BIOMOLECULES & THERAPEUTICS

Invited Review
Biomol Ther 33(1), 18-38 (2025)

New Insights into AMPK, as a Potential Therapeutic Target in Metabolic Dysfunction-Associated Steatotic Liver Disease and Hepatic Fibrosis

Haeun An[1,†], Yerin Jang[1,†], Jungin Choi[1,†], Juhee Hur[1], Seojeong Kim[2] and Youngjoo Kwon[1,2,*]

[1]*College of Pharmacy, Ewha Womans University, Seoul 03760,*
[2]*Graduate School of Pharmaceutical Sciences, Ewha Womans University, Seoul 03760, Republic of Korea*

Abstract

AMP-activated protein kinase (AMPK) activators have garnered significant attention for their potential to prevent the progression of metabolic dysfunction-associated steatotic liver disease (MASLD) into liver fibrosis and to fundamentally improve liver function. The broad spectrum of pathways regulated by AMPK activators makes them promising alternatives to conventional liver replacement therapies and the limited pharmacological treatments currently available. In this study, we aim to illustrate the newly detailed multiple mechanisms of MASLD progression based on the multiple-hit hypothesis. This model posits that impaired lipid metabolism, combined with insulin resistance and metabolic imbalance, initiates inflammatory cascades, gut dysbiosis, and the accumulation of toxic metabolites, ultimately promoting fibrosis and accelerating MASLD progression to irreversible hepatocellular carcinoma (HCC). AMPK plays a multifaceted protective role against these pathological conditions by regulating several key downstream signaling pathways. It regulates biological effectors critical to metabolic and inflammatory responses, such as SIRT1, Nrf2, mTOR, and TGF-β, through complex and interrelated mechanisms. Due to these intricate connections, AMPK's role is pivotal in managing metabolic and inflammatory disorders. In this review, we demonstrate the specific roles of AMPK and its related pathways. Several agents directly activate AMPK by binding as agonists, while some others indirectly activate AMPK by modulating upstream molecules, including adiponectin, LKB1, and the AMP: ATP ratio. As AMPK activators can target each stage of MASLD progression, the development of AMPK activators offers immense potential to expand therapeutic strategies for liver diseases such as MASH, MASLD, and liver fibrosis.

Key Words: AMP-activated protein kinase (AMPK), Metabolic dysfunction-associated steatotic liver disease (MASLD), Metabolic dysfunction-associated steatohepatitis (MASH), Hepatic fibrosis, AMPK activators

INTRODUCTION

More than a quarter of the world's population suffers from metabolic dysfunction-associated steatotic liver disease (MASLD), characterized by the accumulation of hepatic fatty acids exceeding 5% of liver weight in the absence of excessive alcohol consumption or other conditions typically associated with steatosis (Miao *et al*., 2024). MASLD, which replaces the term Non-Alcoholic Fatty Liver Disease (NAFLD), reflects a shift towards in terminology to emphasize metabolic risk factors as primary drivers (Rinella *et al*., 2023). The updated MASLD criteria require hepatic steatosis along with at least one metabolic or cardiovascular risk factor, with nearly complete overlap (99%) between the MASLD and historically defined NAFLD populations (Hagström *et al*., 2024). However, given the extensive use of NAFLD in previous research and clinical diagnoses, this review will reference the term NAFLD to provide continuity and avoid confusion.

While MASLD encompasses a range of liver conditions defined by hepatic steatosis, it can also include more advanced features such as hepatocyte ballooning, inflammation, and lipoapoptotic damage. MASLD can progress from simple steatosis to metabolic dysfunction-associated steatohepatitis (MASH) and fibrosis, potentially advancing to cirrhosis and hepatocellular carcinoma (HCC). MASH is characterized by steatosis, lobular inflammation, and hepatocyte ballooning, with a NAFLD activity score (NAS) of 4 or higher, with or without fibrosis (Marti-Aguado *et al*., 2024). As insulin resistance in-

Open Access **https://doi.org/10.4062/biomolther.2024.188**

Received Oct 16, 2024 **Revised** Dec 8, 2024 **Accepted** Dec 10, 2024
Published Online Dec 20, 2024

***Corresponding Author**
E-mail: ykwon@ewha.ac.kr
Tel: +82-2-3277-4653, Fax: +82-2-3277-2851
[†]The first three authors contributed equally to this work.

creases, elevated levels of serum free fatty acids (FFAs) drive lipogenesis in the liver, leading to hepatic steatosis (Sakurai *et al*., 2021). The accumulation of circulating FFAs activates pro-apoptotic proteins, leading to apoptosis, oxidative stress, and inflammation networks, ultimately contributing to the progression of MASLD to MASH (Flessa *et al*., 2021).

Although the global prevalence of MASH is estimated to be around 5%, it is projected to increase by about 56% worldwide over the next decade (Huang *et al*., 2021). MASH significantly contributes to metabolic syndrome by elevating levels of ALT, AST, cholesterol, and FFAs, and is one of the leading causes of liver transplantation or liver-related mortality (Di Mauro *et al*., 2021). Persistent liver cell damage activates hepatic stellate cells (HSCs), initiating fibrogenesis (Kim *et al*., 2024). Hepatic fibrosis occurs when HSCs, which constitute about 10% of total liver cells, transform into myofibroblasts, leading to altered expression of extracellular matrix (ECM) proteins such as collagen and fibronectin. While fibrosis is a normal reparative response to tissue injury, chronic fibrosis results in excessive ECM protein accumulation, ultimately leading to cirrhosis (Kim *et al*., 2024). Cirrhosis is characterized by hepatic insufficiency and increased resistance to blood flow in the liver, which can result in portal hypertension and the development of HCC (Ginès *et al*., 2021). Interestingly, MASLD-associated HCC can occur even in the absence of fibrosis, driven by inflammation, immune dysregulation, and impaired cell cycle regulation (Polyzos *et al*., 2023).

According to the World Health Organization (WHO), HCC is the sixth most common cancer globally and the third leading cause of cancer-related deaths (Bray *et al*., 2024). Notably, AMP-activated protein kinase (AMPK) has gained attention for its potential to mitigate MASH, MASLD, and liver fibrosis with its multiple mechanisms that address each stage of these pathological conditions. Therefore, in this study, we aim to thoroughly understand the processes of MASH and hepatic fibrosis that led to HCC, systematically review the role of AMPK in these disease pathways, and provide an in-depth examination of emerging AMPK-related therapeutic targets and treatments.

PATHOPHYSIOLOGY OF MASLD AND MASH

To explore promising therapeutic targets for hepatic fibrosis, it is necessary to understand the complex pathophysiological processes of MASLD and MASH, which span from non-fibrotic stages to advanced liver fibrosis, potentially culminating in cirrhosis and organ failure (Powell *et al*., 2021). The pathophysiology of MASLD and MASH is multifaceted, as the term suggests – "liver disease driven by metabolic abnormalities." The development and progression of MASLD is often explained through the "multiple-hit hypothesis." This theory posits that MASLD arises from a combination of factors, including physiological changes driven by genetic predisposition, alterations in the gut microbiome, and metabolic factors such as oxidative stress, inflammation, and adipokine signaling from adipocytes. These interconnected processes activate various molecular pathways that contribute to the onset and advancement of the disease (Tilg *et al*., 2021). In this section, we aim to provide a comprehensive review of the underlying mechanisms that support the multiple-hit hypothesis, highlighting the complex interactions between these factors and their roles in the pathogenesis of MASLD and MASH.

Multiple-hit hypothesis: lipid accumulation and lipotoxicity

Hepatic lipid accumulation is the first factor in the progression of MASLD, leading to hepatic lipotoxicity and rendering the liver more vulnerable to pathophysiological changes (Buzzetti *et al*., 2016). Lipid dysregulation in MASLD can be attributed to several factors, including high-fat diets, obesity, genetic factors, insulin resistance, and disruptions in microbiome balance (Tilg *et al*., 2021).

In the liver, lipids are stored as triglycerides (TGs), which are formed by the condensation of fatty acids (FAs) and glycerol. TGs serve a protective role for hepatocytes by sequestering lipotoxic FFAs and preventing direct liver damage. However, impaired lipid metabolism, typically due to reduced liver function, can result in chronic lipotoxicity and liver damage (Musso *et al*., 2013). The balance of FFAs in the liver is regulated by processes like *de novo* lipogenesis and lipolysis. Excessive accumulation of FFAs can lead to direct (or acute) lipotoxicity, causing organelle damage, particularly in mitochondria and the endoplasmic reticulum, and contributing to liver dysfunction (Geng *et al*., 2021).

In MASLD, hepatic lipogenesis and adipose tissue lipolysis are both elevated, leading to an increased flux of FFAs to the liver (Esler and Cohen, 2023). The overload of FFAs and their toxic metabolites can trigger proinflammatory responses, including the activation of toll-like receptor 4 (TLR4) signaling, which activates the NF-κB pathway. TLR-related inflammatory responses are crucial to MASH progression, as evidenced by several studies. For instance, saturated fatty acids have been shown to activate TLR4 signaling and its downstream effector myeloid differentiation factor-88 (Khanmohammadi and Kuchay, 2022). Similarly, the activation of the nod-like receptor protein 3 (NLRP3) inflammasome by palmitate and TLR2 ligands leads to the release of interleukin-1β (IL-1β) (Paik *et al*., 2021; Prakash *et al*., 2023). High-fat diets (HFDs) further contribute to activation of inflammatory signaling through the cooperative activation of TLR4 and fetuin-A, an endogenous TLR4 ligand (Jensen-Cody and Potthoff, 2021). Additionally, palmitate-induced formation of the TLR4-myeloid differentiation protein-2 complex promotes reactive oxygen species (ROS) generation, increasing inflammation (Kim *et al*., 2017). Excess cholesterol accumulation in Kupffer cells (liver macrophages) can lead to their transformation into activated, lipid-laden foam cells. This transformation is associated with the activation of the NLRP3 inflammasome and the upregulation of cleaved caspase-1, both of which contribute to the development of MASH (Ioannou *et al*., 2017).

Multiple-hit hypothesis: insulin resistance

Under normal physiological conditions, insulin facilitates the uptake of glucose into adipocytes and inhibits lipolysis, effectively reducing circulating glucose and FFA levels (Rahman *et al*., 2021). However, in states of insulin resistance, this regulatory function is impaired, resulting in elevated FFAs in the bloodstream. These excess FFAs accumulate in the liver, where they are stored as TGs. Additionally, selective hepatic insulin resistance disrupts the normal regulation of *de novo* lipogenesis (DNL) by insulin, as elevated circulating glucose and insulin concentrations stimulate hepatic DNL, leading to the conversion of excess carbohydrates into fatty acids in the

liver and further exacerbating lipid accumulation, as observed in individuals with NAFLD (Smith *et al*., 2020). Beyond lipid accumulation, insulin resistance also plays a pivotal role in inflammation and oxidative stress. Adipocytes in an insulin-resistant state release proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and IL-1β, which not only promote hepatic inflammation but also exacerbate insulin resistance, creating a vicious cycle (Jager *et al*., 2007; Rehman and Akash, 2016). Furthermore, the oxidation of FFAs within the liver generates ROS that aggravates inflammation and oxidative stress (Buzzetti *et al*., 2016). Chronic inflammation and oxidative stress are major drivers of HSC activation, a critical step in the development of fibrosis characteristic of MASH. Once activated, HSCs promote liver fibrosis by producing excessive ECM components (Tsuchida and Friedman, 2017). This section highlights the key role of insulin resistance in the progression of MASLD and MASH, linking lipid accumulation, inflammation, and fibrosis in a continuous feedback loop.

Multiple-hit hypothesis: dysregulated autophagy of cellular scavenger organelles

A key factor in the progression of MASLD is the impairment of autophagy in cellular scavenger organelles. Under healthy conditions, autophagy plays a protective role against hepatic steatosis and further liver pathologies by maintaining organelle homeostasis and mitigating oxidative stress, inflammation, and apoptosis (Zhang *et al*., 2022). Autophagy is particularly important for preserving the integrity of cellular antioxidant defenses by removing damaged organelles, such as mitochondria and the endoplasmic reticulum (ER), which are especially vulnerable to lipotoxicity in hepatocytes (Ornatowski *et al*., 2020).

Autophagy is a dynamic process that adjusts to nutrient availability. It is upregulated during fasting to supply essential nutrients and energy and suppressed during feeding when dietary nutrients are abundant (He, 2022). During feeding, autophagy is inhibited by elevated insulin levels and nutrient-sensing pathways, particularly the mechanistic target of rapamycin (mTOR) pathway (Sinha *et al*., 2017). However, in MASLD, defects in mitophagy (the autophagic degradation of damaged mitochondria) lead to the production of excessive ROS. This overwhelms the liver's antioxidant defense system, exacerbates oxidative stress, and impairs cellular repair mechanisms, contributing to further metabolic dysfunction (García-Ruiz and Fernández-Checa, 2018). The mitochondrial damage also leads to the release of mitochondrial DNA, which can activate the NLRP3 inflammasome and stimulate the production of inflammatory mediators, such as interferon regulatory factor 1 (IRF1), promoting disease progression (Fromenty and Roden, 2023; Zong *et al*., 2024).

Reduced autophagy also disrupts normal ER function, particularly in protein folding and lipid metabolism. This disruption contributes to the accumulation of misfolded proteins, leading to ER stress and the upregulation of lipogenesis through activation of sterol regulatory element-binding protein 1c (SREBP1c). Moreover, the impaired assembly and secretion of very-low-density lipoproteins (VLDLs) result in further triglyceride (TG) accumulation in hepatocytes. ER stress also activates the unfolded protein response (UPR) pathways, including inositol-requiring enzyme-1α (IRE-1α), protein kinase RNA-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6α (ATF6α), which can trigger ER stress-induced apoptosis (Song and Malhi, 2019). The combined effects of ER stress and mitochondrial dysfunction lead to further ROS production, activating the NF-κB inflammatory pathway, which contributes to insulin resistance and perpetuates a cycle of metabolic dysfunction and liver damage. Overall, dysregulated autophagy in MASLD plays a central role in exacerbating hepatic inflammation, oxidative stress, and insulin resistance, driving disease progression toward more advanced stages such as MASH and fibrosis.

Multiple-hit hypothesis: chronic inflammatory response with microbial imbalance

Chronic inflammation driven by gut dysbiosis plays a significant role in the progression of MASLD and the development of hepatic fibrogenesis. MASH, a more severe subtype of MASLD, is marked by persistent inflammation, immune cell recruitment, and activation of proinflammatory signaling pathways, particularly those involving macrophages (Kazankov *et al*., 2019; Luci *et al*., 2020). Liver-resident macrophages, known as Kupffer cells, play a central role in initiating and sustaining this inflammation. Metabolic imbalances and the disruption of the gut-liver axis allow toxic metabolites and endotoxins to bind to toll-like receptors (TLR2, TLR4, TLR5, and TLR9) on Kupffer cells. This interaction triggers the release of proinflammatory cytokines (TNF-α, IL-1β, IL-6, IL-12) and ROS, which in turn recruit and activate other immune cells, such as neutrophils and monocyte-derived macrophages (MoMF), further exacerbating liver inflammation (Shi *et al*., 2021; Li *et al*., 2022c; Xu *et al*., 2023).

During acute inflammation, Kupffer cells adopt the M1 phenotype, driving early inflammatory responses through pathways like JNK-AP-1 and IKK-NF-κB. These pathways stimulate the production of proinflammatory cytokines such as TNF-α, IL-6, and MCP-1 (Chen *et al*., 2020). Prolonged exposure to these cytokines aggravates liver inflammation, promoting hepatocyte necrosis, neutrophil infiltration, HSC activation, and formation of Mallory bodies, all of which contribute to liver fibrosis (Xu *et al*., 2022). Chronic inflammation often leads to a shift toward the M2 macrophage phenotype, which, while promoting tissue repair through anti-inflammatory cytokines, also contributes to fibrogenesis and progression to HCC (Tilg *et al*., 2021; Zhao *et al*., 2021).

Gut dysbiosis, characterized by an imbalance in gut microbiota, is another critical factor contributing to MASLD progression. Excessive lipid accumulation compromises the integrity of the gut barrier, allowing gut-derived toxins and microbial products to leak into the bloodstream, exacerbating liver inflammation through the gut-liver axis. Bile produced by the liver facilitates lipid digestion in the small intestine. Primary bile acids synthesized in the liver are converted into secondary bile acids by gut bacteria in the colon. Both primary and secondary bile acids, along with various microbial metabolites, are reabsorbed into the liver via the portal circulation. This enterohepatic circulation of bile acids plays a vital role in lipid digestion and metabolism. Among the dominant gut microbial phyla, *Firmicutes* and *Bacteroidetes* have significant roles in regulating metabolism, and their balance is crucial in the development of obesity and MASLD/MASH. In MASLD, the ratio of *Firmicutes* to *Bacteroidetes* often shifts, with *Firmicutes* generally decreasing and *Bacteroidetes* increasing (Maestri *et al*., 2023). Furthermore, a study on non-obese NAFLD pa-

tients in Asia revealed notable changes in gut bacterial diversity, showing a reduction in *Ruminococcaceae* and an increase in *Veillonellaceae*, which were linked to the severity of liver fibrosis (Lee *et al*., 2020). Patients with MASH or advanced liver fibrosis (stages F2–F4) also exhibit distinct fecal fungal microbiome profiles (Demir *et al*., 2022).

Microbial products like lipopolysaccharides (LPS), a form of endotoxin, can induce intestinal inflammation and weaken the intestinal barrier in some MASH patients. This weakened barrier allows these endotoxins to translocate into the liver and systemic circulation (Tilg *et al*., 2021; Hsu and Schnabl, 2023). Once in the liver, these microbial products influence macrophage polarization, specifically promoting the activation of M1 macrophages, which exacerbates liver inflammation. This inflammatory response plays a pivotal role in the progression of MASLD and MASH.

Microbial-derived metabolites also play a significant role in MASLD pathogenesis. Elevated levels of ethanol, phenylacetate, and trimethylamine-N-oxide (TMAVA) have been linked to hepatic steatosis and oxidative stress, contributing to hepatocyte damage and death (Hoyles *et al*., 2018; Yuan *et al*., 2019; Xu *et al*., 2022). In contrast, short-chain fatty acids (SCFAs), fewer than six carbon atoms, such as acetate, butyrate, and propionate, support intestinal barrier integrity, enhance antimicrobial activity of macrophages, and help regulate T regulatory (Treg) cells. SCFAs also exhibit anti-inflammatory properties by inhibiting NF-κB signaling pathways (Duan *et al*., 2023). Despite their multifaceted roles in maintaining metabolic homeostasis–such as regulating lipid metabolism, enhancing insulin sensitivity, promoting GLP-1 hormone secretion, and inhibiting histone deacetylases (HDACs) to upregulate PPARγ expression (e.g., through butyrate)–, gut dysbiosis can disrupt these benefits (Cani *et al*., 2009; Kim, 2023). SCFAs, through their impact on various metabolic pathways and inflammation, significantly contribute to metabolic balance. Consequently, imbalances in gut microbiota that alter SCFA production and utilization can impair metabolic regulation, exacerbating chronic low-grade inflammation, a key feature of MASLD (Kopczyńska and Kowalczyk, 2024).

