



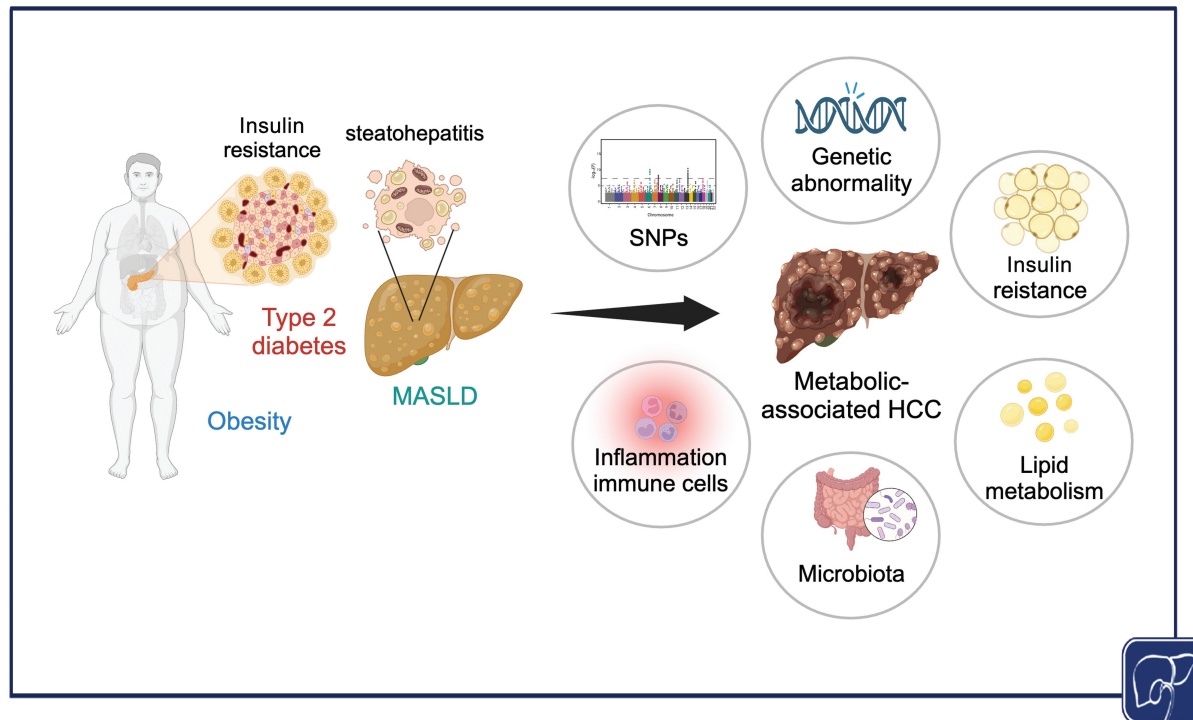
# Molecular Genealogy of Metabolic-associated Hepatocellular Carcinoma

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## Abstract

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, formerly known as nonalcoholic fatty liver disease, 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 in genes such as patatin-like phospholipase domain-containing 3, transmembrane 6

## Keywords

- ▶ MASLD
- ▶ NAFLD
- ▶ dysbiosis
- ▶ lipid metabolism
- ▶ insulin signaling

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superfamily member 2, glucokinase regulator, and membrane-bound O-acyltransferase domain-containing 7 has been observed. The tumor 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.

## 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, formerly known as nonalcoholic fatty liver disease, and type 2 diabetes. We investigated the molecular pathogenesis of this cancer and revealed that it involves certain genetic changes, including single nucleotide polymorphisms, 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.

