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Characteristics of rat bone marrow cells differentiated into a liver cell lineage and dynamics of the transplanted cells in the injured liver

Kazuo Okumoto, Takafumi Saito, Hiroaki Haga, Etsuko Hattori, Rika Ishii, Tetsuru Karasawa, Akihiko Suzuki, Keiko Misawa, Mai Sanjo, Jun-itsu Ito, Kazuhiko Sugahara, Koji Saito, Hitoshi Togashi, and Sumio Kawata

Department of Gastroenterology, Course of Internal Medicine and Therapeutics, Yamagata University School of Medicine, Yamagata 990-9585, Japan

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Background. Bone marrow cells (BMCs) have been shown to differentiate into a liver cell lineage, but little is known about their dynamics following transplantation. BMCs were cultured to investigate the expression of liver-specific genes in vitro and transplanted into in vivo liver-injury models to elucidate their dynamics in the liver. ***Methods.*** The mRNA expression of various liver-specific genes in BMCs cocultured with hepatocytes was analyzed using reverse transcription-polymerase chain reaction. BMCs from transgenic rats expressing green fluorescent protein were transplanted into the spleen of rat liver-injury models induced with 2-acetylaminofluorene (2-AAF) or carbon tetrachloride (CCl_4). BMCs were also transplanted directly into livers treated with CCl_4 to determine which route is better for transplantation. ***Results.*** BMCs differentiated into a liver cell lineage in vitro and expressed mRNAs consistent with mature hepatocytes, including albumin. The transplanted BMCs were found in the liver in the CCl_4-induced injury model, but not in the 2-AAF-induced model. The hepatocyte growth factor and fibroblast growth factor mRNA levels in the liver were significantly higher in the CCl_4-induced model than in the 2-AAF-induced model. Migration of BMCs to the liver was more effective following injection into the liver, rather than into the spleen. ***Conclusions.*** Cultured BMCs differentiated into a liver cell lineage are a potential source for cell transplantation. Transplantation is successful in the severely injured liver with a high level of expression of mRNAs for growth factors. Injection of BMCs directly into the liver is the preferred route of administration.

Key words: transplantation, stem cell, bone marrow, hepatocyte, albumin

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Reprint requests to: T. Saito

Introduction

It has recently been shown that some populations of bone marrow cells (BMCs) can differentiate into hepatocytes,[1–4] and several reports have shown that BMCs can be differentiated into a liver cell lineage in vitro.[5–7] However, little is known about the dynamics of transplanted BMCs in vivo, although we have shown that in vitro BMC differentiation into a liver cell lineage is related to Notch signaling.[8] BMCs that are already differentiated into a hepatocyte lineage are likely to be the best candidate cells for transplantation in liver injury, because cell fusion between BMCs and hepatocytes remains a matter of controversy.[9–11] The detailed expression profiles of liver-specific genes in liver stem cells, including BMCs, also remain unclear. In this study, we analyzed mRNA expression of many liver-specific genes in differentiated BMCs cocultured with mature isolated hepatocytes. The mRNA expression levels of these genes were also analyzed in several differentiated nonstromal liver stem cells from a liver cell lineage, such as oval cells derived from Long-Evans Cinnamon (LEC) rat[12] and hepatic stem-like (HSL) cells derived from Sprague-Dawley (SD) rat.[13]

Using a carbon tetrachloride (CCl_4)-induced liver-injury model, we have recently demonstrated that transplanted BMCs expressing liver cell markers migrate to and stay in the injured liver, following transplantation into the spleen.[14] This suggests that BMC transplantation might be applicable as future therapy for severe liver injury. In the present study, we investigated the dynamics of transplanted BMCs derived from a transgenic rat expressing green fluorescent protein (GFP)[15] after injection into the spleen in two liver-

injury models, which were induced by administration of CCl_4[14] and 2-acetylaminofiuorene (2-AAF),[16] respectively. Migration of the transplanted BMCs to the liver was compared in the two models, with a particular focus on the difference in liver mRNA levels for growth factors such as hepatocyte growth factor (HGF) and fibroblast growth factor (FGF).

We also investigated the injection method that best enhances migration of the transplanted cells to the liver. In heart and vascular diseases, direct injection of BMCs into the affected organ is used for regenerative therapy for treatment of myocardial infarction or arteriosclerosis obliterans.[17–19] Thus, we studied direct injection into the liver as a route of transplantation to determine the effect of this approach on cell migration. Collectively, our results provide a basic understanding of BMC transplantation for liver injury.

