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The Mechanism and Therapeutic Prospect of Autophagy in Metabolic Dysfunction-Associated Steatotic Liver Diseas

Xiaoning Zuo[1,#], Yuhan Wang[2,#], Ning Yao[3,*], Xin Wang[1,*], Jinhui Xu[1], Ying Qin[1], Xin Wang[3], Yajie Chen[1], Yunjuan Wu[1], Ying Su[1]

[1]School of Integrative Medicine, Gansu University of Chinese Medicine, Lanzhou 730000, Gansu, China
[2]Suzhou Medical College, Soochow University, Suzhou 215123, China
[3]The First School of Clinical Medicine, Gansu University of Chinese Medicine, Lanzhou 730000, Gansu, China
[#]*Co-first author*
**Correspondence Author*

Abstract: ***Metabolic dysfunction-associated steatotic liver disease (MASLD) has become the most common chronic liver disease in the world. Metabolic dysfunction-associated steatohepatitis (MASH) is an inflammatory subtype of MASLD, which can further develop into cirrhosis and hepatocellular carcinoma. With the global prevalence of metabolic syndrome, obesity and diabetes, the prevalence of MASLD is increasing year by year, which has brought an increasingly heavy burden to the global economy. Although steady progress has been made in understanding the epidemiology and pathogenesis of the disease, it is still the slowest progress in the treatment field. At present, there is a lack of approved specific therapeutic drugs. Therefore, it is urgent to further analyze the pathogenesis of MASLD and explore new therapeutic targets. In recent years, the role of autophagy in the pathogenesis of MASLD is being extensively studied. It is mainly involved in the occurrence and progression of the disease by regulating multiple factors such as lipotoxicity, mitochondrial dysfunction, oxidative stress, insulin resistance (IR), endoplasmic reticulum stress (ERS), inflammasome activation, and intestinal flora imbalance.***

Keywords: MASLD, Autophagy, MASH, Pathogenesis, Treatment.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) was renamed metabolic dysfunction-associated steatotic liver disease (MASLD) in the multi-school Delphi consensus statement led by the American Association for the Study of Liver Diseases (AASLD). The revised nomenclature better summarizes the link between hepatic steatosis and systemic metabolic disorders, contributes to increased awareness of the disease, and addresses the potentially stigmatizing connotations of NAFLD [1]. With the global epidemics of metabolic syndrome, obesity and diabetes, the number of patients with MASLD has risen dramatically, with a current global prevalence of approximately 32.4% [2], and it has become a major cause of liver transplantation [3]. The mechanisms underlying the pathogenesis of MASLD are extremely complex, and in recent years the theory of "multiple hits" has been widely accepted [4]. This theory suggests that the pathogenesis of MASLD involves multiple parallel events, including IR, mitochondrial dysfunction, ERS, intestinal flora imbalance, inflammatory factor aggregation, and genetic/epigenetic risk factors. Multiple factors synergize and overlap, leading to hepatocellular injury and promoting the onset and progression of MASLD. Despite years of intensive research worldwide, progress in the therapeutic area has been slow, with only one drug, the thyroid hormone beta agonist Rezdiffra (resmetirom), currently approved by the U.S. Food and Drug Administration (FDA) for the treatment of non-cirrhotic adult patients with MASH who have moderate-to-severe liver fibrosis [5]. Therefore, in-depth analysis of the pathogenesis of MASLD in order to discover new therapeutic targets is an urgent problem to be solved at present. Autophagy cleans up redundant intracellular components, and its degradation products can provide raw materials for cells to synthesize, enabling them to maintain a normal physiological state. Dysregulation of autophagy is inextricably linked to many human diseases, and its function is critical for the regulation of cell survival, bioenergetic homeostasis and cell death [6]. More and more studies have recognized that dysfunctional autophagy is closely related to the development of MASLD [7], but the related mechanisms have not been fully understood. In this paper, we review the research progress between autophagy and MASLD in recent years in terms of pathogenesis and treatment.

