Clinicopathological Significance of Cancer Stem Cell-associated ACOT12 Expression in Hepatocellular Carcinoma

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Research Article

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Abstract

The acetyl-CoA pool has been identified using various cell measures in studies related to cell development and mitosis. It is suggested that acetyl-CoA elevates the level of histones at certain qualities of cell development. The relationship between ACOT12 and hepatocellular carcinoma (HCC) is unclear. Here, data on ACOT12 expression were obtained from HPA, GEPIA, TCGA portal, and UALCAN web repositories. Clinicopathological significance of ACOT12 was examined using Kaplan-Meier analysis, OncoLnc, TCGA portal and PROGgeneV2. The association between ACOT12 with cancer stem cells was examined based on GEO datasets GSE111802 and GSE7053. The link between ACOT12 and microRNAs and genes that modulate survival was determined based on Cytoscape, GEPIA, and TargetScan. These analyses revealed that relative to normal liver and paracancerous tissues, ACOT12 expression was downregulated in HCC and favorable liver tumors at both mRNA and protein levels. HCC patients with lower ACOT12 expression exhibited poorer overall and disease-free survival. Additionally, ACOT12 influenced HCC. In particular, low ACOT12 expression was related to the following groups of patients: poorly differentiated HCC, vascular invasion positive, sorafenib untreated, with alcohol consumption and hepatitis infection of HCC. The intersection of differentially expressed genes (DEGs) was examined to assess the underlying mechanism. This prompted the distinguishing proof of five possibly associated genes-GC, KNG1, DNAJC28, CUX2 and KMO. A network containing ACOT12 miR-7, miR-9, and miR-374b was constructed. Taken together, our findings indicate that ACOT12 is decreased in HCC and it might mediate HCC progression via stem cell-related genes.

Introduction

Hepatocellular carcinoma (HCC) has a poor prognosis due to a high metastasis rate, even after radical treatment [1–4]. Thus, understanding the mechanisms of HCC metastasis is crucial for improving HCC outcomes. Cancer stem cells (CSCs) are crucial in cancer development, metastasis, recurrence, and drug resistance [2]. HCC stem cells (HCSCs) exist in two states: mesenchymal-epithelial transition (MET), and epithelial-mesenchymal transition (EMT) which express markers of CSC. The two states can also switch, giving HCSCs high plasticity [3]. During EMT, cancer cells escape intercellular restraint and become migratory and invasive [4]. To improve prognoses and develop targeted HCC therapy, novel biomarkers for EMT and CSCs in HCC are needed.

Acyl-CoA thioesterase 12 (ACOT12) is a key modulator of HCC proliferation. Downregulation of ACOT12 expression in HCC is strongly linked to metastasis and poor survival. There is evidence that ACOT12 modulate cholesterol metabolism b [5]. This research also revealed high activity of ACOT12 during the acute phase but not chronic phase of streptozotocin-induced rodent diabetes, which was abrogated by insulin [5], indicating that ACOT12 drives adaptation to abnormal lipid metabolism. ACOT12 is a member of ACOT family, which is comprised of 13 members. ACOTs drives the breakdown of acyl-thioester CoAs to free fatty acid and CoA, with each ACOT preferring distinct substrates [6]. Through TCGA dataset analysis we found that ACOT7 and ACOT8 upregulation correlates with poor HCC prognosis. ACOT12 is reported to regulate histone acetylation and acetyl-CoA levels in HCC cells, and its downregulation

promotes HCC metastasis by epigenetically driving EMT. However, it is unclear how ACOT12 influences CSCs in HCC are unknown.

Here, we investigated associations between ACOT12 expression and HCC and found that reduced ACOT12 expression in HCC patients was linked to prognosis and clinicopathology[7]. Our data show that ACOT12 regulates HCC via the HCC stem cell-related genes-GC, KNG1, DNAJC28, CUX2 and KMO. As opposed to ACOT12 articulation and endurance patterns, including miR-7, miR-9, and miR-374b, km-plotter was combined with miRNA-ACOT12-mRNAnetwork. This study demonstrates that ACOT12 is associated with CSCs in HCC.

