Hepatic Lipophagy: New Insights Into Autophagic Catabolism of Lipid Droplets in the Liver

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The liver is a central fat-storage organ, making it especially susceptible to steatosis as well as subsequent inflammation and cirrhosis. The mechanisms by which the liver mobilizes stored lipid for energy production, however, remain incompletely defined. The catabolic process of autophagy, a well-known process of bulk cytoplasmic recycling and cellular self-regeneration, is a central regulator of lipid metabolism in the liver. In the past decade, numerous studies have examined a selective form of autophagy that specifically targets a unique neutral lipid storage organelle, the lipid droplet, to better understand the function for this process in hepatocellular fatty acid metabolism. In the liver (and other oxidative tissues), this specialized pathway, lipophagy, likely plays as important a role in lipid turnover as conventional lipase-driven lipolysis. In this review, we highlight several recent studies that have contributed to our understanding about the regulation and effects of hepatic lipophagy. (*Hepatology Communications* 2017;1:359–369)

Introduction

The liver is a central site for the packaging, redistribution, and processing of fatty acids. As such, liver disease or dysfunction can lead to the progressive accumulation of fat content, a condition (hepatic steatosis) that predisposes individuals to significantly more severe sequelae. This is perhaps best exemplified in the case of nonalcoholic fatty liver disease (NAFLD), a spectrum of diseases ranging in severity from simple steatosis to steatohepatitis or cirrhosis.[1] Importantly, it is believed that simple steatosis is completely reversible when patients are placed on an appropriate dietary and weight-loss regimen.[2,3] As over 25% of the world population is estimated to have some form of fatty liver disease,[4] a greater understanding of the natural history of NAFLD is therefore essential.

Under normal conditions, dietary fatty acids released into hepatic portal circulation or those released from the adipose tissue into general circulation can be taken up by the parenchymal cells of the liver, i.e., hepatocytes.[5] Within the hepatocytes, fatty acids are quickly esterified to produce triglycerides (triacylglycerols [TAGs]) and cholesterol esters (CEs) in a cytoprotective mechanism that mitigates hepatocytotoxicity of free fatty acids.[6,7] This process of esterification occurs within the endoplasmic reticulum (ER) membrane through the coordinated efforts of multiple ER-resident enzymes, including diacylglycerol acyltransferases and acylcoenzyme A:cholesterol acyltransferases.[8]

Abbreviations: Atg, autophagy-related; ATGL, adipose triglyceride lipase; CE, cholesterol ester; CMA, chaperone-mediated autophagy; EH, Eps15 homology; ER, endoplasmic reticulum; GEF, guanine exchange factor; GNMT, glycine N-methyltransferase; GTPase, guanosine triphosphatase; HCV, hepatitis C virus; HSC, hepatic stellate cell; Hsc70, heat shock cognate 70; LC3, microtubule-associated protein 1A/1B-light chain 3; LD, lipid droplet; LRP1, low-density lipoprotein receptor-related protein 1; NAFLD, nonalcoholic fatty liver disease; siRNA, small interfering RNA; SOCE, store-operated Ca2 + entry; SOD1, superoxide dismutase 1; TAG, triacylglycerol.

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The net result of these anabolic processes is the deposition of neutral lipids between the ER bilayer within a lens-like microdomain.[7,9] Recruitment of stabilizing proteins, such as seipin,[10-12] and concomitant further rounds of lipid synthesis[13-15] ultimately cause the outer leaflet of the ER bilayer to distend, resulting in the precipitation of unique spherical cytosolic organelles, i.e., lipid droplets (LDs), that are now known to be key sites of hepatic lipid metabolism.[6]

