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REVIEW

The ubiquitin−proteasome system: A potential target for the MASLD

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Therapeutic targets

Abstract Metabolic dysfunction-associated steatotic liver disease (MASLD), the most prevalent chronic liver condition globally, lacks adequate and effective therapeutic remedies in clinical practice. Recent studies have increasingly highlighted the close connection between the ubiquitin−proteasome system (UPS) and the progression of MASLD. This relationship is crucial for understanding the disease's underlying mechanism. As a sophisticated process, the UPS govern protein stability and function, maintaining protein homeostasis, thus influencing a multitude of elements and biological events of eukaryotic cells. It comprises four enzyme families, namely, ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), ubiquitin-protein ligases (E3), and deubiquitinating enzymes (DUBs). This review aims to delve into the array of pathways and therapeutic targets implicated in the ubiquitination within the pathogenesis of MASLD. Therefore, this review unveils the role of ubiquitination in MASLD while spotlighting potential therapeutic targets within the context of this disease.

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1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is recognized as the predominant chronic liver condition globally, affecting approximately 38% of the population[1,2]. To reflect the pathology and etiology of these conditions more accurately, metabolic dysfunction-associated steatotic liver disease (MASLD) was put forward to replace the term NAFLD, defined by the cases with hepatic steatosis and at least one—and only one—of the five cardiometabolic risk factors[3]. MASLD encompasses a spectrum of liver diseases, including simple steatosis (NAFL) and nonalcoholic steatohepatitis (MASH), and MASLD is commonly associated with metabolic disorders including obesity, dyslipidemia, type 2 diabetes, and metabolic syndrome[4,5]. Given the complex pathogenesis of MASLD, substantial effort has been put into exploring novel therapeutic targets. Presently, the most promising targets include cellular mechanisms of death and metabolism, inflammatory processes, interaction between intestine and liver, and direct inhibition of fibroblast activation and extracellular matrix deposition[6]. Despite these efforts, limited therapeutic agents have been officially approved for MASLD treatment.

Accumulating evidence suggested that the ubiquitin-proteasome system (UPS) plays a pivotal role in MASLD pathogenesis, serving as the primary mechanism for protein degradation in eukaryotic cells. It influences various cellular processes including DNA repair, stress response, and cell proliferation[7]. The system consists of ubiquitin, the 26S proteasome, and several enzymes such as E1, E2, E3, and deubiquitinases. The ubiquitin binding to the substrate is carried out through a multi-step cascade composed of E1, E2, and E3 enzymes. The UPS operates through a sequence of steps beginning with the attachment of ubiquitin's carboxyl group to the sulfhydryl group of the ubiquitin-activating enzymes (E1), forming a thioester bond. This activated ubiquitin is then transferred to ubiquitin-conjugating enzymes (E2s), and subsequently, ubiquitin ligase enzymes (E3s) collaborate with charged E2s to attach the ubiquitin to the substrate. The ubiquitinated substrates are eventually recognized by proteasomes and finally decomposed into short peptides or amino acids under the catalysis of proteases[8-10]. In addition, SUMOylation and NEDDylation modifications are two common ubiquitin-like modifications that generally affect protein function or stability and also play a regulatory role in MASLD progression (Fig. 1). As UPS plays a crucial role in the progression of chronic liver disease by regulating protein degradation, it may serve as a potential targeted therapeutic strategy for MASLD[11,12] (Fig. 2).

2. E3 ubiquitin ligase and MASLD

Since E3 ligases are responsible for binding to substrates and determining the specificity of the ubiquitin system, there are a large number of E3 ligases in different organisms, but only a few E1 and E2 enzymes[13,14]. Based on their structure and function, E3 ligases can be classified into several types: Ring finger type,

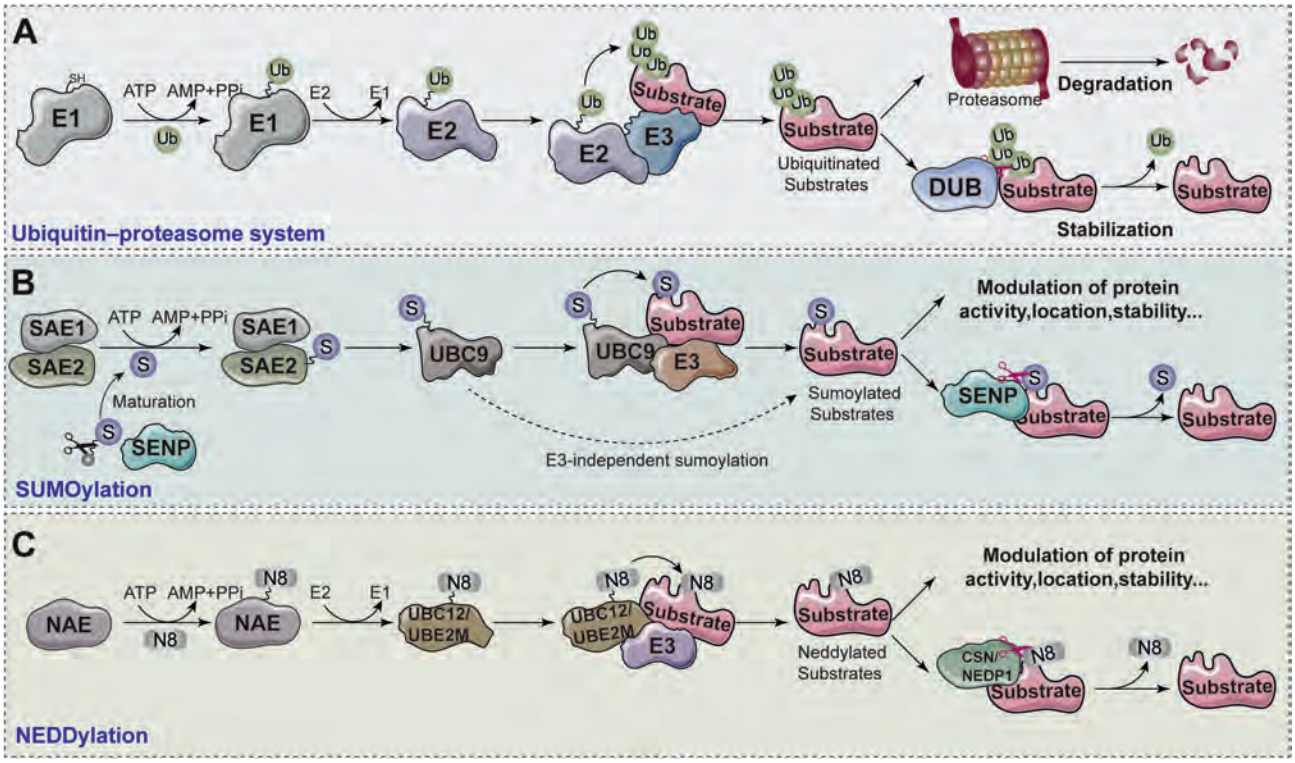

Figure 1 Ubiquitination and ubiquitin-like modification processes. (A) UPS system associated enzymes E1 (ubiquitin activating enzyme), E2 (ubiquitin-conjugating enzyme), E3 (ubiquitin ligase enzymes) and DUB (deubiquitinases) at different steps and the corresponding fate of the substrate. Biochemical process of SUMOylation (B) and NEDDylation (C). SUMOylation process is catalyzed by E1 activating enzymes (including SEA1 and SEA2), E2 binding enzyme UBC9 and SUMO E3 ligase. SENPs can catalyze the removal of SUMO from the substrate. The NEDDylation process is catalyzed by a cascade of NEDD8-activating enzyme (NAE), NEDD8 binding enzyme (UBE2M/UBC12 or UBE2F), and NEDD8 ligase.

