definitive endoderm cells were cultured for 3 days on Matrigel in differentiation hESF-DIF medium supplemented with 20 ng/ml BMP4 (R&D Systems) and 20 ng/ml FGF4 (R&D Systems). Transient overexpression of FOXA2 in the mesendoderm cells is not necessary for establishing HBCs, but it is helpful for efficient generation of the HBCs. The HBCs were first purified from the hESC-derived cells (day 9) by selecting attached cells on a human recombinant LN111 (BioLamina)-coated dish 15 minutes after plating (Takayama et al., 2013). The HBCs were cultured on a human LN111-coated dish (2.0×10^4 cells/cm^2) in maintenance DMEM/F12 medium [DMEM/F12 medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS), 1× insulin/transferrin/selenium, 10 mM nicotinamide, 0.1 μM dexamethasone (DEX) (Sigma), 20 mM HEPES, 25 mM $NaHCO_3$, 2 mM L-glutamine, and penicillin/streptomycin] which contained 40 ng/ml HGF (R&D Systems) and 20 ng/ml epidermal growth factors (EGF) (R&D Systems). The medium was refreshed every day. The HBCs were dissociated with Accutase (Millipore) into single cells, and subcultured every 6 or 7 days. The HBCs used in this study were passaged more than three times.

In vitro hepatocyte and cholangiocyte differentiation

To induce hepatocyte differentiation, the HBCs were cultured on a Matrigel-coated dish (7.5×10^4 cells/cm^2) in Hepatocyte Culture Medium (HCM without EGF; Lonza) supplemented with 20 ng/ml HGF, 20 ng/ml Oncostatin M (OsM) (R&D Systems) and 1 μM DEX. To induce cholangiocyte differentiation, the HBCs were cultured in collagen gel. To establish collagen gel plates, 500 μl collagen gel solution [400 μl type I-A collagen (Nitta gelatin), 50 μl 10× DMEM and 50 μl 200 mM HEPES buffer containing 2.2% $NaHCO_3$ and 0.05 M NaOH] was added to each well, and then the plates were incubated at 37°C for 30 minutes. The HBCs (5×10^4 cells) were resuspended in 500 μl differentiation DMEM/F12 medium [DMEM/F12 medium supplemented with 20 mM HEPES, 2 mM L-glutamine, 100 ng/ml EGF and 40 ng/ml ILGF2 (IGF2)], and then mixed with 500 μl of the collagen gel solution and plated onto the basal layer of collagen. After 30 minutes, 2 ml differentiation DMEM/F12 medium was added to the well.

Inhibition of TGFβ signaling

SB-431542 (Santa Cruz Biotechnology), which is a small molecule that acts as a selective inhibitor of activin receptor-like kinase (ALK) receptors [ALK4, ALK5 and ALK7 (also known as ACVR1B, TGFBR1 and ACVR1C)], was used to inhibit TGFβ signaling in HBCs.

Flow cytometry

Single-cell suspensions of hESC-derived cells were fixed with 2% paraformaldehyde (PFA) at 4°C for 20 minutes, and then incubated with primary antibody (supplementary material Table S1) followed by secondary antibody (supplementary material Table S2). Flow cytometry analysis was performed using a FACS LSR Fortessa flow cytometer (BD Biosciences). Cell sorting was performed using a FACS Aria (BD Biosciences).

RNA isolation and reverse transcription (RT)-PCR

Total RNA was isolated from hESCs and their derivatives using ISOGENE (Nippon Gene). cDNA was synthesized using 500 ng total RNA with the SuperScript VILO cDNA Synthesis Kit (Invitrogen). Real-time RT-PCR was performed with SYBR Green PCR Master Mix (Applied Biosystems) using an Applied Biosystems StemOnePlus real-time PCR system. Relative quantification was performed against a standard curve and the values were normalized against the input determined for the housekeeping gene *GAPDH*. Primers are described in supplementary material Table S3.

Immunohistochemistry

Cells were fixed with 4% PFA. After incubation with 0.1% Triton X-100 (Wako), blocking with Blocking One (Nakalai Tesque) or PBS containing 2% FBS, 2% BSA and 0.1% Triton X-100, the cells were incubated with primary antibody (supplementary material Table S1) at 4°C overnight, followed by secondary antibody (supplementary material Table S2) at room temperature for 1 hour. Immunopositive cells were counted in at least eight randomly chosen fields.

