

Senescent hepatic stellate cells drive inflammation and disease progression in MASH (Review)

ZHIQI HAN^{1*}, YIRAN SHE^{1*}, DI WU^{1*}, NUO ZHANG¹, ZHEYUAN LIU¹,
ZHONGYUAN WANG¹, XIAOYING ZHOU² and SHUO LI²

¹First Clinical Medical College, Nanjing Medical University, Nanjing, Jiangsu 211166, P.R. China; ²Department of Gastroenterology, The First Affiliated Hospital with Nanjing Medical University, Nanjing, Jiangsu 210029, P.R. China

Received September 3, 2025; Accepted January 12, 2026

DOI: 10.3892/etm.2026.13090

Abstract. Metabolic dysfunction-associated steatohepatitis (MASH) is characterized by steatosis, inflammation, hepatocellular injury and fibrosis, with the capacity to progress to cirrhosis and hepatocellular carcinoma. Recent evidence highlights cellular senescence, particularly in hepatic stellate cells (HSCs) as a key regulator of MASH pathogenesis. Senescent HSCs exhibit a context-dependent duality whereby, while transient senescence limits fibrosis through cell-cycle arrest, matrix degradation and enhanced immune clearance, persistent senescence under chronic metabolic and inflammatory stress drives disease progression. Through an expanded senescence-associated secretory phenotype (SASP), senescent HSCs exacerbate inflammation, promote extracellular matrix deposition, alter immune responses and facilitate malignant transformation. The present review summarizes the molecular mechanisms inducing HSC senescence, including lipotoxicity, oxidative stress, DNA damage, mitochondrial dysfunction and impaired autophagy. The mechanisms by which SASP factors

mediate crosstalk between senescent HSCs and other cell types are discussed, including hepatocytes, macrophages, T cells and natural killer cells, collectively altering the inflammatory and fibrotic microenvironment of MASH. Finally, emerging therapeutic strategies targeting cellular senescence are highlighted, such as senolytics, senomorphics and biomarker-guided interventions, which may offer promising avenues for modifying the course of MASH and preventing disease progression.

Contents

1. Introduction
2. HSC activation and dual role in MASH pathogenesis
3. Molecular mechanisms regulating HSC aging and SASP formation
4. Interactions between senescent HSCs and other cells
5. Future directions and outlook
6. Summary

Correspondence to: Dr Xiaoying Zhou or Dr Shuo Li, Department of Gastroenterology, The First Affiliated Hospital with Nanjing Medical University, 300 Guangzhou Road, Nanjing, Jiangsu 210029, P.R. China

E-mail: zhouxiaoying0926@njmu.edu.cn

E-mail: shuoli@njmu.edu.cn

*Contributed equally

Abbreviations: DDR, DNA damage response; ECM, extracellular matrix; FAO, fatty acid oxidation; HSC, hepatic stellate cells; LITAF, lipopolysaccharide-induced tumor necrosis factor; NAFLD, non-alcoholic fatty liver disease; NK, natural killer; PDGF, platelet-derived growth factor; PET, positron emission tomography; RNS, reactive nitrogen species; ROS, reactive oxygen species; SASP, senescence-associated secretory phenotype

Key words: metabolic dysfunction-associated steatohepatitis, senescent cells, hepatic stellate cells, senescence-associated secretory phenotype, liver fibrosis, hepatocellular carcinoma, immune microenvironment, senotherapeutics

1. Introduction

Metabolic dysfunction-associated steatohepatitis (MASH) is characterized by hepatic steatosis, inflammation, hepatocellular injury and progressive fibrosis, becoming a major cause of cirrhosis and hepatocellular carcinoma (HCC) (1,2). Despite the growing global prevalence of MASH, effective pharmacological therapies remain elusive, underscoring the need for a comprehensive understanding of the cellular mechanisms that drive disease progression (3). Among the diverse hepatic cell types involved in MASH, hepatic stellate cells (HSCs) represent the central fibrogenic population, with accumulating evidence highlighting cellular senescence as a critical and context-dependent regulator of HSC behavior (4-6).

Senescent HSCs demonstrate a dual nature. Transient senescence may be protective during acute injury by arresting proliferation, upregulating matrix-degrading enzymes and enhancing immune-mediated clearance (6). However, under the chronic metabolic and inflammatory stress conditions typically associated with MASH, senescent HSCs persist and ultimately acquire an expanded senescence-associated secretory phenotype (SASP). The SASP promotes inflammation,

stimulates extracellular matrix (ECM) deposition, disrupts immune surveillance and contributes to malignant transformation, thereby accelerating disease progression.

This duality reflects the dynamic and multifactorial regulation of HSC senescence, influenced by factors such as lipotoxicity, oxidative stress, mitochondrial dysfunction (5,7), impaired autophagy and alterations in immune surveillance (6,8,9). However, the timing, triggers and downstream consequences of HSC senescence remain incompletely characterized (10), as do the interactions between senescent HSCs, hepatocytes, macrophages and other immune cell populations (11).

The present review summarizes current knowledge regarding both the beneficial and detrimental roles of senescent HSCs in MASH, alongside the molecular pathways leading to HSC senescence and SASP formation. In addition, the mechanisms by which senescent HSCs alter the hepatic inflammatory and fibrotic microenvironment are delineated. Finally, emerging therapeutic strategies targeting cellular senescence, including senolytics, senomorphics and biomarker-based approaches are highlighted, which may offer promising avenues for modulating disease progression and improving outcomes in MASH.

2. HSC activation and dual role in MASH pathogenesis

Dichotomy of senescent HSCs in liver fibrosis. Senescent HSCs function both as a protective mechanism to limit tissue damage and as a deleterious force that drives chronic pathological progression (7,12). Research has shown that HSCs exhibit both an aging-related secretory phenotype that promotes fibrosis and an anti-fibrotic effect through cell cycle arrest (6,13). Therefore, senescent HSCs play a dual role in MASH progression. Certain cytokines and inflammatory factors, including TGF- β , platelet-derived growth factor (PDGF), TNF- α , IL-1 β and IL-6, serve key roles in promoting fibrosis (14).

Related studies propose that TNF- α is a key inflammatory factor regulated by lipopolysaccharide-induced tumor necrosis factor (LITAF) (15-17). Reduced nuclear translocation of LITAF diminishes TNF- α production, thereby inhibiting HSC activation and fibrosis. In addition, TNF- α and IL-17 exhibit a synergistic effect on HSC activity (18). A clinical experiment indicated that IL-17 amplifies the effects of TNF- α on IL-1 β and IL-6 in HSCs and the interaction between hepatocytes and HSCs may modulate the effects of IL-17 and TNF- α on fibrosis-related genes (19).

Senescent HSCs promote inflammation, fibrosis and malignant transformation

SASP is an effective driver of chronic inflammation and fibrosis in MASH. Senescent HSCs are potent drivers of chronic inflammation in MASH through their SASP. The present section examines the mechanisms by which SASP components recruit immune cells, maintain inflammatory signaling and amplify tissue damage. During hepatocyte fibrosis, aged HSCs may secrete a series of specific pro-inflammatory cytokines (such as IL-1 β , IL-6 and TNF- α), chemokines [such as C-X-C motif chemokine ligand (CXCL)-1, CXCL9 and C-C motif chemokine ligand (CCL)-2], growth factors (such as PDGF and TGF- β) and mechanism

remodeling factors, identified as the SASP, leading to chronic inflammation (20-29).

IL-1 β and CCL2 are SASP factors that can recruit and activate Kupffer cells and monocyte-derived macrophages from the bloodstream (30), prompting them to secrete TNF- α and IL-6, thus generating an inflammatory positive feedback loop. Concurrently, chemokines such as CXCL1 promote neutrophil and monocyte infiltration, exacerbating liver inflammation. For example, combined *in vitro* experiments in a mouse model and human MASH-HCC samples revealed notably elevated mRNA levels of IL-1 β , IL-6, CXCL1 and CXCL9 within the secretome of senescent HSCs. These factors amplified the effects of internal and external environmental factors, exacerbating the inflammatory microenvironment of MASH (20).

TGF- β is a core component of the SASP. Within the liver, TGF- β induces cell senescence in both acute and chronic liver injury models. Senescent HSCs release a number of profibrotic factors through the SASP, with TGF- β 1 serving as the core regulatory molecule (31,32). Recent studies indicate that TGF- β 1 markedly inhibits cytoglobin expression through the SMAD2/SMAD3-M1 signaling pathway, thus activating HSCs and promoting collagen deposition, a process positively associated with advanced fibrosis in patients with MASH. Furthermore, TGF- β initiates a positive feedback pathway (oxidative stress-fibrosis) by regulating the antioxidant defense system (33,34).

Soluble urokinase-type plasminogen activator receptor (suPAR), secreted by senescent HSCs as part of the SASP (10), has been shown to co-localize with IL-6 in a CCL4-induced liver fibrosis model, forming a chronic inflammatory microenvironment that activates neighboring HSCs and recruits immune cells. In a MASH mouse model, suPAR secretion has been shown to promote macrophage infiltration and collagen deposition, further aggravating liver fibrosis (35).

SASP induces hepatocyte damage and apoptosis. Studies have shown that reactive oxygen species (ROS) and IL-6 secreted by a SASP induce mitochondrial dysfunction and DNA damage [evidenced by increased γ -H2A.X variant histone-b (γ -H2AXb) marker] by activating the hepatocyte janus kinase (JAK)/STAT3 pathway, indicating that the SASP can induce hepatocyte damage and apoptosis (36-38). This phenomenon may serve a role in the progression of MASH. Concurrently, senescent HSCs may alter the microenvironment and induce DNA damage in hepatocytes by secreting IL-6 and matrix metalloproteinases (MMPs) in the SASP. This interaction aggravates inflammation and fosters an immune-privileged microenvironment conducive to the development of MASH into HCC by inhibiting the function of CD8⁺ T cells (39). This effect was effectively demonstrated in MASH mouse models induced by a choline-deficient high-fat diet or methionine-choline diet alongside programmed death-ligand-1 knockout mouse models (29).

