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Review

Linking fatty liver diseases to hepatocellular carcinoma by hepatic stellate cells

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ABSTRACT

Hepatic stellate cells (HSCs), a distinct category of non-parenchymal cells in the liver, are critical for liver homeostasis. In healthy livers, HSCs remain non-proliferative and quiescent. However, under conditions of acute or chronic liver damage, HSCs are activated and participate in the progression and regulation of liver diseases such as liver fibrosis, cirrhosis, and liver cancer. Fatty liver diseases (FLD), including nonalcoholic (NAFLD) and alcohol-related (ALD), are common chronic inflammatory conditions of the liver. These diseases, often resulting from multiple metabolic disorders, can progress through a sequence of inflammation, fibrosis, and ultimately, cancer. In this review, we focused on the activation and regulatory mechanism of HSCs in the context of FLD. We summarized the molecular pathways of activated HSCs (aHSCs) in mediating FLD and their role in promoting liver tumor development from the perspectives of cell proliferation, invasion, metastasis, angiogenesis, immunosuppression, and chemo-resistance. We aimed to offer an in-depth discussion on the reciprocal regulatory interactions between FLD and HSC activation, providing new insights for researchers in this field.

1. Introduction

1.1. Hepatic stellate cells

Hepatic stellate cells (HSCs) are a distinctive class of non-parenchymal cells found within the liver, residing in the subendothelial Disse's space between hepatocytes and liver sinusoidal endothelial cells (LSECs). They constitute approximately 5–8% of the total liver cell population and make up roughly 33% of the liver's non-parenchymal cells.[1] HSCs play a pivotal role in synthesizing the extracellular matrix (ECM). They are responsible for secreting various ECM components, such as proteoglycan and glycoprotein, and synthesizing collagen, which helps in maintaining the basement membrane.[1] Additionally, they actively regulate microcirculation within the sinusoids through their contractile activity. In healthy livers, HSCs typically display a non-proliferative, quiescent phenotype and contain a large amount of vitamin A lipid droplets, serving as the primary *in vivo* reservoir of vitamin A. Aside from vitamin A storage, quiescent HSCs (qHSCs) perform crucial homeostatic functions, including the secretion of growth factors that support the physiological turnover of hepatocytes and the regulation of hepatic blood flow.[1] Following acute injury, HSCs undergo immediate activation triggered by signals such as transforming growth factor-β (TGF-β) and platelet-derived growth factor B (PDGFB), leading to their trans-differentiation into activated myofibroblast-like HSCs.[2] This activation causes an increase in ECM production and facilitates tissue repair. However, in cases of chronic injury, such as chronic viral infection, alcohol abuse, or non-alcoholic fatty liver disease (NAFLD), activated HSCs (aHSCs) continue to produce excessive ECM, resulting in scar formation and promoting liver fibrogenesis.

1.2. Hepatocellular carcinoma

Liver cancer, notably hepatocellular carcinoma (HCC), represents a major global health challenge and is the third leading cause of cancer-related deaths worldwide.[3] HCC accounts for approximately 80–90% of primary liver cancer cases, predominantly originating from mature hepatocytes.[3] Alarmingly, the annual incidence of HCC is anticipated to exceed one million cases by 2025.[4] The disease predominantly arises

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in the context of chronic liver damage, with over 90% of HCC cases developing in patients with a history of liver diseases.[4] These include chronic viral hepatitis, metabolic disorders, primarily fatty liver diseases (FLD), and exposure to environmental carcinogens like aflatoxins and vinyl chloride. Despite advances in therapeutics, HCC continues to pose significant challenges in patient prognosis, exhibiting a high recurrence rate of over 50%.[5] This underscores an urgent need for continued research and the development of innovative therapeutic approaches to improve patient survival.

Recent studies have underscored the critical influence of the tumor microenvironment (TME) in the progression of HCC and its impact on therapeutic outcomes.[6] The TME in HCC is a complex and dynamic landscape, involving various cellular components like immune cells, fibroblasts, endothelial cells, and a network of signaling molecules. These components interact intricately with HCC cells to promote tumor growth, aid in immune evasion, and foster resistance to therapies. In particular, the role of HSCs within the TME has gained significant attention due to its complex interactions with other components. The aHSCs contribute to fibrosis and create a pro-tumorigenic niche that supports HCC progression through the secretion of growth factors, cytokines, and the modulation of immune responses. They not only facilitate the proliferation and survival of cancer cells but also play a role in the formation of new blood vessels (angiogenesis), crucial for tumor growth and metastasis. Moreover, the interaction between HSCs and immune cells within the TME can lead to an immunosuppressive environment, further complicating treatment strategies.

1.3. Fatty liver diseases

While malignant tumors are a primary concern, HCC-related, precancerous liver diseases also significantly impact human health. The prevalence of viral hepatitis, such as hepatitis B and C, has been effectively mitigated as a result of the increasingly widespread utilization of vaccines, improved safety of blood products, and advancements in antiviral therapies.[7] However, the rise in unhealthy dietary habits and sedentary lifestyles has led to an increase in FLD, closely linked to metabolic syndrome. This condition has become a major public health issue. FLD is generally categorized into two types: NAFLD and alcoholic liver disease (ALD), characterized by distinct triggers that contribute to the development of these conditions.

NAFLD, often associated with metabolic risk factors such as obesity and type 2 diabetes, is characterized by the accumulation of fat in more than 5% of hepatocytes.[8] It occurs in the absence of significant alcohol consumption and excludes other chronic liver diseases.[9] NAFLD encompasses a spectrum of liver conditions, starting from steatosis to nonalcoholic steatohepatitis (NASH), progressing to liver fibrosis, cirrhosis, and potentially leading to HCC.[10] A retrospective study highlighted a substantial global increase in the prevalence of NAFLD, with rates increasing from 25.3% from 1990 through 2006 to 38.0% between 2016 and 2019.[11] Additionally, ALD arises as a consequence of prolonged and/or excessive alcohol consumption, encompassing a range of liver impairments including alcohol-related fatty liver disease (AFLD), alcohol-related steatohepatitis (ASH), liver fibrosis, and cirrhosis, which may eventually progress to HCC.[12] A 2022 meta-analysis indicated that the prevalence of ALD in Asian adults exhibited a notable increase from 3.82% in 2000–-2009 to 6.62% in 2010–2020.[13] About 20–40% of alcohol abusers progress to ASH, and approximately 40% of them develop inflammation and fibrosis.[14] Cirrhosis develops in 10% of heavy drinkers, and within this population, an annual progression rate to HCC ranges from 1 to 2%.[15]

