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Original

Potential involvement of extrahepatic cells in liver regeneration

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Abstract

Extrahepatic cells have been considered a potential cellular resource for liver regeneration in response to liver damage. The genotyping of human living donor liver transplant (LDLT) recipients has reported that cells derived from those recipients of living donor liver transplantation differentiated into multiple types of liver component cells, suggesting that extrahepatic, as well as multipotent, cells contributed to liver regeneration. However, the consequences of the involvement of the extrahepatic cells in terms of clinically observed liver enlargement remain unknown. In the present study, we suggest that cells that might have been of extrahepatic origin contributed to the increase in liver volume, while the hypertrophy of the hepatocytes played an important role in the initial enlargement. Despite the rapid growth of the liver volume, cells in the mitotic phase were not evident. Taken together, these results suggest that: (i) Liver enlargement is achieved through some degree of extrahepatic cellular proliferation via liver progenitor cells, and (ii) cells from the recipient of living donor liver transplantation may contribute to the regeneration of the grafted liver.

Key words: *extrahepatic cells, liver regeneration, stem cells, transplantation*

I. Introduction

The regeneration of the liver from physical damage requires the involvement of various kinds of cells. A number of studies have reported that putative liver progenitor cells are one of the cell types that contribute to liver tissue repair [1-6)]. Recently, it has been reported that sox9-positive intrahepatic cholangiocytes are a major source of hepatocytes for liver tissue homeostasis [7)]. However, it is difficult to apply the human equivalent of the oval cells (i.e., liver progenitor cells) during cell-based therapy in practice because their tissue of origin remains unknown [8)].

On the other hand, it has been proposed that extrahepatic cells, as one of the hepatic lineages that respond to injury, contribute to liver regeneration [9)]. Earlier reports speculated regarding the involvement of extrahepatic

Table 1. Characteristics of donors and recipients of LDLT

Case	Sex(R)	Age(R)	Sex(D)	Age(D)	Graft	Infection	Primary disease	TOB Early/Late
1	F	51	M	24	L + C	(-)	PBC	2w/6m
2	M	59	M	27	R	HCV	LC, HCC	8w/14m
3	F	37	M	37	L	(-)	PSC	3w/6m
4	M	49	F	55	R	HCV	LC, HCC	8w/16m
5	F	57	F	33	L + C	HBV	LC, HCC	3w/6m
6	F	57	F	32	R	(-)	PBC	4w/6m
7	M	57	M	30	ER	HCV	LC, HCC	2w/7m
8	F	56	M	30	R	HCV	LC, HCC	3w/10m
9	F	53	M	25	R	HCV	LF	4w/6m
10	F	60	F	32	R	HCV	LC	7w/8m

R : recipient, D : donor, PBC : primary biliary cirrhosis, PSC : primary sclerosing cholangitis, TOB: Time of Biopsy, L: left lobe, C: caudate lobe, R: right lobe, ER: extended right lobe, LC: liver cirrhosis, HCC: hepatocellular carcinoma, LF: liver failure, w: weeks, m: months.

cells in liver regeneration based on the fact that cellular and genetic chimerism exists in transplanted livers [10-13]. Investigators have also reported that cells from bone marrow are from an extrahepatic cellular source [14-20]. In particular, some recent studies have shown that bone-marrow-derived mesenchymal stem cells (BM-MSCs) contribute to liver regeneration after liver damage [21-24]. However, the consequences of the extrahepatic cells' involvement in liver regeneration are still unclear because it is difficult to keep track of human liver samples histologically. LDLT is one of the rare opportunities in which such pathological time-course samples are available, along with macroscopic images of liver regeneration.

To investigate the histological background against which extrahepatic cells contribute to liver regeneration, we counted the number of hepatocytes per area at three time points after LDLT. The number of hepatocytes indicates the volume of individual cells. Thus, it would be an informative hallmark of cellular hypertrophy or proliferation when it is compared to macroscopic liver volume. Here, we demonstrate how hepatocytes respond during the process of liver regeneration, in which extrahepatic cells may be involved, based on the data from human LDLT specimens.

