J Biomed Sci (2008) 15:615–622
DOI 10.1007/s11373-008-9255-x

ORIGINAL PAPER

Bone marrow transplantation results in donor-derived hepatocytes in an animal model of inherited cholestatic liver disease

Huey-Ling Chen · Renxue Wang · Hui-Ling Chen · Wuh-Liang Hwu · Yung-Ming Jeng · Mei-Hwei Chang · Victor Ling

Received: 19 November 2007 / Accepted: 1 May 2008 / Published online: 29 May 2008

Abstract Cell transplantation is a potential therapy for acquired or inherited liver diseases. Donor-derived hepatocytes (DDH) have been found in humans and mice after bone marrow transplantation (BMT) but with highly variable frequencies in different disease models. To test the effect of liver repopulation after BMT in inherited cholestatic liver diseases, spgp (sister of P-glycoprotein, or bile salt export pump, abcb11) knockout mice, a model for human progressive intrahepatic cholestasis type 2 with defects in excreting bile salts across the hepatocyte canalicular membrane, were transplanted with bone marrow cells from enhanced green fluorescent protein (EGFP) transgenic donor mice after lethal irradiation. One to 6 months later, scattered EGFP-positive DDHs with positive spgp staining were observed in the liver. These hepatocytes had been incorporated into hepatic plates and stained positively with hepatocyte-specific marker albumin. RT-PCR for the spgp gene revealed positive expression in the liver of sgsp knockout mice that had received the transplant. Bile acid analysis of bile samples showed that these mice also had higher levels of total biliary bile acid and taurocholic acid concentration than knockout mice without transplantation, indicating that BMT partially improved biliary bile acid secretion. Our results indicate that bone marrow cells could serve as a potential source for restoration of hepatic functions in chronic metabolic liver disease.

Keywords abcb11 · spgp · Progressive familial intrahepatic cholestasis · Bile salt export pump · Cell transplantation · Cholestasis · Bile acid metabolism

H.-L. Chen · W.-L. Hwu · M.-H. Chang
Department of Pediatrics, National Taiwan University College of Medicine and Hospital, Taipei, Taiwan, ROC

H.-L. Chen
Department of Primary Care Medicine, National Taiwan University College of Medicine and Hospital, Taipei, Taiwan, ROC

R. Wang · V. Ling (✉)
Department of Cancer Genetics, British Columbia Cancer Research Center, British Columbia Cancer Agency, Vancouver, BC, Canada V5Z 1L3
e-mail: vling@bccrc.ca

H.-L. Chen
Department of Hepatitis Research Center, National Taiwan University Hospital, Taipei, Taiwan, ROC

Y.-M. Jeng
Department of Pathology, National Taiwan University College of Medicine and Hospital, Taipei, Taiwan, ROC

Introduction

Orthotopic liver transplantation (OLT) has been the only cure for various forms of inherited and acquired liver diseases. However, due to limited donor availability, many patients have died while waiting for transplantation. Hepatocyte transplantation has recently been evaluated as an alternative to OLT because of the following advantages: it is a non-surgical procedure, there is less morbidity, one donor can supply multiple recipients, and lower medical costs are associated with the procedure. Major limitations to hepatocyte transplantation include the availability of the cell source and a limited repopulation capacity in the recipient liver. Research on stem cell/progenitor cell isolation, in vitro cell expansion, cryopreservation, immortalized hepatocytes, modifying recipient injury with adjuvant drugs, cytokines, and irradiation has therefore been undertaken [1, 2].

Bone marrow cells have the potential to differentiate into liver, muscle, intestine, skin, lung, and CNS cells [3–6]. Bone marrow has been reported as a potential source of hepatic oval cells [7]. It has been shown that multi-organ, multi-lineage engraftment can be achieved by a single bone marrow-derived stem cell [8]. In human recipients of BMT, approximately 1% of hepatocytes are of donor origin [9]. Hepatocytes and epithelial cells of donor origin are also found in human recipients of peripheral-blood stem cells [10].

In the fumarylacetoacetate hydrolase deficient ($fah^{-/-}$) mouse model mimicking human tyrosinemia, purified hematopoietic stem cells are able to rescue the $fah^{-/-}$ mice and restore the biochemical function. At 7 months, 30–50% of the liver mass is observed to be of donor origin and the biochemical parameters of the mice were close to normal [11]. However, a large variation in the quantity of bone marrow-derived hepatocytes was observed by different investigators. Possible causes for the variation include the use of different mouse models, i.e. normal wild-type, toxin induced, or genetically modified; the degree of myeloablation; and the methods for detection [12, 13]. It is therefore necessary that the efficiency of BMT be evaluated for different disease models. It is not known whether bone marrow could serve as a source of cell therapy in other genetic disease models. There has been no report of using BMT in treating cholestatic liver disease.