Materials And Methods

Determination of ACOT12 level

UALCAN (http://ualcan.path.uab.edu) [8], GEPIA (http://gepia.cancer-pku.cn/detail.php) [9], HPA (https://www.proteinatlas.org/) [10] and TCGA portal (http://www.tcgaportal.org) [11] were used to examine ACOT12 expression in various tissues relative to HCC tissues. UALCAN (http://ualcan.path.uab.edu) was then used to investigate methylation of ACOT12 promoter in different molecular types of HCC. cBioPortal (http://www.cbioportal.org) is a free database that may be used to look into various illness genes[12, 13]. The HCC dataset comprised of 428 cases, and the cBioPortal was utilized to analyze the clinical significance of ACOT12. Results were statistically analyzed using Wilcoxon rank sum test.

Relapse and survival analyses

Kaplan-Meier plotter (https://kmplot.com/examination/) was used to examine the relationship between ACOT12 expression and recurrence-free survival (RFS) and overall survival (OS) based on 1809 patient microarrays [14]. The two patient cohorts were analyzed using a Kaplan-Meier survival analysis, hazard ratios, and log rank *p-values*. HCC patients were divided into a high and low expression group based on median ACOT12 expression value and the prognostic value of ACOT12 estimated using OncoLnc (https://www.oncolnc.org/)[15]. PROGgeneV2 Pan cancer prognostics database (http://genomics.jefferson.edu/proggene/) and TCGA portal (https://www.tcgaportal.org) were used to investigate the role of ACOT12 in HCC[16].

Clinicopathological parameters of HCC patients analysis using UCLCAN

UALCAN offers access to publicly available cancer OMICS datasets (TCGA, MET500, and CPTAC), allowing the identification of biomarkers or in silico validation of genes of interest [8]. Tumor stage and grade, hepatitis infection, alcohol consumption, sorafenib treatment, and vascular invasion were among the variables. The prognosis module can identify new HCC prognostic factors. The correlation module was used to examine the relationship between ACOT12 and GC, KNG1, DNAJC28, CUX2, and KMO.

Identification of mRNAs and miRNAs that target ACOT12

GEO (http://www.ncbi.nlm.nih.gov/geo) is a high throughput genomics repository for microarray, gene expression, and chip datasets. Two gene expression datasets [GSE111802[7] and GSE70537[17]] were downloaded from GEO [GPL21282] Phalanx Human OneArray Ver. 7 Release 1, and GPL9115 Illumina Genome Analyzer II (Homo sapiens). Dataset GSE70537 contained two sorts of stem-like cancer cells (HCSCs), Hep3B-C and Huh7-C. Dataset GSE111802 contained a 4-cluster study in which ACOT12 was overexpressed in Huh7 and MHCC97H cells. TargetScan (http://www.targetscan.org/vert 72/) searches for conserved 8mer, 7mer, and 6mer sites that match miRNA seed regions to predict targets genes [18]. These 3 datasets were used to identify miRNAs that are likely to bind to ACOT12 mRNA. Kaplan-Meier analysis was used to determine association between miRNAs and HCC patients' OS and RFS.

Functional enrichment analysis and the miRNA-ACOT12-mRNA network

STRING (https://string-db.org/) can predict direct and indirect protein-protein interactions [19] based on computational expectations, knowledge transfer among organisms, and other datasets. Here, STRING was used to establish an interaction network between ACOT12 and other significant proteins. We examined each of the 5 proteins and 3 miRNAs as a network of ACOT12 connection system. Cytoscape 3.8.0 (https://cytoscape.org/) was used to build a miRNA-ACOT12-mRNA network for these candidates[20].

Results

ACOT12 expression levels in pan-cancer and HCC patients

A flowchart of this study is shown on Fig. 1. The databases used in this study are listed on Table 1. First, we evaluated ACOT12 expression at the mRNA and protein levels, followed by analysis of its clinicopathological impact on HCC. HCC stem cells data was used to investigate ACOT12 upstream and downstream processes.

