Pathogenetic Pathways in Nonalcoholic Fatty Liver Disease: An Incomplete Jigsaw Puzzle

Qin Pan, MD[a,b], Jian-Gao Fan, MD, PhD[b,c,*], Yusuf Yilmaz, MD[d,e,*]

KEYWORDS

• Nonalcoholic fatty liver disease • Nonalcoholic steatohepatitis • Liver fibrosis
• Signaling pathways • Pathophysiology

KEY POINTS

- Non-alcoholic fatty liver disease (NAFLD) has become the most prevalent chronic liver disease worldwide.
- Multiple pathways regulate hepatic steatosis, including Insulin signaling pathway, MAPK signaling pathway, GLP-1R signaling, nuclear receptors, and non-coding RNAs.
- Oxidative stress, endoplasmic reticulum stress, cytokines, gut microbiota contribute to lobular inflammation.
- TGF-beta signaling pathway, nuclear receptors, autophagy, and apoptosis of HSCs involve in the pathogenesis of liver fibrosis.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is characterized by excess liver fat infiltration (steatosis in $\geq$ 5% of hepatocytes) in the absence of significant alcohol consumption or other causes of hepatic steatosis.[1] This condition is regarded as a disease spectrum encompassing different stages, from nonalcoholic fatty liver (NAFL), through nonalcoholic steatohepatitis (NASH), to liver fibrosis, cirrhosis, and even hepatocellular carcinoma.[1] NAFL is the initial stage of NAFLD, which is generally considered benign due to its limited or subclinical impact on hepatic function. According to currently accepted models of disease pathophysiology, NAFL represents the first of the "two-hit system" used to describe the natural history of NAFLD over time. Although

[a] Research Center, Zhoupu Hospital Affiliated to the Shanghai University of Medicine & Health Sciences, Shanghai 201318, China; [b] Department of Gastroenterology, Xinhua Hospital Affiliated to the Shanghai Jiao Tong University School of Medicine, Shanghai 200092, China; [c] Shanghai Key Lab of Pediatric Gastroenterology and Nutrition, Shanghai 200092, China; [d] Department of Gastroenterology, School of Medicine, Recep Tayyip Erdoğan University, Rize 53200, Turkey; [e] Liver Research Unit, Institute of Gastroenterology, Marmara University, İstanbul 34840, Turkey
* Corresponding authors.
E-mail addresses: fanjiangao@xinhuamed.com.cn (J.-G.F.); dryusufyilmaz@gmail.com (Y.Y.)

Clin Liver Dis 27 (2023) 317–332
https://doi.org/10.1016/j.cld.2023.01.013 liver.theclinics.com

accumulation of lipids in the liver is considered benign, it can increase the production of reactive oxygen species (ROS), which in turn elicits an inflammatory response. This second hit can potentially result in the development of lobular inflammation and hepatocellular ballooning (NASH).[1] Thereafter, if the pathogenetic damage continues to persist and/or accumulate, the disease may progress into more advanced stages, namely liver fibrosis and cirrhosis, with the possible concomitant risk of hepatic carcinogenesis.

From an epidemiological standpoint, the burden associated with NAFLD is currently recognized as one of the most pressing issues in public health, with around 32.4% of the global population having this condition.[2] Currently, NAFLD represents the most prevalent cause of chronic liver diseases worldwide and, with the increasing prevalence of unhealthy lifestyles, an epidemic of this condition is anticipated.[1,2] Remarkably, the adverse impact of NAFLD is not limited to liver-related morbidity and mortality but extends over a wide spectrum of extrahepatic manifestations—including cardiometabolic complications and chronic and extrahepatic tumour.[1] Although an international consensus panel has recently recommended metabolic-associated fatty liver disease as a more appropriate term to describe fatty liver disease associated with metabolic dysfunction, a multisociety consensus process concerning disease naming is currently underway.[3] Until the process is completed, the term NAFLD is still being used. Unfortunately, as of now, only modestly effective pharmacological therapies exist for this condition. The uncomplete understanding of the pathophysiology underlying the heterogeneous disease spectrum known as NAFLD remains one of the major obstacles to the development of novel therapeutic approaches. However, growing evidence indicates that NAFLD advances through the interaction between different liver cell types and pathogenic cell-to-cell communication through a host of signaling pathways. The present review compiles current knowledge on the principal pathogenic mechanisms of NAFLD, which are analyzed in relation to the main pathological hallmarks of the spectrum (ie, steatosis, steatohepatitis, and fibrosis). The following sections are not intended to be exhaustive but are aimed to highlight those aspects most relevant to developing effective methods of treatment. It is hoped that these data may inform the identification of novel therapeutic targets and can pave the way for breakthrough treatments of NAFLD.

SIGNALING PATHWAYS INVOLVED IN THE PATHOGENESIS OF HEPATOCYTE STEATOSIS

The liver is a central hub for lipid metabolism, and hepatic steatosis may emerge as an expected consequence whenever this delicate equilibrium is disrupted. Under physiologic conditions, the hepatic fatty acid pool is the result of a balance between fatty acid influx from the diet and adipose tissue lipolysis, *de novo* lipogenesis, and disposal of fatty acid through very-low-density lipoprotein (VLDL) secretion or β-oxidation. Disturbances in these processes can precipitate intracellular lipid accumulation as a result of excess fatty acid content with respect to the oxidative needs of the hepatocytes. Although several different pathways have been involved in hepatic fat deposition and lipid toxicity, the most extensively investigated include insulin signaling, mitogen-activated protein kinase (MAPK) signaling, glucagon-like peptide-1 (GLP-1) signaling, nuclear receptors (NRs) signaling, and noncoding RNA (ncRNA) (**Fig. 1**).

Insulin Signaling Pathway

Hepatic insulin resistance is a key mechanism in the pathogenesis of NAFLD and closely connected to excess lipid accumulation in hepatocytes. Under physiologic