The *spgp* gene knockout mice ($spgp^{-/-}$, *abcb11*) is an animal model for inherited intrahepatic cholestatic liver disease [14]. Spgp belongs to the ATP-binding cassette (ABC) transporter superfamily and is a canalicular-specific bile transporter that mediates the hepatocyte secretion of bile salts. In humans, genetic defects in the *ABCB11* gene, also known as bile salt export pump (BSEP), result in a severe genetic disease called type 2 progressive familial intrahepatic cholestasis (PFIC2), in which total bile acid secretion is only 1% of normal [15]. PFIC2 is an inherited disorder causing jaundice, progressive bile stasis and liver failure in early childhood. Currently, liver transplantation is the only cure for PFIC2. However, lack of donor sources and the risk of whole organ transplantation in young children remain major problems. The $spgp^{-/-}$ mice represent a potential new model system for cell therapy for inherited liver diseases. Here we evaluate whether transplanted donor bone marrow cells can preferentially differentiate into hepatocytes and repopulate the liver of $spgp^{-/-}$ mice.

Subjects and methods

Animals

Mice with targeted inactivation of the spgp gene were generated as previously reported [14]. Mice of backcross generations 7–10 either on a FVB/NJ genetic background or on a C57Bl/6 genetic background were generated. Hybrid mice with a C57Bl/6 and FVB/NJ background were used in the experiment due to the better survival rate of the knockout pups. Only the F1 hybrid mice were used for experiments. Enhanced green fluorescent protein (EGFP) transgenic mice of C57Bl/6 background were obtained from Dr. Okabe (Osaka, Japan) and were mated with wild type FVB/NJ mice to generate EGFP-positive C57Bl/6 and FVB/NJ hybrid donors. Animals were maintained in a 12-h light and dark cycle, at 25°C, with free access to food and water. Experiments were performed using the approved protocols from the Committee on Animal Care, University of British Columbia, and were in accordance with the guidelines set out by the Canadian Council on Animal Care.

Bone marrow cells transplantation after lethal irradiation

Ten adult spgp knockout and four wild-type mice received bone marrow transplantation from donor bone marrow cells obtained from EGFP transgenic mice. In brief, bone marrow was flushed with DMEM high glucose medium containing 2% FBS from the medullary cavities of both femurs and tibias using a 22- or 25-gauge needle. After washing with PBS, the cells were separated using Ficoll (Amersham Biosciences AB) solution, and washed again with PBS and resuspended in DMEM. Recipient mice were given total body irradiation of 9 Gy 24 h prior to transplantation. After that, 100 µl of cell suspension (2.5×10^6 cells) was injected into tail veins. The procedure was performed using aseptic techniques.

Reverse transcription PCR for spgp expression

Total RNA was extracted from four randomly selected areas of recipient liver. Total RNA was reverse transcribed using random primers and reverse transcriptase (Invitrogen). Primers for spgp cDNA (Forward 5′-AGAGAAGAG GCGACAATGGA-3′, Reverse 5′-TCTTGGGTTTCCGTA TGAGG-3′) were designed to amplify the deleted region of the knockout mice. Forty cycles of PCR reaction with 30 s denaturation at 95°C, 30 s annealing at 60°C, and 45 s extension at 72°C were carried out. Wild type mice and knockout mice without transplantation were used as positive and negative controls, respectively. GAPDH was used as a control.

Identifying donor cells in tissue sections

Fresh liver samples were immersed in paraformaldehyde/sucrose solution for 2 h and then embedded in OCT

(Sakura). Frozen sections of 5–7 μM were made, fixed in cool acetone for 10 min, mounted, and viewed with a fluorescence microscope. EGFP positive donor cells with hepatocyte morphology were identified directly using the fluorescence microscope. Donor-derived hepatocytes were identified by green fluorescent polyglonal cells, which were incorporated into surrounding parenchymal cells making up liver cell plates. Ten to twelve tissue sections from four different lobes each were examined. Total numbers of positive donor hepatocytes in all tissue sections were recorded. Total recipient hepatocyte numbers in tissue sections were estimated by counting the number of hepatocyte nuclei in hematoxylin and eosin stained tissues from five randomly selected fields on each slide. Hepatocytes with double nuclei were counted as single cells. Total surface areas of the sections were measured by scanning the glass slides along with a size standard and by analyzing the scanned images with Photoimpact software (Ulead).

Immunofluorescent staning

Frozen sections of liver samples prefixed with paraformaldehyde to preserve GFP fluorescence were used in the following manner. For spgp staining, fresh frozen liver without paraformaldehyde fixation was used. Tissue sections were fixed with pre-cooled acetone for 10 min. Samples were incubated with primary antibodies for 1–2 h at room temperature. Antibodies used were a polyclonal antibody for spgp (IW, 1:100) [14], mouse albumin (1:300, Bethyl Laboratories A90-234A, UK), and a rat monoclonal anti-CD68 (1:100, Serotec MCA1957, UK). After washing with PBS three times for 10 min each, samples were incubated with fluorescent secondary antibodies (Alexa 546, Molecular Probes) at room temperature for 2 h. For double staining, a green fluorescent directly conjugated EGFP antibody (Rabbit anti-GFP, A21311 Molecular Probes) was also added. After washing with PBS, slides were mounted with VECTASHIELD with 4′,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories) and viewed using a fluorescence microscope. Digital images were collected by image capture system (Leica DMRD, CoolSNAP).