HBC colony formation assay

For the colony formation assay, HBCs were cultured at a low density (200 cells/cm^2) on a human LN111-coated dish in maintenance DMEM/F12 medium supplemented with 25 μM LY-27632 (ROCK inhibitor; Millipore).

Transplantation of clonally derived HBCs

Clonally derived HBCs were dissociated using Accutase and then suspended in maintenance DMEM/F12 medium without serum. The HBCs (1×10^6 cells) were transplanted 24 hours after administration of CCl_4 (2 mg/kg) by intrasplenic injection into 8- to 10-week-old *Rag2/Il2rg* double-knockout mice. Recipient mouse livers and blood were harvested 2 weeks after transplantation. Grafts were fixed with 4% PFA and processed for immunohistochemistry. Serum was extracted and subjected to ELISA. All animal experiments were conducted in accordance with institutional guidelines.

ELISA

Levels of human ALB in mouse serum were examined by ELISA using kits from Bethyl Laboratories according to the manufacturer's instructions.

Culture of mouse Dlk1$^+$ cells

Dlk1$^+$ hepatoblasts were isolated from E13.5 mouse livers using anti-mouse Dlk1 monoclonal antibody (MBL International Corporation, D187-4) as described previously (Tanimizu et al., 2003). Dlk1$^+$ cells were resuspended in DMEM/F12 (Sigma) containing 10% FBS, 1× insulin/transferrin/selenium (ITS), 10 mM nicotinamide (Wako), 0.1 μM DEX and 5 mM L-glutamine. Cells were plated on laminin-coated dishes and cultured in medium containing 20 ng/ml HGF, EGF and 25 μM LY-27632 (ROCK inhibitor).

lacZ assay

Hepatoblasts were transduced with Ad-LacZ at 3000 VPs/cell for 1.5 hours. The day after transduction (day 10), 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-Gal) staining was performed as described previously (Kawabata et al., 2005).

Reporter assays

The effects of c/EBPα or c/EBPβ overexpression on *TGFBR2* promoter activity were examined using a reporter assay. An 8 kb fragment of the 5′ flanking region of the *TGFBR2* gene was amplified by PCR using the following primers: Fwd, 5′-CCGAGCTCATGTTTGATGAAGTGTCTAG-CTTCCAAGG-3′; Rev, 5′-GGCTCGAGCCTCGACGTCCAGCCCCT-3′. The fragment was inserted into the *SacI*/*XhoI* sites of pGL3-basic (Promega), resulting in a pGL3-*TGFBR2* promoter region (pGL3-TGFBR2-PR). To generate a *TGFBR2* promoter region containing mutations in the c/EBP binding site, the following primers were used in PCR (mutations are indicated by lowercase letters): Fwd, 5′-CACTAGTATTCAgTG-AtCcgAAAATATGG-3′; Rev, 5′-CACTAGTATTCAgTGAtCcgAAAA-TATGG-3′; this resulted in pGL3-mTGFBR2-PR. HEK293 cells were maintained in DMEM (Wako) supplemented with 10% FBS, penicillin and streptomycin, and 2 mM L-glutamine. In reporter assays, 60 ng pGL3-TGFBR2-PR or pGL3-mTGFBR2-PR was transfected together with 720 ng each expression plasmid (pHMEF5, pHMEF5-c/EBPα and pHMEF5-c/EBPβ) and 60 ng internal control plasmid (pCMV-Renilla luciferase) using Lipofectamine 2000 reagent (Invitrogen). Transfected cells were cultured for 36 hours, and a Dual Luciferase Assay (Promega) was performed according to the manufacturer's instructions.

siRNA-mediated knockdown

Predesigned siRNAs targeting *c/EBPα*, *c/EBPβ* and *TGFBR2* mRNAs were purchased from Thermo Scientific Dharmacon. Cells were transfected with 50 nM siRNA using RNAiMAX (Invitrogen) transfection reagent according to the manufacturer's instructions. As a negative control, we used scrambled siRNA (Qiagen) of a sequence showing no significant similarity to any mammalian gene.