Materials and methods

Culture of BMCs

Preparation and purification of hematopoietic stem cell-enriched BMCs were performed according to our previously described method.[8] Briefiy, BMCs were obtained by fiushing out the femurs of 8-week-old male SD rats. A negative selective magnetic cell separation system was used to separate out the hematopoietic stem cell-enriched BMCs. They were then suspended in phosphate-buffered saline (PBS) at pH 7.2. Normal adult hepatocytes were isolated by the perfusion method of Seglen,[20] with some modifications.[21] The viability of the cells was always greater than 85%, as judged by trypan blue exclusion. Coculture of the sorted BMCs with the hepatocytes was performed in six-well culture plates in which two chambers were separated by a semipermeable membrane (Transwell, Corning Coaster, Cambridge, MA, USA; pore size, 0.4 μm). Hepatocytes (3.0×10^5/cm^2) and sorted BMCs (1×10^5/cm^2) were plated in the upper and lower chambers, respectively. The cells were cultured with Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS), 10^{-7} M dexamethasone, 0.5 μg/ml insulin, and 100 units/ml penicillin. The cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2.

Culture of nonstromal, rat liver stem cells

Oval cells derived from LEC rat[12] and HSL cells derived from SD rat[13] were kind gifts from Dr. Kunihiko Terada and Dr. Toshihiro Sugiyama (Akita University School of Medicine, Japan). Small hepatocytes were isolated from SD rat using a previously described method.[22,23] These cells were cultured for 3 days using DMEM containing 10% FBS, 10^{-7} M dexamethasone, 0.5 μg/ml insulin, and 100 units/ml penicillin, with 20 ng/ml HGF and 20 ng/ml epidermal growth factor (EGF).

RNA isolation

Both the BMCs cultured with hepatocytes and the nonstromal rat liver stem cells cultured with HGF and EGF were harvested. Their total cellular RNAs were extracted using an Isogen kit (Wako Pure Chemical, Osaka, Japan) according to the manufacturer's instructions. RNA concentrations were determined by ultraviolet spectrophotometry (UV-1200 spectrophotometer; Shimadzu, Kyoto, Japan).

Reverse transcription-polymerase chain reaction

The expression of mRNA for liver-specific markers was examined using reverse transcription-polymerase chain reaction (RT-PCR). RT-PCR was carried out using the superscript first-strand synthesis system (Invitrogen, Carlsbad, CA, USA). PCR was performed over 35 cycles at a denaturation temperature of 94°C for 1 min, an annealing temperature of 58°C for 1 min, and an extension temperature of 72°C for 1 min, using a Perkin-Elmer 9600 thermal cycler platform (Perkin-Elmer, Norwalk, CT, USA), and the following primers: for detection of albumin mRNA, 5′-ATACACCCAGA AAGCACCTC-3′ and 5′-CAGAGTGGAAGGTGA AGGTC-3′ (PCR product, 305 bp); for detection of alpha-fetoprotein (AFP) mRNA, 5′-AACAGCAGA GTGCTGCAAAC-3′ and 5′-AGGTTTCGTCCCTCA GAAAG-3′ (PCR product, 686 bp); for detection of tyrosine aminotransferase (TAT) mRNA, 5′-TGA ACAGCACTACCACTGTG-3′ and 5′-AGGCATCC TCCGTCTTCTGC-3′ (PCR product; 380 bp); for detection of tryptophan-2,3-dioxygenase (23TO) mRNA, 5′-CAGCTGAGTACAGCGACAGC-3′ and 5′-TCT ATGGAGGTAAGTGTCAG-3′ (PCR product, 330 bp); for detection of HMF3 β mRNA, 5′-GGA GGACCTTTGGTTCCCAC-3′ and 5′-GACCTGGAT TTCACCATGTC-3′ (PCR product, 310 bp); and for detection of glyceraldehyde-3-phosphate dehydrogerase (GAPDH) mRNA as a control, 5′-ATCACT GCCACTCAGAAGAAGAC-3′ and 5′-TGAGGGA GATGCACAGTGTT-3′ (PCR product, 580 bp).

