Review

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Autophagy in hepatic adaptation to stress

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Summary

Autophagy is an evolutionarily ancient process whereby eukaryotic cells eliminate disposable or potentially dangerous cytoplasmic material, to support bioenergetic metabolism and adapt to stress. Accumulating evidence indicates that autophagy operates as a critical quality control mechanism for the maintenance of hepatic homeostasis in both parenchymal (hepatocytes) and non-parenchymal (stellate cells, sinusoidal endothelial cells, Kupffer cells) compartments. In line with this notion, insufficient autophagy has been aetiologically involved in the pathogenesis of multiple liver disorders, including alpha-1-antitrypsin deficiency, Wilson disease, non-alcoholic steatohepatitis, liver fibrosis and hepatocellular carcinoma. Here, we critically discuss the importance of functional autophagy for hepatic physiology, as well as the mechanisms whereby defects in autophagy cause liver disease.

Keywords: Aggrephagy; Chaperone-mediated autophagy; Lipid droplets; Lipophagy; Mitophagy; Unfolded protein response.

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Introduction

Autophagy is a highly coordinated and phylogenetically conserved cellular mechanism that culminates with the degradation of disposable or potentially toxic cytosolic entities within acidic organelles (*i.e.*, lysosomes in mammalian cells, the vacuole in yeast cells).[1,2] The term autophagy (from the ancient Greek 'αὐτόφαγος', meaning 'self-eating') was originally introduced by the Belgian cytologists Christian de Duve and Robert Wattiaeux in 1966 to distinguish the degradation of intracellular entities from that of extracellular material (which was named 'heterophagy').[3] These observations built on more than a decade of electron microscopy, tissue fractionation and functional studies on the rat liver, leading to the identification of lysosomes as the major site of degradation for both endogenous and exogenous material.[4–12] As a reference, glucagon treatment was shown to boost glycogenolysis, gluconeogenesis and activate protein catabolism during the same period.[13]

As of today, 3 major pathways leading to the lysosomal degradation of cytosolic material have been described (Fig. 1).[2] Microautophagy involves the acquisition of small autophagic substrates by acidic organelles (in mammals, late endosomes) upon direct membrane invagination.[1] Chaperone-mediated autophagy (CMA) relies on the recognition of soluble proteins with a KFERQ motif by heat shock protein family A (Hsp70) member 8 (HSPA8), coupled to the translocation of such KFERQ-bearing substrates across lysosomal membranes by a specific splicing isoform of lysosomal associated membrane protein 2 (LAMP2A).[14] Finally, macroautophagy (Fig. 2) relies on the generation of a double-membraned organelle (the autophagosome) that sequesters cytoplasmic material for degradation and delivers this cargo to lysosomes for degradation.[2]

Endosomal microautophagy (the only form of microautophagy described in mammals) generally degrades cytosolic proteins, either in bulk or selectively.[1] CMA has a multitude of substrates, *de facto* impinging on the regulation of a variety of processes relevant for hepatic homeostasis including bioenergetic metabolism and oncogenesis.[14] Macroautophagy can degrade disposable cytoplasmic entities (including entire organelles or portions thereof) in a rather non-specific manner (*e.g.*, when activated by bioenergetic challenges), as well as with improved selectivity (*e.g.*, when driven by organelle damage).[15] Multiple neologisms have been introduced to refer to specific variants of macroautophagy, including (but not limited to): mitophagy (cargo: mitochondria), aggrephagy (cargo: protein aggregates), pexophagy (cargo: peroxisomes), reticulophagy (cargo: endoplasmic reticulum), lipophagy (cargo: lipid droplets), glycophagy (cargo: glycogen) and xenophagy (cargo: cytoplasmic pathogens).[1,16] Macroautophagy (from here onward referred to as autophagy) has been involved in the regulation of multiple cellular functions with major pathophysiological implications for various organs including the liver.[17,18]

Accumulating evidence indicates that proficient autophagic responses in both hepatocytes and non-parenchymal cells (stellate cells, sinusoidal endothelial cells, Kupffer cells) are key for physiological liver functions.[19] In line with this notion, defects in autophagic substrate degradation contribute to the pathology of a variety of hepatic disorders including alpha-1-antitrypsin deficiency, Wilson disease, non-alcoholic steatohepatitis (NASH), liver fibrosis and hepatocellular carcinoma (HCC).[20] Here, we discuss the role of autophagy in liver health and disease.

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Autophagy in liver physiology

Autophagy occupies a key position in the regulation of multiple liver functions as well as in the preservation of hepatic homeostasis,[19] at least in part reflecting the unique biological features of this organ (Fig. 3). In particular, hepatocytes have limited turnover, with an estimated half-life of 6-to-12 months, which makes them prone to accumulate potentially detrimental cellular byproducts in the absence of proficient autophagy.[21] Although hepatocytes are generally quiescent, they can rapidly resume proliferation in response to injury, which (especially in the presence of autophagy defects) creates a risk for malignant transformation.[22] Along similar lines, the liver mediates central functions in systemic glucose and lipid metabolism, both of which are intimately connected to autophagy.[23,24] Moreover, the liver is highly exposed to xenobiotics and potentially inflammatory mediators from the portal circulation, and autophagy has major cytoprotective and anti-inflammatory effects.[25,26] Finally, the liver is sensitive to infection by multiple hepatotropic viruses, which are the most common cause of hepatic disorders worldwide. In this setting, proficient xenophagic responses constitute a key first line of defence against productive infection.[26] That said, it is important to note that multiple viruses including hepatitis B virus have evolved strategies for hijacking the autophagic machinery to support replication and dissemination.[27,28]

The baseline autophagic flux of the liver is rather elevated compared to that of other organs such as the brain.[19] Moreover, hepatic autophagy fluctuates with regular feeding-fasting behaviour in a circadian fashion, and can be strongly upregulated by prolonged fasting episodes.[29] Thus, in perfused rat livers, autophagy-dependent proteolysis can increase from a basal level of ~1.5% total liver proteins/hour to ~4.5% of total liver protein/hour on starvation, leading to almost ~40% protein loss, if starvation is maintained for 48 hours.[30,31] Similar results have been obtained in cultured primary hepatocytes.[32] These studies were the first to establish the importance of autophagy for hepatic functions in the context of circadian feeding behaviour. Subsequent investigation revealed that multiple cargo-specific variants of autophagy[33] are key for the maintenance of liver homeostasis, as discussed below.

Key points

Baseline autophagy is critical for the proper functionality of the liver in physiological conditions.

