

Autophagy: a new target for nonalcoholic fatty liver disease therapy

Yuqing Mao¹
Fujun Yu¹
Jianbo Wang²
Chuangyong Guo³
Xiaoming Fan¹

¹Department of Gastroenterology and Hepatology, Jinshan Hospital of Fudan University, Shanghai, ²Department of Gastroenterology and Hepatology, The Central Hospital of Lishui City, Wenzhou Medical University, Zhejiang, ³Department of Gastroenterology and Hepatology, Shanghai Tenth People's Hospital, Tongji University, Shanghai, People's Republic of China

Abstract: Nonalcoholic fatty liver disease (NAFLD) has gained importance in recent decades due to drastic changes in diet, especially in Western countries. NAFLD occurs as a spectrum from simple hepatic steatosis, steatohepatitis to cirrhosis, and even hepatocellular carcinoma. Although the molecular mechanisms underlying the development of NAFLD have been intensively investigated, many issues remain to be resolved. Autophagy is a cell survival mechanism for disposing of excess or defective organelles, and has become a hot spot for research. Recent studies have revealed that autophagy is linked to the development of NAFLD and regulation of autophagy has therapeutic potential. Autophagy reduces intracellular lipid droplets by enclosing them and fusing with lysosomes for degradation. Furthermore, autophagy is involved in attenuating inflammation and liver injury. However, autophagy is regarded as a double-edged sword, as it may also affect adipogenesis and adipocyte differentiation. Moreover, it is unclear as to whether autophagy protects the body from injury or causes diseases and even death, and the association between autophagy and NAFLD remains controversial. This review is intended to discuss, comment, and outline the progress made in this field and establish the possible molecular mechanism involved.

Keywords: nonalcoholic fatty liver disease, autophagy, steatosis, steatohepatitis, fibrosis, carcinogenesis

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common hepatic disease in Western countries, and is defined as evidence of hepatic steatosis, either by imaging or histology, with no cause for secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication, or hereditary disorders.¹ The prevalence of NAFLD is 20%–30% in Western countries,² which causes considerable concern with its progression from steatosis to steatohepatitis, cirrhosis, and even hepatocellular carcinoma (HCC; Figure 1). NAFLD is associated with hepatic insulin resistance and type 2 diabetes.³ Recent studies have revealed that patients with NAFLD are at a higher risk of developing coronary atherosclerosis, which might be an independent risk factor.⁴ The mechanisms involved in the onset of NAFLD are complicated and are not entirely clear at present. Recent advances in the understanding of the molecular processes contributing to autophagy have provided insight into the relationship between autophagy and NAFLD. Autophagy might stimulate lipid metabolism,⁵ and thus has therapeutic potential in NAFLD.⁶

Correspondence: Chuangyong Guo
Department of Gastroenterology and Hepatology, Shanghai Tenth People's Hospital, Tongji University, 302 Yanchang Road, Jingan District, Shanghai 200072, People's Republic of China
Tel +86 21 6630 2535
Fax +86 21 6630 3983
Email guochuangyong@hotmail.com

Xiaoming Fan
Department of Gastroenterology and Hepatology, Jinshan Hospital of Fudan University, 1508 Longhang Road, Jinshan District, Shanghai 201508, People's Republic of China
Tel +86 21 3418 9990
Fax +86 21 5794 3141
Email xiaomingfan57@hotmail.com

