Review

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Autophagy in liver diseases

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Autophagy, or cellular self-digestion, is a cellular pathway crucial for development, differentiation, survival, and homeostasis. Its implication in human diseases has been highlighted during the last decade. Recent data show that autophagy is involved in major fields of hepatology. In liver ischemia reperfusion injury, autophagy mainly has a prosurvival activity allowing the cell for coping with nutrient starvation and anoxia. During hepatitis B or C infection, autophagy is also increased but subverted by viruses for their own benefit. In hepatocellular carcinoma, the autophagy level is decreased. In this context, autophagy has an anti-tumor role and therapeutic strategies increasing autophagy, as rapamycin, have a beneficial effect in patients. Moreover, in hepatocellular carcinoma, Beclin-1 level, an autophagy protein, has a prognostic significance. In α-1-antitrypsin deficiency, the aggregation-prone ATZ protein accumulates in the endoplasmic reticulum. This activates the autophagic response which aims at degrading mutant ATZ. Some FDA-approved drugs which enhance autophagy and the disposal of aggregation-prone proteins may be useful in α-1-antitrypsin deficiency. Following alcohol consumption, autophagy is decreased in liver cells, likely due to a decrease in intracellular 5′-AMP-activated protein kinase (AMPk) and due to an alteration in vesicle transport in hepatocytes. This decrease in autophagy contributes to the formation of Mallory-Denk bodies and to liver cell death. Hepatic autophagy is defective in the liver in obesity and its upregulation improves insulin sensitivity.

Definition and molecular machinery of autophagy

Autophagy (Greek for "self eating") is a general term for processes by which cytoplasmic materials, including organelles, reach lysosomes for degradation. Three types of autophagy (macroautophagy, microautophagy, and chaperone-mediated autophagy) have been identified and they differ with respect to their physiological functions and mode of cargo delivery to the lysosome.

This review will focus on macroautophagy (hereafter referred to as autophagy), the major regulated catabolic mechanism that eukaryotic cells use to degrade long-lived proteins and organelles [1]. This pathway is conserved from yeast to mammals (Fig. 1). Upon induction, a small vesicular sac called the isolation membrane or phagophore elongates and subsequently encloses a portion of cytoplasm, which results in the formation of a double-membraned structure, the autophagosome (Fig. 2). Recent data show that the outer membrane of mitochondria participates in autophagosome biogenesis [2]. Then, the outer membrane of the autophagosome fuses with a lysosome (to form an autolysosome), leading to the degradation of the enclosed materials together with the inner autophagosomal membrane. Amino acids and other small molecules that are generated by autophagic degradation are delivered back to the cytoplasm for recycling or energy production. Autophagy occurs at low basal levels in virtually all cells to perform homeostatic functions such as protein and organelle turnover. It is rapidly upregulated through the inhibition of mammalian target of rapamycin (mTOR) when cells need to generate intracellular nutrients and energy, for example, during starvation, growth factor withdrawal, or high bioenergetic demands [1,3]. Subsequently, prolonged starvation reactivates mTOR signaling that both attenuates autophagy and generates proto-lysosomal tubules and vesicles that extrude from autolysosomes and ultimately mature into functional lysosomes, thereby restoring the full complement of lysosomes in the cell [4].

The execution of autophagy involves a set of evolutionarily conserved gene products (initially identified in yeast) known as the Atg proteins that are required for the formation of the isolation membrane and the autophagosome. The process of autophagosome formation involves three major steps described in Fig. 3: initiation with the uncoordinated 51-like kinase 1 (ULK1) complex, nucleation with the Beclin-1-class III phosphatidylinositol 3-kinase (PI3K) complex and elongation of the isolation membrane with a key role of microtubule-associated protein light chain 3 (LC3) lipidation [3,5,6].

Information on the methods for monitoring autophagy can be found elsewhere [7].

Within the past decade, numerous new techniques have been developed, allowing to understand the role of autophagy both in

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Abbreviations: α1AT, alpha-1-antitrypsin; AMPk, 5′-AMP-activated protein Kinase; ATZ, α1AT mutant Z gene; *Atg*, autophagy genes; Bcl, B-cell leukemia/lymphoma; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; LC3, microtubule-associated protein light chain 3; mTOR, mammalian target of rapamycin; Nrf2, nuclear factor erythroid 2-related factor 2; PI3K, phosphatidylinositol 3-kinase; ULK1, uncoordinated 51-like kinase 1.

normal and in pathological conditions [1,5]. The physiological role of autophagy in nutrient and energy metabolism in hepatocytes has been reviewed elsewhere [8]. As detailed in Box 1, this process is particularly crucial in newborn mammals survival [9,10]. Several recent studies have demonstrated the implication of autophagy in major fields of hepatology. This review aims at providing an overview of currently available knowledge on autophagy and liver diseases.

At birth the trans-placental nutrient supply is suddenly interrupted, and neonates face severe starvation until supply can be restored through milk nutrients. Autophagy is immediately upregulated in various tissues, including the liver, after birth and is maintained at high levels for 12 h before returning to basal levels within 2 days [9]. The crucial role of autophagy in neonates is illustrated by the shorter survival time of starved mice deficient for Atg5, which is essential for autophagosome formation, than that of wild-type mice. This survival can be prolonged by forced milk feeding [9]. The amino acids provided by the autophagic degradation of 'self' proteins must contribute to the beneficial role of autophagy for survival during neonatal starvation, since Atg5-deficient neonates exhibit reduced amino acid concentrations in plasma and tissues [9]. Liver heart and muscle glycogen autophagy also occurs in neonatal period and contributes to supplying glucose. The hydrolytic degradation of glycogen in the autophagic vacuoles assists the phosphorolytic degradation of polysaccharide in the cytoplasm to combat hypoglycaemia [10]. Glycogen autophagy is probably required because glucose 6-phosphatase may not be sufficiently active at birth [10].

Autophagy and liver ischemia reperfusion and liver surgery

Liver ischemia/reperfusion injury occurs during liver transplantation, trauma, shock, and elective liver resection. During this process, hypoxic organ damage is accentuated following the return of blood flow and oxygen delivery. The pathophysiology includes direct cellular damage as a result of the ischemic insult and delayed dysfunction and injury resulting from inflammatory pathway activation [11].

As the first known role of autophagy is its action during nutrient starvation, studies on autophagy and liver diseases have rapidly focussed on liver ischemia/reperfusion [12–14]. Two types of experimental protocols have been performed reflecting two different clinical situations: (a) hepatic ischemia induced by occlusion of the portal triad for a duration ranging from 30 to 90 min, followed or not by a reperfusion period ranging from 30 min to 3 h [15–17]; and (b) liver transplantation with 24 h cold ischemia followed by reperfusion [18–20].

Despite very similar protocols, results of studies performed in mice, assessing the impact on autophagy of portal triad occlusion, are highly controversial, some reporting an increase and others a decrease in autophagy protein level [15–17]. Nevertheless, the unique study performed in patients provides interesting information [21]. In this study, 61 patients who underwent liver surgery with total vascular occlusion and preservation of the caval flow after receiving several courses of chemotherapy were studied. For all patients, two liver biopsies were taken, one before the prolonged ischemia required by liver resection and another after the liver reperfusion (median: 88 min; range: 57–125 min), before closure of the abdomen. A unique vascular occlusion had almost no effect on autophagy, since the LC3-II rarely increased and the number of cells containing autophagic vacuoles remained stable. However, a subgroup of patients underwent ischemic preconditioning consisting of 10 min of portal triad clamping followed by 10 min of reperfusion before the prolonged ischemia required by liver resection. In these patients, a frank increase in liver cell autophagy was observed. Even if this study failed to demonstrate a beneficial effect of such ischemic preconditioning in postresection liver injury tests or measure of patient morbidity [21], previous studies including specific groups of patients, such as young patients and patients with liver steatosis or cirrhosis obtained a clinical improvement [22,23]. This suggests that in this context, autophagy enhancement could allow for decreasing liver cell death (Table 1).

