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PATHOGENIC AND THERAPEUTIC ROLES OF MESENCHYMAL STEM CELLS IN LIVER FIBROSIS

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The aim of this study was to conduct a comparative analysis of the bioregulatory role of mesenchymal stem cells (MSCs) in the liver under physiological conditions, in acute and chronic injury with fibrotic remodeling, and during therapeutic correction by implanting exogenous MSCs from healthy tissues into the body. The analysis showed that hepatic MSCs maintain structural homeostasis by interacting with tissue myofibroblasts and migrating immune cells. In acute liver injury that does not deplete adaptive reserves, hepatic (resident) MSCs regulate tissue homeostasis. Chronic injury that depletes adaptive reserves activates both immune cells and hepatic MSCs, leading to liver inflammation and the transdifferentiation of MSCs into myofibroblasts. These activated fibroblasts overproduce extracellular matrix components, thereby driving liver fibrosis progression. Exogenous apoptotic MSCs from healthy auto- or allogeneic tissues, when administered in cases of chronic liver injury, can compensate for deficient regulatory factors and restore metabolic regulation and structural homeostasis through their paracrine and trophic activity. Their therapeutic potential is maximized when their regulatory properties are enhanced prior to administration and when applied in recipients without irreversible liver injury.

Keywords: mesenchymal stem cells, chronic liver injury, liver fibrosis, regenerative medicine, cell therapy, cell-engineered constructs.

INTRODUCTION

The progression of chronic liver disease (CLD) and fibrosis or cirrhosis result from profound impairment of the liver's restorative regenerative capacity, creating conditions for persistent inflammation and ongoing tissue destruction. At present, liver transplantation remains the only effective treatment for irreversible liver damage in patients with CLD [1, 2]. However, the growing shortage of donor organs, coupled with the rising number of patients requiring transplantation, significantly limits access to this life-saving procedure.

Given these constraints, and in light of the limited efficacy of currently available antifibrotic therapies, there is a pressing need to explore alternative, more accessible and physiologically based treatment strategies that can enhance the liver's intrinsic regenerative potential. The use of mesenchymal stem cells (MSCs), derived from autologous or allogeneic human tissues, has emerged as a promising therapeutic approach.

By now, a considerable body of experimental and clinical evidence has demonstrated the beneficial effects of tissue-derived MSCs on liver structure and function in chronic fibrosing injury [3–5]. Several studies have even suggested the potential for regression of established fibrosis following MSC implantation. However, the fibrolytic properties of MSCs remain a subject of debate. Some researchers question these effects and, in contrast, report the possibility of enhanced fibrosis under certain conditions of MSC therapy [6, 7].

Such conflicting outcomes are likely related to insufficient consideration of several critical factors influencing therapeutic efficacy. These include the source of MSCs, the administered dose and frequency of administration, and the intrinsic bioregulatory potential of the cells – whether derived from healthy allogeneic donors or from patients with comorbidities such as chronic renal failure. Particularly important is the degree of reversibility of pre-existing structural (fibrotic) changes in the liver, which are thought to reflect the severity of the accompanying immune imbalance and the progression of immune deficiency, up to the stage of immune paralysis [8].

The inconsistency in outcomes reported for MSC-based therapy in fibrosing liver diseases, together with the need to enhance the therapeutic effectiveness of MSCs in the setting of progressive hepatic injury, prompted us to undertake a comparative assessment. Specifically, we aimed to evaluate the role of liver-resident MSCs in maintaining structural homeostasis during fibrosing in-

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jury and to investigate the therapeutic potential of MSCs derived from healthy tissues for correcting established structural disorders in the liver.

The objective of this study is to perform a comparative analysis of the bioregulatory role of resident liver MSCs in maintaining tissue homeostasis under damaging influences and during progression of destructive fibrotic processes. In addition, the study evaluates the corrective potential of exogenous MSCs derived from healthy tissues, with the aim of identifying factors that may enhance the efficacy of MSC-based antifibrotic therapy.

BIOLOGICAL PROPERTIES OF MSCS IN BODY TISSUES

MSCs are multipotent cells of mesodermal origin with properties characteristic of both stem and progenitor cells. They possess self-renewal capacity and can differentiate into mesodermal derivatives such as chondrocytes, osteoblasts, adipocytes, and skeletal muscle cells. Under specific culture conditions, MSCs may also differentiate into cells of ectodermal and endodermal lineages, including hepatocyte-like cells [5]. Currently, these cells have been described in detail, and the classical characteristic of MSCs is their phenotype [5]. Human MSCs express positive surface markers CD105, CD90, and CD73, while lacking hematopoietic and endothelial markers such as CD45, CD34, CD14, CD19, and HLA-DR. In mice, MSCs are characterized by positive expression of CD105, CD29, CD44, and stem cell antigen-1 (SCA-1), with negative expression of CD45, CD31, and lymphocyte antigen-76 (Ly76). Their multipotent (stem-like) properties are typically confirmed through differentiation into three main lineages: adipocytes, chondrocytes, and osteoblasts.

A unique feature of MSCs is their expression of MHC class I molecules and absence of MHC class II, B7-1, B7-2, CD40, and CD40L. This immunophenotype allows them to evade direct participation in immune responses and to exert immunosuppressive effects.

MSCs are present in virtually all body tissues, with particularly high abundance in mesoderm-derived tissues [9]. They can be efficiently harvested from bone marrow, adipose tissue, placenta, umbilical cord-derived Wharton's jelly, skeletal muscle, and skin, as well as from umbilical cord blood, amniotic fluid, and menstrual blood [5]. MSCs actively proliferate during cultivation, and their cell mass can be increased more than 100-fold without losing their multipotent differentiation potential [9].

