Identify an innovative ferroptosis-related gene in hepatocellular carcinoma

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Abstract
Background: SLC1A5 has been demonstrated to be associated with the progression of other tumors; however, studies are lacking in hepatocellular carcinoma (HCC). Here, we identify SLC1A5, as a novel ferroptosis factor, for HCC patients.

Methods: The core biomarkers were identified by univariate and multivariate Cox regression analysis, and the genes present in liver cancer were validated using the public database. Then, gene set enrichment analysis (GSEA) was performed to explore the underlying molecular mechanisms. In addition, we explore the relationship between SLC1A5 and clinical factors. Finally, we determine the effect of SLC1A5 on HCC cells using real-time PCR, cell scratch analysis, transwell analysis, and CCK8 analysis in molecular biology experiments.

Results: Cox regression model shows that SLC1A5 was an independent risk factor for HCC patients. GSEA results indicated high expression of SLC1A5 related to the fatty acid metabolism pathway. Clinical correlation analysis demonstrates that alpha-fetoprotein (AFP) expression was positively correlated with SLC1A5 ($p = 8e^{-05}$), and the higher tumor stage means the higher expression of SLC1A5 ($p = .02$). In addition, SLC1A5 expression was also positively correlated with vascular infiltration of HCC ($p = .04$). Furthermore, the SLC1A5 function deficiency experiment explored its underlying impact on the biological function of HCC. qPCR, also called quantitative polymerase chain reaction, confirmed that SLC1A5 was highly expressed in liver cancer when compared with normal tissues. Studies have also shown that downregulation of SLC1A5 can inhibit wound healing, invasion, and proliferation of HCC cells.

Conclusion: In conclusion, ferroptosis factor SLC1A5 is a new therapeutic target for hepatocellular carcinoma.

Keywords
ferroptosis, hepatocellular carcinoma, overall survival, prognostic, TCGA
1 | INTRODUCTION

The liver tumor is a familiar tumor around the country, and its morbidity is on the rise. More than 466,100 people are suffering from liver tumors and about 422,100 sacrificed to it every year. Although, surgical, adjuvant radiotherapy, and chemotherapy are the dominant methods of clinical therapy for hepatocellular carcinoma (HCC). However, the prognosis of patients has only slightly improved in recent years, and the effect is not very effective. At the same time, the recurrence rate and metastasis rate of tumors are still high. Therefore, predicting the prognosis of the clinical outcome of HCC patients remains a challenge for clinicians. Although studies have proposed to use different histological parameters to forecast the prognosis of a sick person with a liver tumor, the effect has limitations. In our research, we obtain data from The Cancer Genome Atlas (TCGA) database, which includes genome and clinical information of tumors, and then explored some ferroptosis biomarkers related to HCC. We use the oncoming database, including Gene Expression Omnibus (GEO) datasets, to validate the expression of the hub gene. We hope that these biomarkers will contribute to the diagnosis, treatment, and prognosis of HCC.

In 2012, a new term was coined in the scientific community: ferroptosis. Programmed cell death is a term used to describe a type of iron-dependent cell death caused by the accumulation of lipid-type reactive oxygen elements. This way is balanced in normal people when the body is in a pathological state: cancer. Ferroptosis comes into play. Ferroptosis driving function in liver cancer. For example, one of the ways to prevent iron death in liver cancer is to drive the P62-KEAP1-NRF2 pathway. Studying ferroptosis biomarkers in the prognosis and treatment of HCC patients is important.

SLC1A5 is one of the neutral amino acid transporters in the SLC1 family, solute-carrying cell-surface traffic that regulated the assimilation of neutral amino acids. SLC1A5, as a glutamine transporter, plays a role in human tumor formation. SLC1A5 was also investigated as a ferroptosis factor in HCC. Rene Bernard’s research team has shown that CB-839 and V-9302, as SLC1A5 inhibitors of glutaminase, can effectively inhibit the progression of liver cancer. At present, there are few studies on SLC1A5 as a ferroptosis factor in liver cancer. Our research will focus on the biological role of SLC1A5 in liver cancer.

