Lipid alterations in chronic liver disease and liver cancer

Bichitra Paul,¹ Monika Lewinska,¹ Jesper B. Andersen¹,†,

Summary
Lipids are a complex and diverse group of molecules with crucial roles in many physiological processes, as well as in the onset, progression, and maintenance of cancers. Fatty acids and cholesterol are the building blocks of lipids, orchestrating these crucial metabolic processes. In the liver, lipid alterations are prevalent as a cause and consequence of chronic hepatitis B and C virus infections, alcoholic hepatitis, and non-alcoholic fatty liver disease and steatohepatitis. Recent developments in lipidomics have also revealed that dynamic changes in triacylglycerols, phospholipids, sphingolipids, ceramides, fatty acids, and cholesterol are involved in the development and progression of primary liver cancer. Accordingly, the transcriptional landscape of lipid metabolism suggests a carcinogenic role of increasing fatty acids and sterol synthesis. However, limited mechanistic insights into the complex nature of the hepatic lipidome have so far hindered the development of effective therapies.

Introduction
Liver cancer is the fourth leading cause of cancer-related deaths worldwide,¹ and incidence and mortality rates are steadily increasing.² It is estimated that, by 2025, more than 1 million people will be affected by primary liver cancer annually,³ posing a severe health challenge and societal burden. The most frequent types of primary liver cancer are hepatocellular carcinoma (HCC), accounting for up to 90% of all cases, and cholangiocarcinoma (CCA), accounting for 10-15%.⁴,⁵ The complex heterogeneity of these malignancies makes their early diagnosis and the development of therapies difficult. The common risk factors for liver cancer development are chronic HBV⁴ and HCV infections (whose frequency has decreased considerably due to successful vaccination programmes and antiviral drugs),⁵ alcohol abuse,⁶ and metabolic diseases including non-alcoholic fatty liver disease (NAFLD),⁷ ranging from simple steatosis to non-alcoholic steatohepatitis (NASH),⁸ obesity,¹ and diabetes mellitus.¹ Additional risk factors include aflatoxin exposure in HCC,¹ and inflammation of the biliary tract in CCA,⁹ with underlying causes including primary sclerosing cholangitis (PSC), cholestasis, bile stones and liver fluke infestation.

Metabolic alterations are a well-established hallmark of cancer.¹⁰ The liver is the central organ for metabolism in the body,¹¹–¹⁵; thus, metabolic processes are often highly altered in liver cancer (reviewed in¹⁶). Distinct metabolic alterations have been uncovered in glucose, nucleotide, amino acid, and lipid metabolism in liver cancer.¹⁶ Dysregulation of lipids plays important roles in both the development¹⁷ and the progression¹⁸ of liver cancer, which is a consequence of lipids being a vast and multifarious group of complex structured biomolecules. Lipids are involved in diverse biological processes in the body from energy storage and metabolism,²⁰ to epigenetic regulation,²¹ signal transduction,²² immunoregulation,²³ inflammation,²⁴ and cell-cell recognition.²⁵

The study of the lipidome and its dynamic nature used to pose a significant technical challenge. However, advances in mass spectrometry and chromatography techniques in the past decade have provided deeper insights into the metabolic heterogeneity and biological function(s) of the lipidome in both normal homeostasis and disease.²⁶ In this review, we will highlight the major lipidomic rearrangements that occur in the development and progression of liver cancer, focusing on lipid structural function and roles in energy storage and signal transduction.

The origin and role(s) of hepatic lipids
Fatty acids (FAs), including carboxylic acids with a chain from 2 to 36 carbon atoms,²⁶ and cholesterol, consisting of 4 linked hydrocarbon rings,²⁷ are the fundamental building blocks of all lipids. The hepatic FA pool is mainly dependent on the FA uptake from dietary sources²⁸ (in the fasting state) or adipose tissue lipolysis²⁹,³⁰ (in the fed state) (Fig. 1). However, 15-25% of all FAs originate from a process termed de novo synthesis.

Keywords: Non-alcoholic fatty liver disease; hepatocellular carcinoma; cholangiocarcinoma; metabolomics; lipidomics

Received 15 October 2021; received in revised form 1 March 2022; accepted 7 March 2022; available online 26 March 2022

¹Biotech Research & Innovation Center (BRIC), Department of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
²Contributed equally

* Corresponding author.
Address: Ole Maaløes Vej 5, Copenhagen N, DK-2200 Denmark. Tel.: +45 35325834, fax: +45 72620285.
E-mail address: jesper.andersen@bric.ku.dk (J.B. Andersen).
lipogenesis (DNL). This process allows for FA synthesis up to the Δ9 position, while other FAs need to be taken up from dietary sources. Contrary to FAs, the majority (80%) of cholesterol is synthesised internally, and almost 50% of cholesterol synthesis is controlled by the liver. With a body mass of 70 kg, a human contains around 100 grams of cholesterol with a synthesis rate of 1.2 grams per day. Whereas cholesterol can be sufficiently synthesised, the dietary intake can range from 300-500 mg per day. FA and cholesterol are the backbone of a very diverse group of biomolecules that can be classified based on their structure, chemical properties (such as hydrophobicity or hydrophilicity), and biological function(s) (Fig. 1).

Energy storage
The human body stores energy as fat and carbohydrates. The neutral storage of FAs in the healthy liver is in the form of triglycerides (TGs), which are 3 FAs attached to a glycerol moiety, and sterol esters (SEs), in which FA is esterified to sterol. Neutral lipids (SEs and TGs) are stored in lipid droplets, and in a healthy liver, these lipids should not exceed 5%. FAs stored in TGs and SEs can be utilised at any time during liver homeostasis to generate energy (ATP) via fatty acid oxidation (FAO) or be transported to other organs in very-low-density lipoprotein.

Structural lipids
Glycerophospholipids, sphingolipids, and cholesterol are major building blocks of the cellular membrane (Fig. 1). Glycerophospholipids include phosphatidylcholine (PC), phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, and phosphatidic acid. PC accounts for more than 50% of the phospholipids in most eukaryotic membranes. The second most abundant lipid in the mammalian membrane is cholesterol, which accounts for 30% of lipids, and increases the lipid-packing density to maintain a high membrane fluidity. Lastly,
Sphingomyelin (SM) is the most abundant sphingolipid in mammalian cells and this lipid plays a crucial role in the formation of sterol-enriched ordered membrane domains and in cell-cell signalling.

**Signalling molecules**

Lipids act as first (extracellular) and second (intracellular) messengers in signal transduction and molecular recognition processes (reviewed in 19,41). As such, membrane glycerolipids and spheroglycolipids transduce signals through hydrolysis to generate bioactive molecules: ceramides and sphingosine-1-phosphate (S1P), while steroids (oxysterols, bile acids [BAs], steroid hormones) and FAs interact directly with receptors, such as CD36 (FA translocase) (Fig. 1).

**Lipid alterations in liver diseases associated with the development of liver cancer**

Prominent steatosis is caused by FA uptake and DNL exceeding FAO and secretion, and is a shared feature underlying several risk factors of liver cancer. Accordingly, increased intrahepatic lipid accumulation is observed in viral hepatitis, alcoholic hepatitis, and among individuals suffering from metabolic diseases (obesity, diabetes, and NAFLD), all of which pose a risk for liver cancer development. Conversely, steatosis is rarely observed alongside PSC or primary biliary cholangitis, which are conditions associated with BA deregulation.

**Viral hepatitis**

HBV and HCV infections are important risk factors for liver cancer development. HBV is the main aetiology for HCC in most regions of Asia, Africa, and South America. HCV is the predominant cause in Western Europe, North America, and Japan. Hepatic steatosis is often associated with both HBV and HCV infections, as well as being observed in HBx (HBx protein is crucial in HBV tumorigenesis) transgenic and HCV transgenic mouse models. Hence, viral hepatitis leads to prominent changes in both the serum and hepatic (tumour and tumour-adjacent compartments) lipids (Table 1). Indeed, the blood FA composition is significantly altered in HBV- and HCV-infected patients. As such, serum levels of saturated FAs (SFAs) and monounsaturated FAs (MUfAs) are significantly increased in HBV-positive patients, and increase in parallel with disease severity. Concurrently, the class of polyunsaturated FA (PUfAs) is depleted. This change in FA composition is also observed in mouse HBV-positive liver tumours. Moreover, PUfAs are necessary for HCV particle replication as knockdown of fatty acid desaturase 2 (FADS2) - the first step in PUFA synthesis - impairs HCV virus particle production. Similarly, FAs are involved in stabilisation of the HBx protein. HBx can also increase the cholesterol levels in HCC cells, both in vitro and in vivo. Conversely, in both patients and HCV transgenic mice, cholesterol and lysophosphatidylcholine (LPC) of longer FA chains have been shown to be significantly depleted.

**Alcohol-related liver disease**

Excessive alcohol consumption is the main aetiological factor for liver cancer development in Central and Eastern Europe. In patients with alcohol-related liver disease (ALD), FAs that accumulate in the liver are predominantly released from the adipose tissue. Ethanol increases the uptake of FAs by the liver in vivo and in vitro leading to intrahepatic accumulation of TGs. Abstinence has been shown to reduce serum FA and LPC levels, while TGs stay elevated in patients with ALD (Table 1). Moreover, in several models, FA synthesis pathways are significantly upregulated in mice fed alcohol ad libitum in their drinking water.

**Non-alcoholic fatty liver disease**

NAFLD, ranging from steatosis to its progressive form NASH, is the most common liver disease in the developed world, and is an important risk factor for liver cancer development. NAFLD is associated with prominent changes in both the hepatic and serum lipidosomes at the onset of steatosis, but as the disease progresses to NASH, only certain TGs and steroids change progressively. However, NAFLD-HCC is reflected by a complete rearrangement of the serum lipids. Patients with NAFLD have significantly increased levels of FAs, TGs, ceramides

---

**Table 1. Deregulation of blood (and tissue) lipids as risk factors for liver cancer, HCC and CCA.**

| Lipid     | Serum/plasma | Tissue       |
|-----------|--------------|--------------|
|           | Viral hepatitis | Alcoholic hepatitis | NAFLD | PSC | CCA | HCC | HCC T vs. SL |
| SFA       | 66,68         | 66,68         | 66,68,2,01,02 | – | – | 90,198,122 | 118         |
| MUFA      | 66,68         | 82           | 66,68,2,01,02 | – | – | 90,198,122 | 118         |
| PUFA      | 66,68         | 82           | 66,68,2,01,02 | – | – | 90,198,122 | 118         |
| TG         | 66,68         | 82           | 66,68,2,01,02 | – | – | 90,198,122 | 118         |
| Cholesterol| 86,67         | 86,67         | 86,67,2,01,02 | – | – | 82,122,144,140,449 | 196         |
| BA         | 66,68         | 82           | 66,68,2,01,02 | – | – | 90,198,122 | 118         |
| Cholesterol ester | 76,76 | 82         | 100,103 | – | – | 135 | –         |
| LPC        | 66,68         | 82           | 100,103 | – | – | 135 | –         |
| PC         | 66,68         | 82           | 100,103 | – | – | 135 | –         |
| Ceramide   | 66,68         | 82           | 100,103 | – | – | 135 | –         |
| S1P        | 66,68         | 82           | 100,103 | – | – | 135 | –         |

Data for PSC and CCA are based on single reference (Banales et al.17)† Upregulated metabolites; † downregulated metabolites.