The widespread presence of MSCs in various organs and tissues highlights their fundamental, non-specific role in maintaining structural and functional homeostasis, regulating adaptive and compensatory responses, and promoting both physiological and reparative regeneration [10]. These functions are mediated through direct interactions with neighboring cells in their microenvironment – primarily mesenchymal cells within the tissue and circulating immune cells [5].

In addition to direct cell-to-cell contact, MSCs exert regulatory effects via autocrine and paracrine signaling [11]. Through these mechanisms, they influence evolutionarily conserved pathways of programmed cell death and modulate the activity of key metabolic processes [4, 10–13]. Furthermore, MSCs demonstrate immune-evasive properties, including the ability to avoid innate immune recognition, counteract complement activation [14], and develop immunosuppressive activity in the presence of proinflammatory cytokines [15].

Importantly, MSC behavior is context-dependent. Their immunomodulatory effects vary according to the cytokine microenvironment and the residual adaptive and regulatory reserves of the tissue. Under these conditions, MSCs can exhibit both anti-inflammatory and proinflammatory effects, influencing the activity of innate and adaptive immune cells [9].

Thus, during tissue injury, the outcome of resident MSC activity depends on multiple factors, including the cytokine environment, the extent of tissue reserves, the diversity and coordination of surrounding cell types, and the severity and duration of inflammation. These variables may drive MSCs toward opposing outcomes in their interactions with mesenchymal cells – particularly fibroblasts and myofibroblasts (MFs). In their activated state, MFs are major producers of extracellular matrix (ECM) components.

The interaction between MSCs and MFs during acute exposure to a damaging factor supports tissue homeostasis and restorative regeneration – without fibrotic scarring – provided that the strength and duration of the effect of this factor on the tissue do not exceed the adaptive, compensatory, and regulatory reserves of the tissue. Under these conditions, MSCs contribute to maintaining balance by directly suppressing MF proliferation and the differentiation of other cells into MFs. They also induce expression of pro-apoptotic proteins in MFs [16] and attenuate their activation by inhibiting nuclear factor kappa B (NF-κB) signaling [17], thereby preventing initiation of a sustained inflammatory response.

In contrast, when stress-damaging effects are chronic, recurrent, or of high intensity, surpassing the tissue's evolutionary reserves of adaptation and regulation, the outcome shifts toward progressive fibrosis. In such settings, excessive and repeated tissue injury leads to necrosis and parenchymal dysfunction, often accompanied by apoptotic death of parenchymal cells due to prolonged functional overload. The release of intracellular products from necrotic and apoptotic cells – frequently carrying altered genetic and structural properties – triggers immune activation and recruits innate immune cells to the injury site, thereby sustaining chronic inflammation there.

At the same time, differentiation of resident MSCs (as stem/progenitor cells) into MFs is activated and the

activation of MFs (activated fibroblasts) increases uncontrollably, which is manifested by excessive production of extracellular matrix components and development of tissue fibrosis [9].

THE ROLE OF RESIDENT (LIVER) MSCS IN PREVENTING INFLAMMATION ESCALATION AND MAINTAINING LIVER TISSUE HOMEOSTASIS DURING ACUTE INJURY

In the acute phase of liver injury, when the adaptive and metabolic regulatory reserves of hepatocytes remain preserved, damage-associated molecules released from necrotic and apoptotic hepatocytes – including reactive oxygen species (ROS) and lipid peroxidation products – initiate an acute inflammatory response. These signals promote the recruitment of innate immune cells to the injury site through secretion of chemokines such as CCL-2, CCL-5, CXCL-1, and CXCL-15.

Initiation of the inflammatory cascade is not limited to hepatocytes alone. Other resident liver cell types also contribute significantly, most notably hepatic stellate cells (HSCs) and Kupffer cells (liver-resident macrophages), which enhance chemokine production [9].

It is believed that liver-resident MSCs also contribute to regulation of inflammatory responses. Bone marrow–derived MSCs, for example, have been shown to produce chemokines in response to danger signals such as circulating Toll-like receptor ligands. However, when proinflammatory signals arise at a stage when hepatic adaptive and metabolic reserves are not yet exhausted, MSCs exhibit strong immunosuppressive activity [15], thereby preventing escalation of acute inflammation.

Experimental data demonstrate that stimulation of MSCs with proinflammatory cytokines – including IFN-γ in combination with IL-1β or TNF-α – induces robust production of immunosuppressive molecules such as nitric oxide (NO), indoleamine 2,3-dioxygenase (IDO), prostaglandin E2 (PGE2), and transforming growth factor-β (TGF-β) [9]. These mediators suppress proinflammatory T-cell proliferation and promote the induction of regulatory, anti-inflammatory cell populations. Locally accumulated immunosuppressive factors around activated MSCs form specialized niches within the liver tissue, reshaping the immune microenvironment.

In particular, it has been shown that resident MSCs promote apoptosis of Th1 and Th2 cells, inhibit Th17 differentiation, and enhance regulatory T-cell (Treg) accumulation through high expression of iNOS, IDO, tumor necrosis factor–stimulated gene-6 (TSG-6), and matrix metalloproteinases (MMPs) [9]. These findings align with observations of increased Treg numbers and reduced Th17 infiltration in fibrotic liver tissue following transfusion of intact donor MSCs [18, 19].

Besides T cells, macrophages represent another key component of hepatic immune homeostasis. MSCs can reprogram macrophages toward an anti-inflammatory phenotype during monocyte-to-macrophage differentiation by secreting insulin-like growth factor-2 (IGF-2) [20]. Even under proinflammatory conditions, IGF-2–conditioned macrophages shift toward oxidative phosphorylation and upregulate programmed death ligand-1 (PD-1), acquiring immunosuppressive properties [20]. Interestingly, IGF-2 exerts a dose-dependent effect: at low concentrations, it binds the IGF-2 receptor on monocytes, driving anti-inflammatory macrophage differentiation; at high concentrations, it binds the insulin-like growth factor-1 (IGF-1) receptor, resulting in proinflammatory macrophages [21].

