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Concise Review: Therapeutic Potential of Mesenchymal Stem Cells for the Treatment of Acute Liver Failure and Cirrhosis

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ABSTRACT

Currently, the most effective therapy for acute liver failure and advanced cirrhosis is liver transplantation. However, this procedure has several limitations, including lack of donors, surgical complications, immunological suppression, and high medical costs. The alternative approaches that circumvent the use of a whole liver, such as stem cell transplantation, have been suggested as an effective alternate therapy for hepatic diseases. Mesenchymal stem cells (MSCs), also known as multipotent mesenchymal stromal cells, are self-renewing cells that can be found in almost all postnatal organs and tissues, including liver. During the past decade, great progress has been made in the field of MSC-dependent liver regeneration and immunomodulation. Because of their potential for differentiation into hepatocytes as well as their immunomodulatory characteristics, MSCs are considered as promising therapeutic agents for the therapy of acute liver failure and cirrhosis. In this concise review, we have summarized therapeutic potential of MSCs in the treatment of acute liver failure and cirrhosis, emphasizing their regenerative and immunomodulatory characteristics after engraftment in the liver. We have also presented several outstanding problems including conflicting data regarding MSCs engraftment in the liver and unwanted mesenchymal lineage differentiation in vivo which limits MSC therapy as a mainstream treatment approach for liver regeneration. It can be concluded that efficient and safe MSC-based therapy for acute and chronic liver failure remains a challenging issue that requires more investigation and continuous cooperation between clinicians, researchers, and patients. STEM CELLS *2014;32:2818–2823*

INTRODUCTION

Acute liver failure, which develops secondary to infection, toxin, or immune-mediated attack, is a potentially fatal clinical syndrome characterized by rapid development of hepatocellular dysfunction with diffuse intrahepatic infiltration of inflammatory cells and massive multilobular necrosis. Liver cirrhosis is a chronic disease of the liver, characterized by the loss of functional liver cells and their replacement with fibrous tissue resulted from alcohol abuse, nutritional deprivation, or infection especially by the hepatitis virus B and C (HBV and HCV). Currently, the most effective therapy for acute liver failure and advanced cirrhosis is liver transplantation, but its use is limited because of organ donor shortage, financial considerations, and the requirement for lifelong immunosuppression. An alternative approach such as stem cell transplantation has been suggested as an effective alternate therapy for hepatic diseases [1].

Mesenchymal stem cells (MSCs), also known as multipotent mesenchymal stromal cells, are self-renewing cells that can be found in almost all postnatal organs and tissues, including liver [2]. A potential for differentiation into hepatocytes as well as their immunomodulatory characteristics [3, 4] open the path for the use of MSCs in the therapy of acute and chronic liver diseases.

MSCs: A BRIDGE BETWEEN LIVER REGENERATION AND IMMUNOMODULATION

Previous studies have shown that both rodent [5–8] and human MSCs [9–14], in the presence of growth factors, cytokines, chemical compounds, hepatocytes, or nonparenchymal liver cells (Supporting Information Table S1), are able to differentiate into hepatocytes.

Sai-Nan Shu et al. [5] induced differentiation of rat MSC into hepatocytes using hepatocyte growth factor (HGF) and basic fibroblast growth factor (bFGF). Lange et al. have described hepatocytic gene expression in rat MSC cultured alone [6] or in coculture with hepatocytes [7], both of which required the

