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MASLD development: From molecular pathogenesis toward therapeutic strategies

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Abstract

Metabolic dysfunction-associated steatotic liver disease (MASLD) comprises a spectrum of liver injuries, including steatosis to steatohepatitis (MASH), liver fibrosis, cirrhosis, and relevant complications. The liver mainly comprises hepatocytes, liver sinusoidal endothelial cells (LSECs), Kupffer cells (KCs), immune cells (T cells, B cells), and hepatic stellate cells (HSCs). Crosstalk among these different liver cells, endogenous aberrant glycolipid metabolism, and altered gut dysbiosis are involved in the pathophysiology of MASLD. This review systematically examines advances in understanding the molecular pathogenesis of MASLD, with a focus on emerging therapeutic targets and translational clinical trials. We first delineate the crucial regulatory mechanisms involving diverse liver cells and the gut-liver axis in MASLD development. These cell-specific pathogenic insights offer valuable perspectives for advancing precision medicine approaches in MASLD treatment. Furthermore, we evaluate potential therapeutic targets and summarize clinical trials currently underway. By comprehensively updating the MASLD pathophysiology and identifying promising strategies, this review aims to facilitate the development of novel pharmacotherapies for this increasingly prevalent condition.

Keywords: Metabolic dysfunction-associated steatotic liver disease; Metabolic dysfunction-associated steatohepatitis; Liver fibrosis; Hepatocytes; Liver sinusoidal endothelial cells; Kupffer cells; Immune cells; Hepatic stellate cells

Introduction

Non-alcoholic fatty liver disease (NAFLD), presently known as metabolic dysfunction-associated steatotic liver disease (MASLD), accurately emphasizes steatotic liver diseases associated with metabolic disorders.[1] MASLD is a generic term of clinicopathology that ranges from metabolic dysfunction-associated steatotic liver (MASL) and metabolic dysfunction-associated steatohepatitis (MASH) to liver fibrosis, cirrhosis, and even hepatocellular carcinoma. Currently, its high prevalence and mortality make MASLD a heavy economic burden worldwide. In 2021, the global prevalence of MASLD reached 1234.7 million cases, with China accounting for 287.5 million of these cases.[2] For its high prevalence, MASLD has become the most rapidly growing cause of liver-related mortality worldwide and is emerging as an important cause of end-stage liver disease,[3] primary liver cancer,[4] and liver transplantation. Furthermore, patients with MASLD have increased risks of type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD),[5] chronic kidney disease (CKD),[6] dementia,[7] and sleep apnea.[8]

Although it has been widely studied, the pathogenesis of MASLD remains unclear. Recent findings have confirmed the multiple-hit hypothesis of MASLD, which emphasizes systemic changes related to the liver. The cross-talk between liver cells, the gut–liver axis, and the adipose tissue of multi-organs contributes to the pathogenesis of MASLD.[9] In addition, multiple factors, including endogenous cues (i.e., lipogenesis, lipotoxicity, and insulin resistance), genetic factors, endoplasmic reticulum stress, and the activation of other signaling pathways, are involved in the onset and development of MASLD. As MASLD poses a worldwide public health problem with complex pathogenesis, there is an unmet need for MASLD treatment, including lifestyle modulations and pharmacological strategies. Currently, extensive clinical trials have emerged that focusing on MASLD resolution. Many compounds are also being evaluated in ongoing phase II and III clinical trials.

This review highlights the current understanding of MASLD, mainly regarding its molecular pathogenesis,

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therapeutic targets, and translational trials. In contrast to broader reviews focusing on hepatic signaling pathways, we provide an overview of crucial regulators of different liver cells and the gut–liver axis in the pathogenesis of MASLD and subsequent liver fibrosis. These cell-specific targets will shed light on precision medicine for MASLD treatment. Ongoing clinical trials are also highlighted in this review. This review provides a comprehensive perspective on MASLD and reveals its intricate nature. MASLD-related liver cancer is not the focus of this review, which has been described in other excellent reviews.[10,11]

Molecular Pathogenesis and Key Regulators

There are two predominant liver cell types: parenchymal cells and non-parenchymal cells (NPCs). Parenchymal hepatocytes (HCs) are the predominant components of the liver, accounting for 60% of the total cell amount and 80% of the total liver volume. NPCs constitute 35% of the total cell number and 17% of the total liver volume.[12] These NPCs mainly include Kupffer cells (KCs), liver sinusoidal endothelial cells (LSECs), hepatic stellate cells (HSCs), and other immune cells. In a healthy liver, the crosstalk among these cells maintains sinusoidal homeostasis. However, abnormal communications among these cells are pivotal for liver injury and contribute to the pathogenesis of MASLD and liver fibrosis, including HC injury, inflammatory cell infiltration and dysregulation, LSEC capillarization, HSC activation, and the dysfunctional gut–liver axis.[13] Here, we summarized the key molecular mechanisms and regulators involved in these processes.

HC injury

Normal HCs are crucial for maintaining energy balance because they contain abundant mitochondria. When MASLD occurs, deviant lipid metabolism promotes HC injury and leads to fat deposition and inflammation. These HC injuries are accompanied by damage to mitochondrial DNA and mitochondrial respiratory chain proteins via AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptors (PPARs)[14] [Figure 1].

AMPK in lipid and glucose metabolism

As a highly versatile metabolic organ, the liver plays a central role in regulating systemic metabolism. In HCs, the AMPK signaling pathway maintains lipid metabolism and glucose homeostasis and plays important roles in MASLD and liver fibrosis. AMPK is a serine/threonine kinase composed of catalytic (α1 and α2) and regulatory (β1, β2, and γ1, γ2, γ3) subunits. AMPK can be activated in response to energy deficits (low ATP and reciprocally high AMP and ADP) or an increase in intracellular Ca^{2+}. Once active, AMPK influences cell metabolism (lipids, cholesterol, carbohydrates, and amino acids), mitochondrial function, autophagy, and cell growth by regulating the phosphorylation of numerous key metabolic proteins.[15]

First, AMPK can inhibit fatty acid and cholesterol synthesis by directly phosphorylating and inhibiting acetyl-CoA carboxylase (ACC) and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (HMGCR).[16,17] Cell and animal experiments showed that AMPK reduced hepatic steatosis, HC injury, and hepatocellular carcinoma by inhibiting ACC and HMGCR.[18–20] On the one hand, AMPK can inhibit the conversion of acetyl-CoA to malonyl-CoA by phosphorylating ACC1 at Ser 79 and ACC2 at Ser 212,[18] phosphorylated ACC1 and ACC2 also directly inhibit liver *de novo* lipogenesis (DNL) and hepatocellular carcinoma[20] [Figure 1]. On the other hand, AMPK phosphorylation and inhibition of HMGCR at Ser 871 can prevent cholesterol synthesis, thereby attenuating hepatic steatosis[19] [Figure 1].

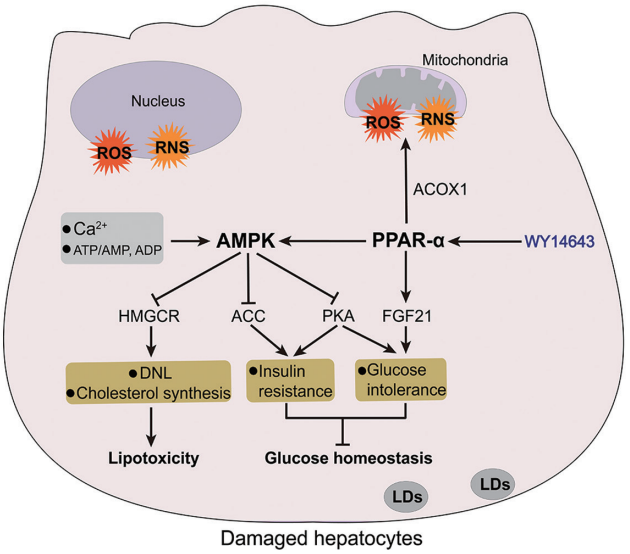

Figure 1: Roles of HCs in MASLD. In MASLD, AMPK is activated by increased levels of intracellular Ca^{2+} and decreased ATP/AMP and ADP levels. Activation of AMPK inhibits HMGCR and ACC, resulting in lipotoxicity and impaired glucose homeostasis. AMPK also antagonizes PKA activation, maintaining glucose homeostasis. ROS and RNS are produced in a PPAR-α manner. ROS and RNS can cause mtDNA, nuclear DNA damage, and lipid peroxidation. ACC: Acetyl-CoA carboxylase; ACOX1: Acyl-CoA oxidase 1; AMP: Adenosine monophosphate; AMPK: AMP-activated protein kinase; ATP: Adenosine triphosphate; DNA: Deoxyribonucleic acid; DNL: *De novo* lipogenesis; FGF21: Fibroblast growth factor 21; HCs: Hepatocytes; HMGCR: 3-Hydroxy-3-Methylglutaryl-CoA reductase; LDs: Lipid droplets; MASLD: Metabolic dysfunction-associated steatotic liver disease; mtDNA: Mitochondrial DNA; PKA: cAMP-mediated protein kinase; PPAR-α: Peroxisome proliferator-activated receptor-α; RNA: Reactive nitrogen species; ROS: Reactive oxygen species.

Second, AMPK inhibited hepatic glucose production. Genetically modified mice and more specific pharmacological studies also revealed that AMPK inhibited hepatic glucose production via both AMPK-dependent and AMPK-independent signaling pathways.[21,22] Specifically, in terms of the AMPK-dependent signaling pathway, gluconeogenesis (GNG) leads to an elevated AMP/ATP ratio.The elevated AMP/ATP ratio subsequently activates AMPK and antagonizes glucagon-stimulated glucose production and cAMP-mediated protein kinase (PKA) activation, thereby maintaining glucose homeostasis and preventing HC injury[21] [Figure 1]. Liver kinase B1 (LKB1) is a primary upstream kinase of AMPK. LKB1 phosphorylates and activates AMPK, which in turn phosphorylates the transcriptional coactivator cAMP regulatory element-binding protein (CREB)-regulated transcription coactivator 2 (TORC2), resulting in its inactivation. The LKB1–AMPK–TORC2 pathway ultimately

leads to a reduction in GNG, thereby decreasing the production of glucose in the liver.[23] While extensive research has been conducted on the role of AMPK in skeletal muscle glucose metabolism, studies focusing on its effects on hepatic glucose metabolism are relatively limited and often contentious.