Taken together, recent evidence indicates that MSCs, when activated by acute inflammatory signals, play a pivotal role in restraining excessive immune responses and preserving liver homeostasis during injury. Proinflammatory macrophages, reprogrammed into anti-inflammatory phenotypes by MSC-derived IGF-2, act as additional regulators of tissue stability. By inhibiting early-stage inflammatory cascades, MSCs not only limit immune cell and cytokine-driven damage but also prevent activation of cells (PSCs, MSCs, and liver fibroblasts) capable of differentiating into macrophages, thereby mitigating fibrosis development.

THE ROLE OF RESIDENT (LIVER) MSCS IN MAINTAINING CHRONIC INFLAMMATION AND DEVELOPMENT OF LIVER FIBROSIS

The transition from acute to chronic inflammation reflects not only the depletion of energy reserves but also the exhaustion of adaptive and regulatory mechanisms in liver cells. Although chronic inflammation is characterized by lower levels of inflammatory mediators, these signals remain sufficient to stimulate MSCs to secrete chemokines and NO, thereby recruiting immune cells to damaged liver tissue [22]. However, the diminished expression of immunosuppressive molecules such as inducible NO synthase (iNOS) and indoleamine 2,3-dioxygenase (IDO) – which are crucial for directing MSC-dependent immunomodulation – limits the ability of MSCs to sustain their suppressive effect. Genetic studies have shown that deletion of iNOS in murine MSCs or IDO in human MSCs reduces the immunosuppressive potential of MSCs triggered by IFN-γ and TNF-α, leading to a more immunostimulatory effect [9]. Even under reduced cytokine levels, which are insufficient for optimal induction of iNOS or IDO, MSCs continue to be activated, secreting chemokines such as CXCL-9 and CCL-5. Under these conditions, MSCs acquire immunostimulatory properties, thereby perpetuating chronic inflammation [22]. Transforming growth factor-β (TGF-β) is recognized as a central cytokine driving liver fibrosis through activation of MFs. Notably, TGF-β sustains immune-mediated inflammation by suppressing iNOS expression induced by inflammatory cytokines in a SMAD3-dependent manner [23].

Chronic liver injury results in a critical reduction of functional hepatocyte mass. This creates conditions for hyperfunction of the remaining hepatocytes, their progressive apoptosis and death, and the persistence of chronic immune-inflammatory reactions coupled with oxidative stress. The latter, characterized by increased production of reactive oxygen species (ROS) and lipid peroxidation, further activates not only immune cells but also other mesenchymal populations in the liver, including hepatic stellate cells (HSCs), resident MSCs, and liver portal fibroblasts. Under conditions of cytokine imbalance, these cells – particularly HSCs and MSCs – upregulate TGF-β, proliferate, and differentiate into MFs. These MFs are the principal producers of ECM and key drivers of fibrogenesis, a pathological process of abnormal hyperplasia of connective tissue in the liver [24, 25].

Mechanisms of involvement of resident (hepatic) MSCs in the pathogenesis of liver fibrosis

In response to liver injury, liver MFs are activated and play a central role in regulating tissue repair and maladaptive remodeling. Multiple mesenchymal cell populations contribute to the pool of hepatic MFs, including HSCs, portal fibroblasts, circulating bone marrow–derived MSCs, and resident liver MSCs [5].

In vitro studies by Mishara et al. [9] showed that under cytokine imbalance, resident MSCs can serve as a source of MF accumulation, driving ECM production and promoting excessive fibrogenesis. Specifically, exposure of MSC cultures to TGF-β – one of the principal profibrotic cytokines – induced expression of α-smooth muscle actin (α-SMA), a hallmark marker of myofibroblastic differentiation. The observed increase in α-SMA–positive MFs, together with enhanced ECM production, provides direct evidence for the transition of MSCs into MFs. These findings suggest that the ability of MSCs to undergo phenotypic transformation and adopt MF functions *in vitro* reflects similar processes occurring *in vivo* under pathological conditions.

Kramann et al. [9] identified Gli-1 as a marker of resident (tissue) MSCs in the liver. In healthy mouse livers, Gli-1^+ MSCs accounted for only 0.02% of resident cells. However, in a carbon tetrachloride–induced model of liver fibrosis, the proportion of Gli-1^+ MSCs increased sharply to 39%. Importantly, their data demonstrated that resident, rather than circulating, Gli-1^+ cells differentiate into MFs during liver injury.

Although resident MSCs contribute significantly to the MF population, the predominant source of MFs (up to 80%) and excessive ECM during fibrosis is attributed to HSCs. In their quiescent state, HSCs exhibit pericyte-like properties and store vitamin A and lipids. Upon chronic liver injury, inflammatory cytokines secreted by hepatocytes and immune cells activate HSCs, leading to upregulation of α-SMA and transition into MFs, which then drive fibrotic ECM deposition [5].

Activation of HSCs and subsequent fibrogenesis are further amplified by proinflammatory cytokines and profibrotic growth factors – including IL-6, IL-1β, TNF-α, TGF-β, and platelet-derived growth factor (PDGF) – secreted by neighboring epithelial and endothelial cells, infiltrating immune cells, and resident fibroblasts [24, 25].

The proposed mechanisms of resident (liver) MSC involvement in the pathogenesis of fibrosis are illustrated schematically in Fig. 1.