Chromatin immunoprecipitation (ChIP) assay

The ChIP assay kit was purchased from Upstate. Cells were crosslinked using formaldehyde at a final concentration of 1% at 37°C for 10 minutes, and then genomic DNA was fragmented by sonicator. The resulting DNA-protein complexes were immunoprecipitated using the antibodies described in supplementary material Table S1 or control IgG as described in supplementary material Table S2. The precipitated DNA fragments were analyzed by real-time RT-PCR using the primers shown in supplementary material Table S4 to amplify the *TGFBR2* promoter region including the c/EBP binding sites or β-actin locus as a control. The results of quantitative ChIP analysis (Fig. 5A) were expressed as the amount of amplified *TGFBR2* promoter region relative to input DNA taken as 100%.

Statistical analysis

Statistical analysis was performed using an unpaired two-tailed Student's *t*-test. All data are represented as mean ± s.d. (n=3).

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Competing interests
The authors declare no competing financial interests.

Author contributions
K. Takayama, K.K. and H.M. developed the concepts or approach; K. Takayama, Y.N., K.O., H.O. and T.Y. performed experiments; K. Takayama, K.K., M.I., K. Tashiro, F.S., T.H., T.O., M.F.K. and H.M. performed data analysis; K. Takayama, K.K. and H.M. prepared or edited the manuscript prior to submission.

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ヒト多能性幹細胞から肝細胞への分化誘導法の開発とその毒性評価系への応用

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要約　肝臓は薬物代謝に関与する主要な臓器であり、ヒト胚性幹細胞（human embryonic stem cells；ヒト ES 細胞）やヒト人工多能性幹細胞（human induced pluripotent stem cells；ヒト iPS 細胞）からヒト初代培養肝細胞に類似した肝細胞を分化誘導できれば、創薬過程における医薬品候補化合物の肝毒性評価などに使用できる。ヒト ES/iPS 細胞を肝細胞へ分化させるためには、生体内での肝発生・分化の環境を模倣してサイトカインや増殖因子などの各種液性因子を作用させる方法が汎用されている。しかしながら、その肝分化誘導効率は不十分であり、さらなる分化効率の向上が要求されている。そこで、筆者らは肝発生に必要な転写因子をヒト ES/iPS 細胞から分化させた細胞に遺伝子導入することによって、肝分化誘導効率を改善させることを試みた。本稿では、筆者らの最近の研究成果を紹介しつつ、ヒト ES/iPS 細胞から肝細胞への分化誘導の現状と毒性評価系への応用の可能性について概説する。

キーワード：　肝細胞、ヒト ES 細胞、ヒト iPS 細胞、毒性スクリーニング

序　　　文

薬物誘発性肝障害（drug-induced liver injury：DILI）は医薬品の市場撤退原因の主要なものであり、米国においては急性肝障害の50％以上を占める。これまでに600種類以上の薬剤が DILI の起因薬剤として報告されている。現在、ヒト初代培養肝細胞（本稿では、凍結ヒト肝細胞を含めてヒト初代培養肝細胞とする）を用いて、医薬品開発の初期段階に DILI を予測することで、安全性の高い医薬品を開発することが試みられている[1]。しかしながら、ヒト初代培養肝細胞は非常に高価であり、ロット差が大きいこと、培養することで薬物代謝能などの肝機能が急速に減弱すること、高い肝機能を有したヒト肝細胞ロットの安定供給が難しいといった問題を有している[2,3]。そこで、無限に増殖するヒト ES/iPS 細胞から分化誘導した肝細胞（分化誘導肝細胞）を、薬物の毒性評価系などへ応用することが期待されている[4,5]。

ヒト ES/iPS 細胞は中内胚葉、内胚葉、肝幹前駆細胞を通して肝細胞へ分化する。そこで、各分化過程で、生体内での肝発生の環境を模倣してサイ

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トカインや増殖因子などの各種液性因子を作用させる肝分化誘導法が開発された（図1）。しかしながら、サイトカインや増殖因子などの各種液性因子の作用のみからなる分化誘導法では、肝分化誘導効率は不十分であり、分化効率の向上が必要であった。そこで、著者らは各々の分化段階の細胞に肝分化に関連する転写因子を順次遺伝子導入することで、肝細胞への分化を飛躍的に促進させる方法を開発した。

本稿では、ヒトES/iPS細胞から肝細胞への分化誘導の現状について、著者らの最新の研究成果を紹介するとともに、分化誘導肝細胞の毒性評価系への応用の可能性について解説する。