PPARs in lipid metabolism and reactive oxygen species (ROS) production

PPARs are nuclear transcription factors of the steroid hormone receptor superfamily that have pleiotropic actions, and are engaged in the transcriptional regulation of glucose homeostasis, lipid metabolism, energy homeostasis, and inflammation.[24,25] The primary function of PPARs in the liver is to regulate fatty acid oxidative metabolism and energy expenditure, thereby affecting the formation of MASLD and liver fibrosis.[26,27] PPARs are ligand-activated transcription factors of the nuclear receptor superfamily with PPAR-α, PPAR-γ, and PPAR-δ isoforms.[28] Among these proteins, PPAR-α is ubiquitously expressed but predominantly expressed in the liver. PPAR-α controls lipid and body energy homeostasis in the liver by regulating three fatty acid oxidative metabolic pathways, namely, peroxisome β-oxidation, mitochondrial β-oxidation, and microsomal ω-oxidation.[26] Lipolysis in adipocytes is associated with the development of MASLD when HCs lack PPAR-α.[29] Similarly, palmitoleic acid can improve metabolic function in fatty liver in a PPAR-α-dependent manner. Palmitoleic acid stimulates glucose uptake and impairs hepatic lipogenesis by activating AMPK and fibroblast growth factor 21 (FGF21)[30] [Figure 1]. In addition to PPAR-α being mainly expressed in the liver, PPAR-γ and PPAR-δ are also differentially expressed in tissues. PPAR-γ is highly expressed in adipose tissue and regulates adipocyte differentiation and energy storage, and PPAR-δ is expressed predominantly in skeletal muscles, where it enhances fatty acid oxidative metabolism and energy uncoupling processes in skeletal muscle and adipose tissue and is involved in the hepatic stress response to starvation.[26] As PPAR-α is mainly expressed in the liver, it is presumable that PPAR-α is a crucial regulator of energy homeostasis and inflammation during MASLD and liver fibrosis.

ROS, which include free radicals and hydrogen peroxide, are byproducts of normal cellular metabolism.[26] PPARs mediate fatty acid oxidation via acyl-CoA oxidase (ACOX), which is one source of ROS. The PPAR-α agonist WY14643 can induce oxidative stress via ACOX.[31] The oxidative metabolism of ACOX1 generates a large amount of ROS and reactive nitrogen species (RNS). Both ROS and RNS have strong oxidative attack properties and can directly attack DNA, organelles, and signaling proteins[26] [Figure 1]. Furthermore, the cytochrome P450A family is another source of ROS in the liver after PPAR-α activation. Increases in catalase and glutathione peroxidase lead to sustained oxygen stress in the liver, thereby leading to oxidative DNA damage in HCs.[26] The ROS and RNS induced by PPAR-α can eventually lead to HC damage and abnormal liver function.[26]

In summary, HCs play predominant roles in glucose and lipid metabolism via the aforementioned signaling pathways. Physiologically, HCs are protected by NPCs to ensure their normal metabolism function. However, various factors lead to abnormal communication in NPCs, exposing HCs to damaging factors and, eventually, HC injuries. Therefore, in addition to protecting HCs, maintaining normal communication among NPCs is also crucial for MASLD progression.

Inflammatory cell infiltration and dysregulation

Inflammation is believed to be the driving force behind the progression of MASL to MASH. The infiltration of various inflammatory cells and inflammatory mediators plays crucial roles in MASLD pathogenesis [Figure 2]. Remarkably, enhanced infiltration of hepatic macrophages, neutrophils, and lymphocytes (T cells, B cells) and inflammasome activation in immune cells have been widely recognized as the main histological features of MASH patients.[32,33] There are complicated interactions between metabolic and immune responses in the pathogenesis and progression of MASH. Briefly, the chemokine C-C motif ligand 2 (CCL2) and C-C motif chemokine receptor 2 (CCR2) axis drive macrophage infiltration, the C-X-C motif chemokine ligand 1 (CXCL1) axis drive neutrophil infiltration, T cells and B cells dysregulation, and inflammasomes in immune cells are supposed to be the key regulators of the progression of MASLD and liver fibrosis.

CCL2–CCR2 axis for macrophage infiltration

Macrophage infiltration is one of the important features of MASLD progression.[34] Thus, deciphering the different types of macrophage infiltration and their mechanisms is an important target for intervention in MASLD. The mechanism by which the CCL2–CCR2 axis drives macrophage infiltration is being progressively deciphered.[35] Previously, CCL2 was shown to be secreted by HSCs, KCs, and macrophages.[36,37] In liver fibrosis, CCL2 was mainly expressed in LSECs.[38] CCR2, a paired receptor for CCL2, has been shown to be expressed in hepatic macrophages.[39] Upon HC or cholangiocyte injury, danger-associated molecular pattern molecules (DAMPs), such as free RNA, mitochondrial DNA, or high mobility group box 1 (HMGB1) protein, activate KCs, which secrete inflammatory cytokines, such as tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β) and release chemokines (CCL2). CCL2 and other chemokines promote the recruitment of $CCR2^{+}$ Ly-$6C^{hi}$ monocytes into the injured liver, where they develop into inflammatory, angiogenic, and fibrotic Ly-$6C^{+}$ macrophages that regulate liver injury and fibrosis[39] [Figure 2].

Targeted therapeutic options for the CCL2–CCR2 axis in MASLD are currently available.[36,40] A recent study showed that Tianhuang Formula ameliorates liver injury, inflammation, and fibrosis by downregulating the expression of the macrophage marker CD68 and inhibiting the CCL2–CCR2 axis and its downstream Mitogen-activated protein kinase (MAPK)/NF-κB signaling pathway.[36] Moreover, CCR2 inhibition by *Ccr2* knockout or cenicriviroc (CVC) has also been shown to reduce hepatic

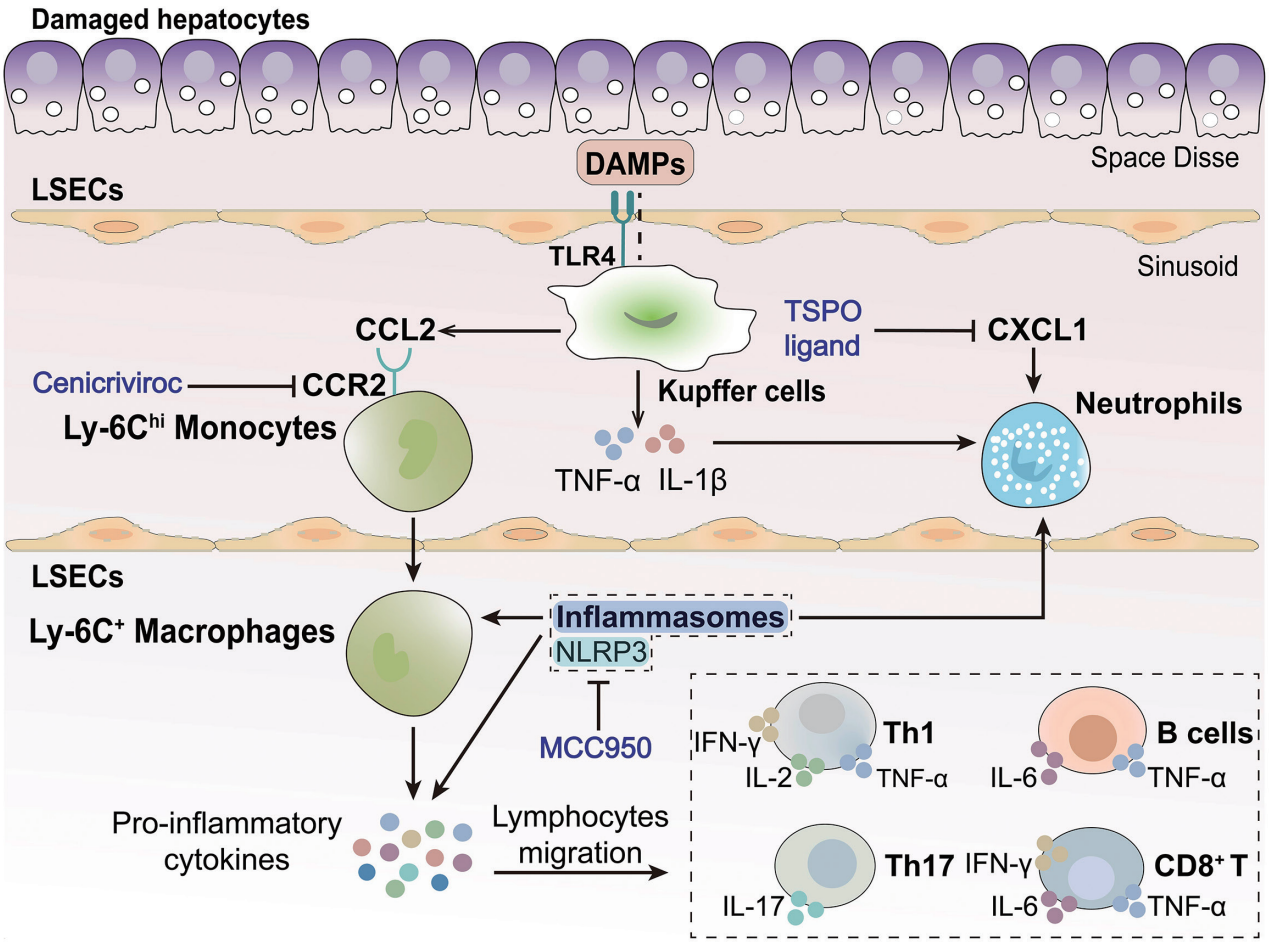

Figure 2: Roles of inflammatory cells and inflammasomes in MASLD. DAMPs activate KCs, leading to the release of TNF-α, IL-1β, and CCL2. CCL2 binding to CCR2 promotes the recruitment of CCR2+ Ly-6C^{hi} monocytes, which subsequently develop into Ly-6C+ macrophages. CXCL1 attracts neutrophils to inflammatory sites. Inflammasomes, such as NLRP3, promote the production of pro-inflammatory cytokines driving neutrophil and macrophage infiltration. The NLRP3 inhibitor MCC950 can suppress hepatic IL-1β and decrease hepatic neutrophil and macrophage infiltration. Dysregulated lymphocytes (T cells, B cells) secrete various pro-inflammatory cytokines, contributing to MASLD/MASH. CCL2: Chemokine C-C motif ligand 2; CCR2: C-C motif chemokine receptor 2; CXCL1: C-X-C motif chemokine ligand 1; DAMPs: Danger-associated molecular pattern molecules; IFN -γ: Interferon-γ; IL-1β: Interleukin 1β; KCs: Kupffer cells; LSECs: Liver sinusoidal endothelial cells; MASH: Metabolic dysfunction-associated steatohepatitis; MASLD: Metabolic dysfunction-associated steatotic liver disease; NLRP3: NOD-like receptor protein 3; Th1: T helper 1; TLR4: Toll-like receptor 4; TNF-α: Tumor necrosis factor α; TSPO: Translocator protein.

