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ORIGINAL ARTICLE

Basic Study

Comprehensive prognostic and immune analysis of sterol O-acyltransferase 1 in patients with hepatocellular carcinoma

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Abstract

BACKGROUND

Sterol O-acyltransferase 1 (SOAT1) is an important target in the diagnosis and treatment of liver cancer. However, the prognostic value of SOAT1 in patients with hepatocellular carcinoma (HCC) is still not clear.

AIM

To investigate the correlation of SOAT1 expression with HCC, using RNA-seq and gene expression data of The Cancer Genome Atlas (TCGA)-liver hepatocellular carcinoma (LIHC) and pan-cancer.

METHODS

The correlation between SOAT1 expression and HCC was analyzed. Cox hazard regression models were conducted to investigate the prognostic value of SOAT1 in HCC. Overall survival and disease-specific survival were explored based on TCGA-LIHC data. Biological processes and functional pathways mediated by SOAT1 were characterized by gene ontology (GO) analysis and the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of differentially expressed genes. In addition, the protein-protein interaction network and co-expression analyses of SOAT1 in HCC were performed to better understand the regulatory mechanisms of SOAT1 in this malignancy.

RESULTS
SOAT1 and SOAT2 were highly expressed in unpaired samples, while only SOAT1 was highly expressed in paired samples. The area under the receiver operating characteristic curve of SOAT1 expression in tumor samples from LIHC patients compared with para-carcinoma tissues was 0.748, while the area under the curve of SOAT1 expression in tumor samples from LIHC patients compared with GTEx was 0.676. Patients with higher SOAT1 expression had lower survival rates. Results from GO/KEGG and gene set enrichment analyses suggested that the PI3K/AKT signaling pathway, the IL-18 signaling pathway, the calcium signaling pathway, secreted factors, the Wnt signaling pathway, the Jak/STAT signaling pathway, the MAPK family signaling pathway, and cell–cell communication were involved in such association. SOAT1 expression was positively associated with the abundance of macrophages, Th2 cells, T helper cells, CD56^{bright} natural killer cells, and Th1 cells, and negatively linked to the abundance of Th17 cells, dendritic cells, and cytotoxic cells.

CONCLUSION
Our findings demonstrate that SOAT1 may serve as a novel target for HCC treatment, which is helpful for the development of new strategies for immunotherapy and metabolic therapy.

Key Words: Sterol O-acyltransferase 1; Hepatocellular carcinoma; Prognostic; Immune

Core Tip: As patients would greatly benefit from early detection of hepatocellular carcinoma, the complementary study of hepatocellular carcinoma-associated proteins in serum samples using state-of-the-art proteomics would be a very attractive direction for future exploration.

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INTRODUCTION

Liver cancer is one of the leading causes of death worldwide. Hepatocellular carcinoma (HCC) is the most devastating type of liver cancer[1], commonly diagnosed at an advanced stage, with a high rate of mortality and aggressive clinical course. The well-known risk factors for HCC include age, sex, alcohol consumption/abuse, environmental toxins, aflatoxin exposure, chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, and non-alcoholic fatty liver disease[2].

Liver transplantation, radical surgical resection, and radiofrequency ablation are commonly used in early-stage HCC. However, the majority of patients do not meet the criteria for radical treatment and are treated with systemic or local treatment instead[3]. Advanced HCC always presents a poor prognosis, although several new treatment modalities, such as immunotherapy and trans-arterial chemoembolization plus systemic treatments, have been proposed[4-6]. Therefore, exploring effective therapeutic targets for HCC is of great importance to both individuals and society.

Sterol O-acyltransferase (SOAT), known as acyl-CoA:cholesterol acyltransferase (ACAT), is located in the endoplasmic reticulum membrane. It plays an important role in cholesterol homeostasis and bile acid biosynthesis by catalyzing the conversion of cholesterol to cholesterol esters[7]. There are two SOAT isoforms in mammals, namely, SOAT1 and SOAT2. SOAT1 is a key enzyme with high expression levels. It is generally expressed in all tissues except the intestine and plays an important role by converting endoplasmic reticulum cholesterol into lipid droplet (LD) stored esters[8,9]. High SOAT1 expression has been shown in several tumor types (such as liver cancer, pancreatic cancer, and prostate cancer[10,11]) and associated with diagnosis and treatment[12-14]. Up-regulation of SOAT1 could further increase the expression levels of inflammatory factors and cause cardiovascular diseases such as atherosclerosis and coronary heart disease[15-17]. Cholesterol ester increases HCC growth by promoting the synthesis of phospholipids and hormones[18-21]. Proteomic evidence from early-stage HBV-HCC patients showed that HCC patients with more aggressive tumors and poor prognosis had disrupted cholesterol metabolism and increased SOAT1 expression[19]. The single nucleotide polymorphisms of SOAT1 have been closely related to cholesterol metabolism[22,23].

However, the relationship of SOAT1 expression with HCC remains unclear. In the current study, we explored whether SOAT1 is involved in the development of HCC, as well as the regulatory mechanisms of SOAT1[17]. Moreover, we further explored various biological processes and signaling pathways *via* which SOAT1 may potentially be involved in the pathogenesis of HCC.

MATERIALS AND METHODS

Microarray data and data processing

The RNA-seq and gene expression data of The Cancer Genome Atlas (TCGA)-liver hepatocellular carcinoma (LIHC) and pan-cancer, including unpaired samples and paired samples, were extracted, filtered to remove missing and duplicated results, and transformed by $\log_2$ (*TPM* + 1) using the Xiantao tool (www.xiantao.love). SOAT1 gene expression was also analyzed using Clinical Proteomic Tumor Analysis Consortium samples. $P < 0.05$ was regarded as significant.

Prognostic value of SOAT1 expression

To investigate the prognostic value of SOAT1 expression, Cox proportional hazard regression models were generated to describe patients' characteristics, including SOAT1 and SOAT2 expression levels and TNM stages. Overall survival (OS) and disease-specific survival (DSS) were also explored based on TCGA-LIHC data. *P* value < 0.05 was regarded as significant. To further investigate the prognostic value of SOAT1 expression, a nomogram and calibration curves were generated.

Diagnostic value of SOAT1 expression

Receiver operation characteristic curve analysis was conducted to explore the diagnostic value of SOAT1 expression in TCGA-LIHC with and without GTEx and the area under the receiver operating characteristic curve (AUC) was calculated using the "*pROC*" package.

