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REVIEW ARTICLE

Relevance of autophagy to fatty liver diseases and potential therapeutic applications

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Abstract Autophagy is an evolutionarily conserved lysosome-mediated cellular degradation program. Accumulating evidence shows that autophagy is important to the maintenance of liver homeostasis. Autophagy involves recycling of cellular nutrients recycling as well as quality control of subcellular organelles. Autophagy deficiency in the liver causes various liver pathologies. Fatty liver disease (FLD) is characterized by the accumulation of lipids in hepatocytes and the dysfunction in energy metabolism. Autophagy is negatively affected by the pathogenesis of FLD and the activation of autophagy could ameliorate steatosis, which suggests a potential therapeutic approach to FLD. In this review, we will discuss autophagy and its relevance to liver diseases, especially FLD. In addition, we will discuss recent findings on potential therapeutic applications of autophagy modulators for FLD.

Keywords Autophagy · Fatty liver disease · Lipophagy · Liver function · Metabolism

Abbreviations

3-MA	3-Methyl adenine
AFLD	Alcoholic fatty liver disease
AMPK	AMP-activated protein kinase
Atg	Autophagy-related proteins
CMA	Chaperone-mediated autophagy
DAMPs	Danger-associated molecular patterns
ECM	Extracellular matrix
ER	Endoplasmic reticulum
ERQC	Endoplasmic reticulum quality control
FAs	Fatty acids
FLD	Fatty liver disease
HCC	Hepatocellular carcinoma
HSC	Hepatic stellate cells
LDs	Lipid droplets
MDB	Mallory–Denk body
mTOR	Mammalian target of rapamycin
NAC	*N*-Acetyl cysteine
NAFLD	Non-alcoholic fatty liver disease
PAMPs	Pathogen-associated molecular patterns
DAMPs	Danger-associated molecular patterns
PI3K	Class III phosphatidylinositol 3-kinase
PKA	Ras/cAMP-dependent protein kinase A
TFEB	Transcription factor EB
ULK1	UNC-5-like autophagy-activating kinase 1
UPR	Unfolded protein response

Introduction

Autophagy (from the Greek, “auto” oneself, “phagy” to eat) is an evolutionarily conserved cellular degradation process that involves the delivery of cytoplasmic cargo (macromolecules or organelles) to the lysosome (Deter and De Duve 1967). It becomes an intensely studied area for its implications in fundamental cell biology as well as its important roles in the pathogenesis including tissue injury, microbial infection, cancer, neurodegeneration, and aging (Shintani and Klionsky 2004). Autophagy has been generally classified into three types including macroautophagy, microautophagy, and chaperone-mediated autophagy (Meijer and Codogno 2004). Macroautophagy is the most active

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form of autophagic process in which cytosolic materials are sequestered into autophagosomes and transported to the lysosome, where they would be degraded (Mizushima and Komatsu 2011). Microautophagy, which is mainly defined in the yeast, but is also observed in mammalian cells, mediates both invagination and vesicle scission into the lumen of lysosomes through direct engulfment of cytoplasmic cargo at a boundary membrane of the lysosome (Mijaljica et al. 2011; Farre et al. 2009). In chaperone-mediated autophagy (CMA), specific proteins are targeted by chaperones, for example, heat-shock cognate protein of 70 kDa (Hsc70), and directly shuttled across the lysosomal membrane for degradation in the lumen (Kaushik and Cuervo 2012).

In this review, we will focus on the signaling and roles of the macroautophagic process, referred to hereafter simply as autophagy, in fatty liver diseases (FLDs). Meanwhile, we will discuss potential therapeutic applications by targeting autophagy in FLDs.

Autophagy: the basic process

Autophagy pathways and regulations

Autophagy occurs when an organelle known as autophagosome is formed. The autophagosome is characterized as a double-membrane cisterna that envelops cytosolic material like proteins and organelles (Lamb et al. 2013). The origin of the autophagosomal membrane and its formation is still not well understood. Various models have been proposed, including the de novo production and the derivation from endoplasmic reticulum (ER), the Golgi apparatus, the mitochondria, the endocytic system or the plasma membrane (Hamasaki et al. 2013; Lamb et al. 2013). The autophagosome then becomes the autolysosome after fusing with the lysosome, where the components from the cytosols are degraded (Kaur and Debnath 2015; Madrigal-Matute and Cuervo 2016).

Several key molecular pathways that regulate the autophagy have been elucidated in the past decade. To date, more than 35 different proteins, named Atg or "autophagy-related" proteins, have been characterized in yeast, including 15 core Atg proteins required for both selective and starvation-induced autophagy are highly conserved in mammal cells (Atg1-10, 12-14, 16, 18) (Nakatogawa et al. 2009; Mizushima et al. 2011). These proteins work together as the main regulators for autophagy. Generally, Atg proteins could be functionally classified into six clusters (Shibutani and Yoshimori 2014; Mizushima et al. 2011).

1. The Atg1/ULK complex is composed of the autophagy-initiating UNC-5-like autophagy-activating kinase 1 (ULK1), FIP200, Atg13L, and Atg101 in mammals.
2. The class III phosphatidylinositol 3-kinase (PI3K) complex consists of VPS34, VPS15, Beclin-1, autophagy/beclin 1 regulator 1 (AMBRA1), and Atg14 (L)/Barkor in mammals.
3. The Atg2–Atg18/WIPI complex includes the PI3P-binding protein Atg18/WIPI and its binding partner Atg2.
4. The Atg12 conjugation system includes Atg12, Atg7, Atg10, Atg5, and Atg16L1/2 in mammals.
5. The Atg8/LC3 conjugation system includes LC3A/B/C, GABARAP, GABARAPL1/2/3, Atg4A-D, Atg7, and Atg3 in mammals.
6. Atg9 vesicles: Atg9 was detected on small vesicles and tubular structures in mammals.

