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Phosphorylation: new star of pathogenesis and treatment in steatotic liver disease

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Abstract

Steatotic liver disease poses a serious threat to human health and has emerged as one of the most significant burdens of chronic liver disease worldwide. Currently, the research mechanism is not clear, and there is no specific targeted drug for direct treatment. Phosphorylation is widely regarded as the most common type of protein modification, closely linked to steatotic liver disease in previous studies. However, there is no systematic review to clarify the relationship and investigate from the perspective of phosphorylation. Phosphorylation has been found to mainly regulate molecule stability, affect localization, transform molecular function, and cooperate with other protein modifications. Among them, adenosine 5′-monophosphate-activated protein kinase (AMPK), serine/threonine kinase (AKT), and nuclear factor kappa-B (NF-kB) are considered the core mechanisms in steatotic liver disease. As to treatment, lifestyle changes, prescription drugs, and herbal ingredients can alleviate symptoms by influencing phosphorylation. It demonstrates the significant role of phosphorylation as a mechanism occurrence and a therapeutic target in steatotic liver disease, which could be a new star for future exploration.

Keywords Phosphorylation, Steatotic liver disease, NAFLD, Pathogenesis

Introduction

Due to advancements in medicine and changing times, the term nonalcoholic fatty liver disease (NAFLD) is no longer regarded suitable due to its exclusivity and stigma. It has been replaced by metabolic dysfunction-associated steatotic liver disease (MASLD). At 2023 Fidel Consensus Statement of multiple academic groups, it has been suggested to change NAFLD to MASLD [1]. Despite this, the vast majority of patients with NAFLD show consistent progression of MASLD [2, 3]. MASLD and NAFLD remain similar in multiple international cohorts on prevalence and hazard factors [4, 5]. NAFLD, also known as MASLD, is associated with steatotic liver disease (SLD) and is currently estimated to affect roughly one fourth population over the world [6]. SLD involves a spectrum ranging from simple steatosis to steatohepatitis, eventually leading to necroinflammation and accelerated progression of fibrosis, culminating in severe cirrhosis and potentially hepatocellular carcinoma (HCC) [7]. Despite advancements in several clinical trials for SLD, the lack of a comprehensive understanding of its complex pathogenesis and underlying molecular mechanisms has hindered the development of effective therapeutics [8, 9]. Currently, maintaining a healthy lifestyle and achieving weight loss are crucial for prevention and treatment, but in reality, they are not sufficient.

Essentially, proteins are the primary agents of life activities and play a role in regulating diseases. In SLD, various proteins can participate in lipid regulation to influence the development of SLD, such as transmembrane 6 superfamily member 2 (TM6SF2), which is relatively necessary for lipidosis of very-low-density-lipoprotein in the

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Pre-Golgi [10]. and widely involved in NAFLD and other cardiovascular diseases [11]. However, simply researching protein levels seems insufficient for today's needs. By further exploring the protein structure at amino acid sites using high-throughput technology, it may be possible to investigate the deeper and more direct mechanisms of SLD. Phosphorylation as one common modification has been studied wide. It is the process of adding phosphate groups to intermediate metabolites or proteins, serving as a major protein modification mechanism [12].

Although phosphorylation may occur on any molecule, it most commonly occurs in regular cases. Over the past few decades, accumulating evidence has validated an essential relationship between phosphorylation and SLD. Notably, due to the complexity of phosphorylation research in SLD, there is currently no comprehensive systematic review available to provide a further summary. However, phosphorylation is closely associated with the development of SLD. Furthermore, numerous medications can enhance SLD by targeting phosphorylation sites. Therefore, the research on phosphorylation in SLD has been summarized, aiming to provide initial insights for SLD, whether NAFLD or MASLD from the perspective of phosphorylated protein modification.

Roles of phosphorylation for SLD

Phosphorylation is an essential cellular process that involves transferring phosphate groups. It is traditionally regarded as an "on/off switch" that regulates the function of molecules or signaling pathways. In eukaryotes, phosphorylation typically occurs on serine, threonine, and tyrosine residues. In SLD, phosphorylation modifications can be abnormally activated or inhibited by certain triggers, such as nutrition imbalance [13], aging [14], smoking [15], unhealthy diets [16], absent exercise [17], and corresponding metabolic diseases like diabetes [18]. or hypertension [19]. disrupting normal physiological activities and promoting SLD. Although it performs similarly in most diseases, it typically involves three aspects: the protein site modified by phosphorylation, the protein kinase that leads to phosphorylation modification, and the phosphatase that performs dephosphorylation. Subsequently, the normal balance of phosphorylation is disrupted, thereby affecting regular life activities. These effects are preliminarily summarized in the following five aspects.

Regulation

Phosphorylation or dephosphorylation of a site can activate or inhibit the function of downstream molecules or signaling pathways. This can be referred to as the function of regulation. In general, phosphorylation modification can promote signaling pathways, leading to SLD. However, this is not absolute. Based on the literature summarized, it appears that certain molecules have a dual effect, either activating or inhibiting the downstream pathways in SLD. Their harmful or protective effects on SLD have been identified based on experimental evidence. Table 1 summarized the regulations of the most common molecules on signaling pathways/targets via phosphorylation in SLD. On the one hand, these phosphorylated molecules under further sufficient data validation can serve as potential biomarkers for the diagnosis or prognosis of SLD; On the other hand, actively exploring these candidate targets help deepen regulatory mechanisms to better understand and treat SLD. Notably, the AMPK, transforming growth factor kinase 1 (TAK1) and c-Jun N-terminal kinase (JNK) seem to indicate both promotional and inhibitory roles in the downstream targets, which need more validation in future.

Involving molecule stability

The addition of phosphate groups has been suggested to improve the stability of molecules or metabolites. For example, sphingomyelin phosphodiesterase 3 (SMPD3) is modified by a ubiquitin group, which typically leads to its degradation [15]. When phosphorylated by upstream AMPK, the phosphate group on SMDP3 inhibits ubiquitination, ultimately preventing its degradation and enhancing stability. Another example is the enhancement of protein stability in Inositol 1,4,5-trisphosphate receptor type 1 (IP3R1) through palmitic acid-induced phosphorylation at Tyr353. This, in turn, leads to an overload of $Ca2+$, which eventually interferes hepatic cells mitochondrial function in NAFLD [20]. Moreover, this phenomenon is not limited to the molecule that acquires the phosphate group itself, but may also occur in its downstream regulatory targets. For example, in nutrition repletion, the function of AMPK will be inhibited, preventing the addition of the phosphate group to downstream TBC1 domain family member 1 (TBC1D1). This results in the improved stability of downstream peroxisome proliferator-activated receptors (PPAR), thereby promoting the progression of NAFLD [21].

