Regulation of the homeostasis of hepatic endoplasmic reticulum and cytochrome P450 enzymes by autophagy☆

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Abstract

The endoplasmic reticulum (ER) is an intracellular organelle consisting of a continuous network of membranes. In the liver, the ER is highly active in protein modification, lipid metabolism, and xenobiotic detoxification. Maintaining these complicated processes requires elaborate control of the ER lumen environment as well as the ER volume. Increasing evidence suggests that autophagy plays a critical role in regulating the homeostasis of hepatic ER contents and levels of cytochrome P450 (CYP) enzymes via selective ER-phagy. This review will provide an overview of ER-phagy, summarizing the possible roles of recently identified ER-phagy receptor proteins in regulating the homeostasis of hepatic ER and CYP enzymes as well as outlining the various implications of ER-phagy in ER-related liver diseases.

Keywords

Alcoholic liver disease; Endoplasmic reticulum (ER)-phagy; Endoplasmic reticulum (ER) stress; Cytochrome P450 (CYP) enzymes; Liver diseases; Non-alcoholic fatty liver disease (NAFLD); Non-alcoholic steatohepatitis (NASH)

1. Introduction

The endoplasmic reticulum (ER) is an elaborate intracellular organelle composed of a single, continuous phospholipid membrane, segregating the ER lumen from the cytoplasm. Two types of ER exist in the cell: flattened peripheral sheets with ribosomes (rough ER) and a complex network of smooth tubules (smooth ER). Each type contributes differently to the biosynthetic capacity of ER. The rough ER is responsible for the folding and post-translational processing of proteins due to the presence of studded ribosomes, whereas the smooth ER is the designated site for lipid and steroid biosynthesis, calcium storage, and drug detoxification.[1,2] The protein folding process is assisted by an array of ER-luminal

chaperones that prevent the aggregation of partially folded proteins. This process is highly sensitive, and it is dependent upon ER luminal factors such as calcium concentration, redox homeostasis, and oxygen supply.[3] As a result, the ER lumen fosters a highly-oxidized environment with elevated calcium concentration compared to the surrounding cytoplasm in order to meet the demands of protein folding and modification. Any physiological or pathological disruption of this finely balanced environment, such as the disruption of calcium homeostasis or the reduction of the oxidized ER lumen environment will trigger a process called "ER-stress" that cripples the ER protein folding machinery. In response to ER stress, cells can activate an adaptive response to relieve the ER stress known as the unfolded protein response (UPR), which is a tightly orchestrated collection of intracellular signal transduction pathways designed to restore ER homeostasis.[2,4] One of the major purposes of UPR is to shut down the general protein translation program. This will decrease protein production and selectively turn on the transcription program for increasing the ER chaperone proteins, ultimately helping with the proper folding of misfolded proteins. Furthermore, to deal with ER stress, cells activate ER-associated degradation (mainly through the ubiquitin proteasome system) and autophagy (lysosome) pathways.[2,5,6] If the above adaptive response is not sufficient to relieve ER stress, these stressed cells will be eliminated via apoptosis mediated by ER stress-induced activation of CCAAT-enhancer-binding protein homologous protein (CHOP) and the c-Jun N-terminal kinase (JNK) as well as caspase-12 (mouse) and -4 (human).[5–7] This review will discuss the current progress in uncovering the mechanisms and overall role of autophagy in the selective removal of excess ER and possible CYP enzymes in the liver.

2. General autophagy machinery and regulation

Autophagy (or macroautophagy) is a highly conserved lysosomal degradation pathway featuring the formation of double membrane autophagosomes that traffic to fuse with lysosomes via the microtubules. Once fused, autolysosomes are formed, where the enwrapped cargos of autophagosomes are degraded.[8,9] Cargo degradation produces molecular building blocks such as amino acids and fatty acids, which are subsequently recycled back into the cytoplasm for reuse. This especially occurs under nutrient deprivation conditions.[8–10]