BA, bile acids; CCA, cholangiocarcinoma; HCC, hepatocellular carcinoma; LPC, lysophosphatidylcholine; MUFA, monounsaturated fatty acid; NAFLD, non-alcoholic fatty liver disease; PC, phosphatidylcholine; PSC, primary sclerosing cholangitis; PUFA, polyunsaturated fatty acid; S1P, sphingosine-1-phosphate; SFA, saturated fatty acid; SL, surrounding liver; SM, sphingomyelin; T, tumour; TG, triglyceride.
Deregulation of lipid metabolism in liver cancer

Deregulated lipid metabolism has been strongly associated with the onset and progression of HCC in several epidemiological studies, as well as in vitro and in vivo modelling (Table 1). In comparison, CCA lipidomic studies are currently limited to biomarker discovery; thus, a comprehensive investigation of the biliary tract and CCA lipidome landscapes are still lacking.

Lipidomic landscape is deregulated in liver cancer

Several lipidomic studies have investigated the blood lipidome to understand the progressive nature of CCA. Several FAs and PUFA levels are increased during disease progression: chronic hepatitis -> cirrhosis -> HCC (this is not seen in CCA). Several SFAs and MUFAs are increased during disease progression; chronic hepatitis -> cirrhosis -> HCC. Particularly, the MUFAs (16:1) and (18:1) progressively increase during development of viral-associated HCC. However, these observations have not been corroborated in NAFLD-HCC.

Conversely, serum levels of PUFA levels are decreased in the blood of patients with HCC.

Sphingolipids are an important lipid class that is upregulated in HCC, especially LPCs, which have been shown to promote cell proliferation, migration, invasion, and epithelial-to-mesenchymal transition (EMT) in HCC, as well as lymph node metastasis in CCA. Sphingosine-1-phosphate receptor (S1PR) could be a potential therapeutic target in HCC, as it is known to promote HCC invasion and progression (Fig. 2). Similarly, Cer as a biomarker in the serum of patients with HCC, but the function of specific Cer remains unknown and contradictory. Furthermore, an increase of SM (40:1) in mice and SM (18:2/24:1) in patients with HCC, as well as the utility of SM as a biomarker in distinguishing HCC and CCA suggest that sphingolipid metabolism may present a therapeutic target in liver cancer.

Phospholipids are also significantly implicated in hepatocarcinogenesis. MUFAs-PCs accumulate in HCC tumours, while PUFA-PCs and SFA-PCs are depleted. Moreover, MUFAs-PCs are associated with a switch in the proliferative capacity of hepatocytes and with the onset of HCC. Additionally, LPCs, a highly anti-inflammatory class of molecules, are progressively decreased during chronic hepatitis and HCC onset. LPCs are highly upregulated in PSC, but significantly depleted in CCA, which follows the opposite trajectory compared to HCC. We can only speculate that LPCs are increased in PSC as a response to bile duct inflammation.

Several studies have demonstrated that a high-fat, high-cholesterol diet can trigger HCC in mice. Phospholipids are conserved liver function and decreased mortality. A recent population study showed that low serum cholesterol in patients (not using statins) was significantly associated with an increased risk of developing HCC. Interestingly, in patients with HCV-associated HCC, serum cholesterol levels and genes involved in

---

(Cer), and BAs, while phospholipids in the blood are depleted. In NAFLD, the most important metabolic dysregulation is a result of high lipolysis and non-esterified FAs released into the bloodstream. Hepatic FA profiles in patients with NAFLD are severely deregulated. Specifically, SFAs and PUFAs are significantly increased in NAFLD compared to normal livers. Conversely, in murine models, a higher consumption of n-6 FAs leads to the onset of NASH by inducing mitochondrial dysfunction and altered apoptosis. Furthermore, palmitic acid and linoleic acid (LA) have been found to modulate the immune response in murine models of NASH. Palmitic acid and LA stimulate neutrophils as well as macrophages to express and secrete inflammatory proteins (for example, interleukin-6, interleukin-10, chemokine (C-C motif) ligand 2, interferon-c-mitoyltransferase (CPT) leading to increased apoptosis of CD4+ T cells. This LA-mediated loss of intrahepatic CD4+ T cells, but not CD8+ T lymphocytes, results in HCC progression. On a background of NASH, CD8+ T cells promote the incidence of murine HCC because of impaired tumour surveillance and increased tissue damage by lymphocytes.

NAFLD is characterised by a significant increase of TGs in the circulation and liver. These TGs with longer carbon chains and fewer double bonds are preferential substrates in the liver and blood. These are TGs with longer carbon chains and fewer double bonds that are basic markers of de novo Cer synthesis. As such, murine models have shown a decrease in hepatic steatosis when levels of liver Cer are lowered by an increase in acid ceramidase activity or deletion of dihydroceramides that are not basic markers of de novo Cer synthesis. Moreover, several studies have shown that BA levels are increased in the liver, plasma, and faeces of patients with NASH. Elevated plasma levels of glycocholate, taurocholate, and taurochenodeoxycholate are associated with progressive liver deterioration and dysfunction. Furthermore, increased levels of cholic, chenodeoxycholic, and deoxycholic acids are present in liver tissue, leading to altered expression and activity of genes involved in BA, lipid, and carbohydrate metabolism, energy expenditure, and inflammation. Meanwhile, PCs and LPCs (particularly classes that contain PUFAs) are depleted in livers and blood obtained from patients with NAFLD and NASH. Interestingly, sphingolipids, phospholipids, and TGs are putative biomarkers of NAFLD progression. Furthermore, cholesterol promotes NAFLD development.

Primary sclerosing cholangitis

Previously, studies with relatively limited sample sizes (n <30) have investigated lipidomic changes in patients with PSC compared to healthy individuals. Several lipidomic studies have investigated the blood lipidome to understand the progressive nature of CCA. Several FAs and PUFA levels are increased during disease progression: chronic hepatitis -> cirrhosis -> HCC. Sphingosine-1-phosphate receptor (S1PR) could be a potential therapeutic target in HCC, as it is known to promote HCC invasion and progression. Similarly, Cer as a class accumulate in the serum of patients with HCC, but the function of specific Cer remains unknown and contradictory. Furthermore, an increase of SM (40:1) in mice and SM (18:2/24:1) in patients with HCC, as well as the utility of SM as a biomarker in distinguishing HCC and CCA suggest that sphingolipid metabolism may present a therapeutic target in liver cancer.

Phospholipids are also significantly implicated in hepatocarcinogenesis. MUFAs-PCs accumulate in HCC tumours, while PUFA-PCs and SFA-PCs are depleted. Moreover, MUFAs-PCs are associated with a switch in the proliferative capacity of hepatocytes and with the onset of HCC. Additionally, LPCs, a highly anti-inflammatory class of molecules, are progressively decreased during chronic hepatitis and HCC onset. LPCs are highly upregulated in PSC, but significantly depleted in CCA, which follows the opposite trajectory compared to HCC. We can only speculate that LPCs are increased in PSC as a response to bile duct inflammation.

Several studies have demonstrated that a high-fat, high-cholesterol diet can trigger HCC in mice. Phospholipids are conserved liver function and decreased mortality. A recent population study showed that low serum cholesterol in patients (not using statins) was significantly associated with an increased risk of developing HCC. Interestingly, in patients with HCV-associated HCC, serum cholesterol levels and genes involved in...
the cholesterol synthesis pathway are significantly reduced. High serum levels of cholesterol suppress HCC tumorigenesis through the activation of natural killer cells; however, further studies are necessary to understand this disagreement. This may not be the situation in CCA, where high levels of cholesterol have been observed in the sera of patients.

The nature and regulation of BAs is less controversial than that of cholesterol itself. Conjugated primary BAs are significantly elevated at the stage of cirrhosis and continue to increase with the progression of HCC. The role of BAs in the development of HCC is well-established and was extensively reviewed elsewhere. Fundamentally, BAs activate farnesoid X receptor (FXR) and G-protein coupled BA receptor 1 (GPBAR1) that both control numerous oncogenic processes, including inflammation, oxidative stress, and the regulation of many cancer-related genes. BAs act through FXR in different cell

**Fig. 2. The interplay between lipid metabolism and oncogene pathways that leads to tumorigenesis in the liver.** Various lipids influence and are influenced by the recurrently deregulated oncogenic pathways, p53, RAS/MAPK, PI3K/AKT/mTOR signalling axis, Wnt/β-catenin signalling axis, TGF-β signalling axis and myc and TAZ/YAP pathways to cause hepatocarcinogenesis. The inhibited genes are marked with (×) and activated with (√). The recurrent molecular alterations in oncogenes are marked with (*). CL, cardiolipin; CTGF, connective tissue growth factor; DNL, de novo lipogenesis; FA, fatty acid; GlcCer, glucosylceramide; mTOR, mammalian target of rapamycin; NPC1, NPC intracellular cholesterol transporter 1; PI3K, phosphoinositide 3-kinase; PDK1, phosphoinositide-dependent protein kinase 1; S1P, sphingosine-1-phosphate; SL, sphingolipid; TG, triglycerides; TGF-β, transforming growth factor-β; YAP, Yes-associated protein.
types and organs, including the gut, hepatocytes, hepatic stellate cells (HSCs) and immune cells. Conversely, a step-wise increase in plasma-conjugated BAs has been observed in the trajectory from healthy control to benign biliary disease, and further to CCA. Indeed, conjugated BAs promote the growth of CCA through control and activation of the NF-κB pathway and decreased expression of FXR.

Lastly, steroid hormones play an important role in hepatocarcinogenesis. Oestrogen shows a significantly protective effect against HCC, while elevated serum oestrogen and high expression levels of oestrogen-related proteins are associated with CCA and poor clinical outcomes. Thus, tamoxifen may potentially aid CCA therapy. As such, the use of oral contraception is associated with an increased risk of CCA, but not HCC development.

**Lipids reshape oncogenic processes**

While many studies have investigated how alterations of lipids alter transcriptomic processes, there are still gaps in our knowledge of how specific lipids alter the metabolic landscape in liver diseases. Herein, we will highlight how specific lipids, acting as signalling molecules, may promote tumorigenesis.

### Table 2. Ability of various lipids to promote or inhibit tumour growth in HCC and CCA.

| Lipid | Impact | Mechanism | Ref |
|-------|--------|-----------|-----|
| SFA (16:0) | Promote HCC | LPCs are metabolized by phospholipase D to produce LPAs that are potent mitogens, mediating their tumorigenic effect by the PI3K/AKT/mTOR signalling pathway | 175–177,184 |
| LPC (20:4) | Inhibit HCC | Low LPC indicate inflammatory and oxidative stress, through apoptosis induction through the death ligands (Fas and/or TNF-α) pathway | 135,178 |
| MUFA-PC | Promote HCC | Increased lipogenesis, fatty acyl desaturation, de novo synthesis of PC, and PC remodeling and decreased β-oxidation | 134 |
| S1P | Promote HCC and CCA | S1P activates YAP, PI3K/AKT and TGF-β1 production in HCC cells | 125–128 |
| Glucosylceramide | Promote HCC | mTORC2 promotes glucosylceramide accumulation which increases tumorigenesis | 124 |
| (SM) 18:2/24:1 | Promote HCC | Enrichment might be due to a specific diet; more studies are needed | 123,125 |
| C16 | Promote HCC | Long-chain ceramides may have proliferative effects for HCC, RAS activation, regulatory ligand of p53 | 123,133,172 |
| C12:0, 16:0, 18:1 and 24:1 ceramides | Promote HCC | Associated with cannabinoid receptor activation and SCD downregulation | 132 |
| Cholesterol ester | Promote NALFD-HCC | PTE/P3AKT/mTOR signalling pathway is responsible for cholesterol ester accumulation which then leads to tumorigenesis | 249 |
| Cholesterol | Promote HCC | Increased expression of TAZ to promote fibrotic NASH | 114,140 |

CCA, cholangiocarcinoma; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HCC, hepatocellular carcinoma; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; MUFA, monounsaturated fatty acid; NASH, non-alcoholic steatohepatitis; PC, phosphatidylcholine; PUFA, polyunsaturated fatty acid; S1P, sphingosine-1-phosphate; SM, sphingomyelin.
transition and proliferation through the miR21-HMG-box transcription factor 1 (HBP1)-p53 axis. In ALD, hepatic FAs suppress the LAMP-2 (lysosome-associated membrane protein 2) autophagy flux pathway through ER stress signalling and increase hepatic injury (Table 2). Furthermore, palmitic acid treatment leads to transcriptional upregulation of Wnt and transforming growth factor-β (TGF-β) signalling, activating EMT. In CCA, it has been demonstrated in vitro that n-3 PUFAs may inhibit c-myc expression.