The LD represents a cellular organelle of greater complexity than was originally appreciated. Previously thought to represent an inert site of fat sequestration, LDs are now known to have distinct and dynamic proteomes that fluctuate in tandem with the metabolic state of the cell and which may define the composition of the neutral lipid stored within the organelle.[16-23] Significant effort has focused on understanding the biogenesis of these structures to potentially intervene in disorders of lipid dysregulation.[7,24] While substantial inroads have been made toward understanding LD biology (primarily through adipocyte-centric studies[25]), the processes of LD catabolism and synthesis in the liver have remained comparatively understudied. Recent studies have begun to draw similarities in lipid metabolism between the two cell types, demonstrating parallels and distinctions between the canonical lipolytic machinery that assists in adipose tissue lipolysis and hepatic cytosolic lipolysis.[26-28]

Within the past decade, an alternative mechanism for hepatic LD mobilization, autophagy, has begun to materialize. Canonical macroautophagy is a tightly regulated process that involves the envelopment of cellular material within a double-membraned structure referred to as an autophagosome.[29-33] The coordination of autophagosome biogenesis is mediated primarily through an intricate network of greater than 30 known factors (autophagy-related [Atg] proteins), characterized in a host of elegant yeast genetic screens performed over the past 25 years.[34] Chief among these factors are Atg7, an E1-like enzyme, and the E3-like complex of Atg5-Atg12-Atg16, which mediates the conjugation of phosphatidylethanolamine onto the classical autophagosomal marker microtubule-associated protein 1A/1B-light chain 3 (LC3; Atg8), forming a lipidated derivative of LC3 (known as LC3-II).[34-37] Cargo-laden LC3-positive autophagosomes are then targeted for fusion with lysosomes, forming hybrid organelles referred to as autolysosomes. This process results in the delivery of hydrolytic enzymes into the autophagosomal lumen and catabolism of cargo into macromolecular substrates that are then made available for biosynthetic and anabolic processes throughout the cell.

As part of the bulk cargo degradation that occurs during macroautophagy, convincing work has shown that LDs can be efficiently incorporated into autophagosomes, ultimately resulting in the release of free fatty acids that can then be used as fuel for mitochondrial β-oxidation.[38] Importantly, a growing body of evidence suggests that LDs can also be selectively recognized (independently from other cytoplasmic structures) by the autophagic machinery (lipophagy)[39-43] to provide a potential mechanism for effectively regulating hepatocellular lipid levels. This review will focus on recent advances made in the fields of lipophagy and hepatic lipid metabolism.

A Key Role for Lipophagy in Hepatic Lipid Metabolism

In 2009, Singh et al.[38] provided the first concrete evidence for a connection between autophagy and hepatic lipid metabolism. Treatment of hepatocytes

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with the lysosomal inhibitor 3-methyladenine or small interfering RNA (siRNA)-mediated knockdown of the autophagy regulatory gene *Atg5* resulted in increased hepatic TAG content and interfered with subsequent mitochondrial β-oxidation. Additionally, immunofluorescence imaging revealed that key components of the autophagic machinery (i.e., LC3, a classical marker of autophagic membranes, and lysosomal-associated membrane protein 1, a lysosomal marker) exhibited a close association with LDs. Using electron microscopy, it was observed that mice fasted for as short as 6 hours had identifiable lipid content enclosed within double-membrane autophagosomes in hepatocytes, a hallmark of macroautophagic progression. To confirm that these results were representative of hepatic lipophagy *in vivo*, a hepatocyte-specific *Atg7* knockout mouse was examined and found to have an enlarged liver with increased TAG accumulation following starvation when compared to control mice.[38] This study was particularly noteworthy as it was previously assumed that lipid catabolism in the hepatocyte generally mimicked that found in the adipocyte, i.e., using a series of cytoplasmic lipases (i.e., adipose triglyceride lipase [ATGL], hormone-sensitive lipase, and monoglyceride lipase) that sequentially reduce TAG into component-free fatty acids for oxidation within mitochondria.

