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Nonalcoholic fatty liver diseases (NAFLDs), especially nonalcoholic steatohepatitis (NASH), have become a major cause of liver transplant and liver-associated death. However, the pathogenesis of NASH is still unclear. Currently, there is no FDA-approved medication to treat this devastating disease. AMP-activated protein kinase (AMPK) senses energy status and regulates metabolic processes to maintain homeostasis. The activity of AMPK is regulated by the availability of nutrients, such as carbohydrates, lipids, and amino acids. AMPK activity is increased by nutrient deprivation and inhibited by overnutrition, inflammation, and hypersecretion of certain anabolic hormones, such as insulin, during obesity. The repression of hepatic AMPK activity permits the transition from simple steatosis to hepatocellular death; thus, activation might ameliorate multiple aspects of NASH. Here we review the pathogenesis of NAFLD and the impact of AMPK activity state on hepatic steatosis, inflammation, liver injury, and fibrosis during the transition of NAFL to NASH and liver failure.

Nonalcoholic fatty liver disease (NAFLD) is a major complication of metabolic dysfunction, usually a complication of obesity. NAFLD is an umbrella term describing two stages of chronic fatty liver diseases: NAFL (nonalcoholic fatty liver) and NASH (nonalcoholic steatohepatitis). NAFL is characterized by hepatic steatosis, which can be reversed simply by reduced caloric intake and exercise. Advanced stage NASH is characterized by steatosis with hepatic inflammation and liver injury, often accompanied by pericellular fibrosis (1). Although NASH is potentially reversible with diet and exercise, there is no FDA-approved medication to treat this devastating disease. NASH frequently progresses to cirrhosis, liver failure, and hepatocellular carcinoma (HCC) (1–3). The number of individuals with NAFLD in the United States is expected to increase from 83.1 million in 2015 to 100.9 million in 2030, whereas severe cases with advanced fibrosis are anticipated to increase by more than 100% (4).

Metabolic syndrome substantially increases the risk of NASH (5). The occurrence of NAFLD is predominantly associated with obesity and insulin resistance (6), whereas the incidence of NASH strongly correlates with central obesity, defined by the waist/hip ratio (7). Type 2 diabetes represents an independent pathogenic factor for NASH (8, 9). Most individuals with NAFLD exhibit dyslipidemia, including hypertriglyceridemia and hypercholesterolemia (8, 9). Moreover, diverticulosis and overgrowth of the intestinal microbiome have been identified in human NASH. NASH-related liver injury and fibrosis might result from exposure to intestine-derived bacterial products, such as lipopolysaccharide (10). In addition, NAFLD and NASH can also result from a diverse array of pharmacotherapies, including glucocorticoids, tamoxifen, and methotrexate (8, 9) and long-term antiretroviral therapy for HIV (8).

The precise pathogenic mechanisms that give rise to NASH remain unclear. The "two-hit model" proposed that ectopic lipid storage caused by high-fat diet, obesity, and insulin resistance primes hepatocytes for a second insult inducing hepatic inflammation, liver injury, and fibrogenesis, which in turn promotes the progression from NAFL to NASH and cirrhosis (11, 12). However, it is now clear that multiple pathogenic factors may act in parallel and synergistically. A "multiple-hit model" proposes that multiple pathogenic factors act together to induce NAFLD, including but not limited to insulin resistance, inflammation, lipotoxicity, mitochondrial dysfunction, ER stress, oxidative stress, genetic determinants, and epigenetic factors (12).

AMP-activated protein kinase (AMPK) is an important energy sensor that regulates metabolic homeostasis. The activity of AMPK is inhibited by overnutrition during obesity and NAFLD (13–18). A recent study demonstrated that although the loss of AMPK activity does not affect hepatic lipid accumulation, it substantially exacerbates liver injury and hepatic fibrosis (17, 19), both of which could promote the transition from NASH to cirrhosis and HCC. Moreover, reactivation of AMPK improves symptoms of NASH and therapeutically improves liver injury (17, 19–21). We review here the regulation of AMPK activity during the pathophysiology of NAFL and the roles of the protein kinase in the regulation of NASH development.