Bile acid analysis by ESI/MS

Bile samples from transplanted and untransplanted knockout mice and wild-type mice were used. [2,2,4,4-D_4]-taurochenodeoxycholic acid, [2,2,4,4-D_4]-taurocholic acid, [2,2,4,4-D_4]-glycocholic acid, and [2,2,4,4-D_4]-glycochenodeoxycholic acid were synthesized as an internal control. Tandem-mass spectrophotometry was performed using the ABI-3000 Tandem Mass coupled with the Perkin–Elmer HPLC Series 200 System. The instrument was operated in negative ion mode. Samples were resuspended in 60% acetonitrile with 0.05% formic acid. A 10 μl sample was injected into the solvent stream of acetonitrile/water (80:20 vol:vol) flowing at 300 μl/min by means of a small volume HPLC pump. For the quantification of bile acids, the negative MRM scan type was used. The monitored Q1 masses for monohydroxylated, dihydroxylated, trihydroxylated, and tetrahydroxylated bile salts were 482.6, 498.8, 514.8, and 530.8, respectively, for taurine-conjugated molecules, and 432.6, 448.6, 464.6, and 480.7, respectively, for glycine conjugated molecules. The monitored Q1 mass for each deuterium labeled bile acid was 4 amu higher than the original mass. The monitored Q3 mass was 74.1 amu for glycine conjugated bile acids and 80.2 amu for taurine conjugated ones. The quantitative result was calculated by Chemoview software (Applied Biosystem).

Results

Ten spgp knockout mice and four congenic wild-type mice received BMT from EGFP transgenic mice. The recipient mice were sacrificed 1 month ($n = 1$), 2 months ($n = 2$), and 6 months ($n = 8$) after transplantation to assay engraftment.

Full reconstitution of hematopoietic cells of donor origin was noted 1 month or longer after bone marrow transplantation. In frozen liver sections, EGFP-positive donor cells were mainly located in the sinusoids or associated with blood vessels, with the morphology of hepatic Kupffer cells (Fig. 1a and b). Occasionally, scattered cells with hepatocyte morphology could be identified (Fig. 1b–d), which were located in the hepatic lobule, and were distinct from other spindle-shaped cells in the sinusoids. Most of these cells were binucleate.

We found that the majority of the donor cells were CD68 positive, which was characteristic of Kupffer cells and macrophages. The cells with hepatocyte morphology of donor origin stained negatively for CD68 (Fig. 1e–f), but positively with hepatocyte-specific marker albumin (Fig. 2). To confirm that the hepatocytes of donor-origin could express spgp protein, we found positive immunofluorescent staining using spgp antibody of these scattered hepatocytes, in the background of the negatively stained hepatocytes of the spgp knockout mouse liver (Fig. 3). Reverse transcription PCR revealed positive expression of spgp genes in mice that had received the transplant (Fig. 4).

Bile samples from the gallbladder bile from four transplanted mice were used for bile acid analysis. Without transplantation, the spgp knockout mice had lower biliary bile acid levels due to the defect of the major bile acid exporting protein in the hepatocyte canalicular membrane,

