Acta Materia Medica

Review Article

Bioinformatics-based analysis of fatty acid metabolic reprogramming in hepatocellular carcinoma: cellular heterogeneity, therapeutic targets, and drug discovery

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Received: 14 September 2024; Revised: 30 November 2024; Accepted: 5 December 2024

Published online: 27 December 2024

DOI 10.15212/AMM-2024-0057

ABSTRACT

Fatty acid (FA) reprogramming has a significant role in liver cancer. However, the contribution of FA metabolism reprogramming to the heterogeneity of hepatocellular carcinoma (HCC) has not been established. Bioinformatics analysis using single-cell sequencing, a non-negative matrix factorization (NMF) algorithm, and survival analyses were used to investigate FA metabolism reprogramming in HCC patients. Molecular targets and the progress of drug discovery were also analyzed and discussed. Among 13 types of HCC cells, epithelial cells exhibited the highest score for FA metabolic aberrance, while certain lymphocytes, such as B cells, CD8Tcm cells, and Treg cells, exhibited the lowest score. Furthermore, epithelial cells displayed significant diversity in FA metabolism with a wide distribution range (−0.2 to 0.8). Additionally, a low level of FA metabolism was associated with poor prognosis in HCC patients (log-rank test, P=0.0089). Higher oxidase expression was correlated with a lower risk of oncogenesis and higher overall survival. However, enzymes involved in synthesis, oxidation, storage, and release exhibited considerable

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phenotypic diversity in HCC. FA metabolism reprograming was shown to be significantly correlated with the heterogeneity of HCC, which is characterized by a diversity of cancerous cells and enzymes.

Keywords: fatty acids, metabolic reprogramming, hepatocellular carcinoma, heterogeneity

1. INTRODUCTION

Liver cancer ranked as the third most lethal malignancy worldwide in 2022, following lung and colorectal cancers and accounting for 7.8% of all cancer-related deaths [1]. Hepatocellular carcinoma (HCC) constituted approximated 90% of these cases [2]. The primary and secondary treatment options for HCC mainly consist of tyrosine kinase inhibitors and immune checkpoint inhibitors (ICIs). Sorafenib, a novel kinase inhibitor, acts as an inhibitor of tyrosine and serine/threonine protein kinases, inducing autophagy. However, prolonged use of sorafenib leads to resistance in HCC cells due to the loss of E-cadherin [3]. ICIs prevent evasion of tumor cells from T cells but most treated patients have a poor prognosis [4] that is probably explained by the reported marked heterogeneity of HCC.

Therefore, exploring alternative strategies against HCC remains crucial. HCC exhibits abnormal metabolism in glucose, fatty acids (FAs), amino acids, and glutamine pathways [5, 6]. Significant progress has been made in understanding metabolic reprogramming in cancer since the 1920s when Warburg discovered that aerobic glycolysis provides cancer cells with a quick ATP supply for rapid proliferation, enhances *de novo* FA synthesis from acetyl-CoA, and promotes tumorigenesis progression through membrane biosynthesis, energy storage, and release (the Warburg effect) [6, 7]. Inhibiting FA synthesis and release while promoting oxidation and storage may limit the supply of FAs and inhibit cancer cell proliferation [8]. Furthermore, FAs act as precursor signaling molecules that regulate metabolism and promote invasion and angiogenesis in HCC [5]. The alteration in FA metabolism appears to be required to maintain CSC stemness [9]. Therefore, elucidating the relationship between FA synthesis, oxidation, storage, release processes, and HCC pathogenesis may offer a potential avenue for developing novel therapeutic strategies to combat this cancer, taking account HCC heterogeneity. Previous studies have shown that FA metabolism-related diseases, such as obesity [10], hepatitis B virus (HBV) [11], non-alcoholic fatty liver disease (NAFLD), and type 2 diabetes (T2D) [12], contribute to a high risk of developing HCC. Furthermore, upregulation of the immune pathway in HCC is likely induced by the accumulation of unsaturated fatty acids [13] and immunotherapy treatment correlates with escaping FA metabolism dysregulation. The FA metabolism levels in B cells, endothelial cells, monocytes, and tissue stem cells significantly decreased after treatment, while those in hepatocytes and epithelial cells increased, suggesting that a low level of FA metabolism is associated with increased malignancy and a poor prognosis [14]. Our previous studies showed that inhibition of HCC FA biosynthesis enzymes, i.e., adenosine triphosphate citrate lyase (ACLY), acetyl-CoA carboxylase alpha (ACACA), and fatty acid synthase (FASN), lead to apoptosis [15].

Despite these converging data, a comprehensive review addressing the relationship between FA metabolism reprogramming and the heterogeneity of HCC is still lacking.

In this review paper we used bioinformatics on single-cell RNA-sequencing, applied a non-negative matrix factorization (NMF) algorithm, and performed survival analyses to investigate FA metabolism reprogramming and HCC heterogeneity. Additionally, we further analyzed and discussed possible molecular targets and recent progress in drug discovery.

2. EPITHELIAL CELLS EXHIBIT THE HIGHEST SCORE FOR FA METABOLIC ABERRATIONS IN HCC

Thirteen cell types (B, CD8Tcm, cDC1, DC, endothelial, epithelial, fibroblast, M1, M2, mast, monocyte, Tprolif, and Treg) were identified and annotated in the UMAP reduction plot after the Seurat standardization process (**Figure 1A**). The ssGSEA algorithm was utilized to quantify FA metabolism and a color gradient was applied to map the levels of FA metabolism for each cell (**Figure 1B**). The quantified scores of FA metabolism were the highest in epithelial cells and lowest in some lymphocytes, such as B, CD8Tcm, and Treg cells. Additionally, significant heterogeneity in FA metabolism was noted within epithelial cells with a wide distribution range (−0.2 to 0.8; **Figure 1C**). These findings highlight the heterogeneity of FA metabolism among different cell types in the tumor microenvironment of HCC, especially epithelial cells.

3. THE LOW LEVEL OF FA METABOLISM CORRELATES WITH A POOR PROGNOSIS IN HCC PATIENTS

An optimal factorization k value of 3 was selected using the NMF algorithm based on a significant decrease in the magnitude of the cophenetic correlation coefficient at k = 3 (**Figure 2A**). The entire cohort of 371 TCGA-HCC samples (including one patient without no PFS information) was clustered into three groups (C1, C2, and C3) with distinct expression profiles of the FA metabolism-related gene set (**Figure 2B**). The ssGSEA scores for FA metabolism in each tumor sample and Adjacent tissues (normal) in the TCGA-HCC cohort indicate that the levels of FA metabolism were highest in normal tissues and varied among the three identified groups (**Figure 3A**). In addition, a significant difference in progression free survival

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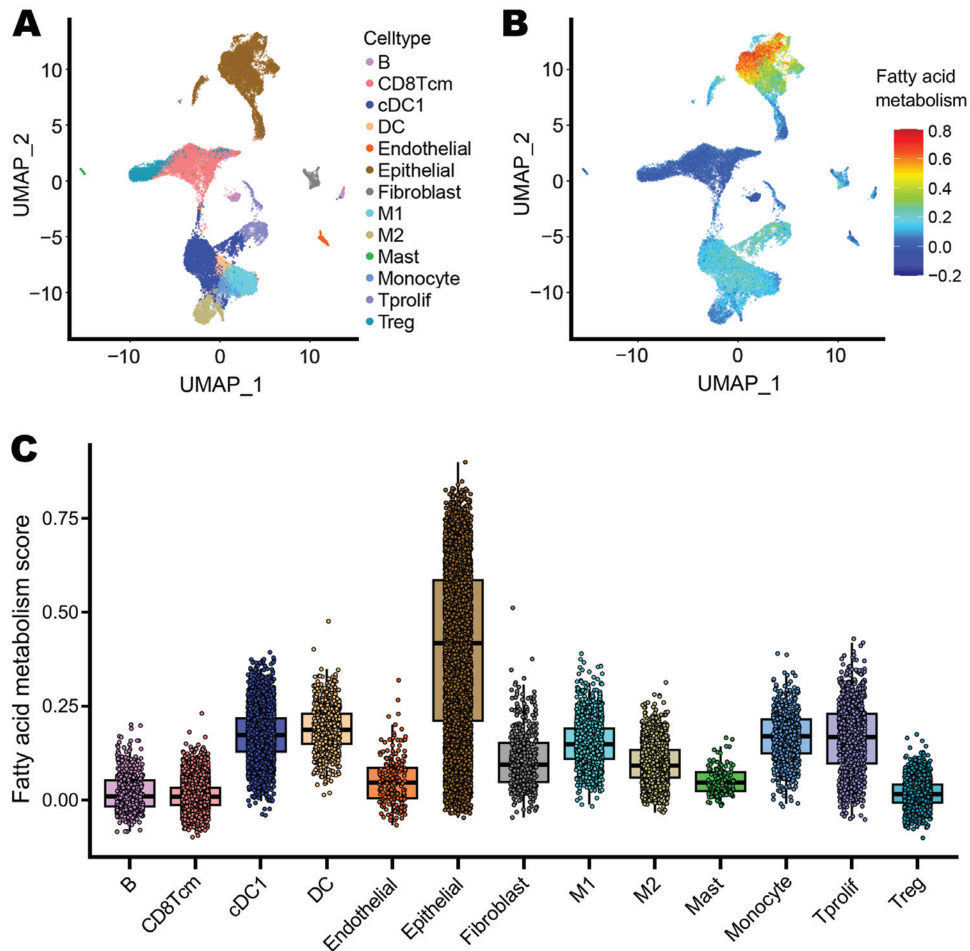

Figure 1 | Aberrant fatty acid metabolism is predominantly observed in epithelial cells in hepatocellular carcinoma. A. Thirteen cell types were identified and annotated in the UMAP reduction plot using single-cell RNA sequencing data of hepatocellular carcinoma. B. A single-sample gene set enrichment analysis algorithm was used to assess the fatty acid metabolism level in different cell types. The color scale ranging from blue-to-red represents the "low-to-high" levels. C. Comparison of the quantified scores of fatty acid metabolism among the 13 cell types.

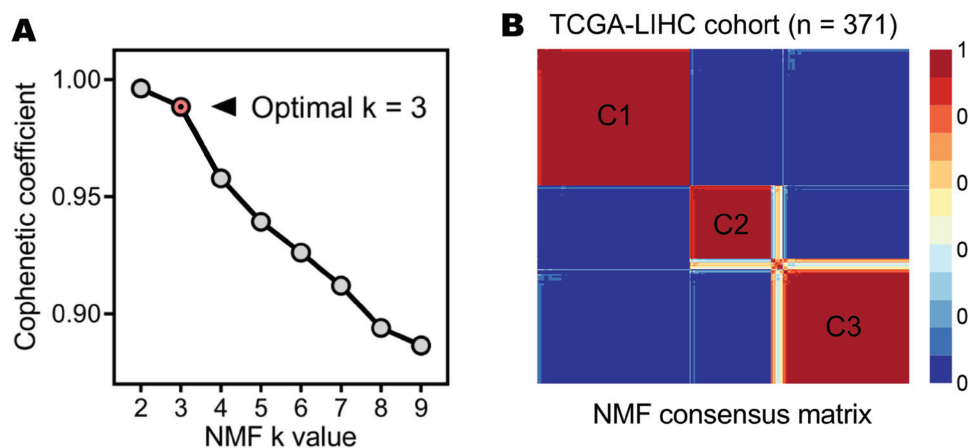

Figure 2 | Three groups were clustered based on the optimal factorization k value. A. Selection of the non-negative matrix factorization (NMF) k value based on the magnitude of the cophenetic correlation coefficient. B. Cohort of 371 samples from *"The Cancer Genome Atlas-Liver Hepatocellular Carcinoma (TCGA-LIHC)"* divided into C1-3 groups.

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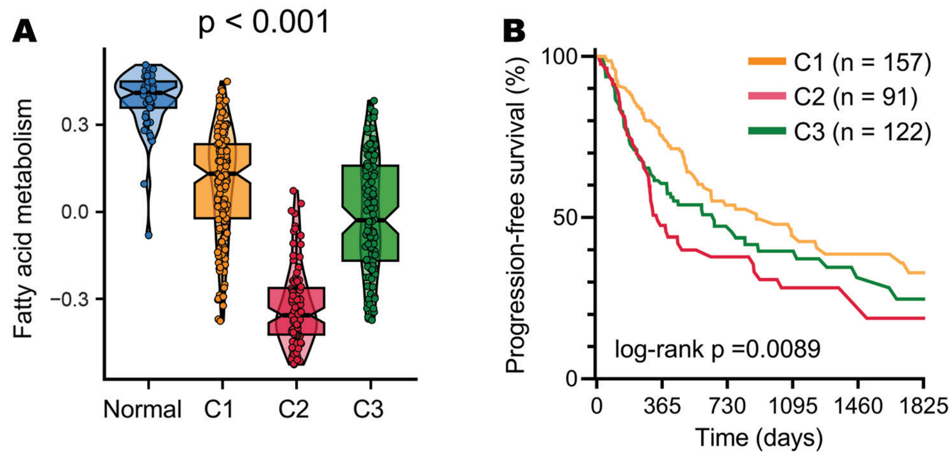

Figure 3 | Different prognoses were observed among the C1, C2, and C3 groups of hepatocellular carcinoma patients identified in Figure 2, i.e., patients with different fatty acid (FA) metabolism levels. A. FA metabolism levels in the three groups compared with adjacent normal tissue. B. Progression-free survival rate in the three groups.