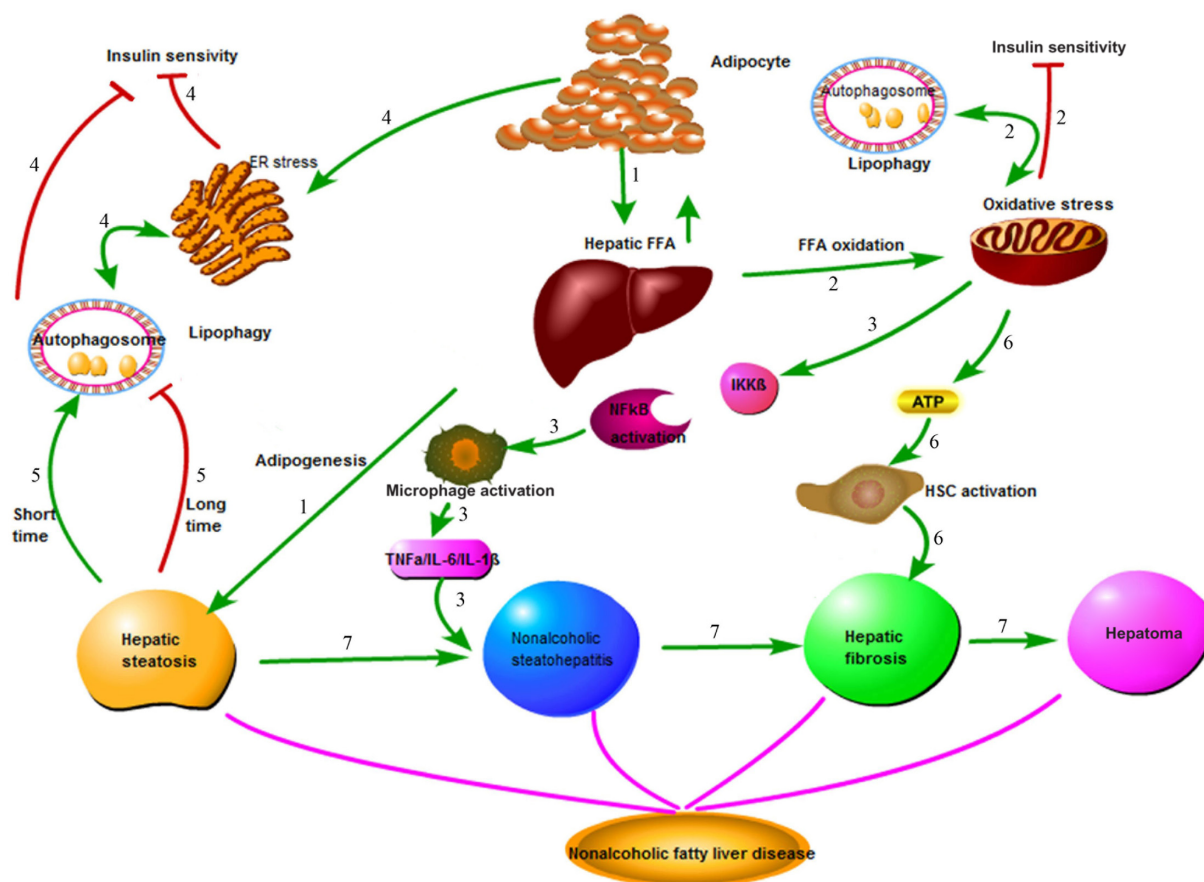


Figure 1 Pathophysiology of nonalcoholic fatty liver disease (NAFLD).

Notes: 1. Adipocyte mobilization increases serum concentration of FFA and de novo synthesis of triglyceride. 2. Increased FFA oxidation in the mitochondria exceeds the capacity of the mitochondria, causing oxidative stress, which impairs insulin sensitivity and autophagy activation to digest defective proteins. 3. Oxidative stress promotes phosphorylation and degradation of mesenchymal IKK β , accompanied by activated NF- κ B translocation into the nucleus, causing inflammatory cascades and liver injury. 4. Obesity causes ER stress, which causes insulin resistance and stimulates autophagy due to an increase in unfolded proteins. Defective autophagy aggravates ER stress. 5. Short-term insulin resistance activates autophagy while long-term lipotoxicity impairs autophagy. 6. Energy produced by autophagy and oxidation fuels HSC activation, which promotes hepatic fibrosis. 7. NAFLD develops from steatosis, steatohepatitis, fibrosis, and hepatoma.

Abbreviations: FFA, free fatty acid; ER, endoplasmic reticulum; HSC, hepatic stellate cells.

Autophagy

Autophagy is a critical intracellular pathway that targets defective organelles to the lysosomes for degradation, and this process is highly preserved in eukaryotic cells. As an initial protective mechanism, autophagy is usually triggered by starvation or damage to maintain survival.

Three different types of autophagy are defined based on how the target substrates are delivered to the lysosomes for degradation: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA).⁷ Macroautophagy is the major type of autophagy. Initially, a small vesicular sac called the isolation membrane is formed to enclose a portion of the cytoplasm or organelles, which results in the formation of a double-membrane structure named the autophagosome⁸ (Figure 2) The autophagosome fuses with the lysosome to form the autolysosome, which is involved in the degradation of defective or excessive organelles for energy recycling⁹ (Figure 3).

More than 30 autophagy-related genes (*Atgs*) have been discovered in yeast and play important roles in autophagy, many of which have mammalian homologs.¹⁰ Many molecules are involved in the formation and working process of autophagy. *Atg8/LC3*, *Atg7*, and *Atg6/Beclin-1* with the class III phosphoinositide 3-kinase (PI3K) complex are the best characterized. The process of autophagosome formation involves three major steps: 1) uncoordinated 51-like kinase (ULK) 1 helps in the double-membrane formation in the initiation phase; 2) nucleation with the Beclin-1/class III PI3K complex; and 3) elongation of the isolation membrane with the help of LC3 lipidation.^{8,11} Conjugation of yeast *Atg8* or mammalian LC3 with phosphatidylethanolamine (PE) during autophagy results in the formation of an insoluble form of *Atg8* (*Atg8-PE*) or LC3 (LC3-II), which contributes to autophagosome formation. *Atg8/LC3* stays on the membrane until it is degraded by lysosomes.

