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Mesenchymal stem cell-derived exosomes as cell-free therapeutics: mechanistic insights and engineering strategies for liver disease treatment

Shihang Yu[1†], Defu Kong[1†], Beike Lu[2†], Yixiao Pan[1], Zhaokai Zeng[1], Yuan Fu[3], Zhicong Zhao[1], Kang He[1*], Ruqi Tang[2*] and Qiang Xia[1*]

Abstract

Liver diseases pose a global health crisis, with liver transplantation remaining the only definitive therapy yet constrained by donor scarcity and complications. Mesenchymal stem cells (MSCs), emerging as stromal cells with immunomodulatory and regenerative capacities, face translational challenges. By contrast, MSC-derived exosomes (MSC-Exos) offer a cell-free approach with lower immunogenicity, minimal tumorigenic risk, and intrinsic hepatic tropism, mediating intercellular communication via protein and microRNA (miRNAs) cargo. To provide a focused synthesis, this review establishes a unified four-pillar framework of MSC-Exos mechanisms across liver pathologies, including (i) maintaining immunological-stromal homeostasis, (ii) reprogramming metabolic circuitry, (iii) determining cell fate, and (iv) intercepting oncogenic signaling. We further discuss advances in engineered MSC-Exos from preparation and application, aiming at enabling precision delivery and the mechanism-to-translation process. While MSC-Exos represent a transformative frontier in hepatology, addressing heterogeneity, scalable production, and cargo standardization remains critical to accelerate clinical translation.

Keywords Mesenchymal stem cell-derived exosome, Cell-free therapeutics, Liver disease, Exosomal miRNA delivery, Mechanistic insight, Immunomodulation, Regeneration, Metabolic reprogramming, Engineered exosomes

†Shihang Yu, Defu Kong, and Beike Lu have contributed equally to this work.

*Correspondence:
Kang He
hekang929@163.com
Ruqi Tang
ruqi_tang@126.com
Qiang Xia
xiaqiang@shsmu.edu.cn
[1]Department of Liver Surgery, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China
[2]Division of Gastroenterology and Hepatology, Key Laboratory of Gastroenterology and Hepatology, Ministry of Health, State Key Laboratory for Oncogenes and Related Genes, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai Institute of Digestive Disease, Shanghai 200001, China
[3]Fudan University Shanghai Cancer Center, Institutes of Biomedical Sciences, New Cornerstone Science Laboratory, State Key Laboratory of Genetic Engineering, Department of Biochemistry and Biophysics, School of Life Sciences, Shanghai Key Laboratory of Radiation Oncology, and Shanghai Key Laboratory of Medical Epigenetics, Shanghai Medical College of Fudan University, Shanghai 200032, China

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Introduction

Liver diseases constitute major global health challenges, with escalating prevalence imposing growing burdens on public health systems and economies worldwide. According to WHO estimates, hepatic disorders contribute to over two million annual deaths, representing 3.5% of global mortality [1]. Pharmacotherapy for liver diseases like metabolic steatohepatitis is only emerging, leaving substantial unmet needs despite the recent approval of resmetirom [2]. If the treatment is neglected, progressive hepatic dysfunction may culminate in end-stage liver disease (ESLD) [3]. While liver transplantation remains the sole definitive therapy for ESLD, critical donor shortages (> 90% demand unmet globally), suboptimal long-term outcomes (< 50% 18-year survival), and exorbitant costs underscore the urgency for developing novel therapeutic strategies targeting both the pathogenesis and progression of hepatic diseases [4].

Decades of translational research have established MSCs as promising therapeutic candidates in regenerative medicine applications [5]. Defined by self-renewal capacity and multilineage differentiation potential, this heterogeneous stromal cell demonstrates particular efficacy in attenuating inflammatory responses and promoting regeneration [6, 7]. Nevertheless, critical barriers impede its clinical translation, such as phenotypic instability, risks of immunological rejection, limited in vivo persistence, as well as pulmonary microvascular entrapment, while MSCs' prominent differentiation capacity also raises oncogenic safety concerns [8]. Moreover, clinical outcomes exhibit significant variability depending on administration routes and expansion cycles, compromising treatment reproducibility [9, 10]. These inherent limitations underscore the rationale for developing cell-free alternatives that retain therapeutic benefits while circumventing cellular risks.

Although direct functions of MSCs in hepatic regeneration and detoxification have been demonstrated [11], emerging evidence highlights their paracrine-based signaling mechanisms, with exosomes emerging as key bioactive effectors [12]. These nanoscale vesicles range from 40 to 160 nm in size, secreted by parental cell exocytosis for intercellular communication [13], serve as multimodal signaling platforms containing various proteins, lipids, metabolites, and nucleic acids [14]. Compared with whole-cell transplantation, MSC-Exos present distinct advantages that enable therapeutic applications across multiple organ systems, including hearts [15], lungs [16], bones [17], and kidneys [18], with particular promise in hepatic pathologies [19]. Their intrinsic hepatic tropism following systemic administration permits targeted cellular communication [20], and the engineering potential allows targetable biodistribution and development as precision drug delivery systems, positioning MSC-Exos as transformative agents for optimizing liver disease therapeutics [21]. While these multimodal effects range from immunomodulation and pro-regenerative activity, precise mechanistic pathways remain elusive. This review systematically analyzes the mechanistic underpinnings of MSC-Exos-mediated hepatic repair, synthesizes their disease-modifying mechanisms across hepatic disorders, and critically evaluates engineered delivery systems alongside emerging clinical translation progress, thereby bridging mechanistic insights with therapeutic innovation to overcome current clinical bottlenecks in hepatology.

Exosomes

Exosomes, defined as evolutionarily conserved vesicular nanostructures, are incapable of replication and originate from endosomal compartments across various cell types [22, 23]. Which were initially perceived as a mechanism for waste disposal, these entities are unveiled as profound role in intercellular communication, serving as essential mediators of post-transcriptional regulation by shuttling a diverse array of functional molecules, including miRNAs and proteins, thereby orchestrating complex cellular processes [24]. Despite the historical interchangeability of the terms "exosome" and "extracellular vesicle" in many publications due to limitations in characterization methods [25], exosomes are now regarded as distinct yet significant components of extracellular vesicles (EVs). EVs are conventionally categorized into three groups based on size and origin: apoptotic bodies (> 1000 nm), microvesicles (100–1000 nm), and nanosized exosomes (40–100 nm), each with unique biological functions [26].

Biogenesis pathways

Exosome biogenesis involves coordinated interactions between the trans-Golgi network (TGN) and endoplasmic reticulum (ER). The process initiates with plasma membrane invagination forming ESEs, which subsequently mature into MVBs with characteristic late endosomal markers containing ILVs, the direct precursors of exosomes. While MVBs are typically destined for lysosomal degradation, they may alternatively fuse with the plasma membrane for extracellular release of functional exosomes [27, 28]. In addition, the molecular mechanisms governing exosome biogenesis remain incompletely defined. Current evidence suggests a predominant role for the ESCRT system, primarily mediated by four multi-protein complexes (ESCRT-0 to -III) [29], furthermore, parallel ESCRT-independent mechanisms have also been implicated in regulating multiple stages of exosome dynamics [30].

Isolation, storage and characterization

The absence of standardized criteria for identifying exosomes has prompted the ISEV to establish the standard MISEV, which has been updated to MISEV2023, providing recommendations and guidelines for exosome-related studies [31]. According to this document, exosome separation should be fit-for-purpose and anchored to measurable properties like size and surface epitopes. In practice, gold-standard ultracentrifugation possesses defects including time consumption and batch variability [32], and with efforts to enhance specificity, alternative techniques, including ultrafiltration, chromatography, polymer-based precipitation, and affinity capture, are also been developed [33]. Separately, chromatography efficiently removes free proteins while preserving vesicle activity, though it dilutes samples and is column-dependent [34]. Stand-alone ultrafiltration may cause shear or adsorptive loss, which can be solved by being paired with chromatography [35]. Polymer-based precipitation and affinity capture offer scalable concentration with good reproducibility, yet they respectively suffer from high impurity levels and low throughput [36]. Generally, each technique varies in terms of exosome purity and scalability but can be strategically combined to address limitations and meet application-specific requirements [37].

Storage conditions may also affect exosome yields, contents, functionality, and the ratio of single particles and aggregates. For short holding, samples are kept at 4 °C (≤ 24–48 h), and for long-term storage, exosomes are frozen at − 80 °C to minimize freeze–thaw cycles [38]. All storage vessels and their materials should be considered, as exosomes can be lost by attaching to surfaces [39]. Prolonged storage in plain PBS is avoided due to loss of EV recovery, with stabilizers (e.g., BSA or trehalose) used as appropriate [40].

In line with current guidelines, exosome preparations should be characterized using at least two different methods to substantiate identity and function [41]. Molecular cargo can be profiled by western blotting, PCR, or next-generation sequencing. Ultrastructure and morphology are visualized by transmission electron microscopy or atomic force microscopy. Particle concentration and size distribution are quantified by nanoparticle tracking analysis, dynamic light scattering, or resistive pulse sensing. Together, these complementary assays provide orthogonal characterization of exosome identity, composition, and biophysical properties [42, 43].

Contents and signature markers

Exosome cargo composition is dynamically determined by physiological conditions and cellular origin. These nanosized vesicles mirror parental cell topology while serving as versatile mediators of intercellular communication through their biomolecular cargos, in which proteins and nucleic acids are the most significant [44]. Evolutionarily conserved proteins functionalize most exosomes, including tetraspanins (CD9/CD63/CD81) mediating intercellular adhesion, HSPs maintaining proteostasis, and trafficking machinery components ALIX and TSG101 [45]. Among bioactive cargo, noncoding RNAs (miRNAs/lncRNAs/circRNAs) constitute a functionally preeminent minority, governing cellular metabolism, signaling cascades, and transcriptional regulation in recipient cells [46]. Notably, Nobel-recognized miRNAs (2024 Physiology/Medicine) exemplify master post-transcriptional regulators, while lncRNAs and circRNAs mediate transcriptional and epigenetic modulation, collectively advancing exosome-based therapeutic strategies [47, 48]. While endogenous RNAs face therapeutic limitations due to rapid degradation, exosomes overcome these barriers through nuclease-resistant encapsulation and tissue-tropic delivery [49].

Physiological roles and clinical applications

Generally, exosomes serve as universal intercellular communicators across cell types. For instance, hepatic homeostasis regulation involves exosome secretion from both parenchymal and non-parenchymal cells [50], while tumor-derived exosomes can orchestrate oncogenic programs through cargo-mediated exosomal protumorigenic crosstalk [51]. Exosomes execute diverse functions through three principal mechanisms: membrane receptor binding, direct fusion, and endocytosis, facilitating the transfer of biomolecules to recipient cells [52]. Distinct from cellular therapies, exosomes exhibit unique therapeutic advantages, propelling their application across multiple medical domains. Beyond their established roles in immune modulation and regeneration, exosomes regulate fundamental biological processes spanning angiogenesis, hemostasis, tissue homeostasis, and programmed cell death (PCD) [53]. Furthermore, their unique capacity further positions exosomes as versatile therapeutic agents in managing viral pathogenicity [54], neurodegeneration [55], and even oncological processes, including epithelial-mesenchymal transition (EMT) and metastasis [56]. Additionally, exosomes are emerging as promising non-invasive diagnostic tools due to their unique molecular composition and inherent accessibility in biofluids [57]. Notably, imaging has advanced with NIR-II probes that label exosomes. This allows real-time in vivo tracking and clearer visualization of exosome pharmacokinetics [58]. Moreover, engineered exosomes' surface modification and drug-loading technologies have opened new avenues for targeted delivery, providing critical insights into their therapeutic potential across multiple disease models [59].

MSCs and MSC-Exos

MSCs serve as a class of adult stem cells, which are characterized by self-renewal capacity and multilineage differentiation potential [60, 61]. These clinically valuable cells reside in diverse anatomical niches, including bone marrow, adipose tissue, and umbilical cord, with their therapeutic applicability enhanced by ethical accessibility and exceptional ex vivo expandability [62]. While demonstrating a highly conserved surface phenotype across tissue origins like CD73/CD90/CD105, with the absence of hematopoietic markers, MSCs retain distinct molecular signatures reflective of their native microenvironment [63]. In addition, MSC transplantation exhibits substantial clinical potential owing to low immunogenicity, which reflects reduced MHC-I and absent MHC-II expression [64]. Notably, tissue injury sites orchestrate MSC homing while inducing localized immune tolerance, thereby enabling organ repair through direct differentiation or paracrine modulation [65]. Acting as immunological rheostats, MSCs also exert pleiotropic effects such as anti-inflammatory, anti-apoptotic, and antimicrobial activities [66, 67].

From MSCs to MSC-Exos: key advantages

Given their unique biological properties and momentum from clinical trials, MSCs are regarded as promising therapeutic candidates for various hepatic pathologies, particularly DLC [68]. To address scalability challenges, immortalized MSC lines are established by lentiviral delivery of hTERT to extend proliferative lifespan and preserve MSC features [69]. Nevertheless, integrating lentiviral vectors pose an insertional-oncogenesis risk, and current validation (PCR and in vivo tumorigenicity assays) remains unsystematic, thus, these applications warrant caution [70, 71].

MSC-related oncogenicity and tumorigenicity concerns should not be ignored [72]. In diverse models, MSCs are confirmed to be capable of directly or indirectly enhancing proliferation and cancer stemness, and drive the EMT process, which in turn boosts invasion and metastasis of tumor cells [73]. MSCs can also change tumor microenvironment (TME) to foster tumors' angiogenesis, immune evasion, and resistance against chemotherapy [74]. Significantly, tumor cues can "educate" MSCs toward tumor-associated phenotypes, becoming a plausible cellular source of cancer-associated fibroblasts (CAFs), contributing to extracellular-matrix remodeling and metastatic niche formation, which further highlights the shortcomings of MSC transplantation therapy [75].

Apart from these, preclinical evidence consistently demonstrates MSCs' persistent translational barriers, including erratic differentiation capacity at injury microenvironments and functional dissociation between engraftment sites and therapeutic outcomes [76]. Paradoxically, functional recovery has been observed despite minimal donor MSC persistence [77]. Specifically, after intraventricular delivery of β-gal–labeled human MSCs in mice, only ~ 0.44% of cells were detectable by day 4 [78]. Other studies on systemic infusion claim that, based on clinical improvement, < 1% administered MSCs persist beyond seven days after transplantation, which challenges the direct differentiation-efficacy paradigm and underscores predominant paracrine mechanisms [79, 80].

Mechanistically, MSC secretome profiling reveals > 200 bioactive factors demonstrating therapeutic bioequivalence or superiority to MSC therapy, prompting their redefinition as "medicinal signaling cells" [81]. Accordingly, this shift advocates transitioning from conventional MSC delivery optimization to developing cell-free derivatives like MSC-Exos for advanced therapeutics, particularly towards hepatic diseases.

