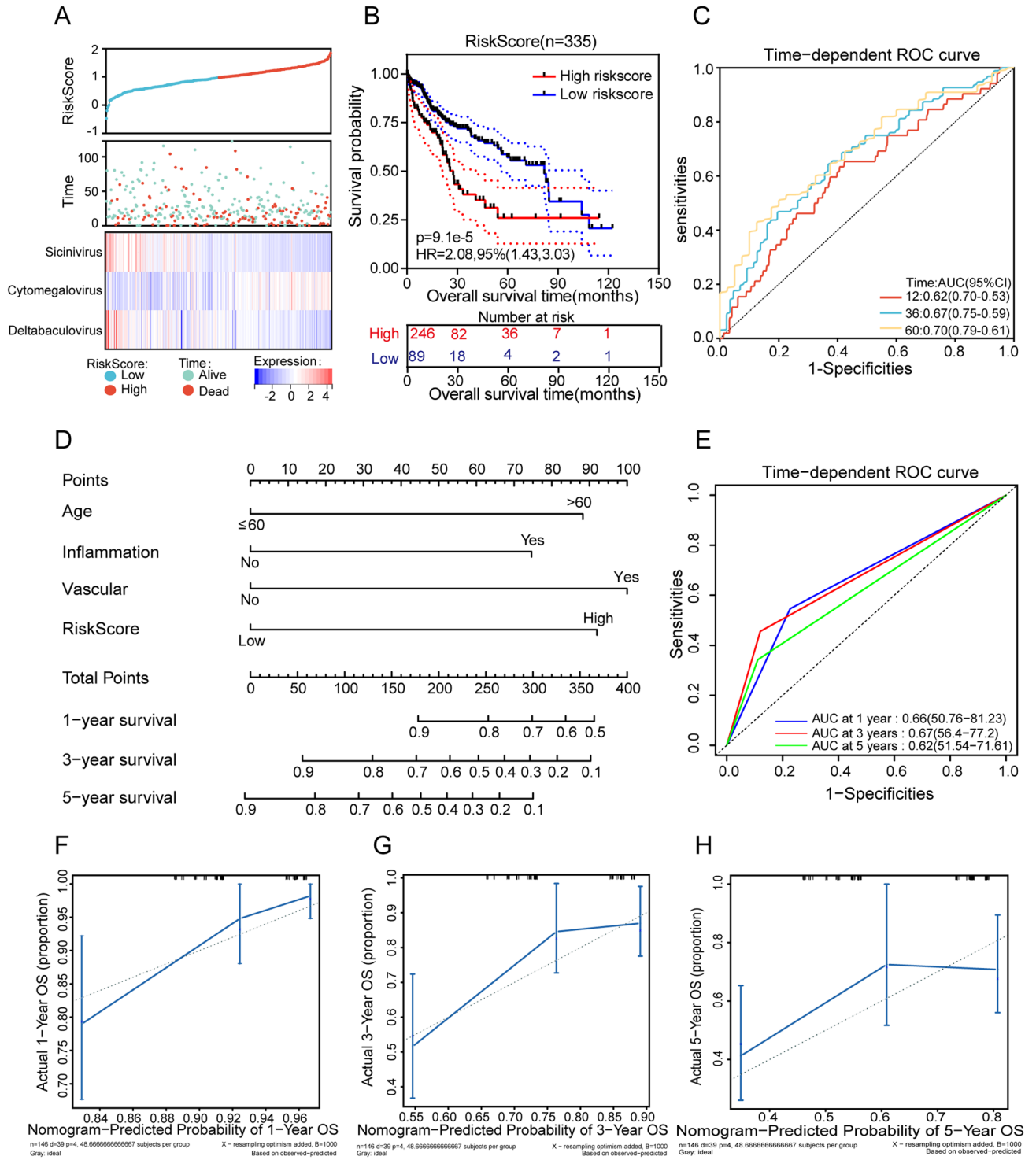

Fig. 2 Viruses signature development and evaluation for HCC and the construction of nomogram scoring system model. **A** Analysis of risk score value and distribution, OS status, and heatmap of 3-viruses signature model. **B**, **C** Kaplan–Meier survival curves of patients in the high- and low-risk score groups (log-rank test, $p = 9.1e{-}5$) and the AUC value of time-dependent ROC curves for OS. The AUC values were 0.62 (1-year), 0.67 (3-year), and 0.70 (5-year). **D** The nomogram scoring system can predict the overall survival in patients with HCC at 1-, 3-, and 5-year. **E**–**H** The time-dependent receiver operating characteristic (ROC) curves of the nomogram for the survival prediction of patients with HCC at 1-, 3-, and 5-year. The AUC values were 0.66 (1-year), 0.67 (3-year), and 0.62(5-year). The calibration curves of the nomogram for 1-, 3-, and 5-year survival probabilities