fibrosis by affecting immune cell landscapes, such as macrophages.[41] Small interfering RNA targeting CCR2 carried by a tetrahedral framework (tFNA-siCcr2) delivery system can attenuate liver fibrosis.[42]

CXCL1 axis for neutrophil infiltration

Neutrophils are also emerging players in the development and progression of MASLD. Liver biopsy revealed excessive hepatic neutrophil infiltration, a prominent histological hallmark of MASLD.[43] Therefore, understanding the mechanisms of neutrophil infiltration is crucial for MASLD intervention. Available evidence has shown that CXCL1 is a key chemokine for neutrophil infiltration and is a hallmark of MASH.[44] Specifically, CXCL1 is a potent chemokine that attracts neutrophils to sites of inflammation or infection and is regulated by the NF-κB signaling pathway[45] [Figure 2]. Furthermore, CXCL1 overexpression in the liver promotes the progression of steatosis in high-fat diet (HFD) fed mice by inducing neutrophil-derived oxidative stress and stress kinases.[44] A recent study revealed that the mitochondrial translocator protein (TSPO) ligand Atriol regulates neutrophil infiltration and attenuates MASH by down-regulating the proinflammatory chemokine CXCL1[45] [Figure 2].

T cells dysregulation

T cells broadly contain CD4+ helper T (Th) cells and CD8+ cytotoxic T cells. T helper 1 (Th1), Th2, Th17, Th22, and regulatory T (Treg) cells are functionally different subpopulations of CD4+ T cells.[46] More concretely, Th1 and Th17 cells exhibit a pro-inflammatory effect, while Treg cells may exert a suppressive effect on the inflammatory process.[46,47] A high fat and high calorie (HFHC) diet was fed in immunodeficient mice engrafted with human immune cells (HIL mice), which developed to liver inflammation, steatosis, and fibrosis with increased numbers of human CD4+ central and effector memory T cells within the liver and in the peripheral blood. Furthermore, *in vivo* exploration showed that depletion of human CD4+ T cells abrogated pro-inflammatory cytokines production and fibrosis.[48] The progression from MASL to MASH

is characterized by an elevated rate of IL-17$^+$ cells in the liver, and an increase in the ratio of Th17/resting Treg and Th2/resting Treg in peripheral blood.[49]

Cytotoxic CD8$^+$ T cells were found to be related to MASH. Hepatic lobular inflammation and ballooning were associated with the accumulation of CD8$^+$ T cells in the liver.[50] Depletion of CD8$^+$ T cells in HFHC diet-induced mice resulted in reduced MASH progression, mainly showing lower hepatic triglyceride (TG) content, lower alanine aminotransferase (ALT) levels, as well as reduced liver fibrosis, and improved MASLD activity scores.[51]

B cells dysregulation

Similar to T cells, the functional diversity of B cells also significantly contributes to the inflammation and progression in MASH. B cells contribute to producing antibodies, antigen presentation, and cytokine secretion. Liver biopsies immunostaining of MASLD patients showed that B-cell accumulations were evident within cell aggregates rich in T-lymphocytes.[52] Pro-inflammatory B cells are characterized by increased secretion of IL-6 and TNF-α accumulated in the MASH liver. However, B-cell deficiency ameliorated inflammation and fibrogenesis during MASH.[53]

Inflammasomes in immune cells

Despite contributing to hepatocellular injury, the inflammasome is also crucial for inflammatory cell infiltration. Inflammasomes promote the production of proinflammatory cytokines such as IL-1β and IL-18, which drive neutrophil and macrophage infiltration[54,55] [Figure 2]. The specific NOD-like receptor protein 3 (NLRP3) inhibitor MCC950 can suppress hepatic caspase-1 and IL-1β and decrease hepatic neutrophil and macrophage infiltration. Eventually, MCC950 alleviated liver inflammation and fibrosis in MASH mice. The pro-infiltration effect of the inflammasome was also observed in cultured cells. *In vitro*, cholesterol crystals activated KCs and macrophages to release IL-1β, while MCC950 reversed this effect and promoted neutrophil migration.[56] Thus, the inflammasome activation, both in HCs and immune cells, is a key regulator of the progression of MASLD and liver fibrosis.

In summary, immune cells are the primary sources of inflammatory molecules for MASLD progression. Current research on MASLD focuses more on macrophages and neutrophils, with relatively less attention paid to T and B cells. Recent single-cell RNA-sequencing and spatial genomics data have revealed that these immune cells can often be categorized into subgroups that regulate the progression of MASLD and fibrosis. Therefore, characterizing these specific subgroups and clarifying their crucial functional molecules may provide new insights for the prevention and treatment of MASLD.

LSEC capillarization

LSECs are specialized endothelial cells with transcellular fenestrae that lack a basement membrane. LSECs localize at the interface between the circulation and the liver parenchyma, determining their predominant role in communicating with other liver cells, exchanging substances, and transporting macromolecules.[57] LSECs play pivotal roles in maintaining intrahepatic sinusoidal homeostasis by regulating vascular tone, inflammation, and thrombosis.[57] During MASLD progression, LSECs lose fenestrae and develop a basement membrane, namely, capillarization [Figure 3]. Capillarized LSECs

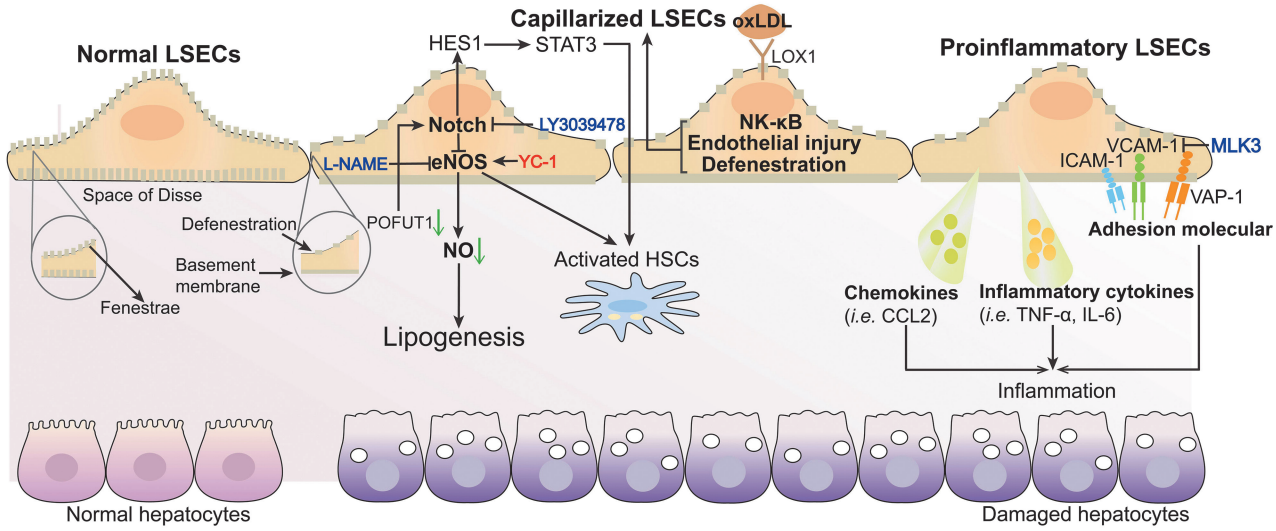

Figure 3: Roles of LSECs in MASLD. Normal LSECs are characterized by the absence of a basement membrane and the presence of open fenestrae, while capillarized LSECs lose fenestrae and develop a basement membrane. During capillarization, eNOS activity is diminished with low production of NO. The Notch-eNOS signaling pathway in LSECs contributes to liver fibrosis. POFUT1 downregulation aggravates liver fibrosis via the Notch/HES1/STAT3 signaling axis. Excessive oxLDL promotes LSEC capillarization via NF-κB activation, endothelial injury, and defenestration. Proinflammatory LSECs overexpress adhesion molecules, chemokines, and cytokines and accelerate inflammation. CCL2: Chemokine C-C motif ligand 2; eNOS: Endothelial NO synthase; HES1: Hairy and enhancer of split 1; HSCs: Hepatic stellate cells; ICAM-1: Intercellular adhesion molecule-1; IL-6: Interleukin 6; L-NAME: N-nitro-L-arginine methyl ester; LOX1: Lectin-like oxLDL receptor 1; LSECs: Liver sinusoidal endothelial cells; MASLD: Metabolic dysfunction-associated steatotic liver disease; NF-κB: Nuclear factor Kappa-light-chain-enhancer of activated B cells; NO: Nitric oxide; Notch: Neurogenic locus notch homolog protein; oxLDL: Oxidized low-density lipoprotein; POFUT1: Protein O-fucosyltransferase 1; STAT3: Signal transducer and activator of transcription 3; TNF-α: Tumor necrosis factor α; VAP-1: Vascular adhesion protein-1; VCAM-1: Vascular cell adhesion molecule-1.

lead to dysfunctional sinusoidal homeostasis and promote steatosis, inflammation, and fibrosis. Thus, the roles of capillarized LSECs and proinflammatory LSECs in the pathogenesis of MASLD and liver fibrosis are summarized in this section.

