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Research Article

Targeting *SOAT1* Ameliorates Hepatocellular Carcinoma by Apoptosis after Comprehensive Analysis for MBOAT Family Genes

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ABSTRACT

Background: Despite the largely number of studies on Hepato Cellular Carcinoma (HCC) over past decades, little development had been made because of futile treatment regimens. Here we discussed the expression level, transcriptional and survival data, mutation, and clinical significance of the MBOATs family in patients with HCC to find *SOAT1* as a scientific evidence for clinical risk management and decisions effectively.

Methods: HCC samples were extracted from the cBioPortal databases; LinkedOmics, Gene Expression Profiling Interactive Analysis (GEPIA), Kaplan-Meier Plotter, The Cancer Genome Atlas (TCGA), and R software (×64 3.6.2) were used to comprehensively analyze the roles of MBOATs. p value below to 0.05 was considered statistically significant. TEM imaging, Tunnel, Cell viability and migration assay and so on are also used in this study.

Results: In total, 369 HCC tissues and 160 paracancerous tissues were included. The expression levels of *MBOAT7*, *SOAT1*, *HHAT*, *DGAT1*, and *PORCN* were higher in HCC tissues than those in normal liver tissue. Gene enrichment analysis revealed that MBOATs played a critical role in apoptosis signaling pathway. Through a comprehensive analysis of the MBOAT family we found a high *SOAT1* expression was obviously related with poor OS and DSS in all of the HCC patients, which seemed consistent with the key role of *SOAT1* in MBOAT family as a tumor promoter. Genetic inhibition of *SOAT1* effectively suppresses tumor growth and induces apoptosis in both *in vitro* and *in vivo*. Eventually, we found that targeting *SOAT1* promoted ROS production will induce mitochondrial damage and apoptosis in tumor cells and markedly suppressed HCC growth.

Conclusions: This is the first time to find the most effective target *SOAT1* in the gene family MBOATs. Our results strongly indicated a crucial role of the MBOAT family in HCC, especially *SOAT1*. *SOAT1* could be potential prognostic and predictive markers, and might also function as a potential therapeutic target in HCC by apoptotic pathway induced by ROS and mitochondrial damage.

Keywords: Hepatocellular carcinoma; TCGA; MBOATs; *SOAT1*; Prognosis

INTRODUCTION

Membrane-bound O-Acyltransferases (MBOATs) comprises more than 7,000 proteins (http://pfam.xfam.org/family/MBOAT). They are a super group of integral trans membrane enzymes both in bacteria and vertebrates [1] with distinct substrate preferences, and can be subdivided into some groups based on their divergent functions. Membrane-bound O-Acyltransferases (MBOATs), a group of integral transmembrane enzymes that perform divergent functions which are attractive drug targets in cancer. 11 of MBOAT proteins (MBOAT1, MBOAT2, *HHAT*L, GOAT, LPCAT3, *MBOAT7*, *SOAT1*, SOAT2, *HHAT*, *DGAT1* and *PORCN*) are located in the progression of various cancer types, especially in HepatoCellular Carcinoma (HCC). Although these proteins

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are important drug targets, the diverse expression patterns and prognostic values have yet to be analyzed. DiacylGlycerol Acyltransferase 1 (*DGAT1*) and Sterol O-AcylTransferase (SOAT)—are responsible for phospholipid remodelling or lipid biosynthesis [2,3]. Ghrelin AcylTransferase (GOAT), Porcupine (*PORCN*) and HedgeHog AcylTransferase (*HHAT*) catalyze essential lipid modifications of secreted proteins respectively, such as hedgehog, WNT and ghrelin [4-10].

MBOAT family members play a significant role in catalysis because of a strictly conserved histidine residue. Mutation of the corresponding histidine residue either abolished or substantially reduced the acyltransferase activities of the enzymes in Diacylglycerol Acyltransferase-1 (*DGAT1*) and Sterol O-Acyltransferase (SOAT), Ghrelin Acyltransferase (GOAT), Porcupine (*PORCN*) and Hedgehog Acyltransferase (*HHAT*) [11-16]. The expression of some MBOAT proteins are deregulated in several human malignancies, including Chronic Lymphocytic Leukemia (CLL), Pancreatic Ductal Adeno Carcinoma (PDAC), prostate cancer, clear cell Renal Carcinoma (ccRCC), Glioblastoma (GBM), breast cancer and Hepatocellular Carcinoma (HCC) [17-23].

Hepatocellular Carcinoma is the fourth most common cause of cancer-related death worldwide and the leading cause of cancer-related death around the world. Early-stage HCC is amenable to potentially curative treatment, which includes local ablation, surgical resection and liver transplantation [24]. HCC has identified six robust subgroups designated as G1–G6 which are associated with specific genetic and clinical characteristics [25,26]. Mutations in the TERT promoter (occurring in 44-65% of patients with HCC and encodin transcription of the catalytic subunit of telomerase), gregulating CTNNB1 (27-40%, -catenin, a proto-oncogene in the WNT signaling pathway) and TP53 (21-31%, the master cell cycle regulator) are the most conventional [27,28]. Despite the headways made in HCC management, including noninvasive radiological diagnosis and more availability treatment options over the past few decades, of which only overall rate of survival after developing this cancer [29]. Due to the heterogeneity of tumor, some boundedness still exists in biomarkers that forecast prognosis. What's more, the data source for analysis should include not only m-RNA data, but also proteome data to evaluate the value of new biomarkers in tumor diagnosis and personalized treatment comprehensively and objectively.

To date, eleven MBOAT family genes relevent with cancer have been selected: MBOAT1, MBOAT2, *HHATL* (*MBOAT3*), GOAT (*MBOAT4*), LPCAT3 (MBOAT5), *MBOAT7*, *SOAT1*, SOAT2, *HHAT*, *DGAT1* and *PORCN*. They are supposed to have luxary and distinct roles in human cancer. MBOAT1 was reported to be involved in CLL17. Liviu Badea et al. [18] reported *MBOAT2* upregulate and cause epithelial mesenchymal transformation in PDAC. *HHATL* (*MBOAT3*) command cell wall integrity and programme cell death [30]. GOAT (*MBOAT4*) is associated with patient's metabolic status, which is a non-invasive biomarker, potentialiy [19]. LPCAT3 (MBOAT5) is a key factor in the control of phospholipid homeostasis and arachidonate availability in myeloid cells and promotes atherosclerosis [31]. *MBOAT7* driven phosphatidylinositol remodeling promotes the progression of clear cell renal carcinoma [20]. *SOAT1* has recently been a potential therapeutic window for pancreatic carcinoma [32]. In addition, it was also reported to promote tumor growth and metastasis, indicating *SOAT1* had an oncogenic role in HCC [23]. Leptin promotes the migration and invasion of breast cancer cells by up regulating ACAT222, indicating that ACAT2 plays an oncogenic role in breast cancer. PKC1-SOX2- *HHAT* signaling cooperate to activate Hedgehog signaling in lung squamous cell carcinoma [33]. Targeting *DGAT1* Ameliorates Glioblastoma by Increasing Fat Catabolism and Oxidative Stress [21]. Madan B et al. [34] demonstrate that dual *PORCN* and PI3K/mTOR inhibition is a potential strategy for treating WNT-driven pancreatic cancers.

Although these proteins have important roles in a variety of human cancer, the diverse expression patterns and prognostic values in HCC have yet to be analyzed, in addition, the underlying mechanism and the distinct functions of the MBOAT family genes in HCC have yet to be fully elucidated. As far as we know, bioinformatics analysis has yet to be applied to explore the role of MBOATs in HCC. As an essential component of biological and biomedical studies RNA and DNA research have been revolutionized with the development of microarray technology [35]. The dysregulated expression levels of MBOAT family genes and their relationship with clinicopathological features and prognosis have been reported in HCC, solely. To the best of our knowledge, there are presently no studies assessing the role of the MBOAT family in HCC systematically to find effective biological target using bioinformatics approach. Herein, we aimed to investigate the expression level, mutation, and clinical significance of the MBOAT family in HCC, and then find a most effective biotarget so as to establish a sufficient evidence for clinical decisions and risk management.

MATERIALS AND METHODS

Ethics statement

This study was approved by the Academic Committee of Academy of Military Medical Sciences, and it was conducted according to the principles expressed in the Declaration of Helsinki. All the datasets came from the published literature, so all written informed consent was getatable.

Gene expression profiling interactive analysis (GEPIA Dataset)

GEPIA is a multidimensional cancer genomics dataset for analyzing the mass RNA sequencing expression data from the The Cancer Genome Atlas and the Genotype-Tissue Expression (GTEx) projects (http://gepia.cancer-pku.cn/). GEPIA provides customizable roles to evaluate the gene expression differences between HCC and normal tissues according to the Analysis of Variance (ANOVA), tumor/normal diversely expression analysis, profiling according to tumor types or pathological stages, patient survival analysis, similar gene detection, correlation analysis, and dissimilarly reduction analysis. The correlation between MBOATs and clinical stage was also evaluated using GEPIA, and the statistical method used was Pearson correlation coefficient.

The cancer genome atlas data and cBioPortal

30 different cancers had both sequencing and pathological data in The Cancer Genome Atlas. Then use cBioPortal (http://www.cbioportal.org/) for further analyses of MBOATs. The genomic profiles included amplifcation, Putative Copy Number Alterations (pCNAs), mRNA expression Z scores (RNA-seq v.2 RSEM), and protein expression Z scores (Reverse Phase Protein Array (RPPA). An overview of genetic alteration of each MBOAT family member was also provided to show all details of each type of mutation in each individual sample. Co-expression and network were calculated according to the cBioPortal's online instructions.

The Kaplan-Meier plotter

The prognostic value of signal transducer and activator of transcription mRNA expression was estimated using an online database, Kaplan-Meier Plotter (www.kmplot.com), which contained different genes expression data and survival information of Hepatocellular Carcinoma patients (http://kmplot.com/analysis/index.php?p=service&cancer=Hepatocellular). To analyze the OS, DSS, PFS and RFS of patients with Hepatocellular Carcinoma, the HCC samples were divided into two groups according to the median expression (high versus low expression). Overall Survival (OS) was considered the time to death or the last follow-up time from the initial diagnosis of HCC, whereas Recurrence Free Survival (RFS) was the time to relapse from the diagnosis. The Hazard Ratio (HR) with 95% Confidence Intervals (CIs) and p value had been labeled.

cBioPortal

As an intuitive Web interface cBioPortal was applied to perform gene variation analysis of HCC (http://www.cbioportal.org/), including mutation, amplification, and copy number variation. An overview of genetic alteration of 11 MBOAT family members was also provided to visualize complete details of each type of mutation in each individual sample.

Pather pathway

Using the online LinkedOmics database, we screened the most relevant genes of 11 MBOAT family members. The top 50 genes significantly associated with MBOATs were screened for further. Pather Pathway enrichment analysis were performed in the DAVID database (https://david.ncifcrf.gov/). Signifcant pathway computing was provided in DAVID. The graph of Pather Pathway analysis was plotted by R packages named ggplot2 in the R software (×64 3.6.2) LinkedOmics database.

Cell lines

The Huh7 and Hep3B were purchased from Punuosai Co., Ltd. (Cellcook, Wuhan, China). Both cell lines were reported to have passed chlamydia and mycoplasma detection.