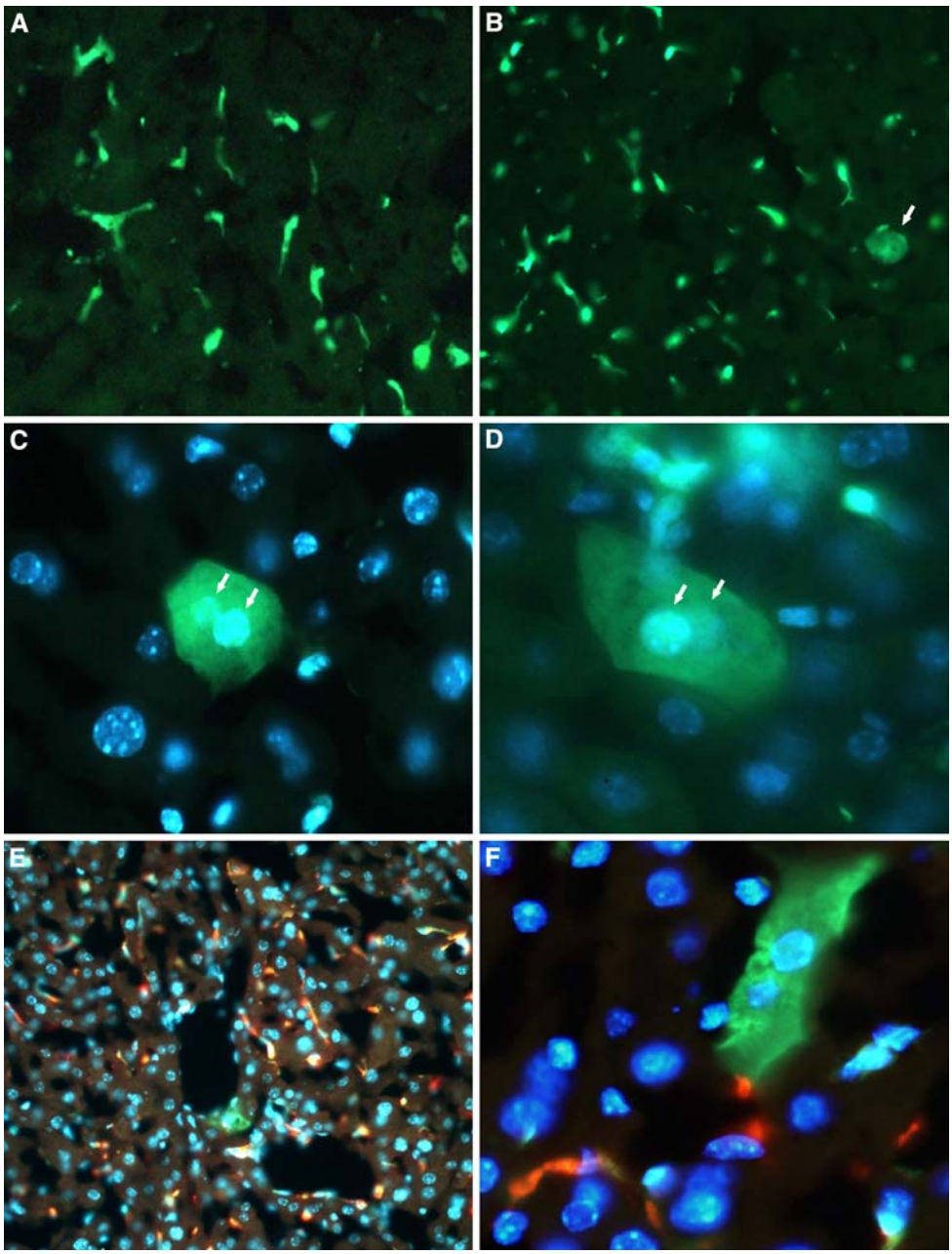

Fig. 1 Six months after bone marrow transplantation, full reconstitution of hematopoietic cells of donor origins was observed. EGFP positive Kupffer cells could be detected easily in liver sections in both wild type and knockout mice. Higher frequencies of donor-derived hepatocyte (arrow) could be seen in the knockout mice (**a**: wild type mice; **b**: knockout mice; 200×). Scattered single hepatocytes of donor origin can be detected (**c**, **d**, 400×; Green: EGFP; Blue: DAPI nucleus stain), some with double nuclei (arrows). Most of the hematopoietic cells stained with Kupffer cell marker CD68, but not the donor hepatocytes (**e** and **f**, Red: CD68; Green: EGFP; Blue: DAPI nucleus stain. 200× and 400×, respectively)

and also had a characteristically higher percentage of tetrahydroxylated bile acids, which were not seen in the wild-type mice. The normal trihydroxycholic acids, mainly muricholic acid, were lower in knockout mice. After bone marrow transplantation, the knockout mice had significantly higher total biliary bile acid concentrations, indicating an improvement in bile acid secretion. There was also a less significant increase in trihydroxycholic acid levels, and lower tetrahydroxycholic acid levels than in knockout mice without transplantation, a trend toward the bile acid profile of wild type mice (Table 1). These results indicate that BMT improved the biliary bile acid secretion in spgp KO mice.

In frozen liver sections, mean frequencies of detectable EGFP positive donor hepatocytes in recipient liver were estimated to be 1 in 43,803 hepatocytes ($2.28 \pm 3.78 \times 10^{-5}$) in the transplanted spgp knockout mice, but only 1 in 171,206 ($0.58 \pm 0.33 \times 10^{-5}$) in wild-type controls, indicating a higher rate of DDH in the spgp knockout mice. The two-sample Wilcoxon rank sum test was used to compare the different proportions of the groups, and the frequency of DDH in spgp knockout mice was higher than wild type mice with a borderline statistical significance ($P = 0.074$).

The H&E stain of liver histopathology revealed cell ballooning, fatty change, and focal necrosis of the knockout liver both with and without bone marrow transplantation.

