

Identification and Validation of a Novel Metabolism-Related Prognostic Signature in Hepatocellular Carcinoma

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Keywords: Hepatocellular carcinoma, Metabolism-related genes, Prognostic signature, Survival

Posted Date: August 19th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-50552/v1>

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**Identification and validation of a novel metabolism-related prognostic signature
in hepatocellular carcinoma**

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Word count: 4979

Number of figures: 8 figures

Abstract

Background: Given that metabolic reprogramming has been recognized as an essential hallmark of cancer cells, this study sought to investigate the potential prognostic values of metabolism-related genes(MRGs) for hepatocellular carcinoma (HCC) diagnosis and treatment.

Methods: The metabolism-related genes sequencing data of HCC samples with clinical information were obtained from the International Cancer Genome Consortium(ICGC) and The Cancer Genome Atlas (TCGA). The differentially expressed MRGs were identified by Wilcoxon rank sum test. Then, univariate Cox regression analysis were performed to identify metabolism-related DEGs that related to overall survival(OS). A novel metabolism-related prognostic signature was developed using the least absolute shrinkage and selection operator (Lasso) and multivariate Cox regression analyses. Furthermore, the signature was validated in the TCGA dataset. Finally, cox regression analysis was applied to identify the prognostic value and clinical relationship of the signature in HCC.

Results: A total of 178 differentially expressed MRGs were detected between the ICGA dataset and the TCGA dataset. We found that 17 MRGs were most significantly associated with OS by using the univariate Cox proportional hazards regression analysis in HCC. Then, the Lasso and multivariate Cox regression analyses were applied to construct the novel metabolism-relevant prognostic signature, which consisted of six MRGs. The prognostic value of this prognostic model was further successfully validated in the TCGA dataset. Further analysis indicated that this

signature could be an independent prognostic indicator after adjusting to other clinical factors. Six MRGs (FLVCR1, MOGAT2, SLC5A11, RRM2, COX7B2, and SCN4A) showed high prognostic performance in predicting HCC outcomes, and were further associated with tumor TNM stage, gender, age, and pathological stage. Finally, the signature was found to be associated with various clinicopathological features.

Conclusions: In summary, our data provided evidence that the metabolism-based signature could serve as a reliable prognostic and predictive tool for overall survival in patients with HCC.

Keywords: Hepatocellular carcinoma, Metabolism-related genes, Prognostic signature, Survival

Background

According to the 2018 Global Cancer Statistics, liver cancer is the sixth most common human malignancies worldwide(841,080 cases/year) and the fourth leading cause of cancer-related deaths(781,631 cases/year)[1]. Hepatocellular carcinoma(HCC) originates from hepatocytes and accounts for the vast majority (approximately 75%-85%) of primary liver cancer. Despite recent advances in early detection and management, the mortality is still high due to its late detection and the low rate of early diagnosis[2]. Therefore, it is urgently necessary to identify effective prognostic biomarkers and therapeutic targets to complement and improve current screening strategies for HCC diagnosis and prognosis.

Recently, metabolic reprogramming has been recognized as a novel and essential hallmark of cancer cells[3]. Cancer cells regulate their metabolism to promote the production of energy, the synthesis of macromolecules and the maintenance of redox balance, so as to support their rapid cell growth and proliferation[4, 5]. Increased glycolysis under normoxic condition (Warburg effect) and glutamine metabolism are the best characterized metabolic change in tumor cells[5, 6]. Accumulating studies have indicated that abnormal metabolism are associated with a poor prognosis of many tumor types, including HCC[7, 8]. Luo et. al [9]screened the combination of phenylalanyl-tryptophan and glycine as liver cancer metabolic markers, and confirmed that their diagnostic sensitivity was superior to AFP. A series of combined markers, such as lactic acid, glycolamine, phenylalanine, aconitric acid and ribose, were screened for identification of patients with and without recurrence[10]. A recent study revealed thousands of metabolism-relevant genes encoding all known human metabolic enzymes and transporters [11], however, the definitive role and underlying mechanisms of them in the development and prognosis of HCC remains poorly understood. More importantly, there are few models based on metabolism-related genes to predict the prognosis of HCC patients. Thus, it is of great clinical significance to establish a novel metabolism-related prognostic signature that can reliably predict the prognosis of HCC. In this study, we identified the differentially expressed MRGs in HCC patients by associating the gene expression profiles with MRGs in TCGA database and the ICGC database. The functional enrichment analysis and prognostic value of the differentially expressed MRGs were also determined. The prognostic model was finally established

based on Cox regression and Lasso regression analyses. To verify the accuracy of the model, the Kaplan-Meier (KM) estimator and the receiver operating characteristic (ROC) curve were applied. Moreover, the prognostic value of our metabolism-related prognostic model was further validated in TCGA database.

Materials and methods

Data collection

The transcriptomic and the corresponding clinical data of patients with hepatocellular carcinoma (HCC) were downloaded from the the International Cancer Genome Consortium(ICGC; <https://icgc.org/>) and The Cancer Genome Atlas (TCGA; <https://portal.gdc.cancer.gov/>) databases. The RNA-seq data, including 243 HCC and 202 adjacent non-tumor cases from ICGC database and 374 HCC and 50 adjacent non-tumor cases from TCGA database were examined. The current study did not require ethics approval because all data had been collected from public availability of data in the ICGC database and TCGA database.

The differentially expressed MRGs screening

2752 metabolism-related genes(MRGs), which have been reported to encode all known human metabolic enzymes and transporters, were obtained from the Possemato's research[11]. The differentially expressed MRGs in HCC and normal tissues were detected using the Wilcoxon test method[12]. $|\log_{2}FC| > 1$ and adjusted $P < 0.05$ were considered as significant. The common differentially expressed MRGs were identified

between the ICGC database and the TCGA database by used FunRich software. These intersection MRGs were selected for further analysis.

Functional enrichment analysis of MRGs

Gene Ontology (GO) [13]and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis[14] were performed by “clusterprofiler” R package and “enrichplot” R package in R software[15]. Functional categories with false discovery rate(FDR) of less than 0.05 were considered statistically significant.

Survival-associated MRGs

We collected clinical information of 232 HCC patients in the ICGC database. A total of 3 patients who were followed for less than 2000 days and more than 30 days were excluded to avoid the interference of irrelevant factors. Survival analysis was performed on 229 patients. To screen out MRGs associated with the prognosis in HCC patients, univariate Cox analysis was implemented by the R “survival” package, and data were visualized using forest plots. Only differentially expressed MRGs a P value < 0.001 were were screened for subsequent analyses.