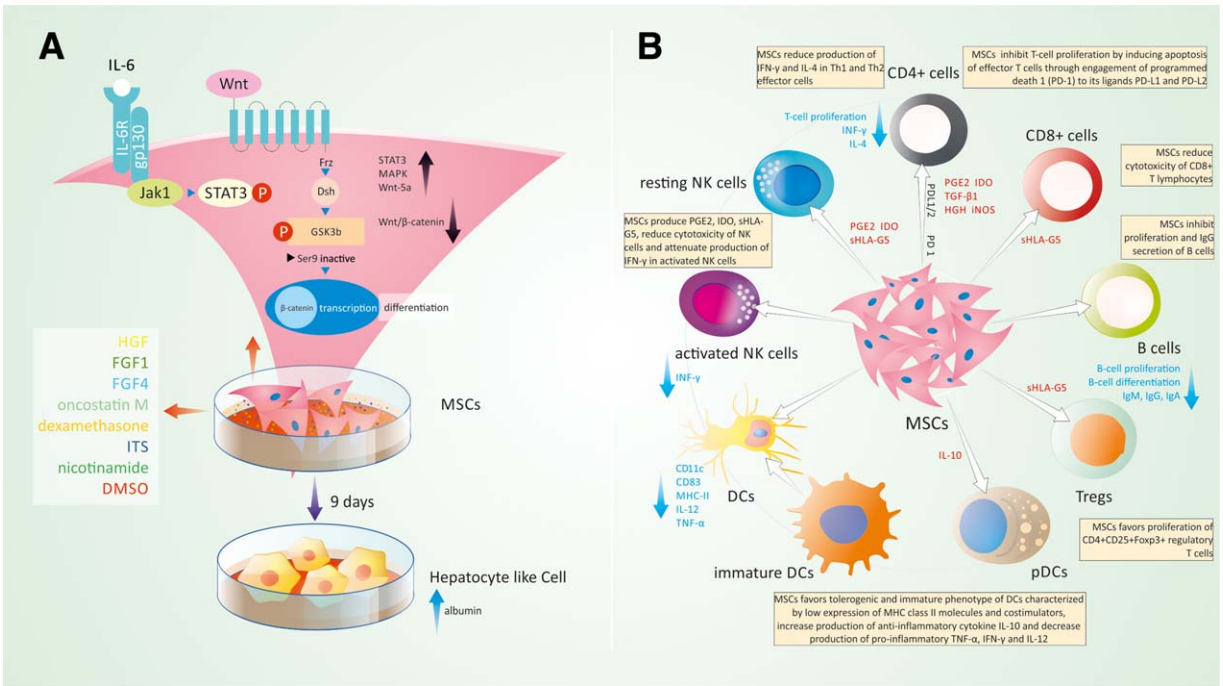

Figure 1. MSC therapy of acute liver failure. Therapeutic effects of MSC in acute liver failure are based on the ability of MSC to differentiate into hepatocytes **(A)** and to alter function of immune cells responsible for acute liver injury **(B)**. Abbreviations: DC, dendritic cell; MSC, mesenchymal stem cell; NK, natural killer.

presence of HGF, FGF-4, and epidermal growth factor (EGF). Luk et al. [8] described differentiation of rat MSC into hepatocytes after coculturing without the addition of growth factors to culture medium, using either normal or injured hepatocytes in the coculture system.

Human MSCs, derived from bone marrow (BM-MSCs), adipose tissue (AT-MSCs), amniotic fluid (AF-MSCs), dental pulp (DP-MSCs), umbilical cord (UC-MSCs), and fetal lung (FL-MSCs), have potential to differentiate into hepatocytes [9–14]. Lee et al. were first to design protocol for differentiation of human BM-MSCs and UC-MSCs into hepatocyte-like cells [9]. Differentiation was induced by treating MSCs with differentiation medium (culture medium supplemented with HGF, bFGF, and nicotinamide) for 7 days, followed by treatment with maturation medium (culture medium containing oncostatin M [OSM], dexamethasone [Dex], and insulin, transferrin, selenium [ITS]). Seo et al. showed that human AT-MSCs are able to differentiate into functional hepatocyte-like cells in vitro by the treatment of HGF, OSM, and dimethyl sulfoxide (DMSO) which has been proven in vivo after successful transplantation in CCl4-injured livers of nonobese diabetes-SCID mice [10]. Similar results were obtained by Banas et al. [11] who observed that 9 days of in vitro cocktail treatment (containing HGF, FGF-1, FGF-4, OSM, Dex, ITS, nicotinamide, and DMSO) induces hepatocyte differentiation of human AT-MSCs that were capable to in vivo regenerate acute injured liver of immunodeficient mice. Zheng et al. managed to differentiate AF-MSCs into hepatocyte-like cells using FGF-4, HGF, trichostatin A, dexamethasone, ITS, and HGF [12] and demonstrated that AF-MSCs had higher hepatic differentiation potential then BM-MSCs. Using HGF, ITS, OSM, and Dex, Ishkitiev et al. differentiated DP-MSCs into hepatocytes [13], while Ling et al. found that FL-MSCs, cultured in the presence of HGF, EGF, and bFGF, could acquired morphologic and functional characteristics of hepatocytes [14]. Hepatocyte-like cells differentiated from BM-MSCs, AT-MSCs, AF-MSCs, DP-MSCs, UC-MSCs, and FL-MSCs exhibited hepatocyte-specific cuboidal morphology, expressed hepatocyte marker genes, and acquired comprehensive in vitro functions specific for liver cells including albumin production, glycogen storage, urea secretion, uptake of low-density lipoprotein, and phenobarbital-inducible cytochrome P450 activity [9–14].