So far, more than 40 autophagy-related genes (Atg) proteins have been identified to regulate several key steps in the autophagy process: (i) The initiation signal of autophagome formation. Uncoordinated 51-like kinase 1 (ULK1), the only kinase of Atg proteins, works as an early signal sensor to initiate autophagy. Two other adaptor molecules, Atg13 and FAK family-interacting protein of 200 kDa (FIP200), interact with ULK1 to form the ULK complex, which is negatively regulated by the nutrient sensor, mechanistic target of rapamycin complex 1 (mTORC1) and positively regulated by the energy sensor AMPK. Nutrient deprivation inhibits mTORC1, which dephosphorylates ULK1 to initiate isolation membrane formation. (ii) The source of the isolation membrane. The exact source of the isolation membrane is still up for debate, but the ER, ER-mitochondria contact site, ER-Golgi intermediate compartment, and plasma membrane (or endocytosis-derived vesicles) have all been proposed as likely contributors to the formation of autophagosomes. Among these membrane sources, the general consensus is that the ER membrane is an important

initiation site. (iii) Autophagosome biogenesis. A series of Atg proteins such as Beclin 1 and vacuolar protein sorting 34 (Vps34) are recruited to the rough ER or ER mitochondria contact site by ER-resident SNARE protein syntaxin 17 (STX17). Vps34 is a mammalian class-III phosphatidylinositol 3-phosphate kinase that promotes phosphatidylinositol 3-phosphate (PI3P) generation. PI3P enrichment recruits more PI3P binding proteins/effectors to initiate autophagosome biogenesis. (iv) Autophagosome membrane expansion and elongation. Two ubiquitin-like conjugation systems, the Atg7-Atg3-Atg8/microtubule-associated protein light chain 3 (LC3) and Atg12-Atg5-Atg16L1 complexes, regulate the phosphatidylethanolamine conjugation with LC3 (called LC3-II), which is essential for autophagosome membrane elongation. Eventually, the autophagosome membrane forms a completely enclosed double membrane vesicle that engulfs cell organelles, misfolded proteins, and/or invading bacterials xenobiotics. The exact mechanisms demonstrating how an autophagosome achieves closure remain unclear. In addition to the two ubiquitin conjugation systems, Atg9, the only Atg protein that has multi-membrane spanning domains, also helps with the expansion of the double membrane autophagosome structure by shuttling and donating membranes from other intracellular organelles to the site of autophagosome biogenesis. (v) Fusion of autophagosomes with lysosomes. The mature autophagosome finally fuses with the lysosome to form an autolysosome in which the autophagosome is degraded by hydrolases. The fusion process is regulated by the fusion machinery soluble N-ethyl-maleimide-sensitive fusion protein (NSF) attachment protein receptor(SNARE) family proteins, such as VAMP7, VAMP8, VAMP9 and STX17/syntaxin 17.[11–13] In addition, LAMP-2A, a lysosomal membrane protein, and RAB7, a RAS-related GTP-binding protein, are required for the fusion of autophagosomes with lysosomes.[14] (vi) Termination of autophagy and lysosome biogenesis. Finally, autophagy is terminated, and lysosomes are regenerated by autophagic lysosome reformation (ALR), a process in which the proto-lysosomes are generated from the tabulation, scission, and budding of autolysosomes.[15] New lysosomes may also be generated via a transcription program mediated by the master lysosomal biogenesis transcription factor EB (TFEB), to meet the needs of autophagic degradation in various tissues/cells including the liver.[16–18]

3. Selective autophagy for ER (ER-phagy)

Autophagic degradation can either be non-selective or selective. Non-selective autophagy generally occurs upon nutrient deprivation, which causes the random breakdown of cytoplasmic components, supplying cells with nutrients for survival purposes. In contrast, selective autophagy specifically targets damaged or superfluous organelles and protein aggregates.[19,20] It has been shown that several organelles, such as mitochondria, peroxisomes, ER, ribosomes, nuclei, and invading bacteria, can be selectively removed by autophagy even under nutrient-rich conditions. Selective autophagy is mediated by receptor proteins that determine the specificity of a given selective autophagy. An autophagy receptor protein generally can bind to cargo and Atg8/LC3 simultaneously, acting as a bridge between the target cargo and nascent autophagosome during the early stages of autophagy. Typically, most receptor proteins share a conserved Atg8/LC3 binding linear peptide region called the LC3-interacting region (LIR) motif. In addition to the autophagy receptor proteins, ubiquitination is another commonly involved cargo recognition mechanism in

selective autophagy. Several autophagy receptor proteins have been proposed to be active in mammals, including p62/Sequestosome 1 (SQSTM1) (hereafter referred to as p62), a neighbor of BRCA1 gene 1 (NBR1), nuclear domain 10 protein 52 kDa (NDP52), optineurin, and Bnip3. Some autophagy receptor proteins such as p62 can directly bind to ubiquitinated cargos through which the target cargos can be delivered to nascent autophagosomes.[20–23]

