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Cell-type specific role of autophagy in the liver and its implications in non-alcoholic fatty liver disease

Hepatic autophagy and NAFLD

Sana Raza, Sangam Rajak, Rajani Singh, Jin Zhou, Rohit A Sinha, Amit Goel

Abstract

Autophagy, a cellular degradative process, has emerged as a key regulator of cellular energy production and stress mitigation. Dysregulated autophagy is a common phenomenon observed in several human diseases, and its restoration offers curative advantage. Non-alcoholic fatty liver disease (NAFLD) more recently rechristened as metabolic dysfunction-associated fatty liver disease (MAFLD) is a major metabolic liver disease affecting almost 30% of the world population. Unfortunately, NAFLD has no pharmacological therapies available to date. Autophagy regulates several hepatic processes including lipid metabolism, inflammation, cellular integrity and cellular plasticity in both parenchymal (hepatocytes) and non-parenchymal cells (Kupffer cells, hepatic stellate cells and sinusoidal endothelial cells) with profound impact on NAFLD progression. Understanding cell type-specific autophagy in the liver is essential to develop targeted treatments for liver diseases such as NAFLD. Modulating autophagy in specific cell types can have varying effects on liver function and pathology, making it a promising area of research for liver-related disorders. This review aims to summarize our present understanding of cell-type specific effects of autophagy and its implication in developing autophagy centric therapies for NAFLD.

Key Words: Autophagy; NAFLD; Hepatocytes; Macrophage; Hepatic stellate cells

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Core Tip: This review presents a succinct overview of the cell-specific distinct effects of autophagy modulation on hepatic pathophysiology and its implication on the progression of Non-alcoholic fatty liver disease (NAFLD). Effects of autophagy alteration on hepatocyte lipid metabolism, macrophage polarization and hepatic stellate cell plasticity are reviewed and discussed in reference to NAFLD pathobiology.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a hepatic manifestation of metabolic syndrome and a risk factor for diabetes, cardiovascular ailments, and hepatocellular cancers (HCC) [1-3]. It is classically defined as hepatic steatosis developed in individuals with no or moderate alcohol consumption. Although, initial clinical presentation of NAFLD includes benign steatosis but this may progress to a more severe form of the disease termed as non-alcoholic steatohepatitis (NASH) [4]. NASH is characterized by increased hepatocyte damage, hepatocyte ballooning, inflammation, and fibrosis [5]. Several factors including high calorie diets, sedentary lifestyle, gut-microbiome, and genetic predisposition, constitute a multiple-hit basis of progression of benign steatosis to NASH in certain individuals[6,7]. NASH is one of the leading causes of liver transplants worldwide [5]. Presently, there are no approved drug therapies of NAFLD and NASH. As physical activity is a key determinant of metabolic control, life-style modifications remain the only available treatment so far [8]. Furthermore, the prevalence of NAFLD, which is currently >30%, has increased significantly in the last ten years with nearly 50% increase from 1990–2006 to 2016–2019 [2]. At the molecular level, the development of NAFLD involves pathogenic alteration in several hepatic cells including hepatocytes, macrophages, hepatic stellate cells (HSCs), endothelial cells and

cholangiocytes[9]. Intracellular changes in the cellular metabolism, mitochondrial energetics, organellar homeostasis, redox hormesis and epigenetic changes in cellular plasticity govern the tissue damage and inflammatory milieu observed during NAFLD progression [10-13].

Autophagy is a cellular quality control process which is activated in response to energy crisis and cellular stress [14-16]. Historically, the liver has been recognised as an organ with high autophagy activity and hepatocytes and Kupffer cells were the first cell types where metabolic role of autophagy and lysosomes were discovered [17,18]. Autophagy serves as a key regulator of hepatocyte, lipid, and carbohydrate metabolism in the liver [19]. Similarly, autophagy in liver macrophages and HSCs differentially regulates their plasticity from a quiescent to activated phenotype [20]. In this review, we uncover the distinct role of cell-type specific autophagy in hepatic physiology and its deregulation in NAFLD.

