



# Autophagy in age-related liver disease

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## Abstract

Aging profoundly impacts liver physiology by disrupting autophagy, a lysosome-dependent degradation pathway essential for maintaining cellular homeostasis. Autophagy declines with aging due to reduced expression of core autophagy-related (ATG) genes/proteins, defective autophagosome fusion, and impaired selective processes such as lipophagy, mitophagy, and chaperone-mediated autophagy. These alterations contribute to lipid accumulation, oxidative stress, inflammation, and mitochondrial dysfunction, thereby accelerating age-related liver diseases including metabolic-associated fatty liver disease (MAFLD), fibrosis, and hepatocellular carcinoma (HCC). Their molecular mechanisms involve deregulation of nutrient-sensing pathways (mTOR complex 1, AMP-activated protein kinase and sirtuin 1 and 3) and context-dependent roles of autophagy-related proteins (ATG5, ATG7, LC3, Beclin-1, LAMP2A). Importantly, the regulatory role of autophagy differs across disease stages related to liver aging. During early phases, it maintains metabolic balance, mitochondrial quality control, and genomic stability in some diseases such as MAFLD and liver fibrosis. Conversely, in advanced disease, particularly in HCC, persistent autophagy supports tumor cell survival, stemness, and immune evasion. Emerging therapies seek to restore autophagic flux through caloric restriction, physical exercise, caloric restriction mimetics (rapalogs, spermidine, metformin), and pharmacological modulators such as Tat-BECLIN-1 peptides or RUBICON-targeted approaches. However, translating these therapies into clinical practice remains challenging due to systemic effects, stage-specific responses, and lack of reliable non-invasive biomarkers for monitoring autophagy in humans. Advances in nanoparticle-based delivery, biomarker-guided stratification, and combination therapies with tyrosine kinase inhibitors or immune checkpoint inhibitors may offer promising strategies. Overall, precision modulation of autophagy could serve as a potent geroprotective approach to preserve liver function, delay age-related metabolic deterioration, and prevent progression to fibrosis and cancer. Achieving this goal requires considering disease stage, systemic interactions, and autophagy's context-dependent duality in aging when implementing these strategies.

**Keywords:** Autophagy, aging, liver disease, MAFLD, liver fibrosis, HCC, geroprotective-approach

## 1. Introduction

Aging disrupts cellular homeostasis and elevates the risk of chronic diseases, with the liver being particularly vulnerable owing to its central role in metabolic regulation. Consequently, age-related hepatic conditions, such as metabolic-associated fatty liver disease (MAFLD), fibrosis, and hepatocellular carcinoma (HCC), are more prevalent among older populations<sup>[1,2]</sup>.

Autophagy, a conserved lysosome-dependent degradation pathway, maintains hepatic homeostasis by eliminating damaged organelles, misfolded proteins, and lipids<sup>[3,4]</sup>. With aging, autophagy declines primarily attributed to three core mechanisms: reduced expression of autophagy-related (ATG) core genes/proteins, defective autophagosome-lysosome fusion, and impaired selective processes such as lipophagy, mitophagy, and chaperone-mediated autophagy<sup>[4,5]</sup>. These alterations collectively drive the onset and progression of multiple age-related liver diseases such as MAFLD, fibrosis and HCC<sup>[6-10]</sup>.

Autophagy efficiency generally declines with aging, contributing to metabolic and age-related disorders such as liver dysfunction<sup>[1,11]</sup>. Global deletion of essential ATG genes is lethal in mice, while tissue-specific knockouts cause localized dysfunction and premature aging-like phenotypes. In rodents, levels of ATG and lysosome-associated membrane protein 2A (LAMP2A) of liver typically decrease with age, and recent studies suggest that their variability depends on strain, sex, and hepatic cell type<sup>[12,13]</sup>. Although direct human evidence remains limited at present, impaired autophagy is consistently observed in age-associated liver diseases such as MAFLD and



hepatic fibrosis<sup>[14,15]</sup>.

In recent decades, numerous studies have highlighted autophagy restoration as a sort of promising therapeutic strategies (lifestyle interventions and pharmacological modulators) for the aging liver in both preclinical and clinical settings<sup>[16]</sup>. Although autophagy is disabled in aging<sup>[17]</sup>, its role remains complex and sometimes controversial. Therefore, when considering autophagy modulation in geromedicine, systemic effects, dosing, and comorbidities requires to be carefully considered<sup>[18]</sup>. The major controversies include the context-dependent nature of autophagy, contradictory findings in loss-of-function models (autophagy-related gene 5 (ATG5), autophagy-related gene 7 (ATG7), Beclin-1), and its dual role of acting as a protective mechanism in early liver disease but potentially promoting tumor progression in advanced HCC.

In this mini review, we summarized current perceptions on autophagy's decline in geriatric liver, its contribution to age-related liver diseases, and emerging therapeutic strategies aimed at modulating this pathway to preserve liver function of the aged.

## 2. Autophagy in the Liver

### 2.1 Basic concepts

Autophagy is a highly selective cellular recycling process that degrades cytosolic proteins and organelles in eukaryotes, and its activity declines with age. Since the early 2000s, research on autophagy has expanded rapidly<sup>[1,3]</sup>.

There are 3 types of autophagy:

- Macroautophagy involves selective and non-selective cytosolic components enclosed in a double membrane-bound vesicle called autophagosome, which subsequently fuses with the lysosome to form an autolysosome<sup>[19]</sup>.
- Microautophagy also implies that selective and non-selective cytosolic components are directly engulfed by the lysosome through membrane invagination<sup>[19]</sup>.
- Chaperone-mediated autophagy (CMA) is a selective process in which soluble proteins with pentapeptide motif for CMA recognition (KFERQ-like motifs) are translocated into lysosomes via LAMP2A<sup>[3,19]</sup>.

