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Review

The ubiquitous role of ubiquitination in lipid metabolism

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Lipids are essential molecules that play key roles in cell physiology by serving as structural components, for storage of energy, and in signal transduction. Hence, efficient regulation and maintenance of lipid homeostasis are crucial for normal cellular and tissue function. In the past decade, increasing research has shown the importance of ubiquitination in regulating the stability of key players in different aspects of lipid metabolism. This review describes recent insights into the regulation of lipid metabolism by ubiquitin signaling, discusses how ubiquitination can be targeted in diseases characterized by lipid dysregulation, and identifies areas that require further research.

Highlights

Lipids have key functions in the assembly of cell structures, serve as an important energy source, and are precursors for signaling molecules.

Ubiquitination is one of the most common post-translational modifications in eukaryotes and is essential for cellular and tissue homeostasis.

Ubiquitination is central in regulating lipid metabolism by controlling the turnover of proteins and lipids involved in lipid metabolism.

Dysregulation of ubiquitination contributes to lipid abnormalities in divergent disorders, and targeting the ubiquitin-proteasome system serves as an interesting therapeutic approach.

Ubiquitination and lipid metabolism

Controlled degradation of proteins by the **ubiquitin** (see Glossary) proteasome system (UPS) (Box 1) is implicated in the breakdown of 80% of intracellular proteins. Accordingly, abnormal UPS function perturbs cellular homeostasis and underpins disease pathology in cancer, metabolic diseases, and neurodegenerative disorders such as Parkinson's disease (PD) and Alzheimer's disease (AD) [1,2]. Emerging evidence indicates that ubiquitination is a hub and driver of cellular lipid metabolism. Lipids are important in preserving membrane structure, regulating energy metabolism, and controlling signal transduction. Hence, balanced lipid homeostasis is crucial for cellular well-being [3,4]. In this review, we discuss current knowledge of implications for ubiquitination in regulating lipid metabolism, highlight links between abnormalities in lipid homeostasis and ubiquitination, and identify areas that require more research. An enormous body of research has been performed on UPS-mediated regulation of lipid metabolism; this review aims to provide a broad and comprehensive overview of existing evidence on this topic. (See Table 1.) (See Box 2.)

UPS-mediated control of cholesterol metabolism

Cholesterol is vital in cellular physiology and metabolism by acting as a major structural component of (intra)cellular membranes and as a precursor for the production of bioactive steroids and derivatives. Imbalances in cholesterol metabolism are linked to cancer and cardiovascular and neurodegenerative diseases [5,6]. Alongside transcriptional regulation, cholesterol homeostasis is maintained via **post-translational mechanisms**. Concerning the latter, ubiquitination is a common post-translational modification controlling cholesterol metabolism (Figure 1). The UPS mainly induces negative regulation of cholesterol metabolism, with high cholesterol load promoting ubiquitination of proteins involved in cholesterol biosynthesis and uptake while reducing that of proteins involved in cholesterol efflux. Vice versa, a decrease in cholesterol levels promotes cholesterol uptake and synthesis by, for example, reducing the ubiquitination of proteins involved in these processes while enhancing the ubiquitination of efflux transporters [7].

Transcriptional regulation of cholesterol metabolism

Essential in preserving cholesterol homeostasis is the transcriptional control of genes involved in the biosynthesis and metabolism of cholesterol. This regulation occurs primarily by the

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Box 1. Protein degradation by the UPS

The UPS plays a fundamental role in maintaining protein homeostasis and is crucial for many central cellular processes, such as apoptosis, cell cycle progression, DNA repair, development, immune responses, and membrane transport. It involves a three-step catalytic cascade in which proteins are marked for degradation by covalent conjugation of ubiquitin, a small, evolutionarily conserved, 76-amino-acid protein. Initially, ubiquitin is activated by an E1 ubiquitin-activating enzyme, generating a high-energy thioester intermediate. Next, ubiquitin is transferred to an E2 ubiquitin-conjugating enzyme that escorts ubiquitin toward an E3 ubiquitin-protein ligase. E3 ligase enzymes mediate the final transfer of ubiquitin toward a protein substrate and can be classified in three different families: RING (really interesting new gene), HECT (homologues to the E6AP carboxyl terminus), and RBR (RING-between-RING) E3 ligases, which differ in their mode of ubiquitin acceptance. Within each group of UPS enzymes, different variants have been identified: 26 for E1, over 105 for E2, and more than 1000 for E3 enzymes, with E3 ligases conferring substrate specificity. E3 ligases recognize a substrate protein by their degron, which is a short amino acid sequence on which ubiquitin is conjugated. Proteins can either be mono- or polyubiquitinated, the latter requiring two or more ubiquitination cycles.

Most commonly, ubiquitinated proteins are recognized by the 26S proteasome for degradation, after which the ubiquitin chain is disassembled by deubiquitinating enzymes, and ubiquitin molecules can be recycled for new ubiquitination cascades [90]. Yet, ubiquitination controls lysosomal degradation, protein trafficking and localization, and cell signaling pathways as well. The fate of a ubiquitinated protein is determined by the ubiquitin-linkage type and the site of ubiquitin attachment. Ubiquitin can be linked through amino- or hydroxyubiquitination, with aminoubiquitination being the most common linkage form regulating diverse aspects, such as protein degradation, activity, and location. Hydroxyubiquitination is less common and has been identified in certain cellular processes, including DNA repair and endocytosis. Ubiquitin can be conjugated to proteins via amide or ester bonds, with amide bonds generally being more stable and involved in proteasomal degradation and the less common ester-linked conjugates being associated with reversible modifications. Finally, the most well-studied and common sites of ubiquitin attachment are linkages to lysin residues, N-terminal amino acids, or cysteine residues. This diversity of enzymes, ubiquitin linkages, and location all contribute to the complexity and versatility of the UPS [91].

transcription factors sterol regulatory element-binding protein (SREBP) and liver X receptor (LXR). Although SREBP predominantly promotes the expression of genes involved in cholesterol and **fatty acid (FA) synthesis** and uptake, LXRs promote the removal of excess cholesterol.

SREBP and its regulatory proteins

SREBPs are a family of membrane-bound transcription factors, with SREBP1a and SREBP1c originating from distinct transcriptional start sites of *SREBF1* and SREBP2 originating from *SREBF2*. Although SREBP1 preferentially activates genes involved in FA synthesis (discussed later in this review), SREBP2 closely regulates genes involved in cholesterol synthesis and uptake. Under conditions of high intracellular cholesterol, trafficking factor SREBP cleavage-activating protein (SCAP) binds to the endoplasmic reticulum (ER)-resident insulin-induced gene (INSIG) protein, which maintains SCAP on the ER. When cholesterol levels are low, SCAP undergoes a conformational change that induces its detachment from INSIG and allows its association with SREBP. By doing so, SCAP transports SREBP from the ER to the Golgi complex, where it is cleaved by proteases. Cleaved SREBP enters the nucleus and binds sterol-regulatory elements (SREs) in the promoter region of sterol-increasing genes such as HMG-CoA reductase (*HMGCR*), squalene epoxidase (*SQLE*), and low-density lipoprotein receptor (*LDLR*) [8].

SREBP2 was shown to be a substrate for UPS-mediated regulation, and ring finger protein (RNF) 139 was identified as the responsible E3 ligase in HepG2 cells. RNF139 has a sterol-sensing domain and binds the SREBP-SCAP complex upon high cholesterol load, thereby hindering its translocation toward the Golgi [9]. Regulatory proteins INSIG and SCAP are substrates for UPS-mediated degradation as well [10–13]. RNF145 is an ER membrane E3 ligase that triggers the ubiquitination of SCAP, thereby inhibiting SREBP shuttling to the Golgi apparatus upon high cholesterol content. RNF145 expression was induced in both human and mouse primary cells and cell lines upon LXR activation and in mice after high-cholesterol diet feeding, thereby providing a molecular link between hepatic cholesterol levels and the reciprocal mechanisms of SREBP and LXR signaling [14]. More recently, ER-anchored E3 ligase RNF5 was demonstrated to mediate

Glossary

26S proteasome: the major protease in eukaryotic cells responsible for degradation of ubiquitinated proteins in both cytoplasm and nucleus.
ATGL: adipose triglyceride lipase, an enzyme critical for the release of FAs from triglycerides on the surface of LDs.
Autophagy: a homeostatic cellular recycling process that mediates the lysosomal delivery and clearance of various cellular components and damaged organelles in double-membraned vesicles termed 'autophagosomes.'
β-oxidation: also called 'fatty acid oxidation,' is the mitochondrial aerobic process of breaking down a fatty acid into acetyl-CoA units.
Cholesterol esterification: the linkage of cholesterol with a fatty acid in a reaction catalyzed by ER-resident acyl-CoA:cholesterol acyltransferases (ACAT), also called 'sterol O-acyltransferases' (SOAT).
Endocytosis: the cellular process by which molecules are brought into the cell.
ERAD: endoplasmic reticulum–associated protein degradation is the degradation of ubiquitinated proteins at the ER.
Fatty acid (FA) synthesis: initiates with the carboxylation of acetyl-CoA to malonyl-CoA, a conversion catalyzed by the rate-limiting enzyme acetyl-CoA carboxylase (ACC). Next, fatty acid synthase (FASN) converts malonyl-CoA to palmitate, which is a substrate for elongation or desaturation by other enzymes, generating fatty acids with different lengths and saturation degrees.
Lipid droplet (LD): ubiquitously expressed organelles in which neutral lipids such as triglycerides and cholesterol esters are sequestered. Emerging evidence indicates that the function of LDs goes beyond mere storage of neutral lipids, as they control cell signaling, gene expression, histone sequestration, and drug activity as well.
Lipolysis: the hydrolysis of LD-associated triglycerides by the consecutive action of cytosolic adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoacylglycerol lipase (MGL), resulting in the release of FFAs and glycerol.
Mass spectrometry: an analytic technique that determines the mass-to-charge ratio of molecules and allows the identification of proteins.

