Review Articles

Contribution of Extrahepatic Cells in Liver Regeneration: Is it real?

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Abstract

Extrahepatic cells, especially bone marrow cells, might contribute to liver repair but not in the normally regenerating liver according to the recent updated literature. The mechanism by which extrahepatic cells express a liver-specific function in a liver, whether transdifferentiation or cell fusion, remains under debate. In this review, we investigate the status of findings on this matter and summarize the recent research.

Introduction

It has been reported that extrahepatic cells, especially bone marrow-derived cells, are mobilized and involved in liver tissue repair, including that after injury.[1-17] However, details regarding how extrahepatic cells are involved and how much they contribute to normal liver regeneration have not been fully elucidated. Even if such involvement is present, it remains unclear whether liver-specific function is achieved through transdifferentiation or cell fusion. In this review, we investigate the status of findings on this matter, and summarize the recent research.

Contribution of extrahepatic cell on liver regeneration or injury

After partial hepatectomy, liver usually restores its mass within 1-2 weeks in rats and 1-3 months in human in order to catch up liver specific function. DNA synthesis and cell division of hepatocytes occurs followed by those of non-parenchymal cells[18, 19]. It has long been believed that only cells in a liver participate in such restoration. However, after new findings in liver transplant recipients who previously underwent bone marrow transplantation, many reports have been focusing on the contribution of bone marrow cells to liver repopulation, especially in liver regeneration after liver damage or partial hepatectomy, but with controversy (Table. 1,2).

In an *in vivo* experiment conducted in 2000, it was first reported that hepatocytes could be derived from bone marrow cells after irradiation in the absence of severe acute injury.[1] Subsequently, Baccarani et al in 2001[2] reported that, in human recipients, replacement of a female liver venous endothelium with male bone marrow showed the possibility of involvement of BM cells in liver rearrangement (Fig. 1) followed by Körbling et al.'s finding of the differentiation of circulating stem cells into mature hepatocytes.[3] From 2002 on, research on this matter has advanced because of the advent of green fluorescent protein (GFP) transgenic mice, which expresses green fluorescent protein throughout their bodies. The GFP-positive cell-transplant model allows researchers to detect transplanted or mobilized cells without complicated molecular biological methods. Using this model after GFP-positive bone marrow (BM) transplantation, Fujii et al. reported that BM cells participated in liver regeneration after hepatectomy, whereas the majority was committed to sinusoidal endothelial cells, probably through endothelial progenitor cell mobilization.[4,5] In 2003, Terai et al., using their GFP/carbon-tetrachloride (CCl4) mouse model, reported that autologous BM cells were an effective treatment for liver failure under persistent liver damage; they found the same results for liver cirrhosis.[6,7] In 2005, am Esch JS 2nd also reported that CD133 (used as hematopoietic stem cell marker) (+) BM stem cells infused into the

portal vein accelerated hepatic regeneration.[8] Very recently, Conzelmann et al., using their reduced-size liver transplantation model, reported that recipient-derived progenitor cells were present and might contribute to liver regeneration in mice.[9] All these reports constitute encouraging data to support the notion that extrahepatic cells, and especially BM cells, are potent therapeutic resources for impaired liver regeneration. In addition, the studies of partial hepatectomy using rats in which liver regeneration was impaired by retrorsine showed some positive results on the matter.[10,11]

However, there is still controversy regarding how much involvement is present and how the cells are involved. We next turn to the studies regarding this matter (Table 3). In 2005 Di Campli et al. reported no evidence of hematopoietic stem cell mobilization in patients who underwent hepatectomy or in patients with acute liver failure. They observed no CD34-positive cells in the blood after hepatectomy for acute decompensation of a cirrhotic liver.[12] Similarly, in 2006, Moritoki et al., using GFP transgenic mice, demonstrated that BM cell transfer seemed not to contribute to the differentiation of cholangiocytes in a chronic cholestasis model. They also found scattered GFP-positive cells in the hepatic parenchyma.[13] In 2007, Tomiyama reported the limited contribution of cells originating from intact extrahepatic tissue in hepatocyte regeneration in transplanted rat livers. They reported that, even in the non-injured liver,