Studies on liver transplantation had also apparently contradictory results. The explanation for such discrepancies must be the solution for cold preservation used. Indeed, a decrease in autophagy was observed in a study using a histidine–tryptophan–ketoglutarate cold-storage solution for 24 h cold preservation [20], while the contrary was reported by authors using the University of Wisconsin (UW) cold-storage solution [18,19]. Electron microscopy analysis of surgical biopsies performed after revascularisation of human liver grafts conserved in UW solution also disclosed the existence of numerous autophagic vacuoles (personal unpublished data). Importantly, UW cold storage solution does not contain amino acids. It is well demonstrated that amino acid depletion rapidly induces autophagy [12] and that anoxia decreases autophagy protein level [16]. This induction of autophagy due to the absence of amino acids, may explain not only the apparent discrepancy between these studies but also the protection of the liver obtained with preservation solution such as the UW solution [11]. Indeed, anoxia/reoxygenation induces mitochondrial dysfunction. Due to the decrease in autophagy proteins induced by anoxia/reoxygenation, autophagy fails to remove dysfunctional mitochondria, so that the mitochondria laden with reactive oxygen species and calcium undergo the mitochondrial permeability transition, which in turn leads to uncoupling of oxidative phosphorylation, energetic failure, ATP depletion, and ultimately cell death. In case of associated nutrient depletion, autophagy is enhanced and facilitates autophagy of damaged mitochondria, leading to cell survival [16].

This hypothesis is supported by the beneficial effect on liver tolerance to ischemia–reperfusion of several strategies aiming at increasing autophagy in murine models: stimulation of PPARγ with rosiglitazone, infusion of nontoxic doses of cisplatin and liver graft hypothermic reconditioning by insufflation of gaseous oxygen via the caval vein during the last 90 min of preservation, [15,17,20]. It is striking to notice that the two studies suggesting that inhibiting autophagy could ameliorate liver tolerance to ischemia [14,18] used non specific inhibitors of autophagy, such as general lysosome protease inhibitors (pepstatin and leupeptin) or PI3K inhibitors (wortmannin or LY294002), known to also have autophagy independent activities.

Currently available studies provide additional information. First, autophagic activity declines in aged organisms which

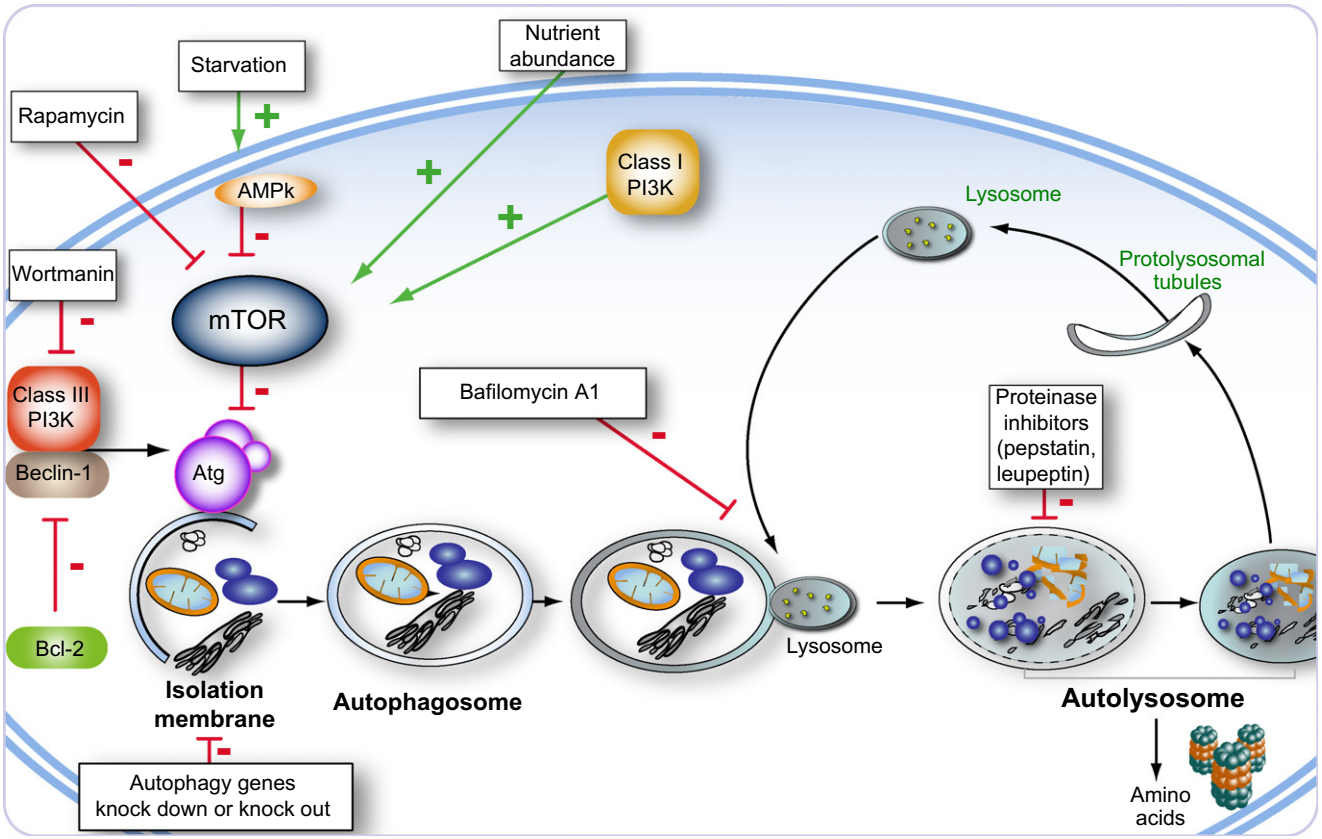

Fig. 1. Cellular and molecular aspects of autophagy. Note that wortmannin inhibits both class I (inhibitory) and class III PI3K, but the overall phenotypic effect is to inhibit autophagy as represented here. *Abbreviations:* AMPk, adenosine monophosphate-activated protein kinase; Atg, autophagy genes; Bcl-2, B-cell leukemia/lymphoma 2; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase.

could explain at least partly the worse tolerance to ischemia reperfusion in aged patients [15,24]. Second, autophagy level decreases following partial hepatectomy suggesting a shift from the physiological steady state between anabolism and catabolism to the positive balance which is required for the compensatory growth of the liver after partial hepatectomy [13]. Third, reperfusion had more effect on autophagy level than ischemia alone [16,19,25], which is in line with the histological lesions observed [11]. Unfortunately, no study has yet specifically evaluated the autophagic pathway in liver sinusoidal endothelial cells. This is a limitation in understand-