Under certain conditions, excess ER contents can be induced in cells. For instance, during the acquired immune response, B cells differentiate into plasma B cells to meet demands necessary for the synthesis and secretion of prodigious amounts of immunoglobulin. This process requires a massive proliferation of rough ER in B cells.[24] Overexpression of HMG-CoA reductase (HMGR) in cultured cells leads to increased proliferation of smooth ER.[25,26] In response to fed, pancreatic acinar cells also experienced increased proliferation of ER to produce more digestive zymogen granules to meet the needs of food digestion by secreting digestive enzymes. Additionally, ER stress-induced activation of UPR leads to increased ER expansion and volume in yeast.[27] In mammals, the liver is the major site for metabolizing xenobiotics, which is mediated by CYP enzymes. This process generally causes proliferation of smooth ER to make the CYP enzymes. For example, 1,4-bis [2-(3, 5-dichloropyridyloxy)] benzene (TCPOBOP) or phenobarbital (PB), two constitutive androstane receptor (CAR) agonists, can increase the proliferation of hepatic ER in rodent livers concomitantly with the induction of several CYP enzymes such as CYP2B and CYP3A.[28–30] After withdrawal of xenobiotics, the proliferating ER can return to normal levels in the liver. It is largely believed that autophagy is involved in the selective removal of the excess ER in these conditions. Below, we will discuss the possible autophagy receptors that may be involved in the selective removal of ER, a process called ER-phagy.

Several mammalian ER-phagy receptors have been identified so far, including family with sequence similarity 134 member B (FAM134B), preprotein translocation factor (Sec62), reticulon domain-containing protein 3 (RTN3), cell-cycle progression gene 1 (CCPG1), p62, and BCL2 interacting protein 3 (Bnip3).[31,32] Additionally, two ER-phagy receptors exist in yeast: Atg39 and Atg40. Atg39 is responsible for the removal of perinuclear ER, whereas Atg40 is more selective for the peripheral ER.[33] Ultrastructural analyses have shown that ER-phagy receptors drive the sequestration of isolated fragments of ER into an autophagosomal lumen defined by discrete, delimiting membrane(s) in the majority of the aforementioned cases.

3.1. ER-phagy receptors in yeast

Two novel Atg proteins, Atg39 and Atg40, have been identified in yeast species *Saccharomyces cerevisiae* as receptors specific to selective autophagy of the ER and the nucleus.[33] Both Atg39 and Atg40 contain the Atg8-family-interacting motif (AIM), which contributes to the interaction between these two proteins with Atg8. The ER system in yeast consists of perinuclear ER (nuclear envelope), cortical ER, and cytoplasmic ER. Interestingly, Atg39 colocalizes with perinuclear ER and induces the autophagic degradation of the nucleus under nitrogen starvation. Depletion of Atg39 significantly suppresses the degradation of nucleolar proteins and decreases cells' viability upon nutrition deficiency. In

contrast, Atg40 is concentrated in the cortical and cytoplasmic ER, and it drives these ER subdomains into autophagosomes. *Atg40* knockout shows a lesser effect on yeast cells' viability during starvation. Knockout of either *Atg39* or *Atg40* partially blocks ER-phagy, while the double knockout almost completely blocks this pathway. These results indicate that different portions of ER may be specifically removed by autophagy using different autophagy receptor proteins. Theoretically, Atg40 is probably the counterpart of mammals' ER-phagy receptor FAM134B (See below discussion).