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Reticulophagy

The shape and volume of the endoplasmic reticulum (ER), the main subcellular compartment involved in protein folding, secretion and lipid biosynthesis, are highly dynamic and responsive to stress conditions, often resulting in increased biogenesis.[34,35] Once reticular homeostasis is resolved, cells dispose of excess ER to recover physiological functions via reticulophagy,[36,37] which also operates at baseline to preserve a normal ER compartment.[38] Additional triggers of reticulophagy include nutrient deprivation and pathogen infection.[38] In reticulophagy, selective cargo recognition largely relies on specific receptors connecting ER proteins to the general autophagic machinery (Fig. 2).[39] Four ER-resident proteins contain at least one LC3-interacting region (LIR) that enables such interactions, namely reticulophagy regulator 1 (RETREG1), reticulon 3 (RTN3), SEC62 homolog, preprotein translocation factor (SEC62), and cell cycle progression 1 (CCPG1) (Fig. 4A).[40–43] RETREG1 and RTN3 also possess a so-called 'reticulon homology domain' (RHD), which favours ER membrane curvature and fragmentation in the course of reticulophagy.[44]

Hepatocytes mount proficient reticulophagic responses to oleic acid,[45] an inducer of non-alcoholic fatty liver disease (NAFLD),[46] as well as to 1,4-bis [2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP) and phenobarbital, both agonists of nuclear receptor subfamily 1 group I member 3 (NR1I3) that drive hepatocyte proliferation.[47] Moreover, RETREG1 has recently been shown to cooperate with the ER chaperone calnexin (CANX) in a reticulophagic response that ensures the quality of procollagen.[48] In particular, RETREG1 interacts with CANX bound to unfolded procollagen in the ER lumen, ultimately directing such proteasome-resistant cargo to reticulophagy for degradation.[48] Thus, reticulophagy stands out as a major suppressor of aberrant collagen secretion (and hence fibrosis) (Fig. 4B).

In summary, baseline and stress-induced reticulophagy contributes to hepatic homeostasis and adaptation by ensuring the optimal functionality of the ER in hepatocytes.

Lipophagy

The liver is the principal organ for lipid metabolism, ensuring not only the first-line transformation of dietary fat from the portal circulation, but also the conversion of lipids that are released in the bloodstream by the adipose tissue.[49] Hepatocytes take up circulating fatty acids (FAs) and rapidly esterify them in the ER to produce triglycerides and cholesterol esters. Upon accumulation within the ER bilayer, neutral lipids form lens-like microdomains that are stabilised by proteins like BSCL2 lipid droplet biogenesis associated, seipin (BSCL2) and fat storage inducing transmembrane protein 2 (FITM2).[50] These lens-like neutral lipid microdomains are unstable and can bud off the ER bilayer due to thermal fluctuation, resulting in the formation of spherical lipid droplets (LDs), which are located in the cytoplasm and are key sites for hepatic lipid metabolism (Fig. 5A).[50] Nascent as well as mature LDs (generally 250–500 nm) are equipped with all the enzymes required for triglyceride synthesis, and their size, physical interaction and functional crosstalk with other organelles are all finely regu-

lated. Thus, depletion of atlastin GTPase 1 (ATL1), a GTPase involved in ER remodelling, or receptor accessory protein 1 (REEP1), a key protein for the generation of ER tubules, drastically affects the size of LDs.[51,52] Moreover, the coatomer protein 1 (COPI) complex regulates the surface tension of LDs by removing phospholipids, *de facto* favouring their fusion with ER membranes.[53] COPI coatomers are also important for the delivery of the cytosolic lipase patatin-like phospholipase domain containing 2 (PNPLA2) to LDs.[54]

On nutrient deprivation, multiple cell types prioritise fatty acid oxidation (FAO, also known as β-oxidation) as a source of energy.[55] FAO involves the mitochondrial uptake of free FAs to locally generate acetyl-CoA and, via the Krebs cycle, $NADH^+$ molecules in support of oxidative phosphorylation.[56] In this scenario, cells can metabolise LDs to form FAs via either of 2 non-mutually exclusive pathways: i) lipolysis, whereby protein kinase A (PKA) drives the phosphorylation-dependent, proteasomal degradation of perilipin 1 (PLIN1), in turn enabling the activation of PNPLA2; and ii) lipophagy (Fig. 5A).[57] The release of FAs from LDs in the course of nutrient deprivation is not only required for hepatocytes to survive in conditions of poor glucose availability, but is also instrumental for the release of FAs and ketone bodies into the bloodstream, serving as energy substrates in other organs.[58]

In line with a central role for lipophagy in hepatic lipid mobilisation, the relatively non-specific autophagy inhibitor 3-methyladenine (3-MA, which targets VPS34)[59] as well as silencing of the essential autophagy gene *ATG5* (Fig. 2) favoured TG and LD accumulation in hepatocytes.[23] Similar observations were made in the hepatic parenchyma of mice subjected to the liver-specific deletion of another autophagy-essential gene, *Atg7*.[60] Glycine N-methyltransferase (GNMT) stands out as a major regulator of hepatic lipophagy, reflecting its ability to limit methionine and *S*-adenosyl-*L*-methionine (SAM) levels and hence prevent the autophagy-inhibitory activation of protein phosphatase 2A (PP2A).[61] Thus, $Gnmt^{-/-}$ livers display impaired autophagic flux, which can be restored by pharmacological PP2A inhibitors.[61] Optimal hepatic lipophagy has also been connected to the Ca^{2+}-driven, forkhead box O1 (FOXO1)-dependent expression of lipase A, lysosomal acid type (LIPA),[62,63] to the activity of small GTPases involved in vesicular trafficking, including RAB7, RAB10 and RAB18,[64–67] as well as to the function of transcription factor EB (TFEB), a master transactivator of genes involved in autophagy and lysosomal biogenesis (Fig. 5B).[68]

Intriguingly, CMA has also been suggested to play a major role in this setting, reflecting the fact that both PLIN2 and PLIN3 possess a KFERQ motif and interact with HSPA8 during starvation.[69] In this case, however, the CMA-dependent degradation of PLIN2 and PLIN3 favours lipolysis, not

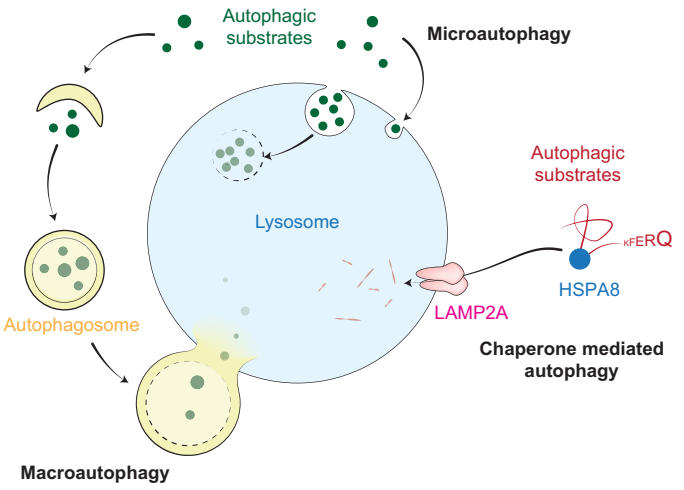

Fig. 1. Major variants of autophagy in mammalian cells. Mammalian cells exhibit 3 main forms of autophagy, which can be discriminated from each other based on i) substrate selection, and ii) mechanism of cargo delivery to lysosomes. Microautophagy is a LAMP2A-independent autophagic response that involves direct membrane invagination at the surface of acidic compartments (lysosomes or late endosomes). Chaperone-mediated autophagy depends on the LAMP2A-dependent translocation of KFERQ-bearing proteins chaperoned by HSPA8 into the lysosome. Macroautophagy relies on the uptake of substrates for degradation by autophagosomes, followed by fusion with lysosomes for cargo delivery. HSPA8, heat shock protein family A (Hsp70) member 8; LAMP2A, lysosomal associated membrane protein 2, splicing isoform A.