Table 1 Comparison between MSC-Exos treatment and MSC transpalntation

	MSC-Exos treatment	MSC transpalntation
Advantage	(1) Low immunogenicity (2) Lack of replication capacity (3) Outstanding ability to target the liver (4) Accumulation of multiple bioactive factors (5) Simplicity of production	(1) High regeneration and differentation ability (2) Strong adaptive capacity (3) Proven efficacy in clinical applications
Disadvantage	(1) High heterogeneity due to insufficient standardization of extraction and purification (2) Short-lived biological activity and difficulties in storage (3) Possibility of contamination	(1) Unstable phenotype (2) Uncertain survival and distribution (3) Potential tumorigenicity (4) Risk of cellular rejection in allogeneic transplantation (5) Complicated production processes (6) Pathological progression induced by aberrant differentiation (7) Vascular occlusion and thrombosis

MSC mesenchymal stem cell, *MSC-Exos* mesenchymal stem cell-derived exosomes

MSC-Exos' therapeutic promise and mechanistic basis

Despite not without limitations, MSC-Exos establish their potential as next-generation biotherapeutics through enhanced efficacy profiles compared to parental MSCs (summarized in Table 1). Therapeutically relevant MSC-Exos originate from diverse natural developmental origins such as bone marrow-derived MSCs (BMSCs), adipose tissue-derived MSCs (AMSCs), umbilical cord-derived MSCs (UCMSCs), and engineered sources like iPSCs (Fig. 1), constituting a versatile biotherapeutic

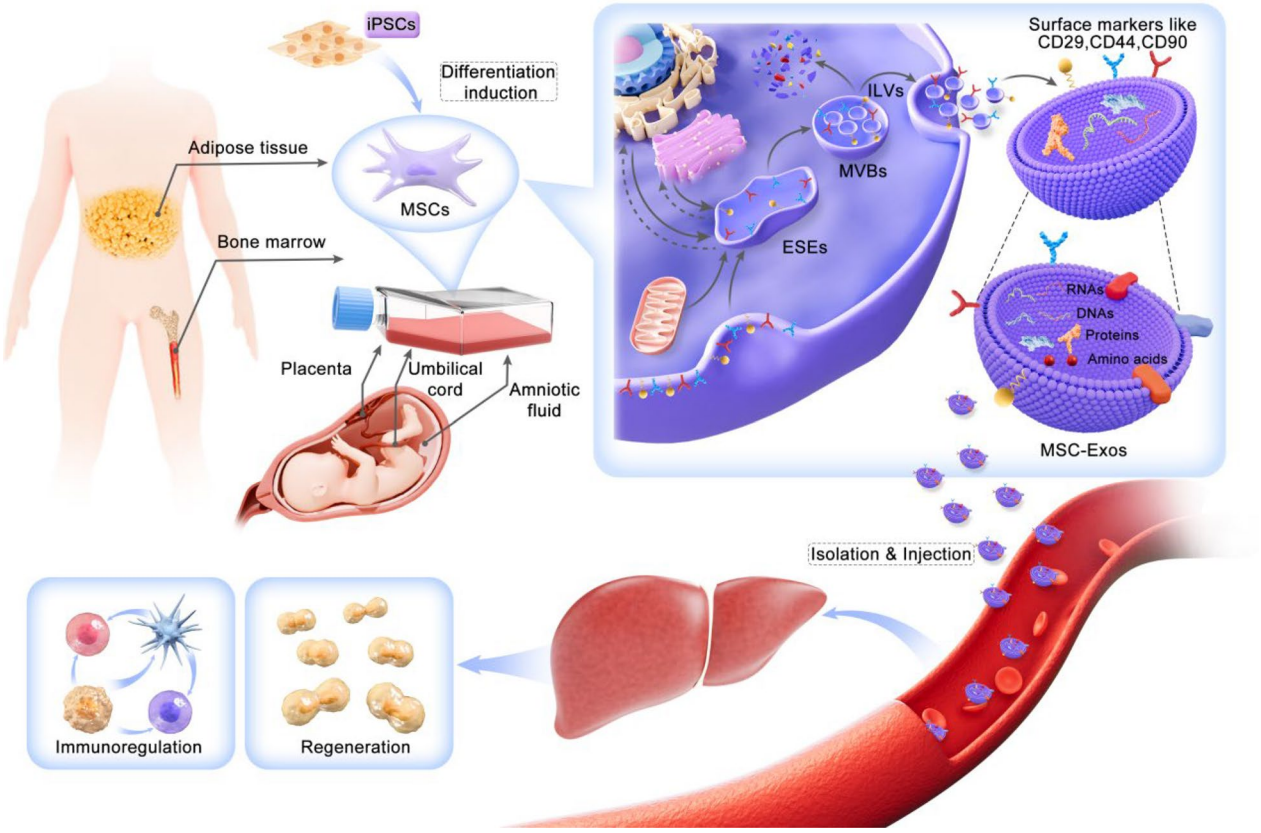


Fig. 1 Multisource MSC-Exos' biogenesis and cargo-mediated hepatic immunoregulation and regeneration. MSC-Exos demonstrate therapeutic potential across liver diseases, offering a viable alternative to liver transplantation. These nanovesicles are obtained from MSC culture supernatants, with cell sources spanning adipose tissue, bone marrow, umbilical cord, placenta, and amniotic fluid, as well as non-natural sources like iPSCs. Biogenesis initiates through ESC-mediated formation of ILVs within MVBs, facilitated by cellular organelles including the TGN, ER, and mitochondria. While MVBs primarily undergo lysosomal degradation, selective plasma membrane fusion enables exosome release. Characteristic surface markers (CD29/CD44/CD90) on MSC-Exos reflect MSC origin, while encapsulated cargo (RNAs, DNAs, and proteins) mediates therapeutic effects. Following systemic administration, MSC-Exos exhibit inherent hepatic tropism, executing targeted immunoregulation and tissue regeneration through bioactive payload delivery. *ER* endoplasmic reticulum, *ESE* early-sorting endosome, *ILV* intraluminal vesicle, *iPSC* induced pluripotent stem cell, *MSC* mesenchymal stem cell, *MSC-Exos* mesenchymal stem cell-derived exosomes, *MVB* multivesicular body, *TGN* trans-Golgi network

platform [82]. MSC-Exos retain source-specific functional determinants from parental MSCs, enabling diverse bioactivity in mitigating injury cascades and activating regenerative processes through target cell reprogramming, surpassing generic exosome functionality [83]. This functional flexibility underpins their therapeutic validation across multisystem pathologies, spanning neurological degeneration, hepatorenal/cardiovascular diseases, to oncological interventions [84–86]. Apart from their therapeutic promise, MSC-Exos face translational bottlenecks requiring resolution, including scalability challenges arising from limited native secretion capacities and complex standardization needs of inherent biological variability and cargo composition [87, 88]. These limitations contrast with their demonstrated biofunctional versatility, underscoring the imperative for engineering solutions to harness their full therapeutic potential.

Proteomic profiling further highlights the functionally biased composition of MSC-Exos (summarized in Table 2) [89]. Roughly half of the MSC-Exos proteins overlap with other exosomes and are significantly linked to vesicle biogenesis and intercellular communication [90]. Beyond canonical markers (CD9/CD63/CD81), MSC-Exos also display MSC membrane markers such as CD29, CD44, CD73, and CD90, indicating their cellular origin [91]. Enzymatic cargos constitute pivotal functional determinants within MSC-Exos, enabling dynamic microenvironmental adaptation through metabolic homeostasis regulation. MSC-Exos also provide enzymatic stabilization and targeted intracellular delivery systems [92], while the adaptation and self-limiting mechanisms prevent therapeutic overdosing by coupling enzymatic activation to injury resolution kinetics [93]. Exosomal RNAs, especially miRNAs, perform an unrivaled role in mediating fundamental pathophysiological processes through post-transcriptional control.

Table 2 Proteomic features of human MSC-Exos [89]

Classification	Representative proteins/classes	Functions	Observation in MSC-Exos vs MSCs
Canonical exosome markers	93/100 ExoCarta top markers like CD9/CD63/CD81	Confirming exosome identity	High overlap, 93/94 for top 100 ExoCarta markers
Extracellular and plasma-membrane proteins	FN1, VTN, COL1A1, etc	Forming ECM and mediating membrane signaling	Enriched in MSC-Exos relative to cells
Proliferation-associated proteins	Extracellular (HGF) and cytoplasmic (CCND1) proteins	Mediating mitogenic properties of MSC-Exos	Not mentioned
Signal transduction proteins	Transporters, peptidases, receptors, GPCRs, ion channels	Suggesting signaling/uptake/processing functions	Higher fractions in MSC-Exos vs cells
Nucleoproteins	Proteins associated with transcription, kinase activity, translation, and phosphatases	Consistent with MSC-Exos' non-nuclear characteristics	Lower fractions in MSC-Exos vs cells
Unique exsomal proteins	ESCRT-associated proteins like ALIX and TSG101	Indicating selective cargo packaging mechanisms	6.7% of proteins unique to MSC-Exos relative to cells

MSC mesenchymal stem cell, *MSC-Exos* mesenchymal stem cell-derived exosomes, *FN1* fibronectin 1, *VTN* vitronectin, *COL1A1* collagen type I alpha 1 chain, *ECM* extracellular matrix, *HGF* hepatocyte growth factor, *CCND1* Cyclin D1, *GPCR* G protein-coupled receptor, *ESCRT* endosomal sorting complex required for transport, *ALIX* ALG-2-interacting protein X, *TSG101* tumor susceptibility gene 101

Mechanistically, hepatic-targeted MSC-Exos engage in multidimensional therapeutic targeting. This functional specialization positions their use as precision RNA-delivery platforms in liver disease [94]. In summary, MSC-Exos are positioned as dynamically regulated biological agents, whose various mechanisms may advance both fundamental research and therapeutic development for hepatic diseases.

The therapeutic mechanisms of MSC-Exos in liver diseases

MSC-Exos have emerged as superior therapeutic agents to MSC transplantation. As cell-free therapies, such exosomes circumvent key limitations of cellular therapies, including immunogenicity, tumorigenicity risks, and so on. Notably, the liver provides a favorable landscape for MSC-Exos-based therapy, like the hepatic immune milieu is uniquely adaptive for MSC-Exos to exert immunomodulatory effects, while intravenously administered EVs can naturally accumulate in the liver, where Kupffer-cell (KC)-rich mononuclear phagocyte systems facilitate uptake, thereby enhancing local exposure [95, 96]. Apart from conventional appraisals focusing on diverse hepatic disease categories, this review provides a mechanistic synthesis of MSC-Exos' therapeutic actions, offering fresh perspectives to advance mechanistic understanding and guide translational research. Accumulating evidence delineates four principal mechanisms underlying their hepatoprotective effects: (1) orchestration of immunological-stromal homeostasis, (2) reprogramming of metabolic circuitry, (3) determination of cell fate, and (4) interception of oncogenic signaling. These effects are dose-dependent within preclinical ranges [97]. Timing sensitivity has also been verified, with earlier administration conferring greater benefit in acute injury models, whereas chronic process typically requires repeated dosing to maintain rebalancing and sustained suppression of damage signaling [98, 99]. The following section systematically examines MSC-Exos' mechanistic pathways in liver disease contexts (Fig. 2).

Orchestration of immunological-stromal homeostasis

As a significant non-parenchymal part of the liver, the immunological network regulates host defense and maintains physiological homeostasis through coordinated interactions between immune components comprising specialized organs, cells, and molecular mediators [100]. As the primary filtration site for nutrients and antigens enriched in portal blood, the liver perpetually balances a delicate equilibrium between inducing immune tolerance and mobilizing defensive cascades [101]. Hepatic stellate cells (HSCs) also constitute an important part of the liver's non-parenchymal cell system, which can undergo metamorphosis from VA-storing sentinels to extracellular matrix (ECM)-overproducing myofibroblasts, disrupting the physiological ECM structure while exacerbating hepatic fibrosis and parenchymal damage [102]. Therefore, dysregulated immunoregulatory and stromal balance may ultimately manifest as cirrhosis, hepatic decompensation, and even carcinogenesis. Positioned as promising modulators for restoring hepatic homeostasis, MSC-Exos represent significant therapeutic potential across the spectrum of liver pathologies.

Innate immune modulation

Accumulating evidence confirmed that MSC-Exos primarily coordinate innate immunity through targeted modulation of KCs, neutrophils, and dendritic cells (DCs) [103]. Mechanistic studies reveal that human UCMSC (hUCMSC)-Exos exert anti-inflammatory effects via miR-455-3p delivery, which attenuates macrophage hyperactivation by targeting PIK3r1 to suppress PI_3K-mediated cytokine synthesis, thereby restoring parenchymal architecture and biofunction [104]. Moreover, NLRP3 inflammasome formed in macrophages is a key driver of acute liver failure (ALF) pathogenesis

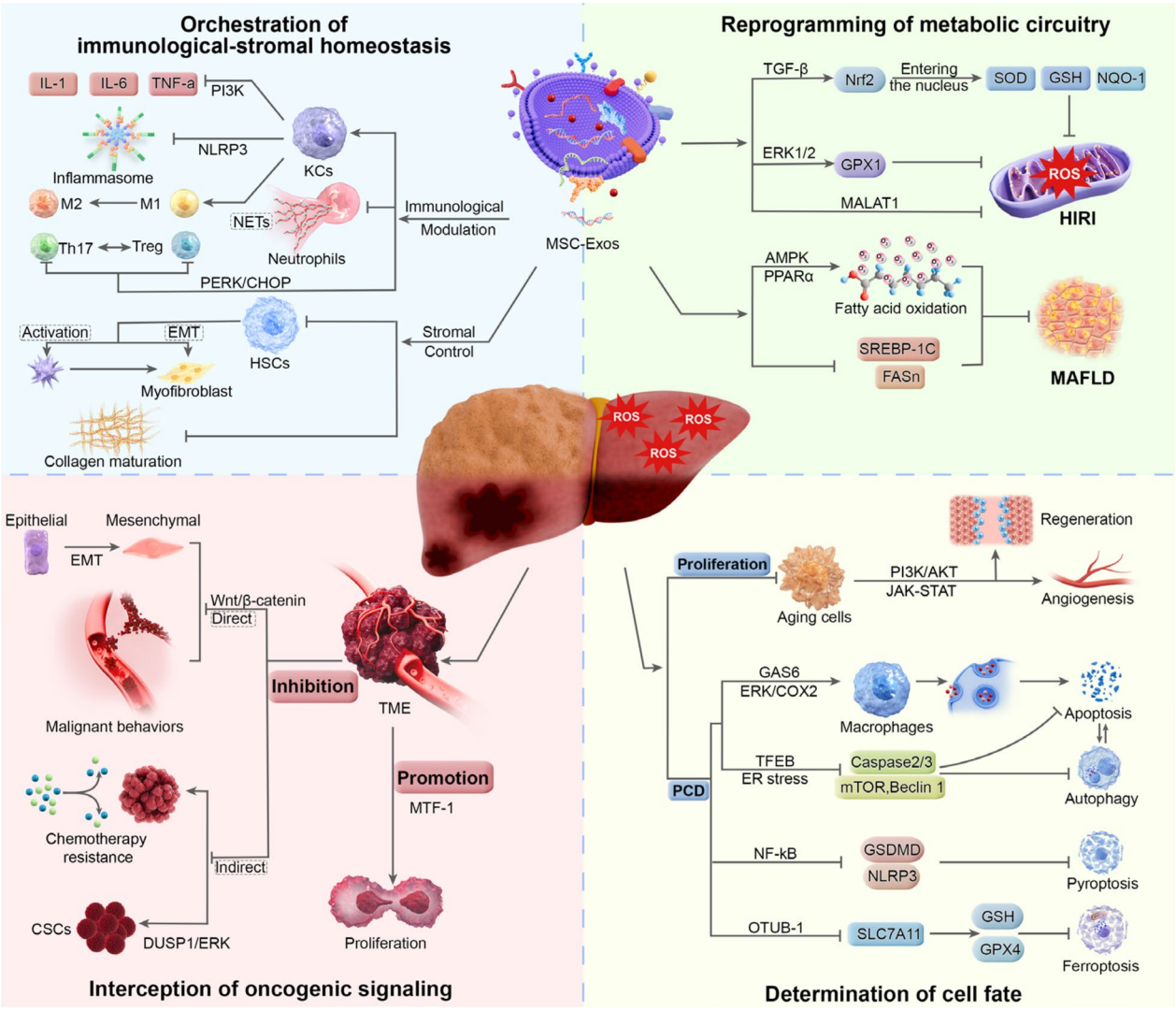


Fig. 2 Multimechanistic therapeutic framework of MSC-Exos in liver diseases. MSC-Exos exhibit multifaceted therapeutic effects in liver diseases through a four-pillar framework of mechanisms: (1) Orchestration of immunological-stromal homeostasis: modulating innate and adaptive immunity (eg., KC polarization, inflammasome/NET control, Th17-Treg transition) and restraining HSC activation/ECM deposition. (2) Reprogramming of metabolic circuitry: restoring redox balance and improving lipid handling to limit oxidative injury and steatosis. (3) Determination of cell fate: promoting regeneration and regulating programmed cell death (apoptosis, autophagy, pyroptosis, ferroptosis). (4) Interception of oncogenic signaling: suppressing EMT/proliferation and enhancing chemosensitivity, while noting context-dependent pro-tumor risks. *AKT* protein kinase B, *AMPK* AMP-activated protein kinase, *CHOP* C/EBP homology protein, *COX2* cyclooxygenase-2, *CSC* cancer stem cell, *DUSP1* dual specificity phosphatase 1, *EMT* epithelial-mesenchymal transition, *ER* endoplasmic reticulum, *ERK* extracellular regulated protein kinase, *FASn* fatty acid synthase, *GAS6* growth arrest-specific protein 6, *GPX1/4* glutathione peroxidase ¼, *GSDMD* gasdermin D, *GSH* glutathione, *HIRI* hepatic IRI, *HSC* hepatic stellate cell, *IRI* ischemia–reperfusion injury, *JAK* Janus kinase, *KC* Kupffer cell, *MAFLD* metabolic dysfunction-associated fatty liver disease, *MALAT1* metastasis-associated lung adenocarcinoma transcript 1, *miRNA* microRNA, *MSC* mesenchymal stem cell, *MSC-Exos* mesenchymal stem cell-derived exosomes, *MTF-1* metal-regulatory transcription factor-1, *mTOR* mammalian target of rapamycin, *NET* neutrophil extracellular trap, *NF-κB* nuclear factor kappa-B, *NLRP3* NOD-like receptor thermal protein domain associated protein 3, *NOX* NADPH oxidase, *NQO-1* NAD(P)H quinone oxidoreductase 1, *Nrf2* nuclear factor E2-related factor 2, *OTUB-1* OTU domain-containing ubiquitin aldehyde-binding protein 1, *PCD* programmed cell death, *PERK* protein kinase R-like ER kinase, *PI_3K* phosphoinositide-3-kinase, *PPARα* peroxisome proliferator-activated receptor alpha, *ROS* reactive oxygen species, *SLC7A11* solute carrier family 7 member 11, *SOD* superoxide dismutase, *SREBP-1C* sterol regulatory element-binding protein 1C, *STAT* signal transducer and activator of transcription, *TFEB* transcription factor EB, *TGF-β* transforming growth factor-β, *Th17* T helper cell 17, *TME* tumor microenvironment, *Treg* regulatory T cell

