89655_Auto_Edited.docx

WORD COUNT 2649
TIME SUBMITTED 16-JAN-2024 03:38PM
PAPER ID 106182902

Name of Journal: *World Journal of Hepatology*
Manuscript NO: 89655
Manuscript Type: ORIGINAL ARTICLE

Basic Study

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

Changjiao Gan, Yue Zheng, Bin Yang, Limin Cao

Abstract

BACKGROUND

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

AIM

To investigate the correlation between of SOAT1 expression with HCC, using RNA-seq and gene expression data of TCGA-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. Overall survival and disease-specific survival were also explored in TCGA-LIHC. Moreover, Biological processes and functional pathways regulated 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, we analyzed the protein-protein interaction network and co-expression of SOAT1 in HCC to better understand the regulatory mechanisms of SOAT1 in HCC.

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. Moreover, patients with higher SOAT1 expression had lower survival rates. Results from GO/KEGG and gene set enrichment analysis (GSEA) 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, $CD56^{bright}$, NK cells, and Th1 cells were 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 and further promote the development of new strategies for immunotherapy and metabolic therapy.

4

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 of HCC include age, sex, alcohol consumption/abuse, environmental toxins, aflatoxin infection, chronic HBV and HCV infection, and non-alcoholic fatty liver disease [2].

Liver transplantation, radical surgical resection, and radiofrequency ablation are commonly used in early-stage disease. However, the majority of patients do not meet the condition for radical treatment and are treated with systemic or local treatment instead [3]. Advanced HCC always presents poor prognosis, although several new treatment modalities has been proposed, such as immunotherapy and trans-arterial chemoembolization plus systemic treatments [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 (LDs) 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 with diagnosis and treatment [12-14]. In addition, up-regulation of SOAT1 could further promote the expression levels of inflammatory factors and cause cardiovascular diseases such as atherosclerosis and coronary heart disease [15-17]. Cholesterol ester increases HCC growth through promoting 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 close related to cholesterol metabolism [22, 23].

However, the relationship of SOAT1 expression with HCC remains unclear. In the current study, we explored if SOAT1 was 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 in which SOAT1 may potentially be involved in the pathogenesis of HCC.

MATERIALS AND METHODS

2.1 Microarray data and data processing

The RNA-seq and gene expression data of TCGA-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.

2.2 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 in TCGA-LIHC. $P < 0.05$ was regarded as significant. To further investigate the prognostic value of SOAT1 expression, the nomogram and calibration curves were used.

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 performed using the "*pROC*" package.

2.4 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. $P < 0.05$ was considered as a cutoff criterion.

2.5 SOAT1 expression association with immune cells

To analysis 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.

2.6 Differentially expressed genes between SOAT1 high and low expression groups

The differentially expressed genes (DEGs) between different SOAT1 expression groups (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.

2.7 Enrichment analysis

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

2.8 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 by Cytoscape version 3.7.1. Protein–protein interaction **(**PPI) network analysis was conducted to obtain the hub genes by the Cytoscape plug-in MCODE.

2.9 Prognostic value of SOAT1 expression in TCGA-LIHC

Lasso regression and risk score analysis was used 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

3.1 SOAT1 was 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 (Fig. 1A, B). The

univariate analysis and multivariate analysis also suggested that SOAT1 expression was an independent risk factor for HCC progression (Fig. 1C; Table S1). SOAT1 expression in pan-cancer, including unpaired and paired samples, was also investigated (Fig. 1D, E).

3.2 The diagnostic and prognostic value of SOAT1 expression

To explore the diagnostic value of SOAT1 expression in HCC, we performed an analysis of receiver operation characteristic curve. 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, suggesting that SOAT1 may be a potential diagnostic biomarker for HCC invasion (Fig. 2A, 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 (Fig. 2C, D). SOAT1 expression was also associated with patient characteristics, for instance, age, gender, histologic grade, T stage, and N stage (Fig. 3). In addition, 1-, 3-, and 5-year OS and DSS analysis demonstrated that higher SOAT1 expression was associated with worse prognosis (Fig. 4).

3.3 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 performed. GO enrichment analysis, KEGG pathway enrichment analysis, and GSEA showed that these DEGs were 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 (Fig. 5).

3.4 SOAT1 expression and immune cells analysis

To analyze the association between SOAT1 expression and immune cells, single sample GSEA was conducted in LIHC, SOAT1 expression was positively associated with 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 (Fig. S2).

3.5 PPI network and the 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*). It might be potential targets for HCC treatment (Fig. 6).

3.6 The 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* (Fig. 7A, B). To further explore the prognostic value of these two hub genes, survival analysis was conducted, which showed that patients with higher expression level of *CYP19A1* and *UGT1A10* had 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 (NAFLD) and related metabolic factors have emerged as the fastest-growing risk factors of HCC in recent years. The relationship between lipid 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, 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. Currently, 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 had 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 to HCC through 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 by the aggregation of cholesterol esters, there 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 is commonly existed 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 insulin and insulin-like growth factor 1 (IGF-1) signaling, which are pro-tumorigenic growth factors [30, 31]. Additionally, Evidence in mice and humans has observed that liver cells without fatty acid synthase (FASN) 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 reported 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 poor prognosis in all HCC cases. Our 1-, 3- and 5-year OS and DSS analyses demonstrated that higher SOAT1 expression was associated with worse prognosis (Fig. 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 integrin and TGF-β signaling pathways. [19]. Consistently, integrin binding was also significantly enhanced, as determined by enrichment analysis of the GO and KEGG pathways of upregulated DEGs in HCC (Fig. 5). Multiple genes, including *CYP19A1*, *CYP2A6*, *CYP1A2*, *CYP1A1*, *UGT1A10*, *KLK3*, *KRT19* and *CEACAM5* (Fig. 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 was consistent with the prognostic analysis of SOAT1 expression. SOAT1 was reported to be expressed by multiple regulatory 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 SOAT1in pancreatic ductal adenocarcinoma [10]. In addition, β-catenin has been reported to be directly bind to the SOAT1 promoter element and promote SOAT1 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 of lipid metabolism, but the effect might be limited. Further studies are warranted to validate our results. The identification of other HCC proteins involved in this multigenic heterogenous cancer type is an important objective for future research. Since early

diagnose 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.

89655_Auto_Edited.docx

ORIGINALITY REPORT

8%
SIMILARITY INDEX

PRIMARY SOURCES

1	bmccancer.biomedcentral.com Internet	102 words — 4%
2	www.frontiersin.org Internet	47 words — 2%
3	www.ncbi.nlm.nih.gov Internet	31 words — 1%
4	Yoon, Sun, Jeong-Min Kim, Jung-Hwa Oh, Yeo-Jin Jeon, Dong-Seok Lee, Joo Kim, Jong Choi, Byung Ahn, Sangsoo Kim, Hyang-Sook Yoo, Yong Kim, and Nam-Soon Kim. "Gene expression profiling of human HBV- and/or HCV-associated hepatocellular carcinoma cells using expressed sequence tags", International Journal of Oncology, 2006. Crossref	20 words — 1%
5	www.aging-us.com Internet	15 words — 1%
6	www.spandidos-publications.com Internet	12 words — < 1%

EXCLUDE QUOTES	ON	EXCLUDE SOURCES	OFF
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 12 WORDS