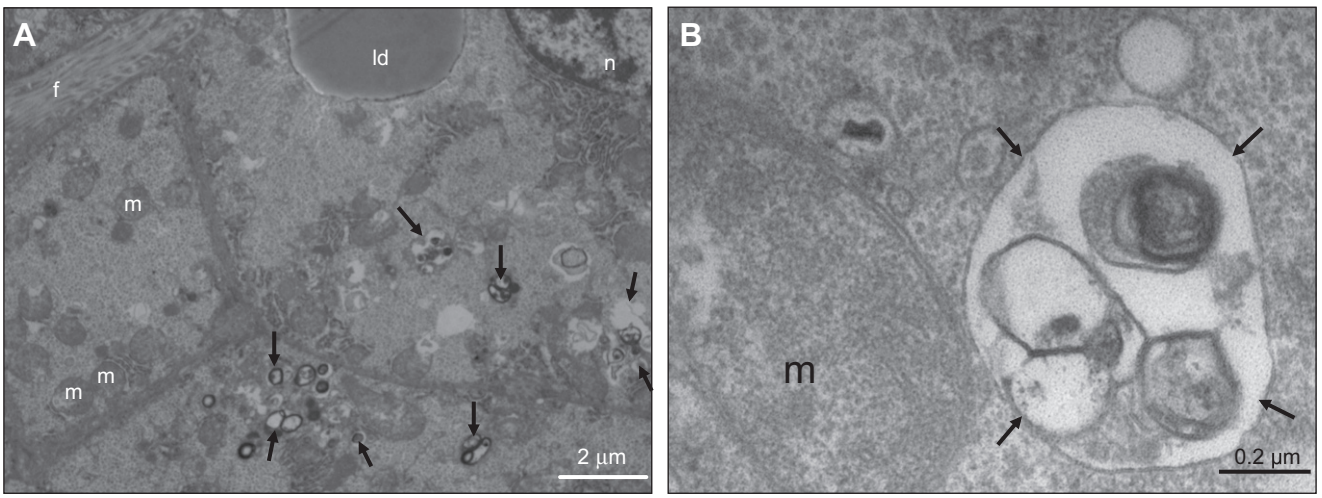

Fig. 2. Electron micrographs showing ultrastructure of hepatocytes from a chronic hepatitis C patient. Black arrows point to autophagic vacuoles. (A) Low-magnification image showing hepatocytes containing several autophagic vacuoles (original magnification, 8000×). (B) Partial view of a hepatocyte containing an autophagic vacuole (original magnification, 100,000×). F, fibrosis; ld, lipid droplet; m, mitochondria; n, nucleus.

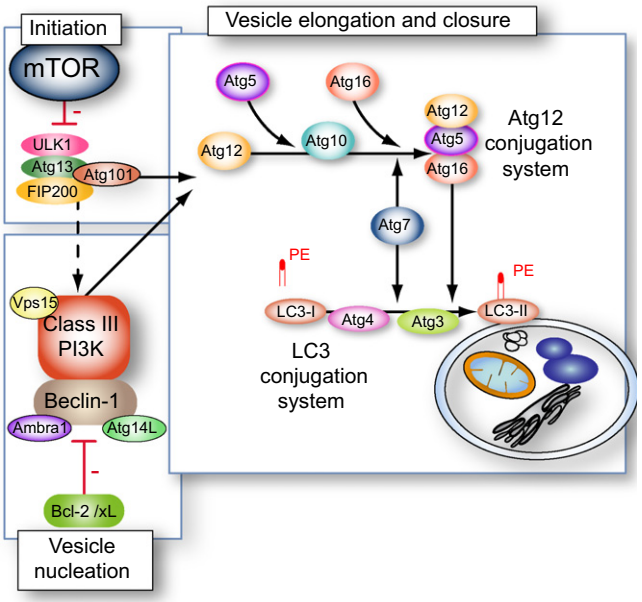

Fig. 3. Basic molecular machinery of autophagy. There are at least three steps in the formation of autophagosomes: initiation, nucleation, and elongation/closure. Autophagy is initiated by the ULK1 complex. This complex is formed by ULK1 Ser/Thr protein kinase, Atg13, and FIP200. Among the initial steps of vesicle nucleation is the activation of the class III PI3K (Vps34) to generate phosphatidylinositol 3-phosphate. This activation depends on the formation of a multiprotein complex that includes Beclin-1, Vps15, Atg14L (Atg14-like protein), and Ambra1. Beclin-1 constitutively interacts with Bcl-2 or its close homolog Bcl-XL and autophagy induction requires the dissociation of Beclin-1 from its inhibitors Bcl-2 or Bcl-XL [6]. The functional relationship between the ULK1 complex (initiation) and Beclin-1-class III PI3K complex (nucleation) complexes remains to be determined [86]. Vesicle elongation, vesicle completion. The membrane formed elongates and closes on itself to form an autophagosome. Two conjugation systems are successively involved. The first involves the covalent conjugation of Atg12 to Atg5, with the help of Atg7 and Atg10. This conjugate is organized into a complex by associating with Atg16 to form the Atg16–Atg5–Atg12 complex. The second involves the conjugation of phosphatidylethanolamine (PE) to a LC3 by the sequential action of the Atg4, Atg7, and Atg3. This lipid conjugation leads to the conversion of the soluble form of LC3 (named LC3-I) to the autophagic vesicle-associated form (LC3-II), allowing for the closure of the autophagic vacuole. *Abbreviations:* Ambra1, activating molecule in Beclin-1-regulated autophagy; Atg, autophagy genes; Bcl, B-cell leukemia/lymphoma; FIP200, 200-kDa focal adhesion kinase family-interacting protein; LC3, microtubule-associated protein light chain 3; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; ULK1, uncoordinated 51-like kinase 1; Vps, vacuolar protein sorting.

ing the effect of autophagy in liver ischemia reperfusion injury since these cells are the most sensitive to ischemia and lesions to these cells are a key event in this context.

Autophagy and viral hepatitis

Besides the physiological function of autophagy in maintaining cellular homeostasis, autophagy is a newly recognized facet of innate and adaptative immunity. Not surprisingly, certain viruses such as hepatitis C virus (HCV) and hepatitis B virus (HBV) have developed strategies to subvert or use autophagy for their own benefit [26] (Table 1).

Several studies have assessed the autophagic pathway in hepatocytes infected with HCV both *in vitro* and in liver biopsies from chronic hepatitis C patients [27–32]. Whatever the approach used (LC3, Atg5 or Beclin-1 immunoblotting, electron microscopy or GFP-LC3 immunofluorescence), these studies consistently demonstrated an accumulation of autophagic vacuoles in HCV-infected hepatocytes. This increase was independent of HCV genotype since it was observed *in vitro* in cells harboring the HCV strain H77 (genotype 1a), Con1 (genotype 1b) and JFH1 (genotype 2a) [27,28,30] and also in patients infected with HCV genotypes 1, 2, 3 and 4 [29]. However, this autophagy was inefficient. Indeed, although HCV JFH1 induced autophagosomes, it was not able to enhance autophagic protein degradation [30].

Contrary to certain viruses such as vesicular stomatitis virus and mutant herpes simplex virus 1, that can be captured and eliminated by the autophagic pathway, HCV has evolved to avoid and subvert autophagy using three strategies (Fig. 4) [26].

First, HCV seems to avoid its recognition by the autophagic machinery. Indeed, both immuno-electron microscopy and immunofluorescence studies revealed no or rare co-localization of HCV proteins with autophagic vacuoles [27,28,30,32].

Second, HCV prevents the maturation of the autophagosome into an autolysosome, as supported by the following elements: (a) the increase in the number of autophagic vacuole without enhancement in autophagic protein degradation [30]; (b) the absence of co-localization of lysosomes (stained with LysoTracker-red) with autophagic vacuoles (GFP-LC3) in HCV-infected cells contrary to starved cells [30]; (c) the reduction in the number of autophagic vacuoles following HCV elimination using interferon alpha for 14 days [31]; (d) the absence of increase in the number of late autophagic vesicles in hepatocytes from chronic hepatitis C patients as compared to controls, while a strong augmentation in the number of autophagic vesicles is observed [29]. This may be related to a lack of fusion between autophagosome and lysosome.

Third, HCV utilizes functions or components of autophagy to enhance its intracellular replication. Indeed, it has been recently shown that autophagy proteins are required for translation and/or delivery of incoming HCV RNA to the cell translation apparatus [28]. However, autophagy proteins are not needed for the translation of progeny HCV once replication is established since down-regulation of autophagy proteins 10 days after transduction had no effect on HCV replication. Therefore, authors hypothesized that, by remodelling endoplasmic reticulum membranes, the autophagy proteins or autophagic vesicles might provide an initial membranous support for translation of incoming RNA, prior to accumulation of viral proteins and the eventual establishment of virus-induced cellular modifications. Alternatively, autophagy proteins might contribute directly or indirectly to the cytoplasmic transport of the incoming RNA to cellular factors or sites that are required for its translation [28]. Importantly, autophagy proteins are required neither for HCV entry nor for HCV secretion [28,32]. Altogether, these data explain the apparent contrast between the results of some *in vitro* studies reporting the implication of autophagy proteins in HCV replication [30–32] and the absence of correlation between the number of autophagic vacuoles or the LC3-II level and the HCV load in chronic hepatitis C patients [29]: autophagy proteins are required only for initial steps of HCV replication, but not once replication is established.

