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Review Article

The Art of Mesenchymal Stem Cells in Liver Fibrosis Management

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ABSTRACT

Liver fibrogenesis is chronic tissue damage characterized by an extracellular accumulation of fibrillar matrix associated with continuous degradation and remodelling. This scientific review describes current concepts on the pathophysiology of liver fibrosis, inflammation as a fundamental concept of liver fibrosis, mechanistic concepts of liver fibrosis, the role of mesenchymal stem cells (MSC) in liver injury, the functional effects of MSC secretome, the advantages of secretome therapy, and the latest research developments on MSC. The role of MSCs has been proven in many liver fibrosis studies involving experimental animals. However, it still requires further research for safety and efficacy aspects.

Keywords: Fibrogenesis, liver fibrosis, mesenchymal stem cells

Introduction

Many studies have been reported over the past 25 years and have focused on several cellular and molecular mechanisms responsible for liver fibrogenesis. In molecular biology terminology, fibrogenesis is a dynamic process characterized by the continuous accumulation of fibrillar ECM (Extracellular Matrix) associated with ongoing degradation and remodelling in the context of chronic tissue damage. Fibrosis appears when there is insufficient degradation [1].

Liver fibrosis is an excess accumulation of the extracellular matrix in the liver parenchyma in response to chronic injury. Virus, autoimmune, cholestatic, toxic, or metabolic disease, including nonalcoholic steatohepatitis, cause injury. The progression of chronic fibrosis from fibrosis to cirrhosis is characterized by the formation of septa and scar tissue in hepatocytes' nodules. The primary mechanism of liver fibrosis is chronic activation in the wound-healing reaction. It is characterized by several biological mechanisms involving cells and a soluble factor to repair a tissue injury. In general, these mechanism and effectors are associated with a sequence corresponding to the previous phase [2,3]. This process causes scar and tissue damage. An organized process of fibrillar matrix deposition and tissue regeneration is the best choice to maintain tissue. Modifications in the ECM composition, especially collagen types I and III have several mechanical, physical, and biochemical implications. Therefore, the modulation of several cellular functions such as growth, migration, and gene expression between ECM components and adhesion molecules cells are urgently needed. These are useful as a reservoir for proinflammatory and profibrogenic mediators [4].

The type of fibrogenic cells in the liver is re presented by hepatic stellate cells (HSC). It is characterized by a physiological ability to store

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retinyl esters in intracytoplasmic lipid droplets by the ultrastructural characteristics of vascular pericytes to regulate sinusoid blood flow. HSC activation and transformation of phenotypes into myofibroblasts play an important role in understanding the hepatic fibrogenic process [5]. Different ECM-producing cells with different localization and immunohistochemical characteristics contribute to the incidence of liver fibrosis [6]. Fibroblasts and myofibroblasts from smooth muscle cells localized to the vessel wall. Furthermore, myofibroblasts located in the centrilobular vein. It has also been shown that the participation of these different cell types is dependent on the development of different fibrosis. Myofibroblasts can be originated from circulating fibroblast-like cells and bone marrow stem cells. It is called fibrocytes [7].

The involvement of oxidative stress plays an important role in chronic wound healing and fibrogenic disorders. These are characterized by chronic tissue damage and overexpression of critical genes associated with inflammation and ECM remodelling. Oxidative stress decreased antioxidant defences' efficiency as a potential toxic consequence of chronic tissue injury, tissue remodelling, and excessive fibrogenesis [8]. Therefore, reactive oxygen species (ROS) or reactive aldehydes (particularly 4-hydroxy-2,3-nonenal; HNE) damaged or activated by neighbouring cells influence myofibroblast to upregulate profibrogenic genes, including procollagen type I, MCP-1 and tissue metallopeptidase-1.9 inhibitors [9]. Oxidative stress is the primary profibrogenic mechanism in chronic alcoholic hepatitis and nonalcoholic steatohepatitis (NASH). Perisinusoidal fibrosis triggers inflammation and tissue necrosis. These are due to ROS, HNE, and acetaldehyde's direct profibrogenic action in chronic alcohol abuse cases [10].

