Fatty Acid Metabolism-Related Genes Are Potential Biomarkers for Prognostic Prediction and Clinical Guidance in Patients with Hepatocellular Carcinoma

Jingze Yang
Huazhong University of Science and Technology
Xin Yang
Huazhong University of Science and Technology
Jinlu Guo
Huazhong University of Science and Technology
Shi Liu (1455019296@qq.com)
Huazhong University of Science and Technology

Research Article

Keywords: Fatty acid metabolism, Prognosis, Immune infiltration, Drug sensitivity, Hepatocellular carcinoma

Posted Date: April 15th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1552212/v1

Abstract

Background: Hepatocellular Carcinoma (HCC) is one of the most common malignant tumors with high mortality. Fatty acid metabolism is associated with the development and treatment of HCC. This study aimed to build a fatty acid metabolism-related gene model to evaluate the prognosis of HCC patients and provide guidance for individualized treatment.

Methods: RNA-sequencing data of HCC from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) database were extracted as the training set and validation set. Fatty acid metabolism-related genes predictive signature was built using univariate and least absolute shrinkage and selection operator (LASSO) Cox regression analyses. The predictive value of prognostic model was evaluated by Kaplan-Meier (K-M) curves, ROC curves, univariate and multivariate Cox regression. In addition, the immune infiltration and drug sensitivity were also explored.

Results: A twelve-gene fatty acid metabolism-related risk signature was constructed. Patients were divided into low-risk and high-risk groups according to risk scores. High-risk patients with the higher risk scores had a poor prognosis. Then, univariate and multivariate Cox regression showed that risk score was an independent risk factor for the overall survival of HCC. K-M and ROC curves further verified the predictive value of the signature. Besides, nomograms were drawn and exhibited the good performance in the prognostic prediction. Finally, the immune cell infiltration and sensitivity to chemotherapy drugs exhibited a correlation with different risk levels.

Conclusion: This novel fatty acid metabolism-related gene predictive signature may be useful for improving prognosis evaluation and individualized treatment for HCC patients.

Background

Liver cancer is reported to be the third leading cause of cancer-related death worldwide, only behind lung cancer and colorectal cancer[1], and even more than 1 million patients will die of live cancer in 2030 from the prediction of World Health Organization[2]. As the most common type of liver cancer, hepatocellular carcinoma (HCC) accounts for 90% of primary liver cancer[3]. Presently, treatment for HCC mainly concludes surgical therapy, chemotherapy, immunotherapy, and radiotherapy, but the actual long-term survival rate of HCC patients remains unsatisfactory[4]. Studies showed the genomic and transcriptional heterogeneity among HCC patients may lead to relapse, drug resistance and the poor prognosis[5]. Therefore, the valid biomarkers are needed for individualized treatment and prognostic prediction for patients with HCC.

Metabolism rewiring can disrupt the cell homoeostasis, cause the excessive cell growth and proliferation, and further promote the cancer. Changes in lipid metabolism acclimatize cancer cells to the various microenvironment by resisting oxidative stresses, adjusting intercellular communication, regulating the immune responses, and sustaining key oncogenic functions[6]. Therefore, lipid profiles are emerging as the biomarkers for the prognostic prediction in patients with cancer. Zhu et al developed a signature

based on lipid metabolism-related genes and verified its good performance for prognostic prediction in patients with bladder cancer[7]. Recently, as the important element of lipid, abnormality of fatty acid metabolism in cancer attracts increasing attention[8], which affects cancer cell biology in various aspects. Fatty acid not only regulates the synthesis of biological membranes, but also takes part in the modulation of oncogenic signaling. In addition, fatty acid also serves as substrates for mitochondrial energy synthesis and the form of energy storage[9]. Lu et al. revealed the contribution of fatty acid metabolism in maintaining HCC cells survival to resist energy deprivation[10], so targeting the fatty acid metabolism may be a promising approach for the treatment of HCC. However, due to the diversity of genotype and oncobiology, it is difficult to stratify in HCC patients and thus limits the drug discovery based on the fatty acid metabolism. Notably, the development of transcriptomics and metabolomics reveal the novel approach in HCC grouping, and may provide clinical guidance targeting the fatty acid metabolism. Ding et al. founded a prognostic model in colorectal cancer with fatty acid metabolism-related genes and showed that low-risk patients were more sensitive to chemotherapeutics[11]. Consequently, targeting the fatty acid metabolism may contribute to novel signature for the prognostic prediction and clinical medication for HCC patients.

Here, we aimed to develop a fatty acid metabolism-related gene signature to measure the risk scores, improve the accuracy of prognosis prediction, explore the drug sensitivity and immune cell infiltration, and further provide the guidance for clinical treatment in patients with HCC.

Methods

Data Collection

The raw RNA sequencing (RNA-seq) data of HCC patients were downloaded from The Cancer Genome Atlas (TCGA) database on 14 Mar 2022. Meanwhile, the clinical information such as gender, age, histological grade, TNM stage, survival time and survival status were also acquired. Besides, the GSE14520 (GPL3921) dataset, including the gene expression and clinical information of HCC patients, was downloaded from the Gene Expression Omnibus (GEO) database. Notably, patients with incomplete clinical data were excluded. Finally, 370 HCC samples from TCGA database were selected as the training set and 221 HCC samples from GEO database were used as the validation set.

Screening Fatty Acid Metabolism-Related Differentially Expressed Genes

Kyoto Encyclopedia of Genes and Genomes (KEGG) gene sets were downloaded from the Molecular Signature Database (MSigDB) and fatty acid metabolic genes were extracted in fatty acid metabolism pathways. A total of 309 fatty acid metabolism-related genes were extracted, and then 137 common genes in TCGA and GEO cohorts were identified (Table S1). Moreover, the differentially expressed fatty acid metabolism-related genes (DEFAMGs) between normal liver tissues and HCC patients were obtained

by the “limma” R package on the basis of following criteria: False Discovery Rate (FDR) < 0.05 and |log FC| ≥ 0.585.

Functional Enrichment Analysis

To further illustrate the major biological features and cell functional pathways, this study made the Gene Ontology (GO) and KEGG pathway enrichment analyses on DEFAMGs using “cluster Profiler” R package. In addition, “enrichplot” and “ggplot2” R packages were employed to visualize the results. P value < 0.05 was considered statistically significant difference.