Construction of metabolism-related signature for HCC

HCC patients in ICGC dataset were used for constructing the COX prognostic signature, and patients in TCGA dataset were used for validating the signature. Lasso and multivariate Cox regression analyses were performed to construct a prognostic

model[16]. To avoid the prognostic signature overfitting and remove highly related survival-related MRGs, Lasso Cox regression was carried out using R “survival” and “glmnet” package. MRGs detected via Lasso algorithm were evaluated by step wise multivariate Cox regression analysis. By weighting the estimated Cox regression coefficients, the model of tumor-related metabolism genes risk was constructed[17]. The prognostic metabolism-related gene signatures were shown as risk score = $\sum (\beta_i \times \text{Exp}_i)$, where β_i , the coefficients, represented the weight of the respective signature and Exp_i represented the expression value. Based on the risk score formula, patients were assigned into low-risk group and high-risk group with the median risk score as the cut-off point. The Kaplan-Meier (K-M) survival curve was used the log-rank test to evaluate the differences in survival rate between the two groups. Furthermore, the receiver operating characteristic (ROC) curve was implemented by R “survivalROC” package[18] and the corresponding area under the ROC curve (AUC) was measured to assess the sensitivity and specificity of the metabolism-related signature.

Validation of metabolism-related signature

To verify the prognostic value of metabolism-related signature, we used the TCGA database as the validation cohort. The same formula was used to calculate the risk scores for each patient. Survival and ROC curve analyses were implemented as described above. In addition, Univariate and multivariate analyses were used to estimate the effect of risk scores on overall survival and the clinicopathologic features. We also explored the correlation between the expression of these MRGs and several

clinical features. For further validation of our analysis, The Human Protein Atlas (HPA) online database (<http://www.proteinatlas.org/>) was applied to identify the expression of these MRGs at a translational level [19].

Statistical analysis

All statistical analyses were performed by version 3.6.1 of R software(<https://www.r-project.org/>) and version 3.1.3 of FunRich software(<http://www.funrich.org/>), along with version 3.7.2 of Cytoscape software (<http://www.cytoscape.org/>). If not otherwise stated, data were considered to be statistically significant with P value <0.05.

Results

Identification of differentially expressed MRGs

A total of 2752 MRGs that encoded all known human metabolic enzymes and transporters were obtained from the Possemato's study[11], and we matched these genes with the sequence data of HCC related mRNA in the ICGC and TCGA databases; the differentially expressed MRGs between HCC and normal samples were identified by Wilcoxon rank sum test. Considering the cutoff criteria (adjusted P value <0.05 and $|\log FC| > 1.0$), 475 differentially expressed MRGs (consisting of 94 downregulated and 381 upregulated genes) were extracted from in the ICGC database(Fig.1a, 1c), 251 differentially expressed MRGs (consisting of 36 downregulated and 215 upregulated genes) were extracted from in the TCGA database(Fig.1b, 1d). Finally, the common differentially expressed MRGs were identified in the two databases, a total of 178

MRGs (consisting of 28 downregulated and 150 upregulated genes) (Fig. 1e) were selected for subsequent analysis.

GO, KEGG and PPI analysis of metabolism-related DEGs

To evaluate the potential molecular mechanisms of MRGs in HCC, the 178 differentially expressed MRGs were further analyzed by GO functional annotation and KEGG pathway enrichment. The results showed the top 10 biological processes GO terms, cellular component GO terms, molecular function GO terms (Fig. 2a), and the top 10 KEGG pathway terms (Fig. 2c). The correlation between the intersection genes and the top 5 biological processes, including organic anion transport, organic acid transport, carboxylic acid transport, lipid catabolic process, and monovalent inorganic cation transport is shown in Figure 2b. The KEGG analysis showed that the intersection genes were associated with material metabolism, especially arachidonic acid metabolism.

Prognostic values of survival-related MRGs

To better define the characteristics of survival-related MRGs, we explored the differentially expressed MRGs associated with survival in HCC samples. In the univariate Cox proportional hazards regression analysis, 17 genes with significant effects on prognosis were identified. The resulting forest plot shown in Figure 3a demonstrates that 15 genes have the characteristics of $HR > 1$, whereas 2 genes have the characteristics of $HR < 1$. This suggests that most survival-related MRGs were risk

factors for HCC prognosis. Furthermore, scatter plots were visualized to display the expression patterns of 17 differentially expressed MRGs between HCC and normal tissues in ICGC database(Fig.3b) and TCGA database(Fig.3c). Scatter plot showing expression patterns of 1 down-regulated genes (MOGAT2) and 16 up-regulated genes(ASRGL1,COX7B2,MTHFD1L,B3GALNT1,ITPKA,SCN4A,PLCB1,NQO1,RRM2,FLVCR1,TMCO3,CAD,TK1,TYMS,SLC5A11 and SPNS1).

Construction of metabolism-related prognostic signature for HCC

To establish the metabolism-associated prognostic signature, the Lasso regression and multivariate Cox proportional hazards regression analyses were conducted. The Lasso regression analysis was applied to exclude genes that may be highly correlated with other genes (Fig.4a, 4b). Then, a prognostic signature model was established based on 6 MRGs selected from further multivariate Cox regression analysis(Fig.4c). Finally, a prognostic model was constructed to evaluate the prognosis of each patient as follows: Risk score = (0.031755×expression value of COX7B2) + (-2.27007×expression value of SCN4A) + (-0.15919 ×expression value of MOGAT2)+(0.164689×expression value of FLVCR1)+(0.02915×expression value of RRM2)+(0.068413×expression value of SLC5A11).

Then, the risk score of each patient was calculated according to this prognostic model. Based on the median risk score, 229 HCC patients were classified into a high risk group (n = 114) and low risk group (n = 115).The risk score, survival status and gene expression heatmap of these prognostic MRGs are presented in Fig 5a-c. Kaplan-meier

log-rank test indicated that patients in the high risk group showed markedly poorer overall survival than those in the low risk group (Fig. 5d). Areas under the curve value of the signature predicting the 1-, 3- and 4-year OS rates were 0.805, 0.803 and 0.94, indicating that this prognostic model exhibited a good sensitivity and specificity (Fig.5e). Since only one patient had a follow-up period of 5 years, we did not plot the ROC curve for 5 years.

Validation of the metabolism-related prognostic signature by TCGA database

The TCGA database including 193 HCC samples were used for the validation of the metabolism-related signature. According to the median risk score, we divided patients into high risk (n = 80) and low risk groups (n = 113). Consistent with the results derived from the ICGC dataset, the Kaplan-Meier curve demonstrated that patients in the high risk group exhibited markedly poorer overall survival than those in the low risk group ($P < 0.001$; Fig. 6d). The risk score, survival status and gene expression heatmap of these prognostic MRGs are shown in Fig 6a-c. The AUCs for 1-, 3- and 5-year OS rates were 0.721, 0.693 and 0.737 (Fig.6e). Univariate and multivariate Cox regression analysis was conducted to evaluate the independent prediction ability of metabolism-related prognostic signature between the signature and other common prognostic factors, including age, gender, histological grade, pathological stage and TNM stage. Although univariate Cox analysis indicated that pathologic stage, T stage and our model were markedly associated with OS (Fig.6f, $p < 0.001$), after the multivariate analysis, only metabolism-related prognostic signature ($p < 0.001$) can be used as an independent

prognostic factor (Fig. 6g). These results demonstrated that this prognostic model exhibited great applicability and stability in predicting the prognosis of HCC patients. To validate the MRGs in this model, the protein expression levels were analyzed using the HPA database. The results showed that FLVCR1, SLC5A11, MOGAT2, and RRM2 protein levels matched their mRNA expression levels (Fig. 7). However, representative images of the SCN4A and COX7B2 protein levels were not available in the HPA database.

