Review of Liver Fibrosis and Inflammation

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Abstract. Liver fibrosis is a common pathological process triggered by chronic liver diseases (such as viral hepatitis, non-alcoholic fatty liver disease, and alcoholic liver disease), posing a serious threat to global public health. This article provides a systematic review of the epidemiology, pathogenesis, major cell types, key signaling pathways, and treatment advances in liver fibrosis. The core of fibrosis lies in the activation of hepatic stellate cells (HSCs) and their sustained synthesis of extracellular matrix (ECM). Macrophages, as important immune regulatory cells, play a bidirectional regulatory role in inflammatory responses and fibrosis reversal. The paper focuses on elucidating the regulatory mechanisms of immune activation mediated by DAMPs/PAMPs, TGF-β/Smad, and PDGF signaling pathways on HSC activation, as well as the polarization dynamics of M1/M2 macrophages. Research has shown that liver fibrosis has a certain degree of reversibility, and early intervention can promote the inactivation or apoptosis of activated HSCs, restoring tissue structure. Based on these mechanisms, drugs targeting key factors such as CCL2/CCR2 and TGF-β, as well as emerging therapeutic approaches like stem cells, gene editing, and gut microbiota modulation, have become key directions for future treatment. Although no specific anti-fibrotic drugs have been approved yet, mechanistic research provides a solid foundation for personalized precision therapy.

1 Overview of Liver Disease: Epidemiological Trends and Health Threats

Liver disease is a significant global public health issue, causing approximately 2 million deaths annually, accounting for 4% of all global deaths[1]. The main types include viral hepatitis, nonalcoholic fatty liver disease (NAFLD), also known as metabolic dysfunction-associated fatty liver disease (MAFLD), and alcohol-related liver disease (ALD)[1]. In the absence of effective intervention, these hepatic disorders have the potential to evolve into progressive stages, including fibrosis, cirrhosis, and hepatocellular carcinoma (HCC), posing substantial risks to human health. Therefore, a systematic understanding of their

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epidemiological characteristics and pathogenesis is crucial for developing effective prevention, control, and intervention measures[1].

Viral hepatitis remains one of the major burdens of liver disease. Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the primary pathogens, with approximately 258 million HBV carriers worldwide[2] and annual HCV-related deaths reaching 1.3 million[3]. HBV is most prevalent in East and Southeast Asia, while HCV is primarily concentrated in countries such as China, India, Pakistan, and Russia[2-3]. Hepatitis D virus (HDV) can co-infect with HBV, significantly exacerbating liver damage[3]. Although vaccination and antiviral therapy have been effective in reducing new infections, a large number of chronic carriers remain at risk of cirrhosis and hepatocellular carcinoma, and the global burden of liver disease remains significant[2-3].

The global surge in obesity and type 2 diabetes has significantly contributed to the increasing prevalence of non-alcoholic fatty liver disease (NAFLD), now recognized as one of the most widespread chronic liver disorders. Current estimates suggest that NAFLD affects nearly 30% of the global population, with particularly high burdens reported in regions including Latin America, the Middle East, South Asia, and East Asia[4]. Some patients progress to non-alcoholic steatohepatitis (NASH) and liver fibrosis, further increasing the risk of cirrhosis and HCC[5]. Additionally, NAFLD is closely associated with chronic diseases such as cardiovascular disease and diabetes, and has become part of a multisystem metabolic disorder, necessitating multidisciplinary comprehensive intervention[6].

Alcohol-related liver disease (ALD) arises predominantly due to sustained or high-level alcohol exposure over time, with a prevalence rate of approximately 3.5% in the general population, significantly higher among high-risk drinkers[7]. In Europe, the Americas, and the Asia-Pacific region, ALD is one of the leading causes of cirrhosis and liver transplantation in young and middle-aged adults[7]. During the COVID-19 pandemic, global high-risk drinking behaviors increased, further exacerbating the burden of ALD. The role of alcohol in liver disease is often underestimated, necessitating enhanced public education and policy management[7].

2 The Pathological Process of Liver Fibrosis

Liver fibrosis (LF) is an important pathological stage common to the progression of various chronic liver diseases. Its causes include viral hepatitis, alcoholic liver disease, NASH, autoimmune hepatitis, cholestatic liver disease, exposure to chemical toxins, and genetic metabolic disorders. Its characteristic features include excessive deposition of extracellular matrix (ECM) within the liver, leading to the formation of fibrous scar tissue, which ultimately results in abnormal liver structure and functional impairment[8].

Without effective intervention, liver fibrosis can progress to cirrhosis and hepatocellular carcinoma, significantly increasing mortality rates. It is estimated that by 2020, the number of liver fibrosis patients in China had reached 142 million, representing a substantial disease burden[8].

Liver fibrosis is a frequent pathological consequence of sustained hepatic injury, representing an aberrant form of the liver's wound-healing response. It is marked by the excessive accumulation of collagen and other extracellular matrix (ECM) components, which progressively disrupt hepatic architecture and can culminate in the formation of cirrhotic scarring[9]. The development of liver fibrosis can be divided into the following stages:

2.1 Initiation of liver fibrosis: the key role of hepatocyte injury and inflammatory response

The development of liver fibrosis begins with sustained damage to hepatocytes caused by various pathogenic factors. These factors include viral infections (such as hepatitis B and C), excessive alcohol consumption, metabolic abnormalities (such as non-alcoholic fatty liver disease [NAFLD] and non-alcoholic steatohepatitis [NASH]), cholestatic diseases (such as primary biliary cholangitis [PBC]), and drug toxicity or environmental toxins (such as carbon tetrachloride)[9]. Following hepatocyte damage, reactive oxygen species (ROS) and damage-associated molecular patterns (DAMPs) are released, signaling the activation of hepatic immune cells, particularly macrophages, and triggering a local inflammatory response[9], as shown in Figure 1[95]. Additionally, intestinal-derived pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), may enter the liver via the portal vein, further exacerbating the inflammatory response[9].