Capillarized LSECs

LSEC capillarization appears very early in MASLD, estimated one week after a choline-deficient, L-amino acid-defined (CDAA) diet in mice[58] and three weeks after a HFD in rats.[59] Nitric oxide (NO) is pivotal for the maintenance of LSEC phenotypes.[60] The fenestrated LSEC phenotype can be maintained via both NO-dependent and NO-independent pathways.[61] Endothelial NO synthase (eNOS) is the major enzyme that synthesizes NO in endothelial cells. During capillarization, the eNOS protein is unchanged, but eNOS activity is diminished with low production of NO owing to increased binding to caveolin, causing eNOS dysfunction and subsequent impairment of the NO-dependent pathway[61] [Figure 3]. Capillarization was observed in a methionine-choline-deficient (MCD) diet-induced MASH mouse model. In this model, endothelial-specific Notch signaling pathway activation can inhibit eNOS transcription.[62] Both the pharmacological eNOS activator YC-1 and the Notch inhibitor LY3039478 can restore LSEC homeostasis and alleviate hepatic steatosis and lipid accumulation[62] [Figure 3]. Additionally, LSECs are regarded as scavengers that internalize oxidized low-density lipoprotein (oxLDL) via lectin-like oxLDL receptor 1 (LOX1), which leads to NF-κB activation, endothelial injury, and defenestration[63] [Figure 3]. Therefore, LSEC capillarization appears very early in MASLD and contributes to MASLD via the Notch–eNOS–NO and NF-κB signaling pathways.

Besides, capillarized LSECs can also promote liver fibrosis by activating neighboring HSCs and promoting their differentiation into a profibrotic phenotype. As previously mentioned, eNOS dysfunction promotes LSEC capillarization in the initial stages of MASLD. LSEC capillarization contributes to liver fibrosis, partly because capillarized LSECs lose the ability to maintain HSC quiescence. Inhibiting eNOS by N-nitro-L-arginine methyl ester (L-NAME) in LSECs abolished the LSEC-mediated suppression of HSC activation.[64] The Notch-eNOS signaling pathway in LSECs also contributes to liver fibrosis [Figure 3]. Endothelial Notch activation aggravated hepatic steatosis, inflammation, and liver fibrosis. In contrast, inhibition of Notch signaling by endothelial-specific recombination signal-binding protein for immunoglobulin kappa J region (RBP-J) knockout in MCD diet-induced MASH mice attenuated basement membrane deposition and induced the formation of more fenestrae, eventually reducing lipid deposition, inflammation, and fibrosis.[62] The protein O-fucosyltransferase 1 (POFUT1) is an essential regulator of the Notch signaling pathway. POFUT1 aggravates injury-induced liver fibrosis by inducing fibrinogen expression in LSECs via the Notch/HES1/STAT3 signaling axis[65] [Figure 3]. Currently, Notch-eNOS-mediated LSEC capillarization is a key mediator of MASLD and liver fibrosis.

Proinflammatory LSECs

During MASLD, LSECs can also exhibit a proinflammatory phenotype, which triggers an inflammatory response and the formation of inflammatory foci.[66–68] Proinflammatory LSECs are characterized by the overexpression of adhesion molecules on their surface, including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and vascular adhesion protein-1 (VAP-1) [Figure 3]. VCAM-1 was the most upregulated adhesion molecule in mouse and human MASH livers. In comparison, VCAM-1 endothelial cell-specific deletion reduces hepatic injury, inflammation, and fibrosis in murine MASH mice. Mechanistically, VCAM-1 in LSECs was reduced by the mitogen-activated protein 3 kinase (MAP3K) mixed lineage kinase 3 (MLK3) inhibitor URMC-099 *in vitro* and in MLK3-deficient MASH mice[66] [Figure 3]. Moreover, increased production of proinflammatory molecules such as TNF-α, IL-6, IL-1, and CCL2 has also been confirmed in activated LSECs of MASH mice.[69] Both CCL2 and its receptor CCR2 are pivotal for inducing the expression of adhesion molecules and recruiting macrophages to inflammatory areas, ultimately accelerating the progression of isolated steatosis to steatohepatitis[70] [Figure 3]. Collectively, both capillarized and proinflammatory LSECs can promote MASLD and liver fibrosis via crucial signaling pathways, including the Notch-eNOS, MLK3-VCAM-1, and CCL2-CCR2 [Figure 3].

In summary, LSECs serve as the first gatekeeper against toxic and inflammatory molecules in the liver. Protecting the normal function of LSECs can potentially reduce the activation of pro-inflammatory and pro-fibrotic angiocrine signaling pathways, thereby decreasing inflammatory cell infiltration, HSC activation, and HC damage. Consequently, targeting dysfunctional LSECs and LSEC-mediates angiocrine signaling may be a new direction for developing therapeutic targets in MASLD and fibrosis in the future.

HSC activation

HSCs play a prominent role in hepatic fibrogenesis via various signaling pathways in MASLD [Figure 4]. Quiescent HSCs can be activated and transdifferentiated into myofibroblasts in response to certain profibrotic stimuli.[71] During MASH, activated HSCs are the primary source of the hepatic extracellular matrix (ECM), which is predominantly composed of type I and III collagen. In addition, HSCs also secrete proinflammatory cytokines and chemokines that recruit immune cells to sites of inflammation.[72] Transforming growth factor β (TGF-β), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) are the key regulators of HSCs.

TGF-β

TGF-β is a crucial cytokine released by several liver cells that initiates the activation of HSCs and drives the process of liver fibrosis. The transcription factor small mothers against decapentaplegic proteins (SMADs) are essential intracellular effectors of the TGF-β signaling pathway[73]

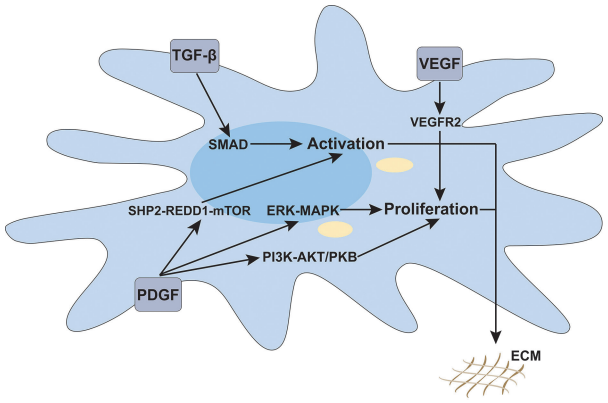

Figure 4: Roles of HSCs in MASLD. In HSCs, TGF-β acts through the SMAD pathway to promote HSC activation. PDGF induces ERK-MAPK and PI3K-Akt/PKB signaling, which promotes HSC proliferation.PDGF also induces HSC activation through the SHP2–REDD1–mTOR pathway via EV release. VEGF induces the proliferation of HSCs via VEGFR2. AKT/PKB: Protein kinase B; ECM: Extracellular matrix; ERK: Extracellular signal-regulated protein kinase; EV: Extracellular vesicles; HSCs: Hepatic stellate cells; MAPK: Mitogen-activated protein kinase; MASLD: Metabolic dysfunction-associated steatotic liver disease; mTOR: Mechanistic target of rapamycin; PDGF: Platelet-derived growth factor; PI3K: Phosphatidylinositol 3-kinase; REDD1: Regulated in development and DNA damage response 1; SHP2: Src homology 2-containing protein tyrosine phosphatase 2; SMAD: Small mothers against decapentaplegic protein; TGF-β: Transforming growth factor β; VEGF: Vascular endothelial growth factor; VEGFR2: Vascular endothelial growth factor receptor 2.

[Figure 4]. In the TGF-β/SMAD signaling pathway, TGF-β1 activates the TGF-β receptor type 1 kinase, which results in the phosphorylation of SMAD2 and SMAD3, ultimately leading to the transcription of target genes, including *Col1a1* and *Pai1*.[74,75] TGF-β1 may play a role in the development of MASH-related advanced fibrosis. The TGF-β1/pSMAD2/SP3-M1 pathway leads to oxidative DNA damage in activated HSCs from patients with MASH.[76] Additionally, crosstalk between PPAR-γ and the TGF-β1-SMAD signaling pathway increases HSC activation, inflammation, and liver fibrosis in MCD diet-fed mice.[77] TGF-β and several signaling pathways may synergistically promote MASLD and liver fibrosis.

PDGF

PDGF is a critical mitogen that drives HSC proliferation and migration.[78] PDGF is a dimeric glycoprotein that includes PDGF-A, PDGF-B, PDGF-C, and PDGF-D and can activate HSCs by binding to their PDGFR-α and PDGFR-β receptors. Liver phosphoenolpyruvate carboxykinase 1 (PCK1) deficiency triggers PDGF-A secretion to exacerbate MASLD progression via the RhoA/PI3K/AKT signaling pathway.[79] In obese patients with T2DM, hypomethylation of the CpG site at the *PDGFA* gene subsequently leads to PDGF-A overexpression. Eventually, it contributes to hepatic insulin resistance and MASLD.[80] Additionally, the binding of PDGF-B and PDGF-D to PDGFR-β can promote HSC proliferation via phosphorylation of extracellular signal-regulated protein kinase/MAPK (ERK/MAPK) and protein kinase B (PKB) within the phosphoinositide-3-kinase (PI3K) signaling pathway[81] [Figure 4]. Moreover, PDGF can also promote HSC activation and liver fibrosis through Src homology 2-containing protein tyrosine phosphatase 2-regulated in development and DNA damage response 1-mechanistic target of rapamycin (SHP2-REDD1-mTOR) pathway-dependent extracellular vesicle (EV) release[82] [Figure 4].

VEGF

VEGF is mainly released from LSECs and HSCs in the liver. VEGF can bind to its receptor (VEGFR) to exert dual effects on both the progression and regression of liver fibrosis.[78] In liver fibrosis progression, VEGF promotes fibrosis through multiple mechanisms, including the promotion of inflammation and direct effects on HSCs.[83] Additionally, VEGF induces both angiogenesis and the proliferation of HSCs in the injured hepatic parenchyma.[78] VEGFR2 blockage attenuates steatosis and inflammation in a diet-induced mouse MASH model.[84] In addition, inhibition of VEGF-B signaling prevents MASLD development by targeting lipolysis in white adipose tissue.[85] Conversely, VEGF can also lead to regression of liver fibrosis. VEGF derived from myeloid cells has been demonstrated to be a critical regulator of ECM degradation. However, myeloid cell VEGF deletion or VEGFR2 inhibition attenuates liver fibrosis.[86] VEGF can also increase liver sinusoidal permeability, which results in monocyte migration and scar-associated macrophage (SAM) accumulation to promote fibrosis resolution via the antifibrotic chemokine CXCL9 and metalloproteinase 13 (MMP13).[87]

Generally, TGF-β, PDGF, and VEGF are critical regulators of HSC activation and liver fibrosis. Other key molecules and pathways involved in HSC activation include connective tissue growth factor (CTGF), the hedgehog (Hh) pathway, and the Notch pathway.[12,72] These key molecules that activate HSCs, such as TGF-β, PDGF, and VEGF, are primarily produced by LSECs and inflammatory cells like macrophages. Thus, it is presumable that HSC activation is a secondary event following LSEC-mediates angiocrine signaling and immune cell infiltration. Therefore, when preventing HSC activation, we should focus more on LSECs and inflammatory cells to keep hepatic sinusoidal homeostasis.