Transplantation of BMCs into the spleen in two rat liver-injury models

The animal study protocol was approved by the Animal Studies Ethics Committee of Yamagata University School of Medicine, Japan. The BMC donors were 8-week-old male transgenic rats expressing GFP (GFP-

Tg), provided by Japan SLC (Hamamatsu, Japan) and used with the kind agreement of Dr. Masaru Okabe (Genome Information Research Center, Osaka University, Japan). The BMC recipients were 8-week-old male SD rats obtained from Japan SLC. Animals were housed in a temperature-monitored environment (20°C to 22°C) with a 12-h light-dark cycle, and fed standard rat chow *ad libitum*. Two kinds of liver-damaged models were constructed in the BMC recipients using CCl_4 and 2-AAF, respectively. In the CCl_4-induced injury model, 0.8 ml/kg of CCl_4 diluted 1:1 with corn oil was administered to three recipient rats by gavage twice a week for 2 weeks before transplantation. BMC transplantation into the spleen of the recipients was performed with a syringe needle. After transplantation, the rats were administered CCl_4 twice a week consecutively for 2 weeks and then killed. In the 2-AAF-induced injury model, 30 mg/kg of 2-AAF was administered to three recipient rats every day for 5 days, and a two-thirds partial hepatectomy was performed when the BMCs were transplanted. The rats were killed 14 days after transplantation. The BMCs for transplantation were isolated from the GFP-Tg donor, and hematopoietic stem cell-enriched, purified BMCs were obtained using a negative selective magnetic cell separation system, as previously described.[8] Each recipient received 5×10^6 cells, which were transplanted into the spleen with a syringe needle, according to the procedure reported by Kobayashi et al.[24,25]

Immunohistochemical study of GFP-positive cells in the recipient liver

To identify the transplanted BMCs in recipient livers, the cells were immunostained using antibodies specific to GFP. Both liver and spleen collected from the recipient were fixed with 4% paraformaldehyde and embedded in paraffin. Four-micrometer-thick serial sections were prepared, deparaffinized, and washed with PBS before immunostaining. An avidin–biotin complex immunoperoxidase staining technique was employed (Vectastain ABC kit: Vector Laboratories, Burlingame, CA, USA). After endogenous peroxidase activity was inhibited by incubating the specimens with 0.3% hydrogen peroxide solution for 30 min at 4°C, the sections were incubated with a rabbit anti-GFP antibody (Chemicon International, Temecula, CA, USA) at 1:200 dilution in a moist chamber at 4°C overnight, followed by incubation with the biotinylated secondary antibody and the avidin–biotin complex according to the manufacturer's instructions. The GFP reaction product was stained brown with a 0.02% solution of 3,3′-diaminobenzidine in 0.05 M Tris-HCl buffer, pH 7.2, containing 0.01% hydrogen peroxide. The slides were counterstained with hematoxylin and mounted. The numbers of GFP-immunopositive cells were counted in five 100-mm^2 fields of specimens of both liver and spleen in the three recipients and expressed as the mean number ± standard deviation (SD).

Quantitation of the HGF and EGF mRNA levels in the 2-AAF- and CCl_4-induced liver-injury models

The total cellular RNA extracted from the liver of SD rats was used as a standard. The methods for RNA isolation and cDNA amplification are described above. To quantitate the HGF and FGF mRNA levels in livers chemically injured with 2-AAF or CCl_4, real-time PCR was performed using a LightCycler quick system 350S (Roche Diagnostics, Tokyo, Japan) according to the manufacturer's instructions. The primers for detection of HGF mRNA in the real-time PCR were 5′-CTACA CAGGGAATCCTCTCG-3′ and 5′- AGCCTTCAGG TCCAATAGACT-3′, and those for detection of FGF mRNA were 5′-TGCCGAGAGGTTCAATCTG-3′ and 5′-AACTCCCGTTCTTCTTGAGG-3′.

Transplantation using an alternative route of injection

Rat liver injury models were made with CCl_4, as described above. A total of 18 rats received 0.8 ml/kg CCl_4 diluted 1:1 with corn oil by gavage twice a week for 2 weeks before transplantation. In nine of these rats, BMC transplantation into the left lobe of the liver was performed, while the remaining nine rats received BMC transplantation into the spleen. CCl_4 was further administered twice a week for a maximum of 3 weeks after transplantation. Three rats in each group were killed on day 14, on day 28, and on day 56 after transplantation, and the liver was fixed with 4% paraformaldehyde and embedded in paraffin. To study the effectiveness of each transplantation route, the numbers of immunopositive transplanted BMCs labeled by GFP antibody in the liver were compared between rats that received BMC transplantation into the left lobe of the liver and those in which BMCs were injected into the spleen.

Statistical Analysis

Statistical analysis was performed using Student's t test, and $P < 0.05$ was considered to be significant.