Senescent HSCs promote the malignant transformation of MASH to HCC. MASH and non-alcoholic fatty liver disease (NAFLD) are recognized as precursors to HCC (38,40). The SASP of senescent HSCs can affect adjacent hepatocytes, which may be in states of fatty degeneration or dysplasia, through paracrine signaling, thereby promoting their malignant transformation (20). A key mechanism underlying this process is the activation of oncogenic signaling pathways in

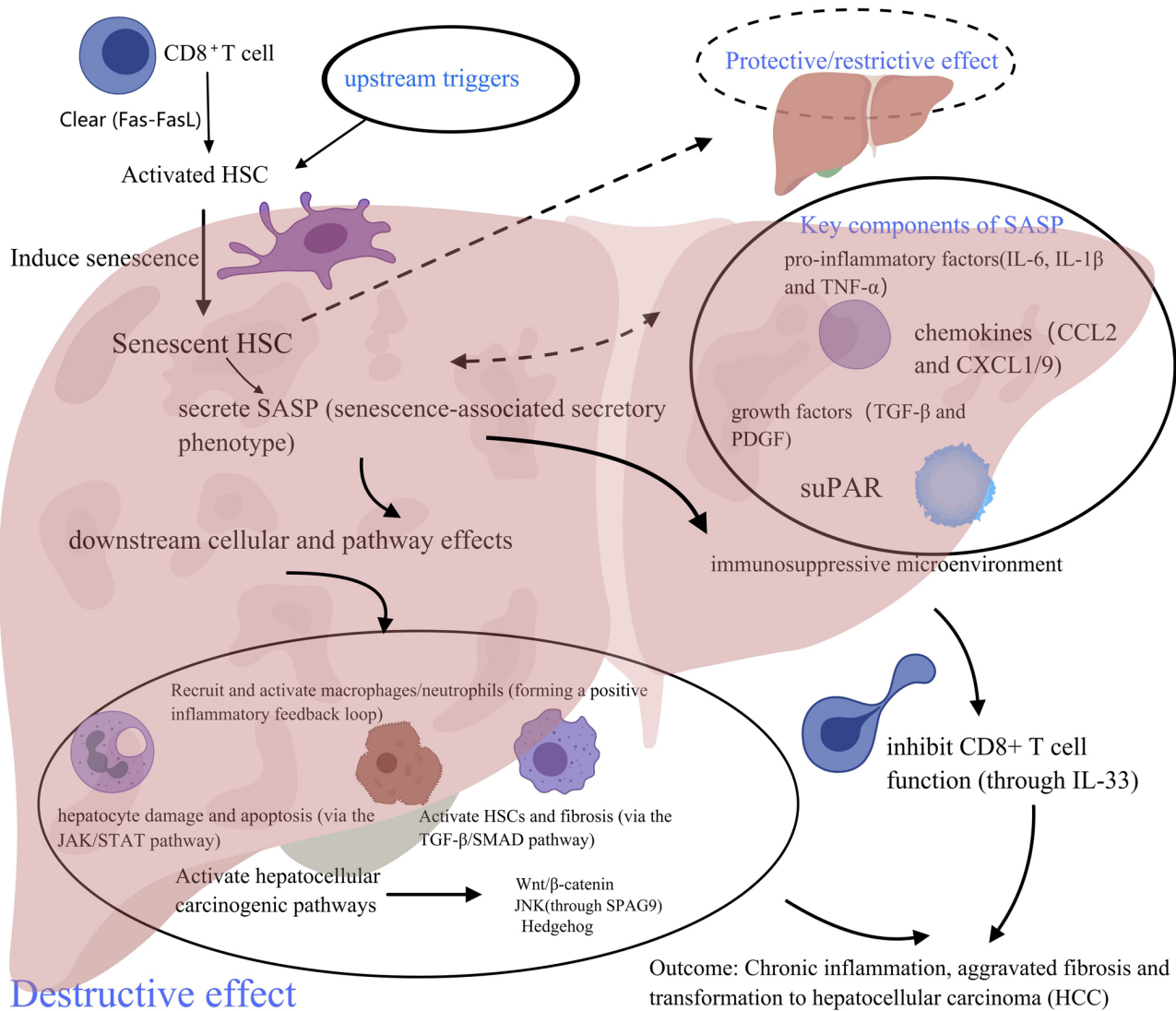


Figure 1. Diagram illustrating the destructive effects of senescent HSCs. HSC, hepatic stellate cells; JAK, janus kinase; SPAG9, sperm-associated antigen 9; suPAR, soluble urokinase-type plasminogen activator receptor; PDGF, platelet-derived growth factor; CCL2, C-C motif chemokine ligand-2; CXCL, C-X-C motif chemokine ligand.

hepatocytes by a SASP, specifically the Wnt/ β -catenin and Hedgehog pathways. In addition, proteomic analysis indicates that sperm-associated antigen-9, secreted by senescent HSCs, influences the growth and metastasis of liver cancer cells through the JNK pathway, while fatty acid binding protein-5 enhances angiogenesis, collectively facilitating the proliferation of cancer cells (20).

In addition to directly promoting cell transformation, a SASP also promotes conditions favorable for tumor growth by reshaping the immune microenvironment (20). In human MASH-related HCC samples, a recent study demonstrated that IL-33, released from senescent HSCs and mediated by gasdermin D, inhibits the anti-fibrotic function of CD8⁺ T cells by binding to ST2 receptors to activate regulatory T cells (41). This immunosuppressive microenvironment may hinder the clearance of activated HSCs, providing a fertile ground for tumor cells to evade the immune response.

SASP factors such as IL-6 and CXCL9, secreted by senescent HSCs driven by obesity-induced intestinal flora metabolites including deoxycholic acid, can recruit

macrophages and neutrophils, amplifying liver inflammation and activating fibroblasts to promote collagen deposition and fibrosis. This ultimately leads to liver cancer development (42). Senescent HSCs may also reduce the secretion of ECM and stimulate the SASP to attract natural killer (NK) cells for cleaning, preventing HSCs from excessive proliferation or transdifferentiation into fibroblasts (43,44), thereby preventing liver fibrosis (7). The co-localization of MMP2/9 and fibrotic areas in liver tissue of patients with MASH, indicates that MMP2/9 degrades normal ECM, while tissue inhibitor of metalloproteinases-1 expression inhibits ECM clearance, leading to an imbalance in fibrotic remodeling (Fig. 1) (38).

Senescent HSCs limit fibrosis and promote tissue repair. A defining characteristic of aging is irreversible cell cycle arrest, predominantly occurring during G₁ phase arrest (45). When activated HSCs enter senescence, they lose their ability to proliferate (25). Gene expression profiling analysis has demonstrated that senescent HSCs downregulate genes associated with cell cycle progression and secretion of

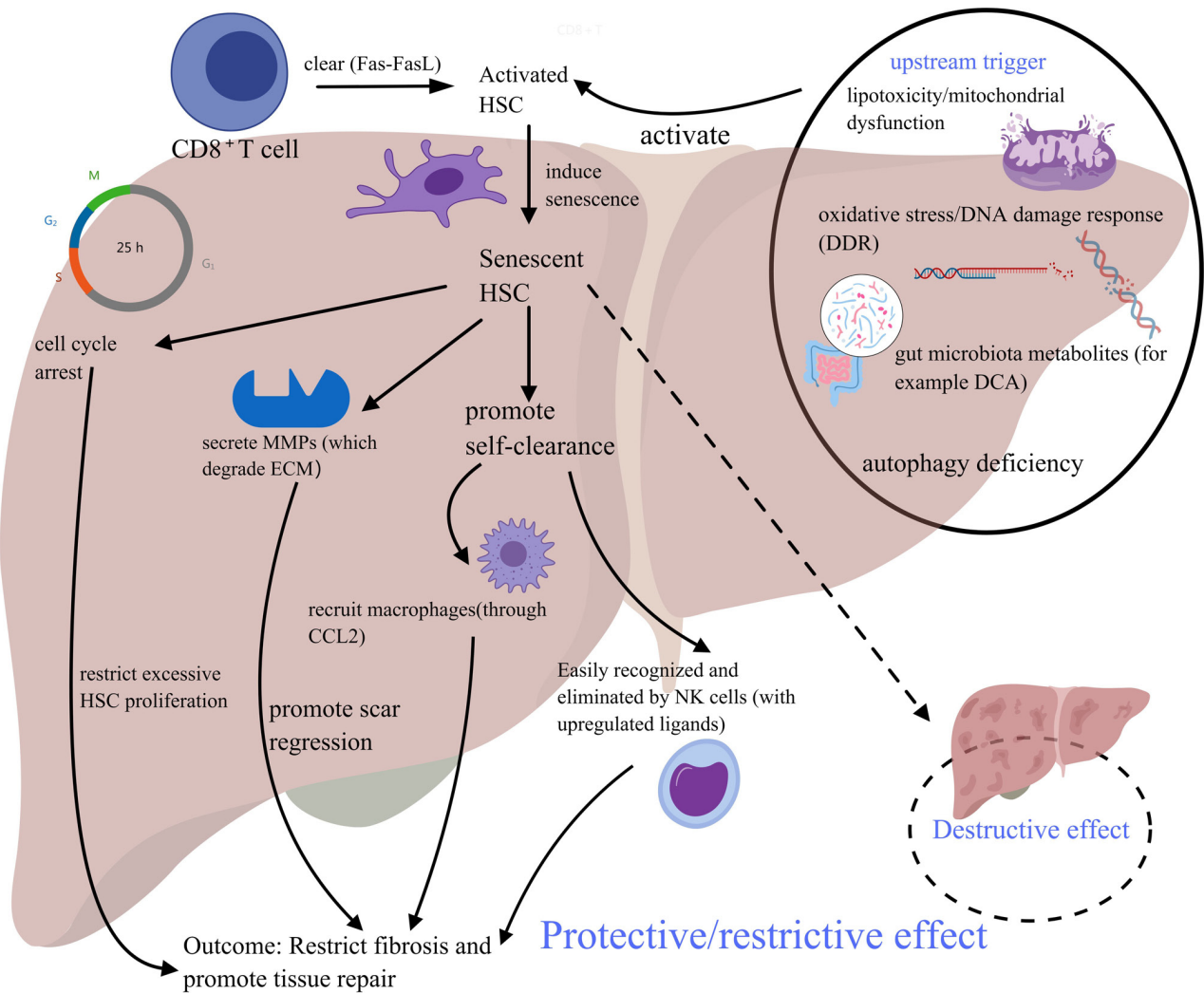


Figure 2. Diagram illustrating the promotive/restrictive effect of senescent HSCs, with upstream triggers shown in detail. HSC, hepatic stellate cells; MMP, matrix metalloproteinases; CCL2, C-C motif chemokine ligand-2; NK, natural killer; DCA, deoxycholic acid; ECM, extracellular matrix.