Here, we assess the presence of SLC1A5 in HCC sicks from the TCGA database and determined that SLC1A5 plays a role of an oncogene in liver cancer. Cox model analysis is used to assess the prognostic worth of SLC1A5 in sicks with HCC. Finally, we verified our conjecture through molecular biology experiments. SLC1A5 acts as a ferroptosis factor. Knockdown of SLC1A5 can suppress the proliferation, migration, and incursion of liver tumors.

2 | MATERIALS AND METHODS

2.1 | Data acquisition and patient characteristics

Download the clinical information of hepatocellular persons from the TCGA project and use Perl language analysis to extract the clinical data of the patients. Patients with incomplete clinical information were deleted, others remained. A total of 374 patients are enrolled in the study, and corresponding clinical data and expression profile data were downloaded. The expression profile data format was fragmented per kilobase per million (FPKM).

2.2 | Exploring DEGs between tumor and normal samples

Analysis of sequencing data of liver cancer patients in TCGA database using the limma package. The threshold is set to FDR < 0.05, |logFC| ≥ 1. Using the avereps function to process genes that appear multiple times, sorted into one line, and took the mean value, and the genes with the expression of zero in all samples were deleted. The mean expression of DEGs in tumor samples and normal samples was obtained by Wilcoxon. Test. A volcano map was drawn to visual DEGs.

2.3 | Differentially expressed ferroptosis-related genes in the TCGA database

Ferroptosis-related genes (FRGs) are obtained from the ferroptosis death database FerrDb. The intersection of FRGs and DEGs was used to extract the expression of differentially expressed ferroptosis-related genes (DE-FRGs) in HCC patients. The DE-FRGs were obtained.

2.4 | Analysis of survival genes

In the TCGA database, the mRNA expression data of genes from multivariate Cox proportional risk regression analysis are displayed as line graphs, the abscissa is grouping information, and the ordinate is Log2 (FPKM+1). Wilcoxon rank-sum test is used to compare two groups (tumor group and normal group). Further, disease-specific survival (DSS) and progress-free interval (PFI) analyses of these genes were performed to obtain genes that are worth studying and interrelated to the prognosis of HCC sicks. Genes conforming to DSS and PFI were included in subsequent studies (p < .05). The expression standard of a survival-related gene in liver cancer was determined in the Oncomine database. The threshold is set up as the following values: p value of .05, the absolute value of log fold change is 1.5, and all genes are sorted. We further explored whether the genes included in this study were related to AFP expression, pathological stage, histological grade, and vascular invasion in HCC.

2.5 | Gene ontology and GSEA

The “cluster profile” R package in Rstudio is used for gene ontology (GO). Analysis based on genes between high risk and low risk (log2FC) ≥ 1, FDR < 0.05. Observe the biological processes, cellular components, and molecular function.
GSEA is a calculation method that can study the prospective molecular mechanism of SLC1A5 in the prognosis of HCC patients.\textsuperscript{19–21} In this study, HCC specimens were distinguished into high expression groups and low expression groups based on the expression pattern of SLC1A5. Subsequently, GSEA was used to evaluate the difference between high and low SLC1A5 expression groups. The data were considered significant when \( p < .05 \), and the error detection rate (FDR) was <25%. According to the normalized enrichment score (NES), enrichment methods related to the biological process of liver cancer were selected.

2.6 | Cell culture

LX2 and HepG2 were obtained from the ATCC. Use DMEM (PM150210) medium containing 10% fetal bovine serum, 1% glutamine, and 1% antibiotic/antifungal solution to culture cells at 37°C and 5% CO\textsubscript{2}.

2.7 | qPCR

Use Trizol reagent (Invitrogen) extraction kit to extract total RNA from LX2 and HepG2 cells. The concentration of the RNA was tested by NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). The PCR reaction conditions are as follows: 95°C for 30 s, 95°C for 10 s, 60°C for 30 s, 70°C for 34 s, a total of 40 cycles. The expression of SLC1A5 was measured using 18s as a reference. The experiment was carried out in triplicate. The primer sequences used for PCR were: SLC1A5 forward, 5′-CAACCTGGTGTCAGCAGCCTT-3′ and SLC1A5 reverse, 5′-GCACCGTCCATGTTGACGGTG-3′.

