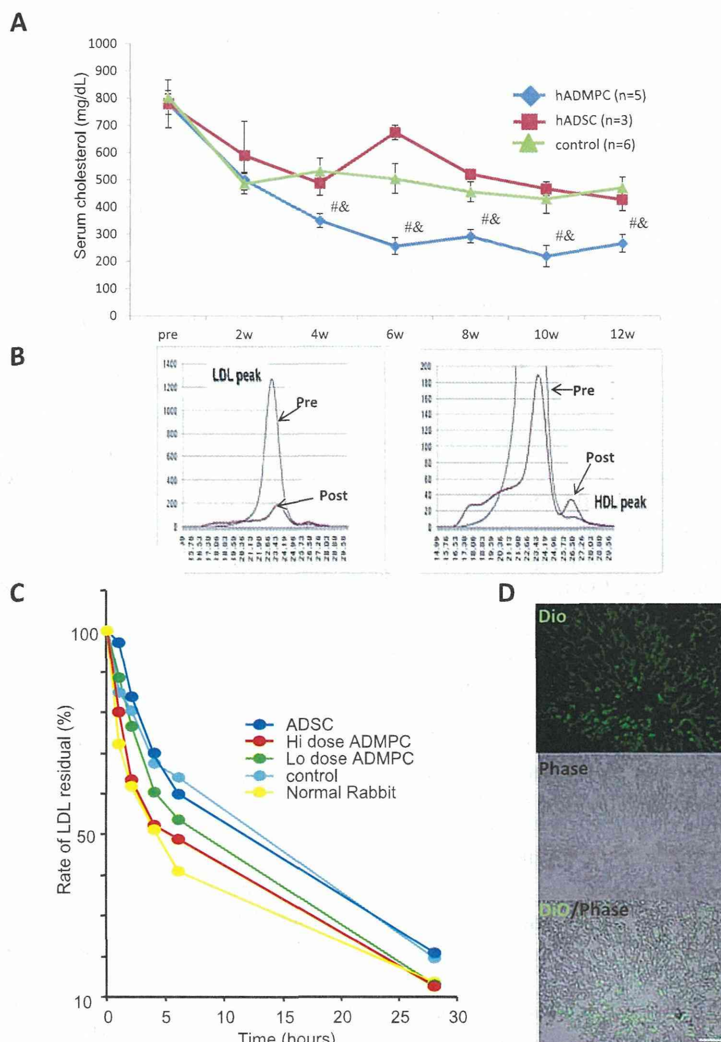


Fig. (4). (**A**) Total serum cholesterol levels. ADMPC transplantation in WHHL rabbits was followed for 12 weeks. Total serum cholesterol was measured in five rabbits that each received 3 x 10^7 ADMPC, three rabbits that each received 3 x 10^7 ADSC, and in six rabbits that received saline (control). Data are mean±SEM. #$P < 0.05$; control vs. the ADMPC-transplanted WHHL rabbit; &$P < 0.05$; the ADSC-transplanted WHHL rabbit vs. the ADMPC-transplanted WHHL rabbit). (**B**) Lipoprotein profiles in a representative WHHL rabbit with ADMP transplantation after gel filtration. Serum samples from the WHHL rabbit before and 4 weeks after transplantation were fractionated. Note the marked reduction in low-density lipoprotein (LDL) peak and appearance of a high-density lipoprotein (HDL) peak. (**C**) Rate of clearance of LDL from the serum of rabbits with and without transplantation of ADMPC. Animals were injected with ^{125}I-labeled human LDL, and the time course of clearance was monitored following trichloroacetic acid precipitation of serum at 5 min, 1 h, 2 h, 4 h, 6 h, and 28 h. Residual ^{125}I-LDL was expressed as a percentage of the signal at 5 min. The panel is the representative of two independent experiments. (**D**) DiO-LDL uptake into ADMPC-derived hepatocytes in the WHHL rabbit liver. Thin slices of recipient liver were incubated with DiO-labeled LDL in the serum-free medium for 24 h. After washing and fixation, the incubated slices were viewed by fluorescence microscopy. DiO-LDL-uptake cells (green) and no-uptake parenchymal cells are observed in the section. Bar = 100 µm.

3.5. *In Situ* Stem Cell Therapy by ADMPC

To determine the effects of ADMPC transplantation on the recipient rabbit lipid profile, serum cholesterol levels were monitored over 12 weeks (Fig. **4**, cited from reference 12 with modification). Significant reductions in total serum cholesterol were observed within 4 weeks of the transplantation, and the reductions were maintained for the entire period (Fig. **4A**). Furthermore, ADMPC-recipient animals showed significantly greater reductions than those in the control group. To determine the effects of ADMPC transplantation on the fractions of high-density lipoprotein and LDL in recipient animals, fractionation by fast protein liquid chromatography was performed (Fig. **4B**). Transplantation of ADMPC resulted in marked reduction of the peak LDL cholesterol and increment of high-density lipoprotein cholesterol fraction (right panel). Next, clearance experiments were performed with human LDL to confirm that the transplanted ADMPC contributed to the fall in serum cholesterol through uptake of LDL via LDL receptors. The rate of LDL clearance was significantly higher in the WHHL rabbits with transplanted ADMPC than WHHL rabbits without transplanted ADMPC (Fig. **4C**). Rabbits with ADMPC transplants showed ~2.4-fold (high-dose; 3 x 10^7 cells/rabbit) and

1.4-fold (low-dose; 5 x 10^6 cells/rabbit) increases in the rate of LDL cholesterol clearance over non-transplanted rabbits. To evaluate the uptake of DiO-LDL by transplants *ex vivo*, thin liver slices of WHHL rabbit were incubated with DiO-labeled LDL for 24 h and the uptake was examined as for the clearance experiments (Fig. **4D**). DiO-LDL was taken up by some but not all of the cells in the WHHL rabbit liver transplanted with ADMPC, with the positive cells observed dispersed, contacting, and integrating among the non DiO-LDL-positive parenchymal cells. This finding suggests that ADMPC differentiated into hepatocytes *in vivo*, thus lowering the serum cholesterol directly via LDL uptake.

