Review Article

Liver regeneration: Influence-factors and mechanism of mesenchymal stem cell transplantation

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Abstract

Mesenchymal stem cells (MSCs) are considered the most promising candidate for therapeutic repair of liver disease due to their effect on immune privilege, self-renewal and multidifferential potency. Recently, there have been a number of advances in the area of the factors that influence liver regeneration as well as the impact of MSCs transplantation on liver regeneration. Moreover, there is important new data on the several factors affect the transplantation efficiency of MSC such as therapeutic pretreatment before MSCs transplantation, the impact of cell number and repeated administration on success, and the implications on the outcome for the various approaches used to transplant the cells. Furthermore, other elements the can influence transplantation success also include transdifferentiation of stem cells, improving the microenvironment for implantation, antioxidant treatments, improving hepatocyte viability and immunomodulation strategies. Similarly, there are a number of important pathways that play a vital role, such as IL-6/STAT3 and Wnt/β-catenin signaling, that can also be modified to improve outcomes. MSCs can effectively contribute to liver regeneration and offer an effective alternative therapy to organ transplantation for the treatment of liver diseases. By developing a better understanding of the factors associated with beneficial outcomes, new strategies for the treatment of liver disease can be developed.

Keywords: Mesenchymal stem cells, Liver regeneration, Liver disease, Transplantation, Antioxidant

INTRODUCTION

Even though only a very minor number of liver cells, 0.0012 to 0.01 %, undergo mitosis under normal conditions, unlike most organs, the liver has a strong regenerative capacity after trauma or surgical resection. Nevertheless, severe damage caused by liver diseases can significantly diminish the proliferative ability of these cells and lead to deleterious outcomes. Although orthotropic liver transplantation is the most therapeutic option for patients with end-stage liver disease, its clinical use is greatly limited due to a number of factors such as, the shortage of appropriate organ donors, the high financial cost associated with this type of surgery and risk of transplant rejection.

With this in mind, there is an urgent need to consider alternative approaches to deal with liver transplantation. Advances in tissue engineering and cell replacement therapy offer some promise for new approaches to dealing with this some difficult area. Moreover, hepatocyte transplantation can be used as an alternative method for liver transplantation, nevertheless, there is also the problem of limited donor resources.

In recent years, mesenchymal stem cells (MSCs) have been suggested as the most promising candidate supporting liver replacement therapy. This is not only because of their self-renewal ability but also their multidifferential potency, in that under certain conditions MSCs can be influenced to differentiate into various cells such as liver cells and also other cell types like adipose and osteoblasts [1,2]. Furthermore, another benefit to MSC transplantation is their immune privilege status that can help reduce the risk of graft rejection [3]. Additionally, it is critically important to maintain the cells that are to be transplanted in a good state and to regenerate the liver cells as quickly as possible to meet the effective liver volume required.

Factors that affect transplantation efficiency

Although the exact mechanisms of MSC transplantation are not fully understood, there are many experiments that suggest that some measures can effectively enhance the transplantation of MSCs.

Pretreatment before MSCs transplantation - *Predifferentiation*

The predifferentiation of MSCs into liver progenitor cells *in vitro* can greatly shorten the time of MSCs differentiation into hepatocytes upon transplantation. This approach supports the clinical needs of patients with severe liver disease and helps to restore liver function as soon as possible through cell transplantation. At the same time, by ensuring the differentiation of the MSC towards liver cells can potentially reduce any unexpected inappropriate tumorigenesis.