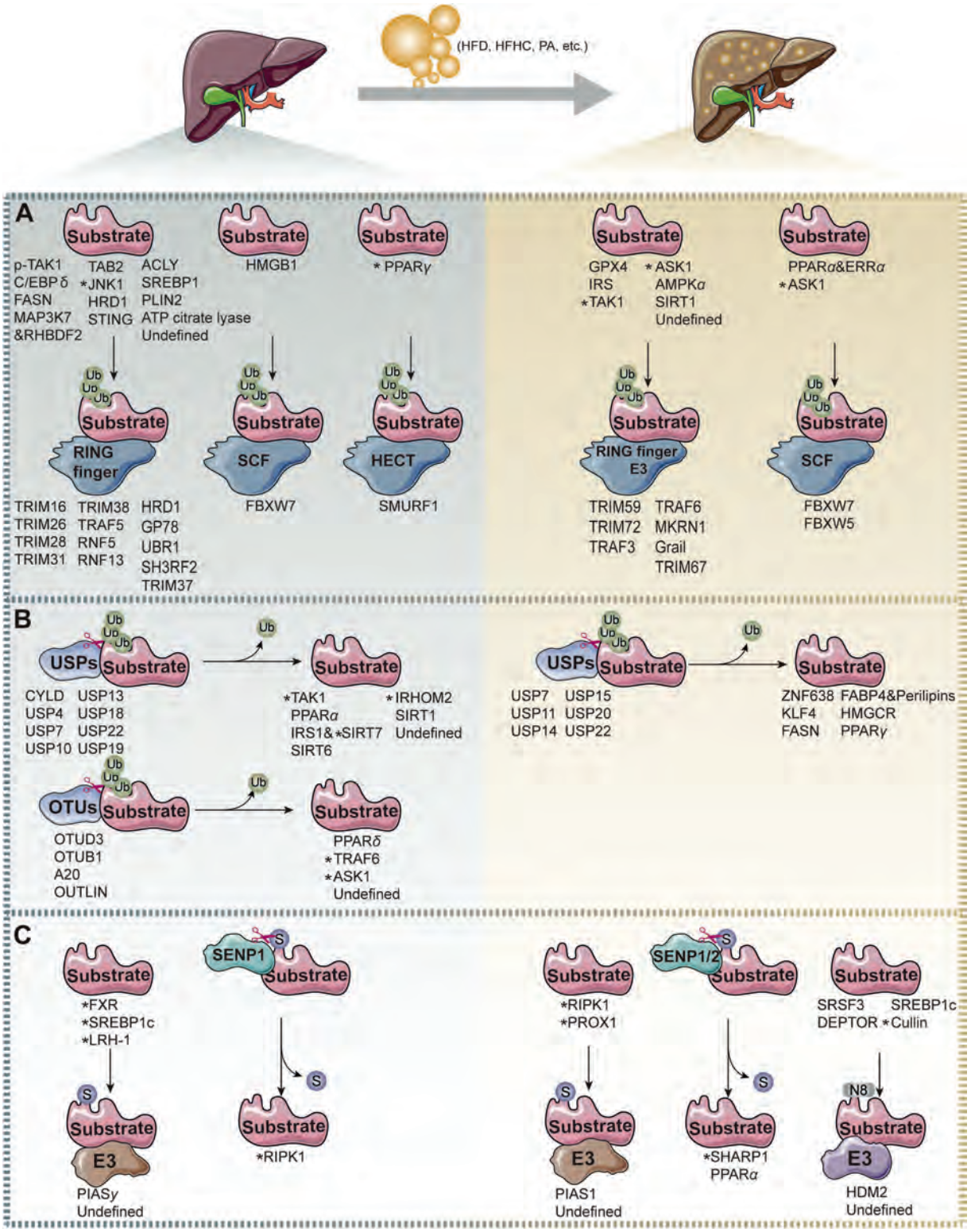

Figure 2 Ubiquitination and ubiquitin-like in MASLD. (A) E3 ubiquitin ligase, (B) deubiquitinases (DUBs) and (C) SUMOylation and NEDDylation in MASLD. Enzymes and corresponding substrates that play a restorative (left) and deteriorative (right) role in MASLD. With * indicates the regulation of substrate activity and without * indicates the regulation of substrate degradation.

Homologous to E6AP C-terminus (HECT), and RING-between-RING (RBR) type (Table 1)[14]. Evidence suggests that the abnormal expression of E3 enzymes is associated to the pathogenesis of MASLD (Table 2[15-40]).

2.1. The RING finger E3 ubiquitin ligases

The Ring finger type represents the most common form of ubiquitin ligase, characterized by a RING or U-box domain that

Table 1 Classification of E3 ubiquitin ligases.

Category	Subclass	Representative member	structural features	Mechanism of action
Single-subunit E3s	RING	MDM2 RNF4	RING domain or U-box domain	Directly interacting with E2 ubiquitin-conjugating enzymes to catalyze ubiquitin transfer
	HETC	Itch Smurf1	HECT domain	Two-step reaction (first forming a thioester bond with ubiquitin, then transferring it to the substrate)
	RBR	ARIH1 PARKIN	Two RING domains and an IBR domain	Two-step reaction
Multi-subunit E3s	SCF		SKP1, CUL1, RBX1, and a variable F-box protein	F-box protein recognizes the substrate
	CUL3-BTB		CUL3, RBX1, and BTB proteins	BTB protein recognizes the substrate
	CUL4-DDB1-DWD		CUL4, DDB1, RBX1, and a variable DWD protein	DWD protein recognizes the substrate
	APC/C		The catalytic core, the platform and the tetratricopeptide repeat (TPR) lobe	

Table 2 Pathophysiological roles of E3 ubiquitin ligases in MASLD.