Among sphingolipids, Cer16 has been shown to be an important activator of the p53 pathway. Cer16 binds the DNA-binding domain of p53 and disrupts its complex with MDM2 (mouse double minute 2), leading to p53 accumulation and transcriptional activation, contributing to the apoptotic process and hepatic liver injury. Indeed, Cer16 is significantly upregulated in serum and tumour tissues obtained from patients with HCC. Moreover, Cer activates KSR1 (kinase suppressor of Ras 1), acting as a positive regulator of the RAS-RAF-MAPK pathway, which is frequently deregulated in liver cancers (Table 2).

Phospholipids can act as signal transducers as well as directly bind to G-protein-coupled receptors, with both oncogenic and tumour suppressive roles. Lysophosphatidic acid (16:0) is a potent regulator of the PI3K/Akt, mTOR (mammalian target of rapamycin), and p38 MAPK signalling pathways, increasing HCC cell migration, invasion, and adhesion. Conversely, LPC (20:4) was shown to induce Fas and tumour necrosis factor-α pathways, resulting in apoptosis and thus playing a tumour suppressive role (Table 2).

Sterol lipids, particularly cholesterol, may play important roles in liver cancer. Increased cholesterol in hepatocytes upregulates the transcriptional regulator TAZ (WWTR1), and thus promotes NASH. Elevated TAZ activity leads to the synthesis and secretion of IHH (Indian hedgehog) and activation of HSCs. Currently, no association has been shown between TAZ and CCA (Table 2).

Collectively, these studies suggest that prominent lipidomic rearrangements occur before carcinogenesis and continue to play a role during liver cancer progression. Several types of lipids have been shown to interact with oncogenes, altering the signalling activity of many pathways, and likely contributing to tumour formation through these interactions. This emphasises possible opportunities for future preventative treatments.

Molecular alterations in liver cancer promote lipid remodelling

Metabolic stress contributes to increased reactive oxygen species levels that may result in mutational processes, with the accumulation of somatic mutations in chronic liver disease eventually leading to cancer. A recent study in patients with ALD and NAFLD showed that 3 master regulators of lipid processing and storage – FOXO1 (forkhead box protein O1), CIDEB (cell death inducing DFFA like effector B) and GPAM (glycerol-3-phosphate acyltransferase, mitochondrial) – are frequent targets of convergent somatic mutations. Recurrent mutations in RAS, TP53, MYC, and CTNNB1 have also been shown to alter the lipidome of liver tumours (Fig. 2).

Mutations in the RAS-RAF pathway are frequent in HCC and CCA, and lead to transcriptional activation of fatty acid synthase (FASN), which promotes lipogenesis. Furthermore, increased Wnt and Myc activities intensify FA desaturation and elevate unsaturated fatty acyl groups in phospholipids in a RAS-dependent manner. Through this metabolic reprogramming, stearoyl-CoA desaturase (SCD) was identified as a putative therapeutic target.

Mutated TP53 is a dominant driver-gene in many cancers, including HCC and CCA, regulating cellular metabolism (reviewed in 183). On the one hand, wild-type p53 is a potent repressor of sterol regulatory element-binding proteins (SREBP-1/2) that regulate DNL and cholesterol synthesis, respectively. On the other hand, wild-type p53 promotes FAO through expression of lipin 1, sirtuin 1, and CPT, maintaining lipid homeostasis in the liver. The role of the Wnt pathway in reprogramming cancer metabolism has been extensively studied and reviewed elsewhere. Many of the recurrent mutations in this pathway are not druggable (though it has received significant attention in the area of small molecule development). However, exploiting the lipidomic changes inflicted by the mutations in Wnt could present an indirect therapeutic strategy. Overall, there is a convincing rationale to target the Wnt/β-catenin pathway in liver cancers; however, the effect on lipid metabolism has received less attention. The activation of the Wnt pathway leads to the release of β-catenin, its translocation to the nucleus and consequently to increased expression of peroxisome proliferator-activated receptor-α (PPARα) resulting in increased FAO in this subset of HCC. Moreover, inhibition of Wnt/β-catenin may lead to downregulation of DNL and FA desaturation, which is frequently upregulated in liver cancer. Thus, further studies to investigate the effects of lipid-targeted therapies are warranted.

Transcriptionally deregulated lipid metabolism pathways

PPARs, SREBPs, and liver X receptors (LXRs) are key hepatic transcriptional regulators of enzymes involved in lipid metabolism (reviewed in 183). As cofactors, lipids can bind directly to transcription factors, modulating the expression of lipid metabolism in a feedback loop. As such, PUFAs and 4-phenyl butyric acid have been shown to directly bind to PPARs, which can contribute to the development of HCC. It has been implied that the PPARα-SCD1 axis is important to maintain the stemness of HCC cells by promoting the nuclear accumulation of β-catenin. Furthermore, LXRs are activated by oxysterols and their activation can trigger lipotoxicity in liver cancer, while their inactivation leads to NAFLD-HCC development. As such, the transcriptomic landscape of lipid metabolism is significantly deregulated in liver cancer (Fig. 3).
De novo lipogenesis and triglyceride synthesis

DNL is significantly upregulated in the proliferative class of HCC and it is predictive of patient prognosis. Therefore, rate-limiting genes of DNL, such as ATP citrate lyase (ACLY), acetyl-CoA carboxylase (ACC), and FASN, are frequently upregulated in HCCs compared to the normal adjacent liver tissue. Interestingly, in CCA, tumour cells show lower dependency on DNL and instead a higher addiction to exogenous FAs. Thus, DNL as a process, and rate-limiting genes in particular, may present an attractive therapeutic option for obesity, NAFLD and HCC, but not CCA. Furthermore, it has been suggested that, in HCC, DNL is glucose-derived and thus, restricting HCC cells access to glucose results in diminished DNL activity. However, a recent study implicated fructose and sucrose, rather than glucose, as substrates in DNL in the healthy liver. Thus, in HCC, the substrates driving DNL require comprehensive studies. Indeed, several HCC studies have focused on DNL inhibition as a therapeutic option, but such work remains in its infancy.

ACLY is the first step in DNL and has been shown to promote HCC transcriptionally by interacting with NONO (non-POU domain-containing octamer binding protein). Overall, ACLY was shown to regulate stemness, migration, and invasion of HCC cells via the Wnt/β-catenin signalling pathway. In CCA, the expression of ACLY is higher in tumour tissues compared to the surrounding tissue, however, its role remains unknown.

Parts of acetyl-CoA are carboxylated to malonyl-CoA by ACC, which is the primary rate-limiting enzyme in this process. Either inhibition of ACC itself or deletion of AMPK-targeted ACC phosphorylation sites lead to a significant decreased tumour burden and attenuated DNL in diethylnitrosamine-induced HCC. Also, in vitro, these modifications result in decreased proliferation and
viability of HCC cells.17 Accordingly, several inhibitors designed to block ACC activity and reduce lipogenesis have led to significant reductions in the accumulation of hepatic TG and activation of HSCs.210,220 Still, liver-specific ACC knockout in mice with diethylamino-hexosamine-induced HCC led to a significant increase in the tumour burden and altered redox regulation.206 Additionally, complete deletion of ACC1 and ACC2 abrogates acetygenic lipogenesis but fails to protect murine livers from increased lipid accumulation, likely due to inhibition of FASO as the compensatory mechanism.221 Taken together, these data suggest that ACC inhibition, but not deletion, could be a beneficial treatment option for patients with HCC.

The next step in DNL is palmitate synthesis, which is catalysed from malonyl-CoA by FASN. The role of FASN in HCC is dependent on model. First, overexpression of FASN alone or in combination with either N-Ras, c-Met, or SCD1 is not sufficient to promote HCC.222 However, FASN expression is essential in the development of HCC in both the AKT222 and AKT/Ras203 models and FASN inhibition delays Pten/c-Met-driven HCC.216 As such, stabilisation of FASN by glyceronephosphate O-acyltransferase promotes DNL and formation of liver tumours in mice.212 Interestingly, FASN expression is dispensable for CCA formation in AKT/Notch intracellular domain 1- and AKT/Ras-driven models,203 and KDM5C-mediated repression of FASN was shown to correlate with reduced CCA cell proliferation and invasion.223

Finally, palmitate, which is the end-product of DNL, can undergo a series of desaturation and elongation reactions that are catalysed by SCD, FADS2, and elongation of very-long-chain fatty acids (ELOVL1-6), respectively. In HCC, upregulation of SCD has been comprehensively described in mice, rats, tumour cells, and patients.194,195,224,225 SCD has been shown to be crucial for proliferation of HCC cells,196,224 for development of HCC in mice,198,225 and is present at higher levels in more aggressive HCCs in patients.198 Additionally, a subset of HCC cell lines is metabolically flexible since they upregulate FADS2 and utilise this as an alternative desaturation pathway when SCD is inhibited,226 suggesting that targeting both desaturation pathways would be necessary to impair HCC growth. Furthermore, suppression of ELOVL6 in HCC cells led to reduced proliferation, tighter cell-cell junctions, and increased lipid accumulation,227 as well as reduced HCC tumour growth in vivo and increased survival.226

The role of TG synthesis in liver cancer development and progression remains elusive. It is implied that TG synthesis is downregulated in HCC.228 The formation of TG from acetyl-CoA and diacylglycerol is catalysed by evolutionarily unrelated enzymes (diglyceride acyltransferase [DGAT1] and DGAT2) that are downregulated in HCC compared with matched normal tissues.228 Higher expression of DGAT2 results in longer overall survival.228 These data were corroborated in vitro and in vivo, demonstrating that overexpression of DGAT2 curbs cell proliferation and diminishes tumour growth.228 While comprehensive studies linking TG synthesis to HCC development and progression are lacking, overexpression of DGAT1 and DGAT2 in hepatocytes229,230 leads to steatosis and lipid accumulation,231 which is one of the key long-term causes of hepatocarcinogenesis. However, DGAT1 is important for the maintenance of HCC in vitro, silencing it reverts HCC cells to a dedifferentiated and stem cell-like phenotype.232 Interestingly, in vitro, if DGAT1 is silenced in HCC cells, the cells compensate by upregulating DGAT2.232 Hence, inhibition of DGAT2 in vivo234 ameliorated liver steatosis. Another less studied enzyme in the TG synthesis pathway is monoacylglycerol O-acyltransferase, which catalyses the synthesis of TGs, may contribute to hepatic steatosis in vivo235,236 and could be an important therapeutic target for the treatment of NAFLD.237

Fatty acid oxidation
CPT1 and CPT2 deliver long-chain FAs to the mitochondria for oxidation and thereby generate ATP and NADPH.238 In both HCC and CCA, the expression levels of CPT1 and CPT2 are downregulated (TCGA,239,240 www.firebrowse.org). In fact, downregulation of CPT2 was shown to protect against lipotoxicity241 in an E2F2-dependent manner in HCC.242 Furthermore, downregulation of acylcarinate translocase (SLC25A20) in the mitochondrial matrix is observed in both HCC and CCA (TCGA, www.firebrowse.org) and was shown to suppress FAO and promote HCC proliferation as well as metastasis.243 Furthermore, enzymes in the FAO process, such as medium-chain acyl-CoA dehydrogenase244 and long-chain acyl-CoA dehydrogenase,245 have potential tumour suppressor roles in HCC.244,245 Therefore, FA utilisation for structural and messenger molecules, rather than storage or energy sources, supports HCC development and progression.