1．分化誘導肝細胞の肝機能評価

肝臓は、生体内に投与された薬物を代謝する主要な臓器であるだけでなく、脂質代謝や糖代謝、胆汁（酸）やアルブミン（ALB）の生産・分泌等、多彩な機能を有する。それゆえ、ヒトES/iPS細胞から分化誘導した肝細胞の機能を評価するにあたっては、様々な機能を検討する必要がある。そこでまず、ヒトES/iPS細胞から肝細胞への分化誘導と毒性評価系への応用について紹介する前に、肝細胞としての機能を評価する場合に必要とされる検討項目について解説する。

分化誘導肝細胞の評価としては、肝細胞の形状、肝関連遺伝子・タンパク質の発現、ALB産生能をはじめとする肝機能の評価が必要である[6-9]。具体的には、分化誘導肝細胞の形状として、肝細胞に特徴的な構造（グリコーゲン顆粒、ゴルジ体の豊富な細胞質、微細胆管構造、頂端側の微絨毛、よく発達した密着結合など）の形成度合が評価される[6]。また、alpha-1-antitrypsin（αAT）、ALB、各種cytochrome P450（CYP）などの遺伝子発現量や、ALB産生、尿素産生[8]、グリコーゲン貯蔵[8,10]、低密度リポタンパク（LDL）の取り込み能[9,10]、インドシアニングリーンの取り込み・排泄能[8,10]の検討も必要であり、これらの機能をヒト初代培養肝細胞と比較検討することが好ましい。これらの評価項目については、これまで発表されてきた論文等で多く実施されている（ヒト初代培養肝細胞と比較検討した例は極めて限られるが）。しかしながら、DILIを引き起こす薬物の多くは、肝臓で薬物代謝酵素により代謝されて反応性代謝物を生じ、肝障害を引き起こすことが知られるため[11]、分化誘導肝細胞を毒性評価に応用する場合には、上述の評価項目に加えて、ヒト初代培養肝細胞に類似

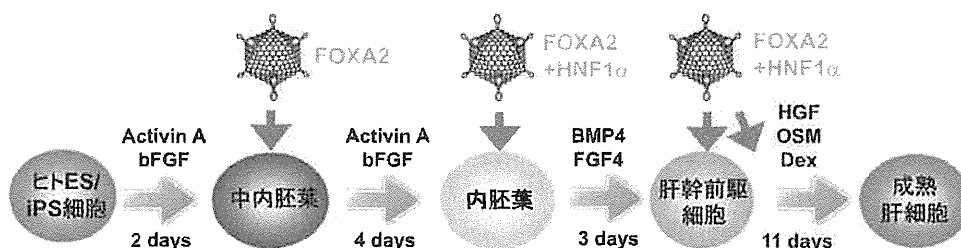

図1　FOXA2、HNF1α遺伝子の導入によるヒトES/iPS細胞から肝細胞への効率の良い分化誘導
ヒトES/iPS細胞は中内胚葉、内胚葉、肝幹前駆細胞を介して、肝細胞へと分化する。ヒトES/iPS細胞から内胚葉への分化にはactivin Aが使用される。内胚葉から肝幹前駆細胞への分化にはBMPおよびFGFが汎用される。肝幹前駆細胞から肝細胞への分化にはHGF、OsM、DEXが使用される。内胚葉分化を促進するために、分化誘導された中内胚葉にFOXA2遺伝子を導入した。肝幹前駆細胞への分化を促進するために、分化誘導された内胚葉にFOXA2、HNF1α遺伝子を導入した。肝細胞への分化を促進するために、肝幹前駆細胞にFOXA2、HNF1α遺伝子を導入した。このように肝細胞分化への適切な時期に適切な転写因子を過剰発現させることで、ヒトES/iPS細胞から肝細胞への効率の良い分化誘導が可能となった。