SCAP ubiquitination in diverse cell lines as well [11]. Finally, INSIG is a target for ubiquitination by gp78 in sterol-depleted cells [12,13]. INSIG degradation releases the SCAP-SREBP complex to travel to the Golgi for subsequent activation and attenuates degradation of HMGCR, the key rate-limiting enzyme of the cholesterol synthesis pathway.

LXRs

LXRs belong to the family of cholesterol-sensing **nuclear receptors**. Upon ligand binding, LXRs undergo a conformational change resulting in recruitment of coactivator complexes to DNA-responsive elements (LXRE). This induces the expression of cholesterol-lowering genes, including ATP-binding cassette (ABC) transporter A1 and G1 (*ABCA1* and *ABCG1*) and apolipoprotein E (*APOE*). LXRs also directly regulate cholesterol homeostasis via the UPS by inducing the transcription of E3 ligases inducible degrader of the LDLR (IDOL) and RNF145 [14,15]. With respect to the former, LXR activation promotes the expression of IDOL, thereby triggering the ubiquitination of LDLR on its intracellular cytoplasmic domain that subsequently directs the receptor toward lysosomal degradation [16]. In parallel, several earlier studies suggested that the UPS controls LXR degradation. With respect to the latter, LXRα ubiquitination reduces ABCG1-mediated cholesterol efflux in interferon-γ (IFNγ)-stimulated macrophages, thereby promoting foam cell formation and atherogenesis [17]. High-density lipoprotein (HDL) tetratricopeptide repeat domain protein 39B (TTC39B) was shown to promote LXR ubiquitination and degradation, attenuating the expression of cholesterol-lowering genes and promoting fatty liver formation in mice [18].

De novo cholesterol synthesis

De novo cholesterol biosynthesis is a complex process involving many intermediates and 22 different enzymes. Accumulation of different intermediates and cholesterol derivates increases the recruitment of INSIG to the sterol-sensing domain of HMGCR to promote its ubiquitination. HMGCR is a multispanning membrane protein, and the extraction of ubiquitinated HMGCR is dependent on its dislocation from UbiA prenyltransferase domain containing 1 (UBIAD1) and subsequent translocation to the cytosol by the 'ATPases associated with diverse cellular activities' (AAA ATPase) valosin-containing protein (VCP)/p97 and recruitment factor UBX domain-containing protein 8 (UBXD8). Released HMGCR is subsequently degraded by the **26S proteasome** [19–21]. Several E3 ligases have been proposed to participate in HMGCR ubiquitination, possibly reflecting the high degree of regulation of this central metabolic enzyme. Meanwhile, HMGCR is one of the most intensely studied cholesterol metabolism substrates of the UPS as it receives major clinical interest by being the target of statins and because it is the key rate-limiting enzyme in the cholesterol synthesis pathway. This might contribute to the fact that divergent E3 ligases are found to regulate HMGCR. With respect to the latter, initial studies defined HMGCR degradation protein 1 (HRD1) as being responsible for basal degradation of HMGCR. Stimulated degradation of HMCGR by lanosterol and other sterol derivatives was proposed to be mediated by gp78 and RNF139. Later, the LXR-responsive RNF145 was also proposed to contribute to HMGCR ubiquitination in diverse cell lines, likely in concert with gp78 and HRD1 [22]. As such, it is possible that the identification of the E3 partaking in HMGCR ubiquitination is context and signal dependent. In parallel, deubiquitination by ubiquitin-specific protease (USP)20 is essential in stabilizing HMGCR levels in the fed state. Specifically, feeding mice a high-sucrose, low-fat diet stimulated mTORC1 to phosphorylate USP20 at S132 and S134. By doing so, mTORC1 enhanced the USP20-HMGCR interaction and prevented HMGCR degradation. Deletion of USP20 attenuated hyperlipidemia and weight gain and was suggested as an attractive therapeutic target to reduce cholesterol levels [23].

A second cholesterol biosynthesis enzyme highly regulated by the UPS is SQLE, which catalyzes the first oxygenation of squalene to mono-oxidosqualene downstream of HMGCR. The regulatory

Nuclear receptors: a group of proteins that sense certain molecules such as steroids and vitamins and respond by regulating the expression of certain genes.
Post-translational mechanisms: any process that happens with a protein after its translation.
Reverse cholesterol transport: movement of cholesterol from the periphery to the liver for elimination out of the body.
PPAR: peroxisome proliferator-activated receptor; a group of nuclear receptors that control FA and glucose metabolism and adipogenesis. The PPAR family consists of PPARα, PPARβ, and PPARγ, which are activated by FFAs, after which they undergo a conformational change that facilitates their heterodimerization with retinoid X receptors (RXRs). The PPAR-RXR complex assembles at PPAR response elements (PPREs) and promotes transactivation of target genes.
Ubiquitin: a small, highly conserved protein that can be reversibly attached to proteins. It regulates substrate degradation, cellular location, activity, or interaction with other proteins.

Table 1. List of ubiquitin ligases which regulate lipid metabolism

E3	Aliases	Substrates	Function of substrate	Refs
RING E3 ligases				
COP1	RNF200, CFAP78, FAP78, RFWD2	ACC	Rate-limiting enzyme of FA synthesis	[59]
		FASN	Involved in FA synthesis	[59]
		ATGL	Hydrolysis of triglycerides	[72]
Cullin 3	CUL3	ABCA1	Cholesterol efflux transporter	[45,46]
FBXW7	SEL-10, FBW7, CDC40, AGO	SREBP1	Regulates the expression of genes involved in cholesterol and FA biosynthesis and uptake	[58]
gp78	AMFR, RNF45, SPG89	INSIG	SREBP-associated molecule	[12,13]
		HMGCR	Key rate-limiting enzyme of cholesterol biosynthesis pathway	[22]
		ACAT2	Catalyzes cholesterol esterification	[38]
HRD1	Synoviolin 1, DER3	HMGCR	See earlier	[22]
		HSD17B4	Enzyme involved in FAO	[69]
		CPT2	Transport of FA into mitochondria	[70]
IDOL	MYLIP	LDLR	Mediates LDL-derived cholesterol endocytosis	[16]
		VLDLR	Involved in cholesterol endocytosis	[16]
		ApoER2	Involved in cholesterol endocytosis in the brain	[27]
MARCHF6	MARCH6, RNF176, TEB4	SQLE	Enzyme in cholesterol biosynthesis pathway	[24,25]
		LDM	Enzyme involved in cholesterol biosynthesis	[26]
		DHCR24	Enzyme involved in cholesterol biosynthesis	[26]
		SC4MOL	Enzyme involved in cholesterol biosynthesis	[30]
		PLIN2	LDAP	
RNF5	RMA1, RING5, NG2	SCAP	See earlier	[11]
RNF20	BRE1A, HBRE1	SREBP1	See earlier	[57]
RNF139	HRCA1, TRC8	SREBP2	Regulates the expression of genes involved in cholesterol and FA biosynthesis and uptake	[9]
RNF145	/[a]	SCAP	SREBP trafficking factor	[14]
		HMGCR	See earlier	[22]
		ADIPOR2	Lipid hydrolase which maintains membrane fluidity	[64]
RNF213	KIAA1618, ALO17	ATGL	See earlier	[73]
		lipid A	Component of lipopolysaccharide in the outer bacterial membrane	[76]
Rsp5	/	Mga2	Transcription factor that regulates membrane fluidity	[63]
Siah2	/	PPARγ	See earlier	[52]
Tul1	/	Phospholipids	Hydrophilic glycerophosphate head linked to two hydrophobic FA tails	[74]
RING-between-RING (RBR) E3 ligases				
Parkin	AR-JP, PARK2, PDJ	CD36	Fatty acid translocase	[66]
HECT E3 ligases				
HUWE1	UREB1, Ib772, KIAA0312	ABCG1	Cholesterol efflux transporter	[43]
NEDD4-1	RPF1	ABCG1	Cholesterol efflux transporter	[43]
		PPARγ	Nuclear receptor involved in regulating FA metabolism	[53]
HECTD1	KIAA1131	ABCA1	Cholesterol efflux transporter	[44]
UBE3A	E6AP	ABCA1	Cholesterol efflux transporter	M. Loix *et al.*, unpublished

[a]/: No existing aliases.