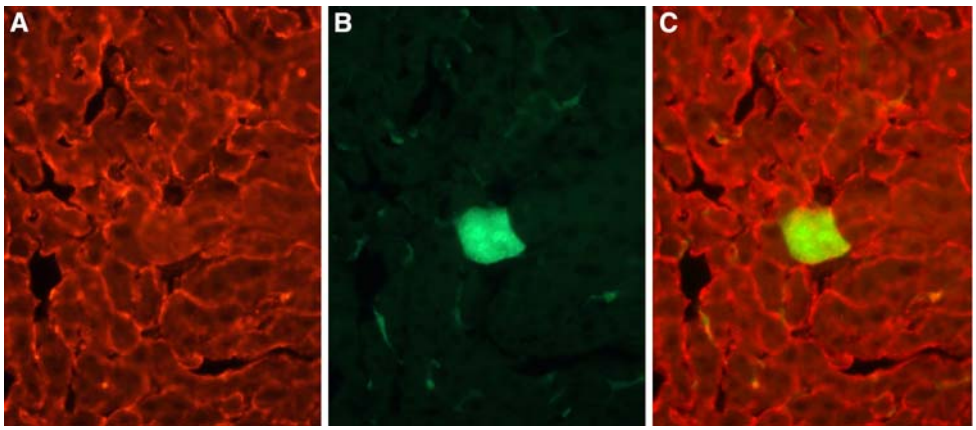

Fig. 2 The EGFP-positive donor hepatocyte stained with albumin, a hepatocyte-specific marker, in the spgp knockout mice transplanted with EGFP-positive bone marrow cells (**a**: albumin stain with red fluorescence; **b**: EGFP; **c**: merge, 400×)

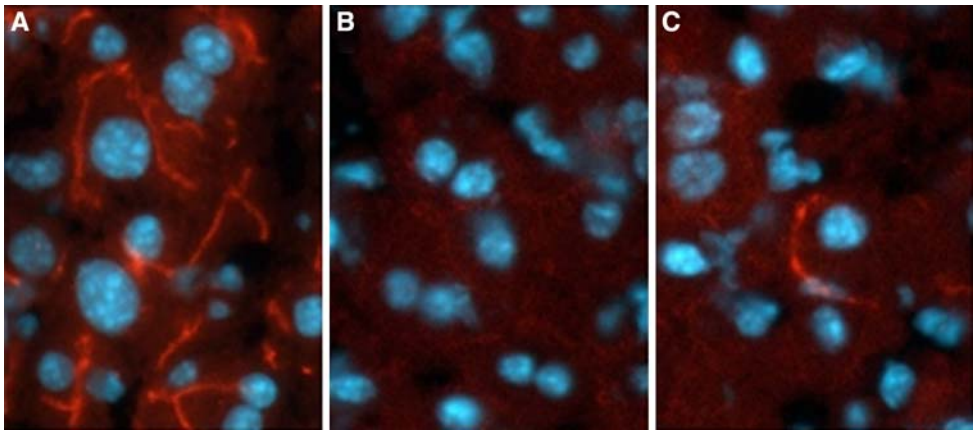

Fig. 3 Immunofluorescent staining using spgp antibody in liver sections. Typical canalicular staining was noted in the hepatocytes of wild-type mice (**a**), no staining was shown in spgp (abcb11) knockout mice without transplantation (**b**), and scattered cells with canalicular staining in the knockout mice liver were noted after bone marrow transplantation (**c**, red, 400×)

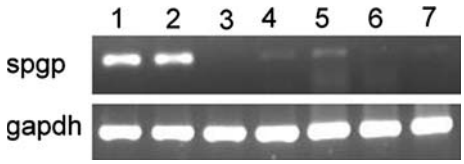

Fig. 4 The expression of spgp was detected by RT-PCR 6 months after transplantation. In the transplanted knockout mice, positive or weak positive spgp expression was found. (lanes 1 and 2: wild-type mice; lane 3: spgp knockout mice without transplantation; lane 4–7: spgp knockout mice after bone marrow transplantation)

There was no significant difference between transplanted and non-transplanted knockout mice in terms of portal/lobular activity and fibrosis scores.

Discussion

In this study, after bone marrow transplantation, DDHs were detected, which were found to express the donor markers EGFP and hepatocyte marker albumin. Expression of spgp, a donor-specific and hepatocyte-specific gene, was also detected. DDHs, although in small numbers, were detected with a higher frequency in the livers of spgp knockout mice than in those of wild-type controls. More importantly, the spgp knockout mice that had received a bone marrow transplant displayed a higher level of biliary bile salt secretion. These results indicate that bone marrow cells could potentially serve as a source for liver repair in inherited cholestatic or metabolic liver diseases.

Table 1 Biliary bile acid analysis in $spgp^{-/-}$ mice after bone marrow transplantation (BMT) in comparison to knockout mice without transplantation

	Total bile acid levels (mM)	% Tri-OH bile acid	% Tetra-OH bile acid
$spgp^{-/-}$ w/o BMT* ($n = 5$)	42.40 ± 7.70	41.16 ± 10.67	56.40 ± 9.30
$spgp^{-/-}$ after BMT* ($n = 4$)	75.08 ± 6.03	67.81 ± 18.40	30.67 ± 18.62
Wild-type mice ($n = 6$)	100.95 ± 46.77	88.11 ± 2.78	1.80 ± 0.67
P value*	<0.001	0.054	0.062