Results

mRNA expression profile for liver-specific genes in liver stem cells

BMCs cultured with mature hepatocytes differentiated into a liver cell lineage expressing mRNAs for albumin,

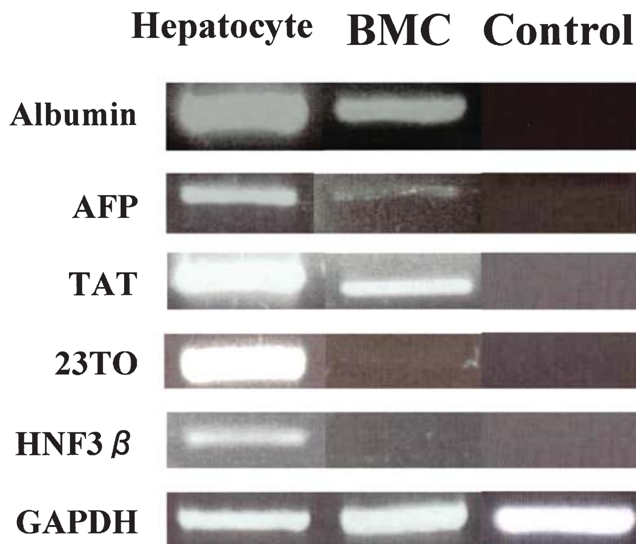

Fig. 1. The mRNA expression profile for liver-specific genes in bone marrow cells (*BMCs*) differentiated into a liver cell lineage after coculturing with isolated *hepatocytes*. Expression of mRNAs for *albumin*, alpha-fetoprotein (*AFP*), and tyrosine aminotransferase (*TAT*) was detectable, but tryptophan-2,3-digoxygenas (*23TO*) and HMF3 β mRNAs were not found. Mature hepatocytes were used as a positive control and expressed mRNA for all the liver-specific genes examined. Reverse transcription-polymerase chain reaction (RT-PCR). BMCs without coculture were used as a negative control for detection of liver-specific genes by polymerase chain reaction. *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase

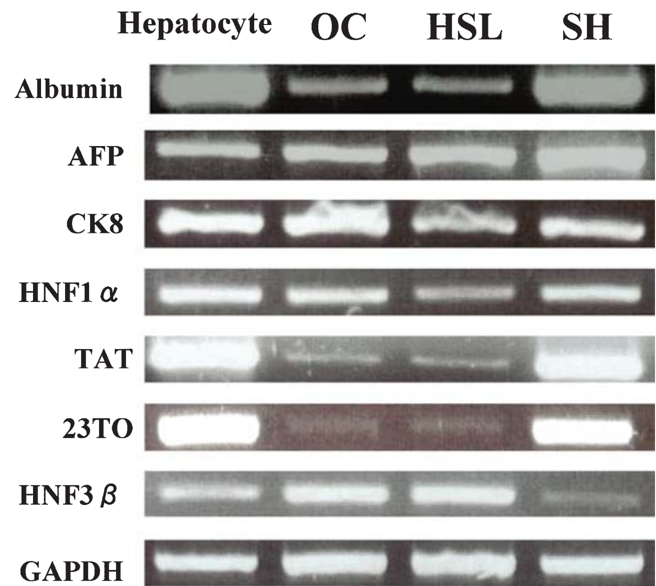

Fig. 2. The mRNA expression profile of liver-specific genes in nonstromal liver stem-like cells, including oval cells (*OC*), hepatic stem-like (*HSL*) cells, and small hepatocytes (*SH*), differentiated into a liver cell lineage after culturing with both hepatocyte growth factor (HGF) and epidermal growth factor (EGF). mRNAs for all the liver-specific genes examined were detected. Mature *hepatocytes* were used as a positive control and expressed mRNA for all the liver-specific genes examined. RT-PCR

AFP, and TAT, but expression of mRNAs for 23TO and HMF3β was not detected in these cells (Fig. 1). Nonstromal liver stem-like cells including oval cells, HSL cells, and small hepatocytes cultured with both HGF and EGF also differentiated into a liver cell lineage expressing mRNAs of all the liver-specific genes examined (Fig. 2). Mature hepatocytes isolated from adult SD rat were used as positive controls and also expressed mRNAs of all the liver-specific genes examined.

Transplantation of BMCs in 2-AAF- and CCl_4-induced liver-injury models

Although BMCs were transplanted into the spleen, no GFP-positive cells were found in the spleen in either the 2-AAF- or CCl_4-induced liver-injury model animals. GFP-positive cells were detected in livers damaged by CCl_4 (Fig. 3A), but not in those damaged by 2-AAF (Fig. 3B). In the CCl_4-induced injured liver, these cells were located in the portal areas of the liver (Fig. 3A). The mean number of GFP-positive cells in five 100-mm^2 fields of specimens from three recipient livers was 48.9 ± 19.2 cells/100 mm^2 (mean ± SD).