Molecular and genetic mechanisms of fibrogenesis

Multiple key molecules are involved in the process of tissue fibrosis [5]. Among these, the most critical are TGF-β, proinflammatory cytokines, integrins, transmembrane receptors, and other signaling factors.

TGF-β is considered the central driver of fibrosis. Following tissue injury, fibroblasts secrete TGF-β, which signals through two types of receptors – TβRI and TβRII – to orchestrate local inflammation, macrophage activation, and immune responses during fibrogenesis. It has also been shown that TGF-β, secreted by macrophages during liver fibrosis, synergizes with other profibrotic mediators, including PDGF and MMPs, to amplify inflammatory signaling and promote progression toward cirrhosis. These effects are mediated primarily via the canonical TGF-β1/Smad pathway as well as the non-canonical PI3K–AKT signaling cascade [5].

TGF-β signaling pathways are in turn coordinated by transcription factors such as PU.1, which activates a broad set of profibrotic genes in fibroblasts and the production of excess ECM [26–28].

Cytokines, particularly the proinflammatory interleukins IL-1 to IL-17, are recognized as important inducers of fibrogenesis, acting synergistically with TGF-β signaling pathways. Among them, IL-17, secreted primarily by $CD4^+$ T lymphocytes, has been shown to play a key role in the development of fibrosis in many tissues, including the liver [29].

In contrast, IL-13, produced by Th2 lymphocytes, promotes fibrosis independently of TGF-β, most likely through its direct stimulatory effect on collagen-producing fibroblasts. The pivotal role of these cytokines in fibrosis development is further supported by experimental findings demonstrating that mice deficient in IL-13, IL-4R, or IL-13Rβ1 show reduced tissue fibrosis following various forms of injury [5].

Integrins, being transmembrane cell receptors, mediate interactions between the ECM and the intracellular cytoskeleton and play a critical role in fibrosis by regulating cellular adhesion, migration, and signaling. Several synergistic mechanisms linking integrin-mediated signaling with TGF-β activation and ECM remodeling, leading to fibrosis activation, have been studied. TGF-β, in its in-

active state, exists as a complex with latency-associated peptide (LAP) and latent TGF-β-binding protein [30]. Certain integrins bind to this latent complex and trigger TGF-β activation. Specifically, integrin αvβ6 has been shown to activate TGF-β through binding to LAP, thereby contributing to fibrotic processes in several organs, including the liver. Furthermore, integrins α8β1 and α11β1 are markedly upregulated in HSCs, where they promote liver fibrosis through TGF-β activation [31].

Disruptions in the gut microbiome have also been shown to influence the progression of liver fibrosis [32]. In patients with CLD, bacterial translocation and the entry of microbial metabolites into the liver occur due to increased intestinal permeability. As a result of intestinal dysbiosis, the liver's innate immune system becomes activated, leading to the production of proinflammatory cytokines that stimulate HSCs and enhance ECM deposition, thereby promoting fibrogenesis [33]. Experimental studies further support this mechanism: blockade of Toll-like receptor 4 (TLR-4) in mice or suppression of intestinal microbial influence using antibiotics slows the development of liver fibrosis.

The progression of liver fibrosis is also associated with alterations in various genetic and molecular regulatory pathways. Previous studies [5] have demonstrated that hepatocyte apoptosis, an initiating event in fibrosis, is mediated by genes involved in programmed cell death, including death receptors (e.g., TRAIL, Bcl-xL, Fas), proapoptotic pathway components (such as caspase-3), and natural killer T (NKT) cells. Furthermore, it has been shown [34] that the transition from fibrosis to cirrhosis is accompanied by significant changes in the mRNA expression of several key genes (tweak, fn14, ang, vegfa, cxcl12, and mmp-9), as well as by altered interactions among them. During the late stage of fibrosis, at the point of transition to cirrhosis, the highest number of correlations between the expression levels of these target genes is still observed. However, as cirrhosis develops, these intergene connections weaken or disappear almost completely, reflecting profound molecular reorganization, metabolic dysregulation in cirrhotic tissue, and the decompensation of the regulatory functions of MSCs.

MECHANISMS OF THE THERAPEUTIC EFFECT OF EXOGENOUS MSCS ON A DAMAGED LIVER

Unlike endogenous (resident) liver MSCs, exogenous MSCs, whether autologous (derived from adipose tissue) or allogeneic/xenogeneic (isolated from healthy donor tissues) [4, 35], retain their full regulatory potential and capacity to maintain tissue homeostasis. Numerous preclinical and clinical studies have shown that the therapeutic effects of healthy exogenous MSCs in fibrosing liver diseases are mediated through activation of evolutionarily conserved mechanisms of stress adaptation and cellular survival. Following transplantation, exogenous MSCs often undergo a spontaneous transition into states of apoptosis or necrosis in response to the new microenvironment [10–13].