Building and Validating the Fatty Acid Metabolism-Related Prognostic Signature

The univariate Cox regression analysis was performed to distinguish the genes significantly related to patient overall survival (OS) from DEFAMGs in the training set. P value < 0.01 was considered significant. Then, least absolute shrinkage and selection operator (LASSO) regression analysis was carried out by “glmnet” R package to avoid overfitting. The finally selected genes were used to build the prognostic signature and the formulae used to calculate the risk scores was as follows:

Risk score = (Coefficient mRNA1 × expression of mRNA1) + (Coefficient mRNA2 × expression of mRNA2) +⋯ + (Coefficient mRNAn × expression of mRNAn)[12]. HCC patients were classified into low-risk and high-risk groups according to the median value of risk scores. Kaplan-Meier (K-M) analysis was conducted using the “survival” and “survminer” R packages to compare the OS difference between low-risk and high-risk groups. Moreover, to assess the predictive performance of risk score model, a time-dependent receiver operating characteristic (ROC) curve was drawn with the “timeROC” package. In addition, the univariate and multivariate Cox regression analyses were carried out to evaluate the independent prognostic value of the signature. Besides, the principal-component analysis (PCA) based on all fatty acid metabolism-related genes and genes used to build prognostic signature model respectively for the low-risk and high-risk groups was performed by “limma” and “ggplot2” R packages. Finally, the fatty acid metabolism-related prognostic signature was further tested by K-M analysis and ROC curve in the validation set.

Establishing Predictive Nomograms

The nomogram model was created to predict the OS for the HCC patients on the basis of the risk score and clinical characteristics (age, gender, histologic grade, and pathological stage) using the “rms” “regplot” and “survival” packages. The calibration curves, univariate and multivariate Cox regression analysis, and ROC curve were used to evaluate the prognostic performance of the nomogram from various aspects.

Exploration of the Risk Model in the Immunity Therapy

Single-sample gene-set enrichment analysis (ssGSEA) was carried out to evaluate the immune-related infiltration of HCC patients by using “GSEABase” and “GSVA” R packages. Then, we calculated the

enrichment scores to assess the relative level of immune-related characteristic expression using the ssGSEA algorithm and compared the difference of enrichment scores between the low-risk and high-risk score groups. In addition, Tumor Immune Dysfunction and Exclusion (TIDE) was applied to predict the feasibility of immunotherapeutic treatment response[13]. P value < 0.05 was indicated statistically significant.

Exploration of Chemotherapy Response Based on Risk Model

In order to predict the therapeutic effect of commonly used chemotherapeutic drugs, including sorafenib, gemcitabine, 5-Fluorouracil, paclitaxel, Lapatinib, the half-maximal inhibitory concentration (IC50) of chemotherapeutic drugs was calculated by using the “pRRophetic” R package. In addition, the correlation between the risk score and IC50 of chemotherapeutic drugs was also performed.

Statistical analysis

All statistical analyses were carried out by using R software (Version 4.1.3). P value < 0.05 was considered statistically significant.

Results

Enrichment analysis of DEFAMGs

Differential expression analysis in TCGA database exhibited that there were 170 DEFAMGs, with 110 genes up-regulated and 60 down-regulated in the HCC samples (Fig. 1A-B; TableS2). Then, GO analysis showed that fatty acid metabolic process, long-chain fatty acid metabolic process, and fatty acid biosynthetic process were highly enriched GO terms (Fig. 2A-B). Meanwhile, the KEGG analysis also exhibited that fatty acid metabolism, degradation, and elongation process were highly enriched KEGG terms (Fig. 2C-D). These findings revealed that fatty acid metabolism played an important role in HCC development.

Building a Fatty Acid Metabolism-Related Risk Signature

The univariate Cox proportional hazard regression analysis showed 42 genes related to OS (p < 0.01; Fig. 3A; Table S3). After the LASSO regression (Fig. 3B-C), 12 fatty acid metabolism-related genes, including PON1, CYP2C9, ACACA, ACADS, ME1, ACAT1, ELOVL1, SMS, UGDH, ADSL, HSP90AA1, and S100A10, were finally selected to build the prognostic risk score model. The risk score formula was as following: risk score = (-0.05089 × expression of PON1) + (-0.0225× expression of CYP2C9) + (0.08121 × expression of ACACA) + (-0.0002 × expression of ACADS) + (0.06192 × expression of ME1) + (-0.0144 × expression of ACAT1) + (0.08595 × expression of ELOVL1) + (0.25743 × expression of SMS) + (0.0033 × expression of UGDH) + (0.05056 × expression of ADSL) + (0.08092 × expression of HSP90AA1) + (0.03604 × expression of S100A10), which was shown in Table S4.

Validating the Fatty Acid Metabolism-Related Risk Signature

The risk scores of HCC patients were calculated according to the above formula. Then, the patients from TCGA database were classified into low-risk (n = 185) and high-risk (n = 185) groups by the median risk score in the training set (Table S5). Correspondingly, patients from GEO database were divided into low-risk (n = 106) and high-risk (n = 115) groups by the median risk score in the validation set (Table S6). The K-M curves showed that patients of high-risk group had a worse prognosis than that of low-risk group both in the training set (P < 0.001; Fig. 4A) and validation set (P = 0.006; Fig. 4E). From the time-dependent ROC, we found the AUCs for 1-, 3-, and 5- year survival was 0.790, 0.685, and 0.698 in the training set (Fig. 4B). Correspondingly, the AUCs for 1-, 3-, and 5- year survival was 0.644, 0.608, and 0.601 in the validation (Fig. 4F). The risk score was also verified as an effective prognostic model both in the univariate (P < 0.001; Fig. 4C) and multivariate Cox regression (P < 0.001; Fig. 4D). Besides, the PCA analysis demonstrated that the prognostic model has a better performance than fatty acid metabolism-related genes in the distinction between the high-risk and low-risk patients (Fig. 4G-H).

Stratification Analysis of the Prognostic Signature

Patients in the TCGA dataset were separated into several subgroups by different clinical parameters and the difference of OS was explored between the low-risk and high-risk patients. From Fig. 5A-J, K-M analysis displayed that patients with high-risk had a worse prognosis under the condition of Stage I–II (P < 0.001), Stage III–IV (P < 0.001), G1 + 2 (P < 0.001), G3 + 4 (P = 0.01), T1 + 2 (P < 0.001), T3 + 4 (P = 0.009), age < 60 (P = 0.039), age ≥ 60 (P < 0.001), male (P < 0.001) except for female (P = 0.059).