2.8 | Cell transfection

SLC1A5 short hairpin oligonucleotide (shRNA, 1 \( \mu \)g) and its negative control (sh-NC, 1 \( \mu \)g) were designed and synthesized by Genesee Biotechnology Co., Ltd. The pcDNA3.1 plasmid (2 \( \mu \)g) used to generate the SLC1A5 construct was provided by Thermo Fisher Scientific. The HepG2 cell line was transfected using Lipofectamine 3000 (Invitrogen) according to the manufacturer’s instructions. All transfected cells were cultured for 48 hours before being used in the experiment.

2.9 | Cell scratch analysis

Resuspend 4 \times 10^5 cells/well in 10 ml medium and inoculate it into 10 cm. Once the cells reach 95% confluence, scratch the cell layer with the tip of a 100\( \mu \)l pipette, making sure that the width of each wound is identical. The scratches were then rinsed with PBS reagent, and the samples were placed in a complete medium containing 1% fetal bovine serum and incubated at 37°C and 5% CO\textsubscript{2}. Observe the width of the cell wounds under an inverted microscope at 0 and 24 h after making the cell scratch.

2.10 | Transwell test

HepG2 cells were resuspended at 2 \times 10^4 cells/ml. Add the cell suspension (200\( \mu \)l) to the upper chamber of the Matrigel-coated serum-free transwell. The lower chamber contains 500\( \mu \)l of complete medium containing 10% FBS. The Transwell system was incubated with 5% CO\textsubscript{2} for 24 h at 37°C. After that, the cells of the lower chamber were stained with 0.1% crystal violet solution for 15 minutes and counted under an optical microscope.

2.11 | CCK8 assay

The cells in the log phase were seeded into a 96-well plate at a density of 1 \times 10^4 cells/well and cultured at 37°C and 5% CO\textsubscript{2} for 0, 12, 24, 48, or 72 h. Next, add 10 \( \mu \)l of cck8 to the medium and incubate the sample at 37°C for 2 h. To construct the cck8 curve, the absorbance at 450nm is set as the ordinate, and the time is set as the abscissa. The results were averaged in three independent experiments.

2.12 | Statistical analysis

GraphPad Prism 8.0 and R (version 3.6.2) software were used for data analysis. Cox regression analysis was used to determine genes related to the prognosis of the disease. A \( p \) value less than .05 indicates a significant difference.

3 | RESULT

3.1 | Identification of DEGs between tumor and normal samples

A total of 374 patients are enrolled in this study (Table 1). Using R language and calling limma package, a total of 10,242 genes were identified, including 9462 upregulated genes, and 780 downregulated genes were calculated for subsequent analysis. Volcano map is used for data visualization (Figure 1A).

3.2 | Ferroptosis genes in the TCGA database

In total, 259 FRGs were obtained from the FerrDb database. FRGs intersected with liver cancer differentially expressed genes in the TCGA database. According to the calculation results of the Wilcoxon test function, 76 DE-FRGs were obtained (Figure 1B).
3.3 | Identification of survival-related DE-FRGs

According to the univariate cox proportional hazards regression analysis, there are 43 prognostic-related FRGs \((p < .05, \text{HR} > 1)\). Four independent prognostic factors were obtained by multivariate Cox proportional risk regression analysis of DE-FRGs, respectively, SLC1A5 \((\text{hazard ratio (HR)} = 1.22; 95\% \text{ confidence interval (CI)} = 1.08 \text{ to } 1.4; p = .001)\), MT3 \((\text{HR} = 1.08; 95\% \text{CI} = 1.00 \text{ to } 1.2; p = .039)\), HSPB1 \((\text{HR} = 1.26; 95\% \text{CI} = 1.10 \text{ to } 1.4; p = .001)\), ZNF419 \((\text{HR} = 1.30; 95\% \text{CI} = 1.12 \text{ to } 1.5; p = .001)\), and all four genes were high-risk genes. \(p < .05\) as threshold (Figure 1C).

