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研究課題名（和文）胆管構造を有する機能的肝組織再構築に関する研究

研究課題名（英文）Functional liver tissue rebuilding with bile duct structure on heterotopic transplantation

研究代表者

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交付決定額（研究期間全体）：（直接経費） 3,600,000 円

研究成果の概要（和文）：ホモジネート肝組織の異所性移植（腸管粘膜下）における脂肪組織由来幹細胞(ADRC)の肝細胞機能維持や肝構成細胞（特に胆管細胞）分化の可能性を検討した。肝組織移植はADRCとの共移植により肝機能保護効果・肝細胞分化を示したが、胆管などの肝構成細胞への分化は認めず、共移植前のADRC分化誘導が必要と考えられた。また3次元培養システムも単離肝細胞保護効果を有し、ADRCとの併用に期待が持たれた。

研究成果の学術的意義や社会的意義

本研究ではADRC・3次元培養システムを活用した異所性肝移植の長期安定化や胆管構築を伴った機能的異所性肝組織再構築の可能性について検討した。残念ながら、ADRCの肝細胞機能維持は確認されたが、胆管細胞分化・組織再構築までの結果を得ることはできず、胆管構造を有する組織再構築には、課題が残ったが、3次元培養システムの可能性や肝組織再構築手法の方向性を示すことはできたと思われる。

研究成果の概要（英文）：The purpose of this study was to establish the procedure of functional liver tissue rebuilding with bile duct structure on heterotopic liver tissue transplantation using adipose-derived regenerative cells (ADRC) and 3D culture system.

We demonstrated the protective effect of ADRC for transplant liver function and the differentiation to hepatocyte like cell, but not the ability to differentiate along multiple lineage pathways for other liver component cell from ADRC. Therefore, the pre-induction protocol of ADRC towards cholangiocyte seemed to be necessary before the combined transplantation. Also, 3D culture system indicated the protective effect of primary hepatocyte culture. But, we could not establish the optimal protocol of cholangiocyte differentiation from ADRC, yet. Consequently, while there were some future problems, the possibility of 3D culture system on liver tissue transplantation and the directionality of liver tissue rebuilding approach could be indicated in this study.

研究分野：医歯薬学、外科系臨床医学、外科学一般

キーワード：異所性肝移植 ADRC 胆管細胞 3次元培養

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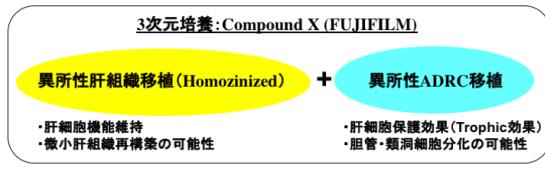
activin□ TGF- β supergene family
follistatin bDb%2II Hepatogastroenterol. 2005,
SHh8I□ H20~21

2007□ □ gB8III
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8G±KS) J Hepatobiliary Pancreat
Sci. 2014III GbG□ □ ADRC c□
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SB(+I(±b)(± b±I
□ OM□ □ (±w±d±I
□ d)I□ b 6×□ □ □
S(S±d)S(b
±v8I OM□

□%□ 2b2I
(1) +(±±)I
+ADRC□
□ ADRC b□
+OMGKS□ 7□ 8 4K SD □
□ 70%J7V □ □ .k/IV 20min □



----- ADRCによる抗炎症・免疫寛容効果
・豊富な血流
・早い細胞周期
・生理的胆汁排泄経路

腸管crypt細胞(腸管幹細胞)の分化・組織再構築の関与?

胆汁排泄機能を有する機能的肝組織の再構築・長期生着

図1：3D培養を用いた異所性肝組織+ADRC移植の可能性

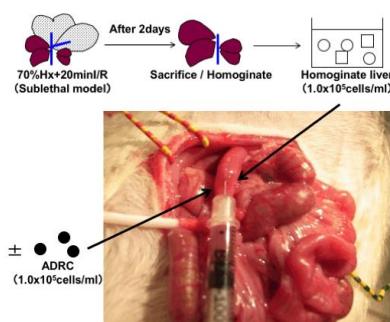


図2：消化管粘膜下への肝組織+ADRC移植モデルの作成

+ADRC1×10⁵ml 1ml 27Gy 5ml 1ml 1ml i)
+ (ii) (iii) (wHE/H/wAH)
□ 2□ □) 10 □ □
4PKC%~70AM/□
D605/□

(2) +□ (ADRC b))□
 □ ADRC b))□
 ADRC b)EMT)HS
 B(+)b)OMSu(+b/7b0s\$□
 7□ 84□ SD □ 5mm 67x□'GvK□ 2mM EDTA+PBS □ 30min □
 □ Medium □Vd□ PBS □wKS□(7EM □VdK□
 PBS □□
 42°NG/Mq□□ Cell strainerde □'(+□ Small intestinal crypt(7M□
 0□ FACS □ stemness marker □ M□