した薬剤代謝酵素活性を有するか否かの確認が最重要である。

2．ヒトES/iPS細胞から肝細胞への分化誘導法

増殖因子やサイトカインの添加だけからなる従来の肝分化誘導法は、肝細胞への分化誘導効率が不十分であり、さらなる分化効率の向上が必要である[12,13]。そこで、筆者らは増殖因子やサイトカインを作用させる従来の分化誘導法で細胞の外部環境を分化に適した状態にした上に、細胞内から強制的に分化を進行させるように肝分化に関連した転写因子を過剰発現させることで、肝分化誘導効率を向上させる方法を開発した[10,14-16]。即ち、ヒトES/iPS細胞から分化誘導した中内胚葉にsry-related HMG box 17（SOX17）遺伝子を[14]、内胚葉から肝幹前駆細胞への分化ステップではhematopoietically expressed homeobox（HEX）遺伝子を[15]、肝幹前駆細胞から肝細胞への分化ステップではhepatocyte nuclear factor 4α（HNF4α）遺伝子を導入することで[10]、高いアルブミン産生能、グリコーゲン貯蔵能、LDL取り込み能、インドシアニングリーン取り込み能等を有した肝細胞を分化誘導することに成功した。さらに最近では、ヒトES/iPS細胞から肝細胞への各分化ステップにおいて7種類の肝関連転写因子（FOXA2、SOX17、HEX、HNF1α、HNF1β、HNF4α、HNF6）を導入し、最も効率良く肝分化を促進できる転写因子を探索した結果、forkhead box protein A2（FOXA2）およびhepatocyte nuclear factor 1homeobox A（HNF1α）遺伝子を組み合わせて発現させることにより、さらに効率良く成熟肝細胞を分化誘導することに成功した[16]（図1）。このようにして作製した分化誘導肝細胞は、80～90％以上の細胞がアシアロ糖タンパク質受容体（成熟した肝細胞のマーカー）陽性であり、ヒト初代培養肝細胞に匹敵する薬物代謝酵素の遺伝子発現レベルを示した。また、CYPなどで代謝される9種類の薬物の代謝プロファイルを調べたところ、分化誘導肝細胞の薬物代謝能はヒト初代培養肝細胞より低いものの（ヒト初代培養肝細胞の1～40％程度の活性）、いずれの薬物に対しても代謝能を有していることが確認された[16]。今後は、より高いCYP活性を有した分化誘導細胞を作製することが必要となる。

3．分化誘導肝細胞の創薬応用の可能性について

筆者らは分化誘導肝細胞を薬剤に対する毒性評価に応用できるか検討した。トログリタゾン、アセトアミノフェンといった肝毒性を起こす薬物を、上述の遺伝子導入を組み合わせた分化誘導法で作製した分化誘導肝細胞に作用させたところ、細胞毒性を生じることを確認した[10]。また、肝毒性を引き起こす20種類以上の薬剤について、筆者らの分化誘導肝細胞を用いて細胞毒性評価試験を実施したところ、*in vitro* 肝毒性評価系に汎用されるHepG2細胞（肝がん細胞由来細胞株）を用いた場合に比べ、より感度良く細胞毒性を示し、かつその細胞毒性はCYPの阻害剤を作用させることで部分的に消失した[17]。したがって、薬剤がCYPで代謝されることで生じた反応性代謝物によって生じた細胞傷害性を、分化誘導肝細胞が検出できることが示唆された[17]。

一般に、薬剤がDILIを引き起こすメカニズムを解明することは困難であるが、DILIによって、肝細胞の細胞小器官（ミトコンドリア[18,19]、小胞体[20]）に障害が生じることが知られている。今後は、薬剤がミトコンドリア毒性や小胞体ストレスに及ぼす影響についても調べることによって、分化誘導肝細胞のより広範な毒性評価への応用が期待される。

お わ り に

ヒト ES/iPS 細胞から肝細胞への分化誘導において、使用する液性因子、低分子化合物、細胞外基質、共培養細胞など様々な培養条件が検討されてきた。本稿で紹介したように、肝分化に関連した転写因子を分化過程の細胞に遺伝子導入することによって、さらなる肝分化誘導効率の向上が可能になったが、ヒト ES/iPS 細胞から肝細胞への分化誘導は現在まさに研究開発途上の技術である。今後、分化誘導肝細胞を作製する技術がさらに向上し、よりヒト初代培養肝細胞に類似した機能を有した細胞が作製されることで、薬物の毒性評価系への応用をはじめとする創薬研究、さらには再生医療への応用に貢献することが期待される。

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Generation of hepatocyte-like cells from human pluripotent stem cells for drug screening

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Abstract

Human embryonic stem (ES) cell and induced pluripotent stem (iPS) cell have ability to differentiate into all body cells, including hepatocytes. Hepatocyte-like cells generated from human ES/iPS cells are expected to be utilized in medical application such as drug screening. However, the existing protocols for hepatic differentiation of pluripotent stem cells are not enough efficient. To promote hepatic differentiation, we developed an efficient method to differentiate hepatocyte-like cells from human ES/iPS cells by overexpression of the hepatocyte-related genes. In this review, we will introduce the present status and feature view of the hepatic differentiation from human ES/iPS cells.

