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REVIEW

The therapeutic potential of bone marrow-derived mesenchymal stem cells on hepatic cirrhosis

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Abstract Hepatic cirrhosis is the end-stage of chronic liver diseases. The majority of patients with hepatic cirrhosis die from life-threatening complications occurring at their earlier ages. Liver transplantation has been the most effective treatment for these patients. Since liver transplantation is critically limited by the shortage of available donor livers, searching for an effective alternative therapy has attracted great interest in preclinical studies. The transplantation of autologous bone marrow-derived mesenchymal stem cells holds great potential for treating hepatic cirrhosis. Mesenchymal stem cells can differentiate to hepatocytes, stimulate the regeneration of endogenous parenchymal cells, and enhance fibrous matrix degradation. Experimental and clinical studies have shown promising beneficial effects. This review is intended to translate the bench study results to the patients' bedside. The potential interventions of mesenchymal stem cells on cirrhosis are illustrated in terms of the cellular and molecular mechanisms of hepatic fibrogenesis.

Contents

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Introduction

Cirrhosis represents the final common pathological outcome for the majority of chronic liver diseases. Most patients with cirrhosis die from one or more clinical complications including ascites, hepatic encephalopathy, and variceal hemorrhage (Bataller and Brenner, 2005). Among the 1.4 million liver disease-related deaths each year worldwide, over 55%, or 796 000, are directly attributable to cirrhosis (Poynard et al., 2003). The majority of chronic liver diseases are initiated by the infection of hepatitis B virus (HBV) and hepatitis C virus (HCV) (Lai et al., 2003; Poynard et al., 2003). Liver transplantation has been the most effective therapy for the patients with advanced liver diseases. Unfortunately, most patients are unable to obtain transplantation due to the limited availability of donor livers. Over 10% of patients die while waiting for liver transplantation. Among the fortunate patients who received liver transplants, the survival rate has been 94% at 3 months, 88% at 1 year and 79% at 3 years (Freeman et al., 2008). Thus, it is of great interest to search for an effective alternate to treat this type of life-threatening disease. Recently, stem cell-based cytotherapy has shown promising benefit on animal models and some clinical patients. This review highlights recent progress in this field, emphasizing the therapeutic potential of bone marrow-derived mesenchymal stem cells (MSCs) on hepatic cirrhosis.

Pathogenesis of liver cirrhosis

Hepatic fibrosis refers to the excessive accumulation of extracellular matrix (ECM) with the formation of scar tissue encapsulating the area of injury. Actually, it is a wound-healing response of the liver to either acute or chronic liver injury (Guo and Friedman, 2007). Cirrhosis, the end-stage of progressive fibrosis, is characterized by septum formation and rings of scar that surround the nodules of hepatocytes. Accumulation of ECM results from both increased synthesis and decreased degradation of ECM.

Cellular sources of ECM in cirrhotic liver

Hepatic stellate cells (HSCs) are the main ECM-producing cells in the injured liver. HSCs reside in the space of Disse and are the major storage sites of vitamin A. When a liver injury occurs (*e.g.*, viral hepatitis), HSCs proliferate and undergo a dramatic phenotypical alteration, which is characterized by the acquisition of a proliferative, contractile, migratory, fibrogenic and inflammatory phenotype. Activated HSCs secrete large amount of ECM proteins, including collagen (I, III, and IV), fibronectin, undulin, elastin, laminin, hyaluronan, and proteoglycans (Bataller and Brenner, 2005). The accumulating interstitial ECM constituents that collectively form the hepatic scar replace the low-density type IV collagen with the normal sub-endothelial space of Disse. These interstitial fibril-forming collagens (especially types I and III collagens) become distributed primarily in the connective septa surrounding the regenerative hepatic nodules. A cirrhotic liver may contain up to six times more collagen and proteoglycan than a healthy organ (Friedman, 2008). In addition to the resident HSCs, periportal fibroblasts, bone marrow-derived fibrogenic cells, epithelial-mesenchymal transition, and possibly circulating fibrocytes can contribute to the fibrogenesis in the liver (Friedman, 2008). The relative importance of each cell type in liver fibrogenesis depends on the origin of liver injury. While HSCs are the main fibrogenic cell type in pericentral areas, periportal fibroblasts predominate when liver injury occurs around portal tracts. Since the majority of patients with chronic liver diseases are induced by HBV and HCV infection (Lai et al., 2003; Poynard et al., 2003), HSC-mediated pericental fibrosis plays an important role in the development of hepatic cirrhosis. Therefore, prevention of HSC activation has been the most promising therapeutic strategy for this disease.