GFP-positive hepatocytes increased by 0.0048% per week, that is, 5×10^3 were generated per day. However, liver injury did not increase the percentage of GFP-positive hepatocytes in their liver transplantation model[14], as Popp reported similar findings in 2007.[15]

Taken together, at present, it seems that limited involvement is possible in normal liver regeneration after partial hepatectomy. However, in the case of impaired liver regeneration, involvement of BM cells may be possible through evidence from an *in vivo* liver injury model. Investigation on liver regeneration using specific model, in which liver cells can not perform cell division using retrorsine, showed that no contribution of multipotent mesenchymal stromal cells in liver regeneration. Whether extrahepatic cells migrated to the regenerating liver function as liver cells or how long they can survive are still under debate and varies among previous reports. Nevertheless, clinical studies have started with autologous bone marrow cells or $CD34^+$ cells to treat liver insufficiency, resulting in moderate effect.[18,19]

Transdifferentiation or Cell fusion

It has been intensely debated whether the mechanism by which bone marrow cells become hepatocytes is transdifferentiation or fusion.

In 2004, Lee et al. reported differentiation of human mesenchymal stem cells into hepatocytes *in vitro*.[20] In the transdifferentiation theory, it has been held that the phenotype of bone marrow cells changes to that of hepatocytes through coordinated changes in the transcriptional activities of many genes. The mesenchymal stem cell component in bone marrow cells or other specific stem cells are candidates for this ability of transdifferentiation. Also, although transdifferentiation of the peripheral blood monocyte-derived subset into hepatic transdifferentiated cells has been reported, the question of which cells are involved in the transdifferentiation has not been answered. Using their mouse model, Brulport reported evidence not for transdifferentiation but instead for a complex situation including partial differentiation and possible horizontal gene transfer.[21] In 2005, Wu also reported minimal evidence of transdifferentiation from recipient bone marrow to parenchymal cells regenerating and long-surviving human allografts.[22]

On the other hand, “cell fusion” between bone-marrow stem cells and hepatocytes was reported and has been believed to be a main mechanism based on an experiment, repeated by many researchers, in which new hepatocytes appear after infusion of bone marrow cells. [23-27]

At present, we still do not know the reason why hepatocytes cannot be more effectively produced through either the “transdifferentiation” or “fusion” mechanism. Those questions and controversies await further research on liver regenerative medicine[28]

In conclusion, to date in the recent literature, extrahepatic cells, especially BM cells, might contribute to injured liver repair but not in the normally regenerating liver. The mechanism by which extrahepatic cells express a liver-specific function, whether transdifferentiation or cell fusion, remains under debate.

Search Strategy

Recent data for this review were collected by PubMed searches.

References

1. Theise ND, Badve S, Saxena R, et al. Derivation of hepatocytes from bone marrow cells in mice after radiation-induced myeloablation. Hepatology 2000;31:235-40

2. Baccarani U, Donini A, Risaliti A, Bresadola F. Replacement of liver venous endothelium. Lancet 2001;357:2137.

3. Körbling M, Katz RL, Khanna A, et al. Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. N Eng J Med 2002;346:738-46.

4. Fujii H, Hirose T, Oe S, et al. Contribution of bone marrow cells to liver regeneration after partial hepatectomy in mice. J Hepatol 36;653-659, 2002.

5. Ferry N, Hadchouel M.Liver regeneration: with a little help from marrow. J Hepatol 2002;36:695-7.

6. Terai S, Sakaida I, Nishina H, Okita K. Lesson from the GFP/CCl4 model--translational research project: the development of cell therapy using autologous bone marrow cells in patients with liver cirrhosis. J Hepatobiliary Pancreat Surg 2005;12:203-7.