ECM components (such as collagen), thus limiting both their proliferation and fibrogenic potential. In addition, senescent HSCs markedly change their secretory properties, increasing the secretion of ECM-degrading enzymes such as MMPs (6). These enzymes exhibit fibrinolytic activity and can actively degrade the deposited fibrotic matrix, thereby promoting the regression of scars and normal tissue remodeling. Concurrently, gene expression profiling indicates that monocyte chemoattractant protein-1 (MCP-1), secreted by senescent HSCs, recruits macrophages to fibrotic areas, promoting the clearance of senescent HSCs and inhibiting inflammation (7). Aging HSCs actively recruit macrophages, including monocyte-derived macrophages, to fibrotic areas by secreting chemokines such as MCP-1 (CCL2) (46). These recruited macrophages perform two primary anti-fibrotic functions, including timely clearance of senescent HSCs, limiting inflammation and chronic accumulation of a SASP (46). Additionally, these macrophages cause active matrix degradation and tissue remodeling. Macrophages involved in fibrosis resolution, typically M2-like or scar-associated macrophages (SAMacs) degrade the ECM by secreting multiple MMPs (such as, MMP-9, -12 and -13)

and releasing anti-fibrotic cytokines (such as IL-10), thereby promoting scar tissue remodeling (21).

Senescent HSCs also upregulate immune surveillance molecules on their surfaces, particularly ligands for the NK cell receptor NK group 2 member D (NKG2D), such as MHC class I-related chain A. This allows for their efficient recognition and preferential elimination by NK cells. Such clearance processes, actively initiated by senescent HSCs, ensure the prompt removal of potentially harmful cells from tissue, ultimately promoting the regression of fibrosis (Fig. 2) (7,47).

Mechanisms underlying the coexistence of two opposing effects in aging HSCs. Senescent HSCs exhibit a dual role, which may be attributed to the decoupling between intrinsic cell cycle arrest mechanisms and extrinsic, environmentally regulated SASP functions, alongside variations in immune clearance efficiency. Current research suggests that early senescence primarily drives fibrosis, while in later stages, it may promote the reversal or stabilization of fibrosis within the context of injury resolution and repair (5,6). This process is also influenced by cytokines such as IL-6, TGF- β and IL-10 at a mechanistic level.

Assessment of the 'age' of HSCs. A variety of methodological approaches have been used to determine the age of HSCs in previous studies (6,48). Telomeres, repetitive DNA sequences at chromosome ends, progressively shorten with each cell division and are regarded as a 'mitotic clock' for gauging cellular replicative senescence and biological age. One study found that in the normal human liver, patterns of telomere shortening differ among cell types, whereby the telomere length of cholangiocytes do not markedly shorten with age, whereas Kupffer cells and HSCs exhibit notable age-related telomere shortening, underscoring the utility of telomere length as an effective metric for assessing chronological age-related changes in HSCs (49).

Primary detection markers and methods.

i) Senescence-associated β -galactosidase (SA- β -gal) activity. As a marker, SA- β -gal is most widely used for senescent cells. The detection principle involves a histochemical stain at a non-physiological pH of 6.0, where the activity of β -gal results in a distinct blue coloration in senescent cells. Optimized protocols for SA- β -gal staining in frozen liver tissue sections are available (50), indicating that this technique is fully applicable for identifying senescent cells, including HSCs.

ii) p16^{INK4a} protein expression. As a cyclin-dependent kinase inhibitor, p16^{INK4a} is notably upregulated in senescent cells, where it maintains the senescent state by inhibiting the cell cycle (51). The expression of p16^{INK4a} can be detected using methods including immunohistochemistry and western blotting. For example, in liver tissue samples from children with end-stage liver disease, p16^{INK4a} expression was found to be elevated, along with SA- β -gal activity, demonstrating its validity as a marker of liver senescence (6).

Expression profiles of associated cytokines. At the cytokine level, pro-inflammatory cytokines constitute a prominent component of the SASP (52). Identified factors include IL-6, TNF- α , IL-1 β , IL-1 α , IL-12 and IFN- γ . Collectively, these factors promote a potent pro-inflammatory microenvironment capable of recruiting and activating immune cells.

Chemokines. Chemokine molecules recruit immune cells to sites of injury or aging, with key examples including MCP-1/CCL2, IL-8, CXCR2 ligands and neutrophil chemoattractant proteins (53). Immunomodulatory cytokines (54), specifically IL-10, is not only secreted by senescent HSCs but has also functions as a signal that induces activated HSCs to enter a senescent state, thereby establishing a complex feedback regulatory loop. In addition to the aforementioned cytokines, the SASP of senescent HSCs includes multiple growth factors (including epidermal growth factors and insulin-like growth factor) and tissue remodeling-associated proteins. Collectively, these influence the actions of senescent HSCs.

The onset of HSC senescence and the establishment of the SASP are dynamic processes rather than instantaneous events. Through *in vivo* models, such as those involving partial hepatectomy, HSCs have been shown to exhibit signs of senescence and begin IL-6 secretion to promote liver regeneration within just 2 days (55). This observation indicates that senescence programs can be initiated quite rapidly during acute repair responses. By contrast, *in vitro* cultures treated with inducers (such as etoposide) typically require 7-10 days for full SASP establishment, during which complex intracellular signaling and gene expression reprogramming

occur, ultimately leading to the sustained release of secreted proteins (56).

Theoretically, SASP can persist as long as senescent cells survive and are not cleared by the immune system. Immune surveillance, including NK cell activity, represents a key clearance mechanism for senescent cells, thereby terminating their SASP. A previous study has indicated that IL-10 mRNA expression in human HSCs can persist for <120 days during long-term culture. However, mRNA levels are not fully associated with sustained protein secretion (57). Overall, precisely quantifying the kinetic profiles (onset, peak and duration) of specific cytokines secreted by senescent HSCs under numerous stimuli remains an unresolved issue requiring further research.

3. Molecular mechanisms regulating HSC aging and SASP formation

Causes of aging: Cellular stress in the MASH environment. A recent study has shown that small extracellular vesicles (sEVs) enriched with LIM domain and actin binding 1 (LIMA1) released from lipotoxic hepatocytes serve a key role in promoting HSC activation in NAFLD-related liver fibrosis by negatively regulating PINK1-mediated mitochondrial autophagy (58). In this study, a high-fat diet (HFD)-induced mouse model was constructed to demonstrate LIMA1 expression. Furthermore, sEV injection was used to assess whether LIMA1 could accelerate HFD-induced liver fibrosis in mice (58). The results showed that LIMA1 was upregulated in sEVs produced by HFD-induced fatty liver and lipotoxic hepatocytes and that these LIMA1-enriched sEVs increased LIMA1 protein expression in HSCs, consequently inducing HSC activation. Moreover, this study further elucidated that LIMA1 further promoted the activation of HSCs by inhibiting mitochondrial autophagy.

ROS and reactive nitrogen species (RNS) are byproducts of mitochondrial dysfunction and inflammatory responses and may cause extensive oxidative damage to cellular macromolecules, especially DNA damage. This damage activates the DNA damage response (DDR) of the cell, marked by the formation of γ H2AX phosphorylation sites. Sustained or severe DDR is a key mechanism underlying cellular senescence (26). Damaged mitochondria function as the main source of ROS and their functional defects, including energy metabolism disorders and can exacerbate cellular stress, perpetuating a continuous cycle (25).

Interweaving with liver disease-related signaling pathways.

An example of a key pathway implicated in liver cancer development is Wnt signaling, with β -catenin as a key protein. β -catenin demonstrates notable interaction potential within the network of senescent HSC secretory proteins. In a previous study, ELISA experiments, immunofluorescence and immunohistochemical staining demonstrated that Wnt signaling pathway activation in tumor tissues was associated with an elevated expression of related genes β -catenin and cellular-Myc, thus demonstrating the role of senescent HSCs in promoting the malignant transformation of individual cells by activating the Wnt signaling pathway (20,59,60).

This study also identified the Hedgehog signaling pathway. The Hedgehog pathway is abnormally activated in chronic

liver injury and MASH and is an important pathway driving fibrosis and tumorigenesis (61). SASP components from senescent HSCs act as potent activators of this pathway (62). Immunofluorescence staining showed activation levels of the pathway's key protein, Gli family zinc finger 1 (Gli-1), initially increased and subsequently decreased throughout MASH-HCC progression, consistent with changes in the number of senescent HSCs (63). Among senescent HSCs, expression levels of key molecules of the Hedgehog signaling pathway (including Gli-1, Patched, cyclin D1 and B cell leukemia-1), were markedly elevated, indicating that the pathway is continuously activated in senescent HSCs. Therefore, the amount of main Hedgehog signaling pathway ligand, sonic hedgehog SHh, also exhibited an upward trend. SHh can directly interact with fibrotic cytokine TGF- β and ECM components (including fibronectin), thereby activating the Hedgehog signaling pathway and promoting the malignant transformation of hepatocytes (20,64,65).

The JAK/STAT signaling pathway is a key downstream mediator of the SASP-induced bystander effect. Key components of the SASP, particularly IL-6, bind to receptors on the surface of adjacent hepatocytes, activating the JAK/STAT3 signaling pathway. Continuous activation of this pathway can lead to mitochondrial dysfunction and DNA damage in hepatocytes (characterized by increased γ H2AX and 53BP1 markers), inducing hepatocyte damage and apoptosis. This process not only exacerbates the inflammatory response but also promotes a continuing cycle of liver damage (26).