In further support of the Singh et al. study, a subsequent report by Kaushik and Cuervo[44] revealed the potential for cooperativity between chaperone-mediated autophagy (CMA) and macroautophagy in the clearance of hepatic LDs. CMA is a mechanism for the direct internalization of particular protein substrates bearing a canonical KFERQ motif.[45-47] In the case of hepatocytes, the LD-resident perilipin proteins PLIN2 and PLIN3, which function as barriers against unregulated lipolysis,[48-51] were identified to contain these motifs and shown to directly interact with heat shock cognate 70 (Hsc70), the CMA-specific chaperone that mediates protein delivery to the lysosome.[44,52] Mutation of this pentapeptide motif in PLIN2 resulted in LD accumulation, suggesting that the removal of the perilipin coat surrounding LDs is a prerequisite for either lipolysis or classical lipophagy.

It now appears evident that coordination and cross-talk exist between the lipolytic and lipophagic pathways. For example, lipase-driven LD breakdown was recently shown to be partially dependent on autophagy.[53] In this study, cold exposure in the mediobasal hypothalamus was observed to drive lipophagy in the liver. The resulting up-regulation of hepatic lipophagy is coupled to lipolysis as the key cytoplasmic lipases ATGL and hormone-sensitive lipase were shown to contain motifs that allow for direct interactions with LC3; mutation of these critical motifs was sufficient to ablate ATGL-driven lipolysis.[53] Likewise, ATGL activity may also represent an important upstream checkpoint in the regulation of lipophagy; overexpression of ATGL increases hepatic lipophagy, possibly through a mechanism involving Sirtuin-1, a nicotinamide adenine dinucleotide-dependent protein deacetylase.[54,55] Clearly, total organismal LD turnover relies to some extent on the autophagic machinery; therefore, a key area of study for hepatocellular lipid metabolism has subsequently been focused on the regulation of lipophagy.

Regulation of Hepatic Lipophagy

As the processes of cellular lipid metabolism and autophagy are so tightly regulated, it is likely that lipophagy is coordinated at multiple fronts. As outlined below, many candidate regulatory factors may modulate this process either indirectly (as with methionine or calcium signaling) or directly (as in the case of the Rab family of small guanosine triphosphatases [GTPases]).

One example of an enzyme that may indirectly influence hepatic lipophagy is glycine *N*-methyltransferase (GNMT). In mice, deficiencies in the expression of GNMT result in increased levels of circulating methionine (as well as its metabolite *S*-adenosyl-L-methionine).[56] This elevation of serum methionine leads to a corresponding reduction in autophagic flux; GNMT-knockout mice exhibited enhanced methionine levels that resulted in autophagic inhibition due to aberrant methylation of the mammalian target of rapamycin-interacting protein phosphatase 2A. Lysosomal inhibition in GNMT-knockout hepatocytes did not result in further increases in cellular TAG levels, indicating that GNMT may play a key role in hepatic lipophagy. The high levels of LD content in individuals with fatty liver and GNMT deficiency may therefore perpetuate an unfavorable cycle whereby liver steatosis is exacerbated by an inability to mobilize LDs via lipophagy.

As with methionine, calcium signaling is another mechanism thought to regulate hepatic lipophagy. It is well established that impaired calcium homeostasis can have broad-reaching effects resulting in the metabolic

dysfunction underlying obesity and diabetes.[57] Impairments in a process of cellular Ca^{2+} influx referred to as store-operated Ca^{2+} entry (SOCE) were recently found to result in hepatic lipid accumulation.[58] SOCE was shown to be required for proper mitochondrial function, primarily at the level of gene expression, controlling a number of critical factors participating in fatty acid oxidation and components of the electron transport chain. Furthermore, cytosolic lipase expression is impaired in SOCE-deficient cells; therefore, mice defective in this process of Ca^{2+} signaling exhibit severe defects in the release of fatty acids from LDs by conventional lipolysis. Interestingly, the cells appear to compensate for the consequent increased intracellular lipid load by up-regulating the process of lipophagy, potentially a lipotoxicity avoidance mechanism. Fibroblasts isolated from patients with loss-of-function mutations in *STIM1* or *ORAI1*, two genes involved in the regulation of SOCE, also exhibited high steady-state levels of LC3-II and enhanced levels of autophagic flux. Enhanced autophagic vacuole formation was also observed in the skeletal muscle of SOCE-deficient mice, reinforcing the importance of Ca^{2+} signaling on lipophagy not only in the liver but likely in other cell types as well.