Overexpression of hepatocyte nuclear factor 3β (HNF3β) as well as stimulation of IL-6/gp130 axis is able to induce the differentiation of human bone marrow-derived-MSCs toward a hepatocyte phenotype through the suppression of Wnt/β-catenin signaling (Fig. 1A). Overexpression of HNF3β suppresses Wnt/β-catenin signaling by altering localization of β-catenin from nuclei and cytoplasm to the cytoplasmic membrane [15], while binding of IL-6 to its receptor triggers the homodimerization of gp130, induces phosphorylation, and activation of STAT3 which upregulates expression of the Wnt-5a, negative modulator of Wnt/β-catenin signaling, and directly reduced β-catenin expression in MSCs leading to hepatocyte differentiation [16].

Although MSC differentiation into hepatocytes has been demonstrated in vitro, it is still controversial whether MSC transplantation can completely regenerate hepatocytes in vivo. The vast majority of recently published studies indicated that therapeutic effects and use of MSCs in acute and chronic liver failure would be primarily based on their release of trophic and immunomodulatory factors [17–21] that alter function of immune cells responsible for liver injury (Fig. 1B). Through production of soluble factors, particularly through the secretion of prostaglandin E2, MSCs alter the secretion profile of dendritic cells (DCs) resulting in increased production of anti-inflammatory cytokine IL-10 and decreased production of tumor

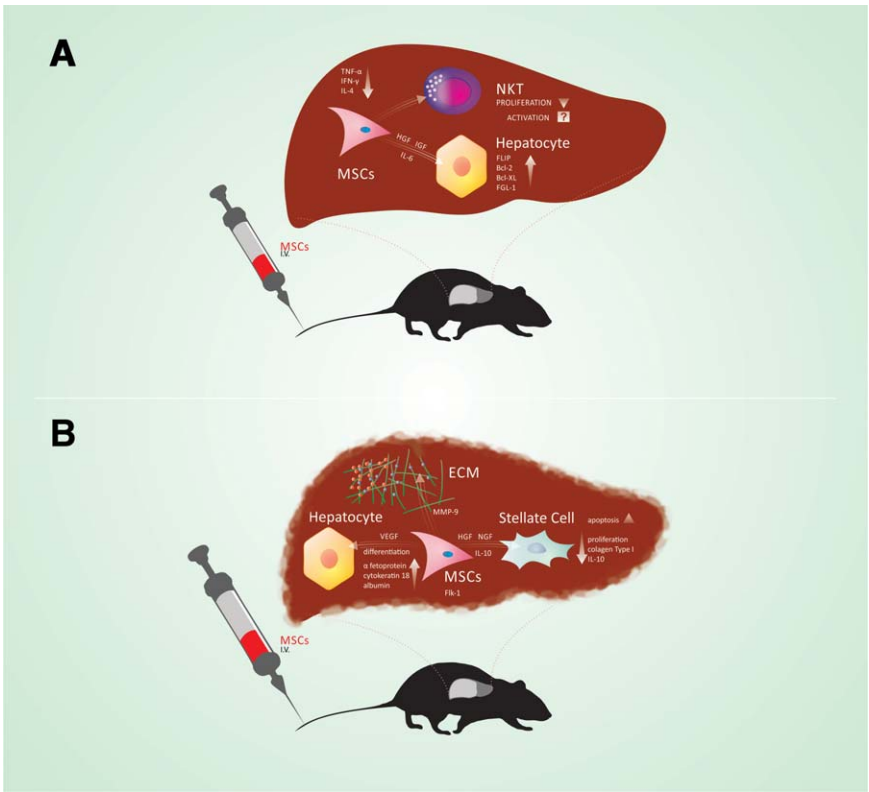

Figure 2. Therapeutic effects of MSCs in acute liver failure and cirrhosis are based on the release of trophic and immunomodulatory factors affecting function of NKT and stellate cells. MSCs engraft in the mice liver, attenuate proliferation of NKT cells, improve recovery of damaged hepatocytes **(A)**, promote apoptosis of stellate cells and reduce synthesis of collagen **(B)**. Abbreviations: ECM, extracellular matrix; MSC, mesenchymal stem cell.

necrosis factor alpha (TNF-α), interferon-gamma (IFN-γ), and IL-12 [3, 4]. Additionally, through secretion of prostaglandin E2, MSCs reduce production of IFN-γ and IL-4 in Th1 and Th2 cells and stimulate proliferation of CD4+CD25+Foxp3+ regulatory T cells (Tregs) [3, 4]. Two other important soluble factors for immunosuppressive effects of MSCs are indoleamine 2,3-dioxygenase (IDO) and soluble human leukocyte antigen-G5 that suppress proliferation of effectors T cells [3, 4]. Through production of prostaglandin E2, IDO, HGF, TGF-β, and IL-10, MSCs can inhibit maturation of DC, proliferation and IgG secretion of B cells, and reduce cytotoxicity of natural killer (NK) cells and CD8+ cytotoxic T lymphocytes [4, 17].