II. Materials and methods

1. Human objects of LDLT

Ten pairs of recipients and donors who underwent LDLT between January 2007 and February 2011 at the Department of Surgery in Iwate Medical University Hospital were subjected to analysis. The subject LDLT pairs were restricted based on the availability of time-course samples. The specimens were 10 livers removed from recipients, 10 zero-point biopsies from donors, and 10 needle biopsies of the grafts. A zero-point biopsy is performed to test the quality of a donor liver before transplantation. The graft biopsies used in the present study were taken at various time points between 5 and 12 months after

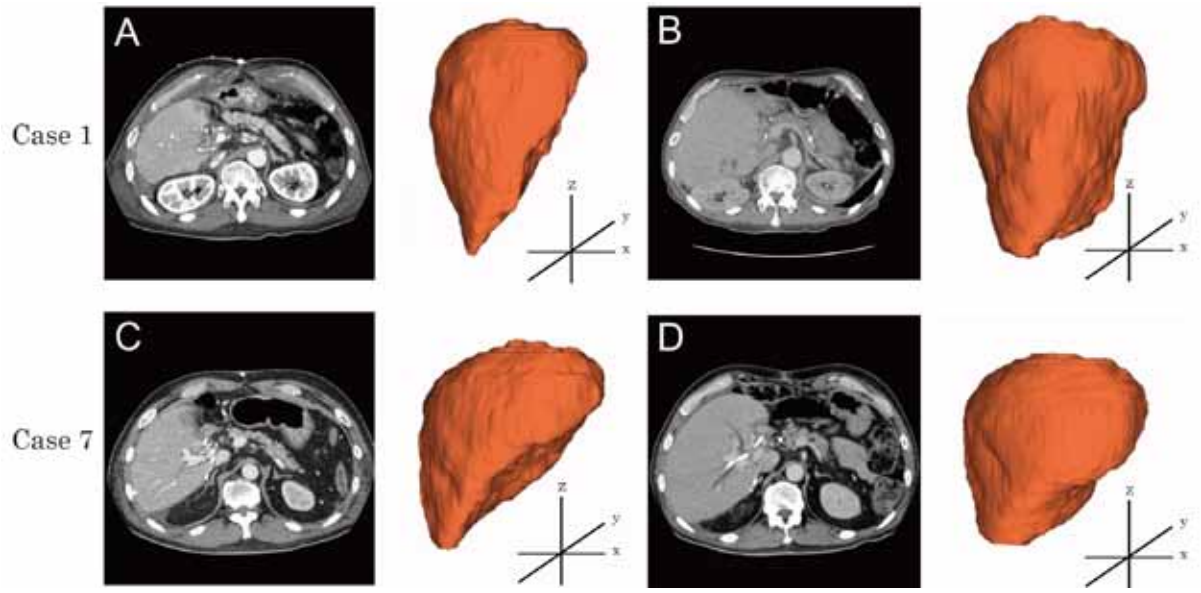

Fig. 1. CT and liver volume rendering. (A) Early and (B) late time points of Case 1; (C) early and (D) late time-points of Case 7. The left and right panel in each box indicate the CT and volume rendering of the regenerating liver, respectively.

LDLT; they were retrospectively reviewed. The study was reviewed and approved by the Ethics Committee of Iwate Medical University School of Medicine (HG H23-13). Informed consent was obtained from all donors/recipients according to the Institutional Review Board guidelines. The details of the LDLT set are shown in Table 1.

2. Macroscopic examinations of liver regeneration

In the process of following up after LDLT, CT volumetry (Toshiba Medical Systems, Tokyo) was performed in the portal phase (30 sec after the injection of the contrasting agent) at early and late time points. A regenerating liver image was constructed from a series of 1-2mm slices from the DCOM file using the SYNAPSE VINCENT program (FUJIFILM, Tokyo) running on an Intel Xenon 2.93GHz personal computer. The outline of the liver was first traced automatically and then fine-tuned manually for all slices. The volumes of the inferior vena cava and extrahepatic portal veins were excluded.

3. Histological examinations

For the human LDLT biopsy specimens, samples were fixed with 10% formalin for pathological examination. Three time-point specimens (i.e., the recipient, zero-point, and graft biopsies) were subjected to H&E staining for hepatocyte number counting (200 × 200 μm^2 per view). Cells were counted in eight arbitrarily selected view fields, and the average number per field was calculated. The cell numbers at each time-point relative to the maximum number of each LDLT set were calculated.

III. Results

1. Macroscopic examinations of graft liver volume

The median weight and volume of the graft liver (before transplantation) were 594g and 635cm^3, respectively. The median volumes of

Table 2. Changes in graft volume after LDLT

Case	Initial volume(cm^3) G	Early (cm^3) V1	Late(cm^3) V2	Initial → Early Δ (%)*	Initial → Late Δ (%)**
1	517	872	1475	168.7	285.3
2	667	981	1343	147.1	201.3
3	429	747	896	174.1	208.9
4	603	831	874	137.8	144.9
5	405	689	874	170.1	215.8
6	560	831	798	148.4	142.5
7	1000	1029	1400	102.9	140.0
8	840	1188	1287	141.4	153.2
9	703	1305	1712	185.6	243.5
10	765	832	1012	108.8	132.3