3.2. FAM134B

In 2001, Tang *et al.*[34] first discovered *FAM134B* gene in esophageal cancer by comparative genomic hybridization analysis. In 2015, FAM134B protein was identified as an ER-anchored autophagy receptor that facilitates autophagic ER degradation.[35] Yeast two-hybrid screenings revealed the interaction of FAM134B protein with the autophagy effectors LC3/GABARAPL2. In mouse embryonic fibroblasts (MEF) cells, both overexpressed and endogenous FAM134B directly bind to LC3-like modifiers through its C-terminal LIR motif, suggesting the potential role of FAM134B in selective autophagy. On the other hand, FAM134B co-localizes with ER-marker proteins and promotes the formation of ER-containing LC3B-positive puncta. LIR motif mutation impairs the ability of FAM134B to drive ER into a LC3-positive autophagosome, demonstrating that FAM134B is a selective ER-phagy receptor. Besides LIR motif, the reticulon homology domain (RHD) located on the N-terminal of FAM134B is also essential for ER-phagy. RHD domain is involved in membrane remodeling and contributes to ER fragmentation, which facilitate the engulfment of ER by the autophagosomal membrane. *In vitro* knockdown of the expression of *FAM134B* leads to a striking ER expansion. Similarly, *FAM134B$^{-/-}$* MEF also show ER expansion and impaired ER turnover, and *FAM134B$^{-/-}$* MEF are more susceptible to stress-induced mitochondrial apoptosis. In *FAM134B$^{-/-}$* MEF, both sheet-like cisternal ER and tubular ER turnover are inhibited under nutrient-deficient conditions. However, the autolysosome number, long-lived protein degradation, LC3 processing, and p62 turnover are constant in *FAM134B$^{-/-}$* MEFs, demonstrating that FAM134B acts as a selective autophagy receptor to mediate ER turnover specifically without affecting the general autophagy. To execute ER breakdown, FAM134B congregates into the ER in which the RHD domain contributes to ER scission, and the LIR motif binds with LC3, the key autophagic effector protein located on the autophagosomal membrane. This interaction tethers fragmented ER to autophagic machinery forming the ER-containing autophagosome structure, which finally fuses with the lysosome for ER turnover. ER is a very dynamic organelle that undergoes continuous fusion reactions. In this way, the ER is very similar to mitochondria, the other organelles known for undergoing continuous fusion and fission. Mitochondrial fission/fragmentation has been thought to favor mitophagy because small, fragmented mitochondria may be easier to become enwrapped by autophagosomes compared with large, elongated mitochondria.[36] Therefore, it is likely that FAM134B-mediated ER fragmentation may also favor selective ER-phagy. Physiologically, FAM134B-mediated ER-phagy is important for maintaining normal neuron function. The observation that aged *FAM134B* knockout mice have significantly decreased sensory neuron numbers compared with wild type mice confirms this assertion. Increased ER expansion is detected in the somata of sensory neurons of *FAM134B* knockout mice, suggesting a defect of ER-phagy in the *FAM134B* knockout

mice.[35] The lack of obvious phenotypes in other organs/tissues in *FAM134B* knockout mice is likely due to the relatively high expression level of FAM134B in the brain. In addition to its role in regulating ER homeostasis in neurons, the FAM134B-mediated ER-phagy has also been suggested to be a protective pathway in viral infection.[37] It has been proven that ER membranes are main sources of flaviviruses such as Dengue virus and Zika virus. ER membranes are utilized to establish viral replication, assembly, and eventual maturation.[37] Consequently, host cells have evolved highly efficient ER turnover processes like ER-phagy to resist virus replication and to maintain cellular homeostasis. Depletion of ER-phagy receptor FAM134B enhances flaviviruses replication *in vitro*, suggesting that FAM134B plays a role in fighting viral infection. Flaviviruses also have correspondingly mechanism to survive themselves. They have the NS3 virally-encoded proteases to directly cleave FAM134B at the RHD domain, which blocks the formation of ER-enriched autophagosomes and provides sufficient membrane sources to support their replication in the host.[37] Whether or not FAM134B plays a role in regulating ER homeostasis in the liver cannot be stated with certainty; however, it seems that FAM134B may be of lesser importance to liver function due to the low expression of FAM134B in the liver.

3.3. CCPG1

CCPG1 is an ER-resident protein whose interaction with the autophagosome-associated Atg8 ortholog GABARAP has been revealed through the unbiased affinity-purification mass spectrometric screening.[38] The cytosolic N-terminal region of CCPG1 contains a canonical LIR motif that binds to autophagic modifier LC3/GABARAP, indicating its potential role as an ER-phagy receptor.[39] Distinctive from other canonical autophagy receptors, Smith *et al.* uncovered the interaction of CCPG1 with FIP200, a component of the autophagy-initiating ULK1 complex. Two discrete but sequence-related FIP200 interacting regions (FIR) have been identified on the N-terminal of CCPG1 that directly bind with FIP200, which enables the cargo to actively initiate autophagy.[39]

In cultured mammalian cells, the expression of CCPG1 is induced by ER stressors and plays a role in the reduction of tubular ER. This phenomenon is dependent upon the interaction of CCPG1 with both mammalian Atg8 and FIP200, and it can be rescued by autophagy inhibition, suggesting that CCPG1 is required for autophagic degradation of tubular ER during ER stress.[39] To further determine the physiological role of CCPG1-mediated ER-phagy, Smith *et al* generated the CCPG1 hypomorphic mice using a gene-trap approach. CCPG1-deficient mice showed defective proteostasis, increased ER stress, and abnormal cell polarization in pancreatic acinar cells, resulting in signs of pancreatitis in aged (48 week-old) mice. Like FAM134B, whether CCPG1 plays a role in ER homeostasis in the liver has yet to be studied.