* Comparisons between $spgp^{-/-}$ mice with and without BMT

Hepatocytes derived from bone marrow cells may originate either from transdifferentiation from hematopoietic cells or from direct fusion of donor bone marrow cells and recipient hepatocytes. Evidence of fusion between bone marrow cells and adult tissue cells has been demonstrated to explain the "transdifferentiation phenomenon" of bone marrow transplantation [16–18]. In a Fah-deficient mouse model, transplantation of hematopoietic stem cells, committed granulocyte-macrophage progenitors, or bone-marrow-derived macrophages, resulted in the robust production of the bone-marrow-derived hepatocytes. These hepatocytes formed clusters or nodules in recipient liver and were able to correct the biochemical dysfunction. The fusion partners of hepatocytes were demonstrated to be myelomonocytic cells and did not require reconstitution of recipient bone marrow [19, 20]. Recently bone-marrow-derived oval cells have also been implicated in liver regeneration in a partial hepatectomy mouse model [21]. The type and severity of injury, and local environment in each animal model determine the efficiency and also the cell source for liver repair. In our study, we did not observe bone-marrow-derived oval cells in the liver. Our data thus appear to indirectly support the fusion theory. In the livers of mice that received bone marrow transplants, we only saw scattered donor-derived hepatocytes, but no clusters or nodules were seen, as one would expect from transdifferentiation.

We speculate that the number of DDHs increased in diseased liver more than in normal liver. Signals of cell damage may facilitate the fusion process. In a model of Bcl-2 transgenic bone marrow transplantation into normal mice, donor-derived clusters of mature hepatocytes expressing Bcl-2 were found when recipients were treated with anti-Fas antibody Jo, but no donor-derived hepatocytes were found without Jo pressure [22]. An increased damage level may result in more frequent fusions and become functionally significant. Our animal model is a chronic, but not progressive cholestatic liver disease model [14], and is not as severe or progressive as human PFIC2. An increased injury level may be required to enhance the donor cell repopulation, such as adding cholic acid in the diet [23]. We speculate that a continuous and significantly high level of hepatocyte injury would be necessary to enhance DDHs in further applications of bone marrow cells as a source of liver repair.

It has been shown that total hematopoietic reconstruction occurs before hepatocyte derivation from bone marrow cells [13]. Bone marrow cell therapy without total reconstruction of bone marrow or hematopoietic systems has been demonstrated in other organ repairs [24, 25]. It has also been reported that bone marrow transplantation without irradiation in a mouse model was used to treat liver cirrhosis [26]. Aside from hematopoietic cells, bone marrow cells have been reported to contribute to hepatic endothelial cells as well as stellate cells [27, 28]. These cells could also contribute to disease corrections. Clinical trials of autologous bone marrow cell infusions have been used to treat adult liver cirrhosis [29]. Although the functional improvement of biliary secretion in our mice was most probably from the donor-derived hepatocytes that express the normal hepatocyte transporters, there is still the possibility that non-parenchymal cells partially contribute to the improvement of the phenotype.

An important question that remains to be addressed is the number of cells that is required to functionally correct a liver disease. Could the low frequency of bone marrow cell derived hepatocytes potentially be useful in treating human diseases? In metabolic diseases, a low percentage of hepatocyte could have a therapeutic effect. For instance, in Crigler-Najjar syndrome, 5% hepatic mass replacement could significantly reduce serum bilirubin levels [30]. In glycogen storage disease, 1% of total hepatocyte mass was found to partially correct the metabolic abnormalities [31]. In our model, which lacks the bile salt transporter function (causing impaired biliary bile acid secretion), a low percentage of donor cells with a positive gene function may have a therapeutic effect, as shown by the improved bile acid profile in our study. The minimum amount of functioning hepatocytes required for restoring bile acid secretion and for correcting liver damage in patients with PFIC-2 remains to be investigated. It is possible that a low quantity of cells repopulated in the recipient liver may be therapeutically effective in treating metabolic liver diseases. In the present model, we have not shown improved clinical parameters such as survival rate, histopathology and liver enzymes in the transplanted mice. These phenotype changes will be further investigated when a higher repopulation frequency can be achieved. In addition to restoring the normal bile excretion function using cell therapy, there are other concerns in the application of cell therapy in PFIC. One is the chronic damage and toxicity of the remaining recipient hepatocytes with deficient bile drainage. If the bile acid toxicity continues in the damaged hepatocytes, the long-term consequences of liver cirrhosis or even carcinogenesis could compromise the effect of cell therapy.

The length of time after transplantation also affects the results. Bone marrow repopulation was a slow and rare event; a previous report showed that donor-derived hepatocytes were first detectable no earlier than 7 weeks after transplantation, and maximal repopulation was observed at 28 weeks [13]. After that, donor bone marrow cells should stably supply the hematopoietic cells in every organ and tissue, including the liver. Our data support this, in that in an inherited liver disease model, in which the endogenous hepatocytes suffer from chronic, continuous injury, donor

bone marrow cells could serve as a continuous source of normal hepatocytes. It is therefore necessary to follow these mice for a longer period of time in the future to observe the duration of the replication of donor cells in the recipient liver.