During the biogenesis of autophagosome, the formation of Atg1/ULK complex is one of the earliest detectable events and is at the most upstream position of the recruitment of Atg proteins (Lamb et al. 2013; Shimizu et al. 2014). In the initial stage (vesicle nucleation), the Atg1/ULK complex activates the PI3K complex, which recruits several Atg proteins to the phagophore. This is followed by the stages of vesicle elongation and completion, which includes the recruitment of Atg12–Atg5–Atg16 complex to the autophagosome membrane and the lipidation of Atg8/LC3 through the conjugation to PE on the autophagosomal membrane. The mature autophagosome then migrates to and fuses with lysosomes to form autolysosome, in which the sequestered cytoplasmic material of the autophagosome is degraded (Liu and Levine 2015).

Other than the aforementioned core pathway of mammalian autophagy, certain subcellular systems, such as the secretory and endocytic pathway, and the cytoskeletal network, may also play important roles during autophagy (He and Klionsky 2009). Meanwhile, autophagy is inhibited or stimulated by multiple stimuli, including the change of nutritional status, hormonal factors, and other environmental differences such as temperature, oxygen concentrations, and cell density (Levine and Kroemer 2008). These stimuli regulate autophagy via different mechanisms. The mammalian target of rapamycin (mTOR) is one of the major inhibitors. mTOR is a master regulator of cellular metabolism and promotes cell growth in response to environmental cues. During the autophagy process, mTOR complex 1 (mTORC1) inhibits ULK complex by phosphorylating Atg13 and ULK1/2, which results in autophagy suppression (Kim and Guan 2015). In addition, mTORC1 has also been suggested to inhibit ULK1 stability by inhibitory phosphorylation of AMBRA1, and to regulate autophagy at the transcriptional level by inhibiting transcription factor EB (TFEB) (Kim and Guan 2015). mTORC1 could be inactivated by an important energy-sensing molecule, AMP-activated protein kinase (AMPK), via phosphorylating

tuberous sclerosis 2 (TSC2) and the mTORC1 component Raptor, which subsequently activate the ULK1 complex (Kim and Guan 2015). Furthermore, AMPK could also phosphorylate and activate ULK1 directly (Kim et al. 2013a). AMPK and mTORC1 also regulate the class III PI3K complex, in which AMPK phosphorylates Beclin1, leading to the activation of the complex, whereas mTORC1 phosphorylates Atg14L, resulting in an inhibitory effect (Kim and Guan 2015). Other nutrient-sensing pathways, such as the Ras/cAMP-dependent protein kinase A (PKA) signaling pathway and the insulin/growth factor pathways are also involved in the regulation of autophagy (He and Klionsky 2009).

Autophagosome formation is also potently induced under various extra- and intracellular stresses, which is beneficial for organisms to rid themselves of damaging cytoplasmic components (He and Klionsky 2009; Levine and Kroemer 2008). These stress stimuli include ER stress, hypoxia, redox stress, pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), and mitochondrial damage (Kroemer et al. 2010). Under these stress conditions, autophagy is regulated through transcriptional reprogramming, activation of autophagy-inducing proteins, cell cycle modulation, and some other unclear events (Kroemer et al. 2010). For example, when the folding capacity is exceeded in the ER, the unfolded protein response (UPR) signaling triggers and mediates the transcriptional activation of the autophagy-related proteins, such as LC3 and Atg5 (Ogata et al. 2006; Ding et al. 2007; Ding and Yin 2008; Kroemer et al. 2010).

Considering the critical role of autophagy in degradative process, its potential regulation mechanisms have been extensively studied. An increasing amount of evidence suggests that autophagy can be regulated at different levels, including transcriptional, post-transcriptional, and post-translational levels to maintain cellular homeostasis (Feng et al. 2015). At the transcriptional level, several transcription factors are involved in the autophagic progress, including the E2F family, FoxO family, p53, STAT1/3, TFEB, FXR, PPARα, GATA, ATF4, etc. (Feng et al. 2015; Seok et al. 2014; Lee et al. 2014). Several of them are also nutrient-sensing nuclear receptors, which may also explain the nutritional regulation of autophagy. TFEB, one target of mTORC1 as mentioned above, has been shown to regulate autophagy process by modulating expression of autophagy-related genes, such as UVRAG, WIPI, MAPLC3B, SQSTM1, VPS11, VPS18, and ATG9B, during starvation (Settembre et al. 2011). Under the condition of nutrient deprivation, forkhead box O 3a (FoxO3a) is phosphorylated by AMPK in the nucleus, which results in transcriptional repression of S-phase kinase-associated protein 2 (SKP2) (Shin et al. 2016). Coactivator-associated arginine methyltransferase (CARM1) protein is increased after the decrease of SKP2 and subsequently increases the dimethylation of histone H3 Arg17 (Shin et al. 2016). Eventually the whole event activates autophagy-related and lysosomal genes through TFEB, which results in autophagy stimulation after nutrient starvation (Shin et al. 2016). Besides transcriptional regulation, autophagy is also regulated at the post-transcriptional or post-translational level. The most typical post-transcriptional regulation is the action of noncoding RNAs, especially microRNAs (miRNA), which target to the core machinery during autophagy process (Feng et al. 2015). Meanwhile, post-translational regulations, such as phosphorylation, ubiquitination, acetylation, epigenetic regulation, and protein–protein interaction, are also essential for the modulation of autophagy (Feng et al. 2015). Accumulating evidence suggests that autophagy modulation could be a potential therapeutic modality for different disease. Some of the aforementioned factors may be considered as therapeutic targets, which will be further discussed below.

Roles of autophagy in metabolic homeostasis

The liver is a gland that plays a critical role in metabolism in the human body (Abdel-Misih and Bloomston 2010). FLD is characterized by hepatic steatosis, inflammation and fibrosis. FLD is clinically classified into two broad entities, including alcoholic fatty liver disease (AFLD) and non-alcoholic fatty liver disease (NAFLD). The previous one is caused by excessive alcohol intake, and the later one is resulted from any non-alcohol etiologies (Reddy and Rao 2006). Disturbance of metabolic homeostasis seems to be one of the key events during the occurrence of FLD. Here we will discuss the roles of autophagy in hepatic metabolic homeostasis.