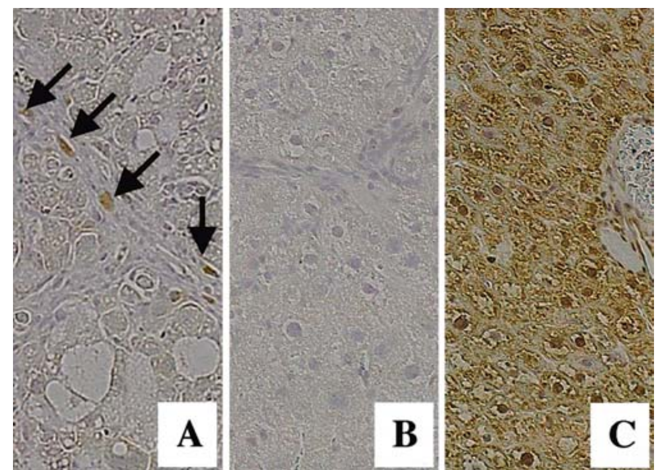

Fig. 3A–C. Immunohistochemistry of transplanted BMCs expressing green fluorescent protein (GFP) in the rat recipient liver. The transplanted GFP-positive cells were detected 14 days after transplantation in the CCl_4-induced injured liver (**A**, *arrows*), but not in the 2-acetylaminofluorene (2-AAF)-induced injured liver (**B**). **A** CCl_4-treated rat liver; **B** 2-AAF-treated rat liver; ×100; **C** positive control of GFP staining in rat donor's liver

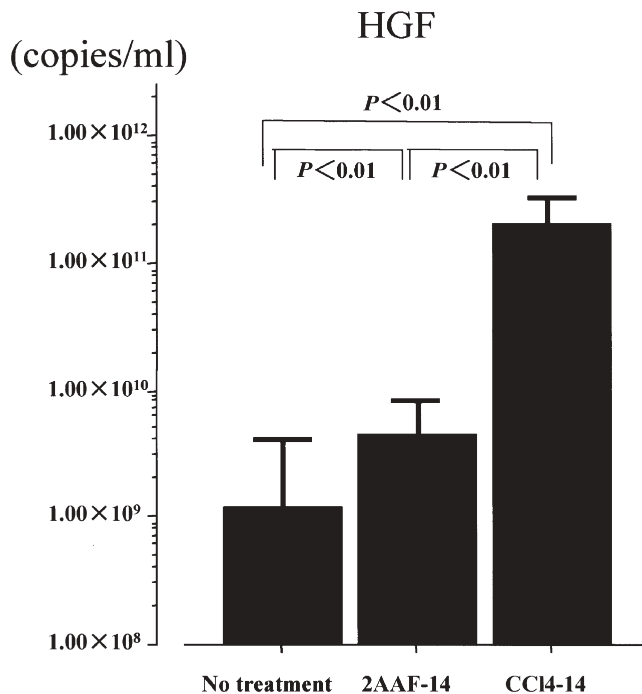

Fig. 4. Quantitation of the HGF mRNA level in liver. The HGF mRNA level on day 14 after BMC transplantation was significantly higher in CCl_4-induced injured liver than in 2-AAF-induced injured liver ($4.52 \pm 3.52 \times 10^9$ copies/ml versus $2.14 \pm 1.41 \times 10^{11}$ copies/ml; $P < 0.01$, $n = 3$) and untreated liver ($4.52 \pm 3.52 \times 10^9$ copies/ml versus $1.02 \times 10^9 \pm 3.34 \times 10^9$ copies/ml; $P < 0.01$, $n = 3$). Real-time quantitative PCR