** Δ =|(V1-G)/G| × 100%, ** Δ =|(V2-G)/G| × 100%.*

Table 3. Number of hepatocytes per unit view (cells/200 × 200 μm^2)

Case	Zero point C0	Early C1	Late C2	Zero → Early Δ (%)*	Zero → Late Δ (%)**
1	56	50	61	− 10.7	8.9
2	70	52	64	− 25.7	− 8.6
3	57	34	46	− 40.4	− 19.3
4	40	39	42	− 2.5	5.0
5	35	34	49	− 2.9	40.0
6	35	28	39	− 20.0	11.4
7	39	25	32	− 35.9	− 17.9
8	35	20	31	− 42.9	− 11.4
9	31	26	27	− 16.1	− 12.9
10	41	32	49	− 22.0	19.5

** Δ =|(C1-C0)/C0| × 100%, ** Δ =|(C2-C0)/C0| × 100%.*

the graft liver at early and late time points were 852 and 1149 cm^3, respectively. The median enlargement rate was 148% at the early time point. Almost all cases showed the enlargement of liver volume, but one case (10%) showed no volume gain, even at the late time point. The rate of enlargement is shown in Table 2. Images of the CT and volume rendering of early and late time points for Cases 1 and 7 are shown (Fig. 1A-D). As shown, the liver volume gradually increased and reached 285% and 216% enlargement in Cases 1 and 7, respectively. Median liver volume enlargement at the late time point for all cases was 177% (Table 2).

2. The number of hepatocytes per field

The number of hepatocytes at the early time points was reduced to 79% of the median value, and it then recovered to almost that of the donor (median 98%, Table 3). Typical H&E images are shown in Figure 2A-F. Almost the entire LDLT dataset demonstrated

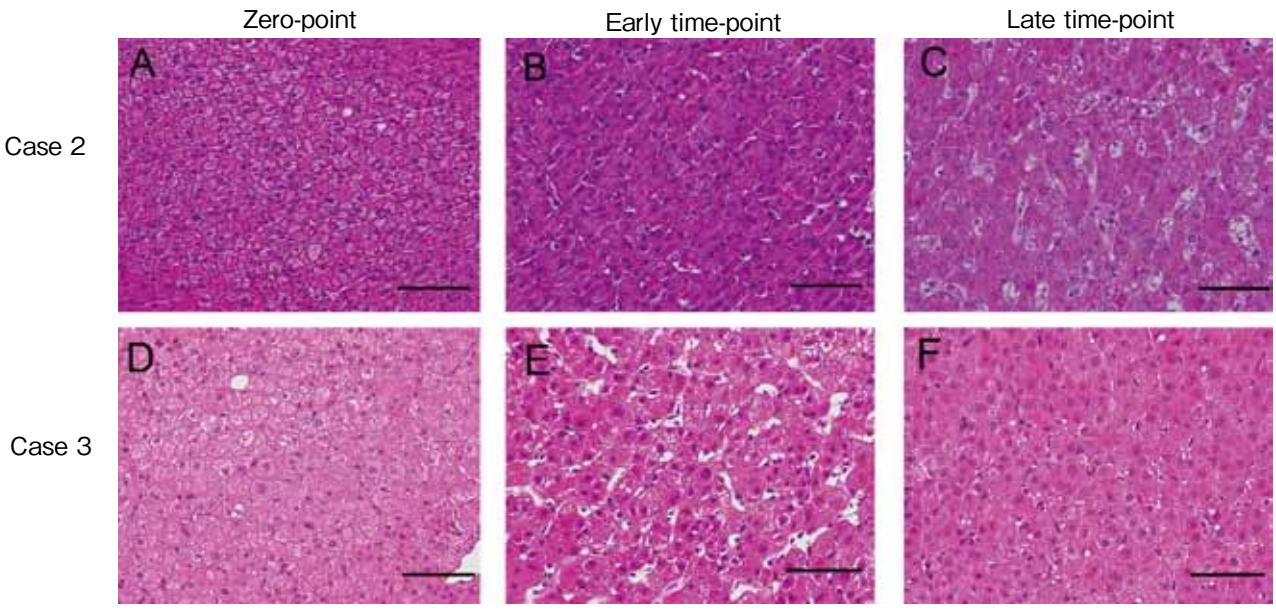

Fig. 2. H&E staining of the liver in the process of regeneration. (A) Zero point, (B) early time point, and (C) late time point of case 2. (D) Zero point, (E) early time point, and (F) late time point of case 3. Scale bar: 20 μm.

the same "v-shape" pattern when plotted, indicating that cells were enlarged shortly after liver transplantation (Fig. 3). The liver volume at the late time point was significantly increased (median 179%), while the number of hepatocytes was not increased (median 98%), suggesting that mechanisms other than early hypertrophy may have been involved.