Hepatic autophagy occurs at the basal level and may be elevated under stressed conditions, thus contributing to the maintenance of normal hepatocyte functions and responding to pathogenic changes in the liver (Yin et al. 2008). Autophagy used to be considered as a bulk degradation process that is non-selective. However, recent evidence suggests that autophagy can be highly selective, especially during such processes as the removal of protein aggregates, damaged organelles, and intracellular pathogens (Okamoto 2014; Stolz et al. 2014). Studies have shown that autophagy not only can modulate cellular energy stores like lipids and carbohydrates, but also could target dysfunctional or superfluous organelles as well as toxic protein aggregates (Mizushima and Klionsky 2007). Based on the targets, several selective types of autophagy have been described, including aggrephagy (targets to aggregated proteins), mitophagy (targets to the damaged mitochondria), reticulophagy or ER-phagy (targets to the ER), pexophagy (targets to peroxisomes), lipophagy (targets to lipid droplets),

ferritinophagy (targets to ferritin), and xenophagy (targets to intracellular microorganisms) (Stolz et al. 2014). Several autophagic adaptor proteins have been found to act as autophagy adaptors, such as p62/SQSTM1, NBR1 (neighbor of BRCA1 gene 1), NDP52 (nuclear domain 10 protein 52 kDa), and OPTN (Optineurin), which bind to the cargo (usually ubiquitinated) and some key components of the autophagy machinery like the LC3 protein (Rogov et al. 2014; Johansen and Lamark 2011; Stolz et al. 2014). Failure to eliminate potentially dangerous substrates in the liver disturbs cellular homeostasis and subsequently results in hepatic diseases (Czaja et al. 2013). In mouse models with hepatic deletion of Atg7 or Atg5, protein aggregates and subcellular organelles are massively accumulated, leading to liver enlargement, severe liver injury, inflammation, fibrosis, cirrhosis, and tumorigenesis (Komatsu et al. 2005; Takamura et al. 2011; Ni et al. 2014).

In general, autophagy plays a role in hepatic metabolic homeostasis in three ways (Fig. 1), promoting nutrient recycle, removing abnormal organelles and toxic protein aggregates, and altering the level of metabolic factors (Christian et al. 2013; Kim and Lee 2014). The liver can actively participate in several parts of lipid metabolisms, such as fatty acids (FAs) uptake, de novo synthesis, and β-oxidation; cholesterol synthesis and biotransformation into bile acid; lipoprotein uptake and secretion (Singh et al. 2009). The storage form of lipids is named lipid droplets (LDs). Accumulating evidence has revealed an important role for autophagy in LDs breakdown. Inhibition of autophagy by either 3-methyladenine (an autophagy inhibitor) or RNA interference against Atg5 or Atg7 increases triglyceride level and LD number and size in both cultured hepatocytes and mouse livers (Singh et al. 2009). Mice deficient in Atg5 in the liver also shows excessive hepatic lipid accumulation (Ni et al. 2014). In addition to the effects on lipid metabolism, the role of autophagy in hepatic carbohydrate metabolism has been shown to affect gluconeogenesis and glycogen storage (Kim and Lee 2014). In hepatic Atg7-deficient mice, the absence of amino acid release by autophagy affects gluconeogenesis during starvation (Ezaki et al. 2011). Atg7 is also necessary for statin-induced gluconeogenesis in the liver (Wang et al. 2015a). Nonetheless, in tissue-specific Atg5-deficient mice, another study suggested that Atg5-related autophagy did not influence gluconeogenesis, but may affect ketogenesis during starvation (Takagi et al. 2016). Other than gluconeogenesis, autophagy is also involved in glycogen breakdown in newborn hepatocytes of the rat and skeletal muscles of *Drosophila melanogaster* (Zirin et al. 2013; Kotoulas et al. 2006). However, a decrease or no change in hepatic glycogen content was observed in other studies with hepatic Atg7- or VPS34-deficient mice (Komatsu et al. 2005; Jaber et al. 2012; Ezaki et al. 2011). These contradictive studies imply differential roles of ATG genes in carbohydrate metabolism, which still need to be clarified for different circumstances.

Carbohydrates supply energy at the beginning of fasting in most organisms, followed with breakdown of proteins to supply substrates for gluconeogenesis (Mizushima and Klionsky 2007). When these stores are exhausted, cells start to employ autophagy to reuse existing macromolecules. During starvation, about 30% of hepatic proteins are decreased in wild-type mice in 24 h of fasting, which becomes insignificant in conditional knockout mice of Atg7 (Mizushima and Klionsky 2007; Komatsu et al. 2005). In fact, protein turnover via autophagy is related to multiple hepatic pathophysiological conditions, including the clearance of misfolded mutant proteins in alpha-1-antitrypsin