Beside paracrine mechanisms, MSCs can suppress immune response through cell to cell contact with immune cells. MSCs can inhibit T-cell proliferation by inducing apoptosis of effector T cells through engagement of programmed death 1 (PD-1) to its ligands PD-L1 and PD-L2 and are able to render T cells anergic by downregulating expression of costimulatory molecules CD80 and CD86 on antigen-presenting cells [3, 4].

MSC Treatment of Acute Liver Failure

Zhu et al. [22] were first to show that systemic infusion of MSCs reduces Concanavalin A (Con A)-induced liver injury, a well-established murine model of fulminant liver failure [23, 24]. Transplantation of 1×10^6 MSCs immediately after injection of Con A significantly reduced liver injury, whereas reduced number (2×10^5) of MSC or later injection of MSCs (8 hours after Con A) had no effects on liver injury. Intravenously injected MSCs managed to engraft in the damaged liver, attenuated inflammation and lymphocyte proliferation, and significantly reduced number of activated NKT cells not only in the liver but throughout the body [22] suggesting that MSC-mediated suppression of lymphocytes was systemic and not limited to the liver area. Infusion of MSCs reduced the levels of major proinflammatory cytokines (TNF-α, IFN-γ, and IL-4) both in serum and the liver but had no effect on the expression of the inducible form of nitric oxide synthase, IL-2, and IL-10 [22]. Transplanted MSCs were detected in injured livers 24 hours after injection but were not found in healthy, undamaged livers indicating that liver damage induced recruitment of MSCs in the liver, particularly in periportal areas. Interestingly, all transplanted MSCs had disappeared from the liver 1 month after injection, indicating that the MSCs have been efficiently cleared from the liver [22].

Similarly to Zhu et al. [22], several recently published studies [17, 25, 26] strongly suggest that therapeutic effects of MSCs in acute liver injury are systemic, based on the release of trophic and immunomodulatory factors. Parekkadan et al. indicated that differentiation of engrafted MSCs into hepatocytes rarely happens [17], while factors secreted by transplanted MSC exert strong beneficial effect on hepatocytes (Fig. 2A). One of defined hepatoprotective MSC-derived factors is the IL-6 [25, 26], which induces the expression of fibroblast-like-protein 1 gene (FGL-1) a key gene for liver regeneration [26] and protects against Fas-mediated death by establishing a critical level of antiapoptotic hepatic proteins:

FLICE-like inhibitory protein, B-cell lymphoma 2 (Bcl-2), and B-cell lymphoma-extra large (Bcl-xl) [25]. Xagorari et al. demonstrated that protective effect of MSC-conditioned medium on hepatic cell apoptosis after carbon tetrachloride (CCl4) induced liver injury is mediated by IL-6 and FGL-1 [20].

Immunomodulatory capacity of human BM-MSC-derived conditioned medium was also shown in an experimental model of acute liver injury caused by hepatotoxin D-galactosamine (Gal-N) [14]. Although no specific mechanism of action has been identified, Parekkadan et al. postulated that soluble factors contained in MSC-conditioned medium (IL-6, VEGF, HGF, and insulin-like growth factor binding proteins) were responsible for both local and systemic effects [17, 27]. There was significantly attenuated mononuclear cell infiltration in the liver, reduced apoptosis, and increased proliferation of hepatocytes accompanied with downregulated serum levels of proinflammatory cytokines IL-1β and TNF-α and increased serum levels of anti-inflammatory IL-10 [17, 27, 28].

MSC–Based Therapy of Liver Cirrhosis

The beneficial effect of MSC transplantation in the treatment of liver cirrhosis was well documented both in animal and clinical studies. Fang et al. [29] showed that systemic infusion of murine BM-MSC expressing Flk-1, a receptor for VEGF, ameliorate CCl-4-induced liver fibrosis in mice. Flk-1+ MSCs managed to engraft into the liver, obtained epithelium-like morphology, expressed albumin at low frequency, initiated endogenous hepatic tissue regeneration, reduced collagen deposition, and significantly improved recovery of damaged hepatocytes [29]. Importantly, it seems that the moment in which cell injection is performed influences the result of cell therapy. Beneficial effects were seen only if MSCs were transplanted immediately after exposure to CCl-4 while there was not any therapeutic effect if MSCs were injected 1 week after fibrosis induction [29].

