PROTEIN TURNOVER IN LIPID HOMEOSTASIS

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Abstract

Lipid droplet (LD) is emerging as a hotbed of metabolic activity as opposed to previously held notion of an “inert” body. The life cycle of a lipid droplet is largely regulated by its resident proteins most of which are turned over by the cellular degradation machineries. To date, two prominent protein degradation machineries have been identified, the ubiquitin proteasome system (UPS) and autophagy. The proteasome machinery turns over short lived proteins while bulk degradation of long lived proteins is usually associated with autophagy. The breakdown of stored lipid at the LD by sequestration of the autophagy machinery is termed lipophagy, a process in which lipids are broken down in acidic lysosomal compartments as opposed to the classical cytosolic breakdown at neutral pH by TG lipases. This review presents the different players fine tuning the proteasomal and autophagic degradation systems in regulating lipid metabolism.

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Introduction

Lipid droplets are dynamic organelles that store neutral lipids like triacylglycerol and cholesteryl esters and serve as nodes of lipid metabolism. Recent findings suggest that the neutral lipids are delivered between leaflets of the endoplasmic reticulum (ER) forming a lens structure that attain a critical mass following which it stems into the cytoplasm from the outer leaflet of the ER (1-3). The dynamism of the lipid droplets is borne by its capacity to bring in toxic lipids like free fatty acids and convert them to TGs (4) or utilize them for energy production (5). Lipid droplets are also involved in membrane biosynthesis (6, 7) and lipid signaling pathways (8, 9). Misregulation of either of these functions can lead to numerous diseases notably fatty liver disease, obesity, diabetes and cardiovascular disease (10, 11).

An emerging theme in lipid metabolism is the pivotal roles of the cellular degradation machineries: ubiquitin proteasome system (UPS) and lipophagy (Fig. 1) (12-14). The role of UPS is emerging to be pivotal in cholesterol metabolism through which proteins are marked for degradation upon specific stimuli. This process of ubiquitin conjugation is a posttranslational modification that maintains homeostatic levels of proteins that regulate key checkpoints of lipid metabolism like synthesis, degradation, uptake and efflux of lipids.

Protein ubiquitylation occurs by activation of Ub by the ubiquitin activating enzyme E1 (15). Ub is covalently attached to the E1 cysteine residue via a thioester linkage with its C-terminal Gly 76 residue (16, 17). There are two E1 enzymes in the vertebrates, Uba1 and Uba6 (16, 18, 19). Post activation by the E1 activating enzyme, Ub is conjugated to the cysteine residue of a second enzyme the Ub conjugating enzyme (E2) as a necessary second step (16).

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Figure 1. Protein degradation machineries in lipid homeostasis. Cholesterol and TG metabolic pathways are regulated by a fine balance of ER and LD resident proteins. These proteins get targeted to the proteasomal and autophagy pathways. The lipid droplet hydrolysis occurs by lipases like ATGL (activated by CGI58 and deactivated by GOS2) or lipophagy initiated by PNPLA5 and orchestrated by Rab7. Players of cholesterol metabolism like HMGCoA Reductase (HMGCR), squalene monooxygenase (SM), SREBP2 and LXR are predominantly degraded at the proteasome. Key members of TG metabolism like ATGL and PLIN2 are targeted to both proteasome and autophagy.

There are nearly 50 E2 ligases in the mammalian genome (20). The final step in the ubiquitin conjugation is brought about by E3 ligases which are diverse with a prevalence of more than 600 in the mammalian genome (21). E3 ligases are broadly classified into three families: 1. RING ligases 2. HECT ligases 3. U-box ligases (22). The diversity of E3 ligases indicates their substrate specificities.

Key E3 ligases in cholesterol metabolism

1. **Fbw7**: SKP1-cullin F-box complex ubiquitin ligases (SCFs) are the largest class of E3 ligases that exist in a complex with Skp1, Cul1, Rbx1 and a F-box protein in mammalian cells that recognize substrates by binding to phosphorylated Ser and Thr residues of specific substrates (23). Fbw7 act on Sterol regulatory

binding proteins (SREBPs) that are basic helix loop helix leucine zipper (bHLH-Zip) family of transcription factors responsible for cholesterol and fatty acid biosynthesis and LDL uptake. The newly synthesized SREBPs are targeted to the ER without any transcriptional activity. When the sterol levels are low, SREBP precursors are transported to the Golgi to get cleaved by proteases following which the N-terminal bHLH-zip domain with the full transcription activity is released from membrane to the nucleus to activate the cholesterol and fatty acid metabolic genes (24). Two SREBP genes, *Srebp1* and *Srebp2* encode three SREBP proteins i.e. SREBP1a, SREBP1c and SREBP2 (25).

2. **Gp78**: Human glycoprotein 78 (gp78) is a 643 amino acid protein composed of multiple conserved domains including the RING finger domain suggesting its multifaceted, complicated

regulations. It is an ER membrane anchored E3 ligase, that degrades several misfolded proteins in the ER by recruiting E2 ubiquitin conjugating enzymes Ubc7/UBE2G2, Ufd1 and p97/VCP (26-28). Several proteins of cholesterol metabolism like 3-hydroxy, 3-methyl glutaryl CoA (HMGCoA) reductase, ApoB100, Insulin induced gene 1&2 (Insig1 and 2) are substrates of gp78.