Mechanism of liver fibrogenesis

Recently, the microenvironment of the liver profibrotic focus on the role of immune cells and the specific subset of macrophages. They play an important role in fibrosis progression or regression, gut microbiota, and the influence of tissue rigidity [11-13]. Moreover, hypoxia in tissues, the establishment of an anaerobic proinflammatory environment, and the effect of epigenetic modification in the development of fibrosis have been reported [14-16]. Among these emerging mechanisms, innate immune mechanisms changes as a systemic proinflammatory and profibrogenic environment affect chronic liver diseases (CLD). The symbiotic relationship between the gut microflora and the human host plays a vital role in modulating immunological homeostasis. In CLD, the combination of dysbiosis (e.g. imbalance between pathogenic and non-pathogenic bacterial species), increased intestinal permeability, altered gut defence, and decreased immunological surveillance triggers bacteria migration or various bacterial products from the intestinal lumen to mesenteric lymph nodes and other extraintestinal organs. Various studies have shown that bacterial translocation contributes to CLD, especially in NASH [17-18].

Bacterial byproducts are known as pathogen-associated molecular patterns (PAMPs). PAMPs are bacterial double-stranded lipoproteins, DNA, and RNA that are recognized by pattern recognition receptors (PRRs) in various cells, including fibroblasts [19]. The interaction between PAMP and PRR serves as the first line of defence during the infection and triggers various proinflammatory cytokines and chemokine responses. Fibroblasts, myofibroblasts, and vascular pericytes, express various PRRs, including Toll-like receptors (TLRs). Moreover, their ligands directly activate these cell types and promote differentiation into collagen-producing myofibroblasts [20,21]. Besides, ligand lipopolysaccharide (LPS) or lipoteichoic acid ligand TLR2 activate mitogen-activated protein kinase pathways, translocate NF-κB, and secrete large amounts of proinflammatory cytokines and chemokines [22]. The interaction between PAMP and PRR, especially TLR, is important for establishing a proinflammatory or profibrogenic in the vascular. The activation of TLR expres HSCs with excessive amounts of PAMP in the liver due to abnormal intestinal permeability under certain conditions, such as chronic alcohol abuse, diabetes, and obesity [23,24].

Inflammation as the primary of liver fibrogenesis

Persistent chronic inflammation is a characteristic of progressive hepatic fibrosis and the development of cirrhosis. Inflammation is a process initiated by tissue-resident immune cells, such as macrophages, especially Kupffer cells, dendritic

cells (DC), mast cells, and others [25,26]. Over the years, molecular understanding of inflammation and its underlying pathways has improved. The liver has unique anatomy and connected to the intestines by the portal vein and bile duct. Therefore, it allows the delivery of products from the intestinal microflora directly to the liver [27]. Liver injury or intestinal tract mucosal damage trigger toxic substances with immunomodulatory activity such as lipopolysaccharides can penetrate the liver. Kupffer cells, a population of macrophages in the liver, are considered the first and most efficient defence to tissue integrity changes or inflammatory signals [28,29].

PAMPs or DAMPs induce Kupffer cells, stellate cells, and hepatocytes to recognize pattern-recognition receptors, including toll-like receptors (TLRs) [30,31]. The activation, expression or secretion of inflammatory cytokines can be initiated, including tumour necrosis factor (TNF)-α, interleukin (IL) -1α/β, IL-6, IL-12, IL-18, granulocyte-macrophage colony-stimulating factor, etc. During the hepatic insult process, endogenous triggers dying cells, such as necrosis, apoptosis, necroptosis [32]. Furthermore, profibrogenic cytokines such as transforming growth factor (TGF) - beta 1, platelet-derived growth factor (PDGF), and endothelial growth factor (EGF) are released by parenchymal and non-parenchyma. These soluble factors initiate hepatic stellate cells (HSC) to phenotypic changes, starting from a non-proliferative, retinoid-storing cell type, a phenotype without retinoids, and fat with proliferation [33]. Therefore, myofibroblast (MFB) increases alpha-smooth muscle actin and produces many ECM components such as collagen. MFB is not only produced almost all ECM components but also synthesizes various cytokines and chemokines. Furthermore, it acquires contractility in response to ligands such as endothelin and nitric oxide. Apart from HSC / MFB, portal fibroblasts and bile duct epithelial cells can participate in the fibrogenesis process, although their fractional contribution remains unclear [34,35].