In addition to significant alterations in hepatocytes, the hepatic tissue microenvironment undergoes substantial modifications during the progression of FLD. Notably, chronic inflammation is concomitant with FLD and plays a pivotal role in its progression to more advanced stages of the disease. Consequently, a two-step "inflammation-carcinoma" progression, commonly observed in various cancers, emerges as a critical pathway in HCC development. However, in HCC, this process is more complex. Liver fibrosis, a specific event in liver disease progression, can be induced by chronic inflammation and subsequently facilitates HCC oncogenesis. Hence, a liver-specific disease progression model—"inflammation-fibrosis-carcinoma"—has been proposed. As a pivotal participant in the pathogenesis of fibrosis, HSCs are intricately involved in this intertwined process. While many reviews have focused on the role of HSCs in HCC development, there is a lack of comprehensive analysis on how HSCs link and mediate the transition from FLD, a metabolic liver disorder, to HCC. This review aims to fill this gap by elaborating the activation and regulatory mechanisms of HSCs in FLD progression. We will explore the molecular mechanisms through which aHSCs facilitate liver tumor development in FLD, examining aspects such as cell proliferation, invasion, metastasis, angiogenesis, immunosuppression, and chemoresistance. Additionally, this review will delve into the intricate interplay between FLD and HSC activation, offering a novel perspective for researchers in this field.

2. Contribution of HSCs in HCC progression

HSCs are widely recognized for their contribution to liver fibrosis, a process that can accelerate the onset of HCC and promote HCC progression. Beyond that, HSCs also actively influence various malignant characteristics of HCC through a range of mechanisms (Fig. 1). These include promoting tumor cell proliferation, facilitating angiogenesis, and modulating the TME. By these means, HSCs not only contribute to liver structural remodeling, but also actively enhance the aggressive nature of HCC, playing a multifunctional role in the progression of liver cancer.

2.1. Tumorigenesis

The association between liver fibrosis and the development of HCC is well-established. Epidemiological data indicate that over 90% of HCC cases arise from fibrotic or cirrhotic livers, which establish a pretumorous microenvironment conducive to carcinogenesis.[16] Numerous studies have identified a strong correlation between high fibrosis indices and liver stiffness (a surrogate marker for liver fibrosis), indicating an increased risk of HCC development.[17] Moreover, liver fibrosis has been linked to a higher recurrence rate of HCC following curative resection.[18] This correlation may be attributed to changes in the elasticity of the ECM, leading to increased organ stiffness, which induces a metabolic switch in both tumor and stromal cells altering cellular machinery that supports tumor growth.[19] Additionally, the ECM in cirrhotic livers is enriched with proteins that encourage epithelial-to-mesenchymal transition (EMT) and enhance TGF-β signaling, both of which are well-established pathways known to accelerate liver cancer progression.[20]

To elucidate the relationship between HSCs and the oncogenic transformation of hepatocytes, extensive research has focused on the molecular mechanisms underlying this process. Much of this research has concentrated on the proteins secreted by aHSCs during fibrosis. These studies have shown that cytokines and chemokines released by aHSCs can elicit chemotaxis of diverse types of cells, such as endothelial cells and immune cells, thereby promoting malignant transformation.[21] Co-culture experiment revealed that HSCs can infiltrate the stroma, thereby enhancing paracrine signaling and converting stable hepatocytes into highly motile cells.[21,22] This pro-tumoral activity may be facilitated by the secretion of vascular endothelial growth factor (VEGF) and matrix metalloproteinases 9 (MMP9) by HSCs.[22] In addition to these pro-inflammatory cytokines, TGF-β secreted by HSCs further promotes the tumorigenic capabilities of transformed hepatocytes.[21] However, it is noteworthy that these studies primarily used cell lines derived from lower-grade HCC or transformed hepatocytes which do not accurately represent normal hepatocytes. The findings imply the necessity for further investigation to comprehensively grasp the influence of HSCs on the

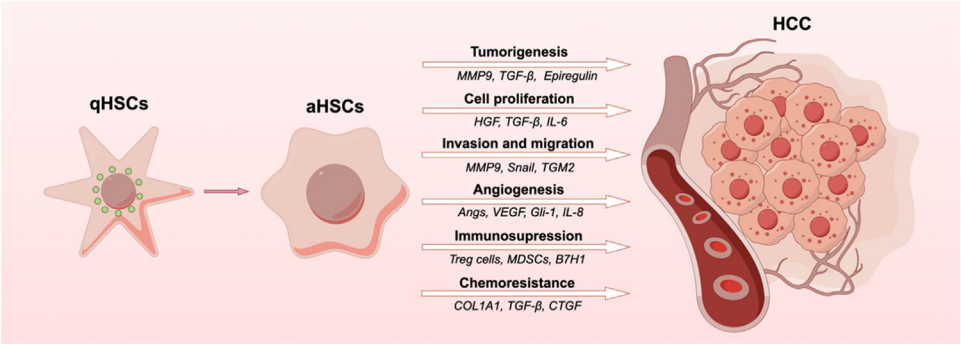

Fig. 1. The multifaceted roles of HSCs in promoting HCC progression. After activation, aHSCs play multifunctional roles in the progression of HCC by promoting tumorigenesis, tumor cell proliferation, invasion and migration, facilitating angiogenesis, and modulating TME to improve the ability of immunosuppression and chemoresistance. HCC, hepatocellular carcinoma; HSCs, hepatic stellate cells; aHSCs, activated HSCs; qHSCs, quiescent HSCs; TME, tumor microenvironment.

tumorigenic transformation of non-malignant liver cells under conditions that are more clinically relevant. Additionally, HSCs were reported as key players in liver tumorigenesis associated with the gut-liver axis and may affect them by secreting epiregulin, which influences toll-like receptor 4 (TLR4) and promotes HCC tumorigenesis.[23,24] This emerging evidence underscores the complex interplay between HSCs and the initiation of liver cancer, emphasizing the need for further investigation into these mechanisms.

2.2. Cell proliferation

The capability for limitless cell division and growth is a defining hallmark of cancer. HSCs have been shown to facilitate the proliferation of HCC cells through various mechanisms. *In vivo*, studies utilizing murine models have demonstrated that co-inoculation of aHSCs with HCC cells augments tumor growth and proliferation.[25] Similarly, *in vitro* experiments have revealed that conditioned media derived from HSC cultures accelerate the growth of tumor spheroids and mitigates central necrosis.[26] Furthermore, it has been established that aHSCs promote the growth of HCC by secreting factors like hepatic growth factor (HGF), TGF-β, and interleukin-6 (IL-6).[21,27–29] Specifically, HGF binds to its receptor c-Met, which is expressed on the surface of HCC cells.[27] This interaction activates signaling pathways such as extracellular signal-regulated kinase (ERK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), thereby promoting tumorigenesis.[27] TGF-β produced by aHSCs fosters tumor formation and augments autocrine TGF-β signaling in tumor cells,[21,30] a process dependent on ERK/mitogen-activated protein kinase (MAPK) signaling.[31] This interaction can accelerate the cell cycle progression and inhibit apoptosis in malignant hepatocytes.[31] Furthermore, IL-6 secreted by aHSCs drives tumor growth through the signal transducer and activator of transcription 3 (STAT3) pathway and potentially exhibits synergistic effects with IL-6 and connective tissue growth factor (CTGF) released by HCC cells.[29]

However, it is worth noting that the role of HGF in HCC development is debatable. Some studies have indicated that HGF expression is actually reduced in HCC tissues compared to adjacent normal tissues, while both the RNA transcript and protein products of c-MET are elevated.[32] Meanwhile, recombinant HGF has been shown to inhibit the growth of most HCC cell lines *in vitro*.[33] Additionally, a recent study published in 2022 identified an HSC subpopulation characterized by HGF secretion that appears to suppress HCC development.[34] The contradictory findings imply that further investigation is necessary to fully understand the precise mechanism through which HSCs promote HCC proliferation.