Notably, cytosolic RNA-sensing protein kinase PKR and eIF2-α phosphorylation regulate virus- and starvation-induced autophagy [33,34]. It is tempting to speculate that recognition of the incoming HCV RNA by RNA-sensing molecules induces autophagy

Table 1. Level and function of autophagy in liver diseases.

Liver disease	Autophagy level in the liver	Function of autophagy
Liver ischemia reperfusion and liver surgery	↑	Cell survival
Hepatitis C viral infection	↑	Autophagy machinery subverted by HCV
Hepatitis B viral infection	↑	Autophagy machinery subverted by HBV
Acute liver injury	↑	Cell survival or death depending on the stimulus
Alpha1 antitrypsin deficiency	↑	Degradation of protein aggregates
Alcoholic liver disease	↓	Degradation of protein aggregates and of damaged organelles
Fatty liver disease	↓	Lipid degradation to fatty acid. Insulin signalling
Hepatocellular carcinoma	↓	Anti-tumor

and hence, favours its initial translation. Alternatively, constitutive basal autophagic vesicle formation might be required for this initial HCV RNA translation.

In conclusion, these data show that autophagy proteins are proviral factors for HCV.

HBV also induces autophagosomes in liver cells, as demonstrated both *in vitro* in several liver derived cell lines and *in vivo* in the liver of transgenic mouse lines harboring low (Tg08) and high (Tg05) replication levels of the HBV DNA. Importantly, this induction was also observed in the liver of an HBV-infected patient but not of a non-infected patient [35]. Contrary to HCV, HBV can enhance the autophagic flux, as late autophagic vacuoles could be detected in mouse hepatocytes using electron microscopy and given the existence of an extensive co-localization of lysosome-associated membrane protein 1 (LAMP1) with GFP–LC3 puncta (Fig. 5). However, without being able to provide

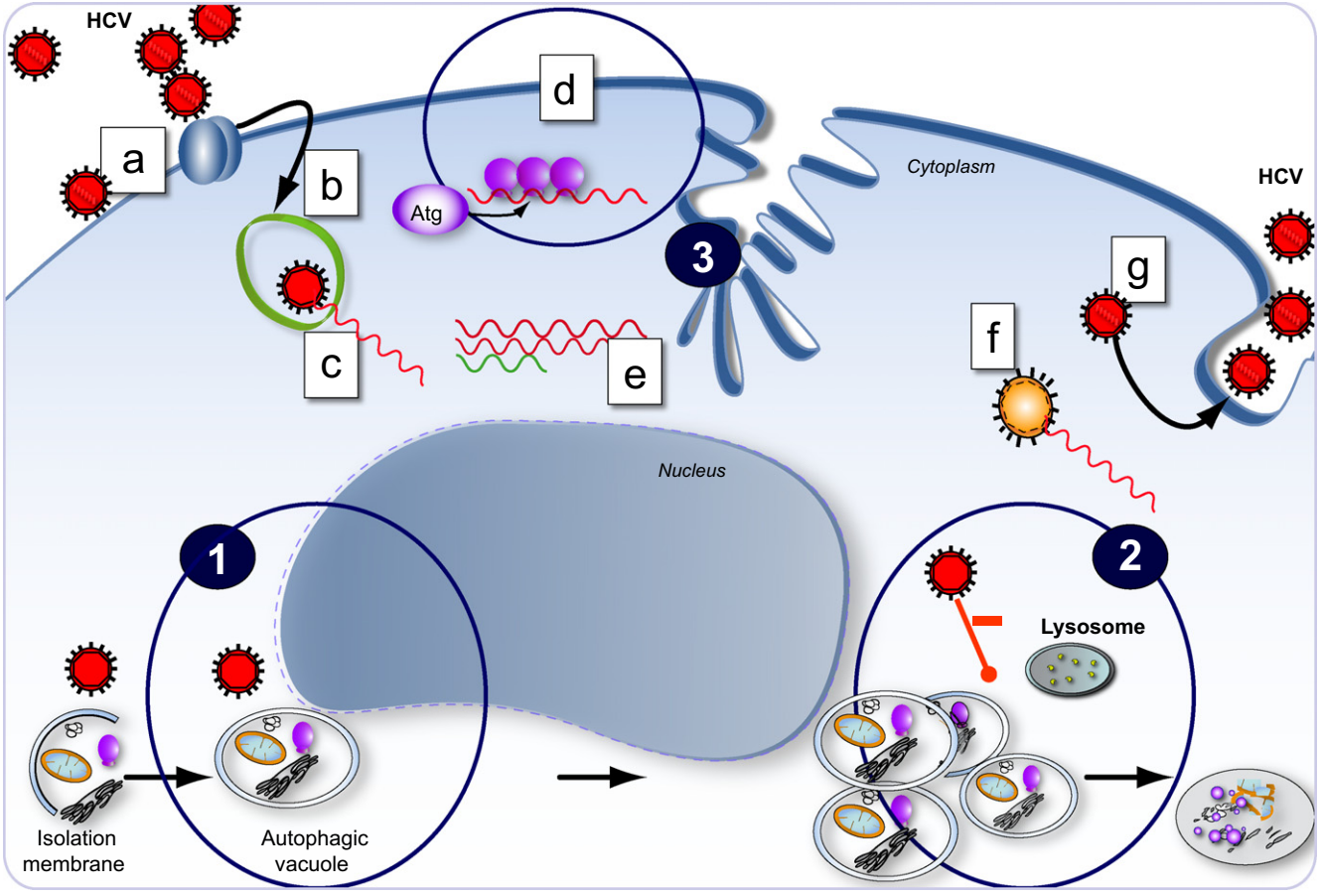

Fig. 4. HCV adaptations to evade and take advantage of autophagy. (1) HCV avoids its recognition by the autophagic machinery. (2) HCV prevents the maturation of the autophagosome into an autolysosome. (3) HCV utilizes functions or components of autophagy to enhance intracellular replication. HCV cycle. (a) HCV binding to cell surface receptors; (b) internalization; (c) fusion with endosomal membranes, allowing release of the plus-strand RNA viral genome into the cytosol; (d) translation of viral RNA; (e) HCV replication; (f) production of progeny viruses; (g) secretion. Atg, autophagy genes.