Construction and Assessment of Predictive Nomograms

As seen from Fig. 6, age, gender, grade, stages and risk score were selected to create the nomogram. Besides, calibration plots exhibited that the observed OS was found to be approximately consistent with the predicted OS. Univariate and multivariate Cox regression analysis showed that nomogram was effective for the prognostic assessment (P < 0.001 and P = 0.001, respectively). Moreover, ROC curves illustrated that the nomogram (AUC = 0.759) had better performance in the prediction of OS than a single marker, such as risk score (AUC = 0.696), age (AUC = 0.587), Gender (AUC = 0.450), Grade (AUC = 0.539), and pathological stage (AUC = 0.663).

Immune-related characteristic in the low- and high-risk score groups

From Fig. 7, we found that the fractions of CD8 + T cells in the low-risk group was higher than that of high-risk group, while the fractions of Macrophages M0 was higher in the high-risk group. In addition, as for the immune-related pathways, the scores of APC co-stimulation, CCR, Check-point, HLA, MHC-class-I, parainflammation, T - cell-co-stimulation in the high-risk scores group was higher than that of low-risk group. However, the score of Type-II-IFN-Response represented higher activity in the low-risk group.

Gene set variation analysis (GSVA)

From Fig. 8, the heatmap analysis showed that a majority of metabolism pathways, such as fatty acid metabolism pathway was enriched in the low-risk score group, while the high-risk score group was

connected with the cancer-related and cell signaling-related pathways (Fig. 8A). As for the TIDE, there was significant difference between the low-risk group and high-risk group, and the patients with high-risk may be more suitable for the immunotherapy. (Fig. 8B). Furthermore, patients with TP53 mutation (P < 0.001) had higher risk scores (Fig. 8C). Besides, low-risk group exhibited a higher degree of progression free survival (P < 0.001; Fig. 8D).

Response to chemotherapy

In addition, we identified the candidate drugs by the fatty acid metabolism-related risk signature. Patients from the high-risk scores group were more sensitive to sorafenib (P < 0.001; Fig. 9A), gemcitabine (P < 0.001; Fig. 9C), 5-Fluorouracil (P < 0.001; Fig. 9E), and paclitaxel (P < 0.001; Fig. 9G), and the sensitivity of chemotherapeutic drugs was negatively associated with the risk score. However, Lapatinib (P < 0.001; Fig. 9I) was more suited for the low-risk groups and there was positive relation between the sensitivity of chemotherapeutic drugs and risk scores.

Discussion

In this study, we analyzed the DEFAMGs between HCC patients and controls in the TCGA database. Then, the prognosis-related genes were screened and a novel prognostic signature for HCC patients was built. Next, we validated the model as an effective biomarker in the GEO database. Furthermore, we demonstrated that the risk score model could predict the OS, identify the response to immunotherapy, select the suited chemotherapy drugs, and provide the guideline for the clinical work.

In this study, a total of 12 genes (PON1, CYP2C9, ACACA, ACADS, ME1, ACAT1, ELOVL1, SMS, UGDH, ADSL, HSP90AA1, S100A10) were used to construct the prognostic signature. PON1 involved in maintaining the function of endothelial cells, inhibiting the adhesion of leukocytes, reducing the chronic inflammation, and suppressing the tumor invasion or metastasis[14]. ACADS was reported to participate in the proliferation and metastasis of HCC[15]. SMS could influence the metabolism process and involved in oncogenesis and drug resistance[16]. Overexpression of ACACA, ME1, ADSL or S100A10 promoted HCC growth, migration or invasion, and was associated with the poor prognosis[17–19]. As for the ACAT1 and CYP2C9, overexpression suppressed the proliferation and migration of tumor cells[20, 21]. As regards the ELOVL1, UGDH and HSP90AA1, studies only showed the correlation with cancer and needed further exploration to elucidate the underlying mechanisms[22–25]. As in our study, the level of PON1, CYP2C9 ACADS, ACAT1 decreased in the HCC and was positively associated with the OS of HCC patients. The expression of SMS, HSP90AA1, ADSL, UGDH, ACACA, ME1, ELOVL1, S100A10 was increased in the tumor tissues and was negatively associated with the OS.

In recent years, concerns have been raised in HCC because of the increasing incidence and mortality. However, the diagnosis and prognosis analysis of HCC patients are main on the basis of the conventional staging, which is not sensitive enough[26]. Therefore, it is necessary to identify reliable prognostic markers to improve the clinical outcomes of HCC. Recently, abnormal metabolism was reported to be closely related to the course of tumorigenesis and metastasis[27], and the altered fatty acid metabolism

in cancer drew the renewed interest in particular[8]. Some studies have revealed that orchestrating fatty acid metabolism can regulate the occurrence and development of HCC[28–30]. Notably, many metabolism-related genes have been revealed to be valuable prognostic biomarkers and metabolism-related risk signatures was built to predict the OS of HCC[12, 31]. However, little study concerning fatty acid metabolism-related gene risk model has been done. Only one study selected 6 fatty acid metabolism-related genes to build a prognostic model of HCC without further drug exploration[32]. Therefore, we screened the fatty acid metabolism-related genes again and finally identified 12 genes to construct the prognostic signature more accurately. We evaluated the predictive capacity of the model and revealed that it may be used as an independent prediction factor in HCC patients. In addition, the prognostic signature showed a higher AUC value and indicated a better predictive power than other clinical characteristics.

Immune system is considered to play an essential role in preventing people from cancer. In recent years, tumor immunotherapy is considered to be the promising adjuvant therapy for HCC[33]. Tumor immunotherapy aimed at strengthening or weakening the abnormal immune state to control tumor growth or kill tumor cells[34]. However, only part of HCC patients was reported to be sensitive to the tumor immunotherapy. Wu et al. found that fatty acid metabolism could regulate the phenotype and function of immune cells, and thus affect the effect of immunotherapy[35]. In this study, according to the fatty acid metabolism-related genes model, high-risk patients responded better to immunotherapy than low-risk patients, which can guide the patient selection for immunotherapy. Besides, tumor mutational burden was regarded as a promising novel biomarker to select patients for the tumor immunotherapy in recent years[36]. TP53 mutation is the most common mutation in HCC, and it influences the progression and prognosis of HCC. Long developed a TP53-associated immune prognostic model for HCC and found the model may have important implications for prediction of OS[37]. In this study, TP53 mutation also showed higher risk scores in HCC patients. Collectively, the fatty acid metabolism-related genes model may provide the valuable information to select the HCC patients fit for immunotherapy.