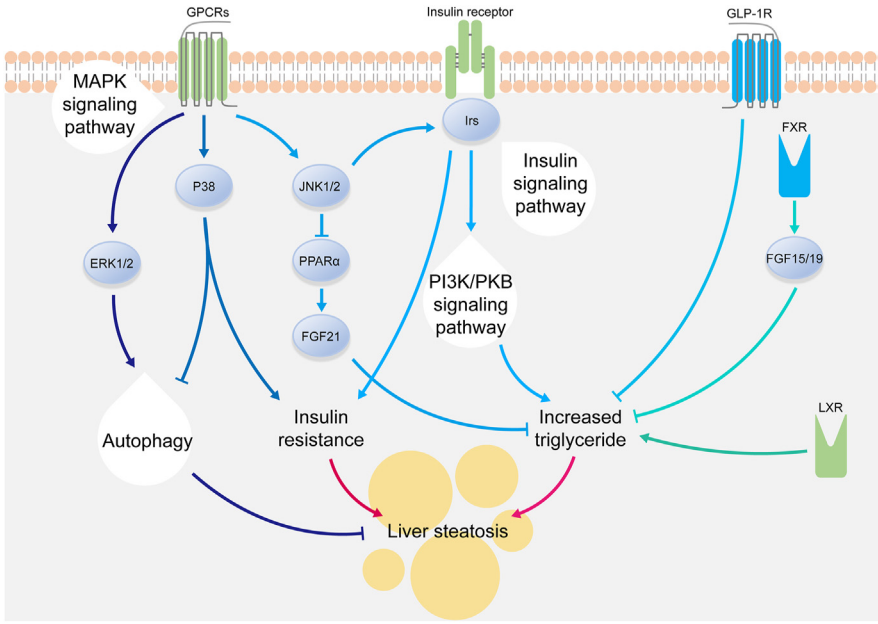

Fig. 1. Pathways associate with hepatocyte steatosis in nonalcoholic fatty liver disease (NAFLD). ERK1/2 of MAPK signaling pathway promotes autophagy of hepatocytes to attenuate liver steatosis. P38 of MAPK signaling pathway simultaneously inhibit autophagy and induce insulin resistance. JNK1/2, another subgroup of MAPKs, increases triglyceride concentration by the downregulation of PPARα-induced FGF21 expression. JNK1 also mediates inhibitory phosphorylation of Irs, leading to the insulin resistance. Insulin signaling pathway cooperates with PI3K/PKB signaling pathway to upregulate TG level, whereas hepatic insulin resistance is thought to involve serine phosphorylation of Irs. In addition, nuclear receptors of LXR and FXR regulate the hepatic triglyceride content in positive and negative manner, respectively. Ligand-activated GLP-1R functions to prevent the triglyceride accumulation in liver. Integration of these pathways finally results in liver steatosis by insulin resistance, autophagy inhibition, and increased triglyceride level. ERK, extracellular signal-regulated kinase; FGF21, fibroblast growth factor 21; FXR, farnesoid X receptor; GLP-1R, glucagon-like peptide-1 receptor; GPCR, G protein–coupled receptor; Irs, insulin receptor substrate; JNK, c-Jun N-terminal kinase; LXR, liver X receptor; PI3K, phosphatidylinositol 3-kinase; PKB, PI3K/protein kinase B; PPARα, peroxisome proliferator–activated receptor α.

conditions, insulin signaling starts with insulin binding to the insulin receptor on the cell surface. On binding, receptor autophosphorylation leads to activation of tyrosine kinase activity—resulting in tyrosine phosphorylation of intracellular insulin receptor substrates (IRS).[4] These effectors bind to a signaling protein complex to activate the MAPK pathway that mediates the mitogenic effects of insulin.[5] IRS is also capable of binding to the regulatory subunit of phosphatidylinositol 3-kinase (PI-3K), which activates the PI-3K/protein kinase B pathway to mediate the metabolic responses to insulin. This signaling arm results in the production of phosphatidylinositol-3,4,5-trisphosphate (PIP3), which recruits both phosphoinositide-dependent kinase (PDK)1/2 and Akt to the plasma membrane, where PDK1/2 phosphorylates and activates Akt.[6] Induction of hepatic insulin resistance is thought to involve serine phosphorylation of IRS proteins[7]; this is deemed to play a role in insulin resistance by decreasing PI-3K interaction with IRS proteins and can also result in IRS1 degradation.[8] In addition to inactivation by

serine phosphorylation, the metabolic arm of insulin signaling is also reversibly modulated by dephosphorylation of the signal mediators by downstream regulatory phosphatases, particularly phosphatase and tensin homolog (PTEN), which dephosphorylates PIP3, and protein tyrosine phosphatase-1B (PTP-1B), which dephosphorylates IR and IRS. Expression of PTP-1B and PTEN is linked to hepatic insulin resistance and VLDL overproduction, as shown in studies with liver-specific deletion of PTP-1B and PTEN.[9,10] It is currently believed that insulin resistance can also be induced by steatosis through the actions of specific lipid species. The main mediator is believed to be diacylglycerol, which activates protein kinase C and interacts with insulin receptor's intracellular domain, consequently rendering the hepatocytes less responsive to insulin signaling.[11] The lipogenic actions of insulin in the liver are mediated through the activation of sterol regulatory element binding protein 1-c (SREBP1-c).[12] A paradoxical hepatic insulin resistance phenomenon is the fact that SREBP-1c and consequently lipogenesis is still activated in parallel with ongoing gluconeogenesis, a phenomenon termed pathway-selective insulin resistance.[13]

Mitogen-Activated Protein Kinase Signaling Pathway

Mammalian MAPKs comprise 3 major subgroups that are classified based on substrate specificity, differential activation by agonists, and sequence similarity. These subgroups include the extracellular signal-regulated kinases 1 and 2 (ERK1/2); c-Jun NH2-terminal kinases 1, 2, and 3 (JNK1/2/3); and p38$\alpha/\beta/\delta/\gamma$ MAPKs.[14–16] The MAPK signaling pathway is a cascade of protein kinases that play a key role in the regulation of hepatic metabolism. ERK1/2 activity has been shown to be compromised in livers of leptin receptor–deficient (db/db) mice, and it may protect against steatosis by promoting autophagy. Activated autophagy could attenuate liver steatosis by sequestering lipid droplets, which are eventually eliminated.[14] MAPK phosphatase-1 is overexpressed in mice with hepatic steatosis fed a high-fat diet (HFD), suggesting that hepatic p38α/β MAPK declines in states of obesity.[17] Similar findings have been reported for JNK. The first evidence implicating JNK signaling in steatosis was JNK1-mediated inhibitory phosphorylation of IRS-1 on the serine-307, which results in insulin resistance.[15] JNK1-null mice have significantly lower degree of steatosis and liver injury than wild-type counterpart.[18] JNK1/2 activation was also observed in liver biopsies from obese patients with hepatic steatosis.[19] Mechanistically, JNK1/2 represses the nuclear hormone receptor peroxisome proliferator–activated receptor α (PPARα) and fibroblast growth factor 21 (FGF21) signaling, in part through regulating nuclear receptor corepressor 1.[20] Hepatic p38α/β expression decreases in livers of HFD-fed mice, leading to increased transcription of lipogenic genes, which is a driver of increased triglyceride levels.[16] Notably, p38γ promotes the phosphorylation of AKT, which in turn phosphorylates AMPK on the inhibitory residues S485 and S491, driving insulin resistance. Insulin resistance is also induced by p38γ/δ activation of p62-mTORC1-S6K1-IRS signaling, which inhibits autophagy.[21] Insulin resistance, autophagy inhibition, and increased triglyceride levels converge to induce hepatic steatosis.