Box 2. Degradative pathways for lipid metabolism

Various cellular degradation systems regulate lipid metabolism. Next to the UPS, autophagy and ERAD are important in maintaining lipid homeostasis. Autophagy is involved in the breakdown and recycling of cellular components, including proteins, organelles, and lipids. A type of selective autophagy, called 'lipophagy,' plays a key role in LD turnover. During this process, parts of LDs are sequestered in autophagosomes, which fuse with lysosomes. This allows the breakdown of LDs, releasing FFAs and glycerol [92]. ERAD is a cellular quality control mechanism that targets misfolded or unassembled proteins in the ER for proteasomal degradation. More than a dozen E3 ligases have been demonstrated to be involved in the ubiquitination of ERAD substrates, including Hrd1 and gp78. ERAD impacts lipid metabolism by regulating the turnover of ER-resident misfolded proteins involved in lipid synthesis and transport. This prevents their accumulation and potential disruption of cellular lipid homeostasis [93]. In summary, the UPS, autophagy, and ERAD are all important in lipid metabolism through different mechanisms. Although the UPS is involved in regulating the degradation of specific lipid-related proteins, autophagy is responsible for LD turnover and recycling, and ERAD ensures the quality control of ER-resident proteins involved in lipid metabolism. Together, these systems maintain cellular lipid homeostasis and prevent the aberrant accumulation of lipids and of damaged or misfolded proteins that can interfere with lipid-related processes.

domain of SQLE is strongly responsive to sterols, and high cellular cholesterol causes SQLE to undergo a conformational change, allowing its ubiquitination. Membrane-associated ring-CH-type finger 6 (MARCHF6) was identified as the responsible E3 in HEK293 cells, which is stabilized by high cholesterol levels itself by inhibiting MARCHF6 autoubiquitination [24,25]. Importantly, silencing of MARCHF6 was demonstrated to stabilize basal HMGCR levels as well. Yet, the contribution of MARCHF6 to HMGCR regulation is not fully clear as sterols were still able to promote HMGCR degradation in a mutant 25-hydroxycholesterol-resistant hamster cell line (SRD-1 cells) [24]. In support of MARCHF6 controlling cholesterol synthesis, follow-up studies demonstrated that it drives the ubiquitination of cholesterol synthesis enzymes lanosterol 14α-demethylase (LDM), dehydrocholesterol reductases (DHCR) 24, and sterol-C4-methyl oxidase-like (SC4MOL) in diverse cell lines [26,27]. Several other cholesterol biosynthesis enzymes, such as DHCR7 and DHCR14, were recently described to be regulated via the UPS, albeit the underlying mechanisms are still poorly understood [28–30].

Cholesterol uptake

As cholesterol biosynthesis is a complex and energy-intensive process, receptor-mediated **endocytosis** often meets cellular cholesterol requirements. Ample evidence demonstrated that the UPS is crucial in regulating key receptors involved in cellular cholesterol uptake, including LDLR, very low-density lipoprotein receptor (VLDLR), and ApoE receptor 2 (ApoER2). As described earlier, LDLR is transcriptionally regulated by SREBP2 when cellular sterol content is low. Vice versa, LXRs reduce LDLR protein abundance when sterol levels rise by inducing IDOL-mediated ubiquitination and degradation [15,31,32]. IDOL also promotes the ubiquitination and subsequent degradation of related receptors VLDR and APOER2 [33]. As these receptors are implicated in neuronal development, IDOL is situated at the crossroads of lipid metabolism and central nervous system (CNS) physiology. Accordingly, loss of IDOL in the CNS of mice protects them from diet-induced obesity, mimicking the phenotype of a whole-body IDOL knockout [34,35]. Therefore, the brain IDOL-VLDLR axis regulates systemic energy homeostasis. As an extra layer of regulation, LDLR was demonstrated to be degraded by proprotein convertase subtilisin/kexin type 9 (PCSK9), a serine protease in which gain-of-function mutations have been linked to familiar hypercholesterolemia. In HEK293T cells, PCSK9 cointernalized with LDLR through endocytosis and induced its ubiquitination. This resulted in lysosomal LDLR degradation instead of recycling to the cell membrane [36]. In aggregate, these studies indicate that the UPS curbs cellular cholesterol levels by driving the degradation of various members of the LDLR family [37].

Cholesterol esterification

Cholesterol esterification is essential for cellular storage and transfer of cholesterol and protects cells from the lipotoxic impact of excess free cholesterol. Cholesterol esterification

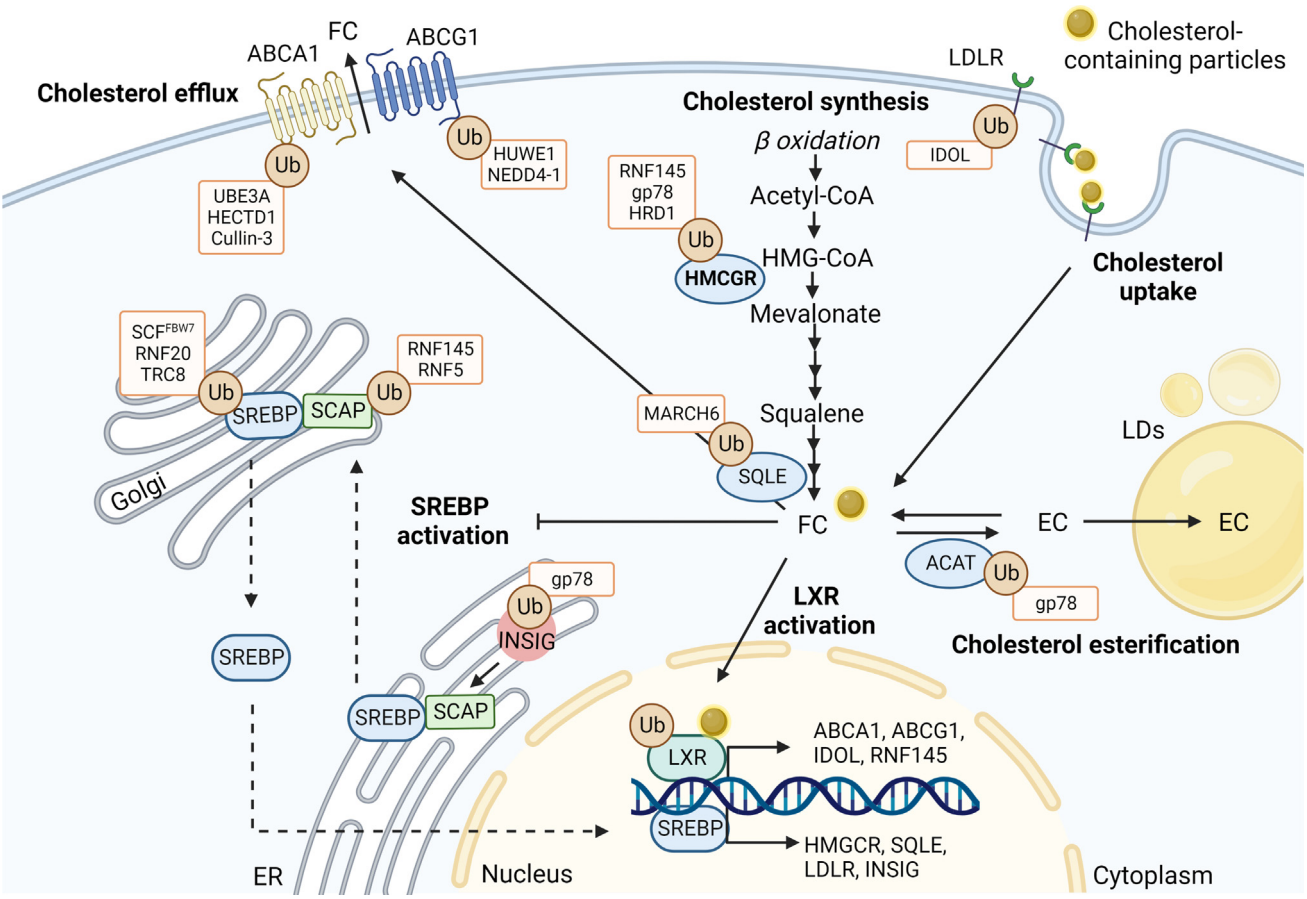

Figure 1. Role of ubiquitination in cholesterol metabolism. Ubiquitination is a common post-translational modification controlling cholesterol metabolism at multiple levels. Key cholesterol metabolism proteins that are described to be targets for ubiquitination are indicated by the orange ubiquitin (Ub) circles. Orange frames list identified E3 ligases involved. At the level of transcription, ubiquitination of sterol regulatory element-binding protein 1 (SREBP) and SREBP cleavage-activating protein (SCAP) hinders its translocation to the nucleus. By contrast, ubiquitination of insulin-induced gene 1 (INSIG) allows the SREBP-SCAP complex to travel from the Golgi to the nucleus. Besides inducing the expression of E3 ligase inducible degrader of low-density lipoprotein receptor (IDOL), liver X receptor (LXR) is degraded by the ubiquitin-proteasome system (UPS) itself. At the level of cholesterol synthesis, both 3-hydroxy-3-methylglutaryl-coa reductase (HMGCR) and squalene monooxygenase (SQLE) are substrates for ubiquitination. Ubiquitination induces the degradation of cholesterol uptake receptor low-density lipoprotein receptor (LDLR) and cholesterol efflux transporters ATP-binding cassette transporter A1 and G1 (ABCA1 and ABCG1). Finally, cholesterol esterification enzyme acyl-CoA:cholesterol acyltransferase (ACAT) was shown to be regulated by E3 ligase gp78. Abbreviations: EC, esterified cholesterol; ER, endoplasmic reticulum; FC, free cholesterol; HECTD1, HECT domain E3 ubiquitin protein ligase 1; HUWE1, HECT, UBA and WWE domain containing E3 ubiquitin protein ligase 1; LD, lipid droplet; MARCH6, membrane-associated ring finger (C3HC4) 6; RNF, ring finger proteins; SCF^{FBW7}, Skp1–Cul1–FBW7 E3 ubiquitin ligase complex; TRC8, translocation in renal carcinoma, chromosome 8 gene; UBE3A, ubiquitin protein ligase E3A. Enzymes in bold denote rate-limiting activity.