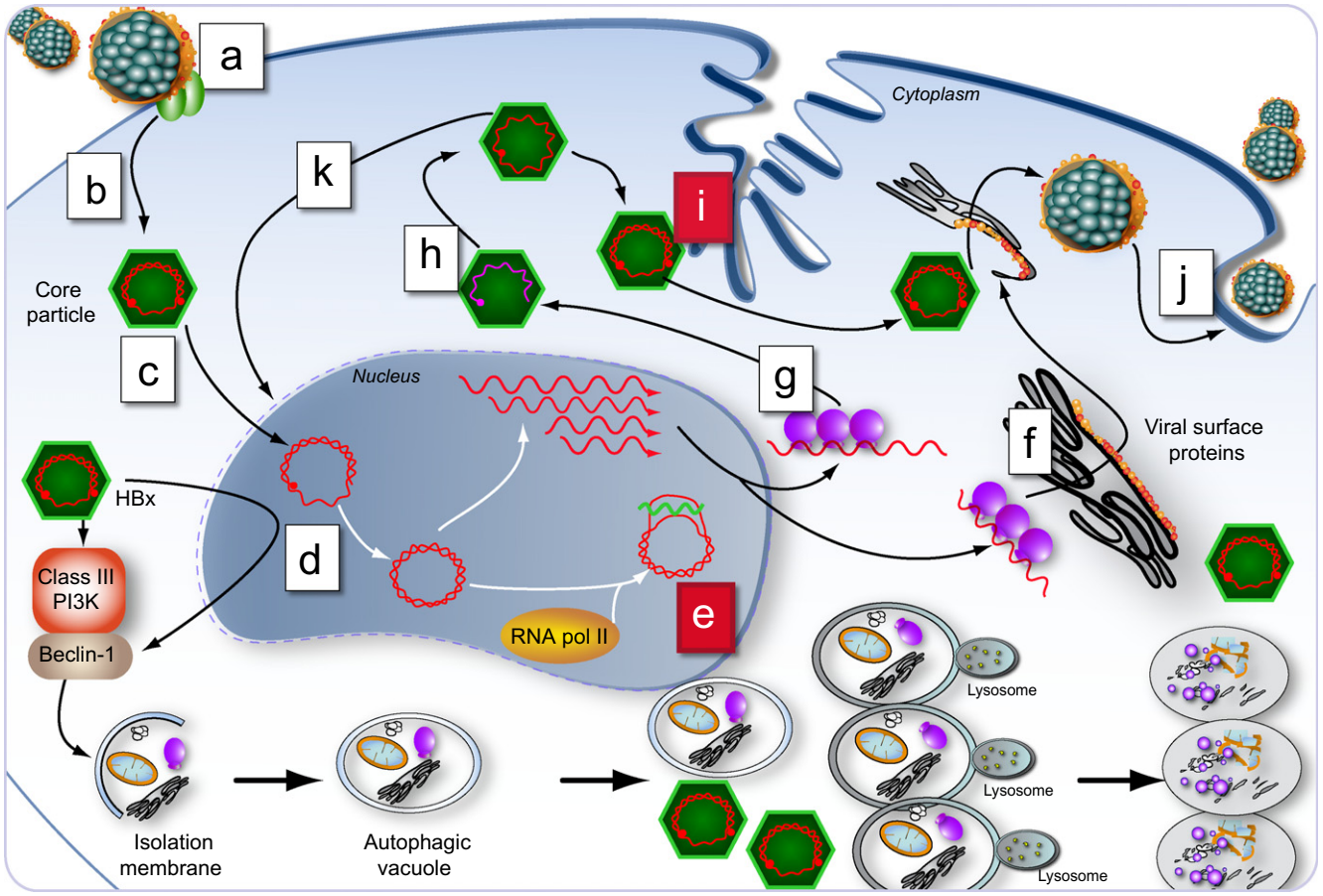

Fig. 5. HBV cycle. (a) HBV binding to cell surface receptors; (b) endocytosis; (c) transfer of the partially double stranded viral DNA to nucleus (d) repair to obtain fully double stranded and transformation into covalently closed circular DNA (cccDNA); (e) transcription; (f) the envelope proteins insert themselves as integral membrane proteins into the lipid membrane of the endoplasmic reticulum; (g) translation; (h) the pregenomic RNA (pgRNA) is packaged together with HBV polymerase and a protein kinase into core particles where it serves as a template for reverse transcription of negative-strand DNA (i); mature, viral nucleocapsids can follow two different intracellular pathways: the formation and secretion of new virions (j), or the amplification of the viral genome inside the cell nucleus (k). Implication of autophagy proteins in HBV life cycle: autophagy proteins are required mainly for HBV DNA replication (i), have a marginal effect on HBV RNA transcription and have no impact on other steps of HBV life cycle. As shown in the lower part of the picture, HBV can induce autophagic vacuole formation.

the reason for it, no significant increase in protein degradation was observed in HBV DNA-transfected cells [35].

An HBV-encoding protein, HBx, plays a crucial role in this HBV-induced autophagy [35,36]. Indeed, transfection of Huh7.5 cells with an HBV unable to express HBx did not enhance autophagy. Moreover, expression of HBx alone was sufficient to induce autophagy; similar results were obtained *in vivo* in transgenic mice [35]. This effect of HBx is due, at least partly, to its ability to bind to class III PI3K, a regulatory molecule that controls autophagy. Although conflicting, HBx may also upregulate the transcription of beclin-1, a protein that forms a complex with class III PI3K and thus sensitizes the cells to starvation-induced autophagy [35,36]. Whether the role of HBx is confined to short nutrient starvation conditions (8 h) or also exists in normal conditions remains controversial [35,36].

If, in the same way as HCV, HBV subverts autophagy, the strategy applied is somewhat different (Fig. 5). Autophagy enhances HBV replication mostly at the step of viral DNA replication, slightly at the step of RNA transcription, and not at other levels [35]. How autophagy may enhance HBV DNA replication remains unresolved.

The question whether HBV could be engulfed in autophagic vacuoles is not fully elucidated despite the observation that HBV core/e antigens and surface antigens partially co-localized with autophagic vacuoles [35]. Immuno-electron microscopy studies would be required to address this issue. However, as HBV seems to benefit from autophagy proteins and as the autophagic protein degradation rate is not increased, this hypothesis seems unlikely and autophagic vacuoles may rather serve as the sites for viral DNA replication and morphogenesis.

Autophagy and acute liver injury

Data regarding autophagy in acute liver injury are scarce. Two classic models of acute liver injury have been used: the concanavalin A and the lipopolysaccharide/D-galactosamine induced acute hepatitis [37–40]. In both models, autophagy was enhanced in mice liver. However, results are discordant for what regards the suggested role of autophagy. In concanavalin A-induced acute liver injury, concanavalin A induced autophagic cell death in hepatocytes and likely also in liver endothelial cells [37–39]. On the contrary, in lipopolysaccharide/D-galactosamine-induced acute liver injury, autophagy may be hepatoprotective. Indeed, lipopolysaccharide/D-galactosamine increased autophagy in the

livers of both wild-type and transgenic mice deficient of pregnane X receptor (PXR). Autophagy level was sustained in the former group but decreased rapidly in the latter. In parallel, the transgenic mice displayed more severe liver injury than wild-type mice [40] (Table 1).

However, the extrapolation of these data to human acute liver injury is uncertain. Indeed, electron microscopy analysis performed on liver biopsies from 5 patients with acute liver disease (2 with autoimmune hepatitis, 1 drug induced acute liver injury, 1 acute fatty liver of pregnancy and 1 Mauriac syndrome) did not show elements suggesting induction of autophagy [41].

Increased autophagy has only been reported in patients with acute liver insufficiency in a context of anorexia nervosa, a rare cause of liver failure. The role of autophagy in anorexia nervosa seems to be dual. As illustrated in Figure 6, initially, when body weight decreases, liver tests abnormalities are moderate, suggesting that autophagy can cope with nutrient starvation. However, when undernutrition reaches a critical level (body mass index at 13 or less), a flare in alanine aminotransferase (ALT) level occurs together with liver insufficiency. At that time, hepatocytes contain numerous autophagic vacuoles and interestingly, some liver cells present with the characteristic features of autophagic cell death, also called type 2 programmed cell death. This type of cell death could explain the contrast between the high serum alanine aminotransferase level (on average 1900 IU/L) and the absence of hepatocyte necrosis or liver cell apoptosis upon histological analysis. Indeed, while apoptosis cell plasma membrane integrity is preserved, during autophagic cell death the permeability increases, so that the aminotrasferase can be released in the blood flow [41]. Data obtained in perfused rat liver may explain this dual action of autophagy, since authors show that the degree of amino acid deprivation determines not only the number of autophagic vacuoles but also the types of cytoplasmic elements engulfed. Autophagic vacuoles initially contain glycogen and smooth endoplasmic reticulum, and subsequently mitochondria, rough endoplasmic reticulum, and free ribosomes [12]. This second step may jeopardize cell survival, particularly in a context of possible hypoxia, given the low cardiac output