The process of inducing the differentiation of MSCs into hepatic progenitor cells and the factors influencing the expansion *in vitro* are still somewhat poorly understood. Growth factors and cytokine can be used to induce the differentiation of MSCs, especially using factors such as FGF-4, HGF and EGF [4]. Additionally, there are other measures that can induce hepatic differentiation of MSC, such as bile acid [7]. Furthermore, the use of extracellular matrix (ECM) plays an important role in the proliferation and differentiation into the various cell types. Bi *et al* [5] indicated that liver-specific ECM promotes hepatic differentiation of MSCs. MSCs promote liver regeneration by altering the microenvironment of target organs. The process of liver regeneration is accompanied by the formation of blood vessels. The degradation of ECM is an important component in developing new blood vessels. ECM mainly involved in the two steps of angiogenesis: endothelial cells invade the stroma after ECM degradation; angiogenesis, ECM deposition composition of vascular lumen.MMP-9 is one of the critical factors for the degradation of ECM. Li *et al* [49] showed that MSCs up-regulated significantly the expressions of VEGF and MMP-9 gene. Laminins, a family of heterotrimeric ECM proteins, play an important role in cell migration and differentiation, were up-regulated in hepatectomized rats after MSCs transplantation [54].

Likewise, Gilsanz *et al* [6] identified that MSCs predifferentiated with collagen (0.1 %) were more beneficial for treating liver fibrosis and greatly improved the outcomes of liver disease. Nevertheless, contrasting this, recent research has compared the MSC derived hepatocyte-like cells and bone marrow MSC (BMSCs) in the treatment of acute liver failure of rats. The results of these experiments showed that both types, induced and un-induced BMSCs, similarly had a positive therapeutic effect on liver regeneration [8].

Genetic modification of cells for therapeutic use

Gene therapy refers to the introduction of exogenous genes into target cells to correct or compensate for diseases caused by gene defects and abnormalities, so as to achieve therapeutic outcomes. Gene modified stem cells have both advantages of stem cell therapy and gene therapy, and become increasingly popular approach for biotherapy.

Homing is the initial and critical step for stem cells and is clearly important in tissue repair. In fact, the main obstacle to repair is if the stem cells cannot efficiently home to the appropriate target tissue. Therefore, improving the homing rate of stem cells is vital for stem cells to be effectively used in disease treatment [9,10]. Approaches that include transfection of genes that promote stem cell migration and adhesion will be beneficial to the homing of stem cells. Chemokines, which are composed of 70 to 100

amino acids, are small molecules secreted by chemotactic cells, which have their functions mediated by chemokine receptors. Chemokines have been shown to be important to the homing behavior of MSCs. The CXC chemokine receptor 4 (CXCR4) is one of the most important chemokine receptors, and is expressed in many types of cells. CXCR4 and its ligand, stromal cell derived factor 1 (SDF-1) and also known as CXCL12, can mediate a variety of signal transduction pathways related to cell chemotaxis, cell survival and proliferation. The interaction of SDF-1 and its receptor CXCR4 has been reported to play a major role in homing of MSCs [11]. Furthermore, cells in injured organs appear to highly express SDF-1. Additionally, overexpression of CXCR4 in MSCs resulted in a significant increase in MSC migration and enhances the cellular release of paracrine factors, such as VEGF, HGF, and IL-6 that contribute to the homing of stem cells [12,13]. Transfection of MSC with antiapoptosis genes or prosurvival genes can increase the survival rate of MSCs Hepatocyte growth factor (HGF) has potent anti-apoptotic effects. Similarly, vascular endothelial growth factor (VEGF) is an important growth and proangiogenic factor. These are important factors during liver regeneration. MSCs over expressing HGF or VEGF can enhance liver regeneration [17,18]. Recently, studies have demonstrated that BMSCs reinforce liver regeneration and have significant suppressive effects on hepatocyte apoptosis [55].

Bcl-2 family proteins are a class of proteins that play a key role in apoptosis. On the mitochondria, Bcl-2 family proteins regulate the stability of mitochondrial structure and function through the synergistic effect with other apoptotic proteins, and play the role of "main switch" of apoptosis. The proportion of Bcl-2 family members is the key factor of apoptosis regulation; especially the Bcl-2/Bax ratio is the molecular switch to initiate cell apoptosis". Zou *et al* [56] revealed that after BMSC treatment, anti-apoptotic factor Bcl-2 expression was increased, whilst the protein expression of pro-apoptotic factor Bax was reduced.