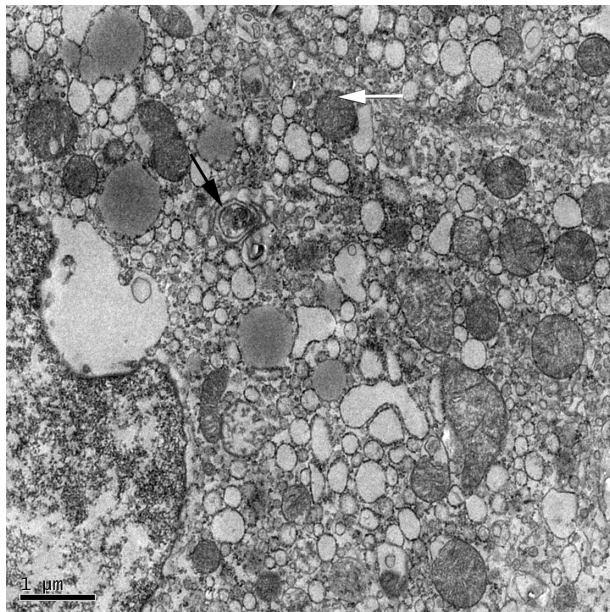


Figure 2 Electron micrograph showing ultrastructure of hepatocytes from a NAFLD mouse model.

Notes: Black arrow indicates autophagic vacuoles with a double-membrane structure that enclose lipid droplets and other defective organelles. White arrow shows autolysosome digesting contents. Original magnification: 100,000 \times .

Abbreviation: NAFLD, nonalcoholic fatty liver disease.

Therefore, *Atg8/LC3* is widely used as a marker for monitoring autophagy.¹²

Several signaling pathways are involved in the regulation of autophagy, among which, the class I PI3K/Akt/mTOR signaling pathway is the most classic one. PI3K promotes the phosphorylation of Akt, which subsequently leads to the activation of mTOR and the inhibition of autophagy. Conversely, inhibiting the activation of Akt induces a high level of autophagy, suggesting that class I PI3K/Akt/mTOR inversely regulates autophagy.¹³ When cells are under normal nutrient conditions, mTOR will be activated and phosphorylates ULK1, which subsequently combines with *Atg13* and FIP200 and results in the inhibition of autophagosome formation (Figure 3). Rapamycin or nutrient deprivation-induced inhibition of mTOR leads to dephosphorylation of ULK1, ULK2, and *Atg13*, and activates ULK to phosphorylate FIP200, resulting in the stimulation of autophagy.^{14,15} Another drug named 3-methyladenine (3-MA) inhibits autophagy by suppressing the class III PI3K/Beclin-1 signaling pathway, which positively regulates autophagy.¹² Furthermore, AMP-activated protein kinase (AMPK) plays a role in the regulation of autophagy. AMPK is activated by elevation of the AMP/ATP ratio¹² in accordance with upregulation of autophagy under conditions of starvation, as described in Figure 3. Initial studies also found that AMPK

is a positive regulator of autophagy both in the yeast¹⁶ and mammalian cells.¹⁷

Autophagy and steatosis

Autophagy reduces lipid droplets via lipophagy

Several studies have demonstrated that autophagy is closely associated with metabolism, and this has caused considerable concern recently. Autophagy is characterized by digesting damaged organelles that are used to generate energy and materials for cell recycling.¹⁸ Degradation of cellular lipid stores through autophagy (lipophagy) is another way to degrade lipid besides cytosolic lipases, which opens up a new understanding of lipid degradation.¹⁹ Increased levels of fatty acids sustain the rates of mitochondrial β -oxidation for generation of ATP.²⁰ However, excessive fatty acids will cause damage to mitochondria if they surpass the oxidation capacity of mitochondria. The increase in autophagy observed in a fatty liver can be confirmed by the presence of lipids enclosed within autophagic vacuoles and elevated expression of LC3-II, a bioprotein marker of autophagy.

Singh et al demonstrated that hepatocyte triglyceride content was significantly increased when challenged with fatty acid supplementation or cultured in methionine and choline-deficient medium with exogenous lipid supplementation treated with 3-MA, a pharmacological inhibitor of autophagy.¹⁹ Conversely, treatment with rapamycin, a pharmacological stimulator of autophagy, significantly decreases lipid droplets.¹⁹ It was reported recently that thyroid hormones, known to be important in promoting metabolism, probably stimulate fatty acid oxidation by induction of hepatic autophagy, which could be blocked by *Atg5* siRNA.²¹ Another study showed that treatment with caffeine reduced cellular lipid accumulation and the serum level of β -hydroxybutyrate, a product of lipolysis, and is accompanied with the increase of LC3-II. However, *Atg5* knockdown in hepatocytes significantly increased intracellular lipids and reduced β -hydroxybutyrate levels.²² Another study demonstrated that pharmacological intervention with rapamycin or carbamazepine (mTOR-independent inducer of autophagy) in high-fat diet (HFD)-fed mice obviously reduced hepatic and blood triglyceride, blood glucose and plasma insulin levels,²³ indicating that modulation of autophagy alleviates NAFLD via an mTOR-dependent or -independent pathway.²⁴ Autophagy removes lipid droplets not only in NAFLD but also in other factor-induced fatty liver diseases such as ethanol-induced steatosis.²⁵

All the studies^{5,12,19,20} mentioned earlier showed similar results that intracellular reduction in lipid droplets was