2.3. Invasion and migration

HSCs are also involved in the advanced stages of HCC, contributing to the formation of a pro-metastatic environment and accelerating disease progression. Many studies have demonstrated that HSCs induce HCC invasion and migration through the following mechanisms.

MMPs secretion: aHSCs secrete matrix metalloproteinases (MMPs), such as MMP9, which degrade the basement membrane and reconstruct the ECM around the tumor, facilitating cancer cell migration across tissue boundaries.[35] The focal adhesion kinase (FAK)/MMP9 pathway is particularly implicated in promoting HCC cell migration and invasion.[35]

EMT induction: aHSCs can promote the activation of the pro-metastasis EMT program in HCC cells, as evidenced by the downregulation of E-cadherin and upregulation of Snail, both of which are indicative of EMT induction.[26,36] Another study hints that the regulation of HSCs on the EMT in HCC was mediated by HGF/c-Met signaling,[36] which requires a dynamic and direct cross-talk between HCC cells and HSCs.

Building a hypoxic-like environment: aHSCs induce transglutaminase 2 (TGM2) expression, leading to von Hippel-Lindau (VHL) protein depletion in HCC cells.[37] This reduction results in the accumulation of hypoxia-inducible factor 1α (HIF-1α), creating a pseudo-hypoxic state that promotes EMT.[37] Additionally, increased production of reactive oxygen species (ROS), along with the secretion of IL-6 and stromal cell-derived factor-1 (SDF-1) by aHSCs, contribute to EMT-mediated metastasis in HCC.[38]

Activation of pro-metastasis signaling: aHSCs activate the Ras/Raf/MAPK (MEK)/ERK and phosphoinositide 3-kinase (PI3K)/AKT pathways, essential for HCC invasion and metastasis. Specifically, the acidic TME leads to ERK phosphorylation and increased osteopontin (OPN) secretion by aHSCs.[39] Furthermore, aHSCs produce laminin-5 (Ln-5) and COMP (carotene oligomeric matrix protein),[40,41] which also activate these signaling pathways. Another study suggests that aHSCs upregulate the expression of nicotinamide N-methyltransferase (NNMT), which promotes HCC invasion and metastasis by mediating histone H3 methylation and activating the transcriptional cluster of differentiation 44 (CD44).[42]

2.4. Angiogenesis

HCC is known for being a highly vascularized tumor, with angiogenesis being essential for the rapid proliferation of malignant hepatocytes. Studies have shown that co-transplantation of HSCs with tumor cells results in a higher microvessel density compared to transplantation of tumor cells alone.[43] Both *in vitro* and *in vivo* experiments have demonstrated that aHSCs express multiple growth factors that interact with surface receptors on endothelial cells, thereby promoting angiogenesis.[44]

Key factors secreted by aHSCs that contribute to angiogenesis include angiotensin I and II (AngI and AngII).[45] These molecules play a crucial role in stabilizing the interaction between endothelial cells and supportive cells during vascular maturation.[45] They also aid in endothelial cell survival, regulate vascular permeability, and induce angiogenesis.[45] Through activating the MAPK pathway, aHSCs secrete VEGF, a critical factor in increasing vascular permeability and promoting endothelial cell mitosis and anti-apoptosis.[46] This action of VEGF is essential in the angiogenic cascade. Co-culture models have further re-

vealed that the interaction between HCC cells and aHSCs creates a pro-angiogenic microenvironment by increasing VEGFA and MMP9 expression in HSCs.[22] Additionally, HSCs may upregulate glioma-associated oncogene homolog 1 (Gli-1) through ROS production,[47] and activate the STAT3 signaling pathway by secreting the pro-inflammatory factor IL-8.[48] These processes collectively contribute to the promotion of angiogenesis in HCC, illustrating the multifaceted role of HSCs in tumor vascularization and highlighting potential therapeutic targets for inhibiting HCC progression.

2.5. Immunosuppression

Immune evasion is a key hallmark of cancer progression, characterized by a reduction in antitumor immune cells and an increase in immunosuppressive cells within the TME. As one of the key cell types in the TME of HCC, aHSCs have been shown to promote liver cancer progression by facilitating immune evasion of HCC cells.

In immunocompetent mice, co-transplantation of HSCs with HCC cells has been shown to diminish the anti-tumor response of the immune cells. This is evidenced by a reduction in the infiltration of $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells in the spleen and tumors following co-transplantation with aHSCs.[43] Moreover, there is a significant induction of $CD4^+CD25^+$ T regulatory (Treg) immunosuppressive cells, known for their role in suppressing anti-tumor immune responses.[49] Furthermore, aHSCs have been found to induce the accumulation of myeloid-derived suppressor cells (MDSCs) through the cyclooxygenases 2 (COX2)/prostaglandin E2 (PGE2)/prostaglandin receptor 4 (EP4) signaling pathway in the bone marrow, spleen, and tumor tissues of tumor-bearing mice.[50] MDSCs are potent inhibitors of T cell proliferation and function, primarily through the increased expression of arginase 1 (Arg-1), inducible nitric oxide synthase (iNOS), and interleukin-4 receptor α (IL-4Rα).[50] Another critical aspect of immune evasion mediated by aHSCs is the upregulation of B7 homolog 1 (B7H1/CD274/PD-L1), a key mediator of immunosuppression. The increased expression of B7H1 in aHSCs within the TME plays a pivotal role in enabling tumor cells to escape host immune surveillance.[51] Its effects include inhibiting T cell activation, inducing T cell apoptosis, and reducing T cell-mediated cytotoxicity.[43] These findings underscore the multifaceted roles of aHSCs in promoting immune evasion in HCC, highlighting their potential as targets for immunotherapeutic strategies aimed at enhancing the anti-tumor immune response.