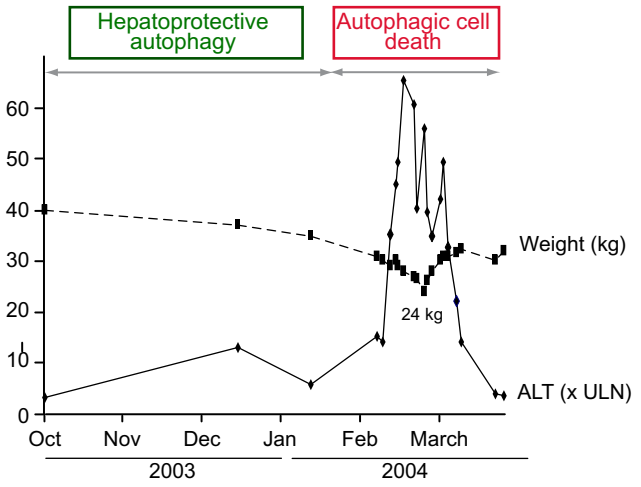

Fig. 6. Anorexia nervosa. Course of alanine aminotransferase (ALT) level (plain line) and body weight (dotted line) in a 24-year old girl (size 1.58 m) with anorexia nervosa. Mild elevations in liver tests were observed several months before onset of acute liver insufficiency. (personal data). *Abbreviation*: ULN, upper limit of normal values.

observed in these patients [42,43]. Altogether, these data suggest that in acute liver injury, autophagy fights to keep cells alive under stressful "life-threatening" conditions, the autophagic cell death being the outcome of failed adaptation.

Autophagy in alpha1-antitrypsin deficiency

The genetic disease alpha-1-antitrypsin (α1AT) deficiency is caused by homozygosity for the α1AT mutant Z gene (ATZ) and occurs 1 in 2000 births. The Z mutation confers an abnormal conformation on the nascent polypeptide, resulting in an accumulation of the mutant protein within the endoplasmic reticulum of hepatocytes rather than the appropriate, highly efficient, secretion of the wild-type protein.

When ATZ accumulates in the ER, it can be degraded by two major mechanisms, the proteasomal and autophagic pathways. The proteasome is probably specialized for the soluble forms of ATZ that accumulates in the ER, presumably bound to multiple chaperones [44]. Several lines of evidence show that the autophagic pathway is specialized for the polymerized/aggregated forms of ATZ (Table 1): (a) an increased accumulation of autophagosomes is observed in fibroblast cell lines engineered for the expression of mutant ATZ, in the liver cells of the PiZ mouse model of α1AT deficiency and in the liver cells of patients with α1AT deficiency [45]; (b) these autophagic vacuoles contain ATZ as demonstrated using both immuno-electron microscopy and immunofluorescence [45,46], but also frequently contain mitochondria, even when compared with liver from patients with other hepatic disorders [47]; (c) in cell lines deleted for the *Atg5* gene, that is necessary for autophagy, the degradation of ATZ is retarded and the characteristic cellular inclusions of ATZ accumulate [46].

This induction of autophagy is specific for the aggregation-prone properties of ATZ because it was not seen in a Saar mouse, a mouse with hepatocyte-specific inducible expression of the AT Saar variant that accumulates in the ER but does not polymerize/aggregate [46].

The mechanisms by which autophagy is activated in the liver in α1AT deficiency are largely unknown. The marked upregulation of regulator of G signaling 16 (RGS16) observed both in the liver of mice that have hepatocyte-specific inducible expression of ATZ and in the liver of patients with α1AT deficiency may be implicated [48]. Indeed, RGS16 binds to and inhibits the heterotrimeric G protein Gαi3, a protein that inhibits hepatic insulin-induced autophagic activity [49]. Therefore, RGS16 might derepress autophagy.

The pathogenesis of liver injury in α1AT deficiency is still incompletely understood. Nevertheless, it has been shown that markers of apoptosis are more pronounced in hepatocytes with greater levels of insoluble ATZ. Furthermore, stimulation of the extrinsic apoptotic pathway with antibody to Fas, resulted in increased apoptosis almost exclusively of the globule-containing cells [50]. Autophagy activation and/or mitochondrial dysfunction present in α1AT hepatocytes must therefore be responsible for this increased apoptotic hepatocellular death.

Interestingly, recent studies have shown that carbamazepin, a well-known food and drug administration-approved drug that enhances autophagy, decreases the hepatic load of ATZ and hepatic fibrosis in a mouse model of AT deficiency-associated liver disease. These results provide a basis for testing carbamazepin in α1AT deficiency patients [51,52].

More details regarding the implication of autophagy in alpha-1-antitrypsin deficiency are provided in a recent review [44].

Autophagy in alcoholic liver disease

Excessive alcohol consumption is the third leading preventable cause of death in the United States [53]. Chronic alcohol use may cause several types of liver injury: fatty liver (also called steatosis), hepatic fibrosis, cirrhosis, and alcoholic hepatitis.

Several direct and indirect arguments suggest that alcohol consumption suppresses liver cell autophagy (Table 1): (a) rats chronically fed with ethanol have a reduced number of autophagic vacuoles in liver cells, as determined morphometrically [54]; (b) chronic ethanol consumption slows down the catabolism of long-lived proteins in the rat liver [54,55]; (c) alcohol abuse is associated with protein accumulation in the liver, as demonstrated in ethanol fed rats [56]; (d) hepatocytes from patients with alcoholic steatohepatitis contain protein aggregates called Mallory-Denk bodies. The major constituents of these cytoplasmic inclusions are cytokeratins 8 and 18, in association with other proteins including ubiquitin and p62 [57,58]. These Mallory-Denk bodies may witness a decrease in autophagy level. Indeed, autophagy participates in the elimination of components of Mallory-Denk bodies, since cytokeratin 8/18 are detected in autophagic vacuoles using immuno-electron microscopy, and since stimulating the autophagic process using rapamycin decreases the number of these protein aggregates [57,59]; (e) loss of autophagy in transgenic mice induces the formation of protein aggregates in hepatocytes, resembling Mallory-Denk bodies [60]; (f) ethanol also suppresses autophagy in cortical neuroepithelial progenitors, suggesting that this effect is not restricted to liver cells [61].

The mechanisms responsible for the decrease in autophagy are not clear, but two explanations can be advanced [62] (Fig. 7). First, ethanol consumption significantly reduces adenosine monophosphate-activated protein kinase (AMPk) activity in the liver [66]. As shown in Figure 7, AMPk suppression reduces autophagy via the mTOR pathway. Second, ethanol is known to alter vesicle transport in hepatocytes. Autophagy requires the action of cytoskeletal elements, including microtubules and microfilaments. Both are necessary for autophagosome formation and fusion with other vesicular bodies, as demonstrated by blocking these processes with specific inhibitors, including nocadazole and vinblastine (microtubules), and cytochalasins (microfilaments) [63,64]. Disruption, by ethanol treatment, of the vesicular movement within the hepatocyte, occurs by mechanisms that are independent of the molecular motors dynein and kinesin, although there is evidence for alterations in the protein dynamin [65].

