Research Square

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# Ferroptosis regulator genes are a favorable biomarker for hepatocellular carcinoma

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**Research**

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# Abstract

Background

In this study, we comprehensively analyzed the relationship between ferroptosis regulator genes (FRGs) and prognosis of hepatocellular carcinoma (HCC), determined the prognostics value of FRGs, established a prediction model, and explored the relationship with immunotherapy for HCC.

Methods

The mRNA transcriptional levels and clinical information of HCC were obtained from The Cancer Genome Atlas (TCGA) database. The 24 FRGs were combined with the differential expression genes (DEGs) of HCC for further analysis. The prognostics values of differential FRGs via the construction of model and validation by the Cox regression analysis.

Result

There were three genes (CARS1, FANCD2, and SLC7A11) were identified as independent risk factors for HCC, and a predictive model was constructed based on CARS1, FANCD2, and SLC7A11. The model showed that the low-risk group HCC patients with a more prolonged overall survival (OS) than the high-risk group (P=0.001). The high-risk group with higher expression of FRGs than the low-risk group. Finally, the relations between FGEs and immune infiltration showed that CARS1, FANCD2, and SLC7A11 had a positive relationship with macrophage infiltration. From these, three genes might be the potential therapeutic targets.

Conclusion

Our study indicated that CARS1, FANCD2, and SLC7A11 might have potential value for therapeutic strategies as practical and reliable prognostic tools for HCC.


# Introduction

HCC is one of the leading digestive cancer, and ranking fifth in cancer-related death, Chinese patients composed more than half of these deaths. In China, since the high incidence and mortality, HCC has become a big challenge for public healthy [1–3]. With the advanced diagnostics and therapeutics methods, the lifespan of HCC has been lengthened via induvial and comprehensive therapy such as surgical resection, liver transplantation, radiofrequency ablation, and chemotherapy according to [4, 5]. However, the rates of recurrence, metastasis, and mortality remain high. Therefore, it urgently needs to understand further the underlying mechanisms of tumorigenesis, progression, recurrence, and therapeutics resistance for HCC.

Induction of tumor cell death is one of the clinical cancer treatment strategies. Apoptosis and necrosis are the two most common forms of cell death. Dr. Stokes reported in 2008 that iron death, a cell death

pattern characterized by the iron-dependent accumulation of reactive lipid oxygen [6]. Because of its morphological, molecular biological, and metabolic characteristics, it is different from other types of cell death and has become one of the research hotspots in cancer. Numbers of studies revealed that iron death could be found in multiple cancer types. Several studies have shown that iron-death was the regulator of tumor cell growth in ovarian cancer, head and neck cancer, liver cancer, cervical cancer, pancreatic cancer, and kidney cancer [7–11]. Iron death may serve as the anti-cancer effect, and there were several studies indicated that accelerated the iron death to promote the death of cancer cells [12, 13]. As the largest digestive gland, the liver is closely related to iron metabolism. Current studies showed two forms (iron metabolism and lipid metabolism) of iron death for HCC tumor cells [14–16]. Sorafenib is one of the commonly used targets therapy drugs for HCC patients. One of the anti-cancer mechanisms of sorafenib is increasing lipid oxidation levels, resulting in cell death closely related to iron death [17]. Furthermore, sorafenib combined with other drugs can enhance iron death for HCC tumor cells [16].

In the present study, we aimed to investigate the relationship between FRGs and the prognosis of HCC and immune cell infiltration. There are two phrases in the present study that including the discovery phase and the verification phase. In the discovery phase, FRGs that are important to the prognosis of HCC were screened out through bioinformatics analysis. In the subsequent validation phase, the relationship between the screened genes and prognosis was evaluated in The Cancer Genome Atlas (TCGA) cohort and the tissue microarray-based cohort. A clinical model was established for internal data validation. Finally, we further evaluated the relationship between selected FRGs

and immune infiltration also explored their predictive value for HCC.

# Materials And Methods

### Data source

We obtained RNA-seq transcriptomic data and relevant clinical data of patients with primary HCC from TCGA (https ://cancergenome.nih.gov/). All RNA-SEQ gene expression data has been standardized using Perl. A total of 370 HCC cases and 50 normal control samples were included for subsequent analysis.

### Key genes screening

There are 24 FRGs, Including cyclin-dependent kinase inhibitor 1 (CDKN1A), heat shock protein family A member 5 (HSPA5), ER membrane protein Complex subunit 2 (TTC35/EMC2), Solute Carrier family 7 member 11 (SLC7A11), nuclear factor, erythroid 2 like 2 (NFE2L2), metallothionein-1G (MT1G), heat shock protein beta 1 (HSPB1), glutathione peroxidase 4 (GPX4), Fanconi anemia complementation group D2 (FANCD2), CDGSH iron sulfur domain 1 (CISD1), farnesyl-diphosphate farnesyltransferase 1 (FDFT1), solute carrier family 1 Member 5 (SLC1A5), spermidine/spermine N1-acetyltransferase 1 (SAT1), transferrin receptor (TFRC), ribosomal protein L8 (RPL8), nuclear receptor coactivator 4 (NCOA4), lysophosphatidylcholine acyltransferase 3 (LPCAT3), glutaminase 2 (GLS2), dipeptidyl-dippeptidase-4

(DPP4), citrate synthase (CS), cysteinyl tRNA synthetase (CARS/CARS1), ATP synthase, H+ transporting, mitochondrial Fo complex subunit C3 (ATP5G3/ATP5MC3), Arachidonate 15-lipoxygenase (ALOX15), and ACyl-coa Synthetase long-chain family member 4 (ACSL4)[18, 19]. We used the "EdgeR" software package to identify FRGs differentially expressed between HCC samples and standard control samples. Univariate Cox regression analysis was used to analyze the prognostic value of differentially expressed genes for HCC, and genes with a P value less than 0.05 were selected.

### Risk Characteristics and Prognosis

To further evaluate the relationship between the above-screened gene expression and HCC patients' prognosis, after determining the independent FRGs and their coefficients, we used a free-based risk score prediction model to divide HCC patients into high-risk and low-risk groups. Receiver operating characteristic (ROC) curves were used to detect the predictive efficiency of the survival model. Significant factors (including age, sex, histological grade, and clinical stage) were screened for HCC. Simultaneously, to further understand the genetic relationship with the immune, using the Timer database (https://cistrome.shinyapps.io/timer/) is analyzed, and explores the value of his and the prognosis of patients with HCC.

### Statistical analysis

For continuous variables, differences between the groups were analyzed using ANOVA, a t-test, or a Wilcoxon rank-sum test following the data's concrete types. A chi-squared test was used to differentiate the rates of different groups. The ROC curves and the AUC measured the diagnostic accuracy of the genes. The Kaplan–Meier method was conducted to calculate the overall survival curves. A log-rank test was used to determine differences in survival rates. These analyses were performed by utilizing MedCalc software (15.2.2; Mariakerke, Belgium). Statistical significance was considered at $P < 0.05$.