3.6. ADMPC as a Promising Tool for Regenerative Medicine

For any successful regenerative medicine program, three issues must be considered: 1) what kind of tissues should be nominated as cell sources, 2) how should the cells be obtained from tissues, and 3) how will the cells behave after transplantation/administration. The following section discusses these considerations with respect to this study.

The source of stem cells for regenerative medicine should be easily and safely accessible as well as free of any ethical issues, thus allowing allogenic as well as autologous cellular therapy applications, and finally, the cell source should be available in large amounts. Adipose tissue is therefore a suitable cell source under these criteria. Liposuction surgery provides a safe method for collection of adipose tissue, harvesting from 100 ml to >3 L of lipoaspirate from material that is routinely discarded, thus avoiding any ethical concerns [16].

As to the second issue of cell processing, methods to isolate cells from adipose tissue were first reported in the 1960s [17-19]. Such methods involved mincing rat fat pads and incubating the resultant tissue fragments with collagenase. The digested material was then centrifuged to separate the floating population of mature adipocytes from the pelleted stromal vascular fraction (SVF). The SVF, which are sometimes referred to adipose tissue-derived regenerative cells (ADRC), consists of a heterogeneous cell population including fibroblasts, endothelial cells, pre-adipocytes, and mesenchymal stem cells [17-19]. However, as mentioned, this non-cultured cell population is too heterogeneous to apply therapeutically, necessitating the isolation of pre-adipocyte and/or mesenchymal stem cell-rich adherent cells from the SVF. The original procedure for this separation step was subsequently modified for the isolation of cells from human adipose tissue SVFs [20-23], and Zuk *et al.* [7] reported that the processed lipoaspirates exhibited mesenchymal stem cell-like features and could differentiate into adipocytes, osteocytes, and chondrocytes. Such cells are currently labeled as adipose tissue-derived stem cells (ADSC). The procedure used for obtaining these stem cells from lipoaspirates was described by Bjorntorp *et al.* [24] to obtain pre-adipocytes.

We have subsequently developed a novel isolation method for stem-like cells according to their adhesion properties [8-12]. As shown in Fig. (**1Aa**), fibroblast- and endothelial-like cells completely adhered onto the culture dish after 24 h in culture following the first-plating of the SVF. Most of the SVF cells were difficult to detach by conventional pipetting after this length of culture (24 h). However, a population of round self-aggregating cells that could only be detached by treatment with EDTA solution showed multi-lineage differentiation potency. In the EDTA-treating method, only cells of their properties with self-aggregation and EDTA-sensitiveness could be selected as ADMPC from plated SVF. In the conventional pre-plating methods to obtain ADSC [7, 24], the cells with EDTA-resistance could not be excluded. ADMPC exhibited high differentiation capacities for osteocytic, adipocytic, and chondrocytic lineages compared with ADSC, and could differentiate into hepatocyte-like cells, insulin-producing cells, and cardiomyoblast-like cells *in vitro* as non-mesenchymal lineages. We therefore named these cells ADMPC.

ADMPC differ from ADSC with regard to gene expression profiling. ADMPC express islet-1, a marker of undifferentiated cells and of cardiac, hepatic, and pancreatic progenitor cells [9-11]. Based on our findings, we propose that the islet-1-expressing ADMPC could be differentiated or reprogrammed into hepatocytes in the recipient liver *in vivo* after transplantation. In other words, the appropriate cells can show *in situ reprogramming* when transplanted and recruited into an appropriate environment.

Finally, we considered how the ADMPC would behave after transplantation/administration *in situ*. Traditionally, stem/progenitor cells are differentiated into terminally differentiated cells prior to transplantation/administration. For example, iPS cells are differentiated into neuronal cells and then applied for neuronal disease therapies. In these processes, researchers could mimic the relevant microenvironment and then differentiate the transplanted cells into the desired cell lineages. In contrast, we hypothesized that *in vitro* or *ex vivo* reprogramming could not sufficiently recapitulate the desired transplant microenvironment. Our concept is that the microenvironment *in situ* might supply cytokines to exert paracrine effects and form appropriate extracellular matrices, thus prompting the progenitor cells toward the desired terminal differentiation. ADMPC express islet-1, indicating that they might be appropriate progenitor cells on their own for *in situ* reprogramming as hepatocytes in the liver microenvironment. If these reprogrammed cells could correct given disease defects, clinical applications for *in situ* stem cell therapy become feasible.

In this review, we propose *in situ* stem cell therapy as a new tool for regenerative medicine and *in situ* reprogramming as a mechanism for the correction of disease. Yamanaka *et al.* [25] presented terminally differentiated cells that could be reprogrammed into the pluripotent state using only four factors. Followers confirmed the fact and named the concept "reprogramming". Melton *et al.* [26] subsequently showed that gene-modified cells alone could direct differentiation along a terminal path, and renamed the mechanism "direct reprogramming", and some cases of *ex vivo* gene therapy might be included in this concept. Here, we presented stem-like cells that could differentiate terminally *in situ* in the appropriate microenvironment.