2. AUTOPHAGY MECHANISMS

+ADw-html+AD4APA-p+AD4-Autophagy, a cellular self-digestion, plays a pivotal role in maintaining cellular homeostasis by recycling damaged or unnecessary cellular components. Autophagy ensures cell survival and contributes to various physiological and pathological processes. To date, three kinds of autophagy have been described: macroautophagy, micro-autophagy, and chaperone-mediated autophagy (CMA) (21). Autophagy involves subcellular membrane trafficking to sequester a portion of cytoplasmic constituents and organelles by a membrane-sac (termed as the phagophore) to form a double-membrane structure termed as the autophagosome. The autophagosome is then transported to the lysosome for bulk protein degradation (proteolysis) of the sequestered intracellular materials by the lysosomal hydrolases. The breakdown products are utilized as an internally derived source of energy. Autophagy may be adaptive or constitutive. Constitutive autophagy is a mechanism of +ACY-lsquo+ADs-cellular housekeeping+ACY-rsquo+ADs- that involves the removal of damaged or senescent organelles and helps to preserve basal energy balance. However,

adaptive autophagy is characterized by recycling of intracellular constituents (proteins, lipids, glycogens, and organelles) to fulfill energy requirements in the event of nutrient deficiency.+ADw-br /+AD4-Macro-autophagy (hereafter referred to as autophagy), is a highly orchestrated process, that can be divided into several key stages: initiation, elongation, maturation, and degradation. The coordinated activity of several regulatory components tightly regulates the process of autophagy from initiation to termination. Autophagy genes, often referred to as autophagy-related genes (Atgs), are a group of genes responsible for regulating and executing the autophagic process within cells (22). More than 30 autophagy related (ATG) proteins have been identified and characterized till now. The autophagic process is initiated by a serine+ACY-ndash+ADs-threonine protein kinase Unc-51 Like autophagy activating kinase 1 (ULK1)(23). The mammalian target of rapamycin (mTOR) is a central regulator of cell growth and metabolism, and known to inhibit autophagy when active. In nutrient-rich conditions, mTOR is activated, preventing autophagy initiation by phosphorylating the autophagy-initiating complex, ULK1/2. This phosphorylation inhibits ULK1/2 and disrupts autophagosome formation. By contrast, AMP kinase (AMPK) is a sensor of cellular energy status. When energy levels are low (e.g., during nutrient deprivation or stress), AMPK is activated. Activated AMPK phosphorylates ULK1/2, relieving the inhibition imposed by mTOR and promoting autophagy initiation. Additionally, AMPK activation stimulates autophagy by inhibiting mTOR directly and by activating transcription factors like transcription factor EB (TFEB), which control the expression of Atgs and various lysosomal genes. When activated, TFEB promotes autophagy by enhancing the production of autophagy-related proteins and lysosome biogenesis +ACY-nbsp+ADs-(24).+ADw-br /+AD4-ULK1/2 complex comprise proteins ULK1, ATG13, ATG101 and FIP200, that play a central role in autophagy initiation (25). The initiation phase is primarily governed by the mTOR and AMPK pathways. When mTOR is inhibited or AMPK is activated in response to nutrient deprivation or stress, ULK1 is phosphorylated and activated, and phosphorylates ATG13 and FIP200 to initiate the process of autophagosome formation (26). Once initiated, autophagy proceeds through