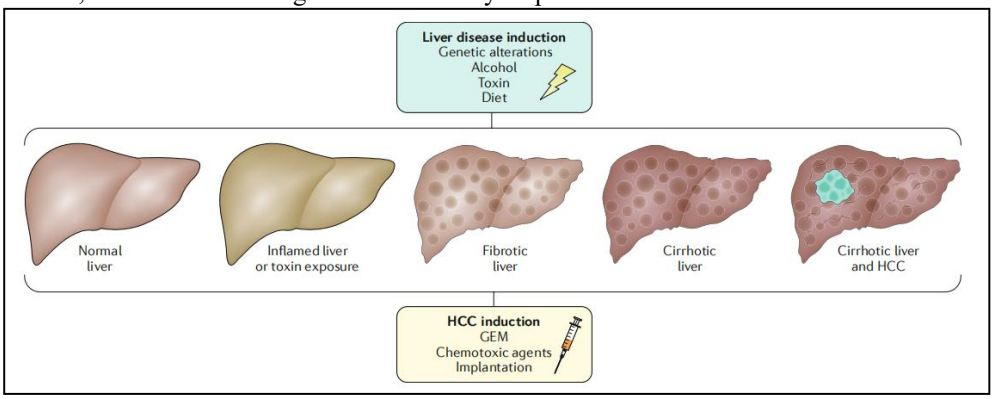

Fig. 1. Induction of Hepatocellular Carcinoma in the Context of Chronic Liver Disease**.**

Damage-associated molecular patterns (DAMPs) are endogenous molecules released into the intercellular space or blood circulation by tissues or cells in response to stress factors such as injury or hypoxia; pathogen-associated molecular patterns (PAMPs) are characteristic molecular signatures conserved across various microorganisms, serving as indicators recognized by the host immune system. Both can be recognized by pattern recognition receptors (PRRs) on the surface or within host cells, such as Toll-like receptors, NLRs, and RIG1-like receptors, thereby initiating the innate immune response and inducing inflammatory reactions[10].

Macrophages (including resident Kupffer cells, KCs) recognize DAMPs and PAMPs through their surface Toll-like receptors and secrete large amounts of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-17, and IL-6, to initiate and amplify the inflammatory cascade reaction[9]. Concurrently, neutrophils release inflammatory factors and extracellular DNA nets to participate in early immune responses, while liver sinusoidal endothelial cells (LSECs) undergo “capillarization” after injury, losing their fenestrated structure and reducing the release of vasodilatory factors (such as nitric oxide, NO), indirectly promoting the activation of hepatic stellate cells (HSCs). This stage establishes a persistent inflammatory microenvironment, laying the foundation for the progression of fibrosis[11].

2.2 Fibrotic Cellular Response: Activation of Hepatic Stellate Cells and Portal Vein Fibroblast

In various types of liver injury, liver fibrosis involves the interaction of multiple cell types, with myofibroblasts playing a primary role in liver fibrosis.

Biliary stasis diseases primarily activate portal vein fibroblasts, which respond to bile duct obstruction or bile acid toxicity by promoting collagen deposition through the TGF-β and IL-25/IL-13 signaling pathways[12]. Additionally, nuclear receptors such as the farnesoid X receptor (FXR) play a key role in regulating bile acid metabolism, and its agonists have been shown to alleviate bile stasis-related liver damage[13].

The development of liver fibrosis is the result of the combined effects of various endogenous and exogenous factors, with core mechanisms including hepatocyte damage, activation of immune responses, and sustained activation of myofibroblasts. Different types of liver injury induce distinct dominant fibrotic mechanisms; for example, hepatotoxic injury primarily activates hepatic stellate cells, while cholestatic injury primarily involves portal vein fibroblasts[14]. Inflammatory responses not only promote fibrotic progression but also harbor the potential for reversal, offering possibilities for clinical intervention.

2.3 Fibrosis Development

The progression of liver fibrosis involves a complex interplay among multiple hepatic and immune cell populations. Signals such as damage-associated molecular patterns (DAMPs), pathogen-associated molecular patterns (PAMPs), reactive oxygen species (ROS), and pro-inflammatory cytokines including IL-17 and TNF-α activate the liver's innate immune system, particularly Kupffer cells. Once activated, these cells release large quantities of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, along with chemokines like CCL2, which promote the recruitment of $CCR2^+$ monocytes from the bloodstream into liver tissue, fostering an inflammatory microenvironment that contributes to fibrogenesis[15-16].

The inflammatory microenvironment can induce hepatic stellate cells (HSCs) in the Disse space to transition from a resting state rich in vitamin A droplets to an activated state expressing α-SMA and secreting type I and III collagen, becoming the primary source of ECM in liver fibrosis[17-18]. Activated HSCs can also secrete factors such as TGF-β1 and PDGF, forming a self-sustaining pro-fibrotic cycle[19].

In addition to HSCs and macrophages, liver sinusoidal endothelial cells (LSECs) undergo "capillarization" changes in liver injury, losing their normal fenestration structure and secreting factors such as TGF-β, IL-6, and PDGF, which indirectly promote HSC activation. LSECs can also enhance immune cell recruitment through the CCL2/CCR2 axis, exacerbating the inflammatory response[20]. Moreover, T lymphocytes and natural killer (NK) cells also participate in regulating fibrosis. Th17 cells secrete IL-17 to promote neutrophil aggregation and HSC activation, while regulatory T cells (Tregs) mitigate inflammation through anti-inflammatory factors such as IL-10; NK cells mediate the clearance of activated HSCs via the NKG2D and TRAIL pathways, exhibiting certain anti-fibrotic effects[21].

In the middle and late stages of liver fibrosis, ECM synthesis continues to increase, while its degradation function gradually decreases. Although matrix metalloproteinases (MMPs) can degrade the ECM, activated HSCs and other cells secrete large amounts of tissue inhibitors of metalloproteinases (TIMPs), such as TIMP-1 and TIMP-2, which inhibit MMP activity, leading to abnormal accumulation of the ECM in the liver. This imbalance between synthesis and degradation is the key mechanism underlying the gradual deposition of fibrous tissue[22].

From a temporal standpoint, liver fibrosis development can be categorized into three distinct phases. During the initial phase, injured hepatocytes release damage-associated molecular patterns (DAMPs), which activate macrophages and trigger an inflammatory cascade. This stage is characterized by the accumulation of inflammatory cells, including monocytes, neutrophils, and lymphocytes, creating a localized repair response. In the intermediate stage, sustained inflammatory stimulation leads to the activation of a large number of HSCs, which synthesize and deposit type I and type III collagen to form fibrous bundles, resulting in the remodeling of liver tissue structure and widening of interlobular septa; LSECs proliferate and undergo capillaryization, laying the foundation for subsequent hemodynamic changes. In the late stage, excessive ECM deposition forms dense fibrous scars, leading to destruction of the hepatic parenchymal structure, the formation of pseudo-lobules, and the liver exhibiting nodular fibrosis characteristics. As the portal venous system is compressed, it often triggers portal hypertension complications, such as splenomegaly and esophageal varices. With vascular neogenesis and sinusoidal remodeling, liver fibrosis enters an irreversible stage of structural and functional damage. If not intervened promptly, it is highly likely to progress to decompensated cirrhosis, significantly increasing the difficulty of clinical treatment[23].

