Bone marrow cell-based regenerative therapy for liver cirrhosis

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Abstract
Bone marrow cells are capable of differentiation into liver cells. Therefore, transplantation of bone marrow cells has considerable potential as a future therapy for regeneration of damaged liver tissue. Autologous bone marrow infusion therapy has been applied to patients with liver cirrhosis, and improvement of liver function parameters has been demonstrated. In this review, we summarize clinical trials of regenerative therapy using bone marrow cells for advanced liver diseases including cirrhosis, as well as topics pertaining to basic in vitro or in vivo approaches in order to outline the essentials of this novel treatment modality.

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Key words: Bone marrow; Liver regeneration; Cirrhosis; Stem cell; Transplantation

Core tip: Bone marrow cells, which include multipotent progenitor cells, are capable of differentiation into liver cells. Autologous bone marrow infusion therapy has been applied to cirrhotic patients, and improvement of liver function parameters has been demonstrated. Although the efficacy of this treatment modality needs to be evaluated in more detail in a large number of patients, regenerative therapy using bone marrow cells for advanced liver diseases has considerable potential.

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INTRODUCTION
Bone marrow cells (BMCs) are capable of differentiating into liver cells[1,2] because they include stem cells known as multipotent adult progenitor cells[3,4]. These cells have been shown to produce albumin when cultured with hepatocyte growth factor (HGF)[5] and various liver-specific proteins, including albumin, when cultured with mature hepatocytes[6]. Using cells obtained with a negatively selective magnetic cell separation system for efficient sorting of rat BMCs enriched with stem cells, we have shown that BMCs differentiate into cells expressing liver-specific genes when cultured with HGF[7].

As there is now much evidence indicating that BMCs can differentiate into cells resembling liver cells in vitro[8-10], the characteristics of such BMCs are of great interest in the context of liver-regenerative medicine[11,12].

Liver cirrhosis is the end stage of chronic liver disease, and is associated with many serious systemic complications resulting from both liver failure and portal hypertension. This condition has a poor prognosis and is difficult to treat. Therefore, development of an effective liver-regenerative therapy for liver cirrhosis is an urgent priority. Liver transplantation is the only curative remedy for cirrhotic patients, but is associated with many problems such as donor shortage, surgical complications,
rejection and high cost. As an alternative approach, regenerative cell therapy using stem cells is now attracting attention. Multipotent stem cells present in bone marrow are a particularly promising candidate for this purpose. In this review, we summarize clinical trials of liver-regenerative therapy using BMCs for advanced liver diseases including cirrhosis, as well as topics pertaining to basic in vivo or in vitro approaches in order to outline the essentials of this novel treatment modality.

**HUMORAL FACTORS BENEFICIAL FOR LIVER REGENERATION AFTER BMC TRANSPLANTATION**

The degree of liver function and fibrosis, as well as survival rate, have been shown to improve significantly after BMC transplantation in animal models of severe liver injury[23,24]. With regard to the mechanisms of liver regeneration resulting from BMC transplantation, many of the physiological and regenerative roles of transplanted BMCs remain unclear. However, it can be said with certainty that humoral factors produced in the liver during the regenerative process after BMC transplantation have a crucial role in both improvement of liver fibrosis and liver cell lineage differentiation of stem cells originating from BMCs and hepatic epithelial stem cells.

Improvement of liver fibrosis results from fibrolysis through the proteolytic action of BMC-induced factors. In this context, matrix metalloproteinase (MMP) activity is particularly noteworthy[25,26]. Sakaida et al[27] showed that BMC transplantation ameliorated liver fibrosis in the CCl4-induced liver-injury model, and that the fibrolytic change was attributable to MMP-9 secreted by BMCs that had migrated to fibrotic areas of the liver.

The liver cell lineage differentiation of BMCs occurs through the cooperative action of a variety of growth factors such as HGF or FGF induced in the injured liver[11,20,26]. Such differentiation may be accompanied by early elevation of the apolipoprotein A1 level in serum and liver[27]. Administration of FGF2 in combination with BMC transplantation synergistically ameliorates liver fibrosis in models of liver injury induced by CCl4[28]. In addition, in severe liver injury where hepatocyte proliferation is strongly inhibited, hepatic stem cells such as oval cells are induced and show differentiation toward a liver cell lineage, thus leading to liver regeneration[29,30].

As BMC transplantation is successfully adaptable to cases of severe liver injury, it has been hypothesized that transplanted BMCs interact with hepatic epithelial stem cells and influence the subsequent proliferation and differentiation of stem cells. Studies of the interaction between BMCs and hepatic stem cells can provide new insight into the mechanisms of recovery from severe liver damage through liver regeneration after BMC transplantation. In this context, in vitro analysis using a system for co-culture of BMCs and an established epithelial hepatic stem cell line has been conducted. Haga et al[31] demonstrated that the expression of FGF2 mRNA was upregulated in BMCs co-cultured with hepatic stem cells, and that expression of mRNAs for both albumin and tyrosine aminotransferase, representative of mature hepatic cells, became detectable in hepatic stem cells after culture with FGF2 protein. Thus, BMCs stimulate both proliferation and differentiation of hepatic stem cells into the hepatocyte lineage, and FGF2 is one of the factors produced by interaction with BMCs, which stimulates

**MIGRATION AND ENGRAFTMENT OF TRANSPLANTED BMCs TO THE INJURED LIVER IN STUDIES USING ANIMAL MODELS**

Although BMCs can show liver cell lineage differentiation in vitro, an understanding of the dynamics of transplanted BMCs in vivo is essential for the development of BMC-based regenerative therapy. In this context, two important issues need to be clarified: (1) How do transplanted BMCs migrate to and engraft in the liver? and (2) Is there a relationship between the degree of liver damage and the extent of migration of transplanted cells? A previous study using model rats with carbon tetrachloride (CCl4)-induced liver damage has demonstrated that transplanted BMCs derived from transgenic rats expressing green fluorescent protein[15] in the spleen migrated to and remained in the periporal area of the recipient’s damaged liver[16]. These transplanted cells expressed liver cell markers such as alpha-fetoprotein as well as Notch signaling markers for stem cells, suggesting that the BMCs retained in the recipient liver possess the potential to differentiate into liver cells.