AMPK

AMPK is a heterotrimeric serine/threonine kinase comprised of three subunits: the α catalytic subunit and the β and γ regulatory subunits. In mammalian cells, the AMPK α subunit has two isoforms, α1 and α2, encoded by the *Prkaa1* and *Prkaa2* genes. The β subunit has β1 and β2 isoforms, encoded by the *Prkab1* and *Prkab2* genes. The γ subunit includes three isoforms, γ1, γ2, and γ3, encoded by *Prkag1*, *Prkag2*, and *Prkag3*. These isoforms have the potential to form 12 different heterotrimeric complexes (15, 22). Although it remains uncertain whether there are functional differences among these isoforms, previous studies have reported different tissue distribution, regulation, subcellular localization, and functions for these complexes (23–25). The γ2 and γ3 isoforms possess

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unique N-terminal regions that may determine the subcellular localization of the holoenzyme (26).

The activity of AMPK is regulated by multiple factors through modulation of different subunits. The phosphorylation of Thr^{172} within the catalytic domain of the α subunit is required for the activation of AMPK (27, 28). Three upstream kinases, liver kinase B1 (LKB1), Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ), and transforming growth factor β (TGFβ)-activated kinase 1 (TAK1), have been shown to phosphorylate residue Thr^{172} (15, 29–34). Moreover, sirtuin 1 (SIRT1), an NAD^+-dependent deacetylase, deacetylates LKB1 to induce its cytosolic localization and thus increases LKB1-dependent AMPK phosphorylation (35, 36). Another study showed that the Src family kinase Fyn phosphorylates LKB1 on Tyr^{265} and Tyr^{365} to increase LKB1 cytosolic localization and AMPK phosphorylation (37). Upon elevated intracellular Ca^{2+} concentrations, CaMKKβ directly phosphorylates AMPK on Thr^{172} to increase its activity (29, 30, 33). Although the mechanism by which TAK1 activates AMPK is still unclear, several studies have demonstrated that the deletion of TAK1 inhibits starvation, metformin, and AICAR-induced AMPK activation (31, 34, 38).

As an important energy sensor, AMPK is allosterically activated by AMP (and ADP to a lesser extent), which binds to the γ subunit, and is repressed by ATP (22, 39). The interaction between AMP and the γ subunit leads to a conformational change, which protects Thr^{172} from dephosphorylation (40–42). Consequently, AMPK senses a high AMP/ATP ratio and responds by increasing lipid oxidation and mitochondrial biogenesis, while reducing lipogenesis and glycogenesis, to increase intracellular ATP levels (43, 44). In addition to ATP, phosphocreatine also allosterically inhibits AMPK activity (45). This finding is consistent with the energy-sensing roles of AMPK.

AMPK activity is controlled by nutrients, including lipids, amino acids, and carbohydrates. High-fat diet feeding reduces AMPK expression and phosphorylation in skeletal muscle, heart, liver, adipose tissue, aortic endothelium, and hypothalamus (18, 46–52). One study suggested that palmitate represses AMPK activity via the ceramide-dependent activation of protein phosphatase 2A (PP2A). High-fat diets rich in palmitate inhibit AMPK activation *in vivo* (53). The increase of cardiac lipid content in Zucker rats and *ob/ob* mice results in the attenuation of AMPK activation. In cultured cardiomyocytes, fatty acids up-regulate the expression of *Ppm1a* (protein phosphatase 2C, PP2C) to inhibit AMPK, representing a feed-forward effect of lipid overload to promote energy storage (48). Excess amino acids have also been demonstrated to suppress AMPK activity (54, 55). High-protein diet or increased protein intake reduces AMPK phosphorylation while increasing mTOR phosphorylation in the hypothalamus and liver. An elevated level of amino acids, especially leucine, results in a decrease of the AMP/ATP ratio and thus represses AMPK activation (56, 57). The AMPKβ subunit also contains a conserved glycogen-binding domain. Glycogen, particularly when in a highly branched state, inhibits AMPK activity. Branched oligosaccharides with a single α1–6 branch allosterically inhibit AMPK phosphorylation by upstream kinases (58). Furthermore, recent studies demonstrated that AMPK activity is modulated by glucose levels. Mechanistically, aldolase senses the glycolytic intermediate fructose 1,6-bisphosphate, and interacts with v-ATPase on the lysosomal surface. Without glucose, the absence of fructose 1,6-bisphosphate causes an altered interaction between aldolase and v-ATPase, which leads to the formation of an AMPK activation complex containing v-ATPase, LKB1, AMPK, AXIN, and Regulator, thus promoting AMPKα Thr^{172} phosphorylation. The presence of glucose disrupts this complex and thus prevents AMPK activation (59).

