Research Article

Mesenchymal Stem Cells: Current Clinical Applications and Therapeutic Potential in Liver Diseases

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Abstract

Mesenchymal stem cells (MSCs) are one of valuable candidates for cell-based therapy which trend to be safe, feasible and promising in the treatment of several diseases. Since, MSCs possess unique biological characteristics which make them variable applying in clinic. These include self-renewal capacity, multi-lineages differentiation potential, homing and migration ability, immunomodulatory properties and paracrine secretion activity. These beneficial advantages lead to increase possibility of using MSCs in several implications such as regenerative medicine, tissue engineering, cells-based therapy and other clinical applications. At present, many clinical trials related to MSCs have been conducted in various diseases including bone defect, myocardial infarction, spinal injury, critical limb ischemia, diabetes and multiple sclerosis based on registered data at http://clinicaltrials.gov. The most of these clinical trials are being under investigation and are in phase I and II. There are several diseases of the liver that cause liver dysfunction due to hepatocytes injury and loss including viral hepatitis, fatty liver disease, drug or toxin induced liver injury, hepatocellular carcinoma, autoimmune-associated hepatic disorders and cirrhosis. In this review, we focus on mesenchymal stem cell (MSCs) toward liver disease treatment by MSCs therapy. In this regard, we summarized characteristics of mesenchymal stem cell (MSCs) including sources, general characteristics, differentiation potential, niches, and biological effects of the cells. We also emphasized on current methods for hepatocyte differentiation and current board clinical applications. The current studies of MSCs both in preclinical and clinical trials related to liver diseases are also discussed in context of possible application in therapeutic purposes in this review. Understanding the basic knowledge of MSCs and their therapeutic capability in preclinical and clinical studies will accelerate therapeutic value of MSCs transplantations for disease treatments, drug screening, stem cell banking and regenerative medicine.

Keywords: Mesenchymal stem cells; Clinical trial; Clinical application; Liver disease

Introduction

Mesenchymal stem cells (MSCs) are multipotent adult stem cells that have self-renewal capacity and differentiation potency into several specialized cell types at least in mesoderm origin. The MSCs were first isolated from rodent bone marrow by Friedenstein and their colleagues in 1976 [1]. They observed that the isolated cells could adhere to plastic culture dishes and had feature as spindle-shape cells like fibroblast. Moreover, the adherent cells demonstrated as clonogenic feature or clonal density form which was defined as colony-forming unit fibroblasts (CFU-F) characteristic. Over the past years, subsequent studies found that these cells could differentiate into mesodermal-lineage cells such as osteoblast, adipocytes and chondrocytes *in vitro* [2-4]. Although there are several terms for defining these cells such as marrow stromal cells, multipotent stromal cells, bone marrow stromal stem cells and multi-potent adult progenitor cells, mesenchymal stem cells are widely mentioned by several studies based on their differentiation potential toward mesodermal tissues or all connective tissues such as adipose, bone and cartilage.

Currently, MSCs have been known as promising tool for therapeutic purpose in clinic based on their several advantages including self-renewal, extensive *in vitro* expansion, immunomodulation property, engraftment capacity, multi-lineages differentiation potential including few ethical concerns as compared to embryonic stem cells. Moreover, increasing evidences have been shown that MSCs can be isolated from various cell types including adipose tissue, dental pulp, peripheral blood, placenta and umbilical cord. These unique biological properties of MSCs highlight great potential in several applications such as regenerative medicine, tissue engineering and cell-based therapy. Here, this review focuses on an overview of MSCs biological

properties including recent clinical trials related to MSCs. In addition, we also discuss the potency of MSCs in liver disease treatment based on accumulating data from *in vitro* and *in vivo* studies. The liver is a vital organ which controls various crucial biological processes in human body. These include several hormones production, glycogen storage, drug and toxin detoxification, metabolism control, urea metabolism and plasma proteins synthesis. In general, the most of physiological properties of liver function are carried out by liver cells or hepatocytes. Thus, the loss of hepatocytes results in failure to compensate major functions of the liver. There are several diseases of the liver that cause liver dysfunction due to hepatocytes injury and loss. These include viral hepatitis, fatty liver disease, drug or toxin induced liver injury, hepatocellular carcinoma, autoimmune-associated hepatic disorders and cirrhosis [5]. During liver disease progression, the end stage of the disease can gradually damage the liver and eventually leads to liver failure. Although orthotopic liver transplantation represents a current optimal treatment in patients with end stage of liver disease, the shortage of donor livers is still an obstacle for routine use. In this

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review, we also highlight a new approach of using MSCs as cells-based therapy in liver disease treatment based on review literatures both preclinical and clinical studies.