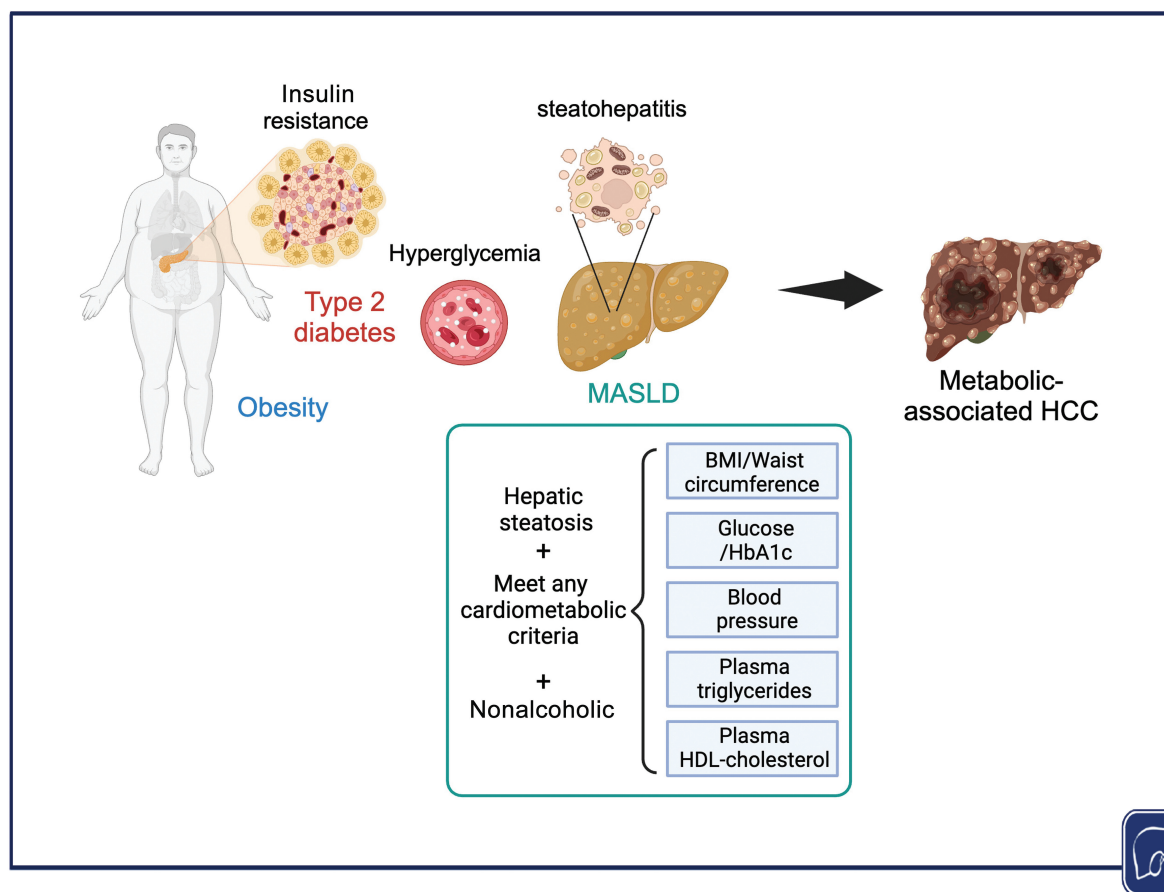
## Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver cancer and is a significant global health concern. Approximately 1 million patients are diagnosed annually with primary liver cancer, and its incidence has increased approximately two-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.<sup>1</sup> HCC exhibits significant geographic variation, with the highest incidence rates occurring in parts of Asia and sub-Saharan Africa.<sup>2,3</sup> Risk factors for HCC include chronic viral hepatitis (primarily hepatitis B and C), alcohol consumption, exposure to aflatoxins, a type of toxin produced by molds, 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.<sup>2,4,5</sup> The increasing preva-

lence 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.<sup>4</sup> 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 signaling 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.

## Epidemiology and Risk Factors in Metabolic-associated Hepatocellular Carcinoma

MASLD, obesity, and type 2 diabetes are closely and directly linked to the development of metabolic-associated HCC (► Fig. 1).<sup>6</sup> 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 cholesterol levels (► Fig. 1).<sup>5,7</sup> 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 1,000 patients examined with proton magnetic resonance spectroscopy.<sup>8,9</sup> Thus, the previous cohort data focusing on NAFLD may apply to MASLD, and to avoid confusion in this review, the new nomenclatures MASLD and metabolic dysfunction-associated steatohepatitis (MASH) were applied when introducing the previous reports of NAFLD and nonalcoholic steatohepatitis (NASH), respectively. The global prevalence of MASLD is approximately 20 to 25%, but it is projected to increase to 55% in 2040.<sup>3,10,11</sup> Approximately 20 to 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



**Fig. 1** Risk factors of metabolic-associated HCC. BMI, body mass index; HCC, hepatocellular carcinoma; HDL, high-density lipoprotein; MASLD, metabolic dysfunction-associated steatotic liver disease.

of HCC is approximately 0.44 per 1,000 person-years among MASLD patients but increases up to 2% per year among MASH-cirrhosis patients.<sup>4,12</sup> A Japanese group also reported that the 5-year incidence of HCC was 11.3% among MASH-cirrhosis patients.<sup>13</sup> 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 United States.<sup>14</sup> Similarly, the proportion of patients with HCC attributed to MASLD is continuously increasing worldwide.<sup>15–17</sup> Importantly, a substantial proportion of MASLD-related HCC patients develop a noncirrhotic liver background. Epidemiological data have shown that 80 to 90% of HCC patients, including those with all various etiologies, have cirrhosis, but this percentage decreases to 50 to 60% among MASLD-HCC patients.<sup>18</sup> Moreover, among noncirrhotic patients, MASLD increases the risk of HCC development five-fold compared to that of hepatitis C virus.<sup>19</sup>