3.4. Sec62

Sec62, an ER-resident protein and a component of the ER translocon, has recently been identified as another autophagy receptor for selective ER-phagy during the recovery phase of post ER stress conditions. Using the bioinformatics analyses, a conserved LIR motif in the C-terminal cytosolic domain of Sec62 is identified.[40] The direct binding of Sec62 with LC3B *in vitro* is further confirmed by surface plasmon resonance, nuclear magnetic

resonance spectroscopy, and peptide array analyses. In cultured cells, the delivery of ER to autolysosomes during recovery from ER stress is inhibited by the silencing of Sec62 but promoted when Sec62 is overexpressed. Interestingly, ER stress does not induce the expression of Sec62. It remains unclear how Sec62, which is already on the ER, would trigger the ER-phagy although it has been proposed that during the recovery phase of ER stress Sec62 may be dissociated from binding with other components of the translocons. It should be noted that the role of Sec62 in ER-phagy has only been demonstrated in cultured cells; its role in ER-phagy *in vivo* is still unknown. However, *Sec62* is a candidate oncogene and is associated with various cancers including hepatocellular carcinoma.[40,41] Therefore, future studies are critical to better understanding the possible role of Sec62 in ER-phagy in the liver.

3.5. RTN3

RTN3, a protein concentrated at ER tubules, has been identified as a specific receptor mediating the autophagic degradation of tubular ER.[42] Two isoforms of RTN3, including long isoform (RTN3L) and short isoform (RTN3S), have been described. Both splicing products share a common C-terminal RHD domain, but the extra N-terminal region of RTN3L contributes to the major functional variations between them. Six discrete LIR motifs have been identified in this extra N-terminal region, which enables the long isoform of RTN3 to interact with autophagic core protein, LC3B. Overexpression of RTN3 in cultured U2OS cells leads to the fragmentation of ER tubules during starvation. This phenomenon is dependent on homo-dimerization of RTN3L but not RTN3S. Under lysosome inhibitor Bafilomycin Al treatment, the overexpressed RTN3 colocalizes with LC3B and leads to the fragmentation of ER, which is abolished in Atg7$^{-/-}$ cells. Upon nutrition deficiency, degradation of tubular ER marker protein is suppressed in RTN3$^{-/-}$ MEFs; meanwhile, the clearance of ER sheet proteins is held constant. The latter appears to be mainly mediated by FAM134B. In addition, general autophagic flux is unchanged in *RTN3* knockout cells, suggesting that RTN3 acts as a selective ER-phagy receptor. Altogether, the study suggests that these two ER-phagy receptors specifically regulate the degradation of distinctive parts of ER: FAM134B is responsible for the turnover of ER sheets whereas RTN3 is culpable for the ER tubules.

3.6. p62/SQSTM1 and hepatic ER-phagy and homeostasis of CYP

Liver is a vital and major organ for drug metabolism in mammals and more than 50% of drugs are metabolized and oxidized by the hepatic CYP enzymes in the liver.[43] In hepatocytes, the ER and microsomes are the central hubs responsible for producing the key metabolic CYP enzymes. Upon exposure to xenobiotics, hepatocytes quickly increase the proliferation of ER and production of CYP enzymes to meet the needs of xenobiotic metabolism. Once the mission of the drug metabolism is accomplished, hepatocytes must get rid of the excess ER to reach cellular homeostasis.

Several decades ago, it was documented that in response to PB or TCPOBOP, rodent livers have increased proliferation of ER and hepatocytes concomitantly with the induction of several CYP enzymes such as CYP2B and CYP3A.[28–30] After the cessation of PB treatment in rat livers, the proliferating ER can return to normal.[29] Morphological analysis revealed