Glucagon-Like Peptide-1 Receptor Signaling

The GLP-1 receptor (GLP-1R) is a G protein–coupled receptor expressed on the surface of many cell types. Its cognate ligand is a 30-residue peptide hormone, GLP-1, which stimulates glucose-mediated insulin production by pancreatic beta cells.[22] Studies have shown a decrease in endogenous GLP-1 secretion in patients with NAFLD.[23] GLP-1R agonists are analogues of GLP-1 and fall under the class of incretin mimetics. In obese mice and in humans, administration of GLP-1R agonists reduces

hepatic steatosis.[24] Apart from its effects on weight loss, GLP-1R agonists might play a direct role in improving hepatic steatosis through upregulation of insulin signaling pathways, fatty acid metabolism, and autophagy-dependent lipid degradation.[25] For example, liraglutide, as an agonist of GLP-1R, has been shown to restore autophagic flux, specifically the transcription factor EB–mediated autophagy-lysosomal pathway.[26] Liu and colleagues[27] have also shown a critical role of liver-derived FGF 21 in mediating the effects of GLP-1, and Farnesoid-X-receptor (FXR) is a multipurpose NR that plays an important role in regulating bile acid homeostasis, glucose and lipid metabolism, and hepatic regeneration.[28]

Nuclear Receptors

NRs comprise a superfamily of ligand-activated transcription factors that act as important cellular sensors. They primarily function through DNA binding and can be activated by several lipid soluble signals. Farnesoid X receptor (FXR) is a multipurpose NR that plays an important role in regulating bile acid homeostasis, glucose and lipid metabolism, and hepatic regeneration. FXR activation represses hepatic lipogenesis via the FXR-SHP-SREBP-1c pathway.[29] In addition, it reduces hepatic fatty acid uptake and promotes lipid oxidation.[30] Obeticholic acid, an agonist of FXR, has been shown to improve histological hepatic steatosis, suggesting a key role of FXR in the pathogenesis of NAFLD.[31] Interestingly, the therapeutic actions of FXR agonism may involve FGF15 and FGF19 as downstream effectors.[32] Other NRs, including liver X receptors (LXRs), are important regulators of intracellular cholesterol and lipids homeostasis. Differently from FXR agonists, LXR agonists can cause hepatic steatosis and dysfunction in part by increasing expression of SREBP1-c, a transcription factor that upregulates fatty acid synthesis.[33] Therefore, FXR- and LXR-related pathways are involved in the pathogenesis of hepatic steatosis in an opposite fashion.

Noncoding Ribonucleic Acid (RNAs)

ncRNAs constitute a vast and diverse family of non–protein-coding transcripts. Through their interaction with DNA, RNA, and proteins, ncRNAs may regulate various biological processes, such as gene transcription, RNA turnover, and mRNA translation that can affect intrahepatic fat accumulation. An increase of the adipocyte-secreted microRNA-34a (miR-34a) is responsible for the decrease of PPAR-α expression and consequent steatosis development.[34] In addition, inhibition of hepatic miR-24 leads to an increase in the Insig1 target, a lipogenesis inhibitor, preventing hepatic lipid accumulation.[35] Another study showed that miR-141/200c deficiency in mice reduced triglyceride accumulation via AMPK activation to promote PPARα-mediated fatty acid β-oxidation.[36] Circular RNA Circ_0057558,[37] LncRNA Blnc1,[38] lncRNA H19,[39] miR-137-3p,[40] circRNA_0046366,[41] and NEAT1/miR-140[42] have all been identified in the literature as potential mediators of hepatic lipogenesis. In general, miRNA expression patterns are complex and dynamic. Emerging data encourage further analysis and characterization of specific miRNAs that may be either downregulated or upregulated aberrantly in hepatic steatosis.

SIGNALING PATHWAYS INVOLVED IN THE PATHOGENESIS OF NONALCOHOLIC STEATOHEPATITIS

In general, accumulation of fat in the liver is not *per se* sufficient to cause parenchymal inflammation, and a “second-hit” is required to trigger liver injury. When different triggers are present concomitantly, the risk of NASH development increases markedly. Currently, the most common triggers of inflammatory changes include oxidative

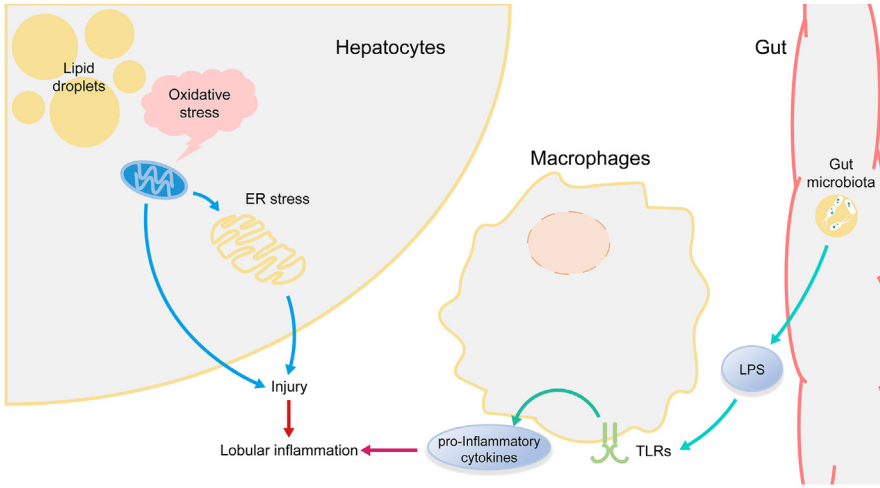

Fig. 2. Pathways regulate lobular inflammation in NAFLD. Liver steatosis provokes lipid peroxidation and successively ER stress, both of which lead to hepatocyte injury. Injured hepatocytes initiate the lobular inflammation in NAFLD. On dysbiosis, endotoxin (eg, LPS) derived from gram-negative gut bacteria activates resident and recruited macrophages via pattern recognition receptors (eg, TLRs). Activated macrophages release proinflammatory cytokines, which disrupt the balance between pro- and antiinflammatory factors, to promote inflammatory response in hepatic lobule. ER, endoplasmic reticulum; LPS, lipopolysaccharide; TLR, Toll-like receptor.

stress, endoplasmic reticulum stress, cytokines, and/or bacterial endotoxin. It has been estimated that 15% to 25% of cases of NAFL will ultimately progress to NASH. The development of NASH is thus a complex phenomenon in which Kupffer cells seem to play a major role.

