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Original Article

Ferroptosis-related Gene Model and Nomogram Predict the Prognosis and Immune Microenvironment for Hepatocellular Carcinoma

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Abstract

Background and objectives: Ferroptosis is a form of programmed cell necrosis with unique mechanisms. However, the ferroptosis-related genes (FRGs) in the prognosis of hepatocellular carcinoma (HCC) have not been clarified.

Methods: In this research, the corresponding clinical data and original mRNA transcripts for HCC patients were acquired from The Cancer Genome Atlas. A new survival model of 10 FRGs was established using the least absolute shrinkage and selection operator regression analysis. The risk scores of these genes and HCC stages were analyzed for independent risk factors for the worst survival. The enrichment scores of different immune cells and immune-related pathways were analyzed by single-sample gene set enrichment analysis (ssGSEA). The prognostic risk characteristics associated with clinical characteristics were analyzed for independent prognostic indicators for the survival of HCC patients.

Results: The 10 gene signatures (*ACACA, SLC7A11, SQSTM1, SRXN1, SLC2A1, SLC38A1, MYB, ZFP69B, G6PD, STMN1*) were used for a prognostic model for HCC. Compared with the low-risk group, the high-risk group had a worse prognosis and lower survival rate. The validation results revealed that the risk model was a better measure for the prognosis of HCC patients. Combined with the clinical characteristics, a nomogram with good prognostic prediction was established. Functional enrichment analysis revealed that immune-related pathways were highly enriched, including the highly enriched T cell-related pathway.

Conclusions: The risk model for ferroptosis-related risk signatures is valuable for the prognosis of HCC patients. However, our findings require further validation.

Keywords: Ferroptosis; Hepatocellular carcinoma; Prognosis; Immune.

Abbreviations: AUC, area under the curve; CI, confidence interval; DEGs, differentially expressed genes; FDR, false discovery rate; FRGs, ferroptosis-related genes; GO, gene ontology; HCC, hepatocellular carcinoma; HR, hazard ratio; ICGC, International Cancer Genome Consortium; KEGG, Kyoto Encyclopedia of Genes and Genomes; K-M, Kaplan-Meier; LASSO, least absolute shrinkage and selection operator; LIHC, liver hepatocellular carcinoma; OS, overall survival; PCA, principal component analysis; ROC, receiver operating characteristic; ssGSEA, single-sample gene set enrichment analysis; TCGA, The Cancer Genome Atlas; t-SNE, t-distributed stochastic neighbor embedding.

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Introduction

Hepatocellular carcinoma (HCC) is the second most deadly cancer with a low 5-year survival rate and poor prognosis.[1] Previous studies have shown that liver cancer has an incidence of approximately 850,000 cases annually.[2] It is well-known that the primary risk fac-

tors for liver cancer include chronic hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, water pollution, and chemical carcinogens. Patients with liver cirrhosis can progress to HCC. Hence, the prevention and prediction of liver cirrhosis are crucial to prevent liver cancer development. A recent transcriptome meta-analysis indicated that targeting the lysophosphatidic acid pathway was the primary chemical preventive measure in >500 patients with liver cirrhosis.[3] Of note, the survival rate of liver cancer has decreased significantly recently, although the 5-year survival rate of advanced liver cancer remains approximately 5%.[4] The prognosis for patients with HCC remains controversial. Therefore, the discovery of biomarkers for the prognosis of HCC is urgently required.[5,6]

Ferroptosis is a novel form of a regulated cell death pathway, and its discovery has helped to explore the role of iron metabolism in the development and progression of cancers. It is characterized by the excessive accumulation of lipid peroxidation products that cause oxidative damage, which is dependent on iron ions and the mitochondrial chain.[7] Several factors contribute to ferroptosis. First, glutathione peroxidase (*GPX4*) can convert lipid peroxides into non-toxic lipid alcohols and its deficiency results in the accumulation of reactive oxygen species (ROC) and a decline in the antioxidant capacity in cells, leading to ferroptosis and oxidative cell death.[8] In addition, ferroptosis is regulated by defective oxidoreductase *FSP1* (*AIFM2*), *GCH1*, and its product tetrahydrobiopterin (BH_4). Functionally, ferroptosis contributes to the pathogenesis of diseases, including various cancers. Significantly, the nuclear receptor coactivator 4 (*NCOA4*) is one of the selective cargo receptors for ferritinophagy. *NCOA4* overexpression increases the degradation of ferritin and promotes ferroptosis.[9,10] Previous research found that many types of tumor cells are prone to drug-induced ferroptosis.[11] Therefore, it is essential to explore and determine the ferroptosis-related biomarkers for the prognosis of HCC patients.

Recently, target therapy has become attractive and benefits HCC patients.[12] Sorafenib can inhibit the growth of HCC by targeting the Raf/MEK/ERK signaling,[13,14] and attenuates tumor angiogenesis by blocking the vascular endothelial and platelet-derived growth factor receptors. Immunotherapy by targeting immune checkpoints is a promising method to kill tumor cells by enhancing T cell activity. Immune checkpoint inhibitors enhance T cell immunity against tumors and benefit patients with hematologic malignancies and those with some types of solid tumors.[15] Therefore, it is vital to discover the immune biomarkers for the response to immunotherapy and the prognosis of HCC patients.

In the present study, the ferroptosis-related genes (FRGs) in HCC patients were screened and identified in the Cancer Genome Atlas (TCGA) database and a prognostic signature was constructed using 10 genes for the prognosis of HCC patients, which was validated in the International Cancer Genome Consortium (ICGC) database. Furthermore, the signature was used to establish a model and determine its value in the prognosis of HCC patients. Then, the immunotumor environment of HCC was analyzed. Finally, a nomogram was constructed using the signature genes to predict the 1-, 3-, and 5-year overall survival (OS) of HCC. Our data indicated that the model was valuable for the prognosis of HCC. Our findings might help to predict the therapeutic response to immunotherapies in HCC patients and provide new insights into the pathogenic role of ferroptosis in the pathogenesis of HCC.

Materials and methods

Data collection

The demographic, clinical, and transcriptomic data of HCC patients were acquired from the TCGA database, which included 374 HCC and 50 adjacent non-tumor samples. The data was validated from the ICGC portal and these samples were from patients with chronic HBV or HCV infection in Japan. The data were analyzed using the algorithm in the limma R package.[16]

Identification of differentially expressed genes

The differentially expressed genes (DEGs) relative to ferroptosis were defined when one gene expression between the control and HCC tissues reached significant ($|\log FC| > 1$ and adjusted $p < 0.05$) with a false discovery rate (FDR) <0.05, determined using the R package limma in R software (version 4.0.5).[17]

Construction and validation of a prognostic ferroptosis-related gene signature

The significance of individual DEGs was determined by univariate Cox analysis for the OS of HCC patients, and only DEGs with a p-value < 0.001 were chosen for the following analysis. Finally, the R package glmnet was applied for least absolute shrinkage and selection operator (LASSO) regression analysis, and the prognostic candidates for ferroptosis-related DEGs were obtained by ten-fold cross-validation lambda (λ) value.[18] The expression levels of different genes were the independent variables for LASSO regression and the survival rate and status were the dependent variables for patients with HCC. A prediction model was established for the analysis of the risk of FRGs using the Cox regression coefficient weighted estimation method.[19] The prognostic FRGs signatures were manifested as:[20]

$$\text{Risk score} = \sum_{i=1}^{n} coefficient * Expressionnn \text{ of FRG.} \quad (1)$$

To better predict the prognosis of HCC, an ROC curve was used to predict the OS of HCC patients.[21] The survival of patients with HCC in TCGA and ICGC was estimated by the Kaplan-Meier (K-M) method and analyzed by the log-rank test. Then, an independent prognostic analysis was performed to evaluate the clinical features of OS-independent prognostic factors.[22] The data were further analyzed by principal component analysis (PCA) to reduce their dimensionality. In addition, the distribution of HCC patients in each group was analyzed by t-distributed stochastic neighbor embedding (t-SNE).[23]