Table 1
Summary of databases used in this study.

Name	Link	This study	Keywords
Oncomine	https://www.oncomine.org/resource/main.html	To analyze the HHEX mRNA expression in different cancers	gene expression
UALCAN	http://ualcan.path.uab.edu/	To analyze the HHEX mRNA expression in different molecular subtypes of BC patients	gene expression; 16 cancer types; survival curve; DNA methylation
GEPIA	http://gepia.cancer-pku.cn/detail.php	To assess the correlations between genes	gene expression; survival curve; isoform details; genes correlation; similar genes detection
TCGAportal	http://www.tcgaportal.org	To investigate the expression and survival of HHEX in human tissues	gene expression; 28 cancer types; survival curve; DNA methylation; mutation
Human Protein Atlas	https://www.proteinatlas.org/	To detect HHEX protein expression and survival	proteins distribution; subcellular localization; impact for survival
Kaplan-Meier Plotter	http://kmplot.com/analysis/index.php?p=background	To analyzed the correlations between genes and miRNAs expression and RFS as well as OS	breast cancer; subtype; survival curve
OncoLnc	https://www.oncolnc.org/	To detect HHEX survival	breast cancer; survival curve

Name	Link	This study	Keywords
PROGgeneV2	http://genomics.jefferson.edu/proggene/		gene expression; breast cancer; subtype; survival curve
cBioportal	http://www.cbioportal.org/	To detect HHEX genetic alterations	genetic alterations; survival curve
GEO	https://www.ncbi.nlm.nih.gov/geo/	To selecte data sets related to breast cancer stem cells to explore further mechanisms	gene expression; datasets details
STRING	https://string-db.org/cgi/input.pl	To obtain the interaction network between HHEX and other important proteins	protein details; interactive network; functional enrichment
starBase v3.0	http://starbase.sysu.edu.cn/index.php	To predict the miRNAs related to HHEX; To perform miRNAs as well as correlation analysis between miRNAs and HHEX mRNA	miRNA target; Pan-cancer

Table 2
Correlation between ACOT12 expression and clinicopathological features.

Gene symbol:ACOT12				
	OS		**RFS**	
characteristic	**HR(95% CI)**	**Log rank P**	**HR(95% CI)**	**Log rank P**
Stage				
1	0.53(0.29–0.99)	**0.0426**	0.49(0.25–0.98)	**0.04**
1 + 2	0.53(0.33–0.86)	**0.0085**	0.46(0.27–0.8)	**0.0043**
2	0.42(0.19–0.94)	**0.029**	0.56(0.24–1.28)	0.16
2 + 3	0.46(0.29–0.76)	**0.0016**	0.73(0.46–1.16)	0.18
3	0.3(0.16–0.57)	**0.000094**	0.58(0.31–1.06)	0.073
3 + 4	0.34(0.18–0.62)	**0.00023**	0.58(0.31–1.06)	0.073
Grade				
1	0.07(0.02–0.23)	**0.000000014**	0.38(0.1–1.45)	0.14
2	0.56(0.33–0.93)	**0.0023**	0.37(0.19–0.73)	**0.0028**
3	0.28(0.11–0.72)	**0.0049**	0.59(0.33–1.05)	0.07
AJCC-T				
1	0.51(0.28–0.91)	**0.021**	0.48(0.25–0.94)	**0.029**
2	0.41(0.19–0.89)	**0.019**	2.2(0.89–5.47)	0.083
3	0.27(0.14–0.51)	**0.000026**	0.44(0.23–0.84)	0.01
Vascular invasion				
none	0.4(0.22–0.74)	**0.0024**	0.43(0.23–0.8)	**0.0065**
micro	0.49(0.23–1.07)	0.068	1.86(0.94–3.68)	0.069
Sorafenib treated	0.22(0.06–0.81)	**0.013**	0.36(0.12–1.04)	0.05
Alcohol consumption				
yes	0.52(0.28–0.98)	**0.038**	0.35(0.18–0.65)	**0.00062**
none	0.54(0.34–0.86)	**0.0089**	0.74(0.45–1.21)	2.20E-01
Hepatitis virus:				
yes	0.39(0.2–0.77)	**0.0047**	0.69(0.42–1.15)	0.16

The bold fonts means the values with statistical significance.