[105], recent advances highlight exosomal miRNA-mediated suppression of this pathway, where AMSC-exosomal miR-17 is delivered to macrophages to reduce pro-inflammatory cytokine release through suppressing TXNIP-mediated inflammasome priming [106]. These coordinated interventions of MSC-Exos demonstrate their capacity to reprogram innate immune responses from destructive inflammation to homeostasis. Outside ALF, MSC-Exos remodel hepatic macrophages by driving macrophage polarization from M1 to M2 in nonalcoholic

steatohepatitis (NASH) models to attenuate metabolic dysfunction-associated fatty liver disease (MAFLD) progression, and via exosomal miR-148a–STAT3 signaling, simultaneously suppress inflammation and promote fibrosis resolution, supporting MSC-Exos' potential in keeping immunological–stromal homeostasis across various liver injury contexts [107–109].

Hepatic DCs function as immunological conductors, coordinating the innate-adaptive interface through antigen presentation and chemotactic networking. MSC-Exos can impair DC-mediated T-cell priming while stabilizing hepatic immune tolerance, primarily mediated by miRNA-21-5p-dependent blockade of the CCR7/CCL21 chemotactic axis [110]. Neutrophil-mediated immunopathology also undergoes exosomal modulation. hUCMSC-Exos can intercept NET formation via mitochondrial transfer. This process reinstates oxidative phosphorylation restoration while suppressing reactive oxygen species (ROS)-driven NETosis, positioning exosomal interventions as precision tools for restoring immunological equilibrium in attenuating ischemia–reperfusion injury (IRI) in the liver [111].

Adaptive immune regulation

Apart from the innate immune responses, adaptive immune orchestration in hepatic inflammation manifests through exosomal regulation of T-cell and B-cell dynamics. MSC-Exos can balance pro-inflammatory Th17 and regulatory Treg subsets undergo precise modulation. For instance, hPMSC-Exos impede Th17-driven fibrogenesis via PERK/CHOP-mediated IκBζ suppression, disrupting IL-17A secretion and the interaction between cholangiocyte and HSCs in sclerosing cholangitis models, resulting in the entire alleviation [112]. Although B cells constitute only ~ 8% of hepatic lymphocytes, emerging evidence highlights their roles in suppressing fibrotic progression through cytokine-mediated humoral regulation [113]. MSC-derived paracrine factors substantially attenuate intrahepatic B cell infiltration. scRNA-seq analyses demonstrate selective downregulation of proliferation-associated pathways like MAPK or NF-κB in hepatic B cells following MSC intervention. Mechanistically, MSC-Exos disrupt critical inflammatory signaling by inhibiting phosphorylation cascades involving ERK1/2 and IKKα/β, thereby rebalancing immune homeostasis in the liver [114].

Remodeling of fibrotic stromal network

While MSCs demonstrate well-established fibrolytic potential, their capacity to differentiate into myofibroblasts remains controversial, potentially undermining therapeutic outcomes by perpetuating fibrotic cascades [115]. This limitation highlights the superiority of MSC-Exos as a cell-free therapeutic agent that synergistically coordinates matrix remodeling by dual regulatory axes: systemic mitigation of inflammatory triggers coupled with targeted suppression of HSCs [116]. The Hh signaling pathway, serving as one of the master modulators of hepatic fibrogenesis, can be selectively silenced by MSC-Exos to alleviate ECM deposition [117]. Crucially, MSC-Exos' cargoes including miR-486-5p (tonsil-derived) and miR-125b (chorionic-derived), directly target SMO to disrupt Hh-driven transcriptional activation of HSCs [118, 119]. As transcriptomic analyses identify miR-148a-5p downregulation as a hallmark of fibrotic livers, MSC-exosomal delivery of this miRNA restores SLIT3-mediated anti-fibrotic signaling in activated HSCs [120].

Additionally, exosomal transfer of circDIDO1 enhances PTEN-mediated cell cycle control through miR-141-3p sequestration [121]. This multi-kinase regulatory framework converges on PI_3K/AKT and NF-κB signaling pathways, effectively suppressing HSC proliferation rather than activation, revealing novel targets against fibrosis. Complementary studies reveal that PMSCs-Exos utilize miR-378c to stabilize epithelial integrity via SKP2-E-cadherin stabilization, providing an additional layer of protection against fibrotic matrix stiffening [122].

Reprogramming of metabolic circuitry

Numerous studies have demonstrated that pathological changes related to several liver diseases are mediated by structural or functional transformations in particular key enzymes [123, 124]. Enzymatic components are capable of efficient structural and functional modifications in the liver, serving as pivotal mediators in MSC-Exos-driven metabolic reprogramming. Primarily towards metabolic liver pathologies, MSC-Exos demonstrate therapeutic potential via dual metabolic interventions, ranging from direct delivery of enzymatic cargos to modulation of recipient cell genetic enzymatic networks.

Metabolic regulation under oxidative stress injury

Physiological ROS support pathogen defense and signaling. When ROS overload occurs, oxidative stress will follow, and lead to lipid/protein/DNA remodeling and loss of mitochondrial membrane integrity [125, 126]. The liver is paradoxically vulnerable to oxidative assault despite dual vascular perfusion. Interruption of hepatic circulation may fuel reperfusion-induced pathophysiological exacerbation, clinically manifesting as hepatic IRI (HIRI) [127]. With novel rigorous preclinical validation, MSC-Exos are potent modulators of this metabolic derangement. They are capable of intercepting oxidative cascades at the mitochondrial-electron transport chain, presenting a novel strategy efficacious in hepatic oxidative injury.

Central to redox homeostasis is the Nrf2-Keap1-p62 pathway. When faced with stimulation, Nrf2 undergoes

nuclear translocation and upregulates antioxidant effectors (SOD, GSH, NQO1) through coordinated promoter engagement [128]. Recent mechanistic insights reveal that MSC-Exos orchestrate multilayered metabolic interventions, including direct potentiation of Nrf2 signaling via Smad/TGF-β pathway modulation, as well as autophagy promotion, positioning MSC-Exos as precision regulators of oxidative stress and hepatic functional restoration [129]. In addition, hUCMSC-Exos can preferentially enhance GPX1 to overcome impaired antioxidant enzyme production. With the help of GPX1-centric defense mechanisms, including ERK1/2-mediated phosphorylation cascades and Bcl-2-dependent anti-apoptotic signaling, such exosomal orchestration can effectively restore hepatocytes from oxidative damage-relevant apoptosis [130]. At the transcriptomic level, MSC-Exos execute metabolic counterregulation by deploying miRNAs like miR-26a-5p to silence MALAT1-mediated antioxidant suppression, thereby intercepting sepsis-associated oxidative cascades and attenuating acute liver injury (ALI) [131, 132]. Collectively, these findings position MSC-Exos as multitarget metabolic engineers capable of reprogramming oxidative stress circuitry, establishing a paradigm shift in treating redox-imbalanced hepatopathies.

Enzymatic control in lipid metabolic homeostasis

MAFLD prevalence is now recognized as a hepatic manifestation of metabolic reprogramming driven by systemic energy surplus, where hepatocellular inflammation and fibrotic remodeling may culminate in ESLD [133]. While obeticholic acid remains the sole approved therapy, MSC-Exos emerge as compelling metabolic modulators capable of multidimensionally intercepting disease evolution through precise interventions [134]. Preclinical models demonstrate that hUCMSC-Exos exert masterful control over reprogramming ectopic lipid deposition through dual-pronged metabolic governance. These nanovesicles reverse diet-induced hepatosteatosis by delivering CAMKK1 to amplify AMPK's role as metabolic sensors, triggering PPARα-driven fatty acid oxidation while suppressing SREBP-1C/FASn-mediated lipogenesis, rewiring lipid flux equilibrium [135]. This multi-nodal metabolic gatekeeping surpasses conventional single-target pharmacological models by holistically resetting hepatic energy substrate partitioning rather than singular pathway modulation.

Enzymatic orchestration of fibrogenesis

Enzymatic dysregulation also constitutes a core pathogenic mechanism in hepatic fibrogenesis, with catalytic byproducts ROS and collagen driving disease progression. AMSC-Exos restore phosphatidylcholine biosynthesis through choline phosphotransferase activation, counteracting PI_3K/AKT/mTOR-driven fibrotic signaling [136]. Crucially, these vesicles target collagen maturation machinery via miRNA-mediated YAP/LOXL2 inhibition, disrupting lysyl oxidase-dependent ECM remodeling [137, 138].

Determination of cell fate

Hepatocyte fate determination by MSC-Exos pivots on transitions from quiescence to proliferation or PCD. This inherent circuitry enables hepatocytes to override G0 arrest through synchronized activation of proliferative checkpoints, while driving irreversibly injured cells to death, which is essential for mass restoration of function post-injury. MSC-Exos emerge as cell cycle reprogrammers against pathological overload, capable of regulating proliferation or PCD relevant markers expression to resurrect proliferative competence while maintaining lineage fidelity, which are positioned as precision tools for hepatic regenerative engineering [139].

Proliferative reprogramming reactivation

Age-imposed regeneration failure stems from G0/S checkpoint entrapment. MSC-Exos act as significant determiners to reprogram epigenetic conversion of mature hepatocytes into EpCAM(+) progenitor states via chromatin remodeling [140], while promoting mitochondrial rejuvenation through DDX5-E2F1-ATG4B axis-driven mitophagic flux normalization to revitalize aged hepatocytes [141], however, the longitudinal fate stability of reprogrammed hepatocytes requires mechanistic elucidation. In respect to liver injury, Psaraki et al. demonstrate that AFMSC-Exos stimulate hepatic progenitor cell proliferation in ALF models through MFGE8, activating PI_3K/AKT signaling to enhance regeneration and suppress apoptosis [142]. Complementarily, hUCMSC-Exos can transport miR-124 to accelerate liver regeneration post-hepatectomy through targeted suppression of Foxg1, a transcriptional repressor of cell cycle progression [143].

Apoptotic signaling modulation

Beyond their regenerative potential, MSC-Exos critically regulate hepatocytes' apoptosis, which is a caspase-dependent PCD characterized by DNA fragmentation and nuclear protein degradation, and functions as a homeostatic mechanism through various pathways. Mechanistic studies identify that AMSC-Exos restore miR-183 levels to suppress ALOX5-mediated apoptosis, thereby serving such exosomes as potential regulators towards HIRI [144]. Proteomic analyses uncover exosomal GAS6 as a critical mediator of macrophage in HIRI. Mechanistically, GAS6 binding to MerTK receptors initiates ERK phosphorylation and COX2 upregulation, potentiating efferocytic clearance of apoptotic cells

and accelerating inflammatory resolution [145]. Additionally, ER stress amplifies apoptotic signaling during the non-alcoholic fatty liver disease (NAFLD)-NASH transition via caspase-2, highlighting a therapeutic entry point [146]. BMSC-Exos mitigate this cascade by miR-96-5p-mediated caspase-2 suppression, normalizing BAX/Bcl-2 and reducing hepatocyte apoptosis in preclinical models [147]. In HIRI, AMSC-Exos downregulate ER stress markers including GRP78, ATF6, IRE1α, and CHOP to restore proteostasis and halt apoptosis [148].

Autophagic cytoprotection regulation

Autophagy, designated as type II PCD, constitutes an evolutionarily conserved cytoprotective mechanism through lysosome-mediated recycling of cellular components. The macroautophagy cascade serves as the predominant autophagy pathway for bulk cytoplasmic renewal, whose dysregulation directly intersects with PCD execution [149]. The crosstalk between autophagy and apoptosis critically determines cellular fate through shared molecular switches, which delineate a sophisticated regulatory network and coordinate the dynamic equilibrium [150]. Lin et al. reveal that MSC-derived exosomal let-7a-5p modulates hepatocyte fate by activating TFEB-mediated autophagic reprogramming. This paracrine signaling converges on MAP4K3-dependent TFEB phosphorylation to intercept PCD cascades in acute-on-chronic liver failure (ACLF) [151]. Complementary evidence from ALF models reveals that BMSC-Exos coordinately upregulate both autophagic machinery and Bcl-2-mediated survival pathways. Significantly, autophagy inhibition reverses these cytoprotective effects, establishing autophagic activation as a prerequisite for apoptosis mitigation [152].

Pyroptotic gateways control

Pyroptosis is a lytic PCD distinct from apoptosis, determining inflammasome-dependent cell fate through gasdermin pore formation. Diverging from apoptotic silent clearance, cleaved GSDMD permeabilizes plasma membranes and amplifies inflammatory effects, providing frontline defense against intracellular pathogens while establishing feedforward loops of tissue injury [153]. Emerging research has highlighted the critical regulatory role of MSC-Exos in pyroptosis-mediated cell fate determination during liver injury. For instance, it's demonstrated that AMSC-Exos attenuate HIRI with PH by suppressing the NLRP3/ASC/caspase-1 axis by inhibiting the NF-κB pathway and effectively mitigating pyroptosis. Notably, this therapeutic outcome is also evidenced by porcine liver injury models [154, 155].

Ferroptotic balance maintenance

As a PCD modality distinguished by iron-dependent lipid peroxidation, ferroptosis induces membrane destabilization through redox imbalance, collectively driving catastrophic oxidative cascades. Central to ferroptosis suppression, the X_C- transporter system (particularly SLC7A11) sustains redox homeostasis by efficient glutathione biosynthesis [156]. Therefore, ferroptosis regulation through SLC7A11 modulation emerges as a pivotal determinant of hepatocyte fate. CCl_4-induced hepatocyte injury demonstrates SLC7A11 suppression potentiating ferroptosis susceptibility, while MSC-Exos intervention restores transporter stability and hinders oxidation via CD44/OTUB1-mediated ubiquitination blockade [157]. Paradoxically, MSC-Exos-derived BECN1 orchestrates dual ferroptotic activation in HSCs to restrain fibrosis through SLC7A11 downregulation and GPX4 inhibition, with genetic BECN1 ablation nullifying anti-fibrotic efficacy [158]. Interestingly, these contrasting SLC7A11 regulatory patterns in multiple liver disorders underscore the condition-dependent nature of exosomal PCD modulation, which necessitates further investigation to confirm the precise mechanisms.