Function enrichment analysis of DEGs in TCGA and ICGC cohorts

To explain the biological enrichment pathway related to the risk score, gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were conducted based on the R package clusterProfiler with high and low-risk groups of DEGs ($|\log 2FC| \geq 1$, FDR < 0.05).[16] The DEGs involved in the immune function-related pathways were further analyzed.[24,25] Finally, the activity of 13 immune-related pathways and 16 types of immune infiltrates were analyzed by the single-sample gene set enrichment analysis (ssGSEA algorithm) using the gsva R package.[26]

Construction of predictive nomogram

A nomogram was established for the prognosis of HCC patients.[27]

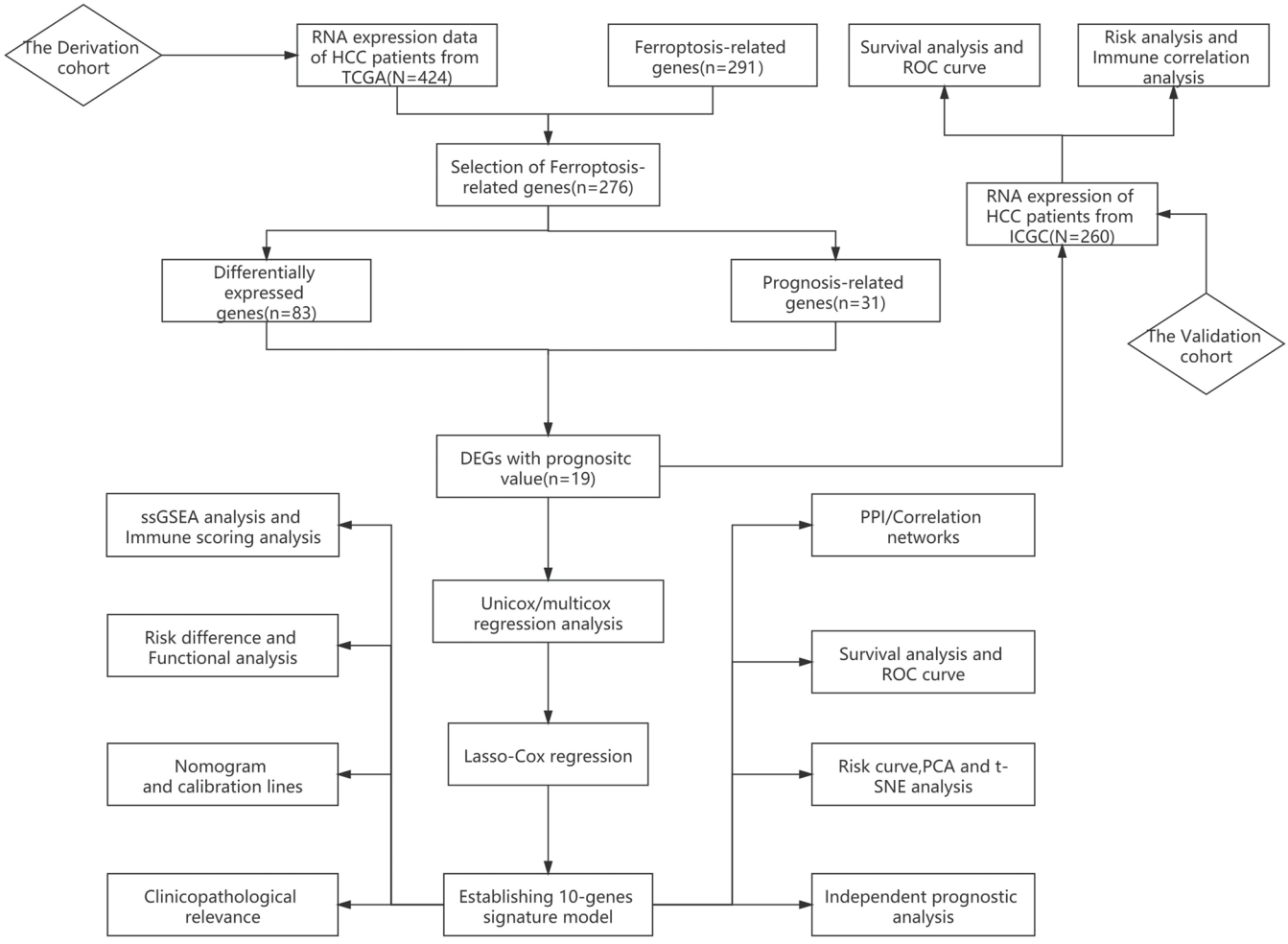

Fig. 1. Flow chart of study design.

The R package rms was used to assess the value of the nomogram in predicting the OS. Finally, the nomogram included risk score and clinical stage and was used to predict 1-, 3-, and 5-year OS of HCC patients. The calibration curves for 1-, 3- and 5-year OS were performed for verification of the power and accuracy of prognosis prediction.[28] The C index was used to evaluate the performance of each model. The different models were compared using the rms R package.

Statistical analysis

The HCC patients in the TCGA training cohort were stratified into high and low-risk groups, based on the median risk score. Univariate and multivariate Cox regression analyses determined the independent prognostic parameters. The survival of HCC patients was estimated by the K-M method and analyzed by a log-rank test using the survival R package. The sensitivity and specificity of the nomogram to predict the OS of HCC patients were analyzed by ROC curves using the R package pROC. The difference between groups was analyzed by a Chi-squared test and Student's t-test. All statistical analyses were performed using R 4.0.5, and a p-value < 0.05 was considered statistically significant.

Results

Research process and patients' characteristics

For simplicity, a flow chart (Fig. 1) was constructed that started with a cohort of 422 HCC patients in the TCGA-LIHC and a validation cohort of 260 HCC patients in the ICGC. Their demographic and clinical data are shown in Table 1. The patients' characteristics included age, tumor Gleason grade, gender, T, M and N stage, and survival status. In addition, 291 FRGs were acquired from FerrDb database (http://www.zhounan.org/ferrdb) and the human gene database (Gene Cards) with the keywords "Ferroptosis" (https://www.genecards.org/).

Identification of prognostic ferroptosis-related DEGs in the TCGA cohort

To determine the association of ferroptosis-related DEGs with the OS of HCC patients using univariate Cox regression analysis, 32 ferroptosis-related DEGs were significantly associated with the OS of HCC patients ($p < 0.001$) (Table 2). Based on the criteria of FDR < 0.05 and |log2FoldChange| > 1, these FRGs were differentially

Table 1. The demographic and clinical characteristics of HCC patients

Case	TCGA cohort	LIRI-TP cohort
	377	260
Age(%)		
≤60	180(47.7%)	55(21.2%)
>60	197(52.3%)	205(78.8%)
Gender(%)		
Female	122(32.4%)	68(26.2%)
Male	255(67.6%)	192(73.8%)
Grade(%)		
Grade 1	55(14.6%)	NA
Grade 2	180(47.8%)	NA
Grade 3	124(32.9%)	NA
Grade4	13(3.4%)	NA
Unknown	5(1.3%)	NA
Stage(%)		
I	175(46.4%)	40(15.4%)
II	87(23.1%)	117(45.0%)
III	86(22.8%)	80(30.8%)
IV	5(1.3%)	23(8.8%)
Unknown	24(6.4%)	0(0.0%)
T(Tumor)(%)		
T1	185(49.1%)	NA
T2	95(25.2%)	NA
T3	81(21.5%)	NA
T4	13(3.4%)	NA
N(Lymph Node)(%)		
N0	257(68.2%)	NA
N1	4(1.1%)	NA
M(Metastasis)(%)		
M0	272(72.1%)	NA
M1	4(1.1%)	NA
Survival status(%)		
Alive	245(65.0%)	214(82.3%)
Dead	132(35.0%)	46(17.7%)

HCC, hepatocellular carcinoma; TCGA, The Cancer Genome Atlas.

expressed between 374 HCC and 50 non-tumor liver samples in the TCGA database. Further univariate Cox regression analyses indicated that these 19 ferroptosis-related DEGs were associated with the OS of HCC patients (Fig. 2e). The forest plot of hazard ratios (HRs) displayed that the majority of these DEGs were risk factors for a worse prognosis in HCC patients (Figs. 2b, 2d). These DEGs connected to form a network (Fig. 2c) and among these genes, *SLC2A1*, *SLC38A1,* and *SLC7A11* were the hub genes (Fig. 2a).