2.6. Chemoresistance

Most HCC patients exhibit resistance to conventional chemotherapeutic agents, and the interactions between cancer cells and the TME, including HSCs, are believed to be crucial in mediating chemoresistance. It has been reported that HSCs can promote resistance to chemotherapeutic drugs like sorafenib and cisplatin in HCC.[26,52] *In vitro* culture experiments have shown that the mixed cell spheres of HSCs and HCC cells are less sensitive to chemotherapy compared to spheres of HCC cells alone.[52] This reduced sensitivity is thought to result from the ECM production and cytokine secretion by HSCs, which establish a protective microenvironment around the tumor.[52] For example, HSCs can increase the expression of collagen 1A1 (COL1A1) in mixed cell spheres, forming tighter and more resistant cellular structures.[52] Additionally, in three-dimensional cell culture models, aHSCs have been shown to express higher levels of pro-fibrotic factors such as TGF-β and CTGF in co-cultures with HCC cells, which also contribute to the chemoresistance of HCC cells.[26]

Furthermore, HSCs can modulate the ubiquitination of fibroblast activating protein (FAP) via the laminin Ln-332/$\alpha 3\beta 1$ integrin axis, thereby contributing to sorafenib resistance in HCC cells.[53] The HGF/c-Met/Akt pathway, in conjunction with the JAK2/STAT3 pathway, represents an additional signaling cascade implicated in HSC-mediated chemoresistance.[54] These pathways are known to modulate various cellular processes including cell survival, proliferation, and angiogenesis, which can collectively contribute to the reduced effectiveness of chemotherapy in HCC. Moreover, HSCs can influence the immune microenvironment of HCC, potentially contributing to chemoresistance. By modulating immune cell infiltration and activity, HSCs can establish an immunosuppressive TME that protects tumor cells from the cytotoxic effects of chemotherapeutic agents. Additionally, the interaction between HSCs and cancer stem cells (CSCs) within HCC might also contribute to chemoresistance, as CSCs are known to be inherently resistant to various chemotherapies.[55]

3. Implication of FLD in HCC development

NAFLD and ALD are closely associated with HCC, with over 90% of HCC cases arising from chronic liver diseases.[4] Chronic alcohol consumption is a well-established pathway leading to ALD, subsequently progressing to cirrhosis and HCC. In addition, NASH, a severe form of NAFLD, has become a prevalent cause of cirrhosis worldwide.[56] Notably, the incidence of viral hepatitis-related HCC is declining globally. In contrast, metabolism-related HCC cases are on the rise, attributed to the increasing prevalence of metabolic syndrome, obesity and insulin resistance.[57] Similarly, the occurrence of ALD-associated HCC (ALD-HCC) is also escalating, reflecting broader trends in lifestyle and health.[57]

3.1. Epidemiology of NAFLD/ALD-HCC

Over the past few decades, the prevalence of NAFLD-associated HCC (NAFLD-HCC) has been on the rise. A comprehensive analysis of 86 studies from 22 countries, covering a total of 8,515,431 individuals, revealed that the incidence of NAFLD-HCC was 0.44 (0.29–0.66) per 1000 people per year.[58] NASH, an advanced stage of NAFLD, only accounts for a small portion of the cases but can lead to a considerable number of HCC cases.[59] The incidence of NASH-HCC varies geographically, from 10 to 12% in North America and Europe to 1–6% in Asia.[59] NASH is expected to become the leading cause of HCC in high-income areas shortly.[4]

In addition to NAFLD, alcohol abuse is also a significant risk factor for HCC development. The 2015 Global Burden of Disease Study reveals that liver cancer affects about 854,000 individuals worldwide, causing 810,000 fatalities.[60] Markedly, alcohol played a role in almost 30% of these cases.[60] Consistent with the risk profile of NAFLD, there is a higher likelihood of developing HCC in the context of ALD. The BRIDGE study highlighted that in North America (21%) and Europe (37%), ALD was responsible for a larger proportion of HCC cases compared to East Asia (4–13%).[59] A retrospective study utilizing 15-year data from the Scientific Registry of Transplant Recipients in the USA, which involved 28,935 HCC patients awaiting liver transplantation, found that the prevalence of ALD-HCC increased from 8.3% in 2002 to 14.2% in 2017.[61] While the annual incidence of NAFLD/ALD-HCC remains relatively low, there has been a substantial increase in recent years.

3.2. Pathological characteristics of NAFLD/ALD-HCC

NAFLD is typically a result of excessive nutrient intake, leading to lipid accumulation in the liver.[62] This excessive lipid accumulation initiates macrophage infiltration in visceral adipose tissue, triggering an inflammatory response and contributing to insulin resistance.[62] In the state of insulin resistance, lipid metabolism is impaired, leading to an overload of free fatty acids in the liver. The excessive accumulation of fat disrupts liver metabolic capacity and disturbs lipid metabolism homeostasis. The resultant lipid metabolic imbalance leads to the production of lipotoxic lipids, causing cellular stresses, including oxidative and endoplasmic reticulum stress.[62] The stresses, in turn, activate inflammasomes and eventually lead to apoptosis. Collectively, these processes exacerbate inflammation, tissue regeneration, and fibrosis, further pro-

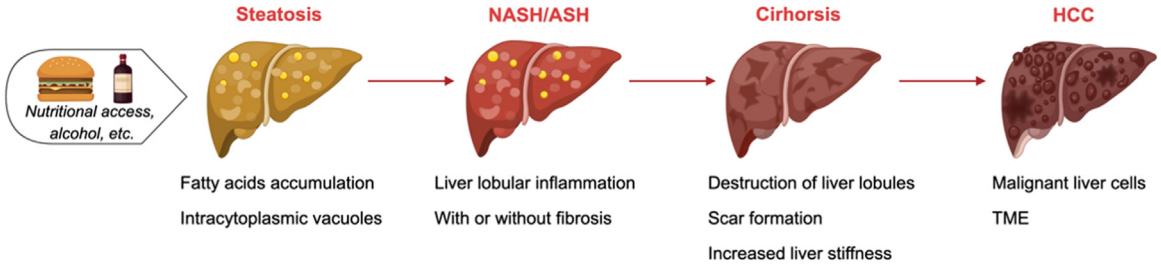

Fig. 2. The transition from FLD to HCC. Steatosis develops in the liver after excessive intake of nutrients and alcohol. As the condition worsens, inflammation develops, leading to NASH and ASH, which can progress to cirrhosis and even HCC. ASH, alcohol-related steatohepatitis; FLD, fat liver disease; HCC, hepatocellular carcinoma; NASH, nonalcoholic steatohepatitis; TME, tumor microenvironment.

moting the progression of NAFLD and increasing the risk of HCC development.