Oxidative Stress

Oxidative stress occurs when ROS are produced in excess of antioxidant defenses. In the liver, oxidative stress can be generated through mechanisms involving mitochondrial dysfunction (leading to progressive impairment of β-oxidation, respiratory chain, and Adenosine Triphosphate synthesis) and increased fatty acid oxidation in either peroxisomes by acyl-CoA oxidase[43] or the endoplasmic reticulum by cytochrome P450 (CYP) enzymes 2E1 and CYP4A isoforms (**Fig. 2**).[44] Mitochondrial structure can also play a role in its dysfunction; in patients with NASH, at least 40% of mitochondria are structurally abnormal. These abnormalities (enlarged mitochondria, loss of mitochondrial cristae, and paracrystalline inclusions) impair the electron transport chain enzyme activity and lead to uncoupling of oxidation from phosphorylation and production of ROS.[45]

Endoplasmic Reticulum Stress

Endoplasmic reticulum (ER) stress (ie, disruption of ER function leading to complex signaling cascades that attempt to ameliorate the stress) can lead to generation of ROS. Two CYP enzymes, CYP2E1 and CYP4A, are found in the ER and are responsible for a variety of detoxification reactions.[46] CYP2E1 catalyzes the ω-1 hydroxylation of long-chain fatty acids, whereas CYP4A catalyzes the ω and ω-1 hydroxylation of medium chain fatty acids (C6-C12). Moreover, CYP2E1 catalyzes

the NADH-dependent reduction of oxygen, leading to lipid peroxidation. Hepatic CYP2E1 expression has been shown to be increased in both rat dietary models of steatohepatitis as well as in patients with NASH.[47] In mice fed a methionine choline–deficient (MCD) diet, steatohepatitis was induced as well as CYP2E1 expression and catalysis of lipid peroxides by hepatic microsomes.[48] This study showed that CYP2E1 can act as an initiator of oxidative stress in steatotic livers; however, when CYP2E1 knockout mice were fed a MCD diet, steatohepatitis and lipid peroxidation were still induced,[48] suggesting there are other catalysts to lipid peroxidation involved in the progression of steatohepatitis. One such alternative catalyst is CYP4A, which was discovered in vitro to play a role in lipid peroxidation in the absence of CYP2E1. In this scenario, targeting a specific enzyme involved in lipid peroxidation may be futile due to the redundant nature of microsomal enzyme expression in lipid store management under conditions of NAFLD. Hanada and colleagues[49] used human hepatoma cells and hepatocytes to show that oxidative stress coupled with limited proteosome inhibition induced ER dysfunction and inclusion formation. Inclusion formations (or Mallory bodies) in hepatocytes are significant markers of many liver diseases, including NASH. Prevention or alleviation of oxidative stress may prevent or reduce Mallory body formation and ER dysfunction in NAFLD.

Cytokines

Cytokines are pleiotropic molecules responsible for the propagation of immune response signal. The liver plays a secretory role in the development of NASH in the context of cytokine release from Kupffer cells.[50] For example, tumor necrosis factor alpha (TNF-α) production occurs early and triggers production of other cytokines that recruit inflammatory cells, kill hepatocytes, and initiate fibrogenesis. Proinflammatory cytokines can upregulate the synthesis of secondary mediators and proinflammatory cytokines by macrophages and mesenchymal cells, stimulate production of acute phase proteins, and attract inflammatory cells.[51] In the context of liver injury, TNF-α production occurs early and triggers production of other cytokines that recruit inflammatory cells, kill hepatocytes, and initiate fibrogenesis.[52] Antiinflammatory cytokines counteract inflammation through direct inhibition of proinflammatory cytokines or through other means. From an anatomical standpoint, both the liver and visceral adipose tissue share proximal associations between metabolic cells (hepatocytes and adipocytes, respectively), immune cells, Kupffer cells, hepatic stellate cells, endothelial cells, or macrophages, with each tissue having immediate access to an extensive network of blood vessels for continuous or dynamic immune and metabolic responses.[53] Data in the fatty livers of Zucker diabetic (fa/fa) rats and leptin-deficient ob/ob mice showed abnormal basal cytokine production after a Kupffer cell–activating endotoxin challenge, which resulted in the development of severe NASH.[54] TNF-α has consistently shown to be increased in patients with NASH and correlates with NASH severity.[55]

Gut Microbiota

Bacterial endotoxin, such as lipopolysaccharides (LPS) derived from gram-negative bacteria, are glycolipids that have the ability to induce an inflammatory response in infected organisms. The liver functions to restrict the entry of LPS from the gut into the systemic circulation. The activation of Kupffer cells in the liver plays a critical role in the hepatic clearance of LPS. Specifically, this activation is mediated through LPS binding protein (LBP), CD14, and Toll-like receptor 4 (TLR-4). LPS binds to LBP in the serum, which transfers LPS to the peripheral monocyte membrane-bound

CD14.[56,57] In Kupffer cells, CD14 expression is relatively low normally but is rapidly upregulated on exposure to agents such as LPS. TLR-4 is a downstream component of CD14 and required for Kupffer cell activation. On activation, Kupffer cells release proinflammatory cytokines.[51] This inflammatory response can induce liver injury, perhaps not via a single cytokine alone but rather through disruption of the balance between pro- and antiinflammatory factors. The composition of gut microflora can have a profound impact on caloric intake in both mice and humans. There are 2 predominant populations of microbiota in both the mouse and human, *Firmicutes* and *Bacteroidetes*. In obese individuals, the proportion of *Firmicutes* is higher than that of lean individuals.[58] The significance of this observation lies in the ability of Firmicutes to encode enzymes that can break down otherwise indigestible dietary polysaccharides, thus increasing caloric absorption. When chronic, this enhanced caloric absorption may lead to increased body weight. Alterations in the composition of gut microflora can influence the integrity of the liver. In one study, Sprague-Dawley rats received either saline, probiotics, *Escherichia coli*, *Salmonella enteritidis*, or gentamicin orally for 7 days. On the eighth day, acute liver damage was induced by intraperitoneal injection of D-galactosamine in all but the control saline group. The probiotic, *Escherichia coli* and gentamicin groups had attenuated liver damage, decreased bacterial translocation, and decreased circulating levels of TNF-α, interleukin-6 (IL-6), IL-10, and IL-12.[59] Overall, the study showed that alterations in gut microflora acted through modifications of bacterial translocation, local gut cytokine expression, and endotoxin to prevent (eg, probiotic, , and gentamicin) or exacerbate (eg, *Salmonella enteritidis*) acute liver injury. The composition of gut microbiota can also influence gut permeability. In a study done by Cani and colleagues, ob/ob mice were treated with a control diet or a control diet fortified with either prebiotic or nonprebiotic carbohydrates. Prebiotic treatment involved incorporation of fermentable dietary fiber (oligofructose), whereas nonprebiotic treatment involved incorporation of nonfermentable dietary fiber (cellulose). Prebiotic treated mice exhibited lower circulating LPS, cytokines, and decreased hepatic expression of inflammatory and oxidative stress biomarkers when compared with nonprebiotic controls.[60]

SIGNALING PATHWAYS INVOLVED IN THE PATHOGENESIS OF LIVER FIBROSIS

The onset and severity of liver fibrosis is the most important predictor of hepatic and extrahepatic outcomes in patients with NAFLD. The main acknowledged cell mediators of profibrotic mechanisms in NAFLD are hepatic stellate cells (HSCs). Activation and transdifferentiation of HSCs toward myofibroblasts and, to a lesser extent, other mesenchymal cells (eg, portal fibroblasts, mesothelia fibroblasts, vascular smooth muscle cells, and scar-associated mesenchymal cells) plays a key role in the deposition of extracellular matrix (ECM) of the liver.