enzyme acyl-CoA:cholesterol acyltransferase 2 (ACAT2) was shown to be regulated by a less common cysteine-linked form of ubiquitination [38]. Here, treatment of multiple mammalian cell types with sterols or FAs resulted in a rapid increase in ACAT2 protein level through ROS-mediated oxidation of the ubiquitin-conjugation site of ACAT2, which allowed efficient storage of sterols in the cell. The authors identified gp78 and its cofactor INSIG as ACAT2 interactors responsible for ACAT2 ubiquitination. In addition, ACAT1 is a target for proteasomal degradation as USP19 was found to enhance cholesterol esterification through deubiquitinating and stabilizing ACAT1 in hepatocellular carcinoma cells [39]. Together, these studies indicate that the UPS controls cholesterol esterification by regulating ACAT1 and ACAT2 levels by divergent E3 ligases.

Cholesterol efflux

As most cell types are unable to metabolize cholesterol, active cholesterol export is essential to reduce intracellular sterol levels and maintain homeostasis. Several ABC transporters, including ABCA1, ABCG1, ABCG5, and ABCG8, have been demonstrated to mediate cholesterol efflux. In mammalian cells, cholesterol efflux is mediated predominantly by ABCA1 and ABCG1, which export free cholesterol toward the extracellular ApoA-I acceptor protein or to HDL, respectively, thereby promoting '**reverse cholesterol transport**' [40]. Proteasomal inhibition increases the abundance of both transporters and cholesterol efflux *in vitro* and *in vivo* in macrophages. Yet, the underlying mechanisms underpinning the latter are poorly understood [41,42]. First, HUWE1 (HECT, UBA, and WWE domain containing E3 ubiquitin protein ligase 1) and NEDD4-1 (neural precursor cell expressed developmentally downregulated protein 4-1)-mediated ubiquitination was demonstrated to control ABCG1 in a human monocyte-derived macrophage cell line [43]. In a follow-up study, ABCA1 was identified as a target for HECTD1 (HECT domain E3 ubiquitin protein ligase 1) ubiquitination in non-cholesterol-loaded macrophages as well, without affecting ABCA1 in cholesterol-loaded cells [44]. Later, cullin-3 was proposed to mediate ABCA1 ubiquitination in response to the activation of thrombin-protease-activated receptor (PAR)1 signaling in macrophages and smooth muscle cells, thereby promoting atherosclerotic lesion formation in $ApoE^{-/-}$ mice [45,46]. Finally, ubiquitin-protein E3 ligase A (UBE3A)-mediated ubiquitination of ABCA1 plays a crucial role in driving myelin-induced foam cell formation in models for CNS repair (M. Loix *et al.*, unpublished). Lipid accumulation induced a more inflammatory macrophage phenotype, which suppresses myelin repair in an *ex vivo* organotypic brain slice model [47–49]. In aggregate, the UPS plays an important role in regulating cellular cholesterol efflux capacity, although underlying mechanisms are still poorly understood. Identification of responsible E3 ligases and metabolic cues can lead to the discovery of new therapeutic targets for diseases involving cholesterol metabolism.

UPS-mediated control of FA metabolism

FAs are pivotal in the human body as they are obligatory constituents of membranes, are the most energy-dense substrate for ATP generation, serve as ligands for nuclear receptors, and affect protein function by post-translational acylation of target proteins. FA homeostasis is governed by their uptake, *de novo* synthesis, and oxidation (**β-oxidation**). As discussed in the following sections, increasing evidence supports UPS-mediated protein degradation acting as a crucial regulator for each of these processes (Figure 2).

Transcriptional regulation of FA metabolism

Peroxisome proliferator-activated receptors (**PPARs**) are crucial for maintaining energy homeostasis by regulating glucose, FA, and insulin metabolism at a transcriptional level. Several studies have shown that the UPS is essential in fine-tuning PPARγ activity by promoting ubiquitination of its ligand-binding domain upon ligand binding, thereby serving as a negative feedback loop that limits transcriptional activity of PPARγ [50]. Follow-up research demonstrated that the inflammatory mediator IFNγ enhances proteasomal degradation of PPARγ in adipocytes, resulting in the development of insulin resistance [51]. E3 ligases that ubiquitinate PPARγ include Siah2 [52] and NEDD4-1 [53]. By contrast, USP22 was found to be involved in PPARγ stabilization, thereby increasing the expression of rate-limiting enzyme acetyl-CoA carboxylase (ACC) and ATP citrate lyase (ACLY), and promoting *de novo* FA synthesis in hepatocellular carcinoma cells [54]. Of note, PPARγ holds E3 ligase activity as well, as its activation induces Lys48-linked ubiquitination of the Rel homology domain region of nuclear factor κB/P65, which halts proinflammatory signaling in cancer tissue [55].

Next to PPARs, SREBP1 plays a key role in regulating FA synthesis. Few studies indicate that the UPS controls SREBP degradation [9,56,57]. First, phosphorylation of transcriptional active

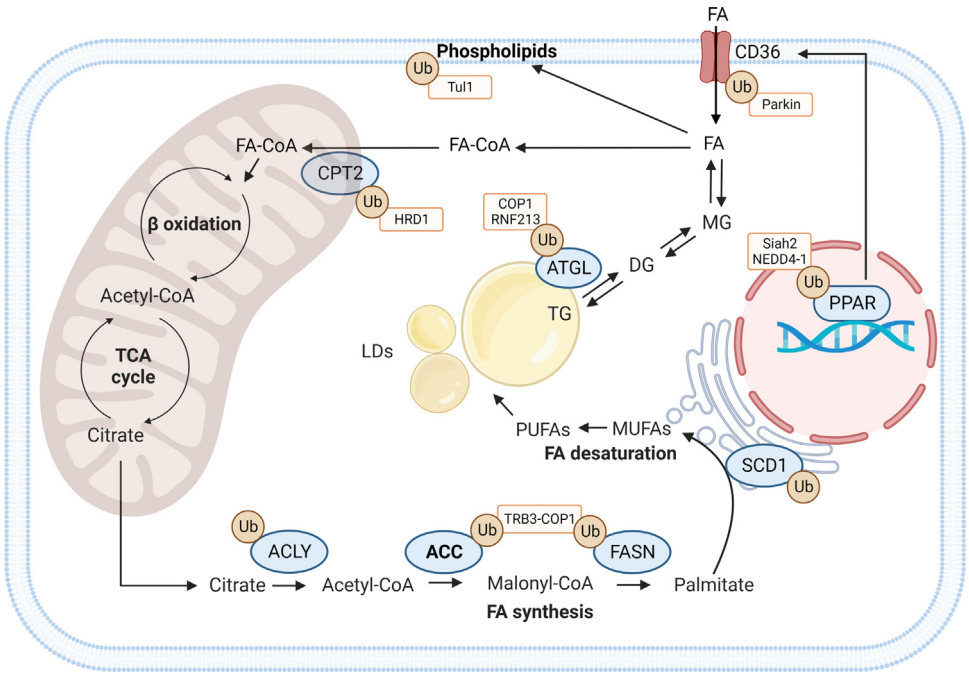

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Figure 2. Role of ubiquitination in fatty acid (FA) metabolism. FA homeostasis is governed by their uptake, *de novo* synthesis, and oxidation (β-oxidation), and ubiquitin-proteasome system (UPS)-mediated protein degradation acts as a crucial regulator for each of these processes. FAs activate SREBP1, which regulates FA synthesis and is a target for ubiquitination by the Skp1-Cul1-FBW7 E3 ligase complex (SCF^{FBW7}) and ring finger protein (RNF) 20. FAs also activate peroxisome proliferator-activated receptors (PPARs) whose activity is fine-tuned by the UPS. PPARs promote the expression of cluster of differentiation 36 (CD36), a receptor involved in the uptake of FAs that is regulated by the UPS in a PARKIN-dependent manner. Internalized free FAs (FFAs) can be converted to monoglyceride (MGs), diglycerides (DGs), and triglycerides (TGs), which are stored in lipid droplets (LDs). Upon energy demand, TGs are hydrolyzed by lipolysis, which requires the action of adipose triglyceride lipase (ATGL) and is subject to ubiquitination. Furthermore, FFAs can be used to generate unsaturated FAs [monounsaturated FAs (MUFAs) and polyunsaturated Fas (PUFAs)], which requires the action of carnitine palmitoyltransferase 2 (CPT2), which is necessary for FA transport into mitochondria, acyl-protein thioesterases (ACLY), acetyl-CoA carboxylase (ACC), fatty acid synthetase (FASN), and stearoyl-CoA-desaturase-1 (SCD1), which are all targets for ubiquitination. Orange frames list the identified E3 ligases involved. Abbreviation: TRB3, Tribbles 3; Ub, ubiquitin. Enzymes in bold denote rate-limiting activity.