Affecting the localization of molecule in cell

Changes in phosphorylation states can impact the cellular localization of molecules. For example, Wilms' tumor 1-associating protein (WTAP) has been reported to be reduced in the liver cell nucleus under phosphorylation by tumor necrosis factor alpha (TNFα) in nonalcoholic steatohepatitis (NASH) condition [22]. In addition, the phosphorylated form of fork head box protein (FOX) can be translocated from the nucleus to the cytoplasm under certain conditions. This translocation leads to decreased expression of downstream targeted genes due to reduced

Table 1 Regulations of different molecules on signaling pathways/targets via phosphorylation

Molecule	Regulation	Signaling pathways/Targets	Phone type	Affection of SLD
p-AMPK [21, 26, 28, 31, 34, 37, 117–119]	Promotion	p-TBC1D1/p-ACC/p-SREBP/p-FAS	Protection from lipogenesis	Protective factor
p-JNK [42, 124, 130–137]		TGFβ1/IL-1β/TNFα/ATF2	Inflammatory reaction/fat deposition/oxidative stress/autophagy/apoptosis	Risk factor
p-P62 [138]		Protein inclusions	ROS accumulation/fibrosis	Risk factor
p-P38 [23, 25, 60–62, 133]		TGF/TNF	Lipid accumulation/glucose metabolism disorder/inflammation	Risk factor
p-ERK [65, 134, 139, 140]		NA	Inflammatory reaction/fibrosis/fat deposition	Risk factor
p-NF-kB [32, 63, 65, 124, 141–143]		NA	inflammatory reaction/oxidative stress	Risk factor
p-P65 [32, 131, 138]		TGFβ1/IL-1β/TNFα	Inflammatory reaction/fat deposition	Risk factor
p-SRSF6 [144]		Alternative splicing to form normally	Healthy mitochondria	Protective factor
p-FAK [65]		p-ERK/p-NF-kB	inflammatory reaction/fat deposition	Risk factor
p-EGFR [145]		NA	Lipid metabolism/inflammation/fibrosis	Risk factor
p-ASK1 [42, 130, 137, 146]		p-JNK/p-P38	Inflammatory reaction/fat deposition	Risk factor
p-LARP1 [147]		NAFLD transforming to HCC	Metastasis/invasion/reproduction	Risk factor
p-IRS [39, 57, 124]		p-AKT/PEPCK	Insulin resistance/glucose metabolism disorders	Risk factor
p-PKM2 [142]		Macrophage phenotype transformation	Macrophage phenotype M1/inflammation	Risk factor
p-PKC [30, 39]		SREBP-1C/ACC/CD36/FASN	Insulin resistance/triglyceride synthesis/fatty acid uptake	Risk factor
p-ErbB [148, 149]		PI3K/p-AKT	Negative regulation of de novo adipogenesis	Protective factor
p-LKB1 [37]		p-AMPK/p-SREBP	Protection from endoplasmic reticulum stress/adipogenesis	Protective factor
p-CaMKK2 [26]		p-AMPK/p-SREBP/p-ACC	Increased FFA oxidation/decreased lipid synthesis	Protective factor
p-MLKL [150]		p-RIPK3/STAT3/TNFα	Necrosis/apoptosis/carcinogenicity	Risk factor
p-TAK1 [25, 60–64]		p-NF-kB/p-JNK/p-P38	Lipid accumulation/inflammation	Risk factor
p-ATGL [151]		CGL-58	Protection from lipogenesis/β-oxidation	Protective factor
p-PKA [46]		p-JDJM3/PPARa	Autophagy for liver normal activities	Protective factor
p-IP3R1 [20]		scr pathway	Mitochondrial Ca2 + overload/dysfunction	Risk factor
p-LXR [30, 45]		Acetylation of H3K27	Reduces progression to inflammation and fibrosis	Protective factor
p-HSF1 [114]		PGC-1a	Protection from steatosis/inflammation/fibrosis	Protective factor
p-caspase6 [152]	Inhibition	Pyrolysis of BID to produce cytochrome C	Protection from hepatocyte death	Protective factor
p-AMPK [153]		p-IKK/p-NF-kB	Protection from Inflammation/metabolic disorder	Protective factor
p-JNK [40, 136]		NRF2/PPAR/p-IRS	Oxidative stress injury/lipid transport/insulin resistance	Risk factor
p-PP2A/p-SP1 [57]		SREBP-1C	Protection from lipogenesis	Protective factor
p-FOX [23, 37, 124, 154]		FOX entering the nucleus	NA	NA
p-TAK1 [89, 93]		p-AMPK	Lipid accumulation/inflammation	Risk factor
p-Pacer [44]		HOPS	Maintain normal autophagy	Protective factor

NA means not available

transcriptional activity in the nucleus, thereby exacerbating NAFLD [23, 24].

Transforming molecular function

Phosphorylation modification often serves as a marker for transforming the function of a molecule. Acetyl-CoA carboxylase (ACC) catalyzes the transformation of acetyl-CoA to malonyl-CoA. As a substrate, malonyl-CoA can improve fatty acid oxidation by allosterically inhibiting carnitine O-palmitoyl transferase 1 (CPT1) [25]. Therefore, acetyl-CoA carboxylase (ACC) is essential for regulating glycolipid metabolism and the tricarboxylic acid cycle to maintain normal metabolic activity. Numerous studies have shown that an increase in ACC content in hepatocytes may induce NAFLD and NASH [26–30]. which further enhances the possibility of conversion to HCC [31]. However, the phosphorylation of ACC can reverse its original harmful effects and subsequently improve NAFLD and NASH [26, 31–34]. Similar findings can be seen for other molecules,such as sterol regulatory element-binding protein (SREBP) [26, 28, 30, 32, 35–37]. and eukaryotic initiation factor 2B (eIF2B) [38, 39]. which regulate in lipid metabolism and endoplasmic reticulum stress, respectively. Phosphorylated and non-phosphorylated forms of the same molecule can be viewed as a regulatory switch governing their respective enzymatic activities, with one state contributing to disease pathology while the other state may mitigate it.

Additionally, interplay among signaling pathways enables phosphate groups to modulate the functional interconversion between different pathways and molecules. Silybin is commonly utilized in NASH, where the activated JNK via phosphorylation is involved in inflammation. Silybin has the capacity to transfer the phosphate group from JNK to Insulin receptor substrate 1 (IRS1), and the subsequently, IRS1 bearing phosphate group can counteract insulin resistance to ameliorate NASH [40]. In the presence of Silybin, the phosphorylation of IRS1 can modulate its activity, leading to potential amelioration of NAFLD.