Hormones and cytokines also modulate AMPK activity in physiological or pathological conditions. Insulin markedly reduces the activity of AMPK, both by increasing glucose uptake and oxidation and through Akt-mediated phosphorylation of AMPKα $Ser^{485/491}$. These phosphorylation events inhibit the activity of the enzyme, leading to a conformational change that exposes the activating Thr^{172} phosphorylation site within the kinase domain in the α subunit. As a result, protein phosphatases, such as PP2A, dephosphorylate Thr^{172} to deactivate AMPK (60). Proinflammatory cytokines, such as tumor necrosis factor α (TNFα), have also been shown to inhibit AMPK activity. TNFα induces the expression of *Ppm1a* (PP2C), which dephosphorylates Thr^{172} to deactivate AMPK in skeletal muscle. Consequently, TNFα reduces ACC phosphorylation and represses fatty acid oxidation (14). Furthermore, TNFα activates TANK-binding kinase 1 (TBK1) in adipocytes. TBK1 phosphorylates Ser^{459} and Ser^{476} residues in the α subunit to inhibit AMPK activity (18). Thus, AMPK activity is regulated by energy status, nutrient availability, and hormone/cytokine levels via various mechanisms, implicating its essential roles in monitoring metabolic processes (Fig. 1).

AMPK and NAFLD

Several studies have reported a strong association between the reduction of AMPK activity and the incidence of metabolic diseases, including obesity, diabetes, and NAFLD (13). AMPK activity is inhibited in both obese rodents and human subjects, mainly attributable to the excess calorie intake and/or lack of exercise as well as increased inflammation (13–16, 18, 52). Hepatic AMPK activity is substantially attenuated in both NAFL and NASH (17, 19, 61). Although the roles of AMPK repression in the pathogenesis of these states remains uncertain, both pharmacological and genetic activation of AMPK in the liver exhibit beneficial effects on multiple aspects of NAFLD (17, 20, 62, 63).

Hepatic steatosis

Ectopic lipid accumulation in the liver causes hepatic steatosis, which is tightly associated with obesity, insulin resistance, and diabetes. The amount of hepatic lipid content is predominantly regulated by four major pathways: *de novo* lipogenesis, fatty acid uptake, lipid oxidation, and very low-density lipoprotein (VLDL) secretion. *De novo* lipogenesis converts acetyl-CoA to fatty acids. The rate of *de novo* lipogenesis is determined by two key enzymes: acetyl-CoA carboxylase and fatty acid synthase (FAS). ACC catalyzes the carboxylation of acetyl-CoA to generate malonyl-CoA, whereas FAS uses acetyl-CoA

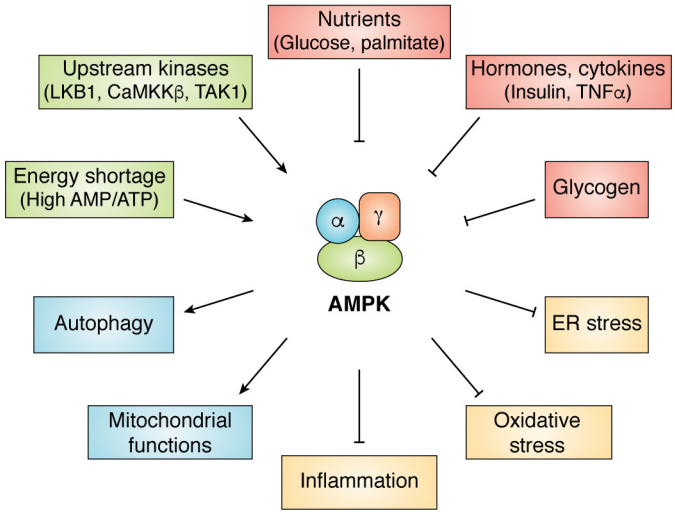

Figure 1. The regulation and function of AMPK

or malonyl-CoA to synthesize fatty acids. Esterification catalyzed by glycerol-3-phosphate acyltransferases, acylglycerol-3-phosphate acyltransferases, and diacylglycerol acyltransferases further converts fatty acids to triglycerides for storage (64). The increase of *de novo* lipogenesis and esterification results in hepatic steatosis. Activation of AMPK inhibits ACC via direct phosphorylation, reducing overall hepatic lipid storage, as seen in a phase 2 clinical trial (65). Hepatic lipid accumulation can also result from fatty acid uptake and subsequent esterification into triglyceride. Hepatic lipid accumulation increases as a function of high levels of serum-free fatty acids, which are determined by the rates of lipolysis in adipose tissue. In this regard, the ability of AMPK activation to improve systemic insulin sensitivity could indirectly lower lipolysis, thus reducing free fatty acid levels and fatty acid re-esterification in liver (66, 67).