2. Overview of Autophagy

Autophagy is a highly conserved eukaryotic recycling process. Autophagy plays an important role in the survival and maintenance of cells through the degradation of macromolecules such as organelles, proteins and lipid droplets and the recycling of decomposition products. According to the different mechanisms of substrate sequestration, the main types of autophagy are divided into macroautophagy, microautophagy and chaperone-mediated autophagy [8]. Macroautophagy (this paper focuses on the introduction, hereinafter referred to as autophagy) can be selective or non-selective. The role of non-selective autophagy is mainly to allow cells to circulate nutrients in an energy-limited environment, while selective autophagy mainly cleans up intracellular organelles to maintain cell structure [9].

Autophagy is regulated by a variety of proteins that are products of autophagy-related genes (Atgs). Autophagosome formation begins with the activation of the unc-51-like kinase 1 (ULK1) complex, which consists of ULK1, ATG13, ATG101, and the FAK family kinase-interacting protein of

200 kDa (FIP200). The ULK1 kinase complex serves as a backbone for recruiting the downstream class III phosphatidylinositol 3-kinase (PI3K) complex (composed of VPS34, Beclin1, VPS15, and ATG14L) recruits to autophagosome formation sites and produces phosphatidylinositol 3-phosphate (PIP3) for autophagic vesicle nucleation and formation. Subsequent extension and closure of the autophagosome is mediated by two ubiquitin-like molecules, Atg12 and microtubule-associated protein 1 light chain 3 (LC3). Atg12 binds to Atg5 and Atg16L to form the Atg12-Atg5-Atg16L complex, ATG4 cleaves LC3 to LC3-I, and then ATG3, ATG7 and Atg12-Atg5- Atg16L together facilitate the coupling reaction of LC3-I with phosphatidylethanolamine to form LC3-II, which is subsequently inserted into the autophagosome. P62/SQSTM1, an autophagy receptor protein, recognizes ubiquitylated cargoes in selective autophagy through direct interaction with LC3-II and connects to autophagosomes, which are matured by fusion with lysosomes to produce autophagic lysosomes in which the selected cargoes are degraded into small molecules within the lysosome and recycled [10-12]. Autophagy is usually maintained at a basal level under physiological conditions, and stress stimuli can significantly enhance autophagy to remove abnormal proteins or organelles from cells and promote cell survival. However, prolonged high levels of autophagy induce cell death [13]. Increasing evidence suggests that dysfunctional autophagy is associated with many diseases, including liver disease, cardiovascular disease, cancer, and neurodegenerative diseases [14].

3. Autophagy is Involved in the Pathogenesis of MASLD

3.1 Autophagy and Mitochondrial Dysfunction

The transition from simple fatty liver to MASH is not only based on steatosis but also driven by mitochondrial dysfunction [15]. Characteristics of mitochondrial dysfunction include ultrastructural damage, abnormal morphological changes, decreased membrane potential, increased membrane permeability, decreased respiratory chain activity, adenosine triphosphate (ATP)depletion, mitochondrial DNA (mtDNA) damage, overproduction of reactive oxygen species (ROS), and impaired mitochondrial β-oxidation [16,17]. ROS involves hydrogen peroxide, superoxide anion, and hydroxyl radicals, among others, that oxidatively damage proteins, lipids, and DNA, causing cellular dysfunction and ultimately leading to apoptosis and necrosis [18]. ROS and the various damage stimuli they trigger mediate the onset of mitochondrial outer membrane permeabilization, and pro-apoptotic factors such as cytochrome c and apoptosis-inducing factor (AIF) are released from mitochondria to the cytoplasm. Among them, cytochrome c activates a cascade reaction in the cytoplasm that leads to caspase-3 activation, thus inducing apoptosis.AIF can localize to the nucleus and cause DNA breaks [19]. ROS also activate the nuclear factor-κB (NF-κB) and NLRP3 inflammasome pathways, which stimulate inflammatory factors such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) production, which promotes the development of inflammation [20]. It has been reported that MASH mouse plasma contains high levels of mtDNA and acts as an injury-associated molecular pattern to activate Toll-like receptor 9 (TLR9) on hepatic Kupffer cells and promotes activation of inflammatory pathways [21], and activated Kuppfer cells secrete a variety of pro-inflammatory factors that initiate and amplify inflammatory signaling, especially transforming growth factor-β (TGF-β) can activate hepatic stellate cells (HSCs), leading to the progression of liver fibrosis.