Mesenchymal Stem Cells (MSCs)

Sources, characteristics and differentiation potential

Since pilot studies by Friedenstein and their colleagues in 1976, the MSCs are extensively interesting in their biology, phenotypes and characteristics. In addition to rodent bone marrow, further studies have been reported that MSCs could be isolated from other mammalian species including monkey, goat, sheep, dog, pig and human [6]. Although MSCs were originally isolated from bone marrow, other adult connective tissues such as adipose tissue [7], muscle [8], dental pulp [9] and peripheral blood [10] have been found as additional sources of MSCs. Furthermore, MSCs can be harvested from fetal origin such as placenta [11], amniotic fluid [12], umbilical cord Wharton's jelly [13] and umbilical cord blood [14]. To date, native location or stem cell niche which MSCs reside is still unclear. In bone marrow, it is well known that MSCs play a role in supportive haematopoietic stem cells niche which is crucial for maintaining haematopoietic stem cells in a quiescent state [15]. In addition to bone marrow, accumulating data revealed that various tissues of post-natal organs have been found as additional sources of MSCs. It has been demonstrated that MSCs may be derived from or identical to pericytes. Pericytes are described as the cells located surrounding small blood vessels (perivascular) including capillaries and microvessels that exist in entire of human body. The widespread of vascularized connective tissues throughout the body seems to be a reason that why can be isolated MSCs from various source of tissues and organs. Pericytes play roles in blood vessel stabilization, tissue regeneration, immune system homeostasis and reconstruction of injured tissue or blood vessel walls. In addition, it has been shown that pericytes were similar to MSCs both in phenotypes and functions. For example, pericytes had ability to secrete diverse growth factors or cytokines as MSCs. Additionally, pericytes could express MSCs markers and exhibited multi-lineages differentiation potential into osteoblast, adipocytes and chondrocytes while still maintained long term viability *in vitro* [16].

In mouse model, it has been reported that the isolated cells from perivascular of capillary, aorta and vena cava had common properties of MSCs. Moreover, the distribution of MSCs-like cells was found in a variety of adult organs including kidney, brain, spleen, liver, bone marrow, lung and muscle [17]. Similarity, perivascular cells purified from human organs including skeletal muscle, pancreas, adipose tissue and placenta exhibited MSCs phenotypes such as mesodermal-lineage differentiation potential and MSCs markers expression [18]. Taken together, these findings suggest that MSCs are widely distributed *in vivo* related with microvasculature system that presents throughout the entire body. The close relationship between pericytes and MSCs makes possibility hypothesis that pericytes may be a native ancestor of MSCs or equivalent of MSCs *in vivo*. Based on this knowledge, perivascular represents as specific location niche of MSCs *in vivo* that reserves precursors of progenitor cells for tissue regeneration especially mesenchymal tissues in responding to tissue injury condition.

Several studies have been reported variable immunophenotype of MSCs due to the fact that each laboratory has been studied MSCs from different sources and culture methods. According to these findings, MSCs are absence of unique specific antigens but present different expression patterns of cell surface antigens depending on their origins which are summarized in Table 1 [19]. To avoid the confusion, the

Tissues	Positive markers	Negative markers
BM-MSCs	CD13, CD44, CD73 (SH3), CD90,	CD14, CD34, CD45
	CD105 (SH2), CD166, STRO-1	
AT-MSCs	CD9, CD13, CD29, CD44, CD54,	CD11b, CD14, CD19, CD31,
	CD73 (SH3), CD90, CD105 (SH2),	CD34, CD45, CD79α, CD133,
	CD106, CD146, CD166, STRO-1, HLA I	CD144, HLA-DR
PB-MSCs	CD44, CD54, CD90, CD105 (SH2), CD166	CD14, CD31, CD34, CD45
WJ-MSCs	CD10, CD13, CD29, CD44, CD90,	CD14, CD31, CD33, CD34,
	CD73 (SH3), CD105 (SH2), HLA I	CD45, CD56, HLA-DR
DP-MSCs	CD13, CD29, CD44, CD59, CD90,	CD11b, CD14, CD24, CD19,
	CD73 (SH3), CD105 (SH2), CD146	CD34, CD45, HLA-DR

Table 1: Cell surface antigen expressions of MSCs isolated from various tissues origins. Modified from Hass's study [19]. BM-MSCs, bone marrow-derived MSCs; AT-MSCs, adipose tissue-derived MSCs; PB-MSCs, peripheral blood-derived MSCs; WJ-MSCs, Wharton's jelly-derived MSCs; DP-MSCs, dental pulp-derived MSCs.

International Society for Cellular Therapy (ISCT) has proposed minimal criteria for defining MSCs in 2006 [20]. The characteristics of MSCs can be described at least 3 definitions as followed. Firstly, MSCs must adhere to plastic culture flasks in standard culture condition. Secondly, they must be positive staining for some cell surface antigens of CD44, CD73, CD90 and CD105 and be negative staining for human haematopoietic stem cells markers such as CD34 and CD45; mature human leukocytes surface antigens such as CD11b, CD14, CD19 and CD79-α as well as human major histocompatibility complex (MHC) class II antigen or HLA-DR. Thirdly, they must be able to differentiate into mesodermal-lineage cells including osteoblasts, adipocytes and chondroblasts *in vitro*.

Based on mesodermal differentiation potential of MSCs *in vitro*, the original mesengenic process pathway has been proposed by Caplan et al. in parallel to haematopoiesis lineage diagrams [21,22]. This pathway attempts to describe function of MSCs *in vivo* that is responsible for maintaining the turnover rate of adult mesenchymal tissues in the body such as bone, cartilage, muscle, fat and other connective tissues. In addition to be as supply source of mesoderm lineage cells including cardiomyocyte [23], MSCs can cross-differentiate into ectoderm and endoderm lineages *in vitro* such as neuron like cells [24,25] and endothelial cells [26,27] in ectoderm lineage as well as insulin-producing cells [28,29] and hepatocyte-like cells [30,31] in endoderm lineage.