Subgroup analysis

To validate the potential effects of SOAT1 expression on TCGA-LIHC progression, SOAT1 expression was determined in subgroups based on age, sex, and tumor stage. The RNA-seq data and related clinical data in level 3 HTSeq-fragments per kilobase per million mapped fragments formats were downloaded from the TCGA database, converted to transcripts per million formats, and then analyzed after log transformation. *P* value < 0.05 was considered as the cutoff criterion.

Association of SOAT1 expression with immune cells

To analyze the relationship between SOAT1 expression and immune cells, single sample gene set enrichment analysis (GSEA) (the "*GSVA*" package in R) was performed, providing a critical assessment and integration of 24 immune cells for RNA-seq samples from TCGA-LIHC.

Differentially expressed genes between SOAT1 high and low expression groups

The differentially expressed genes (DEGs) between groups with different SOAT1 expression (cut-off value: 50%) in TCGA-LIHC were identified. Utilizing Limma, $\log_2$ (*fold change*) > 2 and *P* value < 0.05 were applied as the cut-off criteria.

Enrichment analysis

Gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were conducted to investigate the DEGs between the high and low SOAT1 expression groups in TCGA-LIHC. GSEA was conducted utilizing the "*clusterProfiler*" package in R. *P* value < 0.05 was applied as the cut-off criterion.

Protein–protein interaction and the hub genes

To investigate the proteins that interact with SOAT1, the STRING database (https://string-db.org) was analyzed with a combined score of > 0.4. The nodes were analyzed with Cytoscape version 3.7.1. Protein–protein interaction (PPI) network analysis was conducted to obtain the hub genes using the Cytoscape plug-in MCODE.

Prognostic value of SOAT1 expression in TCGA-LIHC

Lasso regression and risk score analysis were performed to investigate the association between SOAT1 expression, hub genes, and patient status. The association between survival and hub genes was analyzed to further show the prognostic value of SOAT1 expression in TCGA-LIHC.

RESULTS

SOAT1 is highly expressed in LIHC patients

In the TCGA-LIHC cohort, SOAT1 and SOAT2 were highly expressed in unpaired samples, while only SOAT1 was highly expressed in paired samples (Figure 1A and B). The univariate analysis and multivariate analysis suggested that SOAT1 expression was an independent risk factor for HCC progression (Figure 1C; Supplementary Table 1). SOAT1 expression in pan-cancer, including unpaired and paired samples, was also investigated (Figure 1D and E).

Diagnostic and prognostic value of SOAT1 expression

To explore the diagnostic value of SOAT1 expression in HCC, we performed receiver operating characteristic curve analysis. The AUC of SOAT1 expression in tumor samples from LIHC patients compared with para-carcinoma tissues was 0.748, while the AUC of SOAT1 expression in tumor samples from LIHC patients compared with GTEx was 0.676,

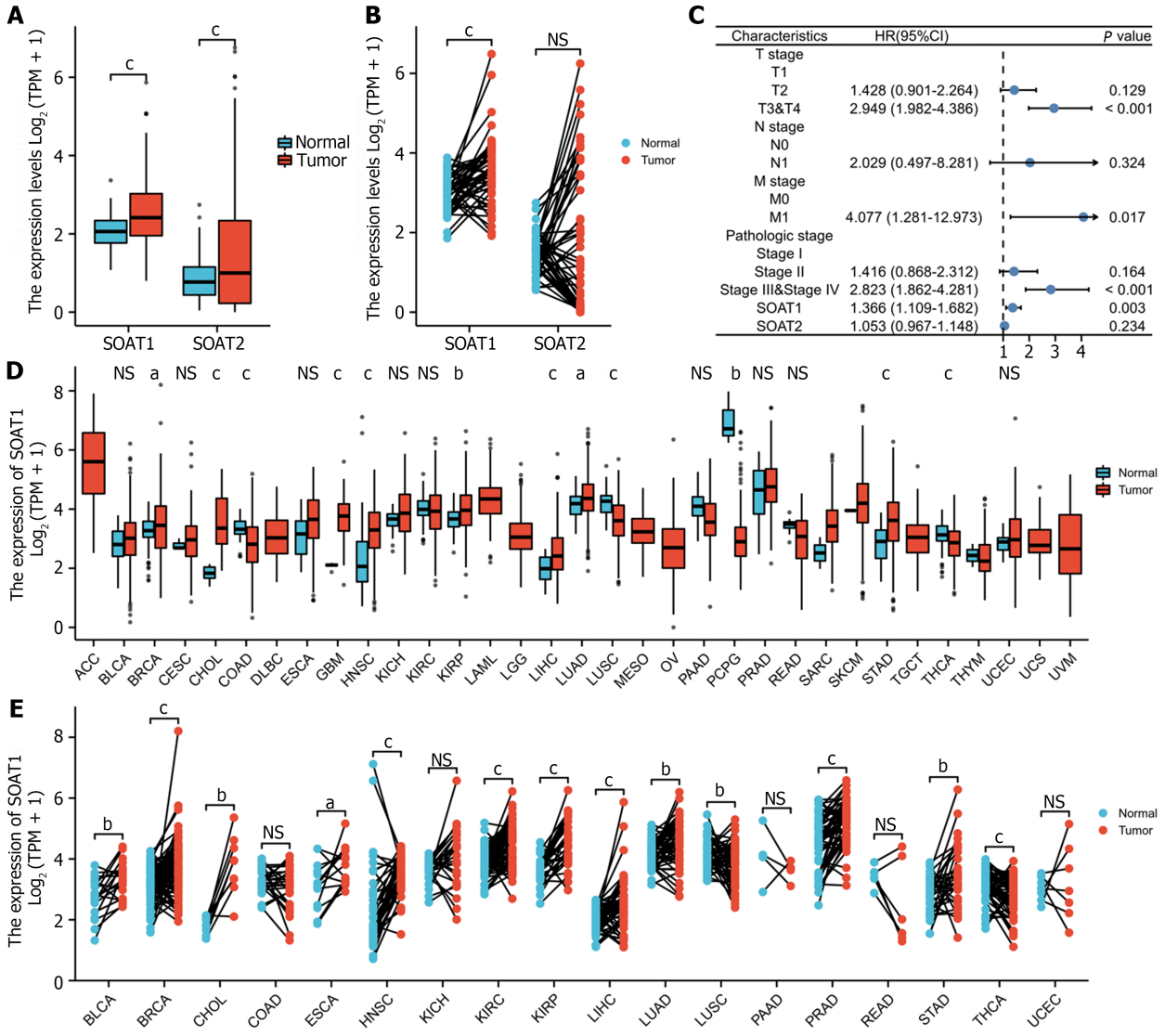

Figure 1 Expression of sterol O-acyltransferase 1 and sterol O-acyltransferase 2 in The Cancer Genome Atlas-liver hepatocellular carcinoma. A: Sterol O-acyltransferase 1 (SOAT1) and SOAT2 expression in the unpaired samples in The Cancer Genome Atlas (TCGA) liver hepatocellular carcinoma TCGA-LIHC; B: SOAT1 and SOAT2 expression in the paired samples in TCGA-LIHC; C: Forest diagram of univariate analysis of patients' characteristics and SOAT1 expression; D: SOAT1 expression in the unpaired samples in TCGA pan-cancer; E: SOAT1 expression in the paired samples (E) in TCGA pan-cancer. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$; NS: Not significant.

suggesting that SOAT1 may be a potential diagnostic biomarker for HCC invasion (Figure 2A and B).