Lysosomal calcium signaling also appears to be involved in the induction of autophagy via the control of key transcriptional regulators, implying the existence of a Ca^{2+}-regulated lysosome-to-nucleus signaling axis that helps the cell respond appropriately to nutrient demand.[59,60] Transcriptional regulation of lipophagy may thus be mediated by factors such as the transcription factor TFE3, which binds to promoters key to the process of lysosomal biogenesis.[61] Lentivirus-mediated overexpression of this transcriptional regulator in hepatoma cells results in an increased association of LC3 with LDs and reductions in total cellular lipid content.[62] It is unknown, however, if similar results are observed *in vivo*. Similarly, knockout of TFEB, another transcriptional regulator of lysosome biogenesis, also resulted in an accumulation of hepatocellular LDs.[63] Notably, overexpression of TFEB was able to rescue both obesity and metabolic syndrome in mice, lending support to the idea that the negative effects of excessive LD accumulation in the liver might be therapeutically addressed by modulation of these key Ca^{2+}-controlled transcriptional regulators.

Another indirect mechanism for hepatocellular lipophagy regulation relates to the consumption of caffeine. A recent study suggested that caffeine promotes autophagic flux in hepatocytes with a possible preference for LDs; electron microscopy revealed lipid content in the autophagosomes and autolysosomes of hepatocytes from caffeine-treated mice.[64,65] Ultimately, the fatty acids liberated as a result of caffeine-stimulated lipophagy undergo β-oxidation within mitochondria, resulting in elevated levels of serum β-hydroxybutyrate, an effect that was inhibited when the lysosomal inhibitor chloroquine was co-administered to mice. The mechanisms whereby caffeine may exert these effects, however, remain undefined.

Other factors have recently been shown to participate in the regulation of hepatic lipophagy. For example, liver-specific knockout of low-density lipoprotein receptor-related protein 1 (LRP1) in mice showed that, following a 24-hour fast, a nearly 7-fold increase in hepatic TAG accumulation was observed as compared to wild-type mice.[66] An accumulation of the autophagic cargo adapter p62 was also noted when isolated $LRP^{-/-}$ hepatocytes were incubated in the presence of palmitate, suggesting that global autophagy is impaired when this receptor is perturbed. However, no accumulation of LC3 was observed, suggesting that LRP1 is not involved in the biogenesis of autophagic membranes but rather that lysosome-mediated degradation of LDs had been compromised.

A similar phenomenon was observed in the livers of fasted mice with a global knockout of superoxide dismutase 1 (SOD1).[67] SOD1 converts superoxide (generated from reactive oxygen species) to hydrogen peroxide as a means of alleviating oxidative stress. Kurahashi et al.[67] found that hepatocytes from SOD1-knockout mice exhibited a ballooned phenotype. Following a 48-hour fast, elevated levels of PLIN2, a marker of enhanced LD content, were also observed. Accumulation of LC3-II in the fasted knockout cells was greater than that observed for fasted wild-type cells, as was p62. Taken together, the above studies reaffirm that hepatic lipophagy can be regulated in a number of different ways. As such, identifying factors that might mediate the targeted control of this process is attractive from a therapeutic standpoint.