It is reported that different patients respond inconsistently to chemotherapy drugs. Although sorafenib is the important first-line chemotherapeutic therapy, some HCC patients showed the sorafenib resistance and therapeutic efficacy was unsatisfying[38]. Meanwhile, the same phenomenon has been seen in other chemotherapeutic drugs[39, 40], which really deteriorated the prognosis of patients. Therefore, the individualized treatment has received great interest and may be an important approach to improve the clinical effect. Ding et al. constructed the fatty acid metabolism-related risk signatures for predicting the effect of 5-Fluorouracil in colorectal cancer and revealed patients in low-risk score group were more sensitive to 5-FU[11]. In our study, we demonstrated that HCC patients from the high-risk scores group were more suitable for sorafenib, gemcitabine, 5-Fluorouracil, and paclitaxel, while Lapatinib was more sensitive to the low-risk groups. Therefore, according to the risk score, appropriate chemotherapy drug can be chosen for the HCC patients in the future.

Study strengths and limitations

The main strength of this study is the construction and validation of a novel prognostic signature based on fatty acid metabolism-related genes, which has good performance in prognostic prediction and provides guidance for clinical medication. However, this study also has limitation without the experimental validation in vivo and vitro. Therefore, further exploration showed be carried out to evaluate the functions of fatty-acid metabolism-related genes in patients of HCC in the future.

Conclusion

In a word, we screened out fatty acid metabolism-related genes by comprehensive bioinformatic analyses in HCC patients. Then, 12 genes involved in prognosis were used to construct and validate the risk signature, which was independently associated with the OS for HCC patients. The findings provided a novel, effective and accurate prognostic predicting model for HCC patients. Moreover, the risk model could provide the guidance for personalized immunotherapy and chemotherapy.

Abbreviations

HCC: Hepatocellular carcinoma; TCGA: The Cancer Genome Atlas; GEO: Gene Expression Omnibus; KEGG: Kyoto Encyclopedia of Genes and Genomes; MSigDB: Molecular Signature Database; DEFAMGs: Differentially expressed fatty acid metabolism-related genes; FDR: False Discovery Rate; GO: Gene Ontology; OS: Overall survival; LASSO: Least absolute shrinkage and selection operator; K-M: Kaplan-Meier; ROC: Receiver operating characteristic; PCA: Principal-component analysis; ssGSEA: Single-sample gene-set enrichment analysis; TIDE: Tumor Immune Dysfunction and Exclusion; GSVA: Gene set variation analysis.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The raw data of this study are downloaded from TCGA database (https://portal.gdc.cancer.gov/) and GEO data portal (https://www.ncbi.nlm.nih.gov/

geo/), which are publicly available databases.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by the National Natural Science Foundation of China

(NO.81770536).

Authors' contributions

J Y and X Y designed the study, analyzed the data and wrote the article. J G analyzed the data and reviewed the manuscript. S L contributed to the concept, designed the study, and reviewed the manuscript.

All authors read and approved the manuscript.

Acknowledgements

Not applicable.

References

1. Sung H, Ferlay J, Siegel R, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: a cancer journal for clinicians.2021; 71:209–249.
2. Villanueva A. Hepatocellular Carcinoma. The New England journal of medicine. 2019; 380:1450–1462.
3. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. Lancet (London, England).2018; 391:1301–1314.
4. Gluer A, Cocco N, Laurence J, Johnston E, Hollands M, Pleass H, et al. Systematic review of actual 10-year survival following resection for hepatocellular carcinoma. HPB : the official journal of the International Hepato Pancreato Biliary Association. 2012; 14:285–290.
5. Xu L, He M, Dai Z, Yu J, Wang J, Li X, et al. Genomic and transcriptional heterogeneity of multifocal hepatocellular carcinoma. Annals of oncology: official journal of the European Society for Medical Oncology. 2019; 30:990–997.
6. Butler L, Perone Y, Dehairs J, Lupien L, de Laat V, Talebi A, et al. Lipids and cancer: Emerging roles in pathogenesis, diagnosis and therapeutic intervention. Advanced drug delivery reviews. 2020; 159:245–293.
7. Zhu K, Xiaoqiang L, Deng W, Wang G, Fu B. Development and validation of a novel lipid metabolism-related gene prognostic signature and candidate drugs for patients with bladder cancer. Lipids in health and disease. 2021: 20:146.