Key words: hepatocytes, human ES cells, human iPS cells, drug screening

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LETTER FROM THE FRONTLINE

Hepatocyte Transplantation Using a Living Donor Reduced Graft in a Baby With Ornithine Transcarbamylase Deficiency: A Novel Source of Hepatocytes

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TO THE EDITORS:

We performed hepatocyte transplantation (HT) in an 11-day-old infant with ornithine transcarbamylase deficiency (OTCD). We used cryopreserved hepatocytes prepared from remnant liver tissue, a byproduct of a hyper-reduced left lateral segment from living donor liver transplantation (LDLT). The patient exhibited hypothermia, drowsiness, and apnea at 3 days of age; these symptoms were accompanied by hyperammonemia (1940 μg/dL at maximum), although there were no abnormalities at birth or an obvious family history (Fig. 1). Further examinations confirmed that the hyperammonemia was the result of OTCD. Multimodal treatments, including alimentotherapy, medications, and continuous hemodiafiltration (CHDF), did not improve the patient's clinical state, and severe hyperammonemia attacks recurred. Because of the patient's small body size (2550 g) and the lack of an available liver donor, HT was indicated. Hepatocytes of the same blood type were chosen from an institutional repository of cryopreserved hepatocytes prepared from the remnant tissue of segment III from unrelated living donors. Thawed hepatocytes were transplanted twice at 11 and 14 days of age with a double-lumen catheter inserted into the left portal vein via the umbilical vein (Fig. 2). The amounts of transplanted hepatocytes were 7.4×10^7 and 6.6×10^7 cells/body, and the viability rates were 89.1% and 82.6%, respectively. The portal flow was kept stable at greater than 10 mL/kg/minute, and the pressure was maintained at less than 20 mm Hg during and after HT. The immunosuppressive treatment followed the same protocol used for LDLT with tacrolimus and low-dose steroids.[1] The patient was weaned from CHDF and the ventilator at 26 and 30 days of age, respectively, with a stable serum ammonia level of 40 μg/dL. The patient was ultimately discharged 56 days after HT. During the 3 months of follow-up, the baby did well with protein restriction (2 g/kg/day), medication for OTCD, and immunosuppression. No neurological sequelae related to hyperammonemia have been observed so far (Fig. 1).

DISCUSSION

For children with metabolic liver disease, HT is indicated as an alternative or bridge to liver transplantation.[2] HT is less invasive than liver transplantation and can be performed repeatedly. Limitations to the widespread application of HT include the poor availability of hepatocytes. Therefore, it is important to find new sources of high-quality hepatocytes. We previously prepared a repository of hepatocytes obtained from remnant liver tissue, a byproduct of hyper-reduced left lateral segmentectomy in LDLT.[1]

The cell donor was an unrelated volunteer with the same blood type who had previously undergone hyper-reduced left lateral segmentectomy. The main unit of segment II was used as a monosegmental liver graft for the primary recipient with end-stage liver disease, and the remnant was used to isolate hepatocytes with fully informed consent. The hepatocytes were isolated according to the collagenase perfusion method, as described elsewhere,[3] with Liberase MTF C/T GMP grade (Roche). All procedures were performed at our cell processing center according to a strictly controlled protocol based on good manufacturing practices. The total number of transplanted live hepatocytes was 1.4×10^8 cells/body; the ammonia removal rate was more than 200 fmol/cell/hour (203.4 and 265.4 fmol/cell/hour with the first and second injections, respectively). The dose was judged to be sufficiently high to obtain therapeutic effectiveness according to our theoretical background.[4]

This work was supported by a grant-in-aid from the National Center for Child Health and Development and the Highway Program for the Realization of Regenerative Medicine (Japanese Science and Technology Agency). This study protocol was approved by institutional review board in National Center for Child Health and Development (reference number 433).