(PFS) was observed among the three groups (log-rank test, *P*=0.0089). The C1 group exhibited the highest FA metabolism scores and had the best PFS, while the C2 group had the lowest scores and the worst PFS (**Figure 3B**). These results suggest that the lowest levels of FA metabolism in HCC patients (C2 group) may be associated with a poor prognosis.

4. FA METABOLISM ENZYMES IDENTIFIED AS POSSIBLE TARGETS FOR ADVANCING DRUG DISCOVERY IN HCC

4.1 Enzymes implicated in the biosynthesis of FAs

ATP citrate lyase (ACLY)

ACLY, the primary enzyme responsible for cytosolic acetyl-CoA synthesis, catalyzes the conversion of citrate and CoA into acetyl-CoA and oxaloacetate, accompanied by ATP hydrolysis to ADP and phosphate. ACLY is essential for HCC cell proliferation [16] and metastasis [17]. Analysis of mRNA RNA-Seq data from the Kaplan-Meier plotter database (https://kmplot.com/) revealed that higher levels of ACLY in HCC patients are associated with a lower median overall survival (OS) [HR=1.74 (1.13-2.69); log rank, *P*=0.011; median survival: 71 months (low expression cohort), 524 months (high expression cohort); number of patients, 364; **Table 1**]. Bempedoic acid (ETC-1002), an ACLY inhibitor, has been reported to lower LDL-cholesterol levels and has received approval from the US FDA for the treatment of heterozygous familial hypercholesterolemia (HeFH) and established atherosclerotic cardiovascular disease (ASCVD). Inhibition of ACLY may suppress HCC metastasis [17].

Acetyl CoA carboxylases (ACCs)

ACCs catalyze the conversion of acetyl-CoA to malonyl-CoA in normal tissue, which is a rate-limiting step in FA synthesis. ACCs are present in two forms (ACC1 and ACC2); *ACACA* encodes ACC1 and *ACACB* encodes ACC2. ACC1 is located in the cytoplasm, while ACC2 is located in the mitochondria. The liver predominantly expresses *ACACA* [18, 19]. *ACACA* was shown to be negatively correlated with the prognosis of HCC patients [HR=1.65 (1.16-2.34); *P*=0.0051]. *ACACA* is upregulated and promotes proliferation, colony formation, migration, invasion, epithelial–mesenchymal transition (EMT), and cell cycle [18] (**Table 1**). Moreover, the expression of *ACACA* facilitates the malignant phenotypes of HCC through aberrant activation of the Wnt/β-catenin signaling pathway. ACC1 has been shown to upregulate genes related to BRL3A cell proliferation in rat liver cell lines (MYCN, JUN, and CCND1) [20]. Hence, *ACACA* may serve as an oncogene and a potential target for HCC therapy. Interestingly, the drug, orlistat, has been shown to restore sensitization of HCC cells to sorafenib and induce apoptosis by inhibiting ACC1 [21]. ND-654, a novel liver-specific ACC inhibitor, phosphorylates and inactivates ACC, thereby inhibiting *de novo* synthesis of liver FAs and the development of HCC [22]. Zinc fingers and homeoboxes 2 (ZHX2), a tumor suppressor associated with liver cancer, inhibits the expression of the FA synthesis regulator, SREBP1c, by upregulating miR-24-3p. Consequently, downstream genes, such as ACC, are inhibited, thereby suppressing the generation of new fat and the development of liver cancer [23].

Fatty acid synthase (FASN)

The FASN complex, which is normally located in the cytoplasm, catalyzes the synthesis of FAs from acetyl-CoA and malonyl-CoA [24]. Excessive FASN metabolism correlates with oncogenesis and liver cancer development. Decreased expression of *FASN* mRNA in HepG2 cells has been shown to inhibit lipid synthesis and induce cell apoptosis [25, 26]. Glyceronephosphate

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Table 1 | mRNA RNA-Seq data for target enzymes in FA metabolic reprograming in HCC in Kaplan-Meier Ppotter database (https://kmplot.com/).

Gene ID	Name	Breast cancer	Triple negative breast cancer (ER-/PR-/HER2-)	Liver cancer	FA metabolic category
31	ACACA			HR=1.65 (1.16-2.34), log rank *P*=0.0051, median survival: 81.9 months (low expression cohort), 45.7 months (high expression cohort), patient number: 364	Synthesis
32	ACACB			HR=0.57 (0.39-0.83), log rank *P*=0.0033, median survival: 30 months (low expression cohort), 70.5 months (high expression cohort), patient number: 364	Synthesis
47	ACLY			HR=1.74 (1.13-2.69), log rank *P*=0.011, median survival: 71 months (low expression cohort), 52 months (high expression cohort), patient number: 364	Synthesis
NA	ACLY	HR=0.52 (0.41-0.64), log rank *P*=2.6e-09, median survival: NA, patient number: 2976			Synthesis
NA	ACLY		HR=0.25 (0.09-0.65), log rank *P*=0.0023, median survival: NA, patient number: 126		Synthesis
2180	ACSL1			HR=0.58 (0.41-0.82), log rank *P*=0.002, median survival: 38.3 months (low expression cohort), 71 months (high expression cohort), patient number: 364	Synthesis
2181	ACSL3			HR=1.61 (1.11-2.34), log rank *P*=0.011, median survival: 71 months (low expression cohort), 33.5 months (high expression cohort), patient number: 364	Synthesis
2182	ACSL4			HR=1.36 (0.93-2), log rank *P*=0.11, median survival: 71 months (low expression cohort), 49.7 months (high expression cohort), patient number: 364	Synthesis
51703	ACSL5			HR=0.72 (0.5-1.06), log rank *P*=0.096, median survival: 37.8 months (low expression cohort), 61.7 months (high expression cohort), patient number: 364	Synthesis
23305	ACSL6			HR=0.58 (0.4-0.83), log rank *P*=0.0027, median survival: 46.2 months (low expression cohort), 81.9 months (high expression cohort), patient number: 364	Synthesis
64834	ELOVL1			HR=1.75 (1.24-2.47), log rank *P*=0.0013, median survival: 81.9 months (low expression cohort), 38.3 months (high expression cohort), patient number: 364	Synthesis
54898	ELOVL2			HR=0.77 (0.51-1.15), log rank *P*=0.2, median survival: 54.1 months (low expression cohort), 61.7 months (high expression cohort), patient number: 364	Synthesis
83401	ELOVL3			HR=2.04 (1.43-2.91), log rank *P*=6.3e-05, median survival: 81.9 months (low expression cohort), 38.3 months (high expression cohort), patient number: 364	Synthesis
6785	ELOVL4			HR=1.25 (0.86-1.82), log rank *P*=0.0013, median survival: 56.5 months (low expression cohort), 56.2 months (high expression cohort), patient number: 364	Synthesis
60481	ELOVL5			HR=0.6 (0.42-0.84), log rank *P*=0.003, median survival: 41 months (low expression cohort), 84.4 months (high expression cohort), patient number: 364	Synthesis
79071	ELOVL6			HR=0.78 (0.54-1.13), log rank *P*=0.19, median survival: 49.7 months (low expression cohort), 71 months (high expression cohort), patient number: 364	Synthesis

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Table 1 | Continued

Gene ID	Name	Breast cancer	Triple negative breast cancer (ER-/PR-/HER2-)	Liver cancer	FA metabolic category
79993	ELOVL7			HR=1.5 (1.05-2.13), log rank *P*=0.023, median survival: 59.7 months (low expression cohort), 38.3 months (high expression cohort), patient number: 364	Synthesis
2194	FASN			HR=1.29 (0.89-1.87), log rank *P*=0.18, median survival: 59.7 months (low expression cohort), 52 months (high expression cohort), patient number: 364	Synthesis
6319	SCD			HR=1.27 (0.9-1.8), log rank *P*=0.018, median survival: 81.9 months (low expression cohort), 54.1 months (high expression cohort), patient number: 364	ER desaturation
10554	AGPAT1			HR=1.47 (1.02-2.13), log rank *P*=0.04, median survival: 70.5 months (low expression cohort), 52 months (high expression cohort), patient number: 364	Storage
NA	AGPAT1	HR=1.32 (1.04-1.67), log rank *P*=0.023, median survival: NA, patient number: 2976			Storage
NA	AGPAT1		HR=1.61 (0.73-3.55), log rank *P*=0.24, median survival: NA, patient number: 126		Storage
10555	AGPAT2			HR=1.42 (1.02-2.13), log rank *P*=0.045, median survival: 81.9 months (low expression cohort), 46.2 months (high expression cohort), patient number: 364	Storage
56894	AGPAT3			HR=0.65 (0.46-0.91), log rank *P*=0.013, median survival: 81.9 months (low expression cohort), 46.2 months (high expression cohort), patient number: 364	Storage
56895	AGPAT4			HR=1.42 (1.02-2.13), log rank *P*=0.045, median survival: 38.3 months (low expression cohort), 84.4 months (high expression cohort), patient number: 364	Storage
55326	AGPAT5			HR=1.87 (1.23-2.83), log rank *P*=0.0029, median survival: 81.9 months (low expression cohort), 46.6 months (high expression cohort), patient number: 364	Storage
137964	AGPAT6			HR=0.67 (0.43-1.03), log rank *P*=0.066, median survival: 52 months (low expression cohort), 108.6 months (high expression cohort), patient number: 364	Storage
84803	AGPAT9			HR=1.29 (0.88-1.89), log rank *P*=0.19, median survival: 71 months (low expression cohort), 52 months (high expression cohort), patient number: 364	Storage
8694	DGAT1			HR=1.16 (0.82-1.64), log rank *P*=0.4, median survival: 70.5 months (low expression cohort), 52 months (high expression cohort), patient number: 364	Storage
NA	DGAT1	HR=1.31 (1.03-1.66), log rank *P*=0.028, median survival: NA, patient number: 2976			Storage
NA	DGAT1		HR=2.09 (0.95-4.6), log rank *P*=0.062, median survival: NA, patient number: 126		Storage

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Table 1 | Continued

Gene ID	Name	Breast cancer	Triple negative breast cancer (ER-/PR-/HER2-)	Liver cancer	FA metabolic category
84649	DGAT2			HR=0.57 (0.39-0.83), log rank *P*=0.0032, median survival: 47.4 months (low expression cohort), 84.4 months (high expression cohort), patient number: 364	Storage
NA	DGAT2	HR=0.78 (0.6-1.01), log rank *P*=0.063, median survival: NA, patient number: 2976			Storage
NA	DGAT2		HR=0.56 (0.26-1.24), log rank *P*=0.15, median survival: 49.9 months (low expression cohort), 63.67 months (high expression cohort), patient number: 126		Storage
33	ACADL			HR=0.5 (0.34-0.75), log rank *P*=0.00056, median survival: 46.2 months (low expression cohort), 81.9 months (high expression cohort), patient number: 364	β-Oxidation
34	ACADM		PT2	HR=0.64 (0.45-0.9), log rank *P*=0.011, median survival: 42.4 months (low expression cohort), 71 months (high expression cohort), patient number: 364	β-Oxidation
35	ACADS			HR=0.44 (0.31-0.64), log rank *P*=7.7e-06, median survival: 27.9 months (low expression cohort), 70.5 months (high expression cohort), patient number: 364	β-Oxidation
51	ACOX1			HR=0.6 (0.42-0.85), log rank *P*=0.0035, median survival: 38.3 months (low expression cohort), 71 months (high expression cohort), patient number: 364	β-Oxidation
NA	ACOX1	HR=0.67 (0.53-0.84), log rank *P*=0.00052, median survival: NA, patient number: 2976			β-Oxidation
NA	ACOX1		HR=0.64 (0.29-1.41), log rank *P*=0.27, median survival: NA, patient number: 126		β-Oxidation
8309	ACOX2			HR=0.55 (0.38-0.78), log rank *P*=0.00092, median survival: 45.7 months (low expression cohort), 84.7 months (high expression cohort), patient number: 364	β-Oxidation
NA	ACOX2	HR=0.57 (0.46-0.71), log rank *P*=6.8e-07, median survival: NA, patient number: 2976			β-Oxidation
NA	ACOX2		HR=0.56 (0.21-1.51), log rank *P*=0.25, median survival: NA, patient number: 126		β-Oxidation
23600	AMACR			HR=0.73 (0.5-1.06), log rank *P*=0.1, median survival: 46.6 months (low expression cohort), 61.7 months (high expression cohort), patient number: 364	β-Oxidation

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Table 1 | Continued

Gene ID	Name	Breast cancer	Triple negative breast cancer (ER-/PR-/HER2-)	Liver cancer	FA metabolic category
NA	AMACR	HR=1.22 (0.97-1.54), log rank *P*=0.087, median survival: NA, patient number: 2976			β-Oxidation
NA	AMACR		HR=1.86 (0.85-4.09), log rank *P*=0.12, median survival: NA, patient number: 126		β-Oxidation
1374	CPT1A			HR=0.65 (0.46-0.94, log rank *P*=0.02, median survival: 45.7 months (low expression cohort), 70.5 months (high expression cohort), patient number: 364	β-Oxidation
1375	CPT1B			HR=0.72 (0.5-1.04), log rank *P*=0.075, median survival: 38.37 months (low expression cohort), 70.5 months (high expression cohort), patient number: 364	β-Oxidation
126129	CPT1C			HR=0.71 (0.47-1.07), log rank *P*=0.0995, median survival: 52 months (low expression cohort), 81.9 months (high expression cohort), patient number: 364	β-Oxidation
1376	CPT2			HR=0.7 (0.49-1), log rank *P*=0.047, median survival: 45.7 months (low expression cohort), 70.5 months (high expression cohort), patient number: 364	β-Oxidation
1892	ECHS1			HR=0.54 (0.38-0.77), log rank *P*=0.00048, median survival: 37.8 months (low expression cohort), 70.5 months (high expression cohort), patient number: 364	β-Oxidation
NA	PHYH	HR=0.58 (0.46-0.72), log rank *P*=1.2e-06, median survival: NA, patient number: 2976			β-Oxidation
5264	PHYH			HR=0.67 (0.46-0.98), log rank *P*=0.036, median survival: 49.7 months (low expression cohort), 59.7 months (high expression cohort), patient number: 364	β-Oxidation
NA	PHYH		HR=0.6 (0.27-1.31), log rank *P*=0.19, median survival: NA, patient number: 126		β-Oxidation
66002	CYP4F12			HR=0.54 (0.38-0.77), log rank *P*=0.00052, median survival: 38.3 months (low expression cohort), 71 months (high expression cohort), patient number: 364	ω-Oxidation
8529	CYP4F2			HR=0.56 (0.38-0.82), log rank *P*=0.0028, median survival: 46.6 months (low expression cohort), 70.5 months (high expression cohort), patient number: 364	ω-Oxidation

Note: Some target enzymes were compared within HCC and breast cancer and the correlations with survival data were determined. Column colors: red color refers to lower risk and higher median overall survival; Green color refers to higher risk and lower median overall survival.