lipophagy.[69] Of note, optimal hepatic lipolysis relies on store-operated Ca^{2+} entry (SOCE) regulated by stromal interaction molecule 1 (STIM1) and ORAI calcium release-activated calcium modulator 1 (ORAI1).[70] Accordingly, defects in STIM1 and ORAI1 (be they imposed experimentally or the consequence of loss-of-function mutations) result in the accumulation of LDs in hepatocytes, a process that is accompanied by the compensatory activation of lipophagy.[70] Thus, the hepatic mobilisation of LDs is a highly coordinated process that involves both CMA and macroautophagy. Consistent with this notion, pharmacological activation of autophagy with caffeine drives hepatic lipid mobilisation in mice, effectively decreasing the size of the LD compartment coupled with FAO.[71] Consistent with this, caffeine alleviates hepatosteatosis in mice receiving a high-fat diet (HFD), an effect that has been largely attributed to autophagy activation.[71,72] That said, coffee contains autophagy inducers other than caffeine, meaning that even decaffeinated coffee stimulates an increase in autophagic flux in the liver,[73] in line with preclinical and epidemiological studies suggesting that coffee intake reduces the incidence of NASH irrespective of its caffeine content.[74–76]

Excessive LD accumulation in hepatocytes can drive a lipotoxic response involving lysosomal-dependent cell death.[77] Experimentally, this phenotype can be established by the hepatocyte-specific deletion of *Lrp1* (encoding LDL receptor related protein 1) combined with FA supplementation.[78,79] Thus, compared to their control counterparts, $Lrp1^{-/-}$ hepatocytes are more sensitive to

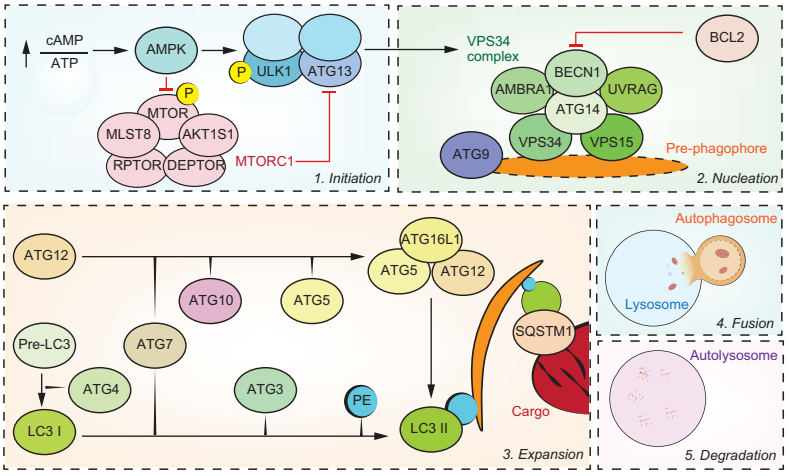

Fig. 2. Core regulation of macroautophagy in mammals. In mammalian cells, non-specific autophagic responses (such as those initiated by nutrient deprivation), can be subdivided into 5 major stages: i) initiation, ii) nucleation, iii) expansion, iv) fusion, and v) degradation. ***Initiation.*** This phase involves the biochemical detection of signs of bioenergetic stress as caused by dwindling nutrient levels, including (but not limited to) ATP depletion coupled to increased levels of cyclic AMP. High cAMP/ATP ratios activate AMPK and hence drive: i) the AMPK-dependent inactivating phosphorylation of MTORC1; and ii) the AMPK-dependent direct activating phosphorylation or indirect activating dephosphorylation (downstream of MTORC1 inhibition) of multiple components of the initiation apparatus, such as ATG13 and ULK1, and the nucleation machinery, including ATG14, AMBRA1, BECN1 and UVRAG. ***Nucleation.*** In this context, ULK1 acquires catalytic activity in the context of a supramolecular complex containing ATG13, ATG101, and RB1CC1 (best known as FIP200). ULK1-, AMPK- and MTORC1-related phosphorylation/dephosphorylation events drive the nucleation of autophagosome precursors (also known as phagophores) at the ER, downstream of PI3P synthesis by a multiprotein class III PI3K activity consisting of PIK3C3 (best known as VPS34), PI3KR4 (best known as VPS15), BECN1, AMBRA1 and/or UVRAG, linked to the recruitment of vesicular ATG9. The VPS34 complex is constitutively inhibited by BCL2, reflecting the ability of the latter to physically bind and inhibit BECN1. ***Elongation.*** Phagophore elongation is mediated by two ubiquitin-like conjugation systems. On the one hand, ATG7 and ATG10 sequentially drive the formation of ATG12-ATG5:ATG16L1 complexes. On the other hand, ATG4, ATG7 and ATG3 enable the cleavage of MAP1LC3B (best known as LC3) and other members of the LC3 family, including GABARAPL1, followed by conjugation to PE and recruitment to forming autophagosomes. LC3, GABARAPL1 and other LC3 homologues provide autophagosomes with the ability to bind LIR-containing autophagy receptors as well as proteins that mediate cargo selectivity, such as SQSTM1 (best known as p62). ***Fusion and degradation.*** Closing autophagosomes fuse with lysosomes to generate autolysosomes, culminating with luminal acidification and activation of hydrolases that catalyse cargo degradation.[2] AMBRA1, autophagy and beclin 1 regulator 1; AMPK, 5' AMP-activated protein kinase; ATG, autophagy related; BCL2, BCL2 apoptosis regulator; BECN1, beclin 1; GABARAPL1, GABA type A receptor associated protein like 1; MAP1LC3B, microtubule associated protein 1 light chain 3 beta; MTORC1, mechanistic target of rapamycin complex 1; P, inorganic phosphate; PE, phosphatidylethanolamine; PI3P, phosphatidylinositol 3-phosphate; PIK3C3, phosphatidylinositol 3-kinase catalytic subunit type 3; PI3KR4, phosphoinositide-3-kinase regulatory subunit 4; RB1CC1, RB1 inducible coiled-coil 1; SQSTM1, sequestome 1; ULK1, unc-51 like autophagy activating kinase 1; UVRAG, UV radiation resistance associated.

FA-driven cell death, a cytotoxic response that is accompanied by increased SQSTM1 levels but unaltered LC3 lipidation (Fig. 2), reflecting a blockade in autophagic flux.[78,79] Similar observations have been made in the liver of *Sod1*$^{-/-}$ mice, which lack a superoxide dismutase involved in antioxidant defences and accumulate LDs as a consequence of impaired lipophagy.[80,81]

Taken together, these observations highlight the key role of lipophagy and CMA in the regulation of hepatic lipid mobilisation.