8. Koundouros N, Poulogiannis G. Reprogramming of fatty acid metabolism in cancer. British journal of cancer. 2020; 122:4–22.
9. Hoy A, Nagarajan S, Butler L. Tumour fatty acid metabolism in the context of therapy resistance and obesity. Nature reviews Cancer. 2021; 21:753–766.
10. Wang M, Han J, Xing H, Zhang H, Li Z, Liang L, et al. Dysregulated fatty acid metabolism in hepatocellular carcinoma. Hepatic oncology. 2016; 3:241–251.
11. Ding C, Shan Z, Li M, Chen H, Li X, Jin Z. Characterization of the fatty acid metabolism in colorectal cancer to guide clinical therapy. Molecular therapy oncolytics. 2021; 20:532–544.
12. Zhao Y, Zhang J, Wang S, Jiang Q, Xu K. Identification and Validation of a Nine-Gene Amino Acid Metabolism-Related Risk Signature in HCC. Frontiers in cell and developmental biology. 2021; 9:731790.
13. Xu F, Zhan X, Zheng X, Xu H, Li Y, Huang X, et al. A signature of immune-related gene pairs predicts oncologic outcomes and response to immunotherapy in lung adenocarcinoma. Genomics. 2020; 112:4675–4683.
14. Ding G, Zhu X, Ji Y, Shi G, Shen Y, Zhou J, et al. Serum PON1 as a biomarker for the estimation of microvascular invasion in hepatocellular carcinoma. Annals of translational medicine. 2020; 8:204.
15. Chen D, Feng X, Lv Z, Xu X, Lu Y, Wu W, et al. ACADS acts as a potential methylation biomarker associated with the proliferation and metastasis of hepatocellular carcinomas. Aging. 2019; 11:8825–8844.
16. Lu H, Zhou L, Zuo H, Le W, Hu J, Zhang T, et al. Overriding sorafenib resistance via blocking lipid metabolism and Ras by sphingomyelin synthase 1 inhibition in hepatocellular carcinoma. Cancer chemotherapy and pharmacology. 2021; 87:217–228.
17. Wang M, Wu H, Fu G, Zhang H, Zhou X, Tang L, et al. Acetyl-coenzyme A carboxylase alpha promotion of glucose-mediated fatty acid synthesis enhances survival of hepatocellular carcinoma in mice and patients. Hepatology (Baltimore, Md). 2016; 63:1272–1286.
18. Nakashima C, Yamamoto K, Fujiwara-Tani R, Luo Y, Matsushima S, Fujii K, et al. Expression of cytosolic malic enzyme (ME1) is associated with disease progression in human oral squamous cell carcinoma. Cancer science. 2018; 109:2036–2045.
19. Jiang T, Sánchez-Rivera F, Soto-Feliciano Y, Yang Q, Song C, Bhuatkar A, et al. Targeting the De Novo Purine Synthesis Pathway Through Adenylosuccinate Lyase Depletion Impairs Liver Cancer Growth by Perturbing Mitochondrial Function. Hepatology (Baltimore, Md). 2021; 74:233–247.
20. Chen L, Peng T, Luo Y, Zhou F, Wang G, Qian K, et al. ACAT1 and Metabolism-Related Pathways Are Essential for the Progression of Clear Cell Renal Cell Carcinoma (ccRCC), as Determined by Co-expression Network Analysis. Frontiers in oncology. 2019; 9:957.
21. Yu D, Green B, Marrone A, Guo Y, Kadlubar S, Lin D, et al. Suppression of CYP2C9 by microRNA hsa-miR-128-3p in human liver cells and association with hepatocellular carcinoma. Scientific reports. 2015; 5:8534.

22. Yamashita Y, Nishiumi S, Kono S, Takao S, Azuma T, Yoshida M. Differences in elongation of very long chain fatty acids and fatty acid metabolism between triple-negative and hormone receptor-positive breast cancer. BMC cancer. 2017; 17:589.
23. Hama K, Fujiwara Y, Hayama T, Ozawa T, Nozawa K, Matsuda K, et al. Very long-chain fatty acids are accumulated in triacylglycerol and nonesterified forms in colorectal cancer tissues. Scientific reports. 2021; 11:6163.
24. Fan H, Liu H, Zhang C, Gao D, Xue Q, Chen J, et al. Comparative proteomics and molecular mechanical analysis in CDA-II induced therapy of LCI-D20 hepatocellular carcinoma model. Journal of cancer research and clinical oncology. 2009; 135:591–602.
25. Shi W, Feng L, Dong S, Ning Z, Hua Y, Liu L, et al. FBXL6 governs c-MYC to promote hepatocellular carcinoma through ubiquitination and stabilization of HSP90AA1. Cell communication and signaling: CCS. 2020; 18:100.
26. Bruix J, Reig M, Sherman M. Evidence-Based Diagnosis, Staging, and Treatment of Patients With Hepatocellular Carcinoma. Gastroenterology. 2016; 150:835–853.
27. Thakur C, Chen F. Connections between metabolism and epigenetics in cancers. Seminars in cancer biology. 2019; 57:52–58.
28. Wu L, Zhang X, Zheng L, Zhao H, Yan G, Zhang Q, et al. RIPK3 Orchestrates Fatty Acid Metabolism in Tumor-Associated Macrophages and Hepatocarcinogenesis. Cancer immunology research. 2020; 8:710–721.
29. Ma A, Yeung C, Tey S, Mao X, Wong S, Ng T, et al. Suppression of ACADM-Mediated Fatty Acid Oxidation Promotes Hepatocellular Carcinoma via Aberrant CAV1/SREBP1 Signaling. Cancer research. 2021; 81:3679–3692.
30. Chen J, Ding C, Chen Y, Hu W, Yu C, Peng C, et al. ACSL4 reprograms fatty acid metabolism in hepatocellular carcinoma via c-Myc/SREBP1 pathway. Cancer letters. 2021; 502:154–165.
31. Hu B, Yang X, Sang X. Construction of a lipid metabolism-related and immune-associated prognostic signature for hepatocellular carcinoma. Cancer medicine. 2020; 9:7646–7662.
32. He D, Cai L, Huang W, Weng Q, Lin X, You M, et al. Prognostic value of fatty acid metabolism-related genes in patients with hepatocellular carcinoma. Aging. 2021; 13:17847–17863.
33. Schoenberg M, Li X, Li X, Han Y, Börner N, Koch D, et al. The interactions between major immune effector cells and Hepatocellular Carcinoma: A systematic review. International immunopharmacology. 2021; 101:108220.
34. Farkona S, Diamandis E, Blasutig I. Cancer immunotherapy: the beginning of the end of cancer? BMC medicine. 2016; 14:73.
35. Wu H, Han Y, Rodriguez Sillke Y, Deng H, Siddiqui S, Treese C, et al. Lipid droplet-dependent fatty acid metabolism controls the immune suppressive phenotype of tumor-associated macrophages. EMBO molecular medicine. 2019; 11:e10698.
36. Galuppini F, Dal Pozzo C, Deckert J, Loupakis F, Fassan M, Baffa R. Tumor mutation burden: from comprehensive mutational screening to the clinic. Cancer cell international. 2019; 19:209.