Cholesterol and BA synthesis
In addition to FA synthesis, other lipogenesis pathways have also been demonstrated to be deregulated during HCC development. The rate-limiting enzyme of cholesterol synthesis (and target of statins) 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR) is upregulated in HCC246 and CCA247 and thus, the use of statins is associated with reduced risk of liver cancer development.142,248 Furthermore, increased expression of squalene epoxidase249 has been implicated in the development of NAFLD-HCC. Interestingly, a decreased ability to utilise circulating cholesterol was associated with increased HCC proliferation and metastasis.250 Furthermore, cholesterol synthesis was shown to support HCC growth in the absence of FASN,246 which indicates crosstalk between DNL and cholesterol synthesis. Similarly, increased cholesterol and BA synthesis, through PPARα activation, cause cholestasis, liver damage, and finally CCA development.251

Diagnostic potential of circulating lipids
Most lipids detected in human serum or plasma remain stable and are well correlated with the liver lipidome.86 Therefore, the lipidome in circulation is an attractive source of biomarkers.16,107 Several studies exploited serum FA as a diagnostic tool.88,252 Recently, it has been shown that a combination of several FAs and PC (18:2) can robustly distinguish patients with NAFLD-HCC from those with HCC of other aetiologies and non-cancerous controls,88 providing a potential tool for non-invasive surveillance.

The increased serum/plasma levels of TGs are a well-known biomarker of liver dysfunction in cholestasis, ALD, NAFLD, and HBV,87,88,103,253-255 but not HCV-associated hepatitis.256 Similarly, increased TG levels have been identified as risk factors for CCA,147,257 Furthermore, diminished levels of circulating TG in patients with HCC are associated with worse overall survival.258 However, due to the unspecific nature of these changes, they remain a generic biomarker of liver dysfunction. On the other hand, the changes in specific TG species may be more useful. The major differences observed with the development of NAFLD and its progression to NASH are increasing serum levels of saturated and monounsaturated TGs,87,103 many of which progressively
increase with the onset of HCC, particularly in the absence of cirrhosis. Conversely, the depletion of cholesterol esters, another energy storage class of lipids, has diagnostic potential in HCC.

Differences in the abundance of structural lipids have also been exploited, with specific sphingolipids having biomarker potential. As an example, following increased levels of C16-ceramides, S1P has been shown to distinguish patients with HCC from those with cirrhosis. The depletion of serum SMs in HCC could distinguish patients with HCC from healthy controls, while SMs were significantly altered between HCC and CCA and could thus distinguish between these malignancies. Furthermore, several LPCs have shown diagnostic value in CCA and HCC.

The increase in lipodomic studies in liver cancer has led to an abundance of novel biomarkers, many of which show significantly higher diagnostic potential than alpha-fetoprotein or carbohydrate antigen 19-9. However, most of these studies lack external validation cohorts. Furthermore, most of these studies lack absolute quantification of metabolites, their reference ranges, and the cut-off values that could be considered as diagnostic. As a result, they have limited clinical value and require further development before clinical use.

**Therapeutic opportunities**

For decades, lipid metabolism has been an attractive target for the development of new therapies that could alleviate the burden of chronic liver diseases and hence reduce the risk of cancer. Furthermore, similar genes orchestrating lipid metabolism in chronic liver diseases have recently been investigated as therapeutic targets in cancer treatment.

**Treatment of underlying diseases to prevent liver cancer**

Several inhibitors of DNL, FAO, or cholesterol synthesis have reached clinical trials and use (Table S1). In DNL, drugs that target ACC have progressed significantly. The ACC inhibitor firsocostat is the most prominent, having shown promising results in mice as well as significantly reducing steatosis in patients with NAFLD in phase II clinical trials. The FASN inhibitor TVB-2640 has also shown promise in humans as has the inhibitor cerulenin in mice. A PPAR inhibitor targeting the intracellular transport of FAs (lobeglitazone) has reached phase VI clinical trials in NAFLD, where it reduced intrahepatic fat content and improved glycemic and lipid profiles in 38 patients with NAFLD and type 2 diabetes. Other PPAR inhibitors such as pioglitazone have had similar promise, but are at the earlier phases of clinical trials. Perhaps the most noteworthy inhibitors in NAFLD and PSC treatment are LXR and FXR agonists such as oltipraz, obeticholic acid, and MET409.

Interestingly, the FXR agonist obeticholic acid has shown promise as a therapeutic target in both NAFLD and PSC. Another noteworthy pathway is the cholesterol biosynthesis pathway; statins, which suppress HMGCR, have been studied in both NAFLD and PSC (Table S1) and successfully lowered LDL cholesterol in NASH. As far as sphingolipid metabolism is concerned, there have been no clinical studies yet, but the SK2 inhibitor K145 and the S1P1R inhibitor fingolimod have ameliorated NAFLD in mouse models.

**Targeting lipid metabolism in liver cancer treatment**

Since lipids partake in liver cancer development and progression through various pathways and there is some progress targeting these in the pre-malignant liver, it is not surprising that there is considerable interest in exploiting lipid metabolism pathways in liver cancer treatment. Many pre-clinical studies have focused on DNL: the ACC inhibitor AICAR showed an anti-cancer growth effect in vitro and ND-654 improved survival of HCC tumour-bearing rats. The FASN inhibitor orlistat has also displayed antitumor activity in vitro and in murine models, along with the inhibitors C75, triclosan and EGCG showing promise in vitro. The SCD inhibitor CAY10566 has also been successful in ameliorating HCC in vitro and in vivo, and the DGAT inhibitor tussilagone reduced TG synthesis in vitro. Interestingly, sorafenib, which is an inhibitor of tyrosine kinases in HCC, targets liver cancer cells by acting on the SCD1 pathway in vitro and in human liver tumours and, in return, SCD inhibition sensitis the tumour to sorafenib treatment.

Targeting FAO by CPT1 inhibition with etomoxir is another pathway that has successfully reduced HCC occurrence in vivo; however these studies are limited to the pre-clinical setting. Furthermore, the SREBP inhibitor betulfin, the FXR agonist INT-767 and simvastatin (a HMGCR and PPAR inhibitor) significantly ameliorate HCC in a pre-clinical setting. Several statins are under investigation in combination therapy for HCC (Table S1). Atoverstatin in phase IV clinical trials for HCC but it is still at the recruiting phase (NCT03024684), while pravastatin in combination with sorafenib failed to improve patient outcomes.

In CCA treatment, targeting sphingolipid metabolism or more specifically the enzyme sphingosine kinase 2 (encoded by the gene SPHK2) with the inhibitor ABC294640 has shown promise in vitro, in vivo, and in a clinical trial setting. In addition, the ASBT inhibitor Bamet-UD has shown significant anticancer effects in a pre-clinical setting, while the role of statins in CCA has not been investigated in a controlled clinical setting. Furthermore, obeticholic acid, which has shown promise in vivo,
in ameliorating NAFLD and PSC, managed to decrease proliferation of CCA cells in vitro.301

**Dietary intervention as preventative and therapeutic strategy**

In addition to drugs, dietary combination strategies may also be helpful.302 n-3 PUFAs have been implicated as a way to reduce hepatic steatosis and inflammation in ALD,109 NAFLD,103,104 and NASH.305 The supplementation of n-3 PUFAs has been shown in a meta-analysis to reduce HCC risk by up to 51%,306 results that have been mimicked in vitro307 and in mice.308 In fact, reducing the ratio (n-6:n-3) FAs in the diet has been shown to impair liver steatosis in vivo.309,310 Therefore, we can speculate that patients with HCC, particularly patients with underlying NAFLD or NASH aetiologies, may benefit from a dietary supplement in combination with their pharmacological therapy.

**Conclusion and future perspectives**

Despite the increasing interest in the investigation of lipidomic rearrangements in liver cancer, targeting lipid metabolism in a therapeutic setting has not been successful. The dynamic nature of the lipidome and lack of mechanistic insights into the role(s) of individual lipids in the development of liver cancer significantly hinder the development of new therapies. Our current knowledge allows us to exploit the human lipidome as a non-invasive diagnostic and prognostic tool; however, dissecting the mechanism of lipid metabolism and homeostasis in liver cancer development and progression is necessary. This review highlights the fact that while we are making great strides to unravel the role of lipids in the development and progression of liver cancer, directed mechanistic studies to understand lipids are necessary (Fig. 4).

**Abbreviations**

ACC, acetyl-CoA carboxylase; ACLY, ATP citrate lyase; ALD, alcohol-related liver disease; BAS, bile acids; CCA, cholangiocarcinoma; Cer, ceramide(s); CPT, carnitine palmitoyltransferase; DNL, de novo lipogenesis; ELOV1-6, elongation of very-long-chain fatty acids; FA, fatty acid; FABP, fatty acid-binding protein; FADS2, fatty acid desaturase 2; FAO, fatty acid oxidation; FASN, fatty acid synthase; FXR, farnesoid X receptor; HCC, hepatocellular carcinoma; HMGR, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; HSCs, hepatic stellate cells; LA, linoleic acid; LPC, lysophosphatidylcholine; LXR, liver X receptor; MUFA, monounsaturated fatty acid; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PC, phosphatidylcholine; PPARG, peroxisome proliferator-activated receptors; PSC, primary sclerosing cholangitis; PUFA, polyunsaturated fatty acid; S1P, sphingosine-1-phosphate; SCD, stearoyl-CoA desaturase; SE, sterol esters; SFA, saturated fatty acid; SM, sphingomyelin; SREBP, sterol regulatory element-binding protein; TERT, telomerase reverse transcriptase; TG, triglycerides; TLR, Toll-like receptor.

**Financial support**

This project has received funding from the European Union’s Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreement No 801481, Danish KfBforskingsfond (FID201481). The laboratory of JBA is supported by competitive funding from the Novo Nordisk Foundation (14040, 0058419), Danish Cancer Society (R167-A10784, R278-A16638), NEYE foundation and the Danish Medical Research Council (1030-00070B).

**Conflict of Interest**

Authors declare no conflicts of interest

Please refer to the accompanying ICMJE disclosure forms for further details.

**Authors’ contributions**

B.P. and M.L. researched data for the article; B.P., M.L, and J.B.A wrote and edited the manuscript before submission.

**Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2022.100479.

**References**

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424.

[2] Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Mariasian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res 2014;74:2913–2921.

[3] Ferlay J, Colombeet M, Soerjomataram I, Mathers C, Parkin DM, Pintos M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer 2019;144:1941–1953.

[4] Akinjemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, et al. Global Burden of Disease Liver Cancer C. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the global burden of disease study 2015. JAMA Oncol 2017;3:1683–1691.

