Transplantation of human matrix metalloproteinase-1 gene-modified bone marrow-derived mesenchymal stem cell attenuates CCL4-induced liver fibrosis in rats

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Abstract. It has been reported that bone marrow-derived mesenchymal stem cells (BMSCs) alleviated liver fibrosis. We investigated whether BMSCs transfected with human matrix metalloproteinase 1 (BMSCs/MMP1) would improve their therapeutic effect in liver fibrosis induced by carbon tetrachloride (CCl_4) in rats. BMSCs were transfected with an adenovirus carrying enhanced green fluorescence protein (GFP) and human MMP1 gene. BMSCs or BMSCs/MMP1 were directly injected into fibrotic rats via the tail vein. GFP-labeled cells appeared in the fibrotic liver after BMSC transplantation. The expression of BMSCs/MMP1 elevated levels of MMP1 *in vitro*. Although BMSC administration reduced liver fibrosis, transplantation of BMSCs/MMP1 enhanced the reduction of liver fibrosis to a higher level. Treatment with BMSCs/MMP1 not only decreased collagen content but also suppressed activation of hepatic stellate cells (HSCs) in fibrotic liver, which led to subsequent improvement of both liver injury and fibrosis. Treatment with BMSCs/MMP1 resulted in an improved therapeutic effect compared with BMSCs alone, which is probably because of the sustainably expressed MMP1 level in the liver. BMSCs/MMP1 transplantation not only improved biochemical parameters but also attenuated progression of liver fibrosis, suggesting that BMSCs may be a potential cell source in preventing liver fibrosis and MMP1 gene may enhance the anti-fibrotic effect of BMSCs.

Introduction

Liver fibrosis is the advanced stage of liver disease accompanying by hepatocyte necrosis and excessive or uncontrolled deposition of extracellular matrix (ECM) under constant stimulation of a variety of pathogenic factors (viral hepatitis, alcohol abuse, metabolic disease and immune injury) (1-3). Great progress has been made in the mechanisms and cell biology of liver fibrosis. Numerous small molecules and biologics have been identified that are reaching preclinical testing of anti-fibrotic agents and strategies, but the effective anti-fibrotic drugs approved for clinical use in advanced liver fibrosis still are scarce (4-6). Although liver transplantation is the only effective treatment to cure liver cirrhosis at present, it is limited by organ donor shortage, surgery-related complications, immunological rejection, and high cost worldwide (7,8).

In recent years, increasing research (9-14) has suggested that stem cell transplantation is an effective alternative therapy for liver fibrosis/cirrhosis. The stem cells, including embryonic stem cells, induced pluripotent stem cells, and adult stem cells, have the potential of differentiation into hepatocyte-like cells both *in vivo* and *in vitro* (15-19). In these stem cells, bone marrow-derived mesenchymal stem cells (BMSCs) are the most abundant source and is most widely used in animal experiments and clinical trials. BMSCs have several advantages, such as easy acquisition, strong proliferative capacities, and immune-modulatory property that are able to migrate to damaged tissues (20). BMSC transplantation therapy alone may not attenuate liver fibrosis completely (21), since it cannot degrade the ECM and fiber scar effectively in cirrhotic tissue which may prevent proliferation of BMSCs, suggesting that the therapeutic efficacy of BMSCs needs improvement. According to recent studies (22-25), BMSCs could be used as a potent ideal vehicle for gene delivery. Gene modified stem cells may maintain the direct differentiation characteristics and secrete exogenous cytokines for the purpose of anti-fibrogenic therapy.

Matrix metalloproteinase (MMP) is the main enzyme responsible for ECM degradation and tissue inhibitor of metalloproteinases (TIMPs) has the ability to inhibit MMPs (26). MMPs secreted by HSCs and Kupffer cells participating in the degradation of ECM, is endogenous proteolytic enzyme family of zinc-calcium ions (27). MMP is the strongest enzyme to degrade collagen fibers, which are the main component of ECM and play an important role in physiological and pathological process. Although some

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studies and cell culture findings suggest that MMP2 promotes hepatic fibrogenesis (28). Moreover, some evidence suggests that MMP2 may be anti-fibrotic in liver disease, which is capable of cleaving type I collagen *in vitro* and limiting HSC activity after liver injury (29-31). MMP1, called fibroblasts type, is the main human interstitial collagenase and reversed liver fibrosis process by degrading collagen type I and III in ECM (32). It has been reported that imbalance between too few MMP1 and too much TIMP1 is an important mechanism of liver fibrosis (33). Iimuro *et al* tried to improve this imbalance by upregulating MMP1 expression in rat and observed liver fibrosis attenuation to some extent (34). Yang *et al* (35) also found that enhancement of the expression of MMP1 in liver tissues of CCl_4-induced hepatic fibrotic rats, which may result in its elevated activity that contributes to fighting against hepatic fibrosis. In the present study, we investigated the therapeutic efficacy of BMSCs overexpressing MMP1 in a rat model of liver fibrosis induced by CCl_4. To assess therapeutic effectiveness, we evaluated changes in liver function, liver histopathology and fibrous protein [hepatic hydroxyproline and α-smooth muscle actin (α-SMA)] after transplantation. We show that therapy with BMSCs/MMP1 resulted in an improved therapeutic effect compared with BMSCs alone, probably because of the sustainably expressed MMP1 level in the liver. Our findings indicate BMSCs/MMP1 transplantation not only improved biochemical parameters but also attenuated progression of liver fibrosis, suggesting that BMSCs may be a potential cell source in preventing liver fibrosis and MMP1 gene may enhance the anti-fibrotic effect of BMSCs.

Materials and methods

Animals. Male Sprague-Dawley (SD) rats were obtained from the Institute of Zoology at the Third Military Medical University (Chongqing, China). The animals were housed in air-conditioned rooms, with controlled temperature and humidity with 12 h light-dark cycles. Food and water were available *ad libitum*. Animal welfare and experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006). The Ethics Committee of Chengdu Military General Hospital approved all of the animal experiments.

Construction of recombinant adenovirus vector. Constructing the recombinant adenovirus vector containing hMMP1 gene with Gateway™ Clone Technology as previously described (36). Briefly, the full-length gene hMMP1 was amplified by using PCR from the pcDNA3.1 plasmid, then it was cut down and connected to the entry vector pENTER™ 1A (both from Invitrogen, Carlsbad, CA, USA). The entry clone and the destination vectors pJTI™ R4 the Dest CMV-IRES/eGFP pA vector (Invitrogen) recombine using the LR reaction to form the expression clone pAd-hMMP1-IRES/eGFP. The linear pAd-hMMP1-IRES/eGFP transfected into HEK293A cells packaging the Ad-hMMP1-IRES/eGFP. The target protein expression was detected by RT-PCR and western blot assay. The adenovirus titre was measured by TCID50 method, and stored at -80˚C in the phosphate-buffered saline (PBS).

BMSCs isolation, culture and gene transduction. Rats BMSCs were isolated from bone marrow and expanded in culture according to previous studies (37,38). For adenoviral transduction, the BMSCs were washed with serum-free Dulbecco's modified Eagle's medium (DMEM) three times and exposed to fresh medium containing Ad-MMP1-eGFP ($1.8x10^{10}$ pfu/ml) and Ad-eGFP ($1.0x10^{10}$ pfu/ml) in 5 ml DMEM at 37˚C for 4 h, according to the multiplicity of infection 50, 100, 200 and 300 (pfu number/cell). The medium was removed, and the cells were washed once with DMEM and re-cultured in normal medium for 24 h, after which cell transplantation was performed.