Paracrine effects

MSCs can secrete a number of bioactive molecules that affect to biological changing of other cells or known as paracrine effect. These paracrine effects are categorized into six main activities as immunomodulation, anti-apoptosis, angiogenesis, supporting the growth and differentiation local stem and progenitor cells, anti-scarring and chemoattraction based on review study of Meirelles Lda et al. [32]. Several studies have shown that MSCs secreted a variety of angiogenic factors including basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), placental growth factor (PlGF), monocyte chemoattractant protein 1 (MCP-1) and interleukin 6 (IL-6). These paracrine factors are shown to promote local angiogenesis that is important for tissue repair process. Additionally, MSCs secreted large amounts of chemokines which play a role in recruitment of leukocytes to the site of injury and further initiating the immune response. MSCs can limit the area of tissue injury by their anti-apoptosis activity. VEGF, hepatocyte growth factor (HGF), insulin-like growth factor 1 (IGF-1), Stanniocalcin-1, transforming growth factor beta (TGF-β), bFGF and granulocyte-macrophage colony-stimulating growth factor (GM-CSF)

were found to reduce apoptosis of the normal tissues around the injured tissues. Anti-scarring or anti-fibrotic is a one activity of paracrine factors secreted by MSCs. It has been demonstrated that HGF, bFGF and adrenomedullin involved in prevention of fibrosis in animal model. Moreover, MSCs could support the growth of haematopoietic stem cells *in vitro* via secretion of paracrine factors including stem cell factor (SCF), leukemia inhibitory factor (LIF), IL-6 and macrophage colony-stimulating factor (M-CSF). Finally, MSCs possess immunomodulatory effects on both the innate and adaptive immune systems by secretion a number of paracrine factors including secreted prostaglandin E2 (PGE-2), TGF-, HGF, indoleamine 2,3-dioxygenase (IDO), LIF, M-CSF, PGE-2, IL-6, IDO, TGF-β and PGE-2. These factors affect on various biological activities of the immune cells such as suppression of T cell proliferation, enhancement of anti-inflammatory cytokines secretion, inhibition of dendritic cell maturation and inhibition of NK cell proliferation [32]. Taken together, all these activities are believed to involve the therapeutic potency of MSCs that make them interesting for cell-based therapy.
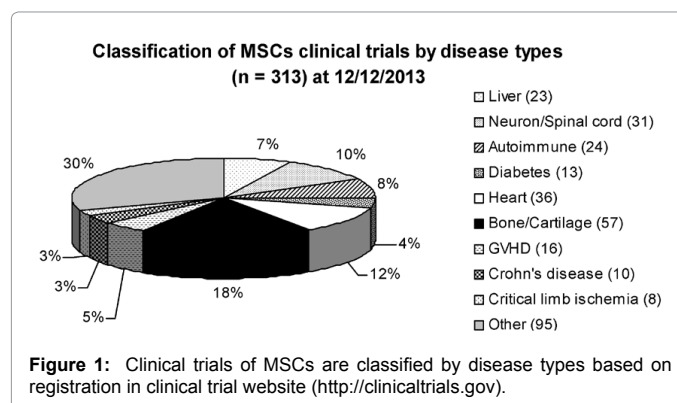
Immunomodulatory effects

MSCs have low immunogenic property because they have natural features as low expression of major histocompatability complex (MHC) class I antigens and lack of MHC class II including co-stimulatory molecules expressions. Additionally, MSCs also have ability to secrete paracrine factors that can regulate the immune systems. Several studies found that MSCs had immunomodulatory effects on both the innate and adaptive immune systems such as inhibition of T cell proliferation and cytokine secretion, inhibition of B cell proliferation and immunoglobulin synthesis including inhibition of monocytes differentiation into dendritic cells. The underlying mechanisms are still unknown, but it has evidences that paracrine factors secreted by MSCs and direct cell-to-cell contacts are involved in these effects. It has been reported that immunomodulatory effects of MSCs on the immune system were exerted by a number of paracrine factors. For example, PGE-2, TGF-β, HGF, IDO and LIF. These factors were not only suppression of T cell proliferation but also enhancement of anti-inflammatory cytokines secretion. The secretion of M-CSF, PGE-2 and IL-6 contributed to inhibition of dendritic cell maturation that led to defect in initiating T cell response. Additionally, MSCs-derived paracrine factors as IDO, TGF-β and PGE-2 also showed the inhibition effect on NK cell proliferation [32]. T lymphocytes or T cells play a major role in cell-mediated immune responses in adaptive immunity. Once stimulation by pathogens, the proliferation of T cells occurs and further releases inflammatory cytokines to destroy the pathogens. It has been demonstrated that MSCs were able to suppress T cells proliferation *in vitro*. This effect was involved cell cycle inhibition via accumulating cell cycle phase in G_0/G_1. A number of paracrine factors secreted by MSCs such as TGF-β, HGF, PGE-2, IDO, LIF, IL-6 and NO as well as cell-to-cell interactions were found to effect on T cells in different manners such as suppression of T cells proliferation, suppression of pro-inflammatory cytokine secretion, inhibition of cytotoxic T cells activation and promotion of anti-inflammatory cytokine secretion. Moreover, MSCs can generate and expand regulatory T cells (Treg) which are important for regulating immune responses by their anti-inflammatory effect. HLA-G5 secreted by MSCs exhibited as a key factor requiring for Treg generation. B lymphocytes or B cells play a role in humoral immune response in order to produce antibodies against specific antigens. Several studies revealed the regulatory effects of MSCs on B cells including inhibition of B cell proliferation and immunoglobulin synthesis. Like T cells, MSCs could arrest B cell proliferation at G_0/G_1 cell cycle phase. Additionally, all of these effects

are believed to associate with the interactions of paracrine factors and direct cell-to-cell contacts [33].