Some other cell types also contribute to the progression of liver fibrosis. These include liver resident cell types (for example, hepatocytes, Kupffer cells, sinusoidal endothelial cells, and bile duct epithelial cells, *etc.*) and non-resident or circulating cells (for example, T and B lymphocytes). They may not secrete ECM proteins directly, but upon being damaged they activate HSCs through a variety of inflammatory mediators, apoptotic bodies, free radicals, and fibrogenic cytokines (Duffield et al., 2005; Canbay et al., 2004; Jarnagin et al., 1994; Sedlaczek et al., 2001).

Molecular regulations of ECM accumulation

Numerous factors are involved in liver ECM synthesis and/or degradation, including growth factors, cytokines, and chemokines. They play direct or indirect roles in fibrogenesis or anti-fibrogenesis depending on their effects and targets. For example, matrix metalloproteinases (MMPs) are directly responsible for matrix breakdown, and an increase of tissue inhibitors of metalloproteinases (TIMPs) indirectly favors the accumulation of ECM. Table 1 lists some liver fibrogenesis-related factors, in which the origin, possible target and mechanism are indicated. The general molecular basis of fibrosis has been summarized in a recent review (Wynn, 2008).

The expression of inflammatory mediators determines the fibrogenic response to liver injury. Hepatocytes are the targets of most hepatotoxic agents, including hepatitis viruses, alcohol metabolites, and bile acids (Higuchi and Gores, 2003). Damaged hepatocytes and involved inflammatory cells release a variety of inflammatory mediators. A number of fibrogenic molecules listed in Table 1 are also inflammatory mediators, such as free radicals, IL-1b, IL-6, IL-10, IL13, IFN-γ, SOCS-1, and osteopontin (Sahai et al., 2004; Bataller and Brenner, 2005). Inflammatory molecules

Table 1 Liver fibrogenesis-related factors

Factors	Origin *	Target	Possible effect	Reference
Fibrogenicz				
TIMPs	N, L	MMP, HSC	inhibit MMPs and activate HSC	Murphy et al., 2002
Apoptotic body	H	HSC	activate HSC	Friedman, 2008
Free radicals	H, K, M	HSC	activate HSC	Bataller et al., 2003
TGF-β1	H, K	TIMP-1,ECM	up-regulate TIMP-1, down-regulate colagenase I	Shek and Benyon, 2004
TNF-α	H, K, B, M	HSC	activate HSC	Bataller and Brenner, 2005
IGF	H, K	HSC	activate HSC	Bataller and Brenner, 2005
PDGF	B, K, P	HSC	activate HSC	Borkham-Kamphorst et al., 2004
FGF	H, D	HSC	activate HSC	Yu et al., 2003
ANG II	M, F	NADPH	activate HSC through free radicals and TGF-ß1	Bataller et al., 2003
Endothelin-1	B	HSC	activate HSC	Bataller and Brenner, 2005
IL-4	L	HSC, TIMP-1	up-regulate TIMP-1, activate HSC	Cheever et al., 1994
IL-5	E	HSC	activate HSC	Cho et al., 2004
IL-6	L,K, HSC	HSC	activate HSC	Hasegawa et al., 2005
IL-13	E, L	HSC	collagen deposition	Reiman et al., 2006
IL-21	L	Macrophage	increase IL-4 and IL-13 receptor expression	Pesce et al., 2006
Anti-fibrogenic				
MMPs	M, D	ECM,HSC	ECM degradation, HSC apoptosis	Wynn, 2007
HGF	H	HSC	HSC apoptosis	Parekkadan et al., 2007a,b
IL-10	L	HSC	suppress the synthesis of type I collagens	Wangoo et al., 1997
IL-12	L	Fibrocyte	inhibit fibrocyte differentiation	Shao et al., 2008
IFN-γ	L	Fibrocyte	inhibit fibrocyte differentiation	Wynn, 2007

* **B**: Biliary Cell; **D**: Endothelial Cell; **E**: Eosinophil; **F**: Fibroblast; **H**: Hepatocyte; **K**: Kupffer Cell; **L**: Lymphocyte; **M**: Macrophage; **N**: Neutrophil; **P**: Platelet.

activate HSCs, and the resulting activated HSCs secrete inflammatory chemokines, express cell adhesion molecules, and modulate the activation of inflammatory cells (Viñas et al., 2003). Therefore, a vicious circle exist between inflammatory and fibrogenic cells. Any approach able to break this circle can presumably lead to the development of an anti-fibrotic treatment.