3.4 | Analysis of survival genes

Figure 2A–D shows that SLC1A5, MT3, HSPB1, and ZNF419 are highly expressed in HCC, and four genes act as oncogenes in HCC. We further performed DSS and PFI survival analysis of these four genes, and the results showed that only SLC1A5 was related to HCC (Figure 3A–H). Therefore, we included SLC1A5 in our subsequent validation study and abandoned MT3, HSPB1, and ZNF419. As shown in Figure 4A–C, the Oncomine database was used to compare the SLC1A5 mRNA expression between LIHC and normal tissue specimens, and the mRNA levels of SLC1A5 in the three data sets (LIHC) were significantly higher. As expected, four genes are rich in antioxidant activity, cellular oxidant detoxification, cellular detoxification, and astrocyte projection. To identify SLC1A5-related

| Characteristic          | Levels     | Overall |
|-------------------------|------------|---------|
| N (%)                   | 374        |         |
| Age (Years)             | ≤60        | 177 (47.5%) |
|                         | >60        | 196 (52.5%) |
| Gender                  | Female     | 121 (32.4%) |
|                         | Male       | 253 (67.6%) |
| Race                    | Asian      | 160 (44.2%) |
|                         | Black or African American | 17 (4.7%) |
|                         | White      | 185 (51.1%) |
| Weight (kg)             | ≤70        | 184 (53.2%) |
|                         | >70        | 162 (46.8%) |
| Height (cm)             | <170       | 201 (58.9%) |
|                         | ≥170       | 140 (41.1%) |
| BMI                     | ≤25        | 177 (52.5%) |
|                         | >25        | 160 (47.5%) |
| Pathologic stage        | Stage I    | 173 (49.4%) |
|                         | Stage II   | 87 (24.9%)  |
|                         | Stage III  | 85 (24.3%)  |
|                         | Stage IV   | 5 (1.4%)     |
| Histologic grade        | G1         | 55 (14.9%)  |
|                         | G2         | 178 (48.2%) |
|                         | G3         | 124 (33.6%) |
|                         | G4         | 12 (3.3%)   |

### TABLE 1 | Baseline characteristic of patients

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### FIGURE 1 | Differentially expression analysis and SLC1A5 gene screen. (A) Volcano plot of the hepatocellular carcinoma differentially expressed genes (DEGs), between hepatocellular carcinoma patients and healthy subjects. Volcano plot of DEGs. Red means upregulated DEGs; Blue means down-regulated DEGs; Black means no different. (B) The number of 76 common ferroptosis-related genes in the TCGA database and ferrDb is shown by the Venn diagram. (C) Four ferroptosis-related genes are significantly associated with overall survival (OS) in hepatocellular carcinoma.

| Gene    | HR  | 95% CI          | P Value |
|---------|-----|-----------------|---------|
| SLC1A5  | 1.22| 1.08 − 1.4      | 0.001   |
| MT3     | 1.08| 1.00 − 1.2      | 0.039   |
| HSPB1   | 1.26| 1.10 − 1.4      | <0.001  |
| ZNF419  | 1.30| 1.12 − 1.5      | <0.001  |

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Signaling access in HCC, we conducted GSEA to determine the difference in SLC1A5 gene expression. Based on the normalized enrichment score (NES), the most significant enrichment signal pathway was determined. The high expression of SLC1A5 was significantly correlated with fatty acid metabolism in HCC (Adjust \( p = 1.98 \times 10^{-6} \), \( ES = 0.652 \)) (Figure 4D).
3.5 | SLC1A5 with clinical factor

We divided the patients into groups with AFP 400 ng/mL as the cutoff value, and the convey of SLC1A5 was positively correlated with the AFP (ng/ml) level of patients with liver cancer (Figure 5A). SLC1A5 is related to the pathological stage, histologic grade, and vascular invasion in HCC patients. The higher the expression of SLC1A5, the higher the pathological stage and histological grade of liver cancer patients. and the expression of SLC1A5 was positively correlated with vascular invasion in HCC patients (Figure 5B–D) and (Table 2).

3.6 | Quantitative reverse transcription-polymerase chain reaction

Real-time PCR results showed that SLC1A5 expression was relatively increased in tumor tissues (Figure 6A).

3.7 | Cell scratch and transwell test analysis

To study whether knocking down SLC1A5 can restrain the migration and invasion of liver cancer cells, we knock down SLC1A5 in HepG2 cells. Through wound healing experiments, we found that the migration ability of knockdown SLC1A5 cells was inhibited. Transwell experiments confirmed that the invasion ability of cells knocked down SLC1A5 was also inhibited (Figure 6B,C).