Th2 polarized CD4 + T cells also cause fibrogenesis. These cells secrete IL-4 and IL-13, which stimulate the differentiation of fibrogenic myeloid cells and macrophages [36]. Th17 cells are induced by TGF-β1, IL-6, and secrete IL-17A. Furthermore, activate myofibroblasts is directly and indirectly, stimulate TGF-β1 via inflammatory cells [37]. Regulatory T cells can be promoted or inhibited fibrogenesis by secreting TGF-β1 (profibrotic) or IL-10 (antifibrotic). Th1 CD4 + cells have an antifibrotic effect [38]. NK cells reduce fibrosis by eliminating activated HSCs and interferon-gamma secretion [39]. Monocytes play an important role in inflammation and fibrosis. They are precursors to fibrocytes, macrophages, and dendritic cells [40]. Macrophages are fibrogenic during fibrosis and fibrotic during reversal [38].

The concept of liver fibrosis

Liver fibrosis is characterized by the progressive accumulation of extracellular matrix (ECM). It damages the physiological changes of the liver [41]. Viral, metabolic, toxic, pathogenic diseases cause hepatocyte damage and infiltration of immune cells. They induce the trans-differentiation of hepatic stellate cells (HSCs) into Collagen-producing myofibroblasts. Antifibrotic mechanisms indicate tissue repair in myofibroblast inactivation, apoptosis, and scar resolution [42]. Conversely, an imbalance of pro-fibrogenic and anti-fibrogenic mechanisms leads to activation of the proliferative, contractile, and migratory processes of myofibroblasts in chronic liver disease. These conditions lead to ECM production [43]. The antifibrotic scar trigger fibrosis and regulated by non-parenchymal cells (NPC), including Kupffer cells and other immune cells [44-46]. Therefore hepatocyte apoptosis and release of damage-associated patterns (DAMPs) by hepatocytes activate HSCs, lymphocytes, and macrophages. They contribute to HSC transdifferentiation and myofibroblast activation by producing pro-inflammatory and pro-cytokines [47,48]. Different macrophage subpopulations participate in fibrosis due to the expression of matrix-metalloproteinases (MMPs). [49,50]. A complex network of cytokine-induced signalling pathways regulate pro-fibrogenic cell interactions. Transforming Growth Factor Beta (TGF-β), Platelet-Derived Growth Factor (PDGF), and the inflammasome pathway (NLRP3) -Caspase1, as well as WNT / beta-catenin signalling, has been suggested to be the major pathways related to HSC activation and development of fibrosis [51-53]. The mechanistic concept of liver fibrosis describes hepatocyte cell death, apoptosis, HSC activation, myofibroblast progenitor cells, liver macrophages, lymphocytes,

gut dysbiosis, and molecular signalling pathways in liver fibrogenesis (PDGF signal, TGF-beta signal), oxidative stress, the inflammasome (NLRP3)-Caspase1 pathway, and the Wnt / beta-Catenin signaling [54].

Role of mesenchymal stem cells (MSC) in liver injury

The use of MSCs in regenerative therapy is a promising therapeutic approach to managing liver injury, modulates the immune response to injury, and enhances liver epithelial repair and regeneration. MSCs are differentiated cells from bone marrow and can be inherited from perivascular cells from the liver [55]. MSCs have an important role in the immune response, including recognition and presentation of antigens, T cell activation, proliferation, and differentiation [56].

Liver MSCs are elongated, spindle-shaped, and express stem cell markers such as vimentin and MSC markers such as CD90. However, Liver MSCs are not expressed hematopoietic stem cell markers, such as CD45, or other liver progenitor cell markers such as CK19. MSCs from bone marrow circulate to the liver when injury [57]. MSCs have several characteristics that contribute to their regenerative properties [58]. First, MSCs can differentiate multilineage into different types of cell types. Furthermore, MSCs have migration, and that allow sequestration to the injury area. [59]. Their capacity for diapedesis across the endothelium can induce cell surface expression of chemokine receptors, adhesion, matrix metalloprotease (MMP), and other proteolytic enzymes [60]. MSCs have immunomodulatory effects both in the innate and adaptive immune systems [61]. Moreover, MSCs release extracellular proteins and vesicles (EV) to directly modulate liver injury [62].