2.4 Regression and Reversibility of Fibrosis

Liver fibrosis is considered a dynamic reversible pathological process, particularly in its early stages, which exhibit significant potential for reversal. When the primary pathogenic factors (such as viral infection, alcohol consumption, or metabolic disorders) are eliminated, the liver can activate its endogenous repair mechanisms to partially or completely clear fibrotic tissue[23]. For example, antiviral therapy or weight-loss interventions can significantly improve tissue structure, suggesting that fibrosis is not irreversible[24]

The core mechanisms of fibrosis reversal primarily include the clearance of activated hepatic stellate cells (HSCs) and the degradation of the extracellular matrix (ECM). After removing the pathogenic stimulus, approximately half of the activated HSCs undergo apoptosis via the FAS or TRAIL pathways, while the remaining portion can be inactivated into a “quiescent-like” state[25] or undergo senescence. Although these inactivated HSCs (iHSCs) exhibit phenotypic similarities to quiescent hepatic stellate cells (qHSCs), there are differences in gene expression, potentially retaining partial “activation memory,” making them more sensitive to fibrotic signals and suggesting they may reactivate upon recurrence of the underlying pathology[25], just like figure 2[96].

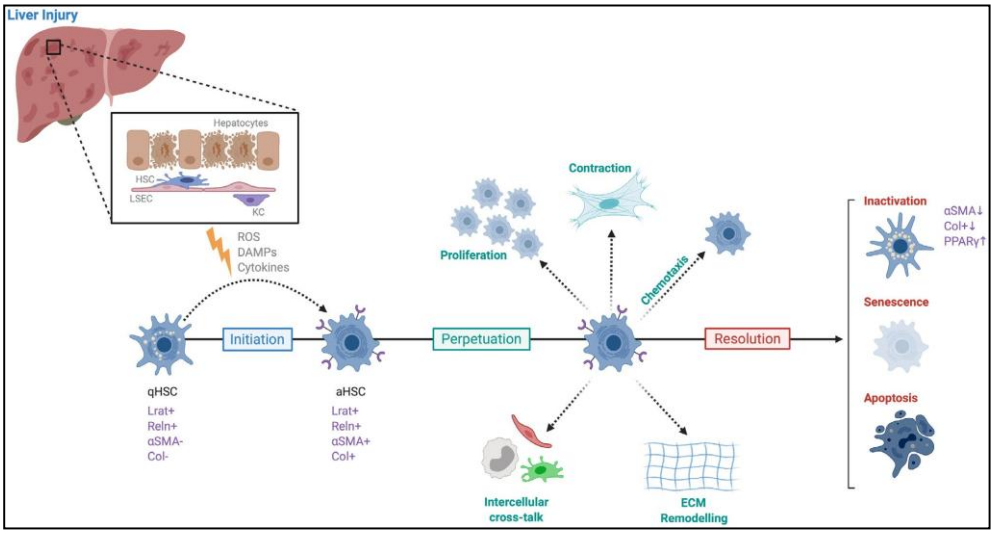

Fig. 2. Phases of Hepatic Stellate Cell Activation and Resolution.

ECM degradation primarily relies on matrix metalloproteinases (MMPs) secreted by macrophages, such as MMP-9, MMP-12, and MMP-13, which effectively degrade fibrous components like collagen and elastin[26]. Concurrently, as myofibroblasts undergo apoptosis or inactivation, the levels of tissue inhibitors of metalloproteinases (TIMPs) they secrete decrease, thereby releasing inhibition of MMPs and enhancing ECM degradation capacity[26]. Additionally, the plasminogen activator system (such as uPA) can indirectly promote ECM degradation and TGF-β activation by activating plasmin, further regulating fiber remodeling[26].

During the regression phase of fibrosis, the involvement of the immune system is particularly critical. Hepatic macrophages can transition from the pro-inflammatory Ly6Chi phenotype to the pro-reparative Ly6Clo phenotype, which secretes large amounts of MMPs and anti-inflammatory factors (such as IL-10) and assists in clearing necrotic tissue and apoptotic cells, thereby enhancing liver tissue regeneration capacity[27-28]. Natural killer (NK) cells and NKT cells directly induce HSC apoptosis by secreting cytokines such as IFN-γ, TRAIL, and TNF-α, thereby inhibiting collagen synthesis and promoting liver tissue repair[26][29]. Studies have also found that regulating molecules such as the E3 ubiquitin ligase RNF-41 can guide macrophages toward a reparative polarization, further accelerating fibrosis reversal[30].

Notably, both experimental models (e.g., CCl_4, alcohol, and bile duct ligation-induced models) and clinical studies have confirmed the feasibility of the aforementioned reversal process[26]. The main characteristics of fibrosis regression include reduced inflammation levels, decreased fibroblast factors, enhanced collagenase activity, and the remodeling and regression of fibrotic scar tissue[26]. Recent lineage tracing and single-cell analysis have shown that approximately half of hepatic fibroblasts can avoid apoptosis after injury resolution and return to the Disse space, restoring a phenotype similar to the resting state[26]. In vitro experiments have also confirmed that activated HSCs can regain resting characteristics in a non-stimulated environment, characterized by reduced expression of fibrosis-related genes such as Col1a1 and α-SMA, and increased expression of resting genes such as PPARγ [26].

The reversal of liver fibrosis is the result of the combined action of multiple cell types and signaling pathways, particularly dependent on the clearance of activated HSCs,

effective degradation of the ECM, functional reprogramming of macrophages, and synergistic regulation by immune cells[31]. These mechanisms work together to enable the gradual restoration of liver tissue structure, providing an important theoretical foundation and practical pathway for clinical intervention.

3 The role of hepatic macrophages in fibrosis

Hepatic macrophages are important immune cells in the liver. Based on their origin, distribution, and surface markers, they are primarily divided into two categories: one category consists of Kupffer cells (KCs), which originate from embryonic yolk sac precursor cells and are resident in the subendothelial space of hepatic sinusoids, accounting for approximately 35% of non-parenchymal cells in the liver; the other type originates from peripheral monocytes derived from bone marrow, which migrate to the liver upon liver injury and differentiate into infiltrating macrophages, also known as monocyte-derived macrophages (MoMϕs), accounting for 5–30% of hepatic macrophages[52]. These two cell types collectively constitute the hepatic macrophage population, one of the richest tissue sources of macrophages in the body[32].