This decline in the autophagic pathway must contribute to the pathological consequences of alcohol ingestion. First, as mentioned above, the decrease in protein catabolism likely contributes to the formation of Mallory-Denk bodies. These inclusions contain the protein p62. Recent studies have demonstrated that accumulation of p62 results in hyperactivation of the transcription factor (nuclear factor erythroid 2-related factor 2 (Nrf2) that causes liver changes such as hepatomegaly, liver cell swelling,

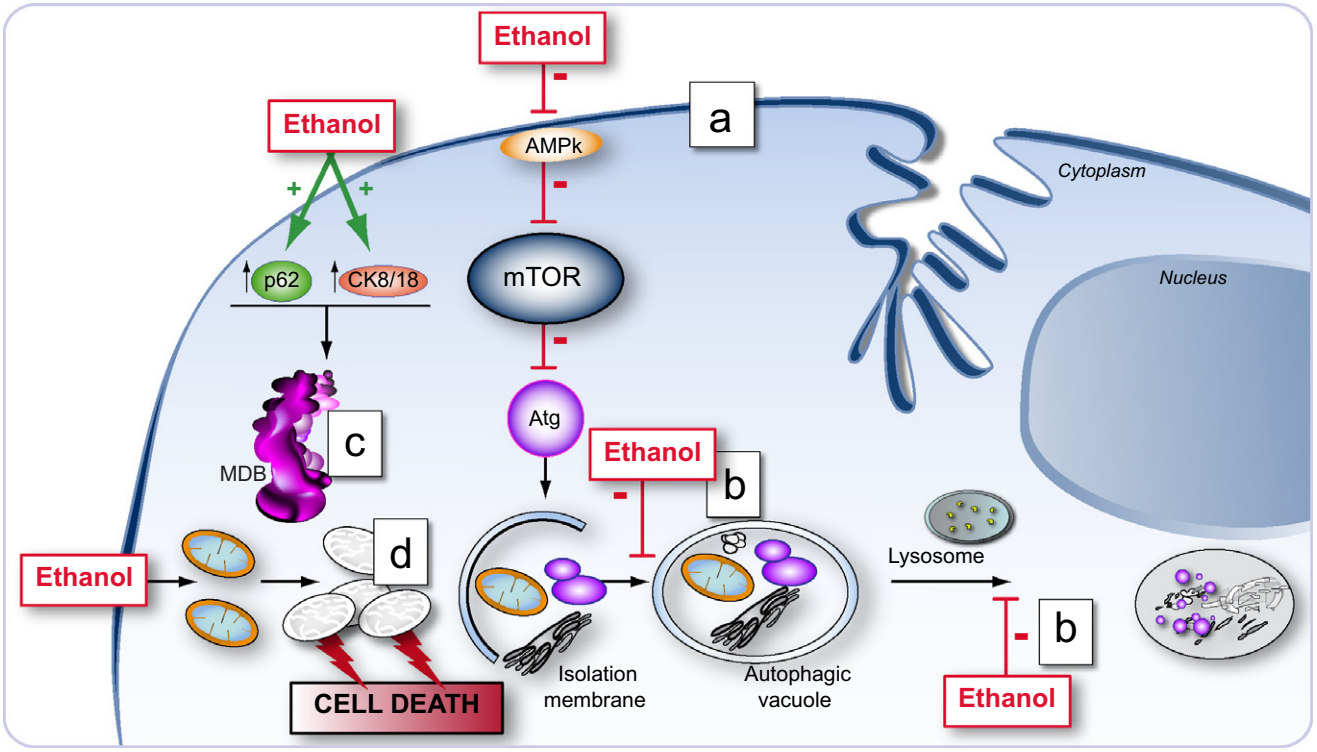

Fig. 7. Ethanol may decrease autophagy level by reducing AMPk activity in the liver (a) and by disrupting vesicular movement within hepatocyte (b). This decrease in the autophagic process results in the accumulation of protein aggregates called Mallory-Denk bodies (c) and of mitochondria damaged following ethanol ingestion (d). Depolarization of the inner membrane of mitochondria may occur leading to mitochondrial permeability transition and cell death. *Abbreviations:* AMPk, adenosine monophosphate-activated protein kinase; CK, cytokeratin; MDB, Mallory-Denk body.

and aminotransferase elevation [67]. Second, ethanol causes mitochondrial damage leading, in certain cases, to depolarization of the inner membrane of mitochondria, called mitochondrial permeability transition. It is crucial that such damaged mitochondria be removed from the cell by engulfment in autophagic vacuoles. The absence of mitochondrial autophagy leads to uncoupling of oxidative phosphorylation and cell death [16,62].

Autophagy and non alcoholic fatty liver disease

The implication of autophagy in hepatocyte lipid metabolism has been recently demonstrated [68]. Besides cytosolic lipases, autophagy regulates intracellular lipid stores through a process called macrolipophagy. Portions of lipid droplets, or even whole droplets, become trapped inside the double-membrane-bound autolipophagosome vesicles and are transported to lysosomes, where they are degraded to fatty acids (Fig. 8). The presence of this alternative lipid degradative pathway in hepatocytes explains their ability to rapidly mobilize large amounts of lipids despite their low levels of cytosolic lipases in comparison with adipocytes [69,70]. In physiological state, both lipolysis and macrolipophagy are inhibited by the hormone insulin [5,8,68,69].

The efficiency of macrolipophagy varies with the nutritional status. In response to a short-term increase in lipid availability, *in vitro* studies have demonstrated that the autophagy level increases, leading to a greater breakdown of stored lipids to supply fatty acids for β-oxidation or other uses [68]. In the same way, hepatocyte-specific atg7-knockout mice had markedly increased hepatic lipid [68].

In contrast, in both genetic (ob/ob mice) and dietary (high fat diet) mouse models of chronic obesity and insulin resistance, a sustained increase in lipid availability results in markedly decreased hepatic autophagy indicators [68,70–73]. Proposed mechanisms are summarized in Figure 8.

This decreased autophagy level impacts on other cellular functions and particularly on ER stress. Indeed, in the liver tissue of lean mice, deficiency of autophagy, induced by suppression of Atg7, results in ER stress [71]. This could be explained by the known role of autophagy in the degradation of unfolded proteins and in the removal of superfluous ER membranes: defect in autophagy may lead to the accumulation of unfolded proteins and thus to ER stress [74]. In obesity, ER function is compromised due to nutrient and energy surplus in an inflammatory milieu [75]. Concomitantly, autophagy level is decreased, suggesting that failure to achieve autophagy may further impair ER function in the face of continuous energy and nutrient stress, engage organelle dysfunction and contribute to insulin resistance, a known consequence of ER stress [71,76]. Interestingly, restoration of Atg7 expression results in significant reduction in obesity-induced ER stress in the liver of ob/ob mice, rescues the defects in insulin receptor signaling, reduces serum insulin level, improves glucose tolerance and whole body insulin sensitivity through the suppression of hepatic glucose production and enhancement of insulin-stimulated glucose disposal in the periphery, and decreases hepatic fatty acid infiltration and liver triglyceride content [68,71]. However, the interest of increasing autophagy, e.g., by pharmacological means, remains uncertain given the opposite role of autophagy in adipocytes: the inhibition, rather than the stimulation, of autophagy in adipocytes gives them a brown-fat-cell-like appearance that favours fatty acid oxidation and increases insulin [68,73].