Reversibility of hepatic cirrhosis

Hepatic cirrhosis is traditionally thought to be irreversible. However, recent evidence from animal studies and human clinical observations indicate that even advanced fibrosis is still reversible (Arthur, 2002; Issa et al., 2004). The most effective intervention in the treatment of liver fibrosis is to remove the causative agents, such as the utilization of anti-viral therapy and the obstruction of alcohol intake. It may take years for significant recovery to be achieved; the time varies depending on the underlying cause of the liver injury and its severity. It is unlikely to reach a complete return to normal histology. So, the term of "regression" is more relevant to the real situation rather than "reversal" (Friedman, 2007). As described above, liver fibrosis results from the imbalance between ECM production and ECM degradation. Theoretically, any approach which decreases ECM synthesis and/or increases ECM degradation could accelerate the regression of hepatic fibrosis/cirrhosis. For example, the inhibition of activated HSCs by modulating their activation and/or proliferation or the promotion of their apoptosis would be a useful strategy.

The potential effects of mesenchymal stem cells on hepatic cirrhosis

Since the bone marrow-derived mesenchymal stem cells (MSC) were found to have differentiative plasticity, there has been great interest in their potential therapeutic application (Jiang et al., 2002). The feature of self-origin and readily *ex vivo* expansion renders MSC autotransplantation a practical approach. There is increasing evidence indicating the therapeutic benefit of MSC transplantation in various disorders characterized by cell injury or cell loss, such as ischemic heart diseases (Assmus et al., 2006) and stroke (Chopp and Li, 2002; Bang et al., 2005). Some encouraging results were also obtained from our own clinical trials on the patients with traumatic brain injury and acute myocardial infarction (Zhang et al., 2008, 2007b). Nevertheless, the investigation of applying MSC to liver diseases is not as advanced as in other fields. Upon liver injury, the typical repair process involves two distinct phases: a regenerative phase, in which injured liver cells are replaced with regenerated hepatocytes; and a phase known as fibroplasias or fibrosis, in which connective tissue replaces normal parenchymal tissue. Although initially beneficial, the repair process becomes pathogenic when it is not controlled appropriately. Extensive accumulation of ECM components can ultimately lead to cirrhosis and liver failure (Wynn, 2008). Moreover, fibronectin, a component of ECM, has been proved to promote the MSC-induced cytoprotection following transplant for liver disease (Kao et al., 2007). The ideal

strategy to treat liver injury is to generate new hepatocytes replacing damaged cells without causing excessive ECM deposition.

MSC replace hepatocytes in injured liver

Hepatocyte-like differentiation

The first demonstration of the existence of putative liver stem cells in the bone marrow was reported by Petersen et al in 1999 (1999). They showed that bone marrow cells transplanted into lethally irradiated mice engrafted in the recipient's liver and differentiated into liver stem cells (oval cells) or mature hepatocytes. These *in vivo* results were confirmed in animal models and in patients who received bone marrow transplantation for hematological disorders (Theise et al., 2000; Alison et al., 2000). Hematopoietic stem cells consist of the majority of stem cell population in the bone marrow, while nonhematopoietic, *i.e.* mesenchymal stem cells (MSC) are only a very small fraction of the population, representing 0.001–0.01% of the nucleated cells in adult human bone marrow (Pittenger et al., 1999). Although these two cell types are reported to differentiate into cell lineages of all three germ layers, *i.e.* ectoderm, mesoderm and endoderm (Yen et al., 2006), MSCs show different characteristics compared to other components in differentiating into hepatocytes (Sato et al., 2005). Schwartz and colleagues (2002) provided direct evidence of *in vitro* hepatogenic differentiation of MSC. A subpopulation of MSC isolated from bone marrow of human, mouse and rat, cultured on Matrigel with FGF-4 and HGF, differentiated into hepatocyte-like cells. These cells express hepatic markers, *i.e.* HNF-3β, GATA4, CK19, transthyretin, α-fetoprotein, albumin and CK18. They also possess functional characteristics of hepatocytes, *i.e.* secreting urea and albumin, having phenobarbital-induced cytochrome p450, taking up LDL and storing glycogen. *In vitro* liver-specific differentiation of MSC can also be induced by co-culture with liver cells (Lange et al., 2005) and pellet culture (Ong et al., 2006a). Although hepatocyte-induction protocols work well in cultured MSC, an organ-specific microenvironment is the most suitable place for them to differentiate into required cell types. Sato et al. (2005) demonstrated the first *in vivo* hepatic differentiation of MSC . In that study, human bone marrow-derived MSCs were directly xenografted to allylalcohol-treated rat liver, and the most human MSC-differentiated hepatocyte-like cells were observed at day 28, as revealed by positive immunostaining for human specific AFP, albumin, CK19, CK18 and asialoglycoprotein receptor. A recent study by Chamberlain et al. (2007) provided further evidence of *in vivo* hepatic differentiation of MSC. Clonal human MSCs were xenotransplanted to fetal sheep liver by intrahepatic injection in their study. A widespread distribution of human MSC-originated hepatocytes throughout the liver parenchyma was exhibited at days 56–70. In addition to the bone marrow-derived MSC, hepatogenic differentiation of MSCs from other sources, such as adipose tissue (Seo et al., 2005), umbilical cord blood (Hong et al., 2005) or commercially available MSCs (Ong et al., 2006b) have also been achieved. It is worth noting that a significant problem for stem cell research is the interpretation of *in vitro* data concerning the content of hepatic differentiation, which is often problematic and controversial (Fox and Strom, 2008). Useful stem cell-derived hepatocytes will need to not only express the genes found in mature liver cells, but the level of the expression need to be at or near those found in the normal liver.