Table 2. Univariate Cox regression analysis of 32 ferroptosis-related DEGs for OS of HCC patients

Id	HR	HR.95L	HR.95H	P value
ABCC1	1.373	1.156	1.630	<0.001
ACACA	1.613	1.229	2.117	<0.001
ATG3	2.333	1.529	3.558	<0.001
ATG7	2.904	1.677	5.027	<0.001
AURKA	1.321	1.120	1.558	<0.001
CARS1	1.685	1.243	2.284	<0.001
EIF2S1	2.462	1.607	3.773	<0.001
FANCD2	1.712	1.293	2.266	<0.001
G6PD	1.416	1.265	1.584	<0.001
GCLM	1.445	1.193	1.750	<0.001
HELLS	1.554	1.196	2.019	<0.001
HILPDA	1.435	1.240	1.661	<0.001
MAFG	1.760	1.385	2,239	<0.001
MT3	1.426	1.164	1.746	<0.001
MYB	3.101	1.618	5.941	<0.001
NCF2	1.314	1.128	1.531	<0.001
NRAS	1.799	1.377	2.350	<0.001
PCBP2	2.179	1.538	3.086	<0.001
PGD	1.502	1.247	1.810	<0.001
PRDX1	1.659	1.303	2.112	<0.001
RRM2	1.417	1.206	1.666	<0.001
SLC1A4	1.456	1.170	1.810	<0.001
SLC1A5	1.364	1.215	1.531	<0.001
SLC2A1	1.533	1.313	1.789	<0.001
SLC38A1	1.333	1.161	1.530	<0.001
SLC7A11	1.466	1.233	1.744	<0.001
SQSTM1	1.381	1.175	1.623	<0.001
SRXN1	1.697	1.343	2.142	<0.001
STMN1	1.426	1.220	1.666	<0.001
TXNRD1	1.361	1.170	1.583	<0.001
ZFP69B	4.076	2.486	6.684	<0.001

DEGs, differentially expressed genes; HCC, hepatocellular carcinoma; HR, hazard ratio; OS, overall survival.

Construction of a prognostic model with good performance in the TCGA cohort

A risk prognosis model was constructed based on 10 FRGs with a risk for worse OS of HCC patients by LASSO regression analysis. To avoid collinearity, the prognostic DEGs were entered into a LASSO regression. Furthermore, a 10-gene signature was generated that was significantly associated with the prognosis with an optimal value of λ (Fig. 3d, e). The coefficient of these 10 genes is exhibited in Table 3. A prognostic model was generated to evaluate the prognosis of each HCC patient as follow:

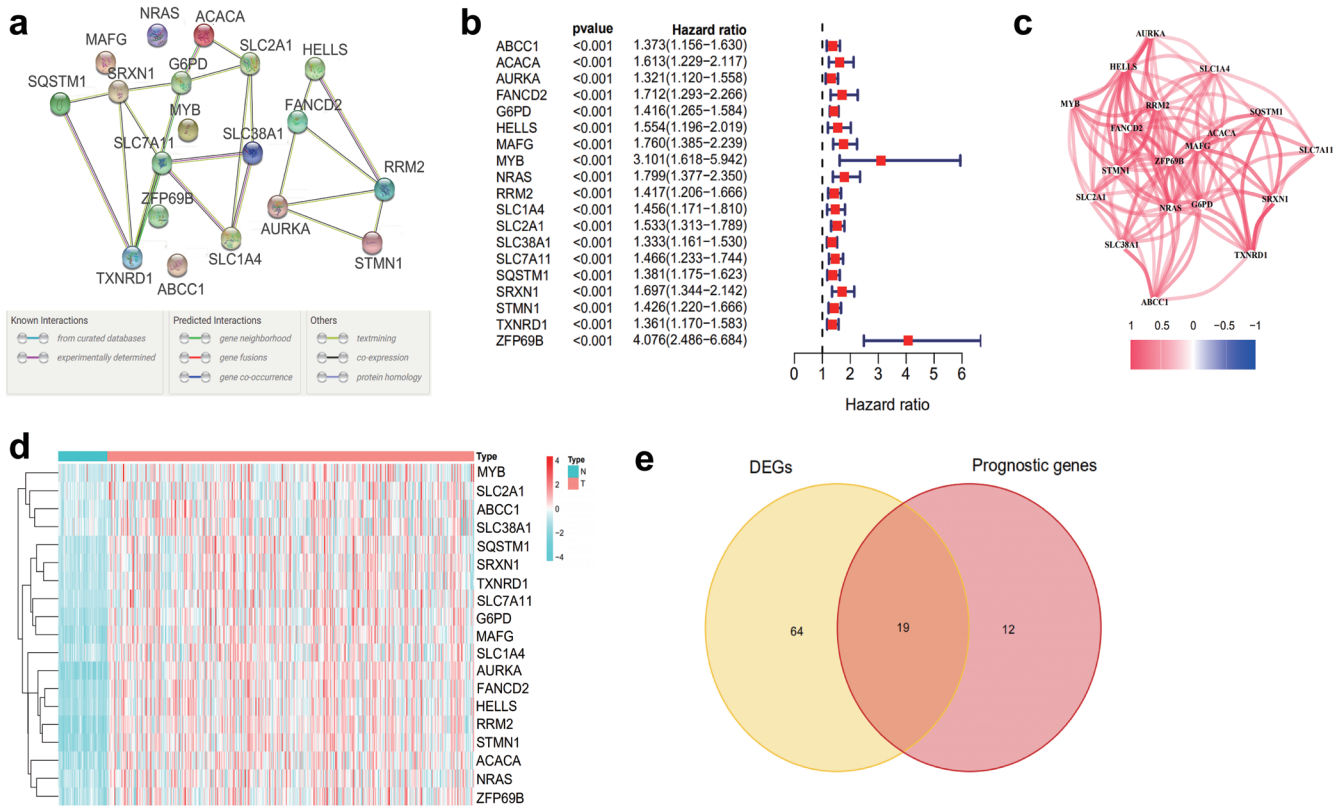

Fig. 2. Screening of candidate FRGs in TCGA cohort. (a) constructing a protein–protein interaction (PPI) network, downloaded from the STRING database (https://string-db.org/) indicated the interactions between the candidate genes;(b) 19 identified candidate genes related to the HCC risks by univariate Cox regression analysis. $p < 0.001$ was statistically significant; (c) correlation network of candidate genes; (d) heatmap of candidate genes expression between groups of HCC patients. The 19 overlapping genes were all upregulated in tumor tissues; and (e) identification of FRGs with prognostic values. FRGs, ferroptosis-related genes; HCC, hepatocellular carcinoma; TCGA, The Cancer Genome Atlas.

Risk score = *ACACA* * 0.004266 + *G6PD* * 0.071354 + *MYB* * 0.117039 + *SLC2A1* * 0.109265 + *SLC38A1* * 0.031264 + *SLC7A11* * 0.121545 + *SQSTM1* * 0.020266 + *SRXN1* * 0.227654 + *STMN1* * 0.126750 + *ZFP69B* * 0.496697.