Name	Expression in MASLD	Inhibit/Promote MASLD	Direct target	Ubiquitin chain types	Ref.
TRIM16	Increase	Inhibit	P-TAK1	K48	15
TRIM31	Decrease	Inhibit	RHBDF2/MAP3K7	K48/K48	16,17
TRIM28	−	Inhibit	FASN	K48	18
TRIM37	−	Inhibit	−	−	19
TRIM38	Decrease	Inhibit	TAB2	−	20
TRIM26	Decrease	Inhibit	C/EBPδ	−	21
TRIM72	Increase	Inhibit	−	−	22
TRAF5	Decrease	Inhibit	JNK1	−	23
RNF13	Increase	Inhibit	STING	−	24
SH3RF2	Decrease	Inhibit	ATP citrate lyase	K48	25
RNF5	Decrease	Inhibit	HRD1	K48 & K33	26
HRD1	Decrease	Inhibit	ACLY	−	27
Gp78	−	Inhibit	SREBP1	−	28
Ubr1	−	Inhibit	Plin2	−	29
TRIM67	Increase	Promote	−	−	30
TRIM59	Increase	Promote	GPX4	−	31
TRAF3	Increase	Promote	TAK1	−	32
TRAF6	−	Promote	ASK1	K63	33
MKRN1	−	Promote	AMPKα	K48	34
Grail	Increase	Promote	Sirtuin 1	K48	35
FBXW5	−	Promote	ASK1	K63	36
FBXW7	Decrease	Promote	PPARα/ERRα	−	37
		Inhibit	HMGB1	−	38
Smurf1	Increase	Inhibit	PPARγ	K63	39
		Promote	SREBP-1c	−	40

facilitates the transfer of activated ubiquitin from the E2 enzyme to the substrate protein without directly binding to ubiquitin molecules[41]. The members of the tripartite motif (TRIM) and the tumor necrosis factor (TNF) receptor-associated factor (TRAF) family subfamilies are important members of the RING E3 family and there is ample evidence indicating that the two subfamilies, as well as other members, play significant roles in regulating the pathogenesis of MASLD.

2.1.1. The TRIM family

TRIM family are distinguished by an N-terminal tripartite RBCC motif that includes one or two BBox domains, a coiled-coil (CC) domain, and a RING-finger domain. The human genome encodes over 70 TRIM family members in humans, which are categorized into 11 subgroups based on their domain architecture[42].

TRIM16 has been shown to reduce lipid accumulation and inflammation in a MASH mouse model by degrading phosphorylated

transforming growth factor-β activated kinase 1 (TAK1) and activating the mitogen-activated protein kinase (MAPK) signaling pathway[15]. Moreover, separate studies have demonstrated that TRIM31 reduces MASLD by promoting the proteasomal degradation of rhomboid 5 homolog 2 (RHBDF2) and mitogen-activated protein kinase kinase kinase 7 (MAP3K7)[16,17].

TRIM28 and TRIM37 are also noted for their protective effects in experimental MASH. Knockout of TRIM37 in mice results in mulibrey nanism, characterized by prenatal-onset growth failure, an increased risk for tumors, fatty liver, and type 2 diabetes[19]. Liver-specific KRAB-associated protein 1 (KAP1, also known as TRIM28), leads to sexually dimorphic phenotypic fatty acid synthase (FASN) shown that sorting nexin 8 (SNX8) could recruit TRIM28 to promote the ubiquitination and degradation of FASN, the rate-limiting enzyme in fatty acid synthesis[18]. Additionally, depletion of TRIM38 in mice exacerbates liver inflammation and fibrosis induced by a high-fat and high-cholesterol diet. Mechanistically, TRIM38 enhances the ubiquitination and degradation of transforming growth factor-β-activated kinase 1 binding protein 2 (TAB2) and suppresses the MAPK signaling cascades[20]. TRIM26 also plays a role in preventing the progression of steatohepatitis by inhibiting the activation of C/EBPδ signaling[21].

Conversely, there are also several studies showing that TRIM family members exacerbate the progression of MASLD. Specifically, TRIM67 protein levels are elevated in obese conditions, which enhances inflammation and facilitates the development of MASLD. This effect is linked to interactions with peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1alpha, a transcription coactivator crucial for regulating metabolic processes[30]. Additionally, TRIM59 has been shown to induce both ferroptosis and steatosis by interacting with glutathione peroxidase 4 (GPX4) and promoting its ubiquitination, highlighting a novel mechanism by which TRIM59 contributes to MASLD pathology[31]. Moreover, TRIM72 plays a pivotal role in insulin resistance, a key component of metabolic syndrome associated with MASLD. It mediates insulin resistance by accelerating the degradation of insulin receptor substrates (IRS and IRS1) in cardiac and skeletal muscle tissues, with its expression markedly increased in these conditions. Intriguingly, physical exercise has been found to enhance muscle insulin sensitivity through the TRIM72/PI3K/Akt/mTOR signaling pathway, offering a potential therapeutic avenue[22].

2.1.2. *The TRAF family*

The TRAF family includes seven characterized members, six of which, with the exception of TRAF1, possess a RING finger domain enabling their function as ubiquitin ligases. Notably, several TRAF family members have been implicated in the pathogenesis of MASLD, through their E3 ligase activities or involvement in inflammatory signaling pathways.

TRAF2 is instrumental in the activation of several downstream pathways, including the canonical and noncanonical nuclear factor kappa B (NF-κB) and c-Jun N-terminal kinase (JNK) cascades. A study has shown that TRAF2 depletion in hepatocytes inhibits glucagon-mediated liver gluconeogenesis in high-fat diet (HFD) mice, although it does not affect insulin sensitivity[43]. This suggests that hepatic TRAF2 acts as a positive regulator of glucagon signaling and hepatic gluconeogenesis in states of overnutrition. Conversely, TRAF3 in hepatocytes enhances the activation of TAK1 through increased ubiquitination and phosphorylation, promoting liver steatosis and insulin resistance[32]. TRAF6 meanwhile, mediates Lys6-associated polyubiquitination of apoptosis signal-regulating kinase 1 (ASK1), activating ASK1 and facilitating the progression of MASH[33]. In contrast, TRAF5 serves as a negative regulator of hepatic steatosis and suppresses JNK1 activity, demonstrating its protective role against hepatic lipid accumulation[23].

2.1.3. *Others*

Ring finger protein 13 (RNF13) interacted with the stimulator of interferon genes protein (STING) and facilitated its proteasomal degradation, eventually inhibiting liver lipid accumulation[24]. Makorin ring finger protein 1 (MKRN1) promotes the ubiquitination and degradation of adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK) and then promotes MASLD, insulin resistance, and obesity associated with HFD[34]. Grail regulates the lipid accumulation in hepatic steatosis *via* interacting with sirtuin type 1 (Sirt1)[35]. SH3 domain containing ring finger 2 (SH3RF2) can promote K48 ubiquitination modification of ATP citrate lyase and promote its ubiquitin-dependent degradation. Hepatocyte SH3RF2 deficiency would aggravate MASLD[25]. Both HMG-CoA reductase degradation 1 (HRD1) and RNF5 were endoplasmic reticulum localized in cells. HRD1 down-regulates the protein level of ATP citrate lyase (ACLY), a key enzyme regulating lipogenesis, thus alleviating MASLD in *db*/*db* mice[27]. RNF5 directly binds to HRD1 and promotes its K48 and K33-linked ubiquitination, then diminishes the palmitic acid and oleic acid (PAOA) lipid deposition[26]. Autocrine motility factor receptor (AMFR, also known as Gp78), a member of RING-H2 E3 ubiquitin ligases, has a Ring figure domain, CUE motif, and E2 binding site[44]. Studies have shown that Gp78 plays a protective role in MASLD and knockout of Gp78 up-regulates unfolded protein response and sterol regulatory element-binding protein 1 (SREBP-1) to increase lipid accumulation[28]. Studies have shown that nutriment could affect the activity of certain E3 ubiquitin ligases, thus affecting the progression of MASLD. Leucine and isoleucine bind to ubiquitin protein ligase E3 component N-recognin 1 (UBR1) and induce its activation, and UBR1 promotes the degradation of perilipin 2 (PLIN2) to ameliorate hepatic steatosis[29].