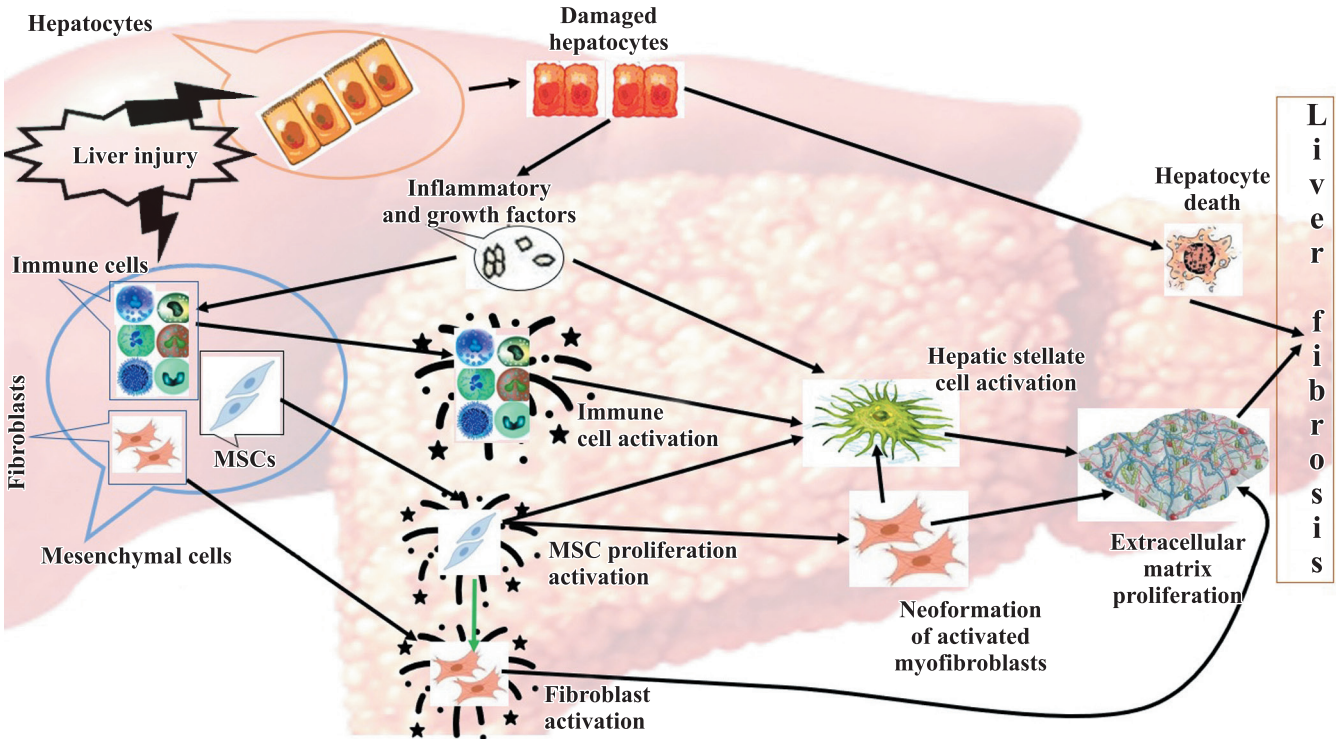

Fig. 1. Schematic representation of the role of resident (hepatic) MSCs in the pathogenesis of liver fibrosis. Arrows indicate the interactions developing between liver cells under chronic damaging effects

During apoptosis, MSCs release a broad spectrum of paracrine and trophic factors, including extracellular vesicles (exosomes, microvesicles, and apoptotic bodies), growth factors, proteases, hormones, diverse RNA species, cytokines, and chemokines [10, 36]. These secreted components exert potent regulatory effects: they protect damaged hepatocytes, suppress the activation of HSCs and MFs, promote the degradation of ECM components, and modulate immune responses, thereby attenuating inflammatory activity within the liver.

A recent meta-analysis evaluating the mechanisms and efficacy of MSC-based therapy in preclinical models of liver fibrosis confirmed that MSCs derived from various tissue sources significantly improve hepatic function, reduce HSC activation through inhibition of the profibrogenic factor TGF-β, and significantly reduce the extent of fibrotic tissue [37]. Notably, the antifibrotic effects were most pronounced when MSCs derived from bone marrow-derived MSCs were used, compared with those obtained from other sources.

Protection of damaged hepatocytes

The pathway by which exogenous MSCs participate in liver regeneration is directly linked to their production of paracrine factors during apoptosis and necrobiosis [38]. Both conditioned medium derived from MSC cultures and MSCs themselves, used alone or in combination with growth factors such as VEGF, have been shown to effectively suppress hepatocyte death and stimulate hepatic regeneration [39, 40].

Exosomes isolated from MSCs can inhibit ferroptosis, a specific form of programmed cell death, by stabilizing SLC7A11 protein levels in hepatocytes during CCl_4-induced liver injury [41]. In addition, MSCs exert hepatoprotective effects via paracrine signaling that activates autophagy, an essential cellular mechanism initiating tissue regeneration [42]. The microRNA Let-7a-5p, delivered through MSC-derived exosomes, has been shown to enhance hepatocyte autophagy and promote regenerative processes in the liver by modulating the mitogen-activated protein kinase-3 (MAP4K3) pathway [43]. Through these tightly regulated molecular pathways, MSCs limit hepatocyte injury, promote survival signaling, and attenuate the progression of liver fibrosis.

Inhibition of HSC and MF activity

To control the progression of fibrosis, one of the principal therapeutic strategies involves suppressing HSCs activation. Consequently, the inhibitory influence of MSCs on HSCs is of particular interest when evaluating the antifibrotic efficacy of MSC-based therapies. Experimental models of liver fibrosis have shown that MSC transplantation can attenuate fibrosis by inhibiting HSC proliferation and inducing their apoptosis [44]. Several other studies have confirmed the suppressive effect of MSCs on HSCs [45, 46]. Umbilical cord–derived MSCs have been shown to downregulate TGF-β1 expression through paracrine signaling [47]. Similarly, adipose tissue–derived MSCs induce cell-cycle arrest of HSCs in the G0/G1 phase of the mitotic cycle , leading to diminished synthesis of profibrogenic proteins and attenuation of fibrotic progression [44, 48]. The antifibrotic effects of MSCs are also mediated through modulation of several signaling pathways, including Notch, Hippo/YAP/Id1 [45, 46], PI3K/AKT/mTOR [44], and p38 MAPK/NF-κB, via regulation of the miR-20a-5p/TGF-βR2 axis [48]. In addition, MSCs inhibit fibrosis by interfering with the Hedgehog/SMO pathway, a central regulator of fibrogenesis [49]. It is believed that human umbilical cord MSCs also suppress HSC proliferation by inhibiting Smad3 protein expression while upregulating Smad7 expression [47].

