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Molecular genealogy of metabolic-associated hepatocellular carcinoma

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This review examines the latest epidemiological and molecular pathogenic findings of metabolic-associated hepatocellular carcinoma (HCC). Its increasing prevalence is a significant concern and reflects the growing burden of obesity and metabolic diseases, including metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as nonalcoholic fatty liver disease (NAFLD), and type 2 diabetes. Metabolic-associated HCC has unique molecular abnormality and distinctive gene expression patterns implicating aberrations in bile acid, fatty acid metabolism, oxidative stress, and proinflammatory pathways. Furthermore, a notable frequency of single nucleotide polymorphisms (SNPs) in genes such as patatin-like phospholipase domain-containing 3 (PNPLA3), transmembrane 6 superfamily member 2 (TM6SF2), glucokinase regulator (GCKR), and membrane bound O-acyltransferase domain-containing 7 (MBOAT7) has been observed. The tumour immune microenvironment of metabolic-associated HCC is characterized by unique phenotypes of macrophages, neutrophils, and T lymphocytes. Additionally, the pathogenesis of metabolic-associated HCC is influenced by abnormal lipid metabolism, insulin resistance, and dysbiosis. In conclusion, deciphering the intricate interactions among metabolic processes, genetic predispositions, inflammatory responses, immune regulation, and microbial ecology is imperative for the development of novel therapeutic and preventative measures against metabolic-associated HCC.

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Molecular genealogy of metabolic-associated hepatocellular carcinoma

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KEYWORDS: MASLD, NAFLD, Dysbiosis, Lipid metabolism, Insulin signalling

Lay summary

This paper provides a review of the latest knowledge about the epidemiology and molecular pathogenesis of metabolic-associated hepatocellular carcinoma (HCC). Its increasing prevalence is a significant concern and reflects the growing burden of obesity and metabolic diseases, including metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as nonalcoholic fatty liver disease (NAFLD), and type 2 diabetes. We investigated the molecular pathogenesis of this cancer and revealed that it involves certain genetic changes, including single nucleotide polymorphisms (SNPs), and inflammatory processes. Altered lipid metabolism, insulin resistance, and an imbalance in gut bacteria, as well as unique phenotypes of macrophages, neutrophils, and T cells, can also influence the development and progression of metabolic-associated HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and is a significant global health concern. Approximately one million patients are diagnosed annually with primary liver cancer, and its incidence has increased ~2-fold since 1990. HCC is often associated with chronic liver disease and typically develops over several years. It is the sixth most common cancer globally and is responsible for a substantial number of cancer-related deaths[1]. HCC exhibits significant geographic variation, with the highest incidence rates occurring in parts of Asia and sub-Saharan Africa[2,3]. Risk factors for HCC include chronic viral hepatitis (primarily hepatitis B and C), alcohol consumption, exposure to aflatoxins, a type of toxin produced by moulds, and metabolic conditions such as obesity, type 2 diabetes and nonalcoholic fatty liver disease (NAFLD). NAFLD has been recently renamed metabolic dysfunction-associated steatotic liver disease (MASLD) due to the inherent drawback of the nomenclature and definition being exclusive and stigmatizing [2,4,5]. The increasing prevalence of metabolic-associated HCC is a substantial concern and multifaceted issue driven by lifestyle and environmental factors, as well as the growing burden of obesity and metabolic diseases[4]. Thus, the importance of public health efforts to address these risk factors and the need for improved

understanding and management of HCC in individuals with metabolic conditions are important. Metabolic-associated HCC is characterized by complex interplay between signalling pathways and genetic alterations. Understanding these mechanisms is crucial for developing targeted therapies and improving the clinical management of metabolic-associated HCC. Here, we comprehensively reviewed the epidemiological findings, risk factors, and molecular abnormalities associated with metabolic-associated HCC.

2. Epidemiology and risk factors in metabolic-associated HCC

MASLD, obesity, and type 2 diabetes are closely and directly linked to the development of metabolic-associated HCC **(Figure 1)**[6]. MASLD, formerly known as NAFLD, is characterized by the accumulation of fat in the liver, metabolic abnormalities and a lack of alcohol consumption. The diagnostic criteria for MASLD include fatty liver disease with at least one of five cardiometabolic risk factors, which includes an increase in body mass index (BMI) or waist circumference, impaired glucose metabolism, high blood pressure, high triglyceride (TG) levels, and low high-density lipoprotein (HDL-C) levels **(Figure 1)**[5,7]. The latest reports showed that more than 99% of NAFLD

patients met the MASLD criteria, and a minimal difference was observed in prevalence between NAFLD (25.7%) and MASLD (26.7%) in a randomly chosen 1000 patients examined with proton magnetic resonance spectroscopy[8,9]. Thus, the previous cohort data focusing on NAFLD may apply to MASLD, and to avoid confusion in this review, the new nomenclatures MASLD and MASH were applied when introducing the previous reports of NAFLD and NASH, respectively. The global prevalence of MASLD is approximately 20-25%, but it is projected to increase to 55% in 2040 [3,10,11]. Approximately 20-30% of MASLD patients progress to MASH, which is characterized by liver inflammation and injury. Over time, MASH can lead to fibrosis and cirrhosis, significantly increasing the risk of HCC. The incidence of HCC is approximately 0.44 per 1000 person-years among MASLD patients but increases up to 2% per year among MASH-cirrhosis patients [4,12]. A Japanese group also reported that the 5-year incidence of HCC was 11.3% among MASH-cirrhosis patients [13]. Due to the rapid increase in the prevalence of MASLD, MASLD is now the first growing cause of HCC in liver transplant recipients in the U.S. [14]. Similarly, the proportion of patients with HCC attributed to MASLD is continuously increasing worldwide [15-17]. Importantly, a substantial

proportion of MASLD-related HCC patients develop a noncirrhotic liver background. Epidemiological data have shown that 80-90% of HCC patients, including those with all various aetiologies, have cirrhosis, but this percentage decreases to 50-60% among MASLD-HCC patients [18]. Moreover, among noncirrhotic patients, MASLD increases the risk of HCC development 5-fold compared to that of HCV[19].

Approximately 0.7 billion adults were obese worldwide in 2020, and this number is projected to increase to more than 1 billion by 2030 according to the World Obesity Federation[20]. In the U.S., one-third of the population was obese in 2012, but approximately half of the total population is projected to be obese by 2030[21]. Obesity is characterized by excess adipose tissue, which can lead to a state of chronic low-grade inflammation[6]. This inflammation triggers the release of tumour-promoting cytokines, such as interleukin-6 (IL-6) and tumour necrosis factor (TNF), which can damage liver tissue and stimulate hepatocyte proliferation over time[6,22]. In obese individuals, adipose tissue can produce hormones and adipokines, such as leptin and adiponectin, which can impact cell proliferation and survival, increasing the risk of HCC[6]. Epidemiological studies have consistently shown a strong association between obesity and an

increased risk of HCC. A meta-analysis of 28 prospective cohort studies with approximately 8 million subjects reported that an increase in BMI was associated with a 69% increased risk of primary liver cancer and a 61% increased risk of liver cancer-related mortality[23]. The risk is particularly elevated in individuals with central or visceral obesity, which is characterized by excess fat around the abdominal organs[24]. Obesity also increases the risk of HCC development 2.6-fold in the presence of diabetes, hypertension, or hyperlipidaemia [3,25]. In the presence of MASLD, obesity (BMI≥30) was associated with a 1.18-fold increase in the risk of HCC development, but this difference was not statistically significant (p=0.06)[26]. A retrospective study of 98,090 patients with MASLD and severe obesity, including 33,435 individuals who underwent bariatric surgery, showed that HCC risk was reduced by up to 52% via surgical weight loss [27].