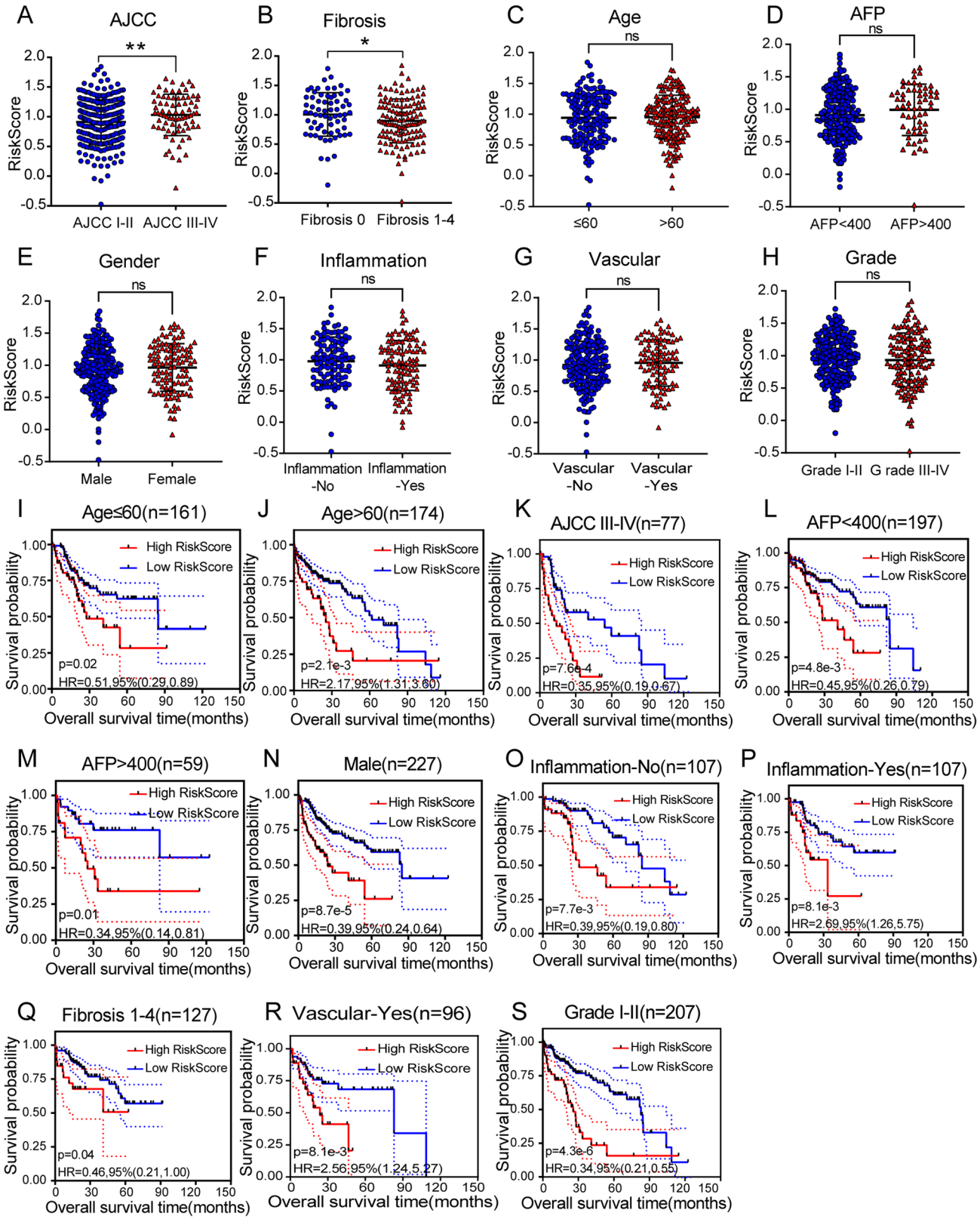

Fig. 3 Clinical correlations and survival analysis of viruses signature in different Clinicopathological features. **A–H** The correlation between risk score and the clinical data. **I–S** Kaplan–Meier survival curves of patients in the high- and low-risk score groups within clinically stratified subgroups, including patients with age ≤ 60 (**I**), age > 60 (**J**), AJCC III-IV stage (**K**), AFP < 400 (**L**), AFP > 400 (**M**), Male (**N**), Inflammation-no (**O**), Inflammation-yes (**P**), the Fibrosis 1–4 (**Q**), Vascular-yes (**R**), the Grade I-II (**S**), respectively. Patients in the low-risk score group had better survival outcomes than those in the high-risk score group across clinically stratified subgroups (log-rank test, *p* value < 0.05). **p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001

(Fig. 4A, B). These results showed that the viral signature prognostic model was closely associated with tumor proliferation and could be a good prognostic model for predicting the survival rate of patients with HCC.