Mitophagy

Mitochondrial damage initiates a cargo-specific form of autophagy that removes mitochondria with depolarised membranes.[82] Indeed, although the ubiquitin-proteasome system is partially involved in the removal of damaged mitochondria,[83] these large organelles require lysosomal degradation for their proper disposal.[82] In mitophagy, selectivity is achieved via 3 different classes of autophagy receptors: i) ubiquitinated receptors, which render the mitochondrial surface prone to recognition by SQSTM1; ii) non-ubiquitinated proteins of the outer mitochondrial membrane (OMM); and iii) lipid receptors.[84]

Arguably, the best characterised variant of mitophagy is driven by mitochondrial depolarisation, and is initiated by the consequent blockage of TOMM complex-dependent protein import.[85,86] This prevents the normal processing and intramitochondrial degradation of PTEN induced kinase 1 (PINK1), resulting in its accumulation at the OMM and autophosphorylation.[87,88] Upon phosphorylation of ubiquitin and other proteins including mitofusin 2 (MFN2),[89,90] PINK1 facilitates the recruitment of parkin RBR E3 ubiquitin protein ligase (PRKN), culminating in the ubiquitination of multiple OMM proteins that serve as receptors for SQSTM1.[87] As an alternative, the autophagic machinery can be recruited to damaged mitochondria by non-ubiquitinated, LIR-containing OMM proteins, including (but potentially not limited to) BH3-containing proteins like BCL2 interacting protein 3 (BNIP3), BCL2 interacting protein 3 like (BNIP3L), BCL2 like 13 (BCL2L13), and FUN14 domain containing 1 (FUNDC1).[91] Finally, the inner mitochondrial membrane (IMM) lipid cardiolipin can translocate to the OMM in the context of mitochondrial damage, where it can physically interact with nascent autophagosomes via lipidated LC3 (Fig. 6A).[92]

During fasting, hepatocytes mobilise lipids for the rest of the organism by lipolysis and lipophagy (as described above), a process that is maximised by the rapid autophagic degradation of otherwise FAO-competent mitochondria.[93,94] In this setting, the selective uptake of mitochondria by autophagosomes can account for up to 85% of autophagic events,[93,94] and can be initiated as early as 30 min after nutrient withdrawal.[93,95] Baseline mitophagy is also key for the maintenance of homeostasis in hepatocytes as it preserves the quality of the mitochondrial network, preventing the generation of reactive oxygen species (ROS) and futile ATP hydrolysis due to mitochondrial membrane depolarisation (Fig. 6B).[94,96,97]

In sum, mitophagy contributes to normal liver functions by preventing the accumulation of damaged mitochondria, which can have major cytotoxic effects,[98] and by tuning mitochondrial metabolism to serve organismal needs.

Pexophagy

Hepatic peroxisome biogenesis and degradation are highly dynamic processes, as suggested by the estimated half-life of rat liver peroxisomal proteins (1.5 days).[99] Pexophagy is particularly

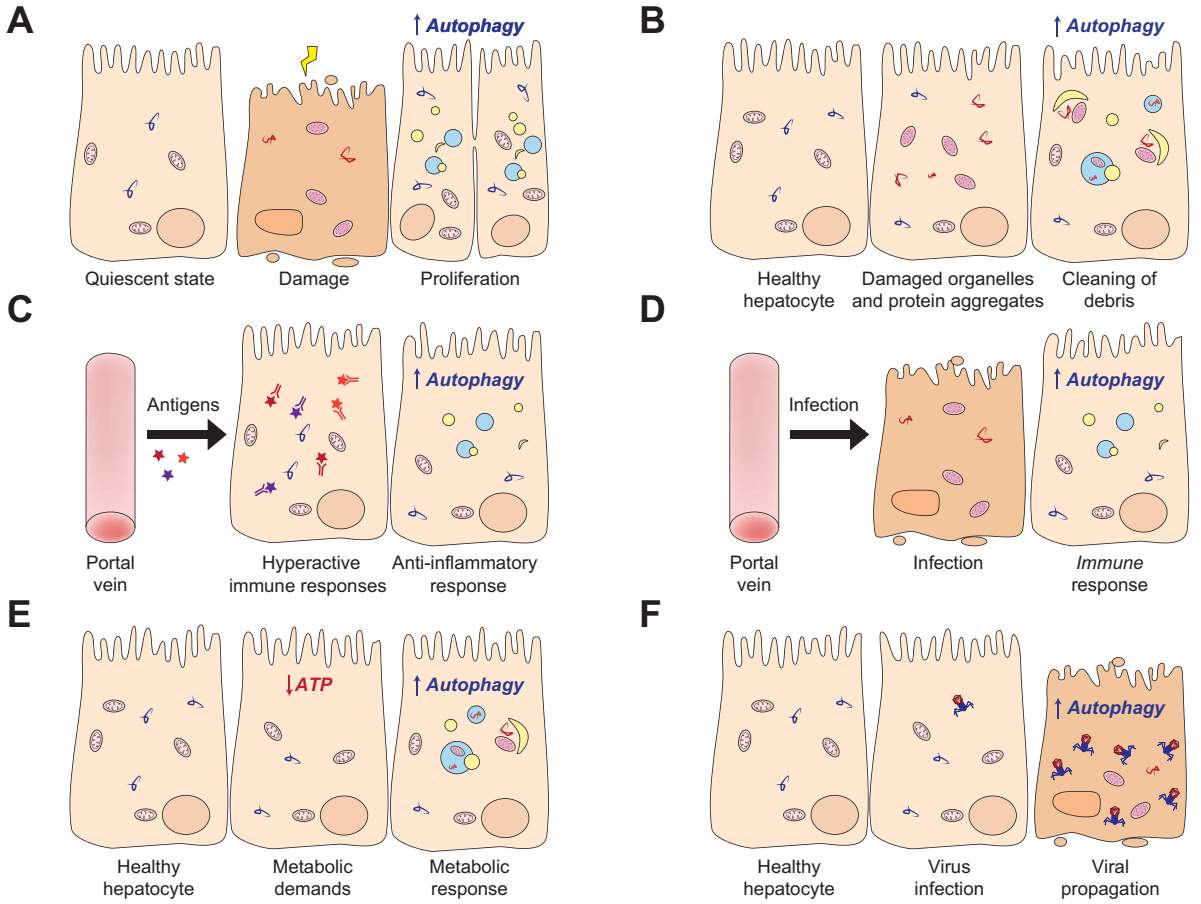

Fig. 3. Key functions of autophagy in liver physiology and pathology. (A) Autophagy occupies a central role in the preservation of liver homeostasis, because it supports the elevated regenerative potential of the organ, (B) clears hepatocytes of potentially cytotoxic byproducts of normal cellular metabolism, (C) limits potentially detrimental inflammatory responses to toxins and antigens from the portal circulation, (D) supports immune responses against invading pathogens, and (E) plays a critical role in the maintenance of local and systemic metabolism. (F) However, several hepatotropic viruses have acquired the ability to harness the autophagic machinery for their own benefit.