### IHC staining

The tumor tissue and adjacent non-tumor tissue were fixed in 10% formalin for 1 week, then embedded in paraffin, and then the tissue specimens were sectioned, deparaffin treated, and the antigen was repaired by EDTA Antigen Retrieval Solution( Solution). The following antibodies were used for immunostaining of CARS (proteintech, 15296-1-AP), SLC7A11 (proteintech, 26864-1-AP), and FANCD2 proteintech, 24006-1-AP).

## Results

### FRGs associated with HCC

Heat map was used to reflect the expression of FRGs in HCC tissues. Compared to normal tissue, GPX4($P < 0.001$), CISD1($P < 0.001$), ATP5MC3($P < 0.001$), CARS($P < 0.001$), CS($P < 0.001$), FANCD2($P < 0.001$), TFRC($P < 0.001$), SLC7A11($P < 0.001$), SLC1A5($P < 0.05$), EMC2($P < 0.001$), HSPB1($P < 0.001$), RPL8($P <$

0.001), HSPA5(P < 0.001), ACSL4(P < 0.001), SAT1(P < 0.05), MT1G(P < 0.001), GLS2(P < 0.001), ALOX15(P < 0.001), NFE2L2(P < 0.01), FDFT1(P < 0.001) and DPP4 (P < 0.001) were different in tumor tissues. CDKN1A, NCOA4 and LPCAT3 showed no significant difference (Figure 1). Further prognostic analysis of differentially expressed genes showed that CARS1 (P= 0.002), FANCD2(P=0.009) and SLC7A11(P < 0.001) were significantly related to the prognosis of HCC (Figure 2).

### Construction and validation of prognostic features

The three genes in the above FRGs (CARS1, FANCD2, and SLC7A11) were used as predictive genes, univariate Cox analysis was conducted, and the coefficients were extracted from them. The risk score formula for OS prediction was as follows: Risk score= (CARS1* 0.049) + (FANCD2* 0.266) + (SLC7A11* 0.073) (Figure3, A), suggesting that FANCD2 has the most significant influence on the prognosis of patients, followed by SLC7A11 and CARS1, which are independent risk factors affecting the prognosis of HCC patients. We used time-dependent ROC analysis to assess the prognostic value of these three risk genes, and the results showed that the area under the ROC curve (AUC) of 1-year, 3-year, and 5-year OS was 0.713, 0.637, and 0.627, respectively (Figure3, B). Patients were divided into high-risk and low-risk groups based on the median risk, and the distribution of risk scores and the survival time and status of patients were shown in Figure 4. Survival analysis showed that patients in the high-risk group had a worse OS than those in the low-risk group (Figure3 C).

The three genes included above were included as independent OS risk factors in the nomogram, which had a total number of 160. The number of points for each factor was determined by drawing a vertical line upward. The sum of the points corresponding to the three factors was the patient's total number of points. The patients' survival probability was 1 year, 3 years, and 5 years when projected to the corresponding points of the total number of points. It can be seen that the more total points, the worse the prognosis. The calibration curves for 1-year, 3-year, and 5-year survival in the validation cohort showed good fitting. (Figure 5).

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Subsequently, the expression of FRGs in HCC immune cells and their prognostic value were further analyzed. The results showed that CARS1 has associated with macrophage (P= 6.43e−10) expression, FANCD2 was related to dendritic cells (P= 5.13e−22) expression, and SLC7A11 was associated with B cells (P= 5.68e−04), macrophages (P= 9.62E −05) and dendritic cells (P= 5.51e−06) expression (Figure 7). In terms of immune-related prognosis, the analysis showed that low expression of CARS1 (P=0.003), FANCD2 (P=0.006), and SLC7A11 (P=0.002) suggested better OS in patients (Figure 8).

We performed immunohistochemical (IHC) to test the expression of CARS1, FANCD2 and SLC7A11 proteins in hepatocellular carcinoma tissues and their counterparts. We found that the expression of FANCD2 and SLC7A11 protein in HCC tissue was higher than that in normal tissue, while the expression of CARS1 protein in HCC tissue was not significantly different from that in normal tissue (Figure 9).

# Discussion

The liver is an essential hub for sugar, lipid, and amino acids' metabolism, the body's three major nutrients. Studies have shown that HCC presents various characteristic metabolic changes [20], thus providing energy and bio-macromolecular synthesis raw materials for rapidly growing and proliferating tumor cells. The discovery of iron death has attracted extensive attention from researchers. During iron death in tumor cells, intracellular iron, lipid, and amino acid metabolism play a significant regulatory role [21, 22]. There is currently a lack of systematic research on the iron death of HCC and its regulatory genes. Studies have identified 24 FRGs through high-throughput sequencing [18]. We attempted to study the value of these genes with HCC prognosis. Therefore, TCGA data were used to compare 24 FRGs in HCC and control samples, and 21 genes were found to have differences in expression. Further analysis was conducted on the relationship with OS, and finally, three genes were found to be prognostic factors for HCC patients. Besides, HCC patients were divided into two groups according to their risk level, and there were significant differences in OS and some clinical features.

In this study, we aimed to obtain HCC related RNA sequences through TCGA data and perform correlation analysis with FRGs to determine THE FRGs related to HCC prognosis. We found that the prognostic model constructed by three genes (CARS1, FANCD2, and SLC7A11) has an excellent predictive function, which can independently predict HCC patients' prognosis. Moreover, these three genes' expression characteristics are not affected by underlying diseases, suggesting that the prognostic model constructed has good population adaptability. Besides, for HCC patients who have completed genetic testing, clinicians can use this model to make better clinical decisions and enhance follow-up or early intervention for patients with high-risk recurrence factors. CARS1 alias CARS, encoding type 1 ammonia acyl tRNA synthetase, in ammonia acyl tRNA synthetase, the gene is located in chromosome 11 p15. 5 on an essential area of the tumor suppressor gene imprinting genes domain near one of several genes, such as Krebs people find expression of HBV specific CARS CD8 (+) T cells can recognize different HBV subtypes, and immune function in HBV transgenic mice transplanted and amplification, can effectively control the replication of HBV quickly, only transient hepatic damage [23]. Besides, Cho et al. found that the special region of antigen-presenting cells was associated with CARS secreted by cancer cells to activate the immune response, thus stimulating a strong humoral and cellular immune response in the body [24] suggesting CARS may be related to immunity. FANCD2 gene is closely associated with DNA damage response. Studies have shown that interference with FANCD2 will affect DNA interstrand cross-linking agents' sensitivity, which is correlated with HCC resistance to chemotherapy [25, 26]. Komatsu et al. found that FANCD2 plays an important role in DNA replication of HCC cells and inhibiting liver cancer [27]. It was also found that FANCD2 expression was up-regulated in HCC tumor tissues, and patients with high

expression had a poor prognosis and were associated with larger tumor size and invasive phenotype. FANCD2 gene knockout can inhibit the proliferation and invasion of liver cancer cells [27]. SLC7A11, also known as xCT, encodes a highly specific all-source of cysteine and glutamate. Studies have shown that blocking SLC7A11 can inhibit HCC cells' growth through the ROS/ autophagy pathway [28]. SLC7A11 can induce iron death when lipid peroxides accumulate excessively [29, 30]. Yue's study found that SLC7A11 expression was associated with the prognosis of liver cancer [31].