The gene signaling pathways in hepatic differentiation are essential for MSC-based therapies for the treatment of cirrhotic liver disease. Hepatic-differentiated cells are characterized by the expression of hepatocyte-specific genes. This specific gene expression is ingeniously regulated by numerous transcription factors and is also influenced by microenvironmental conditions. Costa et al. (2003) elaborated the transcription factors in liver development, differentiation and regeneration. Recently, Yamamoto et al. (2008) demonstrated the hepatic differentiation of human adipose tissue-derived MSCs. They utilized microarray analysis to identify the genes responsible for hepatic differentiation and found evidence of transdifferetiation through mesenchymal-epithelial transition in the process of hepatic differentiation. Further studies identifying the complex network of interaction between gene signals during hepatic differentiation of MSCs may facilitate the development of novel methods of therapeutic intervention in human liver cirrhosis.

Homing and functional integration

The potential therapeutic benefit of MSCs can only be realized through their homing efficiency to the required site. Although the ultimate success or failure of cell therapy will rest on its ability to show clinical efficacy rather than the underlying mechanism, a variety of evidence from clinical and animal studies has indicated that MSC's direct differentiation and indirect effect through its secretion play important roles in promoting tissue recovery. An animal study by Aurich et al. (2007) showed the functional integration of MSC-differentiated hepatocytes in the liver. Human MSCs were pre-differentiated and directly transplanted into immunodeficient mouse liver. Engraftment of transplanted pre-differentiated human MSCs in the mouse liver was observed three weeks after transplantation. Functional hepatic integration was also revealed using undifferentiated human MSCs xenografted directly onto rat liver (Sato et al., 2005).

The following questions have been frequently encountered while pursuing cell-based therapeutic investigations: what is the best method of delivery of cells, how do the cells get to the sites of injury and by what mechanisms are they targeted? As previously discussed, the methods of MSC administration can be classified into three categories, directional or site-specific delivery, semi-directional delivery, and systemic delivery (Zhang et al., 2008). Among above examples, Sato et al. (2005) administered MSCs to the injured liver by intrahepatic injection, which is considered as directional delivery; intrasplenic injection by Aurich et al. (2007) and intravenous infusion by Fang et al. (2004) are examples of semi-directional and systemic deliveries. Under certain circumstances, the combination of more than one method and repeated administration may also be considered. The migration of MSC from the circulation into damaged or pathological tissues is the most crucial step bringing MSC into play. Biological signals released from the injured area and corresponding receptors expressed on the cell surface of MSC are critical determinants in this step.

Xiang et al. (2005) reported that, in rats with CCl_4-induced liver injury, the timing and numbers of MSC homing to the liver are closely related to the presence of liver injury but not to the route of MSC infusion, *e.g.*, through the tail vein or the portal vein. The process of leucocyte homing to specific inflammatory sites in response to inflammatory stimuli is a well characterized sequential process, which involves selectins, chemokines, integrins and other adhesion molecules. As MSCs are known to be selectively recruited to injured tissue it can be reasonably assumed that they utilize comparable mechanisms of recruitment, *i.e.* transendotelial migration directed by chemokine gradient (Fox et al., 2007). This assumption has been evidenced in the studies of myocardial infarction (Abbott et al., 2004) and ischemic brain injury (Wang et al., 2002). The detailed process of transendothelial migration of MSC was elaborated in a recent review (Fox et al., 2007). It is worth noting that MSC chemokine receptor expression has been shown to diminish with *in vitro* culture and decreased receptor expression leads to a corresponding decrease in chemotactic responsiveness of the cells (Honczarenko et al., 2006).