Dysfunctional gut–liver axis

The liver is the largest parenchymal organ of the human body and is composed of parenchymal HCs and NPCs that regulate substance metabolism. Another essential hub for maintaining systemic homeostasis is the crosstalk between the liver and gut, which is anatomically and physiologically linked and termed the "gut–liver axis." The gut–liver axis reflects collective interactions across the liver, gastrointestinal tract, and gut microbial communities.[88] The gut–liver axis, which is connected by portal circulation, the bile tract, and systemic circulation, is increasingly recognized as an important pathway for host metabolic function and the initiation and progression of liver diseases.[89,90] Several mechanisms may explain how alterations in the gut–liver axis modulate MASLD, including metabolites and altered bile acid (BA) profiles produced by the gut microbiome, as well as a damaged gut barrier and increased intestinal permeability resulting in endotoxemia and inflammation.[91] The mechanisms

underlying MASLD and its progression are multifactorial and mainly involve cellular interactions in the liver and an imbalanced gut–liver axis involving various signaling pathways, which includes the gut microbiome, BAs, lipopolysaccharide (LPS)/pathogen-associated molecular pattern (PAMP)-Toll-like receptor 4 (TLR4), and FGF19-FGF receptor 4 (FGFR4) pathways [Figure 5].

The gut microbiome

The gut–liver axis is pivotal for mediating the gut microbiota during the progression of MASLD. Under normal conditions, the gut microbiota plays critical physiological roles in host digestion, immunity, and metabolism. Gut microbiota dysbiosis, which refers to disrupted gut microbiota, contributes to MASLD pathogenesis. Clinical investigations have compared the gut microbiome of patients suffering from MASLD caused by depletion of anti-inflammatory bacteria (including *Ruminococcaceae* and *Coprococcus*) and enrichment of pro-inflammatory bacteria (including *Fusobacterium* and *Escherichia*) with that of healthy controls.[92] Another study recruiting 25 MASLD patients and 22 healthy individuals verified that *Proteobacteria* and *Fusobacteria phyla* were more abundant in MASLD patients and had a lower abundance of *Prevotella* than in healthy controls.[93] Consistently, gut dysbiosis contributes to the pathogenesis of MASLD and liver fibrosis. Additionally, fecal microbiota transplantation (FMT) from MASH patients and healthy controls to germ-free mice fed a standard diet or high fat diet (HFD) showed that the gut microbiota from MASH patients exacerbates hepatic steatosis and inflammation.[94]

The gut microbiota can produce various metabolites involved in MASLD pathogenesis, including short-chain fatty acids (SCFAs), lipopolysaccharide (LPS), bile acids (BAs), choline, trimethylamine-N-oxide, and ammonia.[95] In a recent study, the gut microbiome *Bacteroides xylanisolvens* attenuated smoking-related MASH by degrading gut nicotine.[96] Consistently, in liver tissue biopsies of MASLD patients, several microbial-derived BAs, including 3-succinylated cholic acid (3-sucCA), are negatively associated with liver damage.[97] Gut dysbiosis results in a more permeable bowel, and this increased gut permeability plays a predominant role in the metabolism and transportation of the abovementioned substances. Moreover, increased gut permeability is due to the loss of intestinal barrier integrity and impaired intercellular tight

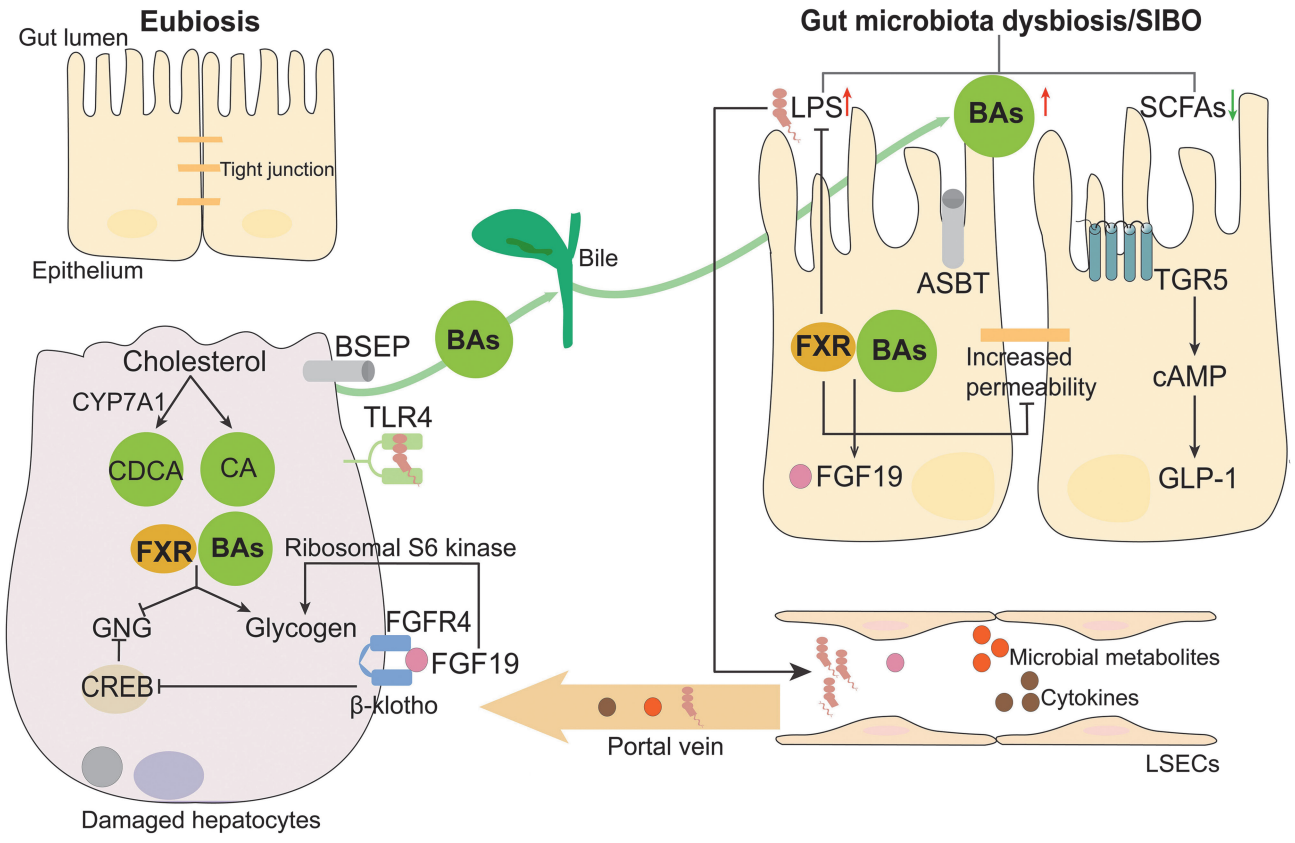

Figure 5: Roles of the gut–liver axis in MASLD. Dysbiosis of the gut microbiota renders the bowel more permeable, allowing the transport of LPS, BAs, and SCFAs. BAs are synthesized from cholesterol in the liver and metabolized in the intestine by the gut microbiome. FXR activation modulates bile synthesis and subsequently promotes glycogen but inhibits GNG. TGR5 activation induces intestinal GLP-1 release and maintains glucose homeostasis and insulin sensitivity. LPS is recognized by TLR4 and is associated with insulin resistance. TGR5 induces intestinal GLP-1 release. FGF19 binding to FGFR4 not only with a cofactor (β-Klotho) inhibits CREB phosphorylation and GNG but also propagates the Ras/ERK/p90 ribosomal S6 kinase signaling pathway and stimulates glycogen synthesis. ASBT: Apical sodium-dependent bile acid transporter; BAs: Bile acids; BSEP: Bile salt export pump; CA: Cholic acid; cAMP: Cyclic adenosine monophosphate; CDCA: Chenodeoxycholic acid; CREB: cAMP regulatory element-binding protein; CYP7A1: Cytochrome P450 7A1; FGF19: Fibroblast growth factor 19; FGFR4: Fibroblast growth factor receptor 4; FXR: Farnesoid X receptor; GLP-1: Glucagon-like peptide-1; GNG: Gluconeogenesis; LPS: Lipopolysaccharide; LSECs: Liver sinusoidal endothelial cells; MASLD: Metabolic dysfunction-associated steatotic liver disease; SCFAs: Short-chain fatty acids; SIBO: Small intestinal bacterial overgrowth; TGR5: Takeda G protein-coupled receptor 5; TLR4: Toll-like receptor 4.

junctions in the intestine.[92,98] Generally, gut microbiota dysbiosis can promote MASLD by leading to a leaky gut, which allows the delivery of various metabolites and inflammatory factors produced by the gut microbiota into the liver.

Bile acids

BAs are synthesized from cholesterol in the liver and metabolized in the intestine by the gut microbiome. BAs are categorized into primary BAs, which include chenodeoxycholic acid (CDCA) and cholic acid (CA), and secondary BAs, which include deoxycholic and lithocholic acids[99] [Figure 5]. The gut microbiome converts primary BAs into more hydrophobic secondary BAs via deconjugation and dihydroxylation.