7. Sakaida I, Terai S, Yamamoto M, et al. Transplantation of bone marrow cells reduces CCl4-induced liver fibrosis in mice. Hepatology 78;83-88:2004.

8. am Esch JS 2nd, Knoefel WT, Klein M, et al. Portal application of autologous CD133+ bone marrow cells to the liver: a novel concept to support hepatic regeneration. Stem Cells 2005;23:463-70.

9. Conzelmann LO, Hines IN, Kremer M, Perry AW, Lemasters JJ, Wheeler MD. Extrahepatic cells contribute to the progenitor/stem cell response following reduced-size liver transplantation in mice. Exp Biol Med (Maywood). 2007;232:571-80.

10. Dahlke MH, Popp FC, Bahlmann FH, et al. Liver regeneration in a retrorsine/CCl4-induced acute liver failure model: do bone marrow-derived cells contribute? J Hepatol 2003;39:365-73.

11. Hunter Best D, Coleman WB. Cells of origin of small hepatocyte-like progenitor cells in the retrorsine model of rat liver injury and regeneration. J Hepatol 2008;48:369-71.

12. Di Campli C, Piscaglia AC, Giuliante F, et al. No evidence of hematopoietic stem cell mobilization in patients submitted to hepatectomy or in patients with acute on chronic liver failure. Transplant Proc 2005;37:2563-6.

13. Moritoki Y, Ueno Y, Kanno N, et al. Lack of evidence that bone marrow cells contribute to cholangiocyte repopulation during experimental cholestatic ductal hyperplasia. Liver Int 2006;26:457-66.

14. Tomiyama K, Miyazaki M, Nukui M, et al. Contribution of cells of intact extrahepatic tissue origin to hepatocyte regeneration in transplanted rat liver. Transplantation 2007;83:624-30.

15. Popp FC, Slowik P, Eggenhofer E, Renner P, Lang SA, Stoeltzing O, Geissler EK, Piso P, Schlitt HJ, Dahlke MH.No contribution of multipotent mesenchymal stromal cells to liver regeneration in a rat model of prolonged hepatic injury. Stem Cells. 2007;25:639-45.

16. Terai S, Ishikawa T, Omori K, et al. Improved liver function in patients with liver cirrhosis after autologous bone marrow cell infusion therapy. Stem Cells 2006;24:2292-8.

17. Eguchi S, Kamlot A, Ljubimova J, Lebow LT, Demetriou AA, Rozga J. A novel experimental animal model of fulminant hepatic faillure. -Survival and effect on biochemistry and liver regeneration. Hepatology 1996:24;1452-1459.

18. Eguchi S, Yanaga K, Sugiyama N, et al. Changes in serum levels of Hepato cyte growth factor in patients undergoing adult-to-adult living donor liver tra nsplantation. Transplantation 2003:76;1769-70.

19. Gordon MY, Levicar N, Pai M, et al. Characterization and clinical application of human CD34+ stem/progenitor cell populations mobilized into the blood by granulocyte colony-stimulating factor. Stem Cells 2006;24:1822-30.

20. Lee KD, Kuo TK, Whang-Peng J et al. In vitro hepatic differentiation of human mesenchymal stem cells. Hepatology2004; 40;1275-1284.

21. Brulport M, Schormann W, Bauer A, et al. Fate of extrahepatic human stem and precursor cells after transplantation into mouse livers. Hepatology 2007;46:861-70.

22. Wu T, Cieply K, Nalesnik MA, Randhawa PS, et al.. .Minimal evidence of transdifferentiation from recipient bone marrow to parenchymal cells in regenerating and long-surviving human allografts. Am J Transplant 2003;3:1173-81.

23. Camargo FD, Finegold M, Goodell MA. Hematopoietic myelomonocytic cells are the major source of hepatocyte fusion partners. J Clin Invest 2004;113:1266-70.