2.2. *SCF (SKP1-CUL1-F-box protein) E3 ubiquitin ligase*

SCF (SKP1-CUL1-F-box protein) E3 ubiquitin ligase is the largest family of ubiquitin ligases. Among its members, F-box and WD repeat domain containing 7 (FBXW7) and F-box and WD repeat domain containing 5 (FBXW5) have shown substantial relevance to MASLD. The role of FBXW7 in lipid metabolism remains ambiguous; however, its function in MASLD has garnered considerable attention due to its regulatory impact on several metabolic transcription factors, including SREBP1/2, the nuclear receptors nuclear receptor subfamily 1, group D member 2 (REV-ERBα, also known as NR1D1), estrogen-related receptor alpha (ERRα, also known as NR3B1), and the co-regulator proteins nuclear receptor coactivator 3 (NCOA3) and PGC-1α[45-47]. A recent study indicates that decreased expression of FBXW7 is a feature of advanced MASH; as FBXW7 regulates the transcription activity of the nutrient-sensing nuclear receptors peroxisome proliferator-activated receptor α (PPARα) and ERRα, thus promoting the progress of MASH[37]. Additionally, FBXW7 ameliorates hepatic inflammation and insulin resistance by diminishing high mobility group box 1 (HMGB1)-mediated innate immune signaling[38]. On the other hand, FBXW5 exacerbates nonalcoholic steatohepatitis by facilitating the addition of Lys63-linked ubiquitin to ASK1, suggesting that drug candidates mimicking FBXW5(S1) or FBXW5(S3) might be viable therapeutic approaches for MASH[36].

Furthermore, CUL4B-DDB1 also plays a role in the development of MASLD. The pancreatic progenitor cell differentiation and proliferation factor (PPDPF) impedes the interaction between Raptor and its E3 ligase CUL4B-DDB1, thereby inhibiting the ubiquitination and activation of Raptor, which results in suppression of the mTORC1−S6K−SREBP1 signaling pathway[48].

2.3. HECT E3 ubiquitin ligases

HECT E3s are classified into 3 groups: the Nedd4 family, the HERC family and others[14]. Within the NEDD4 family, smad ubiquitin regulatory factor 1 (SMURF1) promotes K63-linked polyubiquitination of PPARγ, thereby inhibiting its transcriptional activity and reducing the lipid accumulation in the liver[49]. However, another study has shown that SMURF1 might aggravate lipid accumulation in the liver by stabilizing SREBP-1c in an E3 activity-independent in the late phases of MASLD[40]. This highlights the diverse regulatory roles that HECT E3 ligases play in hepatic lipid metabolism and provides insights into potential therapeutic targets for treating liver diseases.

3. Deubiquitinases (DUBs) and MASLD

Ubiquitination represents a reversible post-translational modification process, where DUBs catalyze the removal of ubiquitin moieties from target proteins or polyubiquitin chains. This results in modifications to the function or stability of these targeted proteins[50]. Based on sequence and domain conservation, seven structurally distinct DUB families have been proposed: the ubiquitin-specific proteases (USPs), the ovarian-tumor proteases (OTUs), the ubiquitin C-terminal hydrolases (UCHs), Machado−Joseph disease protein domain proteases (MJDs), JAMM/MPN domain-associated metallopeptidases (JAMMs), motif interacting with ubiquitin-containing novel DUB family proteases (MINDYs) and zinc finger-containing ubiquitin peptidase 1 (ZUP1)[50-52]. Amongst them, several DUBs have been pinpointed as exerting vital roles in the regulation of cell cycle control, cell signaling, and apoptosis. Some DUBs have also been reported to be involved in the regulation of MASLD. In the sections that follow, we will outline these DUBs and present examples illustrating their roles in the onset and progression of MASLD (Table 3[53-70]).

3.1. The ubiquitin-specific proteases (USPs)

The USP class constitutes the majority of the DUBs, due to a rapid diversification during evolution, possibly in concert with the diversification of E3 ligases[51]. These proteases comprise three subdomains, analogized to the fingers, thumb and palm of a right hand. Most USPs contain a core catalytic domain with insertions and terminal extensions bearing additional protein interaction domains[8,71].

Several studies have shown that USPs play a protective role in MASLD. For example, cylindromatosis-associated DUB (CYLD) functions as a pivotal suppressor in MASLD and relates to metabolic disorders[53]. Mechanistically, hepatocyte CYLD directly interacts with and deubiquitinates TAK1 and suppresses the downstream NF-κB and JNK cascades. Hepatic steatosis was ameliorated in Hepatocyte-specific CYLD transgenic mice but worsened in hepatocyte-specific CYLD-KO mice[53]. Moreover, USP4 and USP18 also deubiquitinate TAK1 and inhibit TAK1 activation[54,56]. In addition to this, USP4 also deubiquitinates and stabilizes PPARα. Oxidized fish oil (OFO) induced the decline of USP4 protein expression, promoting oxidative stress and inducing mitochondrial dysfunction and lipotoxicity[55].

Inactive rhomboid protein 2 (IRHOM2) was a crucial and positive regulator of inflammation-associated diseases and hepatocyte USP13 interacted with IRHOM2 and removed its K63-linked ubiquitination in response to metabolic stresses. When USP13 is knocked out in hepatocytes, liver metabolic homeostasis is disrupted, leading to carbohydrate metabolism disorder, lipid deposition, increased inflammation, and eventually promoting MASH development[57].

Likewise, USP10 has been reported to play a protective role in the pathogenesis of MASLD through multiple mechanisms[58,72,73].

Table 3 Pathophysiological roles of deubiquitinases in MASLD.