3.1 Functional heterogeneity of macrophage subtypes

KCs maintain immune tolerance in hepatic homeostasis, clear pathogens and apoptotic cells from the circulation, and regulate iron and lipid metabolism. When activated by injury signals (such as DAMPs), KCs rapidly respond via pattern recognition receptors (such as TLR4), releasing pro-inflammatory factors like IL-1β and TNF-α, inducing HSC (hepatocyte stellate cell) activation, and initiating the fibrosis process[33-34]. Concurrently, they secrete the chemokine CCL2 to recruit monocytes, further exacerbating inflammation[33]. However, KCs also possess anti-fibrotic potential: they participate in collagen degradation by secreting matrix metalloproteinases such as MMP-9 and promote collagen cross-linking by expressing LOXL2, demonstrating a dual role in both fibrogenesis and degradation[33-34].

MoMφs enter liver tissue in large numbers via chemokine axes such as CCL2/CCR2 and CCL5/CCR5 during liver injury and differentiate into functionally heterogeneous macrophage subtypes, primarily including Ly6Chi (pro-inflammatory, pro-fibrotic) and Ly6Clo (anti-inflammatory, pro-reparative) types[8]. Ly6Chi cells secrete IL-1β, TNF-α, and TGF-β, supporting inflammation maintenance and HSC activation, thereby promoting fibrosis; while Ly6Clo cells release IL-10, MMP-9, MMP-13, etc., inhibiting inflammation, degrading ECM, and repairing tissue[8]. In the early stages of fibrosis progression, Ly6Chi cells clear pathogens and necrotic cells; during the injury resolution phase, some Ly6Chi cells can convert to the Ly6Clo phenotype, promoting fibrosis reversal[8] just like what is shown in Figure 3[97].

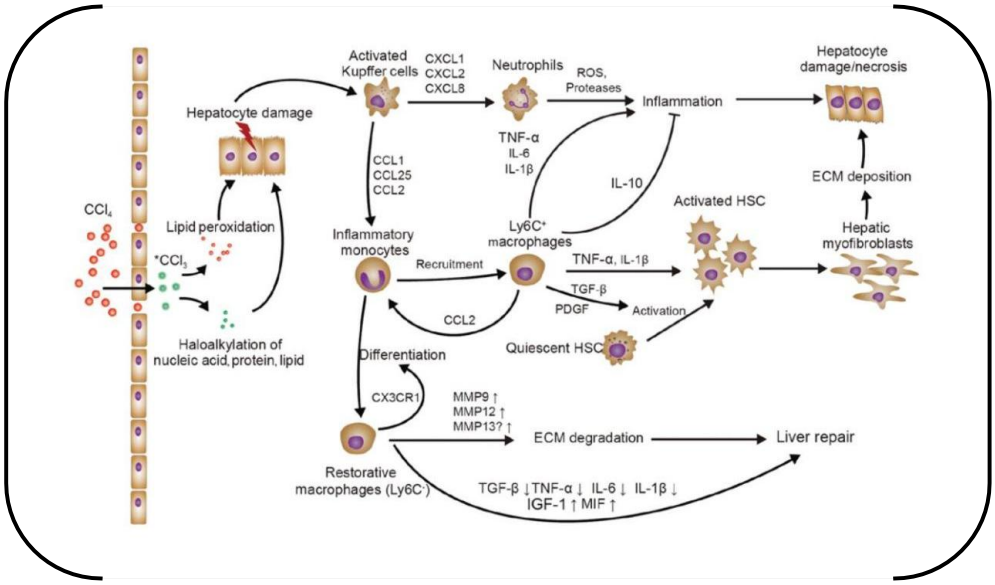

Fig. 3. Dual Roles of Ly6C$^+$ and Ly6C$^-$ Macrophages in CCl_4-Induced Liver Injury and Fibrosis Progression.

3.2 Macrophage polarization, phenotypic plasticity, and their regulatory role in fibrosis

Macrophages play a dual role in the development and reversal of liver fibrosis, with their functions primarily determined by their activation state and phenotypic characteristics[6]. Traditionally, macrophages are classified into M1-type (induced by lipopolysaccharide (LPS) or IFN-γ) and M2-type (induced by IL-4, IL-10, or TGF-β) based on their activation mechanisms[35]. A common PAMP is lipopolysaccharide (LPS), which is present on the cell walls of Gram-negative bacteria. M1-type macrophages express CD80, CD86, TLR2/4, and iNOS, and secrete pro-inflammatory factors such as IL-1β, TNF-α, and IL-12, exhibiting antimicrobial activity and inducing inflammation; in contrast, M2-type macrophages express markers such as CD206 and MHC II, secreting IL-10 and TGF-β, and plays a crucial role in immune regulation, tissue repair, and fibrotic remodeling[8][35].

Although the M1/M2 polarization model provides a foundation for understanding macrophage function, numerous studies have shown that macrophages in vivo are not statically divided into two categories but exhibit continuous phenotypic changes under different microenvironmental stimuli, demonstrating high functional plasticity[8]. This plasticity enables macrophages to adjust their pro-inflammatory or anti-inflammatory characteristics according to the pathological process, thereby influencing the development of fibrosis.

Additionally, the functional heterogeneity of precursor monocytes also influences macrophage behavior in tissues[8]. In mice, Ly6Chi (CCR2hi) monocytes tend to differentiate into M1-like macrophages involved in inflammatory responses, while Ly6Clo (CX3CR1hi) monocytes lean toward M2-like phenotypes, primarily participating in repair processes. In humans, subpopulations such as $CD14^{++}CD16^-$, $CD14^{++}CD16^+$, and $CD14^+CD16^{++}$ are involved in different stages of immune responses and tissue repair[8][35].