Immunotherapy is a tumor therapy that utilizes the human body's immune system to generate an anti-tumor response, providing a new therapeutic approach for the treatment of HCC [32-34], especially the application of immune checkpoint inhibition, with better objective remission rate and longer progression-free survival time for HCC patients [35]. Our study found that FRGs in 3 were associated with high expression of B cells, macrophages, and dendritic cells in high-risk HCC patients and were associated with prognosis, reflecting that iron death may be involved in the regulation of the immune microenvironment. These differences may promote the growth and development of HCC, leading to poor prognosis. In addition, the use of immune checkpoint inhibitors in high-risk HCC patients may be of more significant benefit. Although we found that 3 FRGs are closely related to THE prognosis of HCC, the specific mechanism of action is poorly understood. Therefore, it is necessary further to determine the prognostic significance of these genes in HCC.

In conclusion, we determined that 3 FRGs were associated with the prognosis of HCC and had good predictive value. The model based on these three genes showed good predictive performance. The further immune analysis showed that these three genes may be related to the immunity of HCC and may be potential therapeutic targets.

# Declarations

### Ethical Approval and Consent to participate

All studies were approved by the Ethical Review Committee of the First Affiliated Hospital of Guangxi Medical University. All participants signed an informed consent form.

### Consent for publication

Agree to conditions of submission, BioMed Central's copyright and license agreement and article-processing charge (APC).

### Availability of supporting data

The data and materials used to support the findings of this study are available from the corresponding author upon request.

### Competing interests

The authors declare no potential conflicts of interest.


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### Authors' Contributions

Tao Peng designed the study. Yongfei He analysis of data, interpreted the results and wrote the article. Tianyi Liang, Shuqi Zhao, Zhongliu Wei and Yongguang Wei collected the data and specimens. Xin Zhou conducted immunohistochemical analysis.Chuangye Han contributed to guidance writing. Tao Peng edited and funding the article. All authors discussed the results, and approved the final version of the manuscript.


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## References


[1] Chen W, Zheng R, Baade P D, et al. Cancer statistics in China, 2015 [J]. CA: a cancer journal for clinicians, 2016, 66(2): 115-32.

[2] Akinyemiju T, Abera S, Ahmed M, et al. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the global burden of disease study 2015 [J]. JAMA oncology, 2017, 3(12): 1683-91.

[3] Bray F, Ferlay J, Soerjomataram I, et al. Cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries [J]. CA Cancer J Clin, 2018, 68(6): 394-424.

[4] Forner A, Reig M. Bruix J Hepatocellular carcinoma [J]. Lancet, 2018, 391(10127): 1301-14.

[5] Marrero J A, Kulik L M, Sirlin C B, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases [J]. Hepatology, 2018, 68(2): 723-50.

[6] Yang W S, Stockwell B R. Synthetic lethal screening identifies compounds activating iron-dependent, nonapoptotic cell death in oncogenic-RAS-harboring cancer cells [J]. Chemistry & biology, 2008, 15(3): 234-45.

[7] Gao M, Monian P, Quadri N, et al. Glutaminolysis and transferrin regulate ferroptosis [J]. Molecular cell, 2015, 59(2): 298-308.

[8] Sun X, Ou Z, Xie M, et al. HSPB1 as a novel regulator of ferroptotic cancer cell death [J]. Oncogene, 2015, 34(45): 5617-25.

[9] Song Y, Yang H, Lin R, et al. The role of ferroptosis in digestive system cancer [J]. Oncology letters, 2019, 18(3): 2159-64.

[10] Xia X, Fan X, Zhao M, et al. The relationship between ferroptosis and tumors: A novel landscape for therapeutic approach [J]. Current gene therapy, 2019, 19(2): 117-24.

[11] Chen G, Guo G, Zhou X, et al. Potential mechanism of ferroptosis in pancreatic cancer [J]. Oncology letters, 2020, 19(1): 579-87.

[12] Li J, Dirisala A, Ge Z, et al. Therapeutic Vesicular Nanoreactors with Tumor-Specific Activation and Self-Destruction for Synergistic Tumor Ablation [J]. Angewandte Chemie, 2017, 129(45): 14213-8.

[13] Lin L-S, Huang T, Song J, et al. Synthesis of copper peroxide nanodots for H2O2 self-supplying chemodynamic therapy [J]. Journal of the American Chemical Society, 2019, 141(25): 9937-45.

[14] Louandre C, Ezzoukhry Z, Godin C, et al. Iron-dependent cell death of hepatocellular carcinoma cells exposed to sorafenib [J]. International journal of cancer, 2013, 133(7): 1732-42.

[15] Yuan H, Li X, Zhang X, et al. CISD1 inhibits ferroptosis by protection against mitochondrial lipid peroxidation [J]. Biochemical and biophysical research communications, 2016, 478(2): 838-44.

[16] Bai T, Wang S, Zhao Y, et al. Haloperidol, a sigma receptor 1 antagonist, promotes ferroptosis in hepatocellular carcinoma cells [J]. Biochemical and biophysical research communications, 2017, 491(4): 919-25.

[17] Sun X, Ou Z, Chen R, et al. Activation of the p62-Keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells [J]. Hepatology, 2016, 63(1): 173-84.

[18] Mages K, Grassmann F, Jägle H, et al. The agonistic TSPO ligand XBD173 attenuates the glial response thereby protecting inner retinal neurons in a murine model of retinal ischemia [J]. Journal of neuroinflammation, 2019, 16(1): 1-16.

[19] Liu Z, Zhao Q, Zuo Z-X, et al. Systematic Analysis of the Aberrances and Functional Implications of Ferroptosis in Cancer [J]. Iscience, 2020, 23(7): 101302.