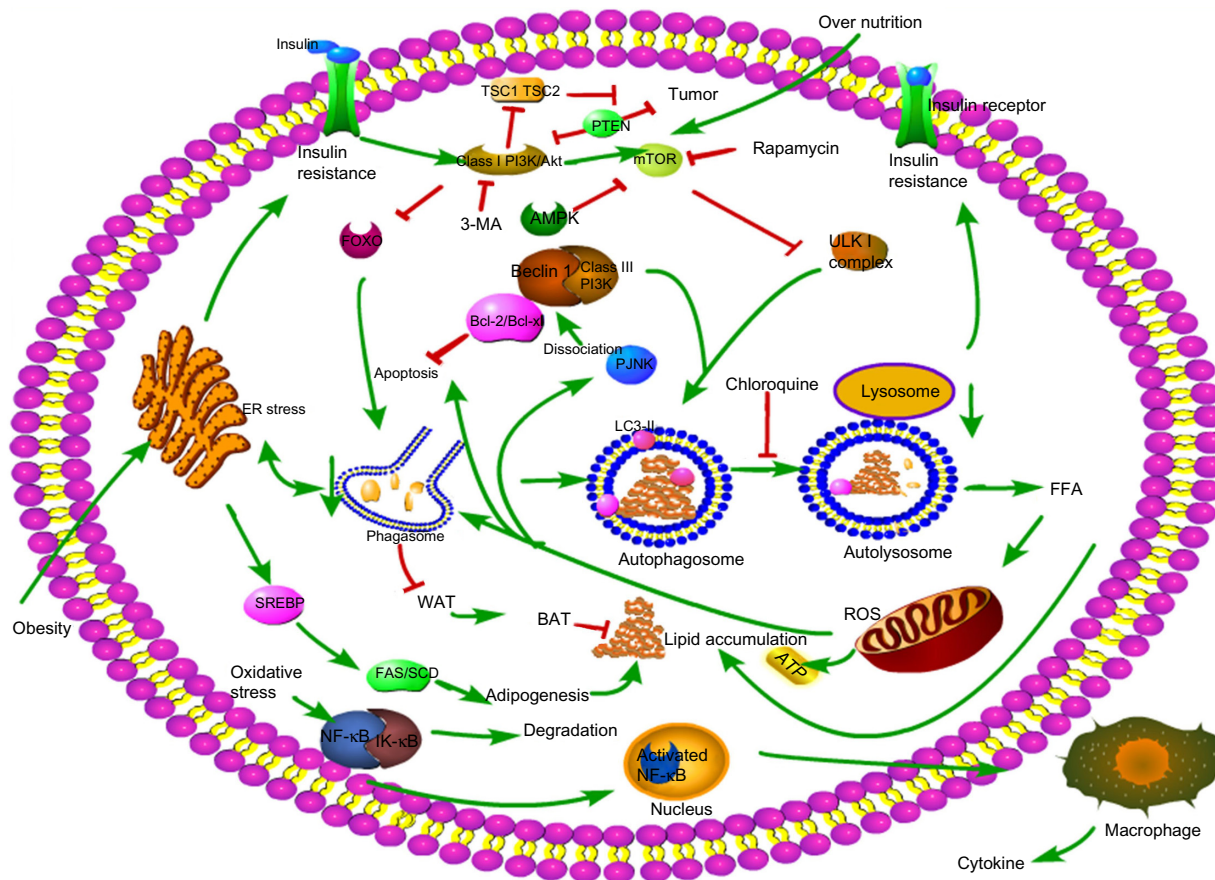


Figure 3 Molecular mechanisms involved in autophagy and NAFLD.

Notes: 1. A small vesicular sac called an isolation membrane is formed to enclose lipid droplets, which results in a double membrane structure called an autophagosome, which subsequently fuses with a lysosome to degrade lipid droplets into FFA. 2. Growth factor activates the PI3K/Akt signaling pathway to activate mTOR, leading to phosphorylation of the ULK1 complex, resulting in inhibition of autophagy formation. Short-term resistance may activate autophagy via inhibition of mTOR, while long-term lipotoxicity impairs autophagy by inhibition of the FOXO pathway. 3. The class III PI3K/Beclin-1 complex positively regulates autophagy. 4. Increased FFA oxidation causes mitochondrial oxidative stress and activates autophagy. ROS also promotes cell apoptosis and JNK phosphorylation, which stimulates Bcl-2 phosphorylation and Bcl-2/Beclin-1 dissociation, resulting in apoptosis and autophagy, respectively. Likewise, ROS promotes NF- κ B translocation into the nucleus, causing inflammatory cascades and liver injury. 5. Obesity causes ER stress, which activates autophagy and promotes adipogenesis-related gene expression such as FAS, SCD, leading to the synthesis of adipocytes. 6. Autophagy may show an inhibitory effect on adipocyte differentiation from WAT to BAT. 7. MP-activated protein kinase (AMPK) inhibits mTOR, and ultimately activates autophagy.