Mitochondrial autophagy is a type of mitochondria-specific autophagy that involves the selective isolation and degradation of damaged mitochondria to maintain mitochondrial functional integrity and cellular homeostasis. Mitochondrial autophagy is a protective mechanism in the development of MASLD, enabling cells to avoid excessive ROS derived from damaged mitochondria and attenuate oxidative stress and inflammatory responses. In addition, mitochondrial autophagy stimulates the catabolism of lipid droplets (LDs) to have the release of free fatty acids (FFAs), which are then translocated to healthy mitochondria for β-oxidation and increased energy release [16]. Dysregulation of mitochondrial autophagy prevents the regeneration of healthy mitochondria, leading to the accumulation of damaged mitochondria, and the accumulation of damaged mitochondria and the overproduction of ROS enhance each other, creating a vicious cycle that leads to a variety of pathologies including metabolic disorders, inflammation, liver injury, and cancer [22]. Several signaling pathways are involved in the regulation of mitochondrial autophagy in MASLD and, if manipulated properly, may restore mitochondrial autophagy in hepatocytes and ultimately improve metabolic outcomes. The most classical is mitochondrial autophagy mediated by the PTEN-induced kinase 1 (PINK1)/parkin signaling pathway. In addition, there are several mitochondrial autophagy receptor-dominated pathways, such as Bcl-2 and adenovirus E1B 19-kDa-interacting protein 3 (BNIP3), Nip3-like protein X (NIX), and FUN14 domain-containing protein 1 (FUNDC1), which are receptor proteins that further recruit autophagosomes into mitochondria through direct interaction with LC3 to initiate mitochondrial autophagy [23]. Corn peptides have been reported to ameliorate damage to rat mitochondrial structures and attenuate oxidative stress and lipid accumulation through enhancement of PINK1/Parkin-mediated mitochondrial autophagy in a 10-week HFD-fed rat model of MASLD [24]. Sirtuin3 (Sirt3) is a NAD+-dependent deacetylase expressed predominantly in the mitochondria, and Li [25] et al. showed that in palmitic acid (PA)-treated primary hepatocytes, Sirt3 overexpression activated the ERK-CREB signaling pathway, which up-regulated Bnip3 expression and its mediated mitochondrial autophagy, thereby attenuating PA-induced mitochondrial damage and inhibiting mitochondria - dependent apoptosis. Predictably, restoration of mitochondrial autophagy is a promising strategy for the treatment of MASLD. However, many other questions remain to be addressed in understanding how mitochondrial autophagy is inhibited and how to restore it.

3.2 Autophagy and ERS

During MSALD progression, IR, hyperglycemia, and lipid accumulation disrupt protein homeostasis and trigger

hepatocyte ERS. At this point, the cell activates a series of complementary adaptive mechanisms in response to the protein folding changes, which is termed the unfolded protein response (UPR). The UPR is regulated by three sensors embedded in the ER membrane, namely double-stranded RNA-activated protein kinase (PKR)-like ER kinase (PERK), inositol-requiring enzyme 1α (IRE1α) and activating transcription factor 6 (ATF6) [26]. Under physiological conditions, these transmembrane proteins bind to the chaperone glucose regulatory protein 78 (GRP78) in the lumen of the endoplasmic reticulum and are inactivated. When unfolded proteins accumulate in the endoplasmic reticulum, GRP78 is released from these complexes to help fold the accumulated proteins. Although the UPR is an adaptive response for cells to restore endoplasmic reticulum homeostasis, severe or prolonged endoplasmic reticulum stress can lead to cell death and tissue damage. Endoplasmic reticulum stress is directly linked to inflammation through the UPR pathway, which regulates transcriptional programs to induce the expression of inflammatory genes. Importantly, endoplasmic reticulum stress-induced inflammation contributes directly to the development of metabolic and inflammatory diseases [27].