Cell surface labeling. BMSCs and MMP1-BMSC phenotypes were analyzed by flow cytometry using a FACSCalibur (Becton-Dickinson Biosciences, Ann Arbor, MI, USA). The cells were re-suspended in phosphate-buffered saline (PBS) at a concentration of $1x10^6$ cells/ml, and were incubated with following fluorescent anti-human antibodies: fluorescein isothiocyanate (FITC)-conjugated CD45 and CD90, phycoerythrin (PE)-conjugated CD105, CD14, CD34 and CD79a (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). The rat immunoglobulin IgG-FITC and IgG-PE was used as the isotype-matched control. The cells were tagged 45 min away from light at room temperature, washed three times with PBS and detected with FACSCalibur flow cytometer.

CCl_4-induced liver fibrosis model and BMSCs transplantation. Seventy male rats were divided into two groups randomly, group A: liver fibrosis model (n=60) and group B: control (n=10). Liver fibrosis was induced by subcutaneous injection of CCl_4 oil solution (1:1 olive oil; Sigma-Aldrich, Steinheim, Germany) at a dose of 1 ml/kg body weight twice per week for 8 weeks. The same volume of saline solution was applied to control rats. The rats were sacrificed to assess the extent of liver fibrosis after withdrawing injection of CCl_4. For cell implantation, CCl_4 treated rats were classified into five groups (n=10): normal control group, rats were treated with olive oil infused with saline; model control group, rats were treated with CCl_4 infused with olive oil; BMSCs group, rats were treated with CCl_4 infused with saline containing untreated BMSCs ($3x10^6$ cells); eGFP/BMSCs group, rats were treated with CCl_4 infused with saline containing BMSCs transduced with Ad-eGFP for 24 h ($3x10^6$ cells); MMP1-eGFP/BMSCs group, rats treated with CCl_4 were infused with saline containing BMSCs transduced with Ad MMP1-eGFP for 24 h ($3x10^6$ cells); transplantation was administered as a single dose. Rats were sacrificed 2 or 4 weeks post-implantation, liver tissue was obtained to observe the expression of GFP by frozen section. Blood was collected from celiac artery for analysis. Liver tissue was fixed in 10% neutral buffered formalin for histopathological and immunohistochemical examination, or stored at -80˚C for future use.

Enzyme-linked immunosorbent assay (ELISA) for MMP1 secreted by adenoviral transducted BMSCs and TIMP1 in the liver. Enzyme-linked immunosorbent assay for MMP1 secreted in both MMP1/BMSCs and BMSCs ($2x10^6$ cells) were transfected with Ad-MMP1-eGFP at optimum MOI of 300 puf/cell in 6-well plates and cultured for 72 h, the culture

supernants were centrifuged at 10,000 x g/min for 10 min and collected for analysis. In addition, the media from untreated BMSCs were collected as the control. Commercial MMP1 ELISA kits (R&D Systems, Minneapolis, MN, USA) were used to detect the content of MMP1 in each group.

The levels of MMP1 and tissue inhibitor of metalloproteinases-1 (TIMP1) in the liver tissue of rats 4 weeks after transplantation were measured using ELISA kits (R&D Systems). Wet liver tissue (100 mg/sample) was homogenized in 1 ml PBS in the presence of 1% protease inhibitors (Sigma-Aldrich). The supernatant fraction of liver homogenate was used to measure MMP1 and TIMP1 levels.

Enzymatic activity of MMP1 in vitro and in vivo. The enzymatic activity of human matrix metalloproteinase 1 secreted in MMP1 gene modified BMSCs and in liver after implantation was tested by fluorescence quantitative kits of MMP1 enzyme activity (GEM, China). The protein of MMP1 was collected from the supernatants in cultured BMSCs and from liver tissue as above. Enzyme activity was performed according to the manufacturer's instructions. Fluorescence microplate reader (Japan) the setting was: excitation wavelength 330 nm, distribution wavelength 400 nm, 37˚C. The data were expressed as the mean [nmol/(mg•min)] ± SD, with n=10/group.

Liver function test. The blood samples were obtained from large artery according to experimental design, and stored at -80˚C. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), albumin (ALB) and prothrombin time (PT) were measured with automatic biochemical analyzer.

Liver histology. The Masson staining method was to detect collagen fibers, the blue areas are considered the collagen area. The livers were harvested at sacrifice, washed in PBS, and fixed in 10% formalin overnight at 4˚C. Tissues embedded in paraffin were cut into 5-μm-thin sections. For Masson's trichrome stain, sectioned samples were placed in Bouin's solution at waterbath of 60˚C for 1 h and washed in running tap water for 5 min, stained in succession with Weigert's working hematoxylin solution for 10 min, Biebrich Scarlet solution for 5 min, phosphomo-lybdic/phosphotungstic-acid for 10 min, transferred directly into Aniline blue for 5 min, 1% acetic acid for 1 min, dehydrate, clear, and coverslip. Sections were examined with a microscope (IX70; Olympus, Tokyo, Japan). For H&E analysis, sectioned samples were stained with Mayer's hematoxylin solution (Sigma-Aldrich) for 5 min followed by Eosin Y (Deventer, The Netherlands) for 5 min.

Hepatic hydroxyproline determination. Hepatic hydroxyproline was tested by improvement of Kivirikko method. Simply, liver samples of rats were obtained from sacrifice, and 20 mg of liver tissue were weighed, frozen and cut into homogeneity. Liver tissue was hydrolyzed with 3 ml 6 N HCl for 24 h at 100˚C. The mixture was centrifuged at 2,000 rpm, 4˚C for 5 min. The content of hydroxyproline (HYP) was determined by colorimetric assay at a wavelength of 560 nm. The quantity of HYP was calculated against a calibration curve obtained using HYP standards. Finally, the HYP content in each sample was quantified with μg/g (liver wet dry).

Western blot analysis for α-SMA of the liver tissue. Liver tissues harvested from rats per group were lysed with RIPA peptide lysis buffer (Shenerg Biocolor, Shanghai, China) with 1% protease inhibitors at 4˚C. Lysate containing 20 μg of protein was separated by electrophoresis on 10% acrylamide sodium dodecyl sulfate (SDS) gels. After electrophoresis, the protein was transferred onto polyvinylidene difluoride membranes (PVDF; Millipore, Billerica, MA, USA). The membrane was incubated with mouse anti-rat α-SMA monoclonal antibody (1:1,000 dilution; Sigma-Aldrich) overnight at 4˚C and horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (1:5,000 dilution; Santa Cruz Biotechnology, Inc.) for 2 h at room temperature in a gyratory shaker. After adequate washes, the membrane was processed using SuperSignal West Pico chemiluminescent substrate (Pierce, Rockford, IL, USA). The mouse anti-rat β-actin monoclonal antibody (1:500 dilution; Santa Cruz Biotechnology, Inc.) as an internal standard. The intensities of α-SMA and β-actin were measured using the quantity.

Statistical analysis. The data are presented as the means ± standard deviation (SD). The differences between mean values of each group were compared by a one-way analysis of variance (ANOVA) and considered to be statistically significant when the adjusted $P<0.05$. All analyses were performed using SPSS version 16.0 statistical software (SPSS, Inc., Chicago, IL, USA). ImageJ was used to analyze the images.