Cooperating with other protein modification

Phosphorylation represents just one facet of protein modification, frequently triggering alterations in conjunction with other groups, thereby fostering interplay between them. Ubiquitination assumes a critical function in protein degradation and governs numerous fundamental processes, including cell division, fate determination, and migration, often exhibiting correlation with phosphorylation [41]. For instance, the attachment of the phosphate group has been documented to alter the transcription of apoptosis signal-regulating kinase 1 (ASK1), recruiting ubiquitination at its 3' end, thereby activating ASK1 to facilitate the progression of NAFLD [42]. Acetylation is widely acknowledged as a common mechanism for regulating molecular transcription, primarily involved in the maintenance of cellular energy balance, regulation of gene expression, and modulation of metabolic pathways [43]. In instances of inadequate nutrition, the phosphorylated histone acetyltransferase Tip60 facilitates the acetylation process, leading to the disruption of autophagy in NAFLD [44]. Furthermore, the process of phosphorylating the oxysterol receptor α (LXRα) at the S196A site has been found to regulate hepatic chromatin acetylation, thereby decreasing the likelihood of developing hepatic inflammation and fibrosis [45]. Phosphorylation is not only associated with ubiquitination and acetylation, but also has been linked to methylation [46]. and glycosylation [30]. The process of protein modification involves intricate interactions between different types of modifications. To fully comprehend the pathological mechanism of SLD, it is essential to examine the interplay among diverse protein modifications in a comprehensive manner.

Signaling pathways for regulating phosphorylation in SLD

Published research on phosphorylation in SLD has primarily concentrated on the phosphorylated AMPK, AKT, and NF-kB, as depicted in Fig. 1. Altered levels of molecular phosphorylation have been linked to various downstream effects, including adipogenesis, steatosis, inflammatory responses, oxidative stress, fibrosis, insulin resistance, autophagy, and mitochondrial dysfunction [7–9]. While these three pathways have been extensively investigated, they are associated with different functions for SLD. AMPK is primarily involved in regulating abnormal fat metabolism in SLD, AKT is mainly associated with insulin resistance and abnormal glucose metabolism, and NF-kB is closely linked to inflammatory responses and immune abnormalities. The phosphorylation modifications mediated by these pathways may ultimately interact to contribute to SLD, and will be further discussed in the subsequent sections.

AMPK

The AMPK pathway is of great value in detecting energy status in eukaryotic cells, initiating energy insufficiency, and thus contributing to the process of cellular metabolism and energy transformation [47]. In Homo sapiens, AMPK predominantly occurs as heterotrimers, comprising one catalytic subunit α and another two regulatory subunits β and γ [48]. Additionally, subunitα exhibits catalytic activity, featuring an activation loop motif in close proximity to its ATP binding site. Typically, kinase domains are rendered active solely when

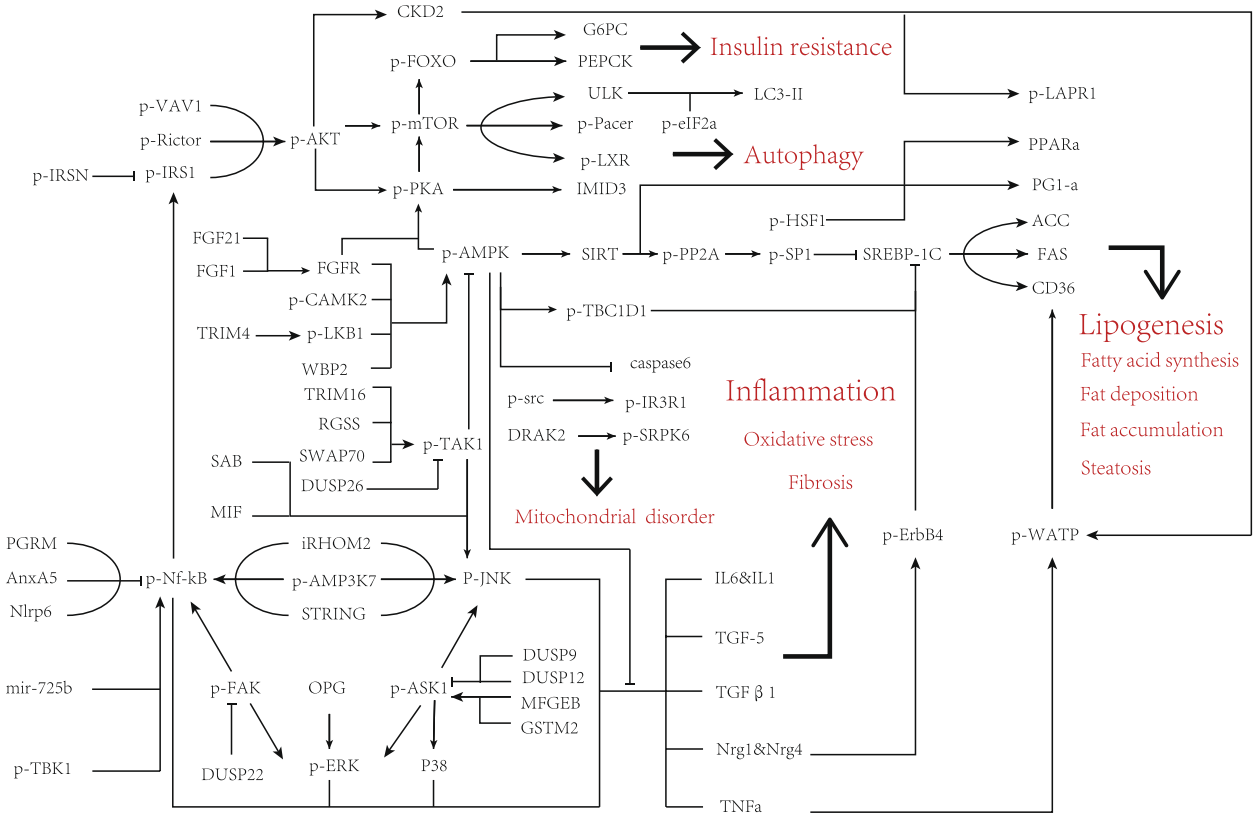

Fig. 1 Core signaling pathways of phosphorylation regulation in SLD

the conserved threonine residues within the activation loop are phosphorylated, serving as a common indicator of AMPK activation [49]. Another area abundant in serine and threonine, known as the ST loop, exists. Certain residues within the ST loop have the potential to undergo phosphorylation under particular conditions, leading to their binding to the kinase domain and creating steric hindrance at the Thr172 site. This, in turn, hinders the activation of AMPK [50].