[20] De Matteis S, Ragusa A, Marisi G, et al. Aberrant metabolism in hepatocellular carcinoma provides diagnostic and therapeutic opportunities [J]. Oxidative medicine and cellular longevity, 2018, 2018(

[21] Angeli J P F, Shah R, Pratt D A, et al. Ferroptosis inhibition: mechanisms and opportunities [J]. Trends in pharmacological sciences, 2017, 38(5): 489-98.

[22] Kagan V E, Mao G, Qu F, et al. Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis [J]. Nature chemical biology, 2017, 13(1): 81-90.

[23] Krebs K, Böttinger N, Huang L-R, et al. All studies published in Gastroenterology are embargoed until 3PM ET of the day they are published as corrected proofs on-line. Studies cannot be publicized as accepted manuscripts or uncorrected proofs [J]. 2013,

[24] Cho S, Kim S B, Lee Y, et al. Endogenous TLR2 ligand embedded in the catalytic region of human cysteinyl-tRNA synthetase 1 [J]. Journal for Immunotherapy of Cancer, 2020, 8(1):

[25] Wu X-Y, Qian J-J, Lin Y, et al. Hepatitis B virus X protein disrupts DNA interstrand crosslinking agent mitomycin C induced ATR dependent intra-S-phase checkpoint [J]. European journal of cancer, 2008, 44(11): 1596-602.

[26] Palagyi A, Neveling K, Plinninger U, et al. Genetic inactivation of the Fanconi anemia gene FANCC identified in the hepatocellular carcinoma cell line HuH-7 confers sensitivity towards DNA-interstrand crosslinking agents [J]. Molecular cancer, 2010, 9(1): 1-15.

[27] Komatsu H, Masuda T, Iguchi T, et al. Clinical significance of FANCD2 gene expression and its association with tumor progression in hepatocellular carcinoma [J]. Anticancer research, 2017, 37(3): 1083-90.

[28] Guo W, Zhao Y, Zhang Z, et al. Disruption of xCT inhibits cell growth via the ROS/autophagy pathway in hepatocellular carcinoma [J]. Cancer letters, 2011, 312(1): 55-61.

[29] Lang X, Green M, Wang W, et al. Radiotherapy and Immunotherapy Promote Tumoral Lipid Oxidation and Ferroptosis via Synergistic Repression of SLC7A11 [J]. Cancer discovery, 2019, 9(12): 1673-85.

[30] Liu T, Jiang L, Tavana O, et al. The deubiquitylase OTUB1 mediates ferroptosis via stabilization of SLC7A11 [J]. Cancer research, 2019, 79(8): 1913-24.

[31] Yue C, Ren Y, Ge H, et al. Comprehensive analysis of potential prognostic genes for the construction of a competing endogenous RNA regulatory network in hepatocellular carcinoma [J]. OncoTargets and therapy, 2019, 12(561.

[32] Prieto J, Melero I, Sangro B. Immunological landscape and immunotherapy of hepatocellular carcinoma [J]. Nature reviews Gastroenterology & hepatology, 2015, 12(12): 681-700.

[33] Tai D, Choo S P, Chew V. Rationale of Immunotherapy in Hepatocellular Carcinoma and Its Potential Biomarkers [J]. Cancers, 2019, 11(12): 1926.

[34] Gopu V, Cai Y, Krishnan S, et al. An accurate aging clock developed from the largest dataset of microbial and human gene expression reveals molecular mechanisms of aging [J]. bioRxiv, 2020,

[35] Finn R, Ryoo B, Merle P, et al. KEYNOTE-240 investigators. Pembrolizumab As Second-Line Therapy in Patients With Advanced Hepatocellular Carcinoma in KEYNOTE-240: A Randomized, Double-Blind, Phase III Trial [J]. J Clin Oncol, 2020, 38(3): 193-202.