Interestingly, impaired hepatic autophagic flux in MASLD patients and mouse models induces elevated ERS and leads to apoptosis [28]. In this study, a link between impaired autophagic flux and increased endoplasmic reticulum stress was also observed in high-fat diet (HFD)-fed rats. Current evidence suggests that autophagy can reduce the level of cellular stress by removing endoplasmic reticulum membranes containing UPR sensors or reduce the magnitude of stress by removing unfolded and misfolded proteins from the endoplasmic reticulum, and it can also block apoptosis induction by inhibiting caspases activation [29,30]. Rubicon acts as a negative regulator of autophagy and inhibits autophagosome-lysosome fusion. In PA-induced HepG2 cells, Rubicon expression was up-regulated and autophagic flux was decreased, accompanied by increased expression of ERS markers PERK, C/EBP homologous protein (CHOP), p-IRE1α, and c-Jun N-terminal kinase (JNK) and apoptosis. In contrast, Rubicon knockdown attenuated autophagy damage and alleviated PA-induced endoplasmic reticulum stress, apoptosis and lipid accumulation [31]. Ding [32] et al. showed that administration of resveratrol and calorie restriction alleviated hepatic steatosis by modulating the SIRT1 autophagy pathway and attenuating the endoplasmic reticulum stress in an animal model of MASLD induced by feeding male rats with a HFD. Degeneration. In conclusion, therapies aimed at restoring autophagic flux and subsequent endoplasmic reticulum stress may attenuate the progression of MASLD.

3.3 Autophagy and Lipotoxicity

The deleterious effects of lipids such as saturated free fatty acids and cholesterol on non-adipose organs have been termed "lipotoxicity", which at the molecular level is characterized by an increase in the amount of toxic lipids in the hepatocytes, leading to organelle dysfunction, cellular stress, and ultimately apoptosis [33]. Lipotoxicity can directly disrupt pancreatic β-cell structure and function, affecting insulin secretion; it can also induce IR by directly interfering with insulin signaling. In the state of IR, lipolysis is up-regulated in adipose tissues, leading to the release of FFAs in the somatic circulation and their uptake by hepatocytes. Fatty acid transporter protein (FATP) and fatty acid translocase (CD36) are the major hepatic plasma membrane proteins involved in hepatic fatty acid uptake and contribute to the intrahepatic lipid pools. Genetic deletion of the FATP members, FATP2 and FATP5, resulted in reduced hepatic steatosis in a diet-induced obese mouse model [34]. Similarly, CD36 expression has been shown to be upregulated in patients with MASLD and shown to correlate with liver injury [35]. Hyperinsulinemia responds to IR in hepatocytes by driving fatty acid synthesis in hepatocytes through a process known as "De novo lipid synthesis". This process is regulated by two transcription factors: sterol regulatory element binding protein 1c (SREBP1c) and carbohydrate response element binding protein (ChREBP), which are activated in response to glucose/fructose-induced insulin release. Lipid intake from fat-rich foods also leads to hepatic lipid accumulation. Inefficiency of hepatocytes in chelating FFAs into triglycerides (TGs) and secreting them into the circulation as very low density lipoprotein particles also leads to increased hepatic lipotoxicity [36]. Accumulation of lipid substances such as cholesterol and ceramides leads to activation of ERS mediators such as ATF4 and CHOP, which initiate pro-apoptotic signaling in hepatocytes [37]. In addition, ERS-activated transcription factors such as X-box binding protein 1 (XBP-1s) can lead to upregulation of de novo lipogenesis in hepatocytes, which can further exacerbate lipotoxicity [38].