Natural killer cells or NK cells are a one of effector cells in innate immunity. NK cells response to destroy the target cells, virus-infected cells and tumor cells, by releasing cytotoxic cytokines in order to destroy the target cells. IDO, TGF-β, HLA-G5 and PGE$_2$ secreted from MSCs could be found to inhibit both proliferation and cytokine secretion of NK cells. It has evidences that MSCs could suppress cytotoxicity function of NK cells through down regulated expression of NK cells receptors. This effect was associated with both paracrine factors as well as direct cell-to-cell contacts. Dendritic cells act as antigen presenting cells that are derived from monocytes. The major role of dendritic cells is initiation of immune response by presenting antigens to naïve T cells which further stimulate T cells activation and proliferation. Several studies demonstrated the inhibition effects of MSCs on dendritic cells including dendritic cells maturation, co-stimulatory receptors expression and inflammatory cytokine secretion. MSCs-derived paracrine factors as M-CSF, PGE$_2$ and IL-6 including direct cell-to-cell contacts were involved in these effects [33]. Taken together, MSCs play a promising role in clinical application based on their immunomodulatory effects on various cells types of the immune system.

MSCs and current clinical applications

MSCs are well known to possess several properties including extensive *in vitro* expansion, broad differentiation potential, immunomodulatory properties and other paracrine effects such as tissue repair and anti-inflammation. These superior effects make MSCs as promising cells for regenerative medicine. In the middle of the 1990s, cultured MSCs have been conducted as cell-based therapy in patients who have received allogeneic haematopoietic stem cells transplantation to reduce acute and chronic graft-versus-host disease (GVHD) [34]. Subsequently, MSCs have been used in a number of clinical trials to test their therapeutic effects on various diseases such as bone defect, myocardial infarction, spinal injury, critical limb ischemia and multiple sclerosis. Currently, several clinical trials with MSCs have been registered on the United States National Institutes of Health's clinical trial website (http://clinicaltrials.gov). Based on this database, MSCs have been conducted for therapeutic purpose as a total number of 313 clinical trials which are categorized by disease types as shown in (Figure 1). Most of these clinical trials are being under investigation and are in phase I and II or phase I/II which aims are to investigate safety and efficacy of the treatment, respectively. Most of used MSCs are isolated from human bone marrow and adipose tissue. Furthermore, around 9% of MSCs clinical trials have entered to phase

Figure 1: Clinical trials of MSCs are classified by disease types based on registration in clinical trial website (http://clinicaltrials.gov).

III which aims are to investigate the efficacy of a new treatment as compared to standard treatment in a large number of patients. Phase III clinical trials included acute myocardial infarction, GVHD, cartilage injury, type 1 and 2 diabetes, liver cirrhosis, spinal cord injury, knee osteoarthritis and crohn's disease. Although around 24% of all clinical trials were completed, the results from some studies are available to access. Thus, this review focuses on the available published results only.

It has been demonstrated that autologous BM-MSCs transplantation could improve regional contractility of chronic myocardial scar in 8 patients with myocardial infarction [35]. Recently, the safety and efficacy of intramyocardial injection with autologous BM-MSCs were observed in patients with stable coronary artery disease and refractory angina as long as one year after treatment. These effects included improvement in exercise capacity, Seattle Angina Questionnaire evaluations, decreasing angina attacks per week and reducing nitroglycerine consumption used for heart disease treatment [36]. Phase I/II clinical trial with allogeneic MSCs transplantation showed effective treatment in patients with acute and chronic GVHD. In acute GVHD, complete response, partial response and no response were found in 1, 6 and 3 out of 10 patients, respectively. In chronic GVHD, 1 out of 8 patients achieved complete response, 3 had a partial response and 4 had no response [37]. Recent study found the safety and improvement in type 2 diabetes patients with islet cell dysfunction after transplantation with human placenta-derived MSCs (PD-MSCs). 40% (4 out of 10) of all patients showed reducing of insulin requirement more than 50% after transplantation for 6 months [38]. Moreover, it has been demonstrated that intramuscular BM-MSCs injection could improve the patients with complications of type 2 diabetes including critical limb ischemia and foot ulcer [39]. Regarding ulcerative colitis treatment, allogeneic BM-MSCs transplantation successfully improved clinical and morphological characteristics of the ulcers as compared to standard therapy. Moreover, this therapy also reduced the cost of treatment by decreasing immunosuppressive drugs use [40]. Currently, umbilical cord mesenchymal stem cell transplantation showed safety and effective improvement of neurological function in patients with sequelae of traumatic brain injury [41]. Taken together, MSCs-based therapy trends to be a safe, feasible and promising for therapy in several diseases. However, MSCs-based therapy seems to be transient and need to be validated in a long run. Long term follow-up is needed to monitor the side effects of MSCs transplantation before this therapy becomes as an alternative treatment in the clinic. Furthermore, multicenter and randomized clinical trial with large sample size will be required in further research in order to investigate the role of MSCs in various diseases.