Results

Characteristics of BMSCs and MMP1 gene-transduced BMSCs. The isolated BMSCs presented uniform morphology, grew in spindles, and arranged in whirlpool shape (Fig. 1A and B). BMSCs were then transfected by recombinant adenovirus at the multiplicity of infection (MOI) of 50, 100, 200 or 300 pfu/cell, respectively. We found that BMSCs/MMP1 expressed green fluorescence from 24 h to 21 days after infection. The fluorescence intensity increased in level with MOI and reached its highest level of 76.43% at MOI 200 pfu/cell 72 h after the infection (Fig. 1C-E).

To identify the origin of these cells, we next detected the expression of both BMSC markers CD90 and CD105 and hematopoietic cell markers CD34, CD45, CD14 and CD79a by flow cytometry. The results showed that BMSCs/MMP1 were 99.6% positive for CD90 and 99.8% for CD105, while only 0.1% positive for CD45, CD14 and CD79a, and 0.3% positive for CD34 (Fig. 2), which indicated that the bone marrow-derived and MMP1 gene modified cells were BMSCs.

BMSCs/MMP1 alleviated CCl_4-induced liver fibrosis. After transplantation of BMSCs, the green fluorescence positive cells distributed around the hepatic vascular, hepatic sinusoid, and hepatic lobule of implantation rats by fluorescent microscope (Fig. 3A), indicating that BMSCs/MMP1 were implanted successfully in the liver. To address the therapeutic effect of BMSCs/MMP1 on liver fibrosis, we injected saline, BMSCs, or BMSCs/MMP1 into rats via the tail vein. In CCl_4-induced fibrotic liver, there were evidently much pseudolobuli surrounded by fibrotic septa joining the central

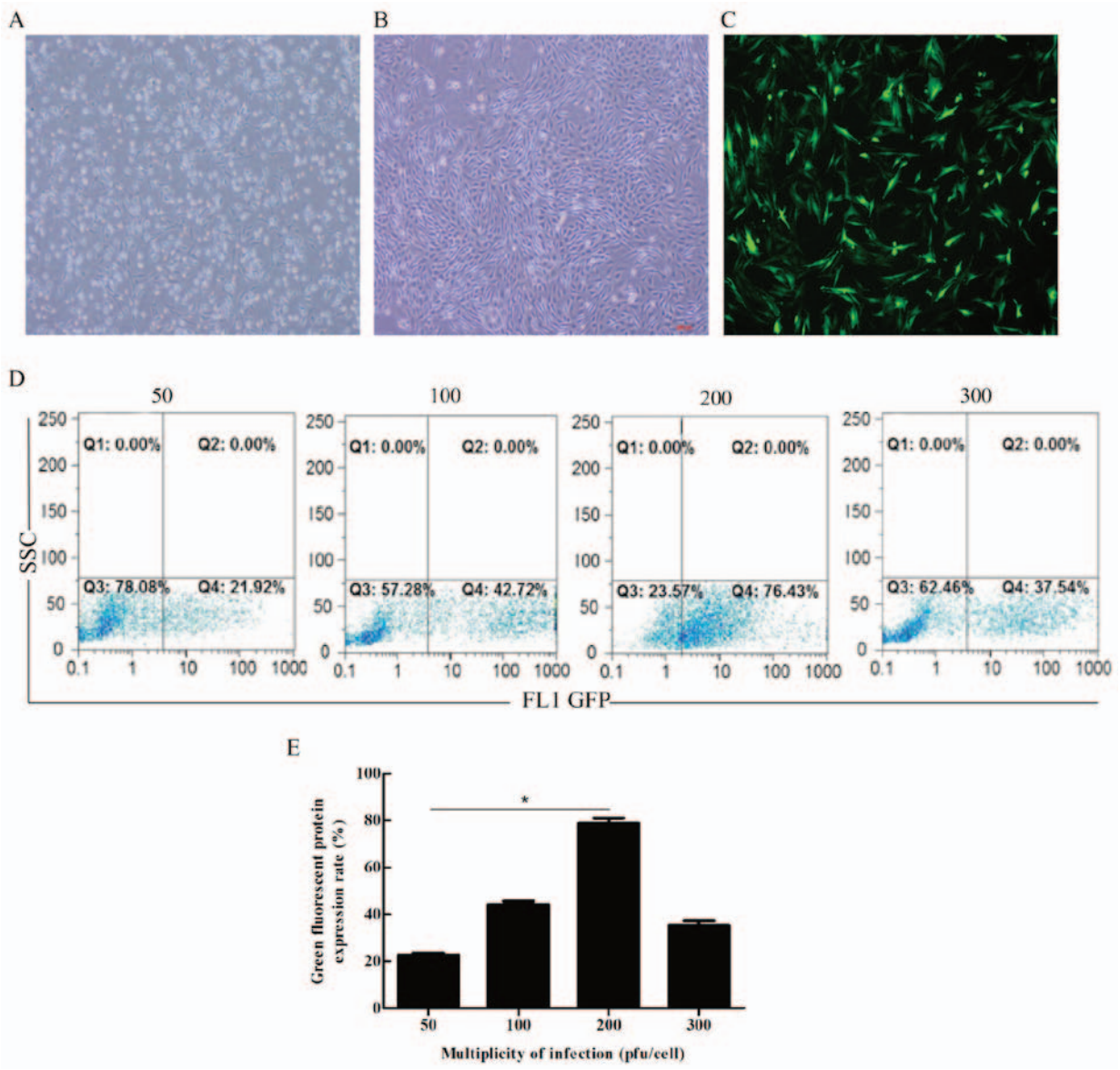

Figure 1. Characteristics of bone marrow-derived mesenchymal stem cells (BMSCs) and BMSCs/matrix metalloproteinase 1 (MMP1). (A and B) BMSCs (passage 16) observed by light microscopy (x100). (C) Green fluorescence protein (GFP)-expressing BMSCs shown by fluorescence microscopy (x100). (D and E) GFP expression rate in gene transfected BMSCs detected by flow cytometry 72 h later at MOI 50, 100, 200 and 300 pfu/cell.

area and was slightly decreased by transplantation of BMSCs, while it was significantly reduced by BMSCs/MMP1 transplantation (Fig. 3B).

Masson staining were performed 4 weeks after cell transplantation to investigate the collagen content in fibrotic liver. The collagen stained area slightly decreased by transplantation of BMSCs and was strongly reduced by BMSCs/MMP1 transplantation, which was consistent with the histological changes (Fig. 3C and D). Intra-hepatic hydroxyproline levels, another indicator of tissue collagen content, showed a similar pattern (Fig. 3E). These results clearly demonstrated that BMSC transplantation degraded hepatic collagen to a certain degree, while BMSCs/MMP1 may enhance the anti-fibrotic effect significantly in liver fibrosis.

The expression of α-SMA represents the activation of hepatic HSCs, a main pro-fibrogenic factor during liver fibrosis. Western blot analysis results showed that expression of α-SMA significantly increased in the model group compared with those of normal group. Transplantation of BMSCs decreased the expression of α-SMA, and transplantation of BMSCs/MMP1 decreased the α-SMA level to a further low level (Fig. 3F and G). Taken together, these data indicated that BMSCs/MMP1 were significantly more effective than BMSCs alone as a therapy for liver fibrosis in rats.