4. CONCLUSIONS

In this review we describe ADMPC, novel adipose tissue-derived cells with stem cell-like properties and higher

differentiation potential than previously reported adipose tissue-derived cells. Not only could ADMPC differentiate into hepatocyte-like cells *in vitro*, but ADMPC *per se* also showed the same capacity in the hepatic environment and the *in situ* reprogrammed cells could correct the metabolic defect of diseased animals. The mechanisms described for *in situ* reprogramming hold great promise for applications in regenerative medicine as "*in situ* stem cell therapy".

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This study was supported in part by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO) and Kobe Translational Research Cluster, the Knowledge Cluster Initiative, Ministry of Education, Culture, Sports, Science and Technology (MEXT).

DISCLOSURE

It should be noted that the authors have previously published much of the material covered in this review article in "Tissue Eng Part C Methods", Volume 17, 2011, Pages 145-154.

REFERENCES

[1] Wagers AJ, Weissman IL. Plasticity of adult stem cells. Cell 2004; 116: 639-48.
[2] Pittenger MF, Mackay AM, Beck SC, *et al.* Multilineage potential of adult human mesenchymal stem cells. Science 1999; 284: 143-7.
[3] Shih DT, Lee DC, Chen SC, *et al.* Isolation and characterization of neurogenic mesenchymal stem cells in human scalp tissue. Stem Cells 2005; 23: 1012-20.
[4] In 't Anker PS, Scherjon SA, Kleijburg-van der Keur C, *et al.* Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. Stem Cells 2004; 22: 1338-45.
[5] Bieback K, Kern S, Klüter H, Eichler H. Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood. Stem Cells 2004; 22: 625-34.
[6] Jiang Y, Jahagirdar BN, Reinhardt RL, *et al.* Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002; 418: 41-9.
[7] Zuk PA, Zhu M, Ashjian P, *et al.* Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002; 13: 4279-95.
[8] Komoda H, Okura H, Lee C-M, *et al.* Reduction of Neu5GC xenoantigen on human ADMSCs lead to them as safer and more useful cell sources for realizing various stem cell therapies. Tissue Eng Part A 2010; 16: 1143-55.
[9] Okura H, Matsuyama A, Lee CM, *et al.* Cardiomyoblast-like cells differentiated from human adipose tissue-derived mesenchymal stem cells improve left ventricular dysfunction and survival in a rat myocardial infarction model. Tissue Eng Part C Methods 2010; 16: 417-25.
[10] Okura H, Komoda H, Saga A, *et al.* Properties of hepatocyte-like cell clusters from human adipose tissue-derived mesenchymal stem cells. Tissue Eng Part C Methods 2010; 16: 761-70.
[11] Okura H, Fumimoto Y, Komoda H, *et al.* Transdifferentiation of human adipose tissue-derived stromal cells into insulin-producing clusters. J Artif Organs 2009; 12: 123-30.
[12] Okura H, Saga A, Fumimoto Y, *et al.* Transplantation of human adipose tissue-derived multilineage progenitor cells reduces serum cholesterol in hyperlipidemic Watanabe rabbits. Tissue Eng Part C Methods 2011; 17: 145-54.
[13] Saga A, Okura H, Soeda M, *et al.* HMG-CoA reductase inhibitor augments the serum total cholesterol-lowering effect of human adipose tissue-derived multilineage progenitor cells in hyperlipidemic homozygous Watanabe rabbits. Biochem Biophys Res Commun 2011; 412: 50-4.
[14] Hosono T, Mizuguchi H, Katayama K, *et al.* RNA interference of PPARgamma using fiber-modified adenovirus vector efficiently suppresses preadipocyte-to-adipocyte differentiation in 3T3-L1 cells. Gene 2005; 348: 157-65.
[15] Yanagita M, Kashiwagi Y, Kobayashi R, Tomoeda M, Shimabukuro Y, Murakami S. Nicotine inhibits mineralization of human dental pulp cells. J Endod 2008; 34: 1061-5.
[16] Katz AJ, Llull R, Hedrick MH, Futrell JW. Emerging approaches to the tissue engineering of fat. Clin Plast Surg 1999; 26: 587-603.
[17] Rodbell M. Metabolism of isolated fat cells. II. The similar effects of phospholipase c (*Clostridium perfringens* alpha toxin) and of insulin on glucose and amino acid metabolism. J Biol Chem 1966; 241: 130-9.
[18] Rodbell M. The metabolism of isolated fat cells. IV. Regulation of release of protein by lipolytic hormones and insulin. J Biol Chem 1966; 241: 3909-17.
[19] Rodbell M, Jones AB. Metabolism of isolated fat cells. III. The similar inhibitory action of phospholipase c (clostridium perfringens alpha toxin) and of insulin on lipolysis stimulated by lipolytic hormones and theophylline. J Biol Chem 1966; 241: 140-2.
[20] Van RL, Bayliss CE, Roncari DA. Cytological and enzymological characterization of adult human adipocyte precursors in culture. J Clin Invest 1976; 58: 699-704.
[21] Björntorp P, Karlsson M, Gustafsson L, *et al.* Quantitation of different cells in the epididymal fat pad of the rat. J Lipid Res 1979; 20: 97-106.
[22] Deslex S, Negrel R, Vannier C, Etienne J, Ailhaud G. Differentiation of human adipocyte precursors in a chemically defined serum-free medium. Int J Obes 1987; 11: 19-27.
[23] Hauner H, Entenmann G, Wabitsch M, *et al.* Promoting effect of glucocorticoids on the differentiation of human adipocyte precursor cells cultured in a chemically defined medium. J Clin Invest 1989; 84: 1663-70.
[24] Bjorntorp P, Karlsson M, Pertoft H, Pettersson P, Sjostrom L, Smith U. Isolation and characterization of cells from rat adipose tissue developing into adipocytes. J Lipid Res 1978; 19: 316-24.
[25] Yamanaka S. Strategies and new developments in the generation of patient-specific pluripotent stem cells. Cell Stem Cell 2007; 1: 39-49.
[26] Gurdon JB, Melton DA. Nuclear reprogramming in cells. Science 2008; 322: 1811-5.