The Therapeutic Potential of Mscs in Liver Diseases

In vitro differentiation of MSCs into hepatocytes

The liver is one of vital organs of the body that performs many essential functions such as producing bile for lipid digestion, generating plasma proteins and metabolic enzymes, detoxifying toxic substances, storing glycogen and regulating blood clotting system. Therefore, numerous effects on the liver can cause the loss of liver functions that lead to liver failure and death. Liver disease is a common term describing any diseases that cause liver inflammation, tissue damage and affect liver functions. To date, orthotopic liver transplantation is proved to be an effective therapeutic choice for patients with end stage of liver disease. Although the patients have benefited from liver transplantation, the shortage of donor organs is still a limitation of this treatment. Therefore, other alternative therapeutic approaches
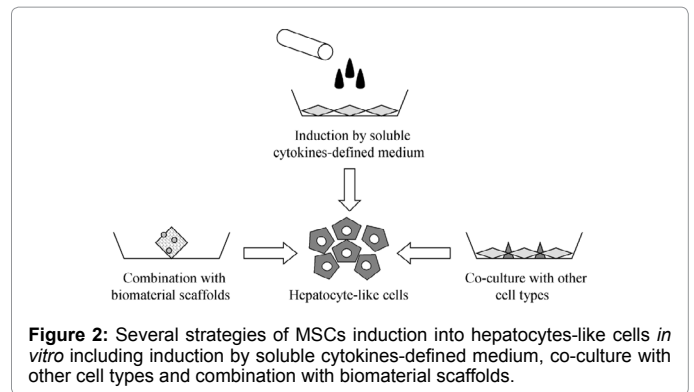
Figure 2: Several strategies of MSCs induction into hepatocytes-like cells *in vitro* including induction by soluble cytokines-defined medium, co-culture with other cell types and combination with biomaterial scaffolds.

are needed. Generation of new hepatocytes or hepatocyte-like cells for replacing the old damaged cells is one alternative choice to overcome the scarcity of donor livers. MSCs are unlimited source due to their self-renewal property. MSCs also have ability to differentiate into many cell types including hepatocytes. To date, numerous studies have successfully generated hepatocyte-like cells *in vitro* by different strategies including induction through soluble growth factors or cytokines-defined medium, co-culture with other cell types and combination with biomaterial scaffolds (Figure 2).

Induction by soluble growth factors or cytokines-defined medium

A number of studies use the sequential exposure of exogenous growth factors or cytokines as a cocktail to induce the differentiation of MSCs into hepatocytes. These factors are applied following the understanding of liver development during mouse and human embryogenesis [42]. The most used factors for the first stage induction are FGF-1, FGF-2 and FGF-4 which are required to initiate early hepatogenesis. Subsequently, the combination of HGF, oncostatin M (OSM) and dexamethasone were widely used for hepatic maturation step. HGF is required for supportive fetal hepatocytes during mid-stage hepatogenesis. OSM is produced by the haematopoietic cells which play an important role in secreting the factor for maturation of fetal hepatocytes. Dexamethasone is a synthetic glucocorticoid hormone which is needed to induce liver enzymes required for gluconeogenesis such as phosphoenolpyruvate carboxykinase (PEPCK) and tyrosine aminotransferase (TAT) [43]. Several studies have successfully generated hepatocyte-like cells from MSCs by various cocktail media and using different induction period ranging from 14-21 days.

Dong et al. demonstrated that FGF-4 and HGF were necessary in the first step of hepatic differentiation. They observed the down-regulation of early hepatic specific genes, α-fetoprotein (AFP) and hepatocyte nuclear factor-3β (HNF3β), after removing these cytokines from induction medium. Moreover, they also investigated that OSM was important for hepatic maturation by observing down regulation of late hepatic specific genes, albumin (ALB) and tyrosine aminotransferase (TAT), after withdrawing OSM from the system [44]. Pournasr et al. added FGF-4 and HGF in the first stage induction and used the cocktail of HGF, insulin-transferrin-sodium selenite (ITS) and dexamethasone in the last stage of differentiation. They successfully generated hepatocyte-like cells from human BM-MSCs after complete induction. The differentiated cells had function as normal hepatocytes including glycogen storage, albumin secretion, urea detoxification, low-density lipoprotein (LDL) uptake and hepatic specific markers expression at both gene and protein levels [45]. Pulavendran et

al. applied a novel technique to gradually deliver HGF to murine BM-MSCs by incorporating HGF into chitosan nanoparticles. This technique successfully induced cells to differentiate into functional hepatocytes via sustainable releasing HGF into the target cells [46]. Saulnier et al. revealed the molecular mechanism underlying the hepatic differentiation of human adipose tissue-derived MSCs (AT-MSCs). The cells were exposed to HGF and FGF-4 in the early stage and the mixture of OSM with nicotinamide in the maturation stage. They found the down-regulation of mesenchymal lineage genes relative to the over expression of epithelial-related genes during differentiation [47]. These results indicate that the transition of molecular pathway occurs during differentiation from mesenchymal to epithelial lineage of hepatocytes. Chivu et al. showed incomplete hepatogenic differentiation of human BM-MSCs when HGF, nicotinamide and dexamethasone were separately added in differentiation medium. Conversely, hepatocyte-like cells features were observed after combination of all these factors in the culture [48].