Alterations in the amount and composition of BAs were associated with MASLD. In patients with MASLD, plasma levels of total BAs are increased and are related to histopathological and genetic determinants of hepatic steatosis progression.[100] Meanwhile, changes in BA transporter expression in the liver and ileum also contribute to MASLD. Both the ATP-binding cassette (ABC) and solute carrier (SLC) families of BA transporters are involved in maintaining the efficient enterohepatic circulation of BAs.[101] The bile salt export pump (BSEP) encoded by the *Abcb11* gene is a major canalicular bile salt transporter that plays an important role in regulating enterohepatic circulation and hepatobiliary lipid metabolism [Figure 5].[102] Several studies have verified that hepatic overexpression of BSEP prevents hepatic lipid accumulation in mice and that decreased BSEP expression is associated with the severity of MASLD.[91,102]

In addition to preserving the balance of enterohepatic circulation via transporters, BAs have also been found to regulate hepatic lipid and glucose metabolism through different receptors.[103] In particular, secondary BAs exert broad metabolic effects via farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5) signaling pathways [Figure 5]. FXR is a BA-activated nuclear receptor highly expressed in the liver and intestine, its activation regulates hepatic bile synthesis, epithelial barrier function, and systemic GNG. In the liver, FXR is protective and helps alleviate steatosis, inflammation, and fibrosis.[104] Consistently, the FXR agonist WAY-362450 markedly reduced hepatic triglyceride accumulation, improved intestinal barrier function, and decreased portal endotoxin levels and circulating TNF-α concentrations in a high-fructose diet mouse MASH model.[105] Controversially, FXR-null mice exhibit severe fatty liver with elevated circulating fatty acids (FFAs) due to elevated serum glucose and impaired glucose and insulin tolerance.[106] Furthermore, FXR activation has been shown to alleviate liver fibrosis. An *in vivo* study showed that the FXR agonist obeticholic acid prevents hepatic fibrosis progression, reverses fibrosis, decreases intrahepatic vascular resistance, and improves portal hypertension.[107] TGR5 is a G protein-coupled BA receptor expressed in brown adipose tissue and muscle. TGR5 increases energy expenditure and attenuates diet-induced obesity. The activation of TGR5 induces intestinal glucagon-like peptide-1 (GLP-1) release, thereby preventing weight gain and hepatic steatosis. TGR5 also protects liver and pancreatic function and maintains glucose homeostasis and insulin sensitivity [Figure 5].[108] Additionally, BAs can promote GLP-1 secretion via TGR5 in a murine enteroendocrine cell line (STC-1) in a dose-dependent manner.[109] Collectively, BAs may contribute to MASLD and liver fibrosis via BA transporters and receptors.

LPS/PAMPs-TLR4

A damaged intestinal barrier is an important prerequisite for transferring highly conserved microbial molecules called pathogen-associated molecular patterns (PAMPs) to the liver. PAMPs are recognized by innate immune receptors, such as Toll-like receptors (TLRs) and NOD-like receptors (NLRPs), in the host and subsequently trigger innate immune responses.[110] Endotoxin is a prototypic PAMP, and LPS is the active component of endotoxin derived from the overgrowth of Gram-negative bacteria.[89] TLR4 can recognize LPS, and the LPS–TLR4 signaling pathway is involved in the inflammation of MASLD. Compared with those in healthy volunteers, the serum levels of TNF-α, endotoxin, and insulin were significantly increased in MASLD patients. In addition, MASLD patients with small intestinal bacterial overgrowth (SIBO) presented significantly increased endotoxin levels, CD14 transcript expression, and TLR4–NF-κB signaling pathway activation.[111] Activation of the LPS–TLR-4 signaling pathway is associated with insulin resistance and MASH [Figure 5].[112]

FGF19–FGFR4

FGFs consist of 22 evolutionarily expressed signaling proteins that regulate diverse biological processes, including cell growth and differentiation, embryonic development, angiogenesis, and wound repair.[113] Importantly, FGF19 is a gut hormone. FGF19 inhibits BA synthesis from cholesterol via cytochrome P4507A1 and insulin-induced hepatic lipogenesis.[114] Lower FGF19 levels may precede MASLD development. FGF19 plays a pivotal role in MASLD by regulating hepatic BA, lipid, and glucose metabolism.[115] Patients with MASLD exhibit lower circulating FGF19 levels than those without MASLD.[116–118] Functionally, FGF19 performs its physiological functions by binding to FGFR4 and requires β-Klotho as a cofactor [Figure 5]. Lower FGF19 levels and elevated hepatic β-Klotho and FGFR4 expression were confirmed in humans with MASH.[119] Additionally, FGF19 has been identified as an important regulator of GNG, which may be the underlying mechanism of glycometabolism in MASLD. In this process, FGF19 binding to FGFR4 in the liver propagates the Ras/ERK/p90 ribosomal S6 kinase signaling pathway and stimulates protein and glycogen synthesis[120] [Figure 5]. Meanwhile, the binding of FGF19 to FGFR4 markedly reduces the phosphorylation of CREB, which regulates CREB dephosphorylation and ultimately leads to the inhibition of GNG in the liver[121] [Figure 5]. Thus, activating the FGF19–FGFR4 signaling pathway may be a therapeutic approach for treating MASH.

In summary, communications among liver cells and the gut–liver axis have exhibited predominant effects on the initiation and progression of MASLD/MASH [Figure 6]. However, the important issue that troubles clinical doctors is that accurate molecular and clinical subtypes of MASLD are still unclear. The progression of MASLD to MASH and fibrosis is difficult to detect in a timely manner, and patients who are at high risk are also hard to distinguish. Therefore, it will be particularly important to determine MASLD subtypes. There are two ways to address this issue. On one hand, multiple omics methods, such as transcriptomics, single-cell sequencing, spatial transcription/proteomics, and organoid methods, will help to determine the in-depth mechanism, crucial regulators, and subtypes of MASLD. On the other hand, the clinical establishment of high-performing non-invasive biomarkers is significant for MASLD diagnosis and predictive analysis.

Therapeutic Strategies

MASLD and liver fibrosis are heterogeneous diseases with complex pathophysiology that involve the production of key disease-relevant regulators. Based on the above summarized molecular mechanisms and key regulators, many compounds are being evaluated in clinical trials. Here, we summarize non-pharmacological interventions, pharmacological treatments, and biotechnology strategies for the clinical treatment of MASLD and liver fibrosis.

Non-pharmacological interventions

Lifestyle modification

MASLD is characterized by abnormal fat accumulation in HCs and is associated with metabolic disorders. According to the American Association for the Study of Liver Diseases (AASLD) and European Association for the Study of the Liver (EASL) guidelines, a healthy diet and regular exercise constitute the fundamental treatment for MASLD, with the ultimate goal being weight loss.[122,123] Key principles are embarking on a low-calorie diet and changing dietary composition.[122] The Mediterranean diet is recommended for patients with MASLD and is associated with cardiovascular benefits.[124] Coffee consumption may also be beneficial.[125] Notably, exercise and diet modification should be added according to the individual physical abilities and preferences of the patient.

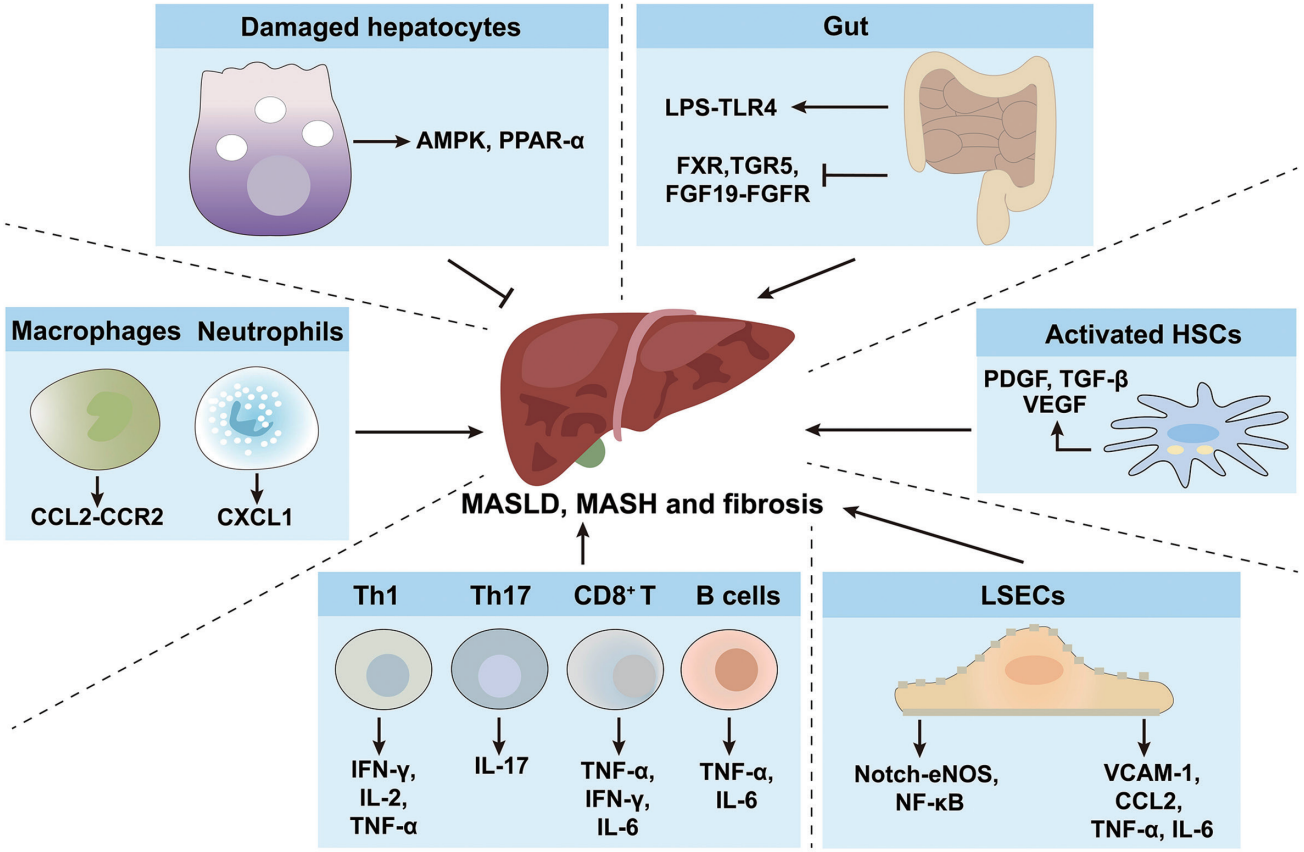

Figure 6: Molecular pathogenesis and key regulators of liver cells and the gut–liver axis in MASLD/MASH. Upon metabolic injury, the close crosstalk among parenchymal, non-parenchymal liver cells, and the gut–liver axis involving multiple molecular regulators and signaling pathways initiates and promotes MASLD development. AMPK: AMP-activated protein kinase; CCL2: Chemokine C-C motif ligand 2; CCR2: C-C motif chemokine receptor 2; CXCL1: C-X-C motif chemokine ligand 1; eNOS: Endothelial NO synthase; FGF19: Fibroblast growth factor 19; FGFR: Fibroblast growth factor receptor; FXR: Farnesoid X receptor; HSCs: Hepatic stellate cells; IFN-γ: Interferon γ; IL-2: Interleukin 2; LPS: Lipopolysaccharide; LSECs: Liver sinusoidal endothelial cells; MASH: Metabolic dysfunction-associated steatohepatitis; MASLD: Metabolic dysfunction-associated steatotic liver disease; NF-κB: Nuclear factor Kappa-light-chain-enhancer of activated B cells; Notch: Neurogenic locus notch homolog protein; PDGF: Platelet-derived growth factor; PPAR-α: peroxisome proliferator-activated receptors α; TGF-β: Transforming growth factor β; TGR5: Takeda G protein-coupled receptor 5; Th1: T helper 1; TLR4: Toll-like receptor 4; TNF-α: Tumor necrosis factor α; VCAM-1: Vascular cell adhesion molecule-1; VEGF: Vascular endothelial growth factor.