There is evidence that MSCs play a key role in the process of ECM degradation [40, 48]. MFs can internalize extracellular vesicles derived from MSCs, resulting in decreased type I collagen mRNA expression, and thus reduced ECM production by MFs [50]. These effects are likely mediated by microRNAs – particularly miR-21 and miR-29c – which interact with key signaling molecules involved in ECM synthesis. MSCs also produce enzymes that promote ECM remodeling, including matrix metalloproteinases (MMP-2 and MMP-9) and their tissue inhibitors (TIMP-1 and TIMP-2), which collectively reduce ECM accumulation in fibrotic areas [9].

Immunomodulation of the inflammatory microenvironment by MSCs

The immunomodulatory properties of MSCs play a crucial role in mitigating liver fibrosis [51]. It was found that through both direct interactions with microenvironmental cells and the secretion of paracrine immunoregulatory factors, such as heme oxygenase-1 (HO-1), nitric oxide (NO), prostaglandin E2 (PGE-2), indoleamine 2,3-dioxygenase (IDO), interleukin-6 (IL-6), and human leukocyte antigen-G5 (HLA-G5), MSCs exert potent immunosuppressive effects. These mechanisms enable MSCs to modulate both innate and adaptive immune responses by influencing the activity of natural killer (NK) cells, regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), neutrophils, macrophages, and T and B lymphocytes [22, 35, 52, 53].

Through these immunoregulatory actions, MSCs attenuate inflammation-induced liver injury [40] and reciprocally regulate immune responses to foster a microenvironment conducive to hepatic regeneration [54]. MSCs have been shown to alter T-lymphocyte phenotypes by increasing the population of $CD4^+CD25\text{^}high\text{^}CD45RA^+$ Tregs and modulating the secretion of cytokines associated with immune tolerance [22, 55]. Activation of autophagy in MSCs enhances their immunosuppressive effects on $CD4^+$ T cells, while in fibrotic liver tissue,

MSCs promote the differentiation of circulating monocytes into macrophages and induce the polarization of proinflammatory M1 macrophages toward the anti-inflammatory M2 phenotype, primarily through PGE-2–dependent mechanisms [9].

Circulating monocytes can mediate the systemic immunomodulatory effects of MSCs by engulfing apoptotic MSCs and transporting their regulatory molecules to sites of inflammation, thereby reinforcing systemic immunosuppression [56]. With the help of immunomodulatory factors, MSCs reduce inflammation-induced liver damage and create conditions for restorative regeneration [40].

Recent evidence also highlights that MSC-derived exosomes replicate the potent immunomodulatory and anti-inflammatory properties of MSCs [51, 57]. The regulatory role of exogenous apoptotic MSCs derived from healthy tissues in liver defibrotic processes is shown in Fig. 2.

WAYS TO ENHANCE THE REGULATORY AND ANTIFIBROTIC ACTIVITY OF EXOGENOUS MSCS

It is well established that the therapeutic efficacy of isolated apoptotic MSCs is not always pronounced, particularly in chronic fibrosing liver diseases. This limitation is primarily attributed to their isolation, poor survival rate, and gradual degradation within the recipient's body. Consequently, MSCs rapidly lose their ability to home to the liver, undergo hepatogenic differentiation, and secrete paracrine and trophic modulatory factors. These shortcomings significantly constrain their broad clinical application.

Preliminary adaptive preparation of MSCs

Numerous attempts have been made to enhance the regulatory potential of MSCs by altering their culture conditions and incorporating various factors into the culture medium that train (adapt) them to the effects of unconventional, deficient, or even unfavorable factors, including fibrogenic ones [58]. Factors used for pre-treatment of MSCs include growth factors, lipids, vitamins, hormones, inflammatory factors, and hypoxic conditions [9, 59].

Pre-co-cultivation of umbilical cord MSCs with Schisandrin B (one of the main components of Schisandra chinensis, which prevents the progression of fibrosis) [60], as well as treatment of MSCs with hepatocyte growth factor (HGF) or fibroblast growth factor 4 (FGF-4) before transplantation [61] contributed to the transdifferentiation of MSCs into hepatocyte-like cells, improved their engraftment, and enhanced the therapeutic effect in mice with CCL4-induced fibrosis. MSCs pre-treated with the hormone melatonin [58, 62] exhibited a high ability to homing to damaged hepatic tissue, preserved hepatocellular glycogen stores, and reduced the accumulation of collagen and lipids in fibrous liver tissue. Similar results were obtained using benzimidazole [63], eugenol [64], vitamin E [65], and L-theanine [66].