(3) 3D culture □ □ primary hepatocyte □ □

3D culture □ □ 80M □

□ □ K □ □

□ □ □ □ □ □ □ □ □ □

□ □ 10% □ □

□ □ □ □ □ □

2D culture LcbrII □ 6well plate □ (±)

8) E) F □ 1x10⁵ □ K □ 3D culture Lc (±)

% T □ 96well U □ plate □ CellSai □ FUJIFILM

3 8 □ b) □ 0.1mg/ml □ (±)

1x10⁵ □ K 8 □ K □ viability x □

□ □ 80M □

7 □ 8 4K C57BL/6J □

0.5 mg/mL □ K S □

0.06 mg/mL collagenase □ □

50 g, 3 min at 4°C x 2 □ □

S □ I □

```

graph LR
    A((ADSCs)) --> B(( ))
    B -- "2 Days" --> C(( ))
    C -- "3 Days" --> D(( ))
    D --> E(( ))
    E -- "5 Days" --> F(( ))
    F --> G(( ))
    G -- "5 Days" --> H(( ))
    H -- "5 Days" --> I(( ))
    I --> J(( ))
    style A fill:#ccc,stroke:#000
    style B fill:#fff,stroke:#000
    style C fill:#fff,stroke:#000
    style D fill:#fff,stroke:#000
    style E fill:#fff,stroke:#000
    style F fill:#fff,stroke:#000
    style G fill:#fff,stroke:#000
    style H fill:#fff,stroke:#000
    style I fill:#fff,stroke:#000
    style J fill:#fff,stroke:#000
    
```

STEP1 (5 days)

CHIR-99021, ITS
DMEM/F12

STEP2 (5 days)

BSA / ITS / BMP2 / FGF4

STEP3 (10 days)

① MEM / NEAA + HGF
② MEM/NEAA+ HGF
/ Oncostatin M / Dexamethasone

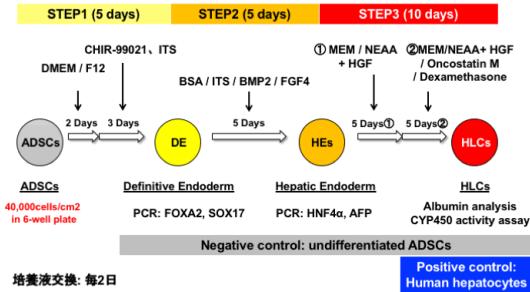


図3：ADRC-hepatocyte like cell 分化プロトコール

(4) ADRC □□□ (⊕) □
 □ (⊕)lb(OKS)
 DMEM □
 GDS(bOMS)
 u ADRC bSB(⊕OKS)
 plate KRbKCC days 8 □ ADRC 40,000cells/cm² □ 6-well
 8d (⊕) (10) □ 3 □
 Step 1: Day1~2; serum-free DMEM/F-12 medium, Day3; DMEM/F-12 containing 2 μM GSK3
 inhibitors Chir99021 □ 0.5 mg/mL albumin fraction V, Day4~5; DMEM/F-12 containing 1% ITS □ 0.5
 mg/mL albumin fraction V
 Step 2: Day6~10; MEM/NEAA containing 1% ITS □ 0.5 mg/mL albumin fraction V □ 20ng/ml BMP2 □
 30ng/ml FGF4
 Step 3: Day11~15; MEM/NEAA containing 1% ITS □ 0.5 mg/mL albumin fraction V □ 20ng/ml HGF
 Day11~15: MEM/NEAA containing 1% ITS □ 0.5 mg/mL albumin fraction V □ 20ng/ml HGF □ 10ng/m
 OSM □ 10-6M Dexamethasone
 +/-% □ Definitive endoderm: DE □ Hepatocyte like cell 8R(+)o □
 /
 in 6-well plate PCR: FOXA2, SOX17 PCR: HNF-4α, AFP CYP450 activity assay
 Negative control: undifferentiated ADSCs Positive control:
 Human hepatocytes

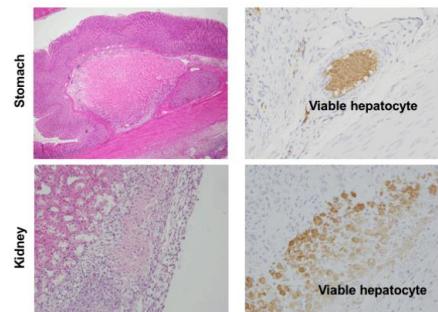


図4：臓器粘膜(被膜)下への肝組織移植モデル(移植14days)