De novo lipogenesis and fatty acid uptake are offset by lipid oxidation, the major catabolic pathway that resolves hepatic lipid storage. β-Oxidation converts fatty acids to acetyl-CoA. The rate-limiting step is catalyzed by carnitine palmitoyltransferases (CPTs), which transport cytosolic acyl-CoA into mitochondria. Acetyl-CoA generated from this process can enter the TCA cycle and then be utilized by mitochondria to produce ATP or heat. Thus, hepatic triglycerides and fatty acids levels are also tightly regulated by mitochondrial number and functions. Finally, hepatic lipid content is also regulated by VLDL packaging and secretion. VLDL is the major circulating vesicle that carries triglycerides from the liver to peripheral tissues (64).

The role of AMPK in hepatic lipid metabolism remains controversial. AMPK deletion does not dramatically affect hepatic steatosis under obesogenic conditions, although activation of AMPK by A-769662 reduced hepatic lipid content in high-fat-diet–induced NAFL (19). Mechanistically, AMPK inhibits *de novo* lipogenesis via directly phosphorylating ACC1 Ser^{79} and ACC2 Ser^{212} to repress the activity of the enzyme (19). The inhibition of ACC reduces malonyl-CoA, which is an allosteric inhibitor of CPT1 (68). Therefore, AMPK activation leads to increased fatty acid oxidation. In addition, sterol regulatory element–binding proteins (SREBPs) regulate both triglyceride and cholesterol synthesis. Maturation and activation of SREBP-1c induce the expression of *Acc* and *Fasn* to increase *de novo* lipogenesis. Activation of SREBP-2 up-regulates the expression of cholesterol synthesis genes, including HMG-CoA reductase (*Hmgcr*), HMG-CoA synthase (*Hmgcs*), farnesyl diphosphate synthase (*Fdps*), and squalene synthase (*Sqs*) (69). AMPK directly phosphorylates and inhibits both SREBP-1 and -2 to reduce *de novo* lipogenesis and cholesterol synthesis in the liver (70). In addition, mTOR increases lipogenesis by promoting transcription and maturation of SREBPs (69). AMPK has been shown to phosphorylate both TSC2 and Raptor to inhibit mTORC1 activity (71–73). Consequently, activation of AMPK inhibits triglyceride and cholesterol synthesis to reduce hepatic steatosis.

The levels of circulating fatty acids are regulated by lipolysis in adipose tissue. Free fatty acids generated by adipocytes are burned through β-oxidation and respiration or secreted into the circulation. Enhanced lipolysis in adipose tissue increases circulating fatty acids, which in turn leads to hepatic fatty acid uptake and promotes steatosis (74, 75). On the other hand, increased fatty acid oxidation and mitochondrial respiration in adipose tissue reduces circulating fatty acids and alleviates hepatic steatosis (76). As a master regulator of metabolism, AMPK directly phosphorylates and activates peroxisome proliferator-activated receptor γ co-activator 1α (PGC1α) to induce mitochondrial biogenesis, hence increasing mitochondrial number (77, 78). In addition, AMPK induces mitophagy via phosphorylating and activating Unc-51–like autophagy activating kinase 1 (ULK1) to clear damaged mitochondria and maintain mitochondrial homeostasis in adipose tissue (79, 80). Although it is still controversial, several studies indicated that AMPK directly phosphorylates hormone-sensitive lipase on Ser^{565} to inhibit lipolysis in adipocytes (66, 81, 82). In summary, the activation of AMPK attenuates hepatic steatosis by modulating *de novo* lipogenesis, fatty acid oxidation, and fatty acid release from adipose tissue (Fig. 2).

Hepatic inflammation

Inflammation is a hallmark for the progression from NAFL to NASH. The recruitment of immune cells, including macrophages, neutrophils, dendritic cells, and T cells, and the production of immune cell–derived cytokines, chemokines, and eicosanoids lead to hepatic inflammation (83). The increased number of recruited macrophages is currently used as a histological marker to determine liver inflammation. During the development of NASH, bone marrow–derived macrophages infiltrate into liver and work together with Kupffer cells to promote inflammation. Kupffer cells are yok sac–derived, self-renewable liver-resident macrophages that localize within hepatic sinusoids (84). A recent study using single-cell RNA-Seq and lineage tracing has revealed that during NASH, resident Kupffer cell partially lose their cell identity and express genes that promote hepatocyte death. Meanwhile, increased bone marrow–derived macrophages acquire some Kupffer cell features and further enhance inflammation (85). Under inflammatory conditions during NASH, Kupffer cells can be induced to

