様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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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

activin TGF-β supergene family
follistatin Hepatogastroenterol. 2005,
2007 SHH H20~21
Surg Today. 2013

adipose tissue-derived
mesenchymal regenelative cell ADRC H22~24 J Surg
Res. 2013, J Hepatobiliary Pancreat Sci. 2014 Epigenetics
H25~27

Transpl Int. 2014 homogenize

Liver Int. 2014

J Exp Clin Cancer Res. 2000

TNF-α T
J Cell Mol Med. 2009 ADRC Trophic
cell-cell contact
VEGF ADRC
J Hepatobiliary Pancreat Sci. 2014 ADRC

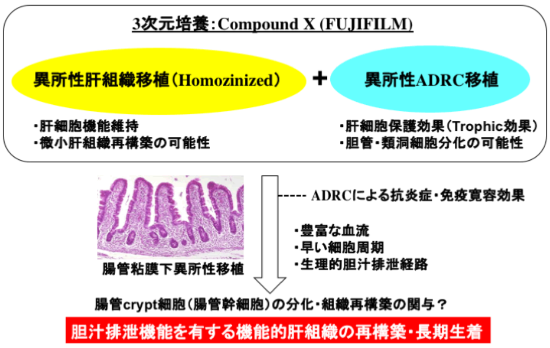

図1：3D培養を用いた異所性肝組織+ADRC移植の可能性

1

homogenize ADRC ADRC

(1)
+ADRC
ADRC
7 8 SD
70% 20min

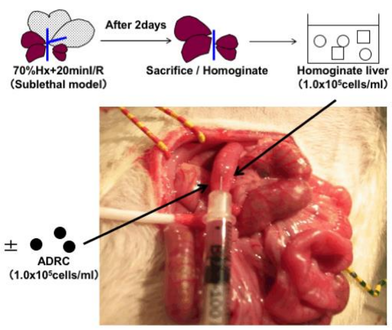

図2：消化管粘膜下への肝組織+ADRC移植モデルの作成

+ADRC1×10 1ml K 27Gy 58 1ml i)
+`(w ii) H(w iii) (wMbH/wA
2
4)PK°5c×%~7o[AM/)× IO
)b0o/

(2) + isolation
)) ADRC b)) ADRC b0MwC(+bS
B(+(b0MSu+`(+b7/bg0sS
7 8 4K SD 5mm 67x+`GvK 2mM EDTA+PBS M 30min M
Medium 7Vd PBS MwKS42°(7+M7VdK PBS I
42°XG/Mq) Cell strainerde `+`(+ Small intestinal crypt(7M
0oc FACS stemness marker M

(3) 3D culture primary hepatocyte H)
3D culture 7(+H)TX80M 7 8 4K C57BL/6J
+7+D44 0.05M 0.5 mg/mL K
K K 0.06 mg/mL collagenase IS ,
M /+wv~7VA(+7%4IO42°M 50 g, 3 min at 4°C x 2
b 10%/ 5 S I
7(+ 8M
2D culture Lcbrr 6well plate (+
8)E)F 1x10^5 K 3D culture Lc(+
%T 96well U plate) CellSaic FUJIFILM
3 8 b) 0.1mg/ml (+
1x10^5 K8 K(+ viability x+
X80oM

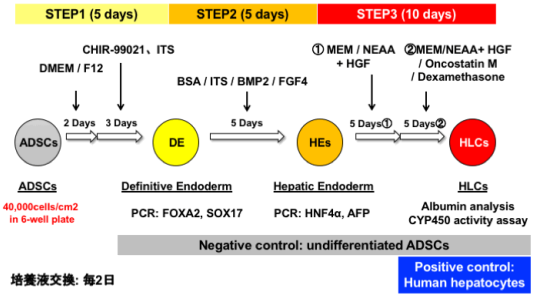

図3：ADRC-hepatocyte like cell 分化プロトコール

(4) ADRC (H(
(+(+lb(1KS
)v))M
GDS(b0MS
u ADRC bSB(+(0KS ADRC 40,000cells/cm^2 6-well
plate KRbvKCc days8 b8
8d+ (+ (IO 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/ml OSM 10-6M Dexamethasone
+/+% Definitive endoderm: DE Hepatocyte like cell 8R(+0o
/

2%2B
(1) +(H)
+ADRC
)(+
+hh)1
14 c%T))ch+H/
+wbs6S 4 ADRC CSFE
1K h+'h
) +ADRC vKCc ADRC '/
dKSG h /□ 14
) +ADRC Lb1 ADRC
c(+(K8S 5
ADRC '/c1(+uSSB(+(gIS))bs1uS
I) +ADRC L 8 ADRC x+`□ (+b(+(+1
b(4c1uS