### 2.2 Autophagy role in liver physiology: lipid metabolism, organelle turnover and detoxification

Autophagy plays a central role in maintaining hepatic homeostasis by regulating nutrient metabolism, organelle turnover, and cellular detoxification through both selective and nonselective mechanisms. Under nutrient deprivation, liver autophagy is primarily induced by mTOR complex 1 (mTORC1) inhibition and AMP-activated protein kinase (AMPK) activation. This induction generates essential metabolic substrates including amino acids, glucose, and free fatty acids (FFAs) by intracellular catabolism of proteins, glycogen (glycophagy), and lipid droplets (lipophagy), respectively, thereby supporting global energy balance<sup>[19,20]</sup>.

Regarding lipids metabolism, selective forms of autophagy such as lipophagy prevent hepatic steatosis by generating FFAs for mitochondrial  $\beta$ -oxidation. Lipophagy is a tightly regulated process that involves ubiquitin-mediated cargo tagging and recruitment of adaptor proteins, including sequestosome 1 (SQSTM1), neighbor of BRCA1 gene 1 (NBR1) and microtubule-associated protein 1 light chain 3 (LC3)/GABA type A receptor-associated protein (GABARAP)-phagophores. To facilitate lipid transport, lipophagy targets lipid droplet-associated proteins, specifically perilipin 2 (PLIN2) and perilipin 3 (PLIN3)<sup>[21]</sup>. Besides, hepatic transcription factors (transcription factor EB and transcription factor E3) enhance autophagic flux via lysosome-associated membrane protein 1 and reduce lipid accumulation<sup>[22]</sup>. Additionally, autophagy promotes mitochondrial  $\beta$ -oxidation by removing inhibitors of peroxisome proliferator-activated receptor alpha, such as nuclear receptor corepressor 1<sup>[23]</sup>.

Autophagy also contributes to intracellular quality control mediated by turnover of selective mitochondria (mitophagy), peroxisomes (pexophagy), and the endoplasmic reticulum (ER-phagy), thus preventing hepatocellular injury. For instance, hepatic mitophagy eliminates dysfunctional mitochondria and mutated mitochondrial DNA through PTEN-induced kinase 1 (PINK1)/Parkin and LC3/mitophagy receptor/BNIP3-like protein complexes, limiting reactive oxygen species (ROS) production and inflammatory signaling<sup>[24]</sup>. Similarly, ER-phagy, mediated by family with sequence similarity 134 member B, SEC62 homolog (ER-phagy receptor), and testis-expressed protein 264 (ER-phagy receptor) receptors alleviate ER stress in an LC3-dependent manner<sup>[25]</sup>. Furthermore, pexophagy regulates peroxisome number and function via ataxia telangiectasia mutated (ATM), SQSTM1/peroxisomal biogenesis factor 5, and LC3-dependent mechanism<sup>[26]</sup>.

Additionally, autophagy modulates hepatic detoxification by regulating redox balance and cytochrome P450 enzyme expression. Under different stress conditions, autophagy is upregulated to influence biotransformation, antioxidant responses, and hepatocyte survival. The SQSTM1-nuclear factor erythroid 2-related factor 2 (NRF2) axis illustrates how selective autophagy enhances cytoprotective gene expression and detoxification by stabilizing NRF2<sup>[27,28]</sup>. Moreover, autophagy activation in response to cellular stressor (lipotoxicity or ER stress) reduces ROS accumulation, preserves organelle function, and limits the activation of Kupffer and hepatic stellate cells (HSCs), protecting against inflammation, fibrosis, and disease progression<sup>[29]</sup>.

Altogether, these selective forms of autophagy not only maintain hepatic homeostasis but also critically modulate the progression of liver diseases. Impaired lipophagy leads to triglyceride accumulation and steatosis<sup>[30,31]</sup>, while defective mitophagy favors ROS production, hepatocyte death, and fibrosis<sup>[24]</sup>. Likewise, dysregulation of ER-phagy and pexophagy exacerbates ER stress and oxidative injury, contributing to inflammation and fibrogenesis<sup>[32,33]</sup>. Thus, selective autophagy pathways are tightly linked to the pathogenesis of MAFLD, fibrosis, and even hepatocarcinogenesis, underscoring their dual role as both protective and potentially pathogenic mechanisms depending on the disease context.

### 3. Age-Related Changes in Hepatic Autophagy

It is known that autophagy efficiency declines with aging, sequentially contributing to liver disorders (Table 1). Nevertheless, recent evidence suggests that the role of autophagy in aging is multifaceted and, at times, controversial<sup>[18]</sup>. This section reviews age-related changes in autophagy, focusing on molecular regulation, mitochondrial dysfunction, nutrient-sensing pathways and emerging interactions with the gut microbiota.

**Table 1.** Key genes and proteins involved in autophagy during age-related liver diseases, their phenotypic effects, and therapeutic strategies under evaluation.

Age-related Liver disorder	Target gene/protein	Experimental approach	Autophagy pathway involved	Phenotypic effect	Disease stage/context	Therapeutic strategy tested	References
MAFLD	RUBICON (overexpression)	HFD, HCV infection	Macroautophagy	Lipid accumulation, apoptosis, inflammation	Steatosis progression	—	[99,105,164]
MAFLD	RUBICON deletion (hepatocyte-specific)	Mouse models	Lipophagy	Reversal of steatosis, ↓ inflammation	Diet-induced MAFLD	siRNA nanoliposomes	[100,123]
MAFLD	RUBICON deficiency	Mouse models	Lipophagy	↑ steatosis (↑ NEFA influx)	Systemic metabolism	—	[106,107]
MAFLD	METTL3 → Rubicon mRNA	Mouse models	Macroautophagy	Suppressed autophagy → lipid accumulation	Steatosis	Epitranscripto mic modulation	[104]
MAFLD	Tat-BECLIN-1 peptide	Mouse models	Macroautophagy	Improved lipid metabolism, ↓ steatosis	Diet-induced MAFLD	Peptide therapy	[52]
Fibrosis	Autophagy in HSCs	HSCs <i>in vitro</i> , mouse fibrosis	Lipophagy	Energy for collagen secretion, fibrosis promotion	Early fibrosis	TGF-β inhibitors	[108-109]
Fibrosis	Autophagy in HSCs	HSC regression models	Macroautophagy	HSC quiescence, ↓ collagen	Regression fibrosis	miR-29 mimics, GATA4 induction	[110,113]
Fibrosis	ECM stiffening	Human, mouse models	Macroautophagy	Profibrotic activation	Chronic fibrosis	LOXL2 inhibitors (mechanotherapy)	[165]
Fibrosis	CD4+ T / macrophages	Human, mouse model	Macroautophagy	↑ inflammation, HSC activation, fibrosis	Fibrogenic	—	[37,48]
HCC	BECLIN-1 and ATG5 (↓ expression)	Human Mouse models	Macroautophagy	↓ tumor suppression, ↑ HCC risk	Premalignant, cirrhosis	—	[117]