Interactions between autophagy and aging. Cellular autophagy degrades damaged or redundant organelles, protein aggregates, pathogen and other cellular components in the cell, breaking them down into smaller molecules for recycling. Autophagy serves a core role in maintaining cell homeostasis, responding to environmental stress (such as nutrient deficiency or oxidative stress) and regulating cell fate. Within hepatocytes, autophagy helps to remove damaged mitochondria and control the intracellular lipidome (66,67), while selective autophagy of mitochondria usually involves distinct signaling pathways, such as the PINK1/Parkin-dependent pathway and receptor-mediated mechanisms involving BNIP3 and NIX (68). By preserving a functional pool of mitochondria, autophagy minimizes ROS production and thus inhibits the activation of intracellular pro-inflammatory factors (69-71). Furthermore, autophagy is key for lipid metabolism as autophagy defects are associated with increased triglyceride accumulation both *in vivo* and *in vitro* (20). Impaired autophagy leads to dysfunctional mitochondria, which reduces the production of ROS and activates the DNA damage response, thereby inducing p53/p21-dependent cell cycle arrest and cell senescence (72). Senescent HSCs further exacerbate MASH-induced fibrosis and promote the progression of MASH. In addition, ROS-dependent alterations in mitochondrial metabolism have been associated with metabolic disorders, particularly insulin resistance (73). Similar to ROS, nitric oxide (NO) and RNS exhibit diverse biological effects, ranging from physiological signaling to pathological nitrosative stress under inflammatory conditions (74). Excessive NO and RNS induce similar impairments in energy metabolism, covalently modifying a large group of proteins and enzymes involved in mitochondrial

respiration including mitochondrial complex IV, ultimately leading to cell death (64-79).

Bidirectional interactions between lipid metabolism and HSC aging. In the early stages of MASH, lipid accumulation (steatosis) in hepatocytes leads to lipotoxicity. This metabolic stress serves as a key initial signal for inducing HSC senescence. Lipotoxic hepatocytes release extracellular vesicles rich in specific proteins such as LIMA1. Upon uptake by HSCs, these vesicles induce HSC senescence and activation through mechanisms that inhibit PINK1-Parkin-mediated mitochondrial autophagy (58,80). Fatty acid oxidation (FAO) is a key metabolic pathway responsible for breaking down fatty acids into acetyl-CoA for energy production, primarily occurring within mitochondria. FAO is important in maintaining the long-term function and quiescent state of HSCs.

Within one notable study, it was demonstrated that the promyelocytic leukemia protein (PML) regulates FAO through the peroxisome proliferator-activated receptor (PPAR)- δ , which is key for sustaining HSC quiescence and functionality (81). In this study, inactivation of the PML-PPAR- δ -FAO axis led to HSC functional exhaustion, demonstrating that FAO is a key metabolic pathway involved in sustaining HSC stemness.

In aging cells, the dynamic equilibrium of lipid droplets is often disrupted. A recent study conducted in 2024 revealed that during cellular senescence, glycerol-3-phosphate accumulation triggers lipid metabolic reprogramming, leading to marked triglyceride accumulation within lipid droplets. Such abnormal lipid storage not only modifies the cellular metabolic state but also activates senescence-associated gene expression programs (82). The formation, growth and degradation of lipid droplets is precisely regulated by a series of lipid droplet-associated proteins, with perilipin (PLIN) family proteins serving as core members. PLIN2, one of the most extensively studied members, is widely expressed in non-adipose cells and regulates lipid droplet stability and lipolysis processes (83-86). Certain aging models exhibit notable alterations in PLIN2 expression. Studies indicate that PLIN2 expression is down-regulated in aged mesenchymal stem cells, which is further associated with changes in lipid droplet content and alterations in β -oxidation capacity (87-89).

4. Interactions between senescent HSCs and other cells

Interactions between senescent HSC and macrophages. Interactions between HSCs and macrophages can promote inflammation and fibrosis in MASH (30,90,91). The SASP of senescent HSCs is rich in chemokines, such as CCL2 (MCP-1) and CXCL1, which recruit a number of circulating monocytes to the liver (25). Once within the liver, these monocytes differentiate into macrophages exhibiting pro-inflammatory and pro-fibrotic phenotypes, facilitated by SASP factors such as IL-1 β and IL-6 (30). In recent years, single-cell RNA sequencing has identified MASH-specific macrophage subsets, such as MASH-associated macrophages or SAMacs, whose formation may be affected by senescent HSCs (90). In return, activated macrophages secrete a number of potent HSC activators, including TGF- β , PDGF and TNF- α . These factors not only activate the remaining quiescent HSCs but also

promote the survival of both activated and senescent HSCs, thus forming a robust, self-amplifying pro-fibrotic positive feedback loop (30).

Interactions between senescent HSCs and NK cells. NK cells selectively eliminate early activated HSCs by recognizing activated ligands on the surface of HSCs, such as NKG2D and Raf-1 proto-oncogene serine/threonine kinase, thereby inhibiting fibrosis (43). Senescent HSCs are more easily cleared by NK cells due to the upregulation of NKG2D ligands and tumor necrosis factor-related apoptosis-inducing ligand receptors, thereby limiting the development of fibrosis (43,44). In addition, NK cells secrete IFN- γ , which induces HSC apoptosis and cell cycle arrest, impeding their capacity to participate in liver repair and proliferation. Disease progression is further aggravated by persistent MASH-related damaging factors (7,43). Gene expression profiling analysis has identified that MCP-1, secreted by senescent HSCs, recruits macrophages to fibrotic areas, promoting the clearance of senescent HSCs and inhibiting inflammation (7). After macrophages are recruited to the liver, Kupffer cells may inhibit NK cell activity by secreting TGF- β , thereby reducing the ability of NK cells to eliminate HSCs. However, TGF- β secreted by senescent HSCs may attenuate NK cell inhibition (7,21,43,92).

Dual role of T cells in MASH. Within MASH, the role of T cells is also dualistic, with senescent HSCs serving an important regulatory role. T cells inhibit HSC fibrosis during the regression of MASH fibrosis. CD8⁺ memory T cells have been shown to attract HSCs through the C-C chemokine receptor type 5 (CCR5) and induce HSC apoptosis through Fas-FasL, promoting the reversal of fibrosis (30). In addition, the persistence of senescent HSCs can effectively orchestrate an immune escape strategy, inhibit the activity of CD8⁺ T cells, hinder the clearance of senescent HSCs, promote collagen deposition and promote an immune-exempt microenvironment for subsequent tumorigenesis.

Anti-fibrotic T cell subsets: HSC clearance mechanism based on the Fas-FasL pathway. Anti-fibrotic T cell subsets mainly promote HSC apoptosis through specific molecular pathways. CD8⁺ memory T cells are directed to the vicinity of HSCs through increased expression of the chemokine receptor CCR5, directly triggering HSC apoptosis through the Fas-FasL death signaling pathway. Simultaneously, liver-enriched $\gamma\delta$ T cells also induce programmed death of HSCs through the same Fas-FasL molecular mechanism. This synergistic effect based on specific molecular markers (including CCR5) and signaling pathways (including Fas-FasL) constitutes a key immune mechanism that promotes fibrosis reversal (93).

Pro-fibrotic T cell subsets: IL-33-suppression of tumorigenicity 2 (ST2)-mediated immunosuppressive mechanism. Pro-fibrotic regulatory T cells hinder HSC clearance by establishing an immunosuppressive microenvironment. This activation depends on the specific binding of IL-33 released by senescent HSCs through the gasdermin D protein pore to the ST2 receptor. Activated regulatory T cells form an immunosuppressive circuit based on the IL-33-ST2 molecular

axis by inhibiting the antifibrotic function of CD8⁺ T cells, ultimately leading to the accumulation of senescent HSCs, increased collagen deposition and therefore an increased risk of tumorigenesis (94,95).

Factors that can reverse the aging process of hematopoietic stem cells

Inducing senescence to terminate fibrosis. i) Pharmacological induction. Doxazosin, the α -1 adrenergic receptor antagonist, has been shown to reverse the activation of HSCs by inducing senescence (96). Experiments have demonstrated that doxazosin treatment upregulates senescence markers p53 and p21, thereby inhibiting HSC proliferation and the expression of pro-fibrotic genes.

ii) Modulation of the FoxO3a/S-phase kinase associated protein 2 (SKP2)/p27 pathway. Notably, the soluble egg antigen of *Schistosoma japonicum*, has been demonstrated to induce HSC senescence by activating the transcription factor FoxO3a, which subsequently inhibits SKP2 (an E3 ubiquitin ligase) expression, leading to the accumulation of the cell cycle inhibitor p27 (97).

iii) Inhibition of endogenous hydrogen sulfide (H₂S). Inhibition of endogenous H₂S production induces HSC senescence and reduces HSC activation primarily through the PI3K-Akt signaling pathway. This inhibition leads to cell cycle arrest via upregulation of p53 and p21, and promotes the SASP, thereby reversing the fibrogenic activity of HSCs (89,94).

Promoting phenotypic reversion to a quiescent state. i) Genetic regulation. In HSCs, specific knockout of Delta-like 1 homolog, which is highly expressed upon HSC activation (98), has been shown to markedly reverse the activated phenotype, restoring these cells to a quiescent or differentiated state. This effect is achieved by inhibiting the Wnt signaling pathway and upregulating PPAR- γ activity, thereby providing evidence that targeting DLK1 via genetic engineering could be a potential therapeutic strategy for reversing HSC activation (98).

ii) Signaling pathways and small-molecule compounds. PPAR- γ is a key transcription factor for maintaining the quiescent state of HSCs (99). The synergistic interactions between retinoic acid (a derivative of vitamin A) and PPAR- γ agonists can be considered effective strategies for promoting the reversal of liver fibrosis. Their combined action may suppress HSC activation markers and restore their quiescent phenotypes.

Selective clearance of senescent HSCs. Given the potential risks associated with the long-term presence of senescent cells, senotherapy, the use of senolytic drugs to selectively induce senescent cell death, has emerged as a new research avenue (100).

Effects of aging HSCs on liver regenerative capacity

A pro-regenerative role. Notably, a previous study reshaped the understanding of the role of senescent HSCs in liver regeneration. Using a mouse model of 2/3 partial hepatectomy, this study demonstrated that in the early phase of regeneration following acute liver injury (~2 days post-surgery), HSCs rapidly enter a transient state of senescence (101). This phenomenon is not a pathological accumulation, but a programmed physiological response key for regeneration.