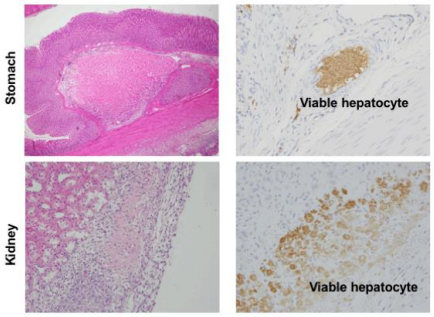

図4：臓器粘膜（被膜）下への肝組織移植モデル（移植14days）

14 % CSFE ADRC
6
ADRC
ADRC 06 ADRC

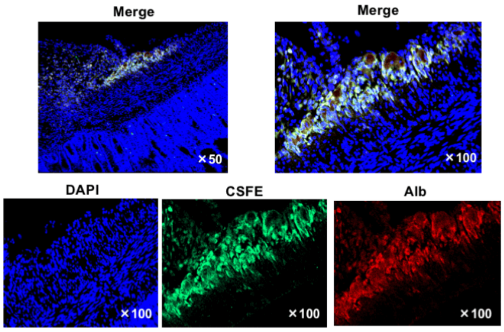

図5：胃粘膜下への肝組織+ADRC移植モデル（移植14days）

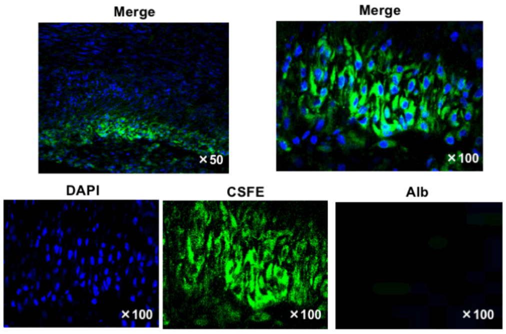

図6：十二指腸粘膜下への肝組織+ADRC移植モデル（移植14days）

ADRC

(2)
isolation
8 SD 5mm
2mM EDTA+PBS 30min
Medium PBS
42° PBS
42° Cell strainerde
Small intestinal crypt
7
crypt

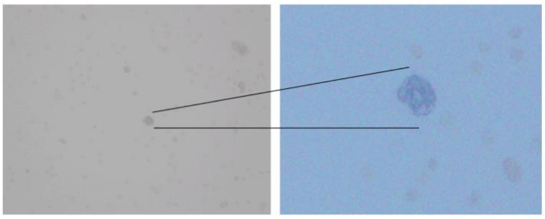
図7：小腸幹細胞isolationによるCrypt様細胞採取

(3) 3D culture primary hepatocyte
CellSaic 3D culture 48 3.5 /well
8 PI viability 3D culture 9 80% cell viability
2D culture 3 9 50%
7, 9 3D culture
Day9 3D culture vs. 2D culture 95.5±14.7 vs. 28.7±11.2 9

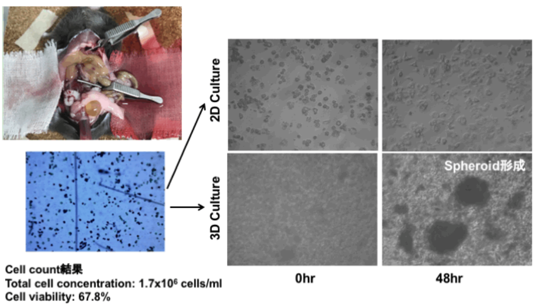

図8：3D培養によるマウスprimary hepatocyte機能維持効果

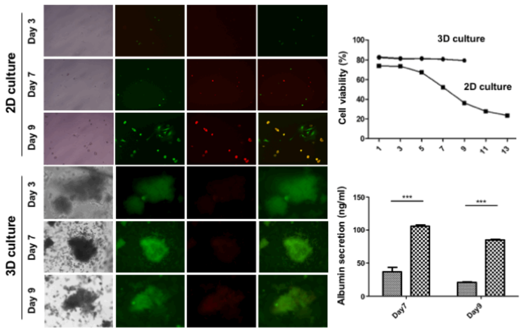

図9：3D培養によるマウスprimary hepatocyte保護効果

3D culture
ADRC

(4) ADRC
ADRC 10
DE ADRC Sox2 / Oct4
SOX17 CYP3A4
11 ADRC

Ò 3D culture 8(⊞(+(X80Mv□ I□
(+(+(%214u6□

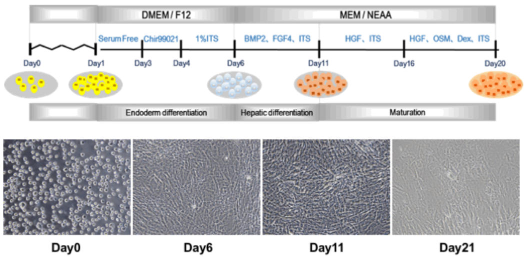

図10：ADRC-hepatocyte like cell 分化プロトコール

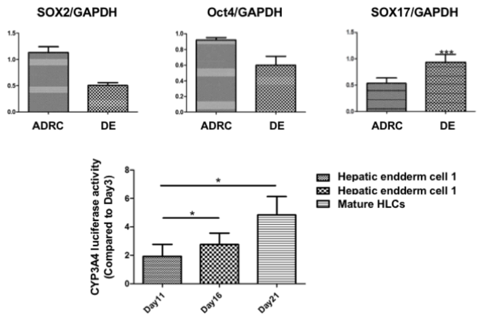

図11：ADRC-hepatocyte like cell 分化・機能評価

Vb0)d))8□ ADRC v(+MGc□
ADRC b(+□ I□ (1Mvbb(+bSB(+lb(1))□
S(c+N□ ADRC b□ □ (1SIKC06Sr□
S□ 3D culture GMG-))b6×2M+g□
IS□ ADRC bGd))□ □ S(c□ k! 018KgIS□

3□ ,z/Ic□ □
7$1e8£ 12 ȯ

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