Individuals with type 2 diabetes often exhibit insulin resistance, in which cells do not respond effectively to insulin, leading to hyperinsulinaemia. Insulin resistance can promote liver fat accumulation and stimulate the growth of liver cells (details are described in chapter 7). The global prevalence of diabetes mellitus (DM) is approximately 9%, and it is projected to affect 300-400 million

people by 2030[28]. Individuals with type 2 diabetes are at a significantly greater risk of developing HCC. A meta-analysis showed that people with diabetes had a 2.5-fold greater risk of HCC than did those without diabetes [28]. Among MASH-cirrhosis patients, the presence of diabetes was associated with a 4.2-fold increase in HCC risk [29]. Even among the MASLD cohort, which mostly consisted of nonfibrotic patients, diabetes was the strongest independent risk factor for HCC development, with a 3.03-fold increase [26,30]. The risk increases with the duration of diabetes and is more pronounced in individuals with poorly controlled blood glucose levels.

Importantly obesity, type 2 diabetes, and MASLD frequently occur together and therefore may synergistically increase the risk of HCC.

3. Genomic abnormalities and molecular pathways in metabolic-associated HCC (Figure 2)

With the advancement of next-generation sequencing technology, global collaborative projects involving cancer genome sequences have been conducted, and genetic abnormalities in more than 1000 HCC genomes across aetiologies have been reported[31]. The most frequently occurring mutations

associated with HCC patients are *TERT* promoter mutations, followed by mutations in *TP53* and *CTNNB1*, and such mutations are considered the 3 major drivers of HCC[31]. Telomerase activation occurs in ~80% of HCC cases via *TERT* promoter mutations, viral insertions, chromosomal translocations or gene amplifications, allowing hepatocytes to overcome senescence and become immortal[2,32,33]. In MASH-HCC, the telomere maintenance pathway was reported to be dysregulated in 56% of patients[34]. The WNT/β-catenin signalling pathway is activated in 30-50% of HCC cases by gain-of-function mutations in *CTNNB1* or loss-of-function mutations in *AXIN1* or *APC*[2]. In the absence of these mutations, cellular β-catenin forms a complex with *APC* and *AXIN1* and undergoes proteosomal degradation[35]. In the presence of these mutations, β-catenin avoids degradation and translocates into the nucleus. There, it activates transcription factors such as T-cell factor (TCF) and the lymphoid enhancer-binding protein family (LEF), which in turn transcribe genes that positively regulate cellular survival and proliferation[35]. In MASH-HCC, the WNT/β-catenin pathway was reported to be dysregulated in 42% of patients[34]. The tumour suppressive role of p53 has been ubiquitously described, but it is dysregulated in 18% of MASH-HCC patients[34]. In addition, genes involved in

the cell cycle (*RB1, CCNA2, CCNE1, CCND1, CDKN2A, RPS6KA3*), oxidative stress (*NFE2L2, KEAP1*), chromatin remodelling (*ARID1A, ARID2, ARID1B*), TGFβ signalling (*ACVR2A, ACVR1B, TGFBR2*), mitogen-activated protein kinase (*MAPK*) and phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (*PI3K/AKT/mTOR*) pathways (*MET, FGFR1, FGF19, HGF, PTEN, PIK3CA*) are known to be dysregulated by mutations or copy number alterations at a relatively lower frequency in HCC[31,36,37]. Importantly, hundreds or thousands of other genes were also mutated at a very low frequency, creating tremendous intratumor and intertumoral genomic heterogeneity in HCC. In general, the pattern of mutated genes does not vary significantly for each background liver disease[37]. However, a unique higher incidence of *ACVR2A* mutations (10% vs. 3%, p = 0.02) was reported in MASH-HCC than in viral/alcohol-related HCC[34]. We also analysed the molecular abnormalities of 113 nonviral HCC patients and detected a high frequency of mutations in *ACVR2A* (29.5%) together with *KMT2C* (42.8%) in MASH-HCC patients[38]. The gene expression of *ACVR2A* was downregulated in MASH-HCC patients harbouring the *ACVR2A* mutation, and its knockdown increased cellular proliferation, indicating the role of ACVR2A as a tumour suppressor [34].

However, the specific role of ACVR2A in HCC in the context of the MASH background has not yet been determined. Biological signature analysis revealed a marked increase in activity related to bile acid and fatty acid metabolism, oxidative stress, and inflammation in MASH-HCC patients[34]. A mutational signature analysis also revealed a unique signature characterized by a greater frequency of C>T and C>A transitions in MASH-HCC patients, especially in female patients[34].

3. Germline genetic associations for metabolic-associated HCC (Figure 2)

Genome-wide association studies (GWAS) have identified a variety of genetic variants associated with MASH and MASH-HCC. Single nucleotide polymorphisms (SNPs) in patatin-like phospholipase domain-containing protein 3 (PNPLA3) are the most well-known SNPs driving MASH. The presence of the *PNPLA3* variant rs738409 impairs triglyceride lipolysis and promotes hepatic steatosis[39,40]. Individuals with this variant have a 2.05-fold greater risk of MASH than healthy control individuals[41] and a 2.26-fold greater risk of metabolic-associated HCC than does patients with MASLD without this variant[42].The presence of the transmembrane 6 superfamily member 2

(TM6SF2) variant rs58542926 is known to increase cholesterol and fatty acid biosynthesis, leading to the increase in hepatic triglyceride levels[43]. Individuals with this variant have a 1.61-fold greater risk of MASH than healthy control individuals[41] and a 1.92-fold greater risk of metabolic-associated HCC than does patients with MASLD without this variant[44]. The presence of the glucokinase regulator (GCKR) variant rs1260326 induces glycolysis, de novo lipogenesis, and insulin resistance[45]. This variant increases the disease severity of MASH only under diabetic conditions but protects against fibrosis under nondiabetic conditions[46]. Individuals with this variant have a 1.55-fold greater risk of MASH than healthy control individuals[47] and a 1.84-fold greater risk of metabolic-associated HCC than does the general population[48]. The presence of the membrane-bound o-acyltransferase domain containing 7 (MBOAT7) variant rs641738 increases hepatic triglyceride levels and promotes steatosis[49]. Individuals with this variant have a 2.1-fold greater risk of metabolic-associated HCC than does those with MASLD without this variant [50].

It should be noted here that racial disparity exists in the frequency and the effect of these SNPs, which may in part explain the racial difference in the prevalence

of MASLD. The race with the highest frequency of the *PNPLA3* rs738409[G] variant is Peruvian(72%), followed by Mexican ancestry in Los Angeles (55%), Japanese (42%), and Colombian (41%), while the lowest is African ancestry such as Luhya in Kenya (9%), followed by Gambians (11%), Mende in Sierra Leone (11%) and Yoruba in Nigeria (12%)[51]. The *MBOAT7* rs641738[T] variant was associated with MASLD severity in Europeans but inversely associated in obese Hispanic children in the U.S [52].

Very recently, GWAS on HCC was conducted in large numbers of individuals from European-descent populations [53]. Significant association with the risk of non-viral HCC was found in *MOBP* variant rs9842969 (OR:0.51), *TERT* variant rs2242652 (OR: 0.70), *TM6SF2* variant rs58542926 (OR:1.49), *MAU2* variant rs58489806 (OR: 1.53), and *PNPLA3* variant rs738409 (OR:1.66). A combination of homozygous mutations in *PNPLA3* and *TERT* showed a 6.5-fold higher risk of non-viral HCC compared to individuals without these genotypes.

4. Inflammation and immune dysregulation in metabolic-associated HCC (Figure 2)

Compared with HCC patients with other aetiologies, MASLD-related HCC

patients exhibit high levels of inflammatory markers, such as C-reactive protein and serum amyloid A[54]. According to previous meta-analyses, the risk of developing HCC is more than 10-fold greater in MASH patients than in patients with simple steatosis according to the MASLD criteria[55]. These data indicate that inflammation may promote metabolic-associated HCC. Indeed, MASH pathogenesis includes metabolic dysfunctions, lipotoxicity, oxidative stress, and gut dysbiosis, all of which weaken an inherent immunotolerant hepatic environment, inducing chronic inflammation[10]. While chronic liver injury fosters cell proliferation, inflammation increases the production of reactive oxygen species (ROS). ROS induce DNA damage and mutations, promoting hepatocarcinogenesis[56]. The major molecular signalling pathways involved in inflammation include the NF-kB, JAK-STAT, and c-Jun-JNK pathways[56]. Obesity-induced inflammation triggers NF-kB, leading to insulin resistance through a mechanism involving phosphotyrosine signalling[57]. NF-kB also has protumourigenic effects, promoting HCC cell growth, survival, and invasion[58]. The JAK-STAT pathway is activated by cytokines and growth factors, affecting genes involved in cell growth and the immune response[58]. STAT3 is frequently activated in HCC tumours by IL-6 and linked to aggressive cancer.