Clinical value of prognostic signature

To further evaluate the clinical value of MRGs, the relationship between MRGs prognostic indicators and clinical features were investigated, and the results indicated that FLVCR1, MOGAT2, RRM2, SCN4A, and COX7B2 were differentially expressed in patients with various clinical features (Fig. 8). To validate the clinical value of the metabolism-related prognostic signature, the association between the risk score and clinical characteristics were subsequently assessed, and the results demonstrated that high risk scores were positively associated with histological grade, and survival status in patients with HCC (Fig. 8).

Discussion

As a typical metabolic disease, the metabolic changes and regulatory mechanism of hepatocellular carcinoma are very complex, which has not been fully defined. HCC is different from normal liver tissue in glucose metabolism, lipid metabolism and protein

metabolism, and also different from other tumors. These metabolic differences provide a theoretical basis for therapeutic strategies targeting tumor metabolism for HCC. Till date, there is no report on MRGs-based prognostic signatures for HCC patients. There is an urgent need to determine reliable metabolic biomarkers and predictive models to predict the prognosis of HCC.

In the current study, based on the analysis of ICGC dataset and TCGA dataset, 178 differentially expressed MRGs were screened. In order to better understand how differentially expressed MRGs are involved in biological processes and signal transduction processes, GO and KEGG enrichment analyses were conducted. The results of the GO enrichment revealed that the above genes were primarily related to metabolism response. The KEGG pathways were mainly focused on metabolism-related pathways. In univariate regression analysis on the differentially expressed metabolism-related genes, 17 genes were detected to display significant association with OS. Then, the metabolism-related prognostic model based on six prognostic MRGs (FLVCR1, MOGAT2, RRM2, SCN4A, SLC5A11, and COX7B2) was constructed by the Lasso and multivariate Cox regression analyses. Using the model, every HCC patient was assigned a risk score. The differences in survival between patients with low and high scores were significant in both the ICGC cohorts and the TCGA cohorts. The ROC curves and AUCs indicated that models of the ICGC and TCGA cohorts both performed well. In addition, we further confirmed the important roles of MRGs in HCC through immunohistochemistry.

Six metabolism related genes which constitute the prognosis model were identified as potential biomarkers of HCC. SLC5A11, an inositol-specific sodium-dependent glucose cotransporter responsible for inositol uptake, has been reportedly related to anaplastic thyroid carcinoma, but the relationship between SLC5A11 and HCC has not been described previously[20, 21]. In this study, our results indicated SLC5A11 may be as a potential oncogene in HCC. Ribonucleotide reductase M2 subunit (RRM2) plays an important role in the proliferation, invasion and metastasis of tumor cells, and thereby is involved in many types of malignancy including HCC[22-24]. A study by Liu et al revealed that HBV induced the upregulation of RRM2 and promoted the development of HCC, while suppression of RRM2 inhibited the growth of HCC [25]. In accordance with these studies, the high expression of RRM2 may serve as a poor prognostic factor for HCC. Monoacylglycerol O-acyltransferase 2 (MOGAT2), a member of MOGAT gene family, plays an important role in the monoacylglycerol pathway for triacylglycerol synthesis and is highly expressed in the small intestine and liver of human[26, 27]. Previous studies have shown that MOGAT2 is an attractive target for the treatment of obesity, the treatment of obesity, type 2 diabetes mellitus(T2DM), and nonalcoholic steatohepatitis(NASH)[28, 29]. Wen et al. reported that MOGAT2 could have applications as a novel biomarker affecting the metastasis of colorectal cancer treatment[30]. However, little is known about the existence of molecular links between MOGAT2 and HCC. Sodium voltage-gated channel alpha subunit 4(SCN4A), encoding a skeletal muscle voltage-gated sodium channel, Nav1.4[31], has been reportedly associated with neuromuscular disorders like hypo-

and hyperkalaemic periodic paralyses (hypoPP and hyperPP), paramyotonia congenita (PC), sodium channel myotonias (SCM) and congenital myasthenic syndrome[32]; however, its molecular mechanisms in cancers have not been explored. Liang et al. reported that the single nucleotide polymorphism (SNPs) of the cytochromooxidase VIIb 2(COX7B2) gene was associated with familial nasopharyngeal carcinoma[33]. A study by Al et al revealed that COX7B2 is a susceptibility gene for type 2 diabetes in an extended Arab family[34]. In addition, little is known about the roles of COX7B2 in human cancers and other diseases. Feline leukemia virus subgroup C receptor 1(FLVCR1) encoding a ubiquitous heme exporter was demonstrated to promote the proliferation and tumorigenicity through inhibiting apoptosis and autophagy[35, 36]. Recently, Xian et al. reported that FLVCR1 showed the intracellular overproduction in HCC cell lines and that FLVCR1 expression was significantly associated with survival in HCC patients, which is consistent with our study[37].

Although our research provides new insights into the treatment of HCC, there are limitations to our study. First, the diagnostic efficiency and prognostic value of key genes were analyzed and verified only in TCGA dataset. Therefore, predictive performance need to be validated in other independent databases. Second, the signature was established based on pure bioinformatics analysis. Thus, further experimental studies are needed to validate the predictive value of the six MRGs based on tumor samples and clinical data. In addition, in vivo and in vitro experiments should be conducted in order to explore the functional role of the critical genes in HCC.

Conclusions

In this study, we assessed the metabolism-related genes expression profiles based on ICGC database and TCGA database. Moreover, a novel metabolism-related prognostic model was constructed, which could be as as an independent prognostic predictor for HCC. The prognostic value of metabolism-related prognostic model was further validated in TCGA database. Taken together, the findings herein may provide a novel prognostic model that would be effective in monitoring metabolism and predicting the prognosis in HCC patients.

Abbreviations

HCC:hepatocellular carcinoma; TCGA:The Cancer Genome Atlas (database); ICGC:the International Cancer Genome Consortium; MRGs;metabolism-related genes; OS:overall survival; ROC:Receiver operating characteristic; AUC:The area under the ROC curve; TNM:Tumour size/lymph nodes/distance metastasis, a tumour staging system used in oncology and constructed by the American Joint Committee on Cancer and the Union for International Cancer Control; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; FDR: False discovery rate.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data used in this study were acquired from The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium(ICGC) portal.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was supported by a Grant from the National Natural Science Foundation of China (Grant 81700012; to Shenghui Qin)

Authors' contributions

Shenghui Qin conceived of and directed the project, wrote the manuscript. Zhihao Wang performed data bioinformatics analyses. Kidane Siele Embaye et.al helped with part of English writing and checking. All authors read and approved the manuscript.