Another problem to overcome in this and many other disease models is that donor hepatocyte proliferation or cluster formation was not observed. Theise et al. reported that up to 0.5–1% of total hepatocytes were of donor origin, as determined by FISH for Y-chromosome in female mice receiving bone marrow transplantation from male mice [4] without overt hepatocyte injury. Kanazawa et al. used liver injury models, including carbon tetrachloride treatment. After 4–6 weeks of bone marrow transplantation, only 5 in 4.1×10^5 hepatocytes were of donor origin by Y chromosome in Situ hybridization [12]. In two other models of albumin-urokinase transgenic mice and hepatitis B transgenic mice, there were no detectable donor hepatocytes when using a GFP or LacZ donor marker. In these models, no proliferation of hepatocytes was noted. There could be strong inhibitory factors or signals that prevented the donor-derived cells from repopulating or dividing. On the other hand, cluster formation and clonal expansion of donor hepatocytes have been found in the $fah^{-/-}$ mouse model. Most bone-marrow-derived cells were single cells or present in small 1–5 cell clusters. Only infrequently were large confluent nodules of hepatocytes observed in the $fah^{-/-}$ mice, which might result from monoclonal or oligoclonal expansion [11]. The highly expandable clones might originate from the donor bone marrow cells fused with $fah^{-/-}$ cells with high regeneration potential, or from a small number of hematopoietic stem cells from donor bone marrow fused with adult recipient hepatocytes, transforming the recipient hepatocytes into progenitor-like cells. The former theory was supported by the fact that $fah^{-/-}$ mice and human tyrosinemia patients have a strong tendency to develop tumors.

Adult hepatocytes are known for their high regeneration potential in human organs. With this high endogenous regeneration activity, together with low levels of recipient liver injury, extensive bone marrow cell repopulation in the liver was hard to achieve. Recently, cell–cell competition between donor and recipient hepatocytes had been proposed to be an important factor in establishing donor repopulation. It has been shown that repopulation is based on the stronger proliferative activity of transplanted cells and reduced apoptosis of their progeny compared with host hepatocytes. A successful repopulation also depends on increased apoptosis of host hepatocytes immediately adjacent to transplanted cells [32].

In conclusion, we have demonstrated that DDHs were detected 1–6 months after bone marrow transplantation in an inherited intrahepatic cholestastic liver disease model. Although present only at a low frequency, donor-derived hepatocytes were found expressing the impaired gene, and had partially rescued the defects in biliary secretion. We suggest that bone marrow cells could serve as a potential source for liver repair in chronic metabolic liver disease. Further investigations aiming to enhance donor repopulation and inhibit recipient cell regeneration by optimizing liver injury levels are required.

Acknowledgements This project was in part supported by grant from the Canadian Institute of Health Research (MOP 42560), National Taiwan University Hospital (NTUH 93A12-1), and the National Science Council, Taiwan, ROC (NSC 96-2752-B-002-010-PAE). The authors thank Kevin Kao, Camila Alverez, Rebecca Ng, Yu-Jung Liu, and Shi-Ping Chou for technical assistance.

References

1. Fausto N (2004) Liver regeneration and repair: hepatocytes, progenitor cells, and stem cells. Hepatology 39:1477–1487
2. Fox IJ, Roy-Chowdhury J (2004) Hepatocyte transplantation. J Hepatol 40:878–886
3. Brazelton TR, Rossi FM, Keshet GI, Blau HM (2000) From marrow to brain: expression of neuronal phenotypes in adult mice. Science 290:1775–1779
4. Theise ND, Badve S, Saxena R, Henegariu O, Sell S, Crawford JM, Krause DS (2000) Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. Hepatology 31:235–240
5. Gussoni E, Soneoka Y, Strickland CD, Buzney EA, Khan MK, Flint AF, Kunkel LM, Mulligan RC (1999) Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature 401:390–394
6. Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, Nadal-Ginard B, Bodine DM, Leri A, Anversa P (2001) Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci USA 98:10344–10349
7. Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, Boggs SS, Greenberger JS, Goff JP (1999) Bone marrow as a potential source of hepatic oval cells. Science 284:1168–1170
8. Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, Sharkis SJ (2001) Multi-organ, multilineage engraftment by a single bone marrow-derived stem cell. Cell 105:369–377
9. Theise ND, Nimmakayalu M, Gardner R, Illei PB, Morgan G, Teperman L, Henegariu O, Krause DS (2000) Liver from bone marrow in humans. Hepatology 32:11–16
10. Korbling M, Katz RL, Khanna A, Ruifrok AC, Rondon G, Albitar M, Champlin RE, Estrov Z (2002) Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. N Engl J Med 346:738–746
11. Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, Wang X, Finegold M, Weissman IL, Grompe M (2000) Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med 6:1229–1234
12. Kanazawa Y, Verma IM (2003) Little evidence of bone marrow-derived hepatocytes in the replacement of injured liver. Proc Natl Acad Sci USA 100(Suppl 1):11850–11853
13. Wang X, Montini E, Al-Dhalimy M, Lagasse E, Finegold M, Grompe M (2002) Kinetics of liver repopulation after bone marrow transplantation. Am J Pathol 161:565–574