RNA interference and transfection

To explore the inhibition efficiency of *SOAT1* expression by siRNA, we used small interfering RNA. *siSOAT1-1* (5'-GCAGAGGAAUUGAAGCCAUTT tt-3'sense, and 5'-AUGGCUUCAAUUCCUCUGCTT tt-3'antisense) *siSOAT1-2* (5'-GCACACUUGUAGUAGAUUATT tt-3'sense, and 5'-UAAUCUACUACAAGUGUGCTT tt-3'antisense) *siSOAT1-3* (5'-GGACCUGGUGGAUCAUGUUTT tt-3'sense, and 5'-AACAUGAUCCACCAGGUCCTT tt-3'antisense) were purchased from Jima Pharmaceutical Technology Co., LTD (Jimapharmatech, Suzhou, China). 50 nmol/L siRNA was transfected into Huh7 and Hep3B cells using INTERFER in (Polyplus transfection, Suzhou, China) according to the company's protocol.

Western blot analysis

Total protein was incubated with RIPA lysis buffer containing protease inhibitor (Shenggong, Shanghai, China) (Jima,Suzhou, China), and the protein was quantified with Nucleic acid protein analyzer (Biochrom, Simplinano, China). The protein was quantified and then used to perform western blot as usual. Antibodies against *SOAT1* and GAPDH were purchased from Merck and Shenggong.

Cell Counting Kit-8 (CCK-8) analysis and cell migration assay

Cell viability was analyzed by cell counting kit-8 (DOJINDO, Kyushu, Japan) according to the matched protocol. In short, Huh7 and Hep3B were seeded in the 96-well plates with 10000 cells/well and 8000 cells/well incubated for overnight, respectively. At 0 h, 24 h, 48 h, 72 h, 10 μl CCK-8 solutions was added to each well, and the cells were incubated for 60 min at 37 °C. Using an IMARK micro plate will help reader (BIO-RAD) to obtain the absorbance at 450 nm. For cell migration assay, we choose coat polycarbonate filters (8 mm; Corning, NY, USA). Incubate cells at 37 °C overnight, and 3 × 105 cells were seeded into the upper chamber with 200 μl serum-free DMEM. 800 μl DMEM with 10% FBS was added to the lower chamber. Then the cultured cells at 37 °C in a 5% CO_2 are used in hydrosphere atmosphere. 24 hours later, the upper chamber was fixed with paraformaldehyde and stained with 0.5% crystal violet. Non-migration cells were washed, and the cells on the lower surface were counted microscopically.

Univariable and multivariable risk analysis

All the MBOATs that had prognostic impact (OS or RFS) were screened as candidates for univariable and multivariable Cox proportional-hazard regression analysis. HRs and 95% Confidence Interval (CI) were calculated for each factor. SPSS software (IBM SPSS 18.0 for Windows, SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Correlation analyses

Correlation between every two MBOATs was assessed using a Pearson's correlation coefficient. Statistical analysis and the graph were dealt with R software (×64 3.6.2). p value below to 0.05 was considered as significant correlations.

RESULTS

mRNA expression levels of 11 MBOAT family genes in patients with HCC

We selected eleven common MBOAT family genes by using the

TCGA and published database to contrast the mRNA levels of 11 MBOATs in tumors with paracancerous samples (Table 1). The transcriptional levels of MBOAT1, MBOAT2, *HHATL*, GOAT, LPCAT3, *MBOAT7*, *DGAT1*, *PORCN* MBOAT1 have no visible difference in all eight datasets. In Roessler's dataset, 39 *SOAT1* was found overexpressed in HCC (fold change=1.781). SOAT2 was overexpressed in Wurmbach 36 dataset (fold change=1.697). Noticeably *HHAT* were obviously up regulated in patients with hepatocellular carcinoma in one datasets, (fold change=3.256) [36].

mRNA expression levels of 11 MBOATs in HCC

The GEPIA database was used to determine the mRNA expression levels of 11 MBOATs in HCC (Figure 1A). 369 HCC tissues and 160 paracancerous tissues were included. Compared to paracancerous tissues, the expression levels of *MBOAT7*, *SOAT1*, *HHAT*, *DGAT1*, and *PORCN* were significantly elevated ($p<0.05$). There was no signifcant difference in expression of the other MBOAT family members (MBOAT1, 2, 4, *HHATL*, LPCAT3, and SOAT2).

Correlation between MBOATs transcriptional level and clinicopathological parameters of the patients with HCC

We compared transcriptional level of MBOAT family genes between HCC and normal liver tissues with the GEPIA. The results indicated that the expression levels of *MBOAT7*, *SOAT1*, *HHAT*, *DGAT1* and *PORCN* were higher in HCC tissues than in normal tissues ($p<0.05$) (Figure 1B). By using GEPIA (Gene Expression Profiling Interactive Analysis) dataset (http://gepia.cancer-pku.cn/), we found MBOAT2, GOAT, *MBOAT7* have significant relationship between expression and Tumor stage in HCC patients ($p<0.05$). No significant correlations were observed between other MBOATs and tumor stage (Figure 2). We performed RNA-seq to test MBOAT RNA expression in HCC. RNA-seq data is reported as median FPKM (number Fragments per Kilo base of exon per Million reads), generated by the The Cancer Genome Atlas (TCGA). We found the average FPKM of *LPCAT3*, *SOAT1*, *SOAT2*, *MBOAT7* and DGAT up to 4.7, 4.9, 5.2, 10.1, 20, respectively (Figure 3).

Diagnostic and prognostic value of MBOATs with HCC

The key efficiency of MBOATs in relationship between tumor stage and the survival of patients with HCC were examined using the GEPIA database and publicly available datasets Kaplan-Meier Plotter tools. (2015 version; http://kmplot.com/analysis/index.php?p=service&cancer=liver). The raised MBOAT1, 2, 7, *SOAT1*, *PORCN* mRNA levels and the declined *HHATL*, *HHAT* and LPCAT3 mRNA levels were obviously associated with the Overall Survival (OS), Relapse Free Survival (RFS), Distant Metastasis Free Survival (DSFS) and Post-Progression Survival (PFS) ($p<0.05$) in the whole patients with hepatocellular carcinoma veiled by the Kaplan-Meier curve and log rank test analyses (Figure 4). The patients with hepatocellular carcinoma with high mRNA levels of the MBOAT1, 2, 7, *SOAT1*, *PORCN* factors or inferior mRNA levels of *HHATL*, *HHAT* and LPCAT3 were predicted to have high OS, RFS, PFS and DSS. Figure of the whole patients with HCC (Figure 4). The patients with hepatocellular carcinoma with high mRNA levels of the MBOAT1, 2, 7, *SOAT1*, *PORCN* factors or inferior mRNA levels of *HHATL*, *HHAT* and LPCAT3 were forecasted to have high OS, RFS, PFS and DSS.

Predicted functions and pathways of the changes in MBOATs factors and their frequently altered neighbor genes in HCC

In addition to diagnostic and prognostic value using the Kaplan–Meier Plotter, we also evaluated variations, correlations, networks and the alters in MBOAT family genes and their Frequently Altered Neighbor Genes in Patients with HCC using cBioPortal (The Cancer Genome Atlas, Provisional; (http://www.cbioportal.org/index.do?session_id=5b4c1773498eb8b3d566f7b8).

MBOATs were changed in 289 (20%) of 1461 sequences patients (1461 total) (Figures 5 and 6). We also calculated the correlations of MBOATs with each other by analyzing their mRNA expressions (RNA sequencing (RNA seq) version (vs.) 2 RSEM) *via* the cBioPortal online tool for HCC (The Cancer Genome Atlas, Provisional), and Pearson's correction was included.

Table 1: The Significant mRNA changes of MBOAT expression between different types of hepatocellular carcinoma and normal liver tissues.

Proteins	Type of Hepatocellular Carcinoma versus Normal Liver Tissue	Samples	p Value	t Test	Fold Change	Source and/or Reference
MBOAT1	Hepatocellular Carcinoma	75	0.474	0.065	1.014	Wurmbach Liver Statistics [36]
	Hepatocellular Carcinoma	185	2.11E-09	6.439	1.077	Guichard Liver Statistics [37]
	Hepatocellular Carcinoma	212	1.72E-07	5.477	1.102	TCGA
	Hepatocellular Carcinoma	197	0.292	0.549	1.049	Chen Liver Statistics[38]
	Hepatocellular Carcinoma	445	1	-3.554	-1.059	Roessler Liver Statistics [39]
	Hepatocellular Carcinoma	115	0.999	-3.528	-1.194	Mas Liver Statistics[40]
	Hepatocellular Carcinoma	52	0.009	2.507	1.025	Guichard Liver 2 Statistics [37]
	Hepatocellular Carcinoma	212	4.66E-04	3.416	1.037	TCGA

MBOAT2	Hepatocellular Carcinoma	197	0.957	-1.726	-1.186	Chen Liver Statistics [38]
	Hepatocellular Carcinoma	75	0.822	-0.963	-1.123	Wurmbach Liver Statistics [36]
	Hepatocellular Carcinoma	445	0.991	-2.384	-1.062	Roessler Liver Statistics [39]
	Hepatocellular Carcinoma	115	0.945	-1.625	-1.077	Mas Liver Statistics [40]
	Hepatocellular Carcinoma	185	0.344	0.404	1.002	Guichard Liver Statistics37
HHATL	Hepatocellular Carcinoma	52	0.575	-0.191	-1.002	Guichard Liver 2 Statistics [37]
	Hepatocellular Carcinoma	212	0.901	-1.296	-1.015	TCGA
	Hepatocellular Carcinoma	75	0.869	-1.183	-1.016	Wurmbach Liver Statistics [36]
	Hepatocellular Carcinoma	52	0.998	-3.135	-1.075	Guichard Liver 2 Statistics [37]
GOAT	Hepatocellular Carcinoma	212	1	-8.673	-1.274	TCGA
	Hepatocellular Carcinoma	185	1	-9.59	-1.155	Guichard Liver Statistics [37]
	Hepatocellular Carcinoma	197	0.016	2.177	1.214	Chen Liver Statistics [38]
	Hepatocellular Carcinoma	445	0.01	2.328	1.1	Roessler Liver 2 Statistics [39]
	Hepatocellular Carcinoma	43	0.754	-0.696	-1.083	Roessler Liver Statistics [39]
LPCAT3	Hepatocellular Carcinoma	115	0.998	-3.149	-1.295	Mas Liver Statistics [40]
	Hepatocellular Carcinoma	52	0.845	-1.036	-1.013	Guichard Liver 2 Statistics [37]
	Hepatocellular Carcinoma	75	0.99	-2.588	-1.614	Wurmbach Liver Statistics [36]
	Hepatocellular Carcinoma	212	0.993	-2.515	-1.042	TCGA
	Hepatocellular Carcinoma	185	0.997	-2.811	-1.026	Guichard Liver Statistics [37]
	Hepatocellular Carcinoma	197	1.88E-08	5.753	1.471	Chen Liver Statistics [38]
	Hepatocellular Carcinoma	43	2.30E-05	4.6	1.402	Roessler Liver Statistics [39]
	Hepatocellular Carcinoma	75	0.004	2.874	1.263	Wurmbach Liver Statistics [36]
MBOAT7	Hepatocellular Carcinoma	445	9.59E-14	7.61	1.267	Roessler Liver 2 Statistics [39]
	Hepatocellular Carcinoma	52	0.046	1.735	1.01	Guichard Liver 2 Statistics [37]
	Hepatocellular Carcinoma	212	0.004	2.684	1.032	TCGA
	Hepatocellular Carcinoma	115	0.825	-0.944	-1.062	Mas Liver Statistics [40]
	Hepatocellular Carcinoma	185	0.18	0.919	1.009	Guichard Liver Statistics [37]
	Hepatocellular Carcinoma	52	4.03E-07	6.467	1.107	Guichard Liver 2 Statistics [37]
	Hepatocellular Carcinoma	185	3.57E-19	10.938	1.113	Guichard Liver Statistics [37]
	Hepatocellular Carcinoma	197	0.002	2.067	1.169	Chen Liver Statistics [38]