14 % □ CSFE □ KS
 □ 6 □ S(□ T□ C□)
 □ ADRC b□ MG□ 06
 □ ADRC b□ MG□

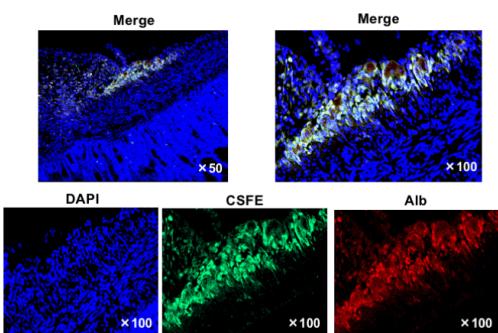


図5：胃粘膜下への肝組織+ADRC移植モデル(移植14days)

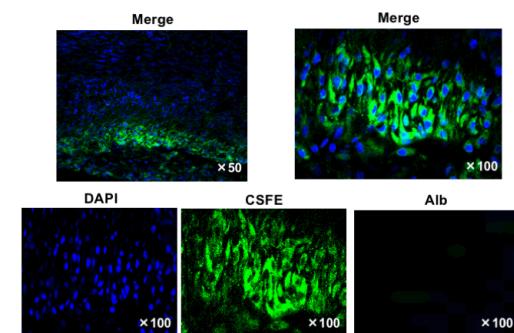


図6：十二指腸粘膜下への肝組織+ADRC移植モデル(移植14days)

bg0608
 nd□ ADRC □ +/□% f□
 O□E□
 06S□

(2) +□ G□G□
 □ isolation□
 84K SD □ 5mm 67x□'□
 GvK□ 2mM EDTA+PBS □ 30min □
 K□ Medium □ PBS □ wKS□
 42°(7EMVdK□ PBS □
 42°XG/K□ Cell strainerde □'
 (□ Small intestinal crypt□(7AS)
 □ 7□□
 K□ KGb□ crypt □ SB(±□ (□ □ c□ Gb0E□ k□
 g□ A□ S□ □

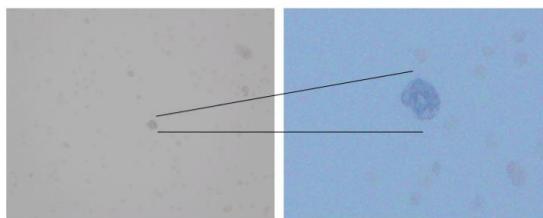


図7：小腸幹細胞isolationによるCrypt様細胞採取

(3) 3D culture □ primary hepatocyte □
 CellSaic □ 3D culture Lc8□ 48 6□ 3.5 □ /well bgBK8S
 8□PI □ viability b0E□ 3D culture Lc8□ 9 □ 80% b cell viability
 JTK8S□ 2D culture Lc8□ 3 □ 8□ 9 □ 50%□
 K8S□ f8S□ 7, 9 b8W 3D culture LwJ□
 8S□ Day9□ 3D culture □ vs. 2D culture □ 95.5±14.7 vs. 28.7±11.2 □ 9 □

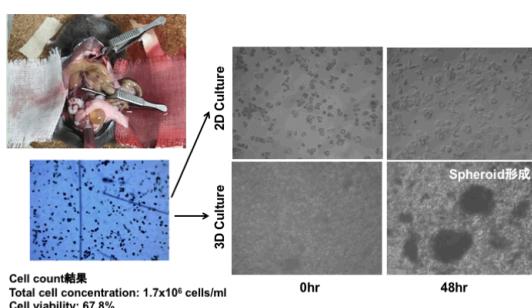


図8：3D培養によるマウスprimary hepatocyte機能維持効果

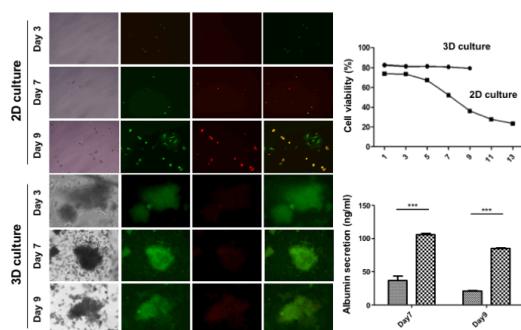


図9：3D培養によるマウスprimary hepatocyte保護効果

GbGE 3D culture b□b(±□Mv6□
 (+□IR(□KS ADRC □ +/□% b8E□S(□
 M3□6□ S□

(4) ADRC □ □ (□
 ADRC c(±□ggM(±□KS
 fS DE c ADRC bK□ Sox2 / Oct4 b□EK□
 □ SOX17□MKS□ CYP3A4 q□
 MKS □ 11 □ KK□Z28□ ADRC □
 1CAS□