[5] DeOliveira MI, Cunningham SC, Cameron JL, Kamangar F, Winter JM, Lillemoe KD, et al. Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. Ann Surg 2007:245:755–762.

[6] Nakeeb A, Pitt HA, Sohn TA, Coleman J, Abrams RA, Pantadosi S, et al. Cholangiocarcinoma. A spectrum of intrahepatic, perihilar, and distal tumors. Ann Surg 1996;224:463–473, discussion 473–465.

[7] Kanwail F, Kramer J, Asch SM, Chayanupatkul M, Cao Y, El-Serag HB. Risk of hepatocellular cancer in HCV patients treated with direct-acting antiviral agents. Gastroenterology 2017;153:996–1005 e1001.

[8] Estes C, Razavi H, Loomba R, Younossi Z, Sanaj AM. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. Hepatology 2018;67:123–133.

[9] Banales JM, Marin JG, Lamarca A, Rodriguez PM, Khan SA, Roberts LR, et al. Cholangiocarcinoma 2020: the next horizon in mechanisms and management. Nat Rev Gastroenterol Hepatol 2020;17:557–588.

[10] Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. J Gen Physiol 1927;8:519–530.

[11] Magnusson I, Schumann WC, Barsch GE, Chandramouli V, Kumaran K, Wahren J, et al. Noninvasive tracing of Krebs cycle metabolism in liver. J Biol Chem 1991;266:6975–6984.

[12] Diraison F, Large V, Brunengraber H, Beylot M. Non-invasive tracing of liver intermediary metabolism in normal subjects and in moderately hyperglycaemic NIDDM subjects. Evidence against increased gluconeogenesis and hepatic fatty acid oxidation in NIDDM. Diabetologia 1998;41:212–220.

[13] Large V, Brunengraber H, Odeen M, Beylot M. Use of labeling pattern of liver glutamate to calculate rates of citric acid cycle and gluconeogenesis. Am J Physiol 1997;275:E51–E58.

[14] Jones GC, Solomon MA, Cole SM, Sherry AD, Malloy CR. An integrated (1)H and (13)C NMR study of gluconeogenesis and TCA cycle flux in humans. J Am Physiol Endocrinol Metab 2001;281:E848–E856.

[15] Magnusson I, Schumann WC, Barsch GE, Chandramouli V, Kumaran K, Wahren J, et al. Noninvasive tracing of Krebs cycle metabolism in liver. J Biol Chem 1991;266:6975–6984.

[16] Diraison F, Large V, Brunengraber H, Beylot M. Non-invasive tracing of liver intermediary metabolism in normal subjects and in moderately hyperglycaemic NIDDM subjects. Evidence against increased gluconeogenesis and hepatic fatty acid oxidation in NIDDM. Diabetologia 1998;41:212–220.

[17] Large V, Brunengraber H, Odeen M, Beylot M. Use of labeling pattern of liver glutamate to calculate rates of citric acid cycle and gluconeogenesis. Am J Physiol 1997;275:E51–E58.

[18] Jones GC, Solomon MA, Cole SM, Sherry AD, Malloy CR. An integrated (2)H and (13)C NMR study of gluconeogenesis and TCA cycle flux in humans. J Am Physiol Endocrinol Metab 2001;281:E848–E856.

[19] Magnusson I, Schumann WC, Barsch GE, Chandramouli V, Kumaran K, Wahren J, et al. Noninvasive tracing of Krebs cycle metabolism in liver. J Biol Chem 1991;266:6975–6984.
the discovery of diagnostic and prognostic biomarkers. Oncotarget 2018;9:5032–5043.

[19] Querschner J, Rössinger C, Thiele C. Imaging of lipid biosynthesis: how a neutral lipid enters lipid droplets. Traffic 2008;9:338–352.

[20] Nierman KM, Kenny HA, Penicka CV, Ladanyi A, Buell-Guthbrod R, Zillhardt MR, et al. Alipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. Nat Med 2011;17:1498–1503.

[21] Sun D, Zhao T, Long K, Wu M, Zhang Z. Triclosan down-regulates fatty acid synthesis through microRNAs in HepG2 cells. Eur J Pharmacol 2021;977:112651.

[22] Chen SZ, Ling Y, Yu LX, Song YT, Chen XF, Gao QQ, et al. 4-phenylbutyric acid promotes hepatocellular carcinoma via initiating cancer stem cells through activation of PPAR-alpha. Clin Transl Med 2021;11:e379.

[23] Baek AE, Yu YA, He S, Wardell SE, Chang CY, Kwon S, et al. The cholesterol metabolism 27 hydroxysterol facilitates breast cancer metastasis through its actions on immune cells. Nat Commun 2017;8:8624.

[24] Fu H, Tang B, Lang J, Du Y, Cao B, Jin L, et al. High-fat diet promotes macrophage-mediated hepatic inflammation and aggravates diethylnitrosamine-induced hepatocarcinogenesis in mice. Front Nutr 2020;7:58306.

[25] Kojima N, Hakomori S. Synergistic effect of two cell recognition systems: glycosphingolipid-glycosphingolipid interaction and integrin receptor interaction with pericellular matrix glycoprotein. Glyobiology 1991;1:623–630.

[26] Han X, Gross RW. Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics. J Lipid Res 2003;44:1071–1079.

[27] Gensch J, Eijkel K, de Vos M, Shevchenko A, Simon K, et al. Accumulation of raft lipids in T-cell plasma membrane domains engaged in TCR signalling. EMBO J 2009;28:466–476.

[28] Schmelzer K, Fahy E, Subramaniam S, Dennis EA. The lipid maps - an atlas of lipid biosynthesis: alterations in hepatic lipid homeostasis. Gastroenterology 2010;130:1245–1258.

[29] Roche TT, Iqbal A, Grimes LL, Bender S. The role of orphan nuclear receptors in the regulation of cholesterol homeostasis. Annu Rev Cell Dev Biol 2005;21:134–151.

[30] Diraison F, Moulin P, Beylot M. Contribution of hepatic de novo lipogenesis to hepatic triglyceride fatty acids: quantitative study. Biochimie 1976;58:345–367.

[31] Rieckenhoff IG, Holman RT, Burr GO. Polyethenoid fatty acid metabolism. Arch Biochem 1949;34:331–340.

[32] Turley SD, Andersen JM, Dietschy JM. Rates of sterol synthesis and up-regulation of hepatic fatty acid transporter protein 5 in vivo reverses diet-induced non-alcoholic fatty liver disease and improves hyperglycemia. J Biol Chem 2018;293:21186–21192.

[33] Bornstein SR. Top-down lipidomics reveals ether lipid de novo synthesis induced by activated PPAR-alpha. Oncotarget 2014;5:5043–5054.

[34] Roche TT, Iqbal A, Grimes LL, Bender S. The role of orphan nuclear receptors in the regulation of cholesterol homeostasis. Annu Rev Cell Dev Biol 2005;21:134–151.
[65] Haberl EM, Weiss TS, Pesichel G, Weigand K, Kohler N, Pauling JK, et al. Liver lipids of patients with hepatitis B and C and associated hepatocellular carcinoma. J Mol Sci 2021:19:3019.

[66] Arain SQ, Talpur FN, Channa NA, Ali MS, Afridi HL. Serum lipid profile as a marker of liver impairment in hepatitis B Cirrhosis patients. Lipids Health Dis 2017;16:51.

[67] Gao R, Cheng J, Fan C, Shi X, Cao Y, Sun B, et al. Serum metabolomics to identify the liver disease-specific biomarkers for the progression of hepatitis to hepatocellular carcinoma. Sci Rep 2015;5:18175.

[68] You M, Fischer M, Deeg MA, Crabb DW. Ethanol induces fatty acid synthesis pathways by activation of sterol regulatory element-binding protein (SREBP). J Biol Chem 2002;277:29342–29347.

[69] Oei GJ, Meikle PJ, Huynh K, Earnest A, Roberts SK, Kemp W, et al. Hepatic lipidomic remodeling in severe obesity manifests with steatosis and does not evolve with non-alcoholic steatohepatitis. J Hepatol 2021;75:524–535.

[70] Evans R, Crespo J, Martinez-Arranz I, Banales JM, Arias M, Minchol E, et al. Metabolomic-based noninvasive serum test to diagnose nonalcoholic steatohepatitis: results from discovery and validation cohorts. Hepatol Commun 2018;2:807–820.

[71] Lewinska M, Santos-Laso A, Arretxe E, Alonso C, Zhuravleva E, Jimenez-Aguero R, et al. The altered serum lipidome and its diagnostic potential for Non-Alcoholic Fatty Liver (NAFL)-associated hepatocellular carcinoma. EBioMedicine 2021;73:103561.

[72] Caussy C, Ajmera VH, Puri P, Hsu CL, Bassirian S, Mgsyam M, et al. Serum metabolites detect the presence of advanced fibrosis in validation and derivation cohorts of patients with non-alcoholic fatty liver disease. Gut 2019;68:1884–1892.

[73] Lewinska MS-LA, Arretxe E, Alonso C, Zhuravleva E, Jimenez-Aguero R, Eizaggirre E, et al. The altered serum lipidome and its diagnostic potential for Non-Alcoholic Fatty Liver (NAFL)-associated hepatocellular carcinoma. 2021.

[74] Puri P, Daita K, Joyce A, Mirshahi F, Santhekadur PK, Cazanave S, et al. The presence and severity of nonalcoholic steatohepatitis is associated with specific changes in circulating bile acids. Hepatology 2018;67:534–548.

[75] Puri P, Baille RA, Wiest MM, Mirshahi F, Choudhury J, Cheung O, et al. Lipidomic analysis of nonalcoholic fatty liver disease. Hepatology 2007;46:1081–1090.

[76] Bugianesi E, Gastaldelli A, Vanni E, Gambino R, Cassader M, Baldi S, et al. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. Diabetologia 2005;48:634–642.

[77] Schuster S, Johnson CD, Hennebelle M, Holtmann T, Taha AV, Kirpiach IA, et al. Oxidized linoleic acid metabolites induce liver mitochondrial dysfunction, apoptosis, and NLRP3 activation in mice. J Lipid Res 2018;59:1597–1609.

[78] van der Windt DJ, Sud V, Zhang H, Varley PR, Gowari J, Vazdani HO, et al. Neutrophil extracellular traps promote inflammation and development of hepatocellular carcinoma in nonalcoholic steatohepatitis. Hepatology 2018;68:1347–1360.

[79] Brown ZJ, Fu Q, Ma C, Kruhlak M, Zhang H, Luo J, et al. Carnitine palmitoyltransferase gene upregulation by linoleic acid induces CD4(+) T cell apoptosis promoting HCC development. Cell Death Dis 2018;9:620.

[80] Ma C, Keswara AH, Eggert T, Medina-Echeverz J, Klein DE, Jin P, et al. NAFLD causes selective CD4(+) T lymphocyte loss and promotes hepatocarcinogenesis. Nature 2016;531:253–257.

[81] Pflster D, Nunez NG, Pinyol R, Gouveia O, Pinter M, Szylowska M, et al. NASH limits anti-tumour surveillance in immunotherapy-treated HCC. Nature 2021;592:450–456.

[82] Kotronen A, Velagapudi VR, Yetukuri L, Westerbacka J, Bergholm R, Ekroos K, et al. Serum saturated fatty acids containing triacylglycerols are better markers of insulin resistance than total serum triacylglycerol concentrations. Diabetologia 2009;52:684–690.

[83] Oresic M, Hyotylainen T, Kotronen A, Gopalacharyulu P, Nygren H, Arola J, et al. Prediction of non-alcoholic fatty liver disease and liver fat content by serum molecular lipids. Diabetologia 2013;56:2266–2274.