# Figures

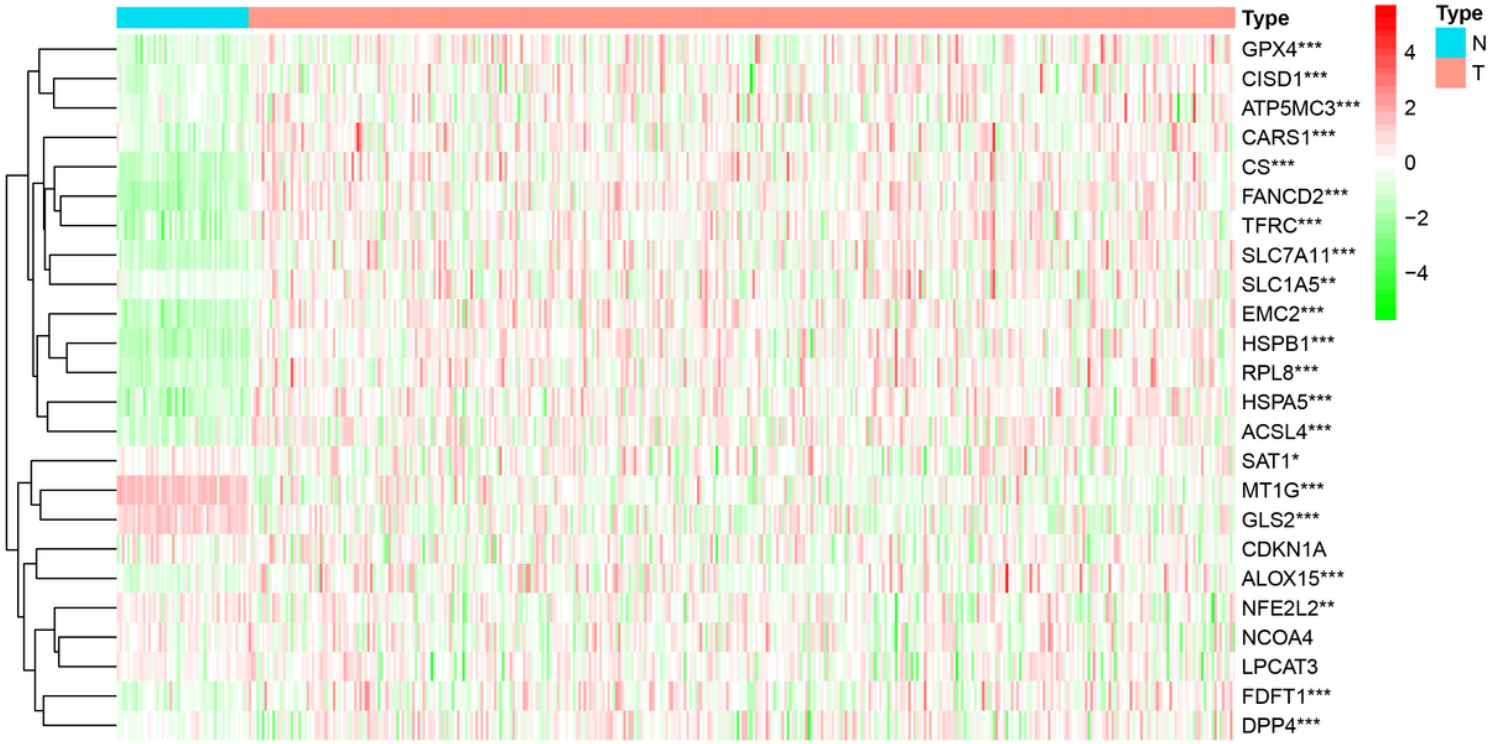
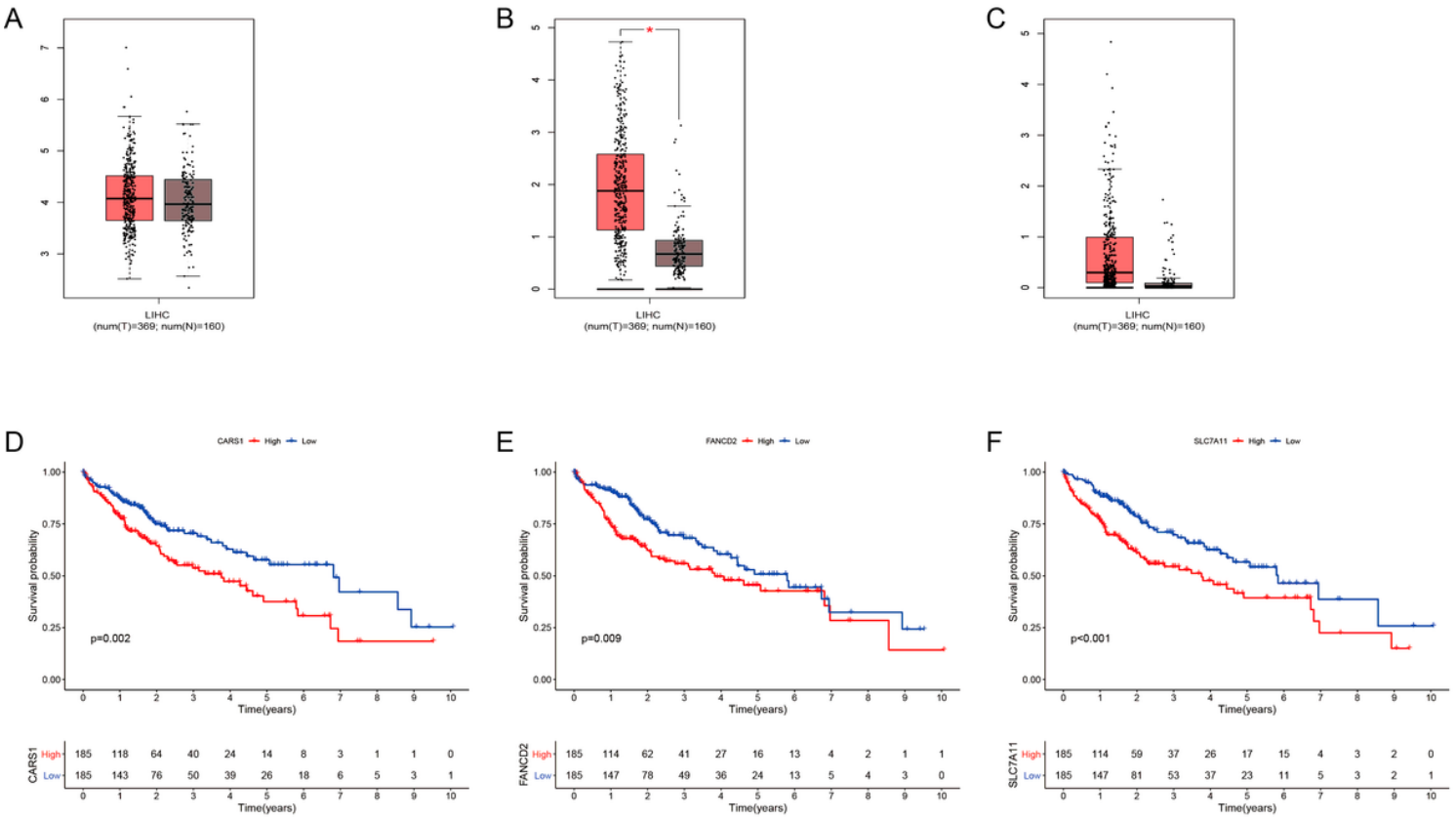


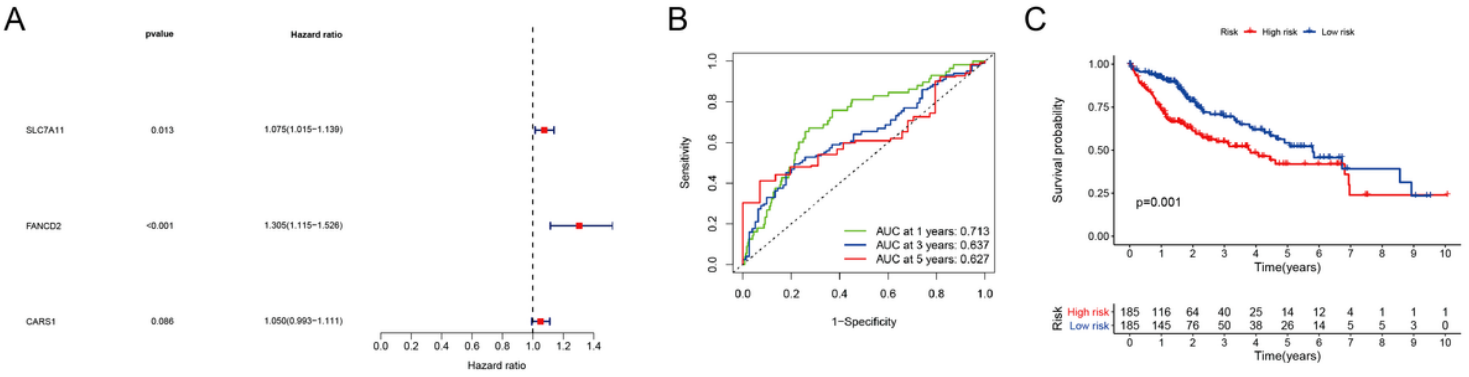

**Figure 1**

heat map of FRGs expression in HCC tumor tissues and normal tissues (up-regulated in red and down-regulated in green). *P < 0.05, **P < 0.01 and ***P < 0.001)