HCC	ATG5 (↑ in tumors)	Human	Macroautophagy	Poor differentiation, metastasis, ↓ survival	Advanced HCC	—	[116]
HCC	ATG5/7 deletion (hepatocyte-specific)	Mouse models	Macroautophagy	Inflammation, fibrosis, NRF2 activation	Pre-malignant HCC	—	[118]
Aging liver	ATG5 expression	Mouse models	Macroautophagy	↑ lifespan, improved aging markers	General aging	—	[19,34]
Aging liver	ATG7 decline	Mouse models	Macroautophagy, mitophagy	Steatosis, fibrosis, defective mtDNA clearance	Aging liver	mTOR inhibition (partial)	[41-44]
Aging liver	ATG7 rare variants	Human	Macroautophagy	Severe MAFLD, fibrosis, HCC	Human aging	—	[39-40]
Aging liver	LC3 decline	Aged hepatocytes	Lipophagy	↓ autophagosomes, steatosis	Aging liver	—	[13,46-48]
Aging liver	Beclin-1/F121A mutation	Mouse models	Macroautophagy	↑ basal autophagy, lifespan extension; overactivation → fibrosis	Aging liver	—	[51-52]
Aging liver	LAMP2A decline	Aged mouse models	CMA	Impaired lysosomal fusion, TG accumulation	Aging liver	—	[5,23,53-54]
Aging liver	PINK1, Parkin loss	Mouse models	Mitophagy	Dysfunctional mitochondria, ↑ ROS, senescence, steatosis	Aging liver	—	[61-62]
Aging liver	SIRT1 decline	Mouse models	Macroautophagy	Impaired autophagosome maturation, ↑ inflammasome	Aging,	SIRT1 activators	[68-69,88-89,47-49]
Aging liver	SIRT3 loss	Mouse models	Mitophagy	Hyperacetylation, defective autophagy	Aging	—	[65,67]
Hepatotoxicity	—	<i>In vitro</i> (HepG2 cells)	Macroautophagy, mitophagy	↓ Damage-associated hepatotoxic stress by APAP	Aging-associated hepatotoxic stress	Derived-Postbiotic supplementation ( <i>L. fermentum</i> , HV110)	[94]
Hepatotoxicity	ATG5 (↑ expression)	Mouse models	Macroautophagy	↓ Damage-associated hepatotoxic stress by D-Galactose	Aging-associated hepatotoxic stress	Probiotic supplementation ( <i>L. plantarum</i> NJAU-01)	[95]
Aging liver	ATG5, ULK1,	Mouse	Macroautophagy	↓ Steatosis;	Diet-induced	Apple Polyphenol	[96]

	BECLIN-1 (↑ expression)	models		microbiota remodeling	MAFLD	Extract supplementation
Aging liver	—	Mouse models	Macroautophagy (indirect effects)	↑ anti-aging metabolites (liver and systemic Spermidine)	General aging	Pro/Postbiotic supplementation ( <i>A. muciniphila</i> ) [98]

APAP: acetaminophen; ATG5: autophagy-related gene 5; ATG7: autophagy-related gene 7; BECN1: beclin-1; CD4: cluster of differentiation 4; CMA: chaperone-mediated autophagy; ECM: extracellular matrix; GATA4: GATA binding protein 4; HCC: hepatocellular carcinoma; HCQ: hydroxychloroquine; HCV: hepatitis C virus; HFD: high-fat diet; HSC: hepatic stellate cell; HV110: *Lactobacillus fermentum* BGHV110; LAMP2A: lysosome-associated membrane protein 2A; LC3: microtubule-associated protein 1 light chain 3; LOXL2: lysyl oxidase-like 2; MAFLD: metabolic-associated fatty liver disease; METTL3: methyltransferase-like 3; NEFA: non-esterified fatty acids; NRF2: nuclear factor erythroid 2-related factor 2; PINK1: PTEN-induced kinase 1; ROS: reactive oxygen species; SIRT1: sirtuin 1; SIRT3: sirtuin 3; TG: triglycerides; TGF: transforming growth factor  $\beta$  1; ULK1: unc-51 like autophagy activating kinase 1; ↑: increased; ↓: decreased.

### 3.1 Molecular evidence of impaired autophagy in the aged liver

Aging impairs autophagic function in hepatocytes by transcriptionally downregulating autophagy-related genes like Atg5, Atg7, Beclin-1, LC3, and LAMP2A<sup>[45]</sup>. Pyo and colleagues demonstrated that Atg5 deficiency in hepatocytes promotes hepatic inflammation<sup>[34]</sup>. In fact, moderate Atg5 overexpression enhances autophagic flux in liver, improves systemic aging markers, and extends lifespan, though liver-specific benefits appear to be context-dependent<sup>[34]</sup>. In aged mice, macrophage/dendritic-specific Atg5 deletion also skews polarization toward a pro-inflammatory phenotype, aggravating tissue injury<sup>[35,36]</sup>, while T cell-specific deletion exacerbates fibrosis<sup>[37]</sup>. Conversely, the injury of hepatocyte-targeted Atg5 knockout (KO) mice is mitigated by defective autophagic flux, sustained activation of NRF2 and compensatory proliferation<sup>[38]</sup>.