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.<sup>20</sup> In the United States, one-third of the population was obese in 2012, but approximately half of the total population is projected to be obese by 2030.<sup>21</sup> Obesity is characterized by excess adipose tissue, which can lead to a state of chronic low-grade inflammation.<sup>6</sup> This inflammation triggers the release of tumor-promoting cytokines, such as interleukin (IL)-6 (IL-6)

and tumor necrosis factor (TNF), which can damage liver tissue and stimulate hepatocyte proliferation over time.<sup>6,22</sup> 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.<sup>6</sup> 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.<sup>23</sup> The risk is particularly elevated in individuals with central or visceral obesity, which is characterized by excess fat around the abdominal organs.<sup>24</sup> Obesity also increases the risk of HCC development 2.6-fold in the presence of diabetes, hypertension, or hyperlipidemia.<sup>3,25</sup> In the presence of MASLD, obesity (BMI  $\geq$  30) was associated with a 1.18-fold increase in the risk of HCC development, but this difference was not statistically significant ( $p = 0.06$ ).<sup>26</sup> 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.<sup>27</sup>

Individuals with type 2 diabetes often exhibit insulin resistance (IR), in which cells do not respond effectively to insulin, leading to hyperinsulinemia. IR can promote liver fat accumulation and stimulate the growth of liver cells (details

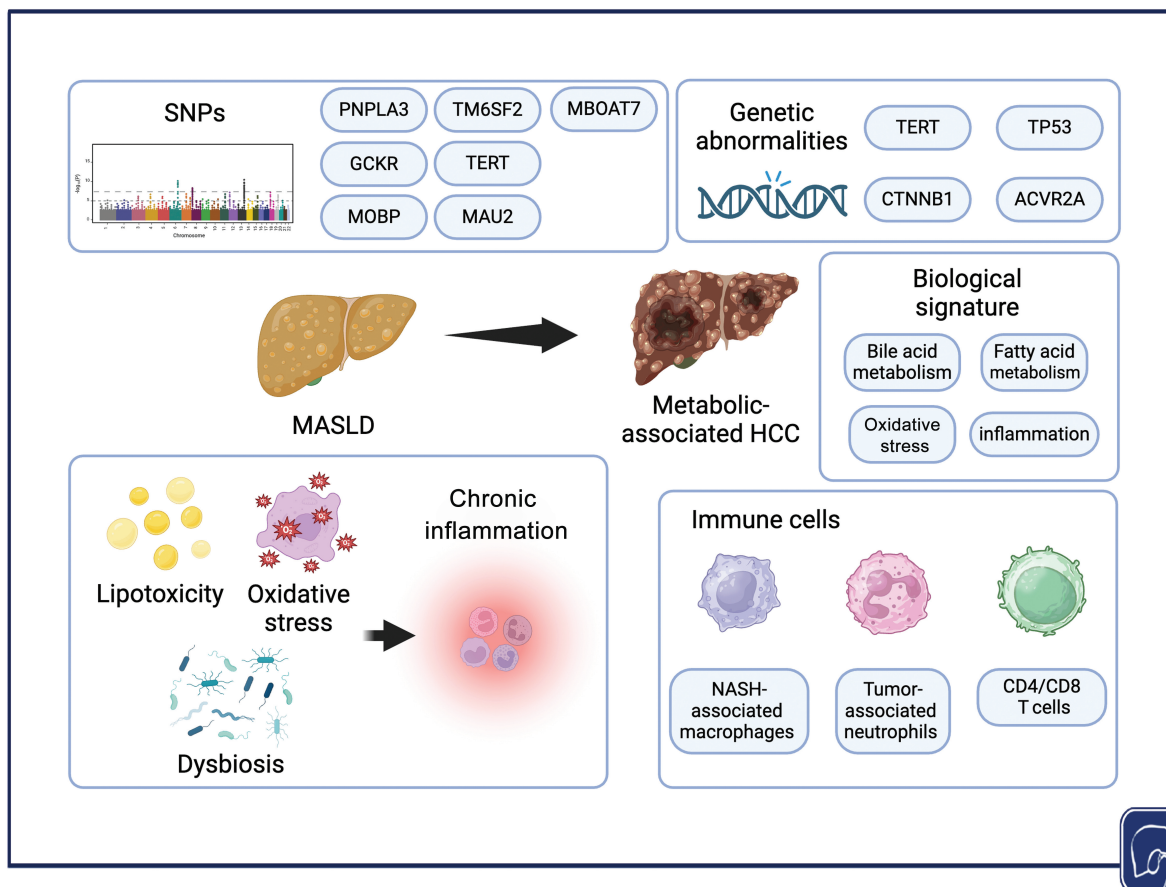
are described in chapter 7). The global prevalence of diabetes mellitus (DM) is approximately 9%, and it is projected to affect 300 to 400 million people by 2030.<sup>28</sup> Individuals with type 2 diabetes are at a significantly greater risk of developing HCC. A meta-analysis showed that people with diabetes had a 2.5-fold greater risk of HCC than those without diabetes.<sup>28</sup> Among MASH-cirrhosis patients, the presence of diabetes was associated with a 4.2-fold increase in HCC risk.<sup>29</sup> 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.<sup>26,30</sup> 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.