The underlying mechanism can be attributed to the SASP of these senescent HSCs, which secrete IL-6 and ligands for the chemokine receptor CXCR2 (including CXCL1 and CXCL2). These secreted factors exert a marked effect on adjacent hepatocytes, driving their proliferation by activating downstream STAT3 and YES-associated protein signaling pathways, thereby promoting the restoration of liver mass. Experimental evidence shows that specific elimination of these senescent HSCs during the early regenerative phase notably impairs the regenerative capacity of the liver, thereby demonstrating the positive role of transient senescence in the acute repair process (102).

An inhibitory role. By contrast with their beneficial role in acute injury, the persistent presence and accumulation of senescent HSCs in chronic liver injuries, or the naturally aged liver, is often associated with decreased regenerative capacity and pathological progression.

First, in the aged liver, the quantity of quiescent HSCs increases, coupled with phenotypic changes, such as increased accumulation of lipid droplets. This alteration in the basal state may compromise the ability of HSCs to maintain homeostasis of the sinusoidal microenvironment (103).

Second, during chronic injury (for example, chronic hepatitis and NAFLD), HSCs are continuously activated and may enter senescent states. The SASP exhibited by long-existing senescent HSCs is more complex, including pro-inflammatory factors and numerous pro-fibrotic factors such as TGF- β . This chronic, low-grade inflammatory and fibrotic environment inhibits normal hepatocyte proliferation. A number of studies have demonstrated that factors secreted by senescent cells (including HSCs and neutrophils) can suppress hepatic progenitor cell activation and proliferation, thereby obstructing compensatory regenerative pathways (104-106).

5. Future directions and outlook

Development of biomarkers. Within aging-related therapies, the primary challenge remaining is the lack of reliable, non-invasive biomarkers. This gap makes patient selection, efficacy tracking and dose optimization during human trials challenging, meaning diseases cannot be accurately diagnosed, the burden of senescent HSCs cannot be quantified and challenges remain regarding treatment response evaluation, subsequently hindering clinical translation (107). Although liver biopsy is the established standard for diagnosing MASH, the invasive nature of this procedure imposes limitations (108). Therefore, the development of biomarkers is key in addressing these challenges (109).

Identifying molecules in the blood that reflect the aging state of the liver remains an active area of research. While the SASP can be detected in plasma, studies reveal that its association with frailty in liver transplant patients is marginally notable, often lacking specificity as it is also associated with systemic inflammation (110,111). The expression of senescence-related genes, including p16^{INK4a} and p21^{CIP1}, in circulating T cells is associated with frailty and increased duration of hospitalization in patients undergoing liver transplants, rendering them viable replacement markers for systemic aging burdens (112). The anti-aging effects of acyl-CoA-binding protein (ACBP) neutralization extend

across a number of cell types. Elevated plasma ACBP levels have been documented in aging and liver injury models induced by a Western diet and repeated CCl₄ injections. Neutralization of ACBP prevents cellular senescence, indicating its potential as a valuable biomarker (113).

Ongoing research continues to explore non-invasive imaging biomarkers. Elastic imaging techniques, such as transient elastography and magnetic resonance elastography, are used to assess the degree of fibrosis by quantifying the shear wave velocity or tissue displacement generated by ultrasound or physical pulses. However, their application is limited by an unclear optimal cutoff point, inability to assess obese patients, poor diagnostic accuracy in the early stages of fibrosis and the non-specificity exhibited in senescent HSC detection (114). In addition, positron emission tomography (PET) technology is highly promising due to its high sensitivity and specificity. PET tracers targeting aging-related SA- β -gal have demonstrated successful applications in preclinical models including liver cancer, having also entered preliminary human trial stages (115).

Potential of anti-aging drugs. Given the integral role of senescent HSCs in MASH pathogenesis, senescent cell scavengers and phenotype regulators have arisen as new therapeutic strategies. Senescent cell scavengers are a class of drugs that selectively induce apoptosis in senescent HSCs. Targeting the pro-survival pathways, these cells upregulate to resist their own SASP toxicity (116).

The combination of dasatinib and quercetin (D + Q) is a widely studied senescent cell scavenger regimen. Studies demonstrate that D + Q effectively clears senescent HSCs in the liver, reduces liver fat deposition and markedly lowers expression levels of key pro-fibrotic factor TGF- β 1 (116,117), with validation in a metabolic dysfunction-associated fatty liver disease (a reclassified terminology for MASH) model established using medaka fish (118). Additionally, ABT-263, an inhibitor of BCL-2 family proteins, has been shown to effectively eliminate senescent HSCs and hepatocytes, reduce expression of SASP factors and improve mitochondrial function in a mouse model of liver injury. Furthermore, it can facilitate the clearance of senescent HSCs in liver regeneration models (119). However, the thrombocytopenia associated with senescent cell removal and the tumor-promoting effects of D + Q in HCC models reflect the limitations of this particular therapy (119-124).

Senescence phenotype regulators constitute an alternative therapeutic strategy that does not directly eliminate senescent HSCs, instead regulating their phenotype primarily through inhibition of harmful SASP production and secretion of SASP factors (125). Potential targets for senescence phenotype regulators include transcription factors such as GATA binding protein 4 (an upstream regulator of NF- κ B) as well as mTOR and p38/MAPK signaling pathways. Analogs including rapamycin, ruxolitinib, glucocorticoids and metformin are also considered to exhibit senescence phenotype regulatory effects (126). However, since these signaling pathways are also essential for the physiological function of normal cells, their inhibition may lead to significant off-target effects and toxicity (127,128).

Other treatment and prevention methods. Lifestyle interventions constitute a foundational strategy for preventing and managing MASH (129). An unhealthy diet rich in fat and sugar can promote intestinal flora imbalance, leading to the translocation of lipopolysaccharides and other pathogenic molecular patterns to the liver, triggering liver inflammation and aggravating MASH. Healthy dietary interventions primarily target obesity reduction, improve dyslipidemia and attenuate MASH progression (130). Supplementation with obeticholic acid may also provide additional benefits in inhibiting liver inflammation and preventing disease progression (131). Therefore, treatments that restore healthy intestinal microecology, such as probiotics, prebiotics, synbiotics and fecal microbiota transplantation, may serve as innovative treatment strategies (132).

6. Summary

Senescent HSCs occupy a central and multifaceted role in the pathophysiology of MASH. The most notable feature of senescent HSCs is their dual role, as during acute injury and repair, they protect the body by arresting the cell cycle and facilitating immune-mediated clearance. However, in the chronic and persistent pathological state of MASH, a reduction in immune clearance mechanisms allows long-term persistence of senescent HSCs, continually secreting harmful SASP factors that drive chronic inflammation, fibrogenesis and malignant transformation towards liver cancer in MASH. Impaired autophagy induces senescence, while SASP promotes pathological processes through key signaling pathways, such as Wnt and Hedgehog. Simultaneously, the senescent SASP promotes a microenvironment conducive to its survival and disease development through interactions with immune cells. It is recommended that future anti-aging therapeutic strategies should evolve from broad-spectrum pathway inhibitors to target the clearance or inhibition of pathogenic senescent HSCs. Focus should remain on the specific identification and clearance of pathogenic senescent HSCs phenotypes or the fine-tuning of their SASP components. In the future, development of refined treatment strategies based on a deeper understanding of the complex interaction networks of senescent HSCs should be prioritized.

Acknowledgements

Not applicable.

Funding

The present review was supported by the National Natural Science Foundation of China (Youth Fund; grant no. 82300666) and the College Students' Innovation and Entrepreneurship Training Program of Nanjing Medical University (grant no. 202510312047).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

ZH, YS and DW contributed to research design, literature review, core content drafting and analysis within the present study. DW acquired the funding. NZ, ZL and ZW assisted with literature retrieval, checking citations of the key signaling pathways and participated in writing the first draft. XZ guided research direction, reviewed mechanism analysis logic and coordinated author division of labor. XZ also participated in writing and proofreading the manuscript. SL led the review design, determined the framework, guided key issue analysis, finalized the manuscript, served as the data contact and also acquired funding for the present study. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, Romero D, Abdelmalek MF, Anstee QM, Arab JP, *et al*: A multi-society Delphi consensus statement on new fatty liver disease nomenclature. *J Hepatol* 79: 1542-1556, 2023.
- Huang DQ, El-Serag HB and Loomba R: Global epidemiology of NAFLD-related HCC: Trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 18: 223-238, 2021.
- Riazi K, Azhari H, Charette JH, Underwood FE, King JA, Afshar EE, Swain MG, Congly SE, Kaplan GG and Shaheen AA: The prevalence and incidence of NAFLD worldwide: A systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 7: 851-861, 2022.
- Tsuchida T and Friedman SL: Mechanisms of hepatic stellate cell activation. *Nat Rev Gastroenterol Hepatol* 14: 397-411, 2017.
- Ferreira-Gonzalez S, Rodrigo-Torres D, Gadd VL and Forbes SJ: Cellular senescence in liver disease and regeneration. *Semin Liver Dis* 41: 50-66, 2021.
- Krizhanovsky V, Yon M, Dickins RA, Hearn S, Simon J, Miething C, Yee H, Zender L and Lowe SW: Senescence of activated stellate cells limits liver fibrosis. *Cell* 134: 657-667, 2008.
- Aravinthan A, Scarpini C, Tachtatzis P, Verma S, Penrhyn-Lowe S, Harvey R, Davies SE, Allison M, Coleman N and Alexander G: Hepatocyte senescence predicts progression in non-alcohol-related fatty liver disease. *J Hepatol* 58: 549-556, 2013.
- Hernández-Gea V, Ghiassi-Nejad Z, Rozenfeld R, Gordon R, Fiel MI, Yue Z, Czaja MJ and Friedman SL: Autophagy releases lipid that promotes fibrogenesis by activated hepatic stellate cells in mice and in human tissues. *Gastroenterology* 142: 938-946, 2012.
- Allaire M, Rautou PE, Codogno P and Lotersztajn S: Autophagy in liver diseases: Time for translation? *J Hepatol* 70: 985-998, 2019.
- Kale A, *et al*: Senescent cells and therapeutic targeting in liver fibrosis. *Gut*. 2020.
- Friedman SL, Neuschwander-Tetri BA, Rinella M and Sanyal AJ: Mechanisms of NAFLD development and therapeutic strategies. *Nat Med* 24: 908-922, 2018