Declining Atg7 expression in aged hepatocytes not only diminishes autophagic activity but also impairs mitochondrial DNA clearance, compromising quality control. Baselli and other authors have described that rare human Atg7 variants (p.P426L, p.V471A) correlate with severe MAFLD, fibrosis, and HCC progression<sup>[39,40]</sup>. In mice, Atg7 ablation exacerbates MAFLD induced by high-fat diet (HFD)<sup>[41,42]</sup>. Accordingly, hepatocyte-specific Atg7 deletion *in vivo* causes acute hepatitis, defective regeneration, fibrosis, and disrupted lipid metabolism<sup>[43,44]</sup>. Some of them are partially reverted by mTOR inhibition and afterwards the survival rates can be enhanced<sup>[43]</sup>. Recent controversial results demonstrated that both systemic and liver-specific Atg7 ablation protects against asparaginase-induced hepatic steatosis<sup>[45]</sup>. This can be explained by the distinct pathophysiological contexts and metabolic demands. Collectively, these results highlight the dual and context-dependent role of Atg7 in liver physiology, underscoring the need for further studies to clarify its precise contribution.

Alterations in Atg8 (LC3) system are key features of hepatic aging. With aging, LC3B lipidation, autophagosome formation, and lipophagy are reduced<sup>[4]</sup>. Unlike extrahepatic tissues, the liver fails to maintain LC3-dependent flux<sup>[46]</sup>. Single-cell data demonstrate more prominent downregulation of Atg8 in hepatocytes than in Kupffer or HSC cells, and further modulation by inflammation and cellular shifts<sup>[13,47]</sup>. Of note, LC3B-II alone is an imperfect marker, as non-canonical functions such as LC3-associated phagocytosis remain active, exerting anti-inflammatory and anti-fibrotic effects, whereas their inhibition worsens fibrosis<sup>[48]</sup>. Another non-canonical pathway, Atg8ylation described by Deretic and Lazarou, involves the covalent attachment of Atg8 proteins to membranes and is altered with aging<sup>[49,50]</sup>, but its role in the liver remains unclear.

Modulation of Beclin-1 yields mixed benefits. Disruption of the Beclin-1-BCL2 interaction (*Becn1<sup>F121A/F121A</sup>* mice) and beclin-1 peptides *in vivo* enhance hepatic autophagy and extends lifespan<sup>[51,52]</sup>. However, aged hepatocytes fail to mount sufficient Beclin-1-driven responses to protect against age-related liver phenotype<sup>[46]</sup>. Thus, Beclin-1 plays a pivotal role in triggering autophagy in the aging livers.

Additionally, Cuervo and others have demonstrated that age-related LAMP2A loss impairs autophagosome-lysosome fusion and CMA, reducing fatty acid oxidation and promoting lipid accumulation<sup>[5,23,53]</sup>. This decline is more pronounced in aged males than in females<sup>[54]</sup>, but it is not universal, as CMA activity can remain stable or increase in certain genetic backgrounds (UM-HET3 and C57BL/6J, respectively)<sup>[55,56]</sup>. Recent studies also indicate sex-specific differences in hepatic autophagic responses under metabolic stress: female rats display impaired autophagic flux (mediated by LAMP2A) and reduced antioxidant activation compared to males<sup>[57]</sup>. Notably, in mice overexpressing specific cytochrome CYB5R3, females, but not males, exhibit enhanced mitochondrial turnover (mitophagy) and differential regulation of autophagy markers, further highlighting the sex-dependent nature of autophagic adaptation<sup>[58]</sup>. These findings suggest that CMA vulnerability to aging is context-dependent, shaped by genetic and endocrine factors, thereby complicating the development of broadly applicable autophagy-targeted therapies.

Finally, it is important to emphasize that all of the above-mentioned alterations in ATG5, ATG7, Beclin-1, LC3, and LAMP2A are not isolated events, but occur within a collaborative regulatory network. Autophagosome biogenesis is initiated by the assembly of a Beclin-1-dependent complex, which recruits the ATG5-ATG12-ATG16L1 conjugation system and ATG7 to promote LC3 lipidation and membrane expansion, culminating in LAMP2A-mediated lysosomal fusion. Consequently, age-related decline in any single component can disrupt the entire cascade, amplifying defects in autophagic flux and exacerbating liver aging phenotypes<sup>[59,60]</sup>.

### 3.2 Mitochondrial dysfunction in the aging liver

Mitochondrial quality maintained through mitophagy, but declines with aging. In the liver, mitochondrial impairment is characterized by reduced mitophagy flux, which disturbs redox homeostasis due to the accumulation of depolarized mitochondria and excessive ROS production<sup>[64]</sup>. Under this stress condition, key regulators such as PINK1, Parkin, BNIP3, and sirtuin 1 (SIRT1) become dysfunctional, thereby exacerbating the liver injury<sup>[62]</sup>. Nonetheless, Parkin-independent, p62/SQSTM1-mediated hepatic mitophagy remains partially active, providing a compensatory mechanism that delays mitochondrial collapse<sup>[63]</sup>. Liver-specific Atg7 knockout models underscore the role of basal autophagy in preserving mitochondrial DNA integrity<sup>[64]</sup>.

Mitochondrial dysfunction also reflects impaired dynamics in the aged liver. One common process is the hyperacetylation of fusion/fission proteins (MFN2, PINK1) as reduced SIRT1 and SIRT3 activity disturb morphology and ATP production<sup>[65,66]</sup>. SIRT3 loss, in combination with ATG4B deficiency, further compromises autophagosome maturation<sup>[67]</sup>. Moreover, increased ROS production associated with hepatic SIRT1 loss induces bioenergetic failure and proinflammatory responses, including activation of the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome and ER stress, thereby promoting fibrosis and inflammaging<sup>[68,69]</sup>. Importantly, mitochondrial autophagy alterations are not limited to hepatocytes, but also involve endothelial dysfunction, loss of zonation, and HSCs cell activation, highlighting the multicellular nature of hepatic aging<sup>[10,70]</sup>.