To clarify the prognostic value of SOAT1 expression in HCC, OS and DSS were analyzed. Patients with higher SOAT1 expression had lower survival rates (Figure 2C and D). SOAT1 expression was also associated with age, gender, histologic grade, T stage, and N stage (Figure 3). In addition, 1-, 3-, and 5-year OS and DSS analysis demonstrated that higher SOAT1 expression was associated with a worse prognosis (Figure 4).

DEGs between groups with high and low SOAT1 expression

After log transformation, DEGs between the group with high and low expression of SOAT1 in LIHC were identified. GO enrichment analysis, KEGG pathway enrichment analysis, and GSEA showed that these DEGs are mainly involved in the PI3K/AKT signaling pathway, the IL-18 signaling pathway, the calcium signaling pathway, secreted factors, the Wnt signaling pathway, the Jak/STAT signaling pathway, the MAPK family signaling pathway, and cell–cell communication (Figure 5).

SOAT1 expression and immune cell analysis

Compared with healthy controls, patients with primary tumor showed significantly increased protein expression of SOAT1 (Supplementary Figure 1). To further analyze the association between SOAT1 expression and immune cells, single sample GSEA was conducted in LIHC, which showed that SOAT1 expression was positively associated with the abundance of macrophages, Th2 cells, T helper cells, $CD56^{bright}$ natural killer (NK) cells, and Th1 cells and negatively associated with the abundance of Th17 cells, dendritic cells, and cytotoxic cells (Supplementary Figure 2).

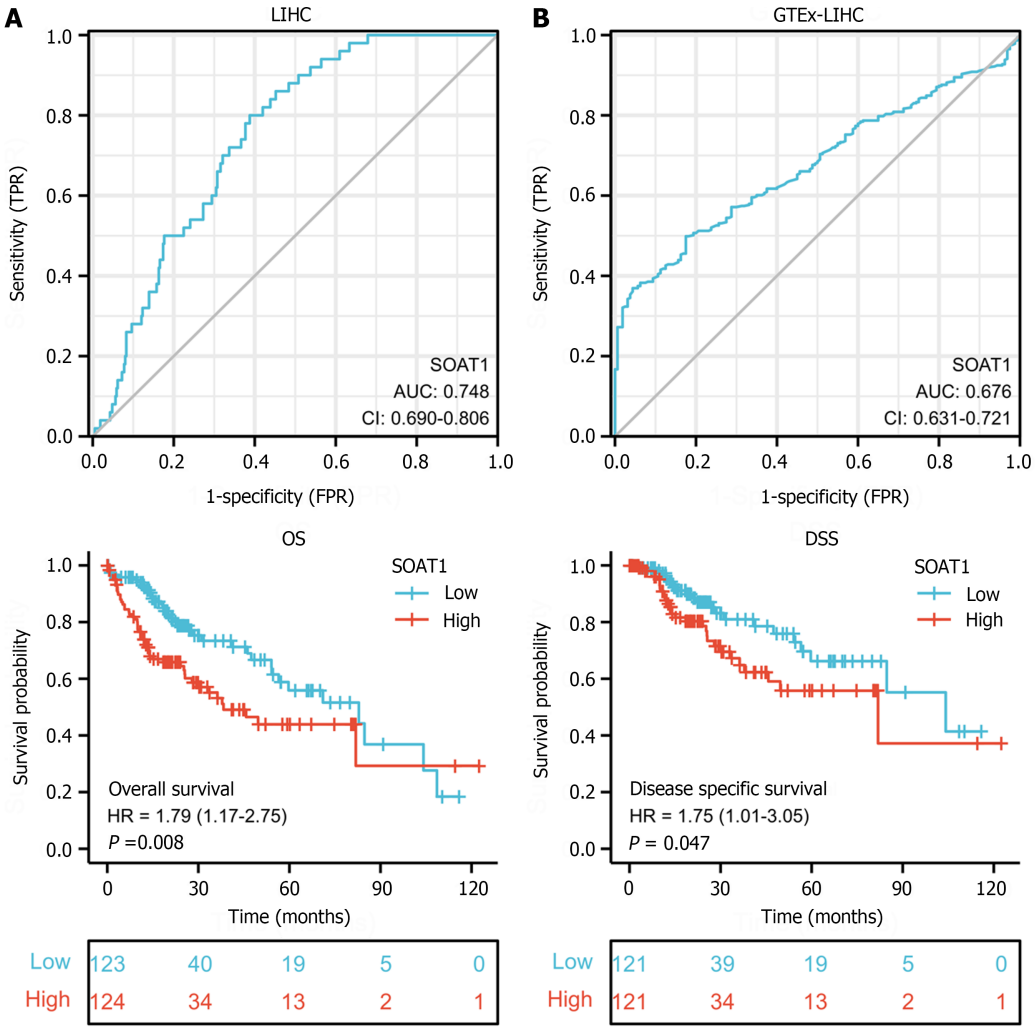

Figure 2 Diagnostic and prognostic value of sterol O-acyltransferase 1 expression in The Cancer Genome Atlas-liver hepatocellular carcinoma. A: Area under the curve (AUC) of sterol O-acyltransferase 1 (SOAT1) expression in tumor samples from liver hepatocellular carcinoma (LIHC) patients compared with para-carcinoma tissues; B: AUC of SOAT1 expression in tumor samples from LIHC patients compared with GTEx; C: Association between OS and SOAT1 expression demonstrated the prognostic value of SOAT1 expression in The Cancer Genome Atlas (TCGA)-LIHC; D: Association between DSS and SOAT1 expression demonstrated the prognostic value of SOAT1 expression in TCGA-LIHC.