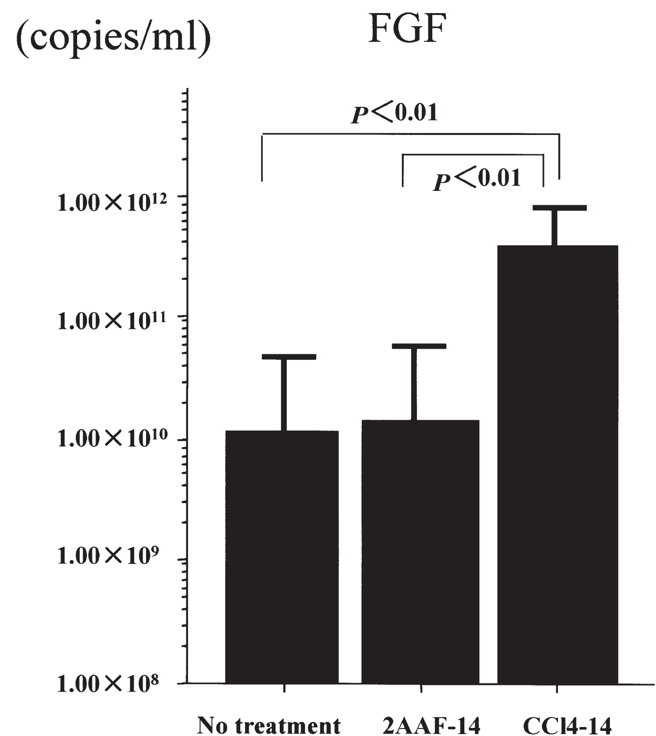

Fig. 5. Quantitation of the fibroblast growth factor (*FGF*) mRNA level in liver. The FGF mRNA level on day 14 after BMC transplantation was significantly higher in CCl_4-induced injured liver than in 2-AAF-induced injured liver ($2.88 \pm 6.45 \times 10^{11}$ copies/ml versus $1.58 \pm 4.57 \times 10^{10}$ copies/ml; $P < 0.01$, $n = 3$) and untreated liver ($2.88 \pm 6.45 \times 10^{11}$ copies/ml versus $1.30 \times 10^9 \pm 3.31 \times 10^9$ copies/ml; $P < 0.01$, $n = 3$). Real-time quantitative PCR

Comparison of the liver HGF and FGF mRNA levels in the two liver-injury models

A comparison of the liver HGF and FGF mRNA levels in the two liver-injury models showed that the HGF mRNA level was significantly higher in the CCl_4-induced model than in the 2-AAF-induced model on day 14 after BMC transplantation ($4.52 \pm 3.52 \times 10^9$ copies/ml versus $2.14 \pm 1.41 \times 10^{11}$ copies/ml; $P < 0.01$, $n = 3$) (Fig. 4). The FGF mRNA level was also significantly higher in the CCl_4 model than in the 2-AAF model on day 14 after BMC transplantation ($2.88 \pm 6.45 \times 10^{11}$ copies/ml versus $1.58 \pm 4.57 \times 10^{10}$ copies/ml; $P < 0.01$, $n = 3$) (Fig. 5). The HGF and FGF mRNA levels in untreated rats were $1.02 \times 10^9 \pm 3.34 \times 10^9$ copy/ml and $1.30 \times 10^9 \pm 3.31 \times 10^9$ copy/ml, respectively; both levels were significantly lower than those in CCl_4-induced injured livers ($P < 0.01$, $n = 3$).

A better injection route for cell engrafting

The results of a histological examination of CCl_4-induced injured livers are shown in Fig. 6. Submassive hepatic necrosis with fibrosis was seen in the liver architecture by day 14 after BMC transplantation (Fig. 6A). Liver regeneration appeared by day 28 after transplantation (Fig. 6B), and recovery from liver injury was apparent on day 56 after transplantation (Fig. 6C). When BMCs were injected into the liver of the recipient, transplanted GFP-positive BMCs were found in the portal areas of the recipient liver on each day, irrespective of the injection site (Figs. 6D, E, F). The number of GFP-positive cells increased by day 28 after transplantation, and then decreased by day 56 after transplantation. The numbers of transplanted GFP-positive cells were significantly higher when BMCs were injected into the liver, compared with injection into the spleen, on day 14 (155.5 ± 28.1 versus $47.0 \pm 20.6/100\,mm^2$; $P < 0.01$, $n = 3$, mean ± SD) and day 28 (202.9 ± 40.1 versus $89.7 \pm 34.5/100\,mm^2$; $P < 0.05$, $n = 3$, mean ± SD) (Fig. 7).

Discussion

In recent years BMC transplantation has received increasing attention as a promising future therapy for

