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REVIEW

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The emerging role of E3 ubiquitin ligases and deubiquitinases in metabolic dysfunction-associated steatotic liver disease

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Abstract

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common chronic liver disease worldwide, with a prevalence as high as 32.4%. MASLD encompasses a spectrum of liver pathologies, ranging from steatosis to metabolic dysfunction-associated steatohepatitis (MASH), fibrosis, and, in some cases, progression to end-stage liver disease (cirrhosis and hepatocellular carcinoma). A comprehensive understanding of the pathogenesis of this highly prevalent liver disease may facilitate the identification of novel targets for the development of improved therapies. E3 ubiquitin ligases and deubiquitinases (DUBs) are key regulatory components of the ubiquitin–proteasome system (UPS), which plays a pivotal role in maintaining intracellular protein homeostasis. Emerging evidence implicates that aberrant expression of E3 ligases and DUBs is involved in the progression of MASLD. Here, we review abnormalities in E3 ligases and DUBs by (1) discussing their targets, mechanisms, and functions in MASLD; (2) summarizing pharmacological interventions targeting these enzymes in preclinical and clinical studies; and (3) addressing challenges and future therapeutic strategies. This review synthesizes current evidence to highlight the development of novel therapeutic strategies based on the UPS for MASLD and progressive liver disease.

Keywords E3 ubiquitin ligases, Deubiquitinases, MASLD, MASH, Therapeutics

Background

Given the popularity of Western lifestyles and the aging global population, the prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) has increased rapidly in recent years, with a worldwide prevalence of 32.4% (95% CI 29.9–34.9) [1, 2]. MASLD is a liver manifestation of metabolic syndrome and is often accompanied by various metabolic disorders, including obesity, insulin resistance (IR), type 2 diabetes mellitus (T2DM), and cardiovascular disease (CVD) [3]. MASLD is a progressive liver pathology that begins with increased lipid deposition in hepatocytes (steatosis), which leads to steatotic liver disease. With the deterioration of steatosis, the emergence of portal and lobular inflammation, and hepatocyte injury, MASLD can progress to metabolic dysfunction-associated steatohepatitis (MASH). Inflammation and liver damage in MASH can cause progressive fibrosis, which can progress to cirrhosis and hepatocellular carcinoma (HCC) [4]. Early studies have proposed a "two-hit" theory describing the pathogenesis of MASLD. The "first hit" is the intrahepatic accumulation of lipids,

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which increases the vulnerability of the liver to many insults that constitute the "second hit" and promote inflammation and fibrosis [5]. However, with the in-depth study of MASLD, the traditional "two hit" theory is no longer sufficient to explain the complex pathogenesis of MASLD, and a new "multiple hit" concept has emerged. The "multiple hit" hypothesis, which encompasses multiple hits, including IR, adipokine deregulation, endoplasmic reticulum (ER) stress, changes in the gut microbiota, and genetic and epigenetic variables, provides a more accurate explanation of MASLD pathogenesis [6, 7].

Although the underlying mechanisms of MASLD pathogenesis are poorly understood, recent studies have shown that abnormal posttranslational modifications (PTMs) are involved in MASLD and its progression to MASH. Among PTMs, ubiquitination and deubiquitination are important contributors to MASLD pathogenesis. Ubiquitination involves an ATP-dependent enzymatic cascade of ubiquitin molecules covalently linked to lysine residues of the substrate, a process mediated by multiple enzymes, including E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme), and E3 (ubiquitin ligase) [8–10]. Ubiquitin, a highly conserved 76 amino acid protein, is activated at its carboxyl-terminal glycine residue to form a high-energy thiol ester intermediate in a reaction catalyzed by E1. After activation, the E2 enzyme transfers the activated ubiquitin from E1 to a member of the E3 ligases. Finally, the E3 ligase attaches the ubiquitin protein via its C-terminal glycine to the lysine site of the target substrate, leading to ubiquitination of the substrate protein [11, 12]. In accordance with the number of ubiquitin molecules linked to a lysine residue, ubiquitination occurs via monomers known as monoubiquitins and in the form of isopeptide-linked polymers, designated polyubiquitin. Polyubiquitin can be linked at seven different sites, as the ubiquitin molecule possesses seven lysine residues (K6, K11, K27, K29, K33, K48, and K63). Typically, K48-linked polyubiquitylation is a canonical recognition signal for ubiquitin-dependent proteasomal degradation [13], whereas K63-linked polyubiquitin chains usually represent a nondegradative fate, including protein–protein interactions, activation, or DNA repair [14, 15]. Deubiquitination, the reverse reaction that removes ubiquitin modifications from substrates, is performed by deubiquitinating enzymes (DUBs) [16]. The UPS system and classification of E3 ubiquitin ligases and deubiquitinating enzymes are shown in Fig. 1. In recent years, the involvement of E3 ubiquitin ligases and DUBs in the regulation of hepatic steatosis and inflammation in MASLD has received increasing attention.

In this review, we searched PubMed, Web of Science, and Google Scholar for the terms "E3 ubiquitin ligase", "deubiquitinating enzyme", "ubiquitin proteasome system", "MASLD (NAFLD)", MASH (NASH)", or combinations thereof to identify the most recent research, systematically reviewed and profiled the role and mechanism of two major ubiquitination players, E3 ubiquitin ligases and DUBs, in the occurrence and development of MASLD, with the goal of providing more evidence for potential strategies targeting E3 ubiquitin ligases and DUBs for the treatment of MASLD.

E3 ubiquitin ligases and MASLD

E3 ubiquitin ligases are numerous and exhibit substrate specificity [17]. More than 600 E3 ubiquitin ligases have been identified in the human genome [18]. E3 ubiquitin ligases are classified into three major subfamilies on the basis of their characteristic domains and ubiquitin transfer patterns: the truly interesting new gene (RING), which is homologous to the E6AP carboxyl terminus (HECT), and RING-between-RING (RBR) [19]. Among them, RING-type E3 ubiquitin ligases are the largest class [20, 21]. They catalyze the direct transfer of ubiquitin molecules from E2-ubiquitin to substrate proteins [22–24]. HECT-type E3 ligases transfer ubiquitin via a two-step process. The HECT domain first receives ubiquitin on a cysteine residue from E2-ubiquitin and then transfers ubiquitin to the lysine residues of substrate proteins [22, 23, 25]. RBR-type E3 ubiquitin ligases possess RING domains but have a ubiquitin transfer mode similar to that of HECT-type E3 ligases [19, 23]. Here, we summarize the E3 ligases involved in the pathogenesis or progression of MASLD/MASH and classify them according to their pathways or functions (Fig. 2; Table 1).

E3 ligases targeting the MAPK signaling pathway

The mitogen-activated protein kinase (MAPK) signaling pathway plays a crucial role in various physiological and pathological processes by regulating cellular stress, apoptosis, and inflammatory responses [26]. The MAPK signaling pathway consists of three main cascades of kinases, mitogen-activated protein kinase kinase kinase (MAPKKK), mitogen-activated protein kinase kinase kinase (MAPKK), and MAPK, which are activated sequentially to form a cascade [27]. The classical MAPK signaling pathways include the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 pathways. The relationships between E3 ligases and DUBs and the MAPK pathway have been well studied and summarized in cancer [28], but the related summary in MASLD/MASH is still incomplete. Therefore, we conclude that E3 ligases and DUBs (see below for details) affect the MAPK signaling pathway in MASLD/MASH.

Transforming growth factor-β-activated kinase 1 (TAK1, also known as MAP3K7) is a member of the MAP3K family that can activate the downstream JNK and p38 pathways through MAPKK and IκB kinase

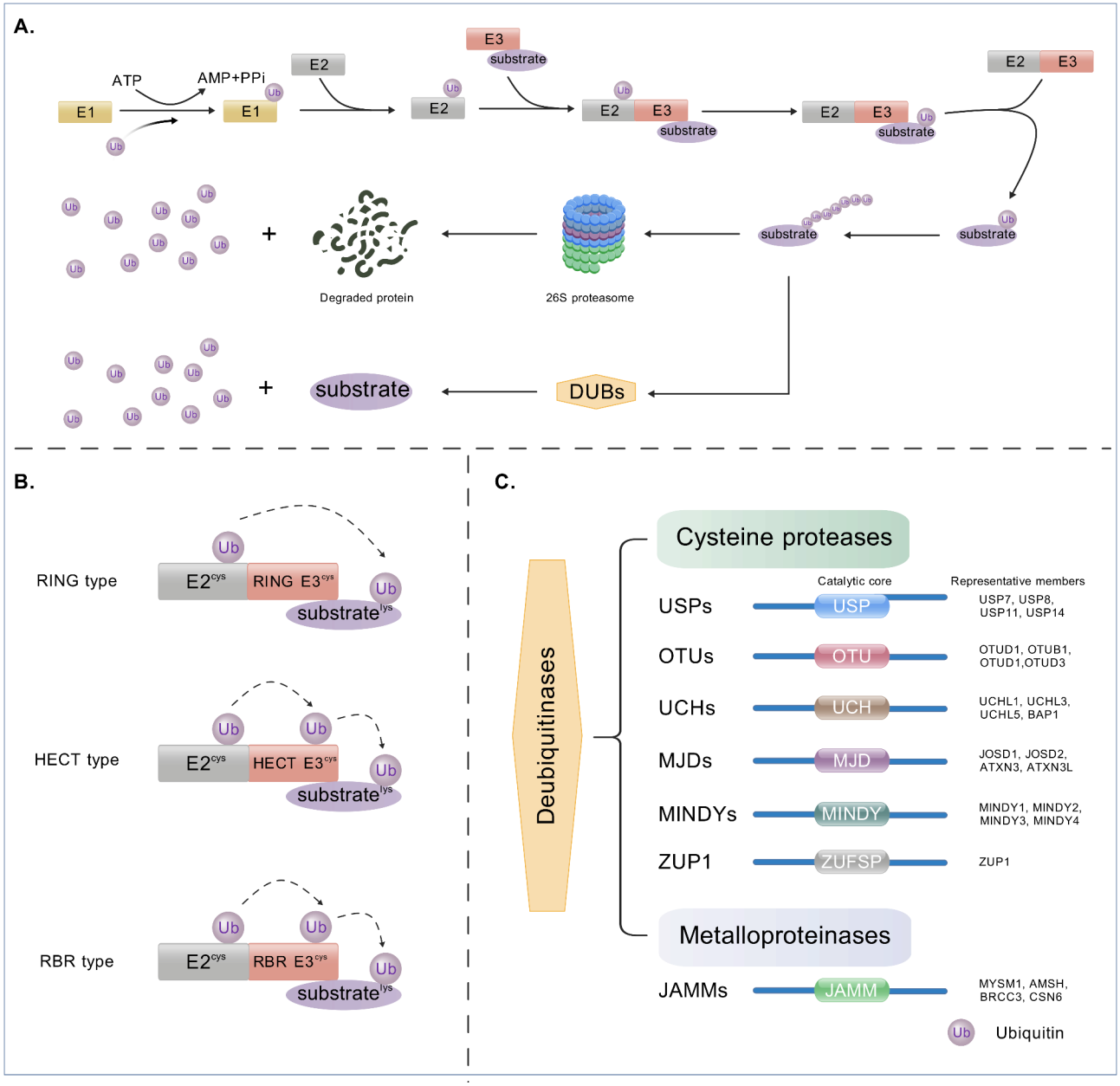

Fig. 1 Overview of the UPS and classification of E3 ubiquitin ligases and DUBs. **A**. Ubiquitination is a cascade of reactions that involves E1, E2, and E3 ligases. Substrate proteins labeled with ubiquitin are degraded by the 26 S proteasome or reversed by DUBs. The grafted ubiquitin monomers are then re-utilized. **B**. E3 ubiquitin ligases are categorized as RING-type, HECT-type, or RBR-type ligases on the basis of their structure and ubiquitin transfer pathway. **C**. DUBs can be divided into two main categories on the basis of their structure: cysteine proteases and catalytic zinc ion metalloproteases (metalloproteinases). Cysteine proteases can be subdivided into six classes on the basis of their catalytic core: USPs, OTUs, UCHs, MJDs, MINDYs, and ZUFSP/ZUP1s. Metalloproteases refer only to JAMMs. (Created with BioGDP.com [254])

(IKK). Many E3 ligases regulate the JNK/p38 pathway by targeting TAK1.