PPI network and hub genes

To clarify the proteins that interact with SOAT1 in TCGA-LIHC, the nodes with a comprehensive score more than 0.4 were studied using the STRING database. The hub genes were obtained from the Cytoscape plug-in MCODE, which included two modules in the network (including *CYP19A1*, *CYP2A6*, *CYP1A2*, *CYP1A1*, *UGT1A10*, *KLK3*, *KRT19*, and *CEACAM5*). These genes might be potential targets for HCC treatment (Figure 6).

Effects of SOAT1 and hub genes on LIHC

To investigate the role of SOAT1 expression in LIHC progression, Lasso regression and risk score analysis were utilized. SOAT1 expression was highly correlated with survival time and with the expression of two hub genes, namely, *CYP19A1* and *UGT1A10* (Figure 7A and B). To further explore the prognostic value of these two hub genes, survival analysis was conducted, which showed that patients with higher expression of *CYP19A1* and *UGT1A10* had a worse prognosis, which was consistent with the prognostic value of SOAT1 expression.

DISCUSSION

Historically, chronic viral hepatitis was the main etiologies of HCC; however, nonalcoholic fatty liver disease and related metabolic factors have emerged as the fastest-growing risk factors for HCC in recent years. The relationship between lipids and HCC is complex, so more investigations are anticipated to continue over the next decade. Understanding the role of cholesterol in HCC development will contribute to developing new therapies. One way to further our understanding of the mechanisms that promote carcinogenesis is through analysis of the proteome[24]. Previously, a system-wide approach was adopted to reveal changes in DNA, protein expression, and phenotype in liver cancer tissue,

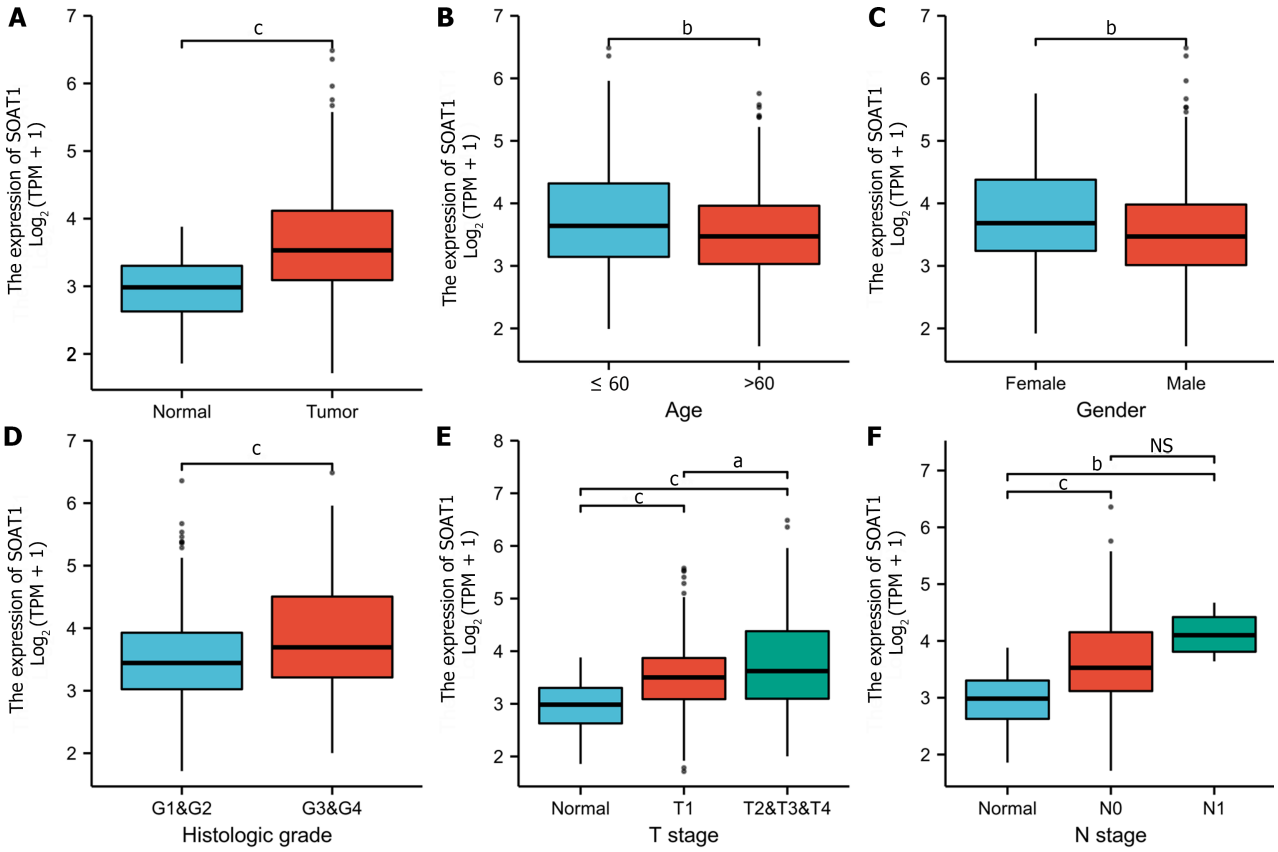

Figure 3 Subgroup analysis of sterol O-acyltransferase 1 expression in liver hepatocellular carcinoma. A: Sterol O-acyltransferase 1 (SOAT1) expression in tumor and normal tissues; B: Association between SOAT1 expression and age; C: Association between SOAT1 expression and gender; D: Association between SOAT1 expression and histologic grade; E: Association between SOAT1 expression and T stage; F: Association between SOAT1 expression and N stage (F). $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$; NS: Not significant.

identifying SOAT1 as a potential biomarker for early-stage HCC. SOAT1 was found to be overexpressed in HCC and to be an independent risk factor for HCC progression[19]. In fact, an increasing body of evidence demonstrates a strong relationship of the tumor metabolic microenvironment with immune microenvironment. In the current study, we found that in HCC, SOAT1 expression was positively linked to the abundance of macrophages, Th2 cells, T helper cells, CD56^{bright} NK cells, and Th1 cells, and negatively associated with the abundance of Th17 cells, dendritic cells, and cytotoxic cells.