double-membrane autophagosomes enveloped with ER membranes in the rodent hepatocytes, suggesting a possible role of autophagy in the removal of the excess ER.[28,29] Using GFP-LC3 transgenic mice and liver-specific *Atg5* knockout mice, it is shown that autophagy is required to remove the excess ER in mouse livers after withdrawal of TCPOBOP, a potent CAR agonist.[32] The contents of ER and CYP2B10 increased from Day 1 and reached their peaks at Day 3 and 6, finally declining to near-basal level at Day 9 after the cessation of TCPOBOP. More importantly, TCPOBOP increased the number of GFP-LC3 puncta in mouse hepatocytes and isolated purified autophagosomes and autolysosomes contained ER proteins and CYP2B10. These data clearly indicate that excess ER and CYP2B10 can be enveloped by autophagosomes. Electron microscopy analysis revealed increased fragmentation of ER and enwrapping of fragmented ER by autophagosomes as shown in Fig. 1. Pharmacological inhibition of hepatic autophagy by chloroquine or genetic deletion of Atg5 in mouse livers both lead to the sustainment of increased ER contents and CYP2B10, demonstrating the critical role of autophagy in the removal of excess ER and CYP2B10 via autophagy. As discussed above, ER-phagy may require specific autophagy/ER-phagy receptors. p62 is an autophagy receptor protein that can bind LC3 and ubiquitinated proteins via its LIR and UAB domain. TCPOBOP increased the levels of ubiquitin on isolated hepatic ER and increased recruitment of p62 to the ER. It should be noted that in contrast to preceding ER-phagy receptors (Atg40, Atg39, FAM134B, Sec62, CCPG1 and RTN3), p62 does not have a transmembrane domain and is not an ER resident protein. It is proposed that the increased ER recruitment of p62 is likely a secondary effect due to its ability to bind ubiquitinated proteins to the ER. Once on the ER, p62 may further recruit LC3B positive autophagosomes to the ubiquitin-p62 decorated ER to initiate selective ER-phagy. More importantly, adenovirus shRNA-mediated knockdown of *p62* increased the ER contents in primary cultured mouse hepatocytes. Moreover, *p62* knockout mice have increased hepatic ER contents and CYP2B10 levels compared with wild type mice after withdrawal of TCPOBOP. These results clearly indicate that p62 can serve as a selective ER-phagy receptor. As discussed above, the hepatic expression levels of other ER-phagy receptors (such as FAM134B, Sec62, CCPG1 and RTN3) are unclear; thus, future studies are needed to determine whether other ER-phagy receptors also play a role in selective ER-phagy in the liver.

3.7. ER-phagy in other liver pathophysiology (Fig. 2)

In addition to the removal of excess ER and CYP enzymes during xenobiotic metabolism, ER-phagy in the liver may also be influential in other liver pathogenesis related to ER accumulation or ER stress. Alpha-1-antitrypsin (AT) deficiency causes liver inflammation and carcinogenesis, which affects l in l800 live births in Northern European and North American populations.[44] The pathogenesis of AT-induced liver injury is due to a mutation in the AT gene resulting in the misfolding of the mutant protein which is then stuck in the ER in its aggregated form.[45] Pharmacological activation of autophagy to remove these ER with aggregated AT has been shown to be beneficial in improving the liver pathology in an AT mouse model. Similar to AT deficiency, hypofibrinogenemia is another liver ER-storage disease resultant from a mutant form of fibrinogen named Aguadilla γD that forms aggregates in the hepatic ER.[46] While the ER-associated protein degradation (ERAD) pathway can help to remove some of the mutant forms, autophagy has been shown to

degrade excess aberrant polypeptides form aggregates within the ER.[46] Another highly common liver metabolic disease is non-alcoholic fatty liver disease (NAFLD), which involves both prominent ER stress and impaired autophagy.[47,48] In addition to supporting drug metabolism, the liver performs key roles in lipid and glucose homeostasis. The ER provides not only the major site for lipid droplet synthesis but also many enzymes and regulators required by these metabolic pathways.[49] Several studies demonstrated that lipid overload in the liver induces ER stress, which in turn promotes hepatic steatosis and lipogenesis.[50–54] Moreover, numerous evidence supports that the UPR signaling cascade may contribute to the inflammatory response of nonalcoholic steatohepatitis (NASH).[55,56] As one of the effector mechanisms of the UPR, autophagy can be induced to alleviate ER stress through the elimination of overloaded lipid contents as well as dysfunctional ER, ultimately protecting against NAFLD. Impaired autophagic and lysosomal function have been identified in livers from NAFLD patients.[47,48] In several mouse NAFLD and alcoholic fatty liver disease (AFLD) models, genetic overexpression of Atg7 or TFEB and/or pharmacological activation of autophagy or TFEB, all result in the alleviation of hepatic lipid metabolic stress and liver injury.[16,57–59] However, whether selective ER-phagy or any of the above mentioned ER-phagy receptors play a role in liver ER-storage diseases, NAFLD, NASH, and/or alcoholic liver disease remain to be studied in the future. The current known ER-phagy receptors and its potential implications in liver physiology and pathogenesis were summarized in Fig. 2.