Tripartite motif 8 (TRIM8), a ubiquitously expressed E3 ubiquitin protein ligase, has been linked to many biological processes, including the innate immune response, carcinogenesis, apoptosis, and inflammation [29, 30]. TRIM8 expression is increased in the livers of MASLD/MASH patients and in the MASH mouse model [31]. Indeed, the downregulation of TRIM8 expression led to a decrease in IR, hepatic lipid accumulation, inflammation, and fibrosis in both high-fat diet (HFD)-induced and gene-deficient (ob/ob)-induced MASH mice. Further studies revealed that the activation of TAK1 is required for TRIM8-regulated steatohepatitis. TRIM8 directly binds to TAK1 and induces TAK1 ubiquitination, leading to the activation of the downstream JNK/p38 and nuclear

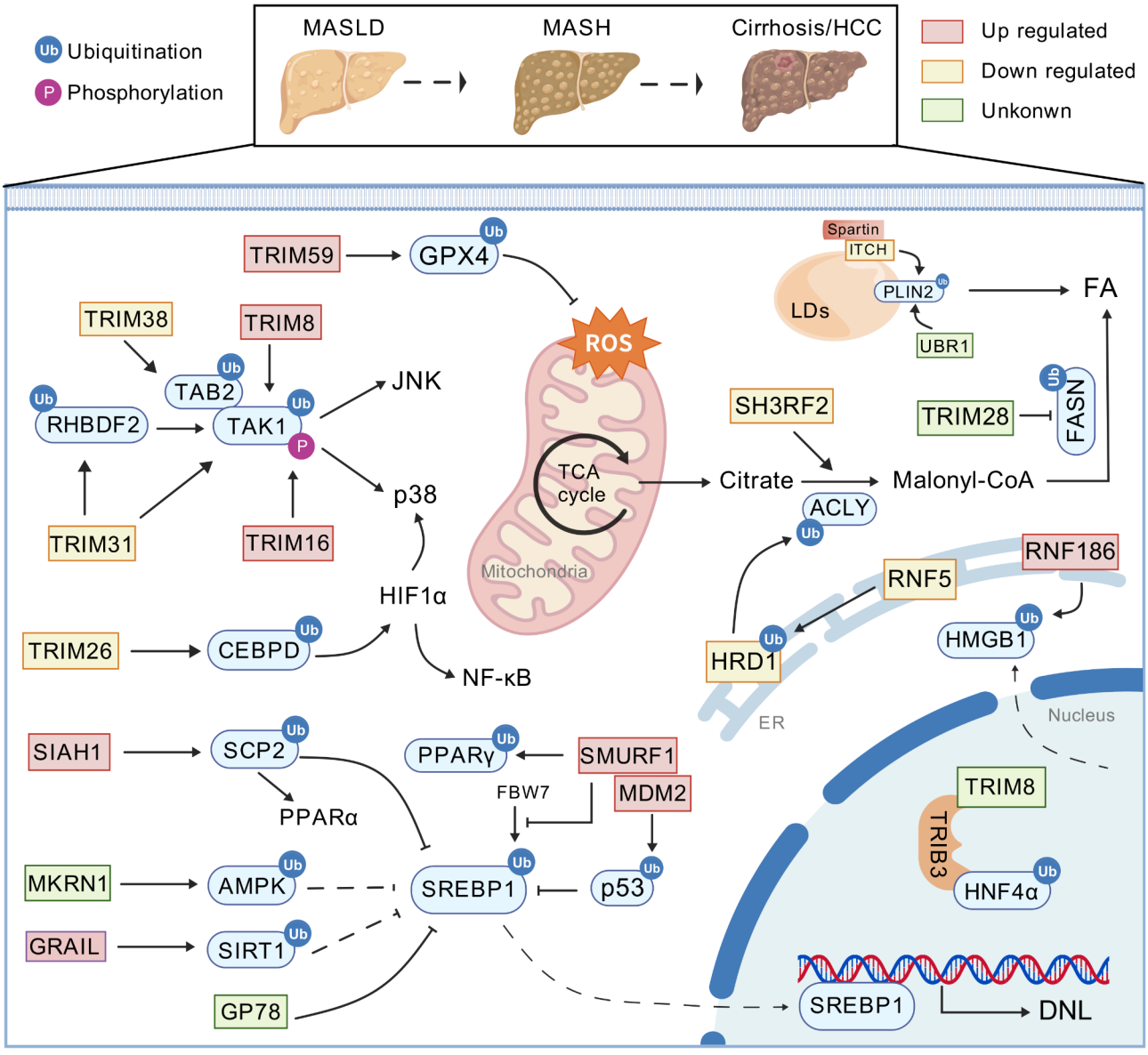

Fig. 2 Role of E3 ubiquitin ligases in MASLD/MASH. Multiple upregulated and downregulated E3 ligases are involved in MASLD/MASH pathogenesis (pink, upregulated; yellow, downregulated; green, unknown changes). Among them, TRIM8, TRIM38, TRIM16, and TRIM31 act through the MAPK pathway. MKRN1 affects the AMPK pathway, and SIAH1 affects the PPAR pathway. SMURF1, HRD1, and TRIM28 have been shown to regulate DNL, hence influencing hepatic lipid deposition. Upregulation of TRIM59 exacerbates MASLD/MASH progression by promoting mitochondrial dysfunction

factor kappa B (NF-κB) signaling pathways. The ability of TRIM8 to activate TAK1 depends on its E3 ligase activity. Given that K63-linked polyubiquitination of TAK1 is reportedly required for its activation [32], whether TRIM8 activates TAK1 via K63-linked polyubiquitination requires further exploration. In adipocytes with insulin resistance induced by lipopolysaccharides (LPS), the level of TRIM8 is increased. Silencing TRIM8 inhibits inflammation and IR by upregulating dual-specificity phosphatase 14 (DUSP14) to dephosphorylate the MAPK signaling pathway [33]. Therefore, targeting TRIM8 to regulate the MAPK pathway may play a role in various diseases.

Tripartite motif 31 (TRIM31) is involved in a wide range of biological processes, particularly the innate immune response, and in cancer and inflammation [34, 35]. In liver samples from mice and humans with MASH, hepatic TRIM31 expression is downregulated [36]. Moreover, TRIM31 mRNA levels in the liver are negatively correlated with fatty liver severity. Mechanistic studies revealed that TRIM31 mitigates MASLD by suppressing rhomboid 5 homolog 2 (RHBDF2)-MAP3K7 signaling, the activation of which has been reported to contribute

Table 1 Role of E3 ubiquitin ligases in MASLD/MASH

Name	Alteration in MASLD	Targeting substrate	Ubiquitination site	Ending	Cell	Experimental MASLD model	Transgenic mice model	Disease phenotype	Effect	Reference
TRIM8	Up-regulation in hepatocytes	TAK1	/	Phosphorylation activation	Primary hepatocytes	HFD, Ob/ob	Trim8-HOE, Trim8-HKO	MASLD, MASH	Pro-insulin resistance; Pro-steatosis; Pro-inflammation; Pro-fibrosis	[31]
TRIM31	Down-regulation in hepatocytes	RHBDF2	K48	Degradation	Primary hepatocytes, L02, THKO-L02, HEK293T	HFD, Ob/ob, HFHF	Trim31-HKO, Trim31-HOE, Rhbdf2-HKO, Trim31 and Rhbdf2 double HKO (DHKO)	MASLD, MASH	Anti-steatosis; Anti-inflammation; Anti-fibrosis; Anti-insulin resistance	[36]
TRIM31	Down-regulation in hepatocytes	MAP3K7	K48	Degradation	Primary hepatocytes, HepG2, SMMC-7721, L02, LX2, HepG2, SMMC-7721	HFHC, WTDF, Ob/ob, HFMCD, HFHC + DEN	Trim31-HKO, Trim31-HOE, Map3k7-HOE, Map3k7-Trim31-HOE	MASH, HCC	Anti-steatosis; Anti-inflammation; Anti-fibrosis; Anti-insulin resistance; Anti-tumor	[38]
TRIM16	Up-regulation in hepatocytes	p-TAK1	K48	Degradation	Primary hepatocytes, L02, HEK293, HEK293T	HFD, HFHC	Trim16-HKO, Trim16-OE	MASLD, MASH	Anti-steatosis; Anti-inflammation; Anti-fibrosis; Anti-insulin resistance	[47]
TRIM38	Down-regulation in hepatocytes	TAB2	/	Degradation	Primary hepatocytes, HEK293T	HFD, HFHC	Trim38-KO	MASLD, MASH	Anti-steatosis; Anti-inflammation; Anti-fibrosis; Anti-insulin resistance	[48]
MKRN1	/	AMPKα	K48	Degradation	MEF, HepG2, HEK293T	HFD	Mkrn1-KO,	MASLD	Pro-steatosis; Pro-insulin resistance	[57]
SIAH1	Up-regulation in hepatocytes	SCP2	/	Degradation	Hepa1-6, AML12, HEK293T	HFD	Siah1-OE, Siah1-KO	MASLD	Pro-steatosis	[70]
SMURF1	/	PPARγ	K63	Transcription inhibition	Primary hepatocytes, Hep3B, AML12	HFD	Smurf1-KO	MASLD	Anti-steatosis	[74]

Table 1 (continued)

Name	Alteration in MASLD	Targeting substrate	Ubiquitination site	Ending	Cell	Experimental MASLD model	Transgenic mice model	Disease phenotype	Effect	Reference
SMURF1	Up-regulation in hepatocytes	SREBP1C	/	Degradation inhibition	Primary hepatocytes, HepG2, HEK293T	HFD	Smurf1-KO	MASLD	Pro-steatosis; Pro-insulin resistance	[82]
SMURF1	Up-regulation in hepatocytes	MDM2	/	Degradation inhibition	Primary hepatocytes, HepG2, HEK293T	HFD	Smurf1-KO	MASLD	Pro-steatosis	[85]
GP78	/	/	/	/	HepG2, THLE-3, MEF	/	Gp78-KO	/	Anti-steatosis; Anti-inflammation; Anti-fibrosis; Anti-tumor	[90]
SH3RF2	Down-regulation in hepatocytes	ACLY	K48	Degradation	Primary hepatocytes	HFD HFHC	Sh3rf2-HKO	MASLD	Anti-steatosis	[95]
RNF5	Down-regulation in hepatocytes	HRD1	K48 K33	Degradation	Primary hepatocytes, L02, HEK-293T	HFD, HFHC	Rnf5-HKO	MASLD, MASH	Anti-steatosis; Anti-inflammation; Anti-fibrosis	[99]
HRD1	Down-regulation in hepatocytes	ACLY	/	Degradation	Primary hepatocytes, HEK-293T, HepG2, Hepa1-6	Db/db, Ob/ob	/	MASLD	Anti-steatosis; Anti-insulin resistance	[100]
TRIM28	/	FASN	K48	Degradation	Primary hepatocytes, L02, HEK293T	HFHC	Snx8-HOE, Snx8-KO	MASLD	Anti-steatosis	[103]
TRIM26	Down-regulation in hepatocytes	CEBPD	/	Degradation	Primary hepatocytes, L02	HFHC, WTDF, HFMCD	Trim26-HKO, Trim26-HOE, Cebpd-HKO, Trim26 and Cebpd double HKO (DHKO)	MASH	Anti-steatosis; Anti-inflammation; Anti-fibrosis; Anti-insulin resistance	[112]
GRAIL	Up-regulation in hepatocytes	SIRT1	K48	Degradation	Primary hepatocytes, HepG2, AML12, THLE-2	HFD	Grail-KO	MASLD	Pro-steatosis; Pro-hepatocellular injury	[118]

Table 1 (continued)

Name	Alteration in MASLD	Targeting substrate	Ubiquitination site	Ending	Cell	Experimental MASLD model	Transgenic mice model	Disease phenotype	Effect	Reference
UBR1	/	PLIN2	K48	Degradation	Drosophila Schneider 2 (S2), Spodoptera fruiperda 9 (Sf9), HEK293S, HEK293T, HepG2, Hela, Huh7, HCCLM3, L02	HFD, Ob/ob	/	MASLD	Anti-steatosis	[127]
RNF186	Up-regulation in hepatocytes	HMGB1	K48 K63	Degradation	Primary hepatocytes, L02	HFD	Rnf186-KO, Rnf186-OE	MASLD	Pro-steatosis	[138]
TRIM8	/	HNF4α	K48	Degradation	Primary hepatocytes, HepG2, HEK-293T	HFF, CDAHFD	Trib3-HKO, Trib3-HOE	MASLD	Pro-insulin resistance; Pro-steatosis	[139]
ITCH	Down-regulation in hepatocytes	/	/	/	/	MCD, DEN + HFD	Itch-KO	MASH, HCC	Anti-steatosis; Anti-inflammation; Anti-fibrosis	[148]
TRIM59	Up-regulation in hepatocytes	GPX4	/	Degradation	L02, AML12	HFD	Trim59-KO	MASLD	Pro-steatosis; Pro-inflammation; Pro-ferroptosis	[157]
TRIM67	Up-regulation in hepatocytes	/	/	/	HepG2	HFD	Trim67-KO	MASLD, MASH	Anti-steatosis; Anti-inflammation; Anti-fibrosis	[161]

to the occurrence of MASLD [37]. TRIM31 interacts with RHBDF2 and promotes its degradation via K48-linked polyubiquitination, which results in a decrease in MAP3K7 phosphorylation and downstream inflammatory signaling, thus alleviating MASLD.