During liver fibrosis, the different polarization states of macrophages are closely associated with their pro-fibrotic or anti-fibrotic effects. In terms of pro-fibrotic effects, Kupffer cells (KCs) perceive injury signals (such as LPS) via TLR4, activate the NLRP3 inflammasome, and further initiate signaling pathways such as TGF-β and PDGF, promoting the activation and proliferation of hepatic stellate cells (HSCs), thereby

enhancing extracellular matrix (ECM) deposition and scar formation[36]. Concurrently, KCs secrete chemokines such as CCL2 and CXCL16 to recruit monocyte-derived macrophages (MoMφs) and NKT cells, and activate the NF-κB pathway, thereby amplifying the local inflammatory response. Furthermore, macrophages can exacerbate local hypoxia by disrupting the integrity of hepatic microvasculature and depositing iron ions produced by erythrocyte phagocytosis, thereby stimulating HSC activation[37].

Conversely, during anti-fibrosis processes, macrophages can degrade excessively deposited ECM by secreting matrix metalloproteinases (MMPs) such as MMP-1, MMP-9, and MMP-13, thereby promoting fibrosis regression. Among these, MMP-9 has been shown to play a key role in antifibrosis, and its deficiency significantly impairs fibrotic degradation capacity[8]. Additionally, as the inflammatory environment subsides, macrophages tend to polarize from M1 to M2, reducing the release of ROS and pro-inflammatory factors, thereby alleviating liver tissue damage[8]. They also protect hepatocyte function by clearing apoptotic cells and activating autophagy signaling pathways to inhibit the sustained expression of inflammatory factors such as IL-1β[38]. Notably, M1-type macrophages can also induce HSC apoptosis through TRAIL signaling, further inhibiting fibrosis progression[8].

3.3 The recruitment effect of macrophages on hepatic stellate cells (HSCs)

Following liver injury, Kupffer cells (KCs) and infiltrating macrophages rapidly release key chemokines, such as CCL2 and CCL5, promoting the aggregation of large numbers of monocytes and hepatic stellate cells (HSCs) at the site of injury[39]. First, CCL2 secreted by macrophages directly promotes HSC activation by binding to the CCR2 receptor on the HSC surface[40]. Concurrently, monocytes entering the liver release inflammatory mediators, further driving the transformation of HSCs into a myofibroblast-like phenotype and exacerbating the fibrotic process[41].

Furthermore, CCL5 exerts its effects through two receptors, CCR1 and CCR5, but CCR5 plays a dominant role in regulating HSC migration and activation. Studies have shown that after binding to CCR5, CCL5 can promote HSC phosphorylation and activation by activating the ERK signaling pathway, thereby influencing the extent of fibrosis development[42-43]. Notably, activated HSCs can also secrete CCL2 and CCL5, forming a positive feedback loop that continuously recruits more unactivated HSCs and promotes the expansion of fibrotic areas[44].

Therefore, intervening in these two key signaling pathways—CCL2/CCR2 and CCL5/CCR5—especially by using CCR2/CCR5 dual inhibitors, can effectively reduce the accumulation of monocytes and HSCs in the liver, lower HSC activation levels, and inhibit the expression of type I collagen and TIMP family proteins[45-46]. This intervention strategy demonstrates significant anti-fibrotic effects in in vivo experiments, providing potential targets for the treatment of liver fibrosis.

3.4 Macrophage involvement in HSC activation and fibrosis regulation

The activation of hepatic stellate cells (HSCs) is a core component of liver fibrosis development. During the fibrotic process, macrophages release various pro-fibrotic factors, including TGF-β, Galectin-3, and IL-6, which can directly induce HSCs to transition from a quiescent state to a myofibroblast-like phenotype, significantly enhancing their ability to synthesize collagen and other extracellular matrix (ECM) components, thereby promoting scar tissue formation and accelerating fibrotic progression[39].

However, macrophages exhibit bidirectional regulatory effects at different stages. After the removal of liver injury factors, the originally recruited Ly6Chi inflammatory monocyte-

macrophages (MoMø) can gradually transition to the Ly6Clo "repair-type" phenotype[47]. These cells can upregulate matrix metalloproteinases such as MMP-9, MMP-12, and MMP-13 to degrade excessively deposited ECM, while secreting anti-inflammatory factors such as IGF-1 and CX3CR1 to promote tissue repair and reversal of fibrosis[48]. Concurrently, resident Kupffer cells (KCs) and newly generated monocyte-derived macrophages in the liver can also produce immunoregulatory factors such as IL-10 and TGF-β1, synergistically completing liver tissue remodeling[32].

In different experimental liver injury models, macrophages exhibit complex and context-dependent functions. For example, in the bile duct ligation (BDL) model, the high inflammatory state induced by bile acids and LPS prompts KCs to secrete large amounts of TNF-α and IL-6, which although exacerbate the inflammatory response, still contribute to the resolution of fibrosis through antibacterial clearance and MMP3/8/9 expression[49]. In the thioacetamide (TAA) model, KCs induce inflammation through the HMGB1-TLR4 pathway while also promoting liver tissue regeneration through NO release, demonstrating their dual role[50]. In the acetaminophen (APAP) overdose model, KCs, Ly6C^{hi}, and Ly6C^{lo} MoMø macrophages sequentially dominate neutrophil apoptosis, hepatocyte proliferation, and ECM remodeling, presenting a dynamic and orderly synergistic process[32].

In the progression of non-alcoholic fatty liver disease (NAFLD), obesity-associated M1-polarized KCs downregulate PPARγ expression via miR-155, induce IL-1β production, inhibit fat oxidation, and lead to lipid droplet accumulation in the liver. Subsequently, the secreted MCP-1 drives the recruitment of Ly6Chi macrophages, which further release TNF-α and IL-1β, promoting HSC activation and triggering fibrosis[51]. Conversely, M2-polarized KCs can induce M1 macrophage apoptosis by secreting IL-10, thereby alleviating liver inflammation and fibrosis[52].

The key role of macrophages in regulating liver fibrosis is particularly evident in their plasticity-driven phenotypic switching mechanism. Studies have shown that during disease progression, a large number of Ly6Chi inflammatory macrophages accumulate in the liver, but after the removal of pathogenic factors, these cells can switch to the Ly6Clo phenotype, promoting collagen degradation and the clearance of scar tissue[27]. Therefore, targeting and regulating this conversion process, such as using CCR2 antagonists to block the recruitment of Ly6Chi macrophages, has emerged as a promising anti-fibrotic therapeutic strategy.