37. Long J, Wang A, Bai Y, Lin J, Yang X, Wang D, et al. Development and validation of a TP53-associated immune prognostic model for hepatocellular carcinoma. EBioMedicine. 2019; 42:363–374.
38. Xu J, Ji L, Liang Y, Wan Z, Zheng W, Song X, et al. CircRNA-SORE mediates sorafenib resistance in hepatocellular carcinoma by stabilizing YBX1. Signal transduction and targeted therapy. 2020; 5:298.
39. Yu X, Gao X, Mao X, Shi Z, Zhu B, Xie L, et al. Knockdown of FOXO6 Inhibits Glycolysis and Reduces Cell Resistance to Paclitaxel in HCC Cells via PI3K/Akt Signaling Pathway. OncoTargets and therapy. 2020; 13:1545–1556.
40. Bekaii-Saab T, Markowitz J, Prescott N, Sadee W, Heerema N, Wei L, et al. A multi-institutional phase II study of the efficacy and tolerability of lapatinib in patients with advanced hepatocellular carcinomas. Clinical cancer research: an official journal of the American Association for Cancer Research. 2009; 15:5895–5901.

Figures

Figure 1

Visualization of differential expressed fatty acid metabolism-related genes. (A) The heatmap (B) The volcano plot.

Figure 2

Enrichment analysis of differentially expressed fatty acid metabolism-related genes. (A-B) GO enrichment analysis (C-D) KEGG enrichment analysis

Figure 3

Construction of the fatty acid metabolism-related prognostic signature in the training cohort. (A) Univariate Cox regression (B-C) LASSO regression analysis.

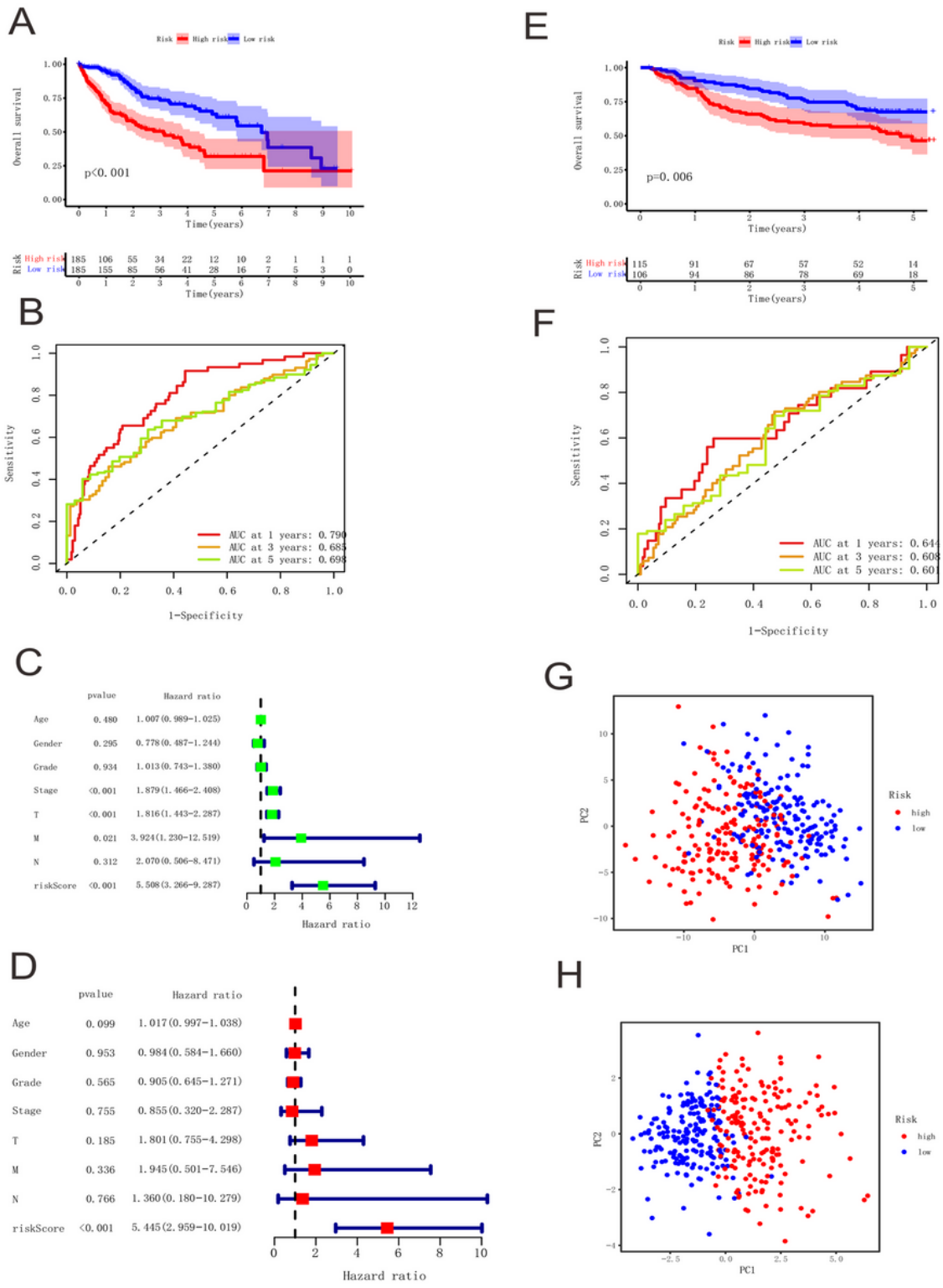

Figure 4

The predictive performance of fatty acid metabolism score model in survival status of HCC patients. (A, E) K-M analysis of the overall survival in the low-risk and high-risk groups in the training set and validation set. (B, F) The ROC analysis in the training set and the validation set. (C, D) The univariate and multivariate Cox regression analysis in the training set. (G) Principal-component analysis based on the all

fatty acid metabolism-related genes. (H) Principal-component analysis based on genes used to build the risk model.

Figure 5

The Kaplan-Meier curves displayed the survival probability between the low-risk and high-risk patients under the condition of (A) Stage I-II, (B) Stage III–IV, (C) G1+2, (D) G3+4, (E) T1+2, (F) T3+4, (G) age 60, (H) age ≥ 60, (I) female, (J) male.