The association between AMPK and SLD is intimate. In response to external stimuli, phosphorylation of the Thr172 site on subunitα can be induced, leading to enhanced de novo lipogenesis and mitigation of SLD. Triptolide [51]. ginsenoside Rg1 [52]. green tea polyphenols [53]. alogliptin [54]. and aerobic exercise [17]. have currently been reported that they can participate in regulating the phosphorylation of AMPK at Thr172 site, effectively improving SLD. Furthermore, certain kinases, including liver kinase B1 (LKB1), TAK1, and the tripartite motif-containing protein (TRIM) family, as well as the fibroblast growth factor (FGF) family, have the capacity to modulate the phosphorylation of AMPK at the Thr172 site. This thus leads to the regulation in downstream signaling molecules, such as ACC and SREBP, primarily involving in de novo lipid synthesis and abnormal fatty accumulation. Currently, there is a dearth of research on the association between AMPK regulatory subunits and SLD. Further investigation is warranted to ascertain whether the regulatory subunits are implicated in the stability of AMPK phosphorylation conformation.

AKT

Phosphatidylinositol 3-kinase 3 (PI3K) is a dimer consisting of one subunit known as p85 and one catalytic subunit referred to as p110. Its primary function involves modifying the protein structure of AKT, thereby activating or inhibiting downstream substrates through phosphorylation. This process plays a crucial role in regulating cellular life processes, such as proliferation, differentiation, apoptosis, migration and others.

Upon receiving phosphorylation groups from activated PI3K, AKT typically facilitates the regulation of downstream signaling pathways. For insulin resistance in skeletal muscle, AKT has been documented to primarily exert its effects on the mammalian target of rapamycin (mTOR) [27, 55]. and FOX families [23, 24]. to promote insulin resistance; Simultaneously, the excessive release of insulin molecules return to active the phosphorylation

of IRS, which subsequently triggers the phosphorylation of AKT [39, 56, 57]. creating a self-perpetuating cycle. Furthermore, the phosphorylation of AKT can induce irregularities in cell cycle proteins, thereby contributing to cell apoptosis and influencing SLD [58]. In contrast to AMPK, which predominantly controls the synthesis of lipids and breakdown of molecules, the activation of the phosphorylated AKT are primarily associated with insulin resistance and apoptosis in SLD.

NF-kB

NF-kB proteins typically form heterodimeric complexes with p65 and p50, which are rendered inactive in the cytoplasm due to their association with the NF-kB inhibitor epsilon (IkB). Upon activation of upstream signaling factors, IkB is phosphorylated by IkB kinase, leading to its dissociation from the trimer [59]. Consequently, NF-kB is able to expose its nuclear localization sequence (NLS), facilitating its rapid translocation from the cytoplasm to the nucleus binding with specific DNA sequences, thereby promoting the expression of downstream molecule. Literature suggests that the NF-kB primarily causes the activation of inflammation and immune dysregulation in SLD.

The identification of the phosphorylation of TAK1 [25, 60–64]. and focal adhesion kinase (FAK) [65]. has been documented to enhance the function of a range of inflammatory cytokines, such as TNFα, transforming growth factor β (TGFβ), and interleukin family, leading to the activation of NF-kB, inducing inflammatory reactions and immune irregularities. Furthermore, in research investigating the use of metformin to mitigate SLD [66, 67]. it has been attributed to the ability of metformin to stimulate the phosphorylation of AMPK, thereby reducing subunit p65 and suppressing the NF-kB inflammatory response.

Therapeutic progress targeted in phosphorylation of SLD

Owing to the absence of specific drugs for SLD, current mainstream treatment approaches primarily involve lifestyle modifications and weight reduction. Nevertheless, these interventions may not yield favorable outcomes for all individuals [68, 69]. Phosphorylation is a significant factor in the development of SLD, suggesting that therapies directed at phosphorylation processes could have a substantial impact on alleviating the condition. Consequently, this outlines the predominant treatment strategies and underlying fundamental mechanisms related to the phosphorylation of SLD.

Lifestyle intervention

The primary approach for managing SLD involves lifestyle intervention, which encompasses dietary modifications and physical activity. These interventions have been shown to impact the phosphorylation of SLD patients, indicating their potential as therapeutic targets. Further details are provided in Table 2.

Dietary modifications have the potential to regulate phosphorylation levels in NAFLD. Adjusting dietary composition and habits can yield significant effects in treatment or prevention of NAFLD, with the potential mechanisms primarily associated with AMPK phosphorylation. For instance, the consumption of beans [16]. and tomatoes [70]. has been shown to notably ameliorate NAFLD by reducing body weight and inflammatory responses. Additionally, a blend of lard and soybean oil is recognized for its ability to lower cholesterol levels and shield the liver from inflammation [71]. Furthermore, the consumption of Ishige okamurae, an edible seaweed, may also offer beneficial support in the treatment and prevention of NAFLD [72]. Moreover, supplementing with a specific amount of carbon during pregnancy has been associated to reduced occurrence of NAFLD in offspring [73]. In terms of dietary habits, fasting is a crucial measure for alleviating and preventing NAFLD [46]. More specifically, alternate-day fasting is considered to hold significant value in enhancing the cognitive function of NAFLD patients by reducing oxidative stress and mitigating microglial over-activation in the central nervous system [74].

Physical activity potentially reduce fatty accumulation and inflammation in the liver, making it a viable strategy for the treatment of NAFLD and NASH [69]. Additionally, exercise has been shown to ameliorate metabolic abnormalities such as insulin resistance and hypertriglyceridemia to some extent [75, 76]. Evidence from liver biopsy confirms that exercise can mitigate or improve hepatitis in NASH patients [77]. The primary mechanism through which exercise exerts these benefits is by increasing phosphorylated AMPK, thereby inhibiting genes associated with metabolic disorders and fatty accumulation.

Prescriptions

While there are no authorized pharmaceutical drugs for NAFLD and NASH, various prescription medications have shown promising results in clinical settings, particularly those targeting phosphorylation regulation. Table 3 provides an overview of the advancements in utilizing phosphorylation-regulating prescriptions for the management in NAFLD and NASH.