ESTIMATE analysis showed that the risk score negatively correlated with the stromal and ESTIMATE scores (Fig. 4C). Macrophage infiltration was significantly higher in patients with low-risk scores than in those with high-risk scores (Fig. 4D). Subsequently, we analyzed the correlation between risk and TIDE scores. Risk score positively correlated with myeloid-derived suppressor cells (MDSCs), revealing that patients with high-risk scores had a poor prognosis due to a diminished anti-tumor immune response through increasing MDSC infiltration. The Risk score negatively correlated with CD274 (also commonly referred to as PDL1), revealing that patients with high-risk scores had a poor prognosis due to a diminished anti-tumor immune response through decreasing the expression of PDL1 (Fig. 4E). Furthermore, correlation analysis between the risk score and immune checkpoint-related genes showed that the risk score positively correlated with VEGFA and HMGB1 and negatively correlated with IL10 and IFA1 (Fig. 4F). Collectively, the results showed a significant correlation between the risk score and tumor microenvironment (TME) immune characteristics in patients with HCC, which may provide a basis for suitable treatment alternatives for patients with HCC.

3.5 Functional enrichment analysis of *Sicinivirus* and *Deltabaculovirus*

Based on the Lasso-Cox regression analysis results, *Deltabaculovirus* and *Sicinivirus* were favorable indicators for the prognosis of patients with HCC; therefore, we selected them as major research factors in subsequent analysis. GSEA suggested that the complement and coagulation cascades, amino acid metabolism (glycine, serine, and threonine metabolism; tryptophan metabolism; valine, leucine, and isoleucine degradation; and arginine and proline metabolism), allograft rejection, xenobiotic metabolism, bile acid metabolism, and complement system-related signaling pathways were enriched in patients with high *Deltabaculovirus* expression (Fig. 5A). Similarly, ether lipid metabolism, cell cycle, taste transduction, and glycerophospholipid metabolism were enriched in patients with low *Sicinivirus* expression (Fig. 5B).

Correlation analysis of *Deltabaculovirus* and signaling pathways showed that *Deltabaculovirus* positively correlated with amino acid metabolism (histidine metabolism; valine, leucine, and isoleucine degradation; glycine, serine, and threonine metabolism; tyrosine metabolism; beta-alanine metabolism; phenylalanine metabolism; tryptophan metabolism; and lysine degradation), energy metabolism signaling pathways (fatty acid metabolism, adipocytokine signaling pathway, insulin signaling pathway), peroxisomes, and complement coagulation cascades. In contrast, *Deltabaculovirus* expression negatively correlated with olfactory transduction and RNA polymerase expression (Fig. 5C). Except for olfactory transduction and RNA polymerase, the aforementioned signaling pathways positively correlated with the prognosis of patients with HCC (Supplementary Fig. 3A–O). *Sicinivirus* negatively correlated with the cell cycle, DNA replication, spliceosome, base excision repair, pentose phosphate pathway, mismatch repair, nucleotide excision repair, and oocyte meiosis (Fig. 5D). The signaling pathways correlated with *Sicinivirus* negatively correlated with the prognosis of patients with HCC (Supplementary Fig. 4A–H). Moreover, a significant correlation was observed between *Deltabaculovirus* and tumor AJCC stage, fibrosis, inflammation, and vascular invasion in HCC; however, there was no significant correlation between *Sicinivirus* and tumor AJCC stage, fibrosis, inflammation, and vascular invasion. *Deltabaculovirus* was significantly higher in the groups of patients with tumor AJCC stages I–II and fibrosis 1–4, inflammation-yes, and vascular invasion-no. (Supplementary Fig. 4I–P). In conclusion, compared with *Sicinivirus*, *Deltabaculovirus* was superior in predicting the prognosis of patients with HCC.

To further study the correlation between *Deltabaculovirus* and complement cascades, 115 complement cascade-related genes were downloaded from the MSigDB database. Twenty-five complement cascade-related genes positively correlated with *Deltabaculovirus* (Supplementary Fig. 5A). Subsequently, GO functional analysis of the 25 complement cascade-related genes revealed that they were mainly related to complement activation, immune response regulation, acute inflammatory response, complement binding, serine-type endopeptidase activity, and other functions (Supplementary Fig. 5B–D). KEGG enrichment analysis demonstrated significant enrichment of complement and coagulation cascades, *Staphylococcus aureus* infection, and systemic lupus erythematosus. (Supplementary Fig. 5E).