Previous studies have shown lower lipid levels in HCC patients compared to healthy controls[23], suggesting that cholesterol metabolism plays a pivotal role in the development of HCC[25,26]. Evidence from proteomic studies have found that HCC patients with abnormal cholesterol metabolism and high SOAT1 expression seemed to have a worse prognosis[19], suggesting that SOAT1 may have an effect on HCC by regulating lipid metabolism. A recent study has found that extracellular lipid loading promoted glioma-associated macrophage infiltration and new blood vessel formation in tumors, which was increased by an elevated continuous supply of lipids throughout the body[27]. It is direct evidence that LD^{+} glioblastoma cells are related to immunosuppressive glioma-associated macrophage infiltration. Since LDs are formed due to the aggregation of cholesterol esters, it is not surprising that SOAT1 expression is associated with M2 macrophage infiltration in HCC. There is a complex relationship between lipids and HCC. Altered lipid metabolism may be a result of HCC development. Cachexia commonly exists in cancer patients, characterized by reduced fat storage, increased carbohydrate utilization, and elevated protein degradation. The high growth rate of cancer cells may lead to hypoxia and increased energy requirements, ultimately promoting fatty-acid oxidation and depleting fat stores[28,29]. In addition, dysregulation of lipid metabolism may contribute to the development of HCC, due to impaired pro-tumorigenic insulin and insulin-like growth factor 1 signaling[30,31]. Additionally, research in mice and humans has showed that liver cells without fatty acid synthase might support c-MET oncogene-mediated liver tumor formation through up-regulation of SREBP2 *via* the cholesterol synthesis pathway[32].

Studies have demonstrated that SOAT1 plays a carcinogenic role through multiple pathways. Our OS and DSS analyses also showed that higher SOAT1 expression was associated with poor survival in patients with HCC. Therefore, further studies are warranted to explore the prognostic value of SOAT1 in HCC. Indeed, SOAT1 expression is associated with a poor prognosis in all HCC cases. Our 1-, 3- and 5-year OS and DSS analyses demonstrated that higher SOAT1 expression was associated with a worse prognosis (Figure 4), suggesting that SOAT1 may be a potential diagnostic biomarker for HCC invasion. Down-regulation of SOAT1 has been reported to inhibit proliferation and migration of HCC cells by reducing plasma membrane cholesterol content and inhibiting the integrin and TGF-β signaling pathways[19]. Consistently, integrin binding was also significantly enhanced, as determined by enrichment analysis of the GO and

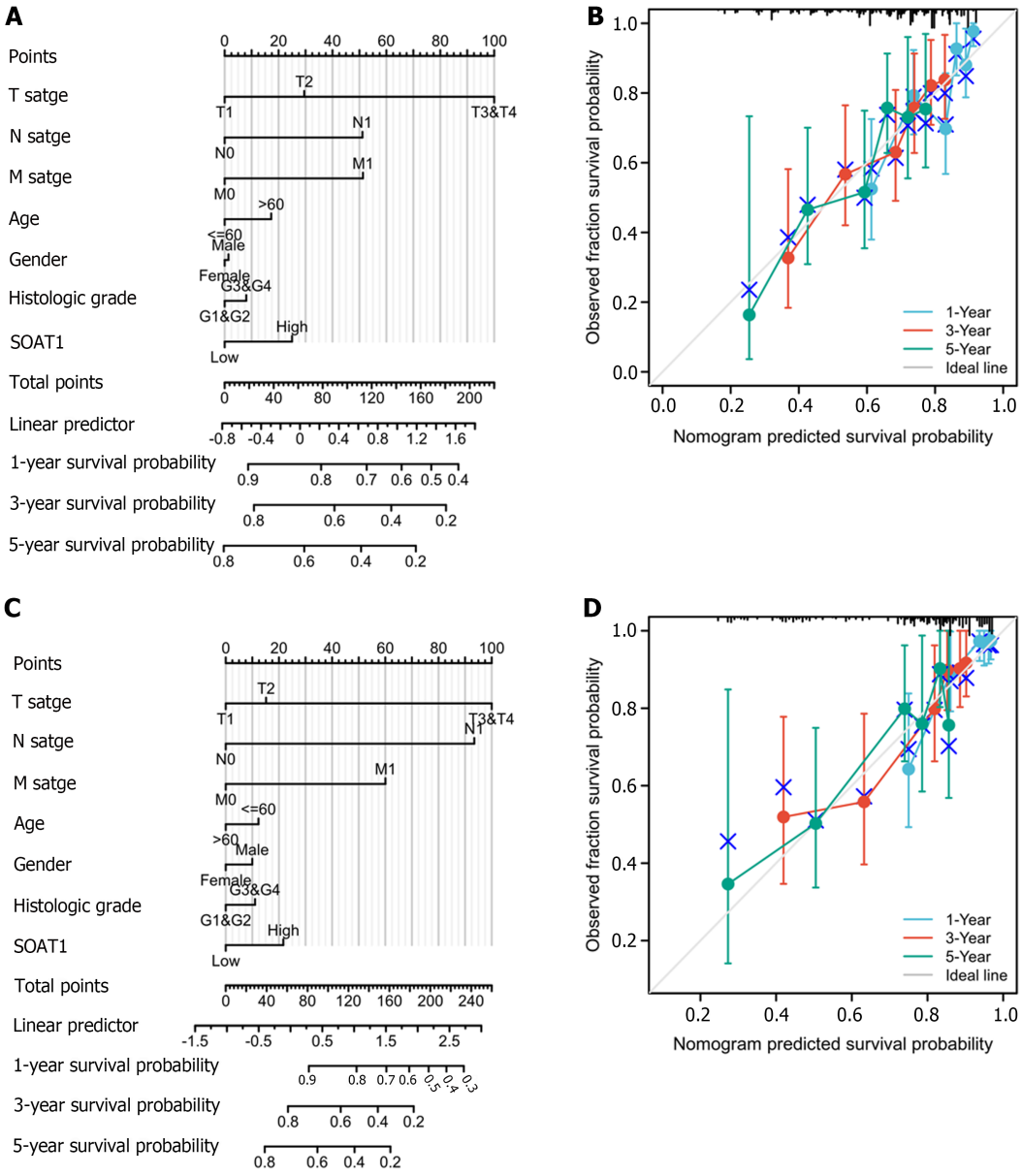

Figure 4 Prognostic value of sterol O-acyltransferase 1 expression in liver hepatocellular carcinoma. A: Nomograms for 1-, 3-, and 5-year overall survival in different subgroups based on sterol O-acyltransferase 1 (SOAT1) expression and other clinical characteristics in liver hepatocellular carcinoma (LIHC); B: Calibration for 1-, 3-, and 5-year overall survival in different subgroups based on SOAT1 expression and other clinical characteristics in LIHC; C: Nomograms for 1-, 3-, and 5-year disease specific survival in different subgroups based on SOAT1 expression and other clinical characteristics in LIHC; D: Calibration for 1-, 3-, and 5-year disease specific survival in different subgroups based on SOAT1 expression and other clinical characteristics in LIHC.