3.8 | CCK8 assay

To investigate whether SLC1A5 knockdown can inhibit the proliferation of HCC cells, we knocked out SLC1A5 in HepG2 cells. Through the CCK8 experiment, we found that the proliferation ability of SLC1A5 cells with knockdown was inhibited (Figure 6D).

4 | DISCUSSION

Liver cancer is a worldwide public health event, so it deserves attention. Some previous studies use bioinformatics techniques to identify tumor biomarkers for other cancers. Here, we also use the above methods to analyze the TCGA database and select SLC1A5 as a new biomarker for HCC.

Conventional parameters such as AFP, tumor stage, tumor grade, and vascular invasion of HCC are helpful to predict the prognosis of
HCC to some extent in clinical. Alpha-fetoprotein (AFP) is a glycoprotein derived from embryonic germ cells and has various biological functions. Healthy adult blood AFP content is low, and tumor (liver cancer, gastric cancer, pancreatic cancer, etc.) in patients with AFP content will be higher than normal. AFP acts a part in the diagnosis and immunotherapy of liver cancer. The data analysis of TCGA liver cancer patients showed that when the AFP content in liver cancer patients was greater than 400 ng/mL, the expression of SLC1A5 was also increased, and it was statistically significant \((p < 0.05)\). Through the above information, we guess that SLC1A5 may play a role in the diagnosis of liver cancer patients with AFP, SLC1A5 may directly or indirectly cause AFP expression in some way. Tumor staging and grading are indicators to evaluate the malignant degree of the tumor. The higher the tumor staging and grading of clinical liver cancer patients are, the worse the prognosis of patients is. We found that SLC1A5 is associated with the stage and grade of HCC patients. The higher SLC1A5 is, the higher the tumor stage and grade of HCC patients are. SLC1A5 may promote the development of liver cancer and lead to a poor prognosis. Vascular invasion is necessary for the growth and metastasis of invasive tumors and is an important part of controlling cancer progression. Invasion of blood vessels by tumor tissue in patients with liver cancer can lead to tumor tissue invasion of peripheral blood vessels, which is related to the poor prognosis of patients.

As a biological compound in the human body, fatty acids play an important role in human metabolism, health, and diseases. The liver is the main place for fatty acid synthesis and metabolism. The abnormal activation of the carcinogenic signaling pathway changes the expression and activity of lipid metabolic enzymes, which leads to the imbalance of fatty acid metabolism. It is increasingly considered to be an important metabolic redistribution phenomenon in tumor cells and then participates in the occurrence and development of liver cancer. SLC1A5 is involved in liver fatty acid metabolism as an iron death factor. Our results show that high expression of SLC1A5 was enriched in the fatty acid metabolism pathway, which suggests that SLC1A5 may promote metabolism disorder in the human body, then promote the cancer process. Cai’s research team found that SLC1A5, as a glutamine transporter (a participant in the fatty acid metabolism pathway), is highly expressed in colorectal signet ring cell carcinoma and mediates glutamine metabolism. Increased expression of SLC1A5 has been found in a variety of cancers, including colorectal cancer and liver cancer, and its high expression has been confirmed to be associated with poor prognosis.
SLC1A5 plays an oncogene role in a variety of cancers. Qingjun Pan’s team discovered that SLC1A5 expression was significantly higher in 86.5% (32/37) of cancer tissues than in adjacent normal tissues. In esophageal cancer cell lines, siRNA knockdown of SLC1A5 significantly reduced cell growth, reduced glutamine transport, inhibited mTORC1 signaling, and resulted in cell cycle arrest and apoptosis. Inhibiting the expression of SLC1A5 in vivo and in vitro, respectively, showed that the inhibitory effect of cetuximab on CRC proliferation was enhanced. Zhang’s team analyzes the OV data in TCGA. By establishing nine models of ferroptosis-related genes, SLC1A5, as a high-risk gene in ovarian cancer, is related to the poor prognosis of patients, and SLC1A5 is related to the immune pathway in ovarian cancer. Our study revealed that SLC1A5 was an independent prognostic factor related to iron death in liver cancer, and we speculated that SLC1A5 might be related to the immune pathway in liver cancer. Other studies have shown that SLC1A5 protects non-serous OC patients from disease recurrence, which may be through a biological mechanism unrelated to cytotoxic drug sensitivity. Therefore, we predict that SLC1A5 plays different roles in different types of liver cancer and may play a protective role in certain subtypes of liver cancer. Inhibition of SLC1A5 by Mir-137 strongly inhibits glutamine decomposition, leading to ferroptosis in cells. Since SLC1A5-mediated glutamine transport plays a key role in tumor cell metabolism, proliferation, and iron death, inhibition of targeted glutamine transport by SLC1A5 is a potential approach for the treatment of solid tumors.