the elongation and maturation stages. Key proteins like autophagy-related protein 5 (ATG5) and ATG12 form complexes that contribute to the elongation (expansion) of the isolation membrane, which eventually seals to form the autophagosome+ACY-mdash+ADs-a double-membraned vesicle that engulfs cellular cargo (27). ATG5 is part of a complex with ATG12 and ATG16L1, which is crucial for the elongation of the phagophore and the closure of the autophagosome. Lipid-+ACY-shy+ADs-conjugated microtubule-associated protein 1A/1B-light chain 3 (LC3+ACY-ndash+ADs-phosphatidylethanolamine), which is localized onto the autophagosomal membranes, plays a central role in the biogenesis and elongation of autophagosomes (28).+ADw-br /+AD4-The autophagy receptor or adaptor proteins facilitate the tethering of target proteins and organelles destined for degradation on to the autophagosome. Sequestosome+ACY-shy+ADs-1, also known as p62/SQSTM1 is a cargo receptor that recognizes ubiquitinated cargo, such as damaged organelles or proteins, and targets them for selective autophagic degradation. P62 contains LC3-interacting regions (LIR) to interact with LC3 on the autophagosome membrane. Once the double-membrane vesicle is formed, it travels along the microtubules to the lysosome, where the outer membrane of autophagosome fuses with lysosomes to form autolysosomes (28). Inside the autolysosomes, the lysosomal enzymes enable the degradation of the the cargo.+ADw-/p+AD4APA-/html+AD4-

3. AUTOPHAGY IN NAFLD

NAFLD is characterized by the accumulation of excess fat (triglycerides) in the liver, not caused by excessive alcohol consumption. Autophagy, which is the cellular process responsible for degrading and recycling damaged or unnecessary cellular components, has several implications in NAFLD. The interaction of cellular autophagy with NAFLD pathogenesis has been derived from several line of evidence as described below:

3.1 *LESSONS FROM AUTOPHAGY (ATG) GENE KNOCKOUT MOUSE MODELS*

Studies performed in liver-specific autophagy gene (*ATG5 & ATG7)* knockouts revealed a lipolytic role of autophagy, and mice deficient in either of these genes showed increased hepatic steatosis (29). The loss of autophagy genes also increased hepatocyte susceptibility to gut endotoxin-induced injury (30). Autophagy is also known to regulate hepatic inflammation. In this regard, hepatic macrophages also known as Kupffer cells derived from *ATG5*$^{-/-}$ mice, acquired a pro-inflammatory phenotype resulting from macrophage polarization when fed with a high-fat diet (HFD) (31).

3.2 *STUDIES INVOLVING PHARMACOLOGICAL/NON-PHARMACOLOGICAL AUTOPHAGY INDUCERS IN ANIMAL MODELS OF NAFLD*

Preclinical experiments performed with a classical autophagy inducer, such as, rapamycin resulted in the reduction of hepatic steatosis and injury in animals fed HFD(32). Similarly, the administration of autophagy inducing hormones such as thyroid hormone, ghrelin, glucagon like peptide-1 (GLP-1) and vitamin D also increased autophagy in mouse liver and reduced steatosis in animals fed high calorie diets (33-38). Similarly, several natural compounds including caffeine, epigallocatechin gallate (EGCG), and resveratrol, together with several herbal extracts derived from traditional Chinese and Indian medicines, have exhibited potent pro-autophagy activity which is associated with their anti-NAFLD effect in animals (39-49). Besides pharmacological agents, lifestyle modifications including intermittent fasting (50, 51) and exercise (52-54) also induce hepatic autophagy as a means to counter NAFLD/NASH pathogenesis.

3.3 ANALYSIS OF LIVER AUTOPHAGY IN HUMAN NAFLD

+ADw-html+AD4APA-p+AD4-Assessment of autophagy in the liver biopsies of patients with progressive degree of severity showed reduced expression of lysosomal cathepsins, accumulation of p62 and decreased autophagy flux (55, 56). Furthermore, the impairment of autophagy strongly correlated with markers of hepatic injury and

inflammation(55, 56). More recently, whole exome sequencing data has revealed pathogenic mutations in human autophagy related genes which increases the susceptibility to develop NAFLD(57, 58). Notably, the defects in autophagy observed in human NAFLD are similar to that observed in murine models of NAFLD wherein early increase in autophagic flux is followed by late block in autophagic flux and concomitant increase in ER-stress(56, 59).+ADw-/p+AD4APA-/html+AD4-