### 3.3 Nutrient sensing and autophagy in the aging liver

Physiological decline associated with aging, including insulin resistance, hormonal imbalance, and chronic low-grade inflammation, markedly influences the metabolism of autophagy regulators. These systemic alterations impair nutrient-sensing pathways such as mTOR, AMPK, and SIRT1, diminishing their ability to properly respond to metabolic cues. Consequently, the progressive changes of these pathways contribute to the heterogeneous decline of autophagic activity observed among elderly populations<sup>[1,68,71-73]</sup>.

The mTOR, AMPK, and SIRT1 pathways that depend on nutrients status can impact autophagy in the aged liver<sup>[74]</sup>. Mild chronic activation of mTORC1 reduces lifespan by approximately 30% and promotes parenchymal injury, inflammation, and senescence features in mice<sup>[74]</sup>, while dysregulated nutrient and hormonal signaling to mTORC1 alters hepatocyte metabolic zonation<sup>[72]</sup>. Persistent mTORC1 hyperactivation suppresses unc-51 like autophagy activating kinase 1 (ULK1)-mediated initiation and lysosomal gene transcription, driving accumulation of damaged organelles<sup>[75,76]</sup>. Although pharmacological mTOR inhibition restores liver autophagy<sup>[77,78]</sup>, it may impair regenerative capacity *in vivo*<sup>[79,80]</sup>, suggesting the need for mTOR-independent strategies such as AMPK-ULK1 activation without compromising regenerative capacity<sup>[81]</sup>.

AMPK, traditionally viewed as a pro-autophagic and longevity-promoting kinase<sup>[82]</sup>, supports mitochondrial<sup>[83]</sup> and lysosomal function<sup>[84]</sup>. However, Park *et al.* indicated that under energy stress, hepatic AMPK can paradoxically inhibit autophagy by suppressing ULK1 activity, contrary to its canonical function<sup>[85]</sup>. Simultaneously it can protect ULK1 and the associated autophagy machinery from caspase-mediated degradation, thus preserving the capacity to resume autophagy upon recovery *in vivo*<sup>[86]</sup>. This dual regulatory capacity suggests that AMPK functions both as a brake, preventing excessive autophagy activation, and as a safeguard, preserving the integrity of its core components.

SIRT1 can control autophagy by deacetylating ATG proteins and transcription factors regulating lysosomal function. Its age-related decline impairs autophagosome maturation, aggravates mitochondrial dysfunction, and enhances inflammasome activation<sup>[69,87]</sup>. Nutrient-induced O-linked  $\beta$ -N-acetylglucosaminylation (post-translational modification) activates SIRT1, protecting hepatic cells from stress<sup>[88]</sup>. Similarly, SIRT1 restoration in HFD-treated mice attenuates liver lipotoxicity<sup>[88,89]</sup>. Nonetheless, in hepatocarcinogenesis, SIRT1 activation under glucose deprivation can reprogram metabolism<sup>[90]</sup> or promote tumorigenic pathways via p62/SQSTM1<sup>[91]</sup>, contributing to liver cancer progression via autophagy-dependent<sup>[92,93]</sup> or autophagy-independent pathway<sup>[91]</sup>, respectively. Thus, SIRT1 exemplifies the duality of nutrient-sensing pathways in the aging liver, acting as both a guardian of metabolic homeostasis and, under pathological conditions, a driver of disease progression.

### 3.4 Microbiota and autophagy in liver aging

The gut microbiota exerts a profound influence on hepatic metabolism and aging, partly by modulating autophagic pathways. Recent studies have explored the interplay between microbial products, autophagy, and liver protection. For instance, Lactobacilli, a genus of beneficial bacterium commonly found in fermented foods, has been shown to modulate autophagy in hepatocytes. A postbiotic compound derived from *Lactobacillus fermentum* BGHV110 (HV110) activates autophagy in HepG2 cells and protects against acetaminophen-induced toxicity<sup>[94]</sup>. Similarly, supplementation with *Lactobacillus plantarum* NJAU-01 protects mouse liver from D-galactose-induced oxidative stress and aging-related damage<sup>[95]</sup>.

Polyphenol-rich dietary components can also modulate the gut-liver axis. For example, apple polyphenol extract (APE) protects the liver of aged mice from HFD-induced damage, promoting autophagy and increasing the Firmicutes/Bacteroidetes ratio and Akkermansia abundance<sup>[96]</sup>. However, the mechanistic link between APE-induced autophagy and microbiota remodeling remains unclear.

Studies in mice with defective hepatic autophagy have revealed that impaired autophagic flux is associated with altered bile acid metabolism, which in turn leads to adaptive changes in microbiota composition<sup>[97]</sup>. Interestingly, pasteurized *Akkermansia muciniphila* increases the hepatic levels of anti-aging metabolites such as N<sup>1</sup>, N<sup>12</sup>-diacetylspermine<sup>[98]</sup>, suggesting that modulation of the microbiota may indirectly influence liver aging through metabolite-mediated autophagy regulation.

In summary, although emerging evidence links microbiota-derived factors and autophagy to hepatoprotection during aging, the underlying causal and mechanistic relationships remain poorly understood. Further studies are needed to elucidate whether microbiota-induced autophagy activation represents a viable strategy for preventing or reversing age-related hepatic decline.

## 4. Implications for Age-Related Liver Disease and Autophagy

Autophagy dysfunction is known to contribute to disease onset and progression of various age-related liver pathologies (Table 1). Reduced ATG gene expression limits the capacity to remove misfolded proteins, promoting aggregate accumulation in MAFLD and fibrosis. Defective fusion exacerbates oxidative stress and mitochondrial dysfunction, accelerating hepatocyte death in MAFLD and fibrosis. Additionally, impaired selective autophagy drives lipid accumulation, a hallmark of MAFLD that predisposes to inflammation, fibrosis, and ultimately HCC<sup>[6-10]</sup>.