A recent study revealed that TRIM31 confers protection against MASH not only by regulating MAP3K7 through RHBDF2 but also by directly inducing K48-linked polyubiquitination of MAP3K7 [38]. MASH-associated HCC was significantly suppressed in hepatic TRIM31-overexpressing (TRIM31-HOE) mice fed a diethylnitrosamine (DEN) or high-fat/high-cholesterol (HFHC) diet, suggesting that TRIM31 is a protective molecule against MASH-related HCC. An early study revealed that upregulated TRIM31 facilitates the progression of hepatocellular carcinoma [39], which is inconsistent with its role in MASH-related HCC; however, the specific mechanism of TRIM31 in MASH-related HCC is unclear and requires further exploration.

The herbal component mulberry has been reported to possess anti-inflammatory and antioxidant properties [40]. Mulberrin treatment of carbon tetrachloride (CCl_4)-induced liver fibrosis upregulates TRIM31, which mediates nuclear factor E2-related factor 2 (Nrf2) signal transduction and exerts anti-inflammatory and antioxidant actions that combat fibrosis [41]. The protective role of TRIM31 in metabolism is conducive to homeostasis.

Tripartite motif 16 (TRIM16), a member of the TRIM family, lacks the RING domain and functions via the B-BOX structure [42]. Previous studies have suggested that TRIM16 has beneficial effects on cardiac hypertrophy, autophagy, and tumor suppression [43–45]. In

diabetic nephropathy (DN), cordyceps cicadae polysaccharides (CCPs) have been reported to protect podocytes from inflammatory, apoptotic, and oxidative stress damage via the miR-30a-3p/TRIM16 axis [46]. TRIM16 was recently recognized as an inhibitor of lipotoxicity [47]. TRIM16 is markedly upregulated in response to lipotoxicity in hepatocytes. The transcription factor early growth response protein 2 (EGR2) has been identified as a mediator of TRIM16 expression in the context of lipotoxicity. Mechanistically, phosphorylated TAK1 (p-TAK1) is an essential target of TRIM16 in MASH. TRIM16 interacts with p-TAK1 and promotes K48-linked polyubiquitination and proteasomal degradation of p-TAK1, leading to inhibition of the JNK/p38 signaling pathway to block MASH. These findings support TRIM16 as a novel suppressor of lipotoxicity and indicate that targeting the TRIM16-p-TAK1 axis represents a promising therapeutic strategy for MASH [47].

The downregulation of tripartite motif 38 (TRIM38) is closely associated with the development of MASH [48]. TRIM38 overexpression promoted the degradation of TAK1-binding protein 2 (TAB2), thus inhibiting the TAK1-MAPK signaling pathway and alleviating MASH progression. TRIM38 suppresses the NF-κB pathway by degrading TAB2 in a lysosomal manner [49]. However, as an E3 ubiquitin protein ligase, the mechanism of action of TRIM38 in MASLD requires further investigation.

E3 ligases targeting the AMPK signaling pathway

AMP-activated protein kinase (AMPK) is a heterotrimeric complex that can sensitively perceive changes in intracellular ATP levels, regulate energy metabolism, and maintain homeostasis [50]. Under conditions such as oxidative stress, glucose deprivation, and mitochondrial damage, AMPK activation promotes ATP synthesis and inhibits ATP breakdown, thereby maintaining energy balance [51]. The AMPK signaling pathway has been well studied in various metabolic diseases, including diabetes mellitus, obesity, MASH, and cancer [52, 53]. Below, we summarize the E3 ubiquitin ligases that target the AMPK signaling pathway in MASLD/MASH.

Makorin ring finger protein 1 (MKRN1) targets various substrates, such as smad nuclear-interacting protein 1 (SNIP1), p53, fas-associated protein with death domain (FADD), and peroxisome proliferator-activated receptor γ (PPARγ), which are involved in neoplasia, cell apoptosis, and adipocyte differentiation [54–56]. Hepatic lipid accumulation in HFD-induced MASLD mice was significantly reduced when MKRN1 expression was ablated by adenoviruses expressing short hairpin RNA (shRNA) targeting MKRN1 (Ad-shMKRN1) [57]. Notably, the substantial reduction in lipid accumulation observed in MKRN1-silenced livers was reversed by hepatic knockdown of the α2 subunit of AMPK. Thus, MKRN1 deficiency protects against HFD-induced MASLD in an AMPK-dependent manner [57]. MKRN1 interacts with AMPK and promotes the ubiquitination and proteasomal degradation of the K48 linker of the AMPKα1 and α2 subunits. Since MKRN1 is present in the liver and adipose tissue, the development of organ-specific competitive inhibitors of MKRN1-AMPK interactions or inhibitors that eliminate the ubiquitination of MKRN1 could provide new therapeutic approaches for metabolic syndrome. PPARγ has been reported to be a substrate of MKRN1 and to play a greater regulatory role in lipid metabolism; however, no changes in PPAR have been observed in the absence of MKRN1, which requires further investigation [56, 58].

E3 ligases targeting the PPAR signaling pathway

The peroxisome proliferator-activated receptor (PPAR), a member of the nuclear receptor superfamily, is involved in the regulation of glycolipid metabolism via a sophisticated signaling network upon ligand binding. To date, three subtypes have been identified: PPARα, PPARγ, and PPARβ/δ [59]. Multiple metabolic disorders manifested in MASLD have led to an increasing number of studies on PPAR [60, 61]. PPAR is widely involved in MASLD/MASH by regulating IR, oxidative stress, adipogenesis, and fibrosis [62, 63]. The development of various PPAR agonists has provided new therapeutic options for metabolic disorders, such as T2DM and MASLD [64–66].

The highly conserved E3 ubiquitin ligase seven in absentia homolog 1 (SIAH1) plays a role in various cancers [67–69]. SIAH1 expression is elevated in patients with MASLD and in mice [70]. SIAH1 knockdown significantly inhibited HFD-induced hepatic lipid accumulation. Ubiquitinomic analysis of cells with or without SIAH1 knockdown revealed that ubiquitinated proteins in the PPAR pathway were significantly enriched. Mechanistically, SIAH1 promoted the ubiquitination and degradation of sterol carrier protein 2 (SCP2) in the PPAR pathway. SCP2 levels further regulate PPARα expression, which is involved in MASLD progression [70].

The E3-ubiquitin ligase smad ubiquitination regulatory factor 1 (SMURF1) plays an important role in osteoblast function, carcinogenesis, autophagy, and cell differentiation [71–73]. Mice deficient in SMURF1 were shown to have considerable lipid droplet (LD) aggregation in the liver as they aged, which was observed only in mice on a mixed black Swiss × 129/SvEv (BL) background but not in those on a C57BL/6 N (B6) background [74]. SMURF1 ablation significantly exacerbated HFD-induced hepatic steatosis in both strains, suggesting that SMURF1 plays a systemic role in regulating lipid accumulation. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of wild-type (WT) and SMURF1-KO mice revealed enrichment of the PPAR signaling pathway. Treatment

of SMURF1-KO mice with the PPARγ antagonist GW9662 protected the mice from hepatosteatosis. In vitro experiments demonstrated that SMURF1 induces K63-linked ubiquitination of PPARγ and suppresses its transcriptional activity [74]. The MASLD phenotypes in SMURF1-KO mice can be attributed to the increased transcriptional activity of PPARγ, which in turn increases the expression of genes involved in lipogenesis and fatty acid transport.

E3 ligases targeting DNL

In healthy individuals, de novo synthesis (DNL) converts excess carbohydrates into triglycerides for storage. During times of high energy demand in the body, stored fat is mobilized and broken down to provide energy to other organs [75]. However, lipogenesis and lipolysis must be balanced to maintain homeostasis. Excessive DNL is an important risk factor for intrahepatic lipid deposition and the consequent development of MASLD. From citrate generated by the tricarboxylic acid (TCA) cycle to the first fatty acid product, succinate, three key enzymes are required in sequence: ATP citrate lyase (ACLY), acetyl-CoA carboxylase 1 (ACC1), and fatty acid synthase (FASN) [76]. Sterol regulatory element-binding protein-1 (SREBP1) is a key transcription factor that mediates fatty acid synthesis (FAS). Its activation promotes downstream transcription of ACC1, FAS, and stearoyl-CoA desaturase 1 (SCD1), which in turn promotes DNL. Aberrant expression of SREBP1, ACLY, ACC1, and FASN has been observed in the livers of MASLD mice [77–79]. Therefore, targeting the key factors of DNL has always been an important strategy for suppressing lipid deposition [80, 81]. Here, we categorized E3 ligases that target SREBP1 and the three rate-limiting enzymes of DNL, potentially providing additional directions for DNL inhibitors.

Regulation of SREBP1

SMURF1-KO mice with a B6 background fed a HFD for 19 weeks are not susceptible to developing liver steatosis [82]. The expression of SREBP1 was also reduced in SMURF1-deficient mice [83]. A mechanistic study revealed that SMURF1 binds to the helix-loop-helix (HLH) structural domain of SREBP1. This HLH structural domain has also been reported to bind to another E3 ubiquitin ligase, F-box and WD repeat domain-containing 7 (FBW7), which promotes SREBP1C ubiquitination and degradation [84]. During the pathogenesis of liver steatosis, SMURF1 preferentially interacts with SREBP1C to prevent SREBP1C from being ubiquitinated and degraded by FBW7. Another possible mechanism through which SMURF1 is involved in MASLD pathogenesis should be mentioned [85]. P53, a crucial tumor suppressor gene, is involved in the role of SMURF1 in MASLD development. Mechanistically, SMURF1 interacts with and stabilizes murine double minute 2 (MDM2), a p53-specific E3 ubiquitin ligase, to promote p53 ubiquitylation and degradation, leading to increased lipogenesis by inducing the transcription of SREBP1C [86].

In conclusion, SMURF1 plays a complex role in MASLD via the PPAR, AMPK, and MDM2-p53 signaling pathways. Interestingly, SMURF1 does not directly degrade its substrate via the proteasome but instead affects downstream proteins by reducing transcription, inhibiting competition, or stabilizing its content. Whether SMURF1 degrades specific proteins in MASLD and has a continuous effect on the entire spectrum of MASLD diseases, from MASH development to hepatocellular cancer, remains unclear. Casein kinase 2 interacting protein-1 (CKIP-1) promotes the ubiquitination and degradation of SMURF1, activates the Nrf2/ARE pathway, and ultimately ameliorates DN fibrosis [87]. SMURF1 also promotes the ubiquitination and degradation of Takeda G-protein-coupled receptor 5 (TGR5) to promote the development of diabetic nephropathy [88]. However, further investigations are needed to elucidate the comprehensive mechanism of SMURF1 in the pathogenesis of metabolic disorders.

GP78 was initially identified as a tumor autocrine motility factor receptor (AMFR) and subsequently as an ER-localized E3 ubiquitin ligase engaged in ER-associated degradation (ERAD) in response to ER stress [89]. GP78-knockout mice at 12 months of age exhibit typical hepatic lipid deposition, liver injury, and fibrosis, recapitulating age-related human MASH [90]. GP78 is a ubiquitin ligase that mediates the degradation of insulin-induced gene 1 (Insig-1), a key negative regulator of sterol-regulated proteolysis of SREBP1 [91]. Another study showed that liver-specific GP78 knockout reduces lipid accumulation and hepatic steatosis by decreasing Insig-1 levels to induce SREBP1 activation [92]. One possible explanation for this contradictory conclusion is that GP78 plays a completely different role in other organs, such as adipose tissue and the intestine, than in the liver. Further clarification of the roles and molecular mechanisms of GP78 in different organs to interpret these two conflicting observations is necessary.