Gene symbol:ACOT12				
none	0.57(0.35–0.92)	**0.02**	0.51(0.3–0.88)	**0.013**
The bold fonts means the values with statistical significance.				

ACOT12 gene expression in various organs was first determined on HPA and UALCAN and compared to expression in various malignancies vs normal tissues. This analysis revealed ACOT12 to be mainly expressed in the liver and to be downregulated in HCC (Fig. 2A-C). GEPIA and TCGA portal analysis of ACOT12 expression in HCC vs normal tissues (Fig. 2D-F) showed that it was significantly downregulated in HCC patients relative to normal tissues. These data demonstrate that ACOT12 may be tumor suppressive in HCC.

Prognostic value of ACOT12 in HCC

To assess the contribution of ACOT12 to the clinical progress of HCC, we carried out survival analyses for ACOT12 utilizing km-plotter, GEPIA, TCGA portal, OncoLnc and PROGgeneV2. Figure 3A shows that patients with high ACOT12 expression levels had better 5-year (60 months/1800 days) survival rates than patients with low ACOT12 expression, and that most HCC patients with low levels of ACOT12 showed poor RFS and OS (Fig. 3A-E). Low ACOT12 mRNA expression [HR 0.51 [0.36–0.72], P = 0.00011] was linked to worse RFS and OS in HCC patients (Fig. 3A). At follow-up durations exceeding 120 months, HCC prognosis in patients with high ACOT12 levels was even worse. HPA data strongly linked reduced ACOT12 protein levels to poorer survival (Fig. 3B).

Impact ACOT12 on clinical features of HCC patients

To assess the clinicopathology impact of ACOT12 in HCC, we performed sub-group analysis of various prognostic parameters on UALCAN and found a strong link between ACOT12 expression and tumor grade. Patients with poorly differentiated cancers (grades 3 and 4) had lower ACOT12 levels than those with well-differentiated tumors (grades 1 and 2), similar to the American Joint Committee on Cancer (AJCC) and TNM stage classification. Additionally, patients with vascular invasion scores tended to have lower ACOT12 levels. Sorafenib-treated HCC patients showed fundamentally higher ACOT12 levels relative to non-Sorafenib treated ones. As expected, alcohol consumption and hepatitis infection showed markedly reduced ACOT12 expression in HCC patients (Fig. 3E). Additionally, there is a critical relationship between ACOT12 expression, histological type, and HCC subtype. UALCAN subclass results were predicted using GEPIA (Fig. 3C). GEPIA analysis revealed strong link between ACOT12 levels and pathological stage of HCC (Fig. 3D).

Kaplan-Meier analysis revealed that ACOT12 expression correlated with tumor stage, vascular invasion, history of sorafenib treatment, alcohol consumption, and hepatitis infection (Table 3). OS and RFS analyses showed that ACOT12 significantly correlates with vascular invasion and history of sorafenib

treatment, alcohol consumption, and no hepatitis infection. Together, these data indicate that ACOT12 influences HCC clinical features and is a promising biomarker for HCC prognosis.

Genetic alterations and promoter methylation of ACOT12 in HCC patients

To investigate the mechanisms underlying ACOT12 association with HCC prognosis, we first investigated ACOT12 genetic mutations and methylation levels. UALCAN analysis showed that ACOT12 promoter methylation was lower in HCC than in the typical group (Fig. 4A). Additionally, HCC nodal metastasis correlated with ACOT12 promoter methylation (Fig. 4B). ACOT12 was changed in 6.7% of queried HCC patients (Fig. 4D). Kaplan-Meier and log rank test analyses showed that patients with ACOT12 alteration had longer OS (Fig. 4C).