### 4.1 Autophagy and MAFLD

The aging liver undergoes progressive decline of autophagy, with lipophagy that is the selective degradation of lipid droplets being particularly compromised. This dysfunction directly contributes to the development of MAFLD. A central regulator of this process is Rubicon, an autophagy inhibitor that impairs autophagosome-lysosome fusion and accumulates in senescent hepatocytes under metabolic stress such as high-fat diets or HCV infection. Rubicon overexpression exacerbates lipid accumulation, apoptosis, and inflammation, whereas hepatocyte-specific deletion reverses steatosis in preclinical models, underscoring its therapeutic relevance<sup>[99,100]</sup>. Mechanistically, Rubicon inhibition enhances lipid flux not only by relieving the autophagosome-lysosome fusion blockade but also by restoring proper lysosomal trafficking of lipid droplet cargo. Loss of Rubicon enables SNARE-mediated vesicle tethering, allowing LC3-decorated autophagosomes to efficiently fuse with cathepsin-active lysosomes<sup>[101]</sup>. Within this compartment, lysosomal acid lipase hydrolyzes triglycerides into fatty acids, which are subsequently directed toward  $\beta$ -oxidation rather than cytosolic re-esterification<sup>[102]</sup>. Importantly, hepatic lipophagy induction reduces lipid accumulation even in ATGL-knockout livers, indicating an ATGL-independent pathway. This lipid clearance occurs without detectable increases in circulating NEFAs, implying that the enhanced turnover favors oxidative disposal over systemic hyperlipolysis<sup>[103]</sup>.

An additional epitranscriptomic layer has been identified: METTL3-mediated m<sup>6</sup>A methylation of Rubicon mRNA suppresses autophagic flux, providing novel regulatory mechanisms and therapeutic targets<sup>[104]</sup>. Viral cofactors exacerbate this dysfunction, as HCV infection enhances Rubicon expression, thereby creating synergy between metabolic and viral insults in aging patients<sup>[105]</sup>. Recent evidence further indicates that rare loss-of-function variants in ATG7 increase susceptibility to severe fatty liver disease, highlighting the role of autophagy-related gene defects in the MAFLD progression<sup>[39]</sup>.

Emerging hepatocyte-targeted therapies include anti-Rubicon small interfering RNA (siRNA) delivered via nanoparticles, which reduces steatosis and inflammation in diet-induced or FFAs-overload models<sup>[99,100]</sup>, and the pro-autophagic Tat-Bec1-1 peptide, which improves MAFLD phenotypes<sup>[52]</sup>. However, a paradox has emerged: systemic Rubicon deficiency worsens fasting-induced steatosis due to uncontrolled peripheral lipolysis and increased circulating NEFAs<sup>[106]</sup>. This underscores the importance of tissue-specific modulation of autophagy regulators, particularly in metabolically vulnerable aging contexts.

This complexity is further compounded by the liver-adipose axis: age-related loss of Rubicon in adipocytes enhances lipolysis, increasing hepatic fatty acid influx and accelerating lipotoxicity<sup>[107]</sup>. Therefore, effective MAFLD management in aging individuals necessitates a dual vigilance—restoring hepatocyte-specific autophagy while carefully monitoring systemic lipid flux to prevent maladaptive lipolytic cascades.

### 4.2 Autophagy and liver fibrosis

Hepatic stellate cells display a context-dependent dual role of autophagy during fibrosis, with disease stage determining whether autophagy promotes or restrains pathology. During early activation, autophagy facilitates the transition to collagen-producing myofibroblasts by mobilizing retinoid and lipid stores to meet energy. This process is reinforced by TGF- $\beta$ /Smad signaling and mechanosensitive responses to extracellular matrix (ECM) stiffness<sup>[108]</sup>. At this stage, pharmacologic inhibition of autophagy-related pathways, such as mTOR activation or ATG5 silencing, selectively disrupts lipid catabolism and impedes myofibroblast differentiation, underscoring the therapeutic potential of targeting autophagic flux in early fibrosis<sup>[109]</sup>.

In contrast, during regression, autophagy promotes HSC deactivation by releasing extracellular vesicles enriched with miR-29, which suppress collagen synthesis and induce quiescence<sup>[110]</sup>. In this case, therapeutic interventions, including activating GATA binding protein 4 (GATA4)/miR-29 and inducing mitophagy, can be employed to enhance selective autophagy, thereby restoring metabolic homeostasis and reinforcing the return to quiescence.

Aging exacerbates fibrogenesis through systemic dysfunction, characterized by impaired autophagy in liver infiltrated CD4<sup>+</sup> T cells. This impairment drives IL-17-mediated type 3 inflammation and the release of profibrotic cytokines that sustain HSC activation<sup>[37]</sup>. Moreover, arsenic-induced fibrosis models demonstrate that autophagy-derived cytosolic cathepsin B activates the NLRP3 inflammasome in HSCs, amplifying fibrogenesis triggered by environmental exposure<sup>[114]</sup>. This generates a self-sustaining circuit resistant to monotargeted therapies.

Recent therapeutic paradigms emphasize the importance of temporal precision. In early-stage fibrosis, inhibitors of TGF- $\beta$ 1-induced autophagy such as salvanolic acid B attenuate collagen deposition by blocking pro-activation signals<sup>[112]</sup>. Conversely, during regression, enhancement of GATA4 activity or miR-29 signaling promotes autophagic clearance of profibrotic mediators<sup>[113]</sup>. Advances in nanoparticle-based delivery systems, enable spatially targeted modulation of autophagy while minimizing off-target effects, which is a critical consideration for polymedicated older patients<sup>[114]</sup>.

Furthermore, age-related ECM stiffening hyperactivates mTOR-dependent profibrotic autophagy in HSCs, suggesting therapeutic synergy between autophagy modulators and mechanotherapy (e.g., lysyl oxidase-like 2 inhibitors)<sup>[115]</sup>. Collectively, these findings emphasize that effective antifibrotic strategies must integrate disease stage, immune senescence, and biomechanical alterations into the aging hepatic microenvironment.