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O-acyltransferase (GNPAT) acetylation stabilizes FASN by repressing FASN ubiquitination and degradation, which promotes tumor progression in murine HCC [27]. FASN also interacts with STAT3 and affects the expression of MMP-2/MMP-9, thereby promoting liver cancer metastasis [28]. However, HCC are heterogenous and the effects of FASN are manifest only in the FASN-dependent phenotype. For example, a FASN inhibitor, TVB3664, inhibits HCC progression when combined with cabozantinib or sorafenib but has no effects on FASN-independent HCC [29]. Additionally, miR-1207-5P, which directly targets FASN, inhibits the growth and invasion of liver cancer cells through the Akt/mTOR signaling pathway [30].

Acyl-CoA synthetase (ACS)

ACS, which is located in the endoplasmic reticulum, mitochondria, and microsomal membranes, catalyzes the conversion of FAs to the active form (fatty acyl-CoA), which is essential for carbon chain extension [31]. There are five subtypes of ACSs in the human body (ACSL1, ACSL3, ACSL4, ACSL5, and ACSL6) [32]. ACSL1, ACSL3, and ACSL4 are highly expressed in liver cancer. Activation of the ACSL1 promoter maintains abnormal lipid metabolism in liver cancer cells, which promotes the proliferation, invasion, and metastasis while evading programmed cell death [26]. ACSL3 likely promotes liver cancer formation by increasing mitochondrial β–oxidation of FAs [33]. ACSL4, a key regulator of ferroptosis, promotes the growth and metastasis of liver cancer cells by upregulating SREBP1c [34]. High expression of ACSL4 is associated with poor prognosis in HCC patients by upregulation of cancer-associated fibroblasts in the tumor immune microenvironment, such as Foxp3^{+} tumor-infiltrating lymphocytes [TILs]) [35]. Sorafenib, a multi-kinase inhibitor, inhibits HCC, in part by targeting highly expressed ACSL4. Conversely, miR-23a-3p, the most prominent miRNA in HCC, is overexpressed in sorafenib non-responders and attenuates the effects of sorafenib by reducing ACSL4 expression, suggesting that miR-23a-3p may confer resistance to sorafenib chemotherapy [36, 37].

Elongases of very long chain FAs (ELOVLs)

ELOVLs, which are located in the endoplasmic reticulum, are rate-limiting enzymes that catalyze the synthesis of long-chain and very long-chain FAs [38]. ELOVLs are classified into two groups. The first group includes ELOVL1, ELOVL3, ELOVL6, and ELOVL7, which act on saturated and monounsaturated FAs. ELOVL1 notably catalyzes the production of very long-chain FAs (VLCFAs) with a carbon chain length of at least 23 or 24 carbons [39]. ELOVL3 acts on FAs with chain lengths ranging from C16-C22, while ELOVL6 acts on C12-C16, and ELOVL7 acts on C16-C20 [40]. The second group includes ELOVL2, ELOVL4, and ELOVL5, which act on polyunsaturated FAs. ELOVL2 elongates arachidonic acid (C20:4n-6), eicosapentaenoic acid (C20:5n-3), docosatetraenoic acid (C22:4n–6), and docosapentaenoic acid (C22:5n–3). ELOVL4 elongates eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3) and (C26-C36, n-3) into very long chain polyunsaturated fatty acids (C28-C38, n-3) [41] and ELOVL5 elongates FAs with chain lengths ranging from C18-C20, including stearidonic acid (C18:4n–3) and c-linolenic acid (C18:3n–6) [42, 43].

ELOVL1 mRNA and protein are overexpressed in HCC tissues compared to adjacent normal liver tissues and ELOVL1 expression is negatively correlated with the survival probability of HCC patients. ELOVL1 participates in the tumorigenesis of HCC via immune evasion [44]. ELOVL3 catalyzes the synthesis of C20-C24 FAs and is upregulated in liver cancer tissues [45]. Elimination of ElOVL3 leads to a decrease in lipid synthesis and decomposition [46] but it appears that this enzyme may be dispensable for metabolic homeostasis [47]. *ELOVL5* appears to be positively correlated with a good OS prognosis in liver cancer (**Table 1**). In contrast, *ELOVL6* expression is increased in the pathologic process of non-alcoholic steatohepatitis (NASH)-related HCC and HCC [48, 49]. Compared to adjacent non-cancerous parenchyma, ELOVL6 expression is upregulated in the cancerous parts of non-B, non-C HCC. Silencing ELOVL6 reduces the stearate-to-palmitate ratio, enhances endoplasmic reticulum stress, reduces lipid droplets, suppresses proliferation, and induces apoptosis of Huh7 and HepG2 cells [50]. Knockdown of ELOVL6 expression may increase the levels of SCD-1 and CPT-1 expression, causing lipid accumulation while also resulting in G1/S arrest, inhibiting cell proliferation, and suppressing *in vivo* tumor growth [51]. Increased mitochondrial fission upregulates the expression of ELOVL6 by activating SREBP1, thus promoting *de novo* FA synthesis and the growth and metastasis of HCC [52]. Furthermore, ELOVL6 promotes IL-1β release by activating the ROS signaling pathway, leading to liver inflammation and damage [53]. Therefore, ELOVL1, ELOVL3, and ELOVL6 may have critical roles in the occurrence of HCC and could prove to be interesting therapeutic targets.

The enzymes implicated in the biosynthesis of FAs are listed in **Figure 4**.

4.2 Enzymes implied in the oxidation of FAs

FA oxidation, a multistep catabolic process that involves the participation of several enzymes and coenzymes, leads to the catabolism of long-chain FAs into acetyl coenzyme A, which is then completely oxidized through the tricarboxylic acid cycle and electron transport chain to produce ATP for cellular use [54]. FA oxidation occurs in different organelles, such as mitochondria, peroxisomes, and/or endoplasmic reticulum. However, aberrant expression of key enzymes and intermediates associated with FA oxidation in these organelles is linked to malignant cancer characteristics, including treatment resistance, metastatic potential, and recurrence (**Figure 4**).

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4.2.1 Mitochondrial β-oxidation. The mitochondrial FA β-oxidation pathway has been characterized at biochemical and molecular biological levels [55]. β-oxidation involves four enzymes (acyl-coenzyme A dehydrogenase, 2-enoyl-coenzyme A hydratase, 3-hydroxy acyl-coenzyme A dehydrogenase, and 3-ketoacyl-coenzyme A thiolase). Multiple enzymes are involved in each step with chain length specificity. The carnitine shuttle system induces three main enzymes (carnitine palmitoyltransferase-1, carnitine-acylcarnitine translocase, and carnitine palmitoyltransferase-2) [5, 56, 57]. The role of mitochondrial FA β-oxidation has not been fully elucidated in HCC due to the diverse histologic features of HCC associated with different underlying diseases [54]. Therefore, it is crucial to investigate the tissue specificities of mitochondrial β-oxidation in HCC and identify potential drug targets by analyzing possible aberrant expressions of critical enzymes and intermediates involved.

Carnitine palmitoyltransferase 1 (CPT1)

CPT1 is a key rate-limiting enzyme of the carnitine shuttle system involved in long-chain FA oxidation in mitochondria. CPT1 is located in the outer mitochondrial membrane. Because the inner mitochondrial membrane cannot accept long-chain CoA FAs, CPT1 controls the entry of FAs into mitochondria by converting acyl CoA esters to acylcarnitine esters. There are three main forms of CPT1 isozymes in humans (CPT1A, CPT1B, and CPT1C). CPT1A is expressed in the liver, heart, spleen, lungs, kidneys, and adipose tissue. CPT1A is associated with severe consequences, such as genetic mutations, metabolic disorders, and cancer [58]. In contrast, CPT1B is primarily found in white adipose tissue, heart, skeletal muscle, and testes [57]. CPT1C is found in the brain and testis and although CPT1C function is not fully understood, recent reports indicate that this isoenzyme has a vital role in the endoplasmic reticulum [59]. Among the three isoforms, CPT1A has a dominant role in the progression of HCC by forming an inactive complex with ACC1 [60]. The avasimibe and etomoxir combination disrupts lipid homeostasis and reduces FA oxidation levels, which simultaneously targets sterol o-acyltransferase 1 and CPT1A and has synergistic anticancer efficacy in HCC *in vitro* and *in vivo* [61]. BCL2 inhibits apoptosis by interacting with CPT1A in mitochondria, which benefits cancer cell growth by antagonizing BAK-dependent apoptosis [62, 63].

Carnitine-acylcarnitine translocase (CACT) and CPT2

Like CPT1, CACT and CPT2 are essential components of the carnitine shuttle system. Acylcarnitine esters of cytoplasmic long-chain FAs penetrate the inner mitochondrial membrane in the presence of CACT. Acylcarnitines are subsequently converted to acyl-coenzyme A by CPT2, which are then oxidized by the subsequent enzymes of the mitochondrial β-oxidation pathway [58]. Dysfunction of CACT and CPT2 directly affects the β-oxidation of long-chain FAs in the mitochondrial matrix. In non-alcoholic fatty liver disease (NAFLD)-associated HCC, mRNA levels and expression of CPT2 protein are significantly downregulated, while CPT1A expression is not significantly changed. Low levels of CPT2 result in a significant accumulation of acylcarnitines, which leads to inhibition of mitochondrial FA β-oxidation and ultimately causing metabolic disorders that promote the proliferation, migration, and invasion of HCC cells [64, 65]. In the context of agents or drug development, the inhibition of CPT2 by perhexiline disturbs NADPH and redox homeostasis, increases reactive oxygen species (ROS) generation, and induces cell apoptosis in gastrointestinal cancer cells following oxaliplatin treatment [66]. However, there has been no application for perhexiline in HCC.

Acyl-CoA dehydrogenases (ACADs)

ACAD gene family products are involved in FA β-oxidation, catalyzing the initial steps of each FA β-oxidation cycle in the cellular mitochondria through the introduction of a trans double bond between C2 (α) and C3 (β) of the acyl CoA thioester substrate [67]. Depending on the preferred acyl CoA substrate, enzymes of the ACAD family can be categorized as short-chain (ACADS and SCAD), medium-chain (ACADM), long-chain (ACADL and LCAD), or very long-chain (VLCAD and ACADVL). Although the different dehydrogenases target FAs of different chain lengths, all types of ACAD are mechanistically similar [68]. ACAD expression in HCC tissues appears lower compared to normal liver tissues and DNA methylation levels are negatively correlated with ACADs expression, as revealed by the UCSC Xena database [69]. Increased ACAD activity in *in vivo* and *in vitro* models of HCC inhibits HCC cell proliferation, migration, and invasion, while inducing HCC apoptosis. ACADM expression is significantly reduced in HCC tissues. Functionally, elevated ACADM expression inhibits HCC cell proliferation, migration, and invasion and reduces triglyceride, phospholipid, and cellular lipid droplet levels [70]. Hypoxia-inducible factor 1 (HIF-1) mediates a metabolic transition and HIF-1 inhibits ACADM and ACADL, thereby reducing ROS levels and promoting proliferation. Blocking ACADL attenuates PTEN expression and significantly affects tumor growth *in vivo* [71]. However, restoring ACADL expression in HCC inhibits nuclear accumulation of the protein kinase, Hippo, and through downstream target gene expression leads to cancer cell cycle arrest and growth inhibition [72]. VLCAD enzymatic activity is also inhibited in human HCC tissues and cells. Interestingly, VLCAD overexpression attenuates HCC by inhibiting glycolysis and mitochondrial oxidative phosphorylation through the PI3K/AKT pathway [73].

Enoyl-CoA hydratase, short chain 1 (ECHS1)

ECHS1, a mitochondrial matrix enzyme responsible for the second step of mitochondrial FA β-oxidation, catalyzes the hydration of short-chain enoyl-CoAs. Compared

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to other β-oxidation enzymes (CPT1s, CACT and CPT2), ECHS1 does not directly catalyze oxidative reactions but prepares for the subsequent dehydrogenation process. As a result of the crucial role in fatty acid metabolism, ECHS1 has been shown to be positively associated with the progression of various cancers. Overexpression of ECHS1 promotes cancers [74-77]. Knockdown of ECHS1 induces autophagy and attenuates HCC by impairing cellular metabolism and activating the AMPK pathway [78], inhibits expression of HCC cell cycle protein D3 and cell cycle protein-dependent kinase 6, enhances the expression of p16 and p21, and augments cisplatin-induced apoptosis in HCC cells [79]. These findings may contradict the role of ECHS1 in the prognosis of HCC according to the online K-M database (**Table 1**), which deserves further investigation.