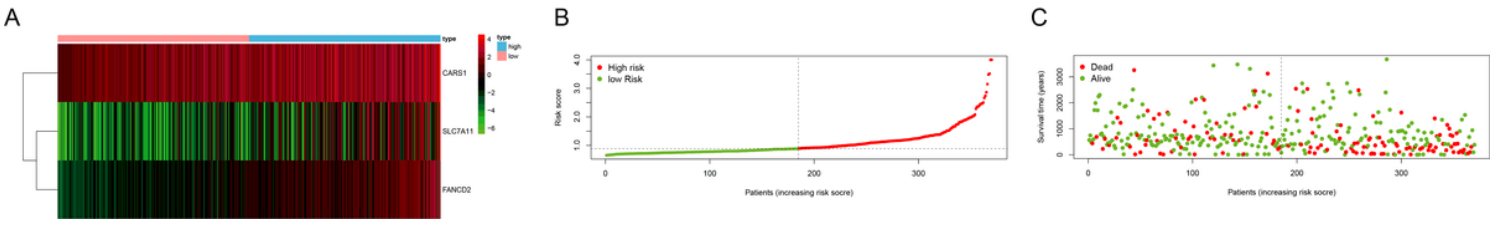

## Figure 2

Expression and prognostic analysis of 3 FRGs in HCC and its corresponding tissues.A and D the expression and prognosis of CARS1 gene in HCC(P=0.002);B and E, the expression and prognosis of FANCD2 gene in HCC (P=0.009); C and F, the expression and prognosis of SLC7A11 gene in HCC (P < 0.001).



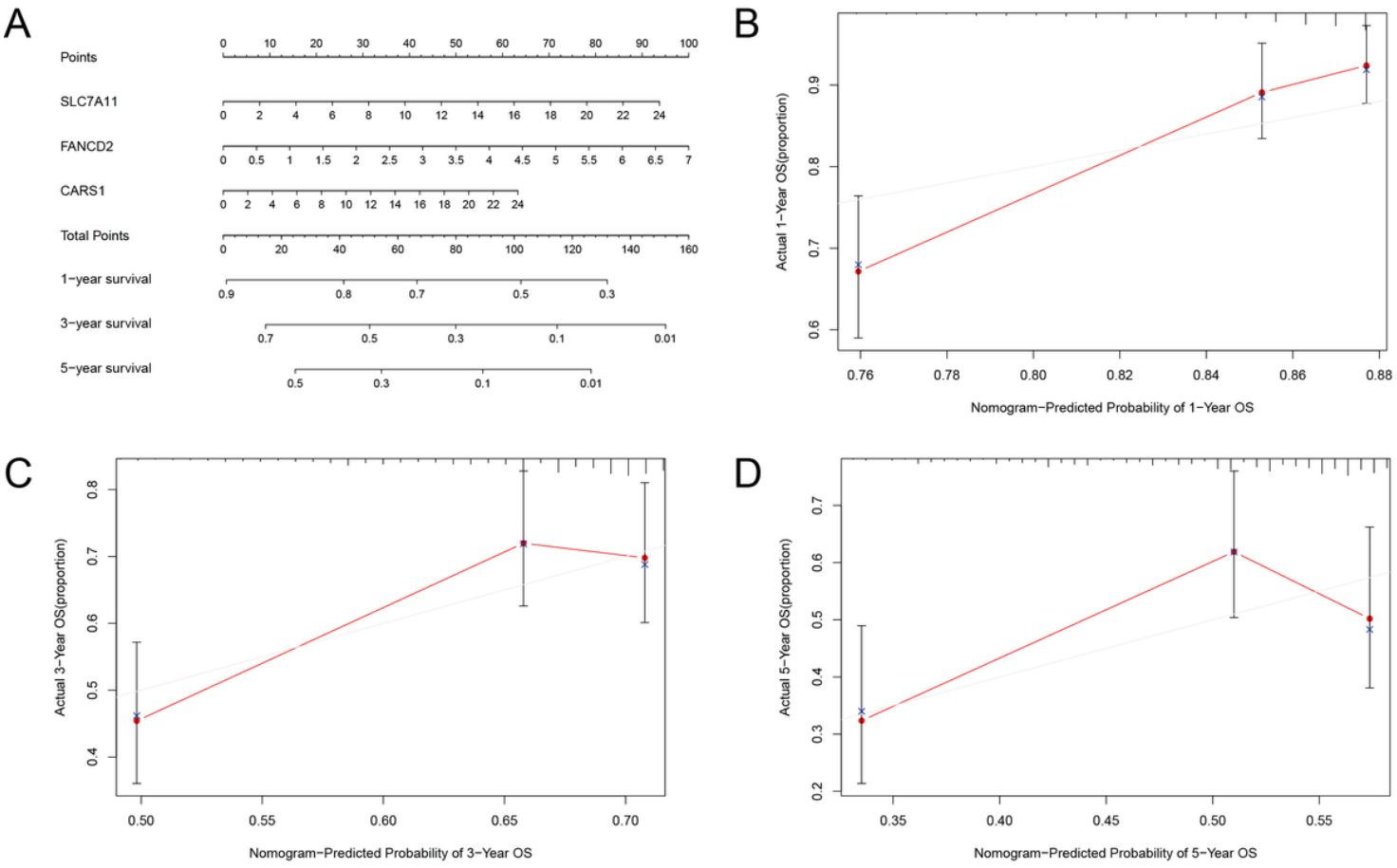

## Figure 3

Influence of FRGs on HCC prognosis. A. Single-factor Cox analysis of the three FRGs; B. ROC curves of 3 FRGs for predicting prognosis of HCC; C. Kaplan-Meier overall survival curve of patients in the TCGA data set was divided into a high-risk group and low-risk group according to the risk score.