SOAT1	Hepatocellular Carcinoma	212	4.38E-20	11.51	1.274	TCGA
	Hepatocellular Carcinoma	75	0.073	1.503	1.236	Wurmbach Liver Statistics [36]
	Hepatocellular Carcinoma	445	6.87E-35	13.78	1.781	Roessler Liver 2 Statistics [39]
	Hepatocellular Carcinoma	43	0.002	3.173	1.461	Roessler Liver Statistics[39]
	Hepatocellular Carcinoma	115	0.68	-0.47	-1.052	Mas Liver Statistics[40]
	Hepatocellular Carcinoma	445	1.98E-11	6.894	1.395	Roessler Liver 2 Statistics[39]
	Hepatocellular Carcinoma	43	0.014	2.325	1.436	Roessler Liver Statistics[39]
	Hepatocellular Carcinoma	75	0.015	2.256	1.697	Wurmbach Liver Statistics[36]
SOAT2	Hepatocellular Carcinoma	52	0.156	1.022	1.009	Guichard Liver 2 Statistics[37]
	Hepatocellular Carcinoma	115	0.769	-0.744	-1.039	Mas Liver Statistics[40]
	Hepatocellular Carcinoma	212	0.343	0.407	1.004	TCGA
	Hepatocellular Carcinoma	185	0.619	-0.304	-1.002	Guichard Liver Statistics[37]
	Hepatocellular Carcinoma	75	2.44E-06	10.324	3.256	Wurmbach Liver Statistics[36]
	Hepatocellular Carcinoma	52	1.09E-06	6..083	1.1	Guichard Liver 2 Statistics[37]
	Hepatocellular Carcinoma	212	3.46E-18	10.619	1.254	TCGA
HHAT	Hepatocellular Carcinoma	197	5.04E-09	6.027	1.524	Chen Liver Statistics[38]
	Hepatocellular Carcinoma	185	3.38E-15	9.157	1.102	Guichard Liver Statistics[37]
	Hepatocellular Carcinoma	445	2.45E-20	9.82	1.328	Roessler Liver 2 Statistics[39]
	Hepatocellular Carcinoma	115	0.046	1.738	1.091	Mas Liver Statistics[40]
	Hepatocellular Carcinoma	43	0.008	2.551	1.203	Roessler Liver Statistics[39]
	Hepatocellular Carcinoma	52	1.47E-05	4.935	1.068	Guichard Liver 2 Statistics[37]
	Hepatocellular Carcinoma	212	2.16E-13	8.367	1.249	TCGA
DGAT1 Hepatocellular Carcinoma		185	1.53E-09	6.356	1.103	Guichard Liver Statistics[37]
	Hepatocellular Carcinoma	43	0.025	2.03	1.298	Roessler Liver Statistics[39]
	Hepatocellular Carcinoma	197	0.113	1.217	1.112	Chen Liver Statistics[38]
	Hepatocellular Carcinoma	445	0.028	1.913	1.098	Roessler Liver 2 Statistics[39]
	Hepatocellular Carcinoma	75	0.049	1.727	1.377	Wurmbach Liver Statistics[36]
	Hepatocellular Carcinoma	115	0.703	-0.536	-1.059	Mas Liver Statistics[40]
PORCN	Hepatocellular Carcinoma	75	0.235	0.731	1.028	Wurmbach Liver Statistics[36]
	Hepatocellular Carcinoma	445	0.304	0.515	1.008	Roessler Liver 2 Statistics[39]
	Hepatocellular Carcinoma	115	0.716	-0.578	-1.029	Mas Liver Statistics [40]
	Hepatocellular Carcinoma	43	0.746	-0.667	-1.041	Roessler Liver Statistics [39]

Note: TCGA: The Cancer Genome Atlas

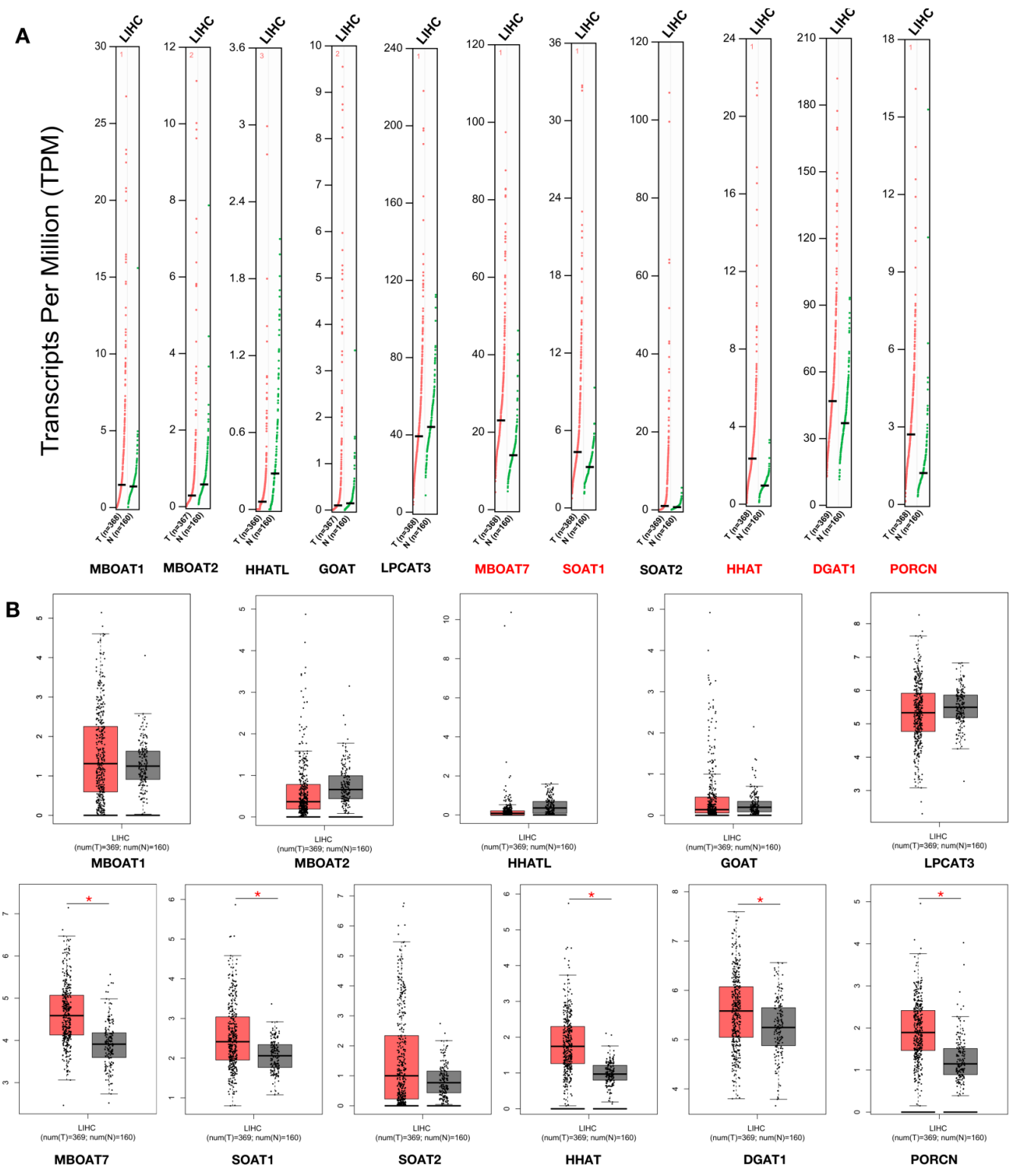


Figure 1: The mRNA Expression of MBOAT family Genes in hepatocellular carcinoma. A: Scatter diagram (____) HCC liver tissue, (____) Normal liver tissue; B: Box plot (■) HCC liver tissue; (■) Normal liver tissue.

Note: Compared transcriptional level of MBOAT family genes between HCC and normal liver tissues with the GEPIA (http://gepia.cancer-pku.cn/) (*p<0.05).

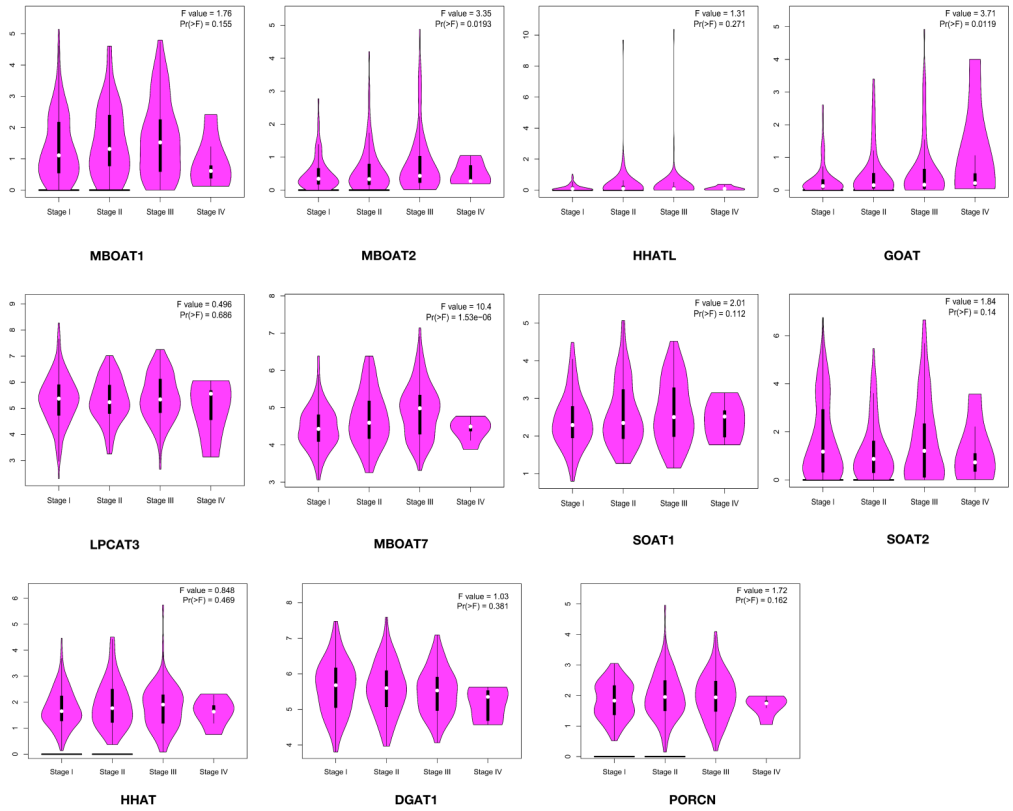


Figure 2: Correlation between MBOAT family genes Expression and Tumour Stage in Hepatocellular Carcinoma Patients (http://gepia.cancer-pku.cn/). MBOAT2, GOAT, *MBOAT7* have significant relationship between expression and Tumor stage in HCC patients.