The current treatment for NAFLD with pharmaceutical interventions primarily focuses on hypoglycemic medications, with metformin and sodium-dependent glucose transporters 2 inhibitors (SGLT-2i) being the most studied. Metformin is frequently utilized in clinical practice

Table 2 Lifestyle interventions in regulation of phosphorylation in SLD

Category	Name	Treatment effect	Phosphorylation action site	Potential mechanism
Dietary content	Tomatoes [70]	Potential treatments against NAFLD	p-AMPK	Ameliorating obesity and hepatic steatosis by regulating lipogenesis via the SIRT1/AMPK pathway
	Beans [16]	Ameliorating obesity and significantly reducing steatosis	p-AMPK	Resulting in inhibition of the downstream SREBP-1c/FAS pathway and an increase β-oxidation to alleviate inflammatory responses via p-AMPK
	Probiotics [35]	Reducing weight, improve glucose tolerance, hyperinsulinemia	p-AKT	Reducing the activation of genes by inhibiting p-AKT
	A lard and soybean oil mixture [71]	Lowering cholesterol and protecting liver	p-AMPK	Stimulation of p-AMPK to downregulate TNF6 to inhibit inflammatory response
	Edible seaweed (Ishige okamurae) [72]	Reducing lip toxicity and triglyceride accumulation	p-AMPK	Stimulating SIRT by up-regulating p-AMPK, thus inhibiting the expression of SREBP, FAS, to alleviate inflammation and adipogenesis
	Carbon Supplement during pregnancy [73]	Reducing the risk of NAFLD in offspring	p-AMPK	Promoting p-AMPK to reduce the expression of related risk genes
Dietary habits	Fasting [46]	Ameliorating obesity and insulin resistance	p-PKA/p-JMJD3	The phosphorylation of PKA and JMJD3 induced by FGF21 leading to the demethylation of H3K27 histone, thus promoting liver autophagy
	Alternate-day fasting [74]	Alleviating obesity and insulin resistance, improving cognition	p-AMPK/p-mTOR	Activating AMPK/ULK1 transduction while inhibiting the phosphorylation of mTOR to reduce oxidative stress and microglial over-activation in the central nervous system
Exercise	Aerobic training (60% of maximum velocity) [75]	Reducing weight, insulin resistance and plasma fatty acid concentration	p-AMPK	Increasing the level of PPARa through p-AMPK to promote fat oxidation
	Voluntary exercise [76]	Improving metabolism and protecting liver	p-AMPK	Increasing p-AMPKα activation with beneficial effects on hepatic and steatosis
	Aerobic training (40–55% of VO2max) [77]	Improve NASH with biopsy-proven	p-AMPK/p-mTOR	modulating the AMPK/mTORC1 pathway in patients with NASH

Table 3 Prescriptions in regulation of phosphorylation in SLD

Category	Name	Treatment effect	Phosphorylation action site	Potential mechanism
Prescription	Metformin (+genistein) [66]	Decreasing body and liver weight/fasting blood glucose/liver triglyceride level	p-GSK-3β/p-AMPK/p-NF-kB	Switching macrophage into M2 phenotype, decreasing macrophage infiltration, reducing pro-inflammatory cytokines via p-GSK3/p-AMPK/p-NF-kB
	Metformin (+chlorogenic acid) [67]	Decreasing fasting blood glucose /hepatic triglyceride level/improving glucose intolerance	p-GSK-3β/p-AMPK	Resulting in the polarization of macrophages to the M2 phenotype, reducing pro-inflammatory cytokines and decreasing protein level of NF-kB via p-AMPK
	Empagliflozin [84]	Potential treatments against NAFLD	p-AMPK	Decreasing the expression of ER transactivating autophagy via increasing p-AMPK, and reducing apoptosis
	Dapagliflozin [27]	Anti-NAFLD/decreasing lipogenic enzyme	p-ACC1/p-mTOR/p-AMPK	Reducing hepatic lipid accumulation via promoting p-ACC1 and inducing autophagy via the AMPK-mTOR pathway
	Silybin [40]	Anti-NAFLD efficacy by antioxidant/anti-inflammatory	p-JNK	Decreasing hepatic injury, lipid metabolism and oxidative stress by CFLAR-JNK pathway
	Ursodeoxycholic Acid [86]	Anti-NAFLD efficacy by antioxidant/anti-inflammatory/preventing mitochondrial dysfunction	p-NF-kB/p-STAT3	Increasing hepatic energy expenditure, mitochondria biogenesis, and incorporation of bile acid metabolism by downregulating p-NF-kB and p-STAT3
	Aspirin [87]	Normalize NAFLD and atherosclerosis	p-AMPK	Inhibiting lipid biosynthesis and inflammation and elevating catabolic metabolism via activation of the PPARδ-AMPK-PGC-1α pathway
	Fenticonazole nitrate [88]	Anti-diabetic and anti-NAFLD efficacies	AKT Ser473/PPARγ Ser273	Activating Adiponectin and GLUT4 by promoting the AKT at Ser473 site and blocking the PPARγ at Ser273 site via phosphorylation

for patients with NAFLD and comorbid obesity and abnormal glucose metabolism due to its favorable effects on weight reduction and blood sugar levels [43, 78]. Its mechanism of action in NAFLD involves phosphorylation regulation targeting AMPK and glycogen synthase kinase 3β (GSK-3β), leading to macrophage polarization, reduction in inflammatory cytokines, and improvement in glycolipid metabolism and weight reduction [66, 67]. Additionally, metformin can ameliorate insulin resistance by regulating the phosphorylated ACC family, thereby mitigating NAFLD and NASH [79]. SGLT-2 inhibitors, a novel class of hypoglycemic drugs, function by reducing sugar absorption in the kidneys and increasing urine sugar excretion [80]. thereby improving cardiovascular and cerebrovascular health, promoting weight loss, and normalizing metabolism, especially in NAFLD patients with diabetes [81, 82]. The phosphorylation regulation of SGLT2i primarily involves AMPK, particularly in the case of Empagliflozin [83, 84]. and Dapagliflozin [27]. These drugs have been reported to enhance downstream targeted molecules by promoting their phosphorylation, thereby reducing fatty accumulation and inhibiting the release of inflammatory cytokines through pathways such as mTOR or NF-kB. However, there is vague on the regulation of glucagon-like peptide (GLP) receptor agonists on the phosphorylation in SLD, likely due to challenges related to dosing inconvenience, heterogeneity, and diversity of types.