The functional recovery of injured liver is proportionally relevant to the amount of hepatic regeneration. At least 2.5 to 5% of a human liver needs to be replaced by healthy cells to reverse a pathological condition (Fox et al., 1998). However, most transplantation studies have indicated that MSC-derived hepatocytes did not comprise more than 1% of the total liver mass. One plausible explanation for this low efficiency is the heterogeneity of MSCs employed in the studies. Due to the xenogeneic nature of the transplantation, a considerable number of inoculated MSCs might have been rejected even in an immunosuppressive state. This viewpoint is consistent with Chamberlain's recent finding in a pre-immune fetal animal model (Chamberlain et al., 2007). Human MSC-derived hepatocytes comprised over 12% of the total liver mass after xenotransplanted into fetal sheep liver. Apparently, homing efficiency of MSC could be greatly improved by autologous transplantation. A recent case report describes the use of autologous unsorted bone marrow stem cells as rescue treatment for hepatic failure in a 67-year-old man ineligible for liver transplantation (Gasbarrini et al., 2007). Apparent rapid improvement in hepatic synthetic function was obtained after the portal venous infusion of the cells. A liver biopsy performed 20 days after cell transplant was reported as showing increased hepatocyte replication around necrotic foci, although transplanted cells were not identifiable as they were not labeled with markers before transplantation. In addition to the direct hepatic differentiation of implanted stem cells in the injured area, MSC paracrine-mediated hepatic regeneration from endogenous liver stem cells may also contribute to the hepatocyte replication and recovery of hepatic function. Parekkadan et al. (2007b) reported the first experimental evidence of therapeutic use of MSC paracrine. A significant survival benefit was observed by MSC-conditioned medium perfusion in Gal-N-induced fulminant hepatic failure rat model. More recently, van Poll et al. (2008) provided further evidence that MSC-derived molecules directly inhibit hepatocellular death, enhance liver regeneration and ultimately improve survival in rats undergoing D-galactosamine-induced fulminant hepatic failure. These investigations validate the therapeutic benefits of MSC-derived molecules on liver disease and may create potential new avenues for the treatment of advanced liver disorders.

MSC attenuate the progression of hepatic fibrogenesis

As described earlier, hepatic fibrosis or cirrhosis results from the imbalance of ECM production and degradation. Any approach that resets the balance could lead to the resolution of fibrogenic liver disorders. The fact that MSC have antifibrosis effects in injured liver has been clearly demonstrated in animal models of liver fibrosis (Aziz et al., 2007; Zhao et al., 2005; Fang et al., 2004). MSC have a significant impact on hepatic fibrogenesis through their ability of inhibiting activated HSC and re-regulating the fibrogenic process.

MSC-induced HSC apoptosis

HSCs are the major source of fibrillar collagens and other ECM proteins that characterize liver fibrosis. Following liver injury, HSCs undergo a phenotypic switch from quiescent, vitamin A-storing cells into proliferative, α-smooth muscle actin positive, myofibroblast-like cells, a process termed activation (Friedman, 2008). Activated HSC is central to liver fibrosis and induction of HSC apoptosis is a potential antifibrotic treatment. This was directly evidenced in a recent study of Parekkadan et al. (2007b). Indirect co-culture of activated HSCs and MSC led to a significant decrease in collagen deposition and cell proliferation, while inducing apoptosis of activated HSCs. The underlying mechanisms in the modulation of HSC activity by MSC were attributed to paracrine mediators, IL-10, TNF-α and HGF. Blockade of MSC-derived IL-10 and TNF-α abolished the inhibitory effects of MSC on HSC proliferation and collagen synthesis; MSC-derived HGF was responsible for the marked induction of HSC apoptosis as determined by antibody-neutralization studies. IL-6 secretion from activated HSCs induced IL-10 secretion from MSC, suggesting a dynamic response of MSC to HSCs in the microenvironment. HSC apoptosis can also be triggered by MSC-secreted nerve growth factor (NGF) stimulation. MSC-originated NGF was directly identified from human MSC culture supernatant by quantitative ELISA measurement (Chen et al., 2002). Trim et al. (2000) found that HSCs express p75, a low affinity NGF receptor, and respond to NGF stimulation by undergoing apoptosis. They also identified the presence of activated HSC in the fibrotic bands of cirrhotic human liver biopsies and claimed p75 as a novel marker of activated HSC. The interactions between NGF and activated HSC have been further demonstrated in mouse models (Oakley et al., 2003; Asai et al., 2006). Zhao et al. (2005) have provided additional evidence that *in vitro* co-culture of MSC and HSC increases the number of HSC in the G_0 phase and reduces the number of HSC in the S phase. Thus, MSC play an inhibitory role in the process of HSC transition from the quiescent state to the activated state. However, it is worth noting the existence of some discrepancies in this field. Russo et al. (2006) reported that bone marrow-derived cells significantly contributed HSC and myofibroblast populations in the cirrhotic mouse liver. These bone marrow-derived cells were found to be active for collagen type I transcription. Also with murine model,