Activation of HSCs and subsequent ECM accumulation in liver fibrosis progression

Liver fibrosis is a chronic disease characterized by an excess production of ECM proteins, primarily type I and III collagens, which leads to scar tissue formation in liver parenchyma and, ultimately, organ failure (Parola and Pinzani, 2019). One of the key drivers in this process is the activation of HSCs, which undergo a transformation from a quiescent state to an activated, myofibroblast-like phenotype. This transformation is a critical factor in hepatic fibrosis and contributes to cell structure distortion through the overproduction of ECM in response to liver injury (Schwabe *et al*., 2020).

Chronic liver damage results in elevated levels of transforming growth factor-β (TGF-β), a key modulator of fibrosis. TGF-β signaling promotes HSC transdifferentiation into activated myofibroblasts, which are highly proliferative and fibrogenic. This transdifferentiation leads to massive hepatocyte cell death, contributing to the advancement of liver fibrosis and, ultimately, cirrhosis (Kitto and Henderson, 2021). In addition to fibrogenesis, the TGF-β/Smad-dependent signaling pathway plays a role in the transition from MASLD to HCC, underscoring the importance of this pathway in both fibrosis and cancer progression (Gough *et al*., 2021). Once activated HSC-derived myofibroblasts upregulate fibrogenic and contractile markers, including ECM proteins, α-smooth muscle actin (α-SMA) and collagen type I (Kim *et al*., 2024). These cells not only amplify matrix production but also recruit bone marrow-derived cells and induce epithelial-to-mesenchymal transition (EMT) in hepatocytes and cholangiocytes, further exacerbating liver fibrosis (Kim *et al*., 2024). Insulin-like growth factor-binding protein 7 (IGFBP7) has been shown to influence HSC activation from their quiescent state, where they typically store vitamin A (Samsuzzaman and Kim, 2023). IGFBP7 interacts with TGF-β1 and activates other signaling pathways, such as ERK and JNK, which further promote ECM production and exacerbate the liver stress response, ultimately driving fibrogenesis (Budi *et al*., 2021; Stanley *et al*., 2021). As ECM composition changes, liver sinusoidal endothelial cells (LSECs) undergo capillarization, a process that interferes with nutrient transport between sinusoidal blood and surrounding hepatocytes. This capillarization contributes to the distortion of hepatic function and worsens the progression of liver fibrosis (Ni *et al*., 2017). In conclusion, the activation of HSCs and the subsequent overproduction of ECM are central processes in the progression of liver fibrosis. Understanding the molecular mechanisms behind HSC activation, including the key roles of TGF-β, IGFBP7, and EMT, is essential for developing targeted therapies to halt or reverse fibrogenesis and prevent the progression of MASLD and MASH to cirrhosis and HCC.

THE ROLE OF AMPK IN MASLD, MASH, AND HEPATIC FIBROSIS

AMPK has emerged as a key therapeutic target for preventing and treating MASLD, MASH, and hepatic fibrosis due to its broad regulatory functions in cellular energy homeostasis and its anti-inflammatory properties. AMPK is a cellular energy sensor that responds to changes in intracellular energy levels and is highly conserved across all eukaryotic cells, including plants, fungi, animals, and humans. Thus, AMPK has been recognized as a kinase that helps cell survival under energy deprivation (Herzig and Shaw, 2018). By detecting low ATP levels and promoting ATP-generating processes, AMPK helps cells survive under conditions of energy deprivation, making it crucial for maintaining metabolic balance in tissues such as the liver (Hardie *et al*., 2012).

The role of AMPK in lipid metabolism and lipotoxicity prevention

AMPK modulates energy metabolism by sensing an increased AMP:ATP ratio under conditions of energy deprivation. This activation is triggered by several upstream kinases, including liver kinase B1 (LKB1), which responds to a decreased ATP to AMP/ADP ratio (Kottakis and Bardeesy, 2012), calmodulin-dependent protein kinase kinase-2 (CaMKK2, also known as CaMKKβ) in response to elevated Ca^{2+} levels (Woods *et al*., 2005), and TGF-β-activated kinase 1 (TAK1) (Neumann, 2018). Once activated, AMPK restores energy balance by inhibiting ATP-consuming anabolic processes and promoting ATP-generating catabolic processes (Xiao *et al*., 2011). In the liver tissue, fatty acids are absorbed across the plasma membrane and converted to fatty acyl-CoA, which can be either stored as TG or oxidized, depending on the liv-

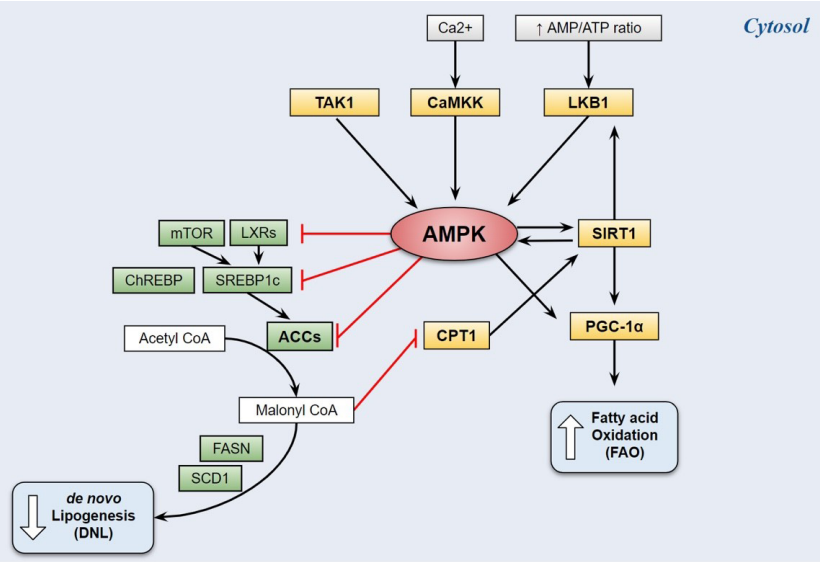

Fig. 1. Schematic diagram illustrating the role of hepatic AMPK in regulating lipogenesis and lipolysis. Hepatic AMPK acts as a critical energy sensor and regulator of lipid metabolism by inhibiting lipogenesis and promoting fatty acid oxidation (FAO). It is activated by main upstream kinases, TAK1, CaMKK2 and LKB1, in response to the increased intracellular calcium concentration and energy state. Upon activation, AMPK inhibits its key substrates, acetyl-CoA carboxylase 1 (ACC1) and acetyl-CoA carboxylase 2 (ACC2). ACC1 catalyzes the conversion of acetyl-CoA to malonyl-CoA in the cytosol, driving de novo lipogenesis (DNL), while ACC2 inhibits FAO by regulating malonyl-CoA levels and controlling CPT1 at the mitochondrial membrane. AMPK suppresses lipogenesis by inhibiting ACC and its upstream transcriptional regulators, including mTOR, LXRs, and SREBP1c. This action reduces lipid accumulation in the liver. Additionally, AMPK enhances FAO by activating key pathways, including SIRT1 and PGC-1α. SIRT1 is upregulated by an increased NAD^+/NADH ratio, which is influenced by AMPK and CPT1. AMPK also boosts SIRT1 activity by increasing NAMPT levels, which further enhances NAD^+ synthesis. Moreover, SIRT1 can reciprocally activate AMPK both directly and indirectly by promoting the translocation of LKB1, creating a positive feedback loop that reinforces AMPK activity and lipid metabolism regulation. AMP-activated Protein Kinase, AMPK; TGF-β-activated kinase 1, TAK1; Calmodulin-dependent protein kinase kinase-β, CAMKK-β; Liver Kinase B1, LKB1; Carnitine Palmitoyl Transferase 1, CPT1; Mammalian Target of Rapamycin, mTOR; Liver X Receptors, LXRs; Sterol Regulatory Element-Binding Protein-1c, SREBP1c; Silent Information Regulator 1, SIRT1.

er's energy status (Alves-Bezerra and Cohen, 2017). During energy deficiency, AMPK inhibits *de novo* lipogenesis (DNL) gene expression by suppressing the acetyl-CoA carboxylase 1 (ACC1)/fatty acid synthase (FASN)/stearoyl-CoA desaturase 1 (SCD1) pathway, while promoting lipolysis through activation of the carnitine palmitoyltransferase 1 (CPT1)/sirtuin1 (SIRT1)/peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) pathway in liver tissue (Pang *et al*., 2021) (Fig. 1).

ACC1, a downstream substrate of AMPK, is an enzyme involved in fatty acid synthesis, catalyzing the carboxylation of acetyl-CoA to produce malonyl-CoA. AMPK inhibits ACC1 through phosphorylation, reducing DNL (Alves-Bezerra and Cohen, 2017). Although ACC1 and ACC2 share about 75% amino acid sequence similarity and both catalyze the conversion of acetyl-CoA to malonyl-CoA, they have distinct physiological roles. ACC1 is predominantly expressed in lipogenic tissues such as the liver and adipose tissues, where it regulates lipogenesis. In contrast, ACC2 is primarily found in oxidative tissues like the heart and skeletal muscles, where it inhibits fatty acid oxidation (FAO) by regulating malonyl-CoA levels and controlling the CPT1 activity at the outer mitochondrial membrane (Schreurs *et al*., 2010; Wang *et al*., 2022). Genetic evidence suggests that the distinct roles of ACC isoforms in regulating DNL and FAO are related to tissue-specific expression profiles (Batchuluun *et al*., 2022). FASN promotes the synthesis of saturated fatty acids by catalyzing the *de novo* synthesis of cytosolic long-chain fatty acids through the condensation of acyl-CoA and malonyl-CoA, both of which are increased by ACC1 and ACC2 (Li *et al*., 2011; Song *et al*., 2018). SCD1, an enzyme located in the ER, converts saturated fatty acids into monounsaturated fatty acids which are precursors of long-chain fatty acids (Ascenzi *et al*., 2021). The inhibition of SCD1, particularly through AMPK activation, enhances lipid autophagy (lipophagy), leading to significant amelioration in hepatic steatosis (Zhou *et al*., 2020). In murine models, alanine knock-in mutations at key phosphorylation sites on both ACC1 (Ser79 and Ser80 in humans) and ACC2 (Ser212 and Ser221 in humans) that block AMPK-mediated phosphorylation prevent the inactivation of ACCs, thereby increasing lipogenesis and reducing FAO. This results in metabolic disorders such as hepatic glucose intolerance, MASLD, and liver fibrosis. These effects cannot be reversed by the indirect AMPK activator metformin or the direct AMPK activator A769662 in ACC1/2-alanine double knock-in mice, confirming that the suppression of hepatic lipogenesis via AMPK depends on its phosphorylation of ACCs (Fullerton *et al*., 2013; Wei *et al*., 2016; Galic *et al*., 2018).

The role of ACC1 in lipogenesis is further regulated by transcription factors such as carbohydrate response element-binding protein (ChREBP) and SREBP1c, both of which are crucial in controlling genes involved in fatty acid and TG synthesis (Ferré *et al*., 2021). AMPK directly binds to and induces inhibitory phosphorylation of SREBP1c and SREBP2. The

main role of SREBP1c is to regulate lipogenesis by activating genes involved in fatty acid and TG synthesis, while SREBP2 regulates cholesterol homeostasis by activating genes required for cholesterol synthesis and absorption (Li *et al*., 2011; Song *et al*., 2018). Once activated, AMPK directly phosphorylates SREBP1c at Ser372, preventing its cleavage and nuclear translocation, thereby decreasing the expressions of key lipogenic genes such as ACC1, ATP-citrate lyase (ACLY), citrate/isocitrate carrier, FASN, and SCD1 in high glucose-exposed hepatocytes. This contributes to the reduction of hepatic steatosis in diet-induced insulin-resistant mice. Additionally, AMPK downregulates the activity of both mTOR and liver X receptors (LXRs), which are upstream transcription factors that activate SREBP1c. By inhibiting these pathways, AMPK reduces lipogenesis and helps alleviate metabolic dysfunctions associated with hepatic steatosis and insulin resistance (Ferré *et al*., 2021). This comprehensive regulation by AMPK underscores its critical role in preventing lipotoxicity and managing lipid metabolism in the liver.

In promoting lipolysis, activated AMPK enhances the CPT1/SIRT1/PGC-1α pathway. CPT1, an upregulator of SIRT1, facilitates the transport of long-chain fatty acyl-CoA into mitochondria (Nie *et al*., 2024), which increases the NAD^+/NADH ratio. This elevation, in turn, activates NAD^+-dependent SIRT1 deacetylase activity, which plays a critical role in inducing FAO (Cantó and Auwerx, 2010). SIRT1 and AMPK are metabolic sensors that mutually regulate each other and share numerous target molecules, providing a fine-tuned amplification mechanism for maintaining energy homeostasis (Cantó *et al*., 2009; Varghese *et al*., 2023).

The enhancement of SIRT1 further increases AMPK activity through both LKB1-dependent and independent pathways. In the LKB1-dependent pathway, SIRT1 deacetylates and stimulates the translocation of LKB1 to the cytoplasm, where it enhances AMPK activity (Cantó *et al*., 2009; Sharma *et al*., 2021). LKB1 is the primary upstream activator of AMPK, phosphorylating the threonine residue (Thr172) on its catalytic α subunit of AMPK under nutrient-deprived conditions in nearly all tissues (Woods *et al*., 2003; Omidkhoda *et al*., 2023). In the LKB1-independent pathway, SIRT1 can also directly activate AMPK via its catalytic function, particularly in response to energy deprivation stimuli (Suchankova *et al*., 2009). SIRT1 itself is a fuel-sensing catalytic enzyme that is activated when the NAD^+/NADH ratio is high (Imai *et al*., 2000).

On the other hand, the activation of AMPK can also increase SIRT1 activity through both nicotinamide phosphoribosyltransferase (NAMPT)-dependent and independent mechanisms. NAMPT is the rate-limiting enzyme in the NAD^+ salvage pathway, catalyzing the conversion of nicotinamide into oxidized NAD^+, a critical substrate for SIRT1. AMPK induces the expression of NAMPT, increasing NAD^+ levels and decreasing nicotinamide, a product of SIRT1-mediated deacetylation (Fulco *et al*., 2008; Sadria and Layton, 2021). As SIRT1 deacetylation activity is driven by cellular NAD^+ abundance and the NAD^+/NADH ratio, NAMPT activity and NAD^+ levels are positively correlated with SIRT1 activation. In a NAMPT-dependent manner, AMPK enhances SIRT1 activity by upregulating NAMPT expression, boosting the NAD^+/NADH ratio and promoting SIRT1 deacetylase activity (Cantó *et al*., 2009). In a NAMPT-independent manner, AMPK stimulates mitochondrial β-oxidation, further increasing the intracellular NAD^+/NADH ratio and driving SIRT1 activation (Sadria and Layton, 2021). PGC-1α, a major player in mitochondrial biogenesis and lipid metabolism, is also activated by AMPK. PGC-1α reduces lipid accumulation and improves mitochondrial function by acting as a co-activator of peroxisome proliferator-activated receptors (PPARs), which are involved in lipid metabolism, antioxidant activity, and anti-inflammatory responses. In patients with MASLD, liver-specific PGC-1α deficiency has been associated with mitochondrial dysfunction, leading to hepatic steatosis (Cheng *et al*., 2024). Thus, AMPK-mediated regulation of the CPT1/SIRT1/PGC-1α pathway underscores its multifaceted role in lipid metabolism, mitochondrial function, and the prevention of lipotoxicity in liver cells.

The role of AMPK in modulating glucose metabolism

Insulin resistance and MASLD exacerbate each other, accelerating the progression of both diseases (Tolman *et al*., 2007; Chen *et al*., 2017). AMPK, as a primary energy status sensor, plays a vital role in regulating glucose metabolism, especially during periods of fasting, thereby improving insulin sensitivity and reducing insulin resistance (Steinberg and Hardie, 2023).

Skeletal muscle is a major site for glucose utilization due to its high energy demands. AMPK activation in skeletal muscle increases the expression of GLUT4, a glucose transporter, which facilitates glucose uptake into muscle cells (Entezari *et al*., 2022). This enhanced glucose uptake lowers serum glucose levels and improves insulin resistance, which, in turn, reduces excessive fatty acid levels, mitigates lipid accumulation, and lessens inflammation in the liver, positively impacting MASLD progression (Steinberg and Hardie, 2023). AMPK also exerts its effect on insulin resistance through the action of its downstream signaling molecule, PGC-1α. PGC-1α contributes to mitochondrial biogenesis and oxidative metabolism, which helps to regulate glucose levels. Notably, PGC-1α improves insulin sensitivity by reducing mitochondrial oxidative stress. Enhanced mitochondrial function and increased glucose metabolism, driven by AMPK activation, facilitate glucose uptake and lower blood glucose levels without promoting gluconeogenesis. This is a key aspect of improving glucose tolerance because it enables efficient energy use without triggering excessive glucose production, which can otherwise exacerbate hyperglycemia (Wu *et al*., 2021).

Insulin is crucial for maintaining metabolic homeostasis by regulating glucose uptake, glycogen synthesis, and lipid metabolism. However, lipid accumulation and inflammation can interfere with this process (Ruderman *et al*., 2013). Excess lipid accumulation in the liver leads to an increase in diacylglycerol (DAG), a lipid metabolite that activates protein kinase C (PKC). This activation inhibits the phosphorylation of insulin receptor substrate (IRS) proteins, reducing insulin signaling and worsening insulin resistance (Samuel and Shulman, 2016; Petersen and Shulman, 2018). By decreasing lipid synthesis and accumulation in the liver, AMPK improves insulin sensitivity and restores normal insulin signaling.

Inflammatory pathways, such as those mediated by NF-κB and JNK, can impair insulin signaling by suppressing IRS proteins. AMPK activation inhibits these inflammatory pathways, reducing proinflammatory cytokines that exacerbate insulin resistance and impair insulin receptor sensitivity in hepatocytes (Olefsky and Glass, 2010). Thus, the ability of AMPK to lower inflammation helps ameliorate insulin resistance, contributing to improved glucose metabolism (Ruderman *et al*., 2013).