3.6 Role of *Sicinivirus* and *Deltabaculovirus* in tumor immune environment characterization

We aimed to study the correlation of *Sicinivirus* and *Deltabaculovirus* with tumor immune environment characteristics. No significant correlation was observed between *Sicinivirus* expression and TIDE score (Fig. 6A, B). However, the TIDE score was lower in patients with high *Deltabaculovirus* expression. *Deltabaculovirus* negatively correlated with TIDE score, exclusion, MDSCs, and tumor-associated macrophages (TAMs) M2, indicating that patients with high *Deltabaculovirus*

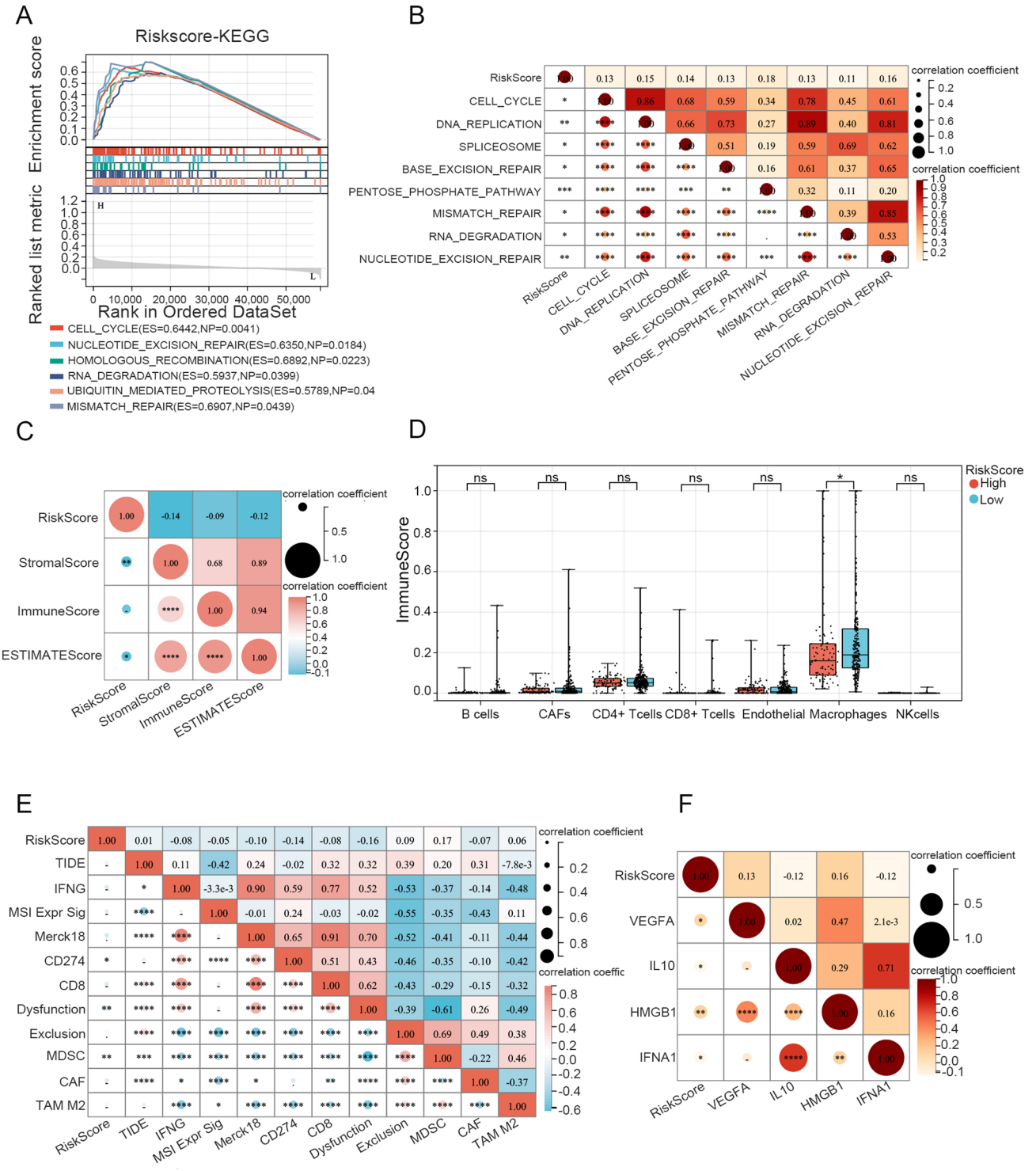

Fig. 4 Gene Set Enrichment Analysis and the correlation between risk score and tumor immune environment characterization. **A** GSEA analysis revealed the signaling pathways of KEGG related to the high-risk score in patients with HCC. **B** Correlation between risk score and KEGG signaling pathways significantly associated with the prognosis of the patients with HCC. **C** Correlation between risk score and ESTIMATE score. **D** Boxplot showed the differential immune infiltrating levels in the high- and low-risk score groups. **E** Correlation between risk score and TIDE score. **F** Correlation between risk score and Immune checkpoint-related genes. The Pearson correlation coefficient was used to determine the correlation. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$