The K-M curve exhibited worse survival in the high-risk group of HCC patients ($p < 0.001$, Fig. 4a). The condition of risk score and survival status in the TCGA cohort is shown in Figure 4c–d. The PCA and t-SNE of the TCGA cohort separated both groups (Fig. 4e–f). Then, the predictive ability of the risk score was estimated for the time-dependent ROC curve in the TCGA cohort, and the areas under the curve (AUCs) reached 0.790, 0.705, and 0.678 for the 1-, 2-, and 3-year OS in this population, respectively (Fig. 4b). Therefore, this risk model had good reliability and specificity.

Validation of the prognostic model in the ICGC cohort

To validate the value of the prediction model from the TCGA database, 260 HCC samples from ICGC were used to validate the ferroptosis-related prognostic model. These HCC patients were stratified into two high and low-risk groups (Fig. 5c). The patients in the high-risk group had significantly worse OS than those in the low-risk group (Fig. 5a). In addition, the ROC curve demonstrated that the AUCs of HCC patients for 1-, 2-, and 3-year OS rates were 0.704, 0.695, and 0.684 (Fig. 5b). Compared with the TCGA cohort, the PCA and t-SNE analysis indicated that patients in both subgroups were different (Fig. 5e–f). In addition, the OS was significantly lower, and the mortality rate was higher in the high-risk group than those in the low-risk group of HCC patients (Fig. 5d).

Functional enrichment analysis of the ferroptosis-related signature

To further investigate the potential functions and pathways related to risk prognosis, GO and KEGG analyses revealed that the DEGs associated with HCC prognosis were enriched in extracellular matrix receptor (ECM-receptor) interaction, rheumatoid arthritis, phagosome, human T cell leukemia virus 1 infection, and others (Fig. 6b–d). Of note, the DEGs in the TCGA cohorts were significantly enriched in many immune-related pathways, such as humoral immune response, immunoglobulin complex, and humoral immune response (adjusted $p < 0.05$, Fig. 6a). In addition, GO pathway analysis indicated that the collagen-containing and integrin-binding were enriched in both cohorts (adjusted $p < 0.05$, Fig. 6a, c). The GO circle graph from the TCGA cohort suggested that the biological functions were related to the FRGs (Fig. 3b, c).

Correlation estimation of the clinical characteristics

Then, the interrelationship between the 10 genes and clinical char-

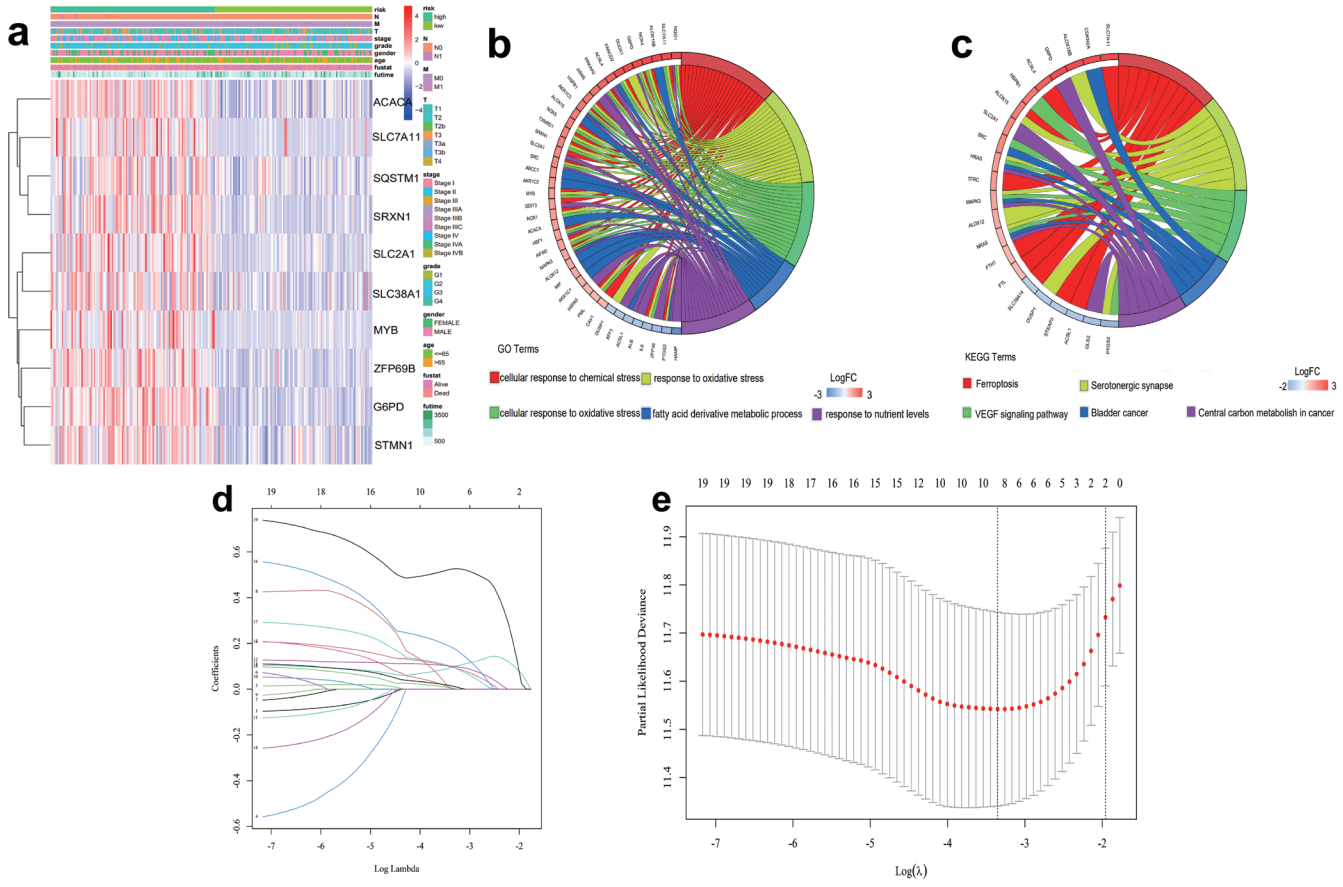

Fig. 3. Correlation of clinical characteristics and LASSO regression analysis in TCGA cohort. (a) heatmap shows the relationship of the 10 genes risk signature with clinic pathological measures; (b and c) GO and KEGG analyses of ferroptosis-related DEGs; (d) screening of optimal parameter (λ) at which the vertical lines were drawn; and (e) LASSO coefficient spectrum of 10 genes in HCC. GO, gene ontology; HCC, hepatocellular carcinoma; KEGG, Kyoto Encyclopedia of Genes and Genomes; LASSO, least absolute shrinkage and selection operator; TCGA, The Cancer Genome Atlas

acteristics (e.g., survival status, survival time, gender, age, tumor Gleason, grade, T, M and N stage) were assessed. We showed the expression difference of high-risk and low-risk groups in different stages (T, M and N) through heat map. In addition, the risk scores for males and HCC patients at >65 years old were elevated (Fig. 3a).

Table 3. LASSO-penalized Cox analysis identifies 10 genes to build a prognostic model

Gene	Coef
ACACA	0.004266
G6PD	0.071354
MYB	0.117039
SLC2A1	0.109265
SLC38A1	0.031264
SLC7A11	0.121545
SQSTM1	0.020266
SRXN1	0.227654
STMN1	0.126750
ZFP69B	0.496697

LASSO, least absolute shrinkage and selection operator.