3-Hydroxyacyl-CoA dehydrogenase (HADH)

HADH participates in the dehydrogenation reaction of mitochondrial β-oxidation, while medium/short-chain hydroxyacyl-CoA dehydrogenase (M/SCHAD) and long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) act on long chain FAs [80]. Downregulation of the HADH gene and protein in poorly differentiated HCC cell lines is associated with higher migration capacity [81], indicating a probable negative role of HADH. LCHAD in the human body is encoded by the *HADHA* gene. *HADHA* expression is significantly reduced in HCC patients and this HADH protein is significantly downregulated in invasive and metastatic HCC [82].

4.2.2 Peroxisomal β-oxidation. Peroxisomes are ubiquitous organelles with varying abundance and function across different cells. Peroxisomes are characterized by a high concentration of matrix proteins (approximately 40 oxidases and peroxidases) [83] that form crystalline inclusions. Very long-chain FAs (VLCFAs) and atypical FAs (branched or with an odd number of carbons) are broken down into short-chain FAs exclusively within the peroxisome in the mammalian liver through a specific β-oxidation system. The shortened FAs are then further metabolized through the mitochondrial β-oxidation pathway, while also providing electrons directly to molecular oxygen to produce hydrogen peroxide (H_2O_2) [79]. Peroxisome β-oxidation has a role in regulating systemic energy expenditure and mitochondrial function [84] (**Figure 1**). The steps involved in peroxisomal β-oxidation are oxidation, hydration, dehydrogenation, and thiolysis [85]. While the pro-tumorigenic functions of peroxisomal β-oxidation have been reported in breast cancer, particularly triple-negative breast cancer (lacking the estrogen receptor, progesterone receptor, and HER2) [86], the mRNA RNA-Seq data from the Kaplan-Meier plotter database (https://kmplot.com/) for liver cancer patients indicates that higher levels of ACOX1, ACOX2, and PHYH are associated with an improved median OS (n=364, $P<0.05$; **Table 1**). Other enzymes involved in peroxisomal β-oxidation include multifunctional enzyme (MFP) and peroxisomal 3-oxo-acyl-CoA thiolase (ACAA1) [85]. Downregulation of MFP expression has been demonstrated in HCC. Mice lacking MFP, when supplemented with the 12-carbon FA, lauric acid, accumulate toxic metabolites, leading to acute hepatocyte necrosis and liver failure. Therefore, MFP deficiency may contribute to the development of HCC [86].

Acyl-CoA oxidases (ACOXs)

ACOXs are the first-rate-limiting enzymes in peroxisomal β-oxidation and catalyze desaturation of acyl-CoA to 2-trans-enoyl-CoA. ACOX is predominantly found in the matrix of peroxisomes as three main isoforms of ACOX [palmitoyl-CoA oxidase (ACOX1), cholesteryl -CoA oxidase (ACOX2), and imido-CoA oxidase (ACOX3)] [87]. ACOX1 dysfunction is associated with peroxisome-related diseases and hepatocarcinogenesis. Peroxisome proliferator-activated receptor α (PPARα), a nuclear receptor, regulates *ACOX1* gene transcript levels. Aberrant upregulation of ACOX1 by PPAR activation stimulates hepatic FA oxidation, leading to H_2O_2 accumulation [88]. H_2O_2 causes oxidative DNA damage and promotes HCC when ACOX1 enters the nucleus. In contrast, ACOX2 is thought to be involved in the metabolism of branched-chain FAs and bile acid intermediates. Recent studies have suggested that deletion of ACOX2 induces HCC in mice. ACOX2-mediated lysine crotonylation regulates hepatic homeostasis in mice. In addition, there is a strong correlation between PPARα and ACOX2 in HCC tissues based on GSEA analysis [89]. PPARα activation inhibits HCC tumorigenesis through anti-angiogenic and anti-inflammatory effects.

α-Methylacyl CoA racemase (AMACR)

AMACR is a peroxisomal and mitochondrial enzyme involved in oxidation of branched-chain FAs and FA derivatives. AMACR is typically found in peroxisomes and mitochondria of renal tubular epithelial cells and hepatocytes. Peroxisomal β-oxidation can only degrade (2)-methyl acyl-CoA esters by converting (2)-methyl acyl-CoA esters to (2S)-methylacyl-CoA, a reaction catalyzed by AMACR [90]. AMACR acts to convert (2R)-methylacyl coenzyme A esters into (2S)-methylacyl coenzyme A stereoisomers. AMACR is highly expressed in HCC but the exact pathologic mechanism by which AMACR contributes to HCC development is unclear. Silencing the expression of AMACR in cancer cells has been shown to reduce cancer cell growth, possibly through reducing H_2O_2 production and DNA damage [91]. Overexpression of AMACR has been closely associated with the presence of CTNNB1 mutations in HCC [92].

Phytanoyl-CoA hydroxylase (PHYH)

PHYH is an enzyme involved in the first step of α-oxidation before β-oxidation. PHYH catalyzes the conversion of phytanoyl-CoA (a racemic mixture of 3R/S diastereomers) into 2-hydroxy-phytanoyl-CoA intermediates. According to the Kaplan-Meier plotter database, PHYH

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is a tumor suppressor in liver cancer that correlates with a high median OS in liver cancer patients (n=364, *P*<0.05; Table 1).

4.2.3 Endoplasmic reticulum ω-oxidation. Endoplasmic reticulum ω-oxidation is a metabolic pathway that involves hydroxylation and oxidation of longer FAs to dicarboxylic acids for energy and other metabolites required during cell growth [93]. This conversion relies on the cytochrome P450 family (CYP4), well-known catalyzers of xenobiotic metabolizations that are notably oxidative. The ω-oxidation pathway is essential, especially in the liver and kidney, for metabolism of FAs that cannot be catabolized by the mitochondria and peroxisome β-oxidation pathways [94]. The ω-oxidation pathway is relevant to tumor cell proliferation, invasion, and metastasis in HCC. In addition, ω-oxidation influences HCC development by regulating inflammatory responses and apoptotic pathways.

Cytochrome P450 gene family 4 (CYP4)

CYP4 allows for ω-hydroxylation of terminal carbons. Members of three CYP4 subfamilies show distinct preferences in metabolizing short (CYP4B), medium (CYP4A), and long (CYP4F) saturated, unsaturated, and branched-chain FAs in mammals [95, 96]. The level of CYP4 subfamily F member 2 (CYP4F2) is downregulated in HCC and CYP4F2 overexpression has been shown to reverse the antioxidant capacity of HCC cells, inhibit HCC cell proliferation and migration, and induce apoptosis [97]. High expression of CYP4F12 inhibits the cancer cell cycle and DNA replication, as well as genes related to the Wnt signaling pathway in HCC. The mechanism of action underlying CYP4A in the development of HCC has not been explored. However, a potential risk factor for HCC in patients with NAFLD is high expression of CYP4A, which is directly correlated with the steatosis phenotype. Inhibition of CYP4A has been shown to ameliorate steatosis and endoplasmic reticulum oxidative stress in a model of hepatic steatosis [98]. It has been shown that diazepam and oxazepam upregulates CYP2B and CYP4A, which leads to liver tumor formation [99].

4.2.4 Endoplasmic reticulum desaturation. Endoplasmic reticulum desaturation catalyzes the enzymatic conversion of saturated FAs into unsaturated FAs. This process is a crucial step in FA oxidation and enables cells to convert FAs into energy. Studies have shown that abnormal functioning of endoplasmic reticulum desaturase in HCC leads to increased endoplasmic reticulum stress and apoptosis, thereby promoting tumor development and progression. Therefore, modulating the activity and function of this enzyme may represent a potential strategy for HCC treatment [100].

Stearoyl-CoA desaturases (SCDs)

SCDs are FA desaturases that are localized to the endoplasmic reticulum membrane. SCDs introduce a carbon-carbon double bond at positions 9-10 of saturated FAs, such as palmitic and stearic acids, resulting in the production of monounsaturated FAs (MUFAs) (palmitoleic and oleic acids) [101, 102]. Unsaturated FAs produced by SCDs promote metabolic reprogramming in cancer through pathways, such as AKT, AMPK, and NF-κB [103, 104]. Four SCD isoforms have been identified in mice (SCD1, SCD2, SCD3, and SCD4) and two isoforms have been identified in humans (SCD1 and SCD5) [105]. A significant elevation in SCD among HCC patients leads to a significant accumulation of free FAs and inhibition of ferroptosis in HCC cells, which subsequently promotes the development of cancer [105, 106]. Furthermore, an SCD1 bioproduct (palmitoleate [C16:1]) promotes HCC cell migration. Inhibition of SCD1 induces apoptosis in HCC cells via the AMPK signaling pathway [107]. Tumor hepatitis B X-interacting protein (HBXIP) is involved in various promotive processes, including growth, metastasis, drug resistance, and metabolic reprogramming. HBXIP induces SCD expression, which leads to the accumulation of free FAs and inhibition of iron oxidation. Sorafenib eradicates HCC cells by disrupting SCD1-mediated MUFA synthesis through the ATP-AMPK-mTOR-SREBP1 signaling pathway [108] and reducing HBXIP expression, leading to ferroptosis mediated by the HBXIP/SCD axis [109]. A cyclic peptidoglycoside (RA-XII), which is isolated from *Rubia yunnanensis* Diels, exhibits antitumor effects on HCC. RA-XII inhibits HCC cells by suppressing the expression of key factors involved in FA metabolism (SREBP, SCD, and FASN), thereby inhibiting tumor growth and lipogenesis [110].

4.3 Enzymes implied in the storage of FAs

The liver is the primary organ responsible for FA metabolism and is where triglyceride (TG)-rich lipoprotein synthesis takes place. Under normal physiologic conditions, the concentration of TGs in the liver remains relatively low and constant. The liver typically stores a small number of FAs as TGs [111]. TGs serve as a neutral storage form of FAs and can be mobilized when energy input is insufficient to enhance the process of gluconeogenesis. In cases of overnutrition and obesity, an excessive amount of FAs flows into the liver, which becomes an important source of liver TGs that are combined with apolipoprotein B and secreted in the form of VLDL particles [112]. FA oxidation, FA absorption, and *de novo* lipogenesis (DNL) are all intricately balanced [111]. The accumulation of TGs mitigate damage caused by an acute increase in FAs [113]. However, when the rate of TG formation in the liver exceeds the combined rate of TG output in very low-density lipoprotein (VLDL) particles and the hepatic oxidation of TG-derived FAs, liver TG content builds up and becomes the primary cause of hepatic steatosis and NASH development [114]. Liposome TGs from adipose tissue provides approximately 60% of the liver FAs in NAFLD, which is an early risk factor promoting the occurrence of HCC [115-117].

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TGs are not the ultimate product of DNL. A comprehensive understanding of the liver lipid profile may reveal that phospholipid metabolism could be a potential therapeutic target for NASH or HCC. The enzyme, 1-acylglycerol-3-phosphate O-acyltransferase (AGPAT), catalyzes the synthesis of phosphatidic acids, which are specific substrates for TGs synthesis. Lysophosphatidic acids (LPAs) undergo additional acylation by a different acyl-CoA through the action of AGPAT, resulting in the formation of phosphatidic acid. Dephospnshorylation then occurs through phosphatidic acid phosphatase (PAP [lipins]), leading to the production of diacylglycerols (DAGs). The final step involves the enzymatic function of diacylglycerol acyltransferase (DGAT), which converts DAGs into TGs through the leveraged acyl-CoA [118]. Thus, these enzymes involved in TG storage are also involved in the generation of important signaling molecule precursors [LPA, DAG, and platelet-aggregation factor (PAF)] and may be potential targets to prevent oncogenesis and/or the development of HCC (Figure 5).