Received: October 24, 2011 Revised: December 2, 2011 Accepted: January 3, 2012

週刊 Journal of Clinical and Experimental Medicine (IGAKU NO AYUMI)
医学のあゆみ
今日の医学動向を伝える総合医学週刊誌

脂肪由来幹細胞の臨床応用への展開

242巻4号 2012年7月28日 p.297-302

脂肪組織由来多系統前駆細胞を用いた重症心不全治療細胞組織加工医薬品の開発

Adipose tissue-derived multi-lineage progenitor cells as promising tool for cardiac regenerative medicine

大倉華雪(写真) 澤 芳樹
Hanayuki OKURA[1,3] and Yoshiki SAWA[1,2]
大阪大学臨床医工学融合研究教育センター[1]，同大学院医学系研究科外科学講座心臓血管外科[2]，先端医療振興財団再生医療研究開発部門[3]

Summary 頻回の虚血障害により残存心筋幹細胞が消失する虚血性心筋症など重症心不全においては，これまでの内科的治療も十分な効果を上げえず，PCI(percutaneous coronary intervention)による血流改善も限定的な効果しかない．これら治療抵抗性の重症心不全end-stageにあっては1年死亡率が75%とされ，新規治療法・医薬品の開発が待たれている．著者らは，大量に簡便・安全・容易に採取可能なヒト皮下脂肪組織から新規間葉系幹細胞として，脂肪組織由来多系統前駆細胞の単離・培養法を確立した．当該細胞から誘導した心筋芽様細胞が慢性心筋梗塞モデルラットに移植した結果，心機能と長期生存率を改善し，被投与細胞を慢性心筋梗塞モデルラット心筋組織内で心筋細胞への分化を組織学的に確認している．非臨床試験として，単回投与毒性試験(経左心室腔内投与・経静脈投与)を中枢・呼吸安全性薬理試験(GLP)下で実施した．毒性を認めず，特殊毒性試験としては，造腫瘍試験，軟寒天コロニー形成試験，核型分析試験をGLPにて終了している．薬理試験のうち安全性薬理コアバッテリー試験では，GLPが終了している．このように著者らは，自らが行ってきた研究の成果を1日でも早く社会に還元するため，当初から薬事開発をめざしている．

Key word 脂肪組織由来多系統前駆細胞(ADMPCs)，心筋再生，*in situ* stem cell therapy，薬事開発，非臨床試験

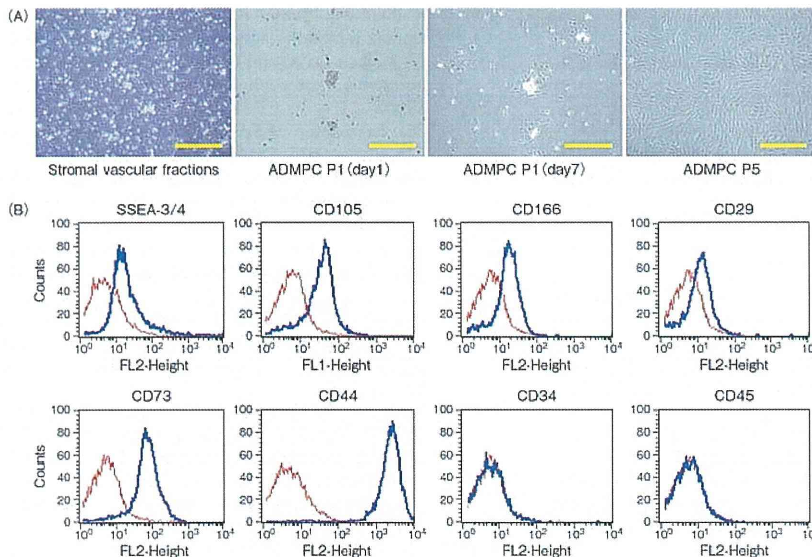


図 1 脂肪組織由来多系統前駆細胞の特性

本誌を購入 | 特集TOPへ戻る

242巻4号 2012年7月28日
週刊(B5判，70頁)
定価 1,050円(本体 1,000円＋税5%)
注文コード：924204
雑誌コード：20474-7/28

買い物カゴへ追加
買い物カゴを見る

Copyright (C) 2013 Ishiyaku Pub,Inc.

Ⅳ．その他

平成24年度
厚生労働科学研究費補助金
再生医療実用化研究事業

研究代表者: 大倉　華雪　（（公財）先端医療振興財団）
研究協力者: 早川　堯夫　（近畿大学　薬学総合研究所）
松田　潤一郎　（（独）医薬基盤研究所）
小浦　美奈子　（（独）医薬基盤研究所）

研究課題　: ライソゾーム病に対する細胞医薬品の開発にむけた
Confidence-in-Mechanism (CIM) 取得のための基礎研究

研究期間　: 平成24年度から2年計画

研究内容　:

ADMPC (脂肪組織由来多系統前駆細胞 Adipose tissue-Derived Multi-lineage Progenitor Cell) 由来再生肝細胞がライソゾーム加水分解酵素を持続的に分泌、全身の細胞組織に供給することを機序とした細胞医薬品の開発を目指し、臨床試験開始に向けた基礎的知見の収集。

期待される成果:

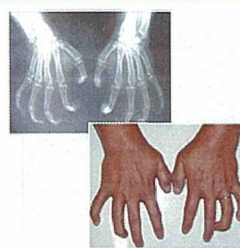
再生肝細胞がライソゾーム加水分解酵素を持続的に分泌、全身の細胞組織に供給することを機序とした細胞医薬品の開発に向けてConfidence-in-Mechanismの取得。