Although we have revealed the role of SLC1A5 as a ferroptosis factor in HCC to some extent through bioinformatics and basic experiments, the limitations of this article cannot be ignored. TCGA
database was selected for this study. If possible, clinical patient specimen data should be collected for analysis and verification of the SLC1A5 expression difference between liver cancer tissues and normal tissues. At the same time, the data collected by ourselves are regarded as external datasets for verification.

5 | CONCLUSION

Our study demonstrated that SLC1A5 expression is higher in tumor tissues compared with normal liver tissues, which means that this gene has potential value to become a candidate therapy biomarker for HCC.

AUTHOR CONTRIBUTIONS

WJ and HGF were involved in the conception and design of the study; HX and ZB analyzed the bioinformatic data; GPF, WW, and WJ drafted the manuscript; WJ reviewed and edited the manuscript. All authors have read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The datasets supporting the conclusions of this article are included within the article.

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REFERENCES

1. Malek NP, Schmidt S, Huber P, Manns MP, Greten TF. The diagnosis and treatment of hepatocellular carcinoma. Dtsch Arztebl Int. 2014;111(7):101-106.
2. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. CA Cancer J Clin. 2016;66(2):115-132.
3. Nakagawa S, Wei L, Song WM, et al. Molecular liver cancer prevention in cirrhosis by organ transcriptome analysis and lysophosphatic acid pathway inhibition. Cancer Cell. 2016;30(6):879-890.
4. Marrero JA, Kulik LM, Sirlin CB, et al. Diagnosis, staging, and Management of Hepatocellular Carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases. Hepatology. 2018;68(2):723-750.
5. Dimitroulis D, Damaskos C, Valsami S, et al. From diagnosis to treatment of hepatocellular carcinoma: an epidemic problem for both developed and developing world. World J Gastroenterol. 2017;23(29):5282-5294.
6. Kerr JF. A histochemical study of hypertrophy and ischaemic injury of rat liver with special reference to changes in lysosomes. J Pathol Bacteriol. 1965;90(2):419-435.
7. Mou Y, Wang J, Wu J, et al. Ferroptosis, a new form of cell death: opportunities and challenges in cancer. J Hematol Oncol. 2019;12(1):34.
8. Sun X, Ou Z, Chen R, et al. Activation of the p62-Keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells. Hepatology. 2016;63(1):173-184.
9. Kanai Y, Hediger MA. The glutamate/neutral amino acid transporter family SLC1: molecular, physiological and pharmacological aspects. Pflogers Arch. 2004;447(5):469-479.
10. Luo M, Wu L, Zhang K, et al. mir-137 regulates ferroptosis by targeting glutamate transporter SLC1A5 in melanoma. Cell Death Differ. 2018;25(8):1457-1472.
11. Jin H, Wang S, Zaal EA, et al. A powerful drug combination strategy targeting glutamine addiction for the treatment of human liver cancer. eLife. 2020;9:e56749.
12. Suwazono S, Arao H. A newly developed free software tool set for averaging electroencephalogram implemented in the Perl programming language. Heliyon. 2020;6(11):e05580.
13. Servant N, Gravier E, Gerstaud P, et al. EMA - a R package for easy microarray data analysis. BMC Res Notes. 2010;3:277.
14. Ritchie ME, Phipson B, Wu D, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43(7):e47.
15. Zhou N, Bao J. FerrDb: a manually curated resource for regulators and markers of ferroptosis and ferroptosis-disease associations. Database (Oxford). 2020;2020;baaa021.
16. Rhodes DR, Yu J, Shanker K, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia. 2004;6(1):1-6.
17. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. Omics. 2012;16(5):284-287.
18. Gene Ontology C. The gene ontology project in 2008. Nucleic Acids Res. 2008;36(Database issue):D440-D444.
19. Ashburner M, Ball CA, Blake JA, et al. Gene ontology: tool for the unification of biology. The gene ontology consortium. Nat Genet. 2000;25(1):25-29.
20. Liberzon A, Birger C, Thorvaldsdottir H, Ghandi M, Mesirov JP, Tamayo P. The molecular signatures database (MSigDB) hallmark gene set collection. Cell Syst. 2015;1(6):417-425.
21. Reimand J, Isserlin R, Voisin V, et al. Pathway enrichment analysis and visualization of omics data using gprofiler, GSEA, Cytoscape and EnrichmentMap. Nat Protoc. 2019;14(2):482-517.
22. Sun W, Shi H, Yuan Z, et al. Prognostic value of genes and immune infiltration in prostate tumor microenvironment. Front Oncol. 2020;10:584055.
23. Ozdemir F, Baskiran A. The importance of AFP in liver transplantation for HCC. J Gastroinest Cancer. 2020;51(4):1127-1132.
24. Wang X, Wang Q. Alpha-fetoprotein and hepatocellular carcinoma staging system with treatment stratification for patients with hepatocellular carcinoma. Gastroenterology. 2014;146(7):1691-700 e3.
25. Folkman J. Role of angiogenesis in tumor growth and metastasis. Semin Oncol. 2002;29(6 suppl 16):15-18.
26. Mokdad AA, Singal AG, Marrero JA, Zhu H, Yopp AC. Vascular invasion and metastasis is predictive of outcome in Barcelona clinic liver
cancer stage C hepatocellular carcinoma. *J Natl Compr Canc Netw.* 2017;15(2):197-204.