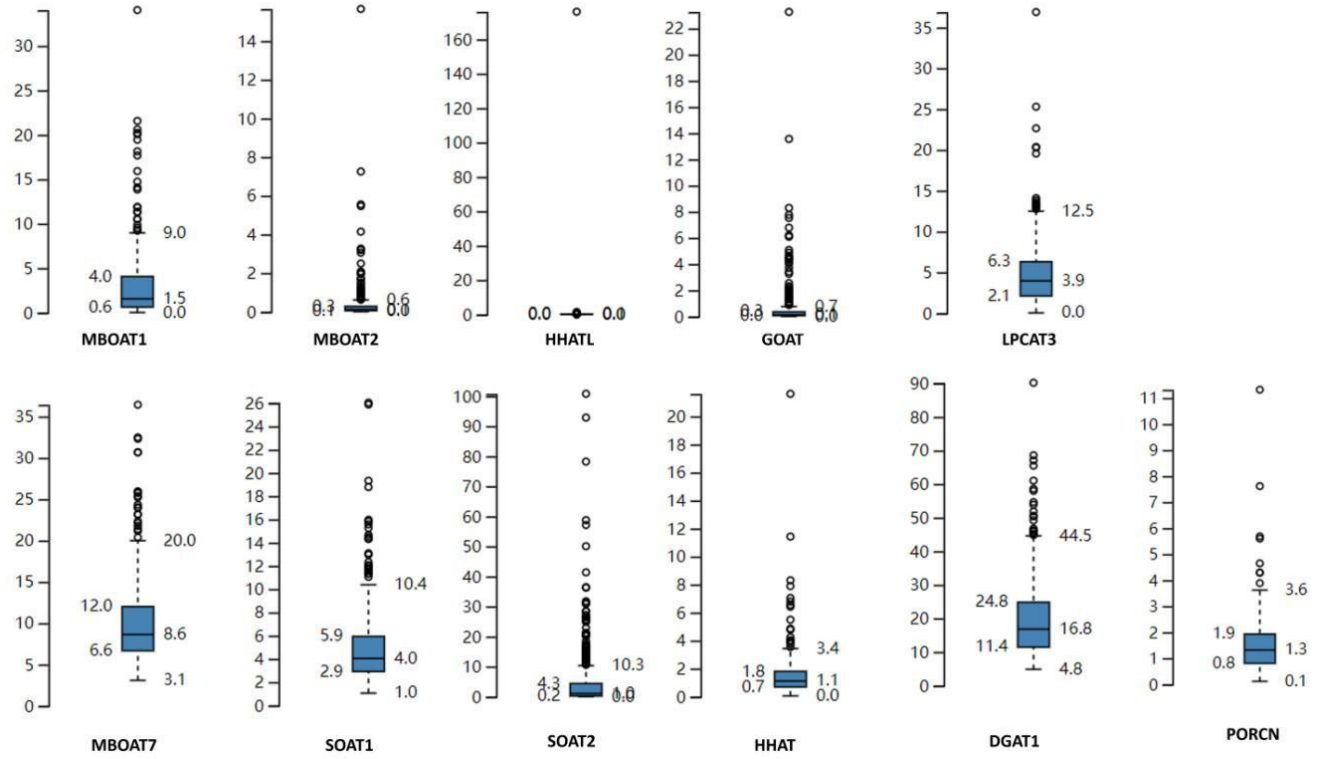
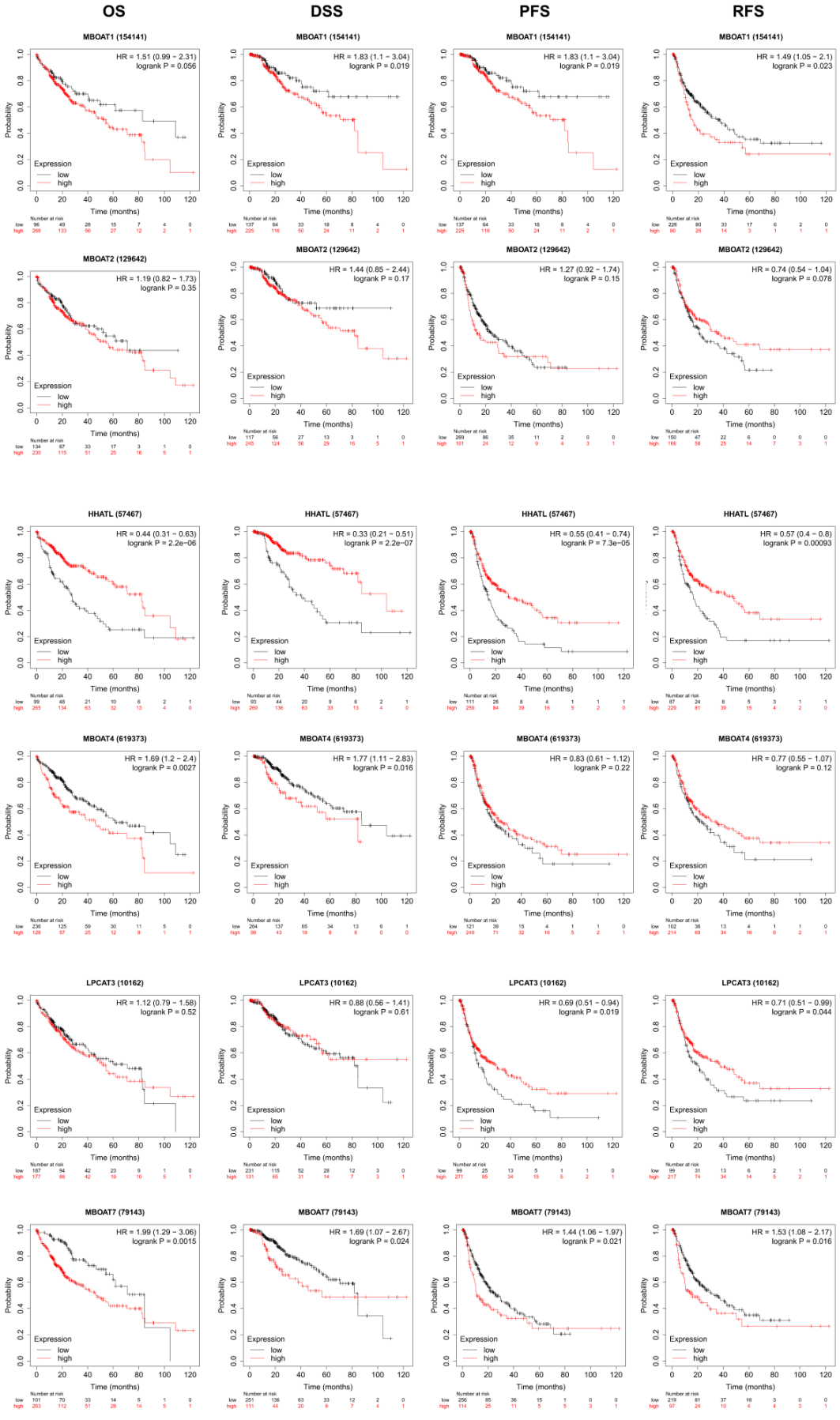


Figure 3: The FPKM (number Fragments per Kilobase of exon per Million reads) generated by the The Cancer Genome Atlas (TCGA) of MBOATs in Hepatocellular Carcinoma (TCGA RNA samples).The average FPKM of *LPCAT3*, *SOAT1*, *SOAT2*, *MBOAT7* and *DGAT* up to 4.7, 4.9, 5.2, 10.1, 20, respectively.


OS
DSS
PFS
RFS
MBOAT1 (154141)
HR = 1.51 (0.99 − 2.31)
logrank P = 0.056
HR = 1.83 (1.1 − 3.04)
logrank P = 0.019
HR = 1.83 (1.1 − 3.04)
logrank P = 0.019
HR = 1.49 (1.05 − 2.1)
logrank P = 0.023
MBOAT2 (129642)
HR = 1.19 (0.82 − 1.73)
logrank P = 0.35
HR = 1.44 (0.85 − 2.44)
logrank P = 0.17
HR = 1.27 (0.92 − 1.74)
logrank P = 0.15
HR = 0.74 (0.54 − 1.04)
logrank P = 0.078
HHATL (57467)
HR = 0.44 (0.31 − 0.63)
logrank P = 2.2e−06
HR = 0.33 (0.21 − 0.51)
logrank P = 2.2e−07
HR = 0.55 (0.41 − 0.74)
logrank P = 7.3e−05
HR = 0.57 (0.4 − 0.8)
logrank P = 0.00093
MBOAT4 (619373)
HR = 1.69 (1.2 − 2.4)
logrank P = 0.0027
HR = 1.77 (1.11 − 2.83)
logrank P = 0.016
HR = 0.83 (0.61 − 1.12)
logrank P = 0.22
HR = 0.77 (0.55 − 1.07)
logrank P = 0.12
LPCAT3 (10162)
HR = 1.12 (0.79 − 1.58)
logrank P = 0.52
HR = 0.88 (0.56 − 1.41)
logrank P = 0.61
HR = 0.69 (0.51 − 0.94)
logrank P = 0.019
HR = 0.71 (0.51 − 0.99)
logrank P = 0.044
MBOAT7 (79143)
HR = 1.99 (1.29 − 3.06)
logrank P = 0.0015
HR = 1.69 (1.07 − 2.67)
logrank P = 0.024
HR = 1.44 (1.06 − 1.97)
logrank P = 0.021
HR = 1.53 (1.08 − 2.17)
logrank P = 0.016
Probability
Time (months)
Expression
low
high
Number at risk