12. Campisi J and d'Adda di Fagagna F: Cellular senescence: When bad things happen to good cells. *Nat Rev Mol Cell Biol* 8: 729-740, 2007.
13. Tchkonina T, Zhu Y, van Deursen J, Campisi J and Kirkland JL: Cellular senescence and the senescent secretory phenotype: Therapeutic opportunities. *J Clin Invest* 123: 966-972, 2013.
14. Xiong J, Dong L, Lv Q, Yin Y, Zhao J, Ke Y, Wang S, Zhang W and Wu M: Targeting senescence-associated secretory phenotypes to remodel the tumour microenvironment and modulate tumour outcomes. *Clin Transl Med* 14: 1772, 2024.
15. Myung PK, Kugel JF and Goodrich JA: The Borrelia burgdorferi p66 protein interacts with the human lipopolysaccharide-induced tumor necrosis factor-alpha factor. *J Bacteriol* 184: 1350-1357, 2002.
16. Tang X, Metzger D, Leeman S and Amar S: LPS-induced TNF-alpha factor (LITAF)-deficient mice show delayed LPS-induced cytokine expression. *Proc Natl Acad Sci USA* 103: 13777-13782, 2006.
17. Ceccarelli S, Panera N, Mina M, Gnani D, De Stefanis C, Crudele A, Rychlicki C, Petrini S, Bruscalupi G, Agostinelli L, *et al*: LPS-induced TNF- α factor mediates pro-inflammatory and pro-fibrogenic pattern in non-alcoholic fatty liver disease. *Oncotarget* 6: 41434-41452, 2015.
18. Beringer A and Miossec P: IL-17 and TNF- α co-operation contributes to the proinflammatory response of hepatic stellate cells. *Clin Exp Immunol* 198: 111-120, 2019.
19. Lemmers A, Moreno C, Gustot T, Maréchal R, Degré D, Demetter P, de Nadai P, Geerts A, Quertinmont E, Vercruyse V, *et al*: The interleukin-17 pathway is involved in human alcoholic liver disease. *Hepatology* 49: 646-657, 2009.
20. Zhou Y, Zhang L, Ma Y, Xie L, Yang YY, Jin C, Chen H, Zhou Y, Song GQ, Ding J and Wu J: Secretome of senescent hepatic stellate cells favors malignant transformation from nonalcoholic steatohepatitis-fibrotic progression to hepatocellular carcinoma. *Theranostics* 13: 4430-4448, 2023.
21. Kuilman T and Peepers DS: Senescence-messaging secretome: SMS-ing cellular stress. *Nat Rev Cancer* 9: 81-94, 2009.
22. Gorgoulis V, Adams PD, Alimonti A, Bennett DC, Bischof O, Bishop C, Campisi J, Collado M, Evangelou K, Ferbeyre G, *et al*: Cellular senescence: Defining a path forward. *Cell* 179: 813-827, 2019.
23. Faget DV, Ren Q and Stewart SA: Unmasking senescence: Context-dependent effects of SASP in cancer. *Nat Rev Cancer* 19: 439-453, 2019.
24. Coppé JP, Patil CK, Rodier F, Sun Y, Muñoz DP, Goldstein J, Nelson PS, Desprez PY and Campisi J: Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol* 6: 2853-2868, 2008.
25. Engelmann C and Tacke F: The potential role of cellular senescence in non-alcoholic fatty liver disease. *Int J Mol Sci* 23: 652, 2022.
26. Li Q and Wang L: Navigating the complex role of senescence in liver disease. *Chin Med J (Engl)* 137: 3061-3072, 2024.
27. Rosenthal SB, Liu X, Ganguly S, Dhar D, Pasillas MP, Ricciardelli E, Li RZ, Troutman TD, Kisseleva T, Glass CK and Brenner DA: Heterogeneity of HSCs in a mouse model of NASH. *Hepatology* 74: 667-685, 2021.
28. Du K, Jun JH, Dutta RK and Diehl AM: Plasticity, heterogeneity and multifunctionality of hepatic stellate cells in liver pathophysiology. *Hepatology Commun* 8: e0411, 2024.
29. Kumar P, Hassan M, Tacke F and Engelmann C: Delineating the heterogeneity of senescence-induced-functional alterations in hepatocytes. *Cell Mol Life Sci* 81: 200, 2024.
30. Carter JK and Friedman SL: Hepatic stellate cell-immune interactions in NASH. *Front Endocrinol (Lausanne)* 13: 867940, 2022.
31. Razdan N, Vasilopoulos T and Herbig U: Telomere dysfunction promotes transdifferentiation of human fibroblasts into myofibroblasts. *Aging Cell* 17: e12838, 2018.
32. Akkız H, Gieseler RK and Canbay A: Liver fibrosis: From basic science towards clinical progress, focusing on the central role of hepatic stellate cells. *Int J Mol Sci* 25: 7873, 2024.
33. Okina Y, Sato-Matsubara M, Matsubara T, Daikoku A, Longato L, Rombouts K, Thanh Thuy LT, Ichikawa H, Minamiyama Y, Kadota M, *et al*: TGF- β 1-driven reduction of cytoglobin leads to oxidative DNA damage in stellate cells during non-alcoholic steatohepatitis. *J Hepatol* 73: 882-895, 2020.
34. Frippiat C, Chen QM, Zdanov S, Magalhaes JP, Remacle J and Toussaint O: Subcytotoxic H₂O₂ stress triggers a release of transforming growth factor-beta1, which induces biomarkers of cellular senescence of human diploid fibroblasts. *J Biol Chem* 276: 2531-2537, 2001.
35. Amor C, Feucht J, Leibold J, Ho YJ, Zhu C, Alonso-Curbelo D, Mansilla-Soto J, Boyer JA, Li X, Giavridis T, *et al*: Senolytic CAR T cells reverse senescence-associated pathologies. *Nature* 583: 127-132, 2020.
36. Nelson G, Wordsworth J, Wang C, Jurk D, Lawless C, Martin-Ruiz C and von Zglinicki T: A senescent cell bystander effect: Senescence-induced senescence. *Aging Cell* 11: 345-349, 2012.
37. Grohmann M, Wiede F, Dodd GT, Gurzov EN, Ooi GJ, Butt T, Rasmiena AA, Kaur S, Gulati T, Goh PK, *et al*: Obesity drives STAT-1-Dependent NASH and STAT-3-Dependent HCC. *Cell* 175: 1289-1306.e20, 2018.
38. Anstee QM, Reeves HL, Kotsiliti E, Govaere O and Heikenwalder M: From NASH to HCC: Current concepts and future challenges. *Nat Rev Gastroenterol Hepatol* 16: 411-428, 2019.
39. Loo TM, Kamachi F, Watanabe Y, Yoshimoto S, Kanda H, Arai Y, Nakajima-Takagi Y, Iwama A, Koga T, Sugimoto Y, *et al*: Gut Microbiota Promotes Obesity-Associated Liver Cancer through PGE2-Mediated Suppression of Antitumor Immunity. *Cancer Discov* 7: 522-538, 2017.
40. Brennan PN, Elsharkawy AM, Kendall TJ, Loomba R, Mann DA and Fallowfield JA: Antifibrotic therapy in nonalcoholic steatohepatitis: Time for a human-centric approach. *Nat Rev Gastroenterol Hepatol* 20: 679-688, 2023.
41. Yamagishi R, Kamachi F, Nakamura M, Yamazaki S, Kamiya T, Takasugi M, Cheng Y, Nonaka Y, Yukawa-Muto Y, Thuy LTT, *et al*: Gasdermin D-mediated release of IL-33 from senescent hepatic stellate cells promotes obesity-associated hepatocellular carcinoma. *Sci Immunol* 7: eabl7209, 2022.
42. Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, Iwakura Y, Oshima K, Morita H, Hattori M, *et al*: Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* 499: 97-101, 2013.
43. Gao B and Radaeva S: Natural killer and natural killer T cells in liver fibrosis. *Biochim Biophys Acta* 1832: 1061-1069, 2013.
44. Park O, Jeong WI, Wang L, Wang H, Lian ZX, Gershwin ME and Gao B: Diverse roles of invariant natural killer T cells in liver injury and fibrosis induced by carbon tetrachloride. *Hepatology* 49: 1683-1694, 2009.
45. Wagner KD and Wagner N: The senescence markers p16INK4A, p14ARF/p19ARF and p21 in organ development and homeostasis. *Cells* 11: 1966, 2022.
46. Jiang Z, Qian B, Xu T, Bai J and Fu W: Immune microenvironment on the molecular mechanisms and therapeutic targets of MAFLD. *Immunotargets Ther* 14: 719-733, 2025.
47. Carter J, Wang S and Friedman SL: Ten thousand points of light: Heterogeneity among the stars of NASH Fibrosis. *Hepatology* 74: 543-546, 2021.
48. Wiemann SU, Satyanarayana A, Tsahuridu M, Tillmann HL, Zender L, Klempnauer J, Flemming P, Franco S, Blasco MA, Manns MP and Rudolph KL: Hepatocyte telomere shortening and senescence are general markers of human liver cirrhosis. *FASEB J* 16: 935-942, 2002.
49. Verma S, Tachtatzis P, Penrhyn-Lowe S, Scarpini C, Jurk D, Von Zglinicki T, Coleman N and Alexander GJ: Sustained telomere length in hepatocytes and cholangiocytes with increasing age in normal liver. *Hepatology* 56: 1510-1520, 2012.
50. Jannone G, Rozzi M, Najimi M, Decottignies A and Sokal EM: An optimized protocol for histochemical detection of senescence-associated beta-galactosidase activity in cryopreserved liver tissue. *J Histochem Cytochem* 68: 269-278, 2020.
51. Serrano M, Lin AW, McCurrach ME, Beach D and Lowe SW: Oncogenic RAS provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* 88: 593-602, 1997.
52. Guo J, Huang X, Dou L, Yan M, Shen T, Tang W and Li J: Aging and aging-related diseases: From molecular mechanisms to interventions and treatments. *Signal Transduct Target Ther* 7: 391, 2022.
53. Friedman SL: Hepatic stellate cells: Protean, multifunctional and enigmatic cells of the liver. *Physiol Rev* 88: 125-172, 2008.
54. De Waal Malefyt R, Abrams J, Bennett B, Figdor CG and de Vries JE: Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: An autoregulatory role of IL-10 produced by monocytes. *J Exp Med* 174: 1209-1220, 1991.