4 Hepatic Stellate Cells (HSCs)

4.1 Roles of Hepatic Stellate Cells (HSCs) in fibrosis

Hepatic stellate cells (HSCs) reside within the space of Disse, situated between hepatic sinusoids and hepatocytes, and constitute roughly 5–15% of the liver's endogenous cell population[8-9]. Under physiological conditions, quiescent HSCs exhibit a spindle-shaped or polygonal morphology, are rich in vitamin A droplets, and primarily function to maintain matrix homeostasis, regulate sinusoidal blood flow, participate in nutrient metabolism, and promote liver regeneration and microcirculatory stability by secreting hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF)[8-9]. Additionally, quiescent HSCs maintain dynamic equilibrium in the liver matrix by synthesizing small amounts of type I and III collagen and laminin, and secreting matrix metalloproteinases (MMPs) and their inhibitors (TIMPs)[8-9]

When the liver is damaged (e.g., by viral infection, alcohol, or fatty toxicity), HSCs are activated from their quiescent state and undergo a three-stage process of "activation–persistence–regression"[53]. During this process, HSCs lose vitamin A, and the expression of GFAP and PPAR decreases, acquiring a myofibroblast phenotype characterized by the

expression of α-smooth muscle actin (α-SMA) and type I collagen, and secreting fibrogenic factors such as transforming growth factor-β (TGF-β)[53-543]. Activated HSCs migrate to the injury site, synthesize and deposit large amounts of extracellular matrix (ECM), forming scar tissue that provides short-term protection but promotes liver fibrosis over the long term[53]. HSC activation is regulated by multiple cytokines, with TGF-β acting as a key driver through the Smad2/3 signaling pathway; IL-6, IL-17, and others enhance COL1A1 transcription via the JAK/STAT3 pathway, while other liver cells (such as Kupffer cells and sinusoidal endothelial cells) can also secrete PDGF, TNF-α, and other factors to stimulate HSC activation[25]. Activated HSCs also release pro-inflammatory and chemotactic factors, recruiting immune cells and forming a pro-fibrotic positive feedback network. Their sustained activation is also associated with metabolic reprogramming, such as enhanced glycolysis[55-56]. Single-cell RNA sequencing reveals that activated HSCs exhibit functional heterogeneity, with some subpopulations primarily involved in ECM synthesis and others in immune regulation[56]. Even in fibrotic liver tissue, a portion of HSCs remain in a quiescent or low-activated state, secreting anti-inflammatory and regenerative factors, and together with highly activated HSCs, they balance the fibrotic process; under specific conditions, some activated HSCs can revert to a quiescent state, providing a theoretical basis for fibrotic therapy[56]. Genetic marker experiments indicate that endogenous HSCs do not transdifferentiate into hepatocytes during liver injury, with their core functions lying in phenotypic conversion, ECM synthesis, and interaction with the immune system[25]. Therefore, hepatic stellate cells are central effector cells in the progression of liver fibrosis, and their activation is a complex process coordinated by multiple factors and pathways[57].

4.2 Activation Mechanisms of Hepatic Stellate Cells (HSCs)

Under conditions of liver injury or chronic inflammation, signals such as reactive oxygen species (ROS), TGF-β, platelet-derived growth factor (PDGF), and interleukin-1β (IL-1β) can rapidly activate quiescent HSCs, transforming them into activated forms with myofibroblastic characteristics[9]. This transformation is accompanied by vitamin A loss, upregulation of α-SMA expression, and extensive synthesis of type I and III collagen and ECM components, making HSCs the primary collagen source for fibrosis[9]. Concurrently, HSCs inhibit MMP activity by upregulating TIMP-1, reduce ECM degradation, and form a positive feedback loop by secreting TGF-β1, thereby continuously amplifying the fibrosis signal[9].

HSC activation is not only regulated by soluble factors but also mediated by direct cell-cell contact (e.g., with hepatic macrophages) and PAMPs/DAMPs[56]. Single-cell RNA sequencing reveals that HSCs express multiple autoreactive receptor-ligand pairs, maintaining their activated state even in advanced fibrosis of diseases like NASH[56]. TGF-β is a central regulatory factor, released by damaged hepatocytes, immune cells, and macrophages (especially after phagocytosis of cell debris); it drives the transformation of HSCs into a pro-fibrotic phenotype by promoting the expression of α-SMA, type I collagen, and connective tissue growth factor (CTGF). Its latent form can be activated by HSCs' αV integrin, and blocking this integrin can reduce fibrosis[56]. Additionally, PDGF promotes HSC proliferation and migration via the PDGFRβ pathway, while VEGF, in addition to promoting angiogenesis, can enhance TGF-β expression and exacerbate ECM accumulation[58-59]. In NAFLD or NASH, lipid accumulation within hepatocytes can stimulate the release of hedgehog proteins and extracellular vesicles, promoting HSC proliferation and scar formation[60-61]. When lipid-overloaded hepatocytes are phagocytosed by macrophages, inflammasomes are activated and release inflammatory factors and TGF-β,

exacerbating fibrosis; bioactive lipids such as lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) have also been shown to stimulate HSC activation[56].

4.3 Cytokine networks and intercellular interaction mechanisms of HSCs in liver fibrosis

Upon activation, HSCs not only undergo phenotypic transformation but also secrete various cytokines, such as TGF-β, IL-10, and PDGF. These factors not only maintain HSC activation but also promote liver fibrosis through interactions with other cells[9][27]. HSCs express multiple chemokine receptors and release chemokines such as CCL2, recruiting immune cells such as monocytes and macrophages to the liver, thereby exacerbating local inflammation[9][27]. HSCs also secrete TIMP-1, which inhibits MMP activity to prevent ECM degradation and suppresses HSC apoptosis, aiding their survival in the fibrotic microenvironment[9]. Additionally, collagen cross-linking enzymes such as LOXL2 produced by activated HSCs promote collagen deposition and scar stabilization, accelerating fibrosis progression[62].

Fibrosis-related cytokines can be broadly categorized into pro-fibrotic stimulatory factors and anti-fibrotic inhibitory factors, with the former being key drivers of liver fibrosis[22]. The first category consists of direct-acting factors, such as TGF-β (a key driver), PDGF, IGF-1, EGF, and FGF, which bind to HSC surface receptors, activate downstream signaling pathways, induce ECM gene transcription, or promote HSC proliferation and phenotypic transformation[22]. The second category includes indirect factors such as TNF-α, IL-4, IL-6, IL-8, and platelet-activating factor, which primarily promote HSC activation indirectly by enhancing inflammatory responses or activating immune cells[22]. The balance between these two categories of cytokines influences the fate of HSCs, and regulating their expression and function is a key strategy for intervening in liver fibrosis[22].