Transforming Growth Factor Beta Signaling Pathway of Hepatic Stellate Cells

The exposure of HSCs to profibrotic mediators, such as Hh, OPN, DAMP, transforming growth factor beta (TGF-β), platelet-derived growth factor, TNFSF12, and IL-1β locating in the sinusoidal space adjacent to Kupffer cells, pit cells, and endothelial cells, function to deposit vitamin A in cytoplasmic lipid droplets.[61] They experience the quiescent-to-activated transition of phenotype after exposing to several fibrogenic agents derived from injured or dead hepatocytes and immune cells. Although lots of pathways exhibit implication in the activation of HSCs, TGF-β signaling pathway has widely been accepted to play a central role. TGF-β_1, one of the key drivers of liver fibrogenesis, released initially in the latent form undergoes activation by local integrin

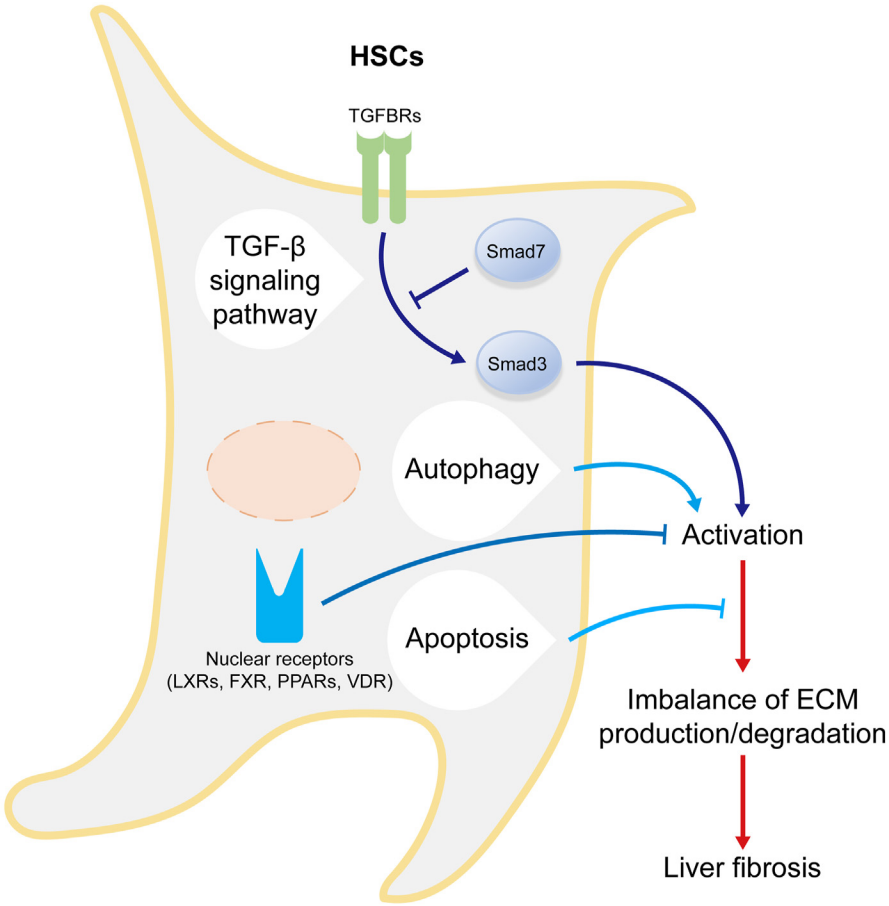

Fig. 3. Pathogenetic pathways underlying hepatic stellate cell (HSC)-induced liver fibrosis in NAFLD. Ligands of TGFBRs activate HSCs through TGF-beta signaling pathway in Smad3-dependent manner, whereas Smad7 antagonizes the process. In similar, autophagy of HSCs promotes their activation. However, nuclear receptors (eg, LXRs, FXR, PPARs, VDR) exert inhibitory role in HSC activation. HSCs apoptosis decreases the population of activated HSCs. Activation of HSCs disrupts the balance of ECM production/degradation and resultantly induces liver fibrosis. ECM, extracellular matrix; ER, endoplasmic reticulum; FXR, farnesoid X receptor; LXR, liver X receptor; PPAR, peroxisome proliferator–activated receptor; TGFBR, transforming growth factor beta receptor.

aV. Then it phosphorylates type I TGF-β receptor to activate the TGF-β signaling pathway in a Smad-dependent way. Phosphorylation of SMAD family member 3 (Smad3) further promotes the transcription of ECM comprising 20 types of fibrillary and nonfibrillar collagen, especially type I and type III collagen. ECM overproduction, together with inhibited degradation, by activated HSCs disrupts the dynamic balance in ECM metabolism and leads to liver fibrosis. Conversely, Smad7 acts as negative regulator to block the fibrogenic response induced by TGF-β signaling pathway (**Fig. 3**).[62] miR-29b, which shows expressive deficiency in activated murine HSCs, exerts inhibitory effect on collagen expression.[63]

Nuclear Receptors of Hepatic Stellate Cells

Except for the imbalanced ECM metabolism, activated HSCs demonstrate phenotypic changes in proliferation, apoptosis, epithelial-to-mesenchymal transition (EMT), chemotaxis, contractility, and so forth. Most of them are revealed to be under the modulation, mainly in the negative pattern, of a group of NRs (eg, LXRs, FXR, PPARs, vitamin D receptor, retinoid X receptor α) associated with glucose and/or lipid metabolism.

Downregulation of these NRs has been recognized in accompany with the activation of HSCs.[64] PPAR-γ deficiency in HSCs exacerbates rodent liver fibrosis in response to carbon tetrachloride (CCl_4) administration. By contrast, miR-16 restores the PPAR-γ expression in a Wnt3a-dependent way and reshapes activated HSCs to the quiescent phenotype.[64] Phenotypic normalization of HSCs abrogates their overexpression of EMT marker (α-SMA), fibrogenic cytokine (TGF-β), and ECM (type I collagen), with an outcome of fibrosis alleviation.[64] Also, VDR ligand inhibits TGF-β–stimulated HSCs activation and subsequently rodent liver fibrosis.[65] HSCs lacking LXR-α/LXR-β suffer from increased cholesterol and retinyl esters, both of which drive their activation through the retinoic acid receptor signaling and predispose LXR-α/β-deficient mice to liver fibrogenesis.[66] Similar phenomena of HSCs activation and fibrosis induction take place in condition of FXR loss.[67] However, treatment of FXR agonist (obeticholic acid) confers clear improvement of fibrosis stage in patients with NAFLD (see **Fig. 3**).[68]

Autophagy of Hepatic Stellate Cells

The pathways of autophagy and apoptosis often act in an antergic way to regulate the activation of HSCs. Autophagy activates HSCs by fatty acids cleaved from retinyl esters of cytoplasmic lipid droplets.[69] In similar, LPS-induced autophagy in HSCs leads to the loss of lipid droplets, dysfunction of retinoic acid signaling, and downregulation of TGF-β pseudoreceptor (Bambi) that sensitize them to fibrotic response stimulated by TGF-β.[70] But autophagy inhibitor (bafilomycin A1) decreases both activation marker of HSCs and their proliferation.[71] HSC-specific deficiency of autophagy-related protein 7 reduces the ECM deposition and fibrogenesis in mice with CCl_4 exposure.[72] Consistently, autophagic inhibition in HSCs and mice attenuates matrix accumulation and live fibrosis (see **Fig. 3**).[73] CircRNA608-miR-222-PINK1 axis has been verified an epigenetic regulation underlying the mitophagy of HSCs in NASH-related liver fibrosis.[74]