Lipophagy is a specific type of intracellular autophagy that prevents intracellular lipid accumulation and lipotoxicity by selectively recognizing lipids, initiating the activation of autophagy-associated molecules to degrade them, and reducing the cytoplasmic storage of TGs in hepatocytes [39]. When lipophagy is activated, the degree of steatosis in the liver is reduced, while the inflammatory response is attenuated, alleviating the condition in the early stages of MASLD. Several pharmacological and non-pharmacological treatments have been shown to upregulate autophagy and reduce hepatic steatosis in preclinical MASLD models. Metformin, one of the most commonly used first-line drugs for the treatment of type 2 diabetes, has been shown to alleviate hepatic steatosis by restoring SIRT1-mediated autophagy through a PRKA-independent pathway [40]. In addition, drugs such as dagliflozin [41], liraglutide [42], and caffeine [43] have been shown to alleviate the progression of MASLD by promoting autophagy, reducing hepatic lipid deposition, and mitigating the effects of lipotoxicity. Improvement of hepatic steatosis was also observed in rapamycin-treated mice. Therefore, inhibition of mammalian target of rapamycin protein complex 1 (mTORC1) is a promising strategy for the treatment of MASLD [44]. Exercise activated the AMP-activated protein kinase (AMPK)/ULK1 pathway to directly enhance adipose autophagy, whereas caloric restriction reversed HFD-induced autophagy inhibition via the protein kinase B (AKT)/mTOR/ULK1 pathway, and the combination of these two therapies was more effective in ameliorating lipid deposition in MASLD. In addition, exercise and dietary interventions ameliorated hepatic aging caused by lipid overaccumulation [45]. It is evident that by targeting

lipophagy, hepatic steatosis can be alleviated, the effects of lipotoxicity can be reduced, and the progression of MASLD can be effectively mitigated.

3.4 Autophagy and Inflammasome Activation

NLRP3 inflammasome activates a series of MASLD-related phenotypes related to steatosis, inflammation and fibrosis progression [46]. It is basically a kind of multimeric protein complex which consists of NLRP3, apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC) and pro-caspase-1. NLRP3 inflammasome is an innate immune response produced by organisms, which can promote the secretion of IL-1β and IL-18 through caspase-1 activation and resist the damage of certain pathogens. However, when NLRP3 inflammasome is overactivated, it can cause various inflammatory diseases, such as inflammatory liver disease and inflammatory bowel disease [47]. Activated caspase-1 can also cut gasdermin D (GSDMD) into GSDMD-N, which mediates the formation of plasma membrane pores and cell osmotic swelling, induces pyroptosis, and leads to a cascade reaction of pro-inflammatory cytokine release [48]. Autophagy can negatively regulate NLRP3 inflammasome activation by removing endogenous inflammasome activating factors (such as damaged mitochondria that produce ROS), removing inflammasome components and inflammatory cytokines. The autophagy mechanism also plays a role in the unconventional secretion of IL-1β, thereby regulating the inflammatory response. In contrast, NLRP3 inflammasome activation regulates autophagosome formation through several different mechanisms. The crosstalk between inflammasomes and autophagy is necessary to balance the required host defense against inflammatory responses and prevent excessive inflammation [49].