Acknowledgements

Not applicable.

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Figure Legends

Fig.1 Differentially expressed metabolism-related genes (MRGs) in hepatocellular carcinoma (HCC).

Heatmap of MRGs between HCC and nontumor tissues in ICGC database(a) and TCGA database (b). The color from blue to red represents the progression from low expression to high expression; Volcano plot of MRGs in ICGC database(c) and TCGA database(d). The red dots in the plot represents upregulated genes and blue dots represents downregulated genes with statistical significance. Black dots represent no differentially expressed genes. (e) Venn diagram showing the gene numbers of the MRGs of HCC in ICGC and TCGA database.

Fig2. The GO and KEGG analysis of differentially expressed MRGs.

(a) the top 10 of biological processes GO terms, cellular component GO terms, molecular function GO terms; (b) The correlation between intersection genes and top 5 biological processes GO terms; (c) The KEGG pathway analysis of differentially expressed MRGs;

Fig3. Identification of survival-related differentially expressed MRGs by univariate Cox regression analysis.

(a) Forest plot of hazard ratios showing survival-related MRGs. P values <0.001 are considered to be statistically significant; The expression of 17 metabolism-related prognostic genes between HCC and normal tissues in ICGC database(b) and TCGA database(c).

Fig.4 Establishment of metabolism-related prognostic signature.

(a) Screening of optimal parameter (λ) at which the vertical lines were drawn. (b) Lasso coefficient profiles of the seventeen MRGs with non-zero coefficients determined by the optimal λ . (c) Multivariate analyses assessing relationship between expression levels of MRGs and overall survival (OS) in patients with HCC.

Fig.5 Construction of the metabolism-based prognostic risk signature in the ICGC cohorts.

(a) The risk score distribution of HCC patients; (b) Survival status and duration of patients; (c) Heatmap of the metabolism-related genes expression; (d) Survival curves for the low risk and high risk groups; (e) Time-independent receiver operating characteristic (ROC) analysis of risk scores for prediction the overall survival in the ICGC set.

Fig.6 Validation of the metabolism-based prognostic risk signature in the TCGA cohorts.

(a) The risk score distribution of HCC patients; (b) Survival status and duration of patients; (c) Heatmap of the metabolism-related genes expression; (d) Survival curves for the low risk and high risk groups; (e) Time-independent receiver operating characteristic (ROC) analysis of risk scores for prediction the overall survival in the TCGA set. (f) Univariate Cox regression analysis of discrete clinical factors ; (g) Multivariate Cox regression analysis of discrete clinical factors.

Fig.7 Verification of hub MRGs expression in HCC and normal liver tissue using the HPA database. (a): FLVCR1, (b): SLC5A11, (c): RRM2, (d): MOGAT2.

Fig.8 Relationships between MRGs expression and clinicopathological factors in HCC ($P < 0.05$).

Figures

Fig.1

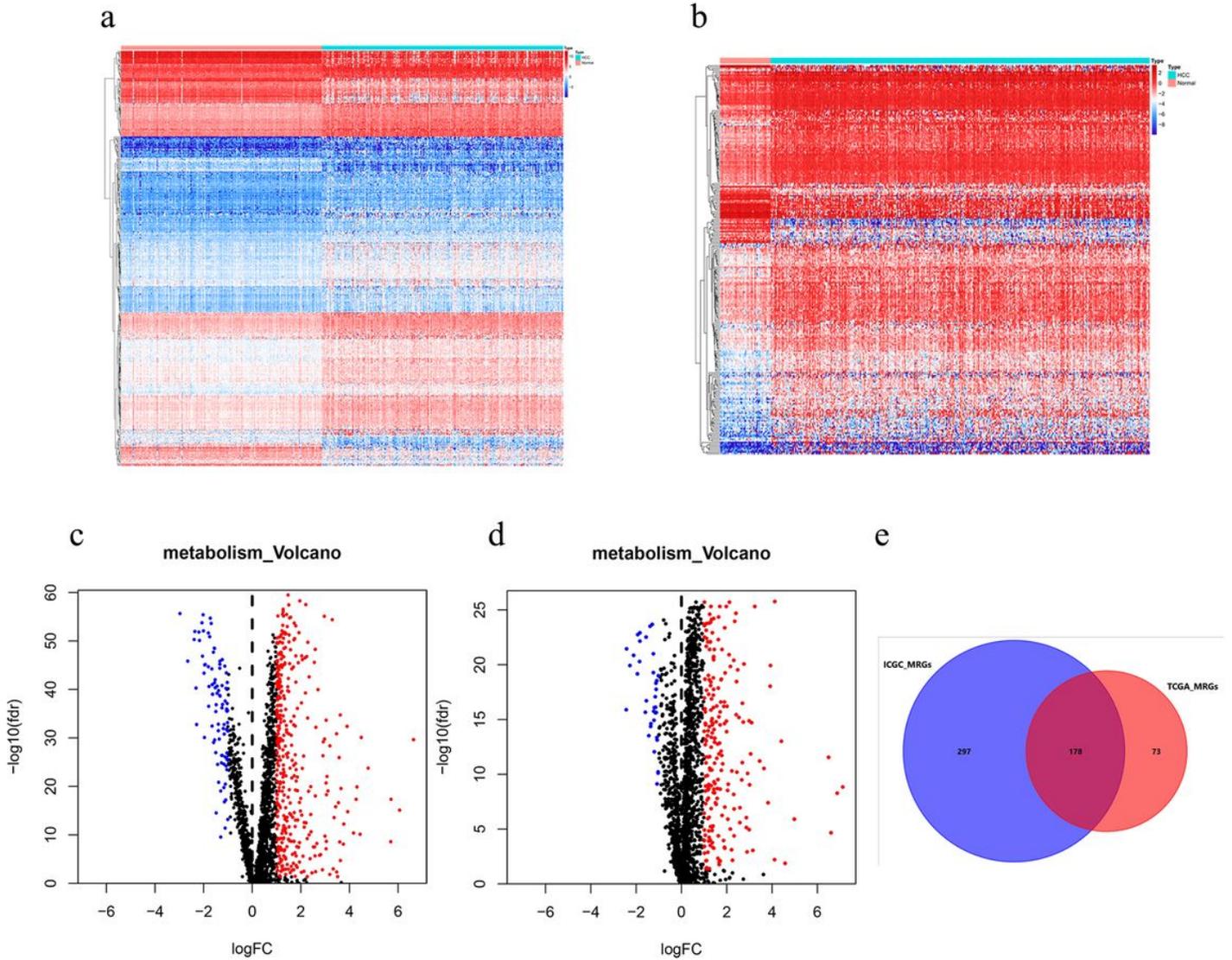


Figure 1

Differentially expressed metabolism-related genes (MRGs) in hepatocellular carcinoma (HCC). Heatmap of MRGs between HCC and nontumor tissues in ICGC database(a) and TCGA database (b). The color from blue to red represents the progression from low expression to high expression; Volcano plot of MRGs in ICGC database(c) and TCGA database(d). The red dots in the plot represents upregulated genes and blue dots represents downregulated genes with statistical significance. Black dots represent no differentially expressed genes. (e) Venn diagram showing the gene numbers of the MRGs of HCC in ICGC and TCGA database.