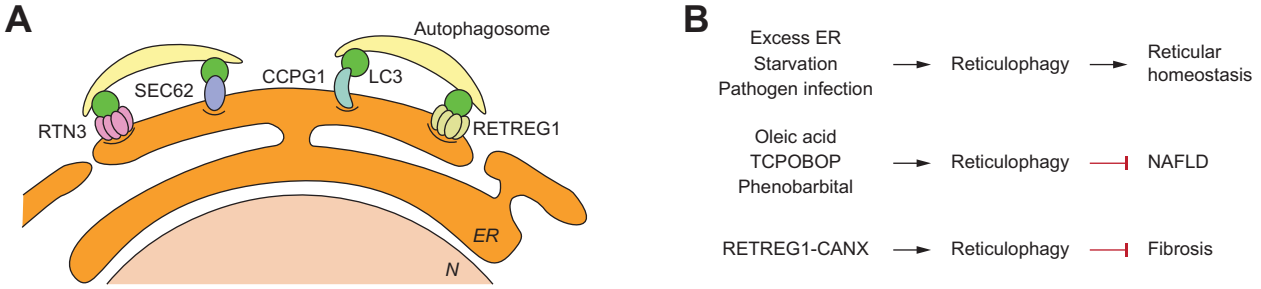

Fig. 4. Reticulophagy in the liver. (A) Reticulophagy is enabled by multiple receptors that directly interact with LC3 on forming autophagosomes, which include RETREG1, RTN3, SEC62 and CCPG1. (B) Reticulophagy ensures the preservation of liver homeostasis by preserving the size and functionality of the ER in the recovery from starvation or viral infection. Moreover, reticulophagy inhibits NAFLD induced by oleic acid, phenobarbital and TCPOBOP. Finally, the reticulophagy receptor RETREG1 negatively regulates aberrant collagen secretion (and hence fibrosis) upon functional interactions with the ER chaperone CANX. CANX, calnexin; CCPG1, cell cycle progression 1; ER, endoplasmic reticulum; N, nucleus; NAFLD, non-alcoholic fatty liver disease; RETREG1, reticulophagy regulator 1; RTN3, reticulon 3; SEC62, SEC62 homolog, preprotein translocation factor; TCPOBOP, 1,4-bis [2-(3,5-dichloropyridyloxy)] benzene.

sensitive to the accumulation of ROS, which have been reported to initiate the autophagic disposal of peroxisomes upon activation of an extranuclear pool of ATM serine/threonine kinase (ATM).[100] In this setting, ATM phosphorylates peroxisomal biogenesis factor 5 (PEX5), favouring PEX5 ubiquitination by PEX2 and leading to the recruitment of autophagosomes via SQSTM1 or NBR1 (Fig. 7A).[100–102] Of note, PEX5 also appears to inhibit autophagy by affecting the phosphorylation status of MTORC1 (Fig. 2) and the activation of TFEB (Fig. 7A).[103] The size of the hepatic peroxisomal compartment is also regulated by oxygen availability. In particular, endothelial PAS domain

Key points

Stress-induced autophagic responses prevent the accumulation of potentially pathogenic material in hepatocytes.

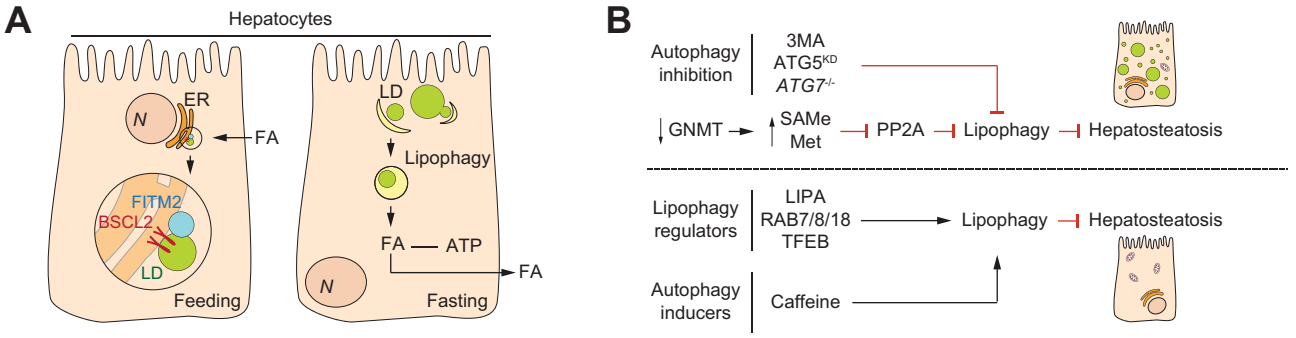

Fig. 5. Lipophagy in hepatic health and disease. (A) Fed hepatocytes accumulate FAs in LDs that are stabilised in association with the ER by proteins including BSCL2 and FITM2. During fasting, LDs are mobilised by lipophagy to support both hepatic and systemic metabolism. (B) GNMT inhibits hepatosteatosis, as it promotes lipophagy by limiting methionine and SAM levels, hence preventing the autophagy-inhibitory activation of PP2A. Further corroborating a beneficial role of lipophagy, autophagy inhibition by 3-MA administration, as well as by the downregulation of *ATG5* or *ATG7*, promotes hepatosteatosis in experimental settings. 3-MA, 3-methyladenine; ATG, autophagy related; BSCL2, BSCL2 lipid droplet biogenesis associated, seipin; ER, endoplasmic reticulum; FA, fatty acid; FITM2, fat storage inducing transmembrane protein 2; GNMT, glycine N-methyltransferase; N, nucleus; PP2A, protein phosphatase 2A; SAM, *S*-adenosyl-*L*-methionine; TFEB, transcription factor EB.

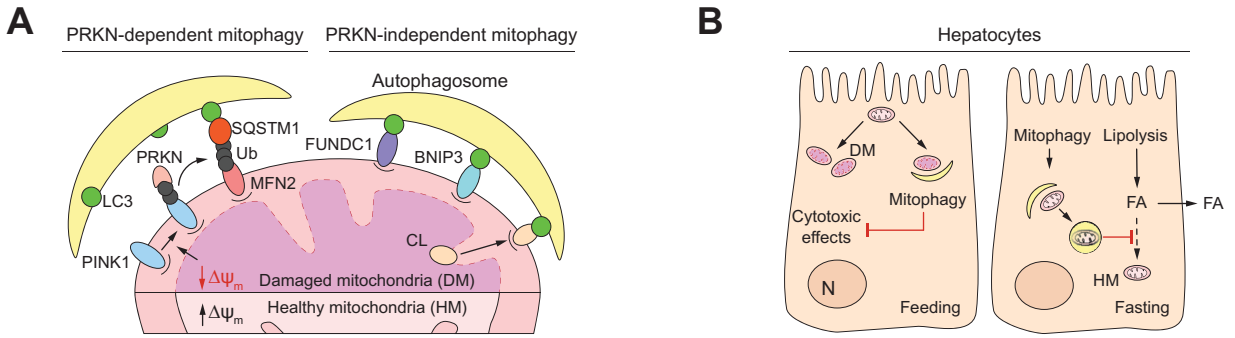

Fig. 6. Role of mitophagy in the preservation of liver homeostasis. (A) DM exhibit reduced mitochondrial transmembrane potential ($\Delta\psi_m$), enabling the accumulation of PINK1 on their surface and PINK1 autophosphorylation, culminating with the recruitment of PRKN. PRKN catalyses the ubiquitination of multiple mitochondrial proteins including MFN2, which serve as receptors that recognise growing autophagosomes via SQSTM1 and LC3. Alternatively, DM can be recognised by LC3 via BNIP3, FUNDC1 and the inner mitochondrial membrane-restricted lipid CL. (B) In fed conditions, mitophagy largely serves as a quality control mechanism for DM. During fasting, however, mitophagy also degrades FAO-competent HM to boost the release of FAs mobilised by lipolysis and lipophagy. BNIP3, BCL2 interacting protein 3; CL, cardiolipin; DM, damaged mitochondria; FAO, fatty acid oxidation; FA, fatty acid; FUNDC1, FUN14 domain containing 1; HM, healthy mitochondria; MFN2, mitofusin 2; PINK1, PTEN induced kinase 1; PRKN, parkin RBR E3 ubiquitin protein ligase; SQSTM1, sequestosome 1.

protein 1 (EPAS1, an oxygen-sensing protein best known as HIF-2α) appears to drive pexophagy in response to low oxygen tension, resulting in alterations of lipid metabolism reminiscent of peroxisomal disorders.[104] These results identify an unsuspected link between oxygen availability and peroxisomal functions.