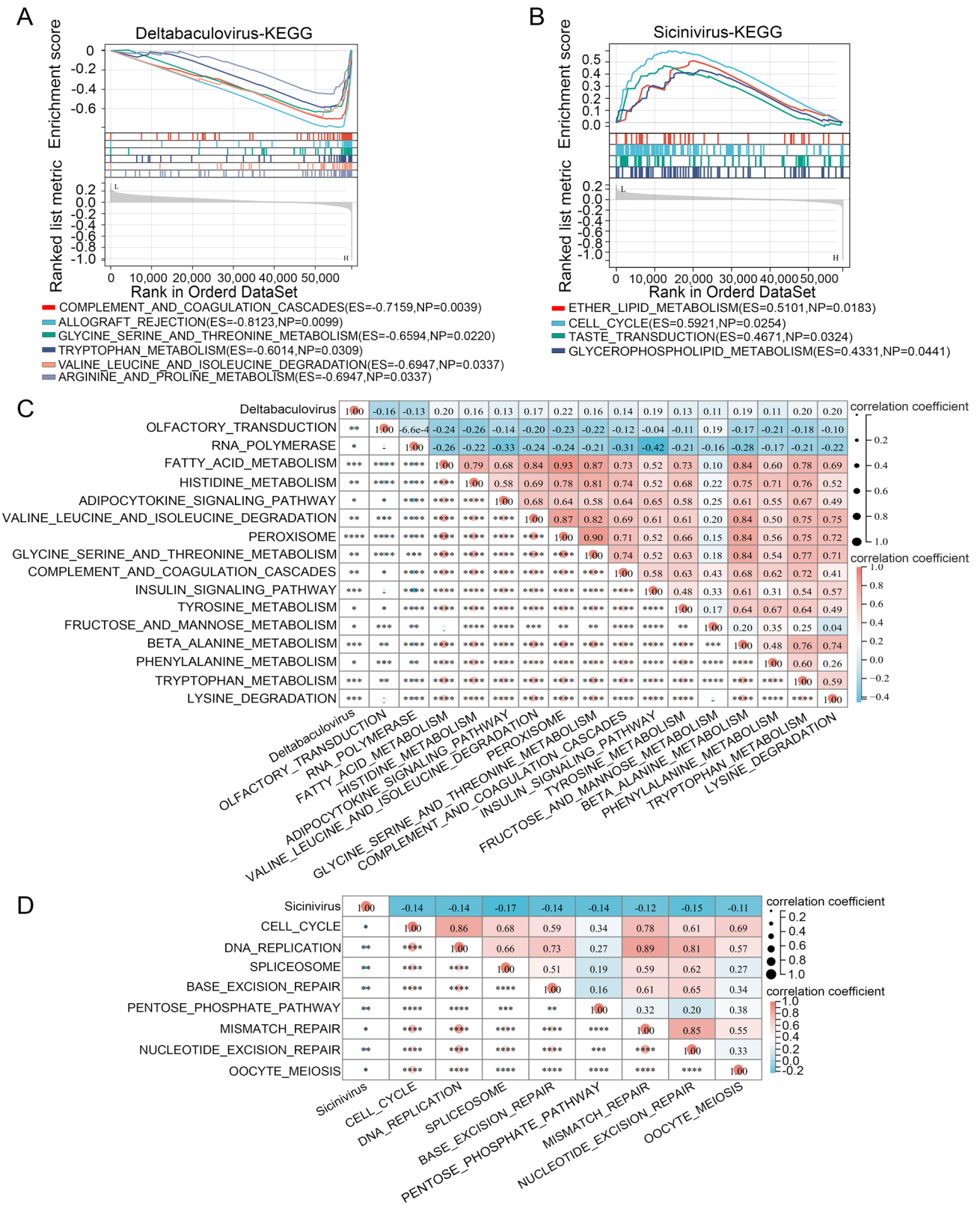

Fig. 5 Functional enrichment analysis and the correlation between Deltabaculovirus and Sicinivirus and KEGG signaling pathways in HCC. **A**, **B** GSEA analysis revealed the signaling pathways of KEGG, related to the high expression of Deltabaculovirus and the low expression of Sicinivirus in patients with HCC. **C**, **D** Correlation between Deltabaculovirus and Sicinivirus and KEGG signaling pathways significantly associated with the prognosis of the patients with HCC. The Pearson correlation coefficient was used to determine the correlation. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$