### 4.3 Autophagy and HCC

Autophagy exerts temporally bidirectional effects in HCC, requiring distinct therapeutic strategies based on disease stage. In premalignant conditions or early tumorigenesis, autophagy exerts tumor-suppressive functions by clearing damaged organelles, mitigating oxidative stress, and maintaining genomic stability. Clinically, low serum Beclin-1 and altered ATG5 expression in cirrhotic patients correlate with increased HCC risk, highlighting autophagy's protective role during malignant transformation<sup>[116,117]</sup>. Consistently, hepatocyte-specific loss of autophagy (e.g., ATG7 or ATG5 deletion) accelerates dedifferentiation toward ductular progenitor-like cells through YAP/TAZ, facilitating malignant initiation<sup>[118]</sup>.

In established tumors, however, autophagy adopts a tumor-promoting role: it recycles nutrients to sustain proliferation, maintains cancer stem cell plasticity by modulating EMT, and facilitates immune evasion through PD-L1 regulation<sup>[119]</sup>. This is reflected in clinical cohorts, where high ATG5 expression predicts poor differentiation, metastasis, and reduced disease-free survival in advanced HCC<sup>[116]</sup>. Notably, macrophage-specific autophagy has been shown to restrain hepatocarcinogenesis by limiting PD-L1 expression and preserving antitumor immunity, underscoring the complexity of cell-type-specific functions<sup>[120]</sup>.

Therapeutic translation thus requires stage-specific precision. In high-risk patients (e.g. cirrhosis from MAFLD), autophagy inducers such as rapalogs may prevent tumor initiation by restoring cellular homeostasis<sup>[121]</sup>. In contrast, in advanced HCC, autophagy inhibitors such as hydroxychloroquine can overcome adaptive resistance to therapy, sensitizing tumors to sorafenib, lenvatinib, and ICIs<sup>[122]</sup>.

The Rubicon-MAFLD/HCC axis introduces a critical dimension: hepatocyte-specific inhibition of Rubicon reduces inflammation and profibrotic remodeling in MASH, potentially lowering HCC risk<sup>[123]</sup>. However, in established tumors, Rubicon deletion could paradoxically enhance pro-survival autophagic flux, necessitating combination therapies with cytotoxic or anti-angiogenic agents.

Emerging clinical frameworks prioritize biomarker-guided strategies, integrating ATG5/Beclin-1 quantification with dynamic PET imaging of autophagy to stratify patients into tailored regimens of autophagy modulators, TKIs, and ICIs<sup>[116,124,125]</sup>. In elderly populations who often present with advanced HCC and metabolic comorbidities, therapeutic strategies must balance efficacy with tolerability, emphasizing sequential or low-toxicity approaches tailored to the distinct pathophysiological features of aging.

Taken together, these findings reinforce the concept that autophagy dysfunction represents a critical turning point in liver pathophysiology during aging. In addition to its roles in lipid metabolism and inflammatory regulation, the progressive loss of this pathway impairs the liver's capacity to manage chronic insults and limits its reparative mechanisms, thereby facilitating the transition toward fibrosis and cancer. Understanding these interactions may help guide therapies that preserve autophagy and slow down age-related liver decline.

## 5. Therapeutic Modulation of Autophagy

### 5.1 Autophagy targeted therapies

Autophagy-targeted therapies emerge as promising alternatives for various diseases (Table 2). Several current interventions are known to regulate autophagy. Caloric restriction (CR) and exercise are widely recommended lifestyle modifications to improve health across many disease conditions<sup>[126,127]</sup>. CR and exercise have proven to be effective in managing hypertension<sup>[128,129]</sup>, metabolic syndrome<sup>[130,131]</sup> and liver diseases<sup>[132]</sup>. The two interventions activate multiple mechanisms including autophagy, which may underlie its beneficial effects<sup>[133,134]</sup>.

**Table 2.** Interventions targeting autophagy activation in age-related liver diseases: Evidence, mechanisms, and safety profiles

Intervention	Evidence	Autophagy activation	Endogenous	Side effects
CR	MASLD <sup>[166,167]</sup>	Macroautophagy and CMA	-	-
Exercise	MASLD <sup>[168,169]</sup>	Macroautophagy and CMA	-	-
Tat-beclin1 peptide	Mitigate MASLD	Direct activator of autophagy	Atg6/Beclin1-derived	Accelerate atheroma plate formation in mice <sup>[170]</sup>
anti-Rubicon siRNA	Mitigate MASLD	↓ Autophagy inhibition	No	Lipodystrophy, infertility and exacerbation of autoimmune diseases <sup>[164]</sup>
CRM Sirolimus	Reduced intrahepatic inflammation in MASLD	Inhibit mTORc1	No	Anemia, thromboembolism, diabetes, dyslipidemia, kidney damage, mucositis and stomatitis, lung problems, angioedema, lymphedema and osteonecrosis <sup>[159]</sup>
Spermidine	Liver fibrosis	Inhibit acetyltransferase EP300	Yes	Safe profile <sup>[158]</sup>
Metformin	MASLD in children and adolescents	Activates AMPK	No	Gastrointestinal side effects including diarrhoea, nausea, vomiting, flatulence, abdominal pain, and loss of appetite <sup>[160]</sup>
Lithocholic acid	↑ Liver healthspan and lifespan	Activates AMPK	Yes	Rosacea-like skin <sup>[171]</sup> ; ↑ coronary atheroma risk <sup>[172]</sup>
Acadesine	Improved maternal hepatic lipid metabolism disorder	Activates AMPK	Purine nucleoside analogue	Safe profile <sup>[173]</sup>

MASLD: metabolic dysfunction-associated steatotic liver disease; CR: caloric restriction; CMA: chaperone-mediated autophagy; Atg6: autophagy-related gene 6; siRNA: small interfering RNA; CRM: caloric restriction mimetics; mTORC1: mechanistic target of rapamycin complex 1; AMPK: AMP-activated protein kinase; EP300: E1A binding protein P300; ↑ : increased; ↓ : decreased.