Independent prognostic analysis of the ten genes signature

To verify whether the prognostic model was independent of other clinical parameters in HCC prognosis, whether the risk score was an independent prognostic parameter for OS was assessed. Univariate and multivariate Cox regression analyses revealed that high-risk scores were significantly associated with worse OS with a higher HR (HR = 3.606) in the TCGA (Fig. 7a) and high-risk score and HCC stage were independent risk factors for worse OS (risk score: HR = 3.294, 95% confidence interval (CI) = 2.294–4.730) and stage (HR = 2.123, 95% CI = 1.453–3.104) (Fig. 7b). Similar findings were achieved in the ICGC cohort (HR = 3.632, 95% CI = 1.627–8.106; Fig. 7c, d).

Analysis of immunological function

To further investigate the relationship between the HCC and prognostic and immune status, the enrichment scores for different immune cell subpopulations were adopted, which related to immune cells and immunity-related functions with ssGSEA. The frequency of antigen-presenting autologous dendritic cells (aDCs), mac-

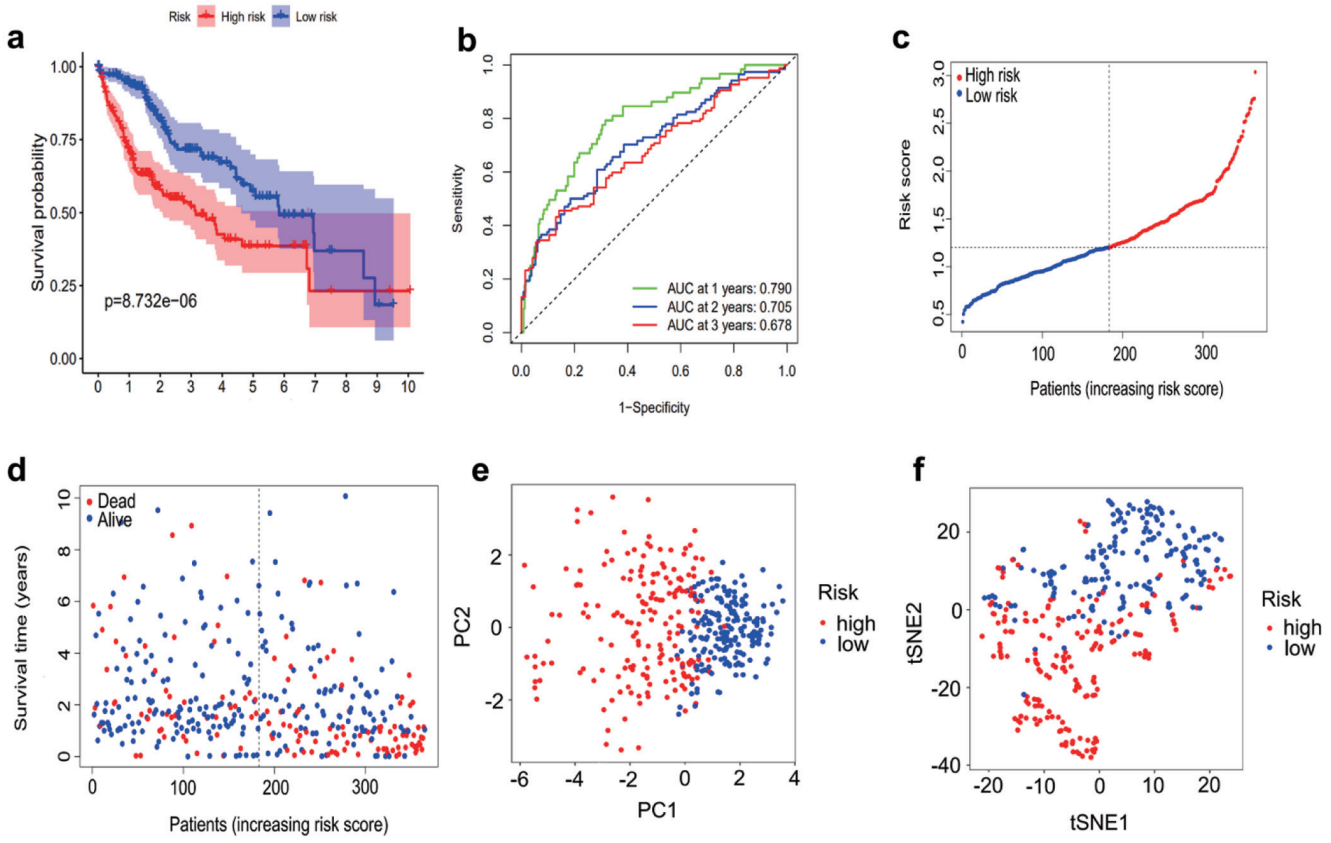

Fig. 4. Construction of the signature model in the TCGA cohort. (a) K-M survival curves of the model for OS of HCC patients; (b) time-dependent ROC curves and AUCs for 1-, 2-, and 3-year OS of HCC patients; (c) distribution and median value of the risk scores; (d) survival status of HCC patients; (e) PCA analysis of HCC patients in the TCGA cohort; and (f) t-SNE of HCC patients in the TCGA cohort. HCC, hepatocellular carcinoma; OS, overall survival; PCA, principal component analysis; ROC, receiver operating characteristic; TCGA, The Cancer Genome Atlas.

rophages (mø), natural killer cells (NK cells), helper T cells 2 (Th2 cells), and regulatory T cells (Tregs) differed significantly between the low and high-risk groups of HCC patients in the TGCA and ICGC cohorts (adjusted $p < 0.05$, Fig. 8a, c). Furthermore, there was a significant difference in the levels of type II interferon (IFN) responses, major histocompatibility complex (MHC I), human leukocyte antigen (HLA), checkpoint, chemokine receptor (CCR) expression and antigen-presenting cell (APC) costimulation between the high and low-risk groups of HCC patients in the ICGC database (adjusted $p < 0.05$, Fig. 8b, d).

Construction and evaluation of nomogram

To strengthen the model's predictive power, a comprehensive prognostic nomogram was generated based on the patients' risk score and clinical characteristics by integrating the metabolic risk signature, age, stage, grade, and gender (Fig. 9a). The nomogram had an excellent agreement with the ideal curve at 1-, 3-, and 5-years survival rates in the TCGA cohort (Fig. 9b–d).

Discussion

Currently, the incidence and mortality rates of HCC remain high, although there has been improvement in the treatment of HCC.[29] Recently, immunotherapy has become a strategy for HCC patients, in addition to surgical resection and target therapies. However, the rate of HCC patients' response to immune checkpoint inhibitors remains low. Therefore, the discovery of reliable biomarkers for evaluating the prognosis of HCC patients is urgently needed.[30,31] Therefore, it is crucial to understand the pathological mechanisms of HCC development and progression.[19,32]

In this study, 291 DEGs were identified related to ferroptosis and HCC patient's OS and generated a prognostic risk model with 10 DEGs after evaluating their association with the prognosis of HCC. These 10 DEGs were associated with an increased risk for worse OS in HCC patients, suggesting that they might contribute to the development and progression of HCC. Of these, *SLC7A11* was of importance for the pathogenesis of HCC. The prognostic risk model was valuable to predict OS in TCGA and ICGC cohorts, because the high-risk score and HCC stage were independent risk factors for worse OS in HCC patients.[33,34] Then, a nomogram was developed by combining the risk scores and clinical features. In addition, the nomogram effectively predicted the 1-, 3-, 5-year OS of HCC patients.[27] Therefore, this nomogram might be valuable for the prognosis of HCC patients in the clinic.