The oxidative hepatic environment in obesity also inactivates the STAT-1 and STAT-3 phosphatase T cell protein tyrosine phosphatase (TCPTP) and increases STAT3 signalling [59]. High c-Jun-JNK activity in the livers of obese patients is linked to hepatic insulin resistance and steatosis. JNK signalling, which is increased by hepatic fat accumulation, increases the expression of the BCL-2 family member BIM, influencing cell death[60]. Additionally, JNK signalling in HCC promotes tumour initiation and inflammation[61].

MASLD is associated with the abnormal growth of harmful bacterial strains that increase the permeability of the mucosal barrier. This disease is known as leaky gut syndrome and promotes the transfer of bacterial products to the liver[62]. The persistence of cellular injury and the influx of pathogen-associated molecular patterns (PAMPs) contribute to chronic inflammation in the liver, leading to fibrosis, cirrhosis, and HCC. PAMPs and damage-associated molecular patterns (DAMPs) are sensed via pattern recognition receptors (PRRs) expressed on Kupffer cells (KCs) in the liver. This triggers their activation and the production of proinflammatory cytokines/chemokines, such as CCL1, TNF-α, and IL-1β, which further recruit proinflammatory immune cells, such as monocyte-derived macrophages[63,64]. NASH-associated

macrophages (NAMs) are characterized by the expression of triggering receptor expressed on myeloid cells 2 (TREM2), the glycoproteins CD9 and NMB (GPNMB), and CD63. NAMs contribute to the liver inflammatory response and promote fibrosis [65-67]. $TREM2^{+}$ macrophages are abundant in the tumour immune microenvironment (TIME) of human HCC and are associated with poor prognosis.

Neutrophils are also recruited to the liver in response to CXCL1/2 and CXCL8 produced by KCs and contribute to hepatic inflammation via the production of ROS, cytokines/chemokines, elastase and myeloperoxidase (MPO)[68]. Neutrophils also produce extracellular traps (NETs), which are web-like structures consisting of DNA, histones, and neutrophil proteases that sustain inflammation and promote hepatocarcinogenesis[69]. The number of neutrophils with an N2-like phenotype increased in the MASH-HCC microenvironment. These cells produced large amounts of TGF-β1, which favours the escape of cancers from immune surveillance. Tumour-associated neutrophils (TANs) isolated from MASH-HCC are specifically characterized by high expression of CXCR2 and carcinoembryonic antigen-associated cell adhesion molecule 8 (CEACAM8)[70]. Interestingly, TANs associated with

MASH-HCC release NETs and reprogram naïve $CD4^+$ T cells into $CD4^+$/$FOXp3^+$ Tregs via Toll-like receptor 4 (TLR4), creating an immunosuppressive TIME[71]. The intrahepatic recruitment of myeloid-derived suppressor cells (MDSCs) was also observed in the MASH liver and MASH-HCC cohorts. MDSCs may produce immunosuppressive factors, such as arginase 1, indoleamine 2,3-dioxyneganase 1 (IDO1), inducible nitric oxide synthase (iNOS) and ROS[72]. In the obese background, high levels of cholesterol induce lipid peroxidation in NKT cells and impair their tumour-suppressive effects, which is cancelled by statin treatment in an experimental mouse model[73].

The contribution of T cells to the pathogenesis of MASH and MASH-HCC has been intensively studied. $CD4^+$ T cells are known to produce interferon-γ (IFN-γ) in MASH patients, and its depletion ameliorates MASH in an experimental model[74,75]. In addition, the MASH-specific subset of hepatic Th17 $CD4^+$ T cells, named ihTh17 cells, expresses CXCR3 and many inflammatory mediators and thus exacerbate MASH[76]. These data suggested the role of $CD4^+$ T cells in the promotion of MASH. Regarding MASH-HCC, $CD4^+$ T-cell depletion was reported to promote MASH-HCC in mice[77]. Meanwhile, Foxp3+ Tregs and Th17 T cells were shown to contribute to MASH-associated HCC in

experimental models[71,78], suggesting the complex roles of $CD4^+$ T cells. A detailed analysis of the role of these genes in each subcluster is necessary. Many studies support the proinflammatory and profibrogenic roles of CD8+ T cells in MASH[79]. A recent report showed that exposure to metabolic stimuli, such as acetate and extracellular ATP, induce MASH-specific $CXCR6^+PD1^+CD8^+$ T cells in the liver, which in turn kill hepatocytes in an antigen-independent autoaggressive manner[79]. Meanwhile, the involvement of $CD8^+$ T cells in MASH resolution has also been recently reported[80]. Tissue-resident memory $CD8^+$ T cells attract activated hepatic stellate cells (HSCs) in a CCR5-dependent manner and kill these cells via Fas ligands[80]. These data suggest the multifaceted role of CD8+ T cells in MASH pathogenesis. Regarding HCC development, further complexity was observed. Depletion of $CD8^+$ T cells limits HCC development according to many reports, but this is not always the case[81,82]. This may be due to the conflicting roles of $CD8^+$ T cells in the MASH-liver, such as the abovementioned proinflammatory and pro-fibrogenic roles that promote hepatocarcinogenesis and the immune-surveillance roles that prevent hepatocarcinogenesis. Recent reports have shown that the number of $PD\text{-}1^+CD8^+$ T cells is increased in the liver of MASH-

HCC patients, and anti-PD-1 therapy induces hepatic damage rather than exerts antitumour effects[25,81]. To support this finding, a meta-analysis of clinical trials showed less benefit of ICI therapy for nonviral HCC patients than for viral HCC patients[4]. We have also recently found that steatotic HCC, characterized by lipid droplet formation inside tumour cells, is characterized by intratumor CD8+ T-cell exhaustion, which is highly responsive to ICI and anti-VEGF therapy in nonviral HCC patients[38]. Taken together, these findings suggest that a steatotic environment, regardless of whether it is intrahepatic or intratumoural, may cause T-cell exhaustion in the liver, resulting in T-cell activation upon ICI therapy[38,81].

Gut dysbiosis and oxidative stress activate B cells via the myeloid differentiation primary response 88 (MyD88) and B-cell receptor (BCR) signalling pathways, which in turn expand INF-$\gamma^{+}CD4^{+}$ helper T cells and exacerbate MASH[74,83,84]. B cells are also known to promote profibrogenic genes, such as TGF-β1 and TIMP-2[85,86]. The presence of B cells was associated with poor prognosis in HCC patients[87]. IgA^{+} plasma cells express PD-L1 and IL-10, inhibiting CD8+ T-cell activation in MASH-HCC patients[82,88].

5. Lipid metabolism and HCC (Figure 3)

Cancer cells reprogram various metabolic pathways to construct cellular components, such as nucleic acids, proteins, and lipids; this process is known as metabolic reprogramming[89]. The activation of lipid synthesis is extremely crucial for rapidly proliferating cancer cells. This is because lipids, like phospholipid bilayers, are fundamental membrane components that enable cell proliferation[90]. Therefore, various tumours activate de novo lipogenesis (DNL), which is a process by which cells produce their own fatty acids, and external lipid uptake regardless of the level of circulating lipids[91]. In addition, altered lipid metabolism is often observed in MASLD patients and is associated with changes in lipid biosynthesis and metabolism pathways[92,93]. Hepatic steatosis develops when the accumulation of fatty acids in the liver, through absorption from the bloodstream and DNL, exceeds the capacity of the liver to metabolize these fats through oxidation and to export them as VLDL triglycerides. In particular, de novo FA synthesis is often upregulated in MASLD patients[93,94]. This increase in lipogenesis may provide the necessary substrates for cell membrane formation and energy production in intrahepatic tumour cells. However, altered lipid metabolism can lead to the generation of

ROS and lipid peroxidation products. These mutations can cause oxidative stress, damage DNA, and promote genetic instability, leading to the accumulation of genetic mutations that also drive HCC development.