Fig.2

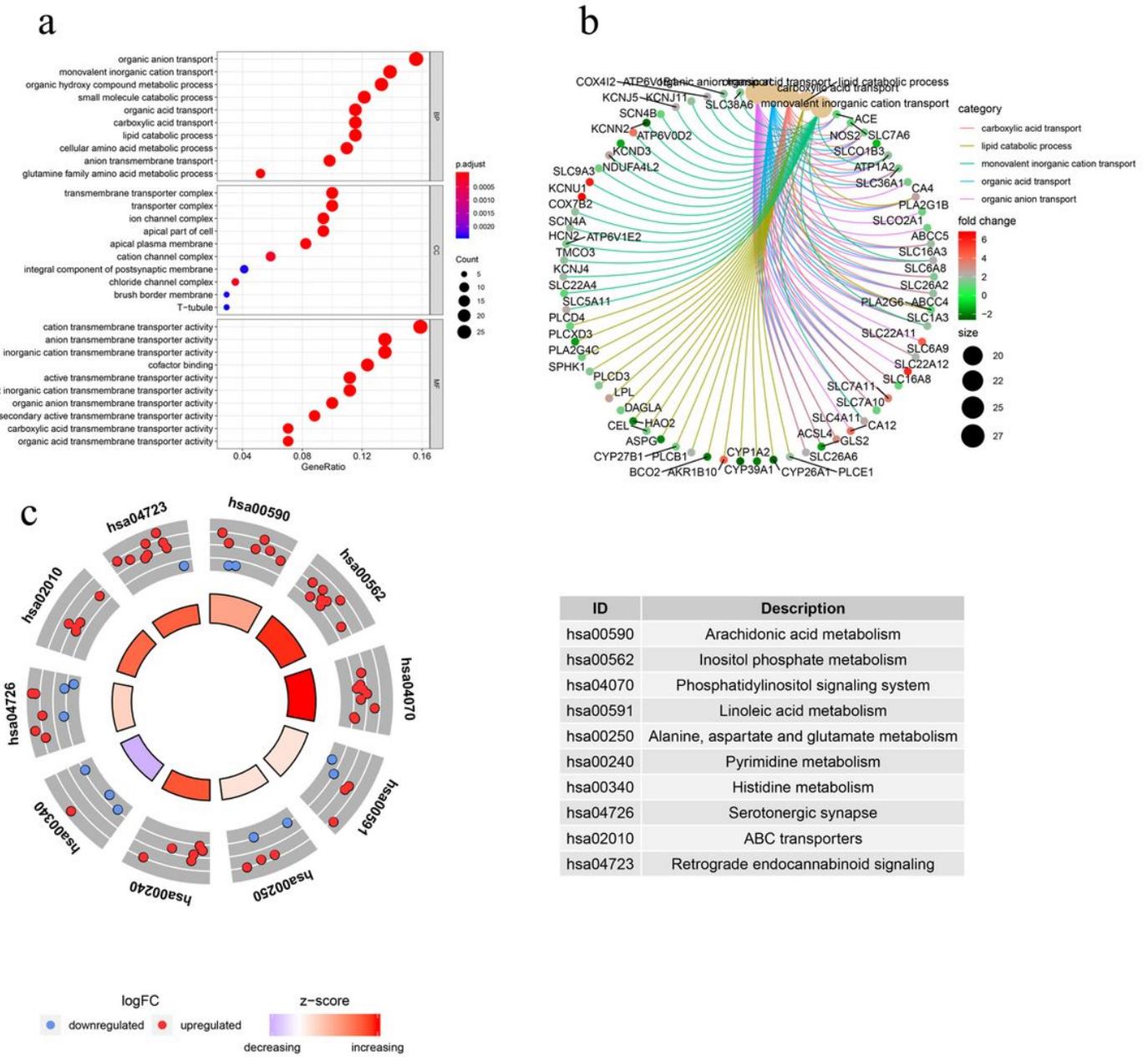


Figure 2

The GO and KEGG analysis of differentially expressed MRGs. (a) the top 10 of biological processes GO terms, cellular component GO terms, molecular function GO terms; (b) The correlation between intersection genes and top 5 biological processes GO terms; (c) The KEGG pathway analysis of differentially expressed MRGs;

