### Title
Liver transplantation for patients with human immunodeficiency virus and hepatitis C virus coinfection with special reference to hemophiliac recipients in Japan.

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Review Articles

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

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Key words: extrahepatic stem cells, liver regeneration, transdifferentiation, cell
fusion
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 cells18, 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 2\(^{nd}\) also reported that CD133 (used as hematopoietic stem cell marker) (+) BM stem cells infused into the
portal vein accelerated hepatic regeneration. 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. 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.

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. 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. 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, $5 \times 10^3$ were generated per day. However, liver injury did not increase the percentage of GFP-positive hepatocytes in their liver transplantation model, as Popp reported similar findings in 2007.

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 \textit{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.

\textbf{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*. 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. In 2005, Wu also reported minimal evidence of transdifferentiation from recipient bone marrow to parenchymal cells regenerating and long-surviving human allografts.

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.
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\textsuperscript{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


Involvement of extrahepatic cells in liver regeneration

Fujii et al. 6

Bone marrow transplantation with Green Fluorescent Protein (GFP)

radiation

70% partial hepatectomy

Bone marrow cells in the regenerated liver

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)**

<table>
<thead>
<tr>
<th>Author</th>
<th>Journal</th>
<th>Patient or Model</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baccarani⁴</td>
<td>Lancet 2001</td>
<td>Human</td>
<td>Replacement liver venous endothelium in livers of BM transplant recipients.</td>
</tr>
<tr>
<td>Fujii²</td>
<td>J Hepatol 2002</td>
<td>GFP transgenic mice</td>
<td>BM cells participated in LR. The majority was committed to sinusoidal endothelial cells.</td>
</tr>
<tr>
<td>Wu¹³</td>
<td>Am J Transplant 2003</td>
<td>Human</td>
<td>Only rare isolated and tentatively identified recipient hepatocytes</td>
</tr>
<tr>
<td>Cantz¹³</td>
<td>Cell Transplant 2004</td>
<td>GFP transgenic mice</td>
<td>No evidence of BM cells in LR.</td>
</tr>
<tr>
<td>Terai⁷</td>
<td>J HBP Surg 2005</td>
<td>GFP transgenic mice</td>
<td>Autologous BM cells was effective for treatment for liver failure.</td>
</tr>
<tr>
<td>Di Campli¹³</td>
<td>Transplant Proc 2005</td>
<td>Human</td>
<td>No evidence of hematopoietic stem cell in LR.</td>
</tr>
</tbody>
</table>

BM: bone marrow, GFP: green fluorescent protein, CCl₄: carbon tetrachloride, LR: liver regeneration

### Table 2
**Relationship between extrahepatic cells and liver regeneration (2)**

<table>
<thead>
<tr>
<th>Author</th>
<th>Journal</th>
<th>Patient or Model</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>am Esch IS²⁰¹⁰</td>
<td>Stem Cells 2005</td>
<td>Human</td>
<td>CD133(+) BM stem cells infused into portal ven accelerating LR.</td>
</tr>
<tr>
<td>Moritoki¹⁴</td>
<td>Liver Int 2006</td>
<td>GFP transgenic mice</td>
<td>BM cells transfer not contribute to the differentiation of cholangiocytes in chronic cholestasis model. Scattered GFP(+) cells in hepatic parenchyma.</td>
</tr>
<tr>
<td>Tomiyama¹⁵</td>
<td>Transplantation 2007</td>
<td>Rat</td>
<td>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.</td>
</tr>
<tr>
<td>Conzelmann¹¹</td>
<td>Exp Biol Med 2007</td>
<td>GFP transgenic mice</td>
<td>Using reduced-size LT, recipient-derived progenitor cells were present and might contribute to LR.</td>
</tr>
<tr>
<td>Beaudry¹²</td>
<td>J Pediatr Surg 2007</td>
<td>GFP transgenic mice</td>
<td>Contribution of circulating endothelial progenitor cells with exogenous vascular endothelial growth factor</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Yes</th>
<th>Journals</th>
<th>Species</th>
<th>Cells differentiated from extrahepatic cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baccarami\textsuperscript{a}</td>
<td>2001 Lancet</td>
<td>Human</td>
<td>Hepatic endothelial cells</td>
</tr>
<tr>
<td>Fujii\textsuperscript{b}</td>
<td>2002 J Hepatology</td>
<td>Rat</td>
<td>Hepatic endothelial cells</td>
</tr>
<tr>
<td>Conzemmann\textsuperscript{11}</td>
<td>2007 Exp Biol Med</td>
<td>Mouse</td>
<td>9% of liver comprised within 28 days</td>
</tr>
<tr>
<td>Beauchy\textsuperscript{12}</td>
<td>2007 J Pediatr Surg</td>
<td>Mouse</td>
<td>Hepatic endothelial cells</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No</th>
<th>Journals</th>
<th>Species</th>
<th>Cells differentiated from extrahepatic cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wu\textsuperscript{13}</td>
<td>2003 Am J Transplant</td>
<td>Human</td>
<td>No endothelial cells from BM cells</td>
</tr>
<tr>
<td>Cantz\textsuperscript{33}</td>
<td>2004 Cell Transplant</td>
<td>Mouse</td>
<td>Limited or no contribution</td>
</tr>
<tr>
<td>De Campi\textsuperscript{14}</td>
<td>2005 Transplant Proc</td>
<td>Human</td>
<td>No evidence of BM mobilization</td>
</tr>
<tr>
<td>Moritoki\textsuperscript{15}</td>
<td>2006 Liver Int</td>
<td>Mouse</td>
<td>No cholangiocyte from BM cells</td>
</tr>
<tr>
<td>Tomiyama\textsuperscript{16}</td>
<td>2007 Transplantation</td>
<td>Rat</td>
<td>Limited contribution</td>
</tr>
</tbody>
</table>

BM: bone marrow