Additionally, medications with hepatoprotective properties such as silybin and ursodeoxycholic acid (UDCA) are integral in the regulation of phosphorylation in NAFLD and are commonly utilized in the treatment of NASH [28, 40, 85, 86]. Silybin, a widely used hepatoprotective drug, demonstrates efficacy in treating elevated levels of glutamic-pyruvic transaminase or glutamic oxaloacetic transaminase. Studies have indicated that silybin can ameliorate NAFLD by modulating caspase 8 and fatty acid synthase-associated protein, thereby improving insulin resistance, mitigating inflammation through inhibition of JNK phosphorylation [37, 40]. UDCA exhibits anti-inflammatory and antioxidant properties, effectively improving mitochondrial dysfunction, particularly under obesity-related conditions, and is suitable for patients with biliary obstruction. Clinical trials have shown that UDCA enhances energy expenditure in hepatic cells, promotes mitochondrial biogenesis, and improves bile acid metabolism by inhibiting NF-kB and signal transducer and activator of transcription 3 (STAT3) phosphorylation, rendering it an effective treatment for NAFLD [85, 86].

Furthermore, aspirin, a typical anti-inflammatory drug, possesses antipyretic, analgesic, and anti-rheumatic properties that have been found to alleviate NAFLD by modulating the AMPK phosphorylation. Aspirin appears to mitigate NAFLD by decreasing lipid biosynthesis and inflammation, thereby promoting catabolic metabolism through the activation of PPARδ and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) [87]. In addition, feniconazole nitrate has been identified as having potential in regulating phosphorylation changes with NAFLD and has been reported to alleviate the condition by activating facilitated glucose transporter member 4 (GLUT4) via the promotion of AKT phosphorylation at the Ser473 site and by blocking PPARγ phosphorylation at the Ser273 site mediated by cyclin-dependent kinases 5 (CDK5), thereby eventually decreasing the expression of adipogenic genes such as ACC. [88]. Although aspirin and feniconazole nitrate are not common in the treatment of NAFLD, their pharmacological mechanisms involve the PPAR pathway, showing crucial function in regulating inflammation, insulin resistance, abnormal fat metabolism and others.

Traditional Chinese medicine

In recent times, Chinese herbal medicine has demonstrated distinctive efficacy in the management of chronic ailments, particularly in the context of SLD. The enigmatic therapeutic properties and interplay of traditional Chinese medicine are increasingly recognized for their significance. Current investigations into traditional Chinese medicine encompass the study of Chinese medicine monomers, Chinese medicine prescriptions, and the active constituents of Chinese medicine, revealing close associations between their efficacy and the modulation of phosphorylation.

In the context of regulating phosphorylation, over 20 traditional Chinese medicines or active components of traditional Chinese medicine have been considered in treating NAFLD or NASH, including: breviscapine [89], anthocyanin [90], coffeeberry [91], cordycepin [92], salidroside [93], resveratrol [36, 94], triptolide [33], berberine [95, 96], morin [97], corosolic acid [98, 99], ginsenoside [100, 101], vine tea polyphenol [102], quercetin [103], aurantio-obtusin [104], patchouli alcohol [105], zingerone [106], scopoletin/umbelliferone [107], astragalus mongholicus polysaccharides [108], lycopus lucidus Turcz. ex Bent [109], gentiana scabra [110], artemisia capillaris [111], mogrosides [112] and Fufang Zhenzhu Tiaozhi formula [113]. The phosphorylation sites and associated regulatory mechanisms of these substances are summarized in Table 4.

The research methodologies and potential regulatory mechanisms of traditional Chinese medicine through phosphorylation appear to align with traditional Chinese medicine theories to some extent. For instance, Coptis chinensis, containing berberine, demonstrates efficacy

Table 4 Traditional Chinese medicines in regulation of phosphorylation in SLD

Category	Name	Treatment effect	Phosphorylation action site	Potential mechanism
Traditional Chinese medicine	Breviscapine [89]	Reducing lipid accumulation/inflammation/liver injury/fibrosis	p-TAK1	Linking the anti-NASH effects of breviscapine was inhibition of p-TAK1 and the subsequent mitogen-activated protein kinase signaling cascade
	Cordycepin [92]	Attenuating aminotransferases and lipid accumulation	p-AMPK	Against hepatic steatosis, inflammation, liver injury, and fibrosis in mice under metabolic stress through activation of the AMPK signaling pathway
	Salidroside [93]	Regulating glucose metabolism dysregulation/lipid accumulation/fibrosis	p-AMPK	alleviated lipid accumulation and inflammatory response in primary hepatocytes via promoting AMPK signaling pathway activation
	Resveratrol [36, 94]	Improving liver histology and reversing serum biochemical abnormalities	p-FOXO3a/p-JNK	Improving insulin resistance, hepatic steatosis, oxidative stress and inflammation, through SIRT1-mediated FOXO3a phosphorylation and NF-kB deacetylation; suppressing oxidative stress by inhibition of p-JNK
	Anthocyanin [90]	Reducing liver fat deposition and triglyceride formation to alleviate NAFLD	p-AMPK/p-ACC	Increasing p-AMPK and p-ACC to reduce SREBP-1c, FAS, PPARγ to relieve inflammation and fat accumulation
	Coffeeberry [91]	Reducing liver fat deposition and inflammation in NAFLD	p-mTOR	Protecting the liver by reducing oxidative stress, activating the CaMKII/CREB/BDNF pathway and improving autophagic and apoptotic
	Triptolide [33]	Revealing a reduction in liver enzymes and bilirubin	p-AMPK	Activating p-AMPK and further led to increasing p-ACC1 to ameliorate hepatic lipogenesis, fatty acid oxidation, and fibrosis of NAFLD
	Oxyberberine /berberine [95, 96]	Attenuating the clinical manifestations of NAFLD	p-IRS-1/p-AMPK	Inhibiting aberrant p-IRS-1 and upregulating PI3K, p-AKT/AKT and p-GSK-3β/GSK-3β to improve hepatic insulin signal transduction, and activating p-AMPK to block inflammation and fibrosis
	Morin [97]	Against hyperlipidemia and steatosis	p-AMPK/p-ACC/p-AKT	Upregulating PPARα and decreasing SREBP-1c, both of which are dependent upon p-ACC, p-AMPK and p-AKT, while suppressing NF-kB and MAPK
	Corosolic acid [98, 99]	Reducing fat accumulation and transaminase serum cholesterol and triglyceride	p-AMPK/p-JNK	Increasing p-AMPK to inhibit SREBP-1c to reduce fat deposition, upregulating p-IkB to reduce NF-kB and p-JNK to block inflammatory reaction and improve insulin resistance
	Ginsenoside [100, 101]	Reducing lipid deposition in liver	p-AMPK	modulating the expression of factors correlated with lipid synthesis and metabolism via activating the p-LKB1 and p-AMPK
	Vine tea polyphenol [102]	Balancing fatty acid oxidation/fat production/liver oxidative stress	p-AMPK	Activating p-AMPK α and subsequently promote PPARα, CPT1A and cytochrome P450 to enhance fatty acid oxidation to relieve NAFLD