4. AUTOPHAGY IN HEPATOCYTES

+ADw-html+AD4APA-p+AD4-Hepatocytes are cells of parenchymal origin, which are the metabolic hub of the liver. These are the primary functional cells of the liver and play a central role in metabolic processes, detoxification, and protein secretion. Not surprisingly, autophagy has been widely studied in these cells under physiological and pathological conditions including NAFLD. Hepatocytes rely on autophagy to remove damaged organelles, manage energy balance, and regulate lipid metabolism. The biological effects of autophagy on hepatocytes and its modulation under NAFLD is described below:+ACY-nbsp+ADsAPA-/p+AD4-

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4.1 *ROLE OF AUTOPHAGY IN HEPATOCYTE LIPID AND CARBOHYDRATE METABOLISM*

Hepatocytes store excess neutral lipids in the form of lipid droplets which are composed of triacylglycerol (TAG). These TAG stores can be degraded by lipases to release free fatty acids (FFAs) as fuel for ATP production. The lipolysis of TAGs mediated by an autophagy-lysosomal pathway was termed “lipophagy” within hepatocytes (29). The sequence of events involved in lipophagy involves the engulfment of lipid droplets by the autophagosomes, followed by their fusion with lysosomes where lipolysis of TAG takes place. The FFAs released from the lysosomes henceforth can be utilized for mitochondrial fat oxidation (29). The key lipase involved in this process is known as lysosomal lipase (LIPA) (29). Defects in hepatocyte lipophagy have

been suspected to be a major cause of early NAFLD development in humans (60-62). Besides the role of autophagy which is the major mediator of lipophagy, chaperon-mediated autophagy (CMA) also plays a key role in the lipolysis of TAGs within hepatocytes (63). In this regard, both LD-associated proteins perilipin 2 (PLIN2) and perilipin 3 (PLIN3) have been identified as CMA substrates and their degradation *via* CMA precedes lipolysis by lipophagy (63). Additionally, lipid degradation by microautophagy termed as "macrolipophagy" has been reported to occur in mouse hepatocytes supplemented with oleate, followed by nutrient starvation(64). Lipophagy has been shown to be activated by MTORC1 inhibition (65) , fibroblast growth factor-21 (FGF-21) (36), as well as by the activation of nuclear receptors including thyroid hormone receptors (THRs), Peroxisome proliferator-activated receptor alpha (PPARα) and transcription factor EB (TFEB) exhibiting anti-steatosis effects (47, 66-69). More recently, the induction of lipophagy was shown to enhance lysosomal mediated lipid exocytosis, thereby ameliorating NASH in animal models (70).

Surprisingly, autophagy and autophagy genes have also been implicated in the assembly of TAGs in hepatocytes. Reports have shown that loss of autophagy genes such as *MAP1LC3* (71), *ATG7* (72) and *FIP200* (30) leads to decreased lipid droplet accumulation in hepatocytes (**Figure 1)**. This totally contrasting effect of autophagy, as described above for lipophagy, presents a paradoxical nature of autophagy in lipid droplet assembly *vs.* degradation may result from the differential effects of *ATG* genes and nutrient status in cells, and is yet to be resolved (73).

Besides its role in lipid metabolism, autophagy also plays a significant role in hepatocyte carbohydrate metabolism by regulating glycogen breakdown (74). The lysosomal α-acid glucosidase (GAA) can hydrolyse glycogen and release free glucose(75). Excessive glycogen deposition in hepatocytes commonly coexist with hepatic injury in both patients with NAFLD (76) and glycogen storage disease type Ia (GSD Ia) (77). GSD Ia is the most common glycogen storage disease. It is caused by the loss-of-function mutation of glucose-6-phosphatase, the enzyme converting glucose-6-phosphate to free glucose. Besides glycogen, GSD Ia is also characterized by excess lipid

accumulation in the liver, and is now considered as a fatty liver-like disease. Recently, the induction of autophagy was shown to attenuate the development of hepatic steatosis and reduce glycogen content in animal model of GSDIa (78). These results, therefore, suggest an intricate interplay between hepatocyte autophagy and glycogenolysis