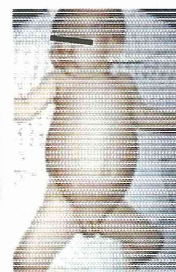
ライソゾーム病 ～病態と治療～

GM1
ガングリオシドーシス

ゴーシェ病

クラッベ病


ムコ多糖症


糖原病

- ○ ライソゾームに存在する加水分解酵素の遺伝的欠損
- ○ その基質がライソゾームに蓄積し、細胞障害、臓器障害を惹起
- ○ ライソゾームへの蓄積物質により病型分類

現在行われている治療法

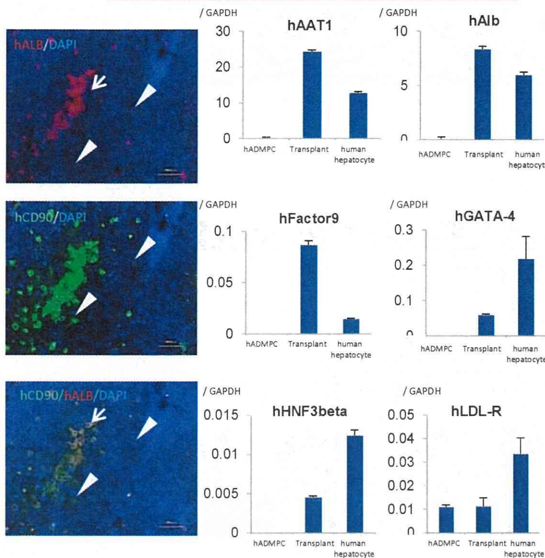
治療法		内容
対症療法	現状	抗痙攣薬、整形外科・脳外科的治療 呼吸管理、栄養管理など
	欠点	症状の緩和のみ。
骨髄移植 臍帯血移植 造血幹細胞移植	現状	移植した骨髄由来細胞が種々の臓器に入り 酵素欠損を補填
		ムコ多糖症I型では早期の移植の 有用性が報告。
	欠点	疾患により効果が限定
		化学療法・放射線照射による臓器障害
		GVHDおよび合併症

治療法		内容
酵素補充療法 (Enzyme replacement therapy)	現状	日本では、ゴーシェ病、ファブリー病 ムコ多糖症I・II・VI型、ポンペ病のみ
	欠点	生涯、週1～2回の点滴治療が必要
		酵素製剤のコストが高い
		中枢神経病変をきたす疾患に対して無効
遺伝子治療	現状	現在までヒトで行われたのは ゴーシェ病のみ
		レトロウイルスベクターを用いたが 成功せず
	欠点	特定の細胞への遺伝子導入の低さ
		挿入変異の可能性

これまでの成果と目的

【研究開始前までの成果】

ADMPCの肝細胞への分化
(rabbit *in vivo*)

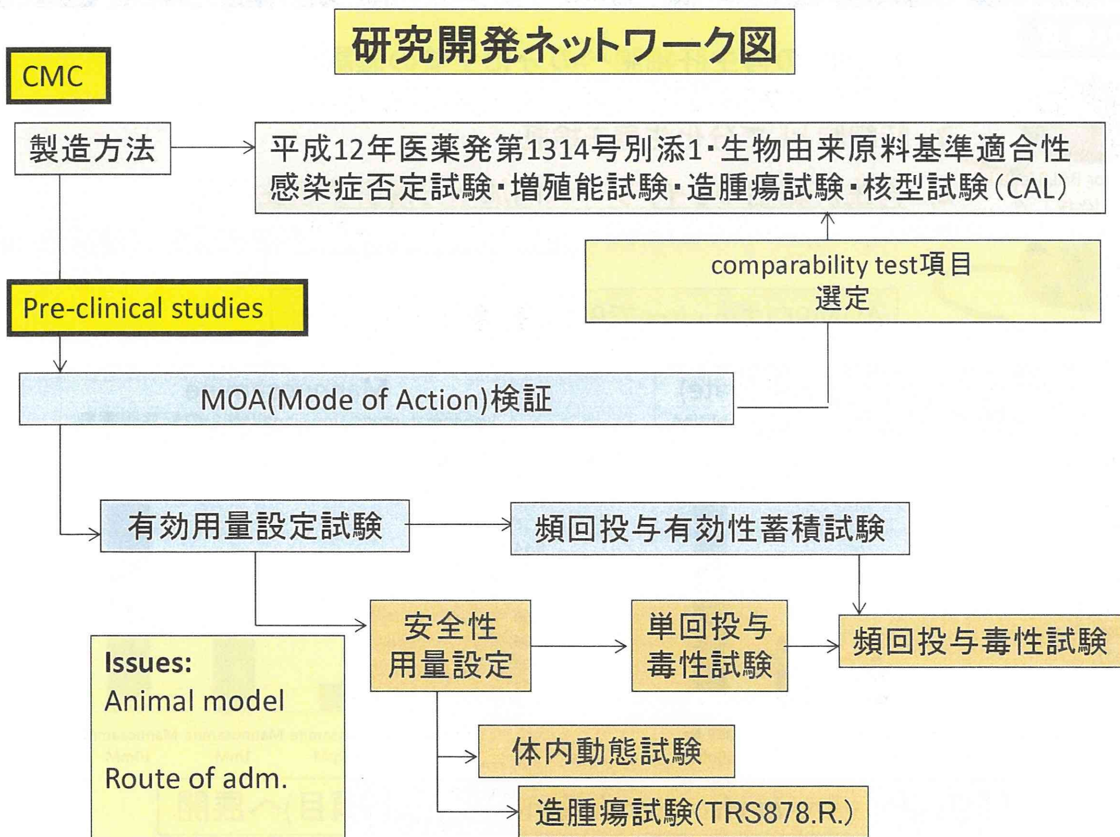


Okura H, et al. Tissue Eng Part C Methods. 2011 17(2):145-54.
Saga A, Okura H, et al. BBRC. 2011 ;412(1):50-4.
Okura H, et al. Current Tissue Engineering. 2012; 1(1): 54-62.