Higashiyama et al. (2007) reported that there were few, if any, bone marrow-derived cells expressing α-smooth muscle actin (αSMA, a marker of activated HSC) in the fibrotic liver. Further studies are required to clarify the controversies.

MSC-mediated re-regulation of fibeogenesis

Collagen turnover and ECM remodeling is regulated by various MMPs and their inhibitors, *i.e.* the tissue inhibitors of metalloproteinases (TIMPs). MMPs and TIMPs are crucial for matrix remodeling processes during hepatic fibrogenesis. The balance of ECM synthesis/ECM degradation is mainly determined by the balance of MMPs/TIMPs. During spontaneous recovery from liver fibrosis, there is a decrease in TIMP expression, an increase in collagenase activity, and increase in apoptosis of HSC. The close correlation between the reduction of TIMP expression and apoptosis of HSC observed *in vivo* highlights a potential role for TIMP in regulating HSC survival. An *in vitro* study of Murphy et al. (2002) indicated that the inhibition of apoptosis of HSC by TIMP-1 is mediated via effects on MMP inhibition. MSC-mediated TIMP-1 reduction was demonstrated in an *in vivo* MSC transplantation study in myocardial infarction rat model (Xu et al., 2005). Comparing to the control, the expression of TIMP-1 was significantly decreased in the infarcted myocardium, along with declined expressions of collagen I, collagen II, TGF-β1, and the protection of cardiac function. Similar molecular mechanisms may apply to liver injury-healing process, though the direct evidence is absent from liver studies. As listed in Table 2, most MSC-secreted and fibrogenesis-related molecules are anti-fibrogenic and this might make MSC favor to ECM degradation rather than accumulation during ECM remodeling. Moreover, the delicate mechanism is very complicated and far from clear, since some MSC-derived molecules are fibrogenic, or favor to ECM accumulation. Di Bonzo et al. (2008) identified a significant number of myofibroblast-like cells of human origin after transplanting human MSCs to the mice with liver injury. It is presumable that the effect of MSC varies with the nature of liver injury, time-frame of MSC application and different experimental models. In a rat model of severe chronic liver injury, MSC failed to reduce fibrosis and improve liver function (Carvalho et al., 2008). Further investigations are required to identify the factors which affect the fate of MSC in the injured area.

Table 2 MSC-secreted and fibrogenesis-related molecules

Molecules	Fibrogenesis-related effect	Reference
HGF	HSC apoptosis	Parekkadan et al., 2007a
IL-6	activate HSC	Liu and Hwang, 2005
IL-10	inhibit HSC proliferation and collagen synthesis	Parekkadan et al., 2007a
LIF	alter inflammatory infiltrate	Linker et al., 2008
NGF	HSC apoptosis	Chen et al., 2002
Nitric Oxide	HSC apoptosis	Ren et al., 2008; Langer et al., 2008
TGF-β2	induce IL-6 secretion	Liu and Hwang, 2005
TIMPs	inhibit MMPs and activate HSC	Liu and Hwang, 2005
TNF-α	inhibit HSC proliferation and collagen synthesis	Parekkadan et al., 2007a
VEGF	stimulate collagen I synthesis in HSC	Liu and Hwang, 2005