Table 4 (continued)

Category	Name	Treatment effect	Phosphorylation action site	Potential mechanism
	Quercetin [103]	Regulating fat production	p-AMPK	Direct anti-lipogenic effect via inhibiting DNL pathway by p-AMPK
	Aurantio-obtusin [104]	Improving adiposity/insulin resistance	p-AMPK	Promoting autophagy and degradation of lipid droplets via p-AMPK, subsequently activating PPAR α and reducing the expression of genes involved in lipid biosynthesis to trigger TFEB to promote SLD
	Patchouli alcohol [105]	Improve insulin resistance/fat deposition	p-AMPK	Increased p-AMPK and SIRT1 to ameliorate inflammation, thereby attenuating skeletal muscle insulin resistance and hepatic steatosis
	Zingerone [106]	Relieving hyperglycemia/hyperlipidemia	p-AMPK	Preventing hepatic deposition, steatosis, and oxidative damage via p-AMPK/Nrf2 axis and concomitant suppression of SREBP1, SREBp2, and NF-kB p65
	Scopoletin/umbelliferone [107]	Attenuating the clinical manifestations of NAFLD	p-JNK	Decreased ER stress and cell death by intermediating p-JNK as well as ROS production
	Astragalus mongholicus polysaccharides [108]	Improving glycolipid metabolism	p-AMPK/p-NF-kB	Reducing fat accumulation related to p-AMPK and PPARα via the decrease of SREBP-1; downregulating TLR4 and p-NF-kB to block inflammation
	Lycopus lucidus Turcz. ex Benth [109]	Decreasing body weight/liver weight/serum ALT, TC, LDL	p-AMPK	Expression of sterol-regulatory element-binding protein 1 decreasing while that of p-AMPK and PPARα increasing
	Gentiana scabra [110]	Anti-inflammation/anti-oxidation/anti-fibrosis	p-TAK1/p-NF-kB	Inhibiting p-TBK1 to block p-NF-kB to block inflammation and macrophage dysfunction
	Artemisia capillaris [111]	Reducing fatty acid synthesis/TG	p-PI3K/p-AMPK	Promoting p- AKT and p-AMPK to inhibit SREBP-1c reducing lipogenesis and lipid accumulation
	Mogrosides [112]	Reducing body weight/liver fat deposition	p-AMPK	Upregulating p-AMPK and SQSTM1 to inhibit reactive oxygen species production and lipid accumulation
	Fufang Zhenzhu Tiaozhi formula [113]	Having an influence on hepatic steatosis and fibrosis in T2DM and coronary heart disease with NASH	p-AMPK	Upregulating the expression levels of p-AMPK and BCL2 and downregulated BAX as to attenuated hepatic steatosis and fibrosis

in improving SLD by reducing blood sugar and fat levels, exhibiting antioxidant properties, and mitigating inflammatory reactions [95, 96]. Berberine's ability to enhance the phosphorylation of various signaling molecules, including IRS, AKT, AMPK, and JNK, contributes to reducing insulin resistance, ameliorating inflammatory responses, alleviating oxidative stress, and diminishing lipid formation [95, 96]. The regulation of phosphorylation may provide a plausible rationale for the diverse effects of individual traditional Chinese medicines or their constituents. While further validation is necessary, this implies that the regulation of phosphorylation holds significant potential in treatment of SLD by traditional Chinese medicine.

Others

There are also some new findings that are important in regulating the phosphorylation of NAFLD and NASH, mainly including medical materials and chemical compounds. A new type of nanoparticle loaded with nifedipine can promote autophagy and reduce liver fat, where it enhances water solubility without modifying the chemical structure while allows prolonged release in vivo. Therefore, by increasing autophagic clearance through Ca2+/calmodulin-dependent kinase II phosphorylation, this nanoparticle leads to suppression of metabolic derangements associated in NAFLD [114]. Additionally, a hepatic-targeted delivery system utilizing oxidized starch-lysozyme nanocarriers to administer resveratrol has been shown to elevate p-AMPK and p-IRS, thereby reducing adipogenesis and insulin resistance [36]. This system achieves precise liver targeting by employing covalently conjugated galactose, recognized by the asialoglycoprotein receptors which is specifically expressed in hepatocytes, and ultimately facilitating the delivery of drugs to modulate phosphorylation.

Furthermore, there have been recent discoveries of newly activated molecules, or synthetic chemicals, that exhibit potential therapeutic properties and are being investigated as potential target for NAFLD and NASH. For instance, SYSU-3d has been found to activate the phosphorylation of heat shock factor 1 (HSF1), thereby promoting PGC-1a to inhibit oxidative stress and inflammation [114]. AdipoRon, the first small molecule adipoR agonist, particularly its subtype Q7, is thought to alleviate NAFLD by enhancing the phosphorylation of AMPK [115]. Additionally, a novel liver-specific ACC inhibitor known as ND-654 mimics the function of ACC phosphorylation and hinders the progression of liver de novo lipogenesis and hepatocellular carcinoma [31]. Moreover, an unexplored type IV collagen inhibitor, Cpd17, influences the phosphorylation of the ATX-LPA axis and holds significant potential in treating NAFLD [116].

Challenges and prospects

As SLD continues to rise, there is a growing global focus on the prevention and management of SLD. However, the precise mechanism of SLD remains unclear, and there is currently no specific pharmaceutical intervention targeting SLD. Proteins play a direct role as downstream molecules in exerting functional effects. Protein modification can directly influence the structure or function of proteins, with phosphorylation being the most extensively studied form of modification. Abnormal regulation of phosphorylation at different amino acid residues and their specific sites can significantly impact the development of SLD. Therefore, investigating the role of phosphorylation in the fundamental nature of SLD is of great importance. Nevertheless, based on current research, the following areas can provide a framework for future research on phosphorylation-related mechanisms in SLD.

Utilizing a combination of multiple omics methodologies and single-cell technology is essential for a comprehensive exploration of phosphorylation

Current research on phosphorylation has been predominantly focused on the effects of specific molecules or phosphorylation sites, thereby elucidating their regulatory role in signaling pathways or phenotypic outcomes. For instance, extensive studies have been conducted on the phosphorylation of AMPK at Thr172, revealing its regulation by various factors and its impact on the development of SLD [21, 26, 28, 31, 34, 37, 117–119].