Regulation of ACLY

The RING-type E3 ubiquitin ligase SH3 domain-containing ring finger 2 (SH3RF2) is involved in cancer and neurological diseases [93, 94]. SH3RF2 is downregulated in hepatocytes from human, mouse, and monkey fatty livers [95]. Both HFD- and HFHC diet-induced MASLD mice with hepatocyte-specific SH3RF2 deletion present increased body weight, hepatic steatosis, glucose intolerance, and lipid metabolic pathway activation, indicating that hepatocyte-specific SH3RF2 ablation is a critical

factor in the progression of diet-induced MASLD. ACLY has been identified as a candidate downstream molecule that mediates the role of SH3RF2 in MASLD. SH3RF2 reduces ACLY protein levels by promoting K48-linked ubiquitination-dependent degradation [95]. A lack of SH3RF2 in hepatocytes increases ACLY expression and the resulting accumulation of acetyl-CoA, leading to increased cholesterol synthesis, which directly promotes lipid deposition. Therefore, SH3RF2/ACLY is a promising therapeutic target for treating MASLD.

ER stress induced by pathological stimuli or chemotherapeutic drugs activates the unfolded protein response (UPR) to alleviate stress and restore ER homeostasis [96]. Ring finger protein 5 (RNF5), an endoplasmic reticulum-based E3 ubiquitin protein ligase, plays a crucial role in the ER stress response and the UPR [97–99]. RNF5 expression is markedly decreased during MASH progression [99]. Mechanistically, RNF5 interacts with HMG-CoA reductase degradation protein 1 (HRD1), an ER-associated ubiquitin ligase that controls cholesterol production by regulating the rate-limiting enzyme HMGCR. RNF5 promotes the K48- and K33-linked ubiquitination of HRD1, leading to its degradation. Both in vitro and in vivo studies have shown that HRD1 is required for the role of RNF5 in MASH pathogenesis [99].

Interestingly, HRD1, an E3 ligase, can directly target ACLY and promote its degradation via ubiquitination [100]. In vitro experiments demonstrated that HRD1 promotes ACLY ubiquitination and subsequent proteasomal degradation, resulting in the inhibition of hepatocyte lipid synthesis. In db/db mice, downregulation of HRD1 expression contributes to ACLY overexpression and its ability to promote MASLD expression. However, this observation was revealed only in db/db mice, which are characterized by obesity, fatty liver, and T2DM due to the loss of leptin receptor function. Another study reported that liver-specific HRD1 knockdown prevents steatosis in mice with diet-induced MASLD. One possible explanation for this inconsistency might be that the pathological mechanisms in high-fat diet-fed and db/db mice are different [101]. Whether RNF5, which targets HRD1, indirectly regulates ACLY is worth exploring.

In DN, epithelial–mesenchymal transition (EMT) of renal tubular epithelial cells is a major cause of renal failure. X-Box binding protein 1 (XBP1)-HRD1 mediates the ubiquitination of Nrf2, thereby promoting ferroptosis of the renal tubular epithelium, exacerbating EMT, and leading to poor prognosis [102]. However, further studies are needed to comprehensively elucidate the roles and mechanisms of HRD1.

Regulation of FASN

As mentioned above, FASN is a key rate-limiting enzyme in DNL that plays an important role in fatty acid synthesis and extension in the liver. In patients with MASLD, a significant upregulation of FASN was detected [103]. Clinical trials involving FASN inhibitors have confirmed their potential as MASLD targets [104, 105]. Notably, FASN is not a unidirectional lipid regulator. The regulatory role of FASN varies significantly under physiological and pathological conditions [106], making the discovery of new FASN targets essential. Sorting nexin 8 (SNX8) was identified as a binding protein for FASN by interactome analysis in FASN-overexpressing cells [103]. SNX8 can act as a scaffold protein by recruiting tripartite motif 28 (TRIM28) to form a ubiquitin ligase complex that promotes K48-linked ubiquitination and degradation of FASN. This process inhibits the downstream elongation of fatty acids and the production of unsaturated fatty acids. Cells and mice with SNX8 knockout present more severe lipid accumulation under steatosis stimulation. Targeting the TRIM28/SNX8-FASN axis is a promising new therapeutic approach for treating MASLD.

Other E3 ligases involved in MASLD/MASH

TRIM26

Tripartite motif 26 (TRIM26) has been shown to be involved in various cancers and the immune response [107–110]. Recent studies have shown that TRIM26 knockout enhances liver regeneration through the Wnt/β-catenin pathway [111]. TRIM26 expression decreases significantly in hepatocytes treated with palmitate/oleic acid (PAOA) [112]. CCAAT/enhancer binding protein delta (CEBPD), a transcription factor involved in inflammation and lipid regulation [113, 114], is a direct target of TRIM26. Mechanistically, TRIM26 promotes polyubiquitination and subsequent proteasomal degradation of CEBPD, thus inhibiting downstream hypoxia-inducible factor-1α (HIF1A) signaling, including p38, NOS2, and p65, and thereby inhibits MASH progression. The results of this study are consistent with previous findings that TRIM26 inhibits CCL_4-induced hepatic fibrosis, confirming the protective effect of TRIM26 on MASH through its ubiquitination function [115].

GRAIL

The gene related to anergy in lymphocytes (GRAIL) is an E3 ubiquitin ligase that plays a role in a variety of biological processes, such as regulating T-cell dysfunction, modulating adipocyte differentiation, and promoting HFD-induced obesity [116, 117]. Upregulated GRAIL expression was observed in the livers of humans and mice with MASLD [118]. HFD-fed GRAIL-KO mice presented considerably low levels of hepatic lipid accumulation [118]. CoIP and ubiquitination experiments

confirmed that GRAIL interacts with sirtuin 1 (SIRT1) and promotes its K48-linked ubiquitination, resulting in decreased SIRT1 protein levels. The role of SIRT1 in MASLD has been reported in multiple studies [119–121], highlighting its importance.

UBR1

Ubiquitin protein ligase E3 component N-recognin 1 (UBR1) is a novel mammalian protein quality control (PQC) system regulator [122]. Cells lacking UBR1 are hypersensitive to ER stress-induced apoptosis. Hepatocytes in MASLD livers are characterized by an abundance of LDs that are highly dynamic in the intracellular environment [123]. These dynamic properties of LDs are regulated by numerous proteins, among which perilipin 2 (PLIN2) is an LD-stabilizing protein that inhibits LD hydrolysis and thus functions as an aggravating factor in hepatic steatosis [124]. A high-protein diet is an effective treatment for MASLD [125, 126]. Dietary amino acid deficiency induces hepatic steatosis by stabilizing PLIN2 and increasing the number of LDs [127]. Supplementation with essential amino acids, especially leucine and isoleucine, induces the degradation of PLIN2, thus ameliorating hepatic steatosis. Leucine and isoleucine directly bind to and activate the E3 ubiquitin ligase UBR1, increasing PLIN2 ubiquitination and subsequent degradation, thereby facilitating hepatic LD clearance and preventing steatosis [127]. Therefore, elevated UBR1 activity is a potential target for the treatment of MASLD. The development of molecular gels to reduce the distance between UBR1 and PLIN2 or the use of proteolysis-targeting chimera (PROTAC) technology to increase PLIN2 degradation is a promising strategy for MASLD treatment.

RNF186

Lipophagy, the degradation of LDs in lysosomes, is a form of selective autophagy that plays an important role in regulating cellular lipid homeostasis [128–130]. A decrease in lipophagy leads to fat accumulation, which triggers steatosis. The relationship between abnormal lipophagy and hepatic steatosis has been increasingly reported [131–133]. Ring finger protein 186 (RNF186) has been shown to have increased expression in MASLD and can induce ER stress, impair insulin sensitivity, and regulate glucose–lipid metabolism through the AMPK–mTOR signaling pathway involved in the MASLD process [134–136]. RNF186 induces autophagy in colonic epithelial cells and regulates intestinal homeostasis [137]. In one study, researchers reported that in HFD-induced MASLD, the deletion of RNF186 destroyed LDs by increasing the expression of the autophagy marker LC3B-II, autophagosome formation, and autophagic flux [138]. Mechanistically, increased RNF186 in MASLD leads to the translocation of high mobility group box 1 (HMGB1) from the nucleus to the cytoplasm and promotes the subsequent K48- and K63-linked ubiquitinated proteasomal degradation of HMGB1. This study elucidates the mechanism by which RNF186 functions in MASLD and provides a theoretical foundation for the treatment of MASLD by targeting E3 ubiquitin ligases.

TRIM8

In another study, TRIM8 formed an E3 ubiquitin ligase complex with tribbles homolog 3 (TRIB3), which was increased in MASH to promote MASH development [139]. TRIB3 serves as a reactor for a variety of stress responses, such as ER stress and oxidative stress, and has been associated with T2DM, cancer, and hepatic fibrosis [140–143]. Under conditions of hepatic-specific TRIB3 deficiency, the lipid degeneration and IR in the liver caused by high-fat and high-cholesterol plus high-fructose/sucrose (HFF) or choline-deficient L-amino acid-defined, high-fat (CDAHF) diets are significantly ameliorated. TRIB3 often interacts with other proteins to participate in various pathological processes [142, 143]. Hepatic nuclear factor 4α (HNF4α) was identified as a substrate of TRIB3, and its level was strongly inhibited by TRIB3. Mechanistically, TRIB3 recruits TRIM8 and forms a complex with it to promote the K48-linked ubiquitinated degradation of HNF4α. Reduced HNF4α promotes hepatic steatosis and fibrosis and accelerates the development of MASH [144, 145].

ITCH

Itchy E3 ubiquitin-protein ligase (ITCH), a HECT-type E3 ubiquitin ligase, was first described in a genetic study on mouse coat color gene mutations in 1998 [146]. Spartin was shown to act as an adaptor protein that activates ITCH. Activated ITCH is recruited to lipid droplets and promotes ubiquitinated degradation of PLIN2 [147]. ITCH expression is downregulated in the liver during MASLD [148]. Elevated levels of branched-chain amino acids (BCAAs) are associated with MASLD development. Transcriptomic analysis of the livers of obese women revealed an association between ITCH and BCAA degradation enzymes [148]. Loss of ITCH results in an increase in circulating BCAA levels during MASLD, supporting a functional role for ITCH in the hepatic regulation of BCAA metabolism in MASLD. Loss of ITCH in the whole body mitigated the MASLD phenotype in methionine-choline-deficient (MCD) diet-fed mice. The ubiquitination of PLIN2 by ITCH in MASLD requires further confirmation.

Notably, knocking out ITCH in apolipoprotein E knockout (ApoE-/-) atherosclerotic mice inhibited the ubiquitination of the silent information regulator sirtuin 6 (SIRT6) and SREBP1, reduced circulating cholesterol,

and thereby alleviated atherosclerosis [149]. Whether the specific molecular mechanisms by which ITCH functions in MASLD are the same as those in atherosclerosis warrants further exploration.

TRIM59

Some studies have suggested a connection between MASLD and ferroptosis. For example, some patients with MASLD show iron deposition in the liver [150], and iron depletion can improve IR and liver damage in these patients [151]. Arbutin and melatonin can inhibit ferroptosis in the liver and ameliorate fatty liver [152, 153]. Tripartite motif 59 (TRIM59) has been reported to be involved in various cancers and immune diseases [154–156]. In MASLD, elevated TRIM59 levels ubiquitinate glutathione peroxidase 4 (GPX4) and reduce its expression [157]. GPX4 is a key enzyme that reduces lipid peroxides in biological membranes and is an important endogenous inhibitor of ferroptosis [158]. TRIM59 knockdown increased GPX4 levels, correcting steatosis and ferroptosis associated with HFD-induced MASLD. The discovery of the TRIM59/GPX4 axis elucidated the role of ferroptosis in MASLD and revealed that inhibiting ferroptosis represents a promising strategy for MASLD treatment.

TRIM67

Unlike other TRIM family members, tripartite motif 67 (TRIM67) is barely expressed in healthy livers and is expressed primarily in the nervous system [159, 160]. However, TRIM67 expression is induced in the livers of obese individuals and those fed a high-fat diet [161]. Both in vivo and in vitro experiments have demonstrated that TRIM67 knockout can reverse the MASLD phenotype induced by a HFD, inhibit ACC1 and SCD1, upregulate PPARα and carnitine palmitoyltransferase 1a (CPT1-α), and alleviate inflammation and fibrosis. The peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) is reported to regulate the expression of TRIM67 in response to obesity. However, further research is needed to elucidate how PGC-1α regulates TRIM67 in MASLD.