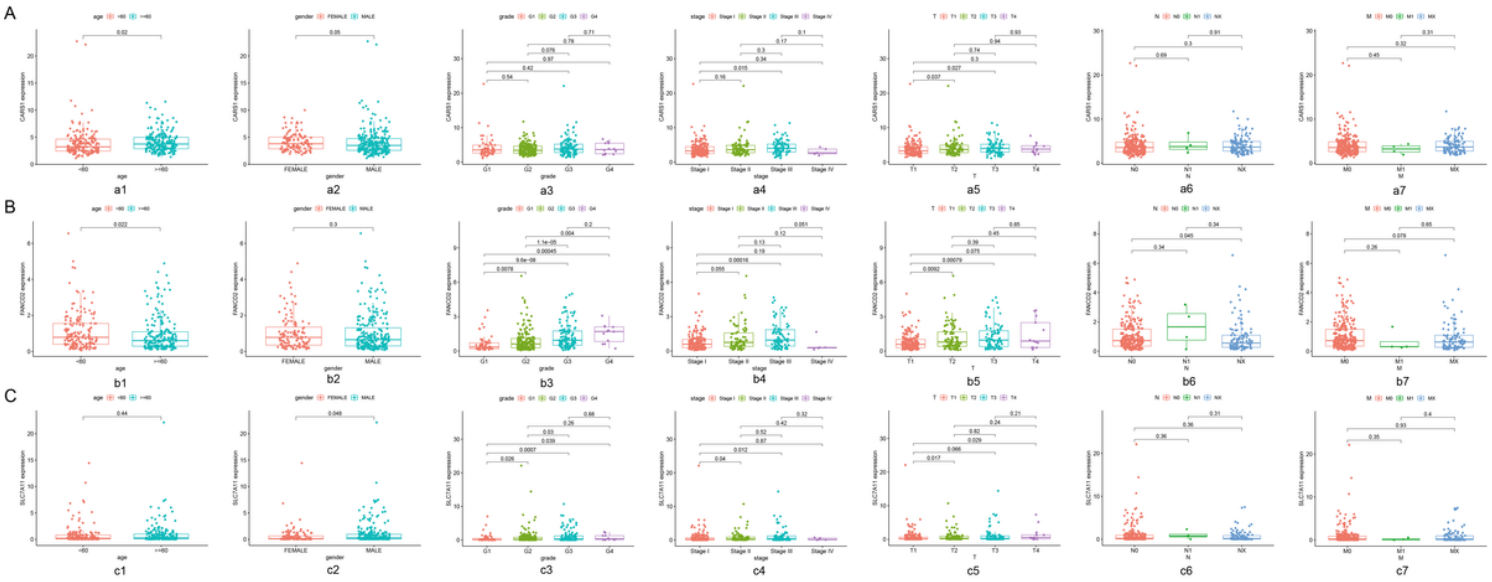

## Figure 4

Influence of risk score on the prognosis of HCC patients. A. The heat map shows the expression of three FRGs (up-regulated in red and down-regulated in green); B. Patients were divided into a high-risk group and low-risk group based on the median risk; C. Distribution of risk scores and survival status of patients.



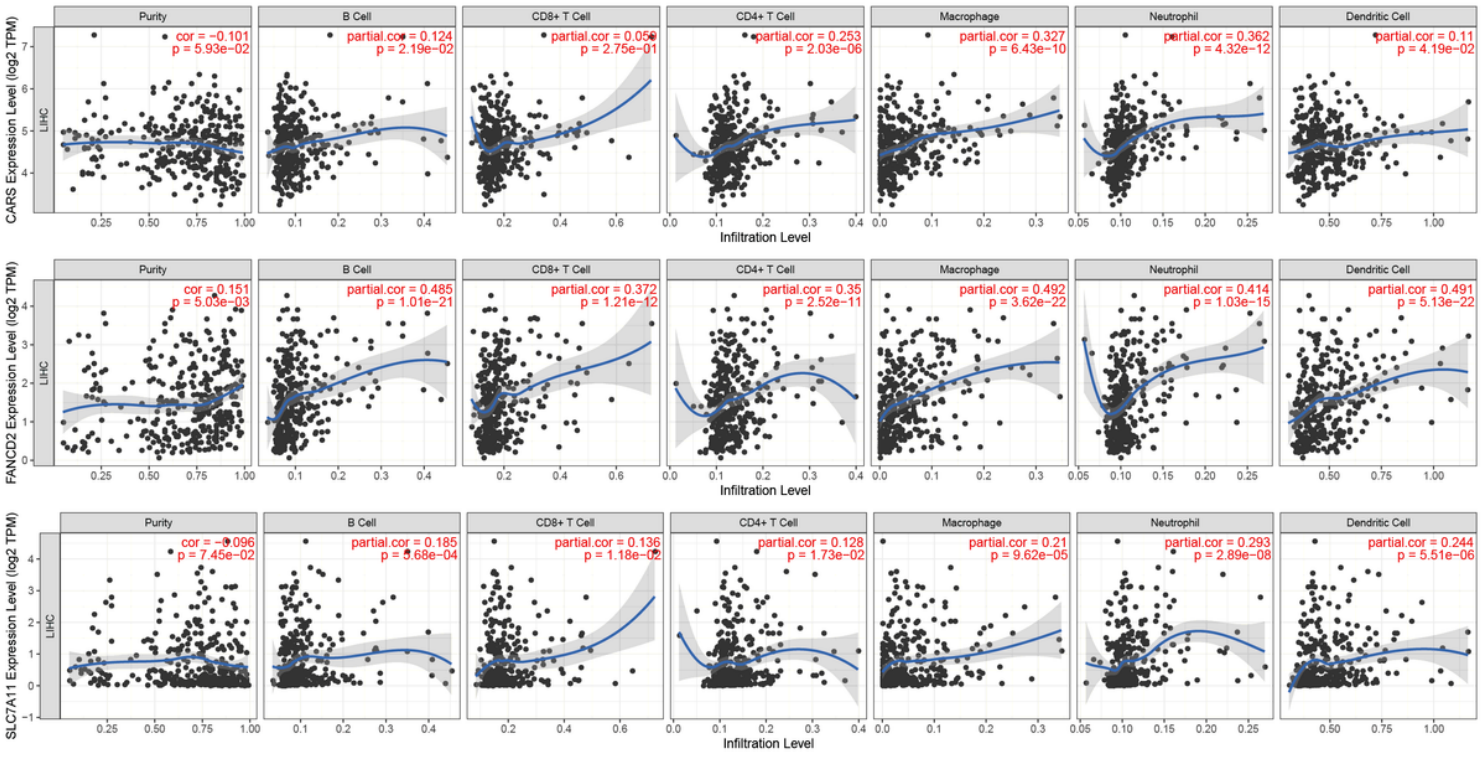

## Figure 5

The nomogram and correction curve of the 3 FRGs predicted OS. A. The nomograms of the 3 FRGs predicted OS; B. 1-year OS prediction probability correction curve; C, 3-year OS prediction probability correction curve; D. Five-year OS prediction probability correction curve.