55. Cheng N, Kim KH and Lau LF: Senescent hepatic stellate cells promote liver regeneration through IL-6 and ligands of CXCR2. *JCI Insight* 7: 158207, 2022.
56. Irvine KM, Skoien R, Bokil NJ, Melino M, Thomas GP, Loo D, Gabrielli B, Hill MM, Sweet MJ, Clouston AD and Powell EE: Senescent human hepatocytes express a unique secretory phenotype and promote macrophage migration. *World J Gastroenterol* 20: 17851-17862, 2014.
57. Thompson KC, Trowern A, Fowell A, Marathe M, Haycock C, Arthur MJ and Sheron N: Primary rat and mouse hepatic stellate cells express the macrophage inhibitor cytokine interleukin-10 during the course of activation in vitro. *Hepatology* 28: 1518-1524, 1998.
58. Li S, Yang F, Cheng F, Zhu L and Yan Y: Lipotoxic hepatocyte derived LIMA1 enriched small extracellular vesicles promote hepatic stellate cells activation via inhibiting mitophagy. *Cell Mol Biol Lett* 29: 82, 2024.
59. Wang Q, Lv Q, Bian H, Yang L, Guo KL, Ye SS, Dong XF and Tao LL: A novel tumor suppressor SPINK5 targets Wnt/ β -catenin signaling pathway in esophageal cancer. *Cancer Med* 8: 2360-2371, 2019.
60. Kordes C, Sawitzka I and Häussinger D: Canonical Wnt signaling maintains the quiescent stage of hepatic stellate cells. *Biochem Biophys Res Commun* 367: 116-123, 2008.
61. Verdelho Machado M and Diehl A: Role of hedgehog signaling pathway in NASH. *Int J Mol Sci* 17: 857, 2016.
62. Ding J, Li HY, Zhang L, Zhou Y and Wu J: Hedgehog signaling, a critical pathway governing the development and progression of hepatocellular carcinoma. *Cells* 10: 123, 2021.
63. Chen Y, Gao WK, Shu YY and Ye J: Mechanisms of ductular reaction in non-alcoholic steatohepatitis. *World J Gastroenterol* 28: 2088-2099, 2022.
64. Shen X, Peng Y and Li H: The injury-related activation of hedgehog signaling pathway modulates the repair-associated inflammation in liver fibrosis. *Front Immunol* 8: 1450, 2017.
65. Hu Y, Peng L, Zhuo X, Yang C and Zhang Y: Hedgehog signaling pathway in fibrosis and targeted therapies. *Biomolecules* 14: 1485, 2024.
66. Brenner C, Galluzzi L, Kepp O and Kroemer G: Decoding cell death signals in liver inflammation. *J Hepatol* 59: 583-594, 2013.
67. Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM and Czaja MJ: Autophagy regulates lipid metabolism. *Nature* 458: 1131-1135, 2009.
68. Pickles S, Vigjié P and Youle RJ: Mitophagy and quality control mechanisms in mitochondrial maintenance. *Curr Biol* 28: R170-R185, 2018.
69. Cui X, Zhou Z, Tu H, Wu J, Zhou J, Yi Q, Liu O and Dai X: Mitophagy in fibrotic diseases: Molecular mechanisms and therapeutic applications. *Front Physiol* 15: 1430230, 2024.
70. Liu S, Wang L, Zhu L, Zhao T, Han P, Yan F, Wang X, Li C, Wang Z and Yang BF: Mechanism and regulation of mitophagy in liver diseases: A review. *Front Cell Dev Biol* 13: 1614940, 2025.
71. Du Y, Zhu S, Zeng H, Wang Z, Huang Y, Zhou Y, Zhang W, Zhu J and Yang C: Research progress on the effect of autophagy and exosomes on liver fibrosis. *Curr Stem Cell Res Ther* 19: 785-797, 2024.
72. Passos JF, Nelson G, Wang C, Richter T, Simillion C, Proctor CJ, Miwa S, Olijslagers S, Hallinan J, Wipat A, *et al*: Feedback between p21 and reactive oxygen production is necessary for cell senescence. *Mol Syst Biol* 6: 347, 2010.
73. Vial G, Dubouchaud H and Leverve XM: Liver mitochondria and insulin resistance. *Acta Biochim Pol* 57: 389-392, 2010.
74. Pacher P, Beckman JS and Liaudet L: Nitric oxide and peroxynitrite in health and disease. *Physiol Rev* 87: 315-424, 2007.
75. Beltrán B, Mathur A, Duchon MR, Erusalimsky JD and Moncada S: The effect of nitric oxide on cell respiration: A key to understanding its role in cell survival or death. *Proc Natl Acad Sci USA* 97: 14602-14607, 2000.
76. d'Adda di Fagagna F: Living on a break: Cellular senescence as a DNA-damage response. *Nat Rev Cancer* 8: 512-522, 2008.
77. Kim KS, Seu YB, Baek SH, Kim MJ, Kim KJ, Kim JH and Kim JR: Induction of cellular senescence by insulin-like growth factor binding protein-5 through a p53-dependent mechanism. *Mol Biol Cell* 18: 4543-4552, 2007.
78. Kortlever RM, Higgins PJ and Bernards R: Plasminogen activator inhibitor-1 is a critical downstream target of p53 in the induction of replicative senescence. *Nat Cell Biol* 8: 877-884, 2006.
79. Merendino N, Costantini L, Manzi L, Molinari R, D'Eliseo D and Velotti F: Dietary ω -3 polyunsaturated fatty acid DHA: A potential adjuvant in the treatment of cancer. *Biomed Res Int* 2013: 310186, 2013.
80. Wu Z, Xia M, Wang J, Aguilar MM, Buist-Homan M and Moshage H: Extracellular vesicles originating from steatotic hepatocytes promote hepatic stellate cell senescence via AKT/mTOR signaling. *Cell Biochem Funct* 42: e4077, 2024.
81. Sun R, Cao M, Zhang J, Yang W, Wei H, Meng X, Yin L and Pu Y: Benzene exposure alters expression of enzymes involved in fatty acid β -oxidation in male C3H/He mice. *Int J Environ Res Public Health* 13: 1068, 2016.
82. Tighanimine K, Nabuco Leva Ferreira Freitas JA, Nemazanyy I, Bankolé A, Benarroch-Popivker D, Brodesser S, Doré G, Robinson L, Benit P, Ladraa S, *et al*: A homeostatic switch causing glycerol-3-phosphate and phosphoethanolamine accumulation triggers senescence by rewiring lipid metabolism. *Nat Metab* 6: 323-342, 2024.
83. Najt CP, Devarajan M and Mashek DG: Perilipins at a glance. *J Cell Sci* 135: jcs259501, 2022.
84. Sztalryd C and Kimmel AR: Perilipin: Lipid droplet coat protein adapted for tissue-specific energy storage and utilization, and lipids cytoprotection. *Biochimie* 96: 96-101, 2014.
85. Marschallinger J, Iram T, Zardeneta M, Lee SE, Lehallier B, Haney MS, Pluvinage JV, Mathur V, Hahn O, Morgens DW, *et al*: Lipid-droplet-accumulating microglia represent a dysfunctional and proinflammatory state in the aging brain. *Nat Neurosci* 23: 194-208, 2020.
86. Mashek DG: Hepatic lipid droplets: A balancing act between energy storage and metabolic dysfunction in NAFLD. *Mol Metab* 50: 101115, 2021.
87. Hosseini MS, Barjesteh F, Azedi F, Alipourfard I, Rezaei Z and Bahreini E: Comparative analysis of β -Estradiol and testosterone on lipid droplet accumulation, and regulatory protein expression in palmitate/oleate-induced fatty HepG2 cells. *BMC Gastroenterol* 25: 263, 2025.
88. Li H and Humphreys BD: Targeting de novo lipogenesis to mitigate kidney disease. *J Clin Invest* 134: e178125, 2024.
89. Conte M, Vasuri F, Trisolino G, Bellavista E, Santoro A, Degiovanni A, Martucci E, D'Errico-Grigioni A, Caporossi D, Capri M, *et al*: Increased Plin2 expression in human skeletal muscle is associated with sarcopenia and muscle weakness. *PLoS One* 8: e73709, 2013.
90. Li H, Zhou Y, Wang H, Zhang M, Qiu P, Zhang R, Zhao Q and Liu J: Crosstalk between liver macrophages and surrounding cells in nonalcoholic steatohepatitis. *Front Immunol* 11: 1169, 2020.
91. Wang YB, Li T, Wang FY, Yao X, Bai QX, Su HW, Liu J, Wang L and Tan RZ: The dual role of cellular senescence in macrophages: Unveiling the hidden driver of age-related inflammation in kidney disease. *Int J Biol Sci* 21: 632-657, 2025.
92. Campisi J: Senescent cells, tumor suppression and organismal aging: Good citizens, bad neighbors. *Cell* 120: 513-522, 2005.
93. Yue B, Gao Y, Hu Y, Zhan M, Wu Y and Lu L: Harnessing CD8+ T cell dynamics in hepatitis B virus-associated liver diseases: Insights, therapies and future directions. *Clin Transl Med* 14: e1731, 2024.
94. Damba T, Zhang M, Serna Salas SA, Wu Z, van Goor H, Arenas AF, Muñoz-Ortega MH, Ventura-Juárez J, Buist-Homan M and Moshage H: Inhibition of endogenous hydrogen sulfide production reduces activation of hepatic stellate cells via the induction of cellular senescence. *Cell Cycle* 23: 629-644, 2024.
95. Ducimetière L, Vermeer M and Tugues S: The interplay between innate lymphoid cells and the tumor microenvironment. *Front Immunol* 10: 2895, 2019.
96. Serna-Salas SA, Arroyave-Ospina JC, Zhang M, Damba T, Buist-Homan M, Muñoz-Ortega MH, Ventura-Juárez J and Moshage H: α -1 Adrenoreceptor antagonist doxazosin reverses hepatic stellate cells activation via induction of senescence. *Mech Ageing Dev* 201: 111617, 2022.
97. Duan Y, Pan J, Chen J, Zhu D, Wang J, Sun X, Chen L and Wu L: Soluble egg antigens of schistosoma japonicum induce senescence of activated hepatic stellate cells by activation of the FoxO3a/SKP2/P27 pathway. *PLoS Negl Trop Dis* 10: e0005268, 2016.
98. Zhu NL, Asahina K, Wang J, Ueno A, Lazaro R, Miyaoka Y, Miyajima A and Tsukamoto H: Hepatic stellate cell-derived delta-like homolog 1 (DLK1) protein in liver regeneration. *J Biol Chem* 287: 10355-10367, 2012.