In the progression of liver fibrosis, HSCs establish a close interactive network with liver sinusoidal endothelial cells (LSECs) and hepatic macrophages (Kupffer cells), jointly shaping a pro-fibrotic microenvironment. Under normal physiological conditions, LSECs maintain HSC quiescence by secreting nitric oxide (NO)[27]. During liver injury, LSECs undergo capillaryization, leading to reduced NO secretion and weakened inhibition of HSCs, thereby promoting their activation[27]. Simultaneously, activated Kupffer cells produce a range of inflammatory and fibrogenic cytokines—such as TGF-β1, PDGF, and IL-1β—which directly stimulate hepatic stellate cell activation and proliferation, enhance ECM deposition, and thereby promote both inflammation and fibrotic progression[27].

The interaction between HSCs and hepatic macrophages is a key driver of liver fibrosis. In the early stages of fibrosis, pro-fibrotic macrophages attract circulating monocytes, inflammatory cells, and quiescent HSCs to the damaged site by secreting chemokines, and release cytokines to directly activate HSCs and promote their proliferation[39]. Subsequently, chemokines secreted by activated HSCs further recruit more macrophages and monocytes, and influence the differentiation and polarization of monocytes into macrophages, forming a positive feedback loop that accelerates fibrosis[39].

4.4 The Dual Role of HSCs: Fibrosis and Regeneration

Although HSCs have become an important target for anti-fibrosis therapy due to their key role in liver fibrosis, recent studies have shown that they also play a positive role in liver injury repair and tissue regeneration, exhibiting complex dual functions[9]. In liver regeneration, activated HSCs secrete a series of bioactive molecules that promote repair through multiple mechanisms, including PDGF and FGF (which activate the fibrinolytic system and release HGF to promote hepatocyte proliferation), TGF-β (early remodeling of

the extracellular matrix, later inhibiting proliferation), VEGF, and sphingolipidase (NE) (involved in angiogenesis and permeability regulation), among other bioactive molecules that promote repair, thereby providing a supportive microenvironment for tissue repair[53]. HSCs not only promote hepatocyte proliferation through factor secretion but also attract stem cell migration and may even induce mesenchymal cells to differentiate into hepatocytes[9].

This bidirectional regulatory role suggests that anti-fibrotic strategies must balance inhibiting their fibrotic activity with preserving their beneficial regenerative effects[9]. At the end of liver regeneration, some activated HSCs return to a quiescent-like state through apoptosis or "deactivation," maintaining tissue homeostasis; these 'deactivated' HSCs retain some activated "memory," but their fibrotic activity is effectively suppressed under normal conditions[53]. Hepatocytes gradually exit the cell cycle under the regulation of integrin and integrin-linked kinase (ILK) signaling. If this regulatory balance is disrupted, it may lead to persistent liver fibrosis or abnormal regeneration[53]. In the reversal of liver fibrosis, the apoptosis or reversion of activated HSCs to a "deactivated" state (regulated by transcription factors such as PPARγ, GATA4/6, and TCF21) is crucial for reducing collagen production[63]. Additionally, γδ T cells, $CD8^+$ T cells, and NK cells can eliminate activated HSCs through FasL-Fas-mediated mechanisms, promoting the reversal of fibrotic tissue[63]. In chronic liver injury, various cytokine-mediated intercellular and cell-matrix interactions activate HSCs and induce excessive ECM synthesis, leading to liver fibrosis[22]. Given the central role of HSCs, most current anti-fibrotic studies target them as the primary therapeutic target, employing strategies such as inhibiting inflammation, alleviating oxidative stress, and regulating key cytokines and their signaling pathways to block HSC activation and proliferation or induce their apoptosis[22]

5 Key molecules and signaling pathways

The initiation and advancement of liver fibrosis represent a multifaceted pathological process characterized by the intricate interplay and regulation of numerous signaling pathways and cytokines. Among these, the TGF-β/Smad pathway is widely recognized as the most central pro-fibrotic mechanism[64]. TGF-β primarily originates from macrophages and hepatic stellate cells (HSCs). Upon binding to the TGF-β receptor, it can activate Smad-dependent and Smad-independent signaling pathways, guiding HSC to transdifferentiate into myofibroblast phenotypes and upregulating the expression of genes such as type I and type III collagen, thereby promoting the massive deposition of extracellular matrix (ECM)[64].

In addition to TGF-β, the PDGF signaling pathway also plays a key role in HSC activation and proliferation. PDGF-BB, secreted by platelets and liver sinusoidal endothelial cells (LSECs), activates downstream pathways such as MAPK and PI3K/Akt, significantly enhancing HSC proliferation capacity and fibrotic potential[65].

Meanwhile, inflammatory factors such as TNF-α, IL-6, and IL-1β are primarily produced by Kupffer cells (KCs) and the mononuclear-macrophage system, maintaining a chronic inflammatory state through pathways such as NF-κB. These factors directly induce HSC collagen synthesis while also promoting immune cell activation and chemotaxis, forming a sustained inflammation-fibrosis positive feedback loop[66].

In the context of chronic liver injury, oxidative stress also plays an important role. Reactive oxygen species (ROS) such as superoxide anions can directly damage mitochondria and DNA, induce hepatocyte apoptosis and the release of damage-associated molecular patterns (DAMPs), and further activate inflammatory pathways such as NF-κB, thereby indirectly promoting HSC activation and the accumulation of fibrotic matrix[67].

The NLRP3 inflammasome is an important PAMP/DAMP receptor. Upon activation in KCs, HSCs, and hepatocytes, it initiates IL-1β and IL-18 maturation and secretion mediated by Caspase-1, and amplifies local inflammatory responses through GSDMD-mediated pyroptosis[68]. This process not only elevates inflammatory levels but also enhances TGF-β expression, converting HSCs into an activated state[68].

Additionally, chemokines such as CCL2 and CCL5 recruit monocytes to liver tissue via their receptors CCR2/CCR5, further releasing inflammatory factors and pro-fibrotic molecules, thereby reinforcing the fibrotic process[41]. Studies indicate that blocking the CCL2-CCR2 pathway significantly reduces macrophage infiltration and alleviates fibrosis severity, suggesting this pathway as a potential therapeutic target[69].