Aerobic and resistance training for 150–200 min per week is recommended.[126]

Bariatric surgery

MASLD/MASH is gradually recognized as a comorbid condition that can benefit from bariatric surgery. Bariatric surgery effectively resolves MASLD/MASH in the majority of patients without cirrhosis and reduces mortality from CVD and malignancy.[122] In a randomized trial with a 1-year follow-up, bariatric metabolic surgery was more effective than lifestyle interventions. In detail, more participants in the Roux-en-Y gastric bypass (56%) and sleeve gastrectomy (57%) groups reached the primary endpoint of histological resolution of MASH without worsening fibrosis than those in the lifestyle modification group (16%).[127]

Pharmacotherapies in MASLD clinical treatment

The efficacy of non-pharmacological interventions, including dietary changes, exercise, and even surgical interventions, may not be consistently high or long-lasting. Therefore, integrating pharmacotherapies with non-pharmacological approaches is crucial for achieving optimal and sustainable outcomes in managing health conditions. Despite numerous potential therapeutic targets identified through cellular and animal studies, substantial drug development challenges remain discouraged. Current pharmacotherapeutic options for MASLD can be broadly categorized into several groups based on their primary mechanism of action, such as those targeting metabolic pathways, inflammation, or fibrosis. Here, we summarize the key targets and current clinical research progress in Table 1.

THR-β agonists

Thyroid hormone receptors (THRs) are found in various tissues throughout the body, with THR-β as the major receptor isoform expressed in the liver. THR-β agonists can reverse steatosis via many mechanisms, including improving hepatic conversion of T4 to T3 and enhancing mitochondrial function, which can eventually reduce low-density lipoprotein cholesterol (LDL-C) and TG in the plasma and liver.[128,129]

Resmetirom is a THR-β agonist. The MAESTRO-NASH trial, a phase III randomized controlled trial of resmetirom, showed that MASH resolution occurred in 25.9% of the 80 mg resmetirom group and 29.9% of the 100 mg resmetirom group, compared to 9.7% of the placebo

Table 1: Main clinical trials (finished) in the treatment of MASLD/MASH.

Class	Drug	Clinical efficacy	Phase	Registration No.	References
THR-β agonists	Resmetirom	Reduces LDL-C; improves liver fibrosis.	III	NCT03900429	[130]
	VK2809	reduces liver fat content.	IIb	NCT04173065	[131]
PPAR agonists	Saroglitazar (PPARα/γ)	Improves ALT, liver fat content, insulin resistance.	II	NCT03061721	[132]
	Lanifibranor (PPARα/δ/γ)	Improves lipid metabolism, inflammation, and fibrosis.	IIb	NCT03008070	[133]
GLP-1/GIP/GCGR agonists	Semaglutide (GLP-1)	Improves MASH resolution rates.	II	NCT02970942	[137]
	Efinopegdutide (GLP-1/GCGR)	Reduces liver fat content.	IIa	NCT04944992	[139]
	Tirzepatide (GLP-1/GIP)	Decreases MASH-related biomarkers.	II	NCT03131687	[140]
	Retatrutide (GLP-1/GIP/GCGR)	Improves blood glucose and reduces body weight.	II	NCT04867785	[141]
SGLT2 inhibitors	Empagliflozin	Reduces liver fat and improves ALT.	/	NCT02686476	[144]
	Dapagliflozin	Improves liver steatosis and fibrosis.	/	UMIN000022155	[145]
FGF analogs	Aldafermin (FGF19)	Improves liver fibrosis.	IIb	NCT03912532	[147]
	Efruxifermin (FGF21)	Improves liver fibrosis and resolves MASH.	IIb	NCT04767529	[148]
FXR agonists	OCA	Improves liver fibrosis and MASH disease activity.	III	NCT02548351	[150]
	Tropifexor	Reduces ALT and hepatic fat fraction.	IIa/b	NCT02855164	[151]
	Vonafexor	Reduces liver fat content, liver enzymes, and body weight.	IIa	NCT03812029	[152]
DGAT2 inhibitors	ION224	Improves liver histology, reduces liver steatosis.	II	NCT04932512	[155]
AMPK activators	PXL770	Reduces liver fat insignificantly.	IIa	NCT03763877	[158]
CCR2/CCR5 antagonists	CVC	Improves liver inflammation and fibrosis.	IIb	NCT02217475	[159]

ALT: Alanine aminotransferase; AMPK: AMP-activated protein kinase; CCR: C-C motif chemokine receptor; CVC: Cenicriviroc; DGAT2: Diacylglycerol acyltransferase 2; FGF: Fibroblast growth factor; FXR: Farnesoid X receptor; GCGR: Glucagon receptor; GIP: Glucose-dependent insulinotropic polypeptide; GLP-1: Glucagon-like peptide-1; LDL-C: Low-density lipoprotein cholesterol; MASH: Metabolic dysfunction-associated steatohepatitis; MASLD: Metabolic dysfunction-associated steatotic liver disease; OCA: Obeticholic acid; PPAR: Peroxisome proliferator-activated receptor; SGLT2: Sodium-glucose cotransporter 2; THR-β: Thyroid hormone receptor β.

group, and fibrosis improvement was achieved in 24.2% of the 80 mg resmetirom group and 25.9% of the 100 mg resmetirom group, compared to 14.2% of the placebo group.[130] The United States Food and Drug Administration (FDA)'s approval of resmetirom on March 14, 2024, marked a significant milestone, as it became the first pharmacological agent to be authorized to manage MASH. Apart from resmetirom, VK2809 is another selective THR-β agonist currently in its phase IIb clinical trial.[131]

PPAR agonists

Multiple target mechanisms of PPARs across various sites are known, dual or even pan-PPAR agonists offer superior efficacy compared to mono-agonists. Saroglitazar is a PPARα/γ agonist. In a phase II trial, 4 mg of saroglitazar significantly improved ALT, liver fat content, insulin resistance, and atherogenic dyslipidemia in participants with MASLD/MASH.[132] Lanifibranor is a pan-PPAR agonist that targets all three isotypes (PPARα/δ/γ). In a phase IIb trial, the percentage of patients who had a decrease of at least 2 points in the SAF-A score (the activity part of the Steatosis, Activity, Fibrosis [SAF] scoring system that incorporates scores for ballooning and inflammation) without worsening of fibrosis was significantly greater among those who received the 1200-mg dose of lanifibranor than among those who received placebo.[133]

GLP-1/GIP/GCGR agonists

Incretins are gastrointestinal hormones that enhance insulin secretion in response to food ingestion. Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are incretins derived from the intestine.[134] Additionally, glucagon has been shown to exhibit incretin-like effects.[135] Given the close interrelationship between T2DM and MASLD/MASH, these targets have also emerged as potential therapeutic directions.[136] A randomized, placebo-controlled phase II trial revealed that semaglutide, a GLP-1 agonist, significantly improved MASH resolution rates, achieving 59% in the 0.4 mg group compared to 17% in the placebo group. However, it has a limited effect on improving fibrosis.[137]

Novel therapies, such as dual GLP-1/GIP receptor agonists and GLP-1/glucagon receptor (GCGR) agonists, demonstrate even more significant potential in managing glycemia and body weight.[138] Efinopegdutide is a GLP-1/GCGR coagonist. In a randomized phase IIa trial, treatment with 10 mg of efinopegdutide weekly led to a significantly reduced liver fat content compared with treatment with 1 mg of semaglutide weekly in patients with MASLD.[139] Tirzepatide is a dual GLP-1 and GIP receptor agonist, significantly decreased MASH-related biomarkers.[140] Retatrutide, a GIP, GLP-1, and GCGR agonist, has already shown positive outcomes in a phase II substudy for patients with T2DM.[141]

SGLT2 inhibitors

Sodium-glucose cotransporter 2 (SGLT2) inhibitors suppress glucose reabsorption by the kidney and can prevent kidney disease and cardiovascular events in patients with T2DM.[142,143] Many patients with MASLD also have T2DM, which exacerbates MASLD progression to MASH or even to cirrhosis.[136] Empagliflozin, an SGLT2 inhibitor, reduced liver fat and improved ALT levels in MASLD patients with T2DM.[144] A 24-week clinical trial demonstrated that the SGLT2 inhibitor dapagliflozin reduced the levels of biomarkers linked to steatosis and fibrosis, such as ALT and gamma-glutamyl transpeptidase (GGT), and contributed to improvements in steatosis and fibrosis.[145]

FGF analogs

FGF19/21 belong to the FGF subfamily that both exhibit similar abilities to increase energy expenditure, reduce hepatic TGs, and improve insulin sensitivity.[146] Thus, FGF19/21 has become attractive potential therapeutic targets for treating MASLD. Aldafermin is an engineered analog of FGF19. In a phase IIb trial, although there was no significant dose-response effect on fibrosis improvement in at least one stage with no worsening of MASH, aldafermin has been demonstrated to have some positive effects on improving fibrosis and several other parameters in MASH patients, with generally good tolerability.[147] Efruxifermin, a bivalent Fc-FGF21 analog, improved liver fibrosis and resolved MASH over 24 weeks in patients with F2 or F3 fibrosis, with acceptable tolerability.[148]