4.2 AUTOPHAGY AND HEPATOCYTE LIPOTOXICITY

Lee *et al* (79), for the first time, termed the harmful effects of lipid species such as saturated free fatty acids (SFAs) and cholesterol in non-adipose organs as "lipotoxicity." At the molecular level, NAFLD/NASH induced lipotoxicity in hepatocytes is characterized with increased oxidative stress, mitochondrial dysfunction, impaired unfolded protein response (UPR), proinflammatory cytokine production, and cell death (80, 81). Intriguingly, basal autophagy inhibition is also observed in response to SFAs such as palmitic acids (82). Chronic SFAs administration impairs autophagosomal-lysosomal fusion, cause disruption of hepatocyte autophagy through suppression of the immune surveillance protein DDX58/Rig-1 (DExD/H box helicase) and stimulates STING-MTORC1 pathway contributing to the autophagy inhibition reported in advanced NAFLD (65, 82, 83). Therefore, restoration autophagic flux has emerged as an important strategy to counter lipotoxicity in hepatocytes (84).

Besides being involved in macromolecular breakdown of lipids, protein and carbohydrates, autophagy is also involved in selective removal of damaged organelles. The autophagic removal of mitochondrial, known as "mitophagy" is a process of mitochondrial pruning that prevents the accumulation of damaged mitochondria resulting from increased oxidative stress (85). Defective mitophagy has been shown to be associated with impaired mitochondrial β-oxidation and increased oxidative stress and lipoapoptosis in both animal models as well as in human NAFLD (86, 87). In hepatocytes, the accumulation of damaged mitochondria resulting from lipotoxicity, may lead to mitochondrial mediated apoptosis as well as activation of inflammasome complex (88). Therefore, the induction of mitophagy ensures both sustained

mitochondrial energetics as well as cell survival (**Figure 1)**. Several mechanisms have been proposed to regulate mitophagy in NAFLD (35, 89-96) . Acyl-CoA:lysocardiolipin acyltransferase-1 (ALCAT1) expression was shown to be elevated in an HFD fed mice, and its silencing restored mitophagy in isolated hepatocytes with observable improvement in mitochondrial architecture and reduced hepatic steatosis in mice (97). Furthermore, the plant flavanol quercetin alleviates HFD-induced hepatic steatosis by activating AMPK-dependent mitophagy (98). Furthermore, sirtuin 3 (SIRT3) overexpression stimulates mitophagy and protects hepatic cells against palmitic acid-induced oxidative stress (99). Mitophagy is also induced by thyroid hormone (100) to both sustain mitochondrial fat oxidation as well as maintain cellular health.

Autophagy also protects hepatocytes against lipotoxicity-induced oxidative stress by degrading kelch like ECH associated protein 1 (KEAP1), which results in nuclear factor, erythroid 2 Like 2 (NRF2/NFE2L2) nuclear translocation and transcription of antioxidant genes (101). Autophagy gene ULK1 was shown to enhance the interaction of autophagy adapter protein p62/SQSTM1 with KEAP1 which results in the autophagy-mediated degradation of KEAP1 and NRF2 mediated protection from lipotoxicity **(Figure 1)** (102).