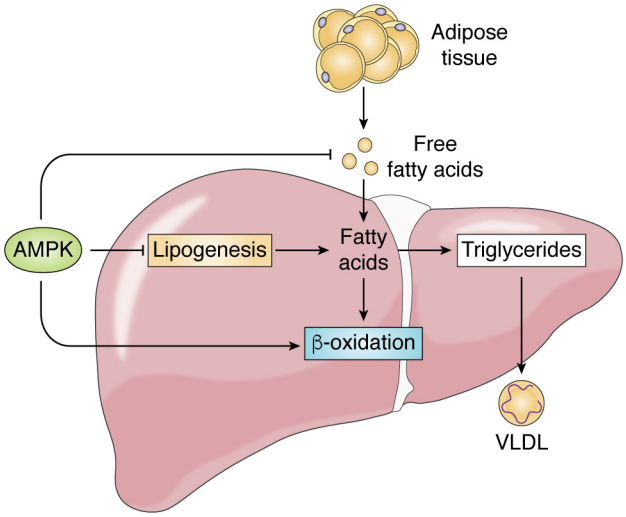

Figure 2. The regulation of hepatic steatosis by AMPK. Activation of AMPK inhibits *de novo* lipogenesis while promoting fatty acid oxidation (β-oxidation) in the livers. In addition, AMPK activation reduces free fatty acid release from adipose tissue to prevent hepatic steatosis.

proliferate and differentiate into different subpopulations (86). Depending on the stimulating signals, both recruited macrophages and Kupffer cells undergo differentiation into M1- or M2-like macrophages (87). In response to proinflammatory stimuli, M1-like macrophages produce various cytokines, including TNFα and IL1β, to induce hepatocellular death and liver injury. In contrast, upon stimulation from signals inducing M2-like differentiation, macrophages secret cytokines like TGFβ to activate hepatic stellate cells (HSCs), and thus promote hepatic fibrosis (88–90). Therefore, hepatic inflammation plays a central role in the progression of NAFLD.

Oxidative stress, ER stress, lipotoxicity and mitochondrial dysfunction are among other pathogenic factors that trigger inflammation (83). Under conditions of oxidative stress, the production of reactive oxygen species (ROS) by mitochondria and NADPH oxidase is substantially up-regulated in the liver (91). The macrophage is a major source of ROS generated by NADPH oxidase (92, 93). Danger-associated molecular patterns are known to induce ROS formation in macrophages (94). ROS activates the NOD–, LRR–, and pyrin domain–containing protein-3 (NLRP3) inflammasome to induce inflammation. Blockage of the NLRP3 inflammasome attenuates hepatic inflammation and fibrosis in NASH (95). Oxidative stress promotes lipid peroxidation in the liver. The products of this non-enzymatic process, such as oxidized phospholipids and 4-hydroxynonenal, further enhance ROS generation to form a vicious cycle (96, 97). Neutralization of oxidized phospholipids prevents mitochondrial damage and protects against amylin diet–induced NASH (96). ER stress caused by the accumulation of unfolded or misfolded protein also promotes the production of ROS by inducing Ca^{2+} release from the ER and thus inducing inflammation (98). Dietary factors, such as fructose, free fatty acids, and cholesterol, are external pathogenic factors triggering inflammation. Fructose is known to induce proinflammatory gene expression and impair β-oxidation in the liver (99–102). The amylin diet, containing fructose, is widely used to establish preclinical NASH mouse models. Elevated levels of saturated fatty acids induce the accumulation of unfolded protein and ER stress (103). High free cholesterol has been associated with mitochondrial dysfunction, ER stress, and oxidative stress (104, 105), all of which contribute to the development of hepatic inflammation.