[84] Westerbacka J, Kotronen A, Fielding BA, Wahren J, Hodson L, Perttula T, et al. Splanchnic balance of free fatty acids, endocannabinoids, and lipids in subjects with nonalcoholic fatty liver disease. Gastroenterology 2010;139:1961–1971 e1961.

[85] Kotronen A, Velagapudi VR, Yetukuri L, Westerbacka J, Kiviluoto T, Arola J, et al. Neutrophil extracellular traps promote inflammation and development of non-alcoholic fatty liver disease: sites and mechanisms. Diabetologia 2005;48:634–642.

[86] Ma C, Keswara AH, Eggert T, Medina-Echeverz J, Klein DE, Jin P, et al. NAFLD causes selective CD4(+) T lymphocyte loss and promotes hepatocarcinogenesis. Nature 2016;531:253–257.

[87] Pflster D, Nunez NG, Pinyol R, Gouveia O, Pinter M, Szylowska M, et al. NASH limits anti-tumour surveillance in immunotherapy-treated HCC. Nature 2021;592:450–456.

[88] Kotronen A, Velagapudi VR, Yetukuri L, Westerbacka J, Bergholm R, Ekroos K, et al. Serum saturated fatty acids containing triacylglycerols are better markers of insulin resistance than total serum triacylglycerol concentrations. Diabetologia 2009;52:684–690.

[89] Oresic M, Hyotylainen T, Kotronen A, Gopalacharyulu P, Nygren H, Arola J, et al. Prediction of non-alcoholic fatty liver disease and liver fat content by serum molecular lipids. Diabetologia 2013;56:2266–2274.

[90] Westerbacka J, Kotronen A, Fielding BA, Wahren J, Hodson L, Perttula T, et al. Splanchnic balance of free fatty acids, endocannabinoids, and lipids in subjects with nonalcoholic fatty liver disease. Gastroenterology 2010;139:1961–1971 e1961.

[91] Kotronen A, Velagapudi VR, Yetukuri L, Westerbacka J, Kiviluoto T, Arola J, et al. Neutrophil extracellular traps promote inflammation and development of non-alcoholic fatty liver disease: sites and mechanisms. Diabetologia 2005;48:634–642.

[92] Ma C, Keswara AH, Eggert T, Medina-Echeverz J, Klein DE, Jin P, et al. NAFLD causes selective CD4(+) T lymphocyte loss and promotes hepatocarcinogenesis. Nature 2016;531:253–257.

[93] Pflster D, Nunez NG, Pinyol R, Gouveia O, Pinter M, Szylowska M, et al. NASH limits anti-tumour surveillance in immunotherapy-treated HCC. Nature 2021;592:450–456.

[94] Kotronen A, Velagapudi VR, Yetukuri L, Westerbacka J, Bergholm R, Ekroos K, et al. Serum saturated fatty acids containing triacylglycerols are better markers of insulin resistance than total serum triacylglycerol concentrations. Diabetologia 2009;52:684–690.

[95] Oresic M, Hyotylainen T, Kotronen A, Gopalacharyulu P, Nygren H, Arola J, et al. Prediction of non-alcoholic fatty liver disease and liver fat content by serum molecular lipids. Diabetologia 2013;56:2266–2274.

[96] Westerbacka J, Kotronen A, Fielding BA, Wahren J, Hodson L, Perttula T, et al. Splanchnic balance of free fatty acids, endocannabinoids, and lipids in subjects with nonalcoholic fatty liver disease. Gastroenterology 2010;139:1961–1971 e1961.

[97] Kotronen A, Velagapudi VR, Yetukuri L, Westerbacka J, Kiviluoto T, Arola J, et al. Neutrophil extracellular traps promote inflammation and development of non-alcoholic fatty liver disease: sites and mechanisms. Diabetologia 2005;48:634–642.

[98] Ma C, Keswara AH, Eggert T, Medina-Echeverz J, Klein DE, Jin P, et al. NAFLD causes selective CD4(+) T lymphocyte loss and promotes hepatocarcinogenesis. Nature 2016;531:253–257.

[99] Pflster D, Nunez NG, Pinyol R, Gouveia O, Pinter M, Szylowska M, et al. NASH limits anti-tumour surveillance in immunotherapy-treated HCC. Nature 2021;592:450–456.

[100] Kotronen A, Velagapudi VR, Yetukuri L, Westerbacka J, Bergholm R, Ekroos K, et al. Serum saturated fatty acids containing triacylglycerols are better markers of insulin resistance than total serum triacylglycerol concentrations. Diabetologia 2009;52:684–690.
Zhou L, Wang Q, Yin P, Xing W, Wu Z, Chen S, et al. Serum metabolomics.
Muir K, Hazim A, He Y, Peyressatre M, Kim DY, Song X, et al. Proteomic.
Bao M, Chen Z, Xu Y, Zhao Y, Zha R, Huang S, et al. Sphingosine kinase 1.
Dasarathy S, Yang Y, McCullough AJ, Marczewski S, Bennett C, Kalhan SC.
Xia JY, Holland WL, Kusminski CM, Sun K, Sharma AK, et al. Sphingosine-1-phosphate.
Guri Y, Colombi M, Dazert E, Hindupur SK, Roszik J, Moes S, et al.
Hirose Y, Nagahashi M, Katsuta E, Yuza K, Miura K, Sakata J, et al.
Ismail IT, Elfert A, Helal M, Salama I, El-Said H, Fiehn O. Remodeling.
Grammatikos G, Schoell N, Ferreiros N, Bon D, Herrmann E, Farnik H,
Chaurasia B, Tippets TS, Mayoral Monibas R, Liu J, Li Y, Wang L, et al.
Dasarathy S, Yang Y, McCullough AJ, Marczewski S, Bennett C, Kalhan SC.
Mouzaki M, Wang AV, Bandsma R, Comelli EM, Arendt BM, Zhang L, et al.
Chavez-Talavera O, Tailleux A, Lefebvre P, Staels B. Bile acid control of
Muir K, Hazim A, He Y, Peyressatre M, Kim DY, Song X, et al. Proteomic.
Bell LN, Wulf J, Cornerford M, Vuppulanchi R, Chalasani N. Serum metabolic
Trottier J, Bialek A, Caron P, Straka RJ, Heathcote J, Milkiewicz P, et al.
Cho Y, Cho EJ, Yoo JJ, Chang Y, Chung GE, Jeong SM, et al. Serum metabolomics.
Muir K, Hazim A, He Y, Peyressatre M, Kim DY, Song X, et al. Proteomic.
Cho Y, Cho EJ, Yoo JJ, Chang Y, Chung GE, Jeong SM, et al. Association between lipid profiles and the incidence of hepatocellular carcinoma: a nationwide population-based study. Cancer (Basel) 2021;13.
Padthaisong S, Phetcharaburanin J, Klinrat P, Li JV, Namwat N, Khuntikeo N, et al. Integration of global metabolomics and lipidomics analyses reveals the molecular mechanisms and the potential biomarkers for postoperative recurrence in early-stage cholangioca. Cancer Metab 2021;9:30.
Ismail II, Elfert A, Helal M, Salama I, El-Said H, Fiehn O. Remodeling lipids in the transition from chronic liver disease to hepatocellular carcinoma. Cancers (Basel) 2020;12.
Vlock EM, Karanj S, Talmon G, Farazi PA. Reduction of polyunsaturated fatty acids with tumor progression in a lean non-alcoholic steatohepatitis-associated hepatocellular carcinoma mouse model. J Cancer 2020;11:5536–5546.
Grammatikos G, Schoell N, Ferreiros N, Bon D, Herrmann E, Farnik H, et al. Serum sphingolipidomic analyses reveal an upregulation of C16-ceramide and sphingosine-1-phosphate in hepatocellular carcinoma. Oncotarget 2016;7:18095–18105.
Curi Y, Colombi M, Dazert E, Hindupur SK, Roszik J, Moes S, et al. mTORC2 promotes tumorigenesis via lipid synthesis. Cancer Cell 2017;32:807–823 e812.
Hirose Y, Nagahashi M, Katsuta E, Yuza K, Miura K, Sakata J, et al. Generation of sphingosine-1-phosphate is enhanced in biliary tract cancer patients and is associated with lymphatic metastasis. Sci Rep 2018;8:10814.
Cheng JC, Wang EY, Yi Y, Thakur A, Tsai SH, Hoodless PA. S1P stimulates proliferation by upregulating CTGF expression through S1PR2-mediated YAP activation. Mol Cancer Res 2018;16:1543–1555.
Boa M, Chen Z, Xu Y, Zhao Y, Zha R, Han S, et al. Sphingosine kinase 1 promotes tumour cell migration and invasion via the S1P/EDG1 axis in hepatocellular carcinoma. Liver Int 2012;32:331–338.
Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, et al. Elevated free fatty acid uptake via CD36 promotes epithelial-mesenchymal transition in hepatocellular carcinoma. Sci Rep 2015;5:14752.

Vul H, Han C, Liu Y, Chen X, Li J, Yu Q, Jij J, Wu J, et al. The gut microbiome-bile acid axis in hepatocarcinogenesis. Biomed Pharmacother 2021;133: 110363.

Huang C, Xin C, Zhang L, Nichols RG, Krausz KW, et al. Intestinal fatty acid receptor signaling promotes nonalcoholic fatty liver disease. J Clin Invest 2015;125:386–402.

Garrido A, Kim E, Tejeiro A, Sanchez Sanchez P, Gallo R, Nair A, et al. Histone acetylation of bile acid transporter genes plays a critical role in cirrhosis. J Hepatol 2021.

Mencarelli A, Renga B, Migliorati M, Cipriani S, Distritti E, Santucci L, et al. The bile acid sensor farnesoid X receptor is a modulator of liver immunity in a rodent model of acute hepatitis. J Immunol 2009;183:6657–6666.

Zhang X, Yang Z, Shi Z, Zhu Li, Da Z, et al. Analysis of bile acid profile in plasma to differentiate cholangiocarcinoma from benign biliary diseases and healthy controls. J Steroid Biochem Mol Biol 2021;205:105775.

Dai J, Wang H, Shi Y, Dong Y, Zhang Y, Wang J. Impact of bile acids on the growth of human cholangiocarcinoma via FXR. J Hepatol Oncol 2011;4:41.

Dai J, Wang H, Dong Y, Zhang Y, Wang J. Bile acids affect the growth of human cholangiocarcinoma via NF-κB pathway. Cancer Invest 2013;31:111–120.

Kemp CJ, Leary CN, Drinkwater NR. Promotion of murine hepatocarcinogenesis by testosterone is androgen receptor-dependent but not cell autonomous. Proc Natl Acad Sci U S A 1989;86:7505–7509.

Petrick JL, McMenamin UC, Zhang X, Zeleniuch-Jacquotte A, Wactawski-Wende J, Simon TC, et al. Exogenous hormone use, reproductive factors and risk of intrahepatic cholangiocarcinoma among women: results from cohort study in the Liver Cancer Screening Project and the UK Biobank. Br J Cancer 2020;123:316–324.

Wei Q, Guo P, Mu K, Zhang Y, Zhao H, Huai W, et al. Estrogen suppresses hepatocellular carcinoma cells through ERβ-mediated upregulation of the NRFLP inflammomass. Lab Invest 2019;95:804–816.

Kawelert W, Sakonsinsiri C, Narmat W, Sawanyawisuth K, Ungarwevittaya P, Khuntikeo N, et al. The importance of CYP19A1 in estrogen-receptor-positive cholangiocarcinoma. Horm Cancer 2018;9:408–419.