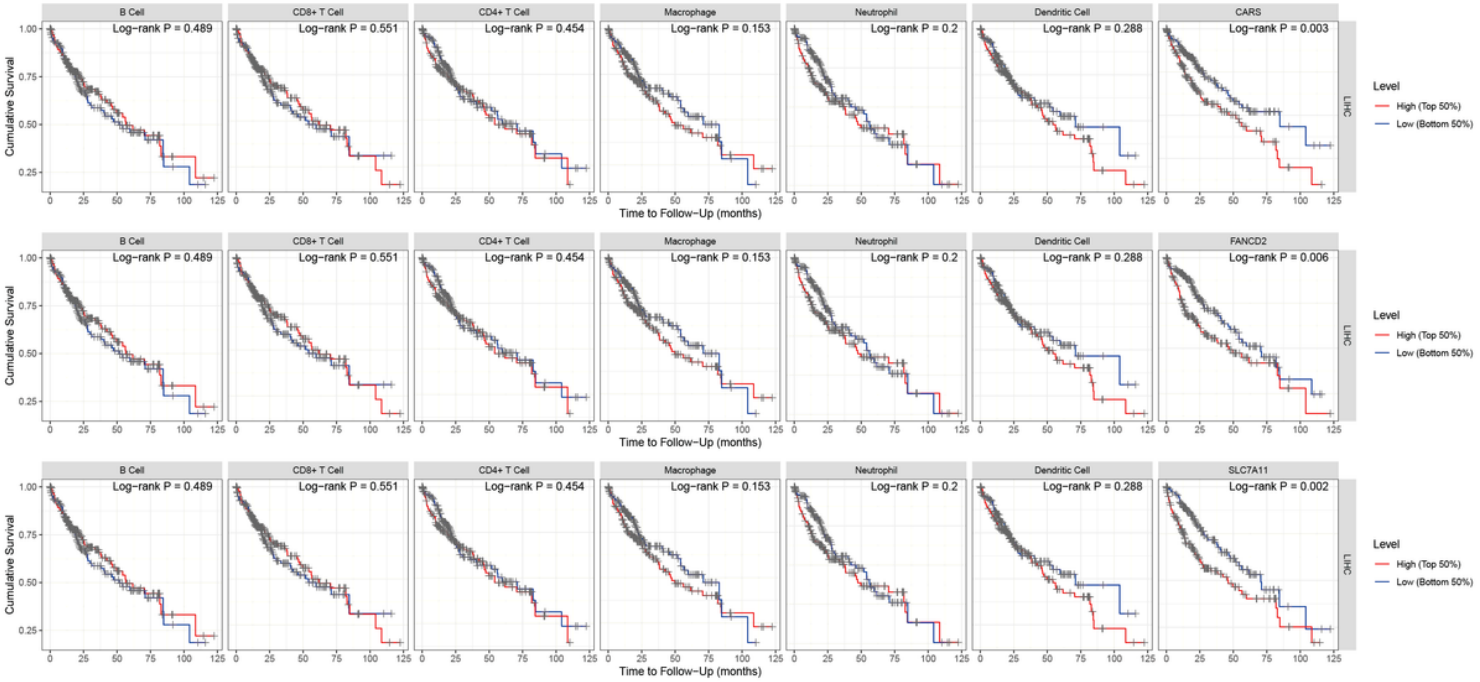

## Figure 6

Expression of the three FRGs in patients with different clinical characteristics of HCC. A CARS; B, FANCD2; C, SLC7A11.



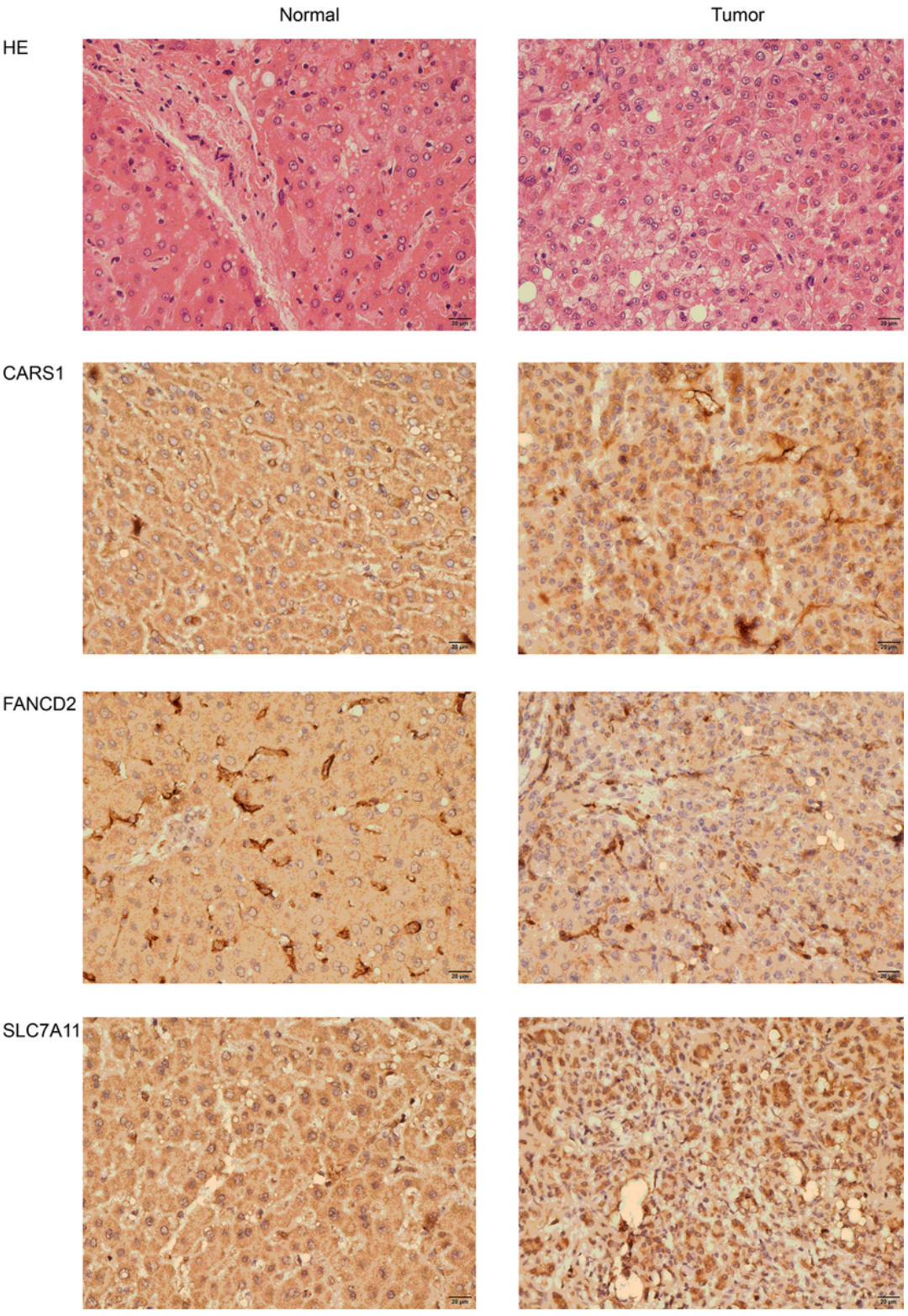

## Figure 7

Expression of 3 FRGs in HCC immune cells.




## Figure 8

Immune prognosis of 3 FRGs in HCC.




## Figure 9

The Expression of 3 FRGs in hepatocellular carcinoma (IHC).