SREBP1 was demonstrated to serve as a degron for ubiquitination by the Skp1-Cul1-FBXW7 E3 ligase complex (SCF^{FBXW7}), resulting in its proteasomal degradation in an HepG2 cell line [56]. Vice versa, acetylation of SREBP1 improves its stability by competing with ubiquitin for SREBP lysine residues [58]. Alongside SCF^{FBXW7}, RNF20 mediates proteasomal degradation of SREBP1 upon nutrient deprivation, thereby inhibiting lipogenesis in primary hepatocytes when precursor metabolites are limited [57].

FA synthesis and remodeling

Early studies already demonstrated an essential role for the UPS in controlling FA synthesis by Tribbles pseudokinase 3 (TRB3)-COP1-mediated ubiquitination of ACC and FA synthase (FASN) in adipose tissue [59]. Furthermore, USP2 and USP38 were lately reported to deubiquitinate and stabilize ACC and FASN [60]. ACC and FASN deubiquitination enhanced triglyceride production in gastric cancer cells, thereby promoting gastric cancer cell growth, migration, and tumorigenesis. Recently, ubiquitination of ACLY, the enzyme responsible for the conversion of citrate toward acetyl-CoA, was demonstrated [61]. ACLY ubiquitination and degradation enhanced FA oxidation (FAO) at the expense of *de novo* FA biosynthesis in regulatory T cell

(Tregs). The authors further showed that this was closely associated with tumor growth factor-β signaling, a pathway involved in Treg differentiation, which alleviated autoimmunity in a mouse colitis model.

Alongside FA synthesis, the UPS also controls FA desaturation. In a murine adipocyte cell line, stearoyl-CoA desaturase 1 (SCD1), a key enzyme that catalyzes the formation of monounsaturated FAs, was demonstrated to be controlled by the UPS, although E3 ligases involved remain to be uncovered [62]. FA desaturation plays a vital role in maintaining cellular membrane fluidity, and increased lipid saturation causes membrane rigidification and lipotoxicity. An interesting connection between membrane fluidity, ER-associated degradation (**ERAD**), and the UPS has been uncovered. In yeast, ERAD was demonstrated to closely regulate membrane fluidity in a ubiquitin-dependent manner [63]**.** Specifically, increased lipid saturation enhanced ubiquitination of the transcription factor Mga2 by E3 ligase RSP5. Consequently, Mga2 undergoes proteasomal processing, resulting in the production of transcriptionally active Mga2. Activated Mga2 promotes the expression of FA cis-Δ9-desaturase OLE1, thereby increasing membrane lipid desaturation and restoring membrane fluidity. Another critical player involved in counteracting saturated FA (SFA)-induced membrane rigidification is lipid hydrolase adiponectin receptor 2 (ADIPOR2), which was identified as a substrate of lipid-sensitive RNF145 [64]. In unsaturated membranes, RNF145 remains stable and ubiquitinates ADIPOR2, inducing its degradation. However, when membranes become enriched with SFAs, RNF145 is autoubiquitinated, leading to its degradation. The latter stabilizes ADIPOR2 and allows restoration of membrane homeostasis.

FA uptake

Alongside biogenesis, cellular FA content is regulated via endocytic processes. The FA translocase cluster of differentiation 36 (CD36) is a well-studied receptor involved in the uptake of FAs and is proposed to be sensitive to UPS-dependent regulation [65–68]. Smith *et al.* [65] were the first to report ubiquitin-mediated regulation of CD36 and found that changes in CD36 abundance upon exposure to FAs and insulin are, at least partially, due to changes in its ubiquitination status. Although insulin exposure reduced CD36 ubiquitination and increased its membrane abundance in Chinese hamster ovary and HEK293 cells, FA treatment enhanced CD36 ubiquitination and reduced CD36 levels [65]. By contrast, ubiquitination was demonstrated to play a role in CD36 stabilization as well. Specifically, Parkin-deficient mice show lower hepatocellular C36 levels and are resistant to weight gain, and reconstitution of Parkin increased CD36 and FA uptake in liver cells [66]. Interestingly, Parkin is defective in patients with early-onset PD. Hence, impaired Parkin-mediated CD36 stabilization may provide a molecular rationale for the observed lipid abnormalities in these patients. Later, deubiquitinating enzymes ubiquitin C-terminal hydrolase 1 (UCHL1) and USP14 were shown to decrease oxidized LDL-induced foam cell formation by reducing CD36 ubiquitination and were suggested to be promising targets for atherosclerosis treatment [67,68].

FA oxidation

To date, few studies demonstrated a role for the UPS in controlling FAO, also called β-oxidation. Using **mass spectrometry**, hydroxysteroid 17-beta dehydrogenase 4 (HSD17B4), and carnitine-palmitoyl-transferase 2 (CPT2), enzymes necessary for FAO and transport into mitochondria, were defined as substrates for the E3 ligase HRD1 in human liver HepG2 cells. Loss of HRD1 induced a metabolic profile that favored FAO, inhibited lipogenesis, and protected mice from diet-induced obesity [69]. Later, ubiquitination of CPT2 by HRD1 was confirmed in human triple-negative breast cancer (TNBC) cells. As a result, HRD1 expression is decreased in TNBC cells, which enhances FAO and tumorigenesis [70]. HRD1 acts as a homeostatic ERAD-associated E3 and therefore has a promiscuous substrate specificity.

Triglyceride metabolism

Excessive levels of free FAs (FFAs) are toxic as their amphipathic structure can perturb membranes. To prevent lipotoxicity, FFAs can be stored in triglycerides, which are formed from the combination of glycerol and three FA molecules, and are generally stored in **lipid droplets (LDs)**. When stored energy has to be mobilized, LD-associated triglycerides can be hydrolyzed by a series of cytosolic lipases in a process called **lipolysis**, releasing glycerol and FFAs [71]. Several recent studies demonstrated UPS-dependent regulation of adipose triglyceride lipase (**ATGL**) [72,73]. First, ubiquitination of ATGL was shown in hepatocytes in a COP1-dependent manner, thereby reducing triglyceride hydrolysis and increasing hepatocellular lipid content. In support of this notion, COP1 inhibition ameliorated hepatic steatosis in high-fat diet–fed mice [72]. Second, RNF213 was demonstrated to ubiquitinate ATGL on LDs, thereby attenuating triglyceride hydrolysis and increasing LD content [73]. Whether the UPS affects other lipases or triglyceride synthesis remains to be explored.

Ubiquitination of lipids

Although ubiquitination was formerly considered a protein-linked modification, in the past decade, various other types of substrates, such as lipids, were found to be targeted by ubiquitination.

Ubiquitination of phospholipids

Phospholipids consist of a hydrophilic glycerophosphate head linked to two hydrophobic FA tails. A recent study by Sakamaki *et al.* showed that ubiquitin can tag phospholipid species [74]. Specifically, endosomal and lysosomal phosphatidylethanolamine (PE) was shown to be ubiquitinated in yeast and human cells by the ubiquitin enzymes Uba1 (E1), Ubc4/5 (E2), and Tul1 (E3). The C-terminal glycine of ubiquitin was found to form a linkage bond with the amino group of PE, resulting in the recruitment of endosomal sorting complex required for transport (ESCRT) components, and was postulated to regulate membrane dynamics and the formation of cellular vesicles. Vice versa, phospholipids control protein ubiquitination. For instance, cardiolipin promotes the autoubiquitination of MARCH5, a mitochondrial membrane-resident E3 ligase important in the regulation of mitophagy [75]. In aggregate, these studies show an intricate relationship between the UPS and phospholipid dynamics.

Ubiquitination of bacterial lipids

In bacteria-infected host cells, ubiquitin is crucial for facilitating targeted xenophagic destruction. Next to tagging bacterial membrane proteins, recent research shows that bacterial lipids are earmarked by ubiquitin as well. Otten *et al.* [76] demonstrated that lipid A, a component of lipopolysaccharide in the outer bacterial membrane, is a target of ubiquitination. They further identified the giant ~600 kDa E3 ligase RNF213 as the responsible enzyme [76]. Hence, lipid regulation by the UPS represents an important defense response against invading pathogenic microorganisms and is crucial in preserving human health.