Hunsawong T, Singsukwasat E, In-chon N, Chawengrattanachot W, Thuwijit S, Sripa B, et al. Estrogen is increased in male cholangiocarcinoma patients’ serum and stimulates invasion in cholangiocarcinoma cell lines in vitro. J Cancer Res Clin Oncol 2012;138:1311–1320.

Pawar P, Ma L, Byon CH, Liu H, Ahn EY, Jhala N, et al. Molecular mechanisms of tamoxifen therapy for cholangiocarcinoma: role of calmodulin. Clin Cancer Res 2009;15:1288–1296.

McGlynn KA, Sahasrabuddhe VV, Campbell PT, Graubard BI, Chen J, Morris JPt, et al. p53 represses the mevalonate pathway to mediate tumor suppression. Proc Natl Acad Sci U S A 2017;114:4300–4305.

Yin X, Zafrullah M, Lee H, Haimovitz-Friedman A, Fuks Z, Kolesnick R. A ceramide-binding C1 domain mediates kinase suppressor of ras membrane translocation. Cell Physiol Biochem 2009;24:219–230.

Shimizu Y, Tamura T, Kemmochi A, Owada Y, Ozawa Y, Hisakura K, et al. Oncogene KRAS activates fatty acid synthase, resulting in specific ERK and lipid signatures associated with lung adenocarcinoma. Proc Natl Acad Sci U S A 2019;116:7509–7514.

Zeng X, Yang Z, Shi Z, Han Q, Zhang Y, et al. Analysis of bile acid profile in plasma to differentiate cholangiocarcinoma from benign biliary diseases and healthy controls. J Steroid Biochem Mol Biol 2021;205:105775.

Xu M, Liu Z, Wang C, Yao B, Zheng X. EDG2 enhanced the progression of hepatocellular carcinoma by LPA1/P3K/PI3K/mTOR signaling. Oncotarget 2017;8:66154–66168.

Zhu B, Shi S, Ma YG, Fan Y, Yao ZZ. Lyposphatidic acid enhances human hepatocellular carcinoma cell migration, invasion and adhesion through β33 MAPK pathway. Hepatogastroenterology 2012;59:785–789.

Sakakima Y, Hayakawa A, Nakao A. Phosphatidylincholine induces growth inhibition of hepatic cancer by apoptosis via death ligands. Hepatogastroenterology 2009;56:481–484.

Wang X, Zheng Z, Caviglia JM, Corey KE, Herfel TM, Cai B, et al. Hepatocyte TAZ2/WWTR1 promotes inflammation and fibrosis in nonalcoholic steatohepatitis. Cell Metab 2016;24:848–862.

Brindley PJ, Bachini M, Ilyas SI, Khan SA, Loukas A, Sirica AE, et al. Cholangiocarcinoma. Nat Rev Dis Primers 2021;7:65.

Gouw AM, Eberlin LS, Margulis K, Sullivan DK, Toal GG, Tong L, et al. Oncogene KRAS activates fatty acid synthase, resulting in specific ERK and lipid signatures associated with lung adenocarcinoma. Proc Natl Acad Sci U S A 2017;114:4300–4305.

Yao Y, Sun S, Wang J, Fei F, Dong Z, Ke AW, et al. Canonical Wnt signaling remodels lipid metabolism in zebrafish hepatocytes following ras oncogenic insult. Cancer Res 2018;78:5548–5560.

Chen LL, Wang WJ. p53 regulates lipid metabolism in cancer. Int J Biol Macromol 2021;185:159–166.

Yahagi N, Shimano H, Matuszaka T, Najima Y, Sekiya M, Nakagawa Y, et al. p53 Activation in adipocytes of obese mice. J Biol Chem 2003;278:25395–25400.

Moon SH, Huang CH, Houlihan SL, Regunath K, Freed-Pastor WA, Morris JPT, et al. p53 represses the mevalonate pathway to mediate tumor suppression. Cell 2019;176:546–580 e519.

Assayli W, Rubinger DA, Wheaton K, Lin Y, Ma W, Xuan W, et al. ROS-mediated p53 induction of Lpin1 regulates fatty acid oxidation in response to nutritional stress. Mol Cell 2011;44:491–501.

Vancraeynest T, Verstraeten V, Vermeire P, Altermanski R, Keyzer E, Buhr J, et al. Increased miR-26a/b expression. Cancer Res 2015;75:1388–1398.

Fekry B, Jeffries KA, Esmaeiliakhooshghazi A, Szulc ZM, Knagge KJ, Kirchner DR, et al. C16-ceramide is a natural regulatory ligand of p53 in cellular stress response. Nat Commun 2018;9:4149.
Nelson ME, Lahiri S, Chow JD, Byrne FL, Hargett SR, Breen DS, et al. In-... 2019;63:1900–1913.

Rebouissou S, Nault JC. Advances in molecular classi-... 2016;34:316–512.

Luo X, Zheng E, Wei L, Zeng H, Wei L, Zeng H, et al. Role of lipogenesis rewiring in hepa-... 2021;12:328.

Anderson GS, Stahl AL, SCLC7 fatty acid transport proteins. Mol Aspects Med 2013;34:316–329.

Li L, Che L, Tharp KM, Park HM, Pilo MG, Cao D, et al. Differential requirement for de novo lipogenesis in cholangiocarcinoma and hepa-... 2018;72:215–220.

Zhou Y, Tao J, Calvisi DF, Chen X. Role of lipogenesis rewiring in hepa-... 2021;12:328.

Cheng G, Palanisamy AP, Evans ZP, Sutter AG, Jin L, Singh I, et al. Cer-... 2013;8:10846.

Li L, Pilo GM, Li X, Cigliano A, Latte G, Che L, et al. Inactivation of fatty acid synthase impairs hepatocarcinogenesis... 2014;3:419–431.

Luo X, Zheng E, Wei L, Zeng H, Wei L, Zeng H, et al. The fatty acid re-... 2021;8:17598.

Su YC, Feng YH, Wu HT, Huang YS, Tung CL, Wu P, et al. Elov6 is a negative clinical predictor for liver cancer and knockdown of Elov6 reduces murine liver cancer progression. Sci Rep 2018;8:6586.

Luo X, Zheng E, Wei L, Zeng H, Wei L, Zeng H, et al. ATP-citrate lyase regulates stemness and metastasis in hepatocellular carcinoma via the Wnt/beta-catenin signaling pathway. Hepatobiliary Pancreat Dis Int 2021;20:251–261.

Kemble G, et al. Fatty acid synthase inhibitor TVB-2640 reduces hepatic steatosis but elevates plasma... 2018;67:1493–1504.

Kim JH, Nispeen LS, Nispeen LS, Tiihonen A, Wahlberg J, et al. Evidence for an alternative fatty acid desaturation pathway increasing... 2019;566:403–406.

Kim JH, Nagappan A, Jung DY, Suh N, Jung MH. Histone demethylase KDM7A contributes to the development of hepatic steatosis by targeting diacylglycerol acyltransferase-2. Int J Mol Sci 2021;22.

Stoilov KD, et al. Fructose- and sucrose- but not glucose-sweetened beverages supports the growth of hepatocarcinoma lesions depleted of fatty acid synthase in mice and humans. Gut 2020;69:177–186.

Stoilov KD, et al. Fructose- and sucrose- but not glucose-sweetened beverages decreases the efficiency of hepatic de novo lipogenesis: a randomized controlled trial. J Hepatol 2021;75:46–54.

Chen J, Shi L, Mao Y, Wang Y, Li Y, Li Z, et al. Genetic inhibition of hepatic acetyl-CoA carboxylase activity increases liver fat and alters global protein acetylation. Mol Metab 2014;3:419–431.

Chen J, Shi L, Mao Y, Wang Y, Li Y, Li Z, et al. Genetic inhibition of hepatic acetyl-CoA carboxylase activity increases liver fat and alters global protein acetylation. Mol Metab 2014;3:419–431.

Yenilmez B, Wetoska N, Kelly M, Echeverria D, Min K, Lifshitz L, et al. Epigenetic programming at the Mogat1 locus may link NAFLD to T2DM. J Clin Invest 2019;129:1751–1761.

Feng YH, Wu HT, Huang YS, Tung CL, Wu P, et al. Elov6 is a negative clinical predictor for liver cancer and knockdown of Elov6 reduces murine liver cancer progression. Sci Rep 2018;8:6586.

Luo X, Zheng E, Wei L, Zeng H, Wei L, Zeng H, et al. ATP-citrate lyase regulates stemness and metastasis in hepatocellular carcinoma... 2021;8:10846.

Han Q, Chen CA, Yang W, Liang D, Lv HW, Lv GS, et al. ATP-citrate lyase regulates stemness and metastasis in hepatocellular carcinoma via the Wnt/beta-catenin signaling pathway. Hepatobiliary Pancreat Dis Int 2021;20:251–261.

Kemble G, et al. Fatty acid synthase inhibitor TVB-2640 reduces hepatic steatosis but elevates plasma... 2018;67:1493–1504.

Kim JH, Nagappan A, Jung DY, Suh N, Jung MH. Histone demethylase KDM7A contributes to the development of hepatic steatosis by targeting diacylglycerol acyltransferase-2. Int J Mol Sci 2021;22.

Stoilov KD, et al. Fructose- and sucrose- but not glucose-sweetened beverages supports the growth of hepatocarcinoma lesions depleted of fatty acid synthase in mice and humans. Gut 2020;69:177–186.

Stoilov KD, et al. Fructose- and sucrose- but not glucose-sweetened beverages decreases the efficiency of hepatic de novo lipogenesis: a randomized controlled trial. J Hepatol 2021;75:46–54.

Chen J, Shi L, Mao Y, Wang Y, Li Y, Li Z, et al. Genetic inhibition of hepatic acetyl-CoA carboxylase activity increases liver fat and alters global protein acetylation. Mol Metab 2014;3:419–431.

Chen J, Shi L, Mao Y, Wang Y, Li Y, Li Z, et al. Genetic inhibition of hepatic acetyl-CoA carboxylase activity increases liver fat and alters global protein acetylation. Mol Metab 2014;3:419–431.
[242] Gonzalez-Romero F, Mestre D, Aurrekoetxea I, O’Rourke CJ, Andersen JB, Woodhoo A, et al. EZF1 and EZF2-mediated repression of CPT2 establishes a lipid tumor-promoting environment. Cancer Res 2021;81:2874–2887.

[243] Yuan P, Mu J, Wang Z, Ma S, Da X, Song J, et al. Down-regulation of SLC25A20 promotes hepatocellular carcinoma growth and metastasis through suppression of fatty-acid oxidation. Cell Death Dis 2021;12:361.

[244] Ma APJ, Yeung CLS, Tey SK, Mao X, Wong SWK, Ng TH, et al. Suppression of ACADM-mediated fatty acid oxidation promotes hepatocellular carcinoma via aberrant CAV1 (SBEBP-1) signaling. Cancer Res 2021.

[245] Zhao X, Qin W, Jiang Y, Yang Z, Yuan B, Dai R, et al. ACADL plays a tumor-suppressor role by targeting Hippo/YAP signaling in hepatocellular carcinoma. NPJ Precis Oncol 2020;4:7.

[246] Liu X, Li M, Wang X, Dang Z, Jiang Y, Wang X, et al. Effect of serum triglyceride level on the prognosis of patients with hepatocellular carcinoma in patients with liver cirrhosis. Anal Chim Acta 2012;743:90–96.