In the regulatory network, Galectin-3 is another important pro-fibrotic factor secreted by macrophages, with significantly elevated expression in diseases such as non-alcoholic fatty liver disease (NAFLD/NASH)[70]. Galectin-3 not only induces hepatic stellate cell (HSC) activation but also enhances TGF-β signaling. Studies have shown that Galectin-3 and β1 integrin exhibit high colocalization on the cell membrane surface (distance <40 nm), and TGF-β1 treatment further enhances this colocalization. This structure facilitates the aggregation and amplification of TGF-β signaling, thereby exacerbating the fibrotic deposition process[71].

The development of liver fibrosis involves the synergistic interaction of multiple signaling pathways and molecules. These pro-inflammatory and pro-fibrotic mechanisms are intertwined, collectively driving HSC activation, ECM accumulation, and sustained inflammation, thereby forming the molecular basis for liver fibrosis development and providing multi-level target support for future intervention strategies.

6 Treatment Progress and Strategies

The treatment of liver fibrosis is becoming increasingly diverse, encompassing etiological control, anti-fibrotic interventions, and cutting-edge technologies such as cellular, immunological, and genetic therapies, gradually moving toward personalized and precision medicine

The core of treatment lies in controlling the underlying causes. For viral hepatitis, antiviral therapy (such as DAAs and nucleoside analogues) can significantly improve liver function and delay fibrosis[72]. NAFLD/NASH relies on lifestyle interventions, with weight loss of 7–10% effectively improving inflammation and fibrosis, and GLP-1 receptor agonists such as liraglutide also showing potential benefits[72-73]. For alcoholic liver disease, abstinence remains the key measure, supplemented by nutritional support and management of complications[7]. Research on direct antifibrotic drugs focuses on key pathways, such as the CCR2/CCR5 antagonist cenicriviroc, which shows potential in slowing inflammation and fibrosis[74]. Inhibitors of pathways such as TGF-β and PDGF are also under investigation, but their efficacy remains uncertain[75-76]. Some candidate drugs, such as Simtuzumab and Emricasan, have unclear efficacy, and research interest has waned[77]. Additionally, factors with hepatoprotective effects, such as IL-22, have also drawn attention[78].

In terms of cell therapy, mesenchymal stem cells (MSCs) can improve liver function through anti-inflammatory and tissue repair mechanisms. Preliminary trial results are positive, but further validation is needed[79]. Engineered cells such as CAR-M and FAP-targeted CAR-T have demonstrated potential in animal models to eliminate pathogenic cells, offering new directions for future treatment[79-80]. The regulation of the gut microbiome and immune system is also being considered in treatment strategies. Probiotics and fecal microbiota transplantation can improve the inflammatory state of the “gut-liver axis” and slow the progression of NAFLD and fibrosis[81]. Immunomodulatory interventions such as

NF-κB and JAK/STAT inhibitors are also used to reduce inflammatory responses[82–84], while multi-target drugs like CCR2/CCR5 inhibitors aim to simultaneously intervene in multiple pathways[85]. In terms of emerging technologies, gene intervention tools such as siRNA and CRISPR/Cas9 can target and inhibit pro-fibrotic genes, demonstrating theoretical potential, but face challenges such as off-target effects and delivery[86-87]. Meanwhile, liver organoid and chip models provide advanced platforms for new drug screening and mechanism research[88].

7 Insights and Challenges

Liver fibrosis is a pathological response triggered by sustained or chronic hepatic injury, with its core mechanism lying in the interaction between damaged hepatocytes, inflammatory cells, and hepatic stellate cells (or myofibroblasts)[89]. This persistent inflammatory and reparative response leads to abnormal deposition of extracellular matrix (ECM), ultimately resulting in liver structural and functional impairment. Therefore, the primary goal of antifibrotic therapy is to eliminate or control the underlying causes of chronic liver injury[89].

Although early studies generally considered liver fibrosis to be irreversible, recent clinical liver biopsy studies have shown that liver fibrosis has a certain degree of reversibility after the underlying cause is effectively removed[24]. For example, patients with secondary biliary fibrosis, hepatitis B and C, non-alcoholic steatohepatitis (NASH), and autoimmune hepatitis experienced significant reduction in fibrosis severity after the underlying cause was controlled[24]. Clinical observations have also shown that abstaining from alcohol can improve fibrosis associated with alcoholic liver disease, while weight-loss surgery or lifestyle interventions that alleviate insulin resistance and metabolic syndrome can help some NASH patients achieve fibrosis reversal[90]. Animal experiments have similarly confirmed that removing pathogenic factors such as CCl_4, alcohol, and bile duct obstruction can induce fibrosis resolution[91].

The reversal of liver fibrosis is accompanied by a series of molecular and cellular changes. With the removal of pathogenic factors, levels of pro-inflammatory factors (such as IL-6, IL-1, and TNF-α) and TGF-β decrease, the number of activated hepatic stellate cells or myofibroblasts significantly reduces, and ECM synthesis ceases[26]. Concurrently, the expression of matrix metalloproteinases (MMPs) is upregulated relative to their inhibitory factors TIMPs, promoting the degradation of deposited collagen fibers. These mechanisms collectively drive structural repair and functional recovery of liver tissue[26].

Currently, treatment strategies for liver fibrosis are evolving toward a more diversified approach. Building on the control of underlying causes, researchers are actively exploring emerging modalities such as direct antifibrotic drugs, cell therapy, and gene therapy. However, no specific antifibrotic drugs have yet been approved. Therefore, mechanism-based clinical research and personalized combination therapy models are considered key directions for future breakthroughs[92].

Furthermore, liver fibrosis constitutes a multifaceted pathological process that engages various hepatic cell types as well as extrahepatic organs[93]. Besides hepatic stellate cells and macrophages, hepatocytes, sinusoidal endothelial cells, and infiltrating immune cells also play roles in fibrosis progression and reversal[94]. Recent studies have also revealed that the onset and resolution of liver fibrosis are influenced by distant organs such as the gut, skeletal muscle, and adipose tissue[26]. Additionally, epigenetic regulation is believed to be involved in the regulatory process of fibrosis[26].

However, the mechanisms underlying the reversibility of liver fibrosis remain largely unknown. For example, the regulatory mechanisms governing the inactivation of myofibroblasts are unclear, the phenotypic transformation of macrophages remains difficult

to precisely manipulate in vivo, and the complex interactions between different cell types and signaling pathways have not yet been fully elucidated[26]. Future research that can deeply elucidate the synergistic mechanisms among various cell types from different organ sources, as well as the molecular basis for reversing liver fibrosis, may provide theoretical support for developing more effective personalized anti-fibrosis treatment strategies and ultimately improve patient outcomes.

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