1-Acylglycerol-3-phosphate O-acyltransferases (AGPATs)
AGPATs catalyze the conversion of lysophosphatidate-to-phosphatidate by adding an acyl group to the *sn*-2 position of the glycerol backbone. The AGPAT family consists of 11 members (AGPAT1-AGPAT11), according

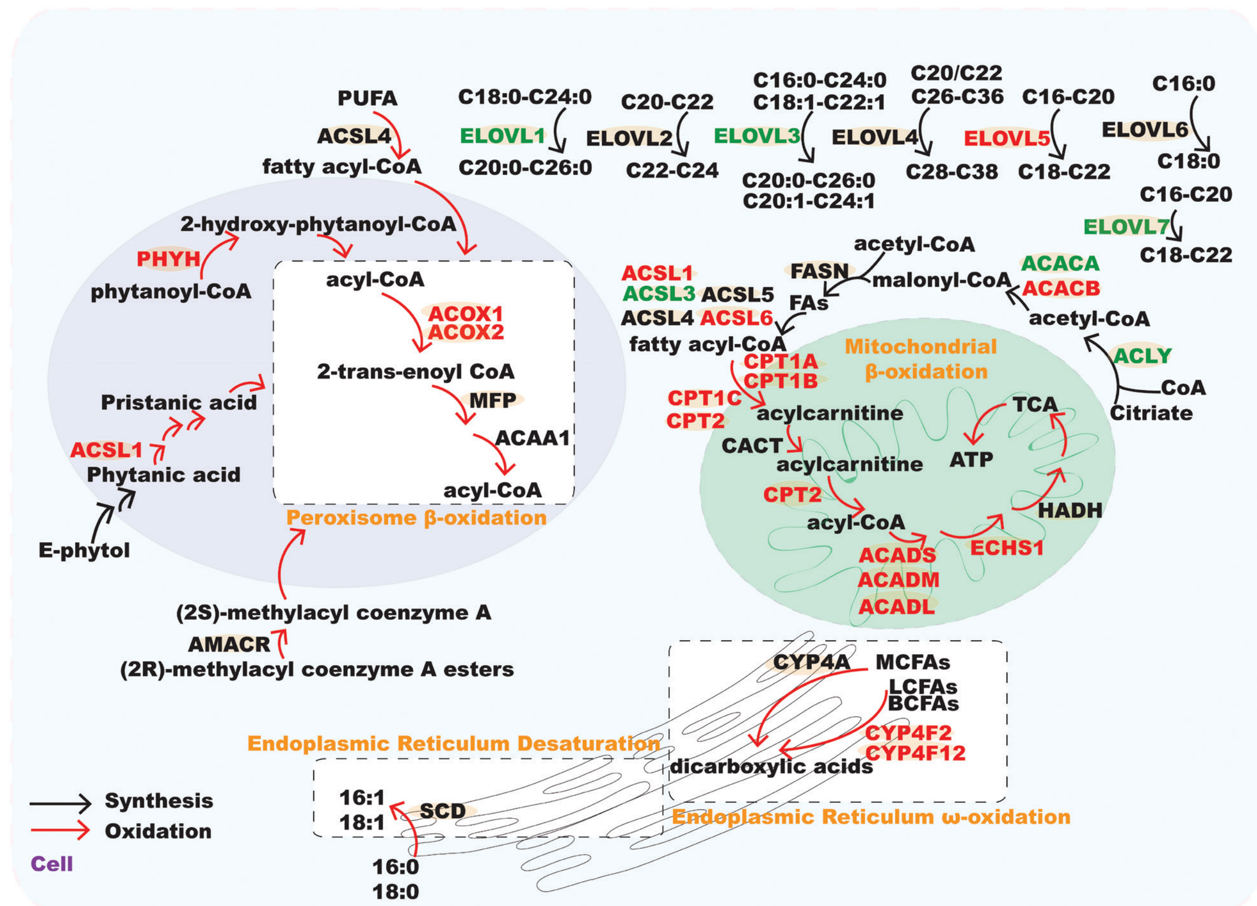

Figure 4 | Synthesis and oxidation of fatty acids (FAs). Enzymes associated with liver cancer are shown in a yellow background. ACAA1, peroxisomal 3-oxo-acyl-CoA thiolase; ACACA, acetyl-CoA carboxylase α; ACACB, acetyl-CoA carboxylase β; ACADS, acyl-CoA dehydrogenase short chain; ACADM, acyl-CoA dehydrogenase medium chain; ACADL, acyl-CoA dehydrogenase long chain; ACLY, ATP citrate lyase; AMACR, α-methylacyl CoA racemase; ACOX1/2, acyl CoA oxidase 1/2; ACSL1/3/4/5/6, acyl-CoA synthetase long chain family member 1/3/4/5/6; BCFAs, branch chain fatty acids; CACT, carnitine-acylcarnitine translocase; CPT1A, carnitine palmitoyl transferase-1A; CPT1B, carnitine palmitoyl transferase-1B; CPT1C, carnitine palmitoyl transferase-1C; CPT2, carnitine palmitoyl transferase-2; CYP4F2, cytochrome P450 family 4 subfamily F member 2; CYP4F12, cytochrome P450 family 4 subfamily F member 12; ELOVL1/2/3/4/5/6/7, very long chain fatty acids elongase 1/2/3/4/5/6/7; ECHS1, enoyl-CoA hydratase short chain 1; FASN, fatty acid synthase; HAD, 3-hydroxyacyl-CoA dehydrogenase; LCFAs, long chain fatty acids; MCFAs, medium chain fatty acids; MFP, multifunctional enzyme; PHYH, phytanoyl-CoA hydroxylase; SCD1/2, stearoyl CoA desaturase 1/2; 16: 0, palmitic acid; 18:0, stearic acid; 16:1, oleic acid; 18:1, linoleic acid. Red words: tumor suppressors; Green words: tumor inducers; Black words: NA.

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to the online database Kaplan-Meier plotter (https://kmplot.com/). AGPAT1 is positively associated with HCC. Higher expression of AGPAT1, AGPAT2, AGPAT3, AGPAT5, and AGPAT9 is correlated with increased risks of HCC and lower OS. For example, AGPAT5 is involved in HCC cell proliferation, migration, and invasion in HCC [119]. Conversely, higher AGPAT4 and AGPAT6 expression is associated with a lower risk of HCC and higher OS [119] (**Table 1**). The liver-specific microRNA (miR-122) is essential for maintaining liver homeostasis and a sensitive biomarker for liver cancer, has been reported to be a suppressor targeting AGPAT1 [120].

Phosphatidic acid phosphatases (PAPs [lipins])

PAPs catalyze the conversion of PA-to-DAG. PAPs consist of three isoforms (lipin 1, lipin 2, and lipin 3) [121]. Lipin 2 is the predominant isoform found in the liver, while lipin 1 is expressed at a low level in the liver and lipin 3 is expressed in bone, the gastrointestinal tract, and liver. Studies suggest that lipins have an important role in breast, lung, prostate, and colon cancer growth. Because lipin 2 negatively regulates SREBP1, it is hypothesized that the lipin inhibitor, propranolol, could increase the sensitivity of HCC cells to the mTOR inhibitor, rapamycin [121].

Diacylglycerol acyltransferase (DGAT)

DGATs include two family members (DGAT1 and DGAT2). Although both enzymes catalyze the conversion of DAG-to-TG in the final synthesis stage, there are different prognoses for patients with varying levels of DGAT1 and DGAT2 expression, according to the online database Kaplan-Meier plotter. Patients with high DGAT1 expression have a poor prognosis with HCC (median survival: 70.5 months for the low expression cohort and 52 months for the high expression cohort; n=364 patients). Conversely, patients with high DGAT2 expression have a positive association with good prognosis for HCC (median survival: 47.4 months for the low expression cohort and 84.4 months for the high expression cohort; n=364 patient; **Table 1**). Taxifolin, a plant flavonoid, inhibits microsomal TG synthesis by 37% by decreasing DGAT activity (35% inhibition for DGAT1 and DGAT2) with effects at the post-transcriptional level [122]. Pradigastat [123] (for NAFLD) and C1q/TNF-related protein 12 [124]

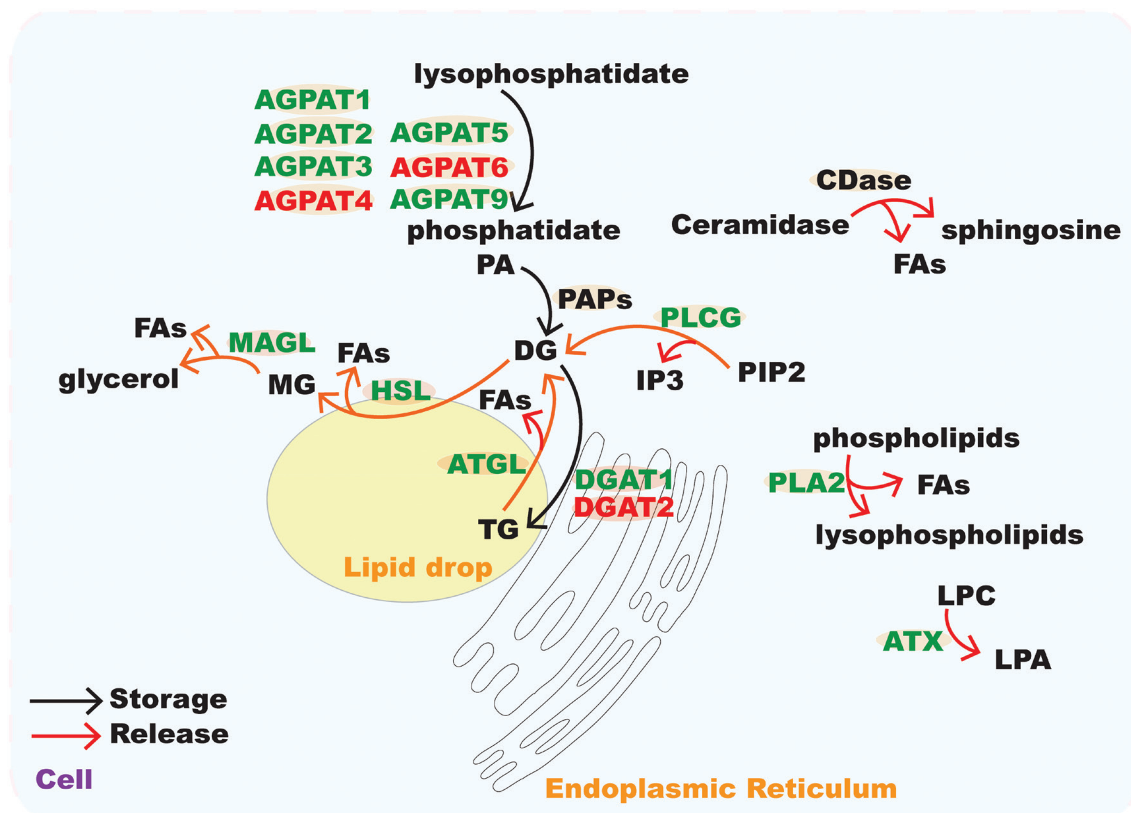

Figure 5 | Storage and release of fatty acids (FAs). Enzymes associated with liver cancer are shown in a yellow background. AGPAT1/2/3/4/5/6/9, 1-acylglycerol-3-phosphate O-acyltransferase 1/2/3/4/5/6/9; ATGL, adipose triacylglycerol lipase; ATX, autotaxin; CDase, ceramidase; DGAT1/2, diacylglycerol acyltransferase 1/2; HSL, hormone-sensitive lipase; MAGL, monoacylglycerol lipase; PAPs, phosphatidic acid phosphohydrolase; PLA2, phospholipase A2; PLCG, phospholipase C-gamma. Red words: tumor suppressors; Green words: tumor inducers; Black words: NA.

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Table 2 | Drugs and agents targeting fatty acid metabolism enzymes.

Drug	Targets	Investigated material	Dose/concentration	Effects	FA metabolic category	References
Aspirin	ACSL1↓	HepG2, Huh7	2.5 and 5 mM	NA	Storage	[132]
Rosuvastatin	SREBP-1c↓; FASN↓; PPARα↑	STAM mice	0.00125%	Inhibits development of hepatic tumors	Oxidation; storage	[133]
Celecoxib	FASN↓;	Wild-type FVB/N mice; HepG2, Huh-7, SMMC-7721 and BEL-7402	125 mg/kg/d, 250mg /kg/d (*in vivo*); 10, 25, 50 μM (*in vitro*)	Delays rapid HCC development	Synthesis	[134]
Oroxyloside	PPARα↑; PPARγ↑; CPT1↑	Male BALB/c nude mice; HepG2, SMMC-7721	90 mg/kg (*in vivo*); 100, 200, 300 μM (*in vitro*)	Inhibits HCC cell proliferation and tumor growth	Oxidation	[135]
Metformin	FASN↓	Wild-type FVB/N mice; HepG2, Hep3B	150, 300 mg/kg/d (*in vivo*); 1, 2 mM (*in vitro*)	Delayed rapid hepatocarcinogenesis in mice, suppressing aberrant lipogenesis	Synthesis	[136]
5-aminoimidazole-4-carboxamide ribonucleotide	ACLY↓; FASN↓	Male C57BL/6 male mice	350 mg/kg	Reduces tumorigenesis	Synthesis	[137]
ND-654	ACC1↓	ACC Knock-in and wild-type mice	10 mg/kg	Suppresses HCC proliferation	Synthesis	[22]
TVB-2640, TVB-3664	FASN↓	C57BL/6L mice; a primary human liver microtissue model	10 mg/kg (*in vivo*); 30 nM, 3 μM (*in vitro*)	Decreases development of HCC tumors	Synthesis	[138]
Orlistat	FASN↓; ACC1↓	Huh7	50 μM	Desensitizes HCC cells to sorafenib and triggers apoptosis	Synthesis	[21]
TVB-3664	FASN↓; SCD1↓; ELOVL5↓; ELOVL6↓	Wild-type FVB/N mice; MHCC97H, HLE, and SNU449	10 mg/kg/day (*in vivo*); 20, 40, 80 μM (*in vitro*)	Suppresses HCC tumor growth, inhibits HCC cell growth	Synthesis	[29]
JZL-184	MAGL↓;	BALB/c node mice; SMMC-7721, HepG2, Huh7, L02	50 mg/kg (*in vivo*); 0.5 μg/μl (*in vitro*)	Inhibits cell proliferation and tumor growth, increases apoptosis	Release	[139]
SI-1	SREBP-1c↓; ACC↓; ACLY↓; FASN↓; ACS↓	Nude mice (xenograft); MHCC97-H, L-02	0.2 mg/kg (*in vivo*);	Enhances the antitumor effects of RFA on xenograft tumors and inhibits aerobic glycolysis of HCC cells	Synthesis	[140]
Spiroketopyrazole derivatives bearing quinoline moieties (7m)	ACC1↓	HepG2	1, 5 and 10 μM	Inhibits cell proliferation and induces G0/G1 arrest and apoptosis	Synthesis	[141]
Canagliflozin	ACC1↓; ACCA1↓; SCD↓	Hep3B, Huh7	10 μM	Inhibits HCC cell proliferation and causes G2/M arrest	Synthesis, oxidation	[142]
Fenofibrate	FASN↓; CPT1↓; CPT2↓	Hep3B	50, 75 and 100 μM	Causes S and G2/M cell cycle arrest and induces cells apoptosis and necroptosis	Synthesis, oxidation	[143]