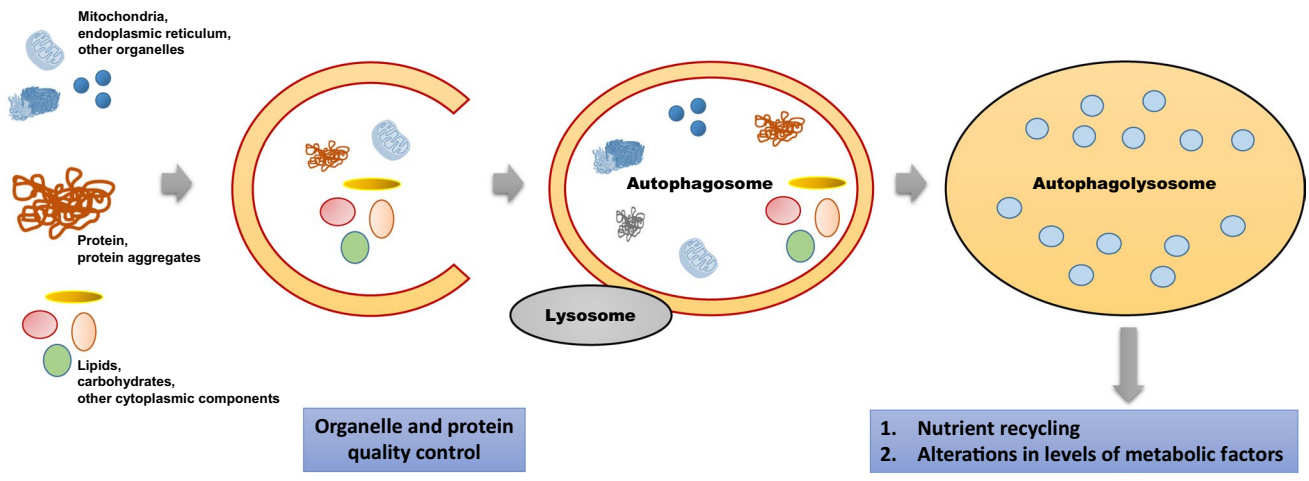

Fig. 1 Roles of autophagy in metabolic homeostasis. Autophagy plays a key role in hepatic metabolic homeostasis in three ways: nutrient recycle, clearance of abnormal organelles and toxic protein aggregates, and regulation of the level of metabolic factors

deficiency (Puri and Chandra 2014; Hidvegi et al. 2010; Yamamura et al. 2014), and in the clearance of the inclusion bodies (Strnad et al. 2008; Zatloukal et al. 2007; Harada et al. 2008).

To maintain nutrients homeostasis, autophagy has been suggested to assist the quality control of organelles, a process also known as organellophagy (Okamoto 2014). As mentioned above, organellophagy could be classified into different forms depending on the target organelles, including mitophagy, reticulophagy, pexophagy, etc. Mitophagy is important to remove defective and unhealthy mitochondria, which can boost cellular oxidative stress and may induce cell death via apoptosis. Recent studies have shown that the PINK1/Parkin pathway of mitophagy is important to mitochondrial homeostasis regulation (Eiyama and Okamoto 2015; Wei et al. 2015; Ding et al. 2010b; Zhang 2013). Another important organellophagy is reticulophagy, also called ER-phagy, which is a process that degrades defective or excessive ER by delivering them to the lysosomes. Xenobiotic substances, like drugs and chemicals, are mostly processed in the liver by a variety of enzymes. These enzymes are associated to hepatocyte ER, the proliferation of ER usually occurs in the liver after being exposed to xenobiotics (Komatsu et al. 2005; Yang et al. 2016). Autophagy is known to be part of the ER quality control (ERQC) system via both micro- or macro-ER-phagy, using p62 as an adaptor or requiring core autophagy-related proteins, respectively (Schuck et al. 2014; Khaminets et al. 2015; Ding and Yin 2008; Lipatova and Segev 2014, 2015; Lipatova et al. 2013). Another common organellophagy is pexophagy, a type of autophagy that participates in the quality control of peroxisomes, which is important to lipid metabolism and hydrogen peroxide production and degradation (Nordgren et al. 2013). Several proteins that are involved in peroxisome biogenesis, e.g., peroxins (encoded by PEX genes), including Pex3, Pex5, and Pex14, are suggested to participate in macropexophagy (Sakai et al. 2006; Manjithaya et al. 2010; Neuhaus et al. 2014; Subramani 2015). An oncoprotein, hypoxia-inducible factor 2α (HIF2α), has also been indicated to induce hepatocyte pexophagy (Walter et al. 2014). All these observations indicate the critical role of autophagy in cellular metabolic homeostasis.

Relevance of autophagy to liver diseases

The liver has the highest protein turnover rate compared to other organs. In mouse livers, protein degradation rate increases from 1.5% of total liver protein per hour to 4.5% under the starving condition, and 40% of total protein would be degraded after a 48-h starvation (Schworer et al. 1981; Mortimore et al. 1983). Previous researches suggested that autophagy assists the degradation of protein in the liver. In mice deficient in Atg5 or Atg7 in the liver, severe hepatomegaly is observed with an abundant amount of polyubiquitinated proteins and with an increased amount of defective mitochondria and peroxisomes (Rusten and Stenmark 2010; Takamura et al. 2011).

Autophagy is important to liver diseases. Recent studies have shown that autophagy is involved in the life cycle of Hepatitis C and B (Virus HCV and HBV). HBV could stimulate autophagy by the X protein (HBx) and the small surface protein (SHBs) (Tang et al. 2009). Inhibition of autophagosome formation could significantly inhibit HBV production, and the induction of autophagy markedly contributed to HBV production (Mao et al. 2011). Meanwhile, HBx could impair lysosomal acidification, possibly through interaction with V-ATPase, which results in a drop in lysosomal degradative capacity and the accumulation of immature lysosomes (Liu et al. 2014). HCV has also been reported to induce autophagy, but to suppress autophagic protein degradation by inhibiting the fusion of autophagosomes and lysosomes (Ait-Goughoulte et al. 2008; Sir et al. 2008). HCV and HBV can also induce intracellular events that trigger the mitochondrial dysfunction leading to mitophagy induction (Kim et al. 2013c).

Autophagy has been suggested to play various roles in tumorigenesis and tumor growth (Brech et al. 2009; White et al. 2011). Hepatic deficiency of Atg5 or Atg7 can lead to the development of hepatocellular adenomas with mitochondrial swelling, accumulation of p62 protein aggregates, oxidative stress, and genomic in the tumor cells (Takamura et al. 2011; Ni et al. 2014). Multiple tumors including liver tumors are also observed in mice with Beclin haplo-insufficiency (Qu et al. 2003; Yue et al. 2003). In contrast, autophagy has been indicated to be an important surviving mechanism for cancer, and in a rat tumor model of *N*-diethylnitrosamine-induced hepatocarcinogenesis, the inhibition of autophagy by chloroquine at the tumor-forming stage could restrain tumor formation (Sun et al. 2013). Autophagy is also critical for the invasion and metastasis of hepatocellular carcinoma (HCC) (Li et al. 2013a; Peng et al. 2013).