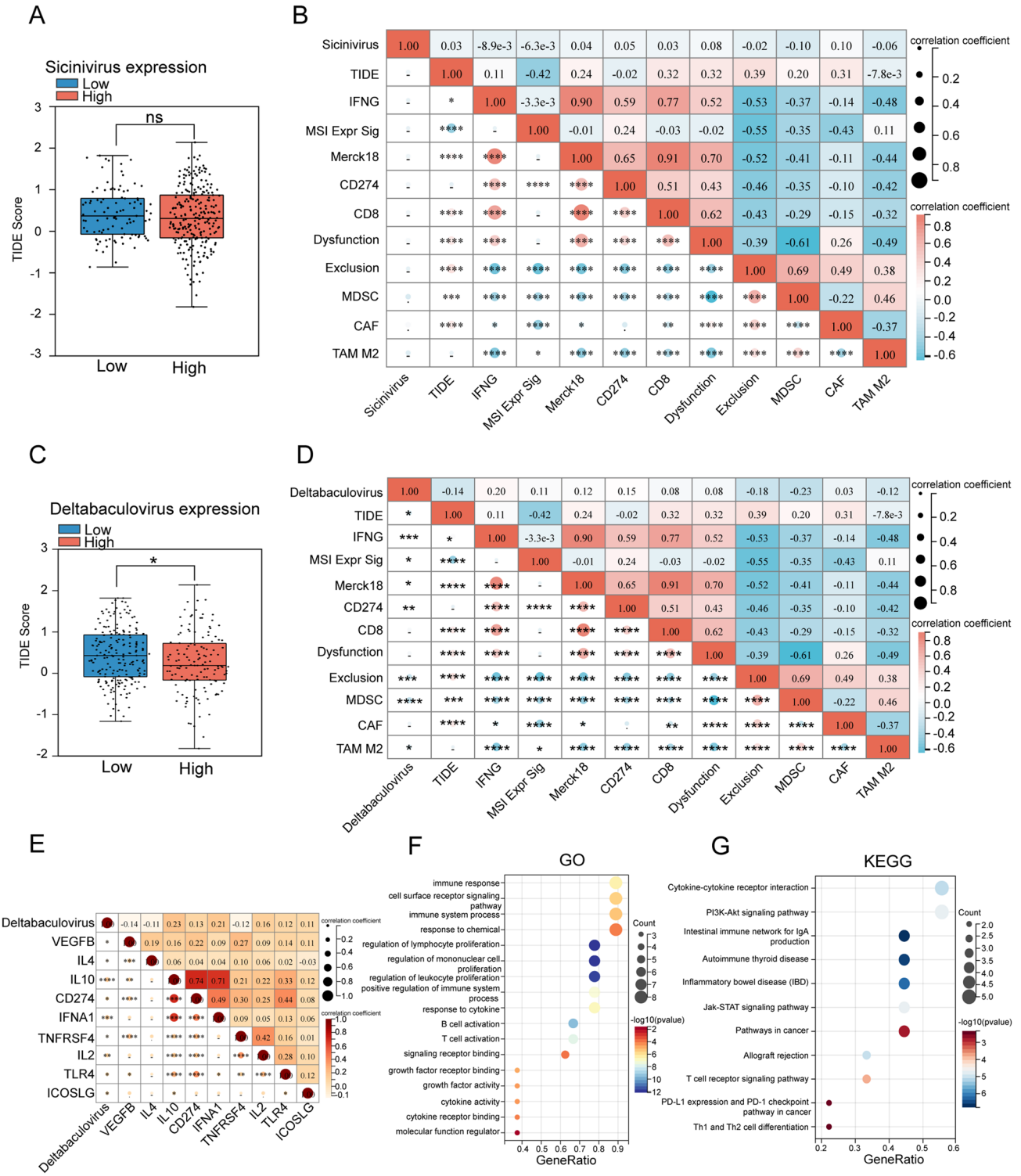

Fig. 6 The correlation between Deltabaculovirus and Sicinivirus and immune cell infiltration in HCC. **A**, **B** Boxplot showed the differential TIDE Score in the high- and low-expression of Sicinivirus groups. Correlation between Sicinivirus and TIDE score. **C**, **D** Boxplot showed the differential TIDE Score in the high- and low-expression of the Deltabaculovirus groups. Correlation between Deltabaculovirus and TIDE score. **E** Correlation between Deltabaculovirus and Immune checkpoint-related genes. **F**, **G** GO functional analysis and KEGG enrichment analysis for the immune checkpoint-related genes that were related to Deltabaculovirus. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$

expression might respond better to immunotherapy. Moreover, *Deltabaculovirus* enhances the anti-tumor immune response by decreasing the invasion levels of MDSCs and TAMs M2. Furthermore, *Deltabaculovirus* positively correlated with IFNG, MSI, and CD274, suggesting it might be possible to enhance the anti-tumor immune response by influencing these factors (Fig. 6C, D). Compared with *Sicinivirus*, *Deltabaculovirus* could more effectively guide the immunotherapy treatment of patients with HCC.