Ubiquitin-dependent regulation of LD dynamics

LDs can be metabolized to release FFAs and free cholesterol. This dynamic process occurs via cytosolic lipolysis or lipophagy, a selective form of **autophagy** of LDs [77]. To date, mechanisms underpinning LD degradation remain poorly understood, but increasing evidence indicates that ubiquitination is involved (Figure 3). With respect to the latter, ubiquitin can associate with light-chain 3 (LC3, autophagosome marker) and sequestosome 2 (SQSTM2) on LDs during lipophagy in foamy macrophages. The authors identified several LD-associated proteins (LDAPs) related to the ubiquitination machinery, such as ancient ubiquitous protein 1 (AUP1) and UBE2G2, to be required for lipophagy and consequent lipid efflux [78]. Similarly, other studies demonstrated that LDAP stability is closely monitored by the UPS machinery, with LD breakdown and biogenesis in

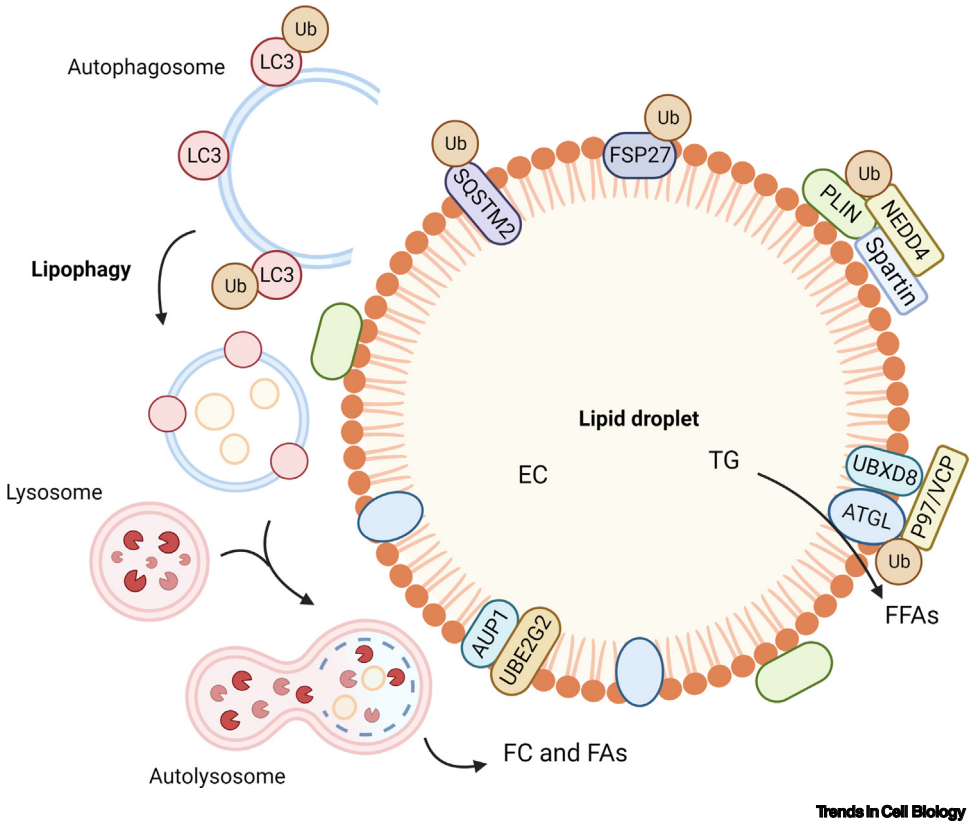

Figure 3. Ubiquitination in lipid droplet (LD) metabolism. Overview of LD and lipophagy proteins that are described to be regulated by ubiquitination. Ubiquitin (Ub) can associate with light-chain 3 (LC3) and sequestosome 2 (SQSTM2) on LDs during lipophagy. Several LD-associated proteins (LDAPs) are related to the ubiquitination machinery, such as ancient ubiquitous protein 1 (AUP1) and ubiquitin conjugating enzyme E2 (UBE2G2). Furthermore, LDAPs adipose triglyceride lipase (ATGL), fat-specific protein 27 (FSP27), and members of the perilipin (PLIN) family are degraded by the ubiquitin-proteasome system (UPS). Abbreviations: EC, esterified cholesterol; FC, free cholesterol; FFA, free fatty acid; TG, triglycerides.

lipid-deprived and -sufficient conditions being associated with increased proteasomal degradation or stability of LDAPs, respectively. Examples of LDAPs that the UPS degrades include perilipin (PLIN) [79], ATGL [80], and LD fusion protein FSP27 (fat-specific protein 27) [81]. In addition, several proteins that function in the ubiquitination pathway or contain ubiquitin-binding motifs have been identified in the LD proteome and can function to regulate UPS-mediated LDAP degradation directly [80,82,83]. For instance, it was demonstrated in mouse embryonic fibroblasts that UBXD8 can recruit VCP/p97 to LDs, thereby inhibiting ATGL activity and increasing LD size [80]. Also, LD membrane-resident Spartin and AUP1 were identified to serve as adaptors for E3 ligases in several cell lines, thereby providing a direct molecular connection between LDs and the ubiquitination machinery [82,83]. All in all, significant evidence argues for an important role of the UPS in the regulation of the LD proteome and dynamics.

Dysregulated ubiquitination in lipid disorders

Altered lipid metabolism is one of the most significant metabolic changes in cancer cells, and dysregulation of ubiquitination of lipid metabolism proteins was demonstrated to affect tumor development. In line with this, most currently available therapies that target the UPS are developed for malignancies and may mediate their effects, at least partly, through intervening with tumor cell lipid metabolism [84]. Atherosclerosis, a metabolic disorder characterized by disrupted lipid

homeostasis and plaque formation, has been linked to altered ubiquitin-mediated regulation of lipid metabolism pathways as well. Here, E3 ligases are shown to be important contributors to the development of this chronic inflammatory vascular disease, and treatment strategies focused at targeting E3 ligases have gained increasing attention in recent years [85]. Furthermore, UPS dysfunction is an important pathological hallmark of several neurodegenerative disorders, such as AD and PD. Specifically, a derangement of the UPS is correlated with toxic amyloid β (Aβ) aggregation and dysregulation of lipid levels in AD [2]. In addition, IDOL is the primary post-translational regulator of LDLR in the brain and was shown to control ApoE-containing HDL particles and Aβ accumulation, thereby increasing plaque burden and neuroinflammation [86]. Finally, mutations in the E3 ligase gene *PARKIN* are associated with PD development, and multiple groups demonstrated that loss of PARKIN leads to lipidomic alterations in the mitochondria of mice in an SREBP-dependent manner [87].

Therapeutic targeting of the UPS

As ubiquitination involves a three-step catalytic pathway with a broad array of proteins involved, multiple strategies could be applied to target the UPS. Bortezomib (trade name Velcade) and its derivates were the first approved UPS-targeting drugs and showed potent effects for the treatment of multiple malignancies [88]. Later, additional UPS-directed drugs were developed, and it was demonstrated that combining different UPS inhibitors was more effective while avoiding the development of drug resistance. Yet, given the complex UPS network, it is more interesting to have a selective approach of UPS targeting. One promising avenue involves intervening with substrate–E3 interactions, which are increasingly recognized as attractive targets with substantial potential. However, this approach has been considered one of the most challenging therapeutic strategies due to several factors. The interaction between substrates and E3 ligases occurs over a large and flat interface area, making it difficult to find small molecular ligands for reference. Additionally, the high molecular weight of protein–protein interactions complicates the development of therapeutics. Alternatively, specificity could be provided by targeting E2 or E3 ligases since these determine, respectively, the nature of the polyubiquitin chain and substrate specificity. Although proteasomal inhibitors are widely used in the clinic for cancer therapy already, small molecules that target specific E2 or E3 ligases are still under development. The identification of these using high-throughput screens is challenging due to the complexity of the ubiquitination reactions and the weak interaction between ligases and their substrates [89,90]. As a result, inhibitors remain poorly defined, and further characterization of their structure and mechanisms should benefit the development of future modulators.

Concluding remarks

Since the discovery of ubiquitination in 1975, significant progress has been made in understanding the molecular action of ubiquitination, and increasing evidence shows a crucial role for ubiquitination events in regulating divergent aspects of lipid metabolism. As a result, dysregulation of ubiquitination actions can lead to aberrant activation or repression of lipid metabolism pathways and has been linked to the development and progression of a number of disorders. Importantly, ubiquitination events are highly context dependent and can vary significantly between cell types, tissues, and organs. Studies investigating ubiquitination typically focus on specific cell types or tissues, which can limit the generalizability of the findings to other contexts. In addition, despite the existence of a broad range of E3 ligases, certain ligases are more often identified as enzymes responsible for ubiquitinating lipid metabolism substrates. Given this complexity and cell-specific nature of ubiquitination, it is crucial to consider these factors when interpreting experimental data. Mass spectrometry–based approaches and bioinformatics have emerged as powerful tools to define ubiquitinated substrates and ubiquitin ligases involved in a high-throughput and more global manner. Further identification of underlying enzymes, targets, and their context will improve

Outstanding questions

Identification of underlying enzymes, targets, and their context will improve our understanding of the role of ubiquitination in lipid metabolism. Which other key proteins of cholesterol, fatty acid, and lipid droplet metabolism are substrates for ubiquitin-mediated regulation?

Certain E3 ligases are shown to target several lipid metabolism substrates. Are there common substrate motives by which E3 ligases impact lipid metabolism?

Although ubiquitination was formerly considered a protein-linked modification, other types of substrates, such as phospholipids, can be a target for ubiquitination as well. In addition to phospholipids, are other lipids ubiquitinated as well?

Enhancing the precision and throughput of identifying E2 and E3 ligases engaged in lipid metabolism holds the key to unraveling cellular processes. How can the identification of E2 and E3 ligases be improved?

Selective targeting of the ubiquitination pathway holds promise for disorders characterized by lipid dysregulation. How can we target ubiquitin-mediated degradation of lipid metabolism proteins in human diseases characterized by disrupted lipid homeostasis?

our understanding of the role of ubiquitination in lipid metabolism and provide more insights into implications for disease development and treatment (see Outstanding questions).

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Declaration of interests

The authors declare no competing interests.