Abbreviations: NAFLD, nonalcoholic fatty liver disease; FFA, free fatty acid; ROS, reactive oxygen species; ER, endoplasmic reticulum; WAT, white adipose tissue; BAT, brown adipose tissue.

associated with a concomitant increase in autophagy flux, suggesting that autophagy plays essential roles in lipolysis, called lipophagy, which may provide a new way to eliminate fat in addition to lipase-mediated lipolysis.^{26,27} In fact, the effect of autophagy on lipid clearance may not be restricted to macrolipophagy, and indirect autophagy of organelles such as mitochondria and peroxisomes are probably involved; however, more investigations are needed to determine the independent effect of macrolipophagy.²⁶

Autophagy regulates adipogenesis and adipose tissue differentiation

Contrary to the lipolysis effect, autophagy may also work on adipogenesis and lipocyte differentiation. It has been found that *Atg7* knockdown of white adipose tissue (WAT) in mice

showed a remarkable phenotype. The mutant mice were smaller than their wild-type counterparts, with larger numbers of smaller adipocytes filled with multilocular lipid droplets, as observed by light microscopy, and higher numbers of mitochondria, as observed by electron microscopy. All the results demonstrate that autophagy is critical for WAT adipogenesis, especially for the formation of the unilocular lipid droplet structure and for the mitochondria homeostasis control.²⁸

Likewise, Ma et al²⁹ discovered that deletion of FIP200, a core subunit of the mammalian autophagy related 1 complex, reduced both HFD- and starvation-induced steatosis and downregulated the gene expression of enzymes involved in de novo lipogenesis, which include ATP-citrate lyase (*Acl*), acetyl-CoA carboxylase (*Acaca*), fatty acid synthase (*Fasn*), and stearoyl-CoA desaturase-1 (*Scd1*). This was consistent

with another study³⁰ which reported that knockdown of *Atg7* in pre-adipocytes inhibited lipid accumulation. Moreover, adipocyte-specific knockout of *Atg7* generated lean mice with decreased WAT and increased brown adipocytes.³⁰ Similar results were demonstrated by other studies where autophagy deficiency exerted a protective role against obesity by inhibiting adipogenesis.^{31–33} In addition to adipocytes, researchers using hepatocytes also demonstrated that intracellular lipid droplets were suppressed by *Atg7* knockdown.³⁴

Autophagy plays essential roles not only in adipogenesis but also in the process of WAT differentiation into mature tissue. Mouse embryonic fibroblasts with *Atg5* deletion failed to complete the differentiation process, with the main cells developing multiple small lipid droplets and eventually dying.³⁵ Although mitochondria are mostly responsible for fatty acid oxidation, their contribution to adipogenesis cannot be neglected. The number of mitochondria is increased in the early stage of adipogenesis and during maturation of WAT, due to the requirement of both energy and the key substrates for lipogenesis such as acetyl-CoA and glycerol 3-phosphate. However, the number of mitochondria was decreased by autophagy, called mitophagy, which may reflect the decreased need for lipogenesis in mature white adipocytes. The most significant features of adipose-specific *Atg* knockout in mice were an increased number of mitochondria and multiple small lipid droplets, compared with mature white adipocytes.³⁶

Autophagy is also involved in the cell-type differentiation of adipocytes. WAT and brown adipose tissue (BAT) are two different types of tissue. In WAT, lipid storage prevents free fatty acid (FFA) lipotoxicity and provides energy when nutrients are restricted. BAT has a reduced capacity for lipid storage but higher rate of oxidation to provide energy.²⁰ Tissue *Atg7* knockout in mice had decreased WAT but increased BAT, along with an increased rate of oxidation and lean body mass, suggesting an inhibitory effect of autophagy on differentiation of WAT to BAT^{30,37} (Figure 3).

Collectively, autophagy is involved in lipolysis, lipogenesis, and differentiation of adipocytes. The two mechanisms are not contradictory, but coexist and coact on lipid regulation. The imbalance between lipogenesis and lipolysis may result in steatosis, and studies focusing on expanding one side, such as lipolysis, may be of interest for NAFLD therapy.

Molecular mechanisms linked with autophagy and steatosis

Effect of lipotoxicity on autophagy varies with time

The effect of autophagy varies with time as well as nutritional status. Autophagy is activated by short-term stimulation of

lipid availability as a self-protective mechanism to degrade lipid droplets.¹⁹ Both ob/ob mice and chronic HFD mice show markedly impaired hepatic autophagy, particularly expression of *Atg7*.³⁸ Furthermore, chronic exposure to lipid challenge has an inhibitory effect on CMA, which is probably associated with accelerated degradation of LAMP-2A.³⁹ Liu et al found that long-term insulin resistance inhibited autophagy by inhibition of the constitutive nuclear form of FOXO1, which controls the transcription of autophagy-related genes, while short-term insulin resistance activates autophagy via the mTOR complex⁴⁰ (Figure 3). Long-term lipid load may change membrane lipid composition and reduce autophagosome/lysosome fusion both in vitro and in vivo, which may partly explain why excessive lipid reduces autophagy and accelerates lipid accumulation.⁴¹

Autophagy and aging

Autophagy declines with aging in parallel with increased lipid accumulation in various organs, which may be associated with the increased prevalence of NAFLD, type II diabetes, and other metabolic disorders. The age-related changes in liver macroautophagic proteolysis have been extensively studied in vitro and in vivo.⁷ Both macroautophagy and CMA decrease with age.^{39,42} What is responsible for the decline in autophagy upon aging? Insulin/IGF-1 is the best characterized pathway that is highly conserved in humans.⁴³ Alterations in the IGF-1 signaling pathway may lead to extension of life and affect the process of autophagy.⁴⁴ IGF-1 may inhibit macroautophagic proteolysis and degradation of mitochondria harboring deleterious mtDNA mutations.⁴⁵ The IGF-1 signaling cascade includes PI3K/Akt pathway and is downstream of mTOR. Downregulation of PI3K/Akt stimulates autophagy, so autophagy may partly account for extension of life.¹³