Fig.3

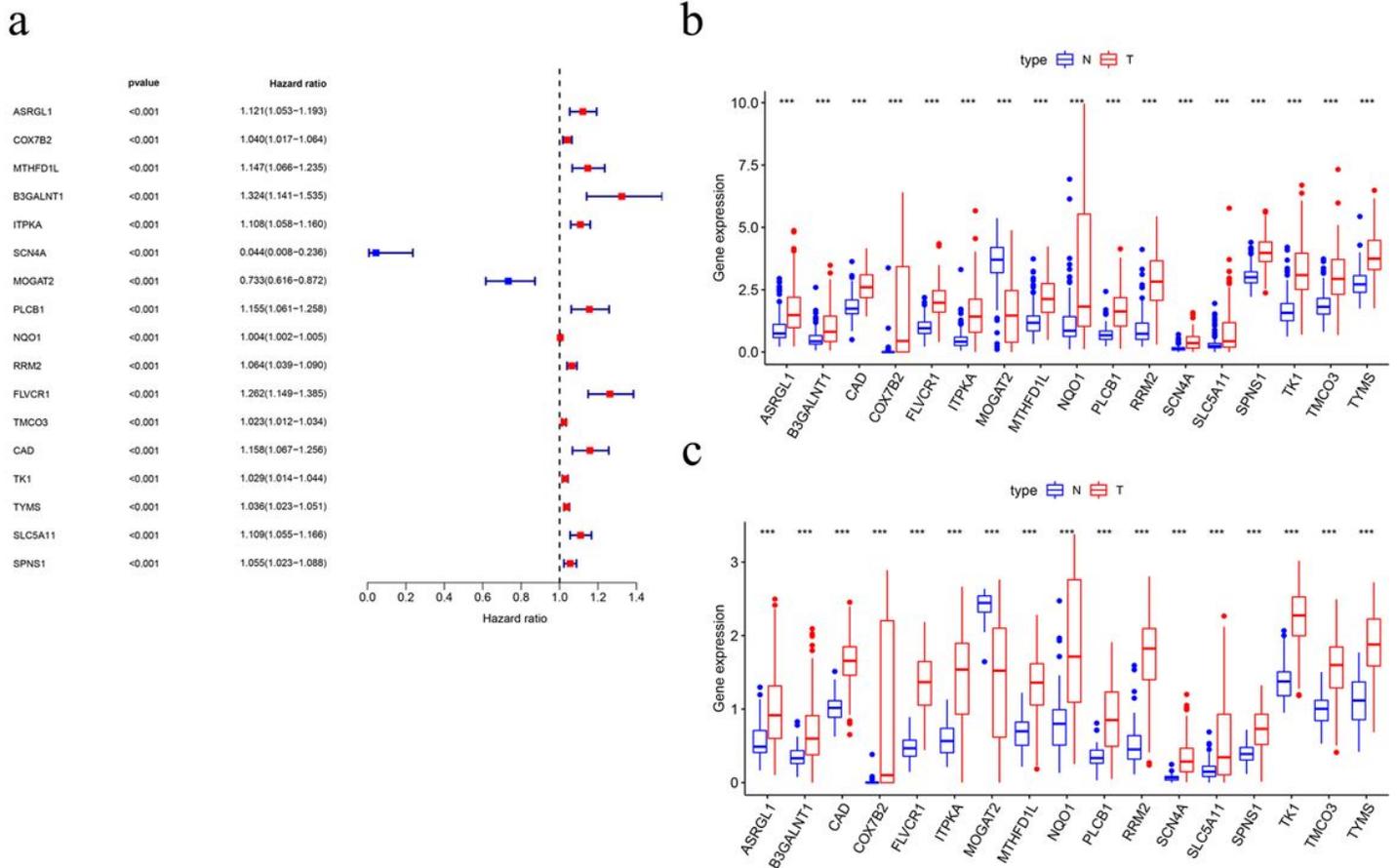


Figure 3

Identification of survival-related differentially expressed MRGs by univariate Cox regression analysis. (a) Forest plot of hazard ratios showing survival-related MRGs. P values <0.001 are considered to be statistically significant; The expression of 17 metabolism-related prognostic genes between HCC and normal tissues in ICGC database(b) and TCGA database(c).

Fig. 4

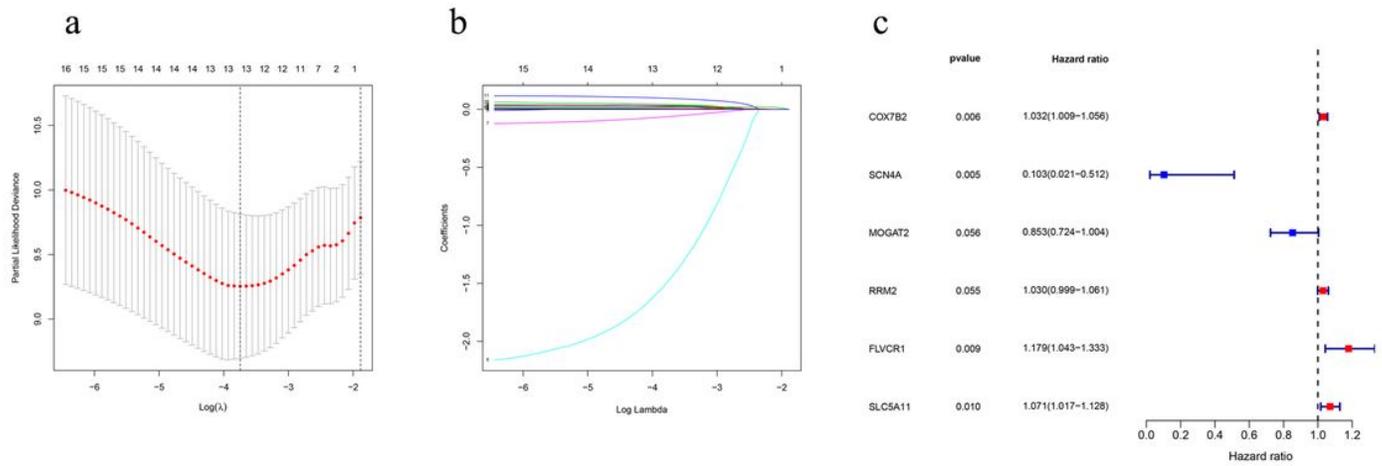


Figure 4

Establishment of metabolism-related prognostic signature. (a) Screening of optimal parameter (λ) at which the vertical lines were drawn. (b) Lasso coefficient profiles of the seventeen MRGs with non-zero coefficients determined by the optimal λ . (c) Multivariate analyses assessing relationship between expression levels of MRGs and overall survival (OS) in patients with HCC.