Autophagy appears to play a beneficial role in survival of eukaryotic cells and research has indicated that MSC transplantation can activate autophagy [15]. Nevertheless, the relationship between liver regeneration and autophagy is controversial. Suppression of autophagy in rat liver might contribute to functional failure of the liver [16]. Likewise, ATG7 is an essential gene for autophagosome formation. Amiri *et al* [14] transfected MSCs with an ATG7-shRNA vector in mice, directed to down regulate ATG7 and

therefore decreasing autophagy. The survival rates of transfection group was higher than the control, suggesting that autophagy suppression in MSCs ameliorates liver regeneration.

Impact of antioxidant treatment on MSC use

Oxidative stress is an important factor that affects the survival of MSCs. Oxidative stress refers to redox dysfunction caused by an increase in reactive oxygen species. Oxidative stress is a pathogenic mechanism in the initiation and progression of liver damage involved in many liver disorders. Cell damage occurs when reactive oxygen species concentration increases in the liver. Due to harsh oxidative stress and inflammatory nature of the transplant microenvironment, the survival of transplanted stem cells at injured sites can be low [19]. Edaravone is a new antioxidant with hydroxyl and radical scavenging activity.

Zeng *et al* have shown that edaravone-treated human umbilical cord mesenchymal stem cells (hUCMSCs) rescued NOD/SCID mice from Gal/LPS-induced death through increased stem cell homing, promoted proliferation, decreased apoptosis and enhanced secretion of hepatocyte growth factor (HGF) under hepatic stress environment. So, elevating levels of antioxidants in can significantly improve hepatic function and promote host liver regeneration [20]. Adult male Wistar rats which were induced with carbon tetrachloride (CCl_4) were injected with xenograft MSCs into the hepatic lobes of the liver, then oxidative stress was attenuated by increased level of GSH content [32].

Hypoxic treatment of MSC to improve their therapeutic potential

Hypoxic culture of MSCs is a commonly used method to ensure appropriate expansion *in vivo* to reduce the requirement for the use of cytokines, such as fibroblast growth factor. However, this approach usually requires continuous exposure to hypoxic conditions. Hypoxic preconditioning for MSCs was first reported by Annabi and colleagues who found that hypoxic culture conditions rapidly induced MSC migration and three-dimensional capillary-like structure formation on Matrigel [21]. Yu *et al* [22] found that hypoxia-conditioned MSCs secreted significantly more VEGF than normally cultured cells, and that these cells reduced liver injury and promoted liver regeneration.

Impact of MSC cell number/dose on transplantation

A variety of different MSC cell numbers have been used in the treatment of liver injury. Lam *et al* [23] delivered 5 x 10^6 MSC cells to D-galactosamine (GalN)-induced acute liver injury model in Sprague Dawley (SD) rats and showed significant reduced in serum levels of alanine aminotransferase, aspartate aminotransferase, and total bilirubin compared to the control group. Engrafted MSCs actively proliferated, differentiated, and further enhanced hepatocyte proliferation activity. However, contrasting this, Alves *et al* [24] reported that about 10^6 MSC were infused into Wistar rats via the portal vein which did not improve liver regeneration rate 3 or 5 days after 70 % hepatectomy in rats. Likewise, the therapy appeared not to significantly affect liver function, proliferative index, or number of mitoses.

Ezquer *et al* [25] transfected 5 x 10^5 MSCs into C57/BL 6 mice via tail vein. MSCs administration improved the hepatocyte proliferative response, PCNA-labeling index, DNA synthesis, liver function, and also reduced the number of apoptotic hepatocytes. However, Kanazawa *et al* [26, 27] used nearly 100 times MSCs (3 x 10^7 and 2 x 10^7 cells) in their two studies, respectively. Higher liver regeneration rates were observed in the male C57/BL6 mice after 70 % hepatectomy compared to the control groups. Perhaps the differences in experimental results were caused by differences in the animal species and models. The maximum dose in rats and mice is determined by the number of cells that do not undergo fatal embolism by transplantation (usually not more than 10 million cells overall) [28]. Generally speaking, most researchers use 10^6 MSCs in rats and 10^5 MSCs in mice.