DUBs and MASLD

DUBs are pivotal regulators of ubiquitin homeostasis. Approximately 100 DUBs have been identified in the human genome, and these DUBs can be divided into two main categories on the basis of their structure: cysteine proteases and catalytic zinc ion metalloproteases (metalloproteinases). Cysteine proteases can be subdivided into six classes: ubiquitin-specific proteases (USPs), ovarian tumor proteases (OTUs), ubiquitin C-terminal hydrolases (UCHs), Machado–Joseph disease proteases (MJDs), motif interacting with ubiquitin (MIU)-containing novel DUB family (MINDY) proteases, and Zn-finger and UFSP domain protein (ZUFSP/ZUP1) proteases, of which the latter two were recently identified. JAB1/MPN/MOV34 metalloproteases (JAMMs) are metalloproteases [162, 163]. DUBs counter the signals induced by E3 ubiquitin ligases by removing ubiquitin from ubiquitylated substrates and regulating their activity and stability. Increasing evidence suggests that deregulated DUBs play crucial roles in the pathogenesis of MASLD/MASH. A thorough understanding of the complex roles of DUBs in MASLD/MASH could provide promising therapeutic strategies on the basis of pharmacological targeting of DUBs. Below, we describe the roles and mechanisms of DUBs in MASLD/MASH (Fig. 3; Table 2).

DUBs targeting the MAPK signaling pathway

Regulation of the TAK1/JNK/p38 pathway

Ubiquitin-specific peptidase 18 (USP18), also known as UBP43, was originally identified as a deISGlase that removes interferon-stimulated gene 15 (ISG15) from substrate proteins and regulates the antiviral activity of interferon against hepatitis C virus [164]. USP18 knockdown can promote the inflammatory response and apoptosis of pancreatic β cells induced by IFN, indicating that USP18 may be a suppressor gene in type 1 diabetes [165]. USP18 also plays a pivotal role in MASLD pathogenesis. Decreased protein levels of USP18 were detected in the livers of both MASH patients and HFD-induced or genetically obese mice [166]. A proteasome inhibitor (MG132), but not a lysosome inhibitor (chloroquine), alleviated the PA-induced reduction in USP18 in hepatocytes, suggesting that the decrease in USP18 in patients with MASH syndrome is due to accelerated protein degradation through the ubiquitin–proteasome pathway. Overexpression of USP18 via recombinant adenovirus in ob/ob mice ameliorated hepatic steatosis, IR, and inflammation. Mechanistically, USP18 alleviates MASLD progression by inhibiting TAK1 activation through its DUB activity, thus suppressing the downstream JNK and NF-κB signaling pathways [166].

Ubiquitin-specific peptidase 4 (USP4) is a negative regulator of cardiac dysfunction, which is dependent on its ability to deubiquitinate TAK1, subsequently blocking TAK1-(JNK1/2)/p38 signaling in response to hypertrophic stress [167]. The known effects of JNK in promoting MASLD progression and USP4 in regulating multiple inflammatory pathways have led to the investigation of the role of USP4 in MASLD [168, 169]. Downregulation of hepatic USP4 was found in both patients with MASLD and different MASLD mouse models [170]. Liver-specific USP4 overexpression increased insulin sensitivity and attenuated steatosis and inflammatory injury. The positive impact of USP4 is based on its capacity to

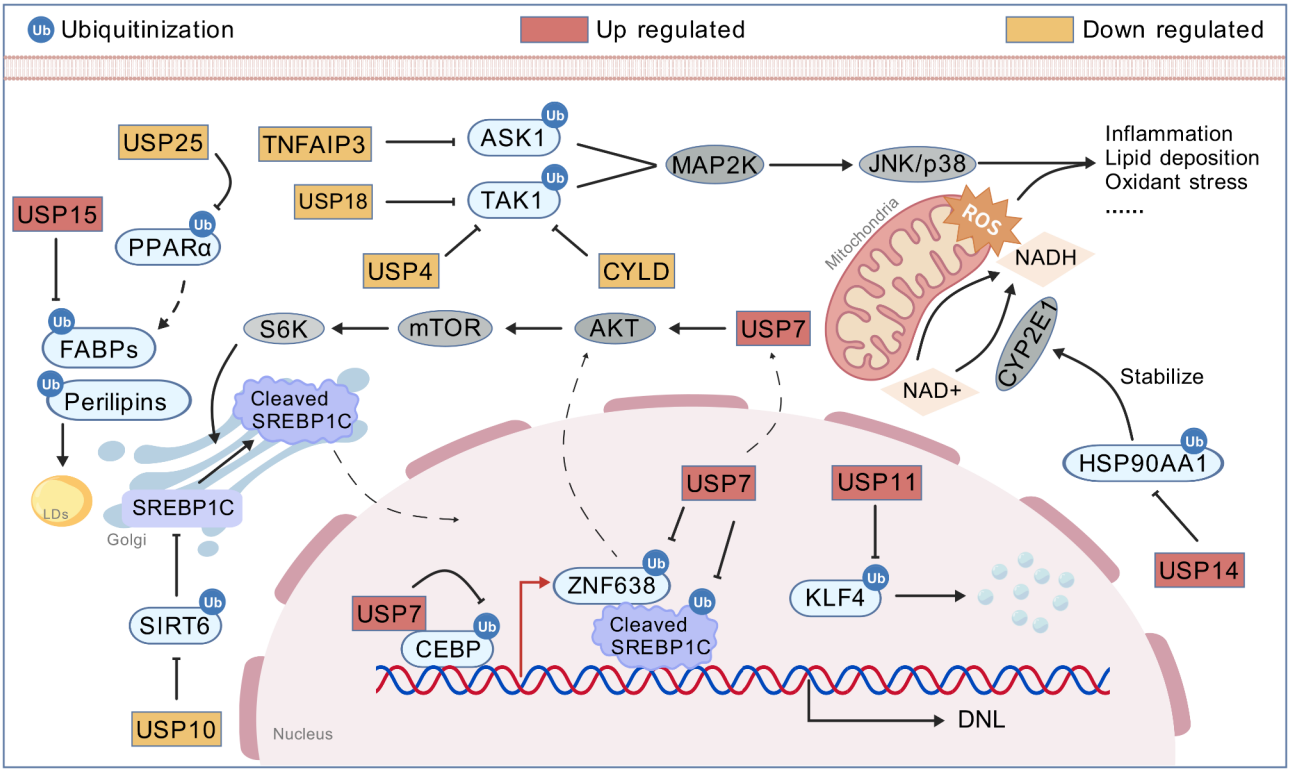

Fig. 3 Role of DUBs in MASLD/MASH. In the MASLD/MASH livers, there are also different changes in DUBs (red for upregulation and yellow for downregulation). USP4, USP18, CYLD, and TNFAIP3 remove ubiquitin from TAK1 or ASK1 and affect the downstream MAPK pathway. USP25 directly influences the ubiquitination of PPARα, which is involved in the disease process. USP7 promotes MASLD/MASH by facilitating the activation of SREBP1C and elevated levels of ZNF638. USP14 stabilizes HSP90AA1 and CYP2E1 and promotes the conversion of NAD + to NADH, leading to ROS accumulation

deubiquitinate TAK1 and inhibit TAK1/JNK signaling activation. Studies by the same group reported that the USP4-TAK1 axis is a potential therapeutic target for cardiac remodeling, a common MASLD-associated cardiac disorder [167]. The role of USP4 in ameliorating IR has been confirmed in T2DM patients [171]. Gastrodin has been reported to alleviate the progression of T2DM. Mechanistically, gastrodin activates the PI3K/AKT signaling pathway, which promotes the phosphorylation of GATA binding protein 1 (GATA1) and increases the transcription of USP4. USP4 further reduces the ubiquitination of insulin receptors and increases their expression. Therefore, USP4 may play an inhibitory role in various diseases associated with IR.

The deubiquitinating enzyme cylindromatosis (CYLD), a member of the USP family, inhibits NF-κB signaling through multiple mechanisms because of its potent deubiquitinating ability, thus playing a pivotal role in the immune response and tumorigenicity [172, 173]. Notably, CYLD functions as a key endogenous suppressor of MASH in both mice and monkeys [174]. Hepatic CYLD is downregulated in MASH model mice and individuals with MASLD or MASH and is associated with the severity of MASLD. E3 ligase tripartite motif 47 (TRIM47) interacts with and ubiquitinates CYLD to induce its degradation, leading to reduced CYLD levels during MASLD progression. Mechanistically, CYLD directly interacts with and removes the K63-linked polyubiquitin chain of TAK1, subsequently inhibiting the downstream TAK1–JNK/p38 pathway in hepatocytes [174]. Collectively, these data support the notion that CYLD is involved in the progression of MASLD/MASH. Thus, increasing the protein level of hepatocyte CYLD by reducing TRIM47 expression or supplementing CYLD could be a viable therapy for MASH in the clinical setting. Nevertheless, the possibility that CYLD in nonhepatic cells, such as Kupffer and hepatic stellate cells, may affect the course of MASH cannot be ruled out in this investigation.

Regulation of the ASK1/JNK/p38 pathway

Apoptosis signal-regulating kinase 1 (ASK1, also known as MAP3K5) belongs to the MAP3K family and activates the downstream JNK/p38 signaling pathway. Tumor necrosis factor alpha-induced protein 3 (TNFAIP3), which belongs to the OTU family, is also known as A20 and has both E3 ubiquitin ligase and deubiquitinating enzyme activities. Several reports have shown that TNFAIP3 is pro-proliferative, anti-inflammatory, and anti-apoptotic after liver injury [175–178]. TNFAIP3 was

Table 2 Role of DUBs in MASLD/MASH

Gene	Alteration in MASLD	Targeting substrate	Deubiquitination site	Ending	Cell	Experimental MASLD model	Transgenic mice model	Disease phenotype	Effect	Reference
USP18	Down-regulation in hepatocytes	TAK1	/	Phosphorylation inhibition	Primary hepatocytes, L02, HEK293T	HFD, Ob/ob	Usp18-HKO, Usp18-HOE	MASLD	Anti-steatosis; Anti-inflammation; Anti-insulin resistance	[166]
USP4	Down-regulation in hepatocytes	TAK1	/	Phosphorylation inhibition	Primary hepatocytes, HEK293T	HFD, Ob/ob	Usp4-HKO, Usp4-HOE	MASLD	Anti-steatosis; Anti-inflammation; Anti-insulin resistance	[170]
CYLD	Down-regulation in hepatocytes	TAK1	K63	Phosphorylation inhibition	Primary hepatocytes, L02, HEK293T	HFD, Ob/ob, HFHC	Cyld-HKO, Cyld-HOE	MASLD, MASH	Anti-steatosis; Anti-inflammation; Anti-fibrosis; Anti-insulin resistance	[174]
TNFAIP3	Down-regulation in hepatocytes	ASK1	K63, K29, K11	Phosphorylation inhibition	Primary hepatocytes, L02	HFD, Ob/ob, HFHC	Tnfaip3-HKO, Tnfaip3-HOE, Ask1-HKO, Tnfaip3- Ask1-DKO	MASLD, MASH	Anti-steatosis; Anti-inflammation; Anti-fibrosis; Anti-insulin resistance	[179]
USP25	Down-regulation in hepatocytes	PPARα	K48	Degradation inhibition	Primary hepatocytes, Huh7, HEK293T	HFD, Ob/ob	Usp25-KO	MASLD	Anti-steatosis	[189]
USP7	Up-regulation in hepatocytes	ZNF638	K48	Transcription promotion	SK-Hep1, Huh-7, NH3T3	30% (w/w) of fructose in drinking water diet	/	Hepatic steatosis model	Pro-steatosis	[191]
USP10	Down-regulation in hepatocytes	SIRT6	/	Degradation inhibition	Primary hepatocytes	HFD, Ob/ob	Usp10-KO, Usp10-OE, Sirt6-KO, Sirt6-OE, Usp10-KO/Sirt6-OE, Usp10-OE/Sirt6-KO	MASLD	Anti-steatosis; Anti-inflammation; Anti-insulin resistance	[196]
USP15	Up-regulation in hepatocytes	FABPs, Perilipins	/	Stability increase	Primary hepatocytes, AML12, HEK293T	HFD, FPCD	Usp15-HKO	MASLD, MASH	Pro-steatosis; Pro-inflammation; Pro-fibrosis; Pro-insulin resistance	[198]
USP14	Up-regulation in hepatocytes	HSP90AA1	K48	Degradation inhibition	Primary hepatocytes, AML12, HEK293T	HFD, HFHC	Usp14-KO, Usp14-OE	MASLD, MASH	Pro-steatosis; Pro-inflammation; Pro-fibrosis	[203]
USP33	Up-regulation in hepatic stellate cells	/	/	/	Primary hepatic stellate cells	HFHC	/	/	Pro-fibrosis	[211]

Table 2 (continued)

Gene	Alteration in MASLD	Targeting substrate	Deubiquitination site	Ending	Cell	Experimental MASLD model	Transgenic mice model	Disease phenotype	Effect	Reference
USP11	Up-regulation in hepatocytes	KLF4	K63	Reduction of stability	HepG2, Hep3B, Sk-Hep1, THLE2, Huh7, SNU423, HEK293T	/	/	/	Pro-steatosis; Pro-tumor	[215]

shown to inhibit the development of MASH by counteracting the hyperactivation of ASK1, the activation of which is known to increase hepatic lipid accumulation and inflammatory responses, mainly by promoting the activation of its downstream JNK–p38 signaling pathway [179]. TNFAIP3 interacts with and selectively removes K11, K29, and K63 polyubiquitin from ASK1 induced by lipid accumulation, thereby suppressing hyperactive ASK1. ASK1 ablation abolished the ability of TNFAIP3 deficiency to potentiate HFD-induced activation of ASK1–p38–JNK1/2 signaling, as well as hepatic steatosis and inflammation, indicating that ASK1 signaling is essential for the protective effect of TNFAIP3 against MASH.