Peroxisomes are critical organelles for hepatic lipid metabolism and bile acid synthesis, pointing to a major role for pexophagy in the maintenance of normal liver functions. Consistent with this notion, studies in mice bearing *Atg7*$^{-/-}$ hepatocytes revealed that 70–80% of hepatic peroxisomes are degraded by autophagy. [105,106] That said, an in-depth characterisation of pexophagy in the liver is lacking.

Glycophagy

Hepatic, cardiac and muscular glycogen is largely degraded by glycogenolysis, a catabolic pathway initiated upon the activation of one of various glycogen phosphorylase variants.[107] Alternatively, glucose can be mobilised following the uptake of glycogen granules by autophagosomes and activation of lysosomal glucosidase alpha, acid (GAA).[108] Glycophagy relies on the LIR-containing protein starch binding domain 1 (STBD1),[109] and can be selectively activated by hypoxia and phlorizin, an inhibitor of the sodium-glucose co-transporters solute carrier family 5 member 1 (SLC5A1) and SLC5A2 (Fig. 7B).[110,111]

Glycophagy plays an important role in hepatic glucose homeostasis. Consistent with this notion, *Gaa*$^{-/-}$*Stbd1*$^{-/-}$ mice fail to exhibit alterations of glycogen metabolism in the heart and skeletal muscle, yet display a 73% reduction in lysosomal glycogen in the liver compared to *Gaa*$^{-/-}$ mice.[112] These findings highlight the critical importance of STBD1 for hepatic glycophagy. Of note, the hepatic levels of multiple autophagic mediators including BECN1, SQSTM1 and GABARAPL1 do not differ between starved *Gaa*$^{-/-}$*Stbd1*$^{-/-}$ and *Gaa*$^{-/-}$

mice,[109] suggesting that the absence of STBD1 imposes a selective defect in glycophagy rather than affecting autophagic responses at large.

In summary, glycophagy stands out as a major regulator of hepatic (and by extension systemic) glucose metabolism, potentially representing a target for the development of novel therapeutic interventions.

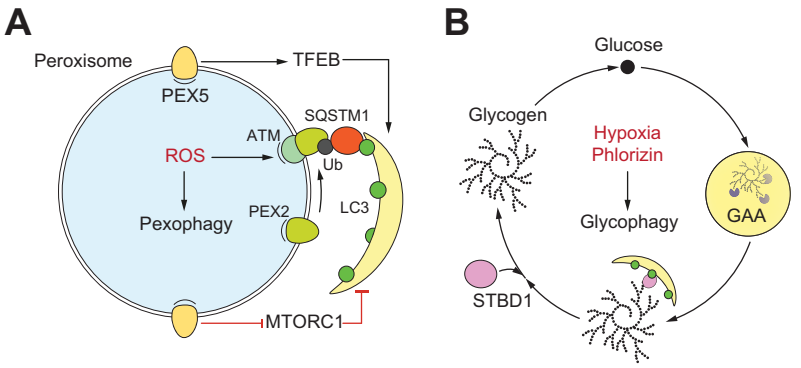

Fig. 7. Pexophagy and glycophagy in the liver. (A) ROS are major drivers of pexophagy, downstream of ATM activation and consequent phosphorylation of PEX5. Phosphorylated PEX5 is a target for PEX2-dependent ubiquitination, hence serving as a receptor for recognition by growing autophagosomes via SQSTM1 and LC3. PEX5 also favours pexophagy by limiting autophagy-inhibitory activity of MTORC1. (B) Hepatic glycophagy, which plays a critical role in the regulation of systemic glucose metabolism, relies on STBD1, which operates as a receptor for LC3 and GAA, facilitating glycogen degradation in lysosomes. Both hypoxia and inhibition of glucose uptake by phlorizin drive glycophagy in hepatocytes. ATM, ATM serine/threonine kinase; GAA, glucosidase alpha, acid; MTORC1, mechanistic target of rapamycin complex 1; PEX5, peroxisomal biogenesis factor 5; ROS, reactive oxygen species; SQSTM1, sequestosome 1; STBD1, starch binding domain 1.

Autophagy in liver disease

Accumulation disorders

Serpin family A member 1 (SERPINA1, best known as alpha-1 antitrypsin or AAT) is an acute-phase glycoprotein with elastase-inhibitory activity, which is predominantly synthesized by hepatocytes and reaches a serum concentration of 85–250 mg/dl in normal healthy individuals.[113] *SERPINA1* is a highly polymorphic gene, and several mutants of pathogenic significance exist, including the common Z, Si, I, S, Br and K mutants.[113] The Z mutation results from a single G->A transition in codon 342, generating a distorted AAT variant (AAT^{E342K}) that forms aggregates retained within the ER of hepatocytes.[113] Accordingly, defects in ER-associated degradation (ERAD) have been linked to increased disease severity in rodent models of AAT deficiency.[114,115] From a clinical perspective, AAT deficiency involves pulmonary and hepatic manifestations, the latter of which include hepatitis, cirrhosis, HCC, and liver failure.[116] The cellular events underlying liver disease in this scenario encompass inflammation, steatosis, loss of hepatocytes and fibrotic alterations.[114,116]

Expression of AAT^{E342K} in *Atg5*$^{-/-}$ cell lines results in exacerbated accumulation of AAT aggregates pointing to a major role for autophagy (over proteasomal degradation) in the control of aberrant AAT deposits.[117] Consistent with this notion, the transgene-enforced, hepatocyte-specific expression of AAT^{E342K} in wild-type mice results in the activation of robust autophagic responses in the liver.[118,119] Moreover, the hepatocyte-specific overexpression of TFEB results in limited AAT accumulation, reduced apoptosis and suppressed fibrosis in mice bearing a pathogenic *SERPINA1* gene.[120] Along similar lines, pharmacological inducers of autophagy including the MTORC1 inhibitor rapamycin and carbamazepine (whose mechanism of action is debated)[59] robustly reduced liver fibrosis in rodent models of AAT deficiency,[121] providing the basis for ongoing phase II clinical testing.[20]

Wilson disease is an inherited disorder of copper metabolism caused by a mutation in *ATP7B* (ATPase copper transporting beta) that promotes copper accumulation in multiple organs, including the liver, kidney and eyes.[122] *Atp7b*$^{-/-}$ cells treated with copper upregulate a panel of 103 genes linked to autophagy and lysosomal functions, and electron microscopy studies confirmed the expansion of the autophagosomal compartment in this setting.[123] Consistent with this, hepatocytes from both patients with Wilson disease and *Atp7b*$^{-/-}$ mice display an increased number of autophagosomes, reflecting the activation of an autophagic response that prevents cell death driven by copper accumulation.[123,124] Indeed, inhibition of autophagy with spautin 1 accelerated the demise of hepatocytes succumbing to copper accumulation, whereas starvation as well TFEB overexpression (both of which boost autophagy) mediate cytoprotective effects.[123] Thus, autophagy stands out as a major mechanism for hepatocytes to preserve homeostasis despite copper accumulation. However, it remains to be investigated whether autophagy inducers such as carbamazepine or rapamycin can be conveniently employed to treat Wilson disease.