Previous studies demonstrated that activation of AMPK decreases the expression of proinflammatory mediators and attenuates inflammation in different conditions (106–108). Liver-specific expression of constitutively active AMPK reduces the expression of inflammatory genes (20). The chemokine CCL2 (monocyte chemoattractant protein 1, MCP-1) is an essential player in the recruitment of macrophages. The expression of *Ccl2* is induced by the activation of multiple proinflammatory signaling pathways, including NFκB- and JNK-mediated pathways (109, 110). AMPK activation by AICAR or by the expression of constitutively active AMPK largely alleviates palmitate- and TNFα-induced NFκB activation (111). Mechanistically, AMPK inhibits the nuclear localization of NFκB to repress the expression of NFκB target genes. Moreover, AMPK activation increases NAD^+ levels, leading to the activation of SIRT1. SIRT1 deacetylates the NFκB RelA/p65 subunit at Lys^{310} to attenuate its transactivation activity (112). Our recent work found that AMPK phosphorylates ULK1 to induce TBK1 phosphorylation. TBK1, in turn, phosphorylates NFκB-inducing kinase (NIK) to induce its degradation. Consequently, AMPK activation leads to NIK degradation, resulting in attenuation of the atypical NFκB pathway, which is aberrantly activated in NAFLD (18, 113). Furthermore, AMPK activation by A-769662 inhibits IL-1β–induced JNK activation (114). As a result, AMPK inhibits proinflammatory signaling pathways to reduce *Ccl2* expression (18, 115). In addition, other studies have found that multiple downstream transcription factors, including FoxO family proteins and PGC1α, could be involved in the anti-inflammatory effects of AMPK through regulating gene expression (108).

In addition to inhibiting proinflammatory signaling, AMPK may ameliorate inflammation via its anti-oxidative functions. ROS plays critical roles in the development of hepatic inflammation. AMPK activation attenuates cytosolic ROS production by down-regulating the expression of NAD(P)H oxidase genes and reducing mitochondrial ROS through an increase in PGC1α target gene expression (116–119). Moreover, the activation of AMPK up-regulates the expression of *Sod2* (superoxide dismutase 2) and *Cat* (catalase) to alleviate oxidative stress (120). The reduction of ROS in turn ameliorates NLRP3 activation to reduce inflammation (121). Studies have demonstrated that antioxidants, such as coenzyme Q_{10} and γ-tocotrienol, activate AMPK and inhibit NLRP3 activation (122, 123). An AMPK-FOXO3 pathway has been shown to induce the expression of thioredoxin (Trx) (124). Trx binds to the thioredoxin-interacting protein (Txnip), blocking the interaction between Txnip and NLRP3. Consequently, AMPK inhibits the activation of NLRP3 inflammasome to prevent hepatic inflammation (108, 125).

Liver injury

In normal liver, hepatocyte apoptosis maintains liver homeostasis, with a strict equilibrium between the loss and

replacement of hepatocytes (110, 126). However, under pathological conditions, such as viral infection, alcoholic or nonalcoholic steatohepatitis, and physical injury, extensive hepatocellular death leads to sustained liver injury, which is responsible for the enhanced scarring, bridging fibrosis, and subsequent development of cirrhosis (127–129). Moreover, hepatocellular death has been recognized as a major contributor to the progression to hepatocellular carcinoma (130). Therefore, understanding the molecular mechanisms of hepatocellular death is crucial for the treatment of liver diseases (131). Currently, hepatocellular death, reflected by increased serum aminotransferase levels, is the most widely used and sensitive parameter to screen for and monitor individuals with liver disease (110, 132). Evaluation of liver injury drives therapeutic decisions and has prognostic value for NASH.

Previous studies suggested that multiple types of cell death may contribute to liver injury in NASH. Although apoptosis plays a vital role to maintain homeostasis in healthy liver, elevated apoptotic stimuli produces extensive apoptosis, resulting in liver injury (126, 127). Whereas early findings suggested that the extensive apoptosis is responsible for NASH-associated liver damage, recent work suggests that other types of cell death also contribute to the pathogenesis of NASH. Necroptosis is a programmed form of inflammatory cell death that is mediated by the activation of receptor-interacting serine/threonine-protein kinase 3 (RIPK3). Activated RIPK3 phosphorylates mixed kinase domain–like protein, which forms pores in the membrane to cause rupture (133, 134). The expression of *Ripk3* is induced in a methionine- and choline-deficient diet (MCD)-induced NASH mouse model. Knockout of *Ripk3* ameliorates liver injury in these mice (135). Ferroptosis is a type of programmed cell death dependent on iron, producing lipid peroxidation-mediated cell death in NASH (136). Although it remains unclear whether pyroptosis, the highly inflammatory form of programmed cell death, occurs during NASH, the pyroptotic effector gasdermin D (GSDMD) and its pyroptosis-inducing fragment GSDMD-N are increased in human NASH. GSDMD deficiency alleviates lipogenesis and inflammation in MCD-induced NASH model (137).