### Genomic Abnormalities and Molecular Pathways in Metabolic-associated Hepatocellular Carcinoma

With the advancement of next-generation sequencing technology, global collaborative projects involving cancer

genome sequences have been conducted, and genetic abnormalities in more than 1,000 HCC genomes across etiologies have been reported.<sup>31</sup> 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 three major drivers of HCC.<sup>31</sup> Telomerase activation occurs in approximately 80% of HCC cases via *TERT* promoter mutations, viral insertions, chromosomal translocations or gene amplifications, allowing hepatocytes to overcome senescence and become immortal.<sup>2,32,33</sup> In MASH-HCC, the telomere maintenance pathway was reported to be dysregulated in 56% of patients (►Fig. 2).<sup>34</sup> The WNT/ $\beta$ -catenin signaling pathway is activated in 30 to 50% of HCC cases by gain-of-function mutations in *CTNNB1* or loss-of-function mutations in *AXIN1* or *APC*.<sup>2</sup> In the absence of these mutations, cellular  $\beta$ -catenin forms a complex with *APC* and *AXIN1* and undergoes proteosomal degradation.<sup>35</sup> In the presence of these mutations,  $\beta$ -catenin avoids degradation and translocates into the nucleus. There, it activates transcription factors such as T-cell factor and the lymphoid enhancer-binding protein family, which in turn transcribe genes that positively regulate cellular survival and proliferation.<sup>35</sup> In MASH-HCC, the WNT/ $\beta$ -catenin pathway was reported to be dysregulated in



**Fig. 2** Molecular abnormality, inflammation and immune dysregulation in metabolic-associated HCC. ACVR2A, Activin A Receptor Type 2A; GCKR, glucokinase regulator; HCC, hepatocellular carcinoma; MASLD, metabolic dysfunction-associated steatotic liver disease; MBOAT7, membrane-bound O-acyltransferase domain-containing 7; PNPLA3, patatin-like phospholipase domain-containing 3; SNP, single nucleotide polymorphism; TM6SF2, transmembrane 6 superfamily member 2.

42% of patients.<sup>34</sup> The tumor-suppressive role of p53 has been ubiquitously described, but it is dysregulated in 18% of MASH-HCC patients.<sup>34</sup> In addition, genes involved in the cell cycle (*RB1*, *CCNA2*, *CCNE1*, *CCND1*, *CDKN2A*, *RPS6KA3*), oxidative stress (*NFE2L2*, *KEAP1*), chromatin remodeling (*ARID1A*, *ARID2*, *ARID1B*), TGF $\beta$  signaling (Activin A Receptor Type 2A [*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*, phosphatase and tensin homolog [*PTEN*], *PIK3CA*) are known to be dysregulated by mutations or copy number alterations at a relatively lower frequency in HCC.<sup>31,36,37</sup> Importantly, hundreds or thousands of other genes were also mutated at a very low frequency, creating tremendous intratumor and intertumor genomic heterogeneity in HCC. In general, the pattern of mutated genes does not vary significantly for each background liver disease.<sup>37</sup> 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.<sup>34</sup> We also analyzed 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.<sup>38</sup> The gene expression of *ACVR2A* was downregulated in MASH-HCC patients harboring the *ACVR2A* mutation, and its knockdown increased cellular proliferation, indicating the role of *ACVR2A* as a tumor suppressor.<sup>34</sup> 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 (FA) metabolism, oxidative stress, and inflammation in MASH-HCC patients.<sup>34</sup> 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.<sup>34</sup>

### Germline Genetic Associations for Metabolic-associated Hepatocellular Carcinoma

Genome-wide association studies (GWAS) have identified a variety of genetic variants associated with MASH and MASH-HCC (►Fig. 2). 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 TG lipolysis and promotes hepatic steatosis.<sup>39,40</sup> Individuals with this variant have a 2.05-fold greater risk of MASH than healthy control individuals<sup>41</sup> and a 2.26-fold greater risk of metabolic-associated HCC in comparison to patients with MASLD without this variant.<sup>42</sup> The presence of the transmembrane 6 superfamily member 2 (*TM6SF2*) variant rs58542926 is known to increase cholesterol and FA biosynthesis, leading to the increase in hepatic TG levels.<sup>43</sup> Individuals with this variant have a 1.61-fold greater risk of MASH than healthy control individuals<sup>41</sup> and a 1.92-fold greater risk of metabolic-associated HCC than patients with MASLD

without this variant.<sup>44</sup> The presence of the glucokinase regulator variant rs1260326 induces glycolysis, de novo lipogenesis (DNL), and IR.<sup>45</sup> This variant increases the disease severity of MASH only under diabetic conditions but protects against fibrosis under nondiabetic conditions.<sup>46</sup> Individuals with this variant have a 1.55-fold greater risk of MASH than healthy control individuals<sup>47</sup> and a 1.84-fold greater risk of metabolic-associated HCC than does the general population.<sup>48</sup> The presence of the membrane-bound O-acyltransferase domain containing 7 (*MBOAT7*) variant rs641738 increases hepatic TG levels and promotes steatosis.<sup>49</sup> Individuals with this variant have a 2.1-fold greater risk of metabolic-associated HCC than does those with MASLD without this variant.<sup>50</sup>

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%).<sup>51</sup> The *MBOAT7* rs641738 [T] variant was associated with MASLD severity in Europeans but inversely associated with obese Hispanic children in the United States.<sup>52</sup>

Very recently, GWAS on HCC was conducted in large numbers of individuals from European-descent populations.<sup>53</sup> Significant association with the risk of nonviral HCC was found in *MOBP* variant rs9842969 (odds ratio (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 nonviral HCC compared to individuals without these genotypes.