4. Conclusion and perspective

In conclusion, ER-phagy is a selective form of autophagy that is mediated by several unique ER-phagy receptor proteins, playing a critical role in maintaining ER homeostasis. In the liver, ER-phagy is vital to effectively regulating the homeostasis of ER contents and drug metabolism of CYP enzymes. Activation of ER-phagy may also be important in alleviating ER-storage liver diseases and metabolic liver diseases such as NAFLD, NASH, and AFLD. However, whether any of the known ER-phagy receptor proteins play an essential role in the pathogenesis of these liver diseases remains to be seen.

Acknowledgements

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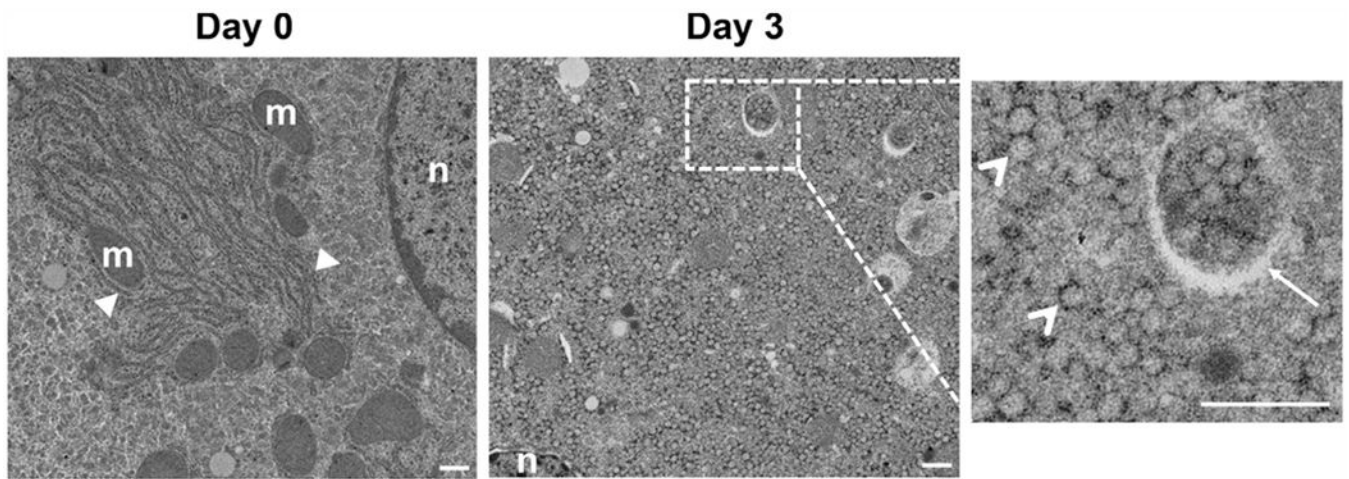
Fig. 1. An illustration for the hepatic ER morphological change and ER-phagy in mouse liver.
Male GFP-LC3 transgenic mice were either administered with vehicle (DMSO) or
TCPOBOP (3 mg/kg) once a day for three consecutive days. Day 0 samples were from mice
that were sacrificed 1 day after withdrawal of DMSO administration. Day 3 samples were
from mice that were sacrificed 3 days after withdrawal of TCPOBOP. Representative
photographs from electron microscopy studies are shown. The right panel is an enlarged
photograph from the boxed area. Solid arrow-heads denote the tubular ER. Open arrow-
heads denote fragmented ER. The arrow denotes an autophagosome that envelops