MSCs are obtained from various anatomical locations, including bone marrow, adipose tissue, Wharton's jelly of the umbilical cord to display a similar immunophenotypic profile [63]. However, MSCs excrete complex active molecules and secretomes. These conditions depend on the age of the host and the niche of the cell. Secretomes of MSCs in the liver have a functional effect [64]. Apart from their beneficial properties, there are some limitations to the use of MSCs as cellular therapy [65]. They have the potential for aberrant differentiation, tumour formation, and low engraftment [66]. Tumor formation or differentiation into undesirable cell types has hindered the adoption, and the use of MSC-based therapeutic approaches are poorly understood [67]. Transplantion of MSCs may be insufficient for tissue regeneration with MSC differentiation [68].

Allogeneic MSCs delivered systemically to accumulate in the lungs within the first 24 hours of transplantation [69]. Elimination by adaptive immune cells contributes to the short half-life of transplanted MSCs [70]. Allogeneic MSCs into target organs may lose their immunity due to the surface expression of the major class II histocompatibility complexes and CD86. Moreover, it is removed from the body due to the formation of anti-donor MSC antibodies. Allogeneic MSCs can be removed by CD8 + cytotoxic T lymphocytes, while transplanted autologous or allogeneic MSCs can be removed by natural killer (NK) cells [71].

Secretome MSC

The limited half-life of the transplanted cells, the tumorigenic potential, and other MSC risks lead to the development of acellular therapy [72]. MSCs can differentiate and contribute to hepatic epithelial replacement. The effects of MSCs are related to paracrine effects. The therapeutic potential of MSCs in liver injury can be utilized primarily via a paracrine mechanism and release dissolved proteins or EVs (Extracellular Vesicles) [73].

The use of MSC secretions as therapeutic agents is an attractive option to avoid some limitations of cell-based approaches. MSC secretions can be used as acellular regenerative and reparative therapy for liver injury and disease. MSC secretomes modulate the local immune microenvironment, reduce injury, and enhance epithelial repair. Undifferentiated MSCs requires to activate T cells. Therefore, the secretome-mediated paracrine play an important contribution to MSCs effects on the modulation of immune cells [74].

The beneficial effect of using CM or EV is to repair an injury. Several studies have reported that EV has been isolated from cell culture supernatant or CM using a classic centrifugation-based approach [76]. Although most studies have not directly evaluated the presence of secreted protein in EV preparations, the isolation approach is expected to eliminate secreted protein content.The use of resin-based EV separation increases a higher secreted protein [52,69].

The function of secretome MSC

The reparative or regenerative properties of MSC secretions contribute to immune modulation, repair of injury, or reduction of fibrosis. Soluble proteins, such as cytokines and chemokines, contribute to several different pathophysiological responses [77]. These include immunomodulatory effects due to some immune cells' direct or indirect effect or their response to cell or tissue injury. Growth factors and cytokines in secretions such as transforming growth factor-beta isoform 3 (TGF-beta 3), hepatocyte growth factor (HGF), IL-10, and tumour necrosis factor-alpha (TNF-alfa) modulate cell signalling in fibrogenesis and hepatic fibrosis [78]. In addition, there are paracrine effects of MSC from the EV.

Extracellular vesicles consists of a heterogeneous group of various size, biogenesis and content. EV derived from express MSC surface markers to modulate immune responses, such as specific tetraspanins, CD63, and CD81 [79]. Furthermore, EVs consist of lipid bilayers, proteins, DNA, and RNA molecules [80]. Extracellular vesicles derived from MSC can be selectively chosen as an antifibrotic and antiapoptotic protein or by non-coding specific RNA [81]. Extracellular vesicles production offers further opportunities for the delivery of specific content targeted for therapeutic applications [82,83].