In the pathogenesis of NASH, these pathogenic factors, including but not limited to oxidative stress, ER stress, lipotoxicity, and mitochondrial dysfunction, all activate signaling that has the capacity to mediate hepatocellular death (138). However, the crucial underlying mechanism for the regulation of hepatocellular death and liver injury during the transition from NAFL to NASH remains unclear. Our recent study found that normal AMPK activity is required to prevent hepatocellular death and liver damage. Liver-specific knockout of AMPKα1/α2 exaggerates liver injury in the choline-deficient high-fat diet (CD-HFD: 60% fat, 0.1% methionine, L-amino acid, no added choline)–induced NASH model. During the development of NASH, caspase-mediated apoptotic signaling pathways are induced in hepatocytes. The cleavage and activation of caspase-6 mediate a feed-forward loop to sustain the activation of apoptotic pathways and thus cause hepatocellular death in NASH. AMPK directly phosphorylates procaspase-6 to inhibit its cleavage and activation and control cell death. The repression of AMPK during obesity and NAFLD unleashes caspase-6 to prime hepatocyte for apoptosis. We further demonstrated that activation of AMPK by A-769662 therapeutically improves liver damage even after NASH onset (17).

Hepatic fibrosis

Hepatic fibrosis is a key feature used to determine severity of NASH. Progressive liver fibrosis frequently results in the progression from NASH to cirrhosis. Fibrosis is a wound-healing process that forms excess fibrous connective tissues to replace normal parenchymal tissues. Normal or mild fibrosis during injury is necessary for tissue repair. Extensive or chronic fibrosis results in the excessive accumulation of collagen and fibers in the extracellular space (62, 128). Hepatic fibrosis is induced upon activation of pathogenic pathways, including inflammation, oxidative stress, and liver injury. Whereas inflammation induces the pericellular fibrosis typically observed in the early stages of NASH, scarring after sustained liver injury leads to progressive fibrosis and subsequent development of cirrhosis (62, 128). The aggravation of liver damage worsens hepatic fibrosis even without changes in hepatic steatosis or inflammation (17). At advanced stages of hepatic fibrosis, the disruption of normal liver architecture and functions possibly results in the liver-associated death (128, 139). Therefore, resolving hepatic fibrosis and restoring liver functions are the ultimate goals for NASH treatment (1, 128, 139).

In chronic liver diseases, HSCs are direct mediators of fibrosis. Growth factors and inflammatory cytokines produced from other cell types, such as TGFβ from macrophages and platelet-derived growth factor from endothelial cells, cause overproliferation and transdifferentiation of HSCs. Upon activation, HSCs transdifferentiate into myofibroblasts, which produce an excessive amount of extracellular matrix (ECM) proteins (62, 140). Consequently, the accumulation of ECM and fibers disturbs hepatic homeostasis and further promotes the progression to cirrhosis or even HCC (141).

The activation of AMPK has been demonstrated to improve liver injury and attenuate hepatic fibrosis in different NASH models. AMPK activation by A-769662 improves liver injury and alleviates fibrosis in CD-HFD–induced NASH (17). Low doses of sorafenib, the first-line treatment for advanced HCC, activates AMPK and attenuates NASH-associated fibrosis in experimental mouse and monkey models (21). The injection of CCl_4 induces liver injury and hepatic fibrosis in a lipid-independent manner. In this mouse model, AMPK activation ameliorates fibrogenesis via inhibiting HSC proliferation and downregulating the expression of fibrogenic genes, including *Nox4*, *Tgfb*, and *Acta2* (142, 143). Mechanistic studies further demonstrated that CCl_4 induces strong oxidative stress and hepatic accumulation of ROS, which in turn promotes hepatocellular death and liver fibrosis. The activation of AMPK prevents ROS production and HSCs activation and thus protects against liver injury and fibrosis (143–145). Furthermore, the fibrogenic cytokine TGFβ is mainly derived from macrophages and induces HSC activation during the fibrogenic process. Multiple studies demonstrated that the induction of AMPK activity represses TGFβ-induced expression of fibrogenic genes in