【目的】

ADMPC由来再生肝細胞は
ライソゾーム加水分解酵素を
持続的に分泌する
細胞医薬品となることを検証

非臨床試験ネットワーク図



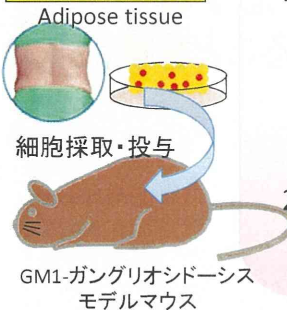
計画

平成24年度

1) **ADMPCの再生肝細胞への分化生着の確認**
モデルマウスに対し、mouse ADMPCを投与し、
肝細胞として分化生着を検証

2) **再生肝細胞のライソゾーム内加水分解酵素産生確認**
message level、蛋白発現、酵素活性から検証

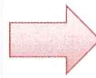
平成25年度



1) **再生肝細胞のライソゾーム内加水分解酵素血中分泌確認**
モデルマウスに対しhuman ADMPCを投与、
肝細胞としての分化、生着を確認。
再生肝細胞のβガラクトシダーゼ産生確認。
再生肝細胞が産生したβガラクトシダーゼの分泌を確認

2) **ライソゾーム内加水分解酵素の肝外組織への供給と治療効果確認**
モデルマウスにおける肝臓以外の組織における
βガラクトシダーゼの存在を免疫組織化学的に検証

Confidence-in-Mechanism
取得



ヒト幹細胞臨床研究指針申請
（H26年以降）

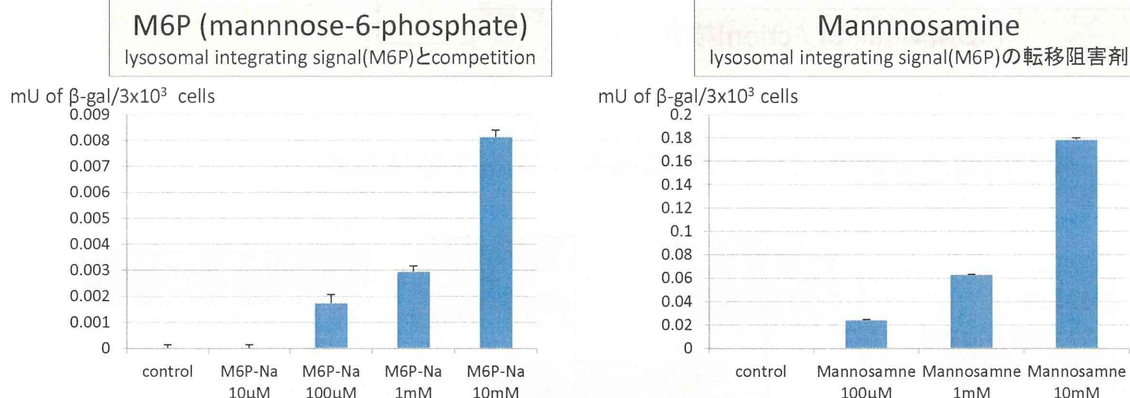
平成24年度成果

平成24年度

1) **ADMPCの再生肝細胞への分化生着の確認**
モデルマウスに対し、mouse ADMPCを投与し、
肝細胞として分化生着を検証

2) **再生肝細胞のライソゾーム内加水分解酵素産生確認**
message level、蛋白発現、酵素活性から検証

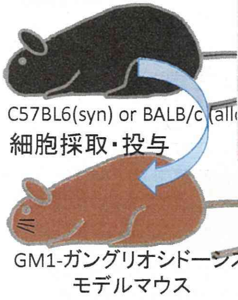
ADMPCは*in vitro*でβ-Galを産生するか？



IVIVC (*in vitro-in vivo* correlation：品質管理項目)へ展開

平成24年度成果

平成24年度



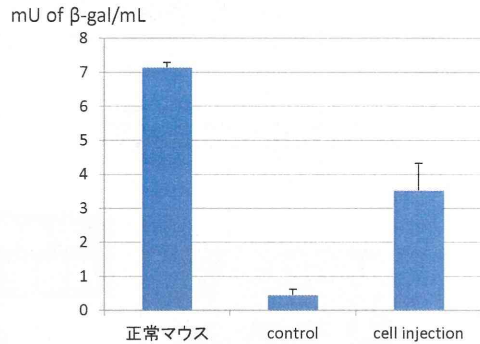
1) **ADMPCの再生肝細胞への分化生着の確認**
モデルマウスに対し、mouse ADMPCを投与し、
肝細胞として分化生着を検証