In the context of ALD, alcohol metabolism in hepatocytes was initially thought to be the primary pathological mechanism. However, current insights suggest that this metabolic process initiates a pathogenic cascade involving inflammatory reactions, oxidative stress, and the impact of intestinal endotoxins.[63] The liver metabolizes the majority of ingested alcohol, primarily oxidizing it into acetaldehyde via alcohol dehydrogenase (ADH).[14] This oxidation process alters the ratio of NADH to NAD^+, thus reducing mitochondrial fatty acid beta-oxidation and leading to fatty liver development.[14] Acetaldehyde also interferes with tubulin, affecting microtubule function and disrupting lipoprotein trafficking in the liver. Chronic and excessive alcohol intake leads to additional alcohol oxidation through modulating cytochrome pathways, particularly by increasing cytochrome P450 levels, which in turn generates ROS. The ROS contribute to oxidative stress, lipid peroxidation, and subsequent alcoholic liver damage.[64] Thus, alcohol induces both direct and indirect toxic effects on the liver, eventually leading to cellular damage, inflammation, fibrosis, and an increased risk of HCC.

Preventing or reversing obesity, diabetes, and other metabolic syndrome will be an effective way to reduce the risk of NAFLD/ALD-HCC. In addition, avoiding alcohol and adjusting diet by increasing the intake of green vegetables that are rich in antioxidants such as vitamin C, vitamin E, and selenium, are also associated with reducing the risk of HCC in NAFLD/ALD patients.[57,65] To be specific, NAFLD and ALD exhibit similar histological features, particularly in terms of steatosis, and both diseases typically progress through stages of inflammation, fibrosis, and ultimately carcinoma (Fig. 2). However, they are influenced by distinct and independent factors. NAFLD-HCC is often associated with a variety of contributing elements, including genetic predispositions, abnormal lipid metabolism, chronic hyperinsulinemia, oxidative and endoplasmic reticulum stress, and mitochondrial dysfunction. These factors collectively exacerbate liver damage and facilitate the carcinogenic process. In contrast, ALD's progression to HCC is marked by distinct mechanisms, notably an exacerbated inflammatory response and accelerated cell proliferation. The metabolism of alcohol in ALD leads to significant oxidative stress, primarily due to the production of ROS. The oxidative stress, coupled with alcohol-induced alterations in gut microbiota and the resulting increase in intestinal permeability, allows for an increased translocation of endotoxins to the liver, thereby further promoting inflammation and fibrosis. Additionally, chronic alcohol consumption in ALD can lead to nutritional deficiencies and immune system dysregulation, which also contribute to liver damage and carcinogenesis.

4. Mutual regulation between NAFLD/ALD and HSC activation promotes disease progression

Both NAFLD and ALD, despite their different mechanisms, converge on liver fibrosis, a pivotal stage in HCC progression. The activation of HSCs during fibrosis is crucial, as it leads to increased ECM production and a carcinogenic environment. Many studies have indicated a bidirectional relationship between HSCs and FLD: NAFLD/ALD progression activates HSCs, which in turn exacerbates NAFLD/ALD (Fig. 3). This complex interplay between HSCs and FLD is key in advancing both conditions and HCC development. A deeper understanding of these dynamics is vital for creating effective treatments for HCC stemming from NAFLD and ALD.

4.1. HSCs are activated in the context of NAFLD/ALD

HSC activation can be triggered by oxidative stress and inflammation, often mediated by signals from adjacent cells like hepatocytes, immune cells, and endothelial cells.[66] For instance, TGF-β, a powerful profibrotic agent sourced from endothelial cells, platelets, and macrophages, activates HSCs. It acts via pathways like ERK, p38, and c-jun N-terminal kinase (JNK), subsequently enhancing SMA and MAD proteins 2/3 (SMAD2/SMAD3) signaling and upregulating NADPH oxidase (NOX1, NOX2, NOX4, and NOX5) expressions.[2,67] Activation of the associated pathways fosters the production of collagen types I and III.[67] Additionally, endothelial cells contribute to HSC activation by secreting fibronectin and thus activating TGF-β, which ultimately modulates inflammation and cellular interactions.[68] LSECs secrete VEGF, promoting HSC proliferation, while platelets release TGF-β, EGF, and PDGF, potent HSC mitogens.[69] Moreover, hepatic macrophages, including tissue-resident Kupffer cells and bone marrow-derived macrophages, play a pivotal role in liver fibrogenesis.[70] They produce multiple pro-inflammatory cytokines that directly activate HSCs. ROS generated by the macrophages initiates signaling pathways like OPN, inducing collagen production and perpetuating aHSCs phenotype.[71] In NAFLD/ALD, these pathological conditions collectively contribute to HSC activation through various mechanisms.

4.1.1. Lipid accumulation-related HSC activation

Lipid accumulation in NAFLD/ALD livers initiates pathological changes, prominently activating HSCs. Palmitic acid, a key product in this process, stimulates NOD-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome activation in HSCs via the TLR4/myeloid differentiation factor 88 (MyD88)/NF-κB pathway.[72] This activation sensitizes cells to lipopolysaccharide (LPS)-induced IL-1β secretion,[73] which in turn up-regulates tissue metalloproteinase-1 inhibitor (TIMP-1),[66] prolonging HSC survival and exacerbating NASH progression to liver fibrosis. Furthermore, toxic lipids like palmitate activate $CD11b^+$ $F4/80^{low}$ macrophages.[74,75] Palmitate binds to monomeric TLR4-myeloid differentiation protein 2 (MD2) complexes, triggering macrophage endocytosis, ROS production, and increasing IL-1β expression in these cells.[74,75]

In hepatocytes during NASH, cholesterol crystals within lipid droplets can activate Kupffer cells/macrophages. This activation leads to NLRP3 inflammasome activation and cytokine production (IL-1β, TGF-β, CCL2), which provokes inflammation and promotes further HSC activation.[76] Concurrently, cholesterol accumulation enhances the tran-