3. **Hrd1:** An ER-resident ubiquitin ligase, uses Ubc 7 as an E2 ligase similar to gp78 (29). They have many common substrates like CD3- δ , CT and CFTR δ F508 (30, 31). The ortholog of Hrd1 in yeast is hrd1/Der3p that ubiquitinates Hmg2p, the yeast isoenzyme of HMGCoA reductase (32) raising the possibility of its role in mammalian cell although it is not shown to be interacting with Insig1(29). This begs for further investigation into the physiological role of Hrd1.

4. **Trc8:** Trc8 is an ER resident multi-pass E3 ligase with a sterol sensing and RING finger domain (33). Unlike Hrd1, Trc8 can interact with both Insig1 and Insig2 and is known to ubiquitylate them (34, 35).

5. **TEB4:** An ER-resident ubiquitin ligase, TEB4 interacts with ubiquitin conjugating enzyme Ubc7 through its RING domain and catalyzes K-48 specific ubiquitin –ubiquitin linkage (36). The yeast homolog of TEB4 is Doa10 that degrades Erg1, a homolog of squalene monooxygenase regulated by lanosterol (37). SREBP2- transcribes squalene monooxygenase gene regulated by sterols (38). The protein levels of squalene monooxygenase is regulated by the cellular cholesterol pool (39).

6. **IDOL:** Inducible degrader of LDLR or IDOL has a FERM (4.1 band, ezrin, radixin and moesin) homology domain at the amino terminus and a RING zinc finger putative ubiquitin ligase domain at the C-terminus(40). It requires the ubiquitin E2 ligase, UBE2D to mediate LDL receptor degradation (41). Cholesterol in LDL is removed from the plasma by the LDL receptors in the liver by receptor mediated endocytosis (42). When the intracellular cholesterol pool decreases the complex of SREBP/SREBP cleavage activating protein (SCAP) move from the ER to the Golgi complex where the resident proteases cleave SREBP2 to release its active form that translocates to the nucleus to upregulate *LDLR* transcription(25). LDLR is also regulated post translationally by Proprotein convertase subtilisin/kexin type9 (PCSK9), expression of which is also regulated by activated SREBP1. The expression of PCSK9 by activated SREBP1 presumably prevents nascent VLDL particles to be taken by hepatocytes thereby routing these to the peripheral tissues (43).

IDOL expression is regulated by LXRs. The *IDOL* gene

promoter is a direct target for binding of LXR/retinoid X receptor heterodimers in both mice and humans (41). Interesting in the absence of ligand LXR gets ubiquitylated by the breast and ovarian cancer susceptibility1 (BRCA1) associated RING domain 1 (BARD1) E3 ligase complex, a process that gets repressed by the LXR ligand (44). Other targets of IDOL mediated degradation are VLDLR and ApoER2 in mammals (44, 44a, 45). The effect of IDOL of LDLR levels act complementarily to the SREBP and PCSK9 pathways. Statins inhibit cholesterol biosynthesis and upregulate SREBP mediated LDLR gene expression (46). Loss of IDOL expression stabilizes elevated LDLR levels (46). PCSK9 that is induced by SREBP to facilitate the degradation of LDLR is able to do so in IDOL deficient cells suggesting a complementary yet independent pathway of LDLR regulation (46).

Proteasomal degradation of lipid droplet proteins

The lipid droplet proteome comprises of many proteins that are substrates of proteasomal degradation. Upon oleate feeding nascent lipid droplets stabilizes many LD resident proteins like perilipins 1 and 2, the TG lipase ATGL, PNPLA3, FSP27 and ATGL inhibitor GOS2 (47-52). Turnover of LD proteins under low lipid levels presumably prevents the hydrolysis of TG stores, a function that goes awry in mice models of fatty liver disease that express the PNPLA3 disease variant (53, 54). The insertion of the LD resident proteins into the lipid droplet monolayer is believed to shield the degradation signals from the proteasomal machinery that might get exposed to the proteasome due to protein crowding that evicts it from the lipid droplet surface during lipolysis (55). The architecture of the lipid droplet is subtly regulated by competition of its resident proteins. For example, PLIN2 stabilizes on lipid droplets on PLIN1 deletion as it lacks a C-terminal region like PLIN1 to sequester CGI-58 for lipolysis (56, 57). PLIN2 has been proposed to be targeted to the proteasome by the N-end rule degron although experimental evidence is lacking (56). A recent report has implicated PLIN2 to be a substrate of chaperone mediated autophagy (58). Recent studies in yeast and mammalian cells show the involvement of ER in degradation of LD proteins suggesting an alternative route for turnover (59, 60). LD proteins sequester soluble ubiquitination factors on the surface; UBXD8 and UBXD2 recruit membrane protein extraction factor VCP (61, 62), AUP1 recruits E2-conjugating enzyme UBE2G2 and spartin (SPG20) recruits E3 ligase AIP4 and AIP5 (63-67).