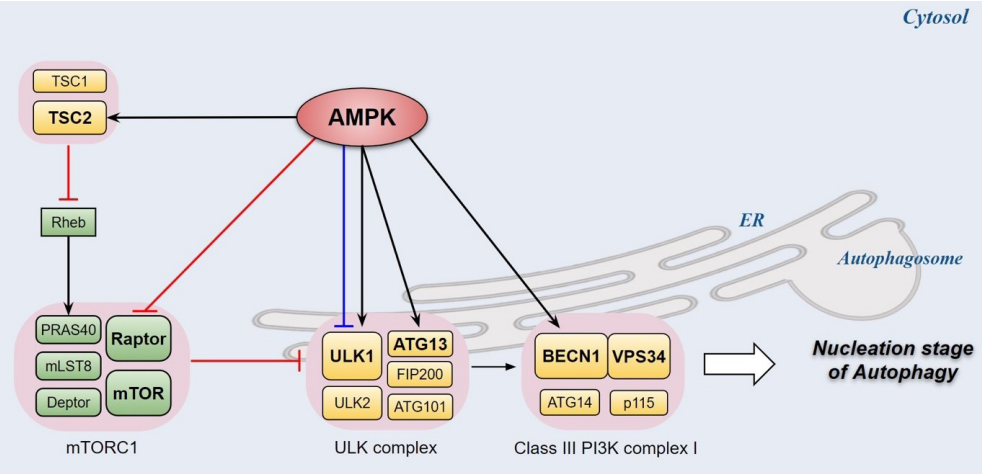

Fig. 2. Mechanisms of autophagy initiation regulated by AMPK and mTORC1. AMPK, as a sensor of energy deficiency, plays a critical role in initiating autophagy by phosphorylating key regulators involved in autophagy and mTORC1 signaling pathways. Under low energy or nutrient-deficient conditions, AMPK phosphorylates TSC2 (in the TSC1/TSC2 complex), Raptor, and Unc-51-like kinase 1 (ULK1), each triggering downstream reactions that promote autophagy. AMPK phosphorylates TSC2, a GTPase-activating protein (GAP) for Rheb. This phosphorylation inhibits Rheb by converting it from its active GTP-bound state to an inactive GDP-bound form, reducing mTORC1 activity, which normally inhibits autophagy. AMPK phosphorylates regulatory-associated protein of mTOR complex (Raptor), an essential regulatory component of mTORC1, thereby inhibiting mTORC1 function. Reduced mTORC1 activity promotes autophagy by allowing ULK1 activation. AMPK directly phosphorylates ULK1, which serves dual roles—either restraining the overactivation of autophagy or stimulating the downstream autophagy machinery. Phosphorylation of ULK1 by AMPK promotes the initiation of autophagy in response to nutrient deficiency. These interconnections illustrate how AMPK, by sensing low energy levels, suppresses mTORC1, and promotes the activation of the autophagy machinery, ensuring cellular homeostasis under stress conditions. AMP-activated Protein Kinase, AMPK; Mammalian Target of Rapamycin, mTOR; Tuberous Sclerosis Complex 2, TSC2; Ras Homolog Enriched in Brain, Rheb.

AMPK enhances metabolic processes by improving insulin sensitivity. It promotes the translocation of GLUT4 to the cell membrane, increasing glucose uptake. Additionally, via the Akt signaling pathway, AMPK inhibits glycogen synthase kinase-3 (GSK-3), which otherwise inhibits glycogen synthesis, thus promoting glycogen synthesis (Huang and Czech, 2007; Tzatsos and Tsichlis, 2007; Chopra *et al*., 2012). This coordinated regulation of glucose uptake and glycogen synthesis highlights the pivotal role of AMPK in glucose metabolism and its therapeutic potential for MASLD and insulin resistance.

The role of AMPK in autophagy

AMPK and mTORC1 are the central regulators of autophagic degradation in mammalian cells, modulating autophagic processes in response to fluctuations in nutrient and energy availability, as summarized in Fig. 2 (Licheva *et al*., 2022). AMPK-driven autophagy is critical for maintaining cellular balance, helping to eliminate damaged organelles and dysfunctional cellular components, even under nutrient-rich conditions. Whereas, during nutrient deprivation, AMPK-mediated autophagy provides essential amino acids through non-selective degradation of cellular materials, ensuring survival under starvation by supporting metabolic balance (Marshall and Vierstra, 2018; Vargas *et al*., 2023).

Under nutrient-rich conditions, mTORC1 is activated to promote anabolic cellular activity and inhibits autophagy by interfering with the interaction between AMPK and Unc-51-like kinase 1 (ULK1) through mTORC1-mediated phosphorylation of ULK1 at Ser757 (758 in human) and autophagy-related protein (ATG) 13, both of which are markers of the early autophagy induction (Kim *et al*., 2011; Park *et al*., 2016). Conversely, under nutrient-poor conditions, AMPK inhibits mTORC1 activity to promote autophagy initiation. Specifically, AMPK phosphorylates key proteins such as Raptor at Ser722 and Ser792, a regulatory component of the mTORC1 complex (Gwinn *et al*., 2008), and TSC2 at Ser1387 and Thr1271, which inhibits the mTOR-activating factor Rheb. These actions lead to the activation of the ULK1 complex, which increases autophagy and results in the accumulation of autophagy receptor proteins like p62 (Bonnet *et al*., 2024). In addition to the mTORC1-dependent mechanism, AMPK stimulates autophagy through modulating each hub signaling components of ULK1 complex and PI3K complex. AMPK directly phosphorylates ULK1 at Ser317, Ser777, and Ser555 as well as Beclin1 (BECN1) at Ser91 and Ser94, both of which are crucial for autophagy initiation. Beclin1 is a component of the downstream Class III PI3K complex along with VPS34 (PI3K) and AMBRA1 (Roach, 2011; Kim *et al*., 2013; Tian *et al*., 2015; Zhang *et al*., 2016). This AMPK-mediated phosphorylation enhances the interaction between BECN1 with VPS34, an autophagy inducer by producing phosphatidylinositol 3-phosphate as lipid kinase. This phosphorylation event shifts the interaction of Beclin1 away from Bcl-2, an anti-apoptotic protein that suppresses autophagic activity (Russell *et al*., 2013).

In HCC cells, AMPK plays a complex role in tumor progression, both oncogenic and anti-cancer effects. For instance, in phosphoserine phosphatase-overexpressed cancer cells, mTORC1 activity can be suppressed through LKB1- or TAK1-mediated AMPK/mTOR/ULK1 signaling in a CaMKK-independent manner. This suppression triggers autophagy initiation, which promotes cellular proliferation and invasion, suggesting that AMPK-driven autophagy can contribute to cancer progression in specific contexts (Zhang *et al*., 2021; Lee *et al*., 2023). Conversely, autophagy-mediated cell death serves as

an anti-tumor activation via AMPK in various types of cancer. In the liver, for instance, deletion of the pseudokinase mixed lineage kinase domain-like (MLKL), a key factor in the necroptotic pathway leading to hepatocarcinogenesis, significantly increases autophagy and inhibits cancer progression (Penugurti *et al*., 2024; Yu *et al*., 2024)

Recent advances in understanding of AMPK's roles in autophagy regulation have revealed that under early glucose-depleted conditions, AMPK-mediated phosphorylation of ULK1 at specific residues, namely Ser556 and Thr660, can actually lead to the suppression of autophagy. Contrary to previous assumptions that phosphorylation at Ser556 in ULK1 would increase autophagy, this finding suggests that p-Ser556 may, under certain conditions, inhibit autophagy while mTOR continues to act as an autophagy inhibitor (Park *et al*., 2023). During prolonged energy deprivation, cells prioritize non-autophagic mechanisms, such as fatty acid oxidation, and only later rely on autophagy as a last resort for energy production. This new model highlights that AMPK preserves ULK1 from caspase-mediated degradation by phosphorylating ULK1 at Ser556 and Thr660 during early glucose deprivation. However, the use of Ser556 phosphorylation as a marker may not accurately reflect autophagy flux *in vivo*, leaving uncertainties about the precise role of AMPK in regulating autophagy. Further research is required to clarify how cells adapt to energy stress via AMPK, integrating both traditional and emerging paradigms (Park *et al*., 2023; Steinberg and Hardie, 2023; Kazyken *et al*., 2024; Kim, 2024).

The role of AMPK in cellular stress and inflammation

Low-grade, chronic inflammation in hepatocytes, triggered by lipotoxicity, metabolic ER stress, and ROS, stimulates innate immune responses, leading to the secretion of proinflammatory cytokines and the recruitment of monocytes, especially into the liver tissue (Rohm *et al*., 2022; Mladenić *et al*., 2024). AMPK mitigates these chronic stress-induced inflammatory responses by indirectly inhibiting NF-κB, a key transcription factor regulating innate immune responses and inflammation, through the activation of SIRT1 and PGC-1α (Salminen *et al*., 2011). In normal physiology, the NF-κB family exists as dimers, composed of various subunits (RelA, c-Rel, RelB, p50, and p52), and remains sequestered in the cytoplasm. The RelA (known as p65)/p50 complex, the most common and well-studied dimer, is bound by an inhibitor κB (IκB) in the cytoplasm of the resting cells. Upon stimulation, IκBs are phosphorylated by the IκB kinase (IKK) complex, leading to their degradation (Fig. 3). AMPK-induced SIRT1 activation inhibits inflammation by deacetylating the RelA/p65 subunit of the NF-κB complex at Lys310, reducing NF-κB's proinflammatory actions (Singh and Ubaid, 2020). Simultaneously, PGC-1α activation, either through AMPK-induced phosphorylation of PGC-1α or AMPK-Sirt1 axis-mediated deacetylation of PGC-1α, blocks NF-κB transcriptional activity and represses proinflammatory signal pathways, such as the p38MAPK and TNF-α pathways, particularly in endothelial cells (Rodgers and Puigserver, 2007; Cantó *et al*., 2009; Alvarez-Guardia *et al*., 2010; Rius-Pérez *et al*., 2020).

Under normal conditions, PGC-1α regulates macrophage polarization from the proinflammatory M1 to the anti-inflam-

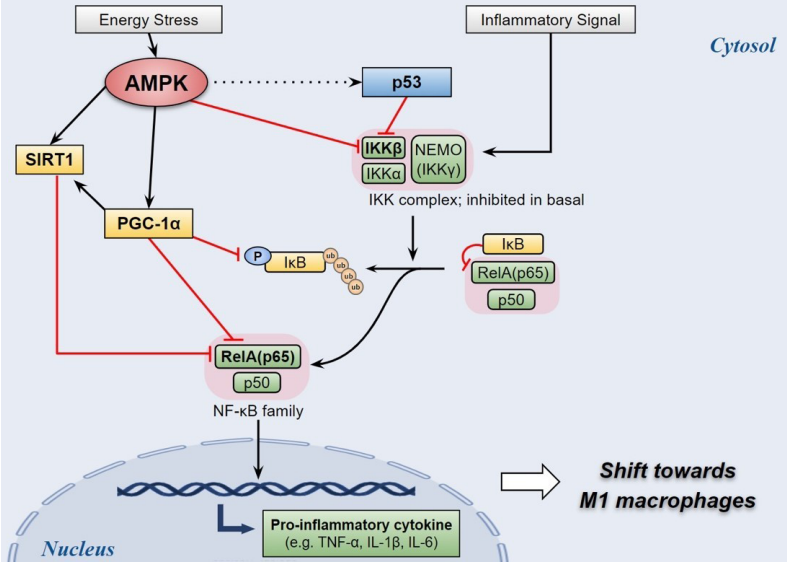

Fig. 3. Regulation of NF-κB signaling by AMPK and its downstream effectors in chronic low-grade inflammation. In chronic low-grade inflammation, upon stimulation by inflammatory cytokines, the IKK complex phosphorylates IκBα, leading to its ubiquitination and proteasomal degradation. Oxidative stress further exacerbates this degradation by phosphorylating IκBα, resulting in the release of NF-κB family members (RelA/p50), which translocate to the nucleus and activate the transcription of target proinflammatory genes. In contrast, under conditions of energy stress, AMPK inhibits this pathway through several mechanisms: directly phosphorylating IKKβ and indirectly activating SIRT1, PGC-1α, and p53. SIRT1 deacetylates the RelA (p65) subunit of NF-κB, while PGC-1α physically blocks its binding to DNA. Additionally, p53 indirectly inhibits IKKβ by suppressing glycolytic activity, which is linked to reduced IKKβ phosphorylation and activity. When glycolysis is downregulated, the *O*-glycosylation of IKKβ (which normally enhances its activity) is reduced, leading to a decrease in IKKβ's ability to phosphorylate IκBα, thereby reducing NF-κB activity. IκB kinase, IKK; Nuclear Factor kappa-light-chain-enhancer of activated B Cells, NF-κB; Silent Information Regulator 1, SIRT1; Peroxisome proliferator-activated receptor Gamma Coactivator 1, PGC-1.

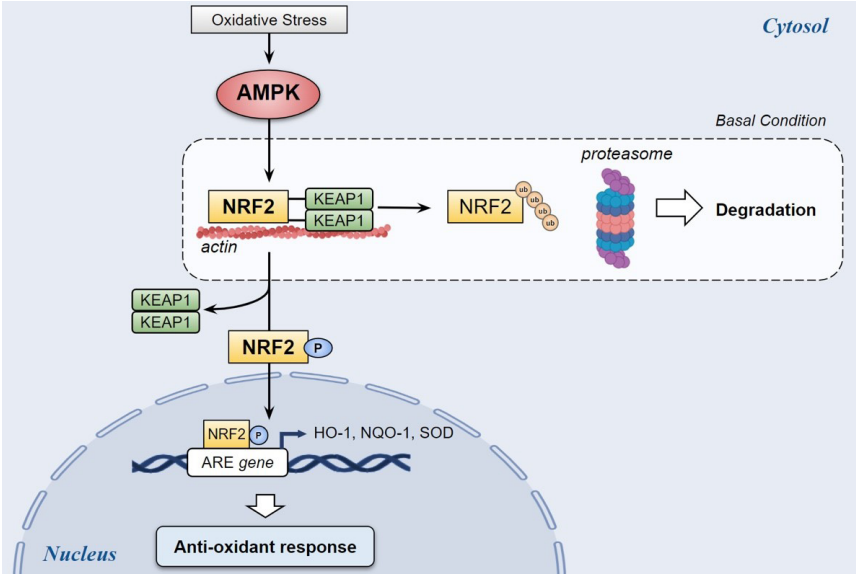

Fig. 4. Antioxidant Response via AMPK/Nrf2/HO-1 Signaling Pathway. Under basal conditions, Nrf2, a master regulator of antioxidant response genes through the Antioxidant Response Element (ARE), is suppressed by KEAP1, which promotes its ubiquitination and degradation via E3 ligase activity. In response to oxidative stress, AMPK is activated and plays a dual role in promoting Nrf2 activity: it directly phosphorylates Nrf2 at specific serine residues, such as Ser448, and indirectly prevents its degradation by inhibiting the GSK-3β/βTrCP axis. This AMPK-mediated activation of Nrf2 leads to the enhanced expression of antioxidant genes, including HO-1, NQO-1, and SOD, which protect cells from oxidative damage caused by carbon monoxide, ROS, and hydrogen peroxide. Nuclear Factor Erythroid 2-Related Factor 2, Nrf2; Kelch-like ECH-associated protein 1, KEAP1; AMP-activated Protein Kinase, AMPK; Glycogen Synthase Kinase-3 Beta, GSK-3β; Beta-transducin repeat-containing protein, βTrCP; Heme Oxygenase-1, HO-1; NAD(P)H Quinone Dehydrogenase 1, NQO-1; Superoxide Dismutase, SOD; Reactive Oxygen Species, ROS.

matory M2 phenotype and modulates proinflammatory cytokines via its physical interaction with the p65 subunit of NF-κB. However, during inflammation, NF-κB downregulates PGC-1α activity, a process exacerbated by high cytokine levels and TNF-α-induced reductions in ROS-scavenging enzymes, disrupting the balance between PGC-1α and NF-κB/P65 interactions (Barroso *et al*., 2018; Zhang *et al*., 2023). In conditions of metabolic stress, such as MASLD, increased glycolytic activity leads to *O*-glycosylation of IKKβ at Ser733, impairing its negative feedback regulation and enhancing IKKβ activity. This upregulates NF-κB signaling by promoting the phosphorylation of IκB at Ser32 and Ser36, leading to its ubiquitination and degradation, thus activating NF-κB with reduced IκB affinity. Conversely, p53 inhibits glycolysis, maintains negative feedback on IKKβ, and thereby reduces IKKβ activity and NF-κB signaling (Kawauchi *et al*., 2008; Salminen *et al*., 2011; Schultze *et al*., 2012).

In addition to its regulation of NF-κB, AMPK also targets nuclear factor E2-related factor 2 (Nrf2), a master regulator of cellular redox homeostasis. Under basal conditions, Nrf2 levels remain low because its Nrf2-ECH homology (Neh1) domain binds to Keap1 protein in the cytoplasm. Keap1, along with the Cullin3-based E3 ubiquitin ligase complex, facilitates the ubiquitination and degradation of Nrf2. AMPK activates Nrf2 through both direct and indirect mechanisms. Direct activation involves the phosphorylation of Nrf2 at specific serine residues, such as Ser550 (Ser558 in human), which facilitates Nrf2's nuclear translocation and dissociation from Keap1 (Joo *et al*., 2016) (Fig. 4). Indirect activation of Nrf2 by AMPK involves the AMPK/GSK-3β/βTrCP axis, wherein AMPK-mediated phosphorylation of GSK-3β at Ser9 inhibits its activity. This inhibition reduces βTrCP2-mediated Nrf2 degradation and prevents FYN-mediated nuclear exclusion (Zimmermann *et al*., 2015). Furthermore, AMPK hyper-phosphorylates Ser374, Ser408, and Ser433 within the Neh6 domain of Nrf2, which enhances its interaction with βTrCP2, improving Nrf2 stability, thereby enhancing the expression of its target genes under energy-deprived conditions. Once Nrf2 is translocated into the nucleus, it regulates the transcription of various antioxidant response element (ARE)-responsive genes, including heme oxygenase-1 (HO-1), NAD(P)H: Quinone Oxidoreductase-1 (NQO-1), and superoxide dismutase (SOD) (Matzinger *et al*., 2020). These genes are critical for cellular defense against oxidative stress and inflammation. By modulating Nrf2 signaling pathways, AMPK plays a vital role in protecting against oxidative stress and chronic inflammation, highlighting its potential as a therapeutic target for metabolic liver diseases such as MASLD (Petsouki *et al*., 2022). Recently, the interplay between AMPK and Nrf2 has been highlighted under metabolic stress conditions. In non-small cell lung cancer, co-occurring mutations in KEAP1 and serine/threonine kinase 11 (STK11, also known as LKB1) establish a double-positive feedback loop between AMPK and SQSTM1 (also known as Sequestosome 1 or p62), promoting the dual activation of AMPK and NFE2 Like BZIP Transcription Factor 2 (NFE2L2, also known as Nrf2). This process involves autophagic degradation of KEAP1, lysosomal complex formation, and SQSTM1 phosphorylation, facilitating metabolic adaptation and supporting tumor growth under stress conditions (Choi *et al*., 2024).