Name	Expression in MASLD	Inhibit/Promote MASLD	Direct target	Regulating effect	Ubiquitin chain types	Ref.
CYLD	Decrease	Inhibit	TAK1	Activity ↓	K63	53
USP4	Decrease	Inhibit	TAK1	Activity ↓	−	54
		Inhibit	PPARα	Degradation ↓	−	55
USP18	Decrease	Inhibit	TAK1	Activity ↓	−	56
USP13	Decrease	Inhibit	IRHOM2	Activity ↓	K63	57
USP10	Decrease	Inhibit	Sirtuin 6	Degradation ↓	−	58
USP19	−	Inhibit	−	−	−	5
OTUD3	−	Inhibit	PPARδ	Degradation ↓	−	59
OTUB1	Decrease	Inhibit	TRAF6	Activity ↓	K63	33
TNFAIP3	Increase	Inhibit	ASK1	Activity ↓	K63/K29/K11	60
OUTLIN	−	Inhibit	−	−	−	61
USP14	Increase	Promote	FASN	Degradation ↓	−	62
USP20	−	Promote	HMGCR	Degradation ↓	−	63
USP15	Increase	Promote	FABP4&perilipins	Degradation ↓	−	64
USP11	Increase	Promote	KLF4	Degradation ↑	K63	65
USP22	−	Inhibit	Sirt1	Degradation ↓	−	66
	−	Promote	PPARγ	Degradation ↓	K48	67
USP7	−	Promote	ZNF638	−	−	68
	−	Inhibit	IRS1	Degradation ↓	−	69
	−	Inhibit	Sirt7	Activity ↓	K63	70

USP10 manages hepatic steatosis by interacting with Sirt6, a negative regulator of inflammation and lipid metabolism, thereby removing its ubiquitin chain and ultimately preventing its degradation[58]. The other study has indicated that USP10 could promote autophagy flux and activate the mTOR/Beclin1 signaling pathways in MCDD-fed mice, reducing histological steatosis, inflammation and fibrosis. Moreover, the autophagy inhibitors, chloroquine or 3-methyladenine, could reduce the suppressive effects of USP10 against steatosis. Thus, USP10 also alleviated hepatic steatosis in an autophagy-dependent manner[72,73]

Several other members of the USP family have been found to promote MASLD development. Both USP2 and USP14 interacted with and stabilized FASN, an essential enzyme for the conversion of dietary carbohydrates to fatty acids[62,74]. Therefore, USP2 and USP14 are regarded to contribute to MASLD progression. USP20 stabilizes HMG-CoA reductase (HMGCR), the rate-limiting enzyme in the cholesterol biosynthetic pathway, and USP20 was phosphorylated by mTORC1 at S132 and S134[63]. Genetic deletion or pharmacological inhibition of USP20 markedly decreased body weight, reduced lipid deposition in the liver, and improved insulin sensitivity[63]. USP15 deubiquitinates and stabilizes fatty acid-binding proteins (FABPs) and perilipins. FABPs are involved in the uptake of long-chain fatty acids and further metabolism, while perilipins are associated with the surface of lipid droplets and control lipid accumulation and inflammation[64]. Therefore, USP15 exerts both pro-inflammatory and pro-lipid accumulation functions in MASLD, indicating its potential as a target for MASLD treatment. Furthermore, *Usp19*-null mice displayed increased glucose tolerance and insulin sensitivity with HFD fed[5]. This suggests therapeutic potential of USP19 for MASLD treatment. USP11 promoted kruppel-like factor 4 (KLF4) instability by deubiquitinating KLF4. Studies have shown that KLF4 level was down-regulated and USP11 level was up-regulated in free fatty acid (FFA)-treated cells. USP11 enhanced hepatic steatosis through KLF4 inhibition[65].

The effect of USP22 on MASLD has been found to be contradictory[66,67]. High expression of USP22 has been associated with abnormal lipid metabolism, promoting lipid accumulation and tumorigenesis in hepatocellular carcinoma (HCC) cells. This is thought to be due to USP22's role in increasing acetyl-CoA carboxylase (ACC) and ACLY expression via the deubiquitination and stabilization of PPARγ[67]. However, a different study has shown that USP22 could reduce lipid accumulation in the liver by modifying mitochondrial respiration in the late phases of MASLD[66]. The Gα12-signaling pathway regulating SIRT1and PPARα appears to be responsible for these effects, as it stabilizes SIRT1 protein *via* the transcriptional induction of USP22 through increased hypoxia inducible factor 1 subunit alpha (HIF-1α)[66]. Further studies were required to elucidate the different mechanisms of USP22 on lipid synthesis.

Another important member of USPs, USP7, is also found to demonstrate the dual roles in MASLD. Under hypoxic conditions, PARP1-mediated poly ADP-ribosylation (PARylation) of HIF-1α is increased by spindle and kinetochore-associated complex subunit 3 (SKA3), leading to a greater association between HIF-1α and USP7 and hence preventing the degradation of HIF-1α and promoting fatty acid synthesis[75]. However, this work was studied for cholangiocarcinoma, and whether the findings are consistent in liver tissue still remains unknown. Zinc finger protein 638 (ZNF638) can increase the cleavage of SREBP1C, hence promoting *de novo* lipogenesis (DNL). USP7 was found to not only interact with and deubiquitylate ZNF638, but also to support the transcription of ZNF638 *via* the stabilization of cAMP-responsive element binding protein (CREB)[68]. USP7 inhibitor significantly inhibited lipid accumulation in fructose-induced hepatic steatosis[68]. However, on the other hand, USP7 has been showing that could deubiquitinate IRS1 and prevent its proteasomal degradation. Deleting phosphate inorganic transporter 1 (PiT1/SLC20A1) inhibited the disassociation of USP7/IRS1 upon insulin stimulation. Hepatocyte-specific *Pit1*-KO protects against obesity induced by a high-fat diet[69]. This study suggests that USP7 may inhibit the development of MASLD. Also, it was shown that USP7 suppressed the forkhead box O1 (*FOXO1*) transcriptional activity and negatively regulated SIRT7 deacetylase activity, thereby attenuating hepatic gluconeogenesis[70,76]. Further studies are required to elucidate these various, and at times contradicting actions of USP22 and USP7 on lipid synthesis and MASLD.

3.2. *The ovarian-tumor proteases (OTUs)*

OTUs are a type of deubiquitinase enzyme family comprising 16 members. OTU deubiquitinase 3 (OTUD3) regulated a series of genes involved in glucose and lipid metabolism and oxidative phosphorylation by stabilizing PPARδ. It has been found that OTUD3 knockout mice are predisposed to obesity and metabolic diseases[59]. As for ovarian tumor associated proteinase B1 (OTUB1), the first identified member of OTUs, it has been found to play a suppressive role in MASH. This is thought to occur through its capacity to deubiquitinate TRAF6 thereby inhibiting the TRAF6-mediated ASK1 activation[33]. As such, targeting the OTUB1−TRAF6−ASK1 axis could be a promising therapeutic approach for MASH[33]. Another member of the OTUs family, tumor necrosis factor-α induced protein (TNFAIP, also known as A20), has been recognized to be a potent anti-inflammatory molecule that inhibits ASK1's polyubiquitination in hepatocytes, thus preventing the onset and progression of MASH[60]. OTU deubiquitinase with linear linkage specificity (OTULIN) was a deubiquitinase involved in the regulation of inflammation, a deficiency of OTULIN in liver parenchymal cells causes steatohepatitis in mice[61]. Further research is required to explore the roles played by the OTUs family in MASLD. Already numerous members have been identified to be involved in inflammatory processes[77], yet much remains to be uncovered.