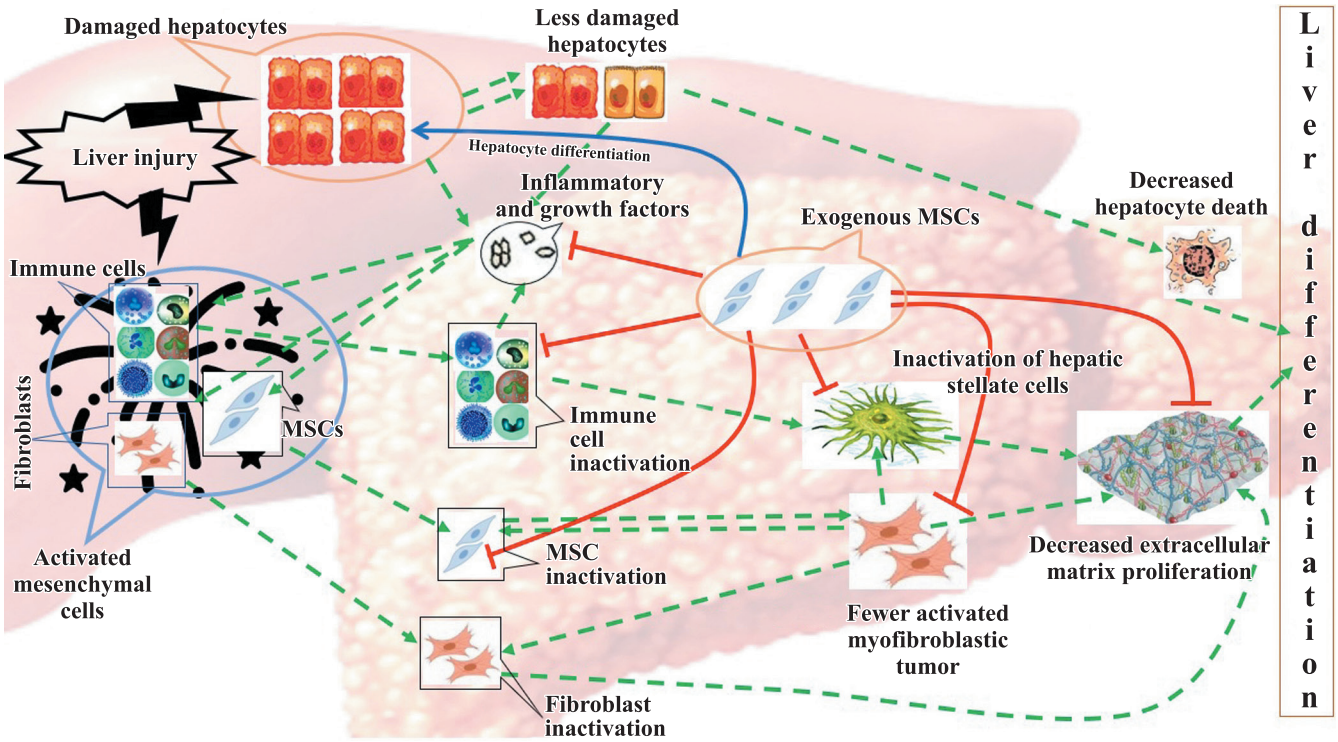

Fig. 2. Diagram showing the involvement of exogenous apoptotic MSCs isolated from healthy tissues in the processes of liver antifibrosis. Details in the text

Combined preparation of MSCs with platelet-rich plasma and HGF [67] significantly enhanced its antifibrotic effect, inhibited HSCs proliferation, inhibiting glycogen synthesis, prolongeing apoptosis, and promoting immunomodulation, transdifferentiation of MSCs into hepatocyte-like cells, secretion of trophic factors, cytokines, chemokines, and more effective regeneration of the damaged liver.

Among the factors that have a pronounced antifibrotic and regenerative effect, it is also worth highlighting preconditioning of MSCs with hypoxia [68], and pretreatment with proinflammatory factors – IFN-α2 [69], TLR4, and IFN-γ [70]. It turned out that pre-treatment of MSCs with pro-inflammatory factors induced higher levels of secretion of CSF-3, IL-8, and the chemokine CCL20 compared to untreated MSCs. In addition, treated MSCs exhibited higher therapeutic activity, and immunohistochemical analysis revealed the accumulation of neutrophils and an increase in MMP-8 activity in the liver [69]. Activation of MSCs via TLR4 and IFN-γ pathways led to downregulation of fibrosis-associated actin-SMA, TGF-β, and TNF-α in the liver.

Hypoxic preconditioning of MSCs also reduced collagen deposition and the number of cells expressing actin-SMA and TGF-β in the liver [68]. Recent studies have further emphasized the synergistic benefits of combining MSCs or their exosomes with chemical agents [67, 68, 71] in experimental models of liver fibrosis.

Genetic modification of MSCs

Genetic modification of MSCs using viral and non-viral vectors has been shown to enhance their capacity for homing, differentiation, and regeneration of fibrotic liver tissue. Modified MSCs overexpress genes that play key roles in liver repair and regeneration, including HGF [72], IGF-1, hepatocyte nuclear factor-4α (HNF-4α), FGF-4 and FGF-21, interleukin-10 (IL-10), and extracellular matrix protein-1 (ECM-1), each of which contributes to improved therapeutic outcomes in liver fibrosis [9].

Expression of the FOXA2 gene in MSCs has been found to promote liver antifibrosis, enhance hepatogenic differentiation, and upregulate several liver-specific genes such as α-fetoprotein, cytokeratin-18 (CK-18), HNF-1, and HGF. In addition to gene overexpression, miRNA modification has also been explored. For instance, upregulation of miR-122 in adipose-derived MSCs enhances their therapeutic efficacy in liver fibrosis by suppressing HSC activation and reducing collagen deposition [73]. IL-10 gene modification in MSCs has been shown to inhibit HSC activity and downregulate TNF-α expression in T lymphocytes and macrophages isolated from fibrotic liver tissue [74].

These findings suggest that modification of specific MSC genes may be a potential new strategy for enhancing the efficacy of liver fibrosis treatment.

Use of cell culture constructs from MSCs (spheroids and cell-engineered constructs)

It is known that three-dimensional (3D) cell cultures exhibit superior phenotypic stability and biological activity compared to traditional two-dimensional (2D) cultures. Studies evaluating the therapeutic potential of MSC spheroids [75] have shown that 3D-cultured MSCs possess enhanced multipotent differentiation capacity, stronger anti-inflammatory and regenerative properties, and secrete higher levels of cytokines. In a mouse model of liver cirrhosis, transplantation of MSC spheroids promoted hepatogenic differentiation, improved liver function, and produced an antifibrotic effect [76].