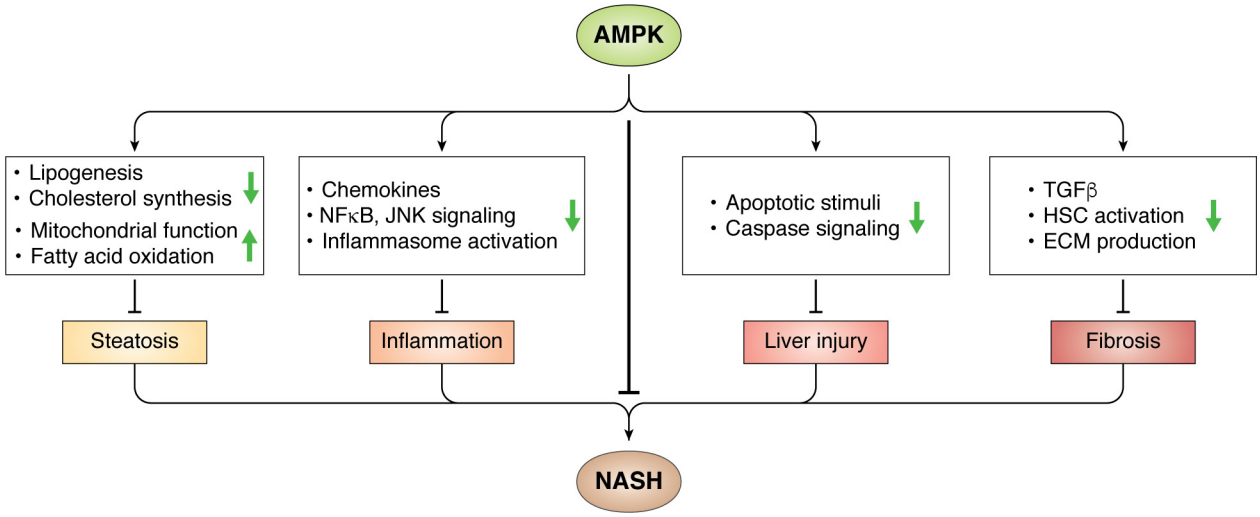

Figure 3. The effects of AMPK on hepatic steatosis, inflammation, liver injury, and fibrosis in NASH.

HSCs (62, 146–149). Both metformin and AICAR down-regulate the expression of *Col1a* and *Acta2* (α-smooth muscle actin) in TGFβ-treated HSCs (150, 151). AMPK disrupts the interaction between Smad3 and its transcriptional coactivator p300 and induces proteasomal degradation of p300 to reduce fibrogenic genes expression in HSCs (151). Additionally, the activation of AMPK by adiponectin induces nitric oxide production to inhibit HSC proliferation and promote HSC apoptosis (149). AMPK activation by macrophage migration–inhibitory factor represses HSC migration and prevents hepatic fibrosis (62, 152). In summary, the activation of AMPK ameliorates hepatic fibrosis through multiple mechanisms, including reducing fibrogenic stimuli, preventing HSC activation/proliferation/migration and inhibiting expression of fibrogenic genes.

Concluding remarks

AMPK is a critical energy sensor that regulates metabolic homeostasis. An increasing body of evidence has demonstrated that AMPK activity is repressed during metabolic disorders, including obesity, diabetes, and NAFLD. The inhibition of AMPK connects lipid dysregulation to inflammation, liver injury, and fibrosis in NAFLD (Fig. 3). Pharmacological activation of AMPK improves NASH in both murine and simian models (21). AMPK activators, such as A-769662, PF-739, or metformin, ameliorate symptoms of NASH-hepatic steatosis, inflammation, liver injury, and fibrosis via different mechanisms. However, global activation of AMPK by MK-8722 results in cardiomyocyte hypertrophy, possibly due to the induction of cardiac glycogen synthesis (153). Further investigation is needed to determine whether cardiomyocyte hypertrophy is a general effect for systemic AMPK activation or a side effect that is specific for MK-8722. In the event that increased AMPK activity results in cardiomyocyte hypertrophy, liver-specific activation of AMPK by liver cell–targeted drug delivery might be of great interest for the treatment of NASH.

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Conflict of interest—A. R. S. and P. Z. are named inventors of patent application related to targeting the AMPK-caspase-6 axis for NASH treatment. A. R. S. is a founder of Elgia Therapeutics.

Abbreviations—The abbreviations used are: NAFLD, nonalcoholic fatty liver disease; NAFL, nonalcoholic fatty liver; NASH, nonalcoholic steatohepatitis; FDA, Food and Drug Administration; HCC, hepatocellular carcinoma; ER, endoplasmic reticulum; AMPK, AMP-activated protein kinase; TNF, tumor necrosis factor; VLDL, very low-density lipoprotein; FAS, fatty acid synthase; CPT, carnitine palmitoyltransferase; SREBP, sterol regulatory element–binding protein; mTOR, mechanistic target of rapamycin; HSC, hepatic stellate cell; ROS, reactive oxygen species; JNK, c-Jun N-terminal kinase; AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide; NIK, NFκB-inducing kinase; MCD, methionine- and choline-deficient diet; GSDMD, gasdermin D; CD-HFD, choline-deficient high-fat diet; TGF, transforming growth factor; ECM, extracellular matrix.

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