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Table 2 | Continued

Drug	Targets	Investigated material	Dose/concentration	Effects	FA metabolic category	References
SFA 22637550	FASN↓	HepG2	28 and 35 μM	Increases sub-G0 population and induces apoptosis	Synthesis	[144]
Sorafenib	SREBP-1c↓; FASN↓; ACC1↓; SCD1↓	BALB/c nu-male mice (xenograft); Huh7.5	20 mg/kg/d (*in vivo*); 10, 20 and 30 μM (*in vitro*)	Induces cell death and suppresses liver cancer growth	Synthesis, oxidation	[108]
Sorafenib	SCD↓	Six-week-old male BALB/c athymic nude mice (xenografts); HCC cell lines HepG2, Hep3B, SMMC-7721, Huh7 and Bel-7402.	30 mg/kg/d (*in vivo*); 5 μM (*in vitro*)	Inhibits tumor growth and promotes iron death in HCC cells	Oxidation	[109]
Etomoxir	CPT1A↓	DEN-induced 15 days old LSL-$Kras^{G12D}$ mice. After 8 weeks, mice were fed HFD until sacrificed. Five weeks after HFD was fed, mice were respectively randomized into two groups and treated with etomoxir [ETO] (40 mg/kg i.p. every other day for 5 weeks) or saline as untreated vehicle	40 mg/kg/d (***in vivo***), i.p.	Ameliorates active Ras-derived HCC via inhibiting CPT1A	Oxidation	[145]

(for diabetes) have been reported as DGAT1 and DGAT2 inhibitors, respectively. However, there is currently no evidence supporting the use of pradigastat or C1q/TNF-related protein 12 as treatment strategies for HCC.

4.4 Enzymes implied in the release of FAs

Metabolic reprogramming in the pathogenesis of HCC involves a complex process of FA release that is influenced by various factors within the tumor microenvironment [125]. Inhibiting lipolysis improves glucose metabolism and insulin sensitivity in adipose tissues, while increasing lipolysis helps attenuates hepatic steatosis in liver tissues [126, 127]. Dysregulation of lipid metabolism commonly occurs in HCC with alterations in lipid droplet dynamics and FA metabolism and may contribute to tumor progression [128, 129]. This complex interplay involves changes in lipid metabolism, modulation of key enzymes, such as adipose triacylglycerol lipase (ATGL), hormone-sensitive lipase (HSL), monoacylglycerol lipase (MAGL), phospholipase A2 (PLA2), phospholipase C-gamma (PLC-γ), autotaxin (ATX), and ceramidase (CDase), as well as an impact of signaling molecules [8, 130, 131] (**Figure 5**). The potential drugs targeting the release enzymes of FAs are listed in **Tables 2** and **3**.

Adipose triacylglycerol lipase (ATGL [PNPLA2])

ATGL, also known as patatin-like phospholipase domain containing 2 (PNPLA2) in humans, has a central role in lipolysis by converting TGs into FAs and glycerol [162]. ATGL regulates the release of FAs from adipose tissue, which can then be used as an energy source in various tissues. Emerging evidence suggests that the physiologic function of ATGL extends beyond adipose tissues and is crucially important in various non-adipose tissues, such as the liver, where ATGL regulates the release of FAs for energy utilization [163]. Liu et al. reported high expression of ATGL in human HCC tissues that positively correlated with tumor size and indicated a poor prognosis [164]. Conversely, Di Leo et al. showed an inverse relationship between the ATGL level and the proliferation rate of HCC-derived cell lines, pointing to a dependence on functional ATGL enzymatic activity [165]. In ATGL-deficient mice, the accumulation of cytoplasmic TGs acts as a major cause of hepatic steatosis [166], while in ATGL-overexpressing mice, the direct release and oxidation of free FAs alleviates hepatic steatosis [167]. ATGL is activated by comparative gene identification-58 and inhibited by G(0)/G(1) switch gene-2 protein [168]. The inhibitory sites for ATGL are residues Y27, V28, G30, A34 G37, V39, and/or L42 [169]. Natural compounds found in various medicinal plants have been identified as potential modulators of lipid metabolism and ATGL activity [170]. Nicotinamide mononucleotide (NMN) is one of the forms of vitamin B3 that suppresses subcutaneous fat mass in diet-induced obese mice by upregulating ATGL and protects the liver against fibrosis and cirrhosis [171]. However, a study showed that NMN induces

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Table 3 | Natural products targeting fatty acid metabolic enzymes.

Natural-product / Compounds	Targets	Subjects	Dose	Results	FA metabolic process	References
Rhizoma Paridis saponins and sorafenib	CPT1A↓	6-8-week-old female Kunming mice (xenograft tumors)	Sorafenib (60 mg/kg); *Rhizoma Paridis saponins* (80 mg/kg)	Reduces mitochondrial damage – inhibits anaerobic glycolysis – Inhibits lipid synthesis	Synthesis; oxidation	[146]
Bergapten	SREBP-1c↓; FASN↓; SCD1↓	Wistar albino rats; HepG2	25 and 50 mg/kg/d (*in vivo*)	Inhibits liver carcinogenesis	Synthesis	[147]
Curcumin	SREBP1c↓; FASN↓	Kunming mice	25 mg/kg/d	Suppresses tumor growth	Synthesis	[148]
Cinobufotalin	SREBP-1c↓; FASN↓; ACC↓; ACLY↓; SCD1↓	BABL/c SCID mice (xenograft); HepG2 and SMMG-7721	2.5 and 5.0 mg/kg/d (*in vivo*); 0.1, 0.2 μM (*in vitro*)	Inhibits tumor growth and lipogenesis; promotes cell apoptosis, induces cell cycle G2-M arrest, and inhibits cell proliferation and lipogenesis	Synthesis	[149]
Clove extract	FASN↓	HepG2	100 mg/L	Inhibits cell proliferation and induces cell cycle S-phase arrest	Synthesis	[150]
Tetrahydrocurcumin	SREBP-1c↓; FASN↓; ACC↓; PPARγ↓; PPARα↑; CPT-1↑	HepG2	10, 25, 50 and 100 μM	NA	Synthesis, oxidation	[151]
Zhiheshouwu ethanol extract	SREBP-1c↓; SCD1↓	Bel-7402	400 and 600 μg/mL	Inhibits HCC cell proliferation and induces intrinsic apoptosis	Synthesis, oxidation	[152]
Emodin	SREBP-1↓; SCD1↓; FASN↓; ACACA↓; ACLY↓	Bel-7402	100 μmol/L	Induces apoptosis	Synthesis, oxidation	[15]
Osthole	FASN↓	Wild-type (WT) FVB/N mice; HepG2, SMMC-7721	244 mg/kg/d (*in vivo*); 60, 120 and 180 μM (*in vitro*)	Suppresses cell proliferation and delays hepatocarcinogenesis	Synthesis	[153]
Berberine	ACC1↓	HepG2	50 and 100 μM	Induces apoptotic and autophagic death	NA	[154]
Fatsioside A	ACC1↓	HepG2	40 and 80 μM	Inhibits the survival and proliferation and induces apoptotic and necrotic death	NA	[155]
Betulin	SREBP-1C↓; ACLY↓; FASN↓; ACC↓; ACS↓	Bal B/c mice with T cell/thymus deletion features(xenograft); MHCC97-H cell	2 mg/kg (*in vivo*); 3 μM (*in vitro*)	Suppresses tumor growth, promotes the antitumor effect of sorafenib; facilitates sorafenib-mediates suppression on metastasis	Synthesis	[156]
Strawberry methanolic extract	ACC1↓	HepG2	10, 50 and 100 μg/mL	Inhibits cell viability	Synthesis	[157]
Mulberry leaf polyphenol extract, Rutin	FASN↓	HepG2	0.25 and 0.5 mg/ mL (*in vitro*); 20 μM (*in vitro*)	Induces autophagy	Synthesis	[158]
Berberine	ATX↓	C57BL/6 mice (primary liver cancer)	0.04% and 0.2% BBR (W/W)	Represses liver tumorigenesis	Release	[159]

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Table 3 | Continued

Natural-product / Compounds	Targets	Subjects	Dose	Results	FA metabolic process	References
Oleiferasaponin A2	SREBP-1c↓; FASN↓; CPT1↑	HepG2	10 μM	NA	Synthesis, oxidation	[160]
Monacolin K	SREBP-1c↓; ACC↓; FASN↓; ATGL↑	HepG2	3, 10, 30 and 100 μM	NA	Synthesis, release	[161]
Rubia yunnanensis extract	SREBP-1↓; FASN↓; SCD↓	HepG2; Female Balb/c nude mice, 6-8 weeks old (xenograft)	0.5, 1 and 2 μM (*in vitro*); 20 and 40 mg/kg(*in vivo*)	Induces G2/M cell cycle arrest and cell death and inhibits tumor growth	Synthesis; oxidation	[110]

Note: ↑, up-regulation; ↓, down-regulation.

autophagy and ferroptosis via the AMPK/mTOR pathway rather than ATGL in HCC [172]. Given that ATGL has a crucial role in lipid metabolism and is implicated in the pathogenesis of HCC, targeting ATGL and related pathways may represent a promising therapeutic approach to HCC treatment with the potential to disrupt lipid metabolism and inhibit tumor growth.

Hormone-sensitive lipase (HSL)

HSL is an intracellular neutral lipase that has a crucial role in lipid metabolism, like ATGL. HSL possesses the ability to hydrolyze various substrates, including TGs, diacylglycerols, monoacylglycerols, cholesteryl esters, and other lipid and water-soluble compounds. HSL acts as a rate-limiting enzyme in the early stages of the lipolysis process [173]. HSL breaks down stored TGs into free FAs and glycerol in response to hormonal signals, such as catecholamines and insulin, thereby modulating energy metabolism and maintaining lipid homeostasis [163]. This enzyme is primarily expressed in adipose and steroidogenic tissues but is typically found at minimal levels in the liver. PKA, PKC, 2,4-dienoyl-CoA reductase (DECR1) [174], and ERK activate HSL via phosphorylation [175]. It has been reported that enhancing FA oxidation and facilitating the direct release of free FAs through liver expression of HSL and ATGL ameliorates steatosis conditions [167, 176]. Individuals with an HSL hereditary deficiency have also been reported to develop fatty liver [177]. Natural compounds that modulate HSL activity might offer promise as supplementary therapies for HCC or as strategies to lessen the development of NAFLD. Notably, sulforaphane, a potential protective phytochemical found in cruciferous vegetables, ameliorates aberrant lipid metabolism by increasing lipolysis through a transcriptional upregulation of ATGL and HSL and by improving mitochondrial function [178]. Despite numerous studies reporting the interest of herbal medicines, such as *Polygonatum stenophyllum* Maxim. Rhizome [175] or *Morus alba* L. (Fructus Mori [mulberry]) water extracts [179] regulate lipid metabolism, promote hepatic lipolysis, and protect against liver steatosis in conditions, such as obesity and diabetes. Evaluations of the effects of herbal medicine targeting HSL in lipid accumulation models of HCC are limited [173].

Monoacylglycerol lipase (MAGL)

MAGL acts as a key enzyme in lipid metabolism by participating in the final step of neutral lipid decomposition and breaking down monoacylglycerol (MG) into FA and glycerol. MAGL-mediated hydrolysis of MGs regulates the levels of bioactive lipids involved in various physiologic processes, including inflammation, pain sensation, and energy homeostasis [139, 180]. Considering the abundance and importance of FAs and lipid metabolism in the liver, MAGL is expected to have a crucial role in the initiation and progression of HCC [139]. Upregulation of MAGL activity enhances cell invasiveness ability in HCC, while several small-molecule inhibitors,

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such as ABX-1431, significantly counteract this effect and exhibit anticancer effects [181]. These inhibitors of MAGL suppress the release of free FAs, thereby impairing tumor growth and metastasis in preclinical models of HCC [139]. Phytochemicals, such as polyphenols, flavonoids, and cannabinoids, have been studied in various cancer models for their ability to inhibit MAGL activity and suppress tumor growth [174]. The diterpene, jewenol A, which is isolated from *Salvia pseudorosmarinus* Epling, is a moderate reversible MAGL inhibitor [182]. The rhizome of *Corydalis yanhusuo* (Y.H.Chou & Chun C.Hsu) W.T.Wang ex Z.Y.Su & C.Y.Wu exerts analgesic effects by inhibiting MAGL activity and thereby inhibiting the hydrolysis of the endocannabinoid, 2-arachidonoylglycerol [183]. There is a limited understanding of the physiologic function of MAGL and its role in HCC pathogenesis. No tests on HCC have been reported for medicinal plants and herbal extracts containing bioactive compounds with specific MAGL.