The de novo fatty acid synthesis pathway starts from ATP-citrate lyase (ACLY). ACLY converts citrate, which is transported from the mitochondria to the cytoplasm, into acetyl-CoA and oxaloacetate[95]. Then, acetyl-CoA carboxylase (ACC) converts acetyl-CoA into malonyl-CoA, which is a precursor for fatty acid synthesis. Malonyl-CoA and acetyl-CoA are condensed by fatty acid synthase (FASN), leading to the generation of palmitic acid and other FA synthesis products[96]. In HCC, these major enzymes related to DNL, including FASN, are often overexpressed, contributing to increased lipogenesis and lipid accumulation in tumour cells[92,97]. In an experimental mouse model, FASN deletion ameliorated HCC through either AKT activation or PTEN deletion[98,99]. Liver-specific ACC inhibitor (ND-654) also suppressed hepatic DNL and the development of HCC [100]. A variety of inhibitors targeting DNL pathways have been tested in clinical trials for MASLD and HCC[92,101]. Stearoyl-CoA desaturase (SCD1) is the rate-limiting enzyme that converts FAs to monounsaturated fatty acids (MUFAs), and its activity is upregulated in

MASLD and HCC[91,102]. Lipogenesis is regulated mainly by sterol regulatory element-binding protein 1 (SREBP-1), which is a transcription factor that is often upregulated in MASLD and HCC[103,104], and by carbohydrate response element binding protein (ChREBP), which is a transcription factor that upregulates lipogenic and glycolytic genes[105]. SREBPs form a complex with SREBP cleavage-activating protein (SCAP), which is further associated with the endoplasmic reticulum (ER) membrane proteins insulin-induced gene 1 (INSIG1) and INSIG2[106]. Normally, when cellular lipid levels decrease, SCAP undergoes structural changes that disrupt its interaction with INSIG proteins. This change leads to the separation of the SREBP/SCAP complex from INSIGs, facilitating their movement from the ER to the Golgi apparatus. In the Golgi, SREBP undergoes cleavage, leading to its activation[106]. Interestingly, Kawamura S et al. recently reported the unexpected observation that the suppression of SREBP through the removal of the SCAP in the MASH liver, despite attenuating liver steatosis, worsened liver damage, fibrosis, and promoted the HCC development [107]. Mechanistically, SREBP inhibition suppresses LPCAT3 and increases membrane lipid saturation, which in turn decreases membrane fluidity, leading to excess ER stress in the liver[107].

Fatty acid uptake and transport are also altered in MASH and HCC patients. This process is mediated by fatty acid translocase (CD36), a cell surface receptor that facilitates the uptake of fatty acids, and fatty acid transport proteins such as FATP2 and FATP5, all of which are known to be upregulated in MASLD[108,109]. An increase in CD36 was also related to EMT in HCC patients[110]. After being absorbed, hydrophobic fatty acids cannot move unaided through the cytosol. Instead, they require transport by specialized fatty acid binding proteins (FABPs), such as FABP1, FABP4 and FABP5. In the liver, the primary form of these proteins is FABP1, which plays a key role in the transportation, storage, and use of FAs. The levels of a variety of FABPs are also increased in MASLD patients[111,112].

The roles of FA β-oxidation (FAO) remain controversial. Due to the inadequate nutrient availability in the core of the tumour caused by insufficient blood vessel development, FAO serves as a key catabolic route to produce ATP and maintain NADPH levels in addition to glycolysis. This process is activated by C/EBPα and AMPK[113-115]. It has also been reported that HCC cells acquire resistance to antiangiogenic agents by activating FA uptake and FAO under hypoxic and nutrient-starved conditions[116]. In contrast, FAO can lead to the

generation of ROS and lipid peroxidation products, which are harmful to cancer cells. Indeed, hypoxia-induced HIF-1 expression facilitates HCC progression by inhibiting FA oxidation and ROS formation via the downregulation of medium-chain acyl-CoA dehydrogenase (MCAD) and long-chain acyl-CoA dehydrogenase (LCAD), both of which are rate-limiting enzymes that catalyse the first step of oxidation in mitochondria[117].

The lipolytic pathway is also sometimes used by HCC cells to generate free FAs from stored lipids. The lipolytic enzyme monoglyceride lipase (MAGL) breaks down monoacylglycerols into free FAs and glycerol, and it facilitates the release of FAs from stored lipids[118]. YAP activation induced MAGL expression in HCC. In addition, lipoprotein lipase (LPL) is also upregulated in HCC and promotes the uptake of extracellular lipoproteins into cells via the hydrolysis of triglycerides[119].

6. Insulin signalling and advanced glycation end-products (AGEs) in metabolic-associated HCC (Figure 3)

Insulin resistance (IR) is a condition in which insulin becomes less effective at managing blood glucose, leading to increased insulin production and related

health issues such as hyperinsulinaemia, type 2 diabetes, obesity, MAFLD, and liver fibrosis. IR and hyperinsulinaemia are two interrelated factors that significantly contribute to the development and progression of HCC, especially in the context of metabolic diseases. High insulin levels increase insulin-like growth factor 1 (IGF-1) production and IRS-1 expression[120]. IR also increases the expression of the growth hormone receptor (GHR), which, in combination with growth hormone (GH), further increases the activation of IGF-1. Consequently, hyperinsulinaemia leads to the hepatic production and release of substantial quantities of IGF-1, which promotes cell growth and prevents cell death, thereby accelerating hepatocarcinogenesis[121]. IRS, bound to insulin or IGF receptor, activates pathways such as the PI3K/AKT and MAPK pathways in liver cells, which can lead to cell growth, fibrosis, and cancer. PTEN serves as a counterbalance to this pathway by deactivating oncogenic PI3K/Akt signalling through the dephosphorylation of phosphatidylinositol 3,4,5-triphosphate (PIP3), which is produced by PI3K[120]. In MASH and HCC patients, PTEN is inactivated for various reasons, resulting in the activation of PI3K/Akt signalling[120]. Mice with a hepatocyte-specific deletion of PTEN develop MASH, which is characterized by increased SREBP-1c and lipogenic gene

expression, and eventually progress to HCC[122]. PTEN loss also promotes MASH-associated liver cancer in combination with SAV1 or TRAF3 deficiency [123,124]. IR also contributes to hepatic steatosis via increased lipolysis in visceral adipose tissue, activation of hepatic DNL, and impairment of hepatic fat oxidation and breakdown[120]. In MASLD, 'there is a notable mismatch between the ability of insulin to reduce hepatic gluconeogenesis and its ability to enhance lipogenesis. This phenomenon, known as selective insulin resistance, manifests as the failure of insulin to decrease gluconeogenesis while still promoting lipogenesis. In conditions of insulin resistance, high levels of insulin in the plasma stimulate lipogenesis via the mTORC1/SREPB1c axis[125]. Furthermore, increased plasma glucose levels, which are a result of excessive hepatic gluconeogenesis, also promote lipogenesis through the activation of ChREBP[126]. Insulin resistance can promote the expression of angiogenic factors, such as vascular endothelial growth factor (VEGF), and promote the formation of new blood vessels in the liver[127]. Insulin signalling can interact with the Ras pathway, particularly in the context of HCC[128].

Very recently, Fan W et al, have proposed the novel hepatocarcinogenesis mechanism in the pre-cirrhotic liver of patients with type 2 DM [129]. AGEs

produced by the non-enzymatic protein glycation accumulated in the matrix and increased ECM viscoelasticity but not stiffness in patients with type 2 DM. Such enhanced viscoelasticity promoted HCC cell proliferation and invasion through an integrin-β1–tensin-1–YAP mechanotransductive pathway.