Liver-specific deletion of Atg5 or Atg7 causes hepatocyte hypertrophy and liver injury in mice (Takamura et al. 2011; Komatsu et al. 2005; Ni et al. 2014). SQSTM1/p62 is significantly increased in the liver of mice with hepatic autophagy deficiency, which weakens the interaction between Kelch-like ECH-associated protein 1 (Keap1) and nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 is thus dissociated from Keap1 and enters into the nucleus, resulting in the transcriptional activation of Nrf2 target molecules, including antioxidant proteins and detoxifying enzymes (Komatsu et al. 2010). Co-deletion of p62/SQSTM1 or Nrf2 in the liver ameliorates the liver injury

from autophagy deficiency (Komatsu et al. 2007, 2010; Ni et al. 2014). However, Keap1 co-deletion further exacerbates the injury (Komatsu et al. 2010). Progressive accumulation of extracellular matrix (ECM) occurs in chronic liver injury due to the activation of hepatic myofibroblasts, which then causes hepatic fibrosis (Mederacke et al. 2013). Hepatic stellate cells (HSC) are suggested to be the dominant contributor to the myofibroblast pool in a variety of hepatic pathological conditions, including toxic, biliary, and fatty liver fibrosis (Mederacke et al. 2013). Autophagy is increased in HSC isolated from murine fibrotic livers, or from liver specimens of patients chronically infected with Hepatitis B Virus (Hernandez-Gea et al. 2012). Autophagy flux is also increased during HSC activation in vitro and pharmacological blockage of autophagy inhibited the HSC activation (Thoen et al. 2011). Additionally, HSCs-specific deletion of Atg7 in mice attenuated fibrosis after carbon tetrachloride or thioacetamide induced sustained liver injury (Hernandez-Gea et al. 2013). All of these pieces of evidence indicate a critical role of autophagy during hepatic fibrosis.

AFLD

Typical pathological features of AFLD include steatosis, inflammation, fibrosis, and cirrhosis, which are considered to be related to oxidative stress resulted from acetaldehyde accumulation, increased NADH/NAD + ratio, or reactive oxidative species (ROS) generation (Lumeng and Crabb 2000; Lieber 2004; Zakhari and Li 2007). Oxidative stress may be involved in the functional and structural changes of mitochondria during the progress of AFLD, leading to the alteration of oxidative phosphorylation, increase of mitochondrial DNA damage, and changes of mitochondrial protein profiles (Bailey and Cunningham 2002; Demeilliers et al. 2002; Mansouri et al. 1999, 2001; Cahill et al. 1999, 2002). The increase of oxygen free radicals, together with lipids accumulation caused by ethanol metabolism, leads to lipid peroxidation that can enhance oxidative damage in AFLD (Wang et al. 2015b). Autophagy induced by ethanol seems to be selective for damaged mitochondria and accumulated lipid droplets, but not long-lived proteins (Ding et al. 2010a). Stimulating or repressing autophagy could correspondingly ease or exacerbate hepatic steatosis in both acute and chronic AFLD (Ding et al. 2010a; Lin et al. 2013).

Effects of autophagy on AFLD can vary in different pathological stages. Hepatic autophagy is activated in vivo and in cultured primary hepatocytes after acute alcohol treatment (Ding et al. 2010a; Thomes et al. 2012). This activation requires ethanol metabolism, reactive oxygen species, the alteration of signaling pathway, and some other mechanisms. Oxidative stress is one stimulus for autophagy in AFLD. Acetaldehyde, a major ethanol metabolite and a pro-oxidant as well, has been indicated to induce autophagy (Thomes et al. 2013), and suppression of ethanol-induced autophagy by anti-oxidants, such as N-acetyl cysteine (NAC), has been also observed (Ding et al. 2010a; Wu et al. 2012). Inhibition of mTOR signaling and activation of AMPK have been indicated to participate in autophagy induced by ethanol under oxidative stress (Sid et al. 2013; Ding et al. 2011). In addition to this, ethanol treatment also activates FoxO3a, which transcriptionally regulates several autophagy genes (Ni et al. 2013). Other autophagy stimulators, including ethanol-induced proteasome inhibition, ER stress, and metal elements like zinc, also participate in autophagy alteration after ethanol treatment (Ding and Yin 2008; Ding et al. 2007; Liuzzi and Yoo 2013).

Autophagy may be suppressed in chronic AFLD. In mice fed with Lieber-DeCarli diet for 4 weeks, autophagy was elevated at a lower dose of ethanol, accounting for 29% of the caloric need, but was inhibited at a higher ethanol dose (accounting for 36% of the caloric need) (Lin et al. 2013). Decrease in both the amount and the function of lysosomes was found in chronic alcohol-treated rat livers, which may contribute to the autophagy suppression (Kharbanda et al. 1996, 1997; Dolganiuc et al. 2012). However, the liver injury is worse when autophagy is suppressed while enhancement of autophagy improves the condition (Lin et al. 2013). A histological character of chronic AFLD is the development of Mallory–Denk body (MDB). MDB is ubiquitin and SQSTM1/p62 positive, and is accumulated more significantly in autophagy deficiency. MDB can be better cleaned using rapamycin, an mTOR inhibitor and autophagy activator, which further supported the connection between MDB formation and autophagy and the suppression of autophagy in chronic AFLD (Harada et al. 2008).

NALFD

NAFLD, which accounts for 75% of all chronic liver diseases, shares several similar pathological changes with AFLD, such as steatosis, inflammation, fibrosis, and cirrhosis (Clark et al. 2003; Ruhl and Everhart 2013). However, it is more prevalent than AFLD, and can occur with obesity and diabetes (Tiniakos et al. 2010). No effective therapeutic approaches for NAFLD have been found. Studies have shown that autophagy can be involved in the pathogenesis of NAFLD. NAFLD can also affect autophagy. Severe hepatic steatosis was observed in mice with a hepatic deletion of either Atg5 or Atg7, and high-fat diet seems to exacerbate the condition (Singh et al. 2009; Ni et al. 2014; Kim et al. 2013b). Hepatic autophagy can be inhibited in both genetic and dietary obesity models, which is partially resulted from a reduction in the expression of

key autophagy molecules like Atg7 (Yang et al. 2010). ER stress is found in both autophagy-deficient MEF cells and in vivo Atg7 know-down mice, which could be improved by the restoration of Atg7 expression (Yang et al. 2010). Hepatic autophagy is also involved in the stimulation of fatty acid β-oxidation via thyroid hormone (TH), and suppression of autophagy dramatically decreased fatty acid β-oxidation in both cultured cells and mouse models (Sinha et al. 2012).