Of interest, the 10 ferroptosis-related DEGs were associated functionally with tumorigenesis and development because they were significantly enriched in many immune-related pathways, such as lymphocyte-mediated immunity, humoral immune response, and immunoglobulin complex.[24] This suggested that ferroptosis might

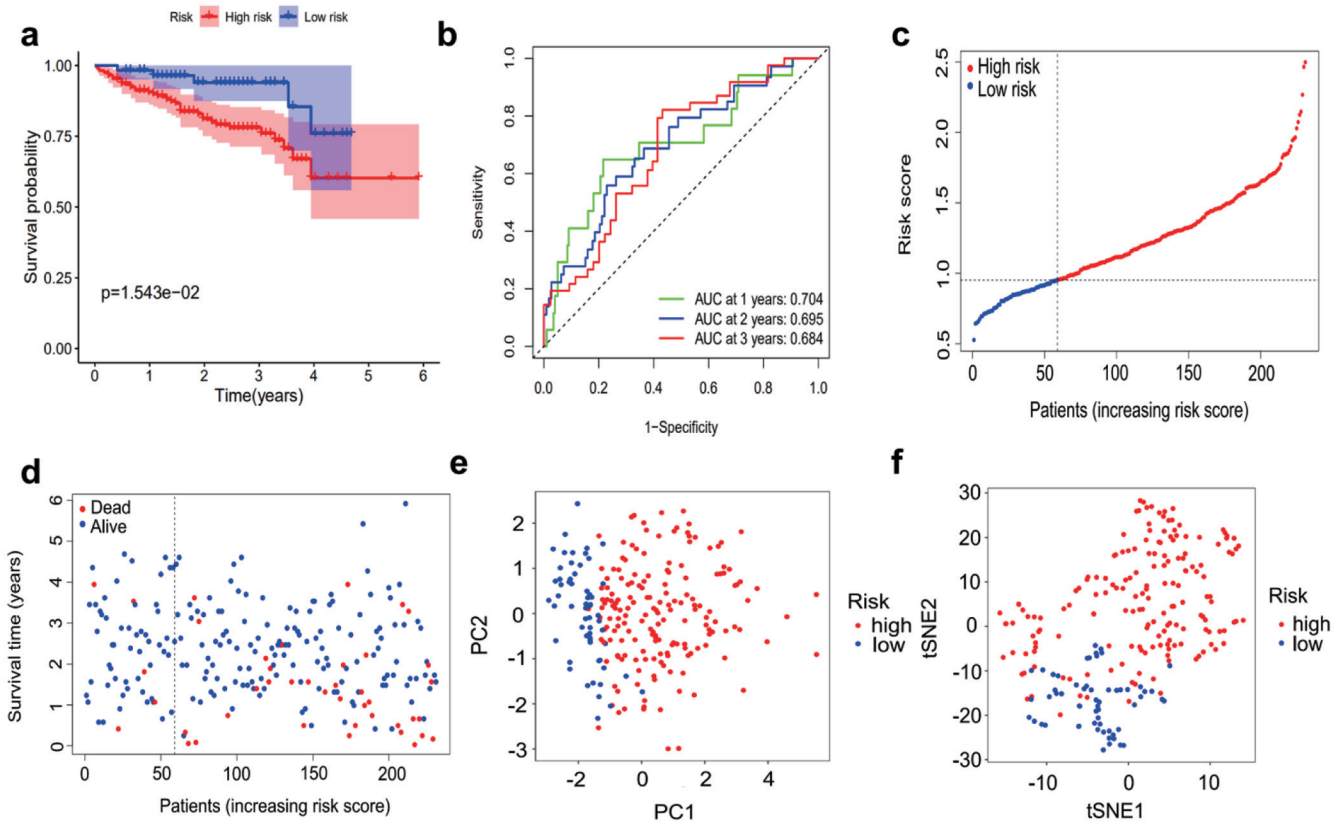

Fig. 5. Validation of the signature model in the ICGC cohort. (a) K-M survival curves of the model for OS of HCC patients; (b) time-dependent ROC curves and AUCs for 1-, 2-, and 3-year OS of HCC patients; (c) distribution and median value of the risk scores; (d) survival status of HCC patients; (e) PCA analysis of HCC patients in the ICGC cohort; and (f) t-SNE of HCC patients in the ICGC cohort. HCC, hepatocellular carcinoma; ICGC, International Cancer Genome Consortium; OS, overall survival; PCA, principal component analysis; ROC, receiver operating characteristic; TCGA, The Cancer Genome Atlas.

be closely related to antitumor immunity, which agreed with a previous observation that antigen presentation-related genes were differentially expressed between the high and low-risk groups.[35] It is well-known that the glutamate/cystine antiporter system Xc- is the most important target for erastin during erastin-induced ferroptosis.[36] Xc- can transport cysteine (the major form of cysteine in the atmosphere) into cells.[37] The inhibition of the Xc- system reduces cellular cysteine, which means that it is unavailable for the synthesis of GSH (glutathione, r-glutamyl cysteingl + glycine), which is an important antioxidant. GSH deficiency in proteins or membranes can lead to an accumulation of reactive oxygen species (ROS) and subsequent ferroptotic cell death. The combination of erastin into *SLC7A5* interferes with cystine uptake by the *SLC3A2/SLC7A11* complex in the trans form. The ferroptosis-related DEGs in HCC were highly enriched in the phagocytosis pathway, which suggested a close relationship between tumor induction and phagocytosis.

The prognostic model was composed of 10 FRGs (*ACACA, SLC7A11, SQSTM1, SRXN1, SLC2A1, SLC38A1, MYB, ZFP69B, G6PD, STMN1*). Significantly, these 10 genes were associated with a worse OS in HCC patients. *ACACA* is the rate-limiting enzyme of fatty acid synthetase (FAS). Inhibition of *ACACA* expression inhibits proliferation and induces apoptosis of prostate cancer LNCaP cells.[38] Previous studies have shown that silencing *ACACA* decreases prostate cancer cell proliferation by affecting the NAD^+/NADH balance. *SLC7A11* is a vital subunit in the Xc-system and is crucial for ferroptosis and oxidation resistance.[39] Furthermore, IFN can downregulate *SLC7A11* expression in tumor cells and a combination of IFN and radiotherapy can decrease cystine transport and antioxidant storage in tumor cells, which leads to ferroptosis.[40] A previous study identified different p62-positive structures in patients with HCC, steatohepatitis, and alcoholic hepatitis, such as Mallory-Denk bodies and intracellular hyaline bodies.[41] *SRXN1* plays a vital regulatory role in the antioxidant response in eukaryotic cells. High expression of *SRXN1* might lead to a poor prognosis in HCC, further reducing the overall survival rate of HCC patients.[42] However, the role of *SRXN1* in HCC remains unclear. *SLC2A1* is a member of the solute carrier family 2 and upregulated *SLC2A1* expression is significantly associated with invasiveness and late clinical stage of gastric cancer.[43] High *SLC38A1* expression is associated with worse survival and was an independent prognostic factor for a poor prognosis in gastric cancer.[44] *MYB* and *ZFP69B* are highly expressed in most tumor tissues. However, the function of *MYB* in HCC requires further research.[45] Glucose-6-phosphate dehydrogenase (*G6PD*) is an important metabolic enzyme in glycolysis, and it can regulate the proliferation and apoptosis of HCC.[46] Increased *G6PD* activity promotes tumor cell growth and proliferation.[47] The activity of *G6PD* is closely related to inflammation, infection, and the progression of the tumor and *G6PD* might be a therapeutic target for cancer therapies. *STMN1* enhances the crosstalk between HCC and hepatic stellate cells by promoting the mesenchymal-o-epithelial transition (MET) process of HCC cells.[48] Therefore, these DEGs are crucial for the development and progression of HCC and are potential therapeutic targets for interventions in HCC.