KEGG pathways of upregulated DEGs in HCC (Figure 5). Multiple genes, including *CYP19A1*, *CYP2A6*, *CYP1A2*, *CYP1A1*, *UGT1A10*, *KLK3*, *KRT19*, and *CEACAM5* (Figure 6), whose encoded proteins may interact with SOAT1 in HCC, were identified *via* PPI network and co-expression analyses, which may be potential targets for HCC treatment. The higher the expression of *CYP19A1* and *UGT1A10*, the worse the prognosis, which is consistent with the prognostic analysis of SOAT1 expression. SOAT1 expression was reported to be regulated by multiple mechanisms in tumors. Runt-related transcription factor 1 promotes SOAT1 expression in squamous cell carcinoma by binding to the promoter region of *SOAT1*[33]. Loss of p53 heterozygosity can promote the expression of SOAT1 by enhancing the transcription of *SOAT1* in pancreatic ductal adenocarcinoma[10]. In addition, β-catenin has been reported to be directly bind to the *SOAT1* promoter element and promote its transcription in colorectal cancer[21], as well.

CONCLUSION

The progression of HCC is complex and several factors are involved, including age, alcohol consumption, environmental toxins, HBV and HCV levels, and diet. In the present study, the prognostic value of SOAT1 in HCC was elucidated. Our findings suggest that SOAT1 may modestly alter the risk for HCC by regulating lipid metabolism, but the effect might be

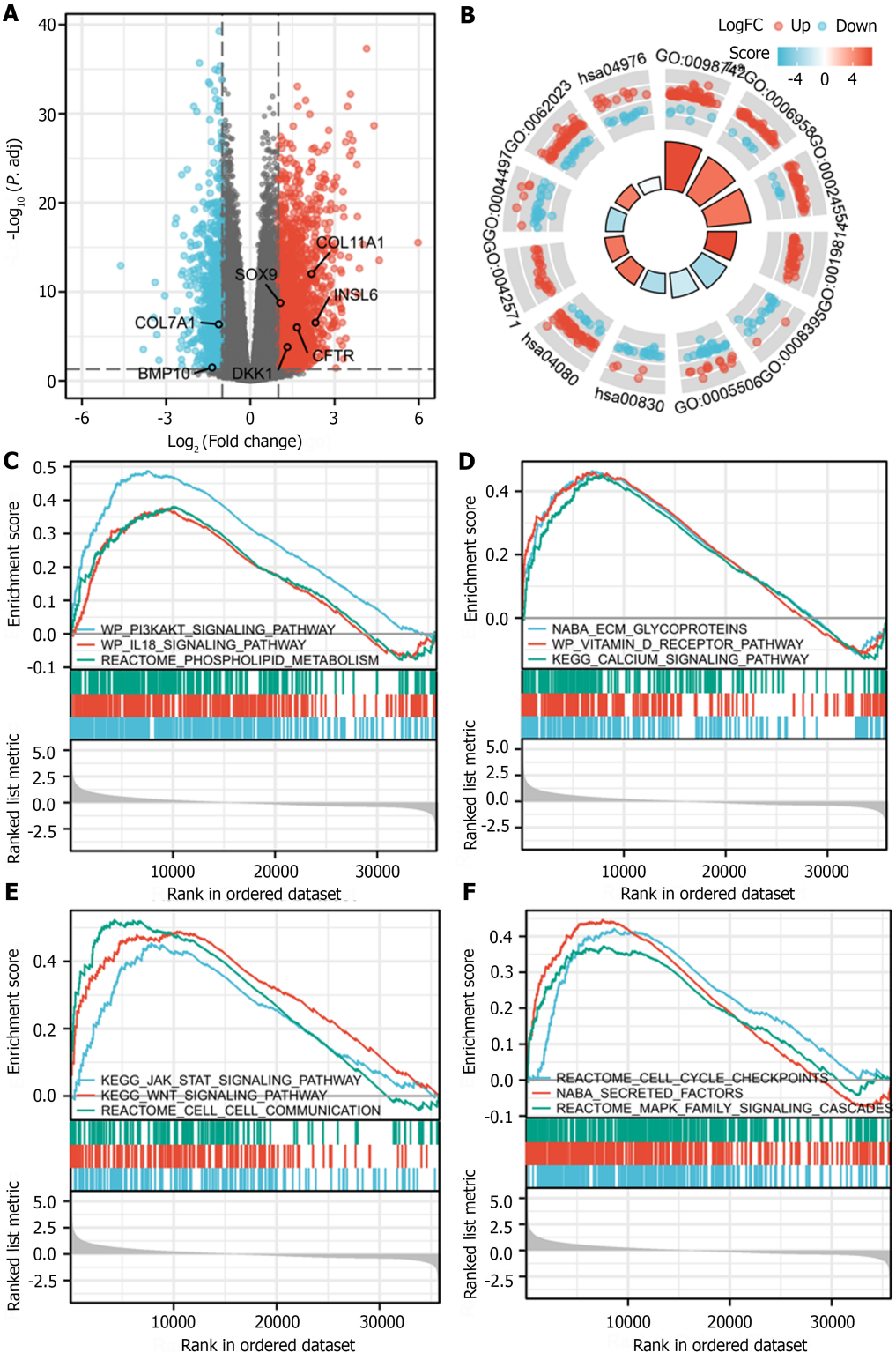

Figure 5 Differentially expressed genes between high and low sterol O-acyltransferase 1 expression groups in liver hepatocellular carcinoma. A: Volcano plot of differentially expressed genes (DEGs) between high and low sterol O-acyltransferase 1 (SOAT1) expression groups in liver hepatocellular carcinoma (LIHC); B: Top GO terms and KEGG pathways enriched by DEGs between high and low SOAT1 expression groups in LIHC; C-F: Gene set enrichment analysis of DEGs between high and low SOAT1 expression groups in LIHC.

limited. Further studies are warranted to validate our results. The identification of other HCC proteins involved in this multigenic heterogeneous cancer type is an important objective for future research. Since early diagnosis of HCC is of great benefit to patients, complementary studies using the most advanced proteomic techniques on HCC-related proteins in serum samples can be a very attractive research direction in the future. That is, SOAT1 may be recognized as a new target to advance the development of immunotherapy and metabolic therapy.