Multiple-dose administration of MSC improves cell homing

One of the most important problems in liver disease cell therapy is the low level of cell homing. Repeated administration of MSCs can lead to protective effects in many diseases [29, 30]. Likewise, repeated administration can induce improved MSCs homing. Transplanting 3 x 10^6 MSCs in three divided doses improved survival, liver fibrosis and necrosis compared with injection of the same number of MSCs in a single dose [31].

The method of transplantation impacts MSC success

There are three main approaches for transplant-

ing MSCs in rodents: delivered by tail vein [25], portal vein [24] and direct hepatic lobes injection [32]. Furthermore, there are others channels for transplanting MSCs. Seki *et al* [33] injected 2 x 10^6 Adipose tissue-derived mesenchymal stem cells (ADSCs) through the penile vein into Wistar rats which were subjected to a 70 % partial hepatectomy.

Tautenhahn *et al* [4] delivered MSCs to the liver by splenic application. Intraperitoneal injection is also a good choice [34]. However, studies up to now have not shown which pathway is most suitable for transplantation. In Kanazawa's studies, many detection indices were significantly up-regulated in the portal vein-BMC group compared with the tail vein-BMC and control groups. He *et al* [35] had the same conclusion. They found that the transplantation via the portal vein is superior to that via the tail vein. However, Mu *et al* [36] found that there was no difference between the two approaches of transplantation. Feng *et al* [37] showed that tail-vein injection of MSCs has a similar therapeutic efficacy but is more convenient compared to liver lobe injection.

Intravenous injection often cause intravenous obstruction and low homing rate limiting its application, but its operation is simple and less damaging, which has attracted the attention of many scholars. Local injection is highly homing effective, but it is necessary to perform surgical operation or catheter insertion and other invasive procedures, so it greatly restricts its clinical application. So far, there are few comparative studies on different cell transport pathways, and further research is needed to find a suitable way of cell delivery, because this directly relates to the efficiency of MSCs homing.

Treatment of targeted tissues to improve MSC engraftment

Although the implantation of mesenchymal stem cells is very sensitive to the lack of serum and oxygen, transient ischemic preconditioning has protective effect on MSCs apoptosis induced by ischemia/reperfusion injury. Therefore, the related treatment of targeted tissues before and after MSC transplantation may increase homing rate. Seki *et al* [33] injected Adipose tissue-derived mesenchymal stem cells (ADSCs) into hepatic ischemia-reperfusion and subsequent hepatectomy in rats.

The parameters of hepatic regeneration, such as hepatic regeneration rate, mitotic index, and anti-proliferating cell nuclear antigen levels, were significantly upregulated in the ADSC transplanted group than the control group. In

addition to changing the external microenvironment, partial addition of chemokines and growth factors is also used to improve the homing and survival of MSCs [38].

Role of transdifferentiation in MSC transplantation

Both clinical and experimental evidence suggest that MSCs are capable of differentiating into functional hepatocyte-like cells, and restoring the liver function in acute and chronic damages [39,41]. Katagiri *et al* [42] demonstrated that only 1–2 % of BM-MSCs called multilineage-differentiating stress-enduring (MUSE) cells could differentiate spontaneously into major liver components, including hepatocytes, cholangiocytes, sinusoidal endothelial cells, and Kupffer cells after transplanted into immune deficient mice of partial hepatectomy model.

Fikry *et al* [43] found that BMSCs contributed to an increase in hepatic oval cells. Hepatic oval cells are a kind of liver stem cells existing in the liver, which have been proved to exist in human and many kinds of animals. Oval cells are located in the bile duct of the liver and have the potential of multi-directional differentiation. MSCs can even induce the conversion of recipient hepatocytes into hepatic oval cells [44]. However, the conclusion of MSCs differentiation has been controversial. In some cases, hepatic differentiation was rare (ranging from less than 0.1 to 0.23 %) [45]. Some report even said MSCs did not differentiate into hepatocytes expressing albumin or alpha-fetoprotein [46].