It has been reported that exposure to perfluorooctanoic acid (PFOA) induces lipid accumulation, increased NLRP3 aggregation, increased IL-1β production, and blocked autophagy flux in mouse liver and L-02 cells. Rapamycin alleviated PFOA-induced lipid accumulation and NLRP3 inflammasome activation by activating autophagy flux. On the contrary, the autophagy inhibitor chloroquine aggravated PFOA-induced lipid accumulation and NLRP3 inflammasome activation [50]. The changes of immune metabolic status in MASLD patients may be reflected by the expression of different circulating peripheral blood mononuclear cells (PBMCs) specific molecular markers. In a cross-sectional study with a sample size of 50 people, pro-inflammatory markers such as iNOS, IL-6, TNF-α, and IL-1β in the serum of MASLD subjects increased. ROS-induced NLRP3 inflammasome marker proteins were up-regulated in PBMCs with the severity of MASLD. The expression of autophagy markers LC3B, Beclin-1 and its regulatory factor p-AMPKα decreased, while p62 increased, and the co-localization of NLRP3 and LC3B in PBMC decreased with the severity of MASLD. It indicates that autophagy impairment in MASLD patients is closely related to the activation of NLRP3 inflammasome, which may aggravate the severity of MASLD [51]. Therefore, inhibiting the activation of NLRP3 inflammasome by restoring autophagy flux may be a potential therapeutic strategy for MASLD.

3.5 Autophagy and Intestinal Flora Disorder

Changes in the abundance and diversity of gut microbiota are linked to the progress of MASLD. Population studies have found that the complexity of intestinal flora in MASH patients is lower than that in normal people [52]. Each stage of MASLD has special intestinal microflora characteristics, and the severity of MASLD is related to the imbalance of microflora and the loss of metabolic function of symbiotic bacteria. It has been reported that at the level of bacterial phylum in MASLD patients, Bacteroidetes decreased, while Firmicutes and Proteobacteria increased [53]. Disturbances in the intestinal flora increase the absorption of monosaccharides and promote hepatic fatty acid and TG synthesis and interfere with choline metabolism in vivo by increasing the activity of acetyl coenzyme A carboxylase and fatty acid synthase, leading to choline deficiency and promoting fatty liver formation [54]. The liver-gut axis plays an important role in the development of MASH, when gut microbial imbalance increases the permeability of the intestinal barrier, intestinal bacteria and their products, such as endotoxins and cytokines, enter the liver through the bloodstream, activate the hepatic immune response, and promote the development of hepatic inflammation. Bacterial products and translocated lipopolysaccharides stimulate the hepatic innate immune system through TLR4 signaling, which mainly acts on HSCs and KCs to produce pro-inflammatory cytokines, such as TGFβ, and promotes the progression of liver inflammation and fibrosis [55].

Autophagy and the gut microbiota interact and are closely related. Defects in autophagy lead to alterations in the composition and diversity of the gut microbiota, resulting in dysbiosis of gut ecology [56]. The intestinal flora may also regulate the autophagic process through a variety of mechanisms [18]. Benjamin [57] et al. demonstrated that oral inoculation of mice with Salmonella typhimurium induced the formation of autophagosomes in small intestinal epithelial cells and immunofluorescent staining showed that most of the autophagosomes co-localized with intracellular bacteria. In addition, intestinal epithelial cell-specific, mice lacking the autophagy-essential gene Atg5 showed increased Salmonella typhimurium populations in both intestinal epithelial cells and extra-intestinal tissues, suggesting that autophagy can limit bacterial populations and spread. It has been reported that prebiotics can regulate the intestinal flora by improving mitochondrial autophagy and thus play a role in the treatment of MASLD [18]. Currently available prebiotics include lactulose, fibers, inulin derivatives, and milk oligosaccharides. In addition, phytochemicals may act as prebiotics in the gut lumen for autophagy inducer. In conclusion, the dysregulation of gut microbiota and bacterial products may promote the development of MASLD through multiple mechanisms [58]. Autophagy can regulate intestinal homeostasis by avoiding intestinal microbiome imbalance, which is of great significance for maintaining intestinal barrier function and can be used as a therapeutic strategy to prevent the progression of MASLD.

4. Mechanism of Autophagic Damage in MASLD

In vivo and in vitro studies have shown that autophagy is enhanced for a short period of time in the initial stages of simple steatosis, which may be a compensatory mechanism to counteract lipid accumulation, but with the progression of MASLD, lipid overaccumulation is observed and autophagy is significantly impaired [59], which may lead to the progression of the disease, and the exact molecular mechanisms are still under investigation.