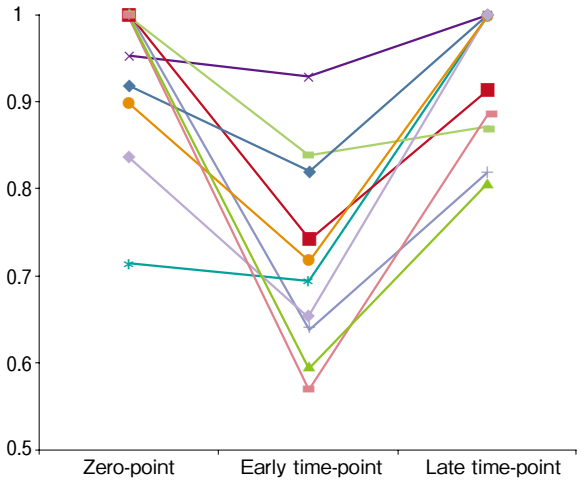

Fig. 3. Changes in the number of hepatocytes. The relative fraction of the maximum number of hepatocytes per area of each time point is plotted. The view area is 200 × 200 μm^2.

IV. Discussion

Here, we demonstrated that hepatocyte proliferation is more dominant in the late time point after LDLT, while hypertrophy is the first trigger of liver regeneration, possibly sharing the process with wound healing. Importantly, the graft liver volume reached 179% during a 6-12-month time period, which should require a similar degree of cell division as is seen in rapidly growing neoplasms. However, cells in the mitotic phase were not evident in the graft samples, indicating that liver regeneration at the late stage requires an extrahepatic origin. Thus, cellular resources should be extrahepatic during the LDLT regeneration process.

Supporting our hypothesis, there have been reports describing the fact that cells of recipient origin were detected in LDLT grafts [11-13]. This observation immediately indicates the involvement of extrahepatic cells. Although the exact origin of the extrahepatic cells has not yet been clarified, some candidate stem cells of bone-marrow mesenchymal stem cell origin are believed to contribute to liver regeneration, such as multilineage-differentiating stress-enduring (Muse) cells [25]. In fact, it has been reported that human Muse cells voluntarily differentiate into AFP-positive cells in vitro [26]. Both AFP and CK19 have been considered to be markers for oval cells, which may differentiate into multiple types of liver tissue components [5]. A specific type of bone-marrow mesenchymal stem cell should be an interesting target to investigate in terms of its application for selected cell-based therapy [22].

It is important to clarify whether any types of liver progenitor cells are present in the process of tissue repair after injury [23,24]. Although the tissue of origin of the liver progenitor cells remains unknown, the prevalence of a recipient signal in each tissue component may suggest that extrahepatic (i.e., recipient) cells that have multipotency, at least partially, may have migrated and differentiated into liver component cells [15]. In the case of male recipients and female liver donors, FISH analysis also revealed that liver component cells showed a Y chromosome signal, which indicates an extrahepatic cellular origin [11-13]. In addition, extrahepatic cells that express the liver progenitor marker may be related to the cells composing hepatocytes and bile-duct-like structures in the periportal area [14]. Based on the present findings and previous reports, we considered extrahepatic cells with differentiation potential to be one of the candidate cells for repairing liver damage. Importantly, in the present study, we suggest that extrahepatic cells may be involved at the late time point after LDLT.

Currently, most liver regeneration studies have been performed using rodent models. The present study, using human LDLD materials, should provide precious insights into developing possible alternative approaches to liver regeneration, such as cell-based therapy using bone marrow cells.

Conflict of interest: The authors have no conflict of interest to declare.

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肝再生における肝外細胞の関与の可能性

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要旨

肝外細胞は肝障害に対する修復に関わる細胞の供給源だと考えられてきた．ヒト生体肝移植（LDLT）のジェノタイピングではレシピエント由来の細胞が複数の種類の肝構成細胞に分化することが報告されている．これは肝外由来かつ多能性を持った細胞が肝再生に貢献していることを示している．しかしながら，臨床的に観察される再生肝の容積の増大の点から肝外細胞の関与による影響は明らかにされていない．本研究では，肝外由来細胞は最終的に肝容積増大に貢献するが，移植後早期の容積増大には肝細胞の肥大が重要な役割を果たすことを示した．また，極めて急速な肝容積の増大にも関わらず分裂期細胞は認められなかった．これらの結果から（i）肝容積の増大は一定数の肝外細胞が肝前駆細胞を経て達成される，（ii）生体肝移植レシピエントの細胞が移植肝の再生に貢献している，ということが示唆された．