Does cell fusion play a role in liver regeneration?

Cell fusion may better explain the coexpression of hepatic and MSC markers. Wang *et al* [47] demonstrated that hepatocytes derived from bone marrow arise from cell fusion and not by differentiation of hematopoietic stem cell through cytogenetic analysis of hepatocytes transplanted from female donor mice into male recipients. It is still controversial whether transplanted MSCs protect and regenerate the liver by cell fusion or by transdifferentiation. Thus, further studies on the fate of MSCs after transplantation are necessary.

Paracrine effects of MSC on hepatocytes

Accumulating evidence has shown that stem cells may exert paracrine effects on endogenous hepatocytes to increase their ability of regeneration [48,49]. MSCs produce and secrete a wide range of cytokines, chemokines and growth factors, including vascular endothelial growth factor (VEGF), hepatocyte growth factor, stromal cell-derived factor-1, interleukin-6 (IL-6) and IL-10, which are involved in tissue regeneration [50-52] (Figure 1). VEGF is regarded as the key factor secreted by MSCs in treating many diseases. MSC upregulated gene expression levels of the cytokine TNF-α, and growth factor HGF, known to be required for initiation of and progression through the hepatocyte cell cycle [34]. However, some research also reported that adipose tissue-derived mesenchymal stem cells (AT-MSCs) transplantation after 70 % partial hepatectomy (PH) did not increase the serum levels of IL-6 and HGF *in vivo* [53].
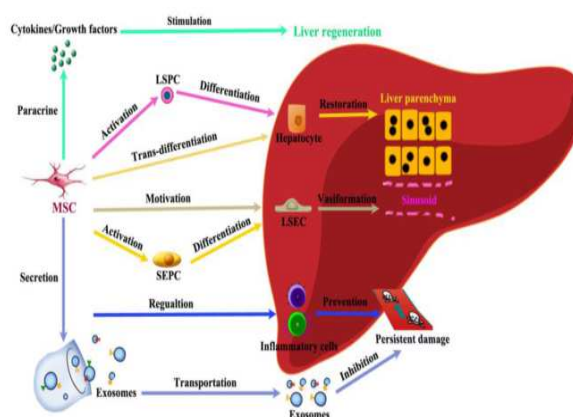
Figure 1: Schematic depicting the contributions of MSCs to liver regeneration**.** Quote from Int. J. Biol. Sci.2016; 12(8): 954-963.Liu WH, Ren LN, Wang T, Navarro-Alvarez N, Tang LJ. The involving roles of intrahepatic and extrahepatic stem/progenitor cells (SPCs) to liver regeneration

MSC immunomodulation in transplantation

In recent years, a number of studies have found that MSCs have an effect of inducing immune tolerance. MSCs low expression of MHC class I molecules, no expression of MHC-Ⅱ type of molecule, also not expressing Fas-L and stimulating molecules CD80, CD86, CD40 and CD40L [57,58]. MSC secretion includes cytokines including IFN- gamma, TNF-α, IL-4, IL-10, TGF-β, IDO, PGE2 and NO [59]. MSCs can also increase expression of regulatory T cells [60], and suppress a variety of the activity of immune cells, including adaptive immune cells (T cells and B cells) and natural immune cells (macrophages, NK cells and dendritic cells, etc.) [61].

Role of IL-6/STAT3 signaling in MSC activity

IL-6 plays an important role in liver regeneration after liver injury. IL -6 binds to the receptor IL-6R,

and IL-6R binds to gpl30 of two subunits to activate JAK's tyrosine kinase. Then, STAT3 is activated by JAK through phosphorylation. STAT3 and other transcription factors (such as C/EBP-p and AP-1) greatly enhanced the expression of some factors (such as c-myc and c-fos) involved with cell proliferation, and coordinated growth factors such as HGF and EGF. They promote the liver cell proliferation together [62]. MSCs promotes IL-6/STAT3 signaling by decreasing the methylation of the IL-6/STAT3 promoters [63], then triggers wound healing, cell migration, and proliferation [23].