Phospholipase A2 (PLA2)

PLA2 catalyzes the hydrolysis of phospholipids, leading to the release of FAs and lysophospholipids [184]. There are several subtypes of PLA2, including cytosolic PLA2, calcium-independent PLA2, and secretory PLA2 (sPLA2), each with distinct physiologic functions and roles in disease processes [184]. PLA2s have essential roles in cellular membrane remodeling, lipid metabolism, and in the generation of bioactive lipid mediators (eicosanoids), such as prostaglandins, leukotrienes, and platelet-activating factor [185]. These lipid mediators are involved in various physiologic processes, including inflammation, immune responses, and cell signaling [186]. Dysregulation of PLA2 expression and activity has been implicated in the pathogenesis and progression of HCC [187]. Increased expression of PLA2s, particularly sPLA2, has been noted in HCC tissues, which generates eicosanoids that correlate with tumor aggressiveness, metastasis, and exacerbation of disease progression [188]. Targeting PLA2 enzymes for therapeutic intervention in HCC remains an area of active investigation. While specific inhibitors of PLA2 are not available for clinical use, conventional drugs, such as corticosteroids and non-steroidal anti-inflammatory drugs, indirectly modulate PLA2 activity and eicosanoid generation, which impacts HCC progression [189, 190]. More recent specific inhibitors that target PLA2 (e.g., FPL67047XX) [191] and 5-lipoxygenase (e.g., zileuton) or antagonize cysteinyl leukotriene receptors (e.g., montelukast) would be interesting to explore in HCC models [192].

Phospholipase C-gamma (PLC-γ)

PLC-γ is an enzyme that has a crucial role in intracellular signaling pathways by catalyzing the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) into the second messengers, inositol 1,4,5-trisphosphate (IP3) and DAG, which are involved in various cellular processes, including cell growth, differentiation, and proliferation [193]. Upon activation by tyrosine kinase receptors (RTKs) or G protein-coupled receptors (GPCRs), PLC-γ hydrolyzes PIP2 to generate IP3, which triggers Ca^{2+} release from intracellular stores and DAG, which activates protein kinase C (PKC) signaling [194, 195]. These signaling events regulate numerous cellular responses, including gene expression, cytoskeletal rearrangement, and vesicle trafficking. Aberrant activation of PLC-γ signaling pathways, often mediated by overexpression or mutations in upstream tyrosine kinase receptors, promotes cancer cell proliferation, survival, and metastasis in HCC [196]. Increased PLC-γ expression in human HCC correlates with unfavorable prognosis of patients, with reports indicating its role in promoting the pathogenic progression of HCC and highlighting the PLC-γ/STAT3 axis as a potential therapeutic target for the disease [197]. Conventional drugs targeting upstream of PLC-γ, such as RTK inhibitors (e.g., sorafenib) and PKC inhibitors or downstream signaling molecules may indirectly modulate PLC-γ activity and impact HCC progression [198]. The combination of sorafenib with emodin, an active component found in plants used in various Chinese materia medica, such as *Rheum palmatum* L. and *Polygonum cuspidatum* Siebold & Zucc (synonym of *Reynoutria japonica* Houtt.), may offer potential therapy for patients with advanced HCC [199]. The traditional Korean herbal prescription, Kyung-Ok-Ko (KOK), is composed of six herbal ingredients {Rehmannia glutinosa *var.* purpurae (Makino) Makino & Nemoto [synonym of *Rehmannia glutinosa*] (Gaertn.) DC.], Lycium chinense Mill., *Aquilaria agallocha* Roxb [synonym of *Aquilaria malaccensis* Lam.], Poria cocos, Panax ginseng L., and honey} and exhibits antiplatelet effects through mechanisms involving inhibition of ATP release, intracellular Ca^{2+} elevation, and phosphorylation regulation of PLC-γ and Akt [200]. Research focusing on the PLC-γ activity of natural compounds utilizing physiologic active substances, has been applied to various disease [201]. However, investigations into the mechanism targeting PLC-γ in HCC models are limited.

Autotaxin (ATX)

ATX, also known as ectonucleotide pyrophosphatase/phosphodiesterase 2 (ENPP2), is an enzyme that has a role in lipid metabolism, specifically in converting lysophosphatidylcholine (LPC), a component of cell membranes, to lysophosphatidic acid (LPA), a potent bioactive lipid mediator [202]. ATX is primarily secreted into the extracellular space and circulates in the bloodstream. LPA acts through a specific G protein-coupled receptor (GPCR) to regulate various cellular processes, including cell proliferation, migration, survival, and angiogenesis [203]. Thus, ATX-mediated production of LPA is involved in diverse physiologic functions, such as embryonic development, tissue repair, the immune response, and vascular homeostasis [204]. Ki16452 (an LPAR1 and LPAR3 antagonist), ONO-8430506 (an ATX inhibitor), and GLPG1690 (an ATX inhibitor and LPAR6 antagonist) function by blocking ATX activity, thereby suppressing LPA production and

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impairing tumor growth and metastasis in preclinical models of HCC [205]. The natural product, berberine, has been reported to inhibit carcinogenesis by antagonizing the ATX-LPA-LPAR2-p38-leptin axis in a mouse hepatoma model [159, 206], while ginsenoside Rk3 has been studied for potential anti-tumor effects in HCC by targeting downstream effectors of ATX/LPA signaling, such as PI3K/Akt inhibitors [207].

Ceramidase (CDase)

CDase catalyzes the breakdown of ceramides into sphingosine and FAs, generating sphingosine and likely, sphingosine 1-phosphate (S1P) within cells, thereby serving as a significant modulator of sphingolipid-mediated signaling [208]. CDase is classified into three families (acid, neutral, and alkaline ceramidase [Acer-1, -2, and -3]) based on pH optima for catalytic activity. The physiologic functions CDase encompasses various cellular processes, including cell growth, differentiation, apoptosis, and signaling pathways related to inflammation and cancer [209, 210]. The role of CDases is complex and context-dependent. Ceramides, which are CDase substrates, are markedly reduced in HCC tissues and some studies suggest that nanoliposomal C6-ceramide administration could be effective in reducing tumor vascularization and proliferation, inducing tumor cell apoptosis, decreasing AKT phosphorylation, and ultimately inhibiting tumor growth, thus offering a possible treatment strategy for HCC [211]. Other studies have proposed that CDase inhibition suppresses HCC growth by inducing apoptosis and inhibiting angiogenesis [212]. Specific inhibitors targeting CDase, currently under exploration for potential therapeutic benefits in HCC, may present a novel strategy for treating human HCC by regulating Acer-2/SMPDL3B and preventing ceramide hydrolysis and S1P production [213]. Additionally, Acer-3, which is implicated in promoting the growth of HCC cells through regulation of S1P/S1PR2/PI3K/AKT signaling, may also offer a promising pathway for HCC treatment [214]. These inhibitors disrupt ceramide metabolism and induce ceramide accumulation, thereby triggering apoptotic pathways in cancer cells [215]. No studies have reported direct activation of CDase by phytochemicals in models of HCC.

5. DISCUSSION

5.1 Heterogeneity of FA metabolism in HCC

HCC is highly heterogeneous malignant disease that most frequently arises in the context of chronic liver inflammation, fibrosis, or cirrhosis. Heterogeneity of HCC is influenced by various factors, including environmental and genetic susceptibilities, such as infections, metabolic injuries, toxic insults, autoimmune reactions, or genomic instability, which lead to deviations in the molecular and signaling network [216]. Therefore, it is crucial to understand the significance of the microenvironmental and molecular targets to develop precision therapies against FA metabolic reprogramming.

The tumor microenvironment is comprised of diverse cell populations, including endothelial, epithelial, hepatic stellate, fibroblast, and immune cells. Thirteen different cell types were analyzed in the current bioinformatic study. The data indicated that epithelial cells exhibit the highest quantified FA metabolism scores, while some lymphocytes, such as B, CD8Tcm, and Treg cells had the lowest scores. These findings provide a unique perspective. Specifically, targeting FA metabolism in lymphocytes rather than epithelial cells may offer a better prognosis in HCC. Furthermore, a significant heterogeneity of FA with a wide distribution range (–0.2 to 0.8, log-rank test, P=0.0089; **Figure 1B**) was observed. This finding indicates the presence of mechanisms contributing to heterogeneity, unmanageability, and recurrence in HCC. Corollary studies are needed to determine the causes and consequences of FA metabolism heterogeneity in epithelial cells.

Although the data herein showed that low FA metabolism correlates with a poor prognosis (**Figure 3**), the four processes of FA metabolism (biosynthesis, oxidation, storage, and release) are associated with different tumor phenotypes. Studies have shown that inhibiting most enzymes of synthesis, storage and release while promoting most those of oxidation limit the supply of free FAs, thereby inhibiting the proliferation, invasion, and angiogenesis in HCC. Higher expression of oxidases (i.e., β-oxidation and ω-oxidation enzymes) is associated with lower risks of oncogenesis and higher OS (**Table 1**). However, the expression of enzymes involved in synthesis, storage, and release display significant diversity according to the HCC phenotype. Even different isoforms within the same enzyme family demonstrated opposite phenotypes in relation to HCC. For example, AGPAT1, 2, 3, and 5 are associated with a lower OS, while AGPAT4 and 9 are associated with a higher OS (**Table 1**, **Figure 5**). Understanding FA metabolic reprograming may provide a novel perspective for combating HCC heterogeneity. However, the prediction of different or opposite prognoses based on different enzyme isoforms suggests the possibility of significant side effects or toxicity when targeting specific isoforms with drugs. In addition, targeting one isoform may lead to overexpression of other enzymes with difficult-to-predict consequences. Further challenges for drug discovery also arise from inconsistent data because the same enzymes may have contradictory roles in different studies. For example, CPT1A promotes HCC via Ras and lipid degradation, while ETO inhibits HCC via downregulation of CPT1A [145] and RBM45 promotes HCC via upregulation of CPT1A [217]. However, in clinical settings CPT1A is positively associated with a good prognosis (n=364; **Table 1**). Therefore, drug discovery strategies require a study with larger cohorts and detailed mechanisms.

5.2 Natural product interest for drug discovery in HCC

Natural medicines, i.e., phytomedicines, have been used for centuries in the treatment of cancer with purported

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low toxicity and multiple effects that could be worth coupling with modern anticancer agents. The saponins of Rhizoma Paridis [the rhizome of *Paris polyphylla* Smith var.*yunnanensis* (Franch) Hand-Mazz. and *Paris polyphylla* Smith var. *chinensis* (Franch) Hara] inhibited CPT1A in combination with sorafenib, leading to enhanced antitumor effects in an HCC mouse model [146]. *Polygonatum stenophyllum* Maxim Rhizome [175] or *Morus alba* L. fruit (Fructus Mori [mulberry]) water extracts [179] targeted HSL in models of lipid accumulation in HCC [173]. Our previous studies showed that emodin, a natural anthraquinone, inhibited FA biosynthesis and induced apoptosis in HCC [15]. **Table 3** lists the natural products targeting FA metabolic enzymes. Although only sorafenib has been reported to be partially effective in patients with HCC or after HCC conventional therapies [218-222] (**Table 4**), the exploration of further natural products, including Chinese medicines, may open new avenues for combating HCC [15, 152, 223].

5.3 Advantages and limitations of scRNA-seq for exploring tumor heterogeneity

As a revolutionary technology for exploring tumor heterogeneity, scRNA-seq enables a deeper understanding of subtle changes in tumor biology by identifying rare cell subpopulations, dissecting the tumor microenvironment, and characterizing cellular genomic mutations. Therefore, different cancer phenotypes associated with poor prognosis and medication resistance are discovered based on vast and complex datasets. This process allows developing a more personalized approach to therapy based on the genetic and transcriptional profile of the tumor, ultimately saving valuable time and preventing potentially harmful side effects. In addition to cost scRNA-seq requires advanced technical expertise and complex data analysis. Errors and biases in scRNA-seq often arise during the preparation of sequence libraries and the assembly of short reads, posing challenges in accurately identifying various subtypes of specific genes [224]. To overcome errors and biases, integrating scRNA-seq with long-read RNA-seq, PacBio circular consensus sequencing (CCS) [224] and spatiotemporal transcriptomics analysis, as well as conventional technologies like histology and genomics should be exploited for high-quality studies [225].

5.4 FA metabolism level and prognosis in HCC

The scRNA-seq data revealed that low FA metabolism level is generally associated with poor prognosis in HCC patients (**Figures 1–3**). Surprisingly, higher oxidase expression was correlated with reduced risks of oncogenesis and improved OS. However, different gene isoforms exhibited varying or opposing functions in FA biosynthesis, storage and release in HCC (**Table 1** and **Figure 6**).

Table 4 | Clinical studies on drugs /natural products targeting HCC.