24. Willenbring H, Bailey AS, Foster M, et al. Myelomonocytic cells are sufficient for therapeutic cell fusion in liver. Nature Med 2004;10:744-8.

25. Quintana-Bustamante O, Alvarez-Barrientos A, et al. Hematopoietic mobilization in mice increases the presence of bone marrow-derived hepatocytes via in vivo cell fusion.. Hepatology 2006;43:108-16.

26. Vassilopoulos G, Wang PR, Russell DW. Transplanted bone marrow regenerates liver by cell fusion. Nature 2003;422:901-4.

27. Wang X, Willenbring H, Akkari Y, et al. Cell fusion is the principal source of bone-marrow-derived hepatocytes. Nature 2003;422:897-901.

28. Kawashita Y, Kawashita Y, Guha C,et al. Liver Repopulation: A New Concept of Hepatocyte Transplantation. Surgery Today 2005;35:705-10.

Figure legend

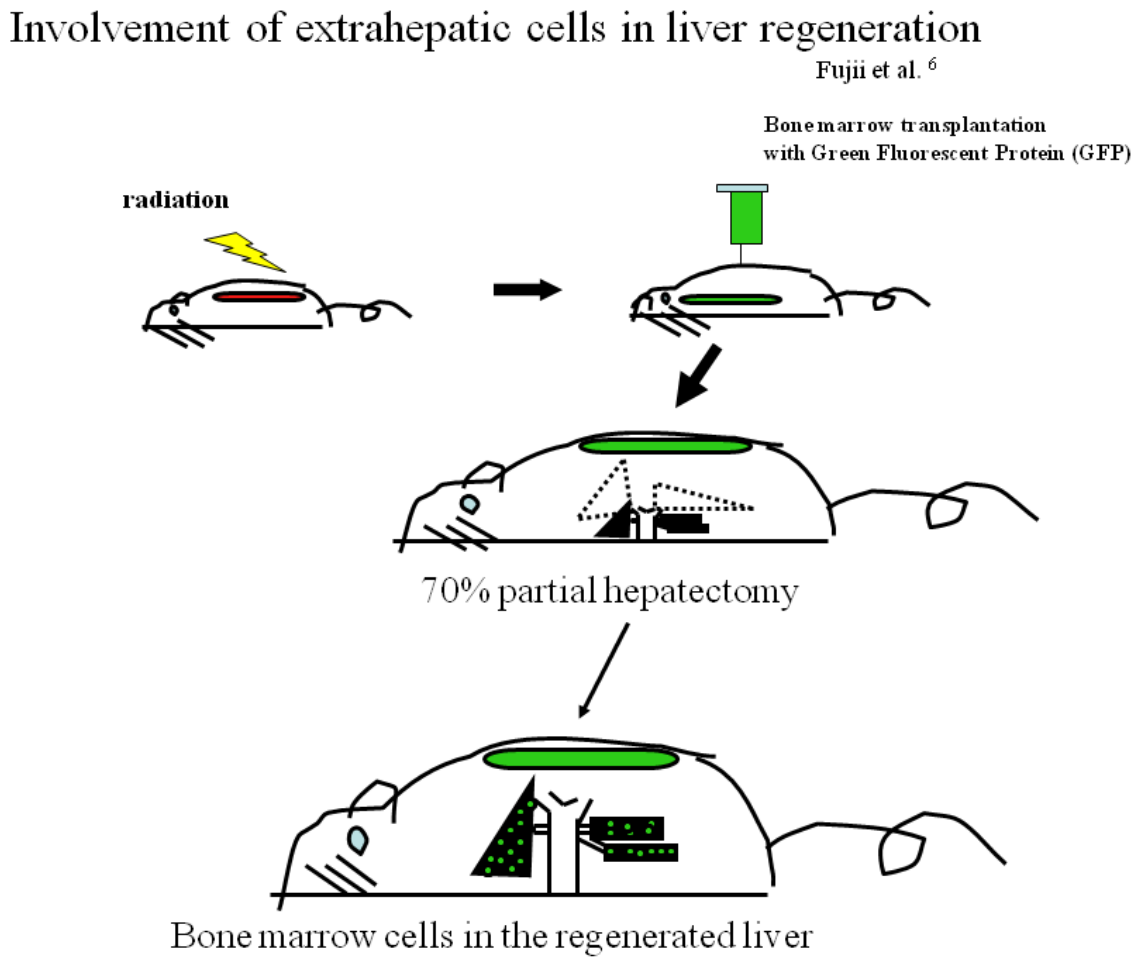

Fig. 1

Transplanted bone marrow cells participated in liver regeneration after partial hepatectomy in rats. The majority was committed to sinusoidal endothelial cells.

Table. 1
Relationship between extrahepatic cells and liver regeneration/impairment (1)

Author	Journal	Patient or Model	Findings
Baccarani[4]	Lancet 2001	Human	Replacement liver venous endothelium in livers of BM transplant recipients.
Fujii[5]	J Hepatol 2002	GFP transgenic mice	BM cells participated in LR. The majority was committed to sinusoidal endothelial cells.
Wu[18]	Am J Transplant 2003	Human	Only rare isolated and tentatively identified recipient hepatocytes
Cantz[13]	Cell Transplant 2004	GFP transgenic mice	No evidence of BM cells in LR.
Terai[8]	J HBP Surg 2005	GFP transgenic mice	Autologous BM cells was effective for treatment for liver failure.
Di Campli[13]	Transplant Proc 2005	Human	No evidence of hematopoietic stem cell in LR.

BM: bone marrow, GFP: green fluorescent protein, CCl_4: carbontetrachloride, LR: liver regeneration