Interception of oncogenic signaling

Hepatocellular carcinoma (HCC), the predominant hepatic malignancy driving global cancer mortality, arises through multistep progressive accumulation of signaling pathway aberrations, where genomic instability intersects with epigenetic reprogramming to sustain malignant transformation [159]. The TME operates as a dynamic mediator where stromal-epithelial crosstalk activates oncogenic pathways through matrix remodeling and paracrine signaling [73]. MSCs serve as central signal integrators in this ecosystem, undergoing differentiation or exosome-mediated pathways to influence tumor development [160]. Emerging evidence positions MSC-Exos as precision interceptors to conduct multi-target modulation on proliferative and chemoresistant signaling nodes while reprogramming EMT or metastatic pathways through cell-free cargo delivery, acting as a double-edged sword to exert promoting or inhibitory effects [161].

Tumor-restraining capacity

As a significant event in tumor progression, the EMT process can be disrupted by miR-374c-5p delivered from BMSC-Exos targeting LIMK1/Wnt/β-catenin signaling [162]. Moreover, MSC-Exos' therapeutic arsenal extends to regulate various malignant behaviors of HCC, where exosomal miR-15 acts in multi-pronged roles to coordinately block proliferation-invasion networks [163]. Beyond direct tumor cell targeting, these vesicles dismantle malignancy driven by CSCs, while BMSC-exosomal regulation of the C5orf66-AS1/miR-127-3p/DUSP1/ERK pathway may effectively neutralize CSC populations to intercept the development of HCC [164]. Given the capacity for overcoming HCC's resistance to

chemosensitization strategies [165], MSC-Exos' role as a multilayered interception of core oncogenic pathways underscores their potential as next-generation HCC therapeutics.

Pro-tumorigenic potential

Intriguingly, considerable attention on MSC-Exos' capacity to facilitate tumor development has emerged nowadays, thereby current MSC therapies remain constrained by potential cancer-promoting capacity. Mechanistically, MSC-derived circ563 acts as an oncogenic switch through competitive binding to miR-148a-3p, thereby derepressing MTF-1-mediated proliferation and metastasis in HCC models, exhibiting strict dose dependency [166]. Interestingly, the pro-tumorigenic exosomal components may hold greater translational value, serving as both therapeutic vulnerability markers and diagnostic targets for HCC stratification. Such functional antagonism underscores the imperative for rigorous investigation and validation of exosomal contents, as well as cargo-specific engineering of MSC-Exos to selectively intercept oncogenic circuitry while preserving tumor-suppressive faculty [167].

Engineered MSC-Exos for liver diseases

Concurrently, there are limitations of native MSC-Exos such as relatively low potency, a lack of selectivity on specific targets, and high demands for industrial technology in preservation or manufacturing efficiency. To make exosomes a more reliable agent for future clinical applications, engineered MSC-Exos have emerged. These bioenhanced vesicles are natural exosomes treated with multi-parametric engineering strategies to optimize therapeutic contents precision, tissue penetrance, and prolonged systemic retention [168], representing a shift in hepatotropic delivery systems. Unlike synthetic nanoparticles, their vesicular architecture with superior biocompatibility confers intrinsic immune-evasive properties and microenvironment-responsive behaviors [169]. Early in 2015, Lou et al. conducted research into miR-122-modified AMSC used for HCC treatment, which was the very first research for engineered MSC-Exos in liver disease [170].

Liver physiology and smart delivery

Actually, the liver is the optimal target organ of exosomes, and the vast majority of systemically administered particles rapidly accumulate in this organ [171]. The phenomenon mainly depends on the unique physiological characteristics of the liver. The microvascular structure in the liver is called liver sinusoids, containing fenestrated endothelium and discontinuous basement membrane [172]. Therefore, the hepatic sinusoidal architecture enables the particles to have easy access to the Space of Disse. In the Space of Disse, there are KCs, HSCs and hepatocytes [173]. As specialized phagocytes in the liver microenvironment, KCs are responsible for removing foreign particles or senescent cells. For therapeutic vesicles targeted at HSCs or hepatocytes, this might be unnecessary; while for targeted macrophage therapies, this is an advantage.

Besides, the liver has its own "immune barrier", which means it maintains immune tolerance to self and foreign antigens [174]. Many studies have identified that both regulatory soluble mediators and antigen-presenting cells in liver sinusoids contribute to a unique hepatic immunoregulatory microenvironment [175, 176].

Through the insight into liver physiology and immunology, more intelligent engineered MSC-Exos can be developed. For instance, inserting immuno-switches on the membrane surface or developing smart-responsive prodrugs can prevent the particle from phagocytosis. Encapsulating targeted immune molecules and cell surface-specific receptors in the formulation may also enable more effective targeted delivery.

Design strategy of engineered MSC-Exos

In the context of the design strategy of engineered MSC-Exos, modifications can achieve enrichment of vesicle content and change of vesicle membrane components [177]. Therefore, functional precision in engineered MSC-Exos can be achieved through dual-axis biofunctionalization, encompassing both membrane engineering and cargo loading strategies.

Membrane engineering

Membrane engineering strategy aims for superior pharmacokinetic parameters by modifying the membranes of these exosomes. Chimeric protein constructs are employed to enable tissue-specific homing while establishing intensified circulation stability. Exemplified by hepatic fibrosis therapy, hUCMSC-Exos engineered with Lamp2b-HTSP1 fusion proteins demonstrate superior phagocytic resistance and collagen-targeting specificity versus native vesicles, translating to significant therapeutic gain ($P < 0.05$) [178, 179]. This engineering approach effectively addresses the inherent mononuclear phagocyte system clearance dilemma while enhancing lesion-site accumulation. Besides LAMP-2B, the transmembrane protein platelet-derived growth factor receptor (PDGFR) and the tetraspanin superfamily CD63/CD9/CD81 are commonly used for membrane display [168].

Cargo loading

Cargo loading strategy potentiates MSC-Exos' therapeutic efficacy through precision integration of endogenous and exogenous pharmacologic agents. In concrete terms,

this strategic augmentation capitalizes on their innate therapeutic capacity to enable combinatorial therapies via small molecule/nucleic acid/protein co-loading. For instance, a novel engineered exosome named MSC-exo/MnO_2@DEX encapsulating DEX within MnO_2-modified MSC-Exos achieves dual-pathway intervention in ALI. This formula utilizes the MnO_2 component to catalyze the decomposition of H_2O_2 into O_2, thereby alleviating hypoxia in liver tissue and promoting tissue repair. In addition, DEX works as an anti-inflammatory agent and protects hepatocytes, while MSC-Exos are SLC7A11/GPX4 antagonists to reduce oxidative stress. This multimodal therapeutic platform synchronously quenches ROS-driven hypoxic stress while intercepting NF-κB-mediated inflammatory cascades [180]. Such multimodal integration demonstrates the transformative potential of MSC-Exos in addressing complex liver pathologies through rational modification and synergistic effects.

Design strategy for distinct liver diseases

Strategies concerning distinct liver disease phenotypes differ. In the context of HCC, BMSC-Exos has adhesion molecules like CD29, which is a hepatocyte-specific ligand. Meanwhile, TME is described as acidosis, and the uptake microenvironment of MSC-Exos has been determined to be acidic, which happens to suit the drug release of the formulation [181, 182]. Delivery of therapeutic nucleic acid drugs by exosomes is also one of the common strategies [170]. Research has shown that ECM remodeling, suppression of aHSCs, inducing immunoregulatory microenvironment and other strategies can achieve reversion of liver fibrosis [183]. HSCs function as the major storage site of vitamin A, and aHSCs maintain the expression of retinol-binding protein receptor, which can be used for target delivery [184]. By administration of MMP, the ECM protein will be cleaved, thus inducing ECM remodeling and weakening of matrix stiffness. For cirrhosis, Baharvand et al. have demonstrated that EVs derived from human embryonic stem cell-derived MSC can ameliorate cirrhosis [185].

Notably, there are no studies into engineered exosomes for cholestasis or NAFLD/MAFLD. Due to the pathogenesis of NAFLD, immune cells of pro-inflammatory phenotype were associated with disease progression. That means engineered MSC-Exos targeted at the hepatic immune microenvironment can probably benefit NAFLD patients [186].

Preparation methods of engineered MSC-Exos

Engineered MSC-Exos exhibit unique engineering plasticity, whose cell-derived characteristics enable progenitor-level bioengineering during vesicle biogenesis. This intrinsic adaptability supports two distinct temporal engineering strategies: (1) Exogenous post-harvest manipulation for precise cargo loading; (2) Endogenous progenitor engineering for constitutive vesicle modification. These two approaches are briefly named post-isolation and pre-isolation modification, collectively enabling spatiotemporal and cargo control over therapeutic vesicle functionality. (summarized in Table 3) (Fig. 3) The choice between these strategies often depends on the specific liver disease context and the desired balance between engineering precision, yield, and probably further concerns about compliance with Good Manufacturing Practice (GMP).

Post-isolation modification

Post-isolation engineering of MSC-Exos employs diverse methods for exogenous cargo integration, categorized as physical (electroporation/extrusion/sonication/freeze–thaw cycles) and chemical (saponin/surfactant-mediated) methods [187]. While physical methods like electroporation achieve superior drug-loading efficiency compared to simple mixing, they risk vesicular aggregation and structural deformation [188]. Chemical techniques enhance membrane permeability yet may compromise membrane integrity and impart cytotoxicity, incurring biosafety concerns [189]. Despite the likelihood of disrupting the positioning of surface biomolecules, targeting modification can be achieved by post-isolation approaches. A novel emerging membrane hybridization pattern, HCQ@VA – Lip – Exo, constructed by the extrusion method on BMSC-Exos and liposomes modified with VA, has been developed to specifically target activated HSCs. This method allows for synergistic retention of parental vesicle functionality and synthetic carrier advantages, contributing to the prevention of liver fibrosis [190].

Except for those post-isolation methods that have been reported to be utilized in liver disease, other post-isolation surface engineering strategies like Click-Chemistry and PEGylation also hold promise for enhancing the targeting and stability of MSC-Exos towards the liver microenvironment. Click chemistry is a technique of modifying the exosomal surface wherein an alkyne group is attached to the isolated exosomes via 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide-N-hydroxysuccinimide condensation reaction. This is then covalently conjugated to the azido group of the targeting moiety in the presence of copper [191]. Due to its high efficacy and mild reaction condition, exosomes will keep their physical properties to the greatest extent. With the help of click chemistry reaction, RGE peptide functionalized exosomes have been developed and exhibited great antitumor ability both in vitro and in vivo [192]. PEGylation is a method that is widely used in bionanotechnology, which prolongs the circulation duration of the formulation and enhances the negative surface potential on the

Table 3 Engineering and retrofitting strategies for MSC-Exos

Categorization	Processing stage	Core molecule	Engineering methods	Strengths	Weaknesses
Post-isolation methods	Exosome level	Exogenous drug molecules	Physical methods (1) Simple mixing (2) Electroporation (3) Hypotonic dialysis (4) Freeze–thaw cycles (5) Sonication (6) Extrusion Chemical methods (1) Transfection reagents (2) Saponin-induced techniques	(1) Relatively high transfection efficiency (2) High targetability (3) Reduced adverse effects (4) Low complexity and low cost	(1) Destruction of membrane structures (2) Exosome aggregation (3) Latent cytotoxicity or toxic excipient (4) High heterogeneity (5) Low stability
Pre-isolation methods	MSC level	Endogenous proteins or RNAs	Genetic engineering	(1) Definite mechanisms (2) Preservation of exosome integrity (3) High stability (4) Good control (5) Efficiency depends on methods	(1) Inability to load small molecule drugs (2) Complicated operation and high cost (3) Possibility of off-targeting (4) Uncertain safety
			Exposure to (1) Hypoxia condition (2) Toxic lipids (3) Complement proteins (4) Chemotherapy agents (5) Other specialized microenvironments	(1) High convenience (2) Direct Enhancement of related functions	(1) Unknown alterations in biological activity (2) Unstable efficacy

MSC mesenchymal stem cell, *MSC-Exos* mesenchymal stem cell-derived exosomes

membrane surface [193]. Nevertheless, there has been doubt about the non-immunogenic intrinsic quality of polyethylene glycol (PEG), indicating that production of anti-PEG antibodies triggers the rapid clearance of subsequent doses of PEGylated materials in mice (called the accelerated blood clearance phenomenon) [194].

Post-isolation methods are based on the preparation of exosomes, obviously making engineered MSC-Exos trapped in the same dilemma as MSC-Exos, such as low yield and heterogeneity between batches. Under the requirements of industrial production, challenges to maintain functional fidelity and scalable manufacturing under GMP standards have to be overcome for clinical translation in liver disease.

Pre-isolation modification

Pre-isolation preparation methods are widely used to achieve constitutive expression of therapeutic macromolecules during vesiculogenesis, enabling genetic reprogramming of MSCs through CRISPR or plasmid-based transcriptional regulation. Genetic engineering of exosomes is a convenient method for imparting exosomes with new properties. This biosynthetic precision ensures the stability and continuity of exosome production, maintaining vesicular integrity while enhancing therapeutic capacity, though confined to biologic agents given inherent limitations in small molecule incorporation. These engineered variants demonstrate multi-faceted functional augmentation. For instance, GPC3-targeting scFv antibodies can be displayed onto the surface of MSC-Exos by genetic modification, cooperating with tumor-suppressive exosomal miR-26a to enable precision HCC targeting and inhibit its malignant behaviors [195]. Despite superior biosynthetic fidelity versus post-harvest methods, this approach demands stringent genomic safety controls and is unable to utilize the established pharmacological effects of small-molecule drugs, significantly increasing the uncertainty. Despite superior biosynthetic fidelity versus post-isolation methods, this approach demands stringent genomic safety controls and is unable to utilize the established pharmacological effects of small-molecule drugs, significantly increasing the uncertainty.