Fig.5

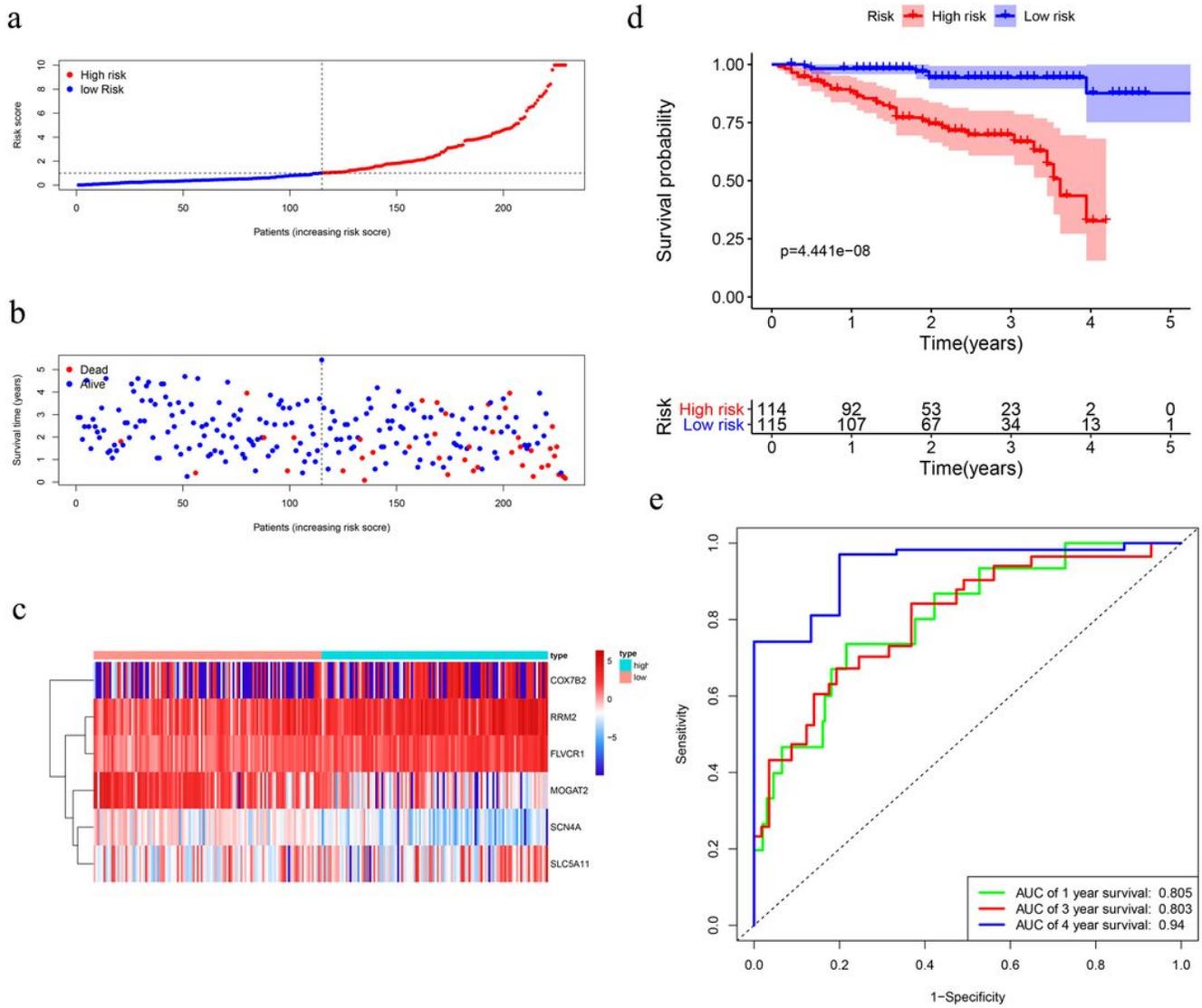


Figure 5

Construction of the metabolism-based prognostic risk signature in the ICGC cohorts. (a) The risk score distribution of HCC patients; (b) Survival status and duration of patients; (c) Heatmap of the metabolism-related genes expression; (d) Survival curves for the low risk and high risk groups; (e) Time-independent receiver operating characteristic (ROC) analysis of risk scores for prediction the overall survival in the ICGC set.

Fig. 6

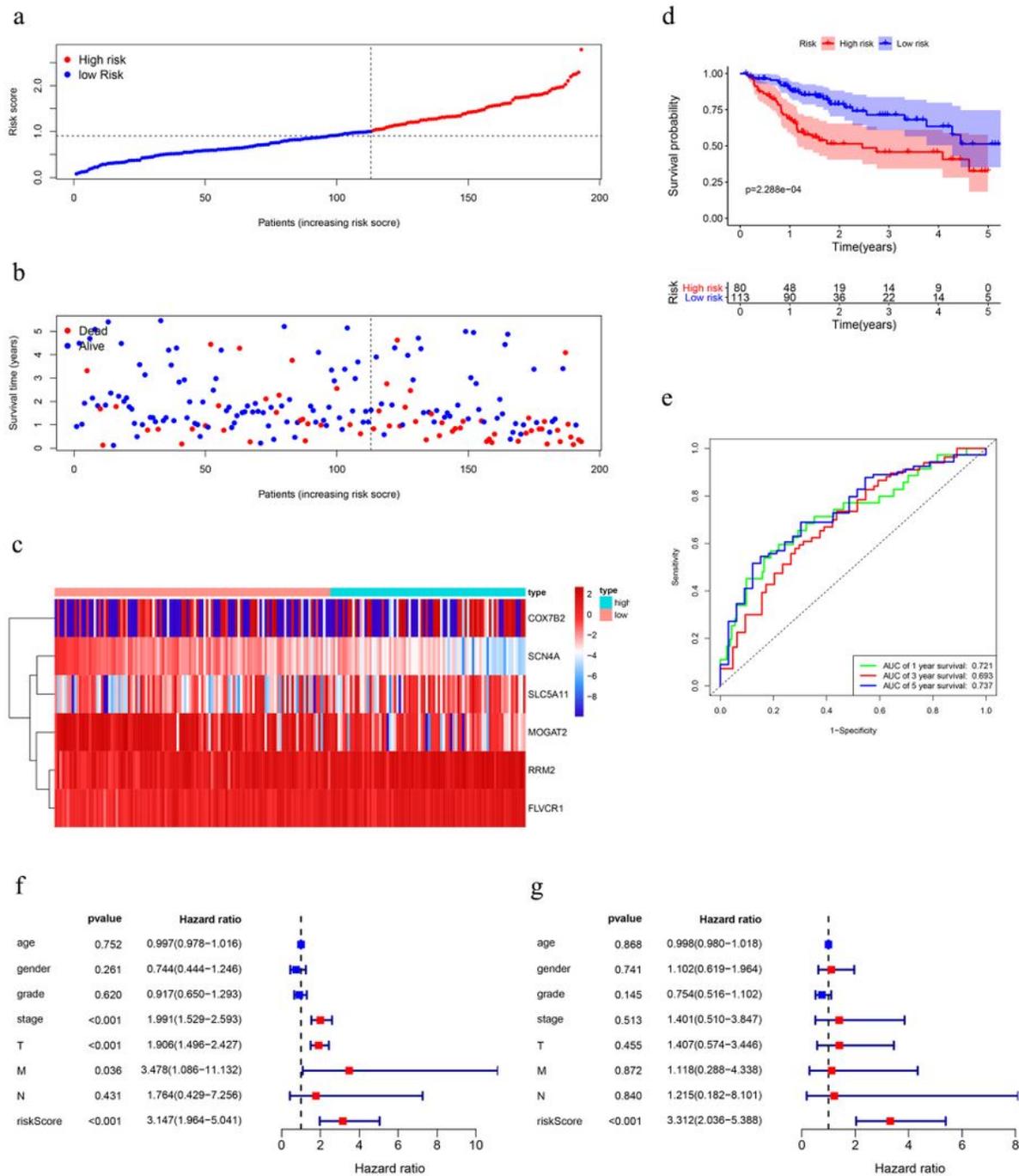


Figure 6

Validation of the metabolism-based prognostic risk signature in the TCGA cohorts. (a) The risk score distribution of HCC patients; (b) Survival status and duration of patients; (c) Heatmap of the metabolism-related genes expression; (d) Survival curves for the low risk and high risk groups; (e) Time-independent receiver operating characteristic (ROC) analysis of risk scores for prediction the overall survival in the

TCGA set. (f) Univariate Cox regression analysis of discrete clinical factors ; (g) Multivariate Cox regression analysis of discrete clinical factors.