Drugs	Deases	Study	Dosage	Treatment group atients	Male:female ratio	Placebo/control group patients	Male:female ratio	Efficacy rate (%)	*P* value	References
Sorafenib	Advanced stage HCC	Phase II	154-800 mg/d, median dosage 400 mg/d	31	24/7	NA	NA	77.40	0.042	[218]
Sorafenib	Advanced HCC in Asian populations	Phase III	400 mg, twice a day in 6-week cycles	150	127/23	76	66/10	35.30	0.014	[219]
Sorafenib	HCC with a complete radiologic response after surgical resection or local ablation	Phase III	577 mg/d	556	451/105	558	461/97	34.89	0.26	[220]
Sorafenib	Sorafenib or placebo plus TACE with doxorubicin-eluting beads for intermediate stage HCC	SPACE trial	150 mg doxorubicin plus sorafenib 400 mg twice daily	154	135/19	153	126/27	89.2	0.072	[221]
Sorafenib	Sorafenib combined with concurrent conventional transarterial chemoembolization (cTACE) vs. sorafenib alone in patients with advanced HCC	Phase III	600 mg/d	170	136/34	169	147/22	60.6	0.29	[222]

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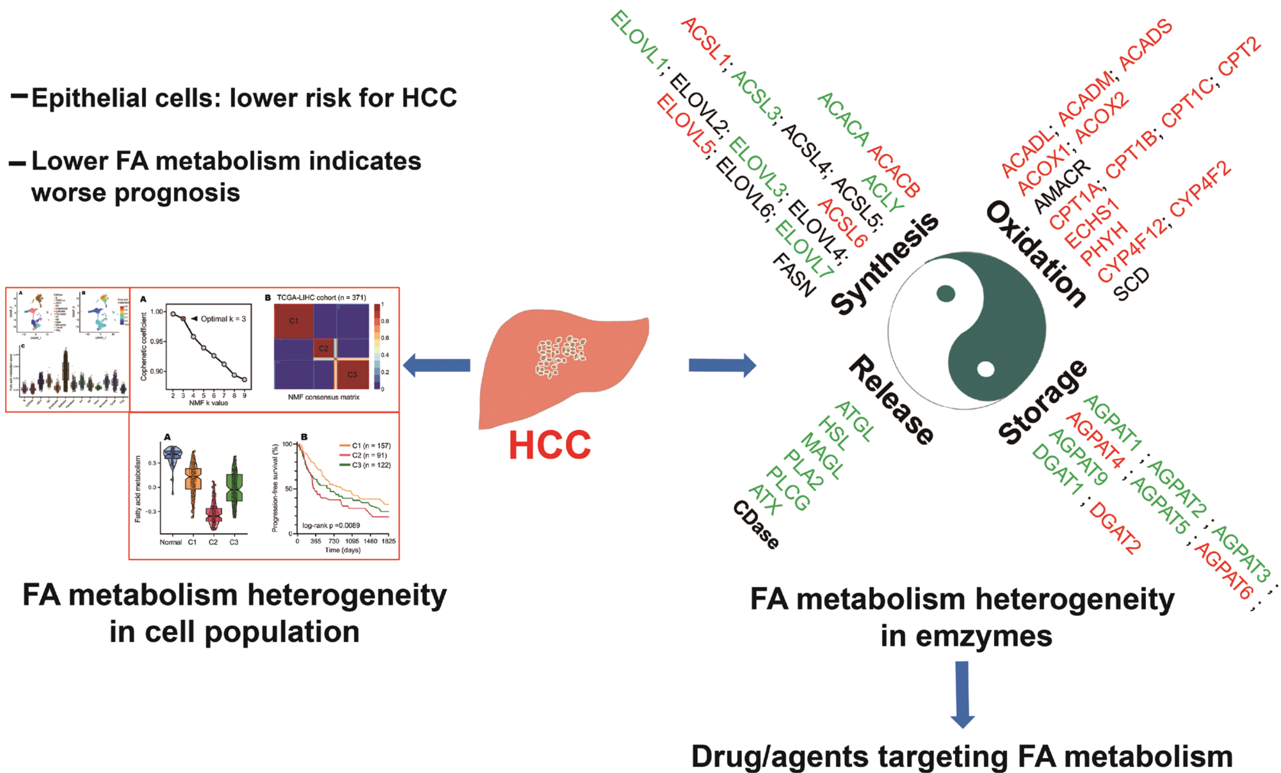

Figure 6 | Graphical abstract of heterogeneity in FA metabolism reprogramming in HCC.

5.5 Limitations of the present study

Considering the limitations of the current study, a comparative analysis should be performed by including gene set enrichment (GSE) data on liver fibrosis and cirrhosis. Additionally, incorporating additional GSE analysis could broaden the sample sources, increase heterogeneity, and thus provide the bases for more comprehensive analyses in HCC [226]. It is important to acknowledge that tumor heterogeneity poses challenges in applying this concept broadly to other cancer types with respect to the generalizability of the FA metabolic subtyping in liver cancer. The role of FA metabolism is particularly distinct in HCC depending on a unique microenvironment that introduces additional complexities to metabolic heterogeneity, which distinguishes HCC from other malignancies. For example, the functions of enzymes involved in biosynthesis and β-oxidation in HCC resemble breast cancer but differ from triple-negative breast cancer, while the scenario changes for enzymes related to storage. The mRNA RNA-Seq data from the Kaplan-Meier plotter database showed that the FA biosynthetic enzyme, ACLY, is a negative factor for patient OS in HCC [HR=1.74 (1.13-2.69); log rank P=0.011; median survival: 71 months (low expression cohort), 52 months (high expression cohort); patient number, 364] and breast cancer [HR=0.52 (0.41-0.64); log rank P=2.6e-09; median survival: NA, patient number, 2976] but a positive factor in triple-negative breast cancer [HR=0.25 (0.09-0.65), log rank P=0.0023, median survival: NA, patient number, 126]. Conversely, the FA β-oxidation enzymes (ACOX1 and ACOX2) may act as suppressors in cancer development in HCC and breast cancer with no significant effect on triple-negative breast cancer. Interestingly, the two isoforms of FA storage (DGAT1 and DGAT2) played different roles among HCC, breast cancer, and triple-negative breast cancer. While DGAT1 is detrimental to OS in HCC and breast cancer patients, DGAT1 is not significant in triple-negative breast cancer. In contrast, DGAT2 was beneficial to OS in HCC and triple-negative breast cancer patients but not significant in breast cancer patients (Table 1). This finding indicates a complex diversity in FA metabolism reprogramming in different cancer types that requires further investigation [227].

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6. CONCLUSION

In conclusion, a low level of aberrant FA metabolism appears associated with a poor prognosis in HCC patients. The heterogeneity of HCC can be attributed to a high diversity of cells present in the tumor and isoforms of possible target enzymes. Epithelial cells have the highest score in FA metabolic reprogramming in HCC. Higher expression of oxidases is associated with reduced oncogenesis risks and improved OS but the products of different gene isoforms involved in FA biosynthesis and storage may exhibit varying or opposing functions, which contribute to the heterogeneity of HCC. Natural products and Chinese medicines offer potential avenues for addressing FA metabolic reprogramming in HCC.

7. MATERIAL AND METHODS

7.1 Single-sample gene set enrichment analysis (ssGSEA)

ssGSEA is a variation of the GSEA algorithm that calculates enrichment scores for individual samples and gene sets rather than groups of samples and sets of genes. In the current study the gene set, "FATTY_ACID_METABOLIC_PROCESS," was obtained from the C5 collection (Gene Ontology gene sets) of the Molecular Signatures Database (MSigDB) [228]. The R package, "GSVA," was used to quantify the ssGSEA score for each sample or cell.

7.2 Single-cell RNA-sequencing (SCRNA-SEQ) analysis

A publicly available scRNA-Seq dataset (GSE166635), including 22,631 cells [229], was studied to investigate the tumor microenvironment components and FA metabolism of HCC. The scRNA-Seq expression matrix (UMI) was processed with the R package, "Seurat." Cluster visualization was performed through UMAP reduction and specific marker genes for each cell type were utilized for cluster annotation. The ssGSEA algorithm was applied to quantify FA metabolism and color gradients were used to map the levels of FA metabolism to each cell.

7.3 Survival analyses

Based on the gene set of FA metabolism retrieved from the MSigDB and the RNA-seq data of the Liver Hepatocellular Carcinoma (LIHC [sample number=371]) cohort downloaded from The Cancer Genome Atlas (TCGA) database, the non-negative matrix factorization (NMF) algorithm was performed to divide the entire cohort into different subgroups with distinct expression patterns. NMF is a machine learning algorithm commonly used for dimensionality reduction, data clustering, and feature extraction, and is particularly effective for analyzing RNA-seq data. The optimal factorization k value of NMF was determined when the magnitude of the cophenetic correlation coefficient started to significantly decrease.

A one-way analysis of variance (ANOVA) was applied to determine the statistically significant difference between the means of three or more independent groups that follow a normal distribution. The PFS information of 370 HCC patients was retrieved from the TCGA portal. Based on the follow-up time and event, the survival curves of each group were plotted using the Kaplan-Meier analysis and the log-rank test was used to compare the survival differences among different FA subgroups. All the statistical analyses in this study were performed using R version 4.2.1 and GraphPad Prism 9.5. A $P<0.05$ is considered statistically significant [229].

7.4 Target enzymes and agents or drugs involved in the synthesis, oxidation, storage, and release of FAs in HCC

Based on bioinformatics data, the genes, thei products, and potential agents or drugs involved in the synthesis, oxidation, storage, and release of FAs in HCC were retrieved and analyzed using online databases, such as PubMed, Web of Science, and PROQUEST. The relationships between the potential target genes of FA metabolism and OS in patients with HCC were also investigated using the online database Kaplan-Meier plotter (https://kmplot.com/).

7.5 Clinical trials and studies on drugs targeting FA metabolism in HCC

To summarize the clinical efficacy of drugs targeting FA metabolism (especially the metabolism enzymes) in HCC, the keywords, "liver cancer," "clinical trial," "clinical study," and "fatty acid metabolism," were retrieved in PubMed and Cochrane Library up to November 2024. For the systematic review of clinical efficacy for HCC, the PRISMA criteria were followed [230] and the two independent reviewers from Hubei Key Laboratory of Wudang Local Chinese Research (Prof. Xuanbin Wang and Ms. Yingying Guo) screened and double-checked the literature using the inclusion criteria as follows: (1) the literature involves the preventive and therapeutic effects of drugs and agents targeting FA metabolism in HCC; (2) the literature includes both chemical entities and traditional medicines (natural products); (3) the study involves a randomized and controlled design; and (4) the efficacy of drugs and/or agents is investigated in clinical settings. The exclusion criteria were as follows: (1) the literature is related to neither chemical entities nor traditional medicines (natural products); (2) the concentration or proportion of drugs and/or agents are not quantified; (3) neither dosage nor concentration are available; (4) the study is not a randomized and controlled design; and (5) the literature involves clinical studies without any ethical approval [230].

ABBREVIATIONS

ACAA1, peroxisomal 3-oxo-acyl-CoA thiolase; ACS, acyl-CoA synthetase; ACSL1/3/4/5/6, acyl-CoA synthetase long chain

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family member 1/3/4/5/6; ACCs, acetyl-CoA carboxylases; ACAD, acyl-CoA dehydrogenase; ACOXs, acyl-CoA oxidases; AGPATs, 1-acylglycerol-3-phosphate O-acyltransferase; ACLY, ATP citrate lyase; AMACR, α-methylacyl CoA racemase; ATGL, adipose triacylglycerol lipase; ATX, autotaxin; CPT1, carnitine palmitoyltransferase-1; CACT, carnitine-acylcarnitine translocase; CDase, ceramidase; CE, cholesteryl esters; CPT2, carnitine palmitoyltransferase-2; CYP4, cytochrome P450 family 4; CYP4F2/12, cytochrome P450 family 4 subfamily F member 2/12; DGATs, diacylglycerol acyltransferases; DG, diacylglycerol; DNL, *de novo* lipogenesis; ECHS1, enoyl-CoA hydratase, short chain 1; EMT, epithelial-mesenchymal transition; ELOVLs, elongase of very long chain fatty acids; ER, endoplasmic reticulum; FASN, Fatty acid synthase; FAs, fatty acids; GPAT, glycerol-3-phosphate acyltransferase; HBXIP, hepatitis B X-interacting protein; FAO, fatty acid oxidation; HCC, hepatocellular carcinoma; HIF-1, hypoxia-inducible factor 1; HSL, hormone-sensitive lipase; ICIs, immune checkpoint inhibitors; INSIG2, insulin-induced gene-2; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; MAGL, monoacylglycerol lipase; MFP, multifunctional enzyme; MG, monoacylglycerols; MUFAs, monounsaturated fatty acids; NAFLD, non-alcoholic fatty liver disease; NMF, non-negative matrix factorization; PA, phosphatidic acid; PAP, phosphatidic acid phosphatase; PPARα, peroxisome proliferator-activated receptor α; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PLA2, phospholipase A2; PLCG, phospholipase C-gamma; PUFAs, polyunsaturated fatty acids; PS, phosphatidylserine phosphate; PHYH, phytanoyl-CoA hydroxylase; PI, phosphatidylinositol; ssGSEA, single-sample gene set enrichment analysis; SCD, stearoyl-CoA desaturase; SFAs, saturated fatty acids; S1P, sphingosine 1-phosphate; SM, sphingolipids; SPT, serine palmitoyltransferase; SREBP1c, sterol regulatory element binding protein 1c; TGs, triglycerides; TILs, tumor-infiltrating lymphocytes; MUFAs, unsaturated fatty acids; VLCFA, very long-chain fatty acids; VLDL, very low-density lipoprotein; ZHX2, zinc fingers and homeoboxes 2.

ACKNOWLEDGEMENTS

We would like to thank Mrs. Ming LIU for technical supports. The study was financially supported by the National Natural Science Foundation of China (82274155, Prof. Xuanbin WANG), the Open Project of Hubei Key Laboratory of Wudang Local Chinese Medicine Research (Hubei University of Medicine [WDCM201918, Dr. Hongliang LI]), and the Key Project of the Department of Science and Technology of Hubei Province (2022EHB046, Prof. Xuanbin WANG). The funders had no role in the study design, data collection and analysis, interpretation of data, writing of the manuscript, or decision to publish, which are solely the responsibilities of the authors.

DECLARATIONS

This study was based on published literature and publicly available datasets and therefore did not require ethical approval and consent.

CONSENT FOR PUBLICATION

All authors have read and approved the manuscript for publication.

AVAILABILITY OF DATA, MATERIALS AND METHODS

The datasets supporting the conclusions of this article are included within the article and its additional files.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest in relation to this study.

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