### Inflammation and Immune Dysregulation in Metabolic-associated Hepatocellular Carcinoma

Compared with HCC patients with other etiologies, MASLD-related HCC patients exhibit high levels of inflammatory markers, such as C-reactive protein and serum amyloid A.<sup>54</sup> 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.<sup>55</sup> These data indicate that inflammation may promote metabolic-associated HCC (►Fig. 2). Indeed, MASH pathogenesis includes metabolic dysfunctions, lipotoxicity, oxidative stress, and gut dysbiosis, all of which weaken an inherent immunotolerant hepatic environment, inducing chronic inflammation.<sup>10</sup> While chronic liver injury fosters cell proliferation, inflammation increases the production of reactive oxygen species (ROS). ROS induce DNA damage and mutations, promoting hepatocarcinogenesis.<sup>56</sup> The major molecular signaling pathways involved in inflammation include the nuclear factor-kappa B (NF- $\kappa$ B), Janus kinase

signal transducer and activator of transcription (JAK-STAT), and c-Jun N-terminal kinases (c-Jun-JN) pathways.<sup>56</sup> Obesity-induced inflammation triggers NF- $\kappa$ B, leading to IR through a mechanism involving phosphotyrosine signaling.<sup>57</sup> NF- $\kappa$ B also has protumorigenic effects, promoting HCC cell growth, survival, and invasion.<sup>58</sup> The JAK-STAT pathway is activated by cytokines and growth factors, affecting genes involved in cell growth and the immune response.<sup>58</sup> STAT3 is frequently activated in HCC tumors 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 and increases STAT3 signaling.<sup>59</sup> High c-Jun-JNK activity in the livers of obese patients is linked to hepatic IR and steatosis. JNK signaling, which is increased by hepatic fat accumulation, increases the expression of the B-cell lymphoma 2 family member BIM, influencing cell death.<sup>60</sup> Additionally, JNK signaling in HCC promotes tumor initiation and inflammation.<sup>61</sup>