Dental pulp mesenchymal cells also had differentiation potency toward hepatic lineage after inducing by common cocktail medium as other studies use. They observed that the differentiated cells acquired the characteristics of hepatocytes both in morphology and function [49]. Amniotic fluid-derived MSCs (AF-MSCs) could be induced into hepatocyte-like cells by using FGF-4 and HGF in early stage and combination of trichostatin A, dexamethasone, ITS and HGF in last stage [50]. Umbilical cord matrix stem cells also had differentiation potential into hepatic lineage after treatment with the mixture of HGF, bFGF, ITS and nicotinamide following by the mixture of OSM, ITS and dexamethasone. The differentiated cells exhibited functional hepatocytes such as hepatic specific markers expression, urea production, glycogen storage and cytochrome P450 (CYP) 3A4 or CYP3A4 activity [51]. Similarly, mesenchymal cells derived from amniotic membrane could differentiate into hepatic lineage after treatment with induction medium of 10% FBS, HGF, bFGF, OSM and dexamethasone. The differentiated cells acquired hepatocytes characteristics including glycogen storage and hepatic lineage expression both at gene and protein levels [52]. Another study successfully promoted hepatic differentiation of mesenchymal stromal cells derived from umbilical cord Wharton's jelly by one step protocol. After induction with 1% FBS, HGF and FGF-4, the differentiated cells expressed hepatic specific markers both at gene and protein levels, stored glycogen as well as had ability to uptake LDL which is one characteristic of functional hepatocytes [31]. Recently, our study successfully induced Wharton's jelly-derived MSCs into hepatocyte-like cells by specified cocktail medium of HGF, FGF-4, OSM, ITS, nicotinamide and dexamethasone in combination with hypoxic condition. The hepatocyte-like cells derived from MSCs exhibited functional hepatocytes features after complete induction including hepatic specific markers expression, glycogen storage, albumin secretion, urea detoxification, and LDL uptake [53]. Our results indicated that hypoxic environment could improve hepatic-lineage differentiation capacity of MSCs in addition to using soluble cytokines cocktail medium alone.

Induction by co-culture with other cell types: Many studies have been shown that MSCs could be induced into hepatocyte-like cells by co-culture together with other cell types such as hepatic stellate cells (HSC) and mature hepatocytes. Secreting factors derived from these cells are assumed to induce hepatic differentiation [54]. Deng et al. observed the hepatocyte-like cells after co-culture rat BM-MSCs with activated HSC. The differentiated cells also had hepatocytes phenotypes both in morphology and function such as glycogen storage capacity [55]. In addition to HSC, mature hepatocytes are commonly used for inducing MSCs into hepatic lineage. Qihao et al. showed the supportive effect of adult liver cells on hepatic differentiation of rat MSCs. They observed spheroid formation of the differentiated cells which could express liver specific markers such as albumin, α-fetoprotein and cytokeratin-18 at gene and protein levels [56]. Moreover, the heterotypic interaction of porcine hepatocytes/MSCs at ratio 2:1 was found as an optimal ratio for hepatic lineage induction. The differentiated cells were proved by liver function tests including albumin secretion, urea production and CYP3A1 activity [57].

Induction by biomaterial scaffolds: Recent studies are focusing on using biomaterial scaffolds in combination with stem cells. Biomaterial scaffolds provide 3 dimensional structures resembling extracellular matrix environment *in vivo* [58]. Several studies observe promising outcomes of using biomaterial scaffolds in association with induction medium to promote MSCS differentiation into hepatocyte-like cells. Alginate scaffold is derived from natural polysaccharide-based biomaterials that provide extracellular matrix structure allowing for cells adhesion. Lin et al. showed the supportive effect of alginate scaffold on hepatic differentiation of rat BM-MSCs. The differentiated cells displayed hepatocytes phenotype and function including albumin secretion, urea production, glycogen storage and liver specific markers expression [59]. However, the variability of materials between lots to lot is still a major disadvantage of natural biomaterials as compared to other materials. In addition to alginate scaffold, nanofibrous scaffold is synthetic polymer-based biomaterials that are widely used for stem cells culture. These scaffolds are made from defined chemical materials allowing easy control the quality and reproducibility of product. Kazemnejad et al. investigated the hepatic differentiation potential of human BM-MSCs seeded on 3 dimensional nanofibrous scaffold with differentiation medium as compared to 2 dimensional culture system. They found that nanofibrous scaffold enhanced cells differentiation into functional hepatocyte-like cells more than 2 dimensional culture systems. The differentiated cells derived from the scaffold could express liver specific markers such as albumin, α-fetoprotein, cytokeratin-18, cytokeratin-19 and CYP3A4 [60]. Another group also studied the hepatic differentiation efficacy of MSCs after seeding on synthetic extracellular matrix, ultraweb nanofibers. They observed significant increasing of albumin, urea and α-fetoprotein levels including CYP3A4 activity in conditioned medium derived from the differentiated cells seeded on ultraweb nanofibers coated plate as compared to those derived from uncoated plate [61]. The effect of collagen-coated poly (lactic-co-glycolic acid) (C-PLGA) scaffold on hepatic differentiation of rat BM-MSCs has been investigated. The researchers found earlier expression of liver specific markers in the differentiated cells derived from C-PLGA scaffolds as compared to the control group, a monolayer culture system. In addition, the differentiated cells derived from C-PLGA scaffolds could store glycogen inside the cells like functional hepatocytes [62].