Highly activated ASK1 has also been identified as a biological marker of metabolism-related obesity and MASH [180]. Although its selective inhibitor selonsertib (GS-4997) has progressed to phase II clinical trials for the treatment of MASH (NCT02466516), its systemic blockade might cause potential unwanted side effects because it also inhibits the physiological function of ASK1. Therefore, identifying the upstream molecular mechanisms of ASK1 hyperactivation could provide a more favorable targeted therapeutic approach [181, 182].

DUBs targeting the PPAR signaling pathway

Ubiquitin-specific peptidase 25 (USP25) plays a role in various metabolic and cancer diseases [183–186]. The correlation between USP25 and IR in adipocytes prompted its study in MASLD [187, 188]. In patients and mice with MASLD, the protein levels of USP25 in the liver decreased. Knocking out USP25 or using its inhibitor, AZ1, can enhance HFD-induced fat deposition [189]. Mechanistically, USP25 inhibits PPARα degradation by removing K48-linked ubiquitin. Low levels of USP25 in MASLD stabilize PPARα and downstream signaling pathways, leading to lipid degeneration. The ability of USP25 to combat IR and to inhibit hepatic lipid deposition requires further investigation.

DUBs targeting DNL

Ubiquitin-specific peptidase 7 (USP7) has been shown to interact with phosphate inorganic transporter 1 (PiT1) to regulate hepatic lipogenesis through glucose metabolism [190]. Recent studies have linked the regulatory role of USP7 in hepatic lipogenesis-associated diseases [191]. USP7 contributes to aberrant DNL through the deubiquitylation of zinc finger protein 638 (ZNF638) in hepatocytes. USP7 can also promote ZNF638 transcription by stabilizing the transcription factor cAMP responsive element-binding protein (CREB). The USP7/ZNF638 axis promotes the nuclear accumulation of cleaved SREBP1C via AKT/mTORC1/S6K signaling, facilitating DNL through the activation of lipogenesis-associated

enzymes. USP7 regulates the levels of cleaved SREBP1C by increasing its stability. The expression of ZNF638, nuclear SREBP1C, and DNL enzymes decreased in hepatic steatotic mice following treatment with GalNAc-conjugated ZNF638-siRNA and a USP7 inhibitor P22077. Consistent with these findings, the USP7/ZNF638 axis may play a crucial role in lipogenesis-associated HCC. However, whether the USP7/ZNF638 axis is involved in the natural progression of steatosis-associated hepatocellular carcinoma or MASLD progression to MASH needs to be further explored in relevant animal models.

Ubiquitin-specific peptidase 10 (USP10), a member of the USP family, is involved in cellular metabolism, cancer, resistance to stress (such as oxidative stress), and inflammation [192–194]. In diabetes-related cardiomyopathy, USP10 is highly expressed under the induction of beneficial cardiac factor follistatin-like protein 1 (FSTL1) and alleviates cardiac fibrosis through NOTCH1 signal transduction [195]. USP10 has recently been reported to be involved in MASLD pathogenesis [196]. Compared with that in normal controls, USP10 expression in the livers of patients with MASLD is lower. USP10 overexpression reverses the MASLD-related phenotype. Further investigation revealed that USP10 attenuates hepatic steatosis by interacting with SIRT6 and reducing its ubiquitination and degradation. Reduced degradation of SIRT6 promotes the expression of lipid synthesis-related factors, such as SREBP1 and FASN, thereby exacerbating MASLD. SIRT6 affects the transcriptional level and activity of SREBP1 in several ways [197]. Therefore, USP10 attenuates MASLD through regulating SIRT6 and SREBP1 expression.

Other DUBs involved in MASLD/MASH

USP15

Ubiquitin-specific peptidase 15 (USP15) regulates the pathogenesis of MASLD and MASH [198]. The expression of USP15 in the liver is markedly upregulated in both MASLD mice and individuals. Mass spectrometry analysis and GST pull-down experiments revealed lipid metabolism-associated proteins such as fatty acid-binding proteins (FABPs) and perilipins as binding partners for USP15. USP15 interacts with FABPs and perilipins to reduce ubiquitination and increase protein stability, which results in lipid accumulation. Hepatic lipid accumulation and the expression of genes related to fatty acid accumulation, such as FABPs and perilipins, are significantly lower in liver-specific USP15 knockout (USP15-LKO) mice than in WT mice. This study provides a scientific basis for the hypothesis that USP15 inhibition may be a promising therapeutic strategy for MASLD/MASH.

Notably, other studies have shown that USP15 can deubiquitinate Keap1, a component of the Cullin RING ubiquitin ligase (CRL), thereby promoting lipogenesis [199, 200]. In DN, USP15 inhibition can activate Nrf2 to counteract podocyte damage and oxidative stress [201]. Thus, targeting USP15 may represent a promising therapeutic strategy for metabolic disorder-related diseases.

USP14

Previous studies have confirmed that ubiquitin-specific peptidase 14 (USP14) can directly interact with FASN, increasing its stability and promoting hepatic steatosis and IR [202]. Further research revealed that USP14 expression is elevated in MASLD and MASH livers and that its knockout downregulates the expression of proteins related to inflammation and fibrosis [203]. Overexpression of USP14 in AML12 cells under PAOA stimulation results in increased oxidative stress. Cytochrome P450 2E1 (CYP2E1) is closely associated with the progression of hepatic steatosis and MASH [204, 205]. USP14 upregulates the protein level of CYP2E1. Mechanistically, USP14 removes K48-linked ubiquitin via heat shock protein 90 alpha family class A member 1 (HSP90AA1). Stable HSP90AA1 acts as a molecular chaperone to further regulate the stability of CYP2E1. The USP14-HSP90AA1-CYP2E1 axis provides a new target for the treatment of MASLD; however, whether HSP90AA1 affects other proteins and whether CYP2E1 is directly regulated by the UPS require further investigation.

Studies have linked USP14 to various diseases. USP14 interacts with sperm-associated antigen 5 antisense RNA1 (SPAG5-AS1), inhibiting the ubiquitination of SPAG5 [206]. SPAG5 mediates the activation of the AKT/mTOR signaling pathway, which inhibits autophagy and promotes podocyte apoptosis [207]. However, USP14 exacerbates diabetic retinopathy by activating the NF-κB signaling pathway and promoting ROS production in Müller cells (a group of glial cells in the retina) [208]. Compared with that in healthy tissues, USP14 is elevated in atherosclerotic tissues [209]. Increased USP14 promotes mTOR/P70S6K signal transduction, leading to the dedifferentiation of vascular smooth muscle cells (VSMCs), which shift from a quiescent phenotype to a proliferative and migratory phenotype. This plastic phenotype is crucial for atherosclerosis development [209]. Another study reported that USP14 is downregulated in endothelial cells (ECs) stimulated with oxidized low-density lipoprotein (ox-LDL) [210]. Overexpression of USP14 deubiquitinates NOD-like receptor family CARD domain-containing 5 (NLRC5), stimulating activation of the NF-κB pathway and thereby exacerbating the inflammatory phenotype in atherosclerosis [210]. In conclusion, USP14 plays a broad role in abnormalities in glycolipid metabolism.

USP33

Ubiquitin-specific peptidase 33 (USP33) expression is elevated in gerbils fed a HFHC diet and in patients [211]. It can promote the activation of stellate cells and glycolysis by upregulating c-Myc. Overexpression of USP33 increases the expression of fibrosis-related proteins such as α-smooth muscle actin (α-SMA) and collagen I (COL1), whereas inhibition of USP33 alleviates fibrosis progression. Bile acid treatment of primary stellate cells promotes the expression of USP33 [211]. Targeting USP33 may inhibit MASLD-related hepatic fibrosis through various mechanisms. Another study revealed that, in skeletal muscle IR caused by liver inflammation, the hepatic factor endoplasmic reticulum aminopeptidase 1 (ERAP1) can inhibit USP33-mediated deubiquitination of the β2-adrenergic receptor (ADRB2), thereby disrupting insulin signaling. Therefore, further research on USP33 in MASLD and other IR-related diseases, such as T2DM, is needed [212].

USP11

Kruppel-like factor 4 (KLF4), a member of the transcription factor KLF family, is a well-studied tumor regulator that inhibits or promotes tumorigenesis, depending on the tissue in which it is located and how it is regulated [213]. Ubiquitination as a posttranslational modification may be the reason for the high turnover rate of KLF4 [214]. Proteomic analysis revealed that ubiquitin-specific peptidase 11 (USP11) is a deubiquitinating enzyme for KLF4, with both proteins interacting with and colocalizing in the nucleus [215]. Mechanistically, USP11 removes the K63-linked ubiquitin of KLF4 and promotes its degradation. The use of shRNAs to reduce elevated USP11 levels in HCC cells resulted in increased levels of KLF4, which inhibited tumor cell proliferation and chemoresistance.

Small-molecule inhibitors of E3 ligases or dubs as therapeutic agents

Currently, no clinical trials have been designed to test E3 ligases or DUB-based therapies specifically for MASLD/MASH. Several small-molecule inhibitors that target E3 ligases or DUBs, which are reportedly involved in MASLD/MASH pathogenesis, have been discovered. Although their inhibition or therapeutic efficacy has been demonstrated in diseases other than MASLD/MASH, these inhibitors have the potential to be used as therapeutic agents for MASLD/MASH (Fig. 4; Table 3).

Inhibitors targeting E3 ubiquitin ligases

SMURF1 inhibitor

Recent studies have shown that SMURF1 is involved in retinal degeneration [216]. Elevated SMURF1 expression was observed in a mouse model of retinal degeneration. A01, a specific SMURF1 inhibitor that disrupts the interaction between SMURF1 and its target protein, significantly alleviated $NaIO_3$-induced acute retinal injury in mice, as revealed by improved retinal structure, decreased cell death, and inflammatory activation [216].

HRD1 inhibitor

HRD1 is essential for flavivirus infection in both mammalian hosts and mosquitoes [217]. LS-102, a small-molecule inhibitor of HRD1, effectively interrupted dengue virus 2 (DENV2) infection in both mice and *Aedes aegypti* mosquitoes and disrupted DENV transmission from infected hosts to mosquitoes [217].

Inhibitors targeting DUBs

USP11 inhibitor

Compared with those in control mice, both USP11 mRNA and protein levels are significantly increased in the obstructed kidneys of unilateral ureteral obstruction (UUO) model mice [218]. Pretreatment with the USP11 inhibitor mitoxantrone (MTX) significantly attenuated the increase in the expression of the TGF-β type II receptor (Tgfbr2), activation of downstream senescence-related signaling pathways, and renal senescence and fibrosis [218].