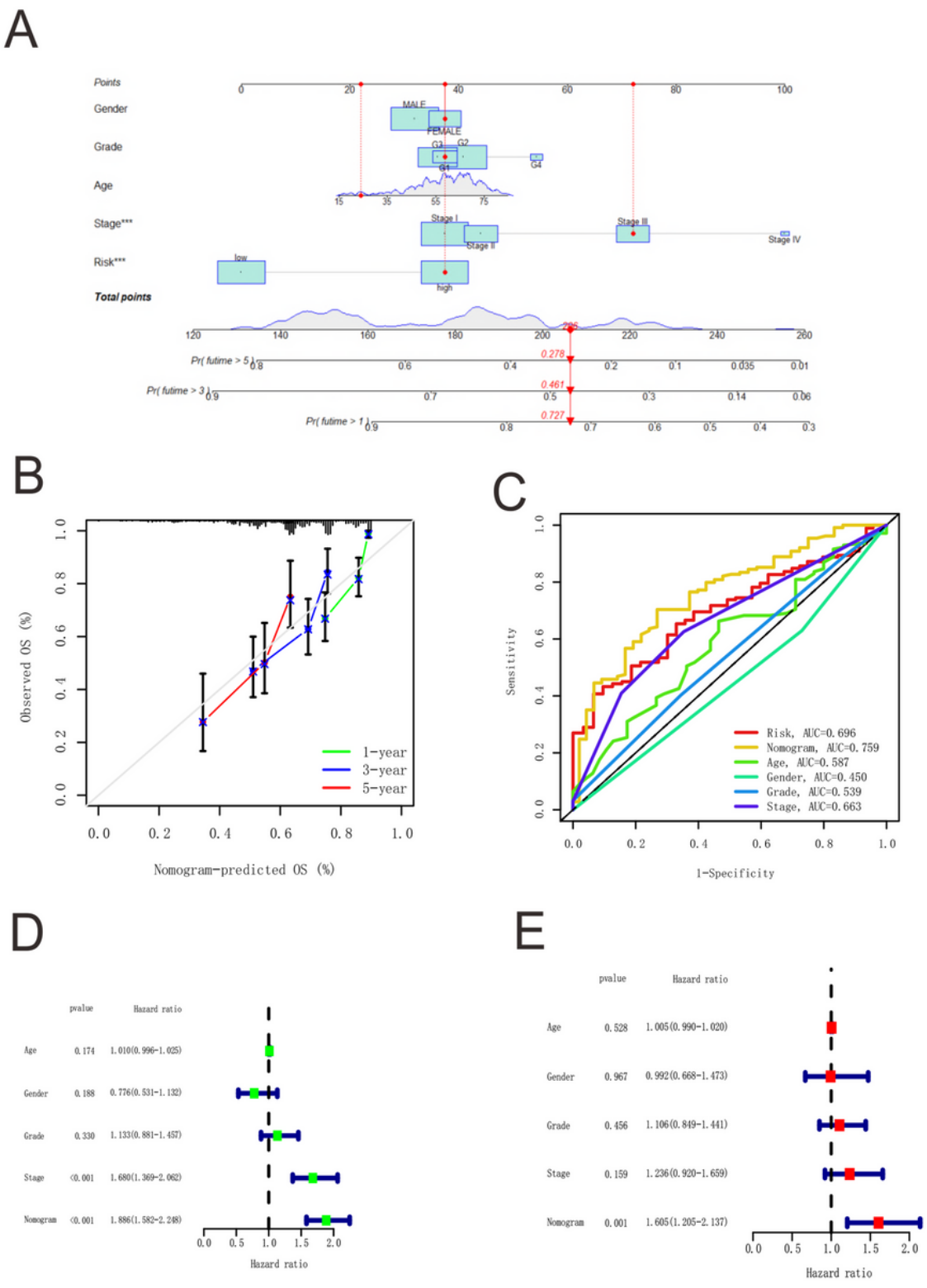

Figure 6

Nomogram to predict overall survival and its validation in HCC patients. (A) Nomograms for predicting the overall survival. (B) Calibration plot analysis. (C) ROC curves. (D) The univariate Cox regression analysis. (E) The multivariate Cox regression analysis.