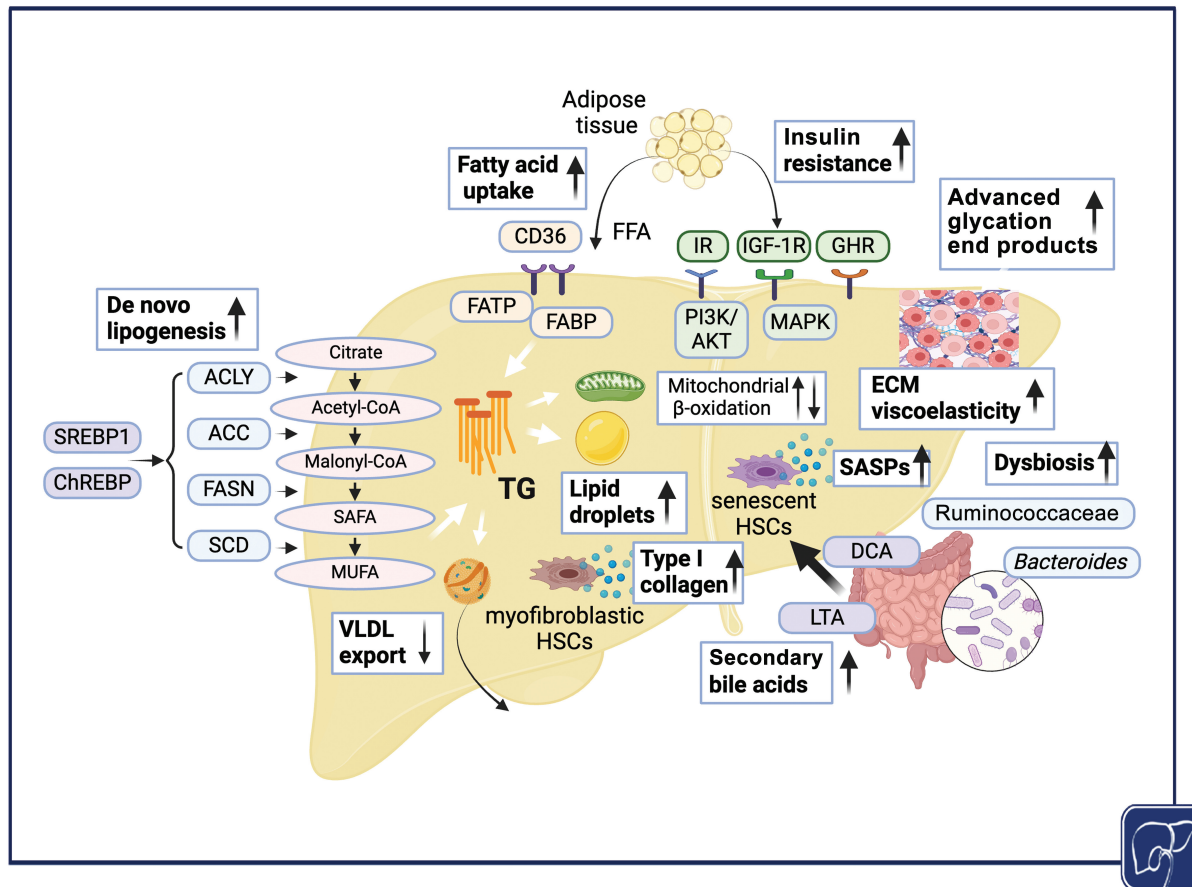
MASLD is associated with the abnormal growth of harmful bacterial strains that increase the permeability of the mucosal barrier. This disease is known as leaky gut syndrome and promotes the transfer of bacterial products to the liver.<sup>62</sup> The persistence of cellular injury and the influx of pathogen-associated molecular patterns (PAMPs) contribute to chronic inflammation in the liver, leading to fibrosis, cirrhosis, and HCC. PAMPs and damage-associated molecular patterns are sensed via pattern recognition receptors expressed on Kupffer cells (KCs) in the liver. This triggers their activation and the production of proinflammatory cytokines/chemokines, such as CCL1, TNF- $\alpha$ , and IL-1 $\beta$ , which further recruit proinflammatory immune cells, such as monocyte-derived macrophages.<sup>63,64</sup> NASH-associated macrophages (NAMs) are characterized by the expression of triggering receptor expressed on myeloid cells 2 (TREM2), the glycoproteins CD9 and NMB, and CD63. NAMs contribute to the liver inflammatory response and promote fibrosis.<sup>65–67</sup> TREM2<sup>+</sup> macrophages are abundant in the tumor 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.<sup>68</sup> Neutrophils also produce extracellular traps (NETs), which are web-like structures consisting of DNA, histones, and neutrophil proteases that sustain inflammation and promote hepatocarcinogenesis.<sup>69</sup> The number of neutrophils with an N2-like phenotype increased in the MASH-HCC microenvironment. These cells produced large amounts of TGF- $\beta$ 1, which favors the escape of cancers from immune surveillance. Tumor-associated neutrophils (TANs) isolated from MASH-HCC are specifically characterized by high expression of CXC motif chemokine receptor (CXCR) 2 (CXCR2) and carcinoembryonic antigen-associated cell adhesion molecule 8.<sup>70</sup> Interestingly, TANs associated with MASH-HCC release NETs and reprogram naïve CD4<sup>+</sup> T cells into CD4<sup>+</sup>/FOXP3<sup>+</sup> Tregs via Toll-like receptor 4 (TLR4), creating an immunosuppressive TIME.<sup>71</sup> 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-dioxygenase 1, inducible nitric oxide synthase, and ROS.<sup>72</sup> In the obese background, high levels of cholesterol induce lipid peroxidation in NKT cells and impair their tumor-suppressive effects, which is cancelled by statin treatment in an experimental mouse model.<sup>73</sup>

The contribution of T cells to the pathogenesis of MASH and MASH-HCC has been intensively studied. CD4<sup>+</sup> T cells are known to produce interferon- $\gamma$  (IFN- $\gamma$ ) in MASH patients, and its depletion ameliorates MASH in an experimental model.<sup>74,75</sup> In addition, the MASH-specific subset of hepatic Th17 CD4<sup>+</sup> T cells, named ihTh17 cells, expresses CXCR3 and many inflammatory mediators and thus exacerbate MASH.<sup>76</sup> These data suggested the role of CD4<sup>+</sup> T cells in the promotion of MASH. Regarding MASH-HCC, CD4<sup>+</sup> T-cell depletion was reported to promote MASH-HCC in mice.<sup>77</sup> Meanwhile, Foxp3<sup>+</sup> Tregs and Th17 T cells were shown to contribute to MASH-associated HCC in experimental models,<sup>71,78</sup> suggesting the complex roles of CD4<sup>+</sup> 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<sup>+</sup> T cells in MASH.<sup>79</sup> A recent report showed that exposure to metabolic stimuli, such as acetate and extracellular ATP, induce MASH-specific CXCR6<sup>+</sup>PD1<sup>+</sup>CD8<sup>+</sup> T cells in the liver, which in turn kill hepatocytes in an antigen-independent autoaggressive manner.<sup>79</sup> Meanwhile, the involvement of CD8<sup>+</sup> T cells in MASH resolution has also been recently reported.<sup>80</sup> Tissue-resident memory CD8<sup>+</sup> T cells attract activated hepatic stellate cells (HSCs) in a CCR5-dependent manner and kill these cells via Fas ligands.<sup>80</sup> These data suggest the multifaceted role of CD8<sup>+</sup> T cells in MASH pathogenesis. Regarding HCC development, further complexity was observed. Depletion of CD8<sup>+</sup> T cells limits HCC development according to many reports, but this is not always the case.<sup>81,82</sup> This may be due to the conflicting roles of CD8<sup>+</sup> T cells in the MASH liver, such as the abovementioned proinflammatory and profibrogenic roles that promote hepatocarcinogenesis and the immune surveillance roles that prevent hepatocarcinogenesis. Recent reports have shown that the number of PD-1<sup>+</sup>CD8<sup>+</sup> T cells is increased in the liver of MASH-HCC patients, and anti-PD-1 therapy induces hepatic damage rather than exert antitumor effects.<sup>25,81</sup> 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.<sup>4</sup> We have also recently found that steatotic HCC, characterized by lipid droplet formation inside tumor cells, is characterized by intratumor CD8<sup>+</sup> T-cell exhaustion, which is highly responsive to ICI and anti-vascular endothelial growth factor (VEGF) therapy in nonviral HCC patients.<sup>38</sup> Taken together, these findings suggest that a steatotic environment, regardless of whether it is intrahepatic or intratumoral, may cause T-cell exhaustion in the liver, resulting in T-cell activation upon ICI therapy.<sup>38,81</sup>