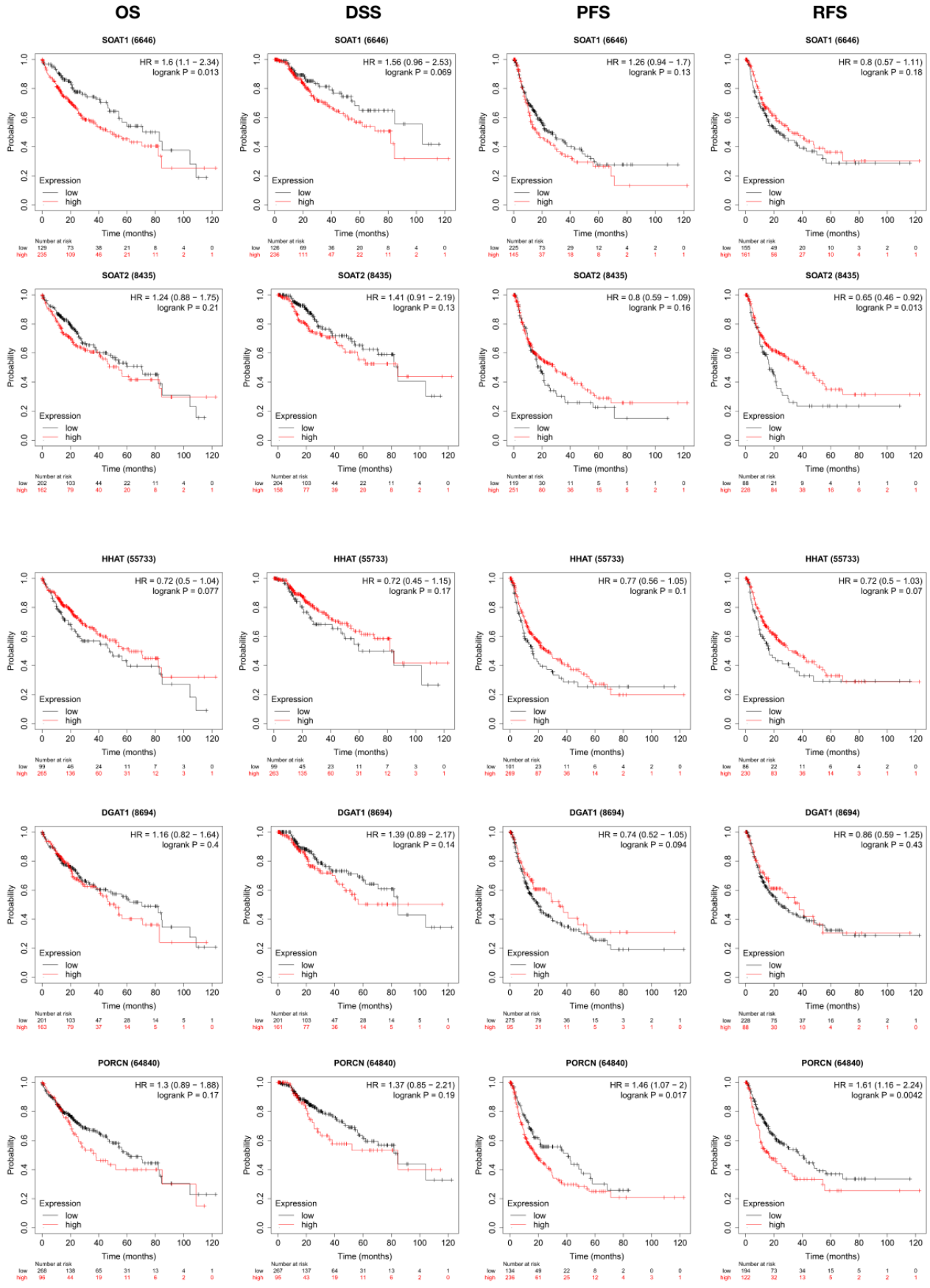


Figure 4: Effect of MBOAT family genes in Hepatocellular Carcinoma Patients on OS, DSS, PFS and RFS (Kaplan-Meier Plotter).

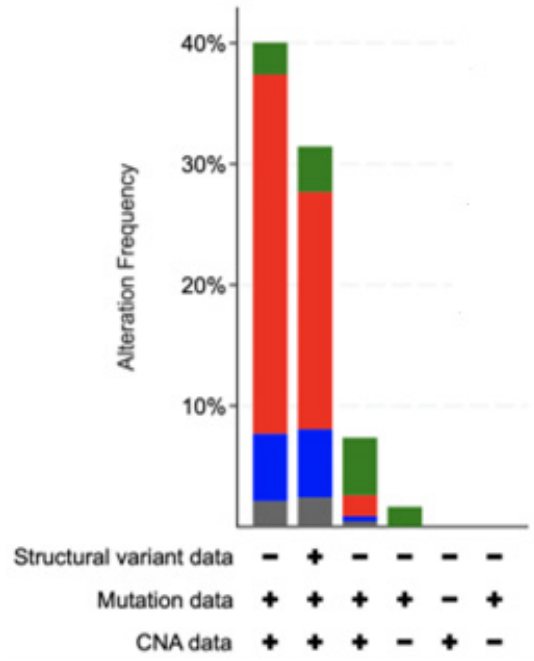


Figure 5: Mutation and Relation Analysis of MBOAT Gene Expression in liver Hepatocellular Carcinoma (cBioPortal). It shows frequency of mutation analysis in hepatocellular carcinoma. **Note:** (■)Mutuation, (■)Amplification, (■)Deep deletion, (■)Multiple alterations.

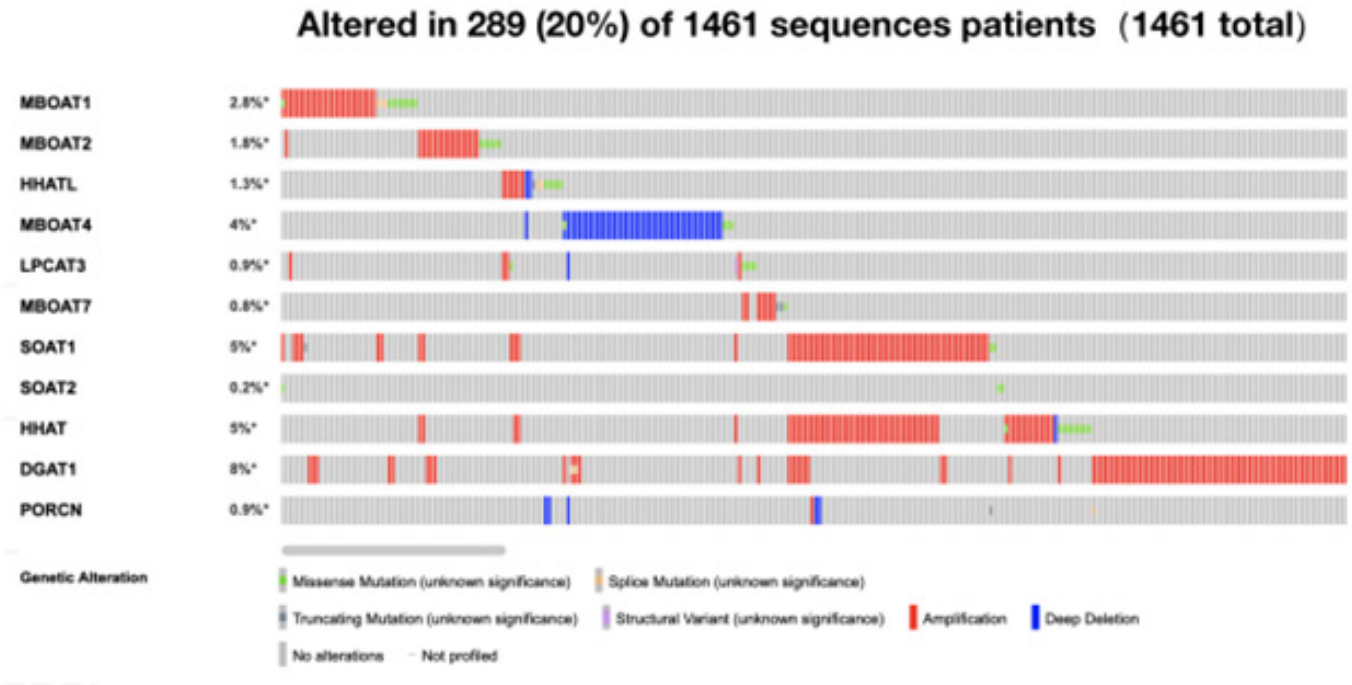


Figure 6: Mutation and Relation Analysis of MBOAT Gene Expression in liver Hepatocellular Carcinoma (cBioPortal). It shows Details of mutation in MBOAT family member. **Note:** (■) Missense mutation (unknown significance), (■) Splice mutation (unknown significance). (■) Truncating mutations (unknown significance), (■) Structural variant (unknown significance), (■) Amplification, (■) Deep deletion, (■) No alterations, (——) Not profiled.

As shown in Table 2, significantly positive correlations were observed between MBOAT1 and MBOAT2, *SOAT1*, *PORCN*; *MBOAT2* and *HHATL*, *MBOAT7*, *SOAT1*, *PORCN*; *MBOAT3* and *MBOAT7*, *PORCN*; GOAT and *MBOAT7*, *SOAT1*; LAPAT3 and SOAT2; *MBOAT7* and SOAT2, *DGAT1*, *PORCN*; *SOAT1* and GOAT; SOAT2 and *DGAT1*; Signifcantly negative correlation was observed between MBOAT1 and *SOAT1*, *DGAT1*; *MBOAT2* and *DGAT1*; GOAT and *MBOAT7*, *SOAT1*; LAPAT3 and *HHAT*, *PORCN*; *MBOAT7* and *HHAT*; *SOAT1* and *DGAT1*; SOAT2 and MBOAT1, *HHAT*, *PORCN*.

The Linked Omics database was used to study the correlated significant genes with the 11 MBOAT members. Then we use the top 50 correlated genes and MBOATs were subject to pather pathway enrichment analysis in the DAVID database. Top processes were shown in Figure 7. Notably, MBOATs were very relevant to P53 signaling pathway and apoptosis signaling pathway (Figure 8). P53 signaling pathway took part in cell cycle, Apoptosis, DNA repair and damage prevention, and p53 negative feedback by acting on FMBOATs.

Proteomics and genomics analysis of the MBOATs in patients with hepatocellular carcinoma

We analyzed the MBOAT family using proteomic data from the published CELL article. The data of proteome can be viewed in NODE (https://www.biosino.org/node) by pasting the accession (OEP000321) into the text search box or through the URL: (https://www.biosino.org/node/project/detail/OEP000321). A total of five proteins were identified in this proteomic data, MBOAT1, LPCAT3, *MBOAT7*, *SOAT1*, *DGAT1*, respectively. We found only *MBOAT7* and *SOAT1* have differences between patients with HCC than normal tissue, significantly (Figure 9). In the hepatocellular carcinoma transcriptome dataset of Nature in 2019, MBOAT1, MBOAT2, LPCAT3, *MBOAT7*, *SOAT1*, SOAT2, *HHAT*, *DGAT1* and *PORCN* were all increased to varying degrees (Figure 9). Gene expression profiles by RNA-seq can be obtained from Gene Expression Omnibus (accession number GSE124535).

SOAT1 knockdown suppressed the proliferation and migration of HCC cell lines

Based on the above results, we further selected the most potential prognostic biomarker for experimental validation. We designed three siRNA based on the *SOAT1* gene sequence. Three siRNAs were transfected into Huh7 cell lines, and sicontrol was transfected into control group. The inhibition efficiency of the si*SOAT1* was detected by Western Blot. The results showed that *siSOAT1-3* had the best inhibition efficiency (Figure 10), and then we only use the most effective siRNA sequence to finish the following proliferation and migration experiments. We use CCK8 assay to examine Huh7 and Hep3B cells proliferation. In this study, the siRNA of *SOAT1* expression could significantly inhibit the proliferation of Huh7 and Hep3B (Figure 11). Cell migration assay was used to evaluate the migration ability of HCC. In the cell migration experiment of Huh7 and Hep3B cells, *SOAT1* knockdown could reduce the migration ability of HCC cells statistically ($p<0.05$) (Figure 12).

Table 2: Mutation and Relation Analysis of MBOAT Gene Expression in liver Hepatocellular Carcinoma. Table is showing correlation between different MBOATs in hepatocellular carcinoma (cBioPortal).