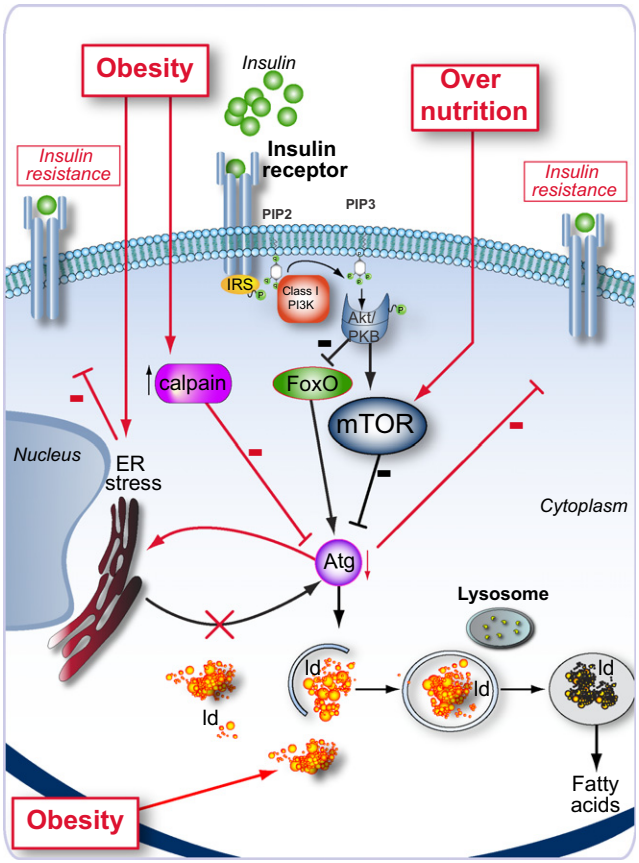

Fig. 8. Fatty liver disease and metabolic syndrome. Under physiological conditions (black arrows), autophagy functions in the basal turnover of lipids by engulfing and degrading lipid droplets. Autophagy is inhibited by the insulin amino acid-mTOR signaling pathway via both short-term and long-term regulation mechanisms. Short-term inhibition can be produced by the mTOR complex. Long-term regulation occurs via the transcription factors FoxO [72], which control the transcription of autophagy genes and become inhibited by insulin-induced activation of protein kinase B. In obesity (red arrows), autophagy level is decreased in hepatocytes. Several mechanisms may account for this decline. First, obesity-induced increase in the calcium-dependent protease calpain 2 leads to down-regulation of Atg7 and then defective autophagy. Acute inhibition of calpain is able to restore Atg7 expression [71]. Second, in obesity, the autophagy inhibitor mTOR is overactivated in the liver, presumably as the result of increased amino acid concentrations following overnutrition [73,87]. Third, although controversial, hyperinsulinemia may also contribute to downregulating autophagy in obese mice. Indeed, inhibiting Akt which is a key molecule in the insulin pathway, increases autophagy in the liver of obese mice [72]. However, destruction of insulin production in β-cells by streptozotocin does not increase autophagy in the liver of obese mice [71], contrary to lean mice [72]. The reasons for these discrepancies are unclear. In obesity, defect in autophagy and its associated decrease in lysosomal degradation rate contribute to further increasing the ER stress induced by nutrient overload in an inflammatory milieu [71,75]. Together, autophagy decline and ER stress lead to insulin resistance [71]. *Abbreviations:* Atg, autophagy genes; ld, lipid droplet; ER, endoplasmic reticulum; mTOR, mammalian target of rapamycin; PKB, protein kinase B.

Autophagy and hepatocellular carcinoma

Early, cancer has been genetically linked to autophagy malfunction. Indeed, the ATG gene beclin-1 is mono-allelically deleted in 40–75% of cases of human breast, ovarian, and prostate cancer [5,77,78]. Moreover, the regulation of autophagy overlaps closely with signaling pathways that regulate tumorigenesis.

Studies assessing autophagy in hepatocellular carcinoma (HCC) have clearly demonstrated *in vitro*, in mice and in patients that, in this context, autophagy is a tumor suppressor mechanism (Table 1).

First, mice with heterozygous disruption of beclin-1 have a high frequency of spontaneous hepatocellular carcinoma. Moreover, crossing beclin-1 +/− mice, with mice, that transgenically express the HBV large-envelope polypeptide under the transcriptional control of the mouse albumin promoter, resulted in the acceleration of the development of hepatitis B virus-induced small-cell dysplasia – an important histopathologic predictor of malignant transformation [79].

Second, expression of several autophagic genes (*ATG5, ATG7* and BECLIN-1) and their corresponding autophagic activity is decreased in HCC cell lines compared to that in a normal hepatic cell line. Similarly, Beclin-1 mRNA and protein levels are lower in HCC tissue samples than in adjacent non-tumor tissues from the same patients [80].

Third, the most aggressive malignant HCC cell lines and HCC tissues with recurrent disease display much lower autophagic levels than less aggressive cell lines or tissues, especially when the anti-apoptotic B-cell leukemia/lymphoma (Bcl)-xL protein is over-expressed [80]. Interestingly, in a tissue microarray study consisting of 300 HCC patients who underwent curative resection, the expression of Beclin-1 was significantly correlated with disease-free survival and overall survival only in the Bcl-xL+ patients. Multivariate analyses revealed that Beclin-1 expression was an independent predictor for disease-free survival and overall survival in Bcl-xL+ patients. In addition, there was a significant correlation between Beclin-1 expression and tumor differentiation in Bcl-xL+ but not in Bcl-xL− HCC patients. These data suggest that autophagy defect synergizes with altered apoptotic activity and facilitates tumor progression and poor prognosis of HCC [80].

The mechanisms responsible for this low autophagy protein level are not elucidated. However, a recent study has demonstrated that HAb18G/CD147, a transmembrane glycoprotein highly expressed in HCC, contributes to this decreased autophagic level in HCC through the class I phosphatidylinositol 3-kinase–Akt pathway upregulation [81]. Other oncoproteins such as the Bcl-2 family proteins may also be implicated in HCC, like in other cancers [77]. Stimulation of hypoxia-inducible factors (HIFs) due to hypoxic stress within HCC may also contribute to autophagy modulation [82].

Fourth, therapeutic approaches aiming at increasing autophagy level have been successfully tested *in vitro* and/or in mice using molecules such as Concanavalin A, a lectin with mannose specificity [38], or cyclo-oxygenase-2 inhibitors [83,84]. Importantly, a large recent study has assessed the survival after liver transplantation according to the immunosuppression protocol administrated. All patients included, 2491 adult recipients of isolated liver transplantation for HCC and 12,167 for non-HCC, remained on stable maintenance immunosuppression protocols for at least 6 months post-transplant. Therapy using rapamycin, a well-known activator of autophagy, was associated with improved survivals after transplantation for HCC. Interestingly, in non-HCC patients, rapamycin showed a trend toward lower rates of survival in non-HCC recipients, confirming the specificity of its beneficial impact for cancer patients [85].

Given the primary prosurvival role of autophagy, this antitumor activity may be surprising. If the mechanisms for this tumor suppressor role of autophagy are not yet clear, the following functions of autophagy have been proposed: (a) limiting chromosomal instability therefore preventing the accumulation of oncogenic mutations; (b) restricting oxidative stress, which is also an oncogenic stimulus; and (c) reducing intratumoral necrosis and local inflammation [77,78].

Conclusion

Autophagy has a Janus-face in that being primarily a survival mechanism it can also, under certain conditions, lead to autophagic cell death. However, in most liver diseases, it seems clear that one of its major functions is to fight to keep cells alive under stressful "life-threatening" conditions. Conversely, during hepatitis viral infection, autophagy is subverted to the benefit of the virus. Hence, taking into account the crucial role of autophagy in liver diseases and the existence of therapies targeting this process, such as rapamycin, makes therapeutic strategies targeting autophagy possible in a near future [8,17,88]

Better understanding of the molecular mechanisms of autophagy and its implication in liver diseases highlight new potential therapeutic targets. Interestingly, some of the drugs that can modulate autophagy are already approved for other human clinical uses. Rapamycin, tamoxifen, carbamazepine, sodium valproate and lithium, cisplatin induce autophagy, while vinblastine, antimalarial compounds chloroquine and hydroxychloroquine as well as the antidepressant agent clomipramine inhibit this process [8,17,88]. However, one of the limitations of these reagents is that their targets are either indirectly or non-exclusively involved in autophagy. Therefore, their use also impinges on independent or parallel systems, potentially leading to unwelcome side effects [88].

Key points

- Autophagy is a crucial physiological process allowing for providing nutrients to maintain vital cellular functions during fasting but also to rid the cell of superfluous or damaged organelles, and misfolded proteins.
- In most liver diseases, autophagy has mainly a protective function allowing for degradating lipid droplets in fatty liver disease, or protein aggregates, such as in a-1 antitrypsin deficiency or in alcoholic liver disease.
- During hepatitis B or C infection, autophagy is subverted by viruses to promote their own replication.
- In hepatocellular carcinoma, autophagy level is decreased within the tumour and therapy aiming at stimulating autophagy, such as rapamycin, may reduce hepatocellular recurrence after liver transplantation.

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Data sources and searches

We searched MEDLINE (1966–2010) for studies on autophagy and liver diseases by using the terms autophagy, autophagosome, liver, and hepatitis. We also reviewed publications in personal reference lists and citation sections of the recovered articles. We performed the final search on July 20 2010.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

There is no financial disclosure.

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