Glycogen storage disease type 1a (GSD1a) is yet another hepatic disorder associated with decreased autophagic flux.[125] GSD1a is an inherited metabolic disorder impacting glycogen storage as a consequence of defects in the enzymatic complex that converts glucose-6-phosphate into glucose, *i.e.*, glucose-6-phosphatase α.[125] These alterations impair intracellular glucose homeostasis, ultimately leading to glycogen and lipid accumulation in hepatocytes, with clinical manifestations ranging from liver failure to hepatomegaly with a high risk for malignant transformation.[125] Thus, GSD1a is accompanied by alterations in AMPK and MTORC1 signalling (Fig. 2), as well as by the downregulation of several core components of the autophagy machinery, pointing to broad defects in transcriptional programmes for autophagy regulation.[126] Consistent with this notion, GSD1a has been associated with defective sirtuin 1 (SIRT1) signalling,[127] which

Key points

Autophagy-based pharmacological interventions for alpha-1-antitrypsin deficiency have reached phase II clinical testing.

compromises TFEB activity. Of note, activating autophagy by pharmacological or genetic means limits the accumulation of glycogen and lipids in both cellular and animal models of GSD1a.[126] However, no therapeutic paradigms based on this approach have been developed so far.

Non-alcoholic fatty liver disease

The current pandemic of obesity and diabetes has led to a considerable increase in the incidence of NAFLD, with clinical manifestations ranging from simple steatosis to NASH and elevated potential for malignant transformation.[49] NAFLD is characterised by lipid accumulation within the ER of hepatocytes,[128] which leads to cell death and acute hepatic injury with a high potential for conversion into chronic liver disease.[49] Accumulating evidence indicates that autophagy (most likely lipophagy) robustly counteract NAFLD and may be disabled, at least in part, during NAFLD pathogenesis.

NAFLD is often associated with the presence of "megamitochondria",[129] most likely reflecting impaired mitophagy.[130] Moreover, multiple ATG proteins and TFEB are downregulated in the hepatocytes of patients with NASH, as well as the hepatocytes of mice receiving an HFD or a methionine/choline-deficient diet.[23,68] Furthermore, the hepatocyte-specific deletion of genes encoding essential autophagy regulators including *Atg7*, *Atg14*, or *Tfeb*, as well as the endothelial or myeloid cell-specific deletion of *Atg5* exacerbate the sensitivity of mice to develop NAFLD accompanied with elevated production of pro-inflammatory cytokines in response to a HFD.[131–133] These observations suggest that autophagy counteracts NAFLD not only by preserving the homeostasis of hepatocytes, but also by limiting the inflammatory potential of liver-infiltrating immune cells.

Key points

Multiple cargo-selective instances of autophagy play a critical role in the preservation of liver homeostasis.

Consistent with the above, transgene-driven TFEB overexpression in hepatocytes reduces disease severity in mice exposed to a HFD.[68] Moreover, multiple pharmacological activators of autophagy have been shown to mediate beneficial effects in cellular or animal models of NAFLD. These include (but may not be limited to): caloric restriction,[134] exercise,[135] trehalose,[136,137] as well as ezetimibe, a lipid-lowering drug with AMPK-activatory potential that is currently being evaluated in clinical trials for its therapeutic activity against NASH.[138,139] It will be interesting to see whether ezetimibe will ultimately be approved for this indication.

Fibrosis

Liver fibrosis involves the excessive accumulation of extracellular matrix (ECM) components including different types of collagen.[140] Hepatic stellate cells (HSCs) are the main source of collage in the liver, and *de facto* underlie fibrogenesis in the context of chronic liver injury.[141] In this setting, HSCs trans-differentiate toward myofibroblast-like cells that migrate to areas of tissue injury and secrete i) ECM components including type I collagen (in support of wound healing), and ii) fibrogenic cytokines such as transforming growth factor beta 1 (TGFB1).[141] Thus, the continued demise of hepatocytes driven by chronic hepatic injury fosters a non-resolving and progressive wound healing response culminating in extensive fibrosis.[142] Autophagy may have a dual impact on hepatic fibrogenesis (Fig. 8).

On the one hand, proficient autophagy appears to be required for the trans-differentiation of HSCs, at least in part owing to its involvement in lipolysis (HSCs lose LDs in the process).[143,144] Consistent with this, HSCs with depleted ATG2A fail to undergo spontaneous trans-differentiation in cell culture.[144] Moreover, the HSC-specific deletion of *Atg5* or *Atg7* renders mice less susceptible to hepatic fibrosis induced by carbon tetrachloride.[145] Apparently at odds with this notion, the autophagic adapter SQSTM1 is downregulated in trans-differentiating HSCs and its ablation accelerates (rather than decelerates) fibrogenesis.[146] However, such activity does not involve autophagic responses, but rather reflects the ability of SQSTM1 to favour the dimerisation-dependent activation of vitamin D receptor and retinoid X receptor.[146]

On the other hand, autophagy in hepatocytes, liver sinusoidal endothelial cells (LSECs) and macrophages mediates cytoprotective, anti-

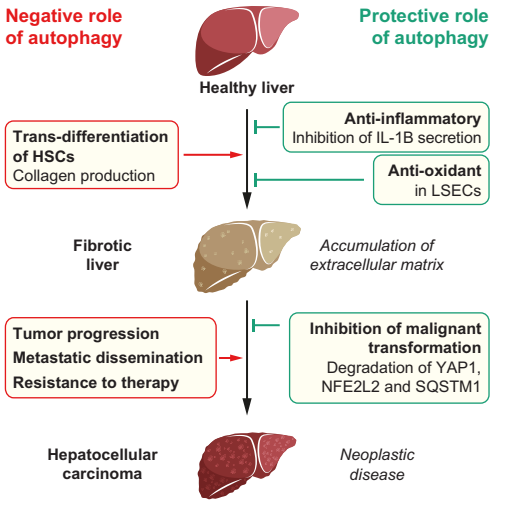

Fig. 8. Dual impact of autophagy on liver fibrosis and hepatocellular carcinoma. On the one hand, autophagy limits hepatic fibrogenesis and malignant transformation by mediating antioxidant and anti-inflammatory functions in macrophages and LSECs, as well as by degrading potentially oncogenic proteins. On the other hand, autophagy supports fibrogenesis by favouring the trans-differentiation of HSCs, and it promotes the progression, dissemination and resistance to therapy of established hepatocellular carcinomas. HSC, hepatic stellate cell; IL1B, interleukin 1 beta; LSEC, liver sinusoidal endothelial cell; NFE2L2, nuclear factor, erythroid 2 like 2; SQSTM1, sequestosome 1; YAP1, Yes associated protein 1.