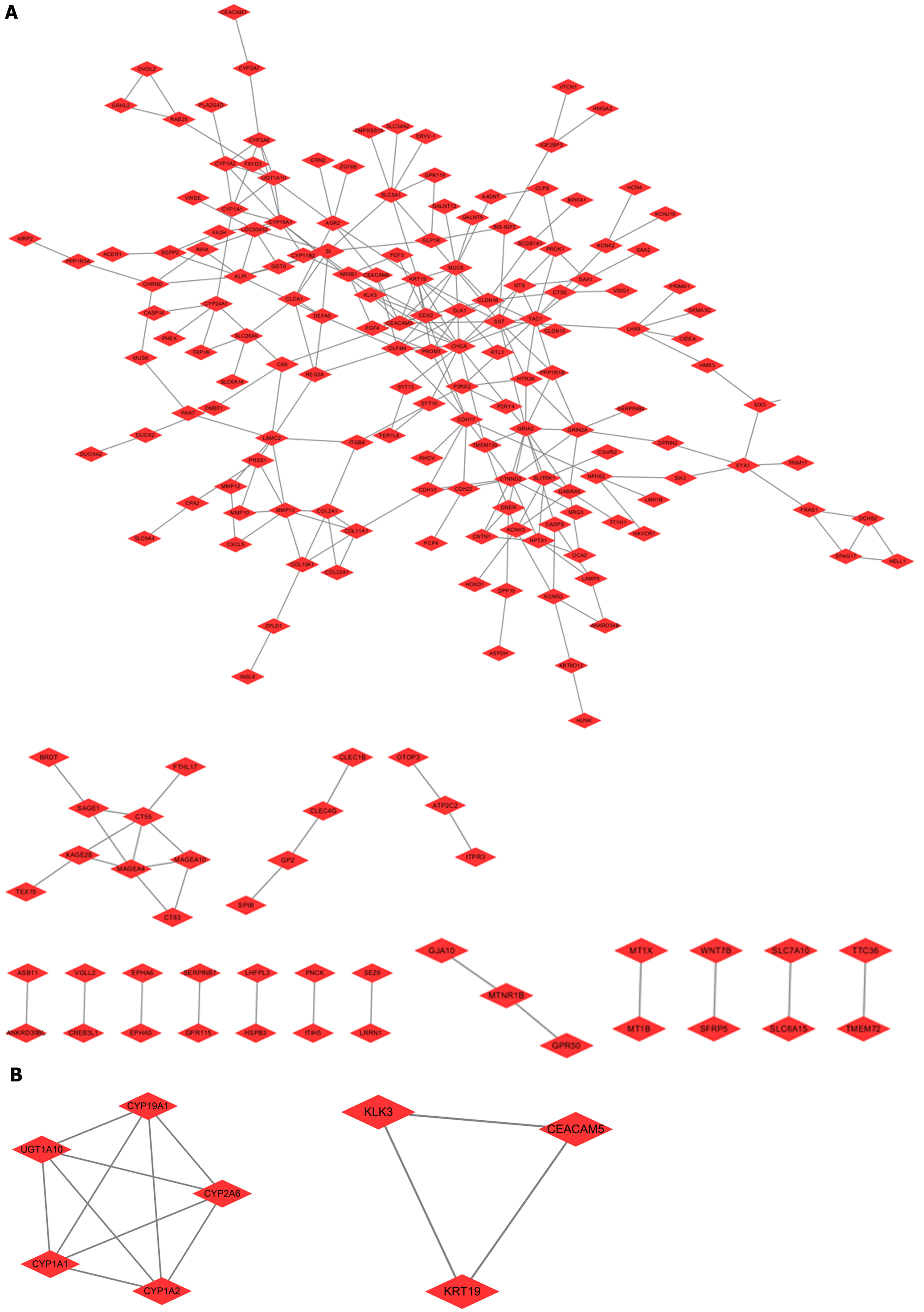

Figure 6 Protein–protein interaction network and hub genes of differentially expressed genes between the high and low sterol O-

acyltransferase 1 expression groups. A: Protein–protein interaction network of differentially expressed genes between high and low sterol O-acyltransferase 1 expression groups; B: Hub genes (two modules) screened using the Cytoscape plugin MCODE.