In vivo studies of MSCs in liver diseases

Preclinical studies in animal models: Based on our knowledge, MSCs exert several strategies to rescue the impaired liver function in animal models which are performed in responding to well understanding in liver disease and treatment. These possible mechanisms are unique capacities of MSCs including engraftment property, paracrine secretion activity and trans-differentiation capacity (Figure 3). Several studies have been hypothesized that paracrine activity of MSCs seems to be a crucial factor for improvement of liver injury in animal models. It has been shown that secreting factors from undifferentiated MSCs were likely to play important roles in regeneration and recovery of the damaged

Figure 3: MSCs exert possible mechanisms to rescue the impaired liver function in animal models with liver disease including engraftment property, paracrine secretion activity and trans-differentiation capacity.
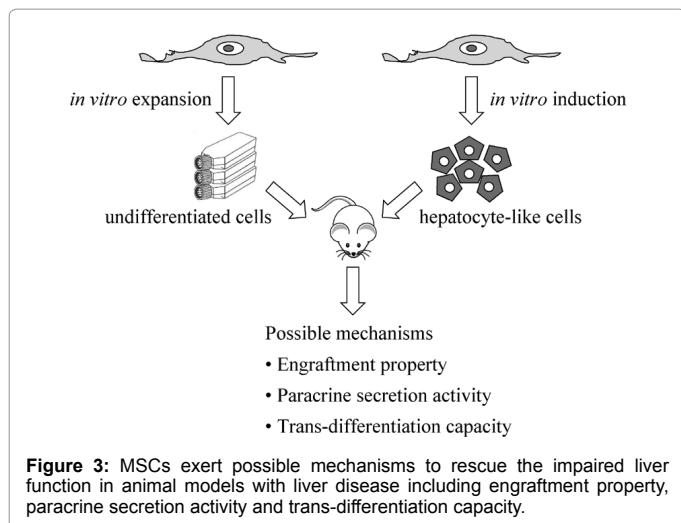
cells by stimulating endogenous cells repair in the recipient. In a model of hepatotoxic chemical-induced acute liver injury, paracrine signals from MSCs exhibited as a major role in improvement the disease. One study demonstrated the effective treatment in rat model with fulminant hepatic failure by using MSCs conditioned medium. This medium consisted of MSCs-derived molecules including growth factors, chemokines and cytokines involved in immunomodulation and liver regeneration which could prevent hepatocytes apoptosis and increased survival rate of rat model [63]. Furthermore, van Poll et al. showed the evidence that systemic infusion of MSCs conditioned medium could promote the survival rate of rat model with acute liver injury. Based on *in vitro* study, they assumed that MSCs-derived molecules mediated therapeutic effects on rats by reducing hepatocellular death and increasing hepatocytes proliferation [64].

In addition to using the conditioned medium, direct infusion with adipose tissue-derived stem cells also had ability to improve liver function in mice with acute liver injury as soon as one day after transplantation. The researchers suggested that paracrine effects derived from undifferentiated cells may exert to ameliorate liver injury [65]. Mice bone marrow stem cells also had ability to home into recipient liver as well as could improve liver function and survival rate of acute liver injury mice [66]. Similarly, Zhang et al. observed the therapeutic treatment in mice model with carbon tetrachloride (CCl_4)-induced acute liver failure by using human MSCs from umbilical cord matrix. This study revealed that the effective treatment was likely to mediate by paracrine activity derived from MSCs which could stimulate endogenous liver regeneration rather than trans-differentiation into hepatocytes [67].

In addition to paracrine effects, trans-differentiation of the engrafted MSCs toward hepatocyte-like cells *in vivo* is shown as a one strategy to improve the liver function. Many studies showed evidences that MSCs could engraft into liver and committed toward hepatic lineage cells *in vivo* ranging from 4-8 weeks post-transplantation. One recent study observed the effective treatment for D-galactosamine-induced fulminant hepatic failure in pig model by transplantation immediately with human BM-MSCs after liver failure induction. The transplanted cells contributed to liver regeneration and trans-differentiation into hepatocytes in recipient liver which accounted for 30% of the total hepatocytes. Moreover, animals in transplanted group survived for more than 6 months while control group died within 96 hours [68]. Similarly, Cao et al. also demonstrated the therapeutic

potential of transplanted human placenta-derived MSCs (PD-MSCs) in pig with D-galactosamine-induced acute liver failure. The transplanted cells could not only differentiate into hepatocyte-like cells *in vivo* but also improving the liver function as well as reducing liver inflammation and increasing liver regeneration in the recipient. The survival time of transplanted group was prolonged significantly as more than 5 months as compared to the control group [69].