Except for directly extracted exosomes, microenvironmental priming emerges as another pre-isolation bioengineering modality, leveraging pathophysiologically relevant stimuli including hypoxia, toxic lipids, cytokines, or chemotherapy towards MSC to program MSC-Exos with enhanced context-dependent therapeutic signatures. This conditioning model orchestrates dual vesicular reprogramming: enhanced biogenesis and stimulus-responsive cargo packaging, resulting in strengthened bioactivity in the resultant vesicular cargos. While offering streamlined operational workflows compared to genetic engineering, stimulus-induced vesicular perturbations may compromise the structural–functional integrity of MSC-Exos, necessitating rigorous biosafety validation [196]. Hypoxia-induced miR-182-5p fosters

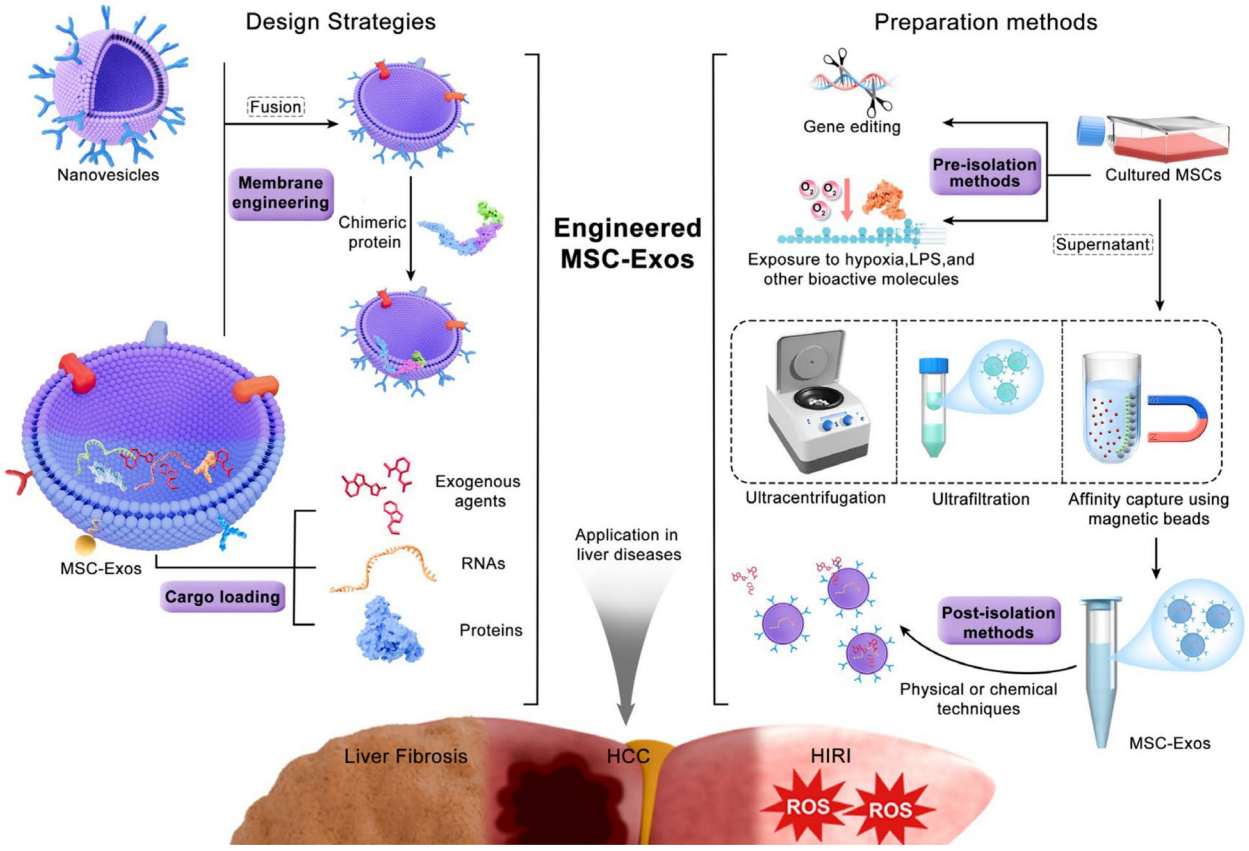


Fig. 3 Engineered MSC-Exos as nanotherapeutic platforms for hepatic disorders. Engineered MSC-Exos present a potent nanotherapeutic platform for hepatic disorders including liver fibrosis, HCC, and HIRI through dual-axis engineering designing strategies. Membrane modification enables enhanced tissue targeting and circulation stability via fusion with artificial nanovesicles and chimeric protein integration, as well as cargo loading potentiates MSC-Exos' therapeutic efficacy through precision integration of endogenous and exogenous pharmacologic agents. Preparation methods also involve two-phase optimization, as pre-isolation methods prime through genetic modification or microenvironment modulation enriches therapeutic cargos. After being isolated from cultured supernatant by diverse techniques, MSC-Exos undergo physical or chemical post-isolation methods to enable controlled drug encapsulation. Clinical translation requires standardized isolation protocols and batch consistency assurance. *HCC* hepatocellular carcinoma, *HIRI* hepatic IRI, *IRI* ischemia–reperfusion injury, *LPS* lipopolysaccharide, *MSC* mesenchymal stem cell, *MSC-Exos* mesenchymal stem cell-derived exosomes, *ROS* reactive oxygen species

M2 polarization to facilitate liver regeneration through FOXO1/TLR4 axis suppression [197]. Moreover, lipopolysaccharide (LPS)-primed BMSCs can derive ATG2B-enriched exosomes to promote mitochondrial quality control via STING pathway inhibition, thereby attenuating septic liver injury [198]. Cytokine-preconditioned MSC-Exos exhibit immunoregulatory specialization, exemplified by IFN-γ-engineered vesicles harboring an ANXA1/LTF-enriched proteome that coordinates macrophage functional polarization, indirectly attenuating fibrotic progression through microenvironmental reprogramming, distinct from direct HSC targeting [199].

One of the biggest challenges of pre-isolation methods is the safety concern of the unknown inner components, for batch differences and uncertain biological processes may lead to suspicions of the amount and quality of the active pharmaceutical ingredient. Therefore, a comprehensive characterization method should be established for evaluation. In the meantime, the separation fragility and storage conditions can be another barrier in clinical application of engineered MSC-Exos.

Application of engineered MSC-Exos in liver diseases

As a novel and promising therapeutic strategy, engineered MSC-Exos have gained much attention, with multiple studies having been conducted (summarized in Table 4).

HCC: targeting & resistance reversal

Engineered MSC-Exos demonstrate strong potential in HCC theranostics. They can eliminate tumor cells through bioengineering of cytotoxic payloads and precision targeting systems. A representative tandem strategy encapsulates norcantharidin into BMSC-Exos via electroporation. This increases cellular uptake and cytotoxicity in HCC cells versus conventional delivery while

Table 4 Researches into engineered MSC-Exos for liver diseases

MSC type	Preparation method	Disease model	Therapeutic agents	Molecular mechanism	Biological effects	Refs.
AMSCs	Plasmid transfection	Nude Balb/c mice bearing HepG2 cells	miR-122	Downregulate the expression of ADAM10, GF1R and CCNG1	Enhanced chemo-sensitivity of HCC	[170]
hUCMSCs	Plasmid transfection	CCl4-induced liver fibrosis in SD rat	–	(1) Inhibit CCL2 secretion from aHSCs (2) Target peptide gene HSTP1 fused with Lamp2b	Anti-inflammation	[179]
BMSCs	Sonication	CCl4-induced ALI in Balb/c mice	DEX MnO2	(1) Stabilize SLC7A11-GPX4 axis (2) Reduce ROS (3) Suppress NF-κB	Anti-inflammation	[180]
BMSCs	Electroporation	HepG2 infected Balb/c mice	NCTD	–	Synergistic effects of antitumor and hepatocyte repair	[181]
BMSCs	Membrane hybridization	TAA-induced liver fibrosis in Balb/c mice	HCQ Vit A	(1) Inhibit the autophagy of aHSCs (2) Vit A specifically binds to RBPR on aHSCs	Anti-inflammation	[190]
BMSCs	Hypoxia pre-conditioned	Partial hepatectomy in C57BL/6 mice	miR-182-5p	Suppress FOXO1/TLR4 axis	Liver regeneration	[197]
BMSCs	LPS pre-treated	CLP induced septic liver injury in C57BL/6 mice	ATG2B	Inhibit macrophage STING signaling	Anti-inflammation	[198]
BMSCs	IFN-γ pre-treated	CCl4-induced liver fibrosis in CX3CR1-EGFP mice	–	Increase the abundance of Treg cells and M2 macrophage	Anti-inflammation	[199]
BMSCs	SiGRP78 overexpressed	HepG2-SR/PLC-SR infected Balb/c nude mice	SiGRP78	Downregulate the expression of GRP78	Enhanced chemo-sensitivity of HCC	[200]
AMSCs	MiR-199a lentivirus infection	PLC/PRF/5 liver orthotopic HCC model in Balb/c nude mice	miR-199a-3p	Suppress mTOR signaling	Enhanced chemo-sensitivity of HCC	[201]
hUCMSCs	Sonication	CCl4-induced liver fibrosis in Balb/c mice	OCA	Activate FXR-Cyp7a1 cascade	ECM remodeling	[203]
BMSCs	Sonication	CCl4-induced liver fibrosis in SD rat	LUT	–	Anti-inflammation	[204]
hUCMSCs	Plasmid transfection	CCl4-induced liver fibrosis in ICR mice	miR-4465	Suppress LOXL2 to prevent HSC activation and collagen expression	Anti-inflammation	[205]
BMSCs	Plasmid transfection	MCD model in rice	miR-124-3p	Downregulate Steap3 to inhibit ferroptosis	Protection of hepatocytes	[207]

ADAM10 ADAM metallopeptidase domain 10, *aHSC* activated hepatic stellate cell, *AMSC* adipose tissue-derived MSC, *ATG2B* autophagy-related 2B cysteine peptidase, *BMSC* bone marrow-derived MSC, *CCL2* C–C motif chemokine ligand 2, *CCNG1* cyclin G1, *CLP* cecal ligation and puncture, *DEX* dexamethasone, *ECM* extracellular matrix, *FXR* Farnesoid X receptor, *HCC* hepatocellular carcinoma, *HCQ* hydroxychloroquine, *hUCMSC* human umbilical cord-derived MSC, *IFN-γ* interferon-γ, *IGF1R* insulin-like growth factor receptor 1, *Lamp2b* lysosomal associated membrane protein 2, *LOXL2* lysyl oxidase-like protein 2, *LPS* lipopolysaccharide, *LUT* luteolin, *MCD* methionine- and choline deficient, *MSC* mesenchymal stem cell, *MSC-Exos* MSC-derived exosomes, *NCTD* norcantharidin, *NF-κB* nuclear factor kappa-B, *OCA* obeticholic acid, *RBPR* retinol-binding protein receptor, *ROS* reactive oxygen species, *SR*, sorafenib resistance *STING* stimulator of interferon gene, *TAA* thioacetamide

synchronously ensuring sustained release, homing properties, and systemic security [181]. In addition, GRP78 is known to be overexpressed to facilitate resistance against Sorafenib in HCC, therefore, MSC-Exos with siGRP78 may reverse such resistance [200]. Complementary approaches employ such bioengineered platforms to overcome drug resistance through overexpressing miR-199a in parental AMSCs. These miRNAs can induce cell cycle arrest and metastasis suppression while restoring chemosensitivity to conventional regimens like DOX or sorafenib [201].

Fibrosis: HSC/ECM remodeling

Engineered MSC-Exos can achieve multimodal antifibrotic intervention through molecular editing of HSC biology. Such strategies synergize MSC-Exos with potent FXR agonist OCA or the plant flavonoid LUT to induce HSC quiescence, resulting in the improvement of liver fibrosis [202]. Mechanistically, combinatorial OCA/LUT-encapsulated exosomal systems simultaneously address ECM remodeling (via MMP/TIMP rebalancing) and fibrogenic signaling nodes, perfectly achieving targeted antifibrotic effects [203, 204]. Additionally, assembled by sonication, miRNA-engineered variants composed of miR4465 and MSC-Exos demonstrate collagenolytic specificity, suppressing LOXL2-driven ECM crosslinking, which establishes engineered MSC-Exos as precision agents for hepatic stromal reprogramming [205].

HIRI: oxidative stress & ferroptosis defense

HIRI in grafts exemplifies a ferroptosis-dependent pathogenesis where lipid peroxidation cascades intersect with oxidative stress amplification [206]. Engineered MSC-Exos counteract this dual assault through precision

molecular interception. HO-1-modified variants deliver miRNA-124-3p to disrupt STEAP3-mediated iron dyshomeostasis, conferring cytoprotection in marginal liver grafts [207]. Complementary redox engineering strategies employ NaHS-biofunctionalized vesicles that simultaneously neutralize ROS/MDA/8-OH-dG triad. They also stabilize MALAT1-mediated mitochondrial apoptosis checkpoints via NF-κB/caspase-3 suppression [208]. These engineering exosomes demonstrate curative capacity to HIRI, establishing a predominant approach for expanding donor organ viability.

Translation: challenges and outlook

Despite the promising future of engineered MSC-Exos, there is a distance from meaningful clinical usage. The very first step is to standardize manufacturing using scalable methods. Except for improvement in industrial production procedure, more practical and credible assessment standards should be constructed, such as using potency for anti-aHSC or reducing α-SMA to evaluate the quality of the drug. Critical quality attributes and critical process parameters should also be early defined to meet contradictory application scenarios and improve scalability [209]. Moreover, humanized liver mouse models can be introduced in preclinical studies to provide more convincing data [210].

Status and prospects

The global hepatology landscape faces a therapeutic imperative driven by escalating liver disease burdens, particularly in high-prevalence regions like China. While MSCs offer regenerative and immunomodulatory potential, their clinical limitations, including oncogenic risks, pulmonary entrapment, and immune rejection, have shifted attention to MSC-Exos as next-generation acellular therapeutics. These vesicles' negligible immunogenicity enables broad compatibility, while their smaller size allows for low thrombosis risk and supports liver-tropic biodistribution. Moreover, MSC-Exos also eliminate tumorigenicity risks inherent to cellular proliferation. Their multimodal therapeutic architecture integrates immunomodulation, redox homeostasis, and metabolic reprogramming via bioactive cargo delivery mechanisms, supporting their use as engineerable platforms for liver therapy management.

Nevertheless, the clinical translation of MSC-Exos confronts several practical challenges, ranging from heterogeneity to hurdles in production and transportation. Manufacturing heterogeneity stemming from unstandardized bioprocessing protocols can alter product features of MSC-Exos across different batches or sources, while optimized isolation and purification techniques may achieve greater consistency. Additionally, despite the chance of disrupting exosomal structure, engineering approaches (pre- and post-isolation modification) are ideal methods to ensure the consistency of MSC-Exos. By contrast, pre-isolation methods offer flexibility and convenience, whereas post-isolation techniques are highly dependent on environmental control, introducing considerable instability in manufacturing.

Regarding the large-scale production of MSC-Exos, despite hUCMSCs' initial scalability advantages, their finite replicative lifespan imposes critical bioproduction bottlenecks. Emerging biomanufacturing innovations (e.g., hydrodynamic fractionation, 3D cultivation, immortalized MSC lineages) demonstrate significant enhancements while maintaining vesicular integrity [211, 212]. Moreover, MSC-Exos exhibit higher environmental sensitivity and lower adaptability than MSCs, thus parallel stabilization strategies employing cryoencapsulation within synthetic matrices can remarkably extend functional half-life under physiological conditions [213]. This technological convergence is being operationalized through standardization frameworks such as China's CRHA 001–2021, which codifies critical quality attributes from vesicle identity profiling to therapeutic potency validation, establishing a global benchmark for acellular therapeutic biomanufacturing.

As the main focus of this review, the mechanisms of diverse exosomal biomolecules are completely elucidated respectively, which can act synergistically or antagonistically. The paradoxical effects of MSC-Exos in hepatology manifest through context-dependent bioactivity, like performing both pro- and anti-cancer properties, or supporting hepatocyte survival with concurrent HSC inactivation. This mechanistic duality necessitates multi-omics deconvolution of the evolutionary conserved miRNA/protein interactome governing hepatic microenvironmental crosstalk. Precision engineering frameworks now enable selective enrichment of desired cargo coupled with antagonistic molecule depletion. Despite these advances, clinical translation remains in translational nascency, underscoring the imperative for mechanistic clarity before therapeutic scale-up. There is only a Phase II trial currently evaluating hUCMSC-Exos interventions in DLC (NCT05871463), planning n = 20 adults with 40 mg hUCMSC-Exos over 3 weeks alongside standard care, whose outcomes include Child–Pugh scores and laboratory measures (ALT, AST, total bilirubin, INR).

In summary, MSC-Exos epitomize a landmark advance for hepatology, enabling simultaneous microenvironmental reprogramming and cellular fate determination. While preclinical validation has illuminated conserved mechanisms, translational goals including manufacturing harmonization, batch-to-batch stabilization, and clinical-grade scalability should also be prioritized to achieve, requiring coordinated work in process standardization and engineering. With their therapeutic promise,

MSC-Exos are uniquely capable of interacting with hepatic biomolecular networks at the nanoscale, standing poised to redefine precision medicine in hepatology.