Potential mechanism underlying ACOT12 function in HCC

Identification of DEGs in EMT and MET states (GSE70537)

HCSCs may influence metastasis, chemoradiotherapy resistance, tumor recurrence, and patient prognosis. Hence, we chose HCSC-related datasets to study deeper mechanisms. Relative to MET, 1388 DEGs, including ACOT12, were identified in GSE111802, implying that ACOT12 is linked to HCSCs.

Identification of DEGs after overexpression of ACOT12 (GSE111802)

To investigate how downstream genes were affected by altered ACOT12 expression, we analyzed the overexpression dataset. To guarantee the consistency of the gene expressio trend, we chose decreased genes with overexpressed ACOT12 to acquire the normal genes. Additionally, we chose upregulated genes with overexpressed ACOT12. Subsequently, we acquired 492 qualities that expressed a similar pattern changed after overexpression of ACOT12 (Fig. 4E).

PPI network and KEGG pathway enrichment analysis of common DEGs

The crossover point of the DEGs linked with HCSCs and the DEGs after changed ACOT12 expression was then used to explorations into mechanisms driving ACOT12 functions. This analysis identified 25 common genes (Fig. 4E). Results of PPI and KEGG pathway enrichment analyses revealed that ACOT12 is associated with JAK-STAT pathway genes, including IFNA4, IL6, IFNL2, IL20 and IL4, and JAK-STAT signaling (Figs. 4F-G), indicating that ACOT12 may influence HCC via JAK-STAT signaling.

Correlations of four-survival genes with ACOT12

Results presented in Fig. 5A-B indicate that 5 qualities - GC, KNG1, DNAJC28, CUX2 and KMO together correlated with survival. GEPIA analysis of the relationship between ACOT12 and the five genes revealed that they have positive connections (Fig. 5C).

ACOT12 regulatory networks in HCC

By predicting miRNAs, we aimed to uncover upstream regulatory mechanisms of ACOT12. Km-plotter analysis identified miR-7, miR-9, and miR-374b as negative modulators of ACOT12 expression and survival (Fig. 5D). Next, we created a miRNA-ACOT12-mRNA regulatory network by which ACOT12 may regulate HCSCs to alter HCC prognosis (Figs. 5E).

Discussion

HCC is associated with high morbidity and mortality and is characterized by a low 5-year survival rate [21]. Due to limited understanding of the characteristics and regulatory mechanisms of HCSCs effective predictors for HCC recurrence and prognosis are limited. ACOT12 suppresses HCC metastasis [7]. Downregulated ACOT12 expression and elevated acetyl-CoA correlate with HCC metastasis, highlighting ACOT12-driven acetyl-CoA elevation as a potential biomarker of HCC metastasis [7]. Recently, acetyl-CoA levels were reported to modulate migration of glioblastoma cells [22]. Therefore, acetyl-CoA contribute to metastasis. FASN and ACC1 have been reported as acetyl-CoA sensitive genes in HCC [23]. Thus, ACOT12 might be a putative CSC-related predictor of HCC prognosis. However, connections between ACOT12, prognosis, and CSCs in HCC are unclear.

Knockdown and upregulation of ACOT12 was reported to modulate lipid metabolism and acetyl-coA level. Here, we found that ACOT12 altered the acetylation of histone H3 without affecting other cytosolic or histone proteins. More research is needed to determine how acetyl-CoA availability in the entire cell influences H3 acetylation. It should be highlighted that alteration to acetyl-CoA level may in turn change substrate selectivity of p300. Moreover, the most beneficial p300 acetylation occurred on H3K9 when the level of acetyl-CoA was highest [24]. KAT2A (GCN5) is primarily involved in H3 acetylation [25], and is physiologically responsive to acetyl-CoA:CoA ratio [26, 27]. We speculate that KAT2A and p300 when acetyl-coA is elevated influences the impact of ACOT12 on H3 acetylation. ACOT12 also has unique effects on H3 acetylation. Acetyl-CoA interact with cofactors, transcription factors and KATs to influence gene expression and locus-explicit H3 acetylation, but the mechanism involved is unknown. More research into the several transcriptional complexes modulated by acetyl-CoA is needed.[7].