Clinical application and perspectives

The translation of preclinical research on MSC to clinical use on cirrhotic patients has generated great interest, due to the growing population of patients with advanced liver diseases and critical shortage of available donor livers. In cardiology, large-scale controlled and double-blinded clinical trials were performed in a number of clinical institutes. Some studies have demonstrated clinical benefits, whereas in others the differences have been less significant (Assmus et al., 2006; Lunde et al., 2006). In most MSC-related clinical trials, bone marrow stem cells or nucleated bone marrow cells, *i.e.*, a mixture of different type of cells, are directionally or systemically delivered to patients. This might be a partial explanation for disparate outcomes in cardiological trials. Rosenzweig (2006) described this as "mixed results from mixed cells". Therefore, improved clinical outcomes can be expected if a pertinent cell population is to be chosen. There are only a handful of clinical trials in the field of hepatology, all of which are small-scale, uncontrolled safety and feasibility studies (Kallis et al., 2007). Terai et al. (2006) implemented a clinical trial on nine patients with decompensated liver cirrhosis. These patients were infused with $5.2 \pm 0.63 \times 10^9$ autologous bone marrow cells from the peripheral vein. At 24 weeks after transplantation, significant improvements were observed. These improvements included total protein, serum albumin, Child-Pugh scores, and α-Fetoprotein and proliferating cell nuclear antigen expression in liver biopsy tissues. Recently, Mohamadnejad et al. performed two small scaled clinical studies. In their first trial, four patients with decompensated liver cirrhosis were infused 31.73×10^6 (mean) MSCs through a peripheral vein (Mohamadnejad et al., 2007a). At the end of follow-up (after 12 months), the model for end-stage liver disease scores of two patients improved by four points and by three points. The mean physical and mental component scales were more than doubled by the end of follow-up. Computed tomography (CT) showed the increase of liver volumes of three patients by the sixth month. However, the results of their second trial (Mohamadnejad et al., 2007b) were not satisfactory. Four patients received 5.25×10^6 (mean) autologous bone marrow-hematopoietic stem cells infused through hepatic artery. Only marginal improvements were observed in some patients. The results of their MSC transplantation were more promising than the study of hematopoietic stem cell transplantation. They also indicated that hepatic artery delivery of stem cells was not a safe procedure. Because of the lack of reliable means of identifying transplanted stem cells in the human body (Pearson, 2006), caution is advised during the evaluation of the clinical outcomes.

Based on the knowledge from preclinical studies and previous clinical trials, following considerations should be addressed during clinical trials for cirrhotic patients: (1)

Utilization of purified MSC population. As discussed in the earlier section, MSC possess the abilities of hepatic engraftment and hepatic differentiation, and in addition, their easy accessibility and quick *in vitro* expansion make MSC an ideal resource for clinical use. Because bone marrow-originated fibrogenic cells play a role in the progression of liver fibrogenesis, the use of purified MSC population could avoid this risk. Peng et al. (2007) successfully isolated and *in vitro* expanded MSC from advanced hepatitis B patients. MSC isolated from patients share the same surface markers and similar biological characteristics to those isolated from healthy humans. Their study reveals the capability of autologous MSC transplantation in patients with advanced liver disorders. About one billion or at least one million/kg body weight of MSCs are required for transplantation at a time (Fox and Strom, 2008); (2) MSC passage. Longer *in vitro* culture and unnecessary manipulation may introduce more unexpected effects to *ex vivo* expanded MSCs. Our experience indicates that 3 to 5-passage cultures are safe and practical. During this culture period MSC retain cytogenetic stability, and enough number of cells can be obtained for transplantation starting from 10 to 15 ml of bone marrow aspirate (Zhang et al., 2007a, 2008); (3) MSC delivery route. Intravenous infusion is the first choice under most circumstances. Although portal vein or hepatic artery delivery may enhance MSC homing efficacy, precautions must be taken when catheterization is applied to cirrhotic patients; (4) Evaluation standard. In order to objectively assess the therapeutic effect of MSC transplantation, a standardized criterion must be established prior to MSC administration. Following aspects should be covered in a practical criterion: I) patient enrollment requirement, *e.g.*, age, gender and type of hepatic disease; II) hepatic function assessment; III) liver parenchyma assessment, *e.g.*, ultrasonography, computed tomography and MRI; and IV) hepatic fibrosis assessment. Liver biopsy is considered to be the gold-standard method for the assessment of liver fibrosis, but the invasive nature and sampling error restrict it as a routine laboratory assay. Recently, Fontana et al. (2008) established a three-variable model to evaluate cirrhosis. Serum hyaluronic acid, TIMP-1 and platelet count are closely correlated with Ishak fibrosis scores based on liver biopsy samples from 513 subjects. This model can be used as a surrogate marker of liver fibrosis. Kuo et al. (2008) and Xu and Liu (2008) described some parameters governing the success of using MSCs and characteristics of various delivery approaches in their recent papers.

Fig. 1 outlines the pathogenesis of hepatic cirrhosis and possible intervention of mesenchymal stem cells. It is likely that mesenchymal stem cells play a part in differentiating to hepatocytes, stimulating the regeneration of endogenous parenchymal cells, and enhancing fibrous matrix degradation.