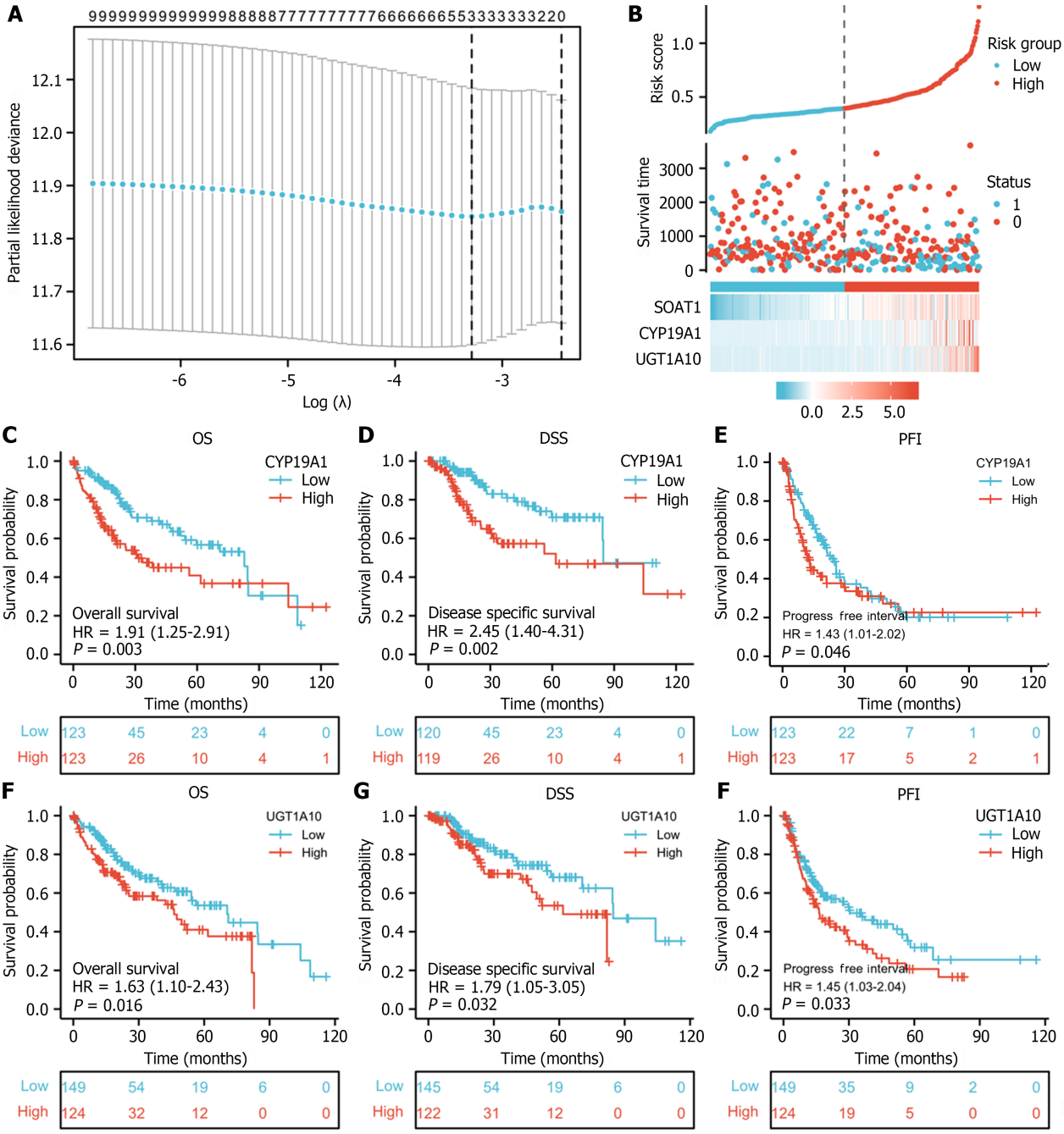

Figure 7 Effects of sterol O-acyltransferase 1 and hub genes on liver hepatocellular carcinoma. A: Lasso regression of survival time and sterol O-acyltransferase 1 (SOAT1) and hub gene expression levels in liver hepatocellular carcinoma (LIHC); B: Risk score analysis of survival time and SOAT1 and hub gene expression levels in LIHC. 1, survival; 0, dead; C: Overall survival (OS) of LIHC patients between high and low CYP19A1 expression groups; D: Disease-specific survival (DSS) of LIHC patients between high and low CYP19A1 expression groups; E: Progression free interval of LIHC patients between high and low CYP19A1 expression groups; F: OS of LIHC patients between high and low UGT1A10 expression groups; G: DSS of LIHC patients between high and low UGT1A10 expression groups: H: Progression free interval of LIHC patients between high and low UGT1A10 expression groups. OS: Overall survival; DSS: Disease specific survival; PFI: Progression free interval.

ARTICLE HIGHLIGHTS

Research background

Hepatocellular carcinoma (HCC) has a poor prognosis and heavy disease burden, but its treatment methods are not satisfactory.

Research motivation

High sterol O-acyltransferase 1 (SOAT1) expression has been shown to be associated with several tumor types (liver cancer, pancreatic cancer, and prostate cancer) and with diagnosis and treatment. However, the relationship between SOAT1 expression and HCC remains unclear. As patients would greatly benefit from early detection of HCC, the complementary study of HCC-associated proteins in serum samples using state-of-the-art proteomics would also be a very attractive direction for future research. Therefore, SOAT1 may serve as a novel target that drives the development of immunotherapy and metabolic therapy.

Research objectives

This study aimed to investigate the correlation between SOAT1 expression and HCC, using RNA-seq and gene expression data of The Cancer Genome Atlas (TCGA)-liver hepatocellular carcinoma (LIHC) and pan-cancer. Our findings demonstrate that SOAT1 may serve as a new target for HCC treatment and promote the development of new strategies for immunotherapy and metabolic therapy.

Research methods

The correlation between SOAT1 expression and HCC was analyzed. Cox hazard regression models were used to investigate the prognostic value of SOAT1. Overall survival and disease-specific survival were also explored in TCGA-LIHC. Moreover, the biological processes and functional pathways regulated by SOAT1 were characterized using gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of differentially expressed genes. To better understand the regulatory mechanism of SOAT1 in HCC, protein–protein interaction network and co-expression analyses of SOAT1 in HCC were conducted.

Research results

SOAT1 and SOAT2 were highly expressed in unpaired samples, while only SOAT1 was highly expressed in paired samples. The area under the receiver operating characteristic curve of SOAT1 expression in tumor samples from LIHC patients compared with para-carcinoma tissues was 0.748, while the area under the curve of SOAT1 expression in tumor samples from LIHC patients compared with GTEx was 0.676. Patients with higher SOAT1 expression had lower survival rates. Results from GO/KEGG and gene set enrichment analyses suggested that the PI3K/AKT signaling pathway, the IL-18 signaling pathway, the calcium signaling pathway, secreted factors, the Wnt signaling pathway, the Jak/STAT signaling pathway, the MAPK family signaling pathway, and cell–cell communication were involved in such association. SOAT1 expression was positively associated with the abundance of macrophages, Th2 cells, T helper cells, $CD56^{bright}$ natural killer cells, and Th1 cells, and negatively linked to the abundance of Th17 cells, dendritic cells, and cytotoxic cells.

Research conclusions

As patients would greatly benefit from early detection of hepatocellular carcinoma, the complementary study of hepatocellular carcinoma-associated proteins in serum samples using state-of-the-art proteomics would be a very attractive direction for future exploration.

Research perspectives

The identification of other HCC proteins involved in this multigenic heterogeneous cancer type is an important objective for future research.

FOOTNOTES

Co-first authors: Chang-Jiao Gan and Yue Zheng.

Co-corresponding authors: Li-Min Cao and Bin Yang.

Author contributions: Gan CJ, Zheng Y, Cao LM, and Yang B conceptualized and designed the research; Gan CJ and Zheng Y performed data analysis; Gan CJ and Zheng Y wrote the paper. All the authors have read and approved the final manuscript. Both Gan CJ and Zheng Y have made crucial and indispensable contributions towards the completion of the project and thus are qualified as the co-first authors of the paper. Both Cao LM and Yang B have played important and indispensable roles in the study design, and manuscript preparation as the co-corresponding authors. Both Cao LM and Yang B applied for and obtained the funds for this research project. Cao LM conceptualized, designed, and supervised the whole process of the project. She searched the literature, and revised and submitted the early version of the manuscript. Yang B was instrumental and responsible for data re-analysis and re-interpretation, figure plotting, comprehensive literature search, and preparation and submission of the current version of the manuscript. This collaboration between Cao LM and Yang B is crucial for the publication of this manuscript and other manuscripts still in preparation.

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Informed consent statement: Consent was not needed as the study was retrospective without exposure to the patients' data.

Conflict-of-interest statement: All the authors declare that they have no conflict of interest to disclose.

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