SFA's induced endoplasmic reticulum stress (ER-stress) and impaired UPR response is also a key feature associated with NAFLD progression in humans (56, 103). SFAs, cause ER stress by increasing di-saturated glycerolipid and saturated phospholipid accumulation in the ER, which causes persistent inositol-requiring enzyme (IRE)-1α, and protein kinase RNA-like ER kinase (PERK) activation in hepatocyte (104, 105). Eventually SFAs-induced hepatocyte lipoapoptosis occurs owing to continuous UPR activation, which results in JNK and CHOP-mediated overexpression of proapoptotic proteins such as p53 upregulated modulator of apoptosis (PUMA) (106). Autophagy serves as key degradative mechanism for misfolded proteins in hepatocytes thus alleviating ER-stress caused by SFA's (107). In this regard, HFD feeding was associated with increased hepatic ER stress and insulin resistance in a study utilizing autophagy defective animals (108). Surprisingly, rescue experiments with *ATG7* gene

overexpression dramatically recovered lipid-induced ER-stress in the mouse liver, as well as hepatic insulin sensitivity (108). Besides degrading specific misfolded proteins, autophagy can also degrade a part of damaged ER by a process known as "ER-Phagy". Although the mechanistic basis of this process is still not very clear in hepatocytes, its role in NAFLD pathogenesis was highlighted by RNA sequencing data revealing numerous ER-phagy receptors such as ATL3, SEC62, and RTN3 as differentially regulated in patients with NAFLD/NASH (107). This data points towards ER-phagy playing an essential role during NASH and underscores its importance as a possible novel strategy for NASH treatment.

SFA exposure in hepatocytes triggers the NLRP3-inflammasone signaling, leading to the activation of IL-1β which causes hepatocyte cell death (109-112). The inhibition of inflammasome activation and hepatocyte pyroptosis is another way of cellular protection conferred by autophagy in hepatocytes (35).

5. AUTOPHAGY IN LIVER MACROPHAGES

The liver is a vital organ with diverse functions, including metabolism, detoxification, and immune regulation. Within the liver's intricate cellular landscape, Kupffer cells, the resident macrophages, are critical players in immune surveillance and tissue homeostasis. Autophagy, a conserved intracellular process, has emerged as a key regulator of Kupffer cell functions and liver physiology. Autophagy in Kupffer cells, plays a pivotal role in maintaining hepatic homeostasis, regulating inflammation, by eliminating misfolded or aggregated proteins, removing damaged organelles and invading pathogens (113).

Macrophages are highly heterogeneous immune cells, which can polarize to diverse phenotypes in response to the surrounding microenvironment (114). During inflammation or injury, macrophage polarization determines the fate of an organ (114). When an organ or a tissue is inflicted with an infection or injury, macrophages are first polarized to their proinflammatory M1 phenotype to facilitate the removal of antigens and necrotic cells by releasing proinflammatory cytokines. Further, the M1

macrophages polarize with the M2 macrophages at the stage of repair, to secrete anti-inflammatory cytokines and suppress inflammation, which promotes tissue repair and remodeling. Autophagy regulates macrophage polarization in NAFLD (31) (115) (116). Macrophage autophagy reduces chronic inflammation and lowers the progression of organ fibrosis by inhibiting M1 macrophage polarization (117) (**Figure 1)**. Impaired macrophage autophagy elevated immune response and chronic hepatic inflammation and injury in obese mice (31). Ubiquitin-specific protease 19 (USP19) induced macrophage autophagy flux promotes anti-inflammatory M2-like macrophage polarization (116). Chronic liver injury results in organ scarring, termed liver fibrosis. Tissue-resident macrophages are the crucial regulators of organ fibrosis (118). Inflammation plays a vital role and may be a cause of fibrosis (119). Macrophage autophagy inhibits macrophage polarization to pro-inflammatory M1 type, and therefore, it may be potential target for organ fibrosis. Macrophage activation and polarization are increasingly being recognized to play an essential role in liver inflammation and fibrosis (120). Autophagy inhibited the release of inflammatory cytokines, particularly interleukin-1 (IL-1), from hepatic macrophages and reduced HSC activation protecting against liver fibrosis in mice (121). Also, the suppression of Atg5 showed increased liver inflammation and fibrosis *via* enhanced mitochondrial ROS/NF-κB/IL-1α/β pathway in autophagy-deficient liver macrophages (122). Macrophage autophagy was reported to downregulate hepatic inflammation by inhibiting inflammasome-dependent IL-1β production (123). Spermine, a polyamine, reduced liver injury by inhibiting the pro-inflammatory response of liver-resident macrophages *via* inducing autophagy (124). LC3-associated phagocytosis (LAP) inhibited inflammation and liver fibrosis by pharmacological as well as genetic interventions. Inhibition of LAP aggravated pro-inflammatory and pro-fibrotic phenotype in the liver (125). Autophagy is also involved in immune regulation in the liver macrophages. It promotes antigen presentation and MHC-II expression, facilitating efficient antigen recognition by T cells. Conversely, defective autophagy can lead to exaggerated inflammatory responses (126). Dysregulation of autophagy in Kupffer cells can have wide-ranging implications for