This study has several limitations. First, the prognostic model was established and validated on public databases, without vali-

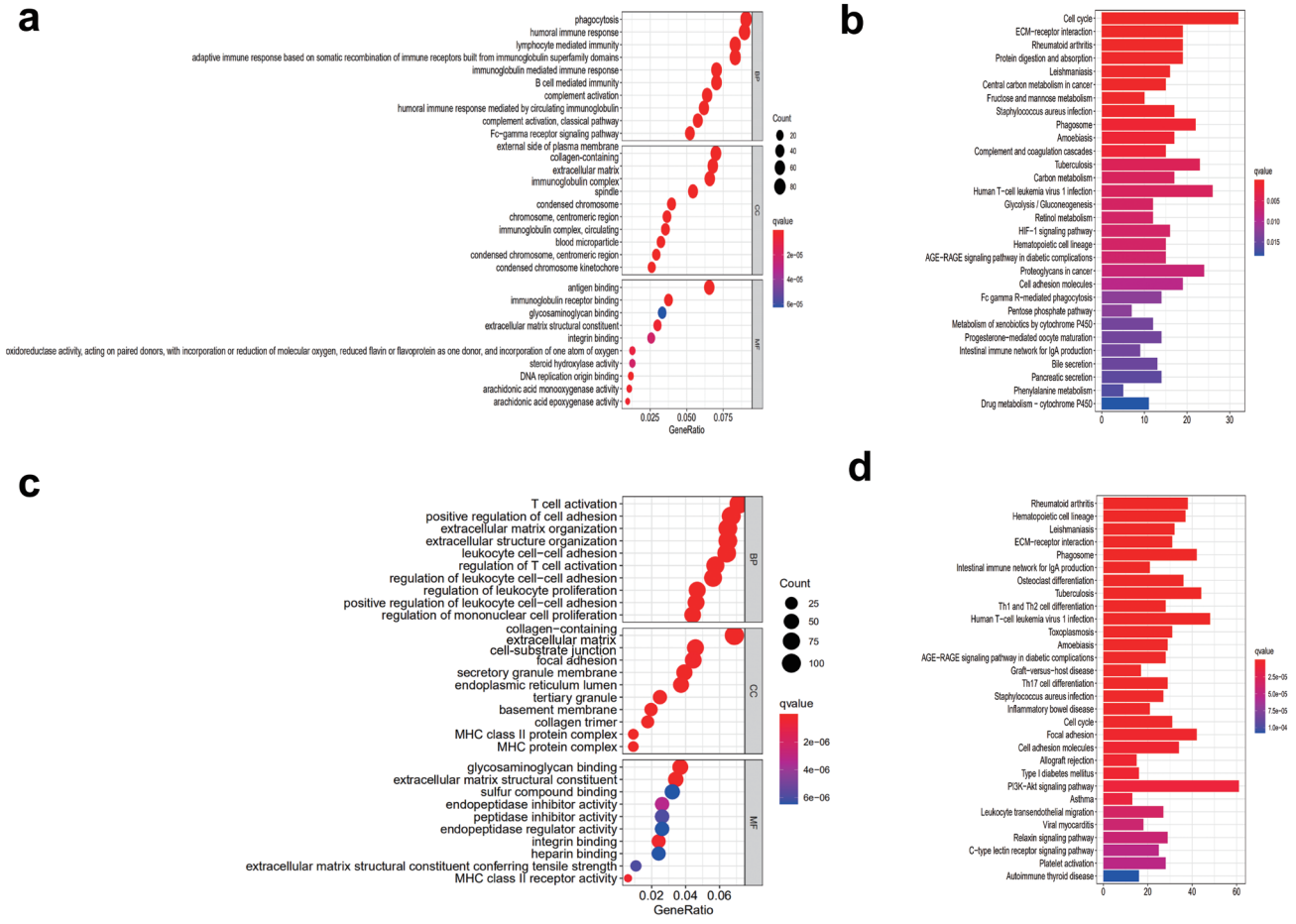

Fig. 6. GO and KEGG enrichment analysis. (a, c) GO enrichment analysis of the risk differential genes in TCGA and ICGC cohorts. BP represents biological process, CC represents cellular component, MF represents molecular function: and (b, d) KEGG enrichment analysis of the risk differential genes in TCGA and ICGC cohorts. GO, gene ontology; ICGC, International Cancer Genome Consortium; KEGG, Kyoto Encyclopedia of Genes and Genomes; TCGA, The Cancer Genome Atlas.

dation in new samples and prospective experimental trials. In addition, the relationship between immune and risk scores was not experimentally confirmed for the prognosis of HCC.

Future directions

Ferroptosis is a new type of cell death with unique properties and recognition functions. Cell ferroptotic death could be significant for the treatment of cancer in the future. This study screened biomarkers related to the prognosis of liver cancer based on ferroptosis-related regulatory genes, to provide help for the clinical treatment of liver cancer in the future. In addition, the prognosis of liver cancer based on ferroptosis-related non-coding RNA will be studied in the future, to explore the regulatory relationship between ferroptosis-related non-coding RNA in the tumor immune microenvironment of liver cancer, and antitumor drugs will be screened to provide help for immunotherapy in liver cancer.

Conclusions

In total, 10 FRGs were identified that were associated with a worse OS in HCC patients. This generated model and nomogram effectively predicted the OS of HCC patients in both databases and might be valuable for the prognosis of HCC in the clinic. In the future, the potential mechanisms that underlie ferroptosis in antitumor immunity against HCC will be investigated.

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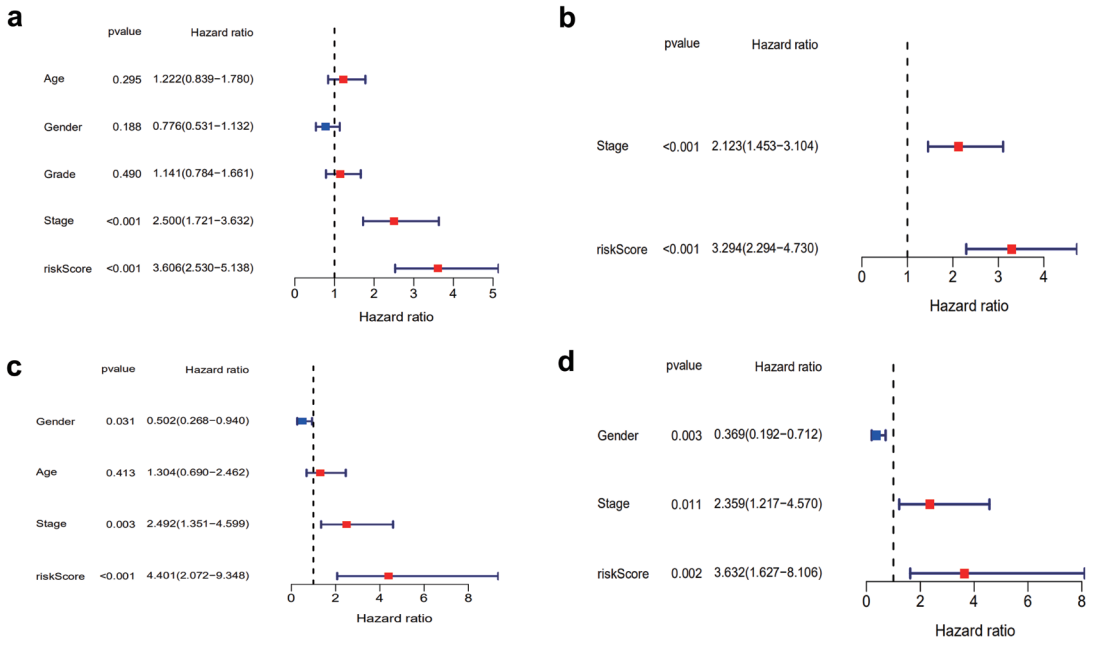

Fig. 7. Univariate and multivariate Cox regression analyses of HCC patients. (a, b) in the TCGA cohort; and (c, d) ICGC cohort (c, d). HCC, hepatocellular carcinoma; ICGC, International Cancer Genome Consortium; TCGA, The Cancer Genome Atlas.