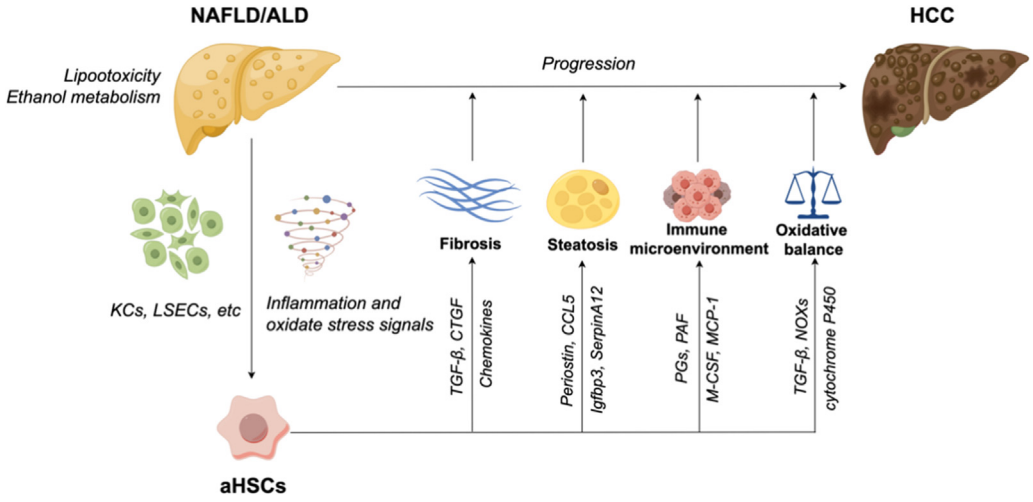

Fig. 3. The interplay between FLD and HSC activation during liver disease progression. The activation of HSCs in the context of NAFLD/ALD promotes the progression of NAFLD/ALD to HCC in various aspects: promoting liver fibrosis and hepatic steatosis, regulating the immune microenvironment and affecting the oxidative balance. ALD, alcohol-related fatty liver disease; FLD, fat liver disease; HCC, hepatocellular carcinoma; HSCs, hepatic stellate cells; aHSCs, activated HSCs; KCs, Kupffer cells; LSECs, liver sinusoidal endothelial cells; NAFLD, nonalcoholic fatty liver disease.

scription and secretion of the pro-fibrotic molecule Indian hedgehog (IHH) by stabilizing the transcriptional regulator TAZ (encoded by the *WWTR1* gene), thereby further driving fibrosis and HSC activation.[77] Oxidized low-density lipoprotein rapidly induces EGFR phosphorylation and ROS production in hepatocytes, facilitating HSC activation and the progression to a pro-fibrotic phenotype in NAFLD.[78] aHSCs in liver injury scenarios express high levels of angiotensin-converting enzyme (ACE) and angiotensin II type 1 receptor (AT1R).[79] The classical renin-angiotensin system (RAS) pathway, through ACE converting AngI to AngII, acts via AT1R, up-regulating TNF-α and TGF-β, thereby promoting HSC activation and NAFLD progression.[79] Elevated AngII levels increase intracellular ROS, leading to the accumulation of lipid peroxidation products and the transformation of qHSCs into aHSCs.[79]

During NAFLD/ALD, stressed hepatocytes release extracellular vesicles (EVs) that are efficiently internalized by HSCs. These EVs contain a plethora of miRNAs, such as miR-27a, which inhibits PTEN-induced putative kinase 1 (PINK1)-mediated mitophagy and is directly linked to liver fibrosis.[80] MiR-128-3p, highly efficient in transfer among lipogenic hepatocyte-derived EVs, inhibits peroxisome proliferator-activated receptor γ (PPARγ), a marker molecule of qHSCs.[81] MiR-192, with increased expression in lipotoxic hepatocyte-derived EVs, promotes the expression of fibrosis markers (α-SMA, TGF-β, COL1A1).[82] Conversely, reduced expression of miR-130a-3p can directly target TGFBR1 and TGFBR2, negatively regulating HSC activation and proliferation during NASH progression.[83] MiR-1297[84] and miR-188-5p[85] inhibit phosphatase and tensin homolog (PTEN), thus activating the PI3K/AKT pathway. These findings highlight the role of hepatocytes in mediating HSC activation through miRNAs in EVs under NAFLD/ALD conditions.

4.1.2. Alcohol-related HSC activation

In ALD, the metabolism of alcohol and its byproduct, acetaldehyde, play pivotal roles in HSC activation. In hepatocytes, ethanol is metabolized into acetaldehyde by ADH and cytochrome P450 2E1 (CYP2E1).[86] This metabolic process results in the production of ROS, leading to oxidative stress.[86] Acetaldehyde affects the regulation of transcription factors involved in fibrosis. It enhances SMAD3 and SMAD4 expression while inhibiting SMAD7.[87] Additionally, acetaldehyde induces the nuclear export and degradation of c-Ski, a suppressor of TGF-β/SMAD signaling, thereby promoting collagen I (Col I) expression and HSC activation.[87]

Ethanol also impacts intestinal integrity, leading to increased leakage and release of endotoxins like LPS.[88] LPS activates NF-κB in Kupffer cells, triggering the production of pro-inflammatory cytokines and liver inflammation.[88] Both ROS and these cytokines act as fibrogenic stimulators, further activating HSCs. Ethanol exacerbates this process by enhancing the synthesis of hyaluronic acid (HA), a key ECM glycosaminoglycan, thus intensifying hepatic pro-fibrotic characteristics.[89] In fatty liver patients, ethanol promotes HA-dependent HSC activation, altering hepatic ECM and accelerating ALD progression.[89]

Ethanol consumption also influences natural killer (NK) cell functions.[90] Chronic ethanol intake hinders NK cell-mediated clearance of aHSCs and induces resistance to NK cell killing via TGF-β1-dependent mechanisms, impeding the anti-fibrotic actions of NK cells.[90] Normally, NK cells release interferon-gamma (IFN-γ) to eliminate aHSCs and promote fibrosis regression. However, ethanol exposure induces suppressors of cytokine signaling (SOCS) proteins and oxidative stress, disrupting IFN-γ/STAT1 signaling in HSCs.[90] Furthermore, ethanol affects methionine metabolism, impacting the availability of S-adenosylmethionine (SAMe) for DNA and histone methylation.[91] Exposure to ethanol leads to upregulation of histone-modifying enzymes like H3K4 methyltransferase mixed lineage leukemia 1 (MLL1) in HSCs, promoting H3K4me3 modification, maintaining an active chromatin state, and resulting in pro-fibrotic gene expression changes, such as elastin, which contribute to HSC activation.[92]

4.1.3. Immune cell-mediated HSC activation

Macrophages are crucial in HSC activation within NAFLD/ALD. In human necrotic livers, CD9$^+$ TREM2$^+$ macrophages, also known as scar/NASH/lipid-associated macrophages, accumulate and express fibrosis-promoting genes in fibrotic niches.[93] These include PDGFB,[94] which enhances HSC proliferation, SPP1,[95] which fosters HSC activation, and IL-1β,[93] which promotes HSC survival and activation. Additionally, apoptotic hepatocytes release TGF-β during egress, contributing to fibrosis when phagocytosed by macrophages, contributes to fibrosis. Interestingly, necrotic hepatocytes produce sonic hedgehog (SHH), a paracrine pro-fibrotic factor, further activating HSCs.[96]