BMSCs/MMP1 attenuated CCl_4-induced liver injury. We subsequently evaluated the effects of cell transplantation on liver injury and liver function. As shown in Fig. 4, levels of ALT (Fig. 4A) and AST (Fig. 4B), which are indicators of liver damage, and PT (Fig. 4C) significantly increased in the CCl_4 model group, and ALB (Fig. 4D), which is a parameter of liver function, markedly decreased compared with those of the normal controls. Transplantations of BMSCs slightly improved these parameters, while BMSCs/MMP1 decreased levels of ALT, AST and PT, and increased ALB level. These results indicated that BMSCs/MMP1 was more effective than

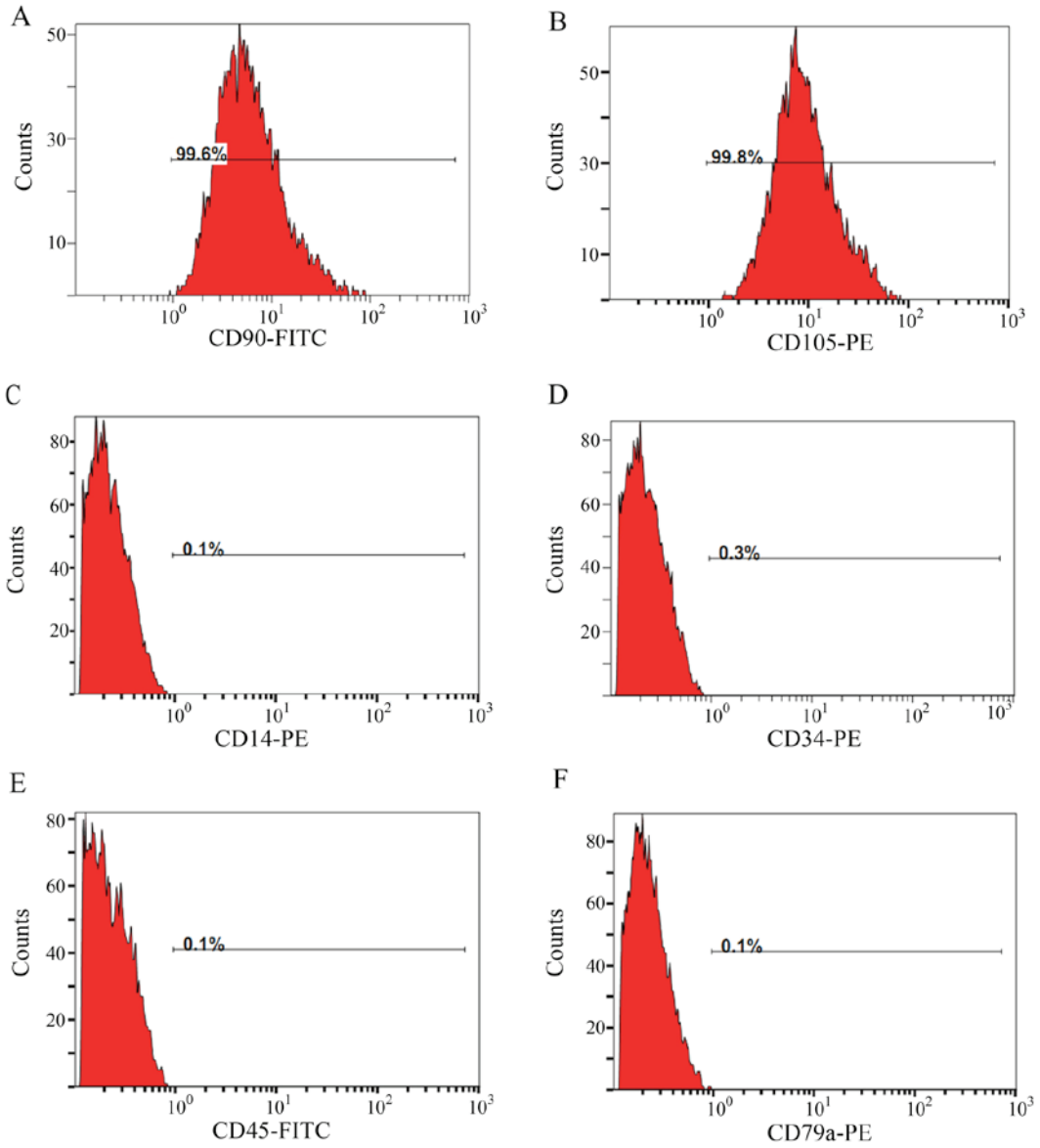

Figure 2. Expression of molecules on cells by flow cytometry analysis. Cells were positive on (A) CD90, and (B) CD105, while negative on (C) CD14, (D) CD34, (E) CD45 and (F) CD79a, indicating that isolated cells were bone marrow-derived mesenchymal stem cells (BMSCs).

BMSCs alone with respect to the attenuation of liver injury and recovery of liver function.

Hepatic MMP1 and TIMP1 levels after transplantation of BMSCs/MMP1. The amount of MMP1 produced by BMSCs/MMP1 was assessed by ELISA. The results showed that the amount of MMP1 from BMSCs/MMP1 increased more than 100 times higher than the amount secreted by BMSCs (Fig. 5A). To investigate the effect of BMSCs/MMP1 on MMP1 and TIMP1 secretion in CCl_4-induced liver fibrosis, we next transplanted the BMSCs/MMP1 in these rats. The results showed that MMP1 level significantly increased in BMSCs/MMP1 group compared with those of BMSC group (Fig. 5B), while TMIP1 level was significantly suppressed in the BMSCs/MMP1 group compared with either model group or normal group (Fig. 5C). In addition, the ratio of the MMP1 to TIMP1 level in model group was lower than that of normal group (data not shown). Transplantation of BMSCs improved the imbalance, while transplantation of BMSCs/MMP1 increased the ratio to a further high level compared with that of BMSCs alone.

We next investigated the enzyme activity of MMP1 produced by BMSCs/MMP1 before and after cell transplantation. The enzyme activity of MMP1, either 72 h after gene transfection or 2 weeks after transplantation was detected. The results showed that enzyme activity of MMP1 [1.3528×10^{-3} nmol/(g•min)] was higher in BMSCs/MMP1 than that of BMSC group (Fig. 5D). After cell transplantation, the enzyme activity of MMP1 was higher in livers of BMSCs/MMP1 injected animals than that of BMSC group (Fig. 5E). These data demonstrated that not only the quantity but also the biological activity of MMP1 produced by BMSCs/MMP1 was elevated either *in vitro* or in the liver.

Discussion

Liver fibrosis is a worldwide disease that may lead to irreversible end-stage liver diseases. There is still no effective drug to reverse liver cirrhosis. Stem cells have the capacity of