Abbreviations

ACLF	Acute-on-chronic liver failure
AFMSC	Amniotic fluid-derived MSC
AKT	Protein kinase B
ALF	Acute liver failure
ALI	Acute liver injury
ALIX	ALG-2-interacting protein X
ALOX5	Arachidonate 5-lipoxygenase
AMPK	AMP-activated protein kinase
AMSC	Adipose tissue-derived MSC
ANXA1	Annexin A1
ASC	Apoptosis-associated speck-like protein
ATF6	Activating transcription factor 6
ATG2/4B	Autophagy-related 2/4B cysteine peptidase
BAX	Bcl-2 associated X protein
Bcl-2	B-cell lymphoma-2
BECN1	Beclin 1
BMSC	Bone marrow-derived MSC
CAF	Cancer-associated fibroblast
CAMKK1	Calcium/calmodulin dependent protein kinase kinase 1
CCL21	C–C motif chemokine ligand 21
CCNG1	Cyclin G1
CCR7	C–C motif chemokine receptor 7
CHOP	C/EBP homology protein
circRNA	Circular RNA
COX2	Cyclooxygenase-2
CRHA	Chinese Research Hospital Association
CRISPR	Clustered regularly interspaced short palindromic repeats
CSC	Cancer stem cell
DAMP	Damage-associated molecular pattern
DC	Dendritic cell
DDX5	DEAD-Box helicase 5
DEX	Dexamethasone
DLC	Decompensated liver cirrhosis
DOX	Doxorubicin
DUSP1	Dual specificity phosphatase 1
E2F1	E2F transcription factor 1
ECM	Extracellular matrix
EMT	Epithelial-mesenchymal transition
EpCAM	Epithelial cell adhesion molecule
ER	Endoplasmic reticulum
ERK	Extracellular regulated protein kinase
ESCRT	Endosomal sorting complex required for transport
ESE	Early-sorting endosome
ESLD	End-stage liver disease
EV	Extracellular vesicle
FASn	Fatty acid synthase
Foxg1	Forkhead box G1
FOXO1	Forkhead box transcription factor 1
FXR	Farnesoid X receptor
GAS6	Growth arrest-specific protein 6
GFP	Good Manufacturing Practice
GPC3	Glypican-3
GPX1/4	Glutathione peroxidase 1/4
GRP78	Glucose-regulated protein 78
GSDMD	Gasdermin D
GSH	Glutathione
HCC	Hepatocellular carcinoma
HCQ	Hydroxychloroquine
HGF	Hepatocyte growth factor
Hh	Hedgehog
HIRI	Hepatic IRI
HO-1	Heme oxygenase-1
hPMSC	Human PMSC
HSC	Hepatic stellate cell
HSP	Heat-shock protein
HTSP1	Human thrombospondin 1
hUCMSC	Human UCMSC
IFN-γ	Interferon-γ
IGF1R	Insulin like growth factor 1 receptor
IKK	Inhibitor of kappa B kinase
IL	Interleukin
ILV	Intraluminal vesicle
iPSC	Induced pluripotent stem cell
IRE1α	Inositol-requiring enzyme 1α
IRI	Ischemia–reperfusion injury
ISEV	International Society for Extracellular Vesicles
IκBζ	Inhibitor of nuclear factor kappa B zeta
KAT2B	Lysine acetyltransferase 2B
KC	Kupffer cell
Keap1	Kelch-like ECH associated protein 1
Lamp2b	Lysosomal associated membrane protein 2
LIMK1	LIM domain kinase 1
lncRNA	Long non-coding RNA
LOXL2	LOX ligand 2
LPS	Lipopolysaccharide
LTF	Lactotransferrin
LUT	Luteolin
MAFLD	Metabolic dysfunction-associated fatty liver disease
MALAT1	Metastasis-associated lung adenocarcinoma transcript 1
MAP4K3	Mitogen-activated protein kinase kinase kinase kinase 3
MAPK	Mitogen-activated protein kinase
MCD	Methionine- and choline deficient
MDA	Malondialdehyde
MerTK	MER proto-oncogene tyrosine kinase
MFGE8	Milk fat globule-EGF factor 8
MHC	Major histocompatibility complex
miRNA	MicroRNA
MISEV	Minimal Information for Studies of Extracellular Vesicles
MMP	Matrix metalloproteinase
MSC	Mesenchymal stem cell
MSC-Exos	MSC-derived exosomes
MTF-1	Metal-regulatory transcription factor-1
mTOR	Mammalian target of rapamycin
MVB	Multivesicular body
NAFLD	Non-alcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NET	Neutrophil extracellular trap
NF-κB	Nuclear factor kappa-B
NIR-II	The secend near infrared
NLRP3	NOD-like receptor thermal protein domain associated protein 3
NQO-1	NAD(P)H quinone oxidoreductase 1
Nrf2	Nuclear factor E2-related factor 2
OCA	Obeticholic acid
OTUB1	OTU domain-containing ubiquitin aldehyde-binding protein 1
PAMP	Pathogen-associated molecular pattern
PCD	Programmed cell death
PEG	Polyethylene glycol
PERK	Protein kinase R-like ER kinase
PH	Partial hepatectomy
PI3K	Phosphoinositide-3-kinase
PIK3r1	PI3K regulatory subunit 1
PMSC	Placental-derived MSC
PPARα	Peroxisome proliferator-activated receptor alpha
PTEN	Phosphatase and tensin homologue
ROS	Reactive oxygen species
scFv	Single-chain fragment variable
scRNA-seq	Single cell RNA sequencing
SKP2	S-Phase kinase-associated protein 2
SLC7A11	Solute carrier family 7 member 11
SLIT3	Slit guidance ligand 3
SMO	Smoothened; SOD, superoxide dismutase
SREBP-1C	Sterol regulatory element-binding protein 1C
STAT3	Signal transducer and activator of transcription 3
STEAP3	Six-transmembrane epithelial antigen of prostate 3
STING	Stimulator of interferon gene
TFEB	Transcription factor EB
TGF-β	Transforming growth factor-β
TGN	Trans-Golgi network

Th17	T helper cell 17
TIMP	Tissue inhibitors of metalloproteinase
TLR	Toll-like receptor
TME	Tumor microenvironment
Treg	Regulatory T cell
TSG101	Tumor susceptibility gene 101
TXNIP	Thioredoxin interacting protein
UCMSC	Umbilical cord-derived MSC
VA	Vitamin A
WHO	World Health Organization
YAP	Yes-associated protein

Author contributions
Shihang Yu and Defu Kong designed the review and prepared Figs. 1, 2, 3. Shihang Yu, Beike Lu, and Yuan Fu synthesized and analyzed relevant studies. Shihang Yu and Beike Lu drafted the manuscript. Defu Kong, Zhicong Zhao, Yixiao Pan, and Zhaokai Zeng provided feedback and guidance. Kang He, Ruqi Tang, and Qiang Xia revised the manuscript. All authors approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

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References

1. Asrani SK, Devarbhavi H, Eaton J, et al. Burden of liver diseases in the world. J Hepatol. 2019;70(1):151–71.
2. Keam SJ. Resmetirom: first approval. Drugs. 2024;84(6):729–35.
3. Carmona C, Claxton L, O'Brien A, et al. Cirrhosis in over 16s: assessment and management-updated summary of NICE guidance. BMJ. 2023;383:2598.
4. Samuel D, Coilly A. Management of patients with liver diseases on the waiting list for transplantation: a major impact to the success of liver transplantation. BMC Med. 2018;16(1):113.
5. Uccelli A, Laroni A, Ali R, et al. Safety, tolerability, and activity of mesenchymal stem cells versus placebo in multiple sclerosis (MESEMS): a phase 2, randomised, double-blind crossover trial. Lancet Neurol. 2021;20(11):917–29.
6. Gazdic M, Arsenijevic A, Markovic BS, et al. Mesenchymal stem cell-dependent modulation of liver diseases. Int J Biol Sci. 2017;13(9):1109–17.
7. Christ B, BRüCKNER S, Winkler S. The therapeutic promise of mesenchymal stem cells for liver restoration. Trends Mol Med. 2015;21(11):673–86.
8. Ridge SM, Sullivan FJ, Glynn SA. Mesenchymal stem cells: key players in cancer progression. Mol Cancer. 2017;16(1):31.
9. Gholamrezanezhad A, Mirpour S, Bagheri M, et al. In vivo tracking of ^{111}In-oxine labeled mesenchymal stem cells following infusion in patients with advanced cirrhosis. Nucl Med Biol. 2011;38(7):961–7.
10. Rombouts WJ, Ploemacher RE. Primary murine MSC show highly efficient homing to the bone marrow but lose homing ability following culture. Leukemia. 2003;17(1):160–70.
11. Kholodenko IV, Kholodenko RV, Yarygin KN. The crosstalk between mesenchymal stromal/stem cells and hepatocytes in homeostasis and under stress. Int J Mol Sci. 2023. https://doi.org/10.3390/ijms242015212.
12. Volarevic V, Nurkovic J, Arsenijevic N, et al. Concise review: Therapeutic potential of mesenchymal stem cells for the treatment of acute liver failure and cirrhosis. Stem Cells. 2014;32(11):2818–23.
13. Kalluri R, Lebleu VS. The biology, function, and biomedical applications of exosomes. Science. 2020. https://doi.org/10.1126/science.aau6977.
14. Xunian Z, Kalluri R. Biology and therapeutic potential of mesenchymal stem cell-derived exosomes. Cancer Sci. 2020;111(9):3100–10.
15. Pan Y, Wu W, Jiang X, et al. Mesenchymal stem cell-derived exosomes in cardiovascular and cerebrovascular diseases: from mechanisms to therapy. Biomed Pharmacother. 2023;163:114817.
16. Feng Y, Guo K, Jiang J, et al. Mesenchymal stem cell-derived exosomes as delivery vehicles for non-coding RNAs in lung diseases. Biomed Pharmacother. 2024;170:116008.
17. Ren S, Lin Y, Liu W, et al. MSC-Exos: Important active factor of bone regeneration. Front Bioeng Biotechnol. 2023;11:1136453.
18. Gang D, Yu CJ, Zhu S, et al. Application of mesenchymal stem cell-derived exosomes in kidney diseases. Cell Immunol. 2021;364:104358.
19. Lou G, Chen Z, Zheng M, et al. Mesenchymal stem cell-derived exosomes as a new therapeutic strategy for liver diseases. Exp Mol Med. 2017;49(6):e346.
20. Wiklander OP, Nordin JZ, O'loughlin A, et al. Extracellular vesicle in vivo biodistribution is determined by cell source, route of administration and targeting. J Extracell Vesicles. 2015;4:26316.
21. Rao D, Huang D, Sang C, et al. Advances in mesenchymal stem cell-derived exosomes as drug delivery vehicles. Front Bioeng Biotechnol. 2021;9:797359.
22. Elsharkasy OM, Nordin JZ, Hagey DW, et al. Extracellular vesicles as drug delivery systems: why and how? Adv Drug Deliv Rev. 2020;159:332–43.
23. Van Niel G, D'angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol. 2018;19(4):213–28.
24. El Andaloussi S, Mäger I, Breakefield XO, et al. Extracellular vesicles: biology and emerging therapeutic opportunities. Nat Rev Drug Discov. 2013;12(5):347–57.
25. Ding J, Wang J, Chen J. Exosomes as therapeutic vehicles in liver diseases. Ann Transl Med. 2021;9(8):735.
26. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol. 2013;200(4):373–83.
27. McAndrews KM, Kalluri R. Mechanisms associated with biogenesis of exosomes in cancer. Mol Cancer. 2019;18(1):52.
28. Mathieu M, Martin-Jaular L, Lavieu G, et al. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. Nat Cell Biol. 2019;21(1):9–17.
29. Hanson PI, Cashikar A. Multivesicular body morphogenesis. Annu Rev Cell Dev Biol. 2012;28:337–62.
30. Andreu Z, YáñEZ-Mó M. Tetraspanins in extracellular vesicle formation and function. Front Immunol. 2014;5:442.
31. Welsh JA, Goberdhan DCI, O'driscoll L, et al. Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. J Extracell Vesicles. 2024;13(2):e12404.
32. De Sousa KP, Rossi I, Abdullahi M, et al. Isolation and characterization of extracellular vesicles and future directions in diagnosis and therapy. WIREs Nanomed Nanobiotechnol. 2023;15(1):e1835.
33. Witwer KW, BUZáS EI, Bemis LT, et al. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. J Extracell Vesicles. 2013. https://doi.org/10.3402/jev.v2i0.20360.
34. Robinson SD, Samuels M, Jones W, et al. Confirming size-exclusion chromatography as a clinically relevant extracellular vesicles separation method from 1mL plasma through a comprehensive comparison of methods. BMC Methods. 2024;1(1):7.
35. Benedikter BJ, Bouwman FG, Vajen T, et al. Ultrafiltration combined with size exclusion chromatography efficiently isolates extracellular vesicles from cell culture media for compositional and functional studies. Sci Rep. 2017;7(1):15297.
36. Lo TW, Zhu Z, Purcell E, et al. Microfluidic device for high-throughput affinity-based isolation of extracellular vesicles. Lab Chip. 2020;20(10):1762–70.
37. Joo HS, Suh JH, Lee HJ, et al. Current knowledge and future perspectives on mesenchymal stem cell-derived exosomes as a new therapeutic agent. Int J Mol Sci. 2020. https://doi.org/10.3390/ijms21030727.
38. Sivanantham A, Jin Y. Impact of storage conditions on EV integrity/surface markers and cargos. Life. 2022. https://doi.org/10.3390/life12050697.
39. Evtushenko EG, Bagrov DV, Lazarev VN, et al. Adsorption of extracellular vesicles onto the tube walls during storage in solution. PLoS ONE. 2020;15(12):e0243738.
40. GöRGENS A, Corso G, Hagey DW, et al. Identification of storage conditions stabilizing extracellular vesicles preparations. J Extracell Vesicles. 2022;11(6):e12238.
41. LöTVALL J, Hill AF, Hochberg F, et al. Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement

from the International Society for Extracellular Vesicles. J Extracell Vesicles. 2014;3:26913.
42. Gurunathan S, Kang MH, Jeyaraj M, et al. Review of the isolation, characterization, biological function, and multifarious therapeutic approaches of exosomes. Cells. 2019. https://doi.org/10.3390/cells8040307.
43. Kreimer S, Belov AM, Ghiran I, et al. Mass-spectrometry-based molecular characterization of extracellular vesicles: lipidomics and proteomics. J Proteome Res. 2015;14(6):2367–84.
44. Pegtel DM, Gould SJ. Exosomes. Annu Rev Biochem. 2019;88:487–514.
45. He C, Zheng S, Luo Y, et al. Exosome theranostics: biology and translational medicine. Theranostics. 2018;8(1):237–55.
46. Valadi H, EKSTRöM K, Bossios A, et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007;9(6):654–9.
47. Huang Z, Zhou JK, Peng Y, et al. The role of long noncoding RNAs in hepatocellular carcinoma [J]. Mol Cancer. 2020;19(1):77.
48. Goodall GJ, Wickramasinghe VO. RNA in cancer. Nat Rev Cancer. 2021;21(1):22–36.
49. Iqbal Z, Rehman K, Mahmood A, et al. Exosome for mRNA delivery: strategies and therapeutic applications. J Nanobiotechnology. 2024;22(1):395.
50. Nojima H, Freeman CM, Schuster RM, et al. Hepatocyte exosomes mediate liver repair and regeneration via sphingosine-1-phosphate. J Hepatol. 2016;64(1):60–8.
51. Liu J, Ren L, Li S, et al. The biology, function, and applications of exosomes in cancer. Acta Pharm Sin B. 2021;11(9):2783–97.
52. Gurung S, Perocheau D, Touramanidou L, et al. The exosome journey: from biogenesis to uptake and intracellular signalling. Cell Commun Signal. 2021;19(1):47.
53. Yáñez-Mó M, Siljander PR, Andreu Z, et al. Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicles. 2015;4:27066.
54. Schorey JS, Harding CV. Extracellular vesicles and infectious diseases: new complexity to an old story. J Clin Invest. 2016;126(4):1181–9.
55. Stuendl A, Kunadt M, Kruse N, et al. Induction of α-synuclein aggregate formation by CSF exosomes from patients with Parkinson's disease and dementia with Lewy bodies. Brain. 2016;139:481–94.
56. Xiao D, Barry S, Kmetz D, et al. Melanoma cell-derived exosomes promote epithelial-mesenchymal transition in primary melanocytes through paracrine/autocrine signaling in the tumor microenvironment. Cancer Lett. 2016;376(2):318–27.
57. Masyuk AI, Masyuk TV, Larusso NF. Exosomes in the pathogenesis, diagnostics and therapeutics of liver diseases. J Hepatol. 2013;59(3):621–5.
58. Ma N, Liu Y, Chen D, et al. In vivo imaging of exosomes labeled with NIR-II polymer dots in liver-injured mice. Biomacromol. 2022;23(11):4825–33.
59. Villa F, Quarto R, Tasso R. Extracellular vesicles as natural, safe and efficient drug delivery systems. Pharmaceutics. 2019. https://doi.org/10.3390/pharmaceutics11110557.
60. Dennis JE, Carbillet JP, Caplan AI, et al. The STRO-1+ marrow cell population is multipotential. Cells Tissues Organs. 2002;170(2–3):73–82.
61. Potten CS, Loeffler M. Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. Development. 1990;110(4):1001–20.
62. Li N, Hua J. Interactions between mesenchymal stem cells and the immune system. Cell Mol Life Sci. 2017;74(13):2345–60.
63. Lan T, Luo M, Wei X. Mesenchymal stem/stromal cells in cancer therapy. J Hematol Oncol. 2021;14(1):195.
64. Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: immune evasive, not immune privileged. Nat Biotechnol. 2014;32(3):252–60.
65. Yu S, Yu S, Liu H, et al. Enhancing mesenchymal stem cell survival and homing capability to improve cell engraftment efficacy for liver diseases. Stem Cell Res Ther. 2023;14(1):235.
66. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat Rev Immunol. 2008;8(9):726–36.
67. Murphy MB, Moncivais K, Caplan AI. Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. Exp Mol Med. 2013;45(11):e54.
68. Shi M, Li YY, Xu RN, et al. Mesenchymal stem cell therapy in decompensated liver cirrhosis: a long-term follow-up analysis of the randomized controlled clinical trial. Hepatol Int. 2021;15(6):1431–41.
69. Chen Q, Jin M, Wang S, et al. Establishing an hTERT-driven immortalized umbilical cord-derived mesenchymal stem cell line and its therapeutic application in mice with liver failure. J Tissue Eng. 2023;14:20417314231200330.
70. Cesana D, Ranzani M, Volpin M, et al. Uncovering and dissecting the genotoxicity of self-inactivating lentiviral vectors *in vivo*. Mol Ther. 2014;22(4):774–85.
71. Gurumoorthy N, Nordin F, Tye GJ, et al. Non-integrating lentiviral vectors in clinical applications: a glance through. Biomedicines. 2022;10(1):107.
72. Antoon R, Overdevest N, Saleh AH, et al. Mesenchymal stromal cells as cancer promoters. Oncogene. 2024;43(49):3545–55.
73. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.
74. Rasmusson I, RINGDéN O, Sundberg B, et al. Mesenchymal stem cells inhibit lymphocyte proliferation by mitogens and alloantigens by different mechanisms. Exp Cell Res. 2005;305(1):33–41.
75. Paunescu V, Bojin FM, Tatu CA, et al. Tumour-associated fibroblasts and mesenchymal stem cells: more similarities than differences. J Cell Mol Med. 2011;15(3):635–46.
76. Vassilopoulos G, Wang PR, Russell DW. Transplanted bone marrow regenerates liver by cell fusion. Nature. 2003;422(6934):901–4.
77. Rani S, Ryan AE, Griffin MD, et al. Mesenchymal stem cell-derived extracellular vesicles: toward cell-free therapeutic applications. Mol Ther. 2015;23(5):812–23.
78. Toma C, Pittenger MF, Cahill KS, et al. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation. 2002;105(1):93–8.
79. Lee RH, Pulin AA, Seo MJ, et al. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell. 2009;5(1):54–63.
80. RINGDéN O, Uzunel M, Rasmusson I, et al. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. Transplantation. 2006;81(10):1390–7.
81. Caplan AI. Mesenchymal stem cells: time to change the name! Stem Cells Transl Med. 2017;6(6):1445–51.
82. Cai J, Wu J, Wang J, et al. Extracellular vesicles derived from different sources of mesenchymal stem cells: therapeutic effects and translational potential. Cell Biosci. 2020;10:69.
83. Wang ZG, He ZY, Liang S, et al. Comprehensive proteomic analysis of exosomes derived from human bone marrow, adipose tissue, and umbilical cord mesenchymal stem cells. Stem Cell Res Ther. 2020;11(1):511.
84. Hade MD, Suire CN, Suo Z. Mesenchymal stem cell-derived exosomes: applications in regenerative medicine. Cells. 2021. https://doi.org/10.3390/cells10081959.
85. Yoon YM, Lee JH, Song KH, et al. Melatonin-stimulated exosomes enhance the regenerative potential of chronic kidney disease-derived mesenchymal stem/stromal cells via cellular prion proteins. J Pineal Res. 2020;68(3):e12632.
86. Zhang Y, Chopp M, Meng Y, et al. Effect of exosomes derived from multipluripotent mesenchymal stromal cells on functional recovery and neurovascular plasticity in rats after traumatic brain injury. J Neurosurg. 2015;122(4):856–67.
87. Chevillet JR, Kang Q, Ruf IK, et al. Quantitative and stoichiometric analysis of the microRNA content of exosomes. Proc Natl Acad Sci U S A. 2014;111(41):14888–93.
88. Kowal J, Arras G, Colombo M, et al. Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. Proc Natl Acad Sci U S A. 2016;113(8):E968–77.
89. Yuan O, Lin C, Wagner J, et al. Exosomes derived from human primed mesenchymal stem cells induce mitosis and potentiate growth factor secretion. Stem Cells Dev. 2019;28(6):398–409.
90. Du Z, Wu T, Liu L, et al. Extracellular vesicles-derived miR-150-5p secreted by adipose-derived mesenchymal stem cells inhibits CXCL1 expression to attenuate hepatic fibrosis. J Cell Mol Med. 2021;25(2):701–15.
91. Shen Z, Huang W, Liu J, et al. Effects of mesenchymal stem cell-derived exosomes on autoimmune diseases. Front Immunol. 2021;12:749192.
92. Chen TS, Lai RC, Lee MM, et al. Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs. Nucleic Acids Res. 2010;38(1):215–24.
93. Lai RC, Yeo RW, Lim SK. Mesenchymal stem cell exosomes. Semin Cell Dev Biol. 2015;40:82–8.
94. Wang C, Zhou H, Wu R, et al. Mesenchymal stem cell-derived exosomes and non-coding RNAs: regulatory and therapeutic role in liver diseases. Biomed Pharmacother. 2023;157:114040.
95. Kang M, Jordan V, Blenkiron C, et al. Biodistribution of extracellular vesicles following administration into animals: a systematic review. J Extracell Vesicles. 2021;10(8):e12085.
96. Zheng M, Tian Z. Liver-mediated adaptive immune tolerance. Front Immunol. 2019;10:2525.
97. Tan CY, Lai RC, Wong W, et al. Mesenchymal stem cell-derived exosomes promote hepatic regeneration in drug-induced liver injury models. Stem Cell Res Ther. 2014;5(3):76.

98. Liang C, Gao S, Gao J, et al. Comparison of effects of HucMSCs, exosomes, and conditioned medium on NASH. Sci Rep. 2023;13(1):18431.
99. Zhang Q, Piao C, Ma H, et al. Exosomes from adipose-derived mesenchymal stem cells alleviate liver ischaemia reperfusion injury subsequent to hepatectomy in rats by regulating mitochondrial dynamics and biogenesis. J Cell Mol Med. 2021;25(21):10152–63.
100. Wisse E, Braet F, Luo D, et al. Structure and function of sinusoidal lining cells in the liver. Toxicol Pathol. 1996;24(1):100–11.
101. Robinson MW, Harmon C, O'Farrelly C. Liver immunology and its role in inflammation and homeostasis. Cell Mol Immunol. 2016;13(3):267–76.
102. Friedman SL, Pinzani M. Hepatic fibrosis 2022: unmet needs and a blueprint for the future. Hepatology. 2022;75(2):473–88.
103. Caplan AI, Correa D. The MSC: an injury drugstore. Cell Stem Cell. 2011;9(1):11–5.
104. Shao M, Xu Q, Wu Z, et al. Exosomes derived from human umbilical cord mesenchymal stem cells ameliorate IL-6-induced acute liver injury through miR-455-3p. Stem Cell Res Ther. 2020;11(1):37.
105. Woolbright BL, Jaeschke H. Role of the inflammasome in acetaminophen-induced liver injury and acute liver failure. J Hepatol. 2017;66(4):836–48.
106. Liu Y, Lou G, Li A, et al. AMSC-derived exosomes alleviate lipopolysaccharide/d-galactosamine-induced acute liver failure by miR-17-mediated reduction of TXNIP/NLRP3 inflammasome activation in macrophages. EBioMedicine. 2018;36:140–50.
107. Lefere S, Tacke F. Macrophages in obesity and non-alcoholic fatty liver disease: crosstalk with metabolism. JHEP Rep. 2019;1(1):30–43.
108. Shi Y, Yang X, Wang S, et al. Human umbilical cord mesenchymal stromal cell-derived exosomes protect against MCD-induced NASH in a mouse model. Stem Cell Res Ther. 2022;13(1):517.
109. Tian S, Zhou X, Zhang M, et al. Mesenchymal stem cell-derived exosomes protect against liver fibrosis via delivering miR-148a to target KLF6/STAT3 pathway in macrophages. Stem Cell Res Ther. 2022;13(1):330.
110. Reis M, Mavin E, Nicholson L, et al. Mesenchymal stromal cell-derived extracellular vesicles attenuate dendritic cell maturation and function. Front Immunol. 2018;9:2538.
111. Lu T, Zhang J, Cai J, et al. Extracellular vesicles derived from mesenchymal stromal cells as nanotherapeutics for liver ischaemia-reperfusion injury by transferring mitochondria to modulate the formation of neutrophil extracellular traps. Biomaterials. 2022;284:121486.
112. Chen W, Lin F, Feng X, et al. MSC-derived exosomes attenuate hepatic fibrosis in primary sclerosing cholangitis through inhibition of Th17 differentiation. Asian J Pharm Sci. 2024;19(1):100889.
113. Faggioli F, Palagano E, di Tommaso L, et al. B lymphocytes limit senescence-driven fibrosis resolution and favor hepatocarcinogenesis in mouse liver injury. Hepatology. 2018;67(5):1970–85.
114. Feng X, Feng B, Zhou J, et al. Mesenchymal stem cells alleviate mouse liver fibrosis by inhibiting pathogenic function of intrahepatic B cells. Hepatology. 2024. https://doi.org/10.1097/HEP.0000000000000831.
115. di Bonzo LV, Ferrero I, Cravanzola C, et al. Human mesenchymal stem cells as a two-edged sword in hepatic regenerative medicine: engraftment and hepatocyte differentiation versus profibrogenic potential. Gut. 2008;57(2):223–31.
116. Meng XM, Nikolic-Paterson DJ, Lan HY. TGF-β: the master regulator of fibrosis. Nat Rev Nephrol. 2016;12(6):325–38.
117. Omenetti A, Choi S, Michelotti G, et al. Hedgehog signaling in the liver. J Hepatol. 2011;54(2):366–73.
118. Kim J, Lee C, Shin Y, et al. SEvs from tonsil-derived mesenchymal stromal cells alleviate activation of hepatic stellate cells and liver fibrosis through miR-486-5p. Mol Ther. 2021;29(4):1471–86.
119. Hyun J, Wang S, Kim J, et al. Microrna125b-mediated Hedgehog signaling influences liver regeneration by chorionic plate-derived mesenchymal stem cells. Sci Rep. 2015;5:14135.
120. Yuan M, Yao L, Chen P, et al. Human umbilical cord mesenchymal stem cells inhibit liver fibrosis via the microRNA-148a-5p/SLIT3 axis. Int Immunopharmacol. 2023;125:111134.
121. Ma L, Wei J, Zeng Y, et al. Mesenchymal stem cell-originated exosomal circDIDO1 suppresses hepatic stellate cell activation by miR-141-3p/PTEN/AKT pathway in human liver fibrosis. Drug Deliv. 2022;29(1):440–53.
122. Zheng W, Bian S, Qiu S, et al. Placenta mesenchymal stem cell-derived extracellular vesicles alleviate liver fibrosis by inactivating hepatic stellate cells through a miR-378c/SKP2 axis. Inflamm Regen. 2023;43(1):47.
123. Esler WP, Cohen DE. Pharmacologic inhibition of lipogenesis for the treatment of NAFLD. J Hepatol. 2024;80(2):362–77.
124. Fuchs CD, Simbrunner B, Baumgartner M, et al. Bile acid metabolism and signalling in liver disease. J Hepatol. 2025;82(1):134–53.
125. Lu SC. Antioxidants in the treatment of chronic liver diseases: why is the efficacy evidence so weak in humans? Hepatology. 2008;48(5):1359–61.
126. Wang B, Van Veldhoven PP, Brees C, et al. Mitochondria are targets for peroxisome-derived oxidative stress in cultured mammalian cells. Free Radic Biol Med. 2013;65:882–94.
127. George J, Lu Y, Tsuchishima M, et al. Cellular and molecular mechanisms of hepatic ischemia-reperfusion injury: the role of oxidative stress and therapeutic approaches. Redox Biol. 2024;75:103258.
128. Arroyave-Ospina JC, Wu Z, Geng Y, et al. Role of oxidative stress in the pathogenesis of non-alcoholic fatty liver disease: implications for prevention and therapy. Antioxidants. 2021. https://doi.org/10.3390/antiox10020174.
129. Al Saihati HA, Badr OA, Dessouky AA, et al. Exploring the cytoprotective role of mesenchymal stem cell-derived exosomes in chronic liver fibrosis: insights into the Nrf2/Keap1/p62 signaling pathway. Int Immunopharmacol. 2024;141:112934.
130. Yan Y, Jiang W, Tan Y, et al. hucMSC exosome-derived GPX1 is required for the recovery of hepatic oxidant injury. Mol Ther. 2017;25(2):465–79.
131. Chen J, Tang S, Ke S, et al. Ablation of long noncoding RNA MALAT1 activates antioxidant pathway and alleviates sepsis in mice. Redox Biol. 2022;54:102377.
132. Cai J, Tang D, Hao X, et al. Mesenchymal stem cell-derived exosome alleviates sepsis- associated acute liver injury by suppressing MALAT1 through microRNA-26a-5p: an innovative immunopharmacological intervention and therapeutic approach for sepsis. Front Immunol. 2023;14:1157793.
133. Younossi ZM. Non-alcoholic fatty liver disease - a global public health perspective. J Hepatol. 2019;70(3):531–44.
134. Mebarki M, Abadie C, Larghero J, et al. Human umbilical cord-derived mesenchymal stem/stromal cells: a promising candidate for the development of advanced therapy medicinal products. Stem Cell Res Ther. 2021;12(1):152.
135. Yang F, Wu Y, Chen Y, et al. Human umbilical cord mesenchymal stem cell-derived exosomes ameliorate liver steatosis by promoting fatty acid oxidation and reducing fatty acid synthesis. JHEP Rep. 2023;5(7):100746.
136. Zhang Z, Shang J, Yang Q, et al. Exosomes derived from human adipose mesenchymal stem cells ameliorate hepatic fibrosis by inhibiting PI3K/Akt/mTOR pathway and remodeling choline metabolism. J Nanobiotechnology. 2023;21(1):29.
137. Pollheimer MJ, Racedo S, Mikels-Vigdal A, et al. Lysyl oxidase-like protein 2 (LOXL2) modulates barrier function in cholangiocytes in cholestasis. J Hepatol. 2018;69(2):368–77.
138. Cheng F, Yang F, Wang Y, et al. Mesenchymal stem cell-derived exosomal miR-27b-3p alleviates liver fibrosis via downregulating YAP/LOXL2 pathway. J Nanobiotechnology. 2023;21(1):195.
139. Huang D, Shen H, Xie F, et al. Role of mesenchymal stem cell-derived exosomes in the regeneration of different tissues. J Biol Eng. 2024;18(1):36.
140. Wu HH, Lee OK. Exosomes from mesenchymal stem cells induce the conversion of hepatocytes into progenitor oval cells. Stem Cell Res Ther. 2017;8(1):117.
141. Zhang J, Lu T, Xiao J, et al. MSC-derived extracellular vesicles as nanotherapeutics for promoting aged liver regeneration. J Control Release. 2023;356:402–15.
142. Psaraki A, Zagoura D, Ntari L, et al. Mfge-8 identified in fetal mesenchymal-stromal-cell-derived exosomes ameliorates acute hepatic failure pathology. iScience. 2023;26(11):108100.
143. Song XJ, Zhang L, Li Q, et al. hUCB-MSC derived exosomal miR-124 promotes rat liver regeneration after partial hepatectomy via downregulating Foxg1. Life Sci. 2021;265:118821.
144. Gong Y, Dai H, Liu W, et al. Exosomes derived from human adipose-derived stem cells alleviate hepatic ischemia-reperfusion (I/R) injury through the miR-183/ALOX5 axis. FASEB J. 2023;37(3):e22782.
145. Miao L, Yu C, Guan G, et al. Extracellular vesicles containing GAS6 protect the liver from ischemia-reperfusion injury by enhancing macrophage efferocytosis via MerTK-ERK-COX2 signaling. Cell Death Discov. 2024;10(1):401.
146. Kim JY, Garcia-Carbonell R, Yamachika S, et al. ER stress drives lipogenesis and steatohepatitis via caspase-2 activation of S1P. Cell. 2018;175(1):133-45.e15.
147. El-Derany MO, Abdelhamid SG. Upregulation of miR-96-5p by bone marrow mesenchymal stem cells and their exosomes alleviate non-alcoholic steatohepatitis: emphasis on caspase-2 signaling inhibition. Biochem Pharmacol. 2021;190:114624.
148. Wang Y, Liu T, Jiao G, et al. Exosomes from adipose-derived mesenchymal stem cells can attenuate liver injury caused by minimally invasive