It seems that therapeutic effects of MSCs in liver cirrhosis are based on the release of trophic and immunomodulatory factors that alter function of hepatic stellate cells which activation is a pivotal event in the development of liver fibrosis. MSCs were able to reduce the proliferation of stellate cells and collagen type I synthesis through the secretion of IL-10 [27], and to promote hepatic stellate cell apoptosis through the secretion of HGF [27] and nerve growth factor (NGF) [30]. Indirect coculture of MSCs and activated hepatic stellate cells led to a significant decrease in collagen deposition and proliferation. Activated hepatic stellate cells produce IL-6 inducing IL-10 secretion from MSCs, which in turn attenuate stellate cell proliferation and collagen synthesis. In addition, MSCs release HGF [27] and NGF responsible for the nuclear factor kappa B and Bcl-xl-dependant induction of apoptosis of hepatic stellate cells [30]. In line with these findings, in vivo study conducted by Oyagi et al. [31] showed that MSCs cultured with HGF are capable of improving serum albumin level and reducing liver fibrosis in rats, an effect that was not observed after transplantation of MSC previously cultured in the absence of HGF.

MSCs can exert antifibrotic effect in liver cirrhosis through the expression of matrix metalloproteinase-9 (MMP-9) that degrades the extracellular matrix [32]. It was recently shown that autologous BM-MSCs can differentiate into hepatocytes and are able to promote liver regeneration after portal vein embolization in cirrhotic rats through improving local microenvironment by upregulating gene expressions of VEGF, HGF, IL-10, and MMP-9 (Fig. 2B) [33].

Clinical Trials Using MSCs for the Treatment of Liver Cirrhosis

Several clinical trials investigate therapeutic potential of MSC for the treatment of liver cirrhosis (Supporting Information Table S3) [21, 34–40].

Results obtained in pilot phase 1 studies, conducted by Mohamadnejad et al. [34] and Kharaziha et al. [35], showed that autologous transplantation of 30–50 million iliac crest-derived-MSCs, injected via peripheral or portal vein, was well tolerated and managed to significantly improve "Mayo End-Stage Liver Disease" (MELD) score in at least 50% of patients.

Amer et al., in phase 2 study [36], showed a significant improvement in Child-Pugh and MELD scores in 20 patients that received autologous BM-MSCs stimulated to hepatic lineage. Interestingly, patients receiving MSCs through intrahepatic route had stronger improvement of MELD and fatigue scores compared to patients that received MSCs through intrasplenic route [36]. Similar results were obtained by Peng et al. [37] who showed that MELD score as well as levels of albumin, bilirubin, prothrombin time was significantly improved in 53 patients with post-HBV liver failure, 2–3 weeks after intrahepatic injection of autologous MSCs isolated from iliac bone aspirates. In another phase 2 trial, El-Ansary et al. [38] noticed significant improvement in serum bilirubin levels, prothrombin time, and MELD score in 15 patients with HCV-related cirrhosis that received 1 million MSCs per kg/b.wt. intravenously.

An open-label trial published by Jang et al. [21] showed beneficial effects of autologous BM-MSC transplantation for the treatment of alcoholic cirrhosis. MSCs (5×10^7 cells) were injected twice, in the weeks 4 and 8, through the hepatic artery. Histological improvement was observed in 54.5% of patients, the Child-Pugh score was improved in 90.9% of patients and the levels of TGF-β1, type 1 collagen, and α-smooth muscle actin significantly decreased after MSCs therapy [21].

A 6-month follow-up of a clinical study conducted by Amin et al. [39] showed that autologous, intrasplenic transplantation of 10^7 BM-MSC managed to improve liver function in 20 patients with post-HCV liver cirrhosis, as determined by significant decrease in the total bilirubin, aspartate transaminase (AST) and alanine transaminase (ALT), prothrombin time as well as significant increase in the albumin level.

MSC–Based Therapy of Liver Diseases: Risk or Benefit?