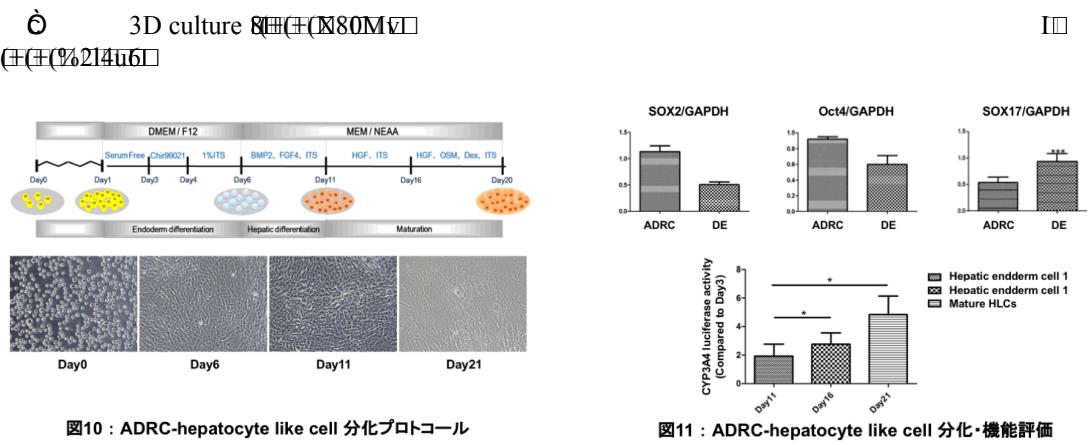


図10 : ADRC-hepatocyte like cell 分化プロトコール

図11：ADRC-hepatocyte like cell 分化・機能評価

Mb0418

ADRC b(+□) □ (IMvbb(+bSB(+lb(D)))□

S(E+N) ADRC b (TSIK006SF)

S 3D culture GMG 66×2M

ISD ADRC 5G D

ADRC v(+)MGc

3 ,z/e

1

7\\$1e8f

12 6

- D Ikemoto T, Morine Y, Imura S, Saito Y, H□ 4 % H□. The protective effect of epigallocatechin 3-gallate on mouse pancreatic islets via the Nrf2 pathway. *Surg Today*. 2019;49(6):536-545. 1w doi: 10.1007/s00595-019-1761-0.

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q a . 田 □ 7 □ . 3 % + □ KS6 □ % (± □)

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.□ 118 G/II

- 14 , □ , □ 5 □ , 3 % . Epigallocatechin-3-gallate c Nrf2 K+□
 186M □ . □ 118 G+□ 2018.
- s 14 One Stop Shop Simulation □ Mixed reality S
 □ Navigation. □ 118 G+□ 2018.
- t 14 , 10 , 5 , 1e Ú 4 □ , 4 % . \$ (LED)66x100
 1000M%2 . □ 36 G+2□ . 2018.5.26 □ .
- u 10 , 100 □ 6 □ , 3 % . Nrf2)2°□ EGCG b+□ . □ 56 G□
 100 . 2018.
- v 1\$, 100 □ 8 □ , 3 % . \$ ROS/ ERK pathway KIS2e, □ LED
 b(+40 . □ 117 G+□ . 2017.
- w 10 , 100 □ 5 □ , 5 % . Epigallocatechin-3-gallate (EGCG) □ Nrf2-Keap1 D
 (KIS+186M0) . □ 72 G+□ . 2017. 5 □ .
- x 1e Ú 7 □ , 3 % . EOB-MRI is useful for 'one stop shop' modality based on functional
 liver volume. □ 29 G+□ 6 G□ R+□ . 2017.
- y 14 , 100 □ 8 □ , 3 % . \$ 100 . 2017.
- z 1\$, 100 □ 12 □ , 3 % . \$ ROS/ ERK pathway KIS2e, □
 LED b(+40 . □ 46 G+□ . 2017.
- 1a , 100 □ 5 □ , 4 % . +PMKb Adipose tissue
 derived stem cells b+ Insulin-producing cells p□ . □ 53 G+□ . 2017.
- 14 , 100 □ 8 □ , 8 % . HDAC 7cc+□ . 116 G+□ . 2016.
- 1a , 100 □ 6 □ , 4 % . \$ 8B6MS □ Insulin-producing cell
 8B6MS □ . □ 71 G+□ . 2016.

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 14 & SAITO, Yu>
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 48 \$7T
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