hemihepatectomy combined with ischemia-reperfusion in minipigs by modulating the endoplasmic reticulum stress response. Life Sci. 2023;321:121618.
149. Mizushima N, Levine B, Cuervo AM, et al. Autophagy fights disease through cellular self-digestion. Nature. 2008;451(7182):1069–75.
150. LóPEZ-OTíN C, Blasco MA, Partridge L, et al. Hallmarks of aging: an expanding universe. Cell. 2023;186(2):243–78.
151. Lin D, Chen H, Xiong J, et al. Mesenchymal stem cells exosomal let-7a-5p improve autophagic flux and alleviate liver injury in acute-on-chronic liver failure by promoting nuclear expression of TFEB. Cell Death Dis. 2022;13(10):865.
152. Zhao S, Liu Y, Pu Z. Bone marrow mesenchymal stem cell-derived exosomes attenuate D-GalN/LPS-induced hepatocyte apoptosis by activating autophagy in vitro. Drug Des Devel Ther. 2019;13:2887–97.
153. Shi J, Zhao Y, Wang K, et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature. 2015;526(7575):660–5.
154. Piao C, Sang J, Kou Z, et al. Effects of exosomes derived from adipose-derived mesenchymal stem cells on pyroptosis and regeneration of injured liver. Int J Mol Sci. 2022. https://doi.org/10.3390/ijms232012065.
155. Wang Y, Piao C, Liu T, et al. Effects of the exosomes of adipose-derived mesenchymal stem cells on apoptosis and pyroptosis of injured liver in miniature pigs. Biomed Pharmacother. 2023;169:115873.
156. Ishimoto T, Nagano O, Yae T, et al. CD44 variant regulates redox status in cancer cells by stabilizing the xCT subunit of system xc(-) and thereby promotes tumor growth. Cancer Cell. 2011;19(3):387–400.
157. Lin F, Chen W, Zhou J, et al. Mesenchymal stem cells protect against ferroptosis via exosome-mediated stabilization of SLC7A11 in acute liver injury. Cell Death Dis. 2022;13(3):271.
158. Tan Y, Huang Y, Mei R, et al. HucMSC-derived exosomes delivered BECN1 induces ferroptosis of hepatic stellate cells via regulating the xCT/GPX4 axis. Cell Death Dis. 2022;13(4):319.
159. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. Lancet. 2018;391(10127):1301–14.
160. Qiao L, Xu Z, Zhao T, et al. Suppression of tumorigenesis by human mesenchymal stem cells in a hepatoma model. Cell Res. 2008;18(4):500–7.
161. Hassanzadeh A, Rahman HS, Markov A, et al. Mesenchymal stem/stromal cell-derived exosomes in regenerative medicine and cancer; overview of development, challenges, and opportunities. Stem Cell Res Ther. 2021;12(1):297.
162. Ding B, Lou W, Fan W, et al. Exosomal miR-374c-5p derived from mesenchymal stem cells suppresses epithelial-mesenchymal transition of hepatocellular carcinoma via the LIMK1-Wnt/β-catenin axis. Environ Toxicol. 2023;38(5):1038–52.
163. Ma YS, Liu JB, Lin L, et al. Exosomal microrna-15a from mesenchymal stem cells impedes hepatocellular carcinoma progression via downregulation of SALL4. Cell Death Discov. 2021;7(1):224.
164. Gu H, Yan C, Wan H, et al. Mesenchymal stem cell-derived exosomes block malignant behaviors of hepatocellular carcinoma stem cells through a lncRNA C5orf66-AS1/microRNA-127-3p/DUSP1/ERK axis. Hum Cell. 2021;34(6):1812–29.
165. Lohitesh K, Chowdhury R, Mukherjee S. Resistance a major hindrance to chemotherapy in hepatocellular carcinoma: an insight. Cancer Cell Int. 2018;18:44.
166. Lyu ZZ, Li M, Yang MY, et al. Exosome-mediated transfer of circRNA563 promoting hepatocellular carcinoma by targeting the microRNA148a-3p/metal-regulatory transcription factor-1 pathway. World J Gastroenterol. 2023;29(46):6060–75.
167. Ha D, Yang N, Nadithe V. Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges. Acta Pharm Sin B. 2016;6(4):287–96.
168. Liang Y, Duan L, Lu J, et al. Engineering exosomes for targeted drug delivery. Theranostics. 2021;11(7):3183–95.
169. Borrelli DA, Yankson K, Shukla N, et al. Extracellular vesicle therapeutics for liver disease. J Control Release. 2018;273:86–98.
170. Lou G, Song X, Yang F, et al. Exosomes derived from miR-122-modified adipose tissue-derived MSCs increase chemosensitivity of hepatocellular carcinoma. J Hematol Oncol. 2015;8:122.
171. Haga H, Yan IK, Takahashi K, et al. Extracellular vesicles from bone marrow-derived mesenchymal stem cells improve survival from lethal hepatic failure in mice. Stem Cells Transl Med. 2017;6(4):1262–72.
172. Kubes P, Jenne C. Immune responses in the liver. Annu Rev Immunol. 2018;36:247–77.
173. Juza RM, Pauli EM. Clinical and surgical anatomy of the liver: a review for clinicians. Clin Anat. 2014;27(5):764–9.
174. Horst AK, Neumann K, Diehl L, et al. Modulation of liver tolerance by conventional and nonconventional antigen-presenting cells and regulatory immune cells. Cell Mol Immunol. 2016;13(3):277–92.
175. Erhardt A, Biburger M, Papadopoulos T, et al. IL-10, regulatory T cells, and Kupffer cells mediate tolerance in concanavalin A-induced liver injury in mice. Hepatology. 2007;45(2):475–85.
176. Thomson AW, Knolle PA. Antigen-presenting cell function in the tolerogenic liver environment. Nat Rev Immunol. 2010;10(11):753–66.
177. Zhai Z, Cui T, Chen J, et al. Advancements in engineered mesenchymal stem cell exosomes for chronic lung disease treatment. J Transl Med. 2023;21(1):895.
178. Wan Z, Zhao L, Lu F, et al. Mononuclear phagocyte system blockade improves therapeutic exosome delivery to the myocardium. Theranostics. 2020;10(1):218–30.
179. Lin Y, Yan M, Bai Z, et al. Huc-MSC-derived exosomes modified with the targeting peptide of ahscs for liver fibrosis therapy. J Nanobiotechnology. 2022;20(1):432.
180. Tian Y, Zhang J, Jia Z, et al. Biomimetic mineralized mesenchymal stem cell-derived exosomes for dual modulation of ferroptosis and lactic acid-driven inflammation in acute liver injury therapy. J Colloid Interface Sci. 2025;687:489–506.
181. Liang L, Zhao L, Wang Y, et al. Treatment for hepatocellular carcinoma is enhanced when norcantharidin is encapsulated in exosomes derived from bone marrow mesenchymal stem cells. Mol Pharm. 2021;18(3):1003–13.
182. Xiao Y, Yu D. Tumor microenvironment as a therapeutic target in cancer. Pharmacol Ther. 2021;221:107753.
183. Kisseleva T, Brenner D. Molecular and cellular mechanisms of liver fibrosis and its regression. Nat Rev Gastroenterol Hepatol. 2021;18(3):151–66.
184. Senoo H, Kojima N, Sato M. Vitamin a-storing cells (stellate cells). Vitam Horm. 2007;75:131–59.
185. Mardpour S, Hassani SN, Mardpour S, et al. Extracellular vesicles derived from human embryonic stem cell-MSCs ameliorate cirrhosis in thioacetamide-induced chronic liver injury. J Cell Physiol. 2018;233(12):9330–44.
186. Moayedfard Z, Sani F, Alizadeh A, et al. The role of the immune system in the pathogenesis of NAFLD and potential therapeutic impacts of mesenchymal stem cell-derived extracellular vesicles. Stem Cell Res Ther. 2022;13(1):242.
187. Tang K, Zhang Y, Zhang H, et al. Delivery of chemotherapeutic drugs in tumour cell-derived microparticles. Nat Commun. 2012;3:1282.
188. Fuhrmann G, Serio A, Mazo M, et al. Active loading into extracellular vesicles significantly improves the cellular uptake and photodynamic effect of porphyrins. J Control Release. 2015;205:35–44.
189. Podolak I, Galanty A, Sobolewska D. Saponins as cytotoxic agents: a review. Phytochem Rev. 2010;9(3):425–74.
190. Zhang YW, Hou LS, Xing JH, et al. Two-membrane hybrid nanobiomimetic delivery system for targeted autophagy inhibition of activated hepatic stellate cells to synergistically treat liver fibrosis. ACS Appl Mater Interfaces. 2023. https://doi.org/10.1021/acsami.3c11046.
191. Salunkhe S, Dheeraj, Basak M, et al. Surface functionalization of exosomes for target-specific delivery and in vivo imaging & tracking: Strategies and significance. J Control Release. 2020;326:599–614.
192. Jia G, Han Y, An Y, et al. NRP-1 targeted and cargo-loaded exosomes facilitate simultaneous imaging and therapy of glioma in vitro and in vivo. Biomaterials. 2018;178:302–16.
193. Kim MS, Haney MJ, Zhao Y, et al. Engineering macrophage-derived exosomes for targeted paclitaxel delivery to pulmonary metastases: in vitro and in vivo evaluations. Nanomedicine. 2018;14(1):195–204.
194. Emam SE, Elsadek NE, Abu Lila AS, et al. Anti-PEG IgM production and accelerated blood clearance phenomenon after the administration of PEGylated exosomes in mice. J Control Release. 2021;334:327–34.
195. Mahati S, Fu X, Ma X, et al. Delivery of miR-26a using an exosomes-based nanosystem inhibited proliferation of hepatocellular carcinoma. Front Mol Biosci. 2021;8:738219.
196. De Jong OG, Verhaar MC, Chen Y, et al. Cellular stress conditions are reflected in the protein and RNA content of endothelial cell-derived exosomes. J Extracell Vesicles. 2012. https://doi.org/10.3402/jev.v1i0.18396.
197. Xu J, Chen P, Yu C, et al. Hypoxic bone marrow mesenchymal stromal cells-derived exosomal miR-182-5p promotes liver regeneration via FOXO1-mediated macrophage polarization. FASEB J. 2022;36(10):e22553.
198. Liu J, Tang M, Li Q, et al. Atg2b upregulated in LPS-stimulated BMSCs-derived exosomes attenuates septic liver injury by inhibiting macrophage STING signaling. Int Immunopharmacol. 2023;117:109931.

199. Takeuchi S, Tsuchiya A, Iwasawa T, et al. Small extracellular vesicles derived from interferon-γ pre-conditioned mesenchymal stromal cells effectively treat liver fibrosis. NPJ Regen Med. 2021;6(1):19.
200. Li H, Yang C, Shi Y, et al. Exosomes derived from siRNA against GRP78 modified bone-marrow-derived mesenchymal stem cells suppress Sorafenib resistance in hepatocellular carcinoma. J Nanobiotechnology. 2018;16(1):103.
201. Lou G, Chen L, Xia C, et al. MiR-199a-modified exosomes from adipose tissue-derived mesenchymal stem cells improve hepatocellular carcinoma chemosensitivity through mTOR pathway. J Exp Clin Cancer Res. 2020;39(1):4.
202. Zivko C, Witt F, Koeberle A, et al. Formulating elafibranor and obeticholic acid with phospholipids decreases drug-induced association of SPARC to extracellular vesicles from LX-2 human hepatic stellate cells. Eur J Pharm Biopharm. 2023;182:32–40.
203. Azizsoltani A, Hatami B, Zali MR, et al. Obeticholic acid-loaded exosomes attenuate liver fibrosis through dual targeting of the FXR signaling pathway and ECM remodeling. Biomed Pharmacother. 2023;168:115777.
204. Ashour AA, El-Kamel AH, Mehanna RA, et al. Luteolin-loaded exosomes derived from bone marrow mesenchymal stem cells: a promising therapy for liver fibrosis. Drug Deliv. 2022;29(1):3270–80.
205. Wang Y, Chen Y, Yang F, et al. MiR-4465-modified mesenchymal stem cell-derived small extracellular vesicles inhibit liver fibrosis development via targeting LOXL2 expression. J Zhejiang Univ Sci B. 2024;25(7):594–604.
206. Eggenhofer E, Groell A, Junger H, et al. Steatotic livers are more susceptible to ischemia reperfusion damage after transplantation and show increased γδ T cell infiltration. Int J Mol Sci. 2021. https://doi.org/10.3390/ijms22042036.
207. Wu L, Tian X, Zuo H, et al. Mir-124-3p delivered by exosomes from heme oxygenase-1 modified bone marrow mesenchymal stem cells inhibits ferroptosis to attenuate ischemia-reperfusion injury in steatotic grafts. J Nanobiotechnology. 2022;20(1):196.
208. Sameri MJ, Savari F, Hoseinynejad K, et al. The hepato-protective effect of H_2S-modified and non-modified mesenchymal stem cell exosomes on liver ischemia-reperfusion injury in mice: the role of MALAT1. Biochem Biophys Res Commun. 2022;635:194–202.
209. Dri DA, Rinaldi F, Carafa M, et al. Nanomedicines and nanocarriers in clinical trials: surfing through regulatory requirements and physico-chemical critical quality attributes. Drug Deliv Transl Res. 2023;13(3):757–69.
210. Walsh NC, Kenney LL, Jangalwe S, et al. Humanized mouse models of clinical disease. Annu Rev Pathol. 2017;12:187–215.
211. Kim JY, Rhim WK, Yoo YI, et al. Defined MSC exosome with high yield and purity to improve regenerative activity. J Tissue Eng. 2021;12:20417314211008624.
212. Cao J, Wang B, Tang T, et al. Three-dimensional culture of MSCs produces exosomes with improved yield and enhanced therapeutic efficacy for cisplatin-induced acute kidney injury. Stem Cell Res Ther. 2020;11(1):206.
213. Mardpour S, Ghanian MH, Sadeghi-Abandansari H, et al. Hydrogel-mediated sustained systemic delivery of mesenchymal stem cell-derived extracellular vesicles improves hepatic regeneration in chronic liver failure. ACS Appl Mater Interfaces. 2019;11(41):37421–33.

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