A number of signal pathways and regulators are found to regulate autophagy in NAFLD, including the insulin-mTOR signaling pathway (Liu et al. 2009). Steatosis can impair autophagy at different levels. SQSTM1/p62 accumulation is observed in the liver of ob/ob mice, and its aggregation is correlated with serum alanine aminotransferase activity and inflammatory activity by NAFLD score (Inami et al. 2011; Fukuo et al. 2014). Further studies have shown a decrease of lysosome proteolytic activity accompanied with the reduction of hepatic levels of cathepsin B and L in both ob/ob mice and NAFLD patients, which suggests that the deficiency of lysosomes may affect autophagic degradation and may contribute to SQSTM1/p62 accumulation (Inami et al. 2011; Fukuo et al. 2014). Additionally, fusion of isolated hepatic lysosomal and autophagosomes was different in mice fed with high-fat diet and regular diet, indicating that an excessive amount of lipids can suppress autophagosome/lysosome fusion (Koga et al. 2010). Furthermore, high-fat diets seem to suppress the expression of certain key ATG genes at the transcriptional level, which may attribute to the activity of transcriptional factors like FoxO1 (Liu et al. 2009; Xiong et al. 2012). Abnormal activation of proteases, such as calpain, may also be involved in the suppression of autophagy by reducing the protein level of certain key autophagy molecules, such as Atg5, Atg7, and Beclin 1 (Yang et al. 2010).

Autophagy can also affect liver injury during NAFLD. Autophagy can be a stress adaptation pathway that avoids cell death and suppresses, although it serves as an alternative cell death pathway in specific cellular settings (Maiuri et al. 2007). In mice with a hepatic deletion of either Atg5 or Atg7, serum alanine aminotransferase activity is increased and inflammatory cells are accumulated in the liver (Ni et al. 2014; Komatsu et al. 2005), which indicates that autophagy is critical to protect hepatocytes against cellular injury. Inhibiting autophagy in cultured hepatocytes by knocking down Atg5 sensitizes cells to death from superoxide-mediated oxidative stress, which may partly attribute to the activation of c-Jun N-terminal kinase (JNK) pathway (Wang et al. 2010). Another study has also shown autophagy could protect hepatocytes against the injury induced by lipopolysaccharide (LPS) in mice with an inducible hepatocyte-specific knockout of Atg7 (Lalazar et al. 2016). The observation may be resulted from a defect in Akt signaling in response to LPS, which sets the condition in which the autophagy-deficient hepatocytes were sensitized to tumor necrosis factor (TNF)-dependent liver damage (Amir et al. 2013; Lalazar et al. 2016). During the development of NAFLD, hepatic JNK and hepatocyte death receptor pathways are activated and autophagy activation can prevent apoptosis from TNF and Fas (Czaja 2016). These pieces of evidence suggest that suppression of autophagy may promote liver injury in NAFLD. However, due to limited evidence of the effects of autophagy on liver injury in in vivo NAFLD models, the precise roles of autophagy in the development of NAFLD and related mechanisms still require further studies.

By affecting innate immunity, autophagy can modulate inflammatory response by sequestering microorganisms and regulating pathogen recognition receptors like the toll-like receptors (TLRs) (Czaja 2016). Macrophage autophagy can be impaired in primary bone marrow-derived macrophages (BMDM) and peritoneal macrophages from mice fed with high-fat diet (Czaja 2016). Mice with Atg5 deficiency in macrophage, when treated with LPS after high-fat diet feeding, developed systemic and hepatic inflammation. Abnormalities in macrophage polarization were observed in BMDM and Kupffer cells with an increase of proinflammatory M1 and a decrease of anti-inflammatory M2 polarization (Liu et al. 2015). This study showed a possible connection between macrophage autophagy and hepatic inflammation in obese mice, which provided a novel view of therapeutic methods for NALFD.

Liver fibrosis usually occurs in the liver with chronic injury, which is mostly attributed to the activation of hepatic myofibroblasts, hepatocytes apoptosis and/or necroptosis, and sustained hepatic inflammation (Mallat et al. 2014). The extent of fibrosis is the most important clinical determinant in patients with non-alcoholic steatohepatitis (NASH) (Czaja 2016). Loss of Atg5 in hepatocytes can induce liver fibrosis in mice (Ni et al. 2014); furthermore, mice with mutations in Atg5 in the myeloid lineage also show more susceptibility to liver fibrosis in chronic carbon tetrachloride treatment (Lodder et al. 2015). In contrast to the effects of autophagy on live fibrosis in hepatocytes and macrophages, autophagy plays a role during stellate cell activation and the development of liver fibrosis (Hernandez-Gea et al. 2012; Thoen et al. 2011). These studies provide a potential connection between autophagy and liver fibrosis. However, the degree of HSC autophagy in murine or human NAFLD is still unclear. Whether autophagy is a friend or foe in liver fibrosis in the pathogenesis of NAFLD still needs to be clarified.

Therapeutic effects of autophagy modulators on FLDs

The excessive accumulation of fatty acids is one of the main pathological features of fatty liver diseases. The reduction of hepatic triglyceride levels and the improvement of hepatic functions by autophagy activation suggest a potential therapeutic application of autophagy regulators for FLD (Singh et al. 2009; Ding et al. 2010a; Lin et al. 2013). Indeed, in both AFLD and NAFLD mouse models, clinically available chemicals that induce autophagy like rapamycin and carbamazepine have shown the anticipated effects (Lin et al. 2013). As more key targets in the autophagy pathway have been identified, the number of novel therapeutic agents targeting autophagy has been increased as well (Cheng et al. 2013). In this part, we will discuss the therapeutic effects of autophagy modulators especially some new agents which have been indicated to affect FLD.