MBOAT Genes	Correlation between MBOAT Gene Expression in liver Hepatocellular Carcinoma										
MBOATI	1	0.15	0.06	0.01	-0.03	-0.08	0.17	-0.13	-0.09	-0.21	0.15
MBOAT2	0.15	1	0.12	0.09	-0.1	0.18	0.17	-0.08	-0.08	-0.12	0.25
HHATL	0.06	0.12	1	-0.02	-0.07	0.01	0.02	-0.03	-0.03	-0.05	0.16
GOAT	0.01	0.09	-0.02	1	0.06	0.19	0.16	-0.08	-0.07	-0.05	0.08
LPCAT3	-0.03	-0.1	-0.07	0.06	1	0.09	0.1	0.26	-0.14	0.09	-0.16
MBOAT7	-0.08	0.18	0.01	0.19	0.09	1	0.04	0.13	-0.18	0.12	0.16
SOATI	0.17	0.17	0.02	0.16	0.1	0.04	1	0.09	0.06	-0.19	0.09
SOAT2	-0.13	-0.08	-0.03	-0.08	0.26	0.13	0.09	1	-0.11	0.21	-0.16
HHAT	-0.09	-0.08	-0.03	-0.07	-0.14	-0.18	0.06	-0.11	1	-0.17	-0.03
DGAT1	-0.21	-0.12	-0.05	-0.05	0.09	0.12	-0.19	0.21	-0.17	1	-0.1
PORCN	0.15	0.25	0.16	0.08	-0.16	0.16	0.09	-0.16	-0.03	-0.1	1
	MBOAT1	MBOAT2	*HHATL*	GOAT	LPCAT3	*MBOAT7*	SOATI	SOAT2	*HHAT*	*DGAT1*	*PORCN*

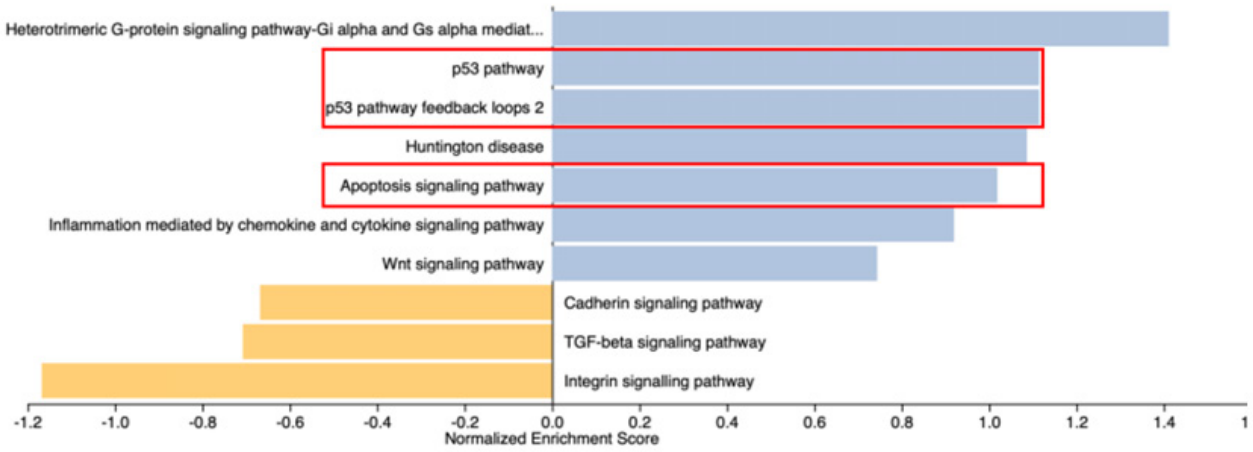


Figure 7: Mutation and Relation Analysis of MBOAT Gene Expression in liver Hepatocellular Carcinoma (cBioPortal). It is showing the functions of 11 MBOAT members and the top 50 correlated genes were predicted by the analysis in the DAVID database. **Note:** ( ) FDR ≤ 0.05, ( ) FDR>0.05.

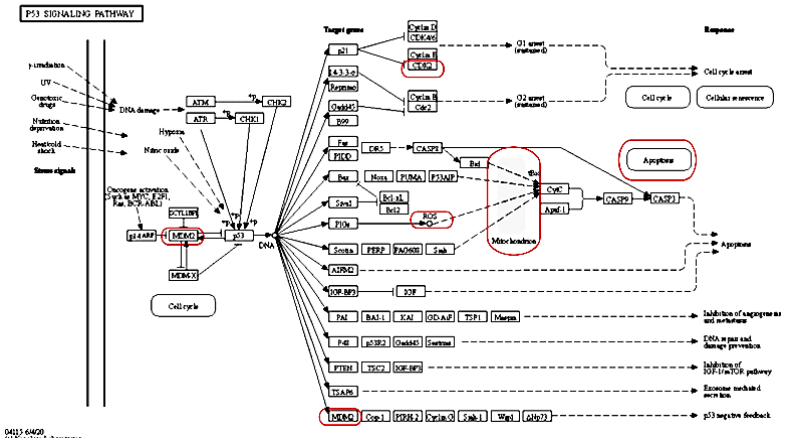


Figure 8: Mutation and Relation Analysis of MBOAT Gene Expression in liver Hepatocellular Carcinoma (cBioPortal). P53 signaling pathway took part in cell cycle, apoptosis, DNA repair and damage prevention and p53 negative feedback by 11 MBOAT members in hepatocellular carcinoma (cBioPortal) are shown.

Figure 9: Expression of MBOAT family in proteomic data and effect of SOAT1 on proliferation and migration of hepatocellular carcinoma cells. MBOATs protein and RNA expression in Hepatocellular Carcinoma (https://www.biosino.org/node).

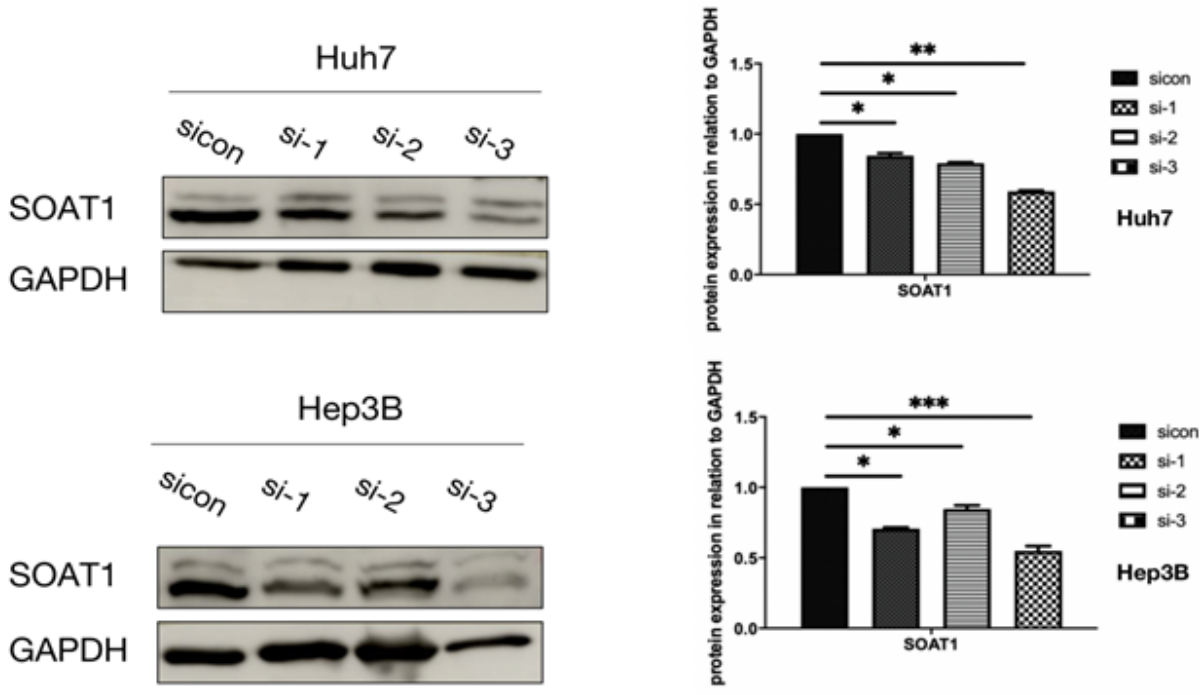


Figure 10: Expression of MBOAT family in proteomic data and effect of *SOAT1* on proliferation and migration of hepatocellular carcinoma cells. The effect of knockdown *SOAT1* with WB in Huh7 and Hep3B cell lines treated with/without *SOAT1* siRNA for 72 h.

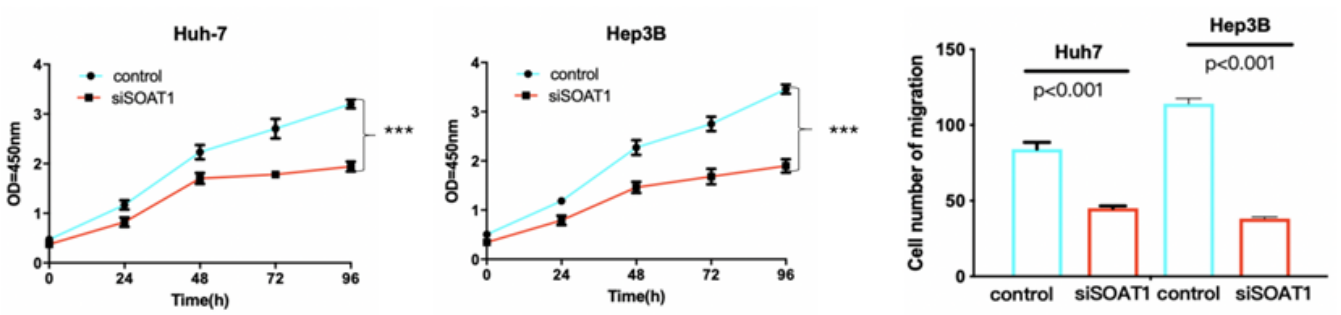


Figure 11: Expression of MBOAT family in proteomic data and effect of SOAT1 on proliferation and migration of hepatocellular carcinoma cells. Cell viability assay of Huh7 and Hep3B cells treated with/without SOAT1 siRNA for 72 h. **Note:** *p<0.05, **p<0.01, ***p<0.001. (●) Control, (■) *siSOAT1*.

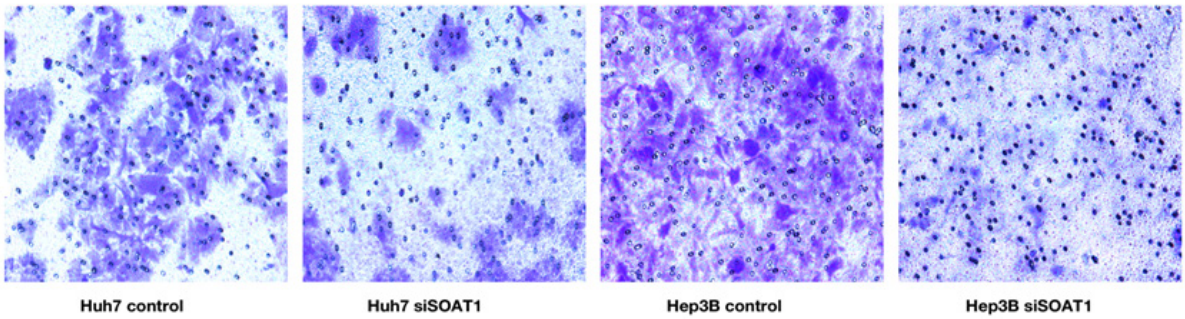


Figure 12: Expression of MBOAT family in proteomic data and effect of *SOAT1* on proliferation and migration of hepatocellular carcinoma cells. Cell migration assay of Huh7 and Hep3B cells treated with/without *SOAT1* siRNA for 72 h.