inflammatory and antioxidant effects that limit hepatic injury and hence suppress fibrosis initiation.[131,147–149] In line with this notion, the hepatocyte-or macrophage-specific deletion of *Atg5*,[147,148] as well as the LSEC-specific deletion of *Atg7*,[131] accelerates disease progression in various rodent models of fibrosis. Of note, in hepatocytes and LSECs, autophagy mostly operates as an antioxidant and cytoprotective mechanism that preserves cellular homeostasis,[131,147,149] whereas in macrophages and Kupffer cells the anti-fibrotic activity of autophagy stems from the inhibition of inflammasome activation and consequent secretion of the pro-inflammatory and fibrogenic cytokine interleukin 1 beta (IL1B).[150,151] Taken together, these observations suggest that autophagy has a context-dependent impact on hepatic fibrosis, which complicates the development of therapeutic paradigms based on autophagy modulation.

Hepatocellular carcinoma

HCC is the most common liver neoplasm, accounting for approximately 90% of all hepatic malignancies.[152] In this setting, malignant transformation can originate from a panel of diverse genetic alterations affecting key signal transduction cascades such as the PI3K → AKT1 → MTORC1, RAS → RAF → MAPK, and WNT → β catenin pathways.[152] Reflecting its key role in the preservation of genetic homeostasis,[153] proficient autophagic responses in hepatocytes limit malignant transformation (Fig. 8).[22] Consistent with this notion, mice bearing a mosaic deletion of *Atg5* or a hepatocyte-specific deletion of *Atg7* spontaneously develop multiple hepatic adenomas as they age.[154] Moreover, autophagy appears to limit malignant transformation in the liver by degrading Yes associated protein 1 (YAP1),[155] a key transducer of Hippo signalling with a major role in hepatic oncogenesis.[156] Apparently at odds with this, SQSTM1 appears to promote, rather than inhibit, hepatic carcinogenesis.[157] The underlying mechanism, however, is unrelated to the activation of autophagy,[28] and rather reflects the ability of SQSTM1 to favour nuclear factor, erythroid 2 like 2 (NFE2L2, best known as NRF2) activation.[158–160]

Conversely, in established HCCs, autophagy supports tumour progression, metastatic dissemination and resistance to therapy.[22,161] Thus, various lysosomal inhibitors including chloroquine and hydroxychloroquine mediate therapeutic activity as standalone agents or combined with chemotherapy, radiation therapy or targeted anticancer agents in a variety of rodent HCC models.[162–164] These findings point to the idea that autophagy could constitute a potential target for the development of novel therapeutic regimens against HCC (Fig. 8).[20] However, most of the data in support of this notion have been obtained in immunodeficient rodents grafted with human cancer cells, and hence fail to consider the key role of autophagy in anticancer immunity.[165,166] Moreover, currently available pharmacological modulators of autophagy are flawed by specificity issues.[59] Thus, additional investigation is required before autophagy modulators can be translated into clinical agents against HCC.

Hyperammonaemia

The liver is a major site for the detoxification of nitrogen-containing products of protein catabolism. Thus, hepatocellular dysfunction as well as genetic defects in enzymes of the urea cycle can lead to systemic hyperammonaemia and hepatic encephalopathy, a condition that confers a high risk of coma and death if untreated.[167] Ammonia triggers a rapid autophagic response[168] that preferentially targets mitochondria in a manner that does not depend on unc-51 like autophagy activating kinase 1 (ULK1),[169] but involves the deacetylase sirtuin 5 (SIRT5),[170] as well as the inhibition of MTORC1.[171] Supporting a key role for autophagy in the clearance of ammonia, mice with a liver-specific deletion of *Atg7* or loss of TFEB function exhibit poor ammonia detoxification.[171] Conversely, autophagy activation in the liver, as induced by transgene-driven TFEB overexpression or rapamycin administration, limits the accumulation of ammonia in the circulation of mice.[171] Thus, autophagy activation stands out as a promising therapeutic approach for both inherited and acquired hyperammonaemia.

Viral infections

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are highly prevalent hepatotropic viruses that can establish chronic infections culminating in cirrhosis and HCC.[172,173] Like many other viruses, both HBV and HCV potently activate xenophagy as a first line of hepatocellular defence.[26] HBV and HCV-driven autophagy is largely initiated by the ER stress response that results from the uncontrolled translation of viral proteins.[174–176] Moreover, HBV X protein (HBx) favours autophagy by promoting *BECN1* transactivation,[177] while multiple proteins encoded in the HCV genome, encompassing p7, NS3/4A and NS4B, drive autophagy by directly or indirectly interacting with core components of the autophagy machinery including BECN1, VPS34, ATG5 and LC3.[178–180] Both HBV and HCV, however, have evolved strategies to harness autophagy for viral replication and dissemination.[28] In line with this notion, autophagy inhibition by pharmacological or genetic intervention has been shown to limit viral yield in a variety of experimental settings.[181–183] In summary, autophagy stands out as a promising target for HBV and HCV infections.

Conclusions and perspectives

The impact of autophagy in liver pathophysiology has just begun to emerge, implying that additional investigations are required to translate promising preclinical data on autophagy modulation into therapeutic strategies that can be used in the clinic.[20] To this aim, it will be extremely important to dissect the complexity of the autophagic network in specific disease models, with particular emphasis on the potentially antagonistic effects of autophagic responses in the different cell types of the hepatic microecosystem. The development of highly specific autophagy modulators and molecular platforms for targeted delivery also stand out as a major challenge for the clinical translation of this paradigm.[20] Despite these and other obstacles, the therapeutic potential of autophagy modulators for the treatment of multiple hepatic disorders remains high, largely reflecting the central role of coordinated autophagic responses in the preservation of liver homeostasis. Our hope is that carbamazepine and ezetimibe will become the first of a long list of autophagy modulators to be used for the treatment of patients with liver disease, thus inaugurating a clinical success story.

Abbreviation

3-MA, 3-methyladenine; CMA, chaperone-mediated autophagy; ECM, extracellular matrix; ER, endoplasmic reticulum; ERAD, endoplasmic reticulum associated degradation; FA, fatty acid; FAO, fatty acid oxidation; GSD1a, glycogen storage disease type 1a; HCC, hepatocellular carcinoma; HFD, high-fat diet; HSC, hepatic stellate cell; IMM, inner mitochondrial membrane; LD, lipid droplet; LIR, LC3-interacting region; LSEC, liver sinusoid endothelial cell; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OMM, outer mitochondrial membrane; PKA, protein kinase A; RHD, reticulon homology domain; ROS, reactive oxygen species; SAM, *S*-adenosyl-*L*-methionine; SOCE, store-operated Ca^{2+} entry; TCPOBOP, 1,4-bis [2-(3,5-dichloropyridyloxy)] benzene.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2019.08.026.

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