Effect of Wnt/β-catenin signaling on MSC

Wnt/β-catenin signaling is a genetically conserved and highly complex signaling pathway. It plays an important role in the development of fetal liver, maintenance of adult liver function, metabolism of liver and regeneration of liver. It over activation is closely related to the occurrence of liver tumors. Wnt/β-catenin signaling also play a vital role in the differentiation of MSCs. Some studies suggested that accompanied by the translocation of β-catenin was observed along the cell membrane and in the cytoplasm, the expression of Wnt/β-catenin-related genes down regulated which can promote the differentiation of MSCs into hepatocytes [64,65].

Doubt about the effectiveness of MSC for liver regeneration

Even though many researchers have shown that MSCs has the effect of promoting liver regeneration, there are still many open questions concerning MSCs therapy for the treatment of liver diseases. Less than 0.1 to 0.23 % MSCs can differentiate into liver cells [45]. Cantz *et al* [66] found that any recipient mouse no EGFP-positive hepatocytes were detected either by analysis of native EGFP fluorescence or by immune fluorescence analysis with anti-EGFP and antidipeptidyl peptidase (DPP) IV antibodies in the liver, heart, intestine, spleen, kidney as well as intestinal tissue. These studies indicate that in vivo differentiation of transplanted MSCs into hepatocytesis rare and quantitatively unsatisfactory event.

In physiological cases, hepatic stellate cells (HSCs) mainly involved in metabolism of vitamin A (Vit A), but in the chronically damaged liver, HSCs is activated by active oxygen and variety of cytokines into activated myofibroblasts (MFB), resulting in cell proliferation, α-SMA expression, and collagen deposition [67]. MSCs also significantly contribute to liver fibrosis by

differentiating into pro-fibrogenic myofibroblast cells and hepatic stellate cells [45,68]. It is crucial to elucidate the relationships that exist not only between MSCs and hepatocytes (regeneration) but also between MSCs, myofbroblasts and stellate cells (fibrogenesis), which might allow more rational use of MSCs therapy in liver diseases.

In spite of some potential markers being used to detect or isolate MSCs in the liver, there are some variations among species. Due to the rare amount of MSCs and lack of uniform sorting markers, separation and purification MSCs remains a challenge. At the same time, the number of cells decreases with age, which increases the difficulty of stem cell therapy to some extent.

Alves *et al* [24] evaluated the effects of MSCs on liver regeneration in rats following a 70 % hepatectomy. Albumin, aminotransaminases (AST, ALT), and Alcaline Phosphatase (AP) levels, proliferative index (ki-67+ straining), and mitotic cell counts were conducted. They found that MSC therapy did not improve liver regeneration rate 3 or 5 days after 70 % hepatectomy in rats. Likewise, the therapy appeared not to affect liver function, proliferative index, or number of mitoses significantly.

CONCLUDING REMARKS

Stem cells therapy has attracted more and more attention from the scientific community and medical community for its advantages. MSCs represent a promising therapeutic strategy for improving liver regeneration. Recent data on the potential to use MSC in liver regeneration has shed a new light on the treatment of liver disease, nevertheless, it still remains that the efficacy of this approach needs to be demonstrated in large randomized clinical trials. Furthermore, it is essential to further clarify the mechanisms of MSCs homing processes. Similarly, it is necessary to understand the interactions between MSCs and the host liver tissue microenvironment in a diseased liver. With further research it is believed that MSCs can be used not only as a great source of cells for liver regeneration, but they also provide as cells for cell therapy, immunotherapy, gene therapy, as well as seed cells for tissue engineering.

DECLARATIONS

Conflict of interest

There is no conflict of interest associated with this work.

Contribution of authors

This manuscript was prepared by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. The first author wrote the manuscript and the corresponding author conceived and designed the study.

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