AMPK and mTORC1 are known regulators of autophagy initiation through activation/inhibition of the ULK1/2 complex, and both are also important regulators of lysosomal function, and therefore of the later steps of the autophagic process, with changes in the activity of both thought to mediate impaired autophagy during MASLD [60]. Autophagy is also disrupted by insulin resistance and concomitant hyperinsulinemia; on the one hand, insulin inhibits autophagy by activating mTORC1 via the PI3K / AKT pathway; on the other hand, phosphorylated AKT also inhibits forkhead box protein O1/3 (FoxO1/3)-mediated transcription of key autophagy genes, such as VPS34, ATG12, and gabarapl1 [61,62]. TFEB, as the a major transcriptional regulator of the autophagy-lysosome pathway, positively regulates the expression of genes related to autophagy and lysosomal biogenesis. In MASLD patients, a negative correlation between the nuclear content of TFEB and steatosis score was observed, suggesting that TFEB activity is impaired in the liver, which may be associated with reduced autophagic activity [63]. Activation of mTORC1 on the lysosomal surface phosphorylates TFEB and promotes its cytoplasmic localization. During nutrient deprivation or lysosomal dysfunction, a decrease in lysosome-derived amino acids can inhibit mTORC1 activity, leading to dephosphorylation of TFEB and translocation to the nucleus. Conversely, TFEB activation upregulates lysosomal activity and restores mTORC1 activity [64]. Chronic increases in cytoplasmic calcium concentration in hepatocytes during obesity and lipotoxicity can attenuate autophagic flux by preventing fusion between autophagosomes and lysosomes. Park [65] et al. used the calcium channel blocker verapamil in the treatment of obese mice, which restored aberrant cytoplasmic Ca2+ levels in hepatocytes and markedly ameliorated impaired autophagosome-lysosome fusion. RUBICON is a BECLIN- 1 interacting protein that inhibits autophagosome-lysosome fusion, which was shown to be upregulated in liver tissues of mice fed HFD and led to the progression of MASLD [31]. Finally, altered lipid availability during obesity has also been suggested to contribute to impaired autophagy. Koga [66] et al. demonstrated that in vitro and in vivo lipid overload impairs autophagosome-lysosome fusion due to the altered lipid composition in both membranes.

5. Summary and Outlook

Autophagy is a complex dynamic process, and its dysfunction may lead to the development of MASLD. In turn, MASLD itself may lead to impaired autophagy, creating a vicious cycle that promotes disease progression. Therefore, strategies targeting autophagy show great potential in the treatment of MASLD. Currently, the supporting evidence for the correlation between autophagy and human MASLD is limited and mainly observational, and how to accurately grasp the "critical value" of the over-activation of autophagy, which may also cause cell damage or apoptosis, is an urgent challenge to be solved. On the other hand, due to the complexity of the pathogenesis of MASLD, drug combinations that include the initiation of autophagy and restoration of blocked degradation processes may be an effective treatment for MASLD; moreover, autophagy can be induced by a variety of pharmacological and non-pharmacological methods, and further research in this direction may broaden the therapeutic strategies for MASLD.

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Author Profile

Xiaoning Zuo male, 1995-, master candidate, research interest: Combination of Chinese and Western medicine to prevent and treat digestive system diseases, Email: 1938901903@qq.com.

Yuhan Wang female, 2006-, bachelor candidate, research interest: Imaging diagnosis and integrated treatment of liver diseases, Email: 1484274517@qq.com.

Ning Yao female, 1978-, doctor of medical sciences candidate, associate professor, postgraduate supervisor, research interests: Pathogenesis and integrated treatment of endocrine and metabolic diseases, E-mail: doctor.ny001@hotmail.com.

Xin Wang male, 1974-, Ph.D., associate professor, postgraduate supervisor, research interests: Combination of Chinese and Western medicine to prevent and treat digestive system diseases, E-mail: doctor.xw001@hotmail.com.