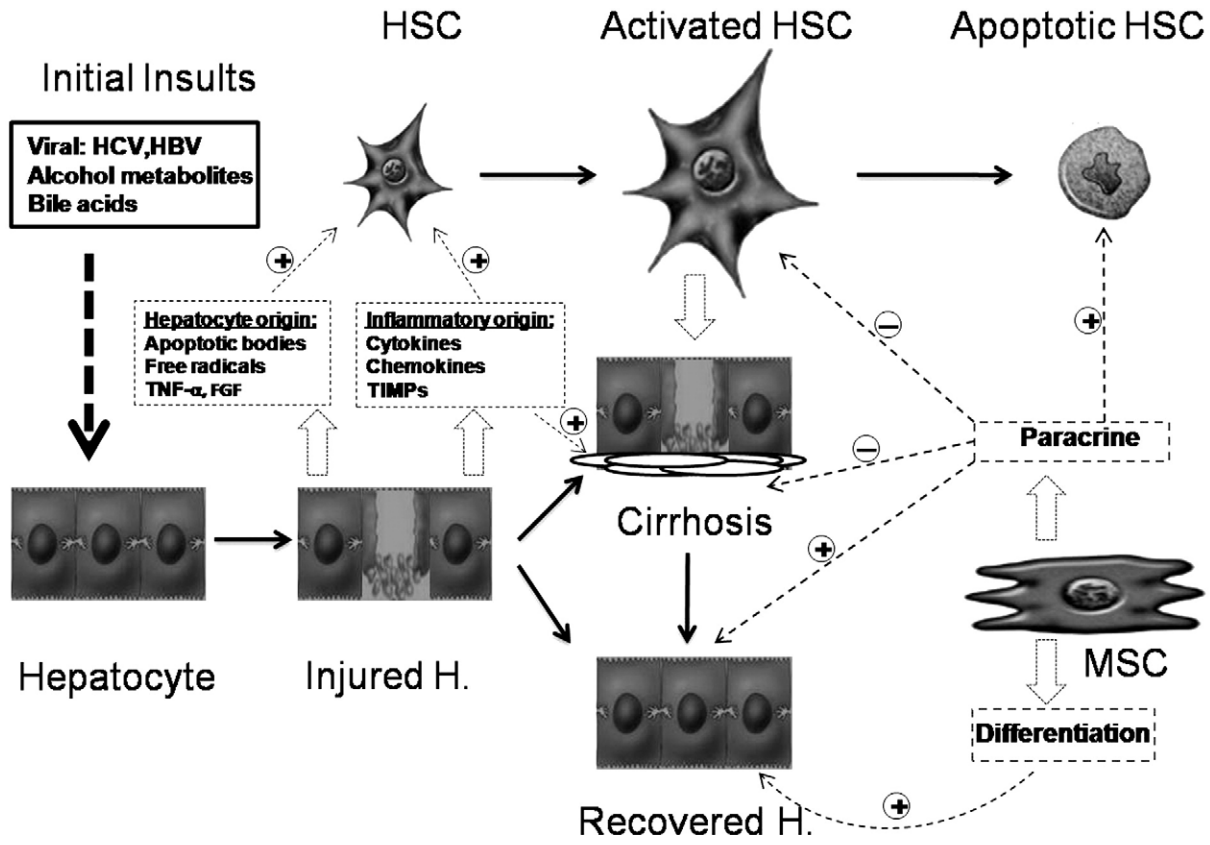

Figure 1 Pathogenesis of hepatic cirrhosis and possible interventions of MSC. Cirrhosis is initiated by hepatic insults-induced injury, and hepatic stellate cells (HSC) dominate the progression of hepatic fibrogenesis. The interventions of mesenchymal stem cells (MSC) include: (1) inhibit HSC proliferation; (2) stimulate HSC apoptosis; (3) inhibit ECM accumulation; (4) stimulate endogenous hepatocyte regeneration; and (5) hepatocyte-like differentiation. Solid arrows point directions of cell change, and dashed arrows indicate possible targets of various factors. (+) and (−) represent stimulation and inhibition, respectively.

Exploring the therapeutic potential of mesenchymal stem cells on hepatic cirrhosis will benefit millions of people who suffer from end-stage of chronic liver diseases. An obvious advantage of using mesenchymal stem cells is their auto-transplantable nature, so as to bypass the ethical hurdles and avoid the use of expensive immunosuppression drugs. It remains unclear about the long-term fate of the engraftment, and some unexpected effects may be encountered during their application. Large-scale controlled and double-blinded clinical trials are required before this cell transplantation becomes a regular therapy.

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