The role of AMPK in fibrosis

Besides its function in energy homeostasis and cellular

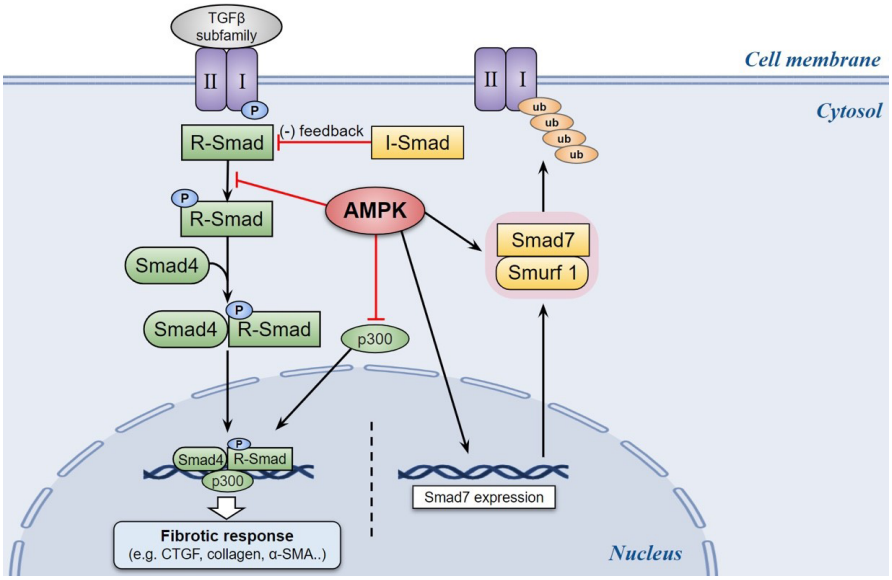

Fig. 5. Schematic diagram of the fibrotic pathway via TGF-β and Smad-dependent signaling, and the effect of AMPK on the pathway. TGF-β signaling is initiated when members of the TGF-β subfamily, such as TGF-β and BMPs, bind to TβRII, leading to the subsequent phosphorylation of TβRI. R-Smads (Smad1/2/3/5/8), phosphorylated by p-TβRII, form a complex with Co-Smad. This complex increases the expression of fibrotic factors in the nucleus with the transcriptional coactivator p300. I-Smads regulate the fibrotic response in two ways: by competing with R-Smads to inhibit complex formation with Smad4, and by promoting TβR1 degradation through Smurf1 in a negative feedback loop. AMPK inhibits the TGF-β pathway through various mechanisms, such as inhibiting R-Smad phosphorylation, suppressing p300 activity, and upregulating Smad7 expression, which leads to increase in TβR1 degradation. Although Smad6 also contributes to TβR1 degradation, studies on the effect of AMPK on Smad6 are lacking. Transforming Growth Factor Beta, TGF-β; Bone Morphogenetic Protein, BMP; Transforming Growth Factor Beta Receptor Type II, TβRII; Transforming Growth Factor Beta Receptor Type I, TβRI; AMP-activated Protein Kinase, AMPK.

stress management, AMPK activation plays a significant role in alleviating fibrosis across various organs, including the liver, heart, kidneys, and lungs (Thakur *et al*., 2015) as summarized in Fig. 5. Hepatocyte growth factor (HGF) has been shown to inhibit TGF-β1-induced ECM deposition and myofibroblastic differentiation by activating AMPK (Cui *et al*., 2011; Cui *et al*., 2013). Since TGF-β signaling accelerates profibrotic responses by activating Smad3, a key regulator of myofibroblast differentiation and ECM production, targeting the Smad pathway–either directly or indirectly–can effectively alleviate fibrosis (Peng *et al*., 2022).

The TGF-β superfamily includes several subfamilies such as TGF-β, activin/inhibin, and bone morphogenetic protein (BMPs). These subfamilies bind to type II receptor kinases (TβRII) and dimerize with type I receptor kinases (TβRI), also known as activin-like receptor kinases (ALKs) to initiate Smad signaling. Within this pathway, Smads are classified into three categories: receptor-regulated Smads (R-Smads: Smad1/2/3/5/8), which are directly phosphorylated and activated by TβRI; co-mediator Smad (Co-Smad: Smad4), which acts with R-Smads to facilitate signal transduction; and inhibitory Smads (I-Smads: Smad6/7), which function in a negative feedback loop to regulate and limit the signal cascade. Smad2 and Smad3 primarily respond to TGF-β signaling, while Smad1, Smad5, and Smad8 respond to BMP signaling, both of which influence the fibrotic process (Shi and Massagué, 2003; Hata and Chen, 2016). I-Smads prevent the formation of the R-Smad and Co-Smad complexes, with Smad6 specifically competing with Smad1/5/8 to inhibit BMP signaling, while Smad7 regulates both the TGF-β and BMP signaling pathways (Shi and Massagué, 2003; de Ceuninck van Capelle *et al*., 2020).

AMPK negatively regulates TGF-β by either inhibiting the phosphorylation of R-Smads or promoting the expression of I-Smads (Park *et al*., 2014; Lin *et al*., 2017; Gao *et al*., 2018). Activated AMPK reduces TGF-β1-induced Smad3 phosphorylation, suppresses TGF-β1 production, and decrease its transcriptional activity (Li *et al*., 2016). Consequently, AMPK inhibits TGF-β1-induced collagen production by inhibiting the Smad3-driven expression of connective tissue growth factor (CTGF) (Lu *et al*., 2015). Additionally, AMPK interferes with the nuclear translocation and transcriptional activity of Smad2/3 by suppressing their phosphorylation in an ALK degradation-independent pathway (Xiao *et al*., 2010). AMPK also diminishes Smad3 acetylation by inhibiting the transcriptional coactivator p300, further reducing Smad-driven fibrotic gene expression without affecting phosphorylation on R-Smads (Mishra *et al*., 2008; Gao *et al*., 2018). Moreover, activated AMPK enhances the expression of I-Smads and Smurf1 (an E3 ubiquitin ligase), promoting the proteasomal degradation of ALK1 and ALK2, thereby inhibiting BMP9 and BMP6 signaling, leading to reduced Smad1/5 phosphorylation (Lin *et al*., 2017; Ying *et al*., 2017; Lin *et al*., 2020). In addition to Smad-dependent pathways, TGF-β signaling activates non-Smad pathways, including MAPK, PI3K/Akt, and RhoA/Rac, which contribute to fibrosis by promoting myofibroblast proliferation and differentiation (Zhang, 2009). AMPK activation alleviates fibrosis by inhibiting these non-Smad pathways, further underlining its antifibrotic potential in conditions such as metabolic liver disease and other fibrotic disorders (Abdelhamid *et al*.,

2021b).

RECENT RESEARCH AND CLINICAL APPROACHES OF AMPK ACTIVATORS

Recent studies on therapeutic agents targeting AMPK for MASLD and liver fibrosis can be classified into two categories: those that directly activate AMPK and those that mimic AMPK's downstream actions. The former directly phosphorylates AMPK through mechanisms such as energy deprivation (e.g., increased AMP: ATP ratio), endocrine regulation, or antioxidants. The latter does not directly activate AMPK. Instead, indirect activators modulate PPAR, SIRT1, Nrf2, mTOR, and TGF-β to activate downstream signaling regulators of AMPK, responsible for each of the AMPK actions: hepatic lipid regulation, autophagy, anti-inflammatory action, and anti-fibrotic processes. Although the exact mechanism of each agent is unclear due to the complexity of AMPK signaling, this session may help navigate current promising agents targeting MASH, MASLD, and liver fibrosis. In this review, we summarize the recent studies on promising AMPK-related therapeutic strategies targeting each facet of the pathophysiological processes involved in MASH, MASLD, and hepatic fibrosis, and introduce various AMPK-related therapeutics in clinical trials.

Structure and modulation of AMPK activity

AMPK is a heterotrimeric protein complex found in mammalian cells, composed of a catalytic α subunit and two regulatory β and γ subunits. Each subunit has multiple isoforms: two α (α1, α2), two β (β1, β2), and three γ (γ1, γ2, γ3) isoforms (Herzig and Shaw, 2018), with tissue-specific expression patterns. Notably, the α1β2γ1 isoform is predominantly expressed in the human liver tissue (Wu *et al*., 2013). The α subunit of AMPK contains an N-terminal kinase domain (KD), followed by an autoinhibitory domain (AID), which keeps the KD in a less active conformation under normal conditions. The γ subunit of AMPK functions as an energy sensor by binding adenosine nucleotides (ATP, ADP, or AMP). When AMP binds to the γ subunit, it induces a conformational change that exposes the KD of the α subunit, thus facilitating allosteric activation of AMPK. This results in a significant increases in the enzymatic activity of AMPK, which can be amplified up to 1000-fold through phosphorylation by upstream kinases, such as LKB1 and CaMKK2 (Steinberg and Carling, 2019). The γ subunit contains four cystathionine β-synthase (CBS) domains that are crucial for nucleotide binding. Specifically, AMP binding to the CBS3 domain of the γ subunit triggers allosteric changes that cause the α-linker to dissociate from the γ subunit, thereby exposing the conserved Thr172 residue in the N-terminal region. This promotes its phosphorylation and ultimately leading to AMPK activation. The AMP-bound γ subunit allows upstream AMPK kinases, such as LKB1 and CaMKK2, to stimulate AMPK through distinct mechanisms. LKB1-dependent phosphorylation of AMPKα at Thr172 is greatly enhanced by AMP binding to the AMPKγ subunit. LKB1 indirectly activates AMPK when the intracellular AMP:ATP ratio increases, while CaMKK2 activates AMPK in response to elevated cytosolic Ca^{2+}, independent of AMP:ATP levels. However, though CaMKK2 activates AMPK primarily in response to increased intracellular Ca^{2+} levels, during ATP depletion, the increase in AMP levels also enhances the interaction between AMPK and CaMKK2, thereby improving AMPK activation (Schmitt *et al*., 2022; Steinberg and Hardie, 2023). The underlying mechanism of LKB1-induced AMPK activation in response to an increased AMP/ATP ratio involves AMP binding, which triggers the formation of the AXIN-AMPK-LKB1 complex, facilitating LKB1's direct tethering to the phosphorylation site of AMPK, thereby activating AMPK (Zhang *et al*., 2013). AMPK can be activated independently of AMP/ADP by sensing the absence of fructose-1,6-bisphosphate (FBP), where unbound aldolase facilitates the assembly of a lysosomal complex involving v-ATPase, ragulator, axin, LKB1, and AMPK, linking glucose availability to AMPK activation (Zhang *et al*., 2017; Li *et al*., 2019).

The AMPKβ subunit regulates kinase activity through diverse mechanisms. One such mechanism involves its carbohydrate-binding module (CBM) that can directly recognize glycogen signals and binds almost exclusively to the α 1-6 branch points of degraded glycogen, inhibiting AMPK activation *in vitro* (McBride *et al*., 2009). Moreover, N-terminal myristoylation of the β subunit is essential for the effect of AMP on the α-Thr172 phosphorylation (Oakhill *et al*., 2011). The AMPK complex also contains a critical regulatory site known as the Allosteric Drug and Metabolite (ADaM) site, located between the α-KD and β-CBM. This site forms a deep cleft that can bind pharmacological activators and long-chain fatty acyl-CoA esters, thereby modulating AMPK activity (Steinberg and Hardie, 2023).

The AMPKγ subunit has four CBS domains, which are responsible for sensing AMP: ATP ratio level and directly interacting with adenine nucleotides to induce its allosteric activation (Scott *et al*., 2007). Each CBS domain holds a helix-loop-strand structure, contributing to in a high degree of connectivity between the nucleotide and the AMPK complex (Xiao *et al*., 2007). CBS1 and CBS3 competitively bind with AMP, ADP, or ATP depending on the cellular adenine nucleotide level, while CBS4 exclusively and permanently binds AMP. This allows the AMPK complex to rapidly sense changes in the AMP: ATP ratio, helping balance the cellular energy supply (Hardie *et al*., 2011). Another key structural element in the AMPKγ subunit is CBS2, which contains a pseudo-substrate sequence (PS) that resembles the sequence of ACC, an AMPK substrate. In the absence of AMP, CBS2 acts as an auto-inhibitor of AMPK by binding to the α subunit. Upon AMP binding, the γ subunit undergoes a conformational change which detaches the PS from the active site of the α subunit (Scott *et al*., 2007).

In summary, the binding of adenosine nucleotides, ADaM site ligands, and CBM phosphorylation affects the conformation of the KD through induced movements of the dynamic domains (Yan *et al*., 2018). Increased AMP:ATP ratio allosterically activates AMPK (Hardie *et al*., 2003), when the AMP:ATP ratio increases due to glucose deprivation, hypoxia, heat shock, or strenuous exercise-induced ATP consumption (Viollet *et al*., 2010). Endocrine and autocrine regulation, including the adipose hormones such as adiponectin and leptin, the adrenergic hormones like catecholamines in adipocytes, and interleukin 6, upregulates AMPK activity (Townsend and Steinberg, 2023). Conversely, sustained hyperglycemia suppresses AMPK activity (Ruderman and Prentki, 2004). Given that AMPK activation can balance energy metabolism and mitigate every stage of hepatic fibrosis progression by modulating the autophagic, inflammatory and fibrotic responses, modifying AMPK function may offer a promising strategy to prevent or

even reverse the progression of MASLD to liver fibrosis (Cusi *et al*., 2021).

Recent research of AMPK activators

Direct activators (Table 1): AMPK has emerged as a novel therapeutic target for MASLD due to its wide-ranging regulatory roles in metabolism. Recent research has uncovered direct AMPK activators that either phosphorylate the α subunit or bind to the ADaM site, located between the α and β subunits. One such molecule is PXL770 (PubChem CID: 72700732), which directly activates AMPK by binding to the ADaM site *in vitro* (Fouqueray *et al*., 2021). According to the results from the NCT03763877 clinical trial updated in October 2021, PXL770 improved metabolic features but did not achieve the primary endpoint of significantly reducing liver fat in a phase 2a study in patients with NAFLD. However, its favorable safety profile and metabolic benefits suggest it could hold promise as part of combination therapies or in specific patient populations (Cusi *et al*., 2021; Wei *et al*., 2024). Salicylate (Pubchem CID: 54675850), a metabolite of aspirin (Pubchem CID: 2244), and A-769662 (Pubchem CID: 54708532) are also representative activators of AMPK that bind to the ADaM site (Steinberg and Carling, 2019). A-769662, as an AMPKβ1 agonist, activates AMPK in the liver, which inhibits caspase-6 activity and mitigates hepatic damage and fibrosis (Zhao *et al*., 2020). AICAR, a direct AMPK activator, enhances Nrf2-regulated hepatic antioxidant capacity and inhibits NLRP3 inflammasome-mediated pyrolysis, thereby protecting rats from sodium taurocholate-induced pancreatitis-associated liver injury (Kong *et al*., 2021). Aspirin and salicylate have been shown to uncouple mitochondrial oxidative phosphorylation in colorectal cancer cells, thereby increasing ADP:ATP ratio, and subsequently activating AMPK (Din *et al*., 2012). In the NCT04031729 phase 2 clinical trial, 80 individuals with MASLD were treated with low-dose aspirin (81 mg) daily for 6 months, resulting in a significant reduction in hepatic fat quantity compared to placebo (Simon *et al*., 2024). Salsalate (Pubchem CID: 5161), the dimer of salicylic acid, activates AMPKβ1-containing heterotrimers (Day *et al*., 2021). Salsalate directly activates AMPK, reducing obese adipose tissue, hepatic macrophage infiltration, inflammation, and adipogenesis gene expression, ultimately ameliorating hepatosteatosis in HFD-fed mice (Li *et al*., 2021). A phase 4 clinical trial (NCT03222206) investigated the effects of salsalate in patients with NAFLD and osteoarthritis. The study has been completed; however, the results have not yet been posted. Ginsenosides, compounds extracted from ginseng, have been validated for their pharmacological activities in improving metabolic disease (Bai *et al*., 2024). Ginsenoside Rh4 (PubChem CID: 21599928) has been shown to strongly bind to AMPKα1, leading to the upregulation of PGC-1α-mediated mitochondrial biogenesis and the downregulation of p38/MAPK/NF-kB-mediated inflammatory responses (Zhang *et al*., 2024). In NAFLD mouse models, Rh4 treatment significantly reduced hepatic steatosis and lobular inflammation. Moreover, Rh4 improved gut microbiota by increasing levels of intestinal SCFAs and bile acids, which are associated with changes in gut flora composition (Yang *et al*., 2023). Magnolol (PubChem CID: 72300), a bioactive compound isolated from Magnolia officinalis, exhibits a broad spectrum of biological activities. A previous study on magnolol demonstrated its effects in promoting activating phosphorylation of AKT and AMPK, inhibitory phosphorylation of ACC, and increasing PPARα expression, while simultaneously inhibiting the activation of the MAPK, NF-κB, and SREBP-1 pathways in oleic acid (OA)-induced steatosis in HepG2 cells (Tian *et al*., 2018). Moreover, magnolol regulates autophagy through the AMPK/mTOR/ULK1 signaling pathway. A recent study showed that magnolol treatment in Alzheimer's disease-induced APP/PS2 mice reversed the pathological changes by increasing phosphorylation at the active sites of AMPK and ULK1, while decreasing phosphorylation of mTOR at its active site (Wang and Jia, 2023). Although the above-mentioned effects of magnolol are associated with AMPK activation, further studies are required to confirm whether magnolol functions as a direct AMPK activator and to assess its therapeutic potential in MASLD through AMPK activation. Cordycepin (PubChem CID: 6303), a compound extracted from *Cordyceps militaris*, has also gained attention as a potential AMPK agonist. Previous studies on direct AMPK agonists, such as A-79662, AICAR (PubChem CID: 17513), and PXL770, have laid the foundation for cordycepin research. Cordycepin has been shown to effectively reduce lipid accumulation and increase p-AMPK levels in HFD-hamsters (Guo *et al*., 2010). Recently, a derivative of cordycepin, V1, identified through structure-activity relationship studies, demonstrated enhanced AMPK activation, bioavailability, and lipid-lowering effects, with its AMPK activation attributed to binding the AMPKγ subunit (Wang *et al*., 2024).