Autophagy inhibitors

Mechanisms of action

The evolutionarily conserved function of autophagy in helping cell survival during metabolic stress has led to therapeutic resistance to cytotoxic therapy (Rubinsztein et al. 2012). Therefore, several studies have undertaken to determine therapeutic targets of autophagy and some of the inhibitors of autophagy are already in clinical trials. The major step in affecting autophagy initiation is the membrane nucleation, which is controlled by Beclin1, Vps34, and ULK complex. Several ULK1 inhibitors such as MRT67307, MRT68921, and SBI-0206965 have been tested in in vitro systems for their autophagy inhibition potency, no detailed information about their application to in vivo autophagy inhibition is available yet (Egan et al. 2015; Petherick et al. 2015). In addition, wortmannin, 3-methyl adenine (3-MA), and LY294002 [2-(4-morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one hydrochloride] are common inhibitors that interfere with the formation of autophagosomes by targeting the activity of Vps34 (Petiot et al. 2000; Pasquier 2016).

In the later stage of the autophagic process, chemicals that inhibit lysosome acidification can block the formation of autolysosomes and, therefore, the autophagic degradation by suppressing the function of lysosome (Rubinsztein et al. 2012). Pepstatin A and Cathepsin proteases inhibitor E64d are the well-known acid protease inhibitors that can effectively suppress autophagy (Li et al. 2013b). In addition, inhibitors like bafilomycin A1 can inhibit V-ATPases by blocking the proton transport and then inhibit the autophagy process (Rubinsztein et al. 2012). Furthermore, a number of preclinical reports now have shown that the two lysosomotropic agents known as chloroquine and hydrochloroquine effectively inhibit autophagy pathway by increasing pH inside the lysosome (Homewood et al. 1972; Pasquier 2016; Slater 1993; O'Neill et al. 1998).

Compounds inhibiting autophagy in liver diseases

Modulation of autophagy can be a novel therapeutic target for the treatment of HCC (Amaravadi et al. 2011). Combination of a number of cancer drugs with autophagy inducers as for example rapamycin, sorafenib (a tyrosine kinase inhibitor), NPC-16, and cannabinoids has been used to target cancer cells. Autophagy was found to be upregulated in metastatic HCC (Peng et al. 2013). Use of inhibitors of autophagy such as chloroquine and hydrochloroquine in combination of therapeutic drugs may be a better option for treating metastatic HCC.

There is evidence showing that autophagy suppression might be harmful in fatty liver diseases (Gracia-Sancho et al. 2014; Ding et al. 2010a; Lin et al. 2013). However, a PI3K–Akt pathway inhibitor, wortmannin, was indicated to have a dual effect on AFLD in mice (Zeng et al. 2012). In this earlier study, low dose of wortmannin significantly reduced the hepatic triglyceride level in an acute ethanol-induced fatty liver mouse model, but aggravated fatty liver at a higher dose treatment. The contradictory findings may be resulted from the effects of the compounds other than autophagy inhibition, but the exact mechanisms still need to be further explored.

Autophagy activators

Discovering new therapeutic interventions to treat lipid disorders is of great interest and the discovery of autophagy as a regulator of lipid metabolism has opened up new avenues for targeting modulators of this pathway. The primary rationales on developing autophagy inducers for the treatment of fatty liver comes from the observation that genetic defect in autophagy gene causes accumulation of fat in hepatocytes (Singh et al. 2009). Meanwhile, autophagy gene therapy via adenovirus-associated viral delivery in liver tissue resulted in significant reduction in hepatic steatosis in Ob/Ob mice model (Yang et al. 2010). Moreover, pharmacological enhancement of autophagy with inducers such as rapamycin and carbamazepine reduced hepatic and blood triglycerides, blood glucose levels and markers of hepatic damage in high-fat-fed rodent model (Lin et al. 2013). All of these observations suggest that upregulation of autophagy could be clinically beneficial.

Autophagy inducers can be divided into two categories—non-pharmaceutical interventions and pharmacological activators. Non-pharmaceutical interventions include the long-term caloric restriction and regular exercise that strongly upregulate autophagy and improve the overall health (Cui et al. 2013). Nutritional supplements such as consumption of coffee and vitamin D may also influence health through autophagy induction. A previous study showed that caffeine could reduce hepatic steatosis by stimulating autophagy in mice with non-alcoholic FLD (Sinha et al. 2014). Pharmacological activators can be those approved by US Food and Drug Administration (FDA) or those identified through high-throughput chemical screening, which are less characterized and not suitable for immediate clinical use.

Mechanisms of action

Various strategies have been utilized for inducing autophagy. Both mTOR-dependent and -independent pathways are considered.

mTOR-dependent autophagy inducers This group of inducers includes rapamycin and rapalogs, such as CCI-779, RAD001, and AP23573. They are basically lipophilic macrolide antibiotics that inhibit mTOR1 Complex 1 (Noda and Ohsumi 1998). Recently, two selective ATP-competitive small molecule mTOR inhibitors, PP242 and Torin 1, have been identified, which directly inhibit both mTORC1 and mTORC2 (Thoreen et al. 2009). Moreover, a new class of stronger inducer of autophagy, PI-103 and NVP-BEZ235 (imidazo[4,5-c]quinoline derivative), has also been reported, which dually inhibits mTORC and class I PI3 K–AKT signaling (Degtyarev et al. 2008; Maira et al. 2008).