7. Microbiota and HSCs in metabolic-associated HCC (fig. 3)

Alterations in microbiota have been reported in MASH and HCC patients[130]. Compared with MASLD patients without HCC, MASLD-related HCC patients exhibited an increase in the abundance of *Bacteroides* and *Ruminococcaceae* that was associated with systemic inflammation and a decrease in *Akkermansia* and *Bifidobacterium*[130,131]. Serum lipopolysaccharide (LPS) levels are also increased in HCC patients and are known to activate Toll-like receptor 4 (TLR4) signalling in hepatocytes, Kupffer cells, and HSCs, which induce inflammation and hepatocarcinogenesis[131]. Secondary bile acids produced by the gut microbiota contribute to MASH and HCC. In the liver, primary bile acids are converted to secondary bile acids, such as deoxycholic acid (DCA) and lithocholic acid. Obesity increases the circulation of DCA and lipoteichoic acid (LTA), triggering a senescence-associated secretory phenotype (SASP) in

HSCs through TLR2[132]. This leads to the secretion of inflammatory and cancer-promoting factors. Moreover, these HSCs produce prostaglandin E2 (PGE2) through COX2 activation, which inhibits the anticancer immune response. Further mechanistic research revealed that LTA induced gasdermin D-mediated release of IL-33 from senescent HSCs and activated Treg cells[133]. Taken together, these findings suggest that obesity-induced dysbiosis promotes HCC development and progression[132-134]. Interestingly, the dual roles of HSCs in hepatocarcinogenesis have been recently clarified. Cytokine-producing quiescent HSCs, especially rich in hepatocyte growth factor, played tumor suppressive roles, while activated myelofibroblastic HSCs producing type I collagen promoted HCC formation via increased stiffness and activation of TAZ and discoidin domain receptor 1 (DDR1). Further research is necessary to clarify the involvement of each HSC subpopulation in metabolic-associated HCC.

TLR-9 in Kupffer cells recruits bacterial and viral DNA and triggers the production of IL-1β, resulting in steatosis, inflammation, and fibrosis[135].

8. Conclusion

Within this scholarly review, we provide a broad overview of the epidemiological and pathogenic findings of metabolic-associated HCC, including the roles of genetics, signalling pathways, and inflammation and immune dysregulation. We also explored the impact of altered lipid metabolism and insulin resistance as well as the influence of the gut microbiota on metabolic-associated HCC. This review emphasizes the urgent need to address the increasing incidence of metabolic-associated HCC due to the increasing incidences of obesity, MASLD, and type 2 diabetes. Elucidating the genomic abnormalities, altered metabolic pathways, inflammation and immune dysregulation, and dysbiosis involved in metabolic-associated HCC is crucial for developing optimal treatments and prevention. Furthermore, there is an urgent need for advanced investigative endeavours to elucidate the complex interactions among metabolic homeostasis, genetic predispositions, and immune dynamics that orchestrate the onset and evolution of metabolic-associated HCC.

Abbreviations

HCC: Hepatocellular Carcinoma, MASLD: Metabolic Dysfunction-Associated Steatotic Liver Disease, NAFLD: Non-Alcoholic Fatty Liver Disease, ACVR2A:

Activin A Receptor Type 2A, GWAS: Genome-wide association studies, SNPs: Single Nucleotide Polymorphisms, PNPLA3: Patatin-Like Phospholipase Domain-Containing Protein 3, TM6SF2: Transmembrane 6 Superfamily Member 2, GCKR: Glucokinase Regulator, MBOAT7: Membrane Bound O-Acyltransferase Domain Containing 7, BMI: Body Mass Index, HDL-C: High-Density Lipoprotein Cholesterol, TG: Triglyceride, MASH: Metabolic Dysfunction-Associated Steatohepatitis, DM: Diabetes Mellitus, IL-6: Interleukin 6, TNF: Tumour Necrosis Factor, PAMPs: pathogen-associated molecular patterns, DAMPs: damage-associated molecular patterns, PRRs: pattern recognition receptors, KCs: Kupffer cells, CCL1, NAMs: NASH-associated macrophages, TREM2: Triggering Receptor Expressed on Myeloid cells 2, GPNMB: glycoproteins CD9 and NMB, MPO: myeloperoxidase, NETs: neutrophil extracellular traps, TANs: Tumour-associated neutrophils, CXCR: CXC motif chemokine receptor, CEACAM: carcinoembryonic antigen-associated cell adhesion molecule, MDSCs: myeloid-derived suppressor cells, IDO1: indoleamine 2,3-dioxyneganase 1, iNOS: inducible nitric oxide synthase, IFN-γ: interferon-γ, IRS-1: Insulin Receptor Substrate 1, AGEs: advanced glycation end-products, IGF-1: Insulin-Like Growth Factor 1, GHR: Growth

Hormone Receptor, GH: Growth Hormone, PTEN: Phosphatase and Tensin Homologue, PIP3: Phosphatidylinositol 3,4,5-Trisphosphate, mTORC1: Mammalian Target Of Rapamycin Complex 1, ChREBP: Carbohydrate Response Element-Binding Protein, VEGF: Vascular Endothelial Growth Factor, LPS: Lipopolysaccharide, TLR4: Toll-Like Receptor 4, DCA: Deoxycholic Acid, LTA: Lipoteichoic Acid, SASP: Senescence-Associated Secretory Phenotype, HSCs: Hepatic Stellate Cells, PGE2: Prostaglandin E2, IL-33: Interleukin 33, TLR2: Toll-Like Receptor 2, TLR-9: Toll-Like Receptor 9, IL-1β: Interleukin 1 Beta

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Conflicts of interest

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References

1. Sung H, Ferlay J, Siegel RL et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021; 71: 209-249
2. Llovet JM, Kelley RK, Villanueva A et al. Hepatocellular carcinoma. Nat Rev Dis Primers 2021; 7: 6
3. Huang DQ, El-Serag HB, Loomba R. Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol 2021; 18: 223-238
4. Llovet JM, Willoughby CE, Singal AG et al. Nonalcoholic steatohepatitis-related hepatocellular carcinoma: pathogenesis and treatment. Nat Rev Gastroenterol Hepatol 2023; 20: 487-503
5. Rinella ME, Lazarus JV, Ratziu V et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol 2023; 79: 1542-1556
6. Kucukoglu O, Sowa JP, Mazzolini GD et al. Hepatokines and adipokines in NASH-related hepatocellular carcinoma. J Hepatol 2021; 74: 442-457
7. Tokushige K. New concept in fatty liver diseases. Hepatol Res 2023; DOI: 10.1111/hepr.14004:
8. Hagstrom H, Vessby J, Ekstedt M et al. 99% of patients with NAFLD meet MASLD criteria and natural history is therefore identical. J Hepatol 2023; DOI: 10.1016/j.jhep.2023.08.026:
9. Song SJ, Lai JC, Wong GL et al. Can we use old NAFLD data under the new MASLD definition? J Hepatol 2023; DOI: 10.1016/j.jhep.2023.07.021:
10. Anstee QM, Reeves HL, Kotsiliti E et al. From NASH to HCC: current concepts and future challenges. Nat Rev Gastroenterol Hepatol 2019; 16: 411-428
11. Le MH, Yeo YH, Zou B et al. Forecasted 2040 global prevalence of nonalcoholic fatty liver disease using hierarchical bayesian approach. Clin Mol Hepatol 2022; 28: 841-850
12. Younossi ZM, Koenig AB, Abdelatif D et al. Global epidemiology of nonalcoholic

fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016; 64: 73-84

13. Yatsuji S, Hashimoto E, Tobari M et al. Clinical features and outcomes of cirrhosis due to non-alcoholic steatohepatitis compared with cirrhosis caused by chronic hepatitis C. J Gastroenterol Hepatol 2009; 24: 248-254
14. Younossi Z, Stepanova M, Ong JP et al. Nonalcoholic Steatohepatitis Is the Fastest Growing Cause of Hepatocellular Carcinoma in Liver Transplant Candidates. Clin Gastroenterol Hepatol 2019; 17: 748-755 e743
15. Dyson J, Jaques B, Chattopadyhay D et al. Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. J Hepatol 2014; 60: 110-117
16. Pais R, Fartoux L, Goumard C et al. Temporal trends, clinical patterns and outcomes of NAFLD-related HCC in patients undergoing liver resection over a 20-year period. Aliment Pharmacol Ther 2017; 46: 856-863
17. Tateishi R, Uchino K, Fujiwara N et al. A nationwide survey on non-B, non-C hepatocellular carcinoma in Japan: 2011-2015 update. J Gastroenterol 2019; 54: 367-376
18. Ertle J, Dechene A, Sowa JP et al. Non-alcoholic fatty liver disease progresses to hepatocellular carcinoma in the absence of apparent cirrhosis. Int J Cancer 2011; 128: 2436-2443
19. Chayanupatkul M, Omino R, Mittal S et al. Hepatocellular carcinoma in the absence of cirrhosis in patients with chronic hepatitis B virus infection. J Hepatol 2017; 66: 355-362
20. Zunica ERM, Heintz EC, Axelrod CL et al. Obesity Management in the Primary Prevention of Hepatocellular Carcinoma. Cancers (Basel) 2022; 14:
21. Ward ZJ, Bleich SN, Cradock AL et al. Projected U.S. State-Level Prevalence of Adult Obesity and Severe Obesity. N Engl J Med 2019; 381: 2440-2450
22. Park EJ, Lee JH, Yu GY et al. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. Cell 2010; 140: 197-208
23. Sohn W, Lee HW, Lee S et al. Obesity and the risk of primary liver cancer: A systematic review and meta-analysis. Clin Mol Hepatol 2021; 27: 157-174
24. Yan LJ, Yang LS, Yan YC et al. Anthropometric indicators of adiposity and risk of primary liver cancer: A systematic review and dose-response meta-analysis. Eur J Cancer 2023; 185: 150-163
25. Kanwal F, Kramer JR, Li L et al. Effect of Metabolic Traits on the Risk of Cirrhosis and Hepatocellular Cancer in Nonalcoholic Fatty Liver Disease. Hepatology 2020;

71: 808-819

26. Kanwal F, Kramer JR, Mapakshi S et al. Risk of Hepatocellular Cancer in Patients With Non-Alcoholic Fatty Liver Disease. Gastroenterology 2018; 155: 1828-1837 e1822
27. Rustgi VK, Li Y, Gupta K et al. Bariatric Surgery Reduces Cancer Risk in Adults With Nonalcoholic Fatty Liver Disease and Severe Obesity. Gastroenterology 2021; 161: 171-184 e110
28. Li X, Wang X, Gao P. Diabetes Mellitus and Risk of Hepatocellular Carcinoma. Biomed Res Int 2017; 2017: 5202684
29. Yang JD, Ahmed F, Mara KC et al. Diabetes Is Associated With Increased Risk of Hepatocellular Carcinoma in Patients With Cirrhosis From Nonalcoholic Fatty Liver Disease. Hepatology 2020; 71: 907-916
30. Alexander M, Loomis AK, van der Lei J et al. Risks and clinical predictors of cirrhosis and hepatocellular carcinoma diagnoses in adults with diagnosed NAFLD: real-world study of 18 million patients in four European cohorts. BMC Med 2019; 17: 95
31. Shibata T, Arai Y, Totoki Y. Molecular genomic landscapes of hepatobiliary cancer. Cancer Sci 2018; 109: 1282-1291
32. Rudolph KL, Hartmann D, Opitz OG. Telomere dysfunction and DNA damage checkpoints in diseases and cancer of the gastrointestinal tract. Gastroenterology 2009; 137: 754-762
33. Nault JC, Calderaro J, Di Tommaso L et al. Telomerase reverse transcriptase promoter mutation is an early somatic genetic alteration in the transformation of premalignant nodules in hepatocellular carcinoma on cirrhosis. Hepatology 2014; 60: 1983-1992
34. Pinyol R, Torrecilla S, Wang H et al. Molecular characterisation of hepatocellular carcinoma in patients with non-alcoholic steatohepatitis. J Hepatol 2021; 75: 865-878
35. Xu C, Xu Z, Zhang Y et al. beta-Catenin signaling in hepatocellular carcinoma. J Clin Invest 2022; 132:
36. Schulze K, Imbeaud S, Letouze E et al. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. Nat Genet 2015; 47: 505-511
37. Cancer Genome Atlas Research Network. Electronic address wbe, Cancer Genome Atlas Research N. Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma. Cell 2017; 169: 1327-1341 e1323

38. Murai H, Kodama T, Maesaka K et al. Multiomics identifies the link between intratumor steatosis and the exhausted tumor immune microenvironment in hepatocellular carcinoma. Hepatology 2022; DOI: 10.1002/hep.32573:
39. BasuRay S, Smagris E, Cohen JC et al. The PNPLA3 variant associated with fatty liver disease (I148M) accumulates on lipid droplets by evading ubiquitylation. Hepatology 2017; 66: 1111-1124
40. BasuRay S, Wang Y, Smagris E et al. Accumulation of PNPLA3 on lipid droplets is the basis of associated hepatic steatosis. Proc Natl Acad Sci U S A 2019; 116: 9521-9526
41. Anstee QM, Darlay R, Cockell S et al. Genome-wide association study of non-alcoholic fatty liver and steatohepatitis in a histologically characterised cohort(☆). J Hepatol 2020; 73: 505-515
42. Liu YL, Patman GL, Leathart JB et al. Carriage of the PNPLA3 rs738409 C >G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. J Hepatol 2014; 61: 75-81
43. Kozlitina J, Smagris E, Stender S et al. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2014; 46: 352-356
44. Liu YL, Reeves HL, Burt AD et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. Nat Commun 2014; 5: 4309
45. Perez-Martinez P, Delgado-Lista J, Garcia-Rios A et al. Glucokinase regulatory protein genetic variant interacts with omega-3 PUFA to influence insulin resistance and inflammation in metabolic syndrome. PLoS One 2011; 6: e20555
46. Kimura M, Iguchi T, Iwasawa K et al. En masse organoid phenotyping informs metabolic-associated genetic susceptibility to NASH. Cell 2022; 185: 4216-4232 e4216
47. Tan HL, Zain SM, Mohamed R et al. Association of glucokinase regulatory gene polymorphisms with risk and severity of non-alcoholic fatty liver disease: an interaction study with adiponutrin gene. J Gastroenterol 2014; 49: 1056-1064
48. Kawaguchi T, Shima T, Mizuno M et al. Risk estimation model for nonalcoholic fatty liver disease in the Japanese using multiple genetic markers. PLoS One 2018; 13: e0185490
49. Mancina RM, Dongiovanni P, Petta S et al. The MBOAT7-TMC4 Variant rs641738 Increases Risk of Nonalcoholic Fatty Liver Disease in Individuals of European Descent. Gastroenterology 2016; 150: 1219-1230 e1216