Degradation independent roles have also been suggested for ubiquitination at lipid droplets (68, 69). VCP, for example, is believed to regulate ATGL activity but not its stability(70). However further studies are warranted to establish the sanguin-

ity of ubiquitylation as a mark to facilitate protein-protein interaction.

Lipophagy

The degradation of fat at the lipid droplets has been classically assigned to the role of lipases associated with lipid droplets (71). Recent studies have reported involvement of the autolysosomal machinery to breakdown lipid stores (58, 72). Early studies hinted at the role of autophagy in lipid droplet degradation through the observation that mutations in the lysosomal acid lipase lead to accumulation lipid droplets in various organs (73-75).

Several proteins are involved in lipophagy induction and regulation. These are (i) PLINs and lipases, (ii) Rab GTPases, and (iii) lipophagy receptors

PLINs: Lipid droplet specific functions are influenced by several resident proteins. Perilipin family of proteins play crucial role in lipid droplet dynamics. There are five members of the PLIN family; PLIN1, PLIN2, PLIN3, PLIN4 and PLIN5 (76). A recent report suggests a role for PLIN2 being targeted to chaperone mediated autophagy by interacting with heat shock cognate protein of 70kD (Hsc70). This is required to sequester ATGL to initiate TG hydrolysis. AMP kinase phosphorylates PLIN2 that primes it for chaperone mediated autophagy (CMA) (58, 77). ATGL has been shown to interact with LC3 via its LIR domain - STFIPV-(145-150) (78). ATGL is necessary for expression of autophagy genes in hepatocytes and enhances interactions of LC3 and lysosomes with lipid droplets (79). ATGL has also been shown to regulate SIRT1 activity, a key player in autophagy (80, 81). ATGL or PNPLA2 is member of the patatin like phospholipase domain containing protein family. Among other members of this family, PNPLA5 and PNPLA8 have been shown to promote autophagy in various cell types (82, 83). Although PNPLA3 was shown to be a substrate of the proteasomal pathway, its specific role in lipophagy is yet to be determined (52, 53).

Rab GTPases: To date, 30 Rab GTPases have been shown to be associated with lipid droplet proteome (84). Among these the prominent ones are:

Rab7

One of the most prominent Rab GTPases to populate the LD surface is Rab7, mutations of which cause a peripheral neuropathy, Charcot-Marie-Tooth Type 2b Disease (85, 86). Rab7 is known to regulate late endocytic transport and plays a key role in autophagosome maturation (87-89). During the process it interacts with a host of effector proteins like FYVE

and coiled-coil domain containing protein1 (FYCO1) or Rab7 interacting lysosomal protein (RILP) to promote plus and minus ended transport of endosomes along microtubules with the help of kinesin and dynein/dynactin motor complex (91-93). Rab7 coordinates essential steps in lipophagy and ethanol induced hepatic steatosis (94, 95). Recent reports suggest modulation of its activity to alter endocytic trafficking as a strategy for therapeutic intervention (96, 97, 96a, 97a).

Rab10

Rab10 has also been implicated in lipophagy. It regulates insulin stimulated GLUT4 vesicle trafficking and ER morphogenesis (98-100).

Rab32

Rab 32 colocalizes with markers on autophagic or lysosomal membranes in the *Drosophila* fat body and is believed to regulate ATGL activity (101, 102).

Rab 18

Rab18 has been shown to have a role in LD homeostasis (103). The transport protein particle (TRAPP-II) and the coatomer protein COP-I function to regulate Rab 18 activity in LD lipolysis (104).

Rab25

Recent studies indicate the involvement of Rab25 in the autophagic turnover of retinyl-ester enriched LDs from hepatic stellate cells (HSCs). ROS generation due to HSC activation increased Rab25 expression suggesting an important role in lipophagy (105).

Lipophagy receptors

Many autophagy receptors have been identified in different cellular organelles like optineurin and NDP52 on mitochondria that interact with autophagosomal marker LC3B to initiate mitophagy (106). Huntingtin mutants cause LD accumulation suggesting a potential role as LD recognition receptor (107).

Lipophagy-mediated LD hydrolysis is yet an unsolved problem. This may be mediated by several mechanisms. Among the PNPLA family members, ATGL has been shown to interact with LC3B which possibly helps in LD degradation or other resident proteins like Rab GTPases may recruit lipophagy receptors to facilitate LD degradation or protein tagging by polyubiquitylation may serve to initiate lipophagy as shown by the recruitment of AUP1 and E2 ubiquitin conjugase G2 to promote ubiquitylation of lipid droplet proteins (64, 69).

Conclusion

Lipid droplet life cycle is determined by the longevity and turnover of the lipid droplet proteome which in turn relies on the fidelity of the UPS and lipophagy. However the degradation mechanisms determining the LD architecture remain to be elucidated. Future studies will focus on unraveling the mechanisms of lipid droplet targeting and removal of proteins to better understand pathophysiology of human metabolic diseases.

Conflict of interest statement

The author certifies that he has no affiliations with or involvement in any organization with any financial interest in the subject matter discussed in this review article.

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