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lecture

再生医療とそれに伴う行政施策

松山晃文*

Regenerative medicine and health policy

近年の生命科学領域の進歩により，その知見を基盤とした再生医学・医療研究が日進月歩で発展している．一方で，再生医療を臨床実現するには，科学的にみても医学的にみてもいまだ不明な点が残されており，体外増幅やiPS細胞化による細胞の腫瘍化，未知ウイルス等の感染症伝播の可能性など，安全性を危惧する声があることは否めない．厚生労働行政に携わった経験から，まず再生医療臨床応用の現状を国際比較するとともに，わが国における再生医療の社会還元にむけた研究開発橋渡しの行政施策を概括し，再生医療の推進にかかる厚生労働省科学研究費補助金事業を俯瞰したい．

"第20回新潟移植再生研究会特別講演" より(2009年12月1日，於 新潟大学医学部有壬記念館)

*Akifumi Matsuyama**

key words：再生医療，薬事法，脂肪組織幹細胞

本日は，第20回新潟移植再生研究会にお招きいただきまして誠に光栄です．このような機会を与えていただきました，代表世話人である新潟大学の荒川正昭先生，高橋公太先生に御礼申し上げます．加えまして，シンポジウム"知的障害者の腎移植"では，県立広島病院の大田敏之先生ほか，新潟大学医歯学総合病院の先生方のお話を聞かせていただきましたが，こんなにおもしろい会はなかなかなくて，食い入るように拝聴させていただきました．

大田先生は功なり名を遂げた先生で，非常にわかりやすく説明していただきましたし，鈴木俊明先生のスライドは非常にクリアカットで，なるほどと合点のいくものでした．池田正博先生の発表では，1人の患者さんを治すことに苦労されていて，医者の鏡だと，私も医者として考え直さなければいけないと思いました．磯貝和也先生は，服薬アドヒアランスの話をされましたが，最初から粉粒にして投与が可能であること，井越寿美子師長からは，患者さんへの思いと，患者さんと同室にすることがドナーのためになるという話に感動しました．

こういうプラットホームをつくられた荒川先生をはじめとして，新潟ってすごいなと感じました．私は長野県飯山市の出身で，新潟県十日町の隣なのですが，ここに来る途中で見た夕焼けが長野の夕焼けとまったく同じで，懐かしく思いました．

僭越ではございますが，本日は厚生労働省で経験させていただいたことをもとにお話をさせていただきます．

再生医療臨床応用の現状

患者自身から細胞を採取して，なんらかのプロセスを経て，心臓や神経あるいは角膜に再生して患者に戻す．現在，日本で行われている再生医療といわれているもののほとんどがこれです．