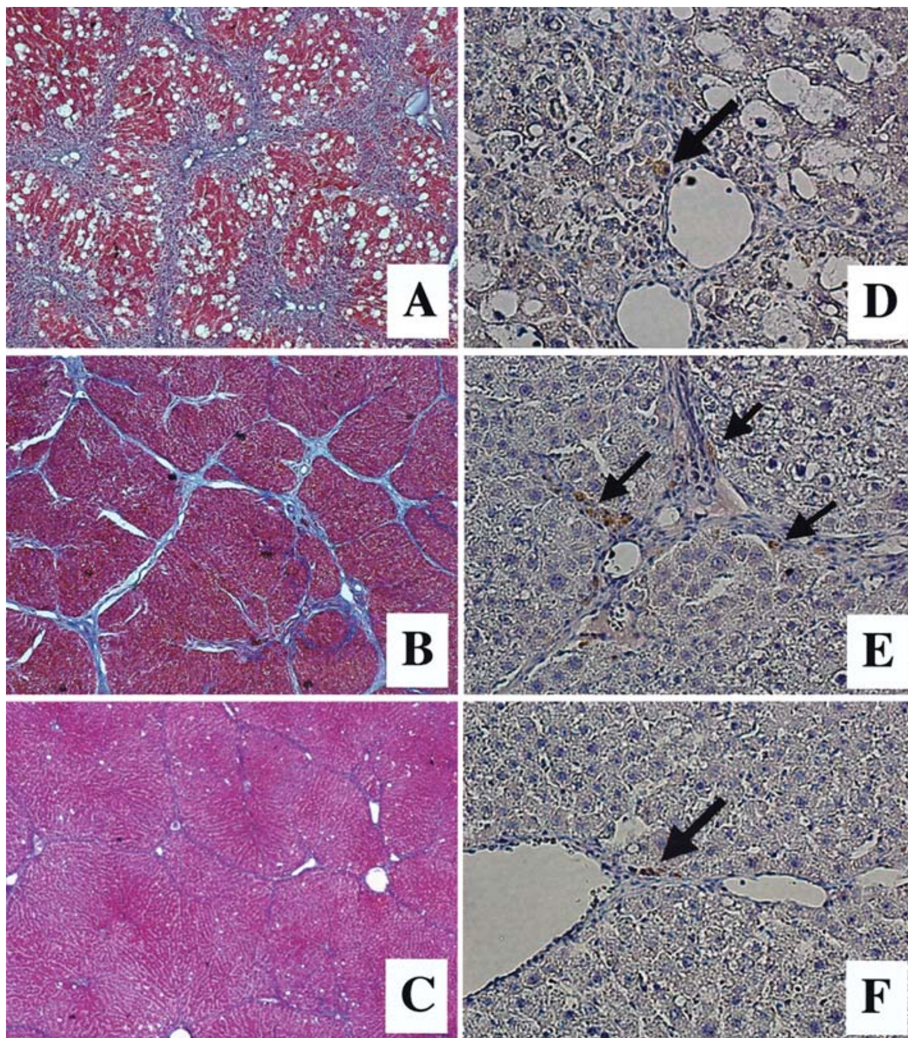

Fig. 6A–F. Liver histology and GFP-positive BMCs in the liver of a CCl_4-induced injury model animal. Submassive hepatic necrosis was seen on day 14 after BMC transplantation (**A**), regeneration appeared on day 28 after transplantation (**B**), and recovery from liver injury was apparent on day 56 after transplantation (**C**). When BMCs were injected into the liver of the recipient, the transplanted GFP-positive BMCs were found in the portal areas of the recipient liver on each day (**D, E, F**). Hematoxylin and eosin staining, **A**–**C**; ×40, **D**–**E**; ×200

severe liver injury. We have previously shown that transplanted BMCs migrate to the injured liver and express certain liver-specific proteins, such as AFP, in vivo.[14] To extend this study, the cell characteristics of BMCs differentiated into a liver cell lineage in vitro and the dynamics of the transplanted cells in the injured liver in vivo were investigated. The results show that (1) not only liver stem cells but also BMCs after culturing with hepatocytes can differentiate into a liver cell lineage expressing mRNA for mature hepatocyte markers, including albumin, in vitro, and (2) transplanted BMCs can be engrafted to the injured liver in a CCl_4-induced injury model, in which severe liver injury with both submassive hepatic necrosis and induction of high levels of HGF and FGF was observed.

Although there are multipotent stem cells in BMCs,[1] little is known about the characteristics of BMCs following differentiation into a liver cell lineage. In this study, we clearly showed that BMCs cultured with hepatocytes can differentiate into cells expressing mRNA for mature hepatocyte markers such as albumin. Liver transplantation has become an established therapy for liver failure, but a lack of donors has been pointed out as a problem, and transplantation using hepatocytes or oval cells has been suggested as an alternative strategy.[26,27] In comparison with mRNA expression in liver stem cells cultured with HGF, the expression of mRNAs for some hepatic markers, including 23TO and HMF3β, was absent in BMCs cultured with hepatocytes. Nevertheless, BMCs differentiated into a liver cell lineage are an attractive source for cell transplantation in liver injury because they are autologous and therefore not subject to immune rejection, and they can be collected safely and easily in sufficient quantities using an established method. In addition, further studies on alternative culture conditions should lead to improvement in the quality of differentiated BMCs with more extensive expression of liver-specific markers in vitro.