Correlation analysis between *Deltabaculovirus* and immune checkpoint-related genes showed that nine were associated with *Deltabaculovirus* (Fig. 6E). GO functional analysis of the nine immune checkpoint-related genes revealed that they were mainly related to the immune response, cell surface receptor signaling pathway, regulation of lymphocytes, mononuclear cells, leukocyte proliferation, positive regulation of immune system processes, response to cytokines, and other functions (Fig. 6F). KEGG enrichment analysis demonstrated significant enrichment of cytokine–cytokine receptor interaction, PI3K-Akt signaling pathway, intestinal immune network for IgA production, autoimmune thyroid disease, Jak-STAT signaling pathway, and pathways in cancer, among others (Fig. 6G).

Correlation analysis between *Deltabaculovirus* and immunomodulation-related genes showed that 27 were associated with *Deltabaculovirus* (Supplementary Fig. 6A). GO functional analysis of the 27 immunomodulation-related genes revealed that they were mainly related to MHC protein complexes, ER–Golgi transport vesicle membranes, positive regulation of immune system processes, cytokine-mediated signaling pathways, signaling receptor binding, and other functions (Supplementary Fig. 6B–D). KEGG enrichment analysis demonstrated significant enrichment of cytokine–cytokine receptor interaction, viral protein interaction with cytokines and cytokine receptors, allograft rejection, intestinal immune network for IgA production, autoimmune thyroid disease, and graft-versus-host disease (Supplementary Fig. 6E). In addition, GSEA indicated that complement and coagulation cascades, intestinal immune network for IgA production, allograft rejection, autoimmune thyroid disease, and graft-versus-host disease were enriched in patients with high *Deltabaculovirus* expression (Supplementary Fig. 6F). Collectively, we demonstrated that *Deltabaculovirus* was significantly related to tumor immune environment characterization, and patients with high *Deltabaculovirus* expression might respond better to immunotherapy.

4 Discussion

In our study, Lasso-Cox regression was performed to identify the five viruses significantly associated with the prognosis of patients with HCC, among which *Deltabaculovirus*, *Sicinivirus*, and *Cytomegalovirus* had the most significant association. These were used to construct a prognostic model for predicting the survival rate of patients with HCC. Patients with HCC in the high-risk score group had poorer overall survival than those in the low-risk score group. Additionally, the time-dependent ROC curve indicated that the viral signature prognostic model performed well in predicting the survival rate of patients with HCC. We constructed a nomogram scoring system to predict survival time in patients with HCC. The results showed that the nomogram scoring system had good predictive ability and effectiveness in predicting the survival rate of patients with HCC. We concluded that *Deltabaculovirus* and *Sicinivirus* were favorable indicators for the prognosis of patients with HCC.

Oncolytic virus immunotherapy is a cancer treatment that uses native or genetically modified viruses that selectively replicate and destroy tumor cells and can induce systemic antitumor immunity. Many viruses have been proposed as vectors for oncolytic viral immunotherapy, and considerable research has been conducted to optimize viral vectors by attenuating pathogenicity and enhancing immunogenicity. Most preclinical studies use adenoviruses and VSV as vectors because of their natural ability to kill or target HCC cells [19, 20]. In addition, various studies also have used other viruses as vectors to treat patients with HCC, including HSV, MeV, NDV, VSV, and VV [21].One study showed that mesenchymal stem cells enabled the delivery of an oncolytic adenovirus specifically to the tumor without posing any risk associated with the systemic administration of naked virions to the host [29]. The newly designed recombinant Ad5sPD1PVR virus significantly enhanced CD8+ T cell-mediated antitumor efficacy with long-term tumor-specific immune surveillance in HCC [30]. In addition, the live attenuated West Nile virus (WNV) vaccine, WNV-poly(A), has been developed as a novel ideal oncolytic agent against several types of cancers. Owing to its high sensitivity to type I interferon, WNV-poly(A) could specifically kill tumor cells rather than normal cells by activating dendritic cells and triggering a tumor antigen-specific response mediated by $CD8^+$ T cells, which inhibits the propagation of original and distal tumor cells [31].