ACOT12 is reported to suppress liver tumor growth and nuclear ACOT12 levels are markedly reduced. ACOT12 overexpression inhibits Hep3B and Huh7 liver cancer cells' migration and invasion. Here, we observed reduced ACOT12 expression in HCC relative to normal liver tissues, similar to existing reports. Low ACOT12 mRNA levels correlate with poor HCC prognosis [7]. We have previously shown that ACOT12 downregulation correlates with better OS and RFS in HCC. Moreover, the prognostic value of ACOT12 was critical regardless of grade, stage, vascular invasion, and smook consumption. We discovered that ACOT12 expression was reduced in HCC, which increased the survival of HCC patients.

With respect to ACOT12 gene alternative and DNA methylation, the impact of ACOT12 mutations on HCC progression has not been sufficiently studied. Here, UALCAN analysis demonstrated that methylation of

ACOT12 promoter was elevated in HCC relative controls. Additionally, nodal HCC nodal metastasis significantly correlated with ACOT12 promoter methylation. ACOT12 was altered in 6.7% of HCC patients showing low OS. Because transcriptomics detect only static mutations, it was necessary to further elucidate the impact of ACOT12 promoter methylation and genetic alterations on HCC.

CSCs present with high clonogenicity and invasiveness. They are crucial for metastasis and clinical recurrence in various cancers. ACOT12-dependent acetyl-CoA changes promote EMT. Thus the connections between CSC-like DEGs and ACOT12-targeted genes was examined.

Dataset GSE70537 is based on the human HCC cell lines, Hep3B and Huh7, that were used to culture stem-like cancer cells and to investigate the regulatory mechanisms of these two types of HCSCs using miRNA and RNA sequencing data. HCSCs are found in EMT and MET states. HCSCs proliferative capacity and invasiveness are linked to different EMT and MET states. HCSCs' plasticity allows them to transition between EMT and MET modes[17]. Thus, we selected datasets to study the link between ACOT12 expression and HCSCs in more detail. GSE70537 yielded 1413 DEGs in the presence of EMT and MET, including ACOT12, indicating that it is associated with HCSCs. This analysis revealed 92 genes in dataset GSE70537 that had similar expression trends for ACOT12 overexpression. The intersection of DEGs of HCSCs and those related to abnormal ACOT12 expression was then examined to determine the mechanisms of ACOT12 activity. Finally, we obtained 25 normal genes: USP49, NAALADL1, PDE4C, ZNF334, HLF, ZIC4, GC, FGF12, SPDYE1, CUX2, FHOD3, HTR2B, SLC2A12, SCN2A, MCOLN3, PRG2, FBXL21, DNAJC28, MCC, KNG1, ROBO2, LOXL4, UNC80, KMO, and CYFIP2.

More than half of these genes are strongly linked to tumor stem-like features [28–36] and the mechanism regulating CSCs can be modulated by EMT, TGF-1, and JAK-STAT signaling. Liver cancer stem-like cells accelerate cancer cell metastasis through PDE4C-induced EMT [28]. In FLT3-ITD, NPM1, and DNMT3A triple-transformed AML, hepatic leukemia factor (HLF) modulator of stem cells [29]. Immunopositive signals like ZIC4 and ZIC1/2/3/5 we previously detected in human cerebellar granule neurons and medulloblastomas [30]. In medulloblastoma, FHOD3 promotes carcinogenesis by driving RhoA/ROCK1/LIMK1 signaling [31]. These genes may be CSC targets and should be investigated further using molecular biology approaches.