Indirect activators (Table 2): As mentioned earlier, LKB1 and CaMMK2 are two critical upstream modulators of AMPK, playing significant roles in its indirect action. One of the primary indirect activators is adiponectin, an adipokine that is abundantly expressed in adipose tissue. Adiponectin ameliorates MASH, MASLD, and liver fibrosis by directly activating the LKB1 and CaMKK2 signaling pathways (Kadowaki *et al*., 2006; Iwabu *et al*., 2010). Plasma adiponectin levels are negatively correlated with visceral obesity and insulin resistance, making it an excellent predictive marker for type 2 diabetes and metabolic syndrome, as it directly enhances insulin sensitivity (Combs and Marliss, 2014). Adiponectin increases fatty acid oxidation while reducing hepatic and serum TG levels. It also downregulates the expression of gluconeogenic enzymes like phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, resulting in decreased insulin secretion (Yamauchi *et al*., 2002; Combs and Marliss, 2014). Full-length adiponectin exerts its effects by binding to its receptors, AdipoR1 and AdipoR2, thereby activating AMPK and PPARα, respectively, as well as potentially other unknown signaling pathways (Yamauchi *et al*., 2007). The effect of adiponectin on AMPK is primarily mediated via the AdipoR1/LKB1/AMPK pathway, as evidenced by the complete abolition of adiponectin-induced suppression of SREBP1c expression in the liver of LKB1-deficient ($LKB1^{-/-}$) mice (Awazawa *et al*., 2009). Additionally, adiponectin activates CaMKK2, another AMPK upstream kinase, by inducing phospholipase C, which increases intracellular calcium levels (Zhou *et al*., 2009). In skeletal muscle-specific AdipoR1 knockout mice, adiponectin treatment leads to the phosphorylation of AMPK, while suppression of CaMKK2 or LKB1 expression significantly decreases the adiponectin-induced activation of AMPK (Iwabu *et al*., 2010). Recent studies on adiponectin and AMPK have unveiled promising therapeutic strategies that enhance or mimic adiponectin action.

Metformin (PubChem CID: 4091), a long-established antidiabetic medication, has gained attention for its beneficial effects

Table 1. List of direct AMPK activators

Potential drug	Mode of action	Effects on the liver	Model	Reference
PXL770	Binds to ADaM site of AMPK	Improves metabolic features with no significant fat reduction	Patients with NAFLD (phase 2a)	NCT03763877, Cusi *et al*., 2021; Wei *et al*., 2024
A-769662	Binds to ADaM site of AMPK	Improves liver damage and attenuates hepatic fibrosis	Liver AMPK-deficient mice & aP2-nSREBP-1c transgenic mice	Steinberg and Carling, 2019, Zhao *et al*., 2020
AICAR	Is converted into ZMP (an AMP mimic) in cells, binds to the γ subunit of AMPK, and promotes AMPK phosphorylation by LKB1	Enhances Nrf2-regulated hepatic anti-oxidant capacity Inhibits NLRP3 inflammasome-mediated pyrolysis Protects rats from sodium taurocholate-induced pancreatitis-associated liver injury.	Sodium taurocholate-induced severe acute pancreatitis rats	Kong *et al*., 2021
Salicylate	Binds to ADaM site of AMPK and increases ADP:ATP ratio	Reduces hepatic fat and improves liver function	Patients with MASLD (phase 2)	NCT04031729, Simon *et al*., 2024
Salsalate	Binds to ADaM site, activates AMPK, and inhibits caspase-6 activity	Reverse metabolic disorders Potential for reducing fatty acids and fibrosis	HFD-fed mice and patients with NAFLD and osteoarthritis (phase 4)	Li *et al*., 2021, NCT03222206
Ginsenoside Rh4	Binds to AMPKα1, upregulates PGC-1α, and downregulates p38/MAPK/NF-κB signal	Decreases hepatic steatosis and lobular inflammation, and improves gut microbiota	Western diet and CCl4-induced NAFLD mice	Yang *et al*., 2023
Cordycepin	Binds to AMPKγ subunit and increases p-AMPK levels	Reduces lipid accumulation	HFD-fed hamsters	Guo *et al*., 2010
V1 (Cordycepin derivative)	Binds to AMPKγ subunit and enhances AMPK activation and bioavailability	Reduces serum LDL and liver TG level	HFD-C57BL/6 mice, HFD-golden hamsters, and rhesus monkeys	Wang *et al*., 2024

Table 2. List of indirect AMPK activators

Potential drug	Pathway that activates AMPK	Effects on the liver	Model	Reference
Adiponectin-related AMPK activators				
Metformin	Increased AMP/ATP and ADP/ATP ratios Activates AMPKα and inhibits NF-κB nuclear binding activity Inhibits TGFβ-Smad3 signaling Inhibits ALK1, leading to inhibition of angiogenesis and neovascularization. Binds to the γ-secretase subunit PEN2, inhibits v-ATPase, and linksthe lysosomal glucose-sensing pathway to AMPK activation without altering cellular AMP levels	Reduces hepatic fat contents Inhibits fibrosis and cancer progression	Primary mouse hepatocytes and CCl_4-treated mice HFD-Ampk–$\beta1^{-/-}$ and Ampk–$\beta2^{-/-}$ mice Intestine- and hepatic-specific PEN2 konckout mice, intestine-specific AMPKα knockout mice HFD-induced obese mice models	Xiao *et al*., 2010; Fullerton *et al*., 2013; Lu *et al*., 2015; Ying *et al*., 2017; Lin *et al*., 2020; Abdelhamid *et al*., 2021a; Ma *et al*., 2022
Antrodan	Increases AMPK phosphorylation	Activates mitochondrial biogenesis and diminishes lipogenesis	High-fat, high-fructose diet male C57BL/6 mice model	Chyau *et al*., 2020
Atractylenolide III	Upregulates hepatic AdipoR1-mediated AMPK/SIRT1 signaling	Improves hepatic enzyme markers indicating reduced oxidative stress, inflammation, and fibrosis	HFD male C57BL/6J mice	Li *et al*., 2022b
Aramchol	Increases adiponectin levels	Reduces steatohepatitis without worsening fibrosis, inhibits hepatic fatty acid synthesis, and increases β-oxidation	Patients with NASH (phase 2b) Patients with MASH (phase 3, ARMOR)	NCT02279524, Ratziu *et al*., 2021, NCT04104321, Bhattacharya *et al*., 2021, Fernández-Ramos *et al*., 2020
Emodin succinate monoethyl ester (ESME)	Upregulates hepatic AdipoR2-mediated AMPK signaling activation	Reduces lipid accumulation in hepatocytes	HFD hamsters and *Apoe*$^{-/-}$ mice with MASLD	Zhao *et al*., 2023
JT003 with V14	Upregulates AMPK signaling as an AdipoR1/2 dual agonist with an EDP inhibitor	Decreases inflammation, oxidative stress, ECM accumulation; increases β-oxidation	Male C57BL/6J mice	Song *et al*., 2023
LKB1-related AMPK activators				
Salusin-α	Activates LKB1 to phosphorylate AMPK at Thr172	Inhibits lipid biosynthesis by suppressing ACC, FASN, and SREBPs	Hepatocyte cell steatosis model	Pan *et al*., 2024
AMP:ATP ratio-related AMPK activators				
Nitazoxanide	Decreases ATP production through mitochondrial uncoupling	Reduces glycogen storage and lipid biogenesis, increases fatty acid oxidation, and improves hepatic steatosis and hyperlipidemia	HFD or WD-induced hepatic steatosis in SPF golden Syrian hamsters, C57BL/6J mice and *Apoe*$^{-/-}$ mice	Li *et al*., 2022a

against fibrosis and cancer progression, primarily through its indirect activation of AMPK, despite the direct targets of its action remaining unidentified (Xiao *et al*., 2010; Fullerton *et al*., 2013; Lu *et al*., 2015; Ying *et al*., 2017; Lin *et al*., 2020; Abdelhamid *et al*., 2021a). AMPK activation induced by high concentrations of metformin is lysosome-independent and occurs through increased AMP/ATP and ADP/ATP ratios. Recently, metformin has been found to activate AMPK by binding to the γ-secretase subunit PEN2, inhibiting the lysosomal proton pump v-ATPase, and linking the lysosomal glucose-sensing pathway to AMPK activation without altering cellular AMP levels (Ma *et al*., 2022).

Antrodan, an upregulator of leptin and adiponectin, stimulates AMPK phosphorylation in high-fat, high-fructose diet C57BL/6 mice. This increase in p-AMPK not only enhances the NAD^+/NADH ratio but also induces SIRT1 expression, improving mitochondrial biogenesis and reducing lipogenesis by inhibiting FASN activity and lowering TG levels through suppression of the PPARγ/SREBP1c axis. Antrodan thus improves serum biochemical markers, including malondialdehyde, total cholesterol, TG, ALT, AST, uric acid, glucose, and insulin (Matsusue *et al*., 2014; Chyau *et al*., 2020). Atractylenolide III (PubChem CID: 155948) upregulates adiponectin receptor expression, counteracting the reduction of hepatic AdipoR1 expression in high-fat diet (HFD) male C57BL/6J mice, and activates AdipoR1 downstream AMPK/SIRT1 signaling in HepG2 cells. Thus, Atractylenolide III significantly alleviates hepatic biochemical markers such as ALT, AST, TGs, total cholesterol, and LDL via the LKB1 pathway, along with reducing oxidative stress, inflammation, and fibrosis (Li *et al*., 2022b).

Aramchol (PubChem CID: 18738120), a conjugate of cholic acid and arachidic acid, is a novel therapeutic agent being investigated for the treatment of NASH. It has been shown to inhibit hepatic SCD1 and upregulate adiponectin levels (Fernández-Ramos *et al*., 2020; Bhattacharya *et al*., 2021). A phase 2b clinical trial (NCT02279524) evaluated the efficacy and safety of aramchol in patients with NASH (Ratziu *et al*., 2021). Aramchol reduces liver TGs and resolves steatohepatitis without worsening fibrosis by increasing adiponectin levels and improving endothelial function (Safadi *et al*., 2014; Ratziu *et al*., 2021). Although the primary endpoint of reducing liver steatosis has not been met, the observed safety profile and other hepatic effects suggest that aramchol has potential for the treatment of MASH. Therefore, a phase 3 clinical trial in patients with MASH and fibrosis (F1-F3) is currently ongoing (NCT04104321).

Emodin succinate monoethyl ester (ESME), a novel anthraquinone compound, activates AdipoR2 and ameliorates hepatic steatosis in hamster and mouse models. The suppression of AdipoR2 expression or AMPK activation eliminates the effect of ESME, confirming that it acts through AMPK phosphorylation (Zhao *et al*., 2023). JT003, an AdipoR1/2 dual agonist, significantly degrades ECM and improves liver fibrosis. However, ECM degradation generates elastin-derived peptides (EDPs), which can exacerbate liver fibrosis. The combination of JT003 with V14, an EDP inhibitor, has shown synergistic benefits in treating NAFLD both *in vitro* and *in vivo* (Song *et al*., 2023).

Salusin-α, a novel bioactive peptide involved in vascular function, blood pressure regulation, and metabolic process, significantly inhibits lipid biosynthesis pathways, including ACC, FASN, and SREBPs. In *in vivo* studies, salusin-α increased the levels of p-LKB1 and p-AMPK. The lipid accumulation-inhibiting effect of salusin-α was hindered when AMPK was inactivated with compound C treatment in the salusin-α-overexpressing group. This suggests that its effect occurs through the LKB1/AMPK pathway and indicates that it indirectly activates AMPK in relation to LKB1 (Pan *et al*., 2024).

Nitazoxanide (PubChem CID: 41684), a broad-spectrum antiparasitic agent (White Jr, 2004), has been demonstrated to decrease ATP production through mitochondrial uncoupling and other mechanisms, leading to AMPK activation (Amireddy *et al*., 2023). This activation enhances autophagy and suppresses lipid biosynthesis, thereby ameliorating hepatic steatosis and fibrosis in HFD or Western diet (WD)-induced hepatic steatosis in SPF golden Syrian hamsters, C57BL/6J mice, and *Apoe*$^{-/-}$ mice via suppressing ACC (Li *et al*., 2022a). These findings underscore its therapeutic potential for modulating cellular energy balance and metabolic processes through AMPK activation.

CONCLUSIONS

The liver is a central organ in metabolism, responsible for detoxifying and regulating energy balance, including controlling blood glucose levels, lipid metabolism, and the clearance of toxic substances from both endogenous and exogenous sources. It also plays a significant role in the efficacy of certain medications used to treat conditions such as obesity, dyslipidemia, hypertension, and diabetes (Samuel and Shulman, 2018). According to recent meta-analyses, MASLD is the most common liver disease globally, with an overall prevalence of 32.4%, making it the leading cause of liver-related morbidity and mortality (Xanthopoulos *et al*., 2019; Riazi *et al*., 2022). Patients with MASLD have been reported to have a lower 1-year survival rate compared to patients who received liver transplants for hepatitis C virus infection or alcohol-associated liver disease (Nagai *et al*., 2019). These patients are more prone to perioperative complications, including infections, malignancies, and cardiovascular and cerebrovascular events (Burra *et al*., 2020). Progressive liver failure presents a more urgent clinical challenge than many other organ diseases because it heightens the risk of fatal cardiovascular events, increases morbidity, and contributes to malnutrition (Kasper *et al*., 2021; Tyczyńska *et al*., 2024). Additionally, liver failure complicates treatment options because the ability of liver to metabolize drugs is impaired, limiting drug use. Despite the prevalence and severity of these conditions, there is currently only one FDA-approved drug, resmetirom (a thyroid hormone receptor beta agonist), for the treatment of noncirrhotic MASH, reflecting the urgent need for further therapeutic development in this area (Keam, 2024).

While significant research has been conducted on alcoholic liver disease, non-alcoholic liver diseases, particularly MASLD, have not been studied to the same extent. Previous studies indicate that the primary drivers of MASLD are excessive oxidative stress due to metabolic imbalances, inflammatory responses, apoptosis, and activation of fibrotic mechanisms. As inflammation escalates due to these toxic factors, healthy hepatocytes die, while fibrotic cells become activated, increasing the proportion of dysfunctional liver tissue over time (Loomba *et al*., 2021). AMPK, which plays a crucial role in

regulating cellular energy metabolism and neutralizing oxidative stress, has emerged as a key regulatory protein capable of counteracting cytotoxicity, reducing inflammation, and inhibiting fibrotic processes. This makes AMPK an ideal therapeutic target for addressing all major causes of MASLD.

Establishing effective MASLD treatment strategies is critical for improving liver function, which in turn enhances patient survival rates, expands treatment options, and improves quality of life, while also reducing healthcare costs. Given that MASLD shares pathophysiological mechanisms with cardiovascular, cerebrovascular, and other fibrotic diseases, AMPK-based therapies for MASLD could also have broader applications. As AMPK is a versatile effector, treatments that mimic the actions of its downstream molecules could provide promising therapeutic targets for MASLD. Therefore, further research into AMPK and its downstream pathways, not only in the liver but also in other fibrotic conditions, will yield insights that may benefit a range of diseases with similar underlying mechanisms.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2018R1A5A2025286) and by the Bio & Medical Technology Development Program of the NRF funded by MSIT (2021M3E5E7024855).

REFERENCES

Abdelhamid, A. M., Youssef, M. E., Abd El-Fattah, E. E., Gobba, N. A., Gaafar, A. G. A., Girgis, S., Shata, A., Hafez, A.-M., El-Ahwany, E. and Amin, N. A. (2021a) Blunting p38 MAPKα and ERK1/2 activities by empagliflozin enhances the antifibrotic effect of metformin and augments its AMPK-induced NF-κB inactivation in mice intoxicated with carbon tetrachloride. *Life Sci.* **286**, 120070.

Abdelhamid, A. M., Youssef, M. E., Abd El-Fattah, E. E., Gobba, N. A., Gaafar, A. G. A., Girgis, S., Shata, A., Hafez, A.-M., El-Ahwany, E., Amin, N. A., Shahien, M. A., Abd-Eldayem, M. A., Abou-Elrous, M. and Saber, S. (2021b) Blunting p38 MAPKα and ERK1/2 activities by empagliflozin enhances the antifibrotic effect of metformin and augments its AMPK-induced NF-κB inactivation in mice intoxicated with carbon tetrachloride. *Life Sci.* **286**, 120070.

Alvarez-Guardia, D., Palomer, X., Coll, T., Davidson, M. M., Chan, T. O., Feldman, A. M., Laguna, J. C. and Vázquez-Carrera, M. (2010) The p65 subunit of NF-κB binds to PGC-1α, linking inflammation and metabolic disturbances in cardiac cells. *Cardiovasc. Res.* **87**, 449-458.

Alves-Bezerra, M. and Cohen, D. E. (2017) Triglyceride metabolism in the liver. *Compr. Physiol.* **8**, 1-8.

Amireddy, N., Dulam, V., Kaul, S., Pakkiri, R. and Kalivendi, S. V. (2023) The mitochondrial uncoupling effects of nitazoxanide enhances cellular autophagy and promotes the clearance of α-synuclein: potential role of AMPK-JNK pathway. *Cell. Signal.* **109**, 110769.

Ascenzi, F., De Vitis, C., Maugeri-Saccà, M., Napoli, C., Ciliberto, G. and Mancini, R. (2021) SCD1, autophagy and cancer: implications for therapy. *J. Exp. Clin. Cancer Res.* **40**, 265.

Awazawa, M., Ueki, K., Inabe, K., Yamauchi, T., Kaneko, K., Okazaki, Y., Bardeesy, N., Ohnishi, S., Nagai, R. and Kadowaki, T. (2009) Adiponectin suppresses hepatic SREBP1c expression in an AdipoR1/LKB1/AMPK dependent pathway. *Biochem. Biophys. Res. Commun.* **382**, 51-56.

Bai, X., Fu, R., Deng, J., Yang, H., Zhu, C. and Fan, D. (2024) New dawn of ginsenosides: regulating gut microbiota to treat metabolic syndrome. *Phytochem. Rev.* **23**, 1247-1269.

Barroso, W. A., Victorino, V. J., Jeremias, I. C., Petroni, R. C., Ariga, S. K. K., Salles, T. A., Barbeiro, D. F., de Lima, T. M. and de Souza, H. P. (2018) High-fat diet inhibits PGC-1α suppressive effect on NFκB signaling in hepatocytes. *Eur. J. Nutr.* **57**, 1891-1900.

Batchuluun, B., Pinkosky, S. L. and Steinberg, G. R. (2022) Lipogenesis inhibitors: therapeutic opportunities and challenges. *Nat. Rev. Drug Discov.* **21**, 283-305.

Bhattacharya, D., Basta, B., Mato, J. M., Craig, A., Fernández-Ramos, D., Lopitz-Otsoa, F., Tsvirkun, D., Hayardeny, L., Chandar, V. and Schwartz, R. E. (2021) Aramchol downregulates stearoyl CoA-desaturase 1 in hepatic stellate cells to attenuate cellular fibrogenesis. *JHEP Rep.* **3**, 100237.