Gut dysbiosis and oxidative stress activate B cells via the myeloid differentiation primary response 88 and B-cell receptor signaling pathways, which in turn expand IFN- $\gamma$ <sup>+</sup>CD4<sup>+</sup> helper T cells and exacerbate MASH.<sup>74,83,84</sup> B cells are also known to promote profibrogenic genes, such as TGF- $\beta$ 1 and TIMP-2.<sup>85,86</sup>



**Fig. 3** Lipid metabolism, insulin signaling, and microbiota in metabolic-associated HCC. ACC, acetyl-CoA carboxylase; ACLY, ATP citrate lyase; ChREBP, carbohydrate response element-binding protein; DCA, deoxycholic acid; FABP, fatty acid-binding protein; FASN, fatty acid synthase; GHR, growth hormone receptor; HSC, hepatic stellate cell; IR, insulin resistance; LTA, lipoteichoic acid; MAPK, mitogen-activated protein kinase; MUFA, monounsaturated fatty acid; PI3K/AKT, phosphatidylinositol 3-kinase/protein kinase B; SCD, stearoyl-CoA desaturase; SREBP1, sterol regulatory element-binding protein 1; TG, triglyceride.

The presence of B cells was associated with poor prognosis in HCC patients.<sup>87</sup> IgA<sup>+</sup> plasma cells express PD-L1 and IL-10, inhibiting CD8<sup>+</sup> T-cell activation in MASH-HCC patients.<sup>82,88</sup>

### Lipid Metabolism and Hepatocellular Carcinoma

Cancer cells reprogram various metabolic pathways to construct cellular components, such as nucleic acids, proteins, and lipids; this process is known as metabolic reprogramming.<sup>89</sup> 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.<sup>90</sup> Therefore, various tumors activate DNL, which is a process by which cells produce their own FAs, and external lipid uptake regardless of the level of circulating lipids.<sup>91</sup> In addition, altered lipid metabolism is often observed in MASLD patients and is associated with changes in lipid biosynthesis and metabolism pathways (►Fig. 3).<sup>92,93</sup> Hepatic steatosis develops when the accumulation of FAs in the liver, through absorption from the bloodstream and DNL, exceeds the capacity of the liver to metabolize these fats through oxidation and

export them as VLDL TGs. In particular, de novo FA synthesis is often upregulated in MASLD patients.<sup>93,94</sup> This increase in lipogenesis may provide the necessary substrates for cell membrane formation and energy production in intrahepatic tumor 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 FA 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.<sup>95</sup> Then, acetyl-CoA carboxylase (ACC) converts acetyl-CoA into malonyl-CoA, which is a precursor for FA 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.<sup>96</sup> In HCC, these major enzymes related to DNL, including FASN, are often overexpressed, contributing to increased lipogenesis and lipid accumulation in tumor cells.<sup>92,97</sup> In an experimental mouse model, FASN deletion ameliorated HCC through either AKT activation or PTEN deletion.<sup>98,99</sup> Liver-specific ACC inhibitor (ND-654) also suppressed hepatic DNL and the

development of HCC.<sup>100</sup> A variety of inhibitors targeting DNL pathways have been tested in clinical trials for MASLD and HCC.<sup>92,101</sup> Stearoyl-CoA desaturase is the rate-limiting enzyme that converts FAs to monounsaturated FAs, and its activity is upregulated in MASLD and HCC.<sup>91,102</sup> 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,<sup>103,104</sup> and by carbohydrate response element-binding protein (ChREBP), which is a transcription factor that upregulates lipogenic and glycolytic genes.<sup>105</sup> 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.<sup>106</sup> 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.<sup>106</sup> Interestingly, Kawamura 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.<sup>107</sup> Mechanistically, SREBP inhibition suppresses LPCAT3 and increases membrane lipid saturation, which in turn decreases membrane fluidity, leading to excess ER stress in the liver.<sup>107</sup>