99. Panebianco C, Oben JA, Vinciguerra M and Paziienza V: Senescence in hepatic stellate cells as a mechanism of liver fibrosis reversal: A putative synergy between retinoic acid and PPAR- γ signalings. *Clin Exp Med* 17: 269-280, 2017.
100. Zhu Y, Tchkonina T, Pirtskhalava T, Gower AC, Ding H, Giorgadze N, Palmer AK, Ikeno Y, Hubbard GB, Lenburg M, *et al.*: The Achilles' heel of senescent cells: From transcriptome to senolytic drugs. *Aging Cell* 14: 644-658, 2015.
101. Wang MJ, Zhang HL, Chen F, Guo XJ, Liu QG and Hou J: The double-edged effects of IL-6 in liver regeneration, aging, inflammation and diseases. *Exp Hematol Oncol* 13: 62, 2024.
102. Ferreira-Gonzalez S, Lu WY, Raven A, *et al.*: Paracrine signaling by senescent cells promotes liver regeneration. *EMBO J* 37: e99195, 2018.
103. Kordes C, Bock HH, Reichert D, May P and Häussinger D: Hepatic stellate cells: Current state and open questions. *Biol Chem* 402: 1021-1032, 2021.
104. Bird TG, Müller M, Boulter L, Vincent DF, Ridgway RA, Lopez-Guadamillas E, Lu WY, Jamieson T, Govaere O, Campbell AD, *et al.*: TGF β inhibition restores a regenerative response in acute liver injury by suppressing paracrine senescence. *Sci Transl Med* 10: eaan1230, 2018.
105. Ritschka B, Storer M, Mas A, Heinzmann F, Ortells MC, Morton JP, Sansom OJ, Zender L and Keyes WM: The senescence-associated secretory phenotype induces cellular plasticity and tissue regeneration. *Genes Dev* 31: 172-183, 2017.
106. Cheng Y, Wang X, Wang B, Zhou H, Dang S, Shi Y, Hao L, Luo Q, Jin M, Zhou Q and Zhang Y: Aging-associated oxidative stress inhibits liver progenitor cell activation in mice. *Aging (Albany NY)* 9: 1359-1374, 2017.
107. Saliev T and Singh PB: Targeting senescence: A review of senolytics and senomorphics in anti-aging interventions. *Biomolecules* 15: 860, 2025.
108. Roeb E: Non-alcoholic fatty liver diseases: Current challenges and future directions. *Ann Transl Med* 9: 726, 2021.
109. Geng Y and Schwabe RF: Hepatic stellate cell heterogeneity: Functional aspects and therapeutic implications. *Hepatology*: May 8, 2025 (Epub ahead of print).
110. Schaenman JM, Rossetti M, Sidharthan S, *et al.*: Increased p16INK4a expression in peripheral blood T cells is associated with frailty in patients evaluated for liver transplantation. *Am J Transplant* 18: 696-703, 2018.
111. Sidharthan S, Wang T, Ying Z, *et al.*: Sarcopenia, frailty, and immunosenescence in liver transplant candidates. *Transplant Direct* 6: e598, 2020.
112. Miller WC, Yousefzadeh MJ, Fisher J, Sarumi H, Kirchner V, Niedernhofer LJ and Pruett T: A brief report on biomarkers of cellular senescence associated with liver frailty and length of stay in liver transplantation. *GeroScience* 47: 5257-5265, 2025.
113. Montégut L, Lambertucci F, Moledo-Nodar L, Fiuza-Luces C, Rodríguez-López C, Serra-Rexach JA, Lachkar S, Motiño O, Abdellatif M, Durand S, *et al.*: Acyl-CoA-binding protein as a driver of pathological aging. *Proc Natl Acad Sci USA* 122: e2501584122, 2025.
114. Yu JH, Lee HA and Kim SU: Noninvasive imaging biomarkers for liver fibrosis in nonalcoholic fatty liver disease: Current and future. *Clin Mol Hepatol* 29 (Suppl): S136-S149, 2023.
115. Xiang X, Dong C, Zhou L, Liu J, Rabinowitz ZM, Zhang Y, Guo H, He F, Chen X, Wang Y, *et al.*: Novel PET imaging probe for quantitative detection of senescence in vivo. *J Med Chem* 67: 5924-5934, 2024.
116. Birch J and Gil J: Senescence and the SASP: Many therapeutic avenues. *Genes Dev* 34: 1565-1576, 2020.
117. Raffaele M, Kovacovicova K, Fröhlich J, Lo Re O, Giallongo S, Oben JA, Faldyna M, Leva L, Giannone AG, Cabibi D and Vinciguerra M: Mild exacerbation of obesity- and age-dependent liver disease progression by senolytic cocktail dasatinib + quercetin. *Cell Commun Signal* 19: 44, 2021.
118. Yakubo S, Abe H, Li Y, Kudo M, Kimura A, Wakabayashi T, Watanabe Y, Kimura N, Setsu T, Yokoo T, *et al.*: Dasatinib and quercetin as senolytic drugs improve fat deposition and exhibit antifibrotic effects in the medaka metabolic dysfunction-associated steatotic liver disease model. *Diseases* 12: 317, 2024.
119. Luo J, Li L, Chang B, Zhu Z, Deng F, Hu M, Yu Y, Lu X, Chen Z, Zuo D and Zhou J: Mannan-binding lectin via interaction with cell surface calreticulin promotes senescence of activated hepatic stellate cells to limit liver fibrosis progression. *Cell Mol Gastroenterol Hepatol* 14: 75-99, 2022.
120. Trussoni CE, O'Hara SP and LaRusso NF: Correction to: Cellular senescence in the cholangiopathies: A driver of immunopathology and a novel therapeutic target. *Semin Immunopathol* 44: 527-544, 2022.
121. Suda M, Paul KH, Tripathi U, Minamino T, Tchkonina T and Kirkland JL: Targeting cell senescence and senolytics: Novel interventions for age-related endocrine dysfunction. *Endocr Rev* 45: 655-675, 2024.
122. Watanabe Y, Abe H, Kimura N, Arao Y, Ishikawa N, Yuichiro M, Setsu T, Sakamaki A, Kamimura H, Yokoo T, *et al.*: Navitoclax improves acute-on-chronic liver failure by eliminating senescent cells in mice. *Hepatol Res* 53: 460-472, 2023.
123. Mohamad Anuar NN, Nor Hisam NS, Liew SL and Ugusman A: Clinical review: Navitoclax as a pro-apoptotic and anti-fibrotic agent. *Front Pharmacol* 11: 564108, 2020.
124. Sharma AK, Roberts RL, Benson RD Jr, Pierce JL, Yu K, Hamrick MW and McGee-Lawrence ME: The senolytic drug navitoclax (ABT-263) causes trabecular bone loss and impaired osteoprogenitor function in aged mice. *Front Cell Dev Biol* 8: 354, 2020.
125. Tang Q, Xiao D, Veviorskiy A, Xin Y, Lok SWY, Pulous FE, Zhang P, Zhu Y, Ma Y, Hu X, *et al.*: AI-driven robotics laboratory identifies pharmacological TNIK inhibition as a potent senomorphic agent. *Aging Dis* 17: 432-51, 2025.
126. Meijnikman AS, Herrema H, Scheithauer TPM, Kroon J, Nieuwdorp M and Groen AK: Evaluating causality of cellular senescence in non-alcoholic fatty liver disease. *JHEP Rep* 3: 100301, 2021.
127. Ryan P and Lee J: In vitro senescence and senolytic functional assays. *Biomater Sci* 13: 3509-3531, 2025.
128. Fei B, Wang H, Ding Y, Shao N, Wen P, Cao Y, Li J, Tanaka M, Wang Z and Li S: Reprogramming cellular senescence of hepatic stellate cells to combat liver fibrosis by targeted nanodrugs. *Mater Today Bio* 33: 101996, 2025.
129. Kakde SP, Mushtaq M, Liaqat M, Ali H, Mushtaq MM, Sarwer MA, Ullah S, Hassan MW, Khalid A and Bokhari SFH: Emerging Therapies for non-alcoholic steatohepatitis (NASH): A comprehensive review of pharmacological and non-pharmacological approaches. *Cureus* 16: e69129, 2024.
130. Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, Abdelmalek MF, Caldwell S, Barb D, Kleiner DE and Loomba R: AASLD Practice Guidance on the clinical assessment and management of nonalcoholic fatty liver disease. *Hepatology* 77: 1797-1835, 2023.
131. Panyod S, Wu W-K, Hu M-Y, Huang H-S, Chen R-A, Chen Y-H, Shen T-CD, Ho C-T, Liu C-J, Chuang H-L, Huang C-C, *et al.*: Healthy diet intervention reverses the progression of NASH through gut microbiota modulation. *Microbiol Spectr* 12: e186823, 2024.
132. Liu Q, Liu S, Chen L, Zhao Z, Du S, Dong Q, Xin Y and Xuan S: Role and effective therapeutic target of gut microbiota in NAFLD/NASH. *Exp Ther Med* 18: 1935-1944, 2019.



Copyright © 2026 Han *et al.* This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.