In a methionine-choline-deficient (MCD) diet-induced NAFLD mouse model and corresponding *in vitro* systems, M2 macrophages boost HSC autophagy by secreting PGE2 and interacting with EP4 receptors on the surface of HSCs.[97] Furthermore, Growth arrest-specific gene 6 (Gas6) and its receptor Axl are highly expressed in ALD patient serum. In normal liver, Gas6 is primarily found in Kupffer cells, while Axl is present

in macrophages and qHSCs.[98] Post-injury, increased Gas6 expression in aHSCs and macrophages indicates that the Gas6/Axl interaction may also contribute to macrophage-HSC-driven HSC activation.[98] In addition to macrophages, other immune cells such as NKT cells play a role in HSC activation.[99] In NASH mice, the activation of the Hedgehog (Hh) pathway leads to NKT cell recruitment and activation in the liver.[99] Conditioned medium from NKT cells can stimulate HSC transdifferentiation into myofibroblasts, potentially promoting HSC activation *in vivo*.[99]

4.2. aHSCs promote disease progression via multiple mechanisms

4.2.1. HSC activation leads to NAFLD/ALD-driven liver fibrosis and HCC development

Fibrosis is the excessive buildup of ECM proteins, like collagen, leading to scarring and tissue hardening. This happens when myofibroblasts, cells that help heal wounds, overact in response to ongoing inflammation and tissue damage. Normally, these cells stop working once healing is done, but if the damage persists, they continue to deposit ECM proteins. This leads to more scarring and thus attracts immune cells like macrophages, which worsen the inflammation. Cytokines and growth factors, especially TGF-β and Wnt1, are crucial in this process.[100] They bind to specific receptors, triggering a cascade of signals that eventually turn on genes for synthesizing additional ECM proteins and transforming more cells into myofibroblasts. As the ECM builds up, the tissue becomes stiffer and loses its normal function. The progressive fibrosis over time can significantly impair the organ's functionality.

In NAFLD/ALD-triggered fibrotic livers, there is an increased deposition of collagen types I and III, alongside other ECM proteins such as fibronectin, laminin, HA, elastin, and proteoglycans. aHSCs are primarily responsible for type I collagen secretion, regulated both transcriptionally and post-transcriptionally.[1] TGF-β1 plays a critical role as the main driver of ECM production in aHSCs through autocrine and paracrine mechanisms. Another significant profibrotic factor is connective tissue growth factor (CTGF/CCN2),[101] whose levels rise in liver injury, stimulating pro-fibrotic activities in HSCs via G protein-coupled receptors (GPCRs).[102] Additional factors influencing fibrosis include chemokines and cellular stressors. OPN activates Col I expression through integrin α(V)β(3) binding, triggering the PI3K/pAkt/NF-κB signaling pathway.[71] HSCs also express various pro-fibrotic chemokine receptors like CXC chemokine receptor 4 (CXCR4),[103] C–C chemokine receptor 1 (CCR1), CCR5,[104] CXCR2,[105] and CCR2,[106] which expand the signaling pathways that contribute to HSC activation. Furthermore, IL-17 promotes fibrosis by activating the STAT3 pathway, which induces Col I production in HSCs.[107] As a result, the aHSC-mediated fibrosis promotes the initiation and progression of HCC as we discussed previously.

4.2.2. aHSCs promote liver steatosis

aHSCs play important roles in NAFLD/ALD beyond fibrosis. In early NAFLD/ALD stages, qHSCs show pro-steatogenic effects. qHSCs store lipids, and upon activation, these lipids break down, releasing fatty acids and exacerbating hepatic lipotoxicity.[108] It has been indicated that inhibiting inhibitor of kappa B kinase ε (IKKε) in HSCs improves glucose and lipid metabolism, reducing steatosis in NAFLD mouse models.[109] Additionally, C–C chemokine ligand 20 (CCL20) from HSCs, upregulated in NAFLD, responds to fatty acid loads, suggesting a lipid-sensing role for HSCs.[110] Genome-wide association studies link a *PNPLA3* gene variant to increased lipid droplets and cytokine production in HSCs, supporting their roles in lipid sensing.[111]

Hepatocytes co-cultured with NASH-HSCs show increased lipid accumulation and PPARγ levels, a lipogenic nuclear receptor facilitating free fatty acid uptake, contributing to hepatic triglyceride (TG) elevation.[112] Overexpressing PPARγ in mice induces hepatic steatosis.[113] Conversely, depleting HSCs with gliotoxin in NASH models reduces steatosis by downregulating PPARγ in hepatocytes.[112] This effect involves HSC-derived prostaglandins like periostin[114] and CCL5.[115] In ALD, HSC-secreted Igfbp3 and SerpinA12 mediate lipid droplet formation in hepatocytes.[116] Igfbp3 enhances lipid droplet formation and lipogenic gene expression via p-Akt, while SerpinA12 combats ethanol-induced steatosis via the p-AMPK pathway.[116] Altered Igfbp3 and SerpinA12 levels are noted in ALD patients' sera. Furthermore, glutamate signaling in HSCs drives ALD progression. Chronic alcohol consumption disrupts the trans-sulfuration pathway, leading to glutathione depletion and compensatory increases in extracellular glutamate via the xCT (SLC7A11) antiporter.[117] Alcohol also upregulates mGluR5 in HSCs, promoting 2-AG production, which activates cannabinoid type-1 receptor (CB1R) in hepatocytes, driving lipogenesis in ALD.[118] HSCs also secrete mediators like FGF1, IL-6, and IL-10, potentially countering hepatic steatosis.[119–121] However, HSC depletion has been shown to protect against hepatic steatosis development,[112] highlighting the complex role of HSCs in liver disease dynamics.

4.2.3. aHSCs reset the immune microenvironment of fatty liver

In the context of FLDs, aHSCs exert significant influence by modulating immune responses and the immune microenvironment. aHSCs secrete various pro-inflammatory factors, including TGF-β, which play a crucial role in shaping the host immune response. These cells respond to liver injury by autocrine and paracrine signaling, with autocrine signaling being particularly significant in the context of NAFLD/ALD. aHSCs also produce prostaglandins such as prostaglandin I 2 (PGI2) and PGE2, which promote macrophage activation by regulating intracellular cyclic adenosine monophosphate (cAMP) levels.[122] Factors like macrophage colony-stimulating factor (M-CSF)[123] and monocyte chemoattractant protein-1 (MCP-1)[124] produced by aHSCs regulate the accumulation and growth of macrophages within the inflammatory milieu of FLD. In addition, aHSCs contribute to neutrophil chemotaxis and activation by secreting platelet-activating factor (PAF), further amplifying neutrophil-driven inflammatory responses in the damaged liver.[125] Additionally, aHSCs secrete cytokines such as CCL21, CCR5, IL-8, IL-10, and RANTES (CCL5), accelerating the recruitment of inflammatory cells to the injured liver and exacerbating liver damage.[126] Multiple Toll-like receptors expressed on aHSCs, including TLR4 and TLR2, are involved upstream in these pro-inflammatory responses, recognizing a range of pathogen-associated molecular patterns (PAMPs) such as LPS, lipoteichoic acid, and N-acetylmuramyl peptide, thereby stimulating the secretion of IL-6, TGF-β, and MCP-1.[127]