4. SUMOylation and MASLD

The small ubiquitin-like modifier (SUMO) family is a newly discovered ubiquitin-like molecule[78]. Currently, at least six SUMO isoforms have been found, including SUMO1, SUMO2, SUMO3, SUMO4, SUMO5 and the newly discovered SUMO7. Among them, SUMO2 and SUMO3 have high homology, and they are often referred to as SUMO-2/3. SUMO2/3 and SUMO1 share only 45% homology. SUMO modification of proteins is very similar to ubiquitination modification, involving a total of three enzymatic reactions: The C-terminal of SUMO is activated ATP-dependent by the E1 activating enzyme (including SEA1 and SEA2) and subsequently transferred to the E2 binding enzyme UBC9 to form E2-SUMO thioester, which is eventually recruited by the SUMO E3 ligase to form complexes with substrates to increase specificity. However, the E2 enzyme also has the function of recognizing substrates in the absence of E3 ligase. SUMO/sentrin-specific proteases (SENPs) can catalyze the removal of SUMO from the target protein linked to SUMO and some

members of this family also participate in the maturation and activation process of SUMO.

SUMOylation is a reversible post-translational modification, as a series of proteases can remove SUMO terminal glycine from the lysine residues of the target protein. These proteases can be classified into three families based on their different subcellular localizations and substrates. The first family includes SENP1 and SENP2, which act on all members of the SUMO family. The second family comprises SENP3 and SENP5, which exert more powerful regulation on SUMO2/3. Furthermore, SENP6 and SENP7 have a similar substrate bias to SENP3 and SENP5. Several studies have reported that the SENP family plays a vital role in the progression of MASLD (Table 4[79-81,83-86,89,90,93,94]).

Receptor-interacting serine/threonine-protein kinase 1 (RIPK1) is implicated in cell death and inflammation in the progression of MASH. SENP1 catalyzes the deSUMOylation of RIPK1, which inhibits its activation[79]. SENP1 is significantly down-regulated in the livers with MASLD or MASH; and liver specific knockout of SENP1 mice will spontaneously form MASH-related phenotypes including liver inflammation, lipid accumulation, liver damage, and fibrosis[79]. Mechanistically, in SENP1-deficient cells, RIPK1 is SUMOylated by protein inhibitor of activated STAT 1 (PIAS1) which alters its ubiquitination and promotes its activation. Additionally, SENP1 can de-SUMOylate Sharp-1, a repressor for PPARγ transcription and adipogenesis, thereby promoting adipocyte differentiation and enhancing adipogenesis[80]. SENP2 and lipid metabolism have been well reported. SENP2 was the most significantly increased SENP in HFD-induced fatty liver and SENP2 deficiency in the liver protects against HFD-induced metabolic disorders and hepatic steatosis[81]. Further research revealed that hepatic SENP2 governed the homeostasis of both liver and adipose tissues, implicating SENP2 in the regulatory cross-talk between these two metabolic tissues. SENP2 induces de-SUMOylation of PPARα which promotes its ubiquitylation and subsequent degradation, causing a significant downregulation of FGF21. SENP2 also exerts control over adipogenesis and adipose lipid storage in different manners[81]. Similarly, SENP3 was predominantly up-regulated in MASLD patients and HFD fed rats. Up-regulated lipid accumulation with FFA treatment was markedly ameliorated with SENP3-siRNA transfection and this conclusion should be further verified in animal models[82].

Compromised SUMOylation of liver receptor homolog-1 (LRH-1) induced the expression of oxysterol binding protein-like 3 (OSBPL3), thus enhancing SREBP1 procession and promoting the development of MASLD[83]. In another case, SUMO2 modification of farnesoid X receptor (FXR) at K277 increased the interaction with NF-κB and decreased the binding of retinoid X receptor alpha (RXRα). FXR modified by SUMO2 repressed NF-κB target inflammatory genes but it didn't affect the FXR/RXRα target genes. In diet-induced obese mice, FXR was highly acetylated and inhibited SUMO2 modification, leading to the transactivation of inflammatory genes[84]. SUMO E3 ligase PIASy induced SREBP1c SUMOylation and increased its proteasome degradation, ultimately leading to a reduction in hepatic lipogenesis when fasting or nutritional deprivation[85]. Prospero homeobox 1 (PROX1), a key transcriptional regulator of lipid metabolism, is SUMOylated on lysine residue 556, thus affecting its ability to regulate cholesterol metabolism transcription. The hepatocyte-selective loss of SUMOylation on PROX1 reduces systemic cholesterol levels[86].

5. NEDDylation and MASLD

Neddylation is a reversible post-translational modification process that involves attaching the ubiquitin-like protein, NEDD8, to a target protein. This process is catalyzed by a cascade of NEDD8-activating enzyme (NAE), NEDD8 binding enzyme (UBE2M/UBC12 or UBE2F), and NEDD8 ligase[87,88]. Studies have found that serum NEDD8 levels were positively associated with the severity of MASLD in patients and pre-clinical mouse models. And MLN4924, a neddylation inhibitor, could boost fatty acid oxidation and reduce hepatic steatosis in pre-clinical mouse models. This suggested that neddylation is an effective intervention for MASLD[89-91]. Cullin-associated and neddylation-dissociated protein 1 (CAND1) also alleviated MASLD by reducing ubiquitinated degradation of acetyl-CoA acyltransferase 2 (ACAA2)[92]. Neddylation of multiple substrate proteins has been reported to influence disease progression in MASLD, such as serine-rich splicing factor 3 (SRSF3), SREBP1c and DEP-domain containing mTOR-interacting protein (DEPTOR). For instance, the neddylation of SRSF3 was found to be crucial for its proteasomal degradation, and inhibition of SRSF3 neddylation could inhibit the MASH progression[93]. Neddylation inhibition increased DEPTOR content and repressed the mTOR signaling, which resulted in enhanced fatty acid oxidation and reduced liver lipid accumulation[89]. Additionally, inhibition of Cullin neddylation was found to reduce IRS degradation, thereby enhancing hepatic insulin signaling and reducing hepatic glucose production[94]. And neddylation of SREBP1c has been reported to compete with ubiquitination, thereby stabilizing its

Table 4 Pathophysiological roles of SUMOylation and NEDDylation in MASLD.

The modified protein	Enzyme	Ubiquitin-like modification type	Regulating effect	Inhibit/Promote NAFLD/NASH	Ref.
RIPK1	SENP1	deSUMOylation	Activity ↓	Inhibit	79
	PIAS1	SUMOylation	Activity ↑	Promote	79
Sharp-1	SENP1	deSUMOylation	Activity ↓	Promote	80
PPARα	SENP2	deSUMOylation	Degradation ↑	Promote	81
LRH-1	−	SUMOylation	Transcription function ↑	Inhibit	83
FXR	PIASy	SUMOylation	Transcription of NF-κB ↓	Inhibit	84
SREBP1c	PIASy	SUMOylation	Transcription function ↑/↓	Inhibit	85
Prox1	−	SUMOylation	Transcription function ↑/↓	Promote	86
SRSF3	−	NEDDylation	Degradation ↑	Promote	93
DEPTOR	−	NEDDylation	Degradation ↑	Promote	89
SREBP1c	HDM2	NEDDylation	Degradation ↓	Promote	90
Cullin	−	NEDDylation	IRS degradation ↑	Promote	94

protein level and promoting hepatic steatosis[90]. Existing evidence suggests that inhibition of neddylation is a potential treatment strategy for MASLD and this highlights the need for further research to fully elucidate the complex roles of neddylation in the pathogenesis and progression of MASLD (Table 4).