CR stands for a reduction in daily caloric intake while preserving all essential nutritional requirements<sup>[127]</sup>. It induces CMA by stabilizing LAMP2A levels in the livers of rats, preventing the age-related decline in autophagy which is observed in these animals<sup>[3]</sup>. It also helps attenuate the decrease in macroautophagy<sup>[135]</sup>. Exercise, for its part, robustly activates both macroautophagy and CMA in liver and muscle tissues of C57BL/6J mice and human, as evidenced by increased autophagic markers and LC3-II/LC3-I ratios<sup>[136,137]</sup>. CR mimetics (CRMs) are compounds that emulate the effects of CR and are considered geroprotective molecules. Several substances are classified as CRMs, including rapalogs (sirolimus), spermidine and metformin<sup>[3,138]</sup>. Since autophagy has been associated with various diseases, and CRMs activate autophagy, these substances possess a potential therapeutic activity<sup>[19]</sup>. Particularly, an inhibitor of autophagy-suppressive acetyltransferase EP300 spermidine, has proven to be effective for preventing liver MALFD/fibrosis through an autophagy-dependent mechanism in mice treated with CCl<sub>4</sub><sup>[139]</sup> and Western diets<sup>[140,141]</sup>. Metformin activated by AMPK and sirolimus inhibited by mTORc1, regulate autophagy and are also classified as CRMs. Their combination protects rat erythrocytes against age-dependent oxidative stress<sup>[142]</sup>. Moreover, both sirolimus<sup>[143]</sup> and metformin<sup>[144,145]</sup> have demonstrated protective effects against MASLD in mice and humans, respectively. Another AMPK activator extends hepatic healthspan and lifespan in preclinical and clinical trial<sup>[84,146]</sup>, and acadesine specifically mitigates the hepatic lipid metabolism disorders, inflammation, and fibrosis caused by environmental contamination exposure during pregnancy<sup>[147]</sup>. More recently, acyl-CoA binding protein/diazepam binding inhibitor has been identified as positively correlated with aging in patients, and its neutralization induces autophagy and produces beneficial effects on aging<sup>[148]</sup> and liver damage<sup>[149-151]</sup>.

As previously noted, Tat-Beclin-1 peptides<sup>[52]</sup> and anti-Rubicon siRNA delivered by nanoliposomes<sup>[123,100]</sup> represent promising autophagy-based strategies for treating age-related liver disease such as MAFLD and fibrosis.

Nevertheless, it is important to highlight that autophagy modulation remains controversial. While autophagy activation shows

beneficial effects in aging and metabolic disorders, it may also promote tumor cell survival in cancer, particularly at advanced stages<sup>[152,153]</sup>.

## 5.2 Targeting autophagy in aged individuals

Given the beneficial effects of autophagy activation on aging-derived problems and other pathologies described in [Table 2](#), targeting autophagy activation appears to be a promising strategy to improve health in elderly populations<sup>[154]</sup>. Ideally, lifestyle interventions such as CR and exercise could be employed to activate autophagy in older adults with existing health issues or in healthy individuals for preventive purposes. However, these interventions are frequently neglected by patients. Efforts have been made to identify barriers to adopting such changes, including psychological and social factors. Unfortunately, most patients tend to prefer medication over lifestyle changes<sup>[155]</sup>.

In this context, CRMs emerge as good alternatives. Spermidine, a natural polyamine widely present in animals, exhibits decreased plasma levels with aging<sup>[156]</sup>. It produced beneficial effects on the brain, kidney, liver, cardiovascular system and immune system<sup>[156,157]</sup>. In a 3-month randomized, placebo-controlled, double-blind phase II clinical trial, elderly patients were supplemented daily with 1.2 mg spermidine derived from wheat extract. The supplementation had produced no effects on vital signs, weight, clinical chemistry parameters, or hematological safety markers, while significantly improving memory performance<sup>[158]</sup>. On the contrary, sirolimus produces a wide range of side effects, including anemia, thromboembolism, diabetes, dyslipidemia, kidney damage, mucositis, stomatitis, lung problems, angioedema, lymphedema and osteonecrosis<sup>[159]</sup>. Metformin causes gastrointestinal side effects in up to 30% of patients, including diarrhea, nausea, vomiting, flatulence, abdominal pain, and loss of appetite<sup>[160]</sup>. Acadesine, an AMPK activator with low specificity, increases uric acid production and favors lactic acidosis<sup>[161]</sup>. The use of liver-specific, such as AMPK activators, may mitigate these potential side effects<sup>[146]</sup>. More studies in humans must be carried out to fully explore the therapeutic potential and associated risks of these compounds.

Ultimately, the clinical translation of these interventions remains challenging because there are no reliable, non-invasive biomarkers to monitor autophagy activity in humans. Commonly used markers, such as LC3 and p62, are difficult to interpret outside controlled experimental settings, since they provide only a static picture of autophagy. In fact, the most reliable *in vivo* approaches require the use of autophagy flux inhibitors, which introduce additional perturbations and limit their applicability in clinical contexts<sup>[162,163]</sup>.

## 6. Conclusions

Autophagy acts as a central regulator of age-related liver diseases, with key genes and proteins such as RUBICON, BECLIN-1, ATG5/7, and sirtuins shaping disease progression. Dysregulation of lipophagy, mitophagy, and lysosomal fusion contributes to hepatic steatosis, fibrosis, and HCC. Notably, autophagy plays a dual role in cancer, suppressing tumor initiation but supporting tumor growth at advanced stages. Interventions including CR, CRMs, exercise, and pharmacologic modulators demonstrate stage-dependent benefits. However, their clinical translation is hindered by systemic effects, comorbidities, and the lack of reliable biomarkers. Future advances will require liver-targeted strategies and integrative approaches to leverage autophagy as a geroprotective therapy.

## Declarations

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### Authors contribution

Palacios-Ramírez P, Francés DE: Writing the manuscript, visualization.

Motiño O: Conceptualization, writing-original draft.

All authors have read and agreed to the published version of the manuscript.

### Conflicts of interest

Omar Motiño García-Miguel is an inventor on a patent covering therapeutic targeting of ACBP/DBI.

### Ethical approval

Not applicable.

### Consent to participate

Not applicable.

### Consent for publication

Not applicable.

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Not applicable.

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