In a model of hepatotoxic chemical-induced chronic liver injury, several studies have been shown anti-fibrosis effect of transplanted MSCs on the recipients. The putative mechanism underlying the anti-fibrotic effect of MSCs may involve in the expression of matrix metalloproteinases (MMPs). MMP-2 and MMP-9 were shown to degrade the extracellular matrix and induced apoptosis of hepatic stellate cells [70,71]. Zhang et al. observed the therapeutic effects of human amniotic membrane-derived MSCs (AM-MSCs) after transplantation into mice model with CCl_4-induced liver cirrhosis. These effects included reducing hepatic stellate cells activation which can enhance fibrosis, decreasing hepatocytes apoptosis and promoting liver regeneration. Moreover, the engrafted cells could differentiate into human albumin or α-fetoprotein-expressing hepatocyte-like cells in the damaged liver [72]. This finding is similar to the results of Jung et al. study. They found that human umbilical cord blood-derived MSCs (UB-MSCs) could improve liver fibrosis in rat model with CCl_4-induced liver cirrhosis by decreasing expression of transforming growth factor-β1 (TGF-β1), alpha-smooth muscle actin (α-SMA) and collagen type I which are mediators of liver fibrosis. In addition, the engrafted cells could differentiate into human albumin or α-fetoprotein-expressing hepatocyte-like cells which may improve the liver function by decreasing serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels [73]. MSCs from human umbilical cord also had therapeutic effect on liver injury mice by decreasing serum AST and ALT levels, inflammation, apoptosis and denaturation of hepatocytes [74]. Similarly, it has been reported that rat BM-MSCs could differentiate toward hepatocyte-like cells in rats with CCl_4-induced liver injury and further improvement of the liver function through increasing albumin level and decreasing ALT level. Moreover, the relation of anti-fibrotic effect and low expression of collagen type I which represents as liver fibrosis marker was also observed [75]. Interestingly, human adipose tissue-derived multilineage progenitor cells infusion could reduce total cholesterol in rabbit model of familial hypercholesterolemia within 4 weeks after transplantation. This effect was maintained for 12 weeks correlated with hepatic differentiation of the engrafted cells in recipient liver [76]. In contrast, Tsai et al. did not observe the trans-differentiation toward hepatocyte-like cells of human MSCs isolated from umbilical cord Wharton's jelly even the percentage of liver fibrosis was significantly reduced in rat model with CCl_4-induced liver fibrosis. This study suggested that bioactive cytokines released from undifferentiated MSCs may play important roles in reducing hepatic inflammation and restoring liver function in recipients [77]. Notably, these studies have shown low percentage of engrafted MSCs at maximum level of 20% in the recipient liver. These results led to the question whether *in vivo* hepatocyte-like cells differentiation of MSCs has enough to compensate and improve the liver function of recipients with liver injury.

Recently, some studies are focusing on using MSCs-derived hepatocyte-like cells for transplantation instead of undifferentiated cells. Several studies generated hepatocyte-like cells *in vitro* by chemically defined cocktail medium before administration *in vivo*. Banas et al. successfully differentiated human AT-MSCs into hepatocyte-like cells under appropriate condition *in vitro*. The

differentiated cells could restore the liver function of acute liver failure mice by decreasing serum ALT, AST and ammonia levels within 24 hours post-transplantation [78]. Similarity, The hepatocyte-like cells clusters derived from human AT-MSCs had hepatic characteristics after exposure to hepatogenic inducer not only *in vitro* but also *in vivo*. The improvement of serum albumin and total bilirubin levels were observed after cells transplantation into non-obese diabetic severe combined immunodeficiency (NOD-SCID) mice with chronic liver injury [79]. Recent study demonstrated the reducing liver fibrosis effect on rats with CCl_4-induced liver fibrosis after intravenous injection with rat bone marrow MSC-derived hepatocyte-like cells. This study suggested that the transplanted cells may induce immune system of recipients to rescue the disease by increasing expression of interleukin 10 (IL-10) which can further decrease the accumulation of liver fibrosis [80]. Hepatic lineage cells derived from MSCs provided promising therapeutic potential in CCl_4-induced liver fibrosis mice. This result was confirmed by reducing liver fibrotic area as well as improving serum levels of bilirubin and alkaline phosphatase (ALP) in transplanted group [81].

Clinical trials in human: MSCs-based therapy has shown as a promising tool for chronic liver diseases especially in cirrhosis condition. Most of these clinical trials are in phase I or II that verified the safety or efficacy of the treatment. All studies have shown the efficacy of MSCs-based therapy without serious side effects or complications. Autologous BM-MSCs were successfully transplanted into patients with decompensated liver cirrhosis. The cells could improve the overall condition by decreasing the end-stage liver disease (MELD) score of recipients. Moreover, the quality of life of all patients was improved shown by the enhancement of mean physical component scale and the mean mental component scale for 1 year post-transplantation [82]. Another study showed the therapeutic effect of autologous BM-MSCs transplantation in patients with end-stage liver disease. The liver function of all patients was improved within 24 weeks after transplantation shown by reductions of MELD score and serum creatinine level [83]. Recently, El-Ansary et al. compared the therapeutic effect of autologous transplantation between undifferentiated BM-MSCs and differentiated cells (hepatocyte-like cells) in patients with liver cirrhosis. The improvement of liver function was observed in patients after transplantation with both undifferentiated and differentiated cells such as increased prothrombin and serum albumin levels and decreased bilirubin and MELD score. Additionally, they did not observe significant difference in clinical improvement between those 2 groups of patients [84]. In consistency with El-Ansary's study, Amer et al. demonstrated the safety and short-term therapeutic effect of autologous transplantation with BM-derived hepatocyte-like cells in patients with end-stage liver failure. Clinical improvement was verified by Child score, MELD score, fatigue scale, performance status and serum albumin level [85]. Recent study investigated the safety and efficacy of umbilical cord-derived MSC (UC-MSC) in patients with decompensated liver cirrhosis. During 1 year follow-up period, the significant reduction of ascites volume and the improvement of liver function were observed in transplanted patients as compared to the control group [86]. Similarly, recent study observed short-term efficacy of autologous BM-MSCs transplantation in liver failure patients caused by viral hepatitis B. The liver function and MELD score were significantly improved in the transplantation group as compared to the control group for 2-3 weeks post-transplantation and these effects could sustain 36 weeks [87]. Currently, autologous BM-MSCs transplantation showed safety, feasibility and efficacy in improving liver function of Egyptian patients with cirrhosis. Thus, MSCs can be used as a regenerative medicine in liver disease especially at end-stage condition, although only short term effect has been observed.

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