Apoptosis of Hepatic Stellate Cells

Compared with their quiescent phenotype, activated HSCs are featured by the resistance to apoptosis.[75] The enhanced proliferation and suppressed apoptosis integrate to expand the population of activated HSCs, which takes essential part in the progression of liver fibrosis (see **Fig. 3**). The activation-related apoptotic insensitivity of HSCs is partly attributed to the significant reduction of miR-130a-3p and resultantly the reactivation of TGF-β signaling pathway by TGFBR1 and TGFBR2 rescue.[76] Expressive loss of miR-16 and miR-15b represents another important mechanism of apoptotic resistance *via* the upregulated level of B-cell lymphoma-2 that prevents mitochondrial apoptosis.[77] Nevertheless, low-level expression of signal transducer and activator of transcription 1 may contribute to this phenotypic characteristics.[78]

SUMMARY

Multiple pathways including insulin signaling, JNK1/2 of MAPK signaling, GLP-1R signaling, and NRs affect to maintain the homeostasis of lipid metabolism in normal

liver. ERK1/2 and P38 of MAPK signaling pathway antagonistically regulate the autophagy of hepatocytes. Functional imbalance of these pathways leads to hepatic steatosis by triglyceride increase and autophagy inhibition, together with insulin resistance based on inhibitory phosphorylation of Irs. Liver steatosis provokes lipid peroxidation and ER stress, which result in hepatocyte injury and lobular inflammation. Besides, gut-derived microbial metabolite (eg, LPS) stimulates the TLR-dependent production of proinflammatory cytokines by macrophages. Both hepatic injury and cytokine-induced inflammatory response initiate the occurrence and development of NASH and related fibrosis. On ligand-based TGFBRs activation, HSCs obtain activated phenotype through the TGF-β signaling pathway. But the activation and population of HSCs can be inhibited by NRs and apoptosis, respectively. Abnormalities in these pathways disrupt the balance of ECM production and degradation of the liver, with an outcome of advanced fibrosis and cirrhosis. Despite the present knowledge of pathogenic pathways, further researches are needed to highlight other ones underlying NAFLD effector cells and related pathological characteristics. Finishing this jigsaw puzzle could make access to the effective prevention and treatment of NAFLD, and keep patients free for NASH and related cirrhosis, and hepatocellular carcinoma.

CLINICS CARE POINTS

- Dysregulation and functional imbalance of pathways (e.g., JNK, ERK, P38, GLP-1R, and insulin signaling) lead to hepatic steatosis.
- Liver steatosis, dysbiosis, and microbial metabolite provokes hepatocyte injury and lobular inflammation.
- Both hepatic injury and inflammatory response induce NASH.
- Quiescent-to-activation transition of hepatic stellate cells results in liver fibrosis and cirrhosis.

FINANCIAL SUPPORT

This work was supported by the National Natural Science Foundation of China (grants 82170588 and 81470859 to Q. Pan; grants 81873565 and 82170593 to J-G. Fan) and the National Key Research and Development Program of China (grant 2021YFC2700802 to J-G. Fan). People's Livelihood Project of PuDong Committee on Science and Technology (PKJ2022-Y44) to Q. Pan.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

1. Fan JG, Wei L, Zhuang H. National workshop on fatty liver and alcoholic liver disease, Chinese society of hepatology, Chinese medical association; fatty liver disease expert committee, Chinese medical doctor association. Guidelines of prevention and treatment of nonalcoholic fatty liver disease (2018, China). J Dig Dis 2019;20(4):163–73.
2. Riazi K, Azhari H, Charette JH, et al. The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol 2022. https://doi.org/10.1016/S2468-1253(22)00165-0. S2468-1253(22)00165-0.

3. Kaya E, Yilmaz Y. Epidemiology, natural history, and diagnosis of metabolic dysfunction-associated fatty liver disease: a comparative review with nonalcoholic fatty liver disease. Ther Adv Endocrinol Metab 2022;13. https://doi.org/10.1177/20420188221139650. 20420188221139650.
4. Matsuzaka T, Kuba M, Koyasu S, et al. Hepatocyte ELOVL fatty acid elongase 6 determines ceramide Acyl-Chain length and hepatic insulin sensitivity in mice. Hepatology 2020;71(5):1609–25.
5. Cordero-Herrera I, Martín MA, Goya L, et al. Cocoa flavonoids protect hepatic cells against high-glucose-induced oxidative stress: relevance of MAPKs. Mol Nutr Food Res 2015;59(4):597–609.
6. Jeong S-H, Kim H-B, Kim M-C, et al. Hippo-mediated suppression of IRS2/AKT signaling prevents hepatic steatosis and liver cancer. J Clin Investig 2018;128(3):1010–25.
7. Yue X, Han T, Hao W, et al. SHP2 knockdown ameliorates liver insulin resistance by activating IRS-2 phosphorylation through the AKT and ERK1/2 signaling pathways. FEBS Open Bio 2020;10(12):2578–87.
8. Jeong O, Kim H-S. Dietary chokeberry and dried jujube fruit attenuates high-fat and high-fructose diet-induced dyslipidemia and insulin resistance via activation of the IRS-1/PI3K/Akt pathway in C57BL/6 J mice. Nutr Metab 2019;16:38.
9. Elchebly M, Payette P, Michaliszyn E, et al. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science (New York, NY) 1999;283(5407):1544–8.
10. Yamamoto-Kataoka S, Ebihara K, Aizawa-Abe M, et al. Leptin improves fatty liver independently of insulin sensitization and appetite suppression in hepatocyte-specific Pten-deficient mice with insulin hypersensitivity. Horm Metab Res 2015;47(3):168–75.
11. Lyu K, Zhang Y, Zhang D, et al. A membrane-bound diacylglycerol species induces PKCε-mediated hepatic insulin resistance. Cell Metab 2020;32(4):654–64.e5.
12. Linden AG, Li S, Choi HY, et al. Interplay between ChREBP and SREBP-1c coordinates postprandial glycolysis and lipogenesis in livers of mice. J Lipid Res 2018;59(3):475–87.
13. Laplante M, Sabatini DM. mTORC1 activates SREBP-1c and uncouples lipogenesis from gluconeogenesis. Proceedings of the National Academy of Sciences of the United States of America 2010;107(8):3281–2.
14. Xiao Y, Liu H, Yu J, et al. Activation of ERK1/2 ameliorates liver steatosis in leptin receptor-deficient (db/db) mice via stimulating ATG7-dependent autophagy. Diabetes 2016;65(2):393–405.
15. Kim T, Wayne Leitner J, Adochio R, et al. Knockdown of JNK rescues 3T3-L1 adipocytes from insulin resistance induced by mitochondrial dysfunction. Biochem Biophys Res Commun 2009;378(4):772–6.
16. Tang P, Low HB, Png CW, et al. Protective function of mitogen-activated protein kinase phosphatase 5 in aging- and diet-induced hepatic steatosis and steatohepatitis. Hepatol Commun 2019;3(6):748–62.
17. Lawan A, Zhang L, Gatzke F, et al. Hepatic mitogen-activated protein kinase phosphatase 1 selectively regulates glucose metabolism and energy homeostasis. Mol Cell Biol 2015;35(1):26–40.
18. Schattenberg JM, Singh R, Wang Y, et al. JNK1 but not JNK2 promotes the development of steatohepatitis in mice. Hepatology 2006;43(1):163–72.