Bonnet, L. V., Palandri, A., Flores-Martin, J. B. and Hallak, M. E. (2024) Arginyltransferase 1 modulates p62-driven autophagy via mTORC1/AMPk signaling. *Cell Commun. Signal.* **22**, 87.

Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R. L., Soerjomataram, I. and Jemal, A. (2024) Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* **74**, 229-263.

Budi, E. H., Schaub, J. R., Decaris, M., Turner, S. and Derynck, R. (2021) TGF-β as a driver of fibrosis: physiological roles and therapeutic opportunities. *J. Pathol.* **254**, 358-373.

Burra, P., Becchetti, C. and Germani, G. (2020) NAFLD and liver transplantation: disease burden, current management and future challenges. *JHEP Rep.* **2**, 100192.

Buzzetti, E., Pinzani, M. and Tsochatzis, E. A. (2016) The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* **65**, 1038-1048.

Cani, P. D., Possemiers, S., Van de Wiele, T., Guiot, Y., Everard, A., Rottier, O., Geurts, L., Naslain, D., Neyrinck, A. and Lambert, D. M. (2009) Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* **58**, 1091-1103.

Cantó, C. and Auwerx, J. (2010) AMP-activated protein kinase and its downstream transcriptional pathways. *Cell. Mol. Life Sci.* **67**, 3407-3423.

Cantó, C., Gerhart-Hines, Z., Feige, J. N., Lagouge, M., Noriega, L., Milne, J. C., Elliott, P. J., Puigserver, P. and Auwerx, J. (2009) AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. *Nature* **458**, 1056-1060.

Chen, J., Deng, X., Liu, Y., Tan, Q., Huang, G., Che, Q., Guo, J. and Su, Z. (2020) Kupffer cells in non-alcoholic fatty liver disease: friend or foe? *Int. J. Biol. Sci.* **16**, 2367.

Chen, Z., Yu, R., Xiong, Y., Du, F. and Zhu, S. (2017) A vicious circle between insulin resistance and inflammation in nonalcoholic fatty liver disease. *Lipids Health Dis.* **16**, 203.

Cheng, D., Zhang, M., Zheng, Y., Wang, M., Gao, Y., Wang, X., Liu, X., Lv, W., Zeng, X., Belosludtsev, K. N., Su, J., Zhao, L. and Liu, J. (2024) α-Ketoglutarate prevents hyperlipidemia-induced fatty liver mitochondrial dysfunction and oxidative stress by activating the AMPK-pgc-1α/Nrf2 pathway. *Redox Biol.* **74**, 103230.

Choi, E.-J., Oh, H.-T., Lee, S.-H., Zhang, C.-S., Li, M., Kim, S.-Y., Park, S., Chang, T.-S., Lee, B.-H. and Lin, S.-C. (2024) Metabolic stress induces a double-positive feedback loop between ampk and sqstm1/P62 conferring dual activation of ampk and nfe2l2/nrf2 to synergize antioxidant defense. *Autophagy* **20**, 2490-2510.

Chopra, I., Li, H. F., Wang, H. and Webster, K. A. (2012) Phosphorylation of the insulin receptor by AMP-activated protein kinase (AMPK) promotes ligand-independent activation of the insulin signalling pathway in rodent muscle. *Diabetologia* **55**, 783-794.

Chyau, C.-C., Wang, H.-F., Zhang, W.-J., Chen, C.-C., Huang, S.-H., Chang, C.-C. and Peng, R. Y. (2020) Antrodan alleviates high-fat and high-fructose diet-induced fatty liver disease in C57BL/6 mice model via AMPK/Sirt1/SREBP-1c/PPARγ pathway. *Int. J. Mol. Sci.* **21**, 360.

Combs, T. P. and Marliss, E. B. (2014) Adiponectin signaling in the

liver. *Rev. Endocr. Metab. Disord.* **15**, 137-147.
Cui, Q., Fu, S. and Li, Z. (2013) Hepatocyte growth factor inhibits TGF-β1-induced myofibroblast differentiation in tendon fibroblasts: role of AMPK signaling pathway. *J. Physiol. Sci.* **63**, 163-170.
Cui, Q., Wang, Z., Jiang, D., Qu, L., Guo, J. and Li, Z. (2011) HGF inhibits TGF-β1-induced myofibroblast differentiation and ECM deposition via MMP-2 in Achilles tendon in rat. *Eur. J. Appl. Physiol.* **111**, 1457-1463.
Cusi, K., Alkhouri, N., Harrison, S., Fouqueray, P., Moller, D., Hallakou-Bozec, S., Bolze, S., Grouin, J., Jeannin Megnien, S. and Dubourg, J. (2021) Efficacy and safety of PXL770, a direct AMP kinase activator, for the treatment of non-alcoholic fatty liver disease (STAMP-NAFLD): a randomised, double-blind, placebo-controlled, phase 2a study. *Lancet Gastroenterol. Hepatol.* **6**, 889-902.
Day, E. A., Ford, R. J., Smith, B. K., Houde, V. P., Stypa, S., Rehal, S., Lhotak, S., Kemp, B. E., Trigatti, B. L. and Werstuck, G. H. (2021) Salsalate reduces atherosclerosis through AMPKβ1 in mice. *Mol. Metabol.* **53**, 101321.
de Ceuninck van Capelle, C., Spit, M. and Ten Dijke, P. (2020) Current perspectives on inhibitory SMAD7 in health and disease. *Crit. Rev. Biochem. Mol. Biol.* **55**, 691-715.
Demir, M., Lang, S., Hartmann, P., Duan, Y., Martin, A., Miyamoto, Y., Bondareva, M., Zhang, X., Wang, Y. and Kasper, P. (2022) The fecal mycobiome in non-alcoholic fatty liver disease. *J. Hepatol.* **76**, 788-799.
Di Mauro, S., Scamporrino, A., Filippello, A., Di Pino, A., Scicali, R., Malaguarnera, R., Purrello, F. and Piro, S. (2021) Clinical and molecular biomarkers for diagnosis and staging of NAFLD. *Int. J. Mol. Sci.* **22**, 11905.
Din, F. V. N., Valanciute, A., Houde, V. P., Zibrova, D., Green, K. A., Sakamoto, K., Alessi, D. R. and Dunlop, M. G. (2012) Aspirin inhibits mTOR signaling, activates AMP-activated protein kinase, and induces autophagy in colorectal cancer cells. *Gastroenterology* **142**, 1504-1515.e3.
Duan, H., Wang, L., Huangfu, M. and Li, H. (2023) The impact of microbiota-derived short-chain fatty acids on macrophage activities in disease: mechanisms and therapeutic potentials. *Biomed. Pharmacother.* **165**, 115276.
Entezari, M., Hashemi, D., Taheriazam, A., Zabolian, A., Mohammadi, S., Fakhri, F., Hashemi, M., Hushmandi, K., Ashrafizadeh, M., Zarrabi, A., Ertas, Y. N., Mirzaei, S. and Samarghandian, S. (2022) AMPK signaling in diabetes mellitus, insulin resistance and diabetic complications: a pre-clinical and clinical investigation. *Biomed. Pharmacother.* **146**, 112563.
Esler, W. P. and Cohen, D. E. (2023) Pharmacologic inhibition of lipogenesis for the treatment of NAFLD. *J. Hepatol.* **80**, 362-377.
Fernández-Ramos, D., Lopitz-Otsoa, F., Delacruz-Villar, L., Bilbao, J., Pagano, M., Mosca, L., Bizkarguenaga, M., Serrano-Macia, M., Azkargorta, M. and Iruarrizaga-Lejarreta, M. (2020) Arachidyl amido cholanoic acid improves liver glucose and lipid homeostasis in non-alcoholic steatohepatitis via AMPK and mTOR regulation. *World J. Gastroenterol.* **26**, 5101.
Ferré, P., Phan, F. and Foufelle, F. (2021) SREBP-1c and lipogenesis in the liver: an update. *Biochem. J.* **478**, 3723-3739.
Flessa, C.-M., Kyrou, I., Nasiri-Ansari, N., Kaltsas, G., Papavassiliou, A. G., Kassi, E. and Randeva, H. S. (2021) Endoplasmic Reticulum stress and autophagy in the pathogenesis of non-alcoholic fatty liver disease (NAFLD): current evidence and perspectives. *Curr. Obes. Rep.* **10**, 134-161.
Fouqueray, P., Bolze, S., Dubourg, J., Hallakou-Bozec, S., Theurey, P., Grouin, J.-M., Chevalier, C., Gluais-Dagorn, P., Moller, D. E. and Cusi, K. (2021) Pharmacodynamic effects of direct AMP kinase activation in humans with insulin resistance and non-alcoholic fatty liver disease: a phase 1b study. *Cell Rep. Med.* **2**, 100474.
Fromenty, B. and Roden, M. (2023) Mitochondrial alterations in fatty liver diseases. *J. Hepatol.* **78**, 415-429.
Fulco, M., Cen, Y., Zhao, P., Hoffman, E. P., McBurney, M. W., Sauve, A. A. and Sartorelli, V. (2008) Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt. *Dev. Cell* **14**, 661-673.
Fullerton, M. D., Galic, S., Marcinko, K., Sikkema, S., Pulinilkunnil, T., Chen, Z.-P., O'neill, H. M., Ford, R. J., Palanivel, R. and O'brien, M. (2013) Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin. *Nat. Med.* **19**, 1649-1654.
Galic, S., Loh, K., Murray-Segal, L., Steinberg, G. R., Andrews, Z. B. and Kemp, B. E. (2018) AMPK signaling to acetyl-CoA carboxylase is required for fasting- and cold-induced appetite but not thermogenesis. *eLife* **7**, e32656.
Gao, J., Ye, J., Ying, Y., Lin, H. and Luo, Z. (2018) Negative regulation of TGF-β by AMPK and implications in the treatment of associated disorders. *Acta Biochim. Biophys. Sin.* **50**, 523-531.
García-Ruiz, C. and Fernández-Checa, J. C. (2018) Mitochondrial oxidative stress and antioxidants balance in fatty liver disease. *Hepatol. Commun.* **2**, 1425-1439.
Geng, Y., Faber, K. N., de Meijer, V. E., Blokzijl, H. and Moshage, H. (2021) How does hepatic lipid accumulation lead to lipotoxicity in non-alcoholic fatty liver disease? *Hepatol. Int.* **15**, 21-35.
Ginès, P., Krag, A., Abraldes, J. G., Solà, E., Fabrellas, N. and Kamath, P. S. (2021) Liver cirrhosis. *Lancet* **398**, 1359-1376.
Gough, N. R., Xiang, X. and Mishra, L. (2021) TGF-β signaling in liver, pancreas, and gastrointestinal diseases and cancer. *Gastroenterology* **161**, 434-452.e15.
Guo, P., Kai, Q., Gao, J., Lian, Z.-q., Wu, C.-m., Wu, C.-a. and Zhu, H.-b. (2010) Cordycepin prevents hyperlipidemia in hamsters fed a high-fat diet via activation of AMP-activated protein kinase. *J. Pharmacol. Sci.* **113**, 395-403.
Gwinn, D. M., Shackelford, D. B., Egan, D. F., Mihaylova, M. M., Mery, A., Vasquez, D. S., Turk, B. E. and Shaw, R. J. (2008) AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol. Cell* **30**, 214-226.
Hagström, H., Vessby, J., Ekstedt, M. and Shang, Y. (2024) 99% of patients with NAFLD meet MASLD criteria and natural history is therefore identical. *J. Hepatol.* **80**, e76-e77.
Hardie, D. G., Carling, D. and Gamblin, S. J. (2011) AMP-activated protein kinase: also regulated by ADP? *Trends Biochem. Sci.* **36**, 470-477.
Hardie, D. G., Ross, F. A. and Hawley, S. A. (2012) AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nat. Rev. Mol. Cell Biol.* **13**, 251-262.
Hardie, D. G., Scott, J. W., Pan, D. A. and Hudson, E. R. (2003) Management of cellular energy by the AMP-activated protein kinase system. *FEBS Lett.* **546**, 113-120.
Hata, A. and Chen, Y. G. (2016) TGF-β Signaling from receptors to Smads. *Cold Spring Harb. Perspect. Biol.* **8**, a022061.
He, C. (2022) Balancing nutrient and energy demand and supply via autophagy. *Curr. Biol.* **32**, R684-R696.
Herzig, S. and Shaw, R. J. (2018) AMPK: guardian of metabolism and mitochondrial homeostasis. *Nat. Rev. Mol. Cell Biol.* **19**, 121-135.
Hoyles, L., Fernández-Real, J.-M., Federici, M., Serino, M., Abbott, J., Charpentier, J., Heymes, C., Luque, J. L., Anthony, E. and Barton, R. H. (2018) Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women. *Nat. Med.* **24**, 1070-1080.
Hsu, C. L. and Schnabl, B. (2023) The gut–liver axis and gut microbiota in health and liver disease. *Nat. Rev. Microbiol.* **21**, 719-733.
Huang, D. Q., El-Serag, H. B. and Loomba, R. (2021) Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. *Nat. Rev. Gastroenterol. Hepatol.* **18**, 223-238.
Huang, S. and Czech, M. P. (2007) The GLUT4 glucose transporter. *Cell Metab.* **5**, 237-252.
Imai, S., Armstrong, C. M., Kaeberlein, M. and Guarente, L. (2000) Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* **403**, 795-800.
Ioannou, G. N., Subramanian, S., Chait, A., Haigh, W. G., Yeh, M. M., Farrell, G. C., Lee, S. P. and Savard, C. (2017) Cholesterol crystallization within hepatocyte lipid droplets and its role in murine NASH [S]. *J. Lipid Res.* **58**, 1067-1079.
Iwabu, M., Yamauchi, T., Okada-Iwabu, M., Sato, K., Nakagawa, T., Funata, M., Yamaguchi, M., Namiki, S., Nakayama, R., Tabata, M., Ogata, H., Kubota, N., Takamoto, I., Hayashi, Y. K., Yamauchi, N., Waki, H., Fukayama, M., Nishino, I., Tokuyama, K., Ueki, K., Oike, Y., Ishii, S., Hirose, K., Shimizu, T., Touhara, K. and Kadowaki, T. (2010) Adiponectin and AdipoR1 regulate PGC-1α and mitochondria by Ca2+ and AMPK/SIRT1. *Nature* **464**, 1313-1319.

Jager, J., Grémeaux, T., Cormont, M., Le Marchand-Brustel, Y. and Tanti, J.-F. (2007) Interleukin-1β-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. *Endocrinology* **148**, 241-251.

Jensen-Cody, S. O. and Potthoff, M. J. (2021) Hepatokines and metabolism: Deciphering communication from the liver. *Mol. Metab.* **44**, 101138.

Joo, M. S., Kim, W. D., Lee, K. Y., Kim, J. H., Koo, J. H. and Kim, S. G. (2016) AMPK facilitates nuclear accumulation of Nrf2 by phosphorylating at serine 550. *Mol. Cell. Biol.* **36**, 1931-1942.

Kadowaki, T., Yamauchi, T., Kubota, N., Hara, K., Ueki, K. and Tobe, K. (2006) Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J. Clin. Invest.* **116**, 1784-1792.

Kasper, P., Martin, A., Lang, S., Kuetting, F., Goeser, T., Demir, M. and Steffen, H.-M. (2021) NAFLD and cardiovascular diseases: a clinical review. *Clin. Res. Cardiol.* **110**, 921-937.

Kawauchi, K., Araki, K., Tobiume, K. and Tanaka, N. (2008) p53 regulates glucose metabolism through an IKK-NF-κB pathway and inhibits cell transformation. *Nat. Cell Biol.* **10**, 611-618.

Kazankov, K., Jørgensen, S. M. D., Thomsen, K. L., Møller, H. J., Vilstrup, H., George, J., Schuppan, D. and Grønbæk, H. (2019) The role of macrophages in nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Nat. Rev. Gastroenterol. Hepatol.* **16**, 145-159.

Kazyken, D., Dame, S. G., Wang, C., Wadley, M. and Fingar, D. C. (2024) Unexpected roles for AMPK in the suppression of autophagy and the reactivation of MTORC1 signaling during prolonged amino acid deprivation. *Autophagy* **20**, 2017-2040.

Keam, S. J. (2024) Resmetirom: first approval. *Drugs* **84**, 729-735.

Khanmohammadi, S. and Kuchay, M. S. (2022) Toll-like receptors and metabolic (dysfunction)-associated fatty liver disease. *Pharmacol. Res.* **185**, 106507.

Kim, C. H. (2023) Complex regulatory effects of gut microbial short-chain fatty acids on immune tolerance and autoimmunity. *Cell. Mol. Immunol.* **20**, 341-350.

Kim, D. H. (2024) Contrasting views on the role of AMPK in autophagy. *BioEssays* **46**, 2300211.

Kim, H. Y., Sakane, S., Eguileor, A., Carvalho Gontijo Weber, R., Lee, W., Liu, X., Lam, K., Ishizuka, K., Rosenthal, S. B., Diggle, K., Brenner, D. A. and Kisseleva, T. (2024) The Origin and Fate of Liver Myofibroblasts. *Cell. Mol. Gastroenterol. Hepatol.* **17**, 93-106.

Kim, J., Kim, Y. C., Fang, C., Russell, R. C., Kim, J. H., Fan, W., Liu, R., Zhong, Q. and Guan, K.-L. (2013) Differential regulation of distinct Vps34 complexes by AMPK in nutrient stress and autophagy. *Cell* **152**, 290-303.

Kim, J., Kundu, M., Viollet, B. and Guan, K.-L. (2011) AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat. Cell Biol.* **13**, 132-141.

Kim, S. Y., Jeong, J.-M., Kim, S. J., Seo, W., Kim, M.-H., Choi, W.-M., Yoo, W., Lee, J.-H., Shim, Y.-R. and Yi, H.-S. (2017) Pro-inflammatory hepatic macrophages generate ROS through NADPH oxidase 2 via endocytosis of monomeric TLR4–MD2 complex. *Nat. Commun.* **8**, 1-15.

Kitto, L. J. and Henderson, N. C. (2021) Hepatic stellate cell regulation of liver regeneration and repair. *Hepatol. Commun.* **5**, 358-370.

Kong, L., Zhang, H., Lu, C., Shi, K., Huang, H., Zheng, Y., Wang, Y., Wang, D., Wang, H. and Huang, W. (2021) AICAR, an AMP-activated protein kinase activator, ameliorates acute pancreatitis-associated liver injury partially through Nrf2-mediated antioxidant effects and inhibition of NLRP3 inflammasome activation. *Front. Pharmacol.* **12**, 724514.