Role for the Rab GTPases in Hepatic Lipophagy

The identification of direct modulators of hepatocellular lipophagy has been the subject of intense recent focus. Promising examples of lipophagy-regulating proteins are the small Rab GTPases, part of the Ras superfamily of monomeric G proteins best known as

master coordinators of membrane trafficking events.[68] Rab GTPases function as molecular switches that transition between GTP-bound ("active") and guanosine diphosphate-bound ("inactive") states with the assistance of guanine exchange factors (GEFs) and GTPase-activating proteins, respectively. All Rab proteins undergo prenylation at C-terminal cysteine residues; this is required for the Rabs to be inserted into target membranes. Once recruited to the site of action, active Rab GTPases interact with specific effector proteins, thus regulating vesicle formation, movement, tethering, and fusion.[68,69] Because lipophagy is a process that relies on coordinated membrane trafficking events, it is not surprising that numerous Rabs have been identified as part of the LD proteome.[18,20,22,70] However, the roles played by many of the LD-associated Rab GTPases remain poorly defined. Below, we discuss a subset of Rab proteins (Rab7, Rab10, Rab18, and Rab32) that have been routinely found in LD proteomic screens and that are likely to play a key role in the lipophagic process.

Rab7 MEDIATES THE RECRUITMENT OF MULTIVESICULAR BODIES AND LYSOSOMES TO LDs DURING LIPOPHAGY

Rab7 is found to reside predominantly on multivesicular bodies and late endosomes and is implicated in cargo trafficking to later endocytic compartments as well as in lysosome biogenesis.[71] Rab7 is also involved in the maturation of autophagic vesicles, making it an attractive candidate to facilitate LD interactions with degradative organelles and thus promote LD breakdown.[72] Recently, Schroeder and coworkers[73] discovered a central role for Rab7 in mediating hepatocellular lipophagy. In their study, the authors found that nutrient deprivation activates Rab7 on LDs and vesicular structures that intimately associate with LDs during starvation. It appears that active Rab7 recruits and mediates the association of multivesicular bodies and lysosomes with LDs. This role of Rab7 is essential for starvation-induced lipophagy as loss of Rab7 function after siRNA-mediated knockdown or expression of a dominant-negative mutant impaired LD breakdown.[73] In a follow-up study, it was observed that chronic ethanol exposure inhibits Rab7 activity, disrupts degradative compartment morphology and motility, and causes reduced LD turnover during the process of lipophagy.[74] These findings provided some additional insights into how alcohol might exacerbate the progression of fatty liver.

RAB10 MEDIATES AUTOPHAGIC ENGULFMENT OF LDs DURING LIPOPHAGY

As with Rab7, Rab10 is a Rab GTPase that repeatedly appears in LD proteomic screens. Rab10 has been previously implicated in basolateral endocytic recycling, insulin-dependent glucose transporter type 4 trafficking to the plasma membrane, and in the regulation of ER tubule dynamics and morphology.[75-77] Li et al.[78] reported a novel function for Rab10 in regulating LD breakdown during lipophagy. These findings suggest that Rab10 is activated during nutrient starvation or autophagy-inducing conditions, ultimately promoting its recruitment to membranous structures surrounding the LD surface that are positive for LC3, Atg16L1, and lysosomal-associated membrane protein 1, thus likely representing nascent autophagic structures. Once Rab10 is activated on autophagic membranes, it binds its effector protein (endocytic adaptor Eps15 homology [EH] domain binding protein 1) as well as the membrane remodeling adenosine triphosphatase EH domain containing 2 protein. The trimeric complex extends the autophagic membranes around the LD surface, eventually resulting in complete LD engulfment within an autophagosome. The function of Rab10 lies, therefore, in promoting the autophagic engulfment of LDs during lipophagic progression. Indeed, the disruption of Rab10 function by siRNA-mediated knockdown or expression of a dominant-negative mutant results in LD accumulation. Interestingly, loss of Rab7 significantly impaired the downstream recruitment of Rab10 to the autophagic membranes surrounding the LD, while localization of Rab7 was not impacted by the absence of Rab10, suggesting that these two lipophagy regulators do not play overlapping roles.[78]