The higher functionality of 3D-cultured MSCs appears to be related to their increased stemness, as well as their anti-inflammatory and immunomodulatory functions, as indicated by the upregulation of stem cell markers (OCT-4, SOX2, NANOG), anti-inflammatory factors (IL-10, TSG-6, IDO), immunomodulatory molecules (HGF, VEGF, and CXCR4) [77], and activation of the TGF-β1/Smad signaling pathway [78]. In rhesus monkeys with experimental liver fibrosis, portal vein infusion of 3D MSC cultures resulted in the preservation of the spheroidal configuration within the liver for up to one hour post-injection. Even after 16 days, although the spheroids had dissociated into individual cells, viable MSCs remained present in the liver in significant quantities, indicating sustained homing ability [78].

To further prolong and enhance MSC functionality in chronic liver failure, researchers have developed and tested cell-engineered constructs (CECs) implanted directly into the liver. These constructs are based on biomimetic ECMs such as collagen-containing hydrogels (spherogels) [79], recombinant spidroin (rS1/9) – an analogue of spider silk proteins [80] – or decellularized liver matrices [81]. The constructs are typically seeded with bone marrow-derived MSCs in combination with hepatocytes at a 1:5 ratio. Multiple implantation of such CECs over a 90-day period resulted in accelerated and robust liver regeneration: by day 30, biochemical liver function parameters normalized, while structural regeneration and antifibrosis (reduction of ECM area) continued throughout the follow-up period.

Recently, a novel cell-engineering technology has been introduced for generating cell sheets–matrices using temperature-responsive culture dishes coated with poly(N-isopropylacrylamide) to promote MSC adhesion. Combining this technique with pre-treatment of MSCs using IC-2 – a derivative of ICG-001, an inhibitor of the Wnt/β-catenin signaling pathway that induces hepatic differentiation – has yielded a new strategy for treating chronic liver diseases [82–84]. Orthotopic implantation of MSCs in the form of engineered MSC–IC-2 sheets into the livers of CCl_4-induced fibrotic mice resulted in elevated expression of MMP-1 and MMP-14, inhibition

of HSC activation, and significant reduction of fibrosis severity.

It should be noted that most scientific studies devoted to developing ECM biopolymer mimetics for CECs generally follow two main approaches. The first focuses on using individual biopolymer components of ECM, such as collagen, hyaluronic acid, and gelatin (a collagen denaturation product), to regulate the morphological characteristics of the cell carrier and enable the incorporation of additional bioactive molecules. The second approach involves obtaining cell carriers from tissues through decellularization, which allows for maximal preservation of the native ECM composition but limits the ability to modify its structure, mechanical properties, and biodegradation rate.

A promising direction lies in combining both strategies to create next-generation carriers that closely replicate the natural ECM composition while possessing tailored physicochemical properties and the capacity for bioactive molecule incorporation. A macroporous morphology throughout the material is particularly favorable for effective cell colonization and vascularization. In this regard, using enzymatic hydrolysates of decellularized liver tissue as the basis for new materials appears especially promising, as cryostructuring can impart the desired structural and mechanical characteristics.

Previous studies have shown that cryostructurates derived from gelatin and hydrolysates can sustain long-term adhesion and proliferation of human adipose tissue-derived MSCs, as well as support albumin and urea synthesis by tissue-specific HepG2 hepatocellular carcinoma cells [85, 86]. This approach may help maintain the viability of MSCs and hepatocytes within CECs after implantation, thereby extending their functional lifespan *in vivo*.

However, it should be emphasized that enhancement of the therapeutic and antifibrotic effects of exogenous MSCs through these methods is feasible only when the recipient's liver has not undergone irreversible damage.

CONCLUSION

A comparative analysis of the regulatory properties of MSCs in healthy tissues, as well as in acute and chronic (fibrosing) liver injury, was conducted. In a healthy body, MSCs are present in virtually all tissues, where they maintain structural homeostasis and physiological tissue regeneration. They interact primarily with tissue myofibroblasts (MFs) and migrating immune cells, which, like MSCs, originate from the mesoderm.

During acute liver injury, when the extent and duration of the damaging factor do not exceed the adaptive capacity of tissue and cellular defense mechanisms, resident liver MSCs continue to regulate and preserve tissue homeostasis. In contrast, chronic (fibrosing) liver injury leads to progressive depletion of these adaptive and regulatory reserves, resulting in the activation of immune inflammatory cells and resident MSCs. Under such conditions, resident MSCs may transdifferentiate into MFs. These activated fibroblasts produce excessive amounts of extracellular matrix, driving hepatic fibrogenesis.

Exogenous MSCs derived from healthy autologous or allogeneic sources, after isolation, typically undergo reversible apoptosis but retain a high capacity to secrete regulatory and adaptive factors. When administered, apoptotic MSCs exert their effects mainly through paracrine and trophic signaling, compensating for deficits in the chronically injured liver and contributing to the restoration of tissue homeostasis.

Reliable restoration of metabolic regulation and structural integrity in the damaged liver using exogenous MSCs is achievable only when their regulatory activity is enhanced – through preconditioning with adaptive agents, genetic modification, or incorporation into bioengineered cell-matrix constructs – and when the residual regulatory capacity of the recipient's liver has not reached the threshold of irreversible damage.

To optimize clinical outcomes, it is essential to develop reliable and convenient criteria for predicting the individual therapeutic efficacy of exogenous MSCs in patients with chronic fibrosing liver diseases.

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The authors declare no conflict of interest.

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