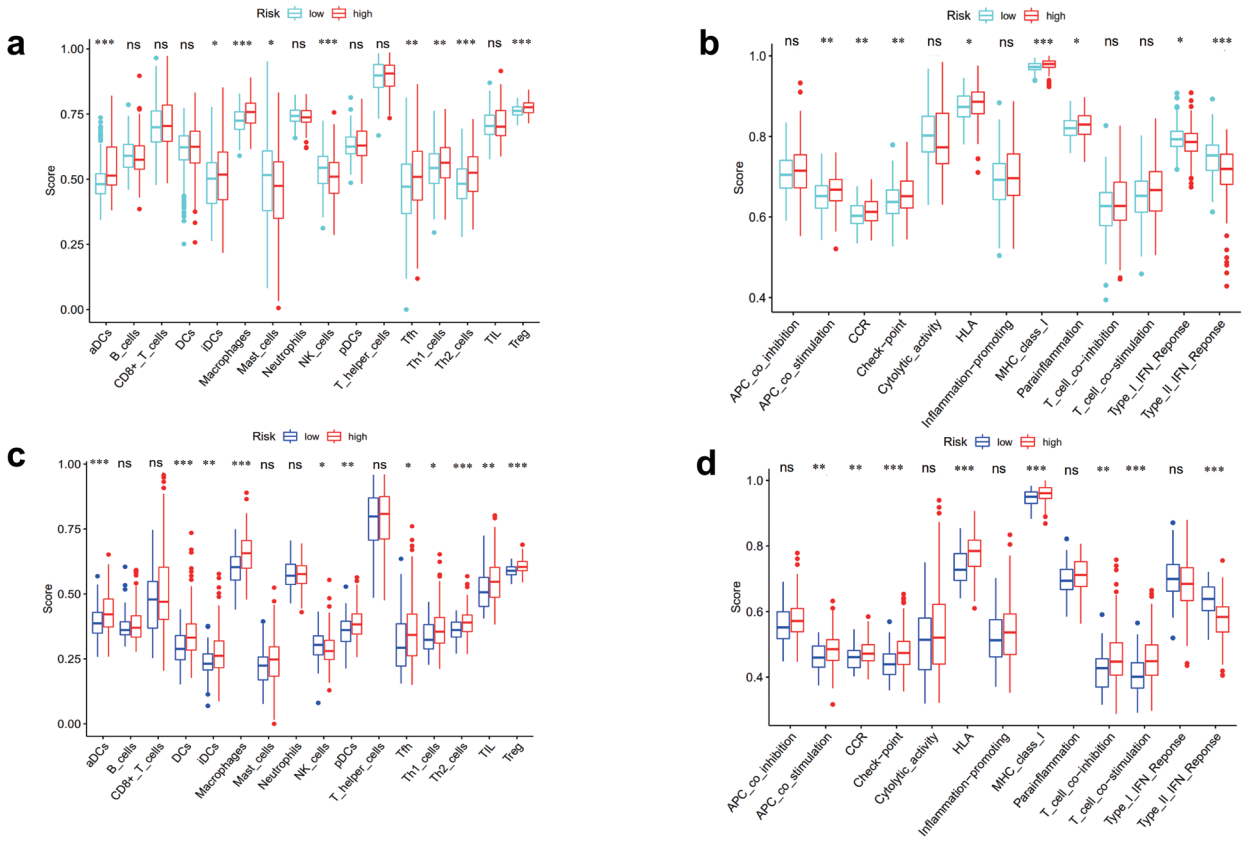

Fig. 8. The ssGSEA scores show different risk groups in the TCGA cohort (a, b) and ICGC validation cohort (c, d). The scores of 16 types of immune cells (a and c) and 13 immune-related functions (b and d) are shown in boxplots. Adjusted *p*-values were shown as: ns, not significant; * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$. ICGC, International Cancer Genome Consortium; ssGSEA, single-sample gene set enrichment analysis; TCGA, The Cancer Genome Atlas.

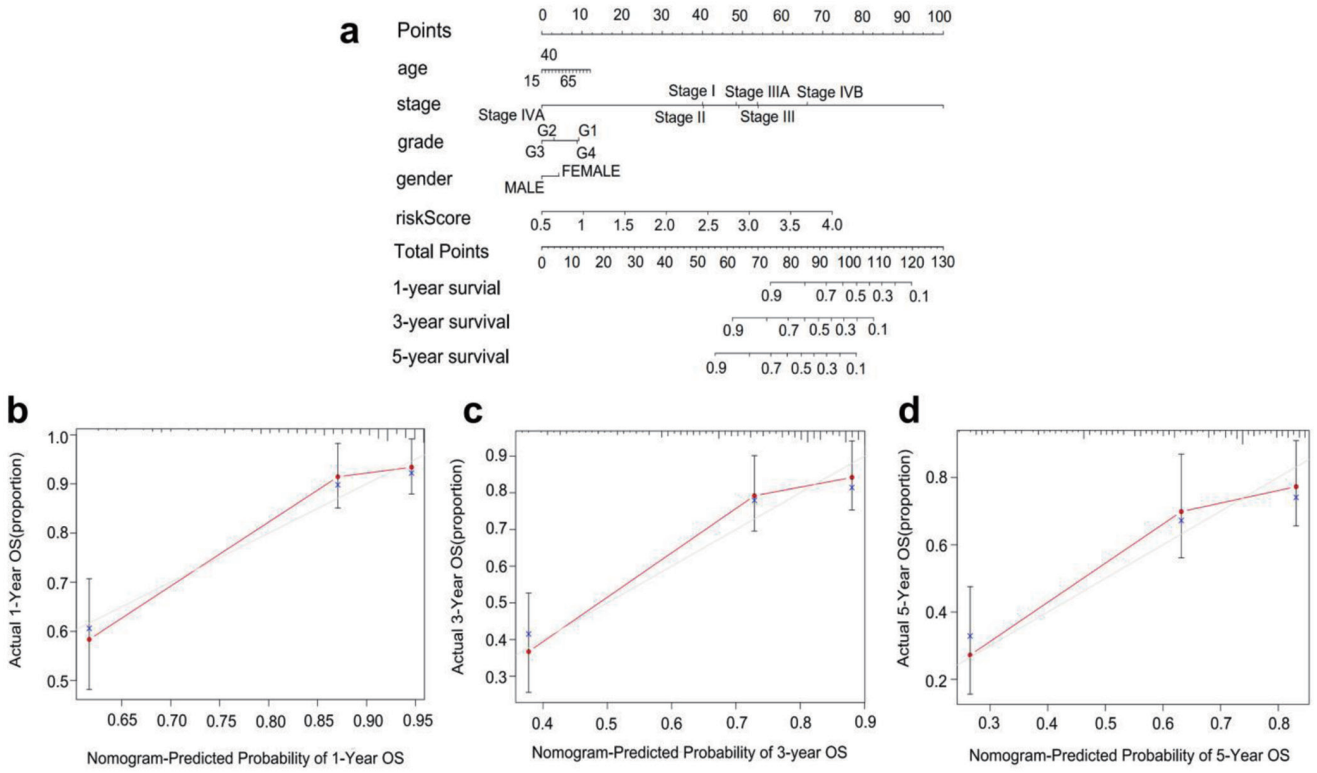

Fig. 9. Nomogram to predict the OS of HCC patients. (a) prognostic nomogram for the HCC patients; the calibration curves for the nomogram at (b) 1-; (c) 3-; and (d) 5-year OS. HCC, hepatocellular carcinoma; OS, overall survival.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Data curation (XSH and ZZ), Funding acquisition (LXL), Investigation (XSH and XLL), Project administration (LXL), Writing – original draft (LXL), Writing – review & editing (ZZ, HL and LXL).

Data sharing statement

The data used to support the findings of this study are included in the article.

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