Rab18: AN EXCLUSIVE LD RESIDENT Rab GTPase

In contrast to Rab7 and Rab10, which associate with LDs as well as with lysosomal and autophagic membranes, respectively, Rab18 is thought to exist solely as an LD resident protein. Two independent groups have simultaneously reported that Rab18 directly associates with the LD monolayer in adipocyte

and nonadipocyte cell models.[79,80] Using both immunofluorescence and immunogold electron microscopy, they showed that fluorescently labeled wild-type or constitutively active (but not dominant-negative) Rab18 mutants localize directly on the LD surface. Both groups also noted a close apposition of the ER network around the LDs in Rab18-expressing cells, proposing that this Rab GTPase may regulate LD–ER associations. Despite its discovery on the LDs more than a decade ago, the role of Rab18 has not been completely established. The observation that Rab18 connects LDs with the ER raises a question whether Rab18 could be involved in regulating LD formation/growth at the ER. Stimulation of adipocytes with either insulin or β-adrenergic agents increased Rab18 recruitment to the LD surface, suggestive of a potential role in mediating adipocellular lipogenic and lipolytic functions.[79,81] Perhaps the most convincing evidence for the role of Rab18 in LD homeostasis comes from patients with Warburg micro syndrome, a neurological disorder caused by mutations in Rab18.[82] Treatment of fibroblasts isolated from these patients with oleic acid for 18 or 24 hours resulted in accumulation of larger LDs compared to control fibroblasts.[83] Most recently, Li et al.[84] discovered that transport protein particle II is recruited to the LD surface after lipid loading where it functions as an LD-associated GEF for Rab18. Interestingly, the consequences for the loss of transport protein particle II GEF activity were comparable to those observed after Rab18 inhibition resulting in impaired lipolysis and accumulation of enlarged LDs. Further studies will prove informative in clarifying the role of Rab18 in LD biology and whether it plays any specific role in hepatic lipophagy.

A POTENTIAL ROLE FOR Rab32 IN THE REGULATION OF LIPID STORAGE DURING AUTOPHAGY

Rab32 has been shown to localize to mitochondria where it functions as an A-kinase anchoring protein and regulates mitochondrial dynamics.[85] It also participates in melanosome biogenesis by regulating post-Golgi trafficking of melanogenic enzymes.[86] The first evidence for the potential role of Rab32 in regulating LD storage came from studies done in larval adipose tissue of *Drosophila*. Wang et al.[87] found that expression of dominant-negative or loss-of-function mutants of Rab32 resulted in a reduction in both LD size and total TAG levels. The authors further reported that Rab32 localized to ring-like structures that were positive for LysoTracker (lysosome) and LC3 markers. Intriguingly, loss of Rab32 function resulted in impaired autophagy, suggesting that Rab32 may exert its role in LD storage by regulating lipophagic LD breakdown. Such a proposed role for Rab32 is not surprising as it has been implicated previously in the formation of autophagosomes.[88] Consistent with the study performed using *Drosophila*, Li et al.[89] showed that depletion of Rab32 in mammalian hepatocyte cell models also results in reduced intracellular LD levels. Although still preliminary, these studies suggest an interesting avenue for further investigations into the role of Rab32 in mediating LD breakdown.

Other Roles for Hepatic Lipophagy

Similar to the effects of caffeine (as described above), acute exposures to alcohol appear to accelerate hepatic autophagy.[90] The macroautophagy induced by ethanol appears to exhibit a particular selectivity toward mitochondria as well as LDs, indicating that mitophagy and lipophagy are up-regulated as part of a comprehensive hepatoprotective response to acute ethanol exposure. This may perhaps be mediated through the activation of transcription factors, such as FoxO3a.[91] Any potentially beneficial function of autophagy in response to short-term alcohol intake may be a double-edged sword, however; the chronic consumption of alcohol appears to be especially detrimental to lipophagy. Livers from patients with a history of chronic alcohol abuse are often identifiable as having microvesicular steatosis, a histologic observation that can indicate the potential for cirrhotic progression.[92,93] Chronic ethanol exposure may interfere with the nuclear localization of transcriptional lipophagic regulators, such as TFEB (as above),[94] or cause the harmful modulation of key mediators of LD interactions with the lipophagic machinery (as with Rab7).[74] Together, these data serve as strong evidence that lipophagy normally serves a hepatoprotective role in response to alcohol consumption. Disruption of this pathway over time, however, may provide an explanation for the prevalence of steatosis that persists with chronic alcohol abuse.