mTOR-independent autophagy inducers Metformin, which is widely used for diabetes treatment, is one of the most popular inducers in this category. Metformin activates AMPK, which in turn increases autophagy through mTOR inhibition and direct ULK1 activation (Alers et al. 2012). Some other reagents, including carbamazepine, lithium, and sodium valproate, are another category of mTOR-independent autophagy inducers. They reduce intracellular levels of inositol and inositol-1, 4, 5-triphosphate (Ins (1, 4, 5) P3) and positively regulate autophagy (Sarkar et al. 2005; Williams et al. 2008). Carbamazepine has been shown to have beneficial effects in mouse models of high-fat diet-induced steatosis, and of α1-antitrypsin deficiency (Lin et al. 2013; Hidvegi et al. 2010). The third group of inducers include FDA approved drugs and pharmacological probes, such as rilmenidine, clonidine, and verapamil, which induce autophagy by targeting the pathways involved in cyclic AMP (Williams et al. 2008; Rose et al. 2010). Other examined inducers of autophagy include BH3 mimetics, resveratrol, trehalose, spermidine, EGFR agonists, and L-NAME (Rubinsztein et al. 2012). Among these inducers, BH3 mimetics is well known to disrupt the inhibitory interaction between the BH3 domain of beclin-1 and BCL-2, stimulating the beclin-1-dependent allosteric activation of the pro-autophagic lipid kinase PIK3C3 (Malik et al. 2011).

Compounds targeting autophagy for liver diseases

Currently there is no approved pharmacological therapy for FLD (Musso et al. 2012). However, a number of novel agents specifically targeting non-alcoholic FLD pathogenesis have entered into clinical trials. Some mTORC1 and mTORC2 inhibitors, including rapamycin, AZD3147, Z1001, rottlerin, and XL388, are in preclinical stage of drug development (Musso et al. 2016). AMPK activators such as monascin, ankaflavin, quercetin, berberine, curcumin, and oltipraz are also in developmental stage for the NAFLD treatment. Among the AMPK activators, oltipraz is currently at phase IIa state of development (Musso et al. 2016). Better understanding of the autophagy role in the pathogenesis of fatty liver (both alcoholic and non-alcoholic) disease and simultaneous preclinical or clinical studies of the current or emerging pharmacological inducers is necessary to develop and identify the promising molecules for applications in FLDs.

Future directions

Although recent studies have elucidated the molecular mechanism of autophagy and the role of autophagy in FLDs, most of recent studies are focusing on potential roles of autophagy in hepatocytes. Only a few studies have explored the effects of autophagy in hepatic non-parenchymal cells on the pathogenesis of FLDs. Accumulating evidences have shown that autophagy in non-parenchymal cells is critical to the pathogenesis of liver diseases, like liver fibrosis (Thoen et al. 2011, 2012; Lodder et al. 2015). Further understanding of the roles of autophagy in hepatic non-parenchymal cells during the pathogenesis of FLDs would be important. Cautions have to be exercised that autophagy may play different roles in hepatocytes and hepatic non-parenchymal cells, which result in opposite effects on FLDs (Czaja 2016). Agents that can target autophagy in a tissue-specific way would be most useful for the treatment of FLD in future.

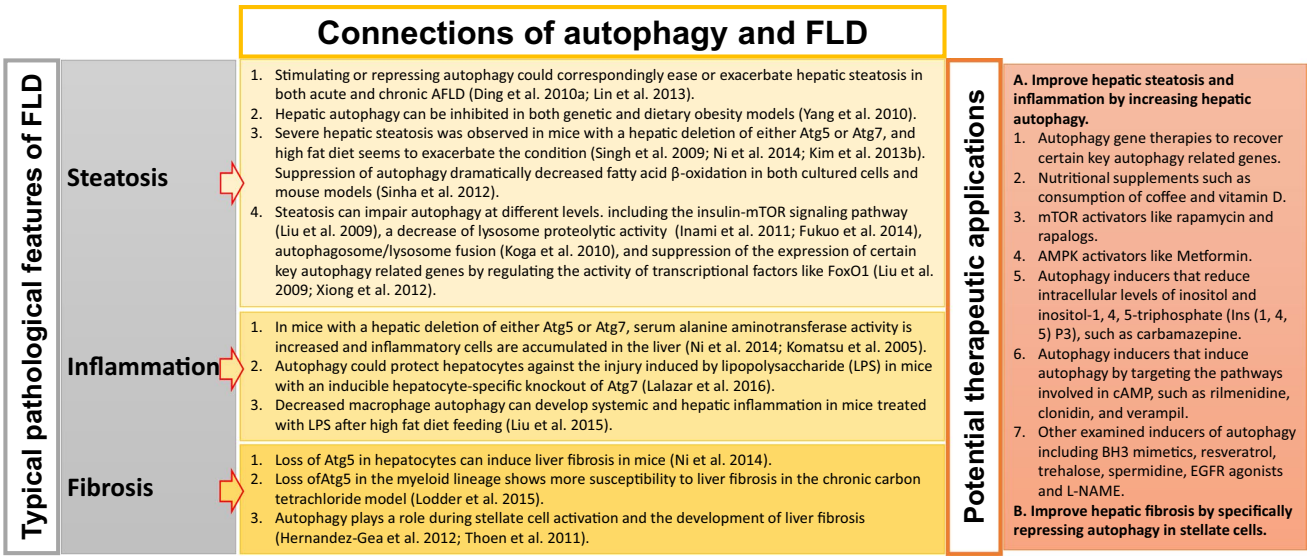

Fig. 2 Relationship of autophagy and fatty liver diseases at different stages, and potential therapeutic applications of autophagy modulators in treating fatty liver diseases

Conclusion

A basal level of autophagy is essential for the maintenance of normal liver function. Accumulating evidence from various FLD models suggests FLDs can affect the autophagic process, and conversely autophagy can affect the pathogenesis of FLDs. Early studies have opened up the avenue for potential therapeutic applications of autophagy regulators in FLDs. Indeed, various modulators of autophagy have been suggested to be beneficial to ameliorate the fatty livers. The connection between autophagy and FLDs at different stages, and potential therapeutic applications of autophagy modulators are summarized in Fig. 2. We hope that this summary will be helpful in the further exploration of the role of autophagy in FLDs, and the use of autophagy modulators to alleviate FLDs.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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