Autophagy and insulin resistance

The pathogenesis of NAFLD involves a two-hit hypothesis.⁴⁶ The first hit is insulin resistance, which is closely associated with the metabolic syndrome. Insulin resistance enhances lipolysis, increasing serum levels of FFA and decreasing very-low-density lipoprotein, which delivers triglycerides and cholesterol from the liver to peripheral organs, and promotes the resynthesis of lipid, thus leading to lipid storage in the liver. At the same time, fatty acid oxidation is increased and impairs mitochondrial oxidation, contributing to oxidative stress⁴⁷ (Figure 1). As previously shown, insulin has an inhibitory effect on autophagy in the liver, but the mechanism remains unknown. This result is in agreement with a former

study in which autophagy flux in rats treated with HFD for 2 weeks was upregulated, but was nearly inhibited after giving HFD for 10 weeks.⁴⁸ FOXO transcription factors are regulators of longevity downstream of insulin signaling, which is involved in promoting expression of autophagy genes and the ubiquitin–proteasome system, known as two cell clearance processes.⁴⁹ Another signaling pathway related to the inhibition of autophagy in the presence of insulin is class I PI3K/AKT that leads to the activation of mTOR and phosphorylation of its downstream p70S6k and autophagy inhibition, which can be blocked by rapamycin, an inhibitor of mTOR (Figure 3).⁵⁰ Furthermore, *Atg7* mutant mice showed impaired glucose tolerance, abnormal levels of insulin, and reduced β -cell mass.⁵¹ Defective hepatic autophagy promotes endoplasmic reticulum stress and causes insulin resistance.³⁸

In contrast, autophagy deficiency in skeletal muscles leads to impaired activity of mitochondrial oxidative phosphorylation and subsequent induction of FGF-21 through ATF4 activation. As a result, autophagy deficiency enhanced oxidation and energy expenditure through browning of WAT and increased insulin sensitivity.³² In conclusion, the relationship between autophagy and insulin resistance is controversial. On the one hand, insulin resistance-induced steatosis reduces autophagy, which in turn aggravates insulin resistance and ER stress (Figure 3), and on the other hand, autophagy deficiency is involved in attenuating insulin resistance.

Autophagy and steatohepatitis

Benign hepatic steatosis can progress to nonalcoholic steatohepatitis (NASH), the state of inflammation and hepatocellular injury that is characterized by elevation of alanine aminotransferase, aspartate aminotransferase, and several adipokines and cytokines. Adiponectin, leptin, TNF- α , and IL-6 play critical roles in the process of inflammation and liver injury⁵² (Figure 1).

Several studies have demonstrated that autophagy exhibits cellular protective effects, leading cells to survival from death stimuli.^{53–56} Cell damage is increased in long-term steatosis and aging in cells and tissues, suggesting that cell damage is correlated with autophagy reduction. Wang et al have discovered that autophagy inhibition by shRNA-mediated knockdown of *Atg5* sensitizes cells to death from menadione by overactivation of JNK and the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) 1/2 signaling pathway.⁵⁶

However, autophagy is a double-edged sword that mediates cell proliferation as well as cell death. Reactive oxygen species (ROS) produced by excessive FFA oxidation

in mitochondria activate the JNK signaling pathway, promoting the dissociation of Bcl-2/Beclin-1 complex and autophagy induction, which is accompanied by apoptosis (Figure 3).^{57,58} The effect of autophagy on life and death of a cell is controversial.

Immune activation and related signaling pathways

Immune activation is a prerequisite for the development of NASH, which exacerbates steatosis, including innate immune pathways.⁵⁹ Salminen et al have discovered that inhibition of autophagic capacity with aging accelerates inflammation via activation of NLR P3.⁶⁰

Activation of JNK and IKKB promotes the synthesis of AP-1 and NF- κ B, leading to an inflammatory reaction.⁵⁹ Insulin resistance and oxidative stress stimulate phosphorylation and subsequent degradation of IKKB, accompanied by translocation of activated NF- κ B to the nucleus, causing inflammatory cascades, which in turn aggravate NF- κ B activation (Figure 1). Extracellular lipid can activate IKKB by engaging TLRs. Mice deficient in hepatocyte TLR4 attenuated severity of steatosis after challenge with an HFD, indicating that TLR4 is an important proinflammatory mediator.⁶¹ TLR may play an important role in pathogen-induced autophagy via a TRIF-dependent, MyD88-independent TLR4 signaling pathway, which has not been recognized before.⁶²

Saitoh et al have shown that *Atg16L1* deficiency disrupts the formation of autophagosome, resulting in more severe damage and higher amounts of inflammatory cytokines such as IL-1 β and IL-18, under stimulation with lipopolysaccharide.⁶³ The mechanism by which autophagy regulates non-pathogen immunity, such as NASH, has not been elucidated and needs further investigation.