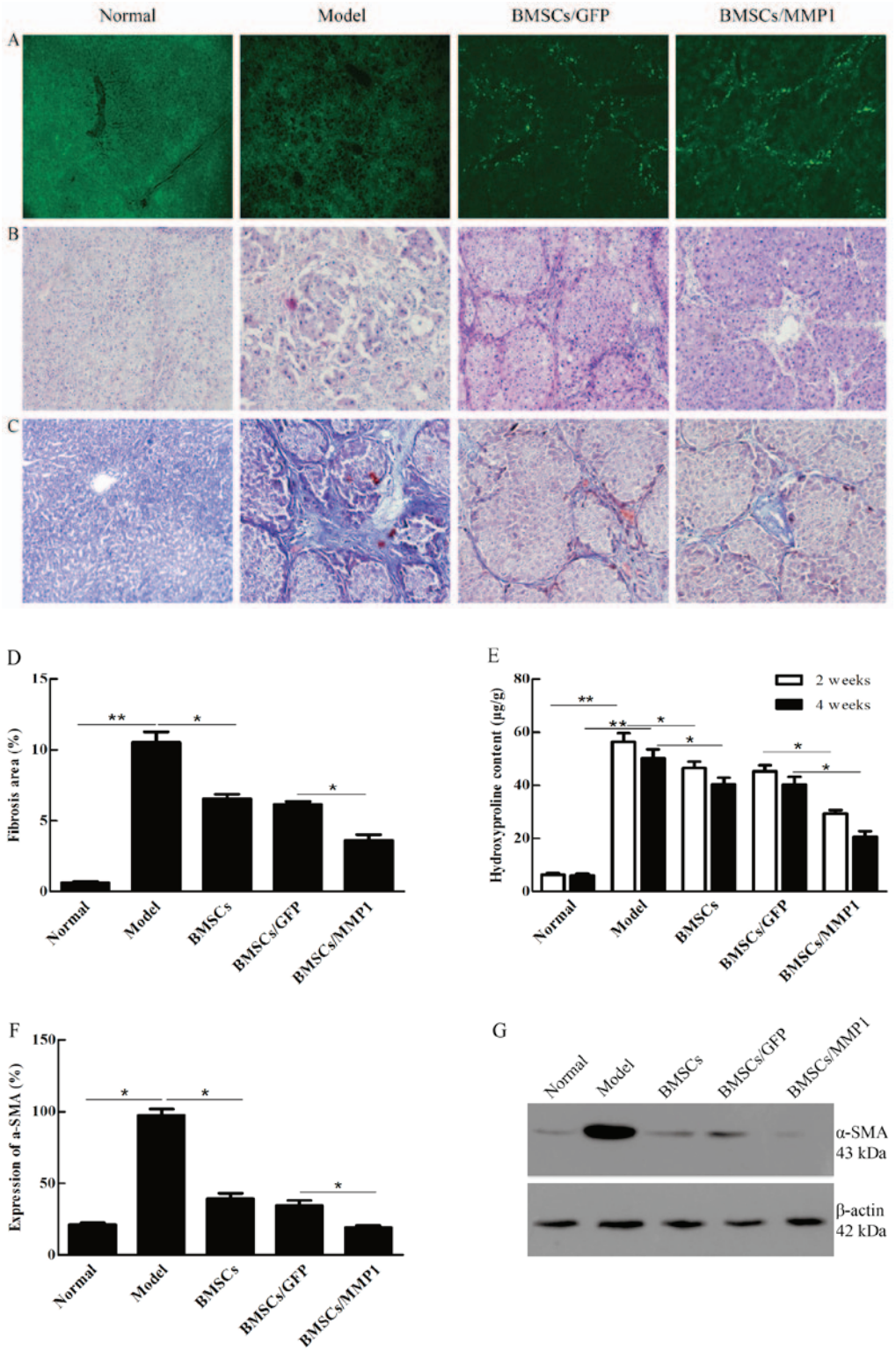

Figure 3. Effect of bone marrow-derived mesenchymal stem cells (BMSCs) on CCl_4-induced liver fibrosis. (A) Tracing of green fluorescence protein (GFP) gene-modified BMSCs in liver. (B) Liver histology after BMSCs/matrix metalloproteinase 1 (MMP1) administration by H&E staining. (C) Collagen content in fibrotic liver by Masson staining. (D) Fibrosis area. (E) Hepatic hydroxyproline content. (F and G) Hepatic α-smooth muscle actin by western blotting. (x100). $^{*}P<0.05$ and $^{**}P<0.01$.

self-renew and differentiation into various cell lines, including hepatocyte-like cells under proper treatments or in the presence of a suitable hepatic microenvironment, and therefore, throw light on therapy in liver diseases (20,39-42). It has been reported that transplantation of stem cells is an effective therapy for hepatic diseases, and BMSCs could improve impaired liver function and participate in the reconstruction of liver architecture (43-46). Nevertheless, other studies indicated that transplantation with BMSCs in liver fibrosis has its limitations due to the imbalance of synthesis and degradation of ECM in liver fibrosis and cirrhosis (21). In cirrhotic liver, recovery of liver functions is extensively inhibited by fiber

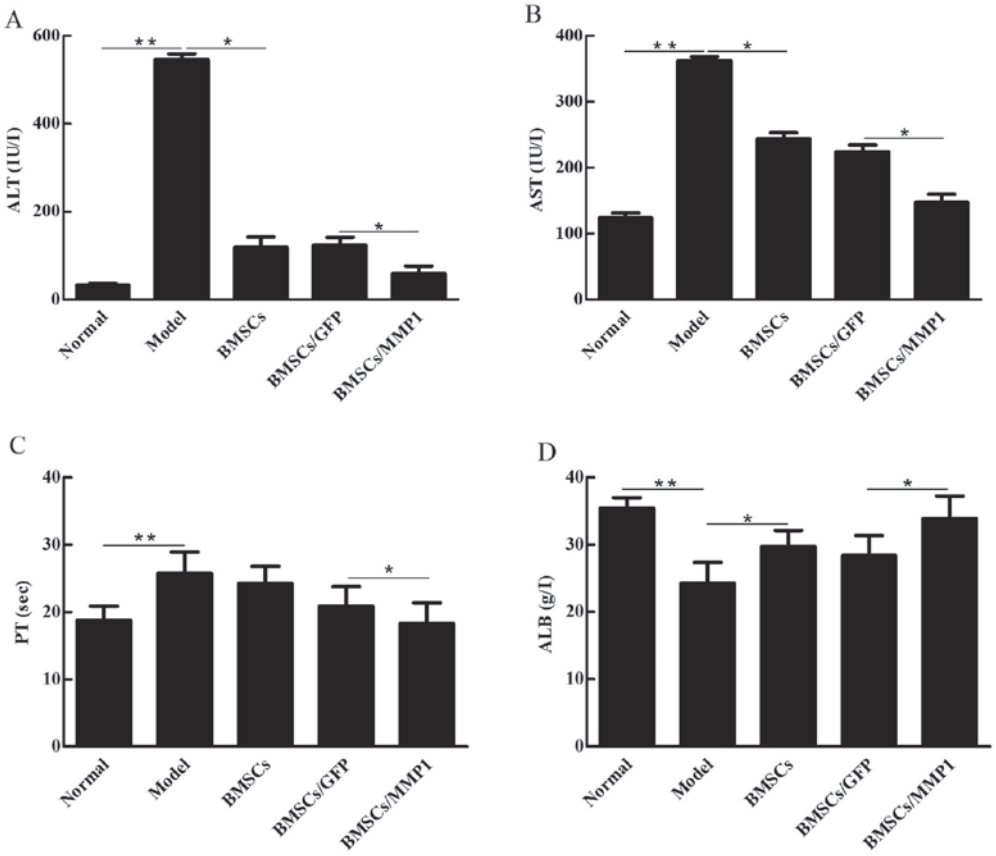

Figure 4. Bone marrow-derived mesenchymal stem cells (BMSCs)/matrix metalloproteinase 1 (MMP1) attenuated CCl_4-induced liver injury. Serum (A) alanine aminotransferase (ALT), (B) aspartate aminotransferase (AST), (C) prothrombin time (PT) and (D) albumin (ALB) levels in normal, CCl_4-treated, CCl_4-treated transfused with BMSCs, CCl_4-treated transfused with BMSCs/green fluorescence protein (GFP), and CCl_4-treated transfused with BMSCs/MMP1 animals. $^{*}P<0.05$ and $^{**}P<0.01$.

tissue which limits hepatocyte regeneration (47). Recent study showed, although BMSCs transplantation can reduce the production of collagen partially by inhibiting the activation of hepatic stellate cells or increasing MMP9, it could not degrade the collagen in fibrotic liver effectively (45). There are still excessive collagens in liver fibrosis after BMSC transplantation, as was also found in the present study.