50. Donati B, Dongiovanni P, Romeo S et al. MBOAT7 rs641738 variant and hepatocellular carcinoma in non-cirrhotic individuals. Sci Rep 2017; 7: 4492
51. Tepper CG, Dang JHT, Stewart SL et al. High frequency of the PNPLA3 rs738409 [G] single-nucleotide polymorphism in Hmong individuals as a potential basis for a predisposition to chronic liver disease. Cancer 2018; 124 Suppl 7: 1583-1589
52. Riazi K, Swain MG, Congly SE et al. Race and Ethnicity in Non-Alcoholic Fatty Liver Disease (NAFLD): A Narrative Review. Nutrients 2022; 14:
53. Hassan MM, Li D, Han Y et al. Genome-wide association study identifies high-impact susceptibility loci for hepatocellular carcinoma in North America. Hepatology 9900; DOI: 10.1097/hep.0000000000000800: 10.1097/HEP.0000000000000800
54. El Jabbour T, Lagana SM, Lee H. Update on hepatocellular carcinoma: Pathologists' review. World J Gastroenterol 2019; 25: 1653-1665
55. Shah PA, Patil R, Harrison SA. NAFLD-related hepatocellular carcinoma: The growing challenge. Hepatology 2023; 77: 323-338
56. Yang YM, Kim SY, Seki E. Inflammation and Liver Cancer: Molecular Mechanisms and Therapeutic Targets. Semin Liver Dis 2019; 39: 26-42
57. Luedde T, Schwabe RF. NF-kappaB in the liver--linking injury, fibrosis and hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 2011; 8: 108-118
58. He G, Karin M. NF-kappaB and STAT3 - key players in liver inflammation and cancer. Cell Res 2011; 21: 159-168
59. Grohmann M, Wiede F, Dodd GT et al. Obesity Drives STAT-1-Dependent NASH and STAT-3-Dependent HCC. Cell 2018; 175: 1289-1306 e1220
60. Litwak SA, Pang L, Galic S et al. JNK Activation of BIM Promotes Hepatic Oxidative Stress, Steatosis, and Insulin Resistance in Obesity. Diabetes 2017; 66: 2973-2986
61. Das M, Garlick DS, Greiner DL et al. The role of JNK in the development of hepatocellular carcinoma. Genes Dev 2011; 25: 634-645
62. Portincasa P, Bonfrate L, Khalil M et al. Intestinal Barrier and Permeability in Health, Obesity and NAFLD. Biomedicines 2021; 10:
63. Heymann F, Tacke F. Immunology in the liver--from homeostasis to disease. Nat Rev Gastroenterol Hepatol 2016; 13: 88-110
64. Schuster S, Cabrera D, Arrese M et al. Triggering and resolution of inflammation in NASH. Nat Rev Gastroenterol Hepatol 2018; 15: 349-364
65. Xiong X, Kuang H, Ansari S et al. Landscape of Intercellular Crosstalk in Healthy and NASH Liver Revealed by Single-Cell Secretome Gene Analysis. Mol Cell 2019; 75: 644-660 e645
66. Daemen S, Gainullina A, Kalugotla G et al. Dynamic Shifts in the Composition of

Resident and Recruited Macrophages Influence Tissue Remodeling in NASH. Cell Rep 2021; 34: 108626

67. Seidman JS, Troutman TD, Sakai M et al. Niche-Specific Reprogramming of Epigenetic Landscapes Drives Myeloid Cell Diversity in Nonalcoholic Steatohepatitis. Immunity 2020; 52: 1057-1074 e1057
68. Hwang S, Yun H, Moon S et al. Role of Neutrophils in the Pathogenesis of Nonalcoholic Steatohepatitis. Front Endocrinol (Lausanne) 2021; 12: 751802
69. van der Windt DJ, Sud V, Zhang H et al. Neutrophil extracellular traps promote inflammation and development of hepatocellular carcinoma in nonalcoholic steatohepatitis. Hepatology 2018; 68: 1347-1360
70. Leslie J, Mackey JBG, Jamieson T et al. CXCR2 inhibition enables NASH-HCC immunotherapy. Gut 2022; 71: 2093-2106
71. Wang H, Zhang H, Wang Y et al. Regulatory T-cell and neutrophil extracellular trap interaction contributes to carcinogenesis in non-alcoholic steatohepatitis. J Hepatol 2021; 75: 1271-1283
72. Ostrand-Rosenberg S, Fenselau C. Myeloid-Derived Suppressor Cells: Immune-Suppressive Cells That Impair Antitumor Immunity and Are Sculpted by Their Environment. J Immunol 2018; 200: 422-431
73. Tang W, Zhou J, Yang W et al. Aberrant cholesterol metabolic signaling impairs antitumor immunosurveillance through natural killer T cell dysfunction in obese liver. Cell Mol Immunol 2022; 19: 834-847
74. Sutti S, Jindal A, Locatelli I et al. Adaptive immune responses triggered by oxidative stress contribute to hepatic inflammation in NASH. Hepatology 2014; 59: 886-897
75. Luo XY, Takahara T, Kawai K et al. IFN-gamma deficiency attenuates hepatic inflammation and fibrosis in a steatohepatitis model induced by a methionine- and choline-deficient high-fat diet. Am J Physiol Gastrointest Liver Physiol 2013; 305: G891-899
76. Moreno-Fernandez ME, Giles DA, Oates JR et al. PKM2-dependent metabolic skewing of hepatic Th17 cells regulates pathogenesis of non-alcoholic fatty liver disease. Cell Metab 2021; 33: 1187-1204 e1189
77. Ma C, Kesarwala AH, Eggert T et al. NAFLD causes selective CD4(+) T lymphocyte loss and promotes hepatocarcinogenesis. Nature 2016; 531: 253-257
78. Gomes AL, Teijeiro A, Buren S et al. Metabolic Inflammation-Associated IL-17A Causes Non-alcoholic Steatohepatitis and Hepatocellular Carcinoma. Cancer Cell 2016; 30: 161-175
79. Dudek M, Pfister D, Donakonda S et al. Auto-aggressive CXCR6(+) CD8 T cells

cause liver immune pathology in NASH. Nature 2021; 592: 444-449
80. Koda Y, Teratani T, Chu PS et al. CD8(+) tissue-resident memory T cells promote liver fibrosis resolution by inducing apoptosis of hepatic stellate cells. Nat Commun 2021; 12: 4474
81. Pfister D, Nunez NG, Pinyol R et al. NASH limits anti-tumour surveillance in immunotherapy-treated HCC. Nature 2021; 592: 450-456
82. Shalapour S, Lin XJ, Bastian IN et al. Inflammation-induced IgA+ cells dismantle anti-liver cancer immunity. Nature 2017; 551: 340-345
83. Bruzzi S, Sutti S, Giudici G et al. B2-Lymphocyte responses to oxidative stress-derived antigens contribute to the evolution of nonalcoholic fatty liver disease (NAFLD). Free Radic Biol Med 2018; 124: 249-259
84. Barrow F, Khan S, Fredrickson G et al. Microbiota-Driven Activation of Intrahepatic B Cells Aggravates NASH Through Innate and Adaptive Signaling. Hepatology 2021; 74: 704-722
85. Thapa M, Chinnadurai R, Velazquez VM et al. Liver fibrosis occurs through dysregulation of MyD88-dependent innate B-cell activity. Hepatology 2015; 61: 2067-2079
86. Novobrantseva TI, Majeau GR, Amatucci A et al. Attenuated liver fibrosis in the absence of B cells. J Clin Invest 2005; 115: 3072-3082
87. Faggioli F, Palagano E, Di Tommaso L et al. B lymphocytes limit senescence-driven fibrosis resolution and favor hepatocarcinogenesis in mouse liver injury. Hepatology 2018; 67: 1970-1985
88. Zhang S, Liu Z, Wu D et al. Single-Cell RNA-Seq Analysis Reveals Microenvironmental Infiltration of Plasma Cells and Hepatocytic Prognostic Markers in HCC With Cirrhosis. Front Oncol 2020; 10: 596318
89. Ward PS, Thompson CB. Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. Cancer Cell 2012; 21: 297-308
90. Sunami Y, Rebelo A, Kleeff J. Lipid Metabolism and Lipid Droplets in Pancreatic Cancer and Stellate Cells. Cancers (Basel) 2017; 10:
91. Budhu A, Roessler S, Zhao X et al. Integrated metabolite and gene expression profiles identify lipid biomarkers associated with progression of hepatocellular carcinoma and patient outcomes. Gastroenterology 2013; 144: 1066-1075 e1061
92. Sunami Y. NASH, Fibrosis and Hepatocellular Carcinoma: Lipid Synthesis and Glutamine/Acetate Signaling. Int J Mol Sci 2020; 21:
93. Esler WP, Cohen DE. Pharmacologic inhibition of lipogenesis for the treatment of NAFLD. J Hepatol 2023; DOI: 10.1016/j.jhep.2023.10.042:

94. Lambert JE, Ramos-Roman MA, Browning JD et al. Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. Gastroenterology 2014; 146: 726-735
95. Icard P, Wu Z, Fournel L et al. ATP citrate lyase: A central metabolic enzyme in cancer. Cancer Lett 2020; 471: 125-134
96. Baenke F, Peck B, Miess H et al. Hooked on fat: the role of lipid synthesis in cancer metabolism and tumour development. Dis Model Mech 2013; 6: 1353-1363
97. Calvisi DF, Wang C, Ho C et al. Increased lipogenesis, induced by AKT-mTORC1-RPS6 signaling, promotes development of human hepatocellular carcinoma. Gastroenterology 2011; 140: 1071-1083
98. Li L, Pilo GM, Li X et al. Inactivation of fatty acid synthase impairs hepatocarcinogenesis driven by AKT in mice and humans. J Hepatol 2016; 64: 333-341
99. Che L, Chi W, Qiao Y et al. Cholesterol biosynthesis supports the growth of hepatocarcinoma lesions depleted of fatty acid synthase in mice and humans. Gut 2020; 69: 177-186
100. Lally JSV, Ghoshal S, DePeralta DK et al. Inhibition of Acetyl-CoA Carboxylase by Phosphorylation or the Inhibitor ND-654 Suppresses Lipogenesis and Hepatocellular Carcinoma. Cell Metab 2019; 29: 174-182 e175
101. Dhanasekaran R, Suzuki H, Lemaitre L et al. Molecular and immune landscape of hepatocellular carcinoma to guide therapeutic decision-making. Hepatology 9900; DOI: 10.1097/hep.0000000000000513: 10.1097/HEP.0000000000000513
102. Kotronen A, Seppanen-Laakso T, Westerbacka J et al. Hepatic stearoyl-CoA desaturase (SCD)-1 activity and diacylglycerol but not ceramide concentrations are increased in the nonalcoholic human fatty liver. Diabetes 2009; 58: 203-208
103. Yamashita T, Honda M, Takatori H et al. Activation of lipogenic pathway correlates with cell proliferation and poor prognosis in hepatocellular carcinoma. J Hepatol 2009; 50: 100-110
104. Li C, Yang W, Zhang J et al. SREBP-1 has a prognostic role and contributes to invasion and metastasis in human hepatocellular carcinoma. Int J Mol Sci 2014; 15: 7124-7138
105. Regnier M, Carbinatti T, Parlati L et al. The role of ChREBP in carbohydrate sensing and NAFLD development. Nat Rev Endocrinol 2023; 19: 336-349
106. Lee SH, Lee JH, Im SS. The cellular function of SCAP in metabolic signaling. Exp Mol Med 2020; 52: 724-729
107. Kawamura S, Matsushita Y, Kurosaki S et al. Inhibiting SCAP/SREBP exacerbates

liver injury and carcinogenesis in murine nonalcoholic steatohepatitis. J Clin Invest 2022; 132:

108. Zhu L, Baker SS, Liu W et al. Lipid in the livers of adolescents with nonalcoholic steatohepatitis: combined effects of pathways on steatosis. Metabolism 2011; 60: 1001-1011
109. Greco D, Kotronen A, Westerbacka J et al. Gene expression in human NAFLD. Am J Physiol Gastrointest Liver Physiol 2008; 294: G1281-1287
110. Nath A, Li I, Roberts LR et al. Elevated free fatty acid uptake via CD36 promotes epithelial-mesenchymal transition in hepatocellular carcinoma. Sci Rep 2015; 5: 14752
111. Westerbacka J, Kolak M, Kiviluoto T et al. Genes involved in fatty acid partitioning and binding, lipolysis, monocyte/macrophage recruitment, and inflammation are overexpressed in the human fatty liver of insulin-resistant subjects. Diabetes 2007; 56: 2759-2765
112. Higuchi N, Kato M, Tanaka M et al. Effects of insulin resistance and hepatic lipid accumulation on hepatic mRNA expression levels of apoB, MTP and L-FABP in non-alcoholic fatty liver disease. Exp Ther Med 2011; 2: 1077-1081
113. Jeon SM, Chandel NS, Hay N. AMPK regulates NADPH homeostasis to promote tumour cell survival during energy stress. Nature 2012; 485: 661-665
114. Yuan H, Wen B, Liu X et al. CCAAT/enhancer-binding protein alpha is required for hepatic outgrowth via the p53 pathway in zebrafish. Sci Rep 2015; 5: 15838
115. Fang C, Pan J, Qu N et al. The AMPK pathway in fatty liver disease. Front Physiol 2022; 13: 970292
116. Iwamoto H, Abe M, Yang Y et al. Cancer Lipid Metabolism Confers Antiangiogenic Drug Resistance. Cell Metab 2018; 28: 104-117 e105
117. Huang D, Li T, Li X et al. HIF-1-mediated suppression of acyl-CoA dehydrogenases and fatty acid oxidation is critical for cancer progression. Cell Rep 2014; 8: 1930-1942
118. Zhang J, Liu Z, Lian Z et al. Monoacylglycerol Lipase: A Novel Potential Therapeutic Target and Prognostic Indicator for Hepatocellular Carcinoma. Sci Rep 2016; 6: 35784
119. Cao D, Song X, Che L et al. Both de novo synthetized and exogenous fatty acids support the growth of hepatocellular carcinoma cells. Liver Int 2017; 37: 80-89
120. Shao G, Liu Y, Lu L et al. The Pathogenesis of HCC Driven by NASH and the Preventive and Therapeutic Effects of Natural Products. Front Pharmacol 2022; 13: 944088

121. Tanaka S, Mohr L, Schmidt EV et al. Biological effects of human insulin receptor substrate-1 overexpression in hepatocytes. Hepatology 1997; 26: 598-604
122. Rajesh Y, Sarkar D. Molecular Mechanisms Regulating Obesity-Associated Hepatocellular Carcinoma. Cancers (Basel) 2020; 12:
123. Shiode Y, Kodama T, Shigeno S et al. TNF receptor-related factor 3 inactivation promotes the development of intrahepatic cholangiocarcinoma through NF-kappaB-inducing kinase-mediated hepatocyte transdifferentiation. Hepatology 2023; 77: 395-410
124. Kodama T, Yi J, Newberg JY et al. Molecular profiling of nonalcoholic fatty liver disease-associated hepatocellular carcinoma using SB transposon mutagenesis. Proc Natl Acad Sci U S A 2018; 115: E10417-E10426
125. Shimano H, Sato R. SREBP-regulated lipid metabolism: convergent physiology - divergent pathophysiology. Nat Rev Endocrinol 2017; 13: 710-730
126. Postic C, Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. J Clin Invest 2008; 118: 829-838
127. Kaji K, Yoshiji H, Kitade M et al. Impact of insulin resistance on the progression of chronic liver diseases. Int J Mol Med 2008; 22: 801-808
128. Cai W, Ma Y, Song L et al. IGF-1R down regulates the sensitivity of hepatocellular carcinoma to sorafenib through the PI3K / akt and RAS / raf / ERK signaling pathways. BMC Cancer 2023; 23: 87
129. Fan W, Adebowale K, Vancza L et al. Matrix viscoelasticity promotes liver cancer progression in the pre-cirrhotic liver. Nature 2024; 626: 635-642
130. Ponziani FR, Bhoori S, Castelli C et al. Hepatocellular Carcinoma Is Associated With Gut Microbiota Profile and Inflammation in Nonalcoholic Fatty Liver Disease. Hepatology 2019; 69: 107-120
131. Liakina V, Strainiene S, Stundiene I et al. Gut microbiota contribution to hepatocellular carcinoma manifestation in non-alcoholic steatohepatitis. World J Hepatol 2022; 14: 1277-1290
132. Yoshimoto S, Loo TM, Atarashi K et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature 2013; 499: 97-101
133. Yamagishi R, Kamachi F, Nakamura M et al. Gasdermin D-mediated release of IL-33 from senescent hepatic stellate cells promotes obesity-associated hepatocellular carcinoma. Sci Immunol 2022; 7: eabl7209
134. Loo TM, Kamachi F, Watanabe Y et al. Gut Microbiota Promotes Obesity-Associated Liver Cancer through PGE(2)-Mediated Suppression of Antitumor

Immunity. Cancer Discov 2017; 7: 522-538

135. Gabele E, Muhlbauer M, Dorn C et al. Role of TLR9 in hepatic stellate cells and experimental liver fibrosis. Biochem Biophys Res Commun 2008; 376: 271-276

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