Apoptosis

Hepatocyte apoptosis due to FFA toxicity, called lipoapoptosis, is an important mechanism in the progression of NAFLD or NASH.^{64–66} Natarajan et al discovered that hepatocyte and cholangiocyte lipoapoptosis is induced by FFAs.⁶⁶ Activation of p38-MAPK, ERK, and JNK signaling pathways is involved in the mechanism of lipoapoptosis,^{65,66} but the exact mechanism involved in the regulation of lipoapoptosis remains controversial.

Autophagy and apoptosis are two major ways of programmed cell death. There is significant cross talk between autophagy and apoptosis, as they can cooperate, antagonize, or assist each other under different conditions.⁶⁷ MCL-1, an antiapoptotic Bcl-2 homolog, is a stress sensor that controls

autophagy and apoptosis, and MCL-1 degradation not only induces apoptosis but also activates autophagy.⁶⁸ It is established that the cross talk between apoptosis and autophagy is mediated, at least partially, by interaction of the Beclin-1 and Bcl-2 complexes.⁶⁹ Bcl-2 functions as an antiapoptotic protein as well as an anti-autophagy protein by inhibitory interaction with Beclin-1, to help maintain autophagy at levels that are compatible with cell survival rather than cell death.⁶⁹ The connection between Bcl-2 and Beclin-1 or Bax is disrupted to induce autophagy and apoptosis, respectively, under conditions of stress.⁵⁷ Localization of Bcl-2 to the ER prevents Beclin-1-mediated, starvation-regulated autophagy by binding to NAF-1, which stabilizes the interaction between Bcl-2 and Beclin-1 at the ER surface.⁷⁰ Proapoptotic Bcl-2 homology (BH)3-only proteins have been described in previous studies to bind with the BH3-binding pocket of Bcl-2 or Bcl-XL, leading to the disassociation of Bcl-2/XL and Beclin-1, resulting in autophagy or apoptosis, respectively.^{71,72} Activation of the JNK1 signaling pathway stimulates the disassociation of Bcl-2 from Beclin-1 via phosphorylating multiple residues at the Bcl-2 regulatory position, leading to inactivated Bcl-2 and autophagy induction⁶⁵ (Figure 3).

There is another type of protein called death-associated protein kinase (DAPK) that is reported to promote disassociation of Bcl-2 from Beclin-1 by phosphorylating Thr119 of Beclin-1.⁷³ HMGB1 is also suggested to be involved in the disassociation of Bcl-2 from Beclin-1.^{74,75} Autophagy is regarded as a cell survival mechanism; however, the interaction of Bcl-2 – an antiapoptotic protein – with Beclin-1 results in the inactivation of Beclin-1 and maintains autophagy to a certain extent. The disassociation of the complex leads to apoptosis and autophagy. The cross talk between autophagy and apoptosis is controversial and needs further investigation.

Autophagy and liver fibrosis

NAFLD is characterized by its course from simple steatosis to steatohepatitis, fibrosis, and cirrhosis. Hepatic fibrosis is the progressive accumulation of extracellular matrix that occurs in various chronic liver diseases,⁷⁶ including NAFLD. Hepatic stellate cells (HSCs) function well in hepatic fibrosis, which may be a possible link with autophagy. HSC activation is critical in liver fibrosis, as it leads to the production of excessive extracellular matrix⁷⁷ (Figure 1). According to a recent study, autophagy was increased in mice treated with CCl₄ or thioacetamide, accompanied by lipid droplet loss and HSC activation. On the contrary, blockade of autophagy with siRNA, resulting in *Atg5* knockdown, reduces fibrosis and matrix accumulation and increases the number and size of

lipid droplets.⁷⁸ Autophagy fuels tissue fibrogenesis not only in liver but also in other organs such as the lungs and kidneys,⁷⁹ and may be a new factor in HSC activation.⁸⁰ Autophagy affects the activation of HSCs not only due to the energy supply by autophagy, but also due to the effect of autophagy on cell growth cycle. 3-MA-induced autophagy inhibition is reported to block the G2 phase of HSC-T6 cells, a rat HSC line, leading to inhibition of cell proliferation.⁸¹ However, the relationship between autophagy and HSC proliferation remains controversial. An early study found that rapamycin – an mTOR target inhibitor and autophagy stimulator – reduced fibrosis,⁸² and that this was due to its inhibition of cell proliferation or its indirect effect on autophagy induction; however, the mechanisms involved remain unclear.

Although autophagy plays essential roles in HSC activation and fibrosis promotion, its effect on antifibrosis and collagen degradation should not be neglected. In α -1-antitrypsin deficiency, a disease in which the α -1-antitrypsin mutant Z protein results in protein aggregates and chronic liver injury, an autophagy-enhancing drug was demonstrated to reduce the hepatic load and reversed fibrosis.⁸³ Both genetic and pharmacological inhibition of autophagy in mice resulted in increased levels of type I collagen in mouse kidneys and primary mesangial cells, suggesting that autophagy promotes intracellular degradation of type I collagen.⁸⁴ However, whether it exhibits the same effect in liver remains unclear. Moreover, autophagy is regarded as a cell survival mechanism; it reduces oxidative stress and ER stress, and inhibits inflammation, thereby leading to fibrosis reduction.