Degradation of ECM is mainly induced by MMPs, which consequently may free up space for hepatic cell proliferation. Iimuro *et al* (34) injected the recombinant adenovirus containing MMP1 gene (Ad-MMP1) to the thioacetamide-induced liver fibrosis in rats and showed that the number of activated HSCs decreased, collagen obviously degraded, hepatocyte partly proliferated, rat liver fibrosis significantly reduced, and liver function improved consequently. Garcia-Banuelos *et al* (48) transplanted hMMP8 gene-modified recombinant adenovirus (Ad-hMMP8) into liver fibrosis of rats induced by CCl_4 injection and bile duct ligation, respectively. The results showed that the degree of liver fibrosis was alleviated and MMP2, MMP3, MMP9 and HGF expression in liver tissue were increased significantly, while transforming growth factor-β1 (TGF-β1) expression was reduced, accompanying by the decrease of the volume of ascites, improvement of liver function, and disappearance of gastric varices. These results imply that therapy with upregulated expression of MMP genes targeted to the liver may be useful as a therapeutic strategy even in advanced liver fibrosis or liver cirrhosis.

With the development of gene therapy, genetically engineered BMSC transplantation has been reported to be beneficial for treatment of bone disease (49), cardiovascular disease (23) and neurological diseases (22). Novel approaches including gene modified BMSCs have been supposed to reverse established liver cirrhosis. In the present study, we transplanted BMSCs/MMP1 into CCl_4-induced liver fibrosis in rats for the first time. Our results showed that exogenous MMP1 stably expressed BMSCs/MMP1 and these cells actively proliferated *in vitro*. Moreover, tracing BMSCs by GFP *in vivo*, we observed that most of the BMSCs planted in the liver successfully after transplantation, distributing around the hepatic vasculature, hepatic sinusoid, and hepatic lobule of implantation rats. BMSCs mainly concentrated in the liver because of its specific homing capacity to the injured organ. Regarding the mechanism of BMSC homing in liver, it was regulated by a variety of molecules, such as Sry-related high-mobility group box 11 (Sox11) (50), stromal-derived factor-1 (SDF-1) (51), vascular endothelial growth factor (VEGF) (52), basic fibroblast growth factor (bFGF) (53), and fibroblast activation protein (FAP) (54). Due to its ease to express exogenous gene and low immunogenicity (55,56), BMSCs may be used as an ideal target for gene therapy and may play an important role in treatment of advanced liver fibrosis or even liver cirrhosis.

In the present study, although inhibition of HSC activation was observed and content of collagen determined both

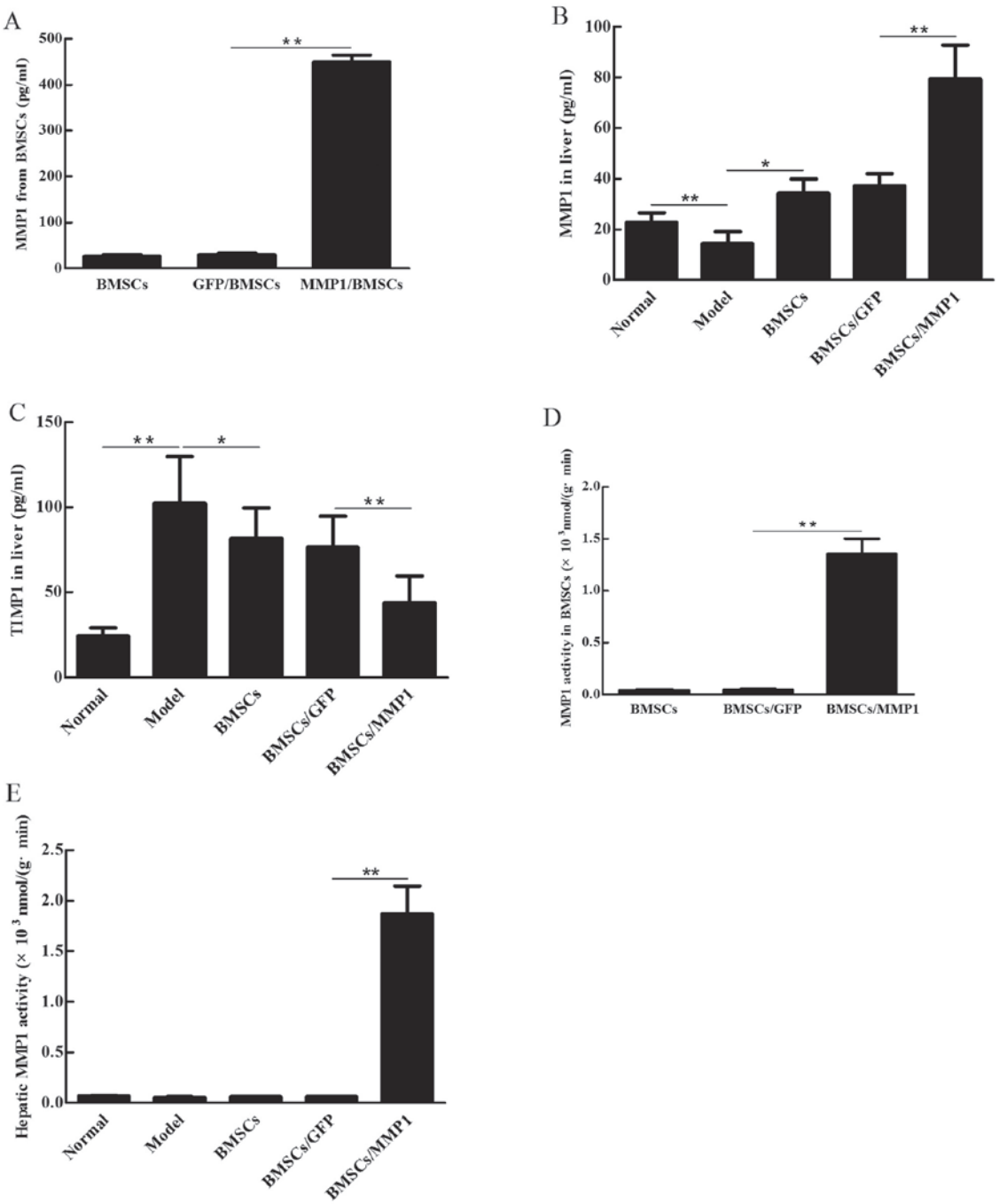

Figure 5. Expression of matrix metalloproteinase 1 (MMP1) and tissue inhibitor of metalloproteinase 1 (TIMP1) from bone marrow-derived mesenchymal stem cells (BMSCs)/MMP1 and liver. (A) Secretion of MMP1 from the BMSCs/MMP1 by enzyme-linked immunosorbent assay (ELISA). (B) Hepatic MMP1 and (C) TIMP1 expression after transplantation of BMSCs/MMP1. (D) Enzyme activity of MMP1 *in vitro* and (E) in liver. $^{*}P<0.05$ and $^{**}P<0.01$.

by the Masson staining and hydroxyproline evaluation in liver fibrosis was partially degraded after BMSC transplantation, the hepatic histology was not improved significantly. Transplantation of BMSCs/MMP1 was more effective than BMSCs alone as a therapy for liver fibrosis in rats. BMSCs differentiated into hepatocytes under the fibrotic liver microenvironment, inhibited HSC activation to reduce collagen deposit, and subsequently improved the liver function. On the other hand, BMSCs/MMP1 sustainably secreted MMP1 to degrade the excessive hepatic collagens. In this study, Masson staining and HE staining showed that collagens were effectively degraded in the liver and distorted architecture of cirrhotic liver was improved obviously after BMSCs/MMP1 transplantation.