liver diseases, making it an attractive target for future therapeutic interventions. Further research into the precise mechanisms and therapeutic potential of autophagy modulation in liver macrophages is warranted to advance our understanding of liver pathophysiology and develop novel treatment strategies.

6. AUTOPHAGY IN HEPATIC STELLATE CELLS (HSCS)

Among several cell types that contribute to liver function and pathology, hepatic stellate cells (HSCs) have emerged as key players in the development of liver fibrosis, a common endpoint in chronic liver diseases. Autophagy, a cellular process of self-digestion and recycling, has gained increasing attention for its role in HSC biology and its implications in liver disease progression. Autophagy in HSCs is intricately involved in maintaining metabolic homeostasis. It ensures an efficient turnover of cellular components, provides energy during stress or activation, and helps regulate key signaling pathways. Dysregulation of autophagy in HSCs can disrupt these metabolic processes and contribute to liver fibrosis and disease progression.

Upon liver injury or inflammation, HSCs undergo activation, transforming into proliferative, fibrogenic myofibroblasts that contribute to fibrous scar formation (127). To role of autophagy in HSC activation remains paradoxical and context specific. Studies performed in HSCs *in vitro & in vivo* showed profibrotic effect of autophagy induction during TGF-β induced HSC activation(128) (**Figure 1)**. Specifically, Autophagy is proposed to induce the activation of HSCs through lipophagy, a selective type of autophagy that degrades lipid droplets(129). In contrast, autophagy plays a critical role in maintaining HSC quiescence and limiting their activation. Inhibition of autophagy in activated HSCs has been associated with increased fibrogenesis, while induction of autophagy can suppress their activation and collagen production(130) (**Figure 1)**. However, HSC autophagy attenuated liver fibrosis by inhibiting the release of extracellular vesicles (EVs) (131). Autophagy in HSC was recently shown to induce the release of miR-29a, whereas autophagy inhibition repressed fibrogenic gene expression through attenuated miR-29a secretion in murine liver fibrosis and hepatitis

C virus patients with chronic liver disease (132). These findings underscore the therapeutic potential of targeting autophagy in HSCs to mitigate liver fibrosis and, consequently, liver disease progression. Autophagy in HSCs has significant implications for liver disease. Understanding these mechanisms holds promise for developing targeted therapies to modulate HSC metabolism and mitigate liver fibrosis. The role of autophagy in maintaining HSC quiescence and limiting fibrogenesis makes it a promising target for therapeutic intervention. Pharmacological agents that regulate autophagy in HSCs are being investigated for their potential to halt or reverse liver fibrosis and alleviate the burden of liver diseases worldwide. Furthermore, strategies to enhance the specificity of these interventions to HSCs hold promise for minimizing their off-target effects.