References

1. Mani, A. and Gelmann, E.P. (2005) The ubiquitin-proteasome pathway and its role in cancer. *J. Clin. Oncol.* 23, 4776–4789
2. Zheng, Q. *et al.* (2016) Dysregulation of ubiquitin-proteasome system in neurodegenerative diseases. *Front. Aging Neurosci.* 8, 303
3. Zechner, R. *et al.* (2012) FAT SIGNALS – lipases and lipolysis in lipid metabolism and signaling. *Cell Metab.* 15, 279–291
4. Yoon, H. *et al.* (2021) Lipid metabolism in sickness and in health: emerging regulators of lipotoxicity. *Mol. Cell* 81, 3708–3730
5. Huang, B. *et al.* (2020) Cholesterol metabolism in cancer: mechanisms and therapeutic opportunities. *Nat. Metab.* 2, 132–141
6. Dai, L. *et al.* (2021) Cholesterol metabolism in neurodegenerative diseases: molecular mechanisms and therapeutic targets. *Mol. Neurobiol.* 58, 2183–2201
7. Sharpe, L.J. *et al.* (2014) The UPS and downs of cholesterol homeostasis. *Trends Biochem. Sci.* 39, 527–535
8. Yan, R. *et al.* (2021) Structural basis for sterol sensing by Scap and Insig. *Cell Rep.* 35, 109299
9. Irisawa, M. *et al.* (2009) The sterol-sensing endoplasmic reticulum (ER) membrane protein TRC8 hampers ER to Golgi transport of sterol regulatory element-binding protein-2 (SREBP-2)/SREBP cleavage-activated protein and reduces SREBP-2 cleavage. *J. Biol. Chem.* 284, 28995–29004
10. Zhang, L. *et al.* (2017) Inhibition of cholesterol biosynthesis through RNF145-dependent ubiquitination of SCAP. *Elife* 6, e28766
11. Kuan, Y.C. *et al.* (2020) Ring finger protein 5 activates sterol regulatory element-binding protein 2 (SREBP2) to promote cholesterol biosynthesis via inducing polyubiquitination of SREBP chaperone SCAP. *J. Biol. Chem.* 295, 3918–3928
12. Gong, Y. *et al.* (2006) Sterol-regulated ubiquitination and degradation of Insig-1 creates a convergent mechanism for feedback control of cholesterol synthesis and uptake. *Cell Metab.* 3, 15–24
13. Lee, J.N. *et al.* (2006) Sterol-regulated degradation of Insig-1 mediated by the membrane-bound ubiquitin ligase gp78. *J. Biol. Chem.* 281, 39308–39315
14. Cook, E.C. *et al.* (2017) Identification of the ER-resident E3 ubiquitin ligase RNF145 as a novel LXR-regulated gene. *PLoS One* 12, e0172721
15. Zelcer, N. *et al.* (2009) LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor. *Science* 325, 100–104
16. Sorrentino, V. and Zelcer, N. (2012) Post-transcriptional regulation of lipoprotein receptors by the E3-ubiquitin ligase inducible degrader of the low-density lipoprotein receptor. *Curr. Opin. Lipidol.* 23, 213–219
17. Dong, M. *et al.* (2021) Interferon-γ decreases ATP-binding cassette subfamily G member 1-mediated cholesterol efflux through small ubiquitin-like modifier/ubiquitin-dependent liver X receptor-α degradation in macrophages. *Biotechnol. Appl. Biochem.* 68, 1412–1420
18. Hsieh, J. *et al.* (2016) TTC39B deficiency stabilizes LXR reducing both atherosclerosis and steatohepatitis. *Nature* 535, 303–307
19. Sever, N. *et al.* (2003) Accelerated degradation of HMG CoA reductase mediated by binding of insig-1 to its sterol-sensing domain. *Mol. Cell* 11, 25–33
20. Sever, N. *et al.* (2003) Insig-dependent ubiquitination and degradation of mammalian 3-hydroxy-3-methylglutaryl-CoA reductase stimulated by sterols and geranylgeraniol. *J. Biol. Chem.* 278, 52479–52490
21. Loregger, A. *et al.* (2017) Haploid mammalian genetic screen identifies UBXD8 as a key determinant of HMGCR degradation and cholesterol biosynthesis. *Arterioscler. Thromb. Vasc. Biol.* 37, 2064–2074
22. Menzies, S.A. *et al.* (2018) The sterol-responsive RNF145 E3 ubiquitin ligase mediates the degradation of HMG-CoA reductase together with gp78 and Hrd1. *ELife* 7, e40009
23. Lu, X.Y. *et al.* (2020) Feeding induces cholesterol biosynthesis via the mTORC1-USP20-HMGCR axis. *Nature* 588, 479–484
24. Zelcer, N. *et al.* (2014) The E3 ubiquitin ligase MARCH6 degrades squalene monooxygenase and affects 3-hydroxy-3-methyl-glutaryl coenzyme A reductase and the cholesterol synthesis pathway. *Mol. Cell. Biol.* 34, 1262–1270
25. Sharpe, L.J. *et al.* (2019) Cholesterol increases protein levels of the E3 ligase MARCH6 and thereby stimulates protein degradation. *J. Biol. Chem.* 294, 2436–2448
26. Scott, N.A. *et al.* (2020) The cholesterol synthesis enzyme lanosterol 14α-demethylase is post-translationally regulated by the E3 ubiquitin ligase MARCH6. *Biochem. J.* 477, 541–555
27. Qian, L. *et al.* (2023) Cholesterol synthesis enzyme SC4MOL is fine-tuned by sterols and targeted for degradation by the E3 ligase MARCHF6. *J. Lipid Res.* 64, 100362
28. Capell-Hattam, I.M. *et al.* (2020) Twin enzymes, divergent control: the cholesterogenic enzymes DHCR14 and LBR are differentially regulated transcriptionally and post-translationally. *J. Biol. Chem.* 295, 2850–2865
29. Prabhu, A.V. *et al.* (2016) Cholesterol-mediated degradation of 7-dehydrocholesterol reductase switches the balance from cholesterol to vitamin D synthesis. *J. Biol. Chem.* 291, 8363–8373
30. Mitsche, M.A. *et al.* (2015) Flux analysis of cholesterol biosynthesis in vivo reveals multiple tissue and cell-type specific pathways. *Elife* 4, e07999
31. Scotti, E. *et al.* (2013) IDOL stimulates clathrin-independent endocytosis and multivesicular body-mediated lysosomal degradation of the low-density lipoprotein receptor. *Mol. Cell. Biol.* 33, 1503–1514
32. Zhang, L. *et al.* (2013) Both K63 and K48 ubiquitin linkages signal lysosomal degradation of the LDL receptor. *J. Lipid Res.* 54, 1410–1420
33. Hong, C. *et al.* (2010) The E3 ubiquitin ligase IDOL induces the degradation of the low density lipoprotein receptor family members VLDLR and ApoER2. *J. Biol. Chem.* 285, 19720–19726
34. Lee, S.D. *et al.* (2019) IDOL regulates systemic energy balance through control of neuronal VLDLR expression. *Nat. Metab.* 1, 1089–1100
35. van Loon, N.M. *et al.* (2018) Inactivation of the E3 ubiquitin ligase IDOL attenuates diet-induced obesity and metabolic dysfunction in mice. *Arterioscler. Thromb. Vasc. Biol.* 38, 1785–1795
36. Chen, Y. *et al.* (2011) Role of ubiquitination in PCSK9-mediated low-density lipoprotein receptor degradation. *Biochem. Biophys. Res. Commun.* 415, 515–518
37. Sorrentino, V. *et al.* (2013) Identification of a loss-of-function inducible degrader of the low-density lipoprotein receptor variant in individuals with low circulating low-density lipoprotein. *Eur. Heart J.* 34, 1292–1297
38. Wang, Y.J. *et al.* (2017) Cholesterol and fatty acids regulate cysteine ubiquitylation of ACAT2 through competitive oxidation. *Nat. Cell Biol.* 19, 808–819