In conclusion, the present study evaluated BMSCs/MMP1 transfusion in established liver fibrosis. We concluded that MMP1 gene sustainably expressed both *in vivo* and *in vitro*, transplanted BMSCs/MMP1 mainly concentrated in fibrotic liver, and consequently both biochemical parameters and hepatic architecture improved, suggesting that BMSCs may be a potential cell source and MMP1 gene may be a target for gene-modified BMSC therapy in chronic liver disease. Although these findings are encouraging for the further development of gene therapeutic approaches in liver cirrhosis,

research should be undertaken to investigate mechanisms that may account for it.

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Availability of data and material

We declared that materials described in the manuscript, including all relevant raw data, will be freely available to any scientist wishing to use them for non-commercial purposes, without breaching participant confidentiality.

Authors' contributions

CD contributed to the data interpretation, drafting, revision and finalization of the manuscript, and funding application. MJ and XW contributed to the data acquisition, analysis and manuscript drafting. JQ, HX and YW contributed to the data acquisition and analysis. YZ and DZ contributed to the conception of the study and manuscript editing. HX contributed to the data acquisition. WZ and SZ contributed to the study concept, experimental design and supervision.

Ethics approval and consent to participate

All animal experiments were approved by the Ethics Committee of Chengdu Military General Hospital.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Friedman SL: Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. J Biol Chem 275: 2247-2250, 2000.
2. Pinzani M, Romanelli RG and Magli S: Progression of fibrosis in chronic liver diseases: Time to tally the score. J Hepatol 34: 764-767, 2001.
3. Malhi H and Gores GJ: Cellular and molecular mechanisms of liver injury. Gastroenterology 134: 1641-1654, 2008.
4. Chen RJ, Wu HH and Wang YJ: Strategies to prevent and reverse liver fibrosis in humans and laboratory animals. Arch Toxicol 89: 1727-1750, 2015.
5. Yoon YJ, Friedman SL and Lee YA: Antifibrotic therapies: Where are we now? Semin Liver Dis 36: 87-98, 2016.
6. Schuppan D: Liver fibrosis: Common mechanisms and antifibrotic therapies. Clin Res Hepatol Gastroenterol 39 (Suppl 1): S51-S59, 2015.
7. Toniutto P, Zanetto A, Ferrarese A and Burra P: Current challenges and future directions for liver transplantation. Liver Int 37: 317-327, 2017.
8. Jadlowiec CC and Taner T: Liver transplantation: Current status and challenges. World J Gastroenterol 22: 4438-4445, 2016.
9. Chang YJ, Liu JW, Lin PC, Sun LY, Peng CW, Luo GH, Chen TM, Lee RP, Lin SZ, Harn HJ, *et al*: Mesenchymal stem cells facilitate recovery from chemically induced liver damage and decrease liver fibrosis. Life Sci 85: 517-525, 2009.
10. Sakaida I, Terai S, Yamamoto N, Aoyama K, Ishikawa T, Nishina H and Okita K: Transplantation of bone marrow cells reduces CCl_4-induced liver fibrosis in mice. Hepatology 40: 1304-1311, 2004.
11. Wu LM, Li LD, Liu H, Ning KY and Li YK: Effects of Guiyuanfang and autologous transplantation of bone marrow stem cells on rats with liver fibrosis. World J Gastroenterol 11: 1155-1160, 2005.
12. Li TZ, Kim JH, Cho HH, Lee HS, Kim KS, Lee SW and Suh H: Therapeutic potential of bone-marrow-derived mesenchymal stem cells differentiated with growth-factor-free coculture method in liver-injured rats. Tissue Eng Part A 16: 2649-2659, 2010.
13. Wang M, Zhang X, Xiong XI, Yang Z, Li P, Wang J, Sun YU, Yang Z and Hoffman RM: Bone marrow mesenchymal stem cells reverse liver damage in a carbon tetrachloride-induced mouse model of chronic liver injury. In Vivo 30: 187-193, 2016.
14. Truong NH, Nguyen NH, Le TV, Vu NB, Huynh N, Nguyen TV, Le HM, Phan NK and Pham PV: Comparison of the treatment efficiency of bone marrow-derived mesenchymal stem cell transplantation via tail and portal veins in CCl_4-induced mouse liver fibrosis. Stem Cells Int 2016: 5720413, 2016.
15. Shu SN, Wei L, Wang JH, Zhan YT, Chen HS and Wang Y: Hepatic differentiation capability of rat bone marrow-derived mesenchymal stem cells and hematopoietic stem cells. World J Gastroenterol 10: 2818-2822, 2004.
16. Si-Tayeb K, Noto FK, Nagaoka M, Li J, Battle MA, Duris C, North PE, Dalton S and Duncan SA: Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. Hepatology 51: 297-305, 2010.
17. di Bonzo LV, Ferrero I, Cravanzola C, Mareschi K, Rustichell D, Novo E, Sanavio F, Cannito S, Zamara E, Bertero M, *et al*: Human mesenchymal stem cells as a two-edged sword in hepatic regenerative medicine: Engraftment and hepatocyte differentiation versus profibrogenic potential. Gut 57: 223-231, 2008.
18. Aurich I, Mueller LP, Aurich H, Luetzkendorf J, Tisljar K, Dollinger MM, Schormann W, Walldorf J, Hengstler JG, Fleig WE, *et al*: Functional integration of hepatocytes derived from human mesenchymal stem cells into mouse livers. Gut 56: 405-415, 2007.
19. Feng Z, Li C, Jiao S, Hu B and Zhao L: In vitro differentiation of rat bone marrow mesenchymal stem cells into hepatocytes. Hepatogastroenterology 58: 2081-2086, 2011.
20. Eom YW, Shim KY and Baik SK: Mesenchymal stem cell therapy for liver fibrosis. Korean J Intern Med 30: 580-589, 2015.
21. Shackel N and Rockey D: In pursuit of the 'Holy Grail' - stem cells, hepatic injury, fibrogenesis and repair. Hepatology 41: 16-18, 2005.
22. Onda T, Honmou O, Harada K, Houkin K, Hamada H and Kocsis JD: Therapeutic benefits by human mesenchymal stem cells (hMSCs) and Ang-1 gene-modified hMSCs after cerebral ischemia. J Cereb Blood Flow Metab 28: 329-340, 2008.
23. Zeng B, Chen H, Zhu C, Ren X, Lin G and Cao F: Effects of combined mesenchymal stem cells and heme oxygenase-1 therapy on cardiac performance. Eur J Cardiothorac Surg 34: 850-856, 2008.
24. Siller-López F, Sandoval A, Salgado S, Salazar A, Bueno M, Garcia J, Vera J, Gálvez J, Hernández I, Ramos M, *et al*: Treatment with human metalloproteinase-8 gene delivery ameliorates experimental rat liver cirrhosis. Gastroenterology 126: 1122-1133, 2004.
25. Hu JJ, Sun C, Lan L, Chen YW and Li DG: Therapeutic effect of transplanting beta(2)m(-)/Thy1(+) bone marrow-derived hepatocyte stem cells transduced with lentiviral-mediated HGF gene into CCl(4)-injured rats. J Gene Med 12: 244-254, 2010.
26. Robert S, Gicquel T, Victoni T, Valença S, Barreto E, Bailly-Maître B, Boichot E and Lagente V: Involvement of matrix metalloproteinases (MMPs) and inflammasome pathway in molecular mechanisms of fibrosis. Biosci Rep 36: e00360, 2016.
27. Brinckerhoff CE and Matrisian LM: Matrix metalloproteinases: A tail of a frog that became a prince. Nat Rev Mol Cell Biol 3: 207-214, 2002.