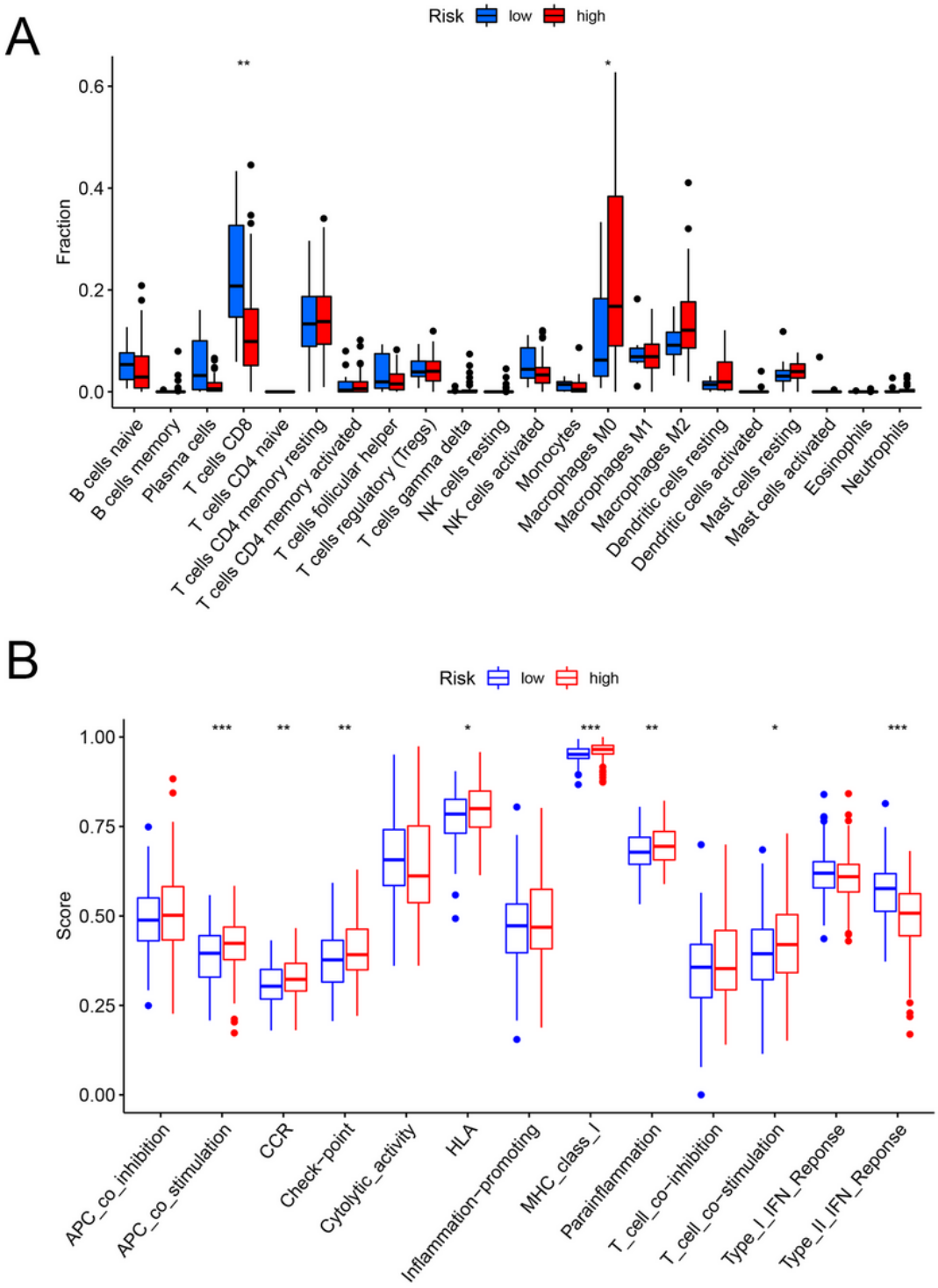

Figure 7

The evaluation of immune cells and immunologic function based on risk scores. (A) Fraction of immune cells in the low-risk and high-risk group. (B) Immunologic function in the low-risk and high-risk group. (* P < 0.05, ** P < 0.01, *** P < 0.001.)

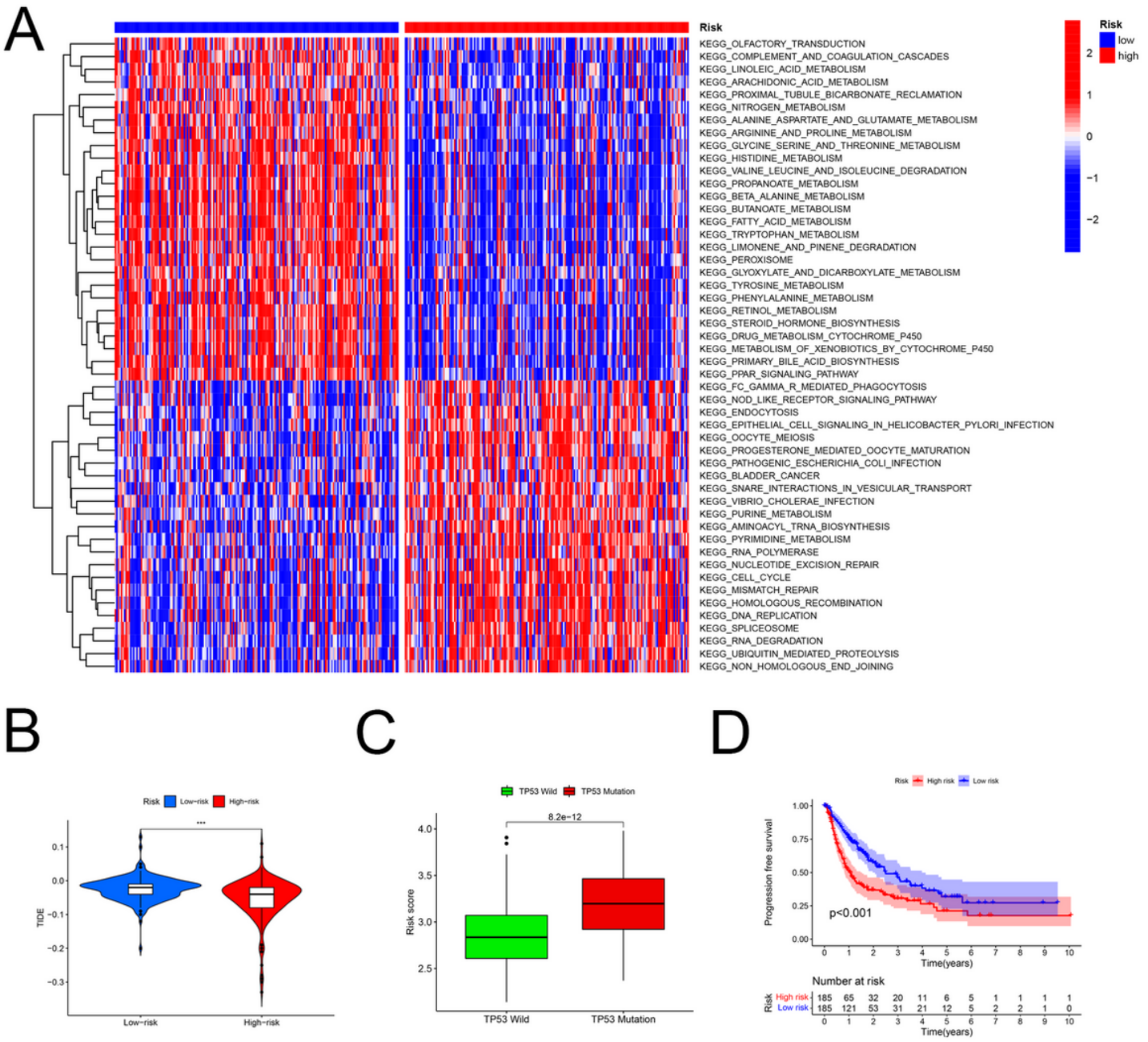

Figure 8

Fatty acid metabolism model in the role of immunotherapy. (A) The heatmap in low-risk and high-risk score groups. (B) Comparison of Tumor Immune Dysfunction and Exclusion between low- and high-risk groups. (C) TP53 mutation in fatty acid metabolism risk score. (D). The comparison of progression-free survival between low-risk and high-risk score groups.

Figure 9

Relationships between the risk scores and chemotherapeutic sensitivity. The difference of IC50 in sorafenib (A), gemcitabine (C), 5-Fluorouracil (E), paclitaxel (G), Lapatinib (I) between low-risk and high-risk score group. The correlation between the risk score and sensitivity of sorafenib (B), gemcitabine (D), 5-Fluorouracil (F), paclitaxel (H), Lapatinib (J).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.xlsx