29. Tvrzicka E, Kremmyda LS, Stankova B, Zak A. Fatty acids as bio-compounds: their role in human metabolism, health and disease—a review. Part 1: classification, dietary sources and biological functions. *Biomed Pap Med Fac Uni Palacky Olomouc Czech Repub.* 2011;155(2):117-130.

30. Hu B, Lin JZ, Yang XB, Sang XT. Aberrant lipid metabolism in hepatocellular carcinoma cells as well as immune microenvironment: a review. *Cell Prolif.* 2020;53(3):e12772.

31. Wang R, Xiang W, Xu Y, et al. Enhanced glutamine utilization mediated by SLC1A5 and GPT2 is an essential metabolic feature of colorectal signet ring cell carcinoma with therapeutic potential. *Ann Transl Med.* 2020;8(6):302.

32. Altman BJ, Stine ZE, Dang CV. From Krebs to clinic: glutamine metabolism to cancer therapy. *Nat Rev Cancer.* 2016;16(10):619–634.

33. Fuchs BC, Finger RE, Onan MC, Bode BP. ASCT2 silencing regulates mammalian target-of-rapamycin growth and survival signaling in human hepatoma cells. *Am J Physiol Cell Physiol.* 2007;293(1):C55-C63.

34. Zhang H, Cui K, Yao S, Yin Y, Liu D, Huang Z. Comprehensive molecular and clinical characterization of SLC1A5 in human cancers. *Pathol Res Pract.* 2021;224:153525.

35. Lin J, Yang T, Peng Z, et al. SLC1A5 silencing inhibits esophageal cancer growth via cell cycle arrest and apoptosis. *Cell Physiol Biochem.* 2018;48(1):397.

36. Ma H, Wu Z, Peng J, et al. Inhibition of SLC1A5 sensitizes colorectal cancer to cetuximab. *Int J Cancer.* 2018;142(12):2578-2588.

37. Yang L, Tian S, Chen Y, et al. Ferroptosis-related gene model to predict overall survival of ovarian carcinoma. *J Oncol.* 2021;2021:6687391.

38. Bjersand K, Seidal T, Sundstrom-Poromaa I, Akerud H, Skirnisdottir I. The clinical and prognostic correlation of HRNPM and SLC1A5 in pathogenesis and prognosis in epithelial ovarian cancer. *PLoS One.* 2017;12(6):e0179363.

39. Kanai Y, Clemenson B, Simonin A, et al. The SLC1 high-affinity glutamate and neutral amino acid transporter family. *Mol Aspects Med.* 2013;34(2–3):108-120.

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