In experimental mouse models, transplanted HSCs have been shown to protect islet allografts from rejection[128] and enhance the engraftment of transplanted hepatocytes,[129] indicating a pro-immune tolerance function of HSCs. When exposed to pro-inflammatory cytokines, HSCs can also act as antigen-presenting cells and express membrane proteins involved in antigen presentation, including human leukocyte antigen (HLA) family members, lipid presentation molecules, and factors involved in T cell activation.[130] Moreover, the co-stimulatory molecule programmed cell death-ligand 1 (PD-L1) is expressed on aHSCs, but not on qHSCs,[131] and it binds to the immunoglobulin superfamily member programmed death 1 (PD-1), which is expressed on various immune cells, including $CD4^+$ T cells.[132] Low level of PD-1 stimulation is sufficient to inhibit T cell activation and cytotoxicity and induce T cell apoptosis.[132] HSCs can also impact B cell immune function through a similar mechanism.[133] These findings suggest that HSCs may have distinct immunoregulatory functions in the context of FLD, and further investigation is needed to elucidate the balance between promoting an inflammatory microenvironment and inhibiting cellular immune function.

Considering the essential role of immune cells in FLD progression, restoring immune homeostasis may be a key to treating FLD. It has been established that certain drugs may effectively alleviate NASH by the anti-inflammation response, including Cenicriviroc (CCR2/5 inhibitor),[134] NOX-E36 (CCL2 inhibitor),[135] and Belapectin (Galectin-3

inhibitor).[136] It will be of interest to know whether these drugs can affect HSC activation, or if the blockage of HSC activation is the direct target of these drugs.

4.2.4. aHSCs regulate oxidative balance and stress

In the landscape of FLD, especially NAFLD, HSCs play a crucial role in modulating oxidative stress, a factor intricately linked to disease progression. One significant hallmark of aHSCs is their propensity to generate ROS, which emerges as a pivotal mechanism in the activation of these cells. ROS production serves as a "feedforward" mechanism for driving fibrogenic cell activation, particularly through the potent mediator, TGF-β1. Within the TGF-β1 signaling pathway, several NADPH oxidases (NOX1, NOX2, NOX4, and NOX5) are upregulated, leading to increased peroxide production and subsequently enhancing collagen synthesis.[67] In the context of NAFLD, aHSCs exhibit a unique facet of their oxidative milieu, closely intertwined with ethanol metabolism. Notably, HSCs express alcohol[137] and aldehyde[138] dehydrogenases, suggesting their involvement in responding to lipid peroxides, hydrogen peroxides, and aldehyde adducts. This implies a potential role for HSCs in the metabolic processes associated with ethanol.

Furthermore, HSCs also express essential components of the nonphagocytic form of NADPH oxidase responsible for peroxide production.[139] Activation of HSCs induces the expression of these components, which generate peroxides upon interaction with AngII receptors.[140] AngII, in turn, phosphorylates p47phox, a regulatory subunit of NADPH oxidase, leading to the generation of ROS.[140] In addition to ROS production, qHSCs harbor multiple cytochrome P450 enzymes, such as CYP2C11, 3A2, 2D1, CYP2S,[141] and CYP3A,[142] which undergo downregulation upon HSC activation.[143] This complex interplay of oxidative processes suggests a model wherein aHSCs may recruit immune cells by producing peroxides, and paracrine peroxides exacerbate hepatocyte injury. Simultaneously, aHSCs may possess mechanisms to mitigate the effects of oxidative stress, thereby maintaining their viability.

In summary, aHSCs exhibit a multifaceted impact in the context of fatty liver diseases. They contribute to the disease progression through the production of pro-inflammatory cytokines and chemokines, the promotion of steatosis, and the recruitment of immune cells to the injured liver. This orchestrated interplay between immune cell recruitment and wound healing processes can lead to persistent inflammation and impaired liver regeneration, both of which constitute pivotal factors in the advancement of NAFLD/ALD. As the disease evolves, it is characterized by the gradual replacement of healthy liver tissue with non-functional scar tissue, culminating in the disruption of organ structure. Ultimately, this pathological progression can result in severe consequences, including the development of HCC and organ failure. The intricate involvement of aHSCs underscores their pivotal role in the intricate landscape of FLD, shedding light on potential therapeutic targets for managing these complex conditions.

5. Concluding remarks

In this review, we summarized the pivotal role of HSCs in the progression of FLD and their association with HCC. As non-parenchymal cells, HSCs exhibit remarkable secretory and regulatory functions that render them central players in disease development, especially during the trajectory of the NAFLD/ALD-HCC pathogenic process. During NAFLD/ALD, accumulation of lipid or ethanol provokes inflammation and oxidative stress, setting in motion a cascade of events including hepatocyte death, immune cell infiltration, and HSC activation. aHSCs then secrete an array of mediators that exacerbate steatosis, inflammation, and oxidative stress, further expediting disease progression. Persistent HSC activation can lead to fibrosis, cirrhosis, and ultimately HCC, through intensive intercellular communications among HSCs, hepatocytes, and immune cells. Understanding these intricate cell-cell interactions during disease pathogenesis may provide potential targets for therapeutic intervention.

The interplay between oxidative stress, inflammation, and fibrosis is a complex area of research, particularly challenging in the context of liver diseases where HSCs are intricately involved. Recent advancements in cell biology have shed light on distinct subpopulations of HSCs with varying physiological and pathological functions. For instance, a 2022 study employing single-cell RNA sequencing identified two HSC subpopulations in fibrotic livers: myofibroblastic HSCs, enriched in ECM-related molecules, and cytokine and growth factor-expressing HSCs with a high cytokine and growth factor expression profile.[34] Notably, these studies revealed shifts in the abundance of these subpopulations in liver disease and their differential roles in hepatocarcinogenesis. These findings suggest that HSCs may possess diverse functions, urging further high-resolution studies to comprehensively elucidate the intricacies of how HSCs contribute to liver disease. These studies may also lead to a new, in-depth understanding of the mutual regulations between oxidative stress, inflammation, and fibrosis.

Targeting the TME, with a specific focus on HSCs, offers a promising avenue for innovative interventions in HCC. Strategies aimed at modulating the TME to disrupt the supportive network for tumor cells, augment immune responses against cancer cells, or directly target the metabolic and signaling pathways of HSCs hold the potential to advance liver cancer management. Personalized approaches, tailored to the unique TME characteristics of individual patients, hold the promise of more effective and targeted therapies, ultimately enhancing the prognosis for HCC patients.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Y.F. and L.C. conceived the original idea and wrote the initial draft. L.Y. and X.Y. gathered the pertinent papers. Y.J. and J.Z. revised the manuscript.

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