Migration of transplanted BMCs to the liver after injection into the spleen has been compared in two models of liver injury induced by administration of CCl4 and 2-acetylaminofluorene (2-AAF)[17], respectively, focusing particularly on differences in levels of liver mRNA for growth factors such as HGF and fibroblast growth factor (FGF), which have been shown to be responsible for efficient liver cell lineage differentiation of BMCs[9,18,19]. Interestingly, transplanted BMCs were found to engraft into CCl4-induced injured liver characterized by submassive hepatic necrosis and induction of high levels of HGF and FGF, but not into liver damaged by 2-AAF[28]. A higher degree of HGF induction is characteristic of more severe liver damage[21,22]. These findings suggest that transplanted BMCs migrate more effectively to a liver with greater damage, and that this transplantation approach would be clinically promising for treatment of advanced liver diseases. However, further studies are needed to clarify the factors produced by both BMCs and hepatocytes that contribute to better differentiation of BMCs into liver cells in vivo, thus improving the effectiveness of BMC transplantation.
such differentiation. Cross-talk between bone marrow stem cells and hepatic epithelial stem cells may underlie the process of liver regeneration, and this is an area of interest for future investigation. Figure 1 shows an overall representation of the putative action of transplanted BMCs in the regeneration of damaged liver.

**CLINICAL TRIALS OF BMC TRANSPLANTATION FOR ADVANCED LIVER DISEASES**

BMC transplantation has received increasing attention as a promising therapy for advanced and severe liver diseases such as cirrhosis. Clinical trials of BMC administration to patients with advanced liver diseases have been performed, and improvement of liver function parameters such as the serum level of albumin, Child-Pugh score or Model for Endstage Liver Disease score have been reported.[32-40] Another study has shown that intraportal administration of autologous CD133+ BMCs and subsequent portal venous embolization of right liver segments resulted in a 2.5-fold increase in the mean proliferation rate of the left lateral segment, in comparison with controls not receiving BM transfusion.[41] These findings suggest that transplanted BMCs have a potential role in liver regeneration and proliferate in the recipient liver. Recently, autologous BMC transplantation - a technique named autologous BMC infusion (ABMi) therapy - has been applied to multi-center patients with liver cirrhosis due to hepatitis C[42], hepatitis B[43] and excess alcohol intake[44] using almost the same protocol, and a series of studies have demonstrated improvement of the serum albumin level, leading to improvement of the Child-Pugh score.

Although BMC administration for advanced liver diseases including cirrhosis is an attractive strategy in the field of cell therapy for liver regeneration, many concerns need to be addressed.[35-47] As in vitro and in vivo experiments have clearly shown, BMCs induce fibrolysis and show hepatocyte differentiation, and they may interact with hepatic epithelial stem cells to aid their differentiation into the hepatocyte lineage. However, it is still unclear how infused BMCs work to improve liver function in humans. A clinical trial of ABMi for patients with cirrhosis demonstrated that the number of AFP-positive cells increased significantly in the liver relative to the situation before ABMi.[42] In addition, ABMi appeared to induce hepatocyte proliferation in the liver, as expression of proliferating cell nuclear antigen, a marker of hepatocyte proliferation, was significantly increased after ABMi in comparison with the pretreatment situation. Although these findings suggest that transplanted BMCs have a potential role in liver regeneration and proliferate in the recipient liver, it remains unknown whether fully functional hepatocytes are induced by ABMi. The characteristics of stem cells present among BMCs that show hepatocyte differentiation require further elucidation.

The factors that determine the difference between effectiveness and non-effectiveness of ABMi are unclear. Collateral circulation resulting from the portal vein disorganization that characterizes liver cirrhosis may affect the flow and effective migration of infused BMCs to the liver, and thus migration of infused cells to the liver may partly depend on the portal venous pressure. In addition, the expression levels of cellular adhesion molecules associated with the attachment of infused cells to liver tissue may vary a great deal among patients. The long-term effectiveness of this therapy in terms of survival rate has not been demonstrated. These issues should be evaluated by a randomized controlled trial involving a large number of patients. Additionally, other issues that impact the efficacy of this therapy, i.e., the long-term culture conditions optimal for stocking BMCs for repeated infusion, the optimal cell population to employ, the optimal number of cells to infuse, the effectiveness of repeated infusion and the optimal route for cell delivery need to be investigated further.

In conclusion, regenerative therapy using BMCs for advanced liver diseases including cirrhosis has considerable potential. Further studies are needed to develop a better method of BMC transplantation that can contribute to improvement of liver function and to clarify the long-term effectiveness of this therapy.

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Saito T et al. Bone marrow infusion for cirrhosis

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