39. Zhu, Y. *et al.* (2023) P53 deficiency affects cholesterol esterification to exacerbate hepatocarcinogenesis. *Hepatology* 77, 1499–1511
40. Calkin, A.C. and Tontonoz, P. (2012) Transcriptional integration of metabolism by the nuclear sterol-activated receptors LXR and FXR. *Nat. Rev. Mol. Cell Biol.* 13, 213–224
41. Ogura, M. *et al.* (2011) Proteasomal inhibition promotes ATP-binding cassette transporter A1 (ABCA1) and ABCG1 expression and cholesterol efflux from macrophages in vitro and in vivo. *Arterioscler. Thromb. Vasc. Biol.* 31, 1980–1987
42. Hsieh, V. *et al.* (2014) Cellular cholesterol regulates ubiquitination and degradation of the cholesterol export proteins ABCA1 and ABCG1. *J. Biol. Chem.* 289, 7524–7536
43. Aleidi, S.M. *et al.* (2015) The E3 ubiquitin ligases, HUWE1 and NEDD4-1, are involved in the post-translational regulation of the ABCG1 and ABCG4 lipid transporters. *J. Biol. Chem.* 290, 24604–24613
44. Aleidi, S.M. *et al.* (2018) The E3 ubiquitin ligase, HECTD1, is involved in ABCA1-mediated cholesterol export from macrophages. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1863, 359–368
45. Raghavan, S. *et al.* (2018) Protease-activated receptor 1 inhibits cholesterol efflux and promotes atherogenesis via cullin 3-mediated degradation of the ABCA1 transporter. *J. Biol. Chem.* 293, 10574–10589
46. Boro, M. *et al.* (2021) Thrombin-Par1 signaling axis disrupts COP9 signalosome subunit 3-mediated ABCA1 stabilization in inducing foam cell formation and atherogenesis. *Cell Death Differ.* 28, 780–798
47. Bogie, J.F.J. *et al.* (2020) Stearoyl-CoA desaturase-1 impairs the reparative properties of macrophages and microglia in the brain. *J. Exp. Med.* 217, e20191660
48. Grajchen, E. *et al.* (2018) The physiology of foamy phagocytes in multiple sclerosis. *Acta Neuropathol. Commun.* 6, 124
49. Matsuo, M. (2022) ABCA1 and ABCG1 as potential therapeutic targets for the prevention of atherosclerosis. *J. Pharmacol. Sci.* 148, 197–203
50. Hauser, S. *et al.* (2000) Degradation of the peroxisome proliferator-activated receptor gamma is linked to ligand-dependent activation. *J. Biol. Chem.* 275, 18527–18533
51. Floyd, Z.E. and Stephens, J.M. (2002) Interferon-gamma-mediated activation and ubiquitin-proteasome-dependent degradation of PPARgamma in adipocytes. *J. Biol. Chem.* 277, 4062–4068
52. Kilroy, G. *et al.* (2012) The ubiquitin ligase Siah2 regulates PPARγ activity in adipocytes. *Endocrinology* 153, 1206–1218
53. Han, L. *et al.* (2013) Upregulation of SIRT1 by 17β-estradiol depends on ubiquitin-proteasome degradation of PPAR-γ mediated by NEDD4-1. *Protein Cell* 4, 310–321
54. Ning, Z. *et al.* (2022) USP22 regulates lipidome accumulation by stabilizing PPARγ in hepatocellular carcinoma. *Nat. Commun.* 13, 2187
55. Hou, Y. *et al.* (2012) PPARγ is an E3 ligase that induces the degradation of NFκB/p65. *Nat. Commun.* 3, 1300
56. Sundqvist, A. *et al.* (2005) Control of lipid metabolism by phosphorylation-dependent degradation of the SREBP family of transcription factors by SCF(Fbw7). *Cell Metab.* 1, 379–391
57. Lee, J.H. *et al.* (2014) Ring finger protein20 regulates hepatic lipid metabolism through protein kinase A-dependent sterol regulatory element binding protein1c degradation. *Hepatology* 60, 844–857
58. Giandomenico, V. *et al.* (2003) Coactivator-dependent acetylation stabilizes members of the SREBP family of transcription factors. *Mol. Cell. Biol.* 23, 2587–2599
59. Qi, L. *et al.* (2006) TRB3 links the E3 ubiquitin ligase COP1 to lipid metabolism. *Science* 312, 1763–1766
60. Zheng, Z. *et al.* (2022) Ubiquitin specific peptidase 38 promotes the progression of gastric cancer through upregulation of fatty acid synthase. *Am. J. Cancer Res.* 12, 2686–2696
61. Tian, M. *et al.* (2021) ACLY ubiquitination by CUL3-KLHL25 induces the reprogramming of fatty acid metabolism to facilitate iTreg differentiation. *Elife* 10, e62394
62. Kato, H. *et al.* (2006) Ubiquitin-proteasome-dependent degradation of mammalian ER stearoyl-CoA desaturase. *J. Cell Sci.* 119, 2342–2353
63. Ballweg, S. *et al.* (2020) Regulation of lipid saturation without sensing membrane fluidity. *Nat. Commun.* 11, 756
64. Volkmar, N. *et al.* (2022) Regulation of membrane fluidity by RNF145-triggered degradation of the lipid hydrolase ADIPOR2. *EMBO J.* 41, e110777
65. Smith, J. *et al.* (2008) Opposite regulation of CD36 ubiquitination by fatty acids and insulin: effects on fatty acid uptake. *J. Biol. Chem.* 283, 13578–13585
66. Kim, K.Y. *et al.* (2011) Parkin is a lipid-responsive regulator of fat uptake in mice and mutant human cells. *J. Clin. Invest.* 121, 3701–3712
67. Xia, X. *et al.* (2020) Deubiquitination of CD36 by UCHL1 promotes foam cell formation. *Cell Death Dis.* 11, 636
68. Zhang, F. *et al.* (2020) Inhibition of USP14 suppresses the formation of foam cell by promoting CD36 degradation. *J. Cell. Mol. Med.* 24, 3292–3302
69. Wei, J. *et al.* (2018) ER-associated ubiquitin ligase HRD1 programs liver metabolism by targeting multiple metabolic enzymes. *Nat. Commun.* 9, 3659
70. Guo, X. *et al.* (2021) HRD1 inhibits fatty acid oxidation and tumorigenesis by ubiquitinating CPT2 in triple-negative breast cancer. *Mol. Oncol.* 15, 642–656
71. Zechner, R. *et al.* (2017) Cytosolic lipolysis and lipophagy: two sides of the same coin. *Nat. Rev. Mol. Cell Biol.* 18, 671–684
72. Ghosh, M. *et al.* (2016) Ubiquitin ligase COP1 controls hepatic fat metabolism by targeting ATGL for degradation. *Diabetes* 65, 3561–3572
73. Sugihara, M. *et al.* (2019) The AAA+ ATPase/ubiquitin ligase mysterin stabilizes cytoplasmic lipid droplets. *J. Cell Biol.* 218, 949–960
74. Sakamaki, J.I. *et al.* (2022) Ubiquitination of phosphatidylethanolamine in organellar membranes. *Mol. Cell* 82, 3677–3692.e11
75. Merklinger, L. *et al.* (2022) Phospholipids alter activity and stability of mitochondrial membrane-bound ubiquitin ligase MARCH5. *Life Sci. Alliance* 5, e202101309
76. Otten, E.G. *et al.* (2021) Ubiquitylation of lipopolysaccharide by RNF213 during bacterial infection. *Nature* 594, 111–116
77. Haidar, M. *et al.* (2021) Lipophagy: a new player in CNS disorders. *Trends Endocrinol. Metab.* 32, 941–951
78. Robichaud, S. *et al.* (2021) Identification of novel lipid droplet factors that regulate lipophagy and cholesterol efflux in macrophage foam cells. *Autophagy* 17, 3671–3689
79. Nguyen, K.T. *et al.* (2019) N-terminal acetylation and the N-end rule pathway control degradation of the lipid droplet protein PLIN2. *J. Biol. Chem.* 294, 379–388
80. Olzmann, J.A. *et al.* (2013) Spatial regulation of UBXD8 and p97/VCP controls ATGL-mediated lipid droplet turnover. *Proc. Natl. Acad. Sci. U. S. A.* 110, 1345–1350
81. Nian, Z. *et al.* (2010) Fat-specific protein 27 undergoes ubiquitin-dependent degradation regulated by triacylglycerol synthesis and lipid droplet formation. *J. Biol. Chem.* 285, 9604–9615
82. Spandl, J. *et al.* (2011) Ancient ubiquitous protein 1 (AUP1) localizes to lipid droplets and binds the E2 ubiquitin conjugase G2 (Ube2g2) via its G2 binding region. *J. Biol. Chem.* 286, 5599–5606
83. Eastman, S.W. *et al.* (2009) A role for ubiquitin ligases and Spartin/SPG20 in lipid droplet turnover. *J. Cell Biol.* 184, 881–894
84. Zhu, S. *et al.* (2022) Regulation of glucose, fatty acid and amino acid metabolism by ubiquitination and SUMOylation for cancer progression. *Front. Cell Dev. Biol.* 10 Published online March 21, 2022. https://doi.org/10.3389/fcell.2022.849625
85. Zhou, Z.X. *et al.* (2021) The role of ubiquitin E3 ligase in atherosclerosis. *Curr. Med. Chem.* 28, 152–168
86. Choi, S.H. *et al.* (2015) Recapitulating amyloid β and tau pathology in human neural cell culture models: clinical implications. *US Neurol.* 11, 102–105
87. Ivatt, R.M. *et al.* (2014) Genome-wide RNAi screen identifies the Parkinson disease GWAS risk locus SREBF1 as a regulator of mitophagy. *Proc. Natl. Acad. Sci. U. S. A.* 111, 8494–8499
88. Cvek, B. and Dvorak, Z. (2011) The ubiquitin-proteasome system (UPS) and the mechanism of action of bortezomib. *Curr. Pharm. Des.* 17, 1483–1499
89. Cohen, P. and Tcherpakov, M. (2010) Will the ubiquitin system furnish as many drug targets as protein kinases? *Cell* 143, 686–693
90. Bedford, L. *et al.* (2011) Ubiquitin-like protein conjugation and the ubiquitin–proteasome system as drug targets. *Nat. Rev. Drug Discov.* 10, 29–46
91. Swatek, K.N. and Komander, D. (2016) Ubiquitin modifications. *Cell Res.* 26, 399–422
92. Singh, R. *et al.* (2009) Autophagy regulates lipid metabolism. *Nature* 458, 1131–1135
93. Stevenson, J. *et al.* (2016) Endoplasmic reticulum-associated degradation and lipid homeostasis. *Annu. Rev. Nutr.* 36, 511–542