Kopczyńska, J. and Kowalczyk, M. (2024) The potential of short-chain fatty acid epigenetic regulation in chronic low-grade inflammation and obesity. *Front. Immunol.* **15**, 1380476.

Kottakis, F. and Bardeesy, N. (2012) LKB1-AMPK axis revisited. *Cell Res.* **22**, 1617-1620.

Lee, G., You, H. J., Bajaj, J. S., Joo, S. K., Yu, J., Park, S., Kang, H., Park, J. H., Kim, J. H. and Lee, D. H. (2020) Distinct signatures of gut microbiome and metabolites associated with significant fibrosis in non-obese NAFLD. *Nat. Commun.* **11**, 4982.

Lee, M. J., Park, J.-S., Jo, S. B. and Joe, Y. A. (2023) Enhancing anti-cancer therapy with selective autophagy inhibitors by targeting protective autophagy. *Biomol. Ther. (Seoul)* **31**, 1-15.

Li, F., Jiang, M., Ma, M., Chen, X., Zhang, Y., Zhang, Y., Yu, Y., Cui, Y., Chen, J., Zhao, H., Sun, Z. and Dong, D. (2022a) Anthelmintics nitazoxanide protects against experimental hyperlipidemia and hepatic steatosis in hamsters and mice. *Acta Pharm. Sin. B* **12**, 1322-1338.

Li, J., Chen, C., Zhang, W., Bi, J. a., Yang, G. and Li, E. (2021) Salsalate reverses metabolic disorders in a mouse model of non-alcoholic fatty liver disease through AMPK activation and caspase-6 activity inhibition. *Basic Clin. Pharmacol. Toxicol.* **128**, 394-409.

Li, M., Zhang, C.-S., Zong, Y., Feng, J.-W., Ma, T., Hu, M., Lin, Z., Li, X., Xie, C. and Wu, Y. (2019) Transient receptor potential V channels are essential for glucose sensing by aldolase and AMPK. *Cell Metab.* **30**, 508-524.e12.

Li, N.-S., Zou, J.-R., Lin, H., Ke, R., He, X.-L., Xiao, L., Huang, D., Luo, L., Lv, N. and Luo, Z. (2016) LKB1/AMPK inhibits TGF-β1 production and the TGF-β signaling pathway in breast cancer cells. *Tumor Biol.* **37**, 8249-8258.

Li, Q., Tan, J.-X., He, Y., Bai, F., Li, S.-W., Hou, Y.-W., Ji, L.-S., Gao, Y.-T., Zhang, X. and Zhou, Z.-H. (2022b) Atractylenolide III ameliorates non-alcoholic fatty liver disease by activating hepatic adiponectin receptor 1-mediated AMPK pathway. *Int. J. Biol. Sci.* **18**, 1594.

Li, W., Chang, N. and Li, L. (2022c) Heterogeneity and function of kupffer cells in liver injury. *Front. Immunol.* **13**, 940867.

Li, Y., Xu, S., Mihaylova, M. M., Zheng, B., Hou, X., Jiang, B., Park, O., Luo, Z., Lefai, E. and Shyy, J. Y.-J. (2011) AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. *Cell Metab.* **13**, 376-388.

Licheva, M., Raman, B., Kraft, C. and Reggiori, F. (2022) Phosphoregulation of the autophagy machinery by kinases and phosphatases. *Autophagy* **18**, 104-123.

Lin, H., Shi, F., Jiang, S., Wang, Y., Zou, J., Ying, Y., Huang, D., Luo, L., Yan, X. and Luo, Z. (2020) Metformin attenuates trauma-induced heterotopic ossification via inhibition of Bone Morphogenetic Protein signalling. *J. Cell. Mol. Med.* **24**, 14491-14501.

Lin, H., Ying, Y., Wang, Y.-Y., Wang, G., Jiang, S.-S., Huang, D., Luo, L., Chen, Y.-G., Gerstenfeld, L. C. and Luo, Z. (2017) AMPK down-regulates ALK2 via increasing the interaction between Smurf1 and Smad6, leading to inhibition of osteogenic differentiation. Biochim. Biophys. *Acta Mol. Cell Res.* **1864**, 2369-2377.

Loomba, R., Friedman, S. L. and Shulman, G. I. (2021) Mechanisms and disease consequences of nonalcoholic fatty liver disease. *Cell* **184**, 2537-2564.

Lu, J., Shi, J., Li, M., Gui, B., Fu, R., Yao, G., Duan, Z., Lv, Z., Yang, Y. and Chen, Z. (2015) Activation of AMPK by metformin inhibits TGF-β-induced collagen production in mouse renal fibroblasts. *Life Sci.* **127**, 59-65.

Luci, C., Bourinet, M., Leclère, P. S., Anty, R. and Gual, P. (2020) Chronic inflammation in non-alcoholic steatohepatitis: molecular mechanisms and therapeutic strategies. *Front. Endocrinol.* **11**, 597648.

Ma, T., Tian, X., Zhang, B., Li, M., Wang, Y., Yang, C., Wu, J., Wei, X., Qu, Q. and Yu, Y. (2022) Low-dose metformin targets the lysosomal AMPK pathway through PEN2. *Nature* **603**, 159-165.

Maestri, M., Santopaolo, F., Pompili, M., Gasbarrini, A. and Ponziani, F. R. (2023) Gut microbiota modulation in patients with non-alcoholic fatty liver disease: effects of current treatments and future strategies. *Front. Nutr.* **10**, 1110536.

Marshall, R. S. and Vierstra, R. D. (2018) Autophagy: the master of bulk and selective recycling. *Annu. Rev. Plant Biol.* **69**, 173-208.

Marti-Aguado, D., Arnouk, J., Liang, J. X., Lara-Romero, C., Behari, J., Furlan, A., Jimenez-Pastor, A., Ten-Esteve, A., Alfaro-Cervello, C. and Bauza, M. (2024) Development and validation of an image biomarker to identify metabolic dysfunction associated steatohepatitis: MR–MASH score. *Liver Int.* **44**, 202-213.

Matsusue, K., Aibara, D., Hayafuchi, R., Matsuo, K., Takiguchi, S., Gonzalez, F. J. and Yamano, S. (2014) Hepatic PPARγ and LXRα independently regulate lipid accumulation in the livers of genetically obese mice. *FEBS Lett.* **588**, 2277-2281.

Matzinger, M., Fischhuber, K., Pölöske, D., Mechtler, K. and Heiss, E. H. (2020) AMPK leads to phosphorylation of the transcription factor Nrf2, tuning transactivation of selected target genes. *Redox Biol.* **29**, 101393.

McBride, A., Ghilagaber, S., Nikolaev, A. and Hardie, D. G. (2009) The glycogen-binding domain on the AMPK β subunit allows the kinase to act as a glycogen sensor. *Cell Metab.* **9**, 23-34.

Miao, L., Targher, G., Byrne, C. D., Cao, Y.-Y. and Zheng, M.-H. (2024) Current status and future trends of the global burden of MASLD. *Trends Endocrinol. Metab.* **35**, 697-707.

Mishra, R., Cool, B. L., Laderoute, K. R., Foretz, M., Viollet, B. and Simonson, M. S. (2008) AMP-activated protein kinase inhibits transforming growth factor-β-induced Smad3-dependent transcription and myofibroblast transdifferentiation. *J. Biol. Chem.* **283**, 10461-10469.

Mladenić, K., Lenartić, M., Marinović, S., Polić, B. and Wensveen, F. M. (2024) The "Domino effect" in MASLD: the inflammatory cascade of steatohepatitis. *Eur. J. Immunol.* **54**, 2149641.

Musso, G., Gambino, R. and Cassader, M. (2013) Cholesterol metabolism and the pathogenesis of non-alcoholic steatohepatitis. *Prog. Lipid Res.* **52**, 175-191.

Nagai, S., Collins, K., Chau, L. C., Safwan, M., Rizzari, M., Yoshida, A., Abouljoud, M. S. and Moonka, D. (2019) Increased risk of death in first year after liver transplantation among patients with nonalcoholic steatohepatitis vs liver disease of other etiologies. *Clin. Gastroenterol. Hepatol.* **17**, 2759-2768.e5.

Neumann, D. (2018) Is TAK1 a direct upstream kinase of AMPK? *Int. J. Mol. Sci.* **19**, 2412.

Ni, Y., Li, J.-M., Liu, M.-K., Zhang, T.-T., Wang, D.-P., Zhou, W.-H., Hu, L.-Z. and Lv, W.-L. (2017) Pathological process of liver sinusoidal endothelial cells in liver diseases. *World J. Gastroenterol.* **23**, 7666.

Nie, T., Wang, X., Li, A., Shan, A. and Ma, J. (2024) The promotion of fatty acid β-oxidation by hesperidin via activating SIRT1/PGC1α to improve NAFLD induced by a high-fat diet. *Food Funct.* **15**, 372-386.

Oakhill, J. S., Steel, R., Chen, Z.-P., Scott, J. W., Ling, N., Tam, S. and Kemp, B. E. (2011) AMPK is a direct adenylate charge-regulated protein kinase. *Science* **332**, 1433-1435.

Olefsky, J. M. and Glass, C. K. (2010) Macrophages, inflammation, and insulin resistance. *Annu. Rev. Physiol.* **72**, 219-246.

Omidkhoda, N., Mahdiani, S., Hayes, A. W. and Karimi, G. (2023) Natural compounds against nonalcoholic fatty liver disease: a review on the involvement of the LKB1/AMPK signaling pathway. *Phytother. Res.* **37**, 5769-5786.

Ornatowski, W., Lu, Q., Yegambaram, M., Garcia, A. E., Zemskov, E. A., Maltepe, E., Fineman, J. R., Wang, T. and Black, S. M. (2020) Complex interplay between autophagy and oxidative stress in the development of pulmonary disease. *Redox Biol.* **36**, 101679.

Paik, S., Kim, J. K., Silwal, P., Sasakawa, C. and Jo, E.-K. (2021) An update on the regulatory mechanisms of NLRP3 inflammasome activation. *Cell. Mol. Immunol.* **18**, 1141-1160.

Pan, J., Yang, C., Xu, A., Zhang, H., Fan, Y., Zeng, R., Chen, L., Liu, X. and Wang, Y. (2024) Salusin-α alleviates lipid metabolism disorders via regulation of the downstream lipogenesis genes through the LKB1/AMPK pathway. *Int. J. Mol. Med.* **54**, 73.

Pang, Y., Xu, X., Xiang, X., Li, Y., Zhao, Z., Li, J., Gao, S., Liu, Q., Mai, K. and Ai, Q. (2021) High fat activates O-GlcNAcylation and affects AMPK/ACC pathway to regulate lipid metabolism. *Nutrients* **13**, 1740.

Park, I.-H., Um, J.-Y., Hong, S.-M., Cho, J.-S., Lee, S. H., Lee, S. H. and Lee, H.-M. (2014) Metformin reduces TGF-β1–induced extracellular matrix production in nasal polyp–derived fibroblasts. Otolaryngol. *Head Neck Surg.* **150**, 148-153.

Park, J.-M., Jung, C. H., Seo, M., Otto, N. M., Grunwald, D., Kim, K. H., Moriarity, B., Kim, Y.-M., Starker, C. and Nho, R. S. (2016) The ULK1 complex mediates MTORC1 signaling to the autophagy initiation machinery via binding and phosphorylating ATG14. *Autophagy* **12**, 547-564.

Park, J.-M., Lee, D.-H. and Kim, D.-H. (2023) Redefining the role of AMPK in autophagy and the energy stress response. *Nat. Commun.* **14**, 2994.

Parola, M. and Pinzani, M. (2019) Liver fibrosis: pathophysiology, pathogenetic targets and clinical issues. *Mol. Aspects Med.* **65**, 37-55.

Peng, D., Fu, M., Wang, M., Wei, Y. and Wei, X. (2022) Targeting TGF-β signal transduction for fibrosis and cancer therapy. *Mol. Cancer* **21**, 104.

Penugurti, V., Manne, R. K., Bai, L., Kant, R. and Lin, H.-K. (2024) AMPK: the energy sensor at the crossroads of aging and cancer. *Semin. Cancer Biol.* **106-107**, 15-27.

Petersen, M. C. and Shulman, G. I. (2018) Mechanisms of insulin action and insulin resistance. *Physiol. Rev.* **98**, 2133-2223.

Petsouki, E., Cabrera, S. N. S. and Heiss, E. H. (2022) AMPK and NRF2: interactive players in the same team for cellular homeostasis? *Free Radic. Biol. Med.* **190**, 75-93.

Polyzos, S. A., Chrysavgis, L., Vachliotis, I. D., Chartampilas, E. and Cholongitas, E. (2023) Nonalcoholic fatty liver disease and hepatocellular carcinoma: Insights in epidemiology, pathogenesis, imaging, prevention and therapy. In Seminars in Cancer Biology, pp. 20-35. Elsevier.

Powell, E. E., Wong, V. W.-S. and Rinella, M. (2021) Non-alcoholic fatty liver disease. *Lancet.* **397**, 2212-2224.

Prakash, A. V., Park, I.-H., Park, J. W., Bae, J. P., Lee, G. S. and Kang, T. J. (2023) NLRP3 inflammasome as therapeutic targets in inflammatory diseases. *Biomol. Ther. (Seoul)* **31**, 395-401.

Rahman, M. S., Hossain, K. S., Das, S., Kundu, S., Adegoke, E. O., Rahman, M. A., Hannan, M. A., Uddin, M. J. and Pang, M.-G. (2021) Role of insulin in health and disease: an update. *Int. J. Mol. Sci.* **22**, 6403.

Ratziu, V., de Guevara, L., Safadi, R., Poordad, F., Fuster, F., Flores-Figueroa, J., Arrese, M., Fracanzani, A. L., Ben Bashat, D., Lackner, K., Gorfine, T., Kadosh, S., Oren, R., Halperin, M., Hayardeny, L., Loomba, R., Friedman, S. and Sanyal, A. J. (2021) Aramchol in patients with nonalcoholic steatohepatitis: a randomized, double-blind, placebo-controlled phase 2b trial. *Nat Med.* **27**, 1825-1835.

Rehman, K. and Akash, M. S. H. (2016) Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? *J. Biomed. Sci.* **23**, 87.

Riazi, K., Azhari, H., Charette, J. H., Underwood, F. E., King, J. A., Afshar, E. E., Swain, M. G., Congly, S. E., Kaplan, G. G. and Shaheen, A.-A. (2022) The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. *Lancet Gastroenterol. Hepatol.* **7**, 851-861.

Rinella, M. E., Lazarus, J. V., Ratziu, V., Francque, S. M., Sanyal, A. J., Kanwal, F., Romero, D., Abdelmalek, M. F., Anstee, Q. M. and Arab, J. P. (2023) A multisociety Delphi consensus statement on new fatty liver disease nomenclature. *Hepatology* **78**, 1966-1986.

Rius-Pérez, S., Torres-Cuevas, I., Millán, I., Ortega, Á. L. and Pérez, S. (2020) PGC-1α, inflammation, and oxidative stress: an integrative view in metabolism. *Oxid. Med. Cell. Longev.* **2020**, 1452696.

Roach, P. J. (2011) AMPK → uLK1 → autophagy. *Mol. Cell. Biol.* **31**, 3082-3084.

Rodgers, J. T. and Puigserver, P. (2007) Fasting-dependent glucose and lipid metabolic response through hepatic sirtuin 1. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 12861-12866.

Rohm, T. V., Meier, D. T., Olefsky, J. M. and Donath, M. Y. (2022) Inflammation in obesity, diabetes, and related disorders. *Immunity* **55**, 31-55.

Ruderman, N. and Prentki, M. (2004) AMP kinase and malonyl-CoA: targets for therapy of the metabolic syndrome. *Nat. Rev. Drug Discov.* **3**, 340-351.

Ruderman, N. B., Carling, D., Prentki, M. and Cacicedo, J. M. (2013) AMPK, insulin resistance, and the metabolic syndrome. *J. Clin. Invest.* **123**, 2764-2772.

Russell, R. C., Tian, Y., Yuan, H., Park, H. W., Chang, Y.-Y., Kim, J., Kim, H., Neufeld, T. P., Dillin, A. and Guan, K.-L. (2013) ULK1 induces autophagy by phosphorylating Beclin-1 and activating VPS34 lipid kinase. *Nat. Cell Biol.* **15**, 741-750.

Sadria, M. and Layton, A. T. (2021) Interactions among mTORC, AMPK and SIRT: a computational model for cell energy balance and metabolism. *Cell Commun. Signal.* **19**, 57.