SOAT1 inhibition results in mitochondrial damage, ROS elevation, and apoptosis

Interestingly, we found that the number of HCC cell death significantly increased after knockdown of *SOAT1* by siRNA in Hep3B cell line (Figures 13 and 14). To identify the leading cause of HCC cell death upon *SOAT1* inhibition, examined whether it led to the production of Reactive Oxygen Species (ROS) in HCC cells. We then Using fluorescence microscope imaging and microplate reader showed that ROS (green) and OD values (450 nm) were markedly elevated upon genetic inhibition of *SOAT1* (Figure 13). According to previous studies, excessive production of ROS can cause mitochondrial damage. We then examined HCC cellular morphology by Transmission Electron Microscopy (TEM). Micrographs showed that the structure of mitochondria was severely disrupted upon genetic transient transfection (siRNA, 24 h) inhibition of *SOAT1*. Mitochondria became round and fragmented and lost cristae in comparison with the lengthy tubular shape of mitochondria in si-control cells (Figure 15). MBOATs were very relevant to apoptosis signaling using the top 50 correlated significant genes with the 11 MBOATs genes subject to pather Pathway enrichment analysis in the DAVID database as above. In order to explore whether the mitochondrial damage is there or not and ROS production caused by *SOAT1*, inhibition can cause cell apoptosis. The experimental verification was performed by Tunnel fluorescence staining. Notably, the number of apoptotic cells increased significantly compare to si-control cells after genetic inhibition of *SOAT1* (Figure 16).

We next examined whether inhibition of *DGAT1* is effective in inhibiting HCC growth using M-NSG xenograft models. We implanted tumor cells in mice flanks and started treatment with the siControl and *si-SOAT1-3* for 21 day. The data showed that the *si-SOAT1* significantly suppressed tumor growth in both xenograft models (Figure 17), as further evidenced by the dramatic reduction in tumor weight. Consistent with the *in vitro* analysis (Figures 16), we also examined the 4 tumor of xenograft models. Moreover, western blot also showed that *SOAT1* inhibition strongly induced apoptosis in HCC cells, as demonstrated by the dramatic upregulation in the cleaved caspase 3 and cleaved caspase 9 proteins as compared with control (Figure 18). Together, these data demonstrate that genetic inhibition of *SOAT1* effectively suppresses tumor growth and induces apoptosis in both *in vitro* and *in vivo*.

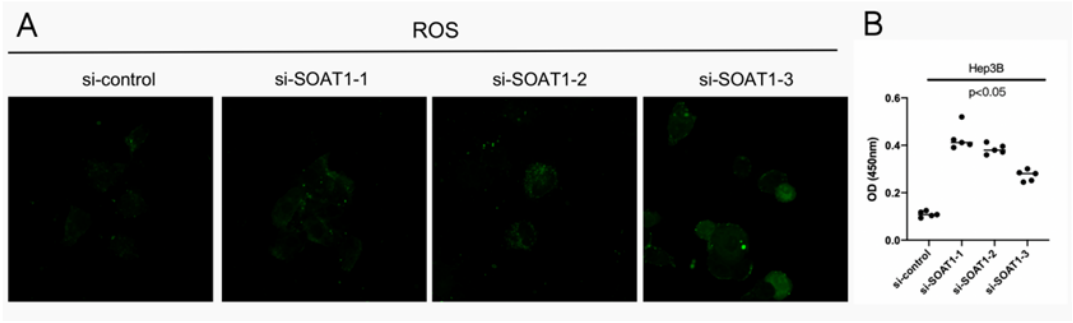


Figure 13: Inhibition of *SOAT1* Causes Mitochondrial Damage, ROS Production, and HCC Cell Apoptosis. A and B represent fluorescence images and micro plate reader of ROS in HCC cells treated with/without *SOAT1* siRNA as in (A) and (B) in the presence or absence of NAC (1 mM). Scale bar, 10 mm. Dead cell percentage was counted after treatment for 72 hours (mean ± SD, n=3) (right bottom panels). **Note:** p<0.05.

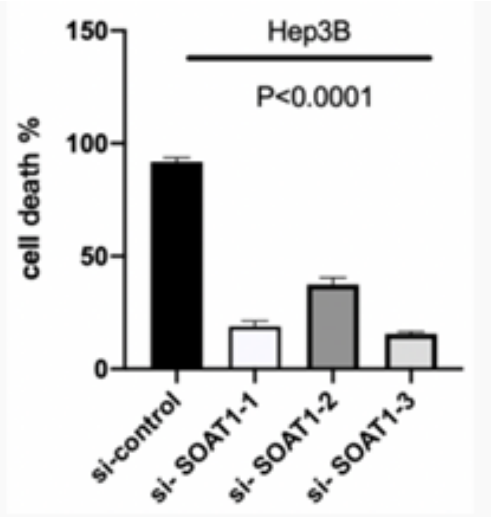


Figure 14: Inhibition of *SOAT1* Causes Mitochondrial Damage, ROS Production, and HCC Cell Apoptosis. Hep3B cells were treated with/without *SOAT1* siRNA, dead cell percentage was counted after treatment for 3 days by Cell viability counter (mean ± SD, n=3). **Note:** p<0.05.

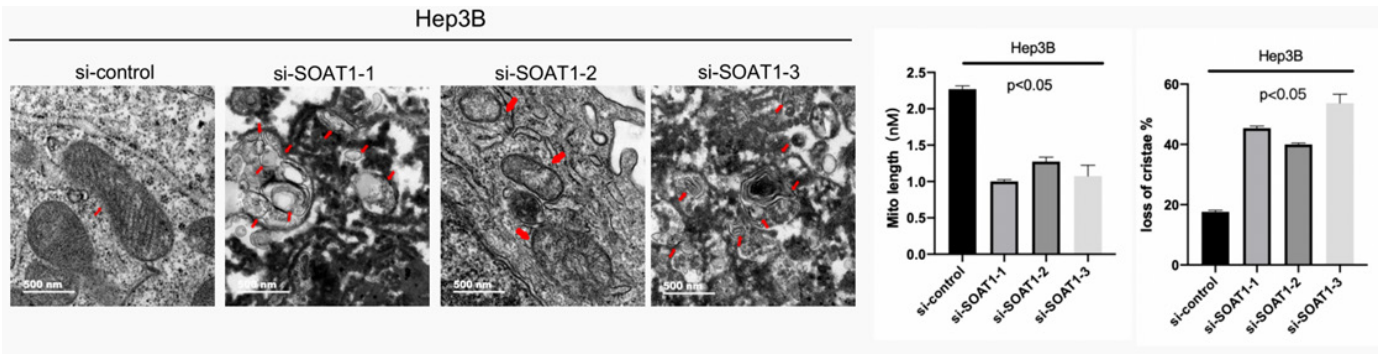


Figure 15: Inhibition of *SOAT1* Causes Mitochondrial Damage, ROS Production, and HCC Cell Apoptosis. Representative TEM imaging of the mitochondria in Hep3B cells treated with/without *SOAT1* siRNA knockdown for 48 h. Scale bar, 500 nm. **Note:** p<0.05. Red arrows indicate mitochondria. More than 10 cells were quantified for tubular or fragmented mitochondria (mean ± SEM).

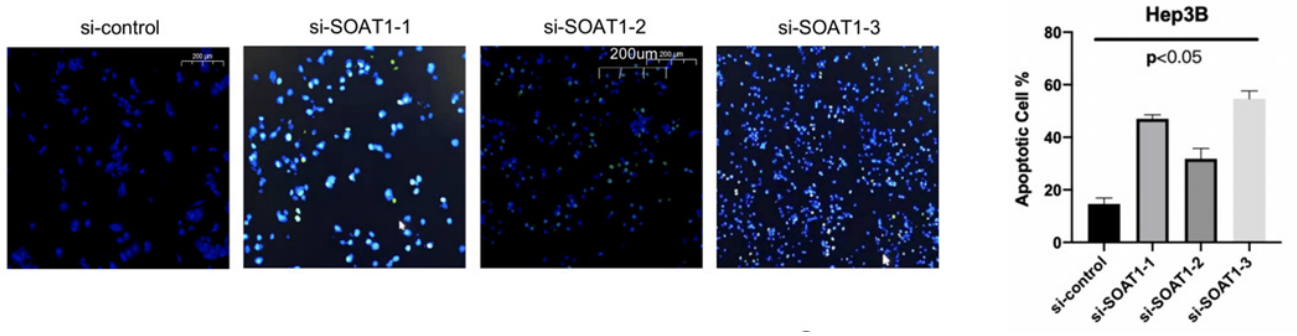


Figure 16: Inhibition of *SOAT1* Causes Mitochondrial Damage, ROS Production, and HCC Cell Apoptosis. Representative Tunnel fluorescence imaging in Hep3B cells treated with siSOAT1. Scale bar, 200 um. **Note:** p<0.05.

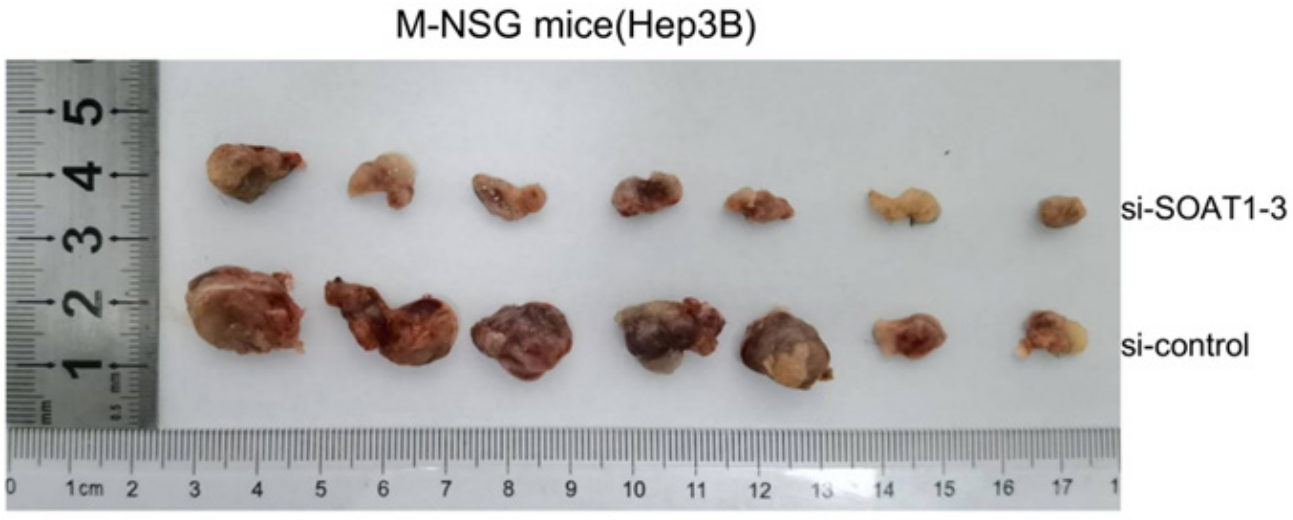


Figure 17: Inhibition of *SOAT1* Causes Mitochondrial Damage, ROS Production, and HCC Cell Apoptosis. The effects of si*SOAT1* in Hep3B-derived subcutaneous models (mean ± SD, n=7).