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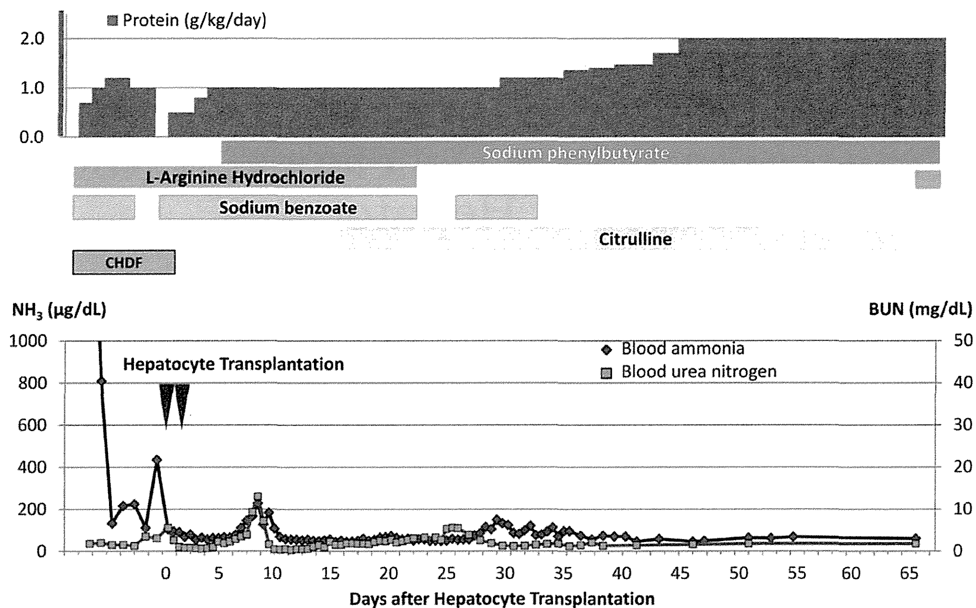

Figure. 1. Treatment schedule (top) and patient condition (bottom). The changes with time for blood ammonia and blood urea nitrogen are shown. The baby was delivered vaginally as a first child. At 3 days of age, hypothermia, low oxygen saturation, and, finally, respiratory arrest occurred. The patient was incubated and given artificial respiration. Concurrently, hyperammonemia (1940 μg/dL) was found, and continuous hemodiafiltration (CHDF) was started in addition to alimentotherapy (protein withdrawal) and medications. Whenever the administration of essential amino acids was restarted, the blood ammonia level became elevated, and at 9 days of age, despite the suspension of essential amino acid administration, the level increased up to 434 μg/dL. At 11 days of age, HT was performed for the first time, and it was performed for the second time at 14 days of age. After HT, amino acid intake was restarted along with the continuation of multimodal treatments, and blood ammonia was controlled well except for episodic increases. The patient was weaned from CHDF and the ventilator at 26 and 30 days of age, respectively, and the patient was ultimately discharged 56 days after HT.

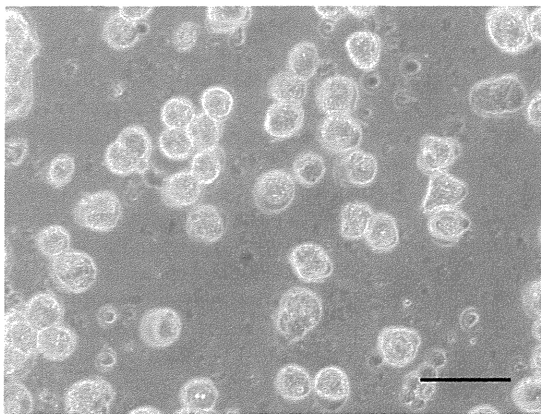

Figure. 2. Hepatocytes transplanted during the first injection. The cells showed a glazed and firm surface. The bar indicates 50 μm.

Because liver transplantation is approved as a treatment for end-stage hepatic failure, donor livers are preferentially allocated for organ transplantation and not for hepatocyte isolation. On rare occasions, the lack of appropriate donor-recipient matching (eg, infant donor livers) provides good-quality hepatocytes.[2] Fetal livers are also considered to be an alternative cell source, although ethical issues remain to be resolved. At present, we have little choice but to use marginal donor tissues, such as livers obtained from donors after cardiac death and organs with steatosis, fibrosis, or a long ischemia time. However, there are unfavorable issues related to the use of marginal donors, including low viability and vulnerability to cryopreservation. In this respect, the remnant liver tissue of hyper-reduction procedures used in LDLT has the same quality as that of left lateral segment grafts. As for availability, there are 5 cases of hyper-reduction per year at our institution on average.[5] The use of remnant liver tissues obtained from hyper-reduced LDLT procedures will, therefore, help to address the shortage of hepatocyte donors.

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