Safadi, R., Konikoff, F. M., Mahamid, M., Zelber-Sagi, S., Halpern, M., Gilat, T. and Oren, R. (2014) The fatty acid-bile acid conjugate Ar-

amchol reduces liver fat content in patients with nonalcoholic fatty liver disease. *Clin. Gastroenterol. Hepatol.* **12**, 2085-2091.e1.
Sakurai, Y., Kubota, N., Yamauchi, T. and Kadowaki, T. (2021) Role of insulin resistance in MAFLD. *Int. J. Mol. Sci.* **22**, 4156.
Salminen, A., Hyttinen, J. M. and Kaarniranta, K. (2011) AMP-activated protein kinase inhibits NF-κB signaling and inflammation: impact on healthspan and lifespan. *J. Mol. Med.* **89**, 667-676.
Samsuzzaman, M. and Kim, S. Y. (2023) Anti-fibrotic effects of DL-glyceraldehyde in hepatic stellate cells via activation of ERK-JNK-caspase-3 signaling axis. *Biomol. Ther. (Seoul)* **31**, 425-433.
Samuel, V. T. and Shulman, G. I. (2016) The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. *J. Clin. Invest.* **126**, 12-22.
Samuel, V. T. and Shulman, G. I. (2018) Nonalcoholic fatty liver disease as a nexus of metabolic and hepatic diseases. *Cell Metab.* **27**, 22-41.
Schmitt, D. L., Curtis, S. D., Lyons, A. C., Zhang, J.-f., Chen, M., He, C. Y., Mehta, S., Shaw, R. J. and Zhang, J. (2022) Spatial regulation of AMPK signaling revealed by a sensitive kinase activity reporter. *Nat. Commun.* **13**, 3856.
Schreurs, M., Kuipers, F. and Van Der Leij, F. R. (2010) Regulatory enzymes of mitochondrial β-oxidation as targets for treatment of the metabolic syndrome. *Obes. Rev.* **11**, 380-388.
Schultze, S. M., Hemmings, B. A., Niessen, M. and Tschopp, O. (2012) PI3K/AKT, MAPK and AMPK signalling: protein kinases in glucose homeostasis. *Expert Rev. Mol. Med.* **14**, e1.
Schwabe, R. F., Tabas, I. and Pajvani, U. B. (2020) Mechanisms of Fibrosis Development in Nonalcoholic Steatohepatitis. *Gastroenterology.* **158**, 1913-1928.
Scott, J. W., Ross, F. A., Liu, J. D. and Hardie, D. G. (2007) Regulation of AMP-activated protein kinase by a pseudosubstrate sequence on the γ subunit. *EMBO J.* **26**, 806-815.
Sharma, A., Anand, S. K., Singh, N., Dwarkanath, A., Dwivedi, U. N. and Kakkar, P. (2021) Berbamine induced activation of the SIRT1/LKB1/AMPK signaling axis attenuates the development of hepatic steatosis in high-fat diet-induced NAFLD rats. *Food Funct.* **12**, 892-909.
Shi, Y. and Massagué, J. (2003) Mechanisms of TGF-β signaling from cell membrane to the nucleus. *Cell* **113**, 685-700.
Shi, Y., Su, W., Zhang, L., Shi, C., Zhou, J., Wang, P., Wang, H., Shi, X., Wei, S. and Wang, Q. (2021) TGR5 regulates macrophage inflammation in nonalcoholic steatohepatitis by modulating NLRP3 inflammasome activation. *Front. Immunol.* **11**, 609060.
Simon, T. G., Wilechansky, R. M., Stoyanova, S., Grossman, A., Dichtel, L. E., Lauer, G. M., Miller, K. K., Hoshida, Y., Corey, K. E., Loomba, R., Chung, R. T. and Chan, A. T. (2024) Aspirin for metabolic dysfunction–associated steatotic liver disease without cirrhosis: a randomized clinical trial. *JAMA* **331**, 920-929.
Singh, V. and Ubaid, S. (2020) Role of silent information regulator 1 (SIRT1) in regulating oxidative stress and inflammation. *Inflammation* **43**, 1589-1598.
Sinha, R. A., Singh, B. K. and Yen, P. M. (2017) Reciprocal crosstalk between autophagic and endocrine signaling in metabolic homeostasis. *Endocr. Rev.* **38**, 69-102.
Smith, G. I., Shankaran, M., Yoshino, M., Schweitzer, G. G., Chondronikola, M., Beals, J. W., Okunade, A. L., Patterson, B. W., Nyangau, E. and Field, T. (2020) Insulin resistance drives hepatic de novo lipogenesis in nonalcoholic fatty liver disease. *J. Clin. Invest.* **130**, 1453-1460.
Song, M. J. and Malhi, H. (2019) The unfolded protein response and hepatic lipid metabolism in non alcoholic fatty liver disease. *Pharmacol. Ther.* **203**, 107401.
Song, N., Xu, H., Wu, S., Luo, S., Xu, J., Zhao, Q., Wang, R. and Jiang, X. (2023) Synergistic activation of AMPK by AdipoR1/2 agonist and inhibitor of EDPs–EBP interaction recover NAFLD through enhancing mitochondrial function in mice. *Acta Pharm. Sin. B* **13**, 542-558.
Song, Z., Xiaoli, A. M. and Yang, F. (2018) Regulation and metabolic significance of de novo lipogenesis in adipose tissues. *Nutrients* **10**, 1383.
Stanley, T. L., Fourman, L. T., Zheng, I., McClure, C. M., Feldpausch, M. N., Torriani, M., Corey, K. E., Chung, R. T., Lee, H. and Kleiner, D. E. (2021) Relationship of IGF-1 and IGF-binding proteins to disease severity and glycemia in nonalcoholic fatty liver disease. *J. Clin. Endocrinol. Metab.* **106**, e520-e533.
Steinberg, G. R. and Carling, D. (2019) AMP-activated protein kinase: the current landscape for drug development. *Nat. Rev. Drug Discov.* **18**, 527-551.
Steinberg, G. R. and Hardie, D. G. (2023) New insights into activation and function of the AMPK. *Nat. Rev. Mol. Cell Biol.* **24**, 255-272.
Suchankova, G., Nelson, L. E., Gerhart-Hines, Z., Kelly, M., Gauthier, M. S., Saha, A. K., Ido, Y., Puigserver, P. and Ruderman, N. B. (2009) Concurrent regulation of AMP-activated protein kinase and SIRT1 in mammalian cells. *Biochem. Biophys. Res. Commun.* **378**, 836-841.
Thakur, S., Viswanadhapalli, S., Kopp, J. B., Shi, Q., Barnes, J. L., Block, K., Gorin, Y. and Abboud, H. E. (2015) Activation of AMP-activated protein kinase prevents TGF-β1–induced epithelial-mesenchymal transition and myofibroblast activation. *Am. J. Pathol.* **185**, 2168-2180.
Tian, W., Li, W., Chen, Y., Yan, Z., Huang, X., Zhuang, H., Zhong, W., Chen, Y., Wu, W. and Lin, C. (2015) Phosphorylation of ULK1 by AMPK regulates translocation of ULK1 to mitochondria and mitophagy. *FEBS Lett.* **589**, 1847-1854.
Tian, Y., Feng, H., Han, L., Wu, L., Lv, H., Shen, B., Li, Z., Zhang, Q. and Liu, G. (2018) Magnolol alleviates inflammatory responses and lipid accumulation by AMP-activated protein kinase-dependent peroxisome proliferator-activated receptor α activation. *Front. Immunol.* **9**, 147.
Tilg, H., Adolph, T. E. and Moschen, A. R. (2021) Multiple parallel hits hypothesis in nonalcoholic fatty liver disease: revisited after a decade. *Hepatology* **73**, 833-842.
Tolman, K. G., Fonseca, V., Dalpiaz, A. and Tan, M. H. (2007) Spectrum of liver disease in type 2 diabetes and management of patients with diabetes and liver disease. *Diabetes Care* **30**, 734-743.
Townsend, L. K. and Steinberg, G. R. (2023) AMPK and the endocrine control of metabolism. *Endocr. Rev.* **44**, 910-933.
Tsuchida, T. and Friedman, S. L. (2017) Mechanisms of hepatic stellate cell activation. *Nat. Rev. Gastroenterol. Hepatol.* **14**, 397-411.
Tyczyńska, M., Hunek, G., Szczasny, M., Brachet, A., Januszewski, J., Forma, A., Portincasa, P., Flieger, J. and Baj, J. (2024) Supplementation of micro-and macronutrients—a role of nutritional status in non-alcoholic fatty liver disease. *Int. J. Mol. Sci.* **25**, 4916.
Tzatsos, A. and Tsichlis, P. N. (2007) Energy depletion inhibits phosphatidylinositol 3-kinase/Akt signaling and induces apoptosis via AMP-activated protein kinase-dependent phosphorylation of IRS-1 at Ser-794. *J. Biol. Chem.* **282**, 18069-18082.
Vargas, J. N. S., Hamasaki, M., Kawabata, T., Youle, R. J. and Yoshimori, T. (2023) The mechanisms and roles of selective autophagy in mammals. *Nat. Rev. Mol. Cell Biol.* **24**, 167-185.
Varghese, B., Chianese, U., Capasso, L., Sian, V., Bontempo, P., Conte, M., Benedetti, R., Altucci, L., Carafa, V. and Nebbioso, A. (2023) SIRT1 activation promotes energy homeostasis and reprograms liver cancer metabolism. *J. Transl. Med.* **21**, 627.
Viollet, B., Horman, S., Leclerc, J., Lantier, L., Foretz, M., Billaud, M., Giri, S. and Andreelli, F. (2010) AMPK inhibition in health and disease. *Crit. Rev. Biochem. Mol. Biol.* **45**, 276-295.
Wang, M., Han, Z., Fan, B., Qu, K., Zhang, W., Li, W., Li, J., Li, L., Li, J., Li, H., Wu, S., Wang, D. and Zhu, H. (2024) Discovery of oral AMP-activated protein kinase activators for treating hyperlipidemia. *J. Med. Chem.* **67**, 7870-7890.
Wang, X. and Jia, J. (2023) Magnolol improves Alzheimer's disease-like pathologies and cognitive decline by promoting autophagy through activation of the AMPK/mTOR/ULK1 pathway. *Biomed. Pharmacother.* **161**, 114473.
Wang, Y., Yu, W., Li, S., Guo, D., He, J. and Wang, Y. (2022) Acetyl-CoA carboxylases and diseases. *Front. Oncol.* **12**, 836058.
Wei, J., Zhang, Y., Yu, T.-Y., Sadre-Bazzaz, K., Rudolph, M. J., Amodeo, G. A., Symington, L. S., Walz, T. and Tong, L. (2016) A unified molecular mechanism for the regulation of acetyl-CoA carboxylase by phosphorylation. *Cell Discov.* **2**, 1-12.
Wei, S., Wang, L., Evans, P. C. and Xu, S. (2024) NAFLD and NASH: etiology, targets and emerging therapies. *Drug Discov. Today* **29**, 103910.
White Jr, A. C. (2004) Nitazoxanide: a new broad spectrum antipara-

sitic agent. *Expert Rev. Anti Infect. Ther.* **2**, 43-49.
Woods, A., Dickerson, K., Heath, R., Hong, S.-P., Momcilovic, M., Johnstone, S. R., Carlson, M. and Carling, D. (2005) Ca2+/calmodulin-dependent protein kinase kinase-β acts upstream of AMP-activated protein kinase in mammalian cells. *Cell Metab.* **2**, 21-33.
Woods, A., Johnstone, S. R., Dickerson, K., Leiper, F. C., Fryer, L. G., Neumann, D., Schlattner, U., Wallimann, T., Carlson, M. and Carling, D. (2003) LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. *Curr. Biol.* **13**, 2004-2008.
Wu, J., Puppala, D., Feng, X., Monetti, M., Lapworth, A. L. and Geoghegan, K. F. (2013) Chemoproteomic analysis of intertissue and interspecies isoform diversity of AMP-activated protein kinase (AMPK). *J. Biol. Chem.* **288**, 35904-35912.
Wu, M., Zhang, C., Xie, M., Zhen, Y., Lai, B., Liu, J., Qiao, L., Liu, S. and Shi, D. (2021) Compartmentally scavenging hepatic oxidants through AMPK/SIRT3-PGC1α axis improves mitochondrial biogenesis and glucose catabolism. *Free Radic. Biol. Med.* **168**, 117-128.
Xanthopoulos, A., Starling, R. C., Kitai, T. and Triposkiadis, F. (2019) Heart failure and liver disease: cardiohepatic interactions. *JACC Heart Fail.* **7**, 87-97.
Xiao, B., Heath, R., Saiu, P., Leiper, F. C., Leone, P., Jing, C., Walker, P. A., Haire, L., Eccleston, J. F. and Davis, C. T. (2007) Structural basis for AMP binding to mammalian AMP-activated protein kinase. *Nature* **449**, 496-500.
Xiao, B., Sanders, M. J., Underwood, E., Heath, R., Mayer, F. V., Carmena, D., Jing, C., Walker, P. A., Eccleston, J. F. and Haire, L. F. (2011) Structure of mammalian AMPK and its regulation by ADP. *Nature* **472**, 230-233.
Xiao, H., Ma, X., Feng, W., Fu, Y., Lu, Z., Xu, M., Shen, Q., Zhu, Y. and Zhang, Y. (2010) Metformin attenuates cardiac fibrosis by inhibiting the TGFβ1–Smad3 signalling pathway. *Cardiovasc. Res.* **87**, 504-513.
Xu, G.-X., Wei, S., Yu, C., Zhao, S.-Q., Yang, W.-J., Feng, Y.-H., Pan, C., Yang, K.-X. and Ma, Y. (2023) Activation of Kupffer cells in NAFLD and NASH: mechanisms and therapeutic interventions. *Front. Cell Dev. Biol.* **11**, 1199519.
Xu, X., Poulsen, K. L., Wu, L., Liu, S., Miyata, T., Song, Q., Wei, Q., Zhao, C., Lin, C. and Yang, J. (2022) Targeted therapeutics and novel signaling pathways in non-alcohol-associated fatty liver/steatohepatitis (NAFL/NASH). *Signal Transduct. Target. Ther.* **7**, 287.
Yamauchi, T., Kamon, J., Minokoshi, Y. a., Ito, Y., Waki, H., Uchida, S., Yamashita, S., Noda, M., Kita, S. and Ueki, K. (2002) Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat. Med.* **8**, 1288-1295.
Yamauchi, T., Nio, Y., Maki, T., Kobayashi, M., Takazawa, T., Iwabu, M., Okada-Iwabu, M., Kawamoto, S., Kubota, N., Kubota, T., Ito, Y., Kamon, J., Tsuchida, A., Kumagai, K., Kozono, H., Hada, Y., Ogata, H., Tokuyama, K., Tsunoda, M., Ide, T., Murakami, K., Awazawa, M., Takamoto, I., Froguel, P., Hara, K., Tobe, K., Nagai, R., Ueki, K. and Kadowaki, T. (2007) Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nat. Med.* **13**, 332-339.
Yan, Y., Zhou, X. E., Xu, H. E. and Melcher, K. (2018) Structure and physiological regulation of AMPK. *Int. J. Mol. Sci.* **19**, 3534.
Yang, S., Duan, Z., Zhang, S., Fan, C., Zhu, C., Fu, R., Ma, X. and Fan, D. (2023) Ginsenoside Rh4 improves hepatic lipid metabolism and inflammation in a model of NAFLD by targeting the gut liver axis and modulating the FXR signaling pathway. *Foods* **12**, 2492.
Ying, Y., Ueta, T., Jiang, S., Lin, H., Wang, Y., Vavvas, D., Wen, R., Chen, Y.-G. and Luo, Z. (2017) Metformin inhibits ALK1-mediated angiogenesis via activation of AMPK. *Oncotarget* **8**, 32794.
Yu, X., Feng, M., Guo, J., Wang, H., Yu, J., Zhang, A., Wu, J., Han, Y., Sun, Z., Liao, Y. and Zhao, Q. (2024) MLKL promotes hepatocarcinogenesis through inhibition of AMPK-mediated autophagy. *Cell Death Differ.* **31**, 1085-1098.
Yuan, J., Chen, C., Cui, J., Lu, J., Yan, C., Wei, X., Zhao, X., Li, N., Li, S. and Xue, G. (2019) Fatty liver disease caused by high-alcohol-producing Klebsiella pneumoniae. *Cell Metab.* **30**, 675-688. e677.
Zhang, C.-S., Hawley, S. A., Zong, Y., Li, M., Wang, Z., Gray, A., Ma, T., Cui, J., Feng, J.-W. and Zhu, M. (2017) Fructose-1, 6-bisphosphate and aldolase mediate glucose sensing by AMPK. *Nature* **548**, 112-116.
Zhang, D., Wang, W., Sun, X., Xu, D., Wang, C., Zhang, Q., Wang, H., Luo, W., Chen, Y. and Chen, H. (2016) AMPK regulates autophagy by phosphorylating BECN1 at threonine 388. *Autophagy* **12**, 1447-1459.
Zhang, J., Lv, W., Liu, X., Sun, Z., Zeng, M., Kang, J., Zhang, Q., Liu, F., Ma, S., Su, J., Cao, K. and Liu, J. (2024) Ginsenoside Rh4 prevents endothelial dysfunction as a novel AMPK activator. *Br. J. Pharmacol.* **181**, 3346-3363.
Zhang, J., Wang, E., Zhang, L. and Zhou, B. (2021) PSPH induces cell autophagy and promotes cell proliferation and invasion in the hepatocellular carcinoma cell line Huh7 via the AMPK/mTOR/ULK1 signaling pathway. *Cell Biol. Int.* **45**, 305-319.
Zhang, S., Peng, X., Yang, S., Li, X., Huang, M., Wei, S., Liu, J., He, G., Zheng, H. and Yang, L. (2022) The regulation, function, and role of lipophagy, a form of selective autophagy, in metabolic disorders. *Cell Death Dis.* **13**, 132.
Zhang, Y.-L., Guo, H., Zhang, C.-S., Lin, S.-Y., Yin, Z., Peng, Y., Luo, H., Shi, Y., Lian, G. and Zhang, C. (2013) AMP as a low-energy charge signal autonomously initiates assembly of AXIN-AMPK-LKB1 complex for AMPK activation. *Cell Metab.* **18**, 546-555.
Zhang, Y., Zhang, L., Zhao, Y., He, J., Zhang, Y. and Zhang, X. (2023) PGC-1α inhibits M2 macrophage polarization and alleviates liver fibrosis following hepatic ischemia reperfusion injury. *Cell Death Discov.* **9**, 337.
Zhang, Y. E. (2009) Non-Smad pathways in TGF-β signaling. *Cell Res.* **19**, 128-139.
Zhao, H., Wu, L., Yan, G., Chen, Y., Zhou, M., Wu, Y. and Li, Y. (2021) Inflammation and tumor progression: signaling pathways and targeted intervention. *Signal Transduct. Target. Ther.* **6**, 263.
Zhao, P., Sun, X., Chaggan, C., Liao, Z., In Wong, K., He, F., Singh, S., Loomba, R., Karin, M. and Witztum, J. L. (2020) An AMPK–caspase-6 axis controls liver damage in nonalcoholic steatohepatitis. *Science* **367**, 652-660.
Zhao, Y., Sun, N., Song, X., Zhu, J., Wang, T., Wang, Z., Yu, Y., Ren, J., Chen, H., Zhan, T., Tian, J., Ma, C., Huang, J., Wang, J., Zhang, Y. and Yang, B. (2023) A novel small molecule AdipoR2 agonist ameliorates experimental hepatic steatosis in hamsters and mice. *Free Radic. Biol. Med.* **203**, 69-85.
Zhou, L., Deepa, S. S., Etzler, J. C., Ryu, J., Mao, X., Fang, Q., Liu, D. D., Torres, J. M., Jia, W. and Lechleiter, J. D. (2009) Adiponectin activates AMP-activated protein kinase in muscle cells via APPL1/LKB1-dependent and phospholipase C/Ca2+/Ca2+/calmodulin-dependent protein kinase kinase-dependent pathways. *J. Biol. Chem.* **284**, 22426-22435.
Zhou, Y., Zhong, L., Yu, S., Shen, W., Cai, C. and Yu, H. (2020) Inhibition of stearoyl-coenzyme A desaturase 1 ameliorates hepatic steatosis by inducing AMPK-mediated lipophagy. *Aging (Albany N.Y.)* **12**, 7350.
Zimmermann, K., Baldinger, J., Mayerhofer, B., Atanasov, A. G., Dirsch, V. M. and Heiss, E. H. (2015) Activated AMPK boosts the Nrf2/HO-1 signaling axis—a role for the unfolded protein response. *Free Radic. Biol. Med.* **88**, 417-426.
Zong, Y., Li, H., Liao, P., Chen, L., Pan, Y., Zheng, Y., Zhang, C., Liu, D., Zheng, M. and Gao, J. (2024) Mitochondrial dysfunction: mechanisms and advances in therapy. *Signal Transduct. Target. Ther.* **9**, 124.