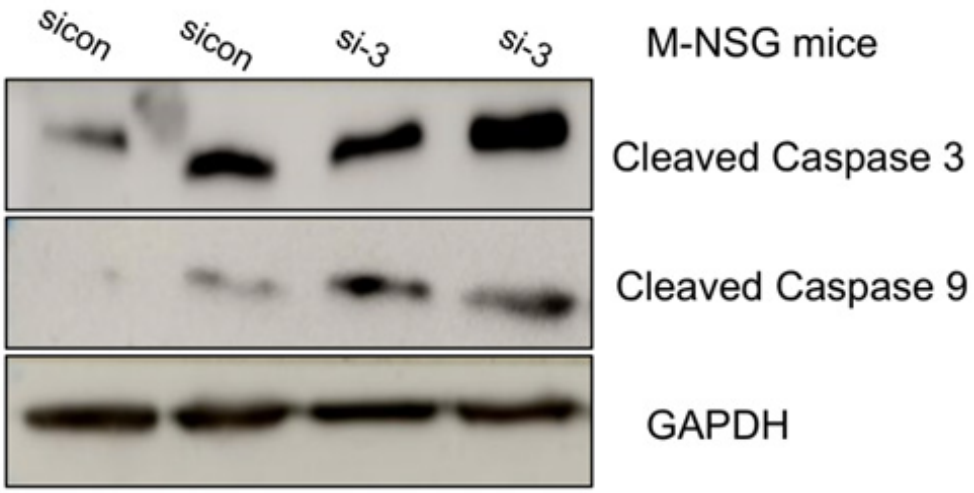


Figure 18: Inhibition of *SOAT1* Causes Mitochondrial Damage, ROS Production, and HCC Cell apoptosis. The effects of si*SOAT1* in Hep3B-derived subcutaneous models (mean ± SD, n=7).

DISCUSSION

Despite the large number of studies on HCC over past decades, little progress had been made because the lack of comprehensive and depth understanding. 11 of MBOAT proteins *(MBOAT1, MBOAT2, HHATL, GOAT, LPCAT3, MBOAT7, SOAT1, SOAT2, HHAT, DGAT1, PORCN)* unusual changes had been reported in many tumors, including Chronic Lymphocytic Leukemia (CLL), Pancreatic Ductal Adenocarcinoma (PDAC), prostate cancer, clear cell Renal Carcinoma (ccRCC), Glioblastoma (GBM), breast cancer and Hepatocellular Carcinoma (HCC) [37-40]. The function of some MBOATs in the prognostic and predictive of cancers has been partially reported, further bio-informatics analysis still need to be confirmed. We use different public databases to comprehensively analyze the mRNA and protein expression, assessing prognostic worth of 11 of MBOAT genes in HCC with the purpose to find the most effective potential biomarkers.

MBOAT1 genes overexpression were significantly associated with a reduced risk of disease progression with HR=2.1, 95% CI: 1.1-3.7, P=.018, indicating that MBOAT1 can surrogate molecular markers for IGHV mutational status in chronic lymphocytic leukemia for predicting time to first treatment [17]. MBOAT1 was higher in cancer than in normal tissues in TCGA. By the Kaplan-Meier Plotter, we found the prognostic value of MBOAT1 in patients with HCC despite it was not correlated with the clinical features. A high MBOAT1 expression was highly associated with poor OS, PFS, RFS, and DSS. The expression and role of *MBOAT2* in HCC was not well known until now. Zhouxiaoxiao et al. [41] reported that Circ-*MBOAT2* knockdown represses tumor progression and glutamine catabolism by miR-433-3p/GOT1 axis in pancreatic cancer. Moreover, Tang Xiaolong et al. [42] reported that *MBOAT2* is highly expressed in both Colorectal Cancer (CRC) tissues and serum samples, and has a relationship with tumor stage. We found the expression of *MBOAT2* in cancer tissues was higher than in normal tissues. What's more, *MBOAT2* expression was correlated with tumor stage in patients with Hepatocellular Carcinoma. However, *MBOAT2* expression was not significantly correlated with poor OS, PFS, RFS, and DSS in all of the patients with hepatocellular carcinoma. *HHATL* is an O-acyl transferase which is required for several cellular processes, relating to apoptosis development, such as rafts integrity and stability, lipid metabolism [43]. We proved that the expression of *HHATL* has no difference and relationship with tumor stage between hepatocellular carcinoma tissues and normal tissues. What's more, the low expression has no correlated with poor OS, PFS, RFS, and DSS in all of the patients with HCC, which seemed that *HHATL* was not an oncogene. *MBOAT4*, a member of the MBOATs super-family, is also named as as Ghrelin O-acyltransferase (GOAT). Gualillo Oreste et al. found drugs that inhibit GOAT might be able to prevent diet-induced obesity and might be an effective therapy for type-2 diabetes [44]. The expression of *MBOAT4* has relationship with tumor stage but has no difference in HCC tissues than in normal. LPCAT3 regulated intestinal stem cells and progenitor cells by stimulating cholesterol biosynthesis; increasing cholesterol in the diet or through genetic manipulation promoted tumorigenesis [45]. LPCAT3 maintains systemic lipid homeostasis by regulating lipid absorption in intestine, lipoprotein secretion, and de novo lipogenesis in liver. Changes in LPCAT activity may be potentially involved in pathological conditions, including nonalcoholic fatty liver disease and cancer [46]. Moreover, Rong Xin et al. [47] discovered that promotion of LPCAT3 activity ameliorates Endoplasmic Reticulum (ER) stress induced by saturated free fatty acids *in vitro* or by hepatic lipid accumulation *in vivo*. Conversely, LPCAT3 knockdown in liver exacerbates ER stress and inflammation.

We demonstrated that transcriptional levels of LPCAT3 in patients with Hepatocellular Carcinoma was higher expressed in Chen Liver (fold change=1.214) [48]. The expression of LPCAT3 was obviously related to poor PFS and RFS in all of the patients with HCC. *SOAT2* is also known as ACAT2 which is reported expressed in several tumors. Pramfalk C et al. [49-52] showed

that HNF4alpha, directly or indirectly (*via* HNF1alpha), can bind to the ACAT2 promoterreported *via* ChIP assays and protein-to-protein interaction studies. Thus lower levels of esterified cholesterol in VLDL-particles and LDL-particles in patients with MODY1 may at least in part-be attributable to lower ACAT2 activity in these patients. Other data indicate that leptin may enhance the proliferation, migration and invasion of breast cancer cells through the PI3K/AKT/SREBP2 signaling pathway *via* ACAT2 up-regulation. Therefore, the leptin/ACAT2 axis may represent an attractive therapeutic target for breast cancer [53]. The mRNA level of SOAT2 was not related with tumor stage but has difference between HCC and normal tissues. *HHAT* was significantly up-regulation in patients with HCC, and has high OS, RFS, PFS and DSS in our report. Asciolla James J et al. [54] reported that *HHAT* serves a dual function as a palmitoyl acyltransferase and a conduit to supply palmitoyl-CoA to the luminal side of the ER. What's more, Regan Joseph L et al using small-molecule inhibitors and RNAi against *HHAT*, demonstrate that non-canonical Hedgehog signaling is a positive regulator of WNT signaling and required for colon CSC survival [55]. This is consistent with our analysis.

PORCN inhibitors that block WNT secretion have proven effective in WNT-addicted preclinical cancer models and are in clinical trials. We found the mRNA expression of *PORCN* in cancer tissues was higher than in normal tissues. *PORCN* is a membrane-bound O-acyltransferase that is required for and dedicated to palmitoylation of WNT ligands, a necessary step in the processing of WNT ligand secretion [56]. *PORCN* inhibitors that block WNT secretion have proven effective in WNT-addicted preclinical cancer models and are in clinical trials [57]. We didn't found significantly mRNA change of *PORCN* in HCC tissues compares with normal tissues. No relationship can be found between the expressions of *PORCN* with tumor stage in patients with HCC. Corbet Cyril showed that upon TGF-β2 stimulation, PKC-zeta-mediated translocation of CD36 promotes the uptake of fatty acids that are either oxidized to generate ATP to fulfill immediate cellular needs or stored as triglycerides in LD through *PORCN*56. The PFS and RFS of *PORCN* have profound meaning in our analysis. In a human biopsied NAFLD cohort, *MBOAT7* is reported associated with fibrosis independent of the presence of histological inflammation [58]. We also found that the transcriptional levels of *MBOAT7* in cancer tissues was higher in three datasets, but has no correlation with tumor stage in cancer patients. A higher *MBOAT7* expression has correlationship with OS, DSS, PFS, and RFS in all of the patients with significantly difference, respectively. In our analysis, no significant difference was found in the mRNA and protein data of *DGAT1*, suggesting that *DGAT1* is not an ideal diagnostic and prognostic marker in HCC.

Targeting gene family has been a steady focus in therapeutic development for cancer. However, as the number of genes in each gene family varies, therapeutically targeting this process in individuals with cancer is limited. Thus, identifying the most effective target in gene family uniquely operating in HCC and other malignancies, while inactive other genes is necessary to develop a specific antitumor therapy. In fact, our findings showing that targeting MBOATs family number of *SOAT1* open up a new opportunity to target this deadly cancer. After comprehensive analysis of the MBOAT family using proteomic data from the published CELL article, we found only *MBOAT7* by saturated free fatty acids *in vitro* or by hepatic lipid accumulation *in vivo*. Conversely, LPCAT3 knockdown in liver exacerbates ER stress and inflammation.

CONCLUSION

SOAT1 has difference between patients with hepatocellular carcinoma than normal tissue, significantly. We found the transcriptional level of *SOAT1* increased in all six of the eight databases. A high *SOAT1* expression was obviously related with poor OS and DSS in all of the HCC patients, which seemed consistent with the role of *SOAT1* as a tumor promoter. In the previous analysis of mRNA data, *MBOAT7* has no correlation with tumor stage in HCC patients. We finally get to the conclusion that the possibility of *SOAT1* as an oncogenic factor. We induced *SOAT1* silencing through transfection siRNAs in two HCC cell lines (Huh7 and Hep3B) to evaluate the role of *SOAT1 in vitro*. The results confirmed that knocking down *SOAT1* decrease proliferation and migration in HCC cells, which further confirm *SOAT1* as a crucial target in HCC. This concept is supported by studies that inhibiting *DGAT1* alone did not subsequently. As has been studied in previous, inhibition of *SOAT1* suppresses glioblastoma growth *via* blocking SREBP-1-Mediated Lipogenesis [49]. Genetic targeting of *SOAT1* impairs cell proliferation *in vitro* and tumor progression *in vivo* and reveals a mevalonate pathway dependency in p53 mutant PDAC cells that have undergone p53 Loss of Heterozygosity (LOH). Some new cholesterol metabolic molecules such as SOAT1, SQLE, and NPC1 have recently emerged as promising drug targets for cancer treatment. Over the past two decades, the prognosis for HCC has remained very dismal despite aggressive treatment. One of the main reasons for this limited progress is a lack of full understanding of HCC biology. This mechanism has the advantage to quickly boost malignant tumor growth. Interestingly, based on our results, targeting *SOAT1* elevated ROS production will induce mitochondrial damage and apoptosis in tumor cells. This is the first time to find the most effective target in the gene family and verify it in HCC. Herein, our work provides a strong basis to translate *SOAT1* inhibition to clinical testing in individuals with HCC and other cancers expressing high levels of *SOAT1*.

AUTHORS CONTRIBUTIONS

Conceptualization, Minghan Wang.; Investigation, Minghan Wang., Junyuan Han.,; Writing Original Draft, Minghan Wang;Writing – Review & Editing, Minghan Wang; Supervision, Minghan Wang, Quanjun Wang; Funding Acquisition, Quanjun Wang., Ying Jiang.

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COMPETING INTERESTS

The authors declare no competing interests

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