Farnesoid X receptor (FXR) agonists

FXR not only controls multiple pathogenetic pathways relevant to MASH, including GNG, lipogenesis, inflammation, and fibrosis, but also controls intestinal barrier integrity and maintains gut microbiota eubiosis.[149] The diverse functions of FXR make it an attractive target for treating MASH. Obeticholic acid (OCA) is a BA-derived FXR agonist. A randomized, global phase III study evaluated the impact of OCA on MASH patients with fibrosis. Based on the interim analysis, the fibrosis improvement endpoint was 23% in the 25 mg OCA group compared to 12% in the placebo group.[150] Tropifexor, another FXR agonist, significantly decreased the ALT level and hepatic fat fraction in MASH patients.[151] Vonafexor, a second-generation, non-BA FXR agonist, also showed positive results in MASH patients.[152]

DGAT2 inhibitors

Acyl-CoA: diacylglycerol acyltransferase (DGAT) enzymes, which contain DGAT1 and DGAT2, can catalyze the final step of TG synthesis,[153] and DGAT2 preferentially utilizes free fatty acids (FFAs) from DNL.[154] To prevent TG accumulation in the liver, blocking TG synthesis via DGAT2 inhibition might be a promising method. ION224 is an antisense oligonucleotide inhibitor of DGAT2. ION224 significantly improved liver histology, as measured by at least a 2-point reduction in the MASLD activity score , and was safe and well tolerated in the study.[155]

In addition, a phase IIa trial investigated the effects of coadministration of an ACC1/2 inhibitor (PF-05221304) and a DGAT2 inhibitor (PF-06865571), which decreased liver fat compared to that of a placebo and mitigated the ACC inhibitor-mediated effect on serum TG.[156] This encouraging result suggested that the coadministration of PF-05221304 and PF-06865571 can address some of the limitations of ACC inhibition alone.

AMPK activators

AMPK is a crucial metabolic regulator that detects energy status and governs energy expenditure and storage.[157] The activity of AMPK is repressed in MASH. PXL770 is a direct AMPK activator. A phase IIa study was performed to assess the efficacy of PXL770 in treating MASH. Although PXL770 did not improve liver fat compared with placebo, it is well tolerated and could still hold potential for further development.[158]

CCR2/CCR5 antagonists

In addition to these metabolic pathway targets, CVC, a dual CCR2/CCR5 antagonist, was developed to attenuate inflammation. In a phase IIb study (CENTAUR; NCT02217475), CVC treatment resulted in an early antifibrotic benefit after one year, especially for the subset of patients with advanced fibrosis.[159] Nevertheless, the further antifibrotic benefit of CVC treatment did not persist for two years, but CVC treatment was well tolerated and safe.[160] Unfortunately, a phase III clinical trial to prove the indication of CVC in fibrotic MASH has been terminated due to a lack of clear efficacy.[161]

Combination therapy

The development of MASH involves complex pathways and potential interactions between them, presenting a significant challenge for creating a single, universally effective medication. Therefore, it may be difficult to develop a one-size-fits-all treatment. Instead, a combination therapy approach that simultaneously targets multiple factors could be the most effective therapeutic strategy. Combinations such as a DGAT2 inhibitor combined with an ACC inhibitor,[156] an FXR agonist combined with an ACC inhibitor or an ASK1 inhibitor,[162] and combinations of a GLP-1 receptor agonist, an FXR agonist, and an ACC inhibitor[163] have yielded positive results. Thus, multiple combination regimens might enhance the therapeutic benefits and mitigate the side effects of single-agent treatments for MASH patients.

Therapeutic strategies have shifted from lifestyle interventions to monotherapy in recent decades. Future combination strategies with proven anti-inflammatory and antifibrotic benefits should pay more attention to MASLD treatment. Multitargeting combinations should be used to enhance the treatment efficacy and safety of MASLD. Additionally, it is gratifying that not only was the first FDA-approved drug regimen expected to be applicable in the clinic but also that substantial clinical studies have been published or are currently registered.

Clinical Research Progress

Ongoing clinical research

In recent decades, more than 20 molecules have been tested for treating MASLD/MASH, providing multiple therapeutic options.[164,165] However, it is worth noting that most of these treatments are not yet licensed to treat MASLD and its complications. Apart from resmetirom recently approved by the FDA, no other FDA- or European Medicines Agency (EMA)-licensed drugs have been approved for treating MASLD due to inadequate clinical endpoint efficacy or undesirable side effects. Nevertheless, there is still hope for further translation of newly developed drugs into clinical applications. Numerous ongoing studies of drugs in development targeting MASH have emerged globally and are registered in different clinical trial stages. Moreover, multitarget therapeutic strategies are also emerging rapidly. We have summarized ongoing registered clinical trials on MASLD treatment. An initial list of 1490 clinical studies was retrieved from the ClinicalTrials.gov database, of which 1120 were interventional trials involving lifestyle and drug interventions and diagnostic tests. After filtration of completed, suspended, terminated, withdrawn, unknown-status studies, and diagnostic trials, 265 ongoing trials were identified (index date: June 11, 2024). The representative ongoing clinical trials worldwide are listed in Table 2.

Precision medicine

In addition to drug development targeting a specific mechanism in the intricate pathophysiology of MASLD/MASH, genetic studies focusing on genetic variations have highlighted inherited determinants in MASLD and metabolic comorbidities. Patatin-like phospholipase domain-containing 3 (PNPLA3), glucokinase regulator (GCKR), transmembrane 6 superfamily member 2 (TM6SF2), and 17-beta hydroxysteroid dehydrogenase 13 (HSD17B13) are associated with MASH development.[166,167] These candidate genes are predominantly involved in insulin regulation, FFAs and TG metabolism, oxidative stress, endotoxin and cytokine activity, and fibrogenesis.[168] Thus, precision medicine that treats individual MASLD patients differentially based on genetic polymorphisms might shed light on the clinical treatment of MASLD and liver fibrosis.

Conclusions

The increasing prevalence of MASLD and its complications, as well as its comorbidities, place heavy burdens on public health and the economy, which has attracted intense attention in the exploration of its pathogenesis and pharmaceutical treatments. Collective evidence has revealed multifactorial interactions among nutrients, metabolic pathways, the gut microbiome, genetic susceptibility, and pro-inflammation/pro-fibrosis in experimental and human MASLD patients. An improved understanding of the pathophysiology of MASLD and extrahepatic complications helps in developing practical and affordable medications.

Table 2: Main ongoing clinical trials (recruiting) in the treatment of MASLD/MASH.

Title	NCT number	Drug	Class	Phase	Locations
Safety, Tolerability, and Efficacy of ASC41 Tablets in Adult Patients with NASH	NCT05462353	ASC41	THR-β	II	Hangzhou, China
Efficacy and Safety of HSK31679 in Chinese Patients with NASH	NCT06168383	HSK31679	THR-β	II	Beijing, China
Effect of Henagliflozein on Hepatic Fat Content in Patients with T2DM and NAFLD	NCT06449833	Henagliflozein	SGLT-2	IIII	Guangzhou, China
Research Study on Whether Semaglutide Works in People with NASH	NCT04822181	Semaglutide	GLP-1	III	Huntsville, United States
A Clinical Study of Efinopegdutide in Participants with Precirrhotic NASH (MK-6024-013)	NCT05877547	Efinopegdutide/ MK-6024-013	GLP-1/GCGR	II	Chandle, United States
Efficacy and Safety of GH509 Versus Placebo in Patients with NASH/NAFLD	NCT05784779	GH509	STKs inhibitor	Ib/II	Hangzhou, China
A Study of ZSP1601 in Adult Subjects with NASH	NCT05692492	ZSP1601	PDE	II	Guangzhou, China
Efficacy and Safety of Lanifibranor in Adult Patients with NASH and Fibrosis Stages F2 and F3	NCT04849728	Lanifibranor/ IVA337	pan-PPAR	III	Anniston, United States
Safety and Effectiveness of Saroglitazar 4 mg in Patients with NAFLD with Comorbidities	NCT05872269	Saroglitazar	PPARα/γ	IIII	Ahmedabad, India
A Study Evaluating Efruxifermin in Subjects with MASH and MASLD	NCT06161571	Efruxifermin	FGF21	III	Birmingham, United States
A Study of LY3885125 in Participants with Dyslipidemia or NAFLD	NCT06007651	LY3885125	SCAP	I	San Antonio, United States
The Impact of Ibutamoren on Non-alcoholic Fatty Liver Disease	NCT05364684	LUM-201	GHSR	II	Boston, United States
Role of Hyperinsulinemia in NAFLD Pathogenesis: Diazoxide Pilot & Feasibility Study	NCT05729282	Diazoxide	KATP-channels	II	NewYork, United States
A Study of LY3849891 in Participants with Non-alcoholic Fatty Liver Disease	NCT05395481	LY3849891	PNPLA3	I	Rialto, United States
A Study of BMN 255 in Participants with NAFLD and Hyperoxaluria	NCT06138327	BMN 255	Glycolic acid oxidase	I	Birmingham, United States

FGF21: Fibroblast growth factor; GCGR: Glucagon receptor; GHSR: Growth hormone secretagogue receptor; GLP-1: Glucagon-like peptide-1; KATP: ATP-sensitive potassium; MASH: Metabolic dysfunction-associated steatohepatitis; MASLD: Metabolic dysfunction-associated steatotic liver disease; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; PDE: Phosphodiesterase; PNPLA3: Patatin-like phospholipase domain-containing 3; PPAR: Peroxisome proliferator-activated receptor; SCAP: Sterol regulatory element binding protein cleavage-activating protein; SGLT-2: Sodium-glucose cotransporter 2; STKs: Serine/threonine protein kinases; T2DM: Type 2 diabetes mellitus; THR-β: Thyroid hormone receptor β.

In conclusion, we highlight the molecular pathogenesis, therapeutic targets, and translational clinical trials of MASLD and liver fibrosis. Although minor medications are approved in the clinic, multi-target treatment of MASLD is a future direction for the clinical treatment of MASLD and liver fibrosis.

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Conflicts of interest

None.

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