USP7 inhibitors

P5091 USP7 plays an essential role in the progression of various cancers [219]. In patients with multiple myeloma (MM), USP7 expression was greater in the bone marrow than in normal controls. P5091, a specific USP7 inhibitor discovered by high-throughput screening of a small-molecule library, exhibited antitumor efficacy both in vitro and in vivo in MM xenograft models [220]. In colorectal cancer (CRC), both the mRNA and protein levels of USP7 are greater in CRC cells than in normal cells. Moreover, the expression of USP7 was negatively correlated with poor CRC prognosis. P5091 has also been reported to inhibit colorectal tumor growth in an HCT116 xenograft mouse model by suppressing proliferation and inducing apoptosis in CRC cells [221].

DHPO USP7 is elevated in gastric cancer (GC) tissues and is involved in gastric cancer progression and drug resistance [222]. Through in silico structure-based screens and cytotoxicity studies, DHPO was identified among the more than 3000 candidates as a potent USP7 inhibitor. Covalent binding of DHPO to USP7 prevents its conjugation to ubiquitin, thus eliminating its ability to act as a deubiquitinating enzyme. In a mouse model of GC, DHPO intervention significantly suppressed GC growth and metastasis by inducing ferroptosis through the regulation of stearoyl-CoA desaturase (SCD) [222].

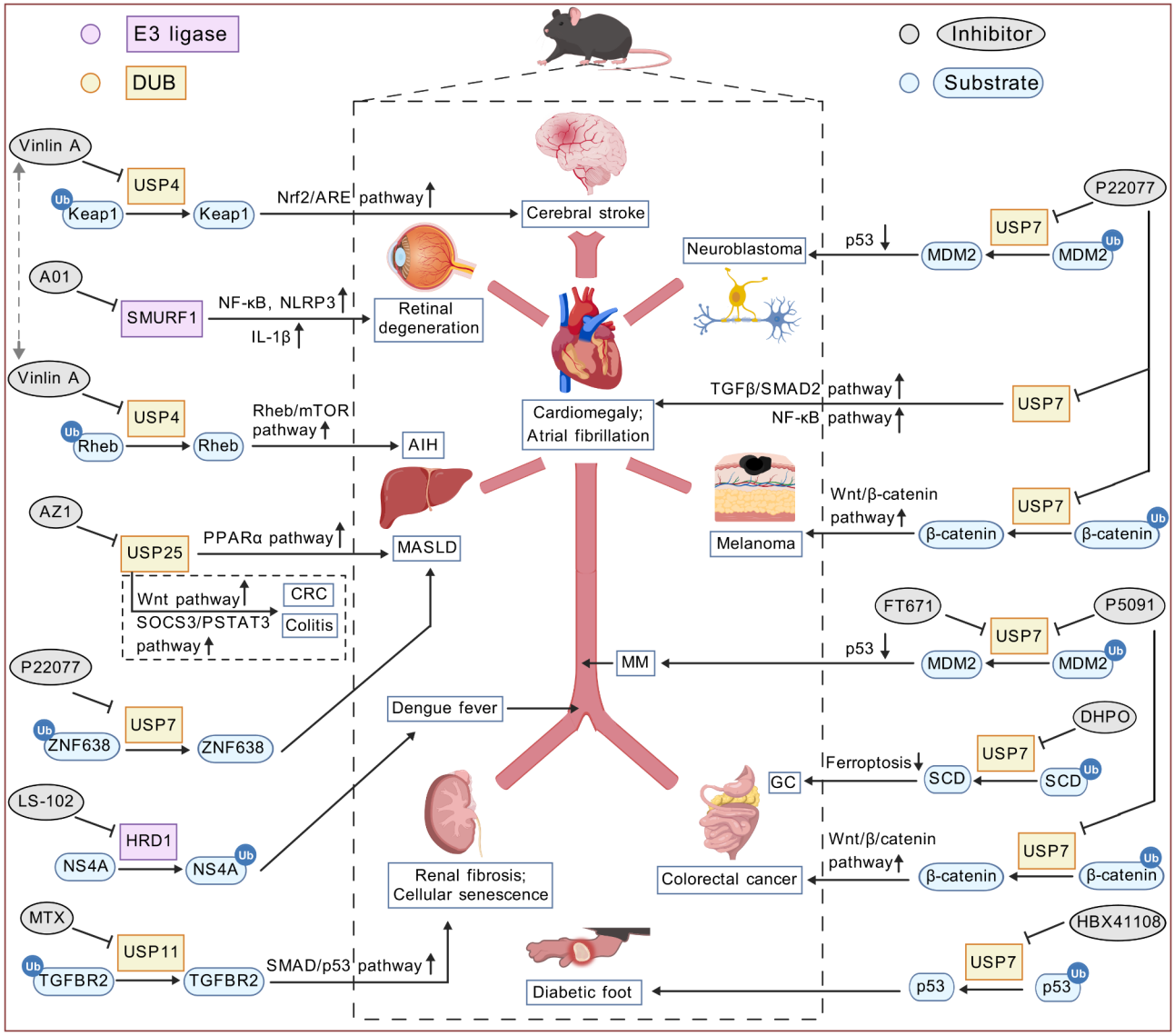

Fig. 4 Mechanisms of small-molecule inhibitors in preclinical studies. Inhibitors targeting the above E3 ubiquitin ligases and DUBs have beneficial effects in various animal models. An inhibitor of USP7, P22077, and an inhibitor of USP25, AZ1, have been used in studies of MASLD mice. The molecular mechanisms by which inhibitors act in different diseases are shown

FT671 FT671, a noncovalent inhibitor of USP7, was identified in compound libraries via a ubiquitin–rhodamine assay [223]. Cocrystal structures revealed that FT671 targets a dynamic pocket near the catalytic center of the autoinhibited apo form of USP7. Administration of FT671 inhibited tumor growth in an MM.1 S xenograft model in a dose-dependent manner by destabilizing USP7 substrates such as the oncogenic E3 ligase MDM2 [224].

P22077 P22077 was identified via activity-based chemical proteomics as an inhibitor of USP7, which can reduce the enzymatic activity of USP7 [225]. In addition to inhibiting MASDL, P22077 has beneficial effects on various disease models. High USP7 expression is associated with poor prognosis in patients with neuroblastoma (NB) [226]. In an in situ NB mouse model, P22077 treatment significantly inhibited tumor growth by inducing HDM2 protein degradation and stabilizing p53 [226]. Similarly, P22077 has been shown to inhibit the growth and metastasis of melanoma tumors [227]. Recent studies have demonstrated that USP7 is involved in cardiac hypertrophy [228, 229]. The expression of USP7 is increased in patients with heart failure (HF) and in mice with angiotensin II-induced cardiac remodeling. Administration of P22077 has shown promising results in alleviating Ang II-induced cardiac hypertrophy, fibrosis, inflammation, and oxidative stress. Additionally, elevated levels of USP7 have been observed in atrial tissues from mice and patients with atrial fibril-

Table 3 Small-molecule inhibitors of E3 ligases or DUBs as therapeutic agents

Name	Target	Disease	Effect	Reference
LS-102	HRD1	Flaviviruses infection	Interrupted Dengue Virus 2 (DENV2) infection in both mice and Aedes aegypti mosquitoes, and significantly disturbed DENV transmission from the infected hosts to mosquitoes owing to reduced viremia.	[217]
SMURF1-IN-A01 (A01)	SMURF1	Age-related macular degeneration	Kept a better retina structure in living imaging and histologic sections, induced less cell death and inflammation activation, alleviated acute retina injury.	[216]
Mitoxantrone (MTX)	USP11	Renal fibrosis	Enhanced the degradation of Tgfbr2 and alleviated renal fibrosis.	[218]
P005091 (P5091)	USP7	1. Multiple myeloma (MM) 2. Colorectal cancers (CRC)	1. Inhibited growth of MM cells and overcame bortezomib-resistance, triggered anti-angiogenic activity in vivo. 2. Inhibited tumor loading as decreased tumor weight and volume.	[220, 221]
DHPO	USP7	Gastric cancer (GC)	Induced ferroptosis in GC and suppressed growth and metastasis of GC cells.	[222]
FT671	USP7	MM	Dose-dependent tumor growth inhibition.	[224]
P22077	USP7	1. MASLD 2.Neuroblastoma (NB) 3. Melanoma 4. Heart failure relevant cardiac hypertrophy and remodeling 5. Atrial fibrillation (AF)	1. Inhibited of hepatic lipid deposition in MASDL. 2. Inhibited tumor growth in vivo and overcame the established chemoresistance in NB cells in vitro. 3. Inhibited melanoma tumor growth, metastasis and invasion. 4. Attenuated Ang II-induced cardiac hypertrophy, cardiac contractile dysfunction, blood pressure, cardiac hypertrophy, fibrosis, inflammation and oxidative stress. 5. Attenuated Ang II-induced inducibility and duration of AF, atrial dilatation, connexin dysfunction, atrial fibrosis, atrial inflammation, and atrial oxidase stress, and then inhibited the progression of AF.	[191, 226–229]
HBX41108	USP7	Diabetic foot	Promoted the healing of ulcerated wounds in rats with diabetic foot.	[230]
Vialinin A	USP4	1. Oxidative stress and neuronal injuries after ischaemic stroke 2. Autoimmune hepatitis	1. Alleviated cerebral ischaemia–reperfusion injury-induced neurological deficits and neuronal apoptosis, ameliorated neurological dysfunction. 2. Attenuated inflammation of S100-induced autoimmune hepatitis and reduced liver fibrosis.	[231, 232]
AZ1	USP25	1. MASLD 2. Colitis and colorectal cancer	1. Inhibited hepatic lipid deposition and alleviated MASLD progression. 2. Inhibited the development of DSS-induced colitis and colorectal cancer.	[186, 189]

lation (AF) [229]. P22077 treatment attenuates the Ang II-induced onset and duration of AF, thus inhibiting the progression of AF.

HBX 41108 The expression level of USP7 is greater in diabetic foot ulcer tissues than in normal tissues. USP7 inhibits the ubiquitination of p53, thereby mediating cellular senescence. The USP7-specific inhibitor HBX 41,108 promotes wound healing in STZ-induced diabetic foot rats [230].

USP4 inhibitor

A recent study indicated that USP4 is a drug target for ischemic stroke [231]. Vialinin A, a natural substance extracted from edible mushrooms, effectively inhibits USP4 activity. Vialinin A treatment significantly reduces ischemia–reperfusion injury in mice with ischemic stroke [231]. Furthermore, USP4 levels are elevated in patients with autoimmune hepatitis (AIH). Treatment of S100-induced AIH mice with Vialinin A significantly attenuates liver inflammation and fibrosis and protects liver function [232].

USP25 inhibitor

The benzylaminoethanol derivative AZ1 is a USP28 inhibitor that was screened and characterized using high-throughput screening (HTS) and in vitro experiments [233]. Given the high similarity between USP28 and USP25, particularly the 57% similarity in their central catalytic regions, it is not surprising that AZ1 was shown to exhibit inhibitory activity against USP25 [234]. In MASLD, AZ1 gavage effectively inhibits USP25 and enhances hepatic lipid deposition [189]. In another study, USP25 was found to be a risk factor for dextran sulfate sodium (DSS)-induced colitis and colorectal cancer. Treatment with AZ1 effectively inhibits both colitis and the spread of bacteria in the gastrointestinal tract and suppresses the development of colorectal cancer [186].

Although these studies identified several inhibitors that target E3 ligases or DUBs associated with MASLD/MASH, their efficacy in MASLD/MASH has not been well investigated. As the regulatory mechanisms of E3 ligases or DUBs can be highly disease specific, future studies should further assess the efficacy of these UPS inhibitors in MASLD/MASH treatment. Additionally, future research is needed to identify more specific inhibitors of previously reported E3 ligases and DUBs.

Conclusion and prospects

The prevalence of MASLD is increasing because of its close association with T2DM and obesity. Although resatinib, the first FDA-approved drug for the treatment of MASH, has been successfully marketed, it is not able to meet clinical needs [235]. Relationships among the TRIM family, chronic liver disease, and insulin resistance have been reported; however, our review focused on MASLD/MASH and covered not only the TRIM family but also other types of E3 ligases and DUBs [236, 237]. In this review, we summarized E3 ubiquitin ligases and DUBs that play nonnegligible roles in the pathogenesis of MASLD/MASH and systematically revealed the impact of the UPS on MASLD/MASH progression, thus providing new and important directions for the treatment of MASH.