Bioinformatics analysis revealed that ACOT12 is a targetable gene with a high degree of gene neighborhood and identified ACOT12-associated miRNAs. These short (20–24 nt) non-coding RNAs modulate gene expression post-transcriptionally and are implicated in cancer [37]. MiR-9-3p, miR-7-5p, and miR-374b-5p can be used as HCC diagnosis and prognosis biomarkers. HCC progression has been linked to specific miRNAs. circMTO1, a circular RNA, suppresses HCC progression by sponging miR-9 [38]. The proliferation and aggressiveness of sorafenib-resistant HCC cells are mediated by a miR-7/growth capture specific 6/TYRO3 axis [39]. ABCA8 is also controlled by miR-374b-5p and inhibits HCC growth and metastasis via the ERK/ZEB1 pathway [40]. These findings indicate that ACOT12 impacts HCC prognosis by influencing the expression of GC, KNG1, DNAJC28, CUX2, and KMO, as well as miR-9-3p, miR-7-5p and miR-374b-5p. Three miRNA inhibitors and three mimics targeting miR-9-3p, miR-7-5p, and

miR-374b-5p on ACOT12 mRNA were transfected into Huh7, HepG2, and MHCC-97H to determine the association between miR-9-3p, miR-7-5p, and miR-374b-5p and ACOT12 expression. miR-9-3p, miR-7-5p, and miR-374b-5p suppressed ACOT12 expression and enhanced cell migration, invasion, and proliferation. miR-9-3p, miR-7-5p, and miR-374b-5p downregulation enhanced ACOT12 expression and suppressed cell migration, invasion, and proliferation. Analysis of how miR-9-3p, miR-7-5p, miR-374b-5p, and ACOT12 contribute to HCC development, proliferation, invasion, and metastasis revealed that miR-9-3p, miR-7-5p, and miR-374b-5p may modulate ACOT12 expression to influence HCC development. However, further studies are needed to uncover the precise mechanism.

Conclusion

Here, an integrated bioinformatics approach identified ACOT12 as a indicator of HCC prognosis and linked it to CSCs. Furthermore, we show ACOT12 downregulation in HCC tissues relative to normal liver tissues. Bioinformatics analyses on various databases suggest that ACOT12 expression is strongly linked to OS and RFS and highlight it as a potential HCC biomarker and therapeutic target. However, further investigation into mechanisms underlying ACOT12 effects on HCC progression is needed.

Declarations

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Conflicts of interest/Competing interests (include appropriate disclosures)

The authors declare no conflict of interest.

Availability of data and material (data transparency)

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Code availability (software application or custom code)

Not applicable.

Authors' contributions (optional: please review the submission guidelines from the journal whether statements are mandatory)

L.C. and C.N. conceived and designed the experiments; W.Z. performed the experiments; L.C., X.J. and J.X. analyzed the data; W.Z. and L.C. contributed reagents and materials.

Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals

Not applicable.

Ethics approval (include appropriate approvals or waivers)

The studies involving human participants were reviewed and approved by all data are from public database on the internet.

Consent to participate (include appropriate statements)

All authors agree to submit articles for publication.

Consent for publication (include appropriate statements)

All authors agree with publication.

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Figures

Figure 1

The flowchart of the analysis process

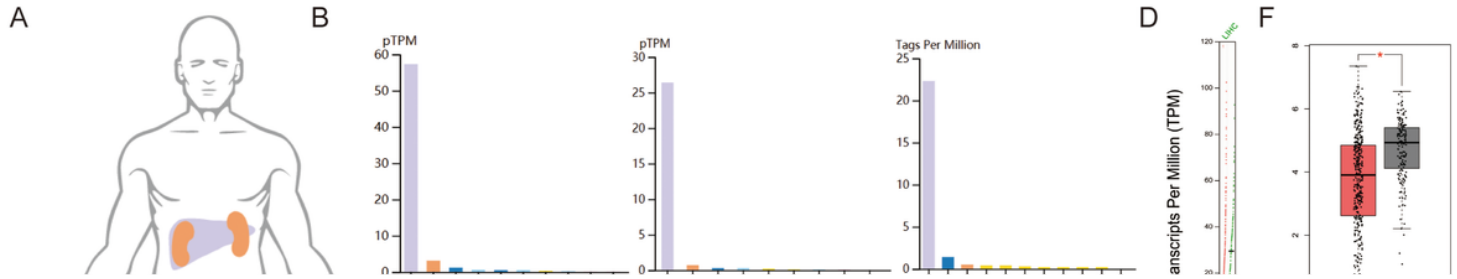

Figure 2

Expression levels of ACOT12 in pan-cancers and liver cancer patients. (A,B,C) Transcriptional levels of ACOT12 in different types of tissues and cancers determined using the HPA and UALCAN databases. (D,E,F) mRNA levels of ACOT12 in HCC determined using GEPIA and TCGAportal.