Based on the Lasso-Cox regression analysis and Kaplan–Meier survival curves analysis, we found that Deltabaculovirus and Sicinivirus were favorable indicators for the prognosis of patients with HCC. On the contrary, Cytomegalovirus was an adverse factor for the prognosis of patients with HCC. Therefore, we selected Deltabaculovirus and Sicinivirus as major

research factors in subsequent analysis. In the study, we found that Deltabaculovirus not only positively correlated with amino acid metabolism, energy metabolism signaling pathways, peroxisomes, and complement coagulation cascades, which positively correlated with the prognosis of patients with HCC, but also significantly correlated with tumor AJCC stage, fibrosis, inflammation, and vascular invasion in HCC. Besides, Deltabaculovirus negatively correlated with TIDE score, MDSCs, and tumor-associated macrophages (TAMs) M2 and positively correlated with IFNG, MSI, and CD274, indicating that patients with high Deltabaculovirus expression might respond better to immunotherapy. Sicinivirus negatively correlated with the cell cycle, DNA replication, spliceosome, base excision repair, pentose phosphate pathway, mismatch repair, nucleotide excision repair, and oocyte meiosis, which negatively correlated with the prognosis of patients with HCC. In addition, there was no significant correlation between Sicinivirus and tumor AJCC stage, fibrosis, inflammation, and vascular invasion. No significant correlation was observed between Sicinivirus expression and TIDE score, revealing that Sicinivirus does not participate in the regulation of tumor immune microenvironment in HCC. Compared with Sicinivirus, Deltabaculovirus is a suitable viral vector for oncolytic virus immunotherapy. In our study, *Deltabaculovirus* was superior in predicting the prognosis of patients with HCC and was significantly related to TME characteristics, suggesting that patients with high *Deltabaculovirus* expression might respond better to immunotherapy. Therefore, *Deltabaculovirus* may be a suitable viral vector for oncolytic virus immunotherapy.

Our study has some limitations. First, although viral signatures can be a novel prognostic indicator for predicting the prognosis and TME immune characteristics in HCC. Currently, there are no effective methods or experimental conditions to clinically detect the expression of these viruses. Therefore, we could not perform further experiments to validate the model. The lack of experimental verification is one of the limitations of this study. The feasible experimental verification methods include qPCR and in situ hybridization in this study. However, the implementation of these two technologies requires the identification of the CDS sequence in which the Deltabaculovirus plays a specific role. At present, we are unable to determine the effective CDS sequence of the Deltabaculovirus, so we will further explore the structure and function of the Deltabaculovirus in the future. Second, the datasets generated for this study can be found in the TCGA-LIHC database. Microbiome signatures and virus expression datasets in HCC were only found in the TCGA database. At present, there is no data set related to viral microorganisms of liver cancer in the GEO dataset. This is also one of the limitations of our study. The study lacked other databases to further verify the predictability and effectiveness of predicting the survival rate of patients with HCC.

5 Conclusion

In conclusion, we constructed and verified a novel prognostic model for patients with HCC based on *Deltabaculovirus*, *Sicinivirus*, and *Cytomegalovirus*. The risk scores generated by this model could be used as an independent prognostic indicator to distinguish patients with different survival outcomes. Moreover, we demonstrated that viral signatures could be a predictive biomarker for survival and guide immunotherapy in patients with HCC. In addition, *Deltabaculovirus* significantly correlated with the immune characteristics of the TME. Compared with *Sicinivirus*, *Deltabaculovirus* is a suitable viral vector for oncolytic virus immunotherapy.

Core tip: This study aimed to construct a virus-based prognostic model using a viral signature derived from microbiome signature data obtained from TCGA-LIHC to predict the prognosis of patients with hepatocellular carcinoma (HCC) via risk score calculation. Deltabaculovirus, Sicinivirus, and Cytomegalovirus were selected as the viruses with prognostic value. Deltabaculovirus positively correlated with amino acid metabolism, energy metabolism signaling pathways, peroxisomes, and complement coagulation cascades. In addition, Deltabaculovirus was significantly associated with immune cell infiltration; therefore, patients with high Deltabaculovirus expression might respond better to HCC immunotherapy. We believe that our study makes a significant contribution to the literature because it identified a promising predictive viral signature for assessing clinical prognosis and guiding immunotherapy in HCC.

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Author contributions SZ. H., M. L., and G. L. carried out bioinformatics analysis, and manuscript preparation. YH.Z., HT. W., D.T., DX. W., B. J. contributed to the data analysis. S.Y., D. W., S. T. designed, supervised and interpreted the study. All authors reviewed the manuscript.

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Data availability Data availability: The datasets generated for this study can be found in the TCGA-LIHC database from the cBioPortal for Cancer Genomics (https://www.cbioportal.org/). Microbiome signatures, virus expression, RNA sequencing (RNA-Seq), and clinical data of TCGA-LIHC patients were downloaded from the cBioPortal for Cancer Genomics (https://www.cbioportal.org/). The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Declarations

Competing interests The authors declare no competing interests.

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