There are contradictory data regarding engraftment of transplanted MSC in the liver and some concerns have been raised regarding their fibrogenic potential and unwanted differentiation into myofibroblasts. It seems that these unwanted effects depend on the time frame and route of MSC injection [40–42]. Di Bonzo et al. [41] showed that intravenously injected BM-MSCs have the potential to migrate into normal or injured liver parenchyma only under conditions of chronic injury and that engraftment of MSCs as well as hepatocyte differentiation rarely happened in acute injured livers. They showed that during acute liver injury significant number of transplanted MSCs

differentiated into myofibroblasts rather then into hepatocytes [41] indicating that the time frame of MSC transplantation in the liver has an important role. It seems that MSCs promote and/or induce fibrogenesis when they are injected during the "liver injury phase," but accelerate healing process when they are transplanted during the "liver resolution phase" [40]. Baertschiger et al. [42] indicated that engraftment and myofibroblastic differentiation of the transplanted MSCs may be influenced by the route of injection. In their view, stable engraftment in the liver could not be achieved after intrasplenic injection. However, after intrahepatic injection, MSCs permanently remained in the liver, but mainly differentiated into myofibroblasts [42]. In order to improve engraftment and to avoid unwanted differentiation, Aurich et al. suggest that human MSCs should be first differentiated into hepatocyte-like cells in vitro and then transplanted in vivo [43].

Poor engraftment in the liver could be a consequence of immune rejection of transplanted MSCs. Autologous stem cell transplantation is difficult to attempt on patients with acute liver failure or in patients with end stage of liver disease, because of a cell preparatory period and cell transplantation timing. Therefore, allogenic stem cell transplantation has more practical therapeutic value in cell based therapy of liver failure. Nevertheless, there are several obstacles for safe allogeneic MSC transplantation. MSCs are permissive for cytomegalovirus (CMV) and herpes simplex virus (HSV) infections in vitro and in contrast to MSC autotransplantation, MSC allotransplantation carry the risk of viral transmission to the recipient [40]. Although no information was available on HSV and CMV transmission by MSCs in vivo, before MSC allotransplantation, both recipient and MSC donor should be screened for CMV and HSV in order to prevent dramatic infections in immunosuppressed patients [40]. Next, allogeneic MSCs are not invisible to the recipient's immune system, and after transplantation, they could trigger immune responses which results with rejection of the transplanted cells, attenuating their engraftment and full therapeutic potential. In line with this observation, 1-year follow-up of randomized placebo-controlled trial conducted in Iran [44] showed that autologous BM-MSC transplantation through peripheral vein had no beneficial effect in cirrhotic patients.

Innovative technologies of reprogramming and derivation of induced pluripotent stem cells (iPSCs) could address this issue. Derived from patient's own somatic cells, iPSCs eliminate the potential for immune rejection. Several laboratories established protocols for differentiation of iPSCs into hepatocytes [40] which are able to restore liver function in animal models of liver failure [45]. However, iPSCs are not yet ready for transplanting into patients. Most iPSCs have been generated with integrating vectors, which may not get silenced efficiently or could disrupt endogenous genes. Furthermore, there is plenty of evidence that iPSCs therapy can lead to tumor formation and before clinical use, researchers need to focus on safety of iPSCs therapy in light of the potential for cancer formation [5, 40].

Some concerns have been raised regarding MSC susceptibility to spontaneously undergo malignant transformation [40]. Although human MSCs are at lower risk of malignant transformation, recently published data suggest that human MSCs may display variable but significant level of genomic instability, concurring with an important level of aneuploidy [46], telomeric deletions at high passages (>170), microsatellite instability, downregulated genes involved in DNA repair, and heteroplasmic point mutations [40]. Although malignant transformation of human MSCs is not reported in clinical trials, in most of them the follow-up was relatively short, and the occurrence of a tumor may take longer to appear. Pan et al. [47] managed to identify number of genes that were differentially expressed between malignant MSCs and their normal parental counterparts and these genes should serve as biomarkers to screen MSC cultures for evidence of early transformation events before clinical use.

Conclusions

Because of their potential for differentiation into hepatocytes as well as their immunomodulatory characteristics, MSCs are promising therapeutic agents for the therapy of acute liver failure and cirrhosis. However, there are still several problems including conflicting data regarding MSCs engraftment in the liver and their long-term efficacy, potential risk of malignant transformation, and unwanted mesenchymal lineages differentiation in vivo which limit their ability to be used as a mainstream treatment approach for liver regeneration.

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Author Contributions

V.V.: conception and design, data analysis and interpretation, and manuscript writing; J.N.: manuscript writing and figure drawing; N.A.: manuscript writing; M.S.: manuscript writing and final approval of manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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