6. Targeting UPS for MASLD treatment

Inhibitors targeting the UPS have shown promising potential in the context of MASLD. There are two main mechanisms of action of these inhibitors: (1) targeting enzymes in the UPS system that function in MASLD and (2) promoting or inhibiting the proteasome degradation of specific proteins that either promote or inhibit the disease (Table 5[36,68,90,91,96-101]). The N-terminus (S1) and C-terminus (S3) of FBXW5 respectively and competitively ablate the function of FBXW5, activating ASK1 and thus blocking ASK1-c-Jun/P38 MAPK signaling[36]. P22077, a selective USP7 inhibitor, inhibited pathological hepatic *de novo* lipogenesis by disturbing the USP7/ZNF638 axis[68]. USP1 inhibitor ML323 prevented adipogenesis and lipid accumulation by decreasing C/EBPβ protein stability and increasing its ubiquitination[95]. The regulatory particle non-ATPase 11 (RPN11) inhibitor capzimin inhibited the RPN11−METTL3−ACSS3 axis and downregulated lipid-related genes[96]. Neddylation inhibitor MLN4924 and SUMOylation inhibitor SP have also shown potential in inhibiting MASLD progression[90,91,97]. MLN4924 reduces excess lipid storage by stimulating fatty acid oxidation and lipid metabolites in liver mitochondria. SUMOylation inhibitors restore FXR signaling, and coadministration with FXR agonists drastically impeded MASH-induced fibrosis. 4-Azidophlorizin promoted the ubiquitin proteasome degradation of geranylgeranyl diphosphate synthase (GGPPS), thus alleviating hepatic steatosis[98]. Similarly, thiolutin (THL), an inhibitor of the JAB1/MPN/Mov34 (JAMM) domain, could potently inhibit NOD-like receptor protein 3 (NLRP3) deubiquitination and inflammasome activation in the model of diet-induced nonalcoholic fatty liver disease[99]. Corylinan, an HSP90β-selective inhibitor, inhibited *de novo* lipid synthesis by promoting mSREBPs ubiquitination and proteasomal degradation[100]. And PROTACI-d promoted the degradation of Keap1 to alleviate oxidative stress and MASLD[101].

7. Conclusions and prospects

Ubiquitination modification of the P38 MAPK signal pathway has been extensively reported in MASLD (Fig. 3). P38 MAPK participates in the progression of MASLD. Oxidative stress and inflammation activate the hepatic P38 MAPK signal pathway, leading to insulin resistance and impairs lipid metabolism[102]. Both ASK1 and TAK1 are significant upstream kinases of P38 MAPK and their activity is largely regulated by ubiquitination. The activity of ASK1 is negatively regulated by deubiquitinases OTUB1 and TNFAIP3 while E3 ligases FBXW5 and TRAF6 promote ASK1 activation. Analogously, DUBs CYLD, USP14 and USP18 inactive TAK1 while E3 ligase TRAF3 actives TAK. Therefore, considering using the P38 MAPK signaling pathway as the therapeutic target for MASLD, inhibition of associated E3 ligase may be an effective strategy while the DUBs involved play a protective role in MASLD.

Additionally, the role of ubiquitin and ubiquitin-like modifications as either protective mechanisms or contributors to disease progression remains ambiguous due to the complexities of these post-translational modifications. Enzymes like FBXW7, Smurf1, USP7, and USP22 have been reported to have differing impacts on disease advancement within different contexts. This inconsistency is largely due to variations in the substrate proteins these enzymes interact with. For instance, USP22's contradictory role in MASLD progression is mainly due to the difference in the substrate: when interacting with PPARγ, it promotes disease progression, but when in connection with Sirt1, it has an inhibitory effect. In addition to this, tissue-specific expression of proteins contributes to the functional disparities.

The UPS systems members participating in MASLD development mentioned above were mostly researched in liver parenchymal cells. Only a few studies have shown that UPS systems play a role in regulating MASLD in other cell types, such as macrophages and adipose tissue. STIP1 homology and U-Box containing protein 1 (STUB1) in adipocytes promoted the degradation of nuclear receptor subfamily 2 group F member 2 (NR2F2) and the transcription of adiponectin (APN), thus alleviating liver steatosis and hepatic stellate cells activation[103]. USP19 also modulated adipogenesis and potentiated high-fat-diet-induced obesity and glucose intolerance[104]. Macrophage USP2A significantly improved insulin sensitivity and prevented age- and/or adiposity-related chronic inflammation, although the mechanism is unclear[105]. Undeniably, macrophages, adipose tissue and hepatic stellate cells play an important role in the pathological process of MASLD, but the function of the USP system in these scenarios remains unclear. Therefore, the multi-omics approach is essential to fully elucidate the functions of ubiquitination and ubiquitin-like modifications in MASLD, these techniques can reveal new significant proteins that regulate disease progression and shed more light on the ubiquitination system's role in MASLD.

Along with the insight into the role of the ubiquitination system in various stages of liver disease has become a growing point of interest, increasing studies have found that the same protein plays

Table 5 Small-molecule inhibitors targeting UPS systems in MASLD.

Inhibitor name	Target molecular	Inhibition molecular	Ref.
S1 or S3 of FBXW5	FBXW5	Competitively ablating the function of FBXW5 on ASK1 activation	36
P22077	USP7	Inhibiting DNL by the inhibition of ZNF638	68
ML323	USP1	Decreasing C/EBPβ protein stability and increasing its ubiquitination	−
Capzimin	RPN11	Inhibiting RPN11−METTL3−ACSS3 axis and downregulating lipid-related genes	96
MLN4924	NEDDylation	Inhibiting the NEDDylation and reducing the levels of SREBP1c	90,91
SP	SUMOylation	Restoring FXR activity and function	97,101
PROTACI-d	Keap1	Promoting the degradation of Keap1 to alleviate oxidative stress and MASLD	101
4-Azidophlorizin	GGPPS	Promoting the degradation of GGPPS by the ubiquitin−proteasome pathway	98
Corylin	HSP90β	Promoting mSREBPs degradation through Akt−GSK3β−FBW7 pathway	100
Thiolutin (THL)	NLRP3	Inhibiting NLRP3 deubiquitination and activation	99