7. AUTOPHAGY IN LIVER SINUSOIDAL ENDOTHELIAL CELLS (LSECS)

Liver sinusoidal endothelial cells (LSECs) form the first barrier of defense in the liver owing to their unique position, lining the sinusoidal lumen. Endothelial dysfunction is known to play a key role in liver injury (133). Autophagy maintains cellular integrity, phenotype and homeostasis and can be found in various cell types, including liver endothelial cells (134). Lowered autophagy has been observed in liver endothelial cells of patients with NASH as compared to patients with simple steatosis or those with normal liver (135). The selective disruption of ATG5 or ATG7 in endothelial cells impairs the endothelial phenotype and favors liver injury, inflammation and fibrosis in mice exposed to prolonged high fat diet feeding or carbon tetrachloride (133, 135) (**Figure 1**).

8. CONCLUSION AND FUTURE PERSPECTIVE

+ADw-html+AD4APA-p+AD4-Autophagy in liver plays key role in hepatic metabolism, immunomodulation, and cellular plasticity with profound effects on NAFLD progression. Further research should be focused to better understand the role of autophagy in inter-cellular crosstalk between various cell types of the liver and its

targeting as a future therapy for NAFLD/NASH in humans. Investigating hepatocyte-specific autophagy mechanisms and their response to various stressors, such as nutrient imbalances, oxidative stress, and toxic insults is crucial to explore the therapeutic potential of autophagy modulation in NAFLD/NASH. Understanding how autophagy affects inflammation and antigen presentation in Kupffer cells could provide insights into liver-related immune disorders and manipulating autophagy in these cells may have implications for treating conditions like liver fibrosis. Additionally, exploring how autophagy contributes to LSEC integrity, angiogenesis, and regulation of blood flow may provide a better understanding of its role in liver health and disease. Furthermore, the deduction of molecular mechanisms by which autophagy influences HSC activation and collagen production can provide insights into therapeutic strategies for liver fibrosis.+ADw-br /+AD4AJg-nbsp+ADs-Given the dynamic sequence of involvement of different cell types and pleiotropic effect of autophagy during NAFLD progression, an optimal therapeutic time-window for targeting autophagy should be identified. Finally, identifying biomarkers of autophagy flux in humans and clinical trial of autophagy modulating drugs for NAFLD/NASH treatment are some keystone goals to be achieved in the near future.+ADw-/p+AD4APA-/html+AD4-

9. AVAILABILITY OF DATA AND MATERIALS

Not applicable.

10. FINANCIAL SUPPORT AND SPONSORSHIP

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11. CONFLICTS OF INTEREST

All authors declared that there are no conflicts of interest.

12. ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

13. CONSENT FOR PUBLICATION

Not applicable.

CONCLUSION

+ADw-html+AD4APA-p+AD4-Autophagy in liver plays key role in hepatic metabolism, immunomodulation, and cellular plasticity with profound effects on NAFLD progression. Further research should be focused to better understand the role of autophagy in inter-cellular crosstalk between various cell types of the liver and its targeting as a future therapy for NAFLD/NASH in humans. Investigating hepatocyte-specific autophagy mechanisms and their response to various stressors, such as nutrient imbalances, oxidative stress, and toxic insults is crucial to explore the therapeutic potential of autophagy modulation in NAFLD/NASH. Understanding how autophagy affects inflammation and antigen presentation in Kupffer cells could provide insights into liver-related immune disorders and manipulating autophagy in these cells may have implications for treating conditions like liver fibrosis. Additionally, exploring how autophagy contributes to LSEC integrity, angiogenesis, and regulation of blood flow may provide a better understanding of its role in liver health and disease. Furthermore, the deduction of molecular mechanisms by which autophagy influences HSC activation and collagen production can provide insights into therapeutic strategies for liver fibrosis.+ADw-br /+AD4AJg-nbsp+ADs-Given the dynamic sequence of involvement of different cell types and pleiotropic effect of autophagy during NAFLD progression, an optimal therapeutic time-window for targeting autophagy should be identified. Finally, identifying biomarkers of autophagy flux in humans and clinical trial of autophagy modulating drugs for NAFLD/NASH treatment are some keystone goals to be achieved in the near future.+ADw-/p+AD4APA-/html+AD4-

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