2) **再生肝細胞のライソゾーム内加水分解酵素産生確認**
message level、蛋白発現、酵素活性から検証

ADMPCは肝臓に生着しβ-Galを血中に分泌するか？

GFP-mADMPC投与　　非投与コントロール

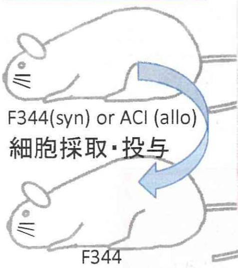
GM1-gangliosidosis
モデルマウス肝臓



Mode of Action (MOA)の検証
Confidence-in-Mechanismの取得

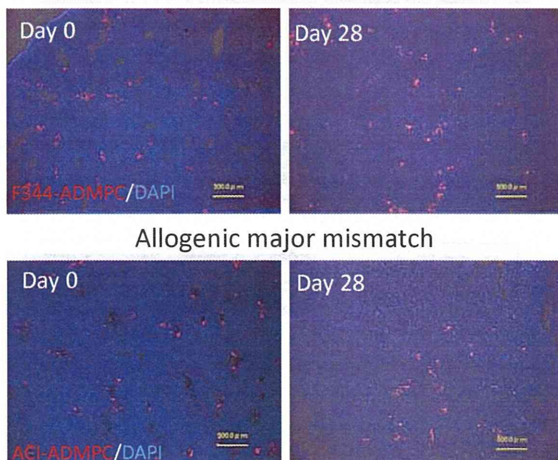
平成24年度成果

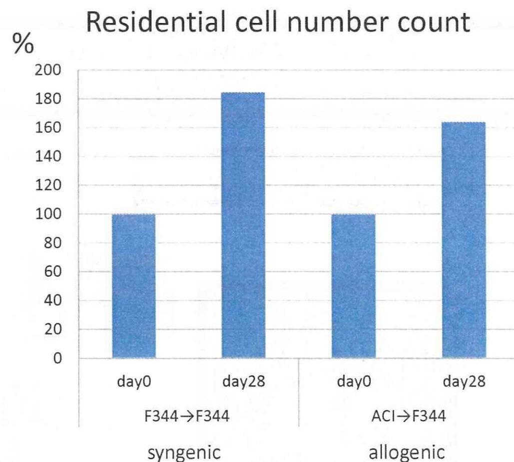
平成24年度



【採択時コメント】移植免疫をどのように回避するのか
【対応】
肝移植では免疫抑制剤を離脱する症例が30%ほどある
肝移植での免疫抑制プロトコールを参考にするため、生体肝移植の
田中紘一先生と相談
　投与時にステロイド、FK506持続投与
　MSCの細胞特性である免疫優位性をもとに6か月程度での離脱を図る