Advantages of secretory therapy

Recent molecular biology research has been reported the biological regulation of communication between cells through the secretome. It is a molecule secreted into the extracellular space. These factors consist of soluble proteins, free nucleic acids, extracellular vesicles, and lipids. Extracellular vesicles can be further divided into apoptotic bodies, microparticles, and exosomes [84].

The secretions of individual cells and tissues are specific. The use of cell-free therapies such as MSC-sourced secretomes in regenerative medicine provides major advantages over stem cell-based applications: (a) the application of secretomes can overcome several safety concerns regarding proliferative and live cell transplantation, population including immune compatibility, tumorigenicity, embolic formation and transmission of infection; (b) Secretomes from MSCs can be evaluated for safety, dosage, and potency by conventional pharmaceutical substances; (c) storage can be carried out without prolonged application of a potentially toxic cryopreservative agent without losing the potency of the product; (d) using secretions derived from MSC, such as conditioned medium (CM), is cheap and more practical for clinical applications because it avoids invasive cell collection procedures; (e) mass production is possible via custom-made cell lines under controlled laboratory conditions, providing a variety of sources of relevant bioactive factors; (f) the time and cost of expanding and maintaining stem cell cultures can be reduced for the treatment of acute conditions such as cerebral ischemia, myocardial infarction, or trauma in the military; (g) Biological products for therapeutic applications may be modified to the desired cell-specific effect [85-88].

Risk factors in stem cell therapy

The type of stem cells used, the site of injection, the level of manipulation, and their culturing history and procurement are all risk factors. Because of the variety of risk factors, the risks associated with various stem cell-based medicinal products may also vary greatly. All significant identified risks, such as theoretical/potential risks such as non-clinical safety concerns that have not been observed in clinical experience, as well as risks or adverse events identified in clinical experience, should be thoroughly evaluated for an adequate benefit/risk assessment of a stem cell-based medicinal product. Such an evaluation at the outset and throughout the development of a stem cell-based therapy may aid in determining the scope and focus of the product development, as well as the extent and safety evaluation plans [67,96].

Several risk factors or hazards and identified risks associated with stem cell-based therapy are classified as intrinsic, extrinsic, and clinical. Intrinsic factors which are cell characteristics, have hazards or risk factors, including: proliferation capacity, origin of cells (autologous vs allogenic, diseased vs healthy donor/tissue), excretion patterns (such as: chemokines, cytokines, and growth factors), long term viability, life span, differentiation status, tumourigenic potential. Toxicity, disease susceptibility, neoplasm formation (benign or malignant), unwanted biological effect (e.g. in vivo differentiation in unwanted cell type), and cell

rejection are among the risks identified in intrinsic factors [67].

Extrinsic factors which include handling and manufacturing, have a variety of hazards or risk factors, such as: culture duration, contamination by adventitious agents (bacterial/viral/fungi/mycoplasma, parasites, prions), conservation (e.g. cryopreservatives), cell handling procedures (e.g. procurement), lack of donor history, non cellular components, plasma derived materials, pooling of allogenic cell populations, raw and starting materials, transport conditions, storage conditions (e.g. human material labelling, failure of traceability), tumourigenic potential (e.g. incomplete removal of undifferentiated cells, culture induced transformation). Identified risks contained in extrinsic factors in the form of: cell line contamination (e.g. with unwanted cells, growth media components, chemicals), disease transmission, neoplasm formation (benign or malignant), reactivation of latent viruses, mix-up of autologous patient material.[67]

Hazards or risk factors are also present in clinical characteristics, such as administration route, exposure duration, underlying disease, indication, use of immunosuppressives, therapeutic use (i.e. homologous or non-homologous), initiation of immune responses, and irreversibility of the treatment.

Research on MSC

The following is a brief outline of various liver injury therapies using MSCs (Table 1).