FA uptake and transport are also altered in MASH and HCC patients. This process is mediated by FA translocase (CD36), a cell surface receptor that facilitates the uptake of FAs, and FA transport proteins such as FATP2 and FATP5, all of which are known to be upregulated in MASLD.<sup>108,109</sup> An increase in CD36 was also related to EMT in HCC patients.<sup>110</sup> After being absorbed, hydrophobic FAs 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.<sup>111,112</sup>

The roles of FA  $\beta$ -oxidation (FAO) remain controversial. Due to the inadequate nutrient availability in the core of the tumor 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/EBP $\alpha$  and AMPK.<sup>113–115</sup> 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.<sup>116</sup> 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- and long-chain acyl-CoA dehydrogenase, both of which are rate-limiting enzymes that catalyze the first step of oxidation in mitochondria.<sup>117</sup>

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.<sup>118</sup> Yes-associated protein (YAP) activation induced MAGL expression in HCC. In addition, lipoprotein lipase is also upregulated in HCC and promotes the uptake of extracellular lipoproteins into cells via the hydrolysis of TGs.<sup>119</sup>

## Insulin Signaling and Advanced Glycation End Products in Metabolic-associated Hepatocellular Carcinoma

IR is a condition where insulin becomes less effective at managing blood glucose, leading to increased insulin production and related health issues such as hyperinsulinemia, type 2 diabetes, obesity, MAFLD, and liver fibrosis. IR and hyperinsulinemia 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 insulin receptor substrate-1 expression.<sup>120</sup> IR also increases the expression of the growth hormone receptor, which, in combination with growth hormone (GH), further increases the activation of IGF-1. Consequently, hyperinsulinemia leads to the hepatic production and release of substantial quantities of IGF-1, which promotes cell growth and prevents cell death, thereby accelerating hepatocarcinogenesis.<sup>121</sup> 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 signaling through the dephosphorylation of phosphatidylinositol 3,4,5-triphosphate, which is produced by PI3K.<sup>120</sup> In MASH and HCC patients, PTEN is inactivated for various reasons, resulting in the activation of PI3K/Akt signaling.<sup>120</sup> 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.<sup>122</sup> PTEN loss also promotes MASH-associated liver cancer in combination with SAV1 or TRAF3 deficiency.<sup>123,124</sup> 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.<sup>120</sup> 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 IR, manifests as the failure of insulin to decrease gluconeogenesis while still promoting lipogenesis. In conditions of IR, high levels of insulin in the plasma stimulate lipogenesis via the mammalian target of rapamycin complex 1/SREBP1c axis.<sup>125</sup> Furthermore, increased plasma glucose levels, which are a result of excessive hepatic gluconeogenesis, also promote lipogenesis through the activation of ChREBP.<sup>126</sup> IR can promote the expression of angiogenic factors, such as VEGF, and promote the formation of new blood vessels in the liver.<sup>127</sup> Insulin signaling can



interact with the Ras pathway, particularly in the context of HCC.<sup>128</sup>

Very recently, Fan et al, have proposed the novel hepatocarcinogenesis mechanism in the precirrhotic liver of patients with type 2 DM.<sup>129</sup> Advanced glycation end products produced by the nonenzymatic protein glycation accumulated in the matrix and increased extracellular matrix viscoelasticity but not stiffness in patients with type 2 DM. Such enhanced viscoelasticity promoted HCC cell proliferation and invasion through an integrin- $\beta$ 1-tensin-1-YAP mechanotransductive pathway.

## Microbiota and Hepatic Stellate Cells in Metabolic-associated Hepatocellular Carcinoma

Alterations in microbiota have been reported in MASH and HCC patients (►Fig. 3).<sup>130</sup> Compared with MASLD patients without HCC, MASLD-related HCC patients exhibited an increase in the abundance of *Bacteroides* and Ruminococcaceae that were associated with systemic inflammation and a decrease in *Akkermansia* and *Bifidobacterium*.<sup>130,131</sup> Serum lipopolysaccharide levels are also increased in HCC patients and are known to activate TLR4 signaling in hepatocytes, KCs, and HSCs, which induce inflammation and hepatocarcinogenesis.<sup>131</sup> 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 in HSCs through TLR2.<sup>132</sup> This leads to the secretion of inflammatory and cancer-promoting factors. Moreover, these HSCs produce prostaglandin E2 through cyclooxygenase-2 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.<sup>133</sup> Taken together, these findings suggest that obesity-induced dysbiosis promotes HCC development and progression.<sup>132–134</sup> 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. Further research is necessary to clarify the involvement of each HSC subpopulation in metabolic-associated HCC.

TLR-9 in KCs recruits bacterial and viral DNA and triggers the production of IL-1 $\beta$ , resulting in steatosis, inflammation, and fibrosis.<sup>135</sup>

## 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, signaling pathways, and inflammation and immune dysregulation. We also explored the

impact of altered lipid metabolism and IR 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 endeavors to elucidate the complex interactions among metabolic homeostasis, genetic predispositions, and immune dynamics that orchestrate the onset and evolution of metabolic-associated HCC.

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