19. Bertran L, Portillo-Carrasquer M, Aguilar C, et al. Deregulation of secreted Frizzled-related protein 5 in nonalcoholic fatty liver disease associated with obesity. Int J Mol Sci 2021;22(13):6895.
20. Vernia S, Cavanagh-Kyros J, Garcia-Haro L, et al. The PPARα-FGF21 hormone axis contributes to metabolic regulation by the hepatic JNK signaling pathway. Cell Metab 2014;20(3):512–25.
21. Duran A, Amanchy R, Linares JF, et al. p62 is a key regulator of nutrient sensing in the mTORC1 pathway. Mol Cell 2011;44(1):134–46.
22. Yang H, Wang S, Ye Y, et al. GLP-1 preserves β cell function via improvement on islet insulin signaling in high fat diet feeding mice. Neuropeptides 2021;85:102110.
23. Liu Y, Wei R, Hong T-P. Potential roles of glucagon-like peptide-1-based therapies in treating non-alcoholic fatty liver disease. World J Gastroenterol 2014;20(27):9090–7.
24. Dong Y, Lv Q, Li S, et al. Efficacy and safety of glucagon-like peptide-1 receptor agonists in non-alcoholic fatty liver disease: a systematic review and meta-analysis. Clinics and Research In Hepatology and Gastroenterology 2017;41(3):284–95.
25. Gupta NA, Mells J, Dunham RM, et al. Glucagon-like peptide-1 receptor is present on human hepatocytes and has a direct role in decreasing hepatic steatosis in vitro by modulating elements of the insulin signaling pathway. Hepatology 2010;51(5):1584–92.
26. Fang Y, Ji L, Zhu C, et al. Liraglutide alleviates hepatic steatosis by activating the TFEB-regulated autophagy-lysosomal pathway. Front Cell Dev Biol 2020;8:602574.
27. Liu D, Pang J, Shao W, et al. Hepatic Fibroblast Growth Factor 21 Is Involved in mediating functions of liraglutide in mice with dietary challenge. Hepatology 2021;74(4):2154–69.
28. Xi Y, Li H. Role of farnesoid X receptor in hepatic steatosis in nonalcoholic fatty liver disease. Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie 2020;121:109609.
29. Carino A, Cipriani S, Marchianò S, et al. BAR502, a dual FXR and GPBAR1 agonist, promotes browning of white adipose tissue and reverses liver steatosis and fibrosis. Sci Rep 2017;7:42801.
30. Gai Z, Krajnc E, Samodelov SL, et al. Obeticholic acid ameliorates valproic acid-induced hepatic steatosis and oxidative stress. Mol Pharmacol 2020;97(5):314–23.
31. Gai Z, Visentin M, Gui T, et al. Effects of farnesoid X receptor activation on arachidonic acid metabolism, NF-kB signaling, and hepatic inflammation. Mol Pharmacol 2018;94(2):802–11.
32. Han X, Cui Z-Y, Song J, et al. Acanthoic acid modulates lipogenesis in nonalcoholic fatty liver disease via FXR/LXRs-dependent manner. Chem Biol Interact 2019;311:108794.
33. Wen F, An C, Wu X, et al. MiR-34a regulates mitochondrial content and fat ectopic deposition induced by resistin through the AMPK/PPARα pathway in HepG2 cells. Int J Biochem Cell Biol 2018;94:133–45.
34. Ng R, Wu H, Xiao H, et al. Inhibition of microRNA-24 expression in liver prevents hepatic lipid accumulation and hyperlipidemia. Hepatology 2014;60(2):554–64.
35. Tran M, Lee S-M, Shin D-J, et al. Loss of miR-141/200c ameliorates hepatic steatosis and inflammation by reprogramming multiple signaling pathways in NASH. JCI Insight 2017;2(21):e96094.

36. Chen X, Tan Q-Q, Tan X-R, et al. Circ_0057558 promotes nonalcoholic fatty liver disease by regulating ROCK1/AMPK signaling through targeting miR-206. Cell Death Dis 2021;12(9):809.
37. Zhao X-Y, Xiong X, Liu T, et al. Long noncoding RNA licensing of obesity-linked hepatic lipogenesis and NAFLD pathogenesis. Nat Commun 2018;9(1):2986.
38. Liu C, Yang Z, Wu J, et al. Long noncoding RNA H19 interacts with polypyrimidine tract-binding protein 1 to reprogram hepatic lipid homeostasis. Hepatology 2018; 67(5):1768–83.
39. Yu Y, He C, Tan S, et al. MicroRNA-137-3p improves nonalcoholic fatty liver disease through activating AMPK. Analytical Cellular Pathology (Amsterdam) 2021; 2021:4853355.
40. Guo X-Y, Sun F, Chen J-N, et al. circRNA_0046366 inhibits hepatocellular steatosis by normalization of PPAR signaling. World J Gastroenterol 2018;24(3):323–37.
41. Sun Y, Song Y, Liu C, et al. LncRNA NEAT1-MicroRNA-140 axis exacerbates nonalcoholic fatty liver through interrupting AMPK/SREBP-1 signaling. Biochem Biophys Res Commun 2019;516(2):584–90.
42. Moreto F, Ferron AJT, Francisqueti-Ferron FV, et al. Differentially expressed proteins obtained by label-free quantitative proteomic analysis reveal affected biological processes and functions in Western diet-induced steatohepatitis. J Biochem Mol Toxicol 2021;35(6):1–11.
43. Liu Y, Liao L, Chen Y, et al. Effects of daphnetin on lipid metabolism, insulin resistance and oxidative stress in OA-treated HepG2 cells. Mol Med Rep 2019;19(6): 4673–84.
44. Vecchione G, Grasselli E, Cioffi F, et al. The nutraceutic silybin counteracts excess lipid accumulation and ongoing oxidative stress in an model of nonalcoholic fatty liver disease progression. Front Nutr 2017;4:42.
45. Song B-J, Akbar M, Jo I, et al. Translational implications of the alcohol-metabolizing enzymes, including cytochrome P450-2E1, in alcoholic and nonalcoholic liver disease. Adv Pharmacol 2015;74:303–72.
46. Ma H-L, Chen S-D, Zheng KI, et al. TA allele of rs2070673 in the CYP2E1 gene is associated with lobular inflammation and nonalcoholic steatohepatitis in patients with biopsy-proven nonalcoholic fatty liver disease. J Gastroenterol Hepatol 2021;36(10):2925–34.
47. Gopal T, Kumar N, Perriotte-Olson C, et al. Nanoformulated SOD1 ameliorates the combined NASH and alcohol-associated liver disease partly via regulating CYP2E1 expression in adipose tissue and liver. Am J Physiol Gastrointest Liver Physiol 2020;318(3):G428–38.
48. Leclercq IA, Farrell GC, Field J, et al. CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine nonalcoholic steatohepatitis. J Clin Investig 2000;105(8):1067–75.
49. Hanada S, Harada M, Kumemura H, et al. Oxidative stress induces the endoplasmic reticulum stress and facilitates inclusion formation in cultured cells. J Hepatol 2007;47(1):93–102.
50. Song K, Kwon H, Han C, et al. Yes-associated protein in kupffer cells enhances the production of proinflammatory cytokines and promotes the development of nonalcoholic steatohepatitis. Hepatology 2020;72(1):72–87.
51. Wree A, McGeough MD, Inzaugarat ME, et al. NLRP3 inflammasome driven liver injury and fibrosis: roles of IL-17 and TNF in mice. Hepatology 2018;67(2): 736–49.