Lipophagy does not appear to be restricted to metabolism of triglyceride-enriched LDs alone; indeed, it also appears to serve an important role in the breakdown of sterol ester-enriched LDs. A number of disorders result in the accumulation of free cholesterol.

Hepatic bile acid metabolism may therefore be another mechanism by which lipophagy is regulated. For example, elevated levels of cholesterol 7α-hydroxylase together with decreased bile acids resulting from cholestyramine feeding were recently shown to result in increased levels of hepatic autophagy. This was shown by Wang et al.[95] to promote the lipophagy-mediated hydrolysis of CEs and the transport of lysosomal cholesterol to the ER.

Additionally, the process of lipophagy plays an important role in more than just the physiology of hepatocytes; another important liver-resident cell type that uses lipophagy is the hepatic stellate cell (HSC). In their quiescent state, HSCs are a principal site of storage for retinol ester-enriched LDs and vitamin A within the liver. As a consequence of tissue damage, HSCs become activated and in this process lose LDs and promote the deposition of extracellular matrix. This loss of LDs is known to partially rely on the lipophagic turnover of LDs.[96,97] Stimulation of liver injury with CCl_4, a compound that promotes fibrosis, was found to increase the autophagic turnover of LDs in HSCs.[97] Stellate cell-specific inhibition of *Atg7* expression was also found to attenuate liver fibrosis *in vivo*.

In addition, lipophagy appears to be central to the replication and function of some viruses. Severe infections with dengue virus often have hepatic involvement. Studies have reported that this particular virus is especially dependent on an up-regulation of autophagy to drive cellular LD turnover and promote replication.[98] Mechanistically, it was recently shown that dengue virus can stimulate lipophagy in HepG2 hepatoma cells via activation of 5′ adenosine monophosphate-activated kinase.[99] This activation subsequently results in inhibition of mammalian target of rapamycin and consequently increased autophagy. siRNA-mediated silencing of 5′ adenosine