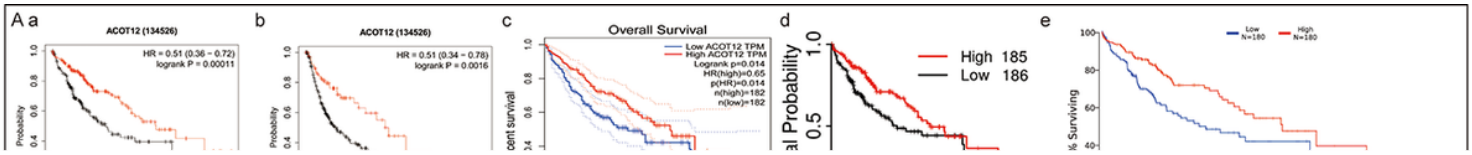

Figure 3

Prognostic value of ACOT12 in hepatocellular carcinoma (HCC) and relationship between ACOT12 and clinicopathological parameters of HCC patients. (A) Kaplan–Meier plotter, GEPIA, TCGAportal, OncoLnc and PROGeneV2 reveal the prognostic value of ACOT12 in HCC. (a) Kaplan–Meier plotter reveals the overall survival curves based on the mRNA level of ACOT12 in HCC patients. (b) Kaplan–Meier plotter reveals relapse-free survival curves based on the mRNA level of ACOT12 in HCC patients. (c) Overall survival curves of ACOT12 in HCC patients determined using GEPIA. (d) Overall survival curves of ACOT12 in HCC patients determined using TCGAportal. (e) Overall survival curves of ACOT12 in HCC patients determined using OncoLnc. (B) Survival curves based on protein level of ACOT12 in HCC patients determined using HPA. (C) Relationship between mRNA levels of ACOT12 with subclasses of HCC determined using UALCAN. (D) Correlation between ACOT12 and the pathological stage of HCC patients determined using GEPIA. (E) Relationship between mRNA levels of ACOT12 and clinicopathological parameters of HCC patients determined using UALCAN. $^{**}P < 0.05$, $^{***}P < 0.001$.

Figure 4

Promoter methylation and genetic alterations of ACOT12 and potential regulatory mechanism of ACOT12 in regulating HCC. (A) Promoter methylation and genetic alterations of ACOT12 in HCC patients. Promoter methylation levels of ACOT12 in HCC determined using UALCAN. (B) Relationship between nodal metastasis status of HCC with the promoter methylation of ACOT12 determined using UALCAN. (C) OS for ACOT12 alterations was analyzed using cBioportal. (D) Genetic alterations of ACOT12 in HCC patients determined using cBioportal. (E) Venn of the DEGs related to HCC stem cells and the DEGs after HHEX altered. (F) KEGG pathway enrichment of the 25 common genes. (G) The neighbor gene network of 25 genes was constructed using STRING. ***P < 0.001.

Figure 5

Potential regulatory mechanism of ACOT12 in HCC. (A,B) Kaplan–Meier plotter reveals the overall survival and Relapse-free survival curves based on the mRNA level of GC, KNG1, DNAJC28, CUX2 and KMO in HCC patients. (C) The positive correlation between ACOT12 expression and GC, KNG1, DNAJC28, CUX2 and by GEPIA. **(D)** Overall survival curves of ACOT12, miR-7, miR-9, and miR-374b in HCC patients determined using Kaplan–Meier plotter. **(E)** The miRNA-HHEX-mRNA potential regulatory network was constructed using Biorender.