Table. 2
Relationship between extrahepatic cells and liver regeneration (2)

Author	Journal	Patient or Model	Findings
am Esch JS 2nd[10]	Stem Cells 2005	Human	CD133(+) BM stem cells infused into portal vein accelerating LR.
Moritoki[14]	Liver Int 2006	GFP transgenic mice	BM cells transfer not contribute to the differentiation of cholangiocytes in chronic cholestasis model. Scattered GFP(+) cells in hepatic parenchyma.
Tomiyama[15]	Transplantation 2007	Rat	Limited contribution of cells of intact extrahepatic tissue origin to LR in transplanted liver. Liver injury did not increase the percentage of GFP(+) hepatocytes using LT model.
Conzelmann[11]	Exp Biol Med 2007	GFP transgenic mice	Using reduced-size LT, recipient-derived progenitor cells were present and might contribute to LR.
Beaudry[12]	J Pediatr Surg 2007	GFP transgenic mice	Contribution of circulating endothelial progenitor cells with exogenous vascular endothelial growth factor.

BM: bone marrow, GFP: green fluorescent protein, LR: liver regeneration, LT: liver transplantation

Table. 3
Controversies on involvement of extrahepatic cells in liver regeneration and repair

Yes	Journals	Species	Cells differentiated from extrahepatic cells
Baccarani[4]	2001 Lancet	Human	Hepatic endothelial cells
Fujii[6]	2002 J Hepatology	Rat	Hepatic endothelial cells
Conzelmann[11]	2007 Exp Biol Med	Mouse	9% of liver comprised within 28 days
Beaudry[12]	2007 J Pediatr Surg	Mouse	Hepatic endothelial cells
No			
Wu[19]	2003 Am J Transplant	Human	No endothelial cells from BM cells
Cantz[13]	2004 Cell Transplant	Mouse	Limited or no contribution
De Campi[14]	2005 Transplant Proc	Human	No evidence of BM mobilization
Moritoki[15]	2006 Liver Int	Mouse	No cholangiocyte from BM cells
Tomiyama[16]	2007 Transplantation	Rat	Limited contribution

BM: bone marrow