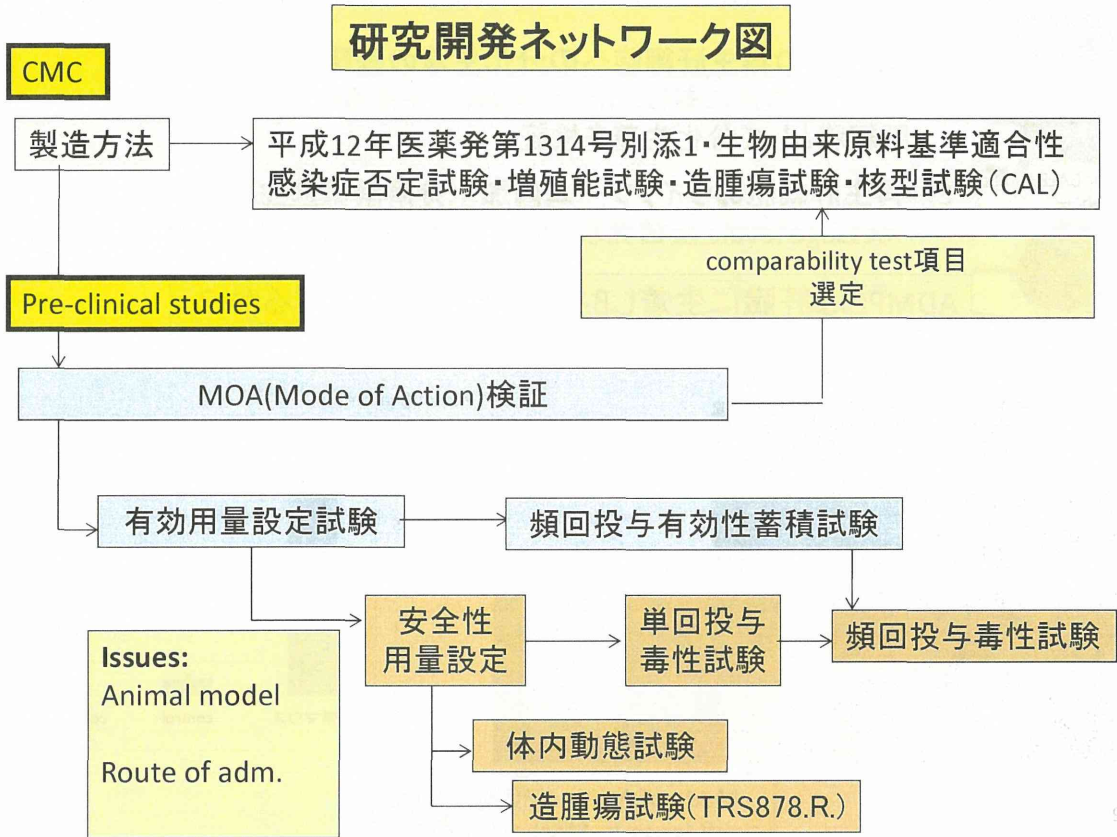
Syngenic



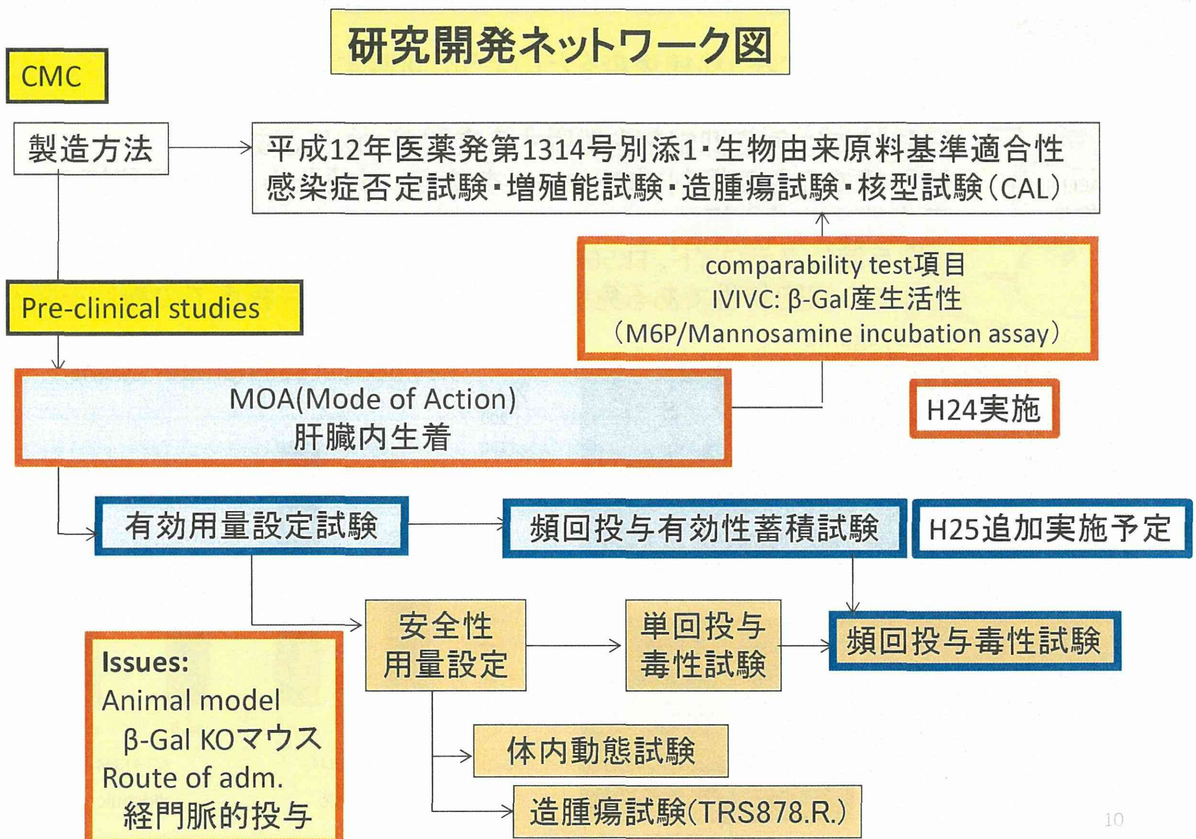


炎症所見認めず

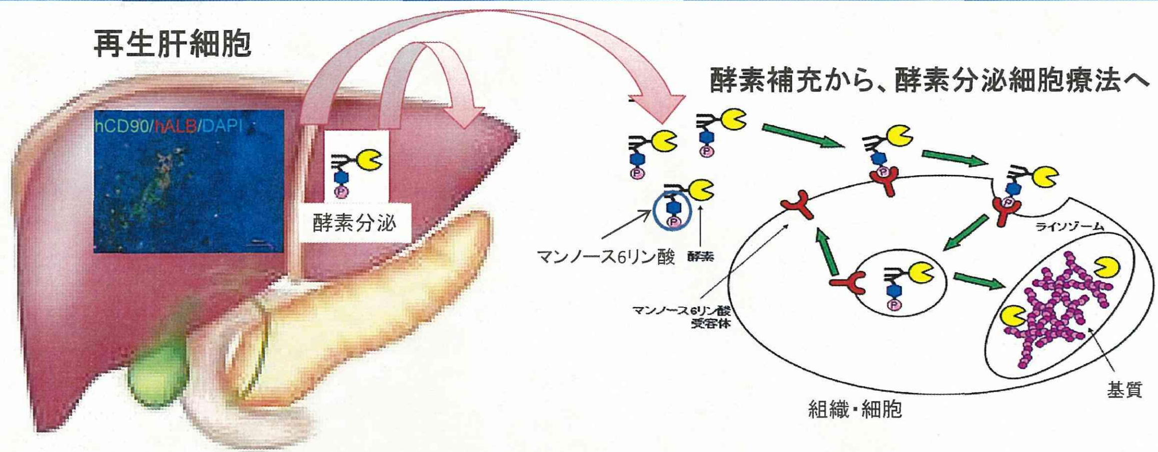
非臨床試験ネットワーク図



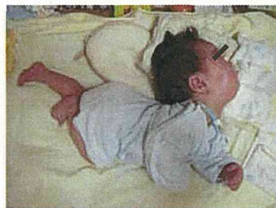
非臨床試験ネットワーク図



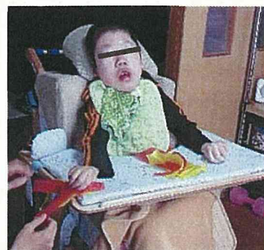
期待される成果



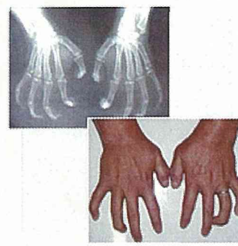
GM1
ガングリオシドーシス



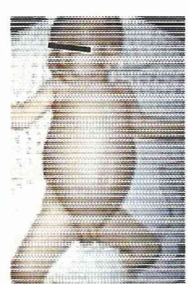
ゴーシェ病



クラッベ病



ムコ多糖症



糖原病

― 患者さんのQOL向上、生命予後改善のために ―