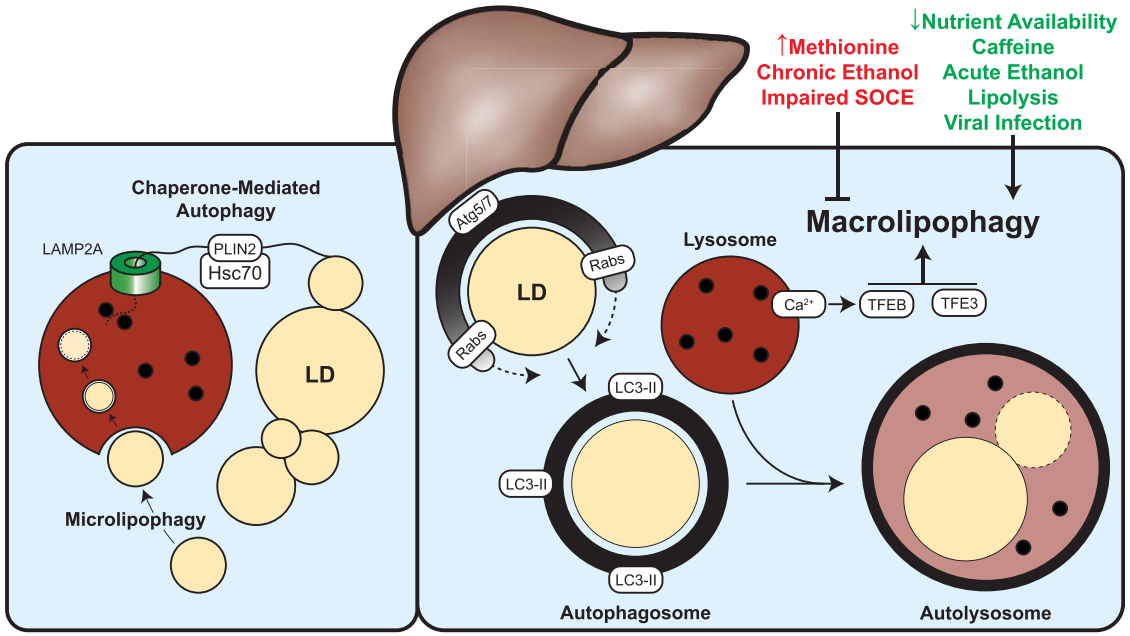

FIG. 1. Hepatic lipophagy can be regulated at many different levels. The machinery associated with CMA appears to prime LDs for further processing in an Hsc70- and LAMP2A-dependent manner. LD-resident perilipin proteins (e.g., PLIN2) appear to be selectively recognized by the Hsc70 chaperone for targeted degradation within the lysosome, exposing the surface of the LD and rendering it susceptible to further processing by cytosolic lipases or alternative components of the autophagic machinery. Microlipophagy, a poorly understood mechanism of direct LD engulfment within the lysosome, may also play an important role in mediating the clearance of accumulated LDs. Classical macrolipophagy (or more generally, lipophagy) is a highly regulated process involving the recruitment of autophagic membranes directly to the LD, which ultimately results in the enclosure of the LD within an autophagosome. Subsequent fusion of an autophagosome with a degradative lysosome results in the formation of a hybrid structure, the autolysosome, in which LDs are ultimately catabolized. As discussed in this review, the initiation and progression of macrolipophagy can be positively and negatively influenced by numerous intracellular and extracellular factors. Abbreviation: Lamp2A, lysosomal-associated membrane protein 2A.

monophosphate-activated kinase resulted in a diminished capacity for catabolism of LDs and suppressed the replication of dengue virus and production of infectious virus particles. Like dengue virus, both hepatitis B[100] and hepatitis C (HCV)[101] viruses upregulate host cell autophagy as a replicative strategy. In particular, HCV appears to hijack lipophagy in order to specifically promote LD formation and accumulation as a mechanism for staging viral assembly. Virally encoded structural (core) and nonstructural (NS5A) proteins are embedded on the LD surface, allowing for efficient HCV virion production.[102-104] HCV also appears to target another host cell lipophagy-related factor, the immunity-related GTPase family M protein, in order to promote efficient replication.[105,106] Reduced immunity-related GTPase family M expression is capable of not only interfering with autophagic progression but also negatively impacting viral replication, suggesting an important role for this lipophagy-related gene in the pathogenesis of hepatitis viruses.

Conclusions

During the past decade, significant progress has been made toward understanding hepatocellular lipophagy (Fig. 1). Key factors and pathways linked to the regulation of this process are being identified but are at an early stage for therapeutic intervention of complex diseases, such as NAFLD. Outstanding questions will no doubt focus on further defining the crosstalk between lipases and the core lipophagic machinery. Additionally, it will be important to reconcile studies supporting a catabolic role for lipophagy with other reports suggesting that inhibition of the autophagic machinery actually reduces hepatic steatosis (see, e.g., Kim et al.[107] and Shibata et al.[108]). The basis for these divergent findings is currently unclear but may be derived from the variety of models, contexts of the inhibition (i.e., whole-animal or tissue-specific knockouts), and methods of quantification used in the evaluation of hepatic steatosis.[109] Furthermore, despite progress made in understanding the interactions between organelles, such as mitochondria and the autophagic machinery, little is known regarding the LD-specific proteins that guide the selective process of lipophagy. Identification of these factors will no doubt fuel a better understanding of the mechanisms underlying LD metabolism in the liver. Finally, it will be important to clarify the relative contributions played by various forms of lysosome-mediated breakdown (i.e., conventional macrolipophagy versus chaperone-mediated lipophagy and microlipophagy) in the context of hepatic lipid metabolism.

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