There is currently no specific elucidation of the involvement of upstream kinases in the regulation of phosphorylation, the influence of phosphorylation modification on protein structure or function, and the validation of novel phosphorylation sites. The emergence of bioinformatics technology has provided opportunities to investigate whether changes in protein-level phosphorylation are implicated in regulating other molecules at the transcriptomic level or in the modulation of protein-protein interactions through high-throughput multi-omics analysis. Furthermore, the examination of potential disparities in the phosphorylation modification of the same protein across different cell types and its impact on various cellular functions or fates, in conjunction with single-cell mass spectrometry technology, may yield insights. For instance, the emerging technology of Cytometry by Time of Flight utilizes metal ions to categorize cell subpopulations for high-throughput exploration of distinct intracellular proteomics and modification sites [120].

Inflammatory signaling pathway specific phosphorylase inhibitors

In the development of SLD, inflammation and various immune irregularities are fundamental mechanisms,

with phosphorylation frequently assuming a central facilitative role, including activation of the NF-kB pathway, JNK pathway, AKT pathway, and others. Currently, while there is a dearth of specific pharmaceutical interventions directly targeting SLD, certain medications have demonstrated the ability to suppress inflammatory signaling pathways and cytokine phosphorylation, thereby mitigating the progression of SLD, such as Silybin [40]. and UDCA [86]. Nevertheless, these medications do not selectively inhibit the phosphorylation of inflammation-related signaling pathways and lack substantial evidence-based support, necessitating further investigation.

Phosphorylation regulation in insulin resistance in SLD

The occurrence of insulin resistance can induce metabolic disorders, further inducing inflammatory reactions and immune abnormalities [121]. At the same time, insulin resistance is regarded as to be closely related to a decrease in muscle and bone content [122, 123], which in turn induces or exacerbates the occurrence of SLD. Although the mechanism research is not yet clear, the regulation of phosphorylation is regarded to be widely involved, mainly through PI3K-AKT signaling and IRS mediated insulin resistance [39, 57, 124]. Inhibiting the phosphorylation of corresponding proteins or targeting AMPK phosphorylation at Th172 through kinase has the potential to reverse insulin resistance, but further research is needed in the future.

Uncoupling protein (UCP) and SLD

UCP may show enormous potential in the treatment of SLD under oxidative phosphorylation. UCP have specific physiological functions, and hibernating and newborn animals can use uncoupling proteins to convert some of the energy originally used for ATP production into heat [125]. On the one hand, the genotype of UCP can be associated with patient prognosis. It is reported that UCP1 (AG + GG) genotype is positively correlated with the severity of hepatic steatosis [126]. On the other hand, UCP has the potential targeting phosphorylation to improve SLD. Although there is no approved drug for SLD, there are many drugs reckoned as good candidates via phosphorylation [127]. For example, thyroxine can promote the expression of UCP, which allows more to join the uncoupling process, thereby increasing heat production and oxygen consumption [128]. Besides, thyroid hormone can increase the number of sodium and potassium pumps on the cell membrane, leading to more ATP consumption and promoting the process of oxidative phosphorylation [128]. At present, the one of new pharmacology in clinical trial for NAFLD is thyroid hormone receptor β agonists targeting to liver, significantly influencing UCP and then reducing liver fatty accumulation and improve NASH [129], which show great potential for future exploration.

Conclusion

The progress of phosphorylation is of great value and shares close association with SLD, whether in pathogenesis or treatment. It is indicated that phosphorylation mainly affects SLD, where AMPK, AKT, and NF-kB are key factors closely related to de novo lipogenesis, metabolic disorders, inflammatory reactions, and abnormal immunity. In terms of treatment, although there are no approved drugs that can treat SLD, many potential drugs that can alleviate SLD through phosphorylation. Further exploration of the mechanism of phosphorylation in SLD can benefit significantly clinical. In addition, more detailed research is necessary for studying phosphorylation in SLD, especially combining multi omics and single-cell technology to accurately explore the pathogenesis of SLD. In all, phosphorylation is of great value as a pathogenesis and therapeutic target for SLD.

Abbreviation

ACC	Acetyl-CoA carboxylase
AKT	Serine/threonine kinase
AMPK	Adenosine 5′-monophosphate activated protein kinase
ASK1	Apoptosis signal-regulating kinase 1
CDK5	Cyclin-dependent kinases 5
CPT1	Carnitine O-palmitoyl transferase 1
eIF2B	Eukaryotic initiation factor 2B
FAK	Focal adhesion kinase
FG F	Fibroblast growth factor
FOX	Fork head box protein
GLUT4	Facilitated glucose transporter member 4
GLP	Glucagon-like peptide
GSK-3β	Glycogen synthase kinase 3β
HCC	Hepatocellular carcinoma
HSF1	Heat shock factor 1
NF-kB	Inhibitor epsilon, IkB
IP3R1	Inositol 1,4,5-trisphosphate receptor type 1
IRS1	Insulin receptor substrate 1
LKB1	Liver kinase B1
JNK	C-Jun N-terminal kinase
LXRα	Oxysterols receptor α
MASLD	Metabolic dysfunction-associated steatotic liver disease
mTOR	Mammalian target of rapamycin
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NF-kB	Nuclear factor kappa-B
NLS	Nuclear localization sequence
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PI3K	Phosphatidylinositol 3-kinase 3
PPAR	Peroxisome proliferators-activated receptors
SGLT-2i	Sodium-dependent glucose transporters 2 inhibitor
SLD	Steatotic liver disease
SMPD3	Sphingomyelin phosphodiesterase 3
STAT3	Signal transducer and activator of transcription 3
TAK1	Transforming growth factor kinase 1
SREBP	Sterol regulatory element-binding protein
TBC1D1	TBC1 domain family member 1
TGFβ	Transforming growth factor β
TM6SF2	Transmembrane 6 superfamily member 2

TNFα	Tumor necrosis factor a
TRIM	Tripartite motif-containing protein
UCP	Uncoupling protein
UDCA	Ursodeoxycholic acid
WTAP	Wilms' tumor 1-associating protein

Supplementary Information

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Additional file 1. 17718536_TiansuLv.docx

Additional file 2. 17698067_TiansuLv.docx

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Authors' contributions
Tiansu Lv wrote the main manuscript text and others assisted in summarizing figure and tables. The entire process was carried out under the guidance of Xiqiao Zhou.All authors reviewed the manuscript.

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