52. Carranza-Trejo AM, Vetvicka V, Vistejnova L, et al. Hepatocyte and immune cell crosstalk in non-alcoholic fatty liver disease. Expet Rev Gastroenterol Hepatol 2021;15(7):783–96.
53. Li H, Zhou Y, Wang H, et al. Crosstalk between liver macrophages and surrounding cells in nonalcoholic steatohepatitis. Front Immunol 2020;11:1169.
54. Diehl AM. Nonalcoholic steatosis and steatohepatitis IV. Nonalcoholic fatty liver disease abnormalities in macrophage function and cytokines. Am J Physiol Gastrointest Liver Physiol 2002;282(1):G1–5.
55. Kugelmas M, Hill DB, Vivian B, et al. Cytokines and NASH: a pilot study of the effects of lifestyle modification and vitamin E. Hepatology 2003;38(2):413–9.
56. Ciesielska A, Matyjek M, Kwiatkowska K. TLR4 and CD14 trafficking and its influence on LPS-induced pro-inflammatory signaling. Cell Mol Life Sci 2021;78(4):1233–61.
57. Tsukamoto H, Takeuchi S, Kubota K, et al. Lipopolysaccharide (LPS)-binding protein stimulates CD14-dependent Toll-like receptor 4 internalization and LPS-induced TBK1-IKKε-IRF3 axis activation. J Biol Chem 2018;293(26):10186–201.
58. Magne F, Gotteland M, Gauthier L, et al. The firmicutes/bacteroidetes ratio: a relevant marker of gut dysbiosis in obese patients? Nutrients 2020;12(5):1474.
59. Li Y-T, Wang L, Chen Y, et al. Effects of gut microflora on hepatic damage after acute liver injury in rats. J Trauma 2010;68(1):76–83.
60. Cani PD, Possemiers S, Van de Wiele T, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 2009;58(8):1091–103.
61. Zhang J, Li Y, Liu Q, et al. Sirt6 alleviated liver fibrosis by deacetylating conserved lysine 54 on Smad2 in hepatic stellate cells. Hepatology 2021;73(3):1140–57.
62. Mu M, Zuo S, Wu R-M, et al. Ferulic acid attenuates liver fibrosis and hepatic stellate cell activation via inhibition of TGF-β/Smad signaling pathway. Drug Des Dev Ther 2018;12:4107–15.
63. Gong X, Wang X, Zhou F. Liver microRNA-29b-3p positively correlates with relative enhancement values of magnetic resonance imaging and represses liver fibrosis. J Biochem 2020;168(6):603–9.
64. Pan Q, Guo C-J, Xu Q-Y, et al. miR-16 integrates signal pathways in myofibroblasts: determinant of cell fate necessary for fibrosis resolution. Cell Death Dis 2020;11(8):639.
65. Wan L-Y, Zhang Y-Q, Li J-M, et al. Liganded Vitamin D receptor through its interacting repressor inhibits the expression of type I collagen α1. DNA Cell Biol 2016;35(9):498–505.
66. Endo-Umeda K, Makishima M. Liver X receptors regulate cholesterol metabolism and immunity in hepatic nonparenchymal cells. Int J Mol Sci 2019;20(20):5045.
67. Yang R, Hu Z, Zhang P, et al. Probucol ameliorates hepatic stellate cell activation and autophagy is associated with farnesoid X receptor. J Pharmacol Sci 2019;139(2):120–8.
68. Younossi ZM, Ratziu V, Loomba R, et al. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. Lancet 2019;394(10215):2184–96.
69. Trivedi P, Wang S, Friedman SL. The power of plasticity-metabolic regulation of hepatic stellate cells. Cell Metab 2021;33(2):242–57.
70. Chen M, Liu J, Yang W, et al. Lipopolysaccharide mediates hepatic stellate cell activation by regulating autophagy and retinoic acid signaling. Autophagy 2017;13(11):1813–27.

71. Wu L, Zhang Q, Mo W, et al. Quercetin prevents hepatic fibrosis by inhibiting hepatic stellate cell activation and reducing autophagy via the TGF-β1/Smads and PI3K/Akt pathways. Sci Rep 2017;7(1):9289.
72. Chen W, Zhang Z, Yao Z, et al. Activation of autophagy is required for Oroxylin A to alleviate carbon tetrachloride-induced liver fibrosis and hepatic stellate cell activation. Int Immunopharm 2018;56:148–55.
73. Meng D, Li Z, Wang G, et al. Carvedilol attenuates liver fibrosis by suppressing autophagy and promoting apoptosis in hepatic stellate cells. Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie 2018;108:1617–27.
74. Xu Z-X, Li J-Z, Li Q, et al. CircRNA608-microRNA222-PINK1 axis regulates the mitophagy of hepatic stellate cells in NASH related fibrosis. Biochem Biophys Res Commun 2022;610:35–42.
75. Bian M, Chen X, Zhang C, et al. Magnesium isoglycyrrhizinate promotes the activated hepatic stellate cells apoptosis via endoplasmic reticulum stress and ameliorates fibrogenesis in vitro and in vivo. Biofactors 2017;43(6):836–46.
76. Wang Y, Du J, Niu X, et al. MiR-130a-3p attenuates activation and induces apoptosis of hepatic stellate cells in nonalcoholic fibrosing steatohepatitis by directly targeting TGFBR1 and TGFBR2. Cell Death Dis 2017;8(5):e2792.
77. Guo C-J, Pan Q, Xiong H, et al. Therapeutic potential of microRNA: a new target to treat intrahepatic portal hypertension? BioMed Res Int 2014;2014:797898.
78. Martí-Rodrigo A, Alegre F, Moragrega ÁB, et al. Rilpivirine attenuates liver fibrosis through selective STAT1-mediated apoptosis in hepatic stellate cells. Gut 2020;69(5):920–32.