[247] Tomacha J, Dokduang H, Papthaisong S, Namwat N, Klanrit P, Rohrbach TD, Asgharpour A, et al. Elafibranor, an agonist of the peroxisome proliferator-activated receptor-alpha and -delta, induces resolution of nonalcoholic steatohepatitis without fibrosis worsening. Gastroenterology 2016;150:1147–1159 e1145.

[248] Harrison SA, Bashir MR, Lee J, Wagner B, et al. A structurally optimized FXR agonist, MET409, reduced liver fat content in mice. J Lipid Res 2019;60:1311–1321.

[249] Stiede K, Miao W, Blanchette HS, Beyens C, Harriman G, Harwood Jr HJ, et al. Acetyl-coenzyme A carboxylase inhibition reduces de novo lipogenesis in C57 Bl/6j liver of nonalcoholic fatty liver disease in mice. J Lipid Res 2019;60:1322–1332.

[250] Loomba R, Mohseni R, Lucas KJ, Gutierrez JA, Perry RG, Trotter JF, et al. TVB-2640 (FASN inhibitor) for the treatment of nonalcoholic steatohepatitis: FASCINATE-1, a randomized, placebo-controlled phase 2a trial. Gastroenterology 2021;161:1475–1486.

[251] Lee YY, Kim JH, Kim SK, Jin HV, Rhee EJ, Cho YM, et al. Lobeglitazone, a novel thiazolidinedione, improves non-alcoholic fatty liver disease in type 2 diabetes: its efficacy and predictive factors related to respon- siveness. J Korean Med Sci 2017;32:60–69.

[252] Yoneda M, Endo H, Nozaki Y, Tomimoto A, Fujisawa T, Fujita K, et al. Life style-related diseases of the digestive system: gene expression in nonalcoholic steatohepatitis patients and treatment strategies. J Pharmacobio-Dyn 2007;30:E151–156.

[253] Franque S, Bedossa P, Ratziu V, Anstee QM, Bugianesi E, Sanyal AJ, et al. Advanced, randomized, controlled trial of the pan-FGER pananofiblaran in NASH. N Engl J Med 2021;385:1547–1558.

[254] Ratziu V, Harrison SA, Franque S, Bedossa P, Lebert P, Serfaty L, et al. Alifranib, an agonist of the peroxisome proliferator-activated receptor-alpha and -delta, induces resolution of nonalcoholic steatohepatitis without fibrosis worsening. Gastroenterology 2016;150:1147–1159 e1145.

[255] Kim W, Kim BG, Lee JS, Lee CK, Yeon JE, Chang MS, et al. Randomised clinical trial: the efficacy and safety of olibipraz, a liver X receptor alpha-inhibitory dithiolethione in patients with non-alcoholic fatty liver disease. Aliment Pharmacol Ther 2017;45:1073–1083.

[256] Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multi-centre, randomised, placebo-controlled trial. Lancet 2015;385:956–965.

[257] Rourke CJ, Andersen JB, Roughton FJW, El Systemy G, et al. A randomized, placebo-controlled, phase II study of obeticholic acid for primary sclerosing cholangitis. J Hepatol 2020;73:94–101.

[258] Corrado C, Condorelli A, Fichera A, Ostor A, et al. Orlistat displays antitumor activity and downregulates pro-inflammatory NF-kB pathway in prostate cancer cells. Sci Rep 2019;9:153.

[259] Harrison SA, Bashir MR, Lee KJ, Shim-Lopez J, Lee J, Wagner R, et al. A randomized, placebo-controlled, phase II study of obeticholic acid for noncirrhotic, non-alcoholic steatohepatitis. J Hepatol 2019;70:709–718.

[260] Shin I, Park H, Jung H, Lee H, Lee K, et al. Antitumor activity of obeticholic acid in patients with advanced hepatocellular carcinoma. J Hepatol 2020;72:1097–1107.

[261] Kowdley KV, Vuppupanchi R, Levy C, Floreani A, Andreone P, Lakuasso NF, et al. A randomized, placebo-controlled, phase II study of obeticholic acid for primary sclerosing cholangitis. J Hepatol 2020;73:94–101.

[262] Tran D, Takeda K, Itoh M, Hara T, et al. Orlistat displays antitumor activity and downregulates pro-inflammatory NF-kB pathway in prostate cancer cells. Sci Rep 2019;9:153.

[263] Harrison SA, Bashir MR, Lee KJ, Shim-Lopez J, Lee J, Wagner R, et al. A randomized, placebo-controlled, phase II study of obeticholic acid for primary sclerosing cholangitis. J Hepatol 2020;73:94–101.

[264] Corrado C, Condorelli A, Fichera A, Ostor A, et al. Orlistat displays antitumor activity and downregulates pro-inflammatory NF-kB pathway in prostate cancer cells. Sci Rep 2019;9:153.
activation of AMPK in p53 positive and negative human hepatoma cells. Mol Nutr Food Res 2009;53:1156–1165.

[284] de Lima Luna AC, Forti FL. Modulation of SCD1 activity in hepatocyte cell lines: evaluation of genomic stability and proliferation. Mol Cell Biochem 2021.

[285] Zhou Y, Zhong L, Yu S, Shen W, Cai C, Yu H. Inhibition of stearyl-Coenzyme A desaturase 1 ameliorates hepatic steatosis by inducing AMPK-mediated lipophagy. Aging (Albany NY) 2020;12:7350–7362.

[286] Park HR, Yoo MY, Seo JH, Kim IS, Kim NY, Kang JY, et al. Sesquiterpenoids isolated from the flower buds of Tuscilago farfara L. inhibit diacylglycerol acyltransferase. J Agric Food Chem 2008;56:10493–10497.

[287] Lencioni R, Marrero J, Venook A, Ye SL, Kudo M. Design and rationale for the non-interventional global investigation of therapeutic decisions in hepatocellular carcinoma and its treatment with sorafenib (GIDEON) study. Int J Clin Pract 2010;64:1034–1041.

[288] Liu G, Kuang S, Cao R, Wang J, Peng Q, Sun C. Sorafenib kills liver cancer cells by disrupting SCD1-mediated synthesis of monounsaturated fatty acids via the ATP-AMPK-mTOR-SREBP1 signaling pathway. FASEB J 2019;33:10089–10103.

[289] Ma MKF, Lau EYT, Leung DHW, Lo J, Ho NPY, Cheng LKW, et al. Stearoyl-CoA desaturase regulates sorafenib resistance via modulation of ER stress-induced differentiation. J Hepatol 2017;67:975–990.

[290] Xu A, Wang B, Fu J, Qin W, Yu T, Yang Z, et al. Diet-induced hepatic steatosis activates Ras to promote hepatocarcinogenesis via CPT1alpha. Cancer Lett 2019;442:40–52.

[291] Merrill CL, Ni H, Yoon LW, Timmenstein MA, Narayanan P, Benavides GR, et al. Etoposide-induced oxidative stress in HepG2 cells detected by differential gene expression is confirmed biochemically. Toxicol Sci 2002;68:93–101.

[292] Ren M, Xu H, Xia H, Tang Q, Bi F. Simultaneously targeting SOAT1 and CPT1A ameliorates hepatocellular carcinoma by disrupting lipid homeostasis. Cell Death Discov 2021;7:125.

[293] Yin F, Feng F, Wang L, Wang X, Li Z, Cao Y. SREBP-1 inhibitor Betulin enhances the antitumor effect of Sorafenib on hepatocellular carcinoma via restricting cellular glycolytic activity. Cell Death Dis 2019;10:672.

[294] Cariello M, Peres C, Zerlotin R, Porru E, Sabba C, Roda A, et al. Enhanced antitumour drug delivery to cholangiocarcinoma through the apical sodium-dependent bile acid transporter (ASBT). J Control Release 2019;216:93–102.

[295] Di Matteo S, Nevi L, Costantini D, Overi D, Carpino G, Safarikia S, et al. The FXR agonist obeticholic acid inhibits the carcinogenic potential of human cholangiocarcinoma. PLoS One 2019;14:e0210077.

[296] Tran Q, Lee H, Kim C, Kong G, Gong N, Kwon SH, et al. Revisiting the Warburg effect: diet-based strategies for cancer prevention. Biomol Res Int 2020;2020:8105735.

[297] Bae JS, Park JM, Lee J, Oh BC, Jang SH, Lee YB, et al. Amelioration of non-alcoholic fatty liver disease with NPC1L1-targeted IgY or n-3 polyunsaturated fatty acids in mice. Metabolism 2017;66:32–44.

[298] Nemoto N, Suzuki S, Kikuchi H, Okabe H, Sassa S, Sakamoto S. Ethyl-eicosapentaenoic acid reduces liver lipids and lowers plasma levels of lipids in mice fed a high-fat diet. In Vivo 2009;23:685–689.

[299] Konuma K, Itoh M, Suganami T, Kanai S, Nakagawa N, Sakai T, et al. Eicosapentaenoic acid ameliorates non-alcoholic steatohepatitis in a novel mouse model using melancortin 4 receptor-deficient mice. PLoS One 2015;10:e0121528.

[300] Gao M, Sun K, Guo M, Gao H, Liu K, Yang C, et al. Fish consumption and n-3 polyunsaturated fatty acids, and risk of hepatocellular carcinoma: systematic review and meta-analysis. Cancer Causes Control 2015;26:367–376.

[301] Kang S, Huang J, Lee BK, Jung YS, Im E, Koh JM, et al. Omega-3 polyunsaturated fatty acids protect human hepatoma cells from developing steatosis through FFAR4 (GPR120). Biochim Biophys Acta Mol Cell Biol Lipids 2018;1863:105–116.

[302] Weylandt KH, Krause LF, Gomolka B, Chiu CY, Bilal S, Nadolny A, et al. Suppressed liver tumorigenesis in fat-1 mice with elevated omega-3 fatty acids is associated with increased omega-3 derived lipid mediators and reduced TNF-alpha. Carcinogenesis 2011;32:897–903.

[303] Jeypal S, Kona SR, Mullapudi SV, Putzka UK, Gurumurthy P, Ibrahim A. Substitution of linoleic acid with alpha-linolenic acid or long chain n-3 polyunsaturated fatty acid prevents Western diet induced nonalcoholic steatohepatitis. Sci Rep 2018;8:10953.

[304] Valenzuela R, Espinosa A, Gonzalez-Manan D, ESpeissailles A, Fernandez V, Videila LA, et al. N-3 long-chain polyunsaturated fatty acid supplementation significantly reduces liver oxidative stress in high fat induced steatosis. PLoS One 2012;7:e46400.

[305] Huang Q, Tan Y, Yin P, Ye G, Gao P, Lu X, et al. Metabolic characterization of hepatocellular carcinoma using nontargeted tissue metabolomics. Cancer Res 2013;73:4992–5002.

[306] Simon J, Ouro A, Ala-Ibanillo B, Presa N, Delgado TC, Martinez-Chantar ML. Sphingolipids in non-alcoholic fatty liver disease and hepatocellular carcinoma: ceramide turnover. Int J Mol Sci 2019;21.

[307] Lim K, Han C, Dai Y, Shen M, Wu T. Omega-3 polyunsaturated fatty acids inhibit hepatocellular carcinoma cell growth through blocking beta-catenin and cyclooxygenase-2. Mol Cancer Ther 2009;8:3046–3055.

[308] Patterson AD, Maurhofer O, Reyoglu D, Lanz C, Krausz KW, Pabst T, et al. Aberrant lipid metabolism in hepatocellular carcinoma revealed by plasma metabolomics and lipid profiling. Cancer Res 2011;71:6590–6600.