28. Takahara T, Furui K, Funaki J, Nakayama Y, Itoh H, Miyabayashi C, Sato H, Seiki M, Ooshima A and Watanabe A: Increased expression of matrix metalloproteinase-II in experimental liver fibrosis in rats. Hepatology 21: 787-795, 1995.
29. Radbill BD, Gupta R, Ramirez MCM, DiFeo A, Martignetti JA, Alvarez CE, Friedman SL, Narla G, Vrabie R, Bowles R, *et al*: Loss of matrix metalloproteinase-2 amplifies murine toxin-induced liver fibrosis by upregulating collagen I expression. Dig Dis Sci 56: 406-416, 2011.
30. Issa R, Zhou X, Constandinou CM, Fallowfield J, Millward-Sadler H, Gaca MDA, Sands E, Suliman I, Trim N and Knorr A: Spontaneous recovery from micronodular cirrhosis: Evidence for incomplete resolution associated with matrix cross-linking. Gastroenterology 126: 1795-1808, 2004.
31. Preaux AM, D'Ortho MP, Bralet MP, Laperche Y and Mavier P: Apoptosis of human hepatic myofibroblasts promotes activation of matrix metalloproteinase-2. Hepatology 36: 615-622, 2002.
32. Visse R and Nagase H: Matrix metalloproteinases and tissue inhibitors of metalloproteinases: Structure, function, and biochemistry. Circ Res 92: 827-839, 2003.
33. Nagase H, Visse R and Murphy G: Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res 69: 562-573, 2006.
34. Iimuro Y, Nishio T, Morimoto T, Nitta T, Stefanovic B, Choi SK, Brenner DA and Yamaoka Y: Delivery of matrix metalloproteinase-1 attenuates established liver fibrosis in the rat. Gastroenterology 124: 445-458, 2003.
35. Yang Q, Xie RJ, Geng XX, Luo XH, Han B and Cheng ML: Effect of Danshao Huaxian capsule on expression of matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1 in fibrotic liver of rats. World J Gastroenterol 11: 4953-4956, 2005.
36. Du C, Jiang MD, Zeng WZ and Zheng SM: Construction of recombinant adenovirus vector for human matrix metalloproteinase-1 gene and detection of collagen type III degradation in vitro. Chin J Tissue Eng Res 7995-8000, 2014 (In Chinese).
37. Nadri S, Soleimani M, Hosseni RH, Massumi M, Atashi A and Izadpanah R: An efficient method for isolation of murine bone marrow mesenchymal stem cells. Int J Dev Biol 51: 723-729, 2007.
38. Soleimani M and Nadri S: A protocol for isolation and culture of mesenchymal stem cells from mouse bone marrow. Nat Protoc 4: 102-106, 2009.
39. Shiota G and Itaba N: Progress in stem cell-based therapy for liver disease. Hepatol Res: May 18, 2016 (Epub ahead of print). doi: 10.1111/hepr.12747.
40. Haldar D, Henderson NC, Hirschfield G and Newsome PN: Mesenchymal stromal cells and liver fibrosis: A complicated relationship. FASEB J 30: 3905-3928, 2016.
41. Matsumoto T, Takami T and Sakaida I: Cell transplantation as a non-invasive strategy for treating liver fibrosis. Expert Rev Gastroenterol Hepatol 10: 639-648, 2016.
42. Raafat N, Abdel AS, Abdo FK and El GN: Mesenchymal stem cells: In vivo therapeutic application ameliorates carbon tetrachloride induced liver fibrosis in rats. Int J Biochem Cell Biol 68: 109-118, 2015.
43. Cho KA, Lim GW, Joo SY, Woo SY, Seoh JY, Cho SJ, Han HS and Ryu KH: Transplantation of bone marrow cells reduces CCl_4-induced liver fibrosis in mice. Liver Int 31: 932-939, 2011.
44. Meier RP, Mahou R, Morel P, Meyer J, Montanari E, Muller YD, Christofilopoulos P, Wandrey C, Gonelle-Gispert C and Bühler LH: Microencapsulated human mesenchymal stem cells decrease liver fibrosis in mice. J Hepatol 62: 634-641, 2015.
45. Kim MD, Kim SS, Cha HY, Jang SH, Chang DY, Kim W, Suh-Kim H and Lee JH: Therapeutic effect of hepatocyte growth factor-secreting mesenchymal stem cells in a rat model of liver fibrosis. Exp Mol Med 46: e110, 2014.
46. Irfan A and Ahmed I: Could stem cell therapy be the cure in liver cirrhosis? J Clin Exp Hepatol 5: 142-146, 2015.
47. Mormone E, George J and Nieto N: Molecular pathogenesis of hepatic fibrosis and current therapeutic approaches. Chem Biol Interact 193: 225-231, 2011.
48. Garcia-Banuelos J, Siller-Lopez F, Miranda A, Aguilar LK, Aguilar-Cordova E and Armendariz-Borunda J: Cirrhotic rat livers with extensive fibrosis can be safely transduced with clinical-grade adenoviral vectors. Evidence of cirrhosis reversion. Gene Ther 9: 127-134, 2002.
49. Dong SW, Ying DJ, Duan XJ, Xie Z, Yu ZJ, Zhu CH, Yang B and Sun JS: Bone regeneration using an acellular extracellular matrix and bone marrow mesenchymal stem cells expressing Cbfa1. Biosci Biotechnol Biochem 73: 2226-2233, 2009.
50. Xu L, Huang S, Hou Y, Liu Y, Ni M, Meng F, Wang K, Rui Y, Jiang X and Li G: Sox11-modified mesenchymal stem cells (MSCs) accelerate bone fracture healing: Sox11 regulates differentiation and migration of MSCs. FASEB J 29: 1143-1152, 2015.
51. Yuan L, Sakamoto N, Song G and Sato M: Low-level shear stress induces human mesenchymal stem cell migration through the SDF-1/CXCR4 axis via MAPK signaling pathways. Stem Cells Dev 22: 2384-2393, 2013.
52. Schichor C, Birnbaum T, Etminan N, Schnell O, Grau S, Miebach S, Aboody K, Padovan C, Straube A, Tonn JC, *et al*: Vascular endothelial growth factor A contributes to glioma-induced migration of human marrow stromal cells (hMSC). Exp Neurol 199: 301-310, 2006.
53. Schmidt A, Ladage D, Schinkothe T, Klausmann U, Ulrichs C, Klinz FJ, Brixius K, Arnhold S, Desai B, Mehlhorn U, *et al*: Basic fibroblast growth factor controls migration in human mesenchymal stem cells. Stem Cells 24: 1750-1758, 2006.
54. Chung KM, Hsu SC, Chu YR, Lin MY, Jiaang WT, Chen RH and Chen X: Fibroblast activation protein (FAP) is essential for the migration of bone marrow mesenchymal stem cells through RhoA activation. PLoS One 9: e88772, 2014.
55. Gebler A, Zabel O and Seliger B: The immunomodulatory capacity of mesenchymal stem cells. Trends Mol Med 18: 128-134, 2012.
56. Holmes C and Stanford WL: Concise review: stem cell antigen-1: expression, function, and enigma. Stem Cells 25: 1339-1347, 2007.