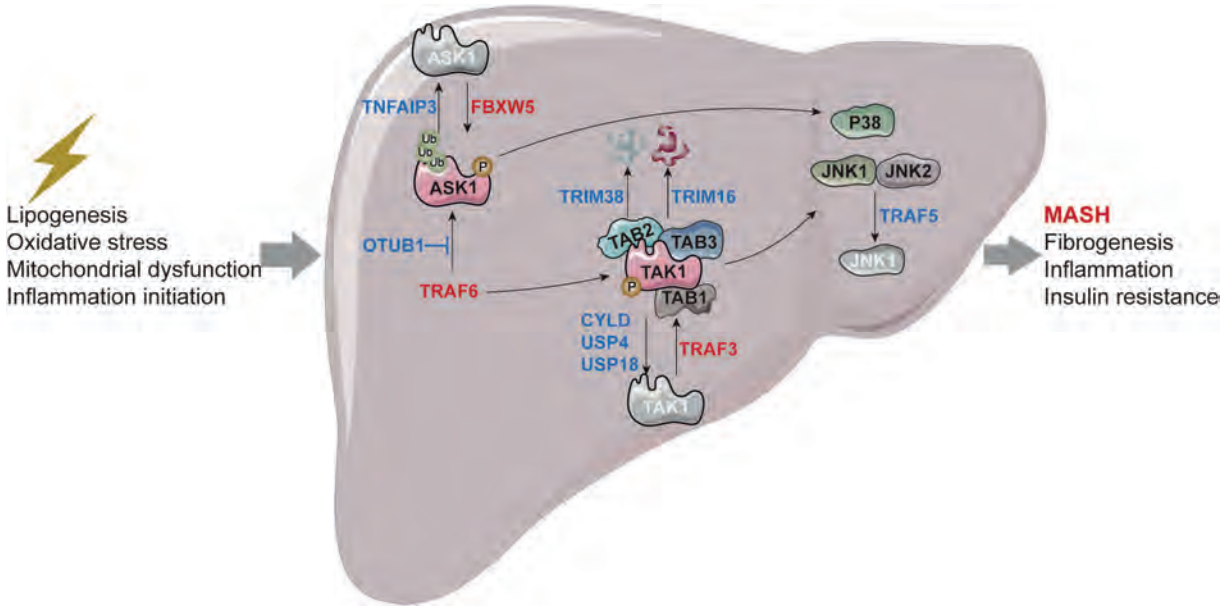

Figure 3 The P38 MAPK signaling pathway is regulated by ubiquitination in MASLD. FBXW5 and TRAF6 stimulate ASK1 activation by increasing ubiquitination and activation could be inhibited by OTUB1 and TNFAIP3. Similarly, TAK1 is activated by TRAF3 and deactivated by CYLD, USP4 and USP18. TRIM38 and TRIM16 promote the ubiquitination degradation of TAB2 and TAK1 respectively. And TRAF5 promotes JAK1 inactivation, resulting in inhibition of the P38 MAPK signaling pathway.

opposite functions in different stages of liver disease. USP10 protects the liver from MASLD but promotes metastasis of HCC in advanced stages of the disease[58,106]. As MASLD is a progressive disease that will eventually progress to HCC, it seems contradictory to play different pathological roles in MASLD and HCC. The underlying reasons may be attributed to the complex pathogenesis of HCC and the substrate selectivity of E3 ligase and DUB under different pathological conditions, and this suggests that elucidating the role of ubiquitin and ubiquitin-like modifications in hepatic diseases remains challenging.

In this review, we proposed that ubiquitin and ubiquitin-like modifications play a crucial role in liver lipid metabolism and glucose metabolism and are deregulated significantly under pathological. We also summarized the expression levels of UPS-related

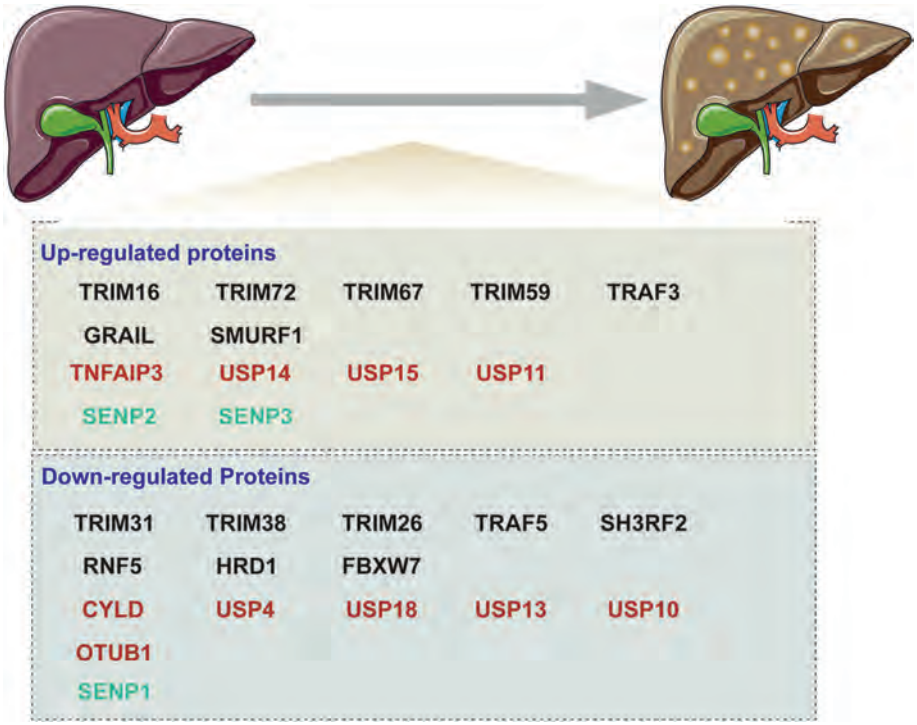

Figure 4 Expression levels of UPS-related proteins in MASLD. The black font represents E3 ubiquitin ligases; The red represents deubiquitinating enzymes and the green represents the SUMO-specific proteases.

proteins in MASLD patients compared to healthy controls (Fig. 4). Given that the regulators of these processes are mostly enzymes, which are more accessible to be targeted by small molecules with high specificity, targeting ubiquitin and ubiquitin-like modification processes may be an effective therapeutic strategy for MASLD. However, several scientific challenges remain in the development of drugs targeting ubiquitin signaling for MASLD. First, the selectivity of these inhibitors remains to be improved, hampering their clinical application. Additionally, research is needed to accurately target the liver to avoid organ toxicity, as the tissue distribution of the target proteins lacks specificity. Furthermore, identifying appropriate biomarkers is critical to determine suitable application scenarios for these inhibitors. Considering that the liver is a key organ for metabolism and that this process heavily relies on mitochondria for energy, enhancing mitochondrial function presents an effective strategy for treating liver diseases. This underscores the importance and feasibility of targeting organelles. Moreover, the endoplasmic reticulum is an essential site for protein synthesis and the clearance of misfolded proteins, playing a crucial role in maintaining protein homeostasis. Therefore, focusing on protein homeostasis within the endoplasmic reticulum could provide more effective targeting strategies for the treatment of MASLD.

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Conflicts of interest

The authors declare that they have no competing interests.

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