Drug development for E3 ligases has become a research topic of great interest in recent years, and several small-molecule inhibitors targeting various E3 ligases have been developed and studied. For example, inhibitors of murine double mimic 2 (MDM2), such as RG7112 and APG-115, have entered phase I clinical trials for hematological and solid tumor studies (NCT No. NCT00559533 and NCT02935907) [238, 239]. Neddylation is required for CRL activation, and MLN4924 (pevonedistat), a neddylation enzyme inhibitor that elicits antitumor effects in various malignancies, is currently undergoing clinical trials [240]. Interestingly, MLN4924 significantly attenuated methionine-choline-deficient diet (MCDD)- and choline-deficient high-fat diet (CDHFD)-induced MASLD in mice, although no relevant clinical trials have been conducted [241]. The efficacy of DUB inhibitors against cancer has also been demonstrated [242]. Among DUB inhibitors, the most studied are USP7 inhibitors, such as P5091 and P22077 [220, 226]. A previous study demonstrated that P22077, by inhibiting USP7, reduces the expression of its substrate ZNF638, contributing to the inhibition of de novo fat synthesis [191]. These findings illustrate the great potential of inhibitors targeting E3 ligases or DUBs in the treatment of MASLD.

Targeting upstream regulators of E3 ligases and DUBs might be an appealing strategy for the treatment of MASLD/MASH. EGR2 binds to the TRIM16 promoter and promotes its transcription [47]. The EGR2 agonist CQMU98 has been shown to have beneficial effects on Vogt–Koyanagi–Harada syndrome (VKH) [243]. In a study of breast cancer, upstream stimulatory factor 2 (USF2) was identified as a negatively regulated transcription factor of SMUFR1/SMURF2. High expression of USF2 promotes tumor development by suppressing SMURFs [244]. CKIP-1 can reduce the level of SMURF1, thereby resisting DN [87]. AKT can activate the deubiquitinating enzyme activity of USP14 via phosphorylation, and AKT inhibitors are promising therapeutic options for treating tumors [245, 246]. In antiviral innate immunity, the oncoprotein p53 binds to the USP4 promoter, promotes its transcription, and plays a role against infection by RNA viruses [247]. Several drugs that target p53 have been used in clinical trials [248]. Strategies targeting upstream regulators of E3 ligases and DUBs should also be emphasized in further studies.

In addition to small-molecule inhibitors, nucleic acid-based drugs are among the most promising fields for drug discovery and development. For example, the nucleic acid drug ARO-HSD (NCT No. NCT04202354), which selectively reduces the expression of HSD17β13 mRNA in hepatocytes via RNA interference (RNAi), has been studied in phase I/II trials for MASH treatment [249]. Therefore, nucleic acid drugs based on dysregulated E3 ligases or DUBs in MASLD can be exploited as potential strategies for MASH treatment. The development of different types of drugs provides the basis for the personalized treatment of patients with MASH.

PROTAC, a newly developed technology, is a revolutionary therapy for various diseases, such as cancer. PROTACs are heterobifunctional molecules that recruit E3 ligases to their substrates, resulting in their degradation via ubiquitination [250, 251]. Recently, a PROTAC designed to induce the degradation of Kelch-like ECH-associated protein 1 (KEAP1) effectively inhibited hepatic steatosis, steatohepatitis, and fibrosis in an MCDD-induced MASLD model [252]. Additionally, lowering the levels of patatin-like phospholipase domain-containing protein 3 (PNPLA3) via PROTAC-mediated degradation reduces liver fat accumulation in mice [253]. The use of PROTAC technology to treat MASLD is a worthy research direction. However, several questions and technical challenges remain to be addressed before progressing to clinical trials of PROTAC-based therapies. Various rounds of experiments are needed to optimize the structures of PROTACs, such as the linkage site, linker length, and E3 ligand of PROTAC. A thorough understanding of the functions and relevance of these E3 ligases will facilitate the development of additional PROTAC-based therapies for MASLD.

In summary, the dysregulation of E3 ligases and DUBs affects target protein stability, expression, and function, contributing to steatosis, inflammation, and fibrosis in the pathogenesis of MASLD/MASH. Recognition of the crucial roles of E3 ligases and DUBs in MASLD/MASH progression has opened exciting avenues for the development of new therapeutic strategies. Given the complex pathogenesis of MASH, further studies are needed to understand how these E3 ligases and DUBs collaborate to regulate this pathological process. However, because E3 ubiquitin ligases/DUBs may have multiple substrates, the adverse effects of targeting them should also be considered. Notably, all of the above studies focused on

hepatocytes, and further studies are needed to elucidate the role of E3 ligases and DUBs in other cells, such as Kupffer cells and stellate cells, which also contribute to the development of this disease. Advances in single-cell omics are likely to help identify dysregulated E3 ligases and DUBs in other cell subsets in MASLD/MASH, thereby contributing to the design of novel UPS-targeted therapies.

Abbreviations

MASLD	Metabolic dysfunction-associated steatotic liver disease
MASH	Metabolic dysfunction-associated steatohepatitis
DUBs	Deubiquitinases
UPS	Ubiquitin–proteasome system
IR	Insulin resistance
T2DM	Type 2 diabetes mellitus
CVD	Cardiovascular disease
HCC	Hepatocellular carcinoma
ER	Endoplasmic reticulum
PTMs	Posttranslational modifications
RING	Really interesting new gene
HECT	Homologous to the E6AP carboxyl terminus
RBR	RING-between-RING
MAPK	Mitogen-activated protein kinase
MAPKKK	Mitogen-activated protein kinase kinase kinase
MAPKK	Mitogen-activated protein kinase kinase
ERK	Extracellular signal-regulated kinase
JNK	C-Jun N-terminal kinase
TAK1	Transforming growth factor-β-activated kinase 1
IKK	IκB kinase
TRIM8	Tripartite motif 8
HFD	High-fat diet
NF-κB	Nuclear factor kappa B
LPS	Lipopolysaccharides
DUSP14	Dual-specificity phosphatase 14
TRIM31	Tripartite motif 31
RHBDF2	Rhomboid 5 homolog 2
DEN	Diethylnitrosamine
HFHC	High-fat/high-cholesterol
CCL_4	Carbon tetrachloride
Nrf2	Nuclear factor E2-related factor 2
TRIM16	Tripartite motif 16
DN	Diabetic nephropathy
CCPs	Cordyceps cicadae polysaccharides
EGR2	Early growth response protein 2
p-TAK1	Phosphorylated TAK1
TRIM38	Tripartite motif 38
TAB2	TAK1-binding protein 2
AMPK	AMP-activated protein kinase
MKRN1	Makorin ring finger protein 1
SNIP1	Smad nuclear-interacting protein 1
FADD	Fas-associated protein with death domain
PPARγ	Peroxisome proliferator-activated receptorγ
shRNA	Short hairpin RNA
SIAH1	Seven in absentia homolog 1
SCP2	Sterol carrier protein 2
LDs	Lipid droplets
KEGG	Kyoto Encyclopedia of Genes and Genomes
WT	Wild type
DNL	De novo lipogenesis
TCA	Tricarboxylic acid
ACLY	ATP citrate lyase
ACC1	Acetyl CoA carboxylase 1
FASN	Fatty acid synthase
SREBP1	Sterol regulatory element-binding protein-1
FAS	Fatty acid synthesis
SCD1	Stearoyl-CoA desaturase 1
SMURF1	Smad ubiquitination regulatory factor 1
HLH	Helix-loop-helix
FBW7	F-box and WD repeat domain-containing 7
MDM2	Murine double minute 2
CKIP-1	Casein kinase 2 interacting protein-1
TGR5	Takeda G-protein-coupled receptor 5
AMFR	Autocrine motility factor receptor
ERAD	ER-associated degradation
Insig-1	Insulin induced gene 1
SH3RF2	SH3 domain-containing ring finger 2
UPR	Unfolded protein response
RNF5	Ring finger protein 5
HRD1	HMG-CoA reductase degradation protein 1
EMT	Epithelial-mesenchymal transition
XBP1	X-Box binding protein 1
SNX8	Sorting nexin 8
TRIM28	Tripartite motif 28
TRIM26	Tripartite motif 26
PAOA	Palmitate/oleic acid
CEBPD	CCAAT/enhancer binding protein delta
HIF1A	Hypoxia-inducible factor-1α
GRAIL	The gene related to anergy in lymphocytes
SIRT1	The silent information regulator sirtuin 1
UBR1	Ubiquitin protein ligase E3 component N-recognin 1
PQC	Protein quality control
PLIN2	Perilipin-2
PROTAC	Proteolysis-targeting chimera
RNF186	Ring finger protein 186
HMGB1	High mobility group box 1
TRIB3	Tribbles homolog 3
HFF	High-fructose/sucrose
CDAHF	Choline-deficient, L-amino acid-defined, high-fat
HNF4α	Hepatic nuclear factor 4α
ITCH	Itchy E3 ubiquitin-protein ligase
BCAAs	Branched-chain amino acids
MCD	Methionine-choline-deficient
ApoE-/-	Apolipoprotein E knockout
SIRT6	The silent information regulator sirtuin 6
TRIM59	Tripartite motif 59
GPX4	Glutathione peroxidase 4
TRIM67	Tripartite motif 67
CPT1-α	Carnitine palmitoyltransferase 1a
PGC-1α	Peroxisome proliferator-activated receptor-γ coativator-1α
USPs	Ubiquitin-specific proteases
OTUs	Ovarian tumor proteases
UCHs	Ubiquitin C-terminal hydrolases
MJDs	Machado–Joseph disease proteases
MINDY	Motif interacting with ubiquitin (MIU)-containing novel DUB family
ZUFSP/ZUP1	Zn-finger and UFSP domain protein
JAMMs	JAB1/MPN/MOV34 metalloproteases
USP18	Ubiquitin-specific peptidase 18
ISG15	Interferon-stimulated gene 15
USP4	Ubiquitin-specific peptidase 4
GATA1	GATA binding protein 1
CYLD	Cylindromatosis
TRIM47	Tripartite motif 47
ASK1	Apoptosis signal-regulating kinase 1
TNFAIP3	Tumor necrosis factor alpha-induced protein 3
USP25	Ubiquitin-specific peptidase 25
USP7	Ubiquitin-specific peptidase 7
PiT1	Phosphate inorganic transporter 1
ZNF638	Zinc finger protein 638
CREB	cAMP responsive element binding protein
USP10	Ubiquitin-specific peptidase 10
FSTL1	Follistatin-like protein 1
USP15	Ubiquitin-specific peptidase 15
FABPs	Fatty acid-binding proteins
CRL	Cullin RING ubiquitin ligase
USP14	Ubiquitin-specific peptidase 14
CYP2E1	Cytochrome P450 2E1
HSP90AA1	Heat shock protein 90 alpha family class A member 1
SPAG5-AS1	Sperm-associated antigen 5 antisense RNA1
VSMC	Vascular smooth muscle cells

ECs	Endothelial cells
ox-LDL	Oxidized low-density lipoprotein
NLRC5	NOD-like receptor family CARD domain-containing 5
USP33	Ubiquitin-specific peptidase 33
α-SMA	α-smooth muscle actin
COL1	Collagen I
ERAP1	Endoplasmic reticulum aminopeptidase 1
ADRB2	β2-adrenergic receptor
KLF4	Krupple-like factor 4
USP11	Ubiquitin-specific peptidase 11
DENV2	Dengue virus 2
UUO	Unilateral ureteral obstruction
MTX	Mitoxantrone
Tgfbr2	TGF-β type II receptor
MM	Multiple myeloma
CRC	Colorectal cancer
GC	Gastric cancer
SCD	Stearoyl-CoA desaturase
NB	Neuroblastoma
HF	Heart failure
AF	Atrial fibrillation
AIH	Autoimmune hepatitis
HTS	High-throughput screening
DSS	Dextran sulfate sodium
MCDD	Methionine-choline-deficient diet
CDHFD	Choline-deficient high-fat diet
VKH	Vogt-Koyanagi-Harada syndrome
USF2	Upstream stimulatory factor 2
RNAi	RNA interference
KEAP1	Kelch-like ECH-associated protein 1
PNPLA3	Patatin-like phospholipase domain-containing protein 3
NLRP3	Nod-like receptor pyrin domain containing 3
IL-1β	Interleukin-1beta
Rheb	Ras homolog enriched in brain
mTOR	Mammalian target of rapamycin
SOCS3	Suppressor of cytokine signaling 3
STAT3	Signal transducer and activator of transcription 3

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SL designed and supervised this study. YZ wrote the manuscript. JY and JM organized the tables. SH and YL revised the manuscript. All the authors have read and approved the final version of the manuscript.

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