Fig. 7

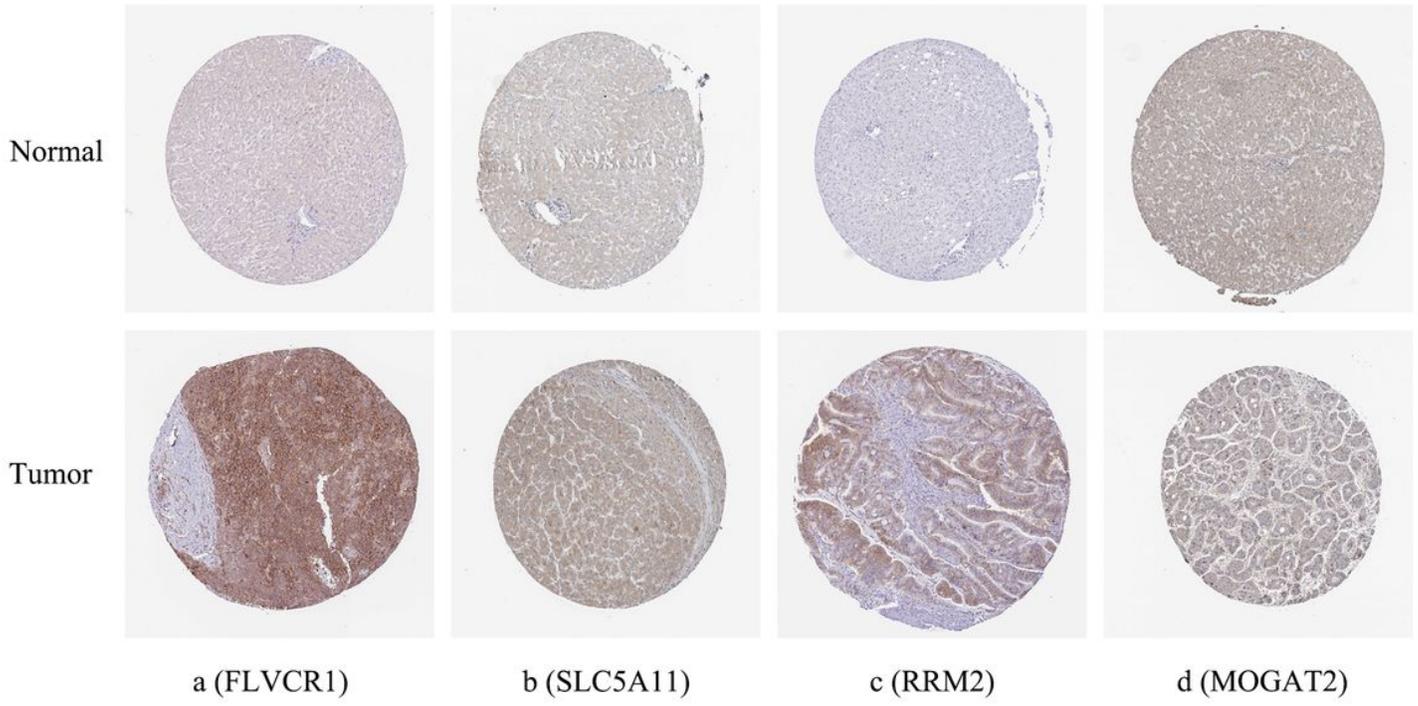


Figure 7

Verification of hub MRGs expression in HCC and normal liver tissue using the HPA database. (a): FLVCR1, (b):SLC5A11, (c): RRM2, (d): MOGAT2.

Fig. 8

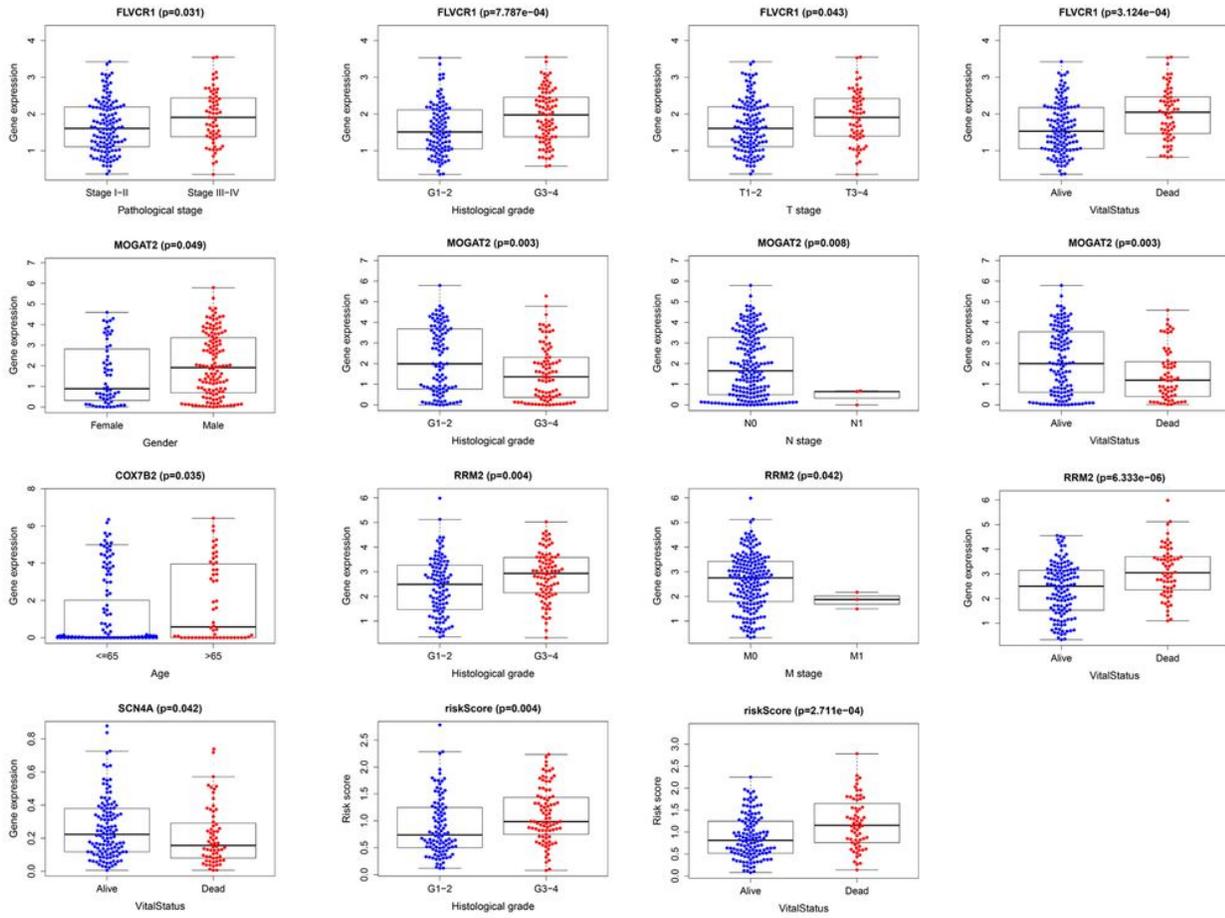


Figure 8

Relationships between MRGs expression and clinicopathological factors in HCC ($P < 0.05$).