fragmented ER. m: mitochondria; n: nucleus. Scale bar, 500 nm.
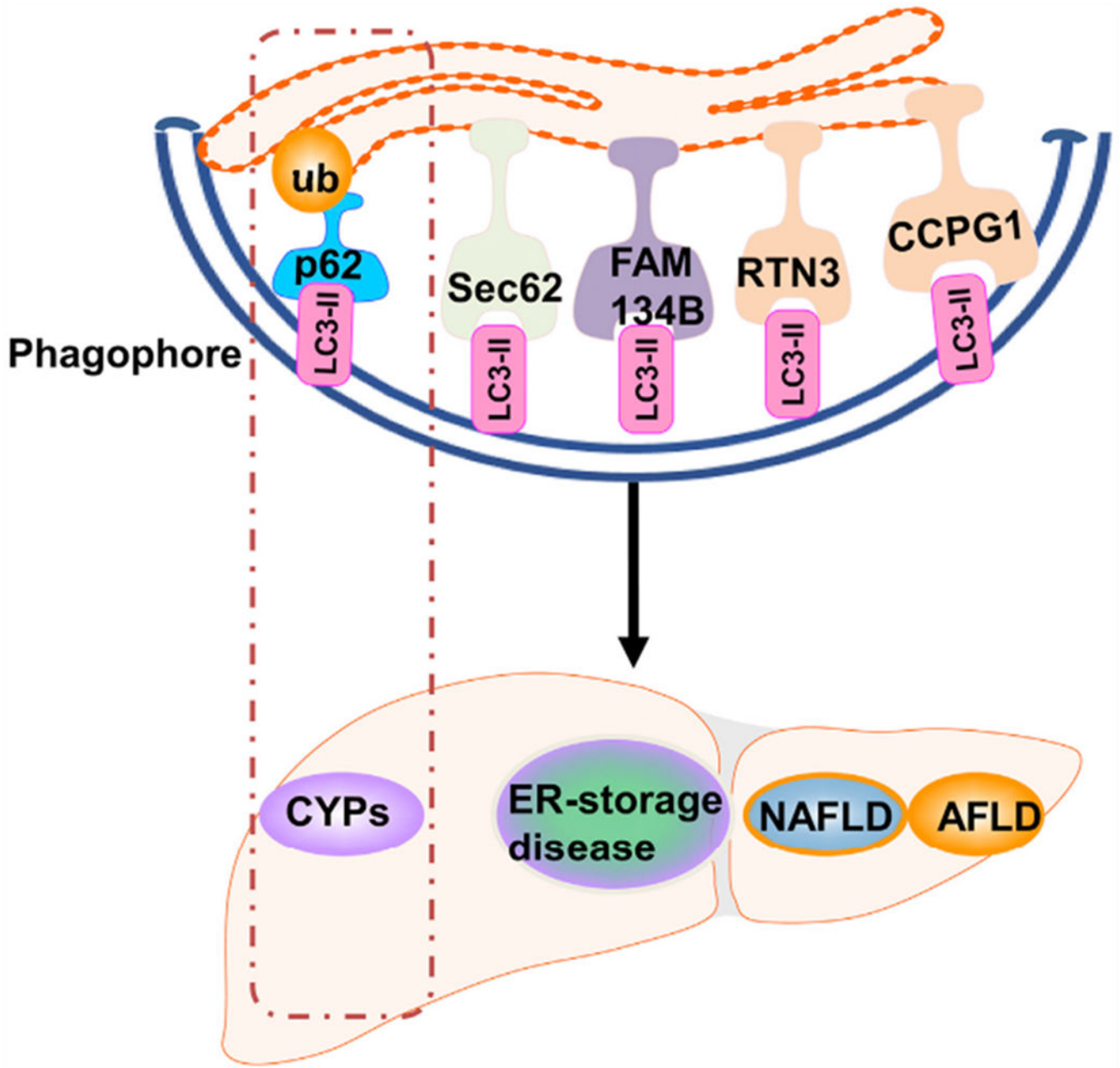
Fig. 2. A simplified scheme for ER-phagy receptors and its potential implications in liver physiology and pathogenesis.

ER-phagy receptors that have been reported in mammalian cells are shown. p62 binds with ubiquitin (ub) on the ER and further recruits LC3-II positive phagophore to the ER. In contrast, FAM134B, Sec62, RTN3 and CCPG1 all have either transmembrane domain or reticulon homology domain to anchor these proteins directly on ER membrane. Like p62, FAM134B, Sec62, RTN3 and CCPG1 also have LIR to interact with LC3-II and recruit the phagophore to the ER. Removal of excess ER by ER-phagy may help to keep the homeostasis of hepatic CYP enzymes for xenobiotic metabolism and may also play roles in possible hepatic ER-storage disease, NAFLD and AFLD. Boxed part indicates that the

known role of p62 in liver ER-phagy. The unboxed part indicates the known ER-phagy receptors for other tissues/cells but their roles in the liver remain to be determined. Abbreviations: ER, endoplasmic reticulum; FAM134B, family with sequence similarity 134 member B; Sec62, preprotein translocation factor; RTN3, reticulon domain-containing protein 3; CCPG1, cell-cycle progression gene 1; CYPs, cytochrome P450 enzymes; LC3, microtubule-associated protein light chain 3; NAFLD, non-alcoholic fatty liver disease; AFLD, alcoholic fatty liver disease.