An understanding of the dynamics of the transplanted BMCs in the liver is also important for future

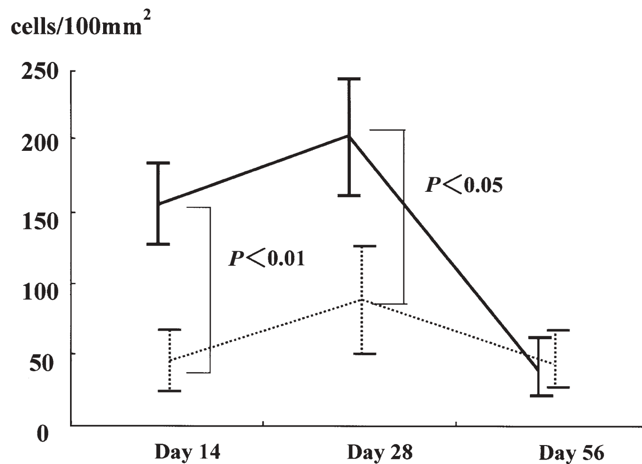

Fig. 7. The number of transplanted BMCs using two injection routes. The number of GFP-positive cells increased on day 28 after transplantation, and decreased by day 56 after transplantation. The numbers of transplanted GFP-positive cells were significantly higher for BMC injection into the liver, compared with injection into the spleen, on day 14 (155.5 ± 28.1 versus $47.0 \pm 20.6/100\,mm^2$ of specimen; $P < 0.01$, $n = 3$, mean ± SD) and day 28 (202.9 ± 40.1 versus $89.7 \pm 34.5/100\,mm^2$ of specimen; $P < 0.05$, $n = 3$, mean ± SD)

improvements. To date, it has been unclear whether transplanted BMCs in vivo migrate to and engraft in the liver,[28–31] and the relationship of the degree of liver damage to the migration effect of the transplanted cells has also been uncertain. In this study, the dynamics of transplanted BMCs in the liver were studied using two chemically induced liver-injury models. Interestingly, transplanted BMCs labeled with GFP were found in livers damaged with CCl_4 but not in livers damaged with 2-AAF. We have shown that both HGF and EGF are effective for the differentiation of BMCs into a liver cell lineage in vitro.[8] Although there are several candidate growth factors that have been reported to support differentiation of BMCs into a liver cell lineage, we measured the mRNA levels of HGF and FGF, which have been shown to be responsible for efficient differentiation of BMCs into a liver cell lineage.[8,32,33] Both the HGF and FGF mRNA levels were higher in the CCl_4-induced injury model than in the 2-AAF model. Further studies are now in progress in our laboratory to clarify what kind of factors from hepatocytes may contribute to the differentiation of BMCs into a liver cell lineage. Since higher levels of induction of HGF are seen in more highly damaged livers,[34,35] these results suggest that transplanted BMCs may migrate more effectively to a liver with greater damage. We have shown that protein expression of AFP and cytoketratin 19 can be detected in the transplanted BMCs using immunohistochemistry.[14] To examine whether the transplanted BMCs from GFP rats express liver-specific genes or their products, including albumin in the injured liver, we need to confirm these expressions using in situ hybridization or further improved immunohistochemical techniques for detecting the transplanted BMCs in the injured liver. Expressions of liver-specific genes or their products in the transplanted BMC in the injured liver are now under investigation.

We note that, in this study, the original BMCs of the recipient were not irradiated, and transplantation of BMCs without irradiation might cause rejection of the transplanted cells. Furthermore, we should use the irradiated rat in order to precisely evaluate dynamics of BMCs that are exogenously transplanted. Thus, the most suitable conditions for BMC transplantation need to be clarified with respect to the settling of the transplanted cells in the liver.

It has been shown that BMC transplantation moderates liver fibrosis in the CCl_4-induced liver-injury model.[36] In this study, relatively small numbers of BMCs were seen only in the transplanted liver; thus, the degree of fibrosis did not seem to be different between the transplantation model and a sham model on day 28 or day 56. Also, we did not measure the serum markers for liver fibrosis in this study. Further studies are needed to clarify whether the moderation of liver fibrosis seen in the BMC transplantation model can be found in this model.

The injection route is another important consideration for improvement of BMC transplantation to the liver. In heart and vascular disease, BMC injection has been performed directly at the injured site,[17,18] and a similar procedure in the present study showed that the number of transplanted BMCs in the liver increased upon direct injection into the liver, compared with injection into the spleen. Alternative injection routes, including venous or portal injection, are worth studying to further improve transplantation.

In conclusion, we have shown that BMCs are a potential source for liver cell transplantation. BMCs migrated more effectively to a highly damaged liver with higher HGF and FGF mRNA levels upon direct injection into the liver. These results are encouraging, but further in vitro and in vivo studies are needed to improve the effectiveness of this transplantation approach for future clinical use.

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