We suggest that if autophagy could be selectively inhibited in HSCs and other fibrotic cells, a special blocker for autophagy would be an attractive candidate for antifibrotic strategies. However, this is challenging, as the tissue of the whole body is a complex of various cell types. Furthermore, since fibrosis is the result of an imbalance between collagen synthesis and degradation, the role of autophagy in collagen degradation is of great interest to the study of its antifibrotic mechanism.

Autophagy and hepatic carcinogenesis

NAFLD has the potential to progress into hepatic carcinoma. The role of autophagy in hepatic carcinogenesis is complex and is regarded as a double-edged sword.⁸⁵ Controversy exists as to whether autophagy inhibits or promotes carcinogenesis. Liang et al first reported that autophagy-related gene Beclin-1 was associated with cancer suppression.⁸⁶ Several tumor suppressor genes such as *PTEN*, *TSC1*, and *TSC2* stimulate

autophagy by inhibiting mTOR pathway (Figure 3). On the contrary, class I PI3K/Akt activates oncogenes and inhibits autophagy by activating mTOR.¹¹ Qu et al⁸⁷ found that *Beclin-1*, a gene closely related to autophagy, was deleted in 40%–75% of the cases of human sporadic breast, ovarian, and prostate cancers. *Beclin-1* gene knockout in mice increased the frequency of spontaneous hepatic malignancies and reduced autophagy.⁸⁷ Similarly, it has been shown that autophagy may function in tumor suppression by mitigating metabolic stress and, in concert with apoptosis, by preventing death by necrosis.⁸⁸ This evidence suggests that defective autophagy is positively correlated with carcinoma development.

However, autophagy is also a cell survival mechanism to help cells live through the environment of stress or nutrient restriction, thus leading to proliferation of tumor cells under stress, and may promote metastasis by enhancing tumor cell survival.^{89,90} Autophagy may suppress the p53 response by limiting DNA damage, oxidative stress, oncogene activation, and tumor cells less sensitive to starvation-induced cell death, thereby protecting cells from stress and promoting tumorigenesis.⁹¹ Furthermore, a recent study demonstrated that autophagy promoted HCC cell invasion by activation of epithelial–mesenchymal transition through a TGF- β /Smad3 signaling-dependent manner.⁹² In addition, LC3 is highly expressed in hepatoma compared with normal tissues, which is regarded as an independent predictor of hepatoma recurrence after surgery.⁹³

As yet, it is undetermined as to whether autophagy promotes or suppresses carcinoma. The double-edged sword effect of autophagy on tumors makes it important as a potential therapeutic option for cancer suppression, if properly controlled. However, several important questions are of concern. How long does it take for autophagy inhibition or activation to suppress an established tumor? Does the inhibition or activation effect depend on the tumor type or the drug concentration? How does autophagy function in tumors deficient in autophagy-related genes? In summary, activation of autophagy may represent a promising and effective strategy for liver cancer therapy, while the inhibition of autophagy may lead to the death of malignant cells due to energy restriction.⁹⁴ Further investigations are needed to determine the dual role of autophagy in carcinoma.

Future perspectives and conclusion

Autophagy is associated with NAFLD. Much work is needed to determine the exact relationship between autophagy and NAFLD, and the results from various studies are controversial. Most studies have provided evidence that autophagy deficiency leads to steatosis, since autophagy digests lipid

droplets, and drugs that increase autophagy may be a potential therapeutic approach in NAFLD.

However, several studies have demonstrated that autophagy is essential in adipogenesis and differentiation. Whether the difference in the results is related to the extent of autophagy or activation of other signaling pathways needs further investigation. It may also be significant to focus on other cell types such as HSCs and Kupffer cells, which are also involved in the progression of NAFLD⁹⁵ in addition to hepatocytes. More attention should be paid to the contribution of other types of autophagy to the pathogenesis of NAFLD; for example, CMA, though macroautophagy is regarded as the major type of autophagy.

There are some other questions that deserve consideration. Is the mechanism of lipid regulation tissue specific in the liver? Upregulation of autophagy protects hepatocytes against lipotoxicity, but cancer may also be promoted since autophagy is regarded as a cellular survival mechanism and helps cells to live through the conditions of stress and nutrient restriction. The answer to whether autophagy is a friend or foe in cancer is controversial. Moreover, the cross talk between apoptosis and autophagy, and the extent autophagy provides a protective effect in cells, need to be investigated. Autophagy induces HSC activation and stimulates hepatic fibrosis; however, autophagy fuels HSC activation and works on collagen degradation and inflammation inhibition, which seems to inhibit fibrosis. It is unclear as to whether the different effects of autophagy on fibrosis are cell type- or tissue-specific. The selective inhibitor of autophagy may be an ideal candidate for antifibrotic medicines.

In conclusion, a better understanding of the bidirectional characters of autophagy and its regulatory mechanisms may allow the practical use of autophagy for therapeutic purposes in the future.

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Disclosure

The authors report no conflicts of interest in this work.

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