14. Wang R, Salem M, Yousef IM, Tuchweber B, Lam P, Childs SJ, Helgason CD, Ackerley C, Phillips MJ, Ling V (2001) Targeted inactivation of sister of P-glycoprotein gene (spgp) in mice results in nonprogressive but persistent intrahepatic cholestasis. Proc Natl Acad Sci USA 98:2011–2016
15. Strautnieks SS, Bull LN, Knisely AS, Kocoshis SA, Dahl N, Arnell H, Sokal E, Dahan K, Childs S, Ling V, Tanner MS, Kagalwalla AF, Nemeth A, Pawlowska J, Baker A, Mieli-Vergani G, Freimer NB, Gardiner RM, Thompson RJ (1998) A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. Nat Gen 20:233–238
16. Terada N, Hamazaki T, Oka M, Hoki M, Mastalerz DM, Nakano Y, Meyer EM, Morel L, Petersen BE, Scott EW (2002) Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. Nature 416:542–545
17. Wang X, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al-Dhalimy M, Lagasse E, Finegold M, Olson S, Grompe M (2003) Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature 422:897–901
18. Vassilopoulos G, Wang PR, Russell DW (2003) Transplanted bone marrow regenerates liver by cell fusion. Nature 422:901–904
19. Willenbring H, Bailey AS, Foster M, Akkari Y, Dorrell C, Olson S, Finegold M, Fleming WH, Grompe M (2004) Myelomonocytic cells are sufficient for therapeutic cell fusion in liver. Nat Med 10:744–748
20. Camargo FD, Finegold M, Goodell MA (2004) Hematopoietic myelomonocytic cells are the major source of hepatocyte fusion partners. J Clin Invest 113:1266–1270
21. Piscaglia AC, Shupe TD, Oh SH, Gasbarrini A, Petersen BE (2007) Granulocyte-colony stimulating factor promotes liver repair and induces oval cell migration and proliferation in rats. Gastroenterology 133:619–631
22. Mallet VO, Mitchell C, Mezey E, Fabre M, Guidotti JE, Renia L, Coulombel L, Kahn A, Gilgenkrantz H (2002) Bone marrow transplantation in mice leads to a minor population of hepatocytes that can be selectively amplified in vivo. Hepatology 35:799–804
23. Wang R, Lam P, Liu L, Forrest D, Yousef IM, Mignault D, Phillips MJ, Ling V (2003) Severe cholestasis induced by cholic acid feeding in knockout mice of sister of P-glycoprotein. Hepatology 38:1489–1499
24. Schatteman GC, Hanlon HD, Jiao C, Dodds SG, Christy BA (2000) Blood-derived angioblasts accelerate blood-flow restoration in diabetic mice. J Clin Invest 106:571–578
25. Yu S, Tanabe T, Dezawa M, Ishikawa H, Yoshimura N (2006) Effects of bone marrow stromal cell injection in an experimental glaucoma model. Biochem Biophys Res Commun 344:1071–1079
26. Sakaida I, Terai S, Yamamoto N, Aoyama K, Ishikawa T, Nishina H, Okita K (2004) Transplantation of bone marrow cells reduce CCl4-induced liver fibrosis in mice. Hepatology 40:1304–1311
27. Baba S, Fujii H, Hirose T, Yasuchika K, Azuma H, Hoppo T, Naito M, Machimoto T, Ikai I (2004) Commitment of bone marrow cells to hepatic stellate cells in mouse. J Hepatol 40:255–260
28. Gao Z, McAlister VC, Williams GM (2001) Repopulation of liver endothelium by bone-marrow-derived cells. Lancet 357:932–933
29. Terai S, Ishikawa T, Omori K, Aoyama K, Marumoto Y, Urata Y, Yokoyama Y, Uchida K, Yamasaki T, Fujii Y, Okita K, Sakaida I (2006) Improved liver function in patients with liver cirrhosis after autologous bone marrow cell infusion therapy. Stem Cells 24:2292–2298
30. Fox IJ, Chowdhury JR, Kaufman SS, Goertzen TC, Chowdhury NR, Warkentin PI, Dorko K, Sauter BV, Strom SC (1998) Treatment of the Crigler-Najjar syndrome type I with hepatocyte transplantation. N Engl J Med 338:1422–1426
31. Muraca M, Gerunda G, Neri D, Vilei MT, Granato A, Feltracco P, Meroni M, Giron G, Burlina AB (2002) Hepatocyte transplantation as a treatment for glycogen storage disease type 1a. Lancet 359:317–318
32. Oertel M, Menthena A, Dabeva MD, Shafritz DA (2006) Cell competition leads to a high level of normal liver reconstitution by transplanted fetal liver stem/progenitor cells. Gastroenterology 130:507–520