Table 1. MSC research on liver injury

Source	Pathways	Modeling Injury	Effects	Mechanisms	Ref.
Human heart	IP MSC-CM injection during injury	Partial hepatectomy	Increased hepatocyte proliferation	Upregulation of TNF-alpha, HGF, TGF-beta, IL-1RA, IL-10	63
Umbilical cord	Secretome injection of hepatocyte-like or undifferentiated	CCl4 and TAA-induced liver fibrosis	Decreased number of activated alpha-SMA + HSCs; reduced collagen deposition.	*Decreased TGF-beta signaling*	81
Human bone marrow	IV CM-MSC injection	D-Gal-induced liver failure	Decreased apoptosis of hepatocytes and reduced serum AST and ALT levels	Increase in circulating serum IL-10; infiltration of TNF-alpha, IL-6, IL-1ra, and attenuated CD45 + leukocytes.	89
Murine bone marrow	CM-MSC injection	Alpha-GalCer-induced acute liver failure	Reduced serum AST and ALT levels, expanded CD4 + CD25 + T cell infiltration and reduced HCT cell-mediated hepatotoxicity	Suppressed T cell proliferation	90

Human umbilical cord	CM-MSC	H2O2-induced hepatocyte injury in vitro	Increased hepatocyte viability	Modulation of Bax and Bcl-2 expression	91
Murine compact bone	Injection of CM-MSC IV	CCl4-induced chronic liver fibrosis and TAA-induced acute liver failure.	Reduced deposition of collagen and alpha-SMA + cells, induces apoptosis of activated HSC in the liver of CCl4 injured mice, decreases apoptosis of hepatocytes, increases cell proliferation.	Reduced hepatic leukocyte infiltration, decreased CD11b + F4 / 80 + and Th-17 macrophages, induced expansion of CD4 + CD25 + Tregs originating from the spleen in CCl4 injury mice.	92
Human adipose tissue	CM-MSC (normoxic or hypoxic precondition)	None	Increased hepatocyte viability (H-CM) enhanced by glycogen and ICG uptake by Hepatocytes		93
Human umbilical cord	MSC co-culture	Injured murine hepatocytes due to CCl4	Increased hepatocyte viability, increased albumin production, increased number of hepatocyte proliferation.		94
Human adipose	Injection of IV ASC-CM (Untreated and LPS-primed)	Partial hepatectomy	Increased number of proliferating cells, accelerated liver regeneration, reduced serum transaminase levels.	Decreased serum TNF-alpha and IL-6 levels; increased expression of hepatic p-STAT3 and PCNA.	95

Noted: a-GalCer galactosylceramine, a-SMA alpha-smooth muscle actin, ALT alanine aminotransferase, AR adrenergic receptor, AST aspartate aminotransferase, BAX Bcl2-associated X protein, Bcl-2 B cell lymphoma 2, BMF Bcl2 modifying protein, CCl4 carbon tetrachloride, CM conditioned media, D-galD-galactosamine, EV extracellular vesicles, Ex exosomes, H hypoxia, H2O2 hydrogen peroxide, HB-EGF heparin binding EGF-like growth factor, hBM-MSC human bone marrow-derived MSC, HGF hepatocyte growth factor, hpucMSC hepatocyte-like umbilical cord-derived MSC, HSC hepatic stellate cells, hucMSC human umbilical cord-derived MSC, ICG indocyanine green, IDO indolamine 2,3 dioxygenase, IL interleukin, IP intraperitoneal, IV intravenous, LPS lipopolysaccharide, N normoxia, NKT natural killer T cells, OSM oncostatin M, PCNA proliferating cell nuclear antigen, p-STAT3 phosphorylated signal transducer and activator of transcription 3, ROS reactive oxygen species, SCF stem cell factor, SITR1 siturin 1, SMAD mothers against decapentaplegic homolog, SOCS3 suppressor of cytokine signaling, TAA thioacetamide, Teff effector T cells, TGF-b transforming growth factor beta, TGFRB1 transforming growth factor beta receptor 1, Th T-helper cell, TIMP tissue inhibitor of metalloproteinases, TNF-a tumor necrosis factor-alpha, Tregs regulatory T cells, ucMSC umbilical cord-derived MSC.

Conclusion

Mesenchymal stem cells (MSC) had an important role in liver injury. MSC increased hepatocyte viability, hepatocyte proliferation, recognized antigens, T cell activation, and differentiation of effector T-cell. Further study should be evaluated the safety and effectiveness aspects in patient of liver fibrosis.

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