一方で，たとえば患児のお父さんやお母さんから細胞を採取して，加工を加えて患児に返す，同種といわれている再生医療もあります．これは臓

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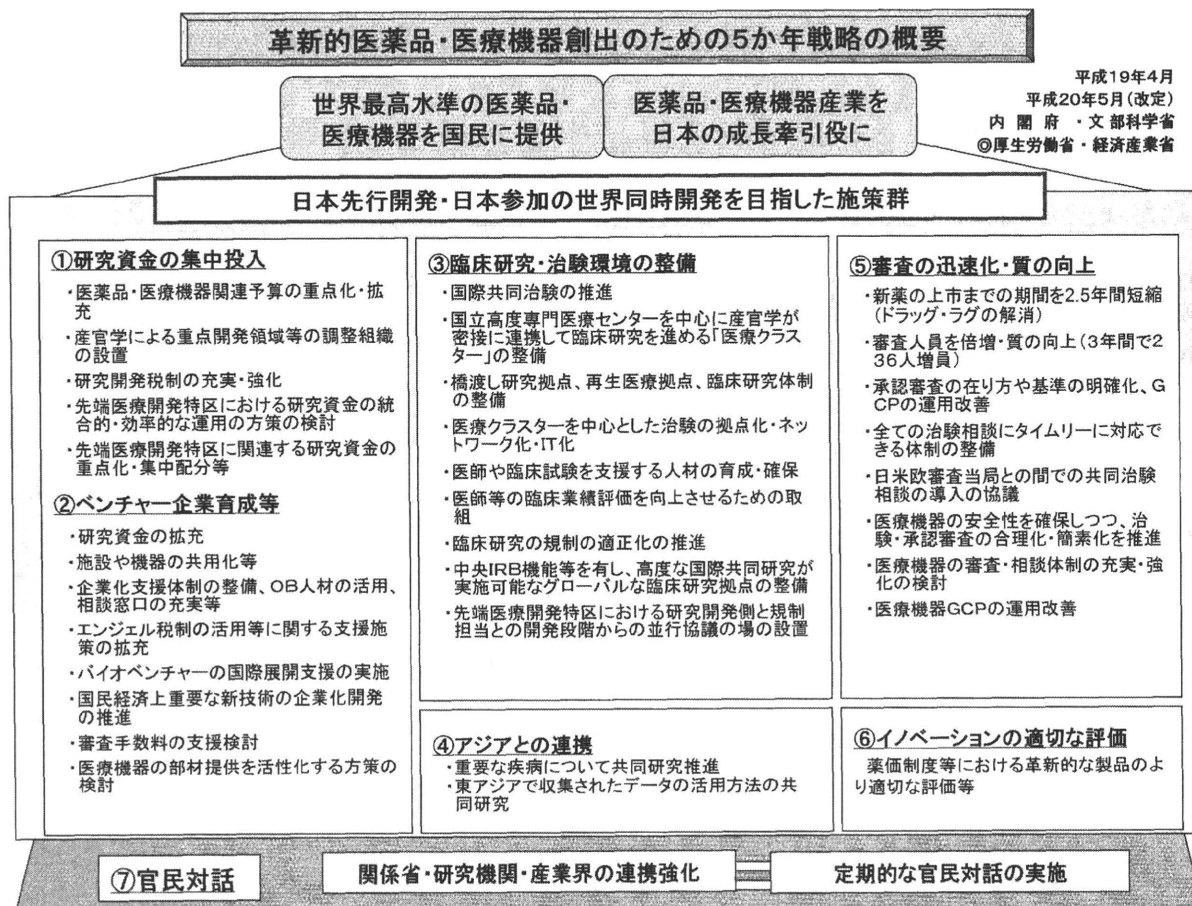

図1　革新的医薬品・医療機器創出のための5カ年戦略
（厚生労働省ホームページ〈http://www.mhlw.go.jp/houdou/2007/04/dl/h0427-3a-1.pdf〉より）

器移植に近いところもあり，なかでも免疫抑制薬の使い方は，臓器移植に携わる方しかわからないこともあって，私たちも協力を仰いでいます．特に，遺伝性疾患を治療しようと思ったら，自身の細胞ではなかなか治せません．iPS細胞（人工多能性幹細胞：induced pluripotent stem cells）による治療が話題となっていますが，たとえばパーキンソン病やハンチントン病の患者はもともと遺伝子変異がありますから，患者の細胞をiPS細胞化して治せるのかという議論があります．

再生医療への期待は大きいのですが，現在のところ再生医療は臓器移植を凌駕することはないといえます．なぜかというと，再生医療では，身体に傷があったとしたらそれを修復することはできますが，臓器自体を修復することは現状ではできないからです．それには臓器移植しかありません．

厚生労働省が進める，イノベーションに向けた政策パッケージのなかで一番の中心が，現在進んでいる“世界最高水準の医薬品医療機器を国民に提供するための革新的医薬品・医療機器創出のための5カ年戦略”です（**図1**）．革新的医薬品には，適応外拡大も含まれます．ですから，臓器移植では新しい免疫抑制薬が別の疾患にも投与できる，たとえば膵島移植では現在，MMF（ミコフェノール酸モフェチル）は保険適用ではありませんが，それを積極的に保険適用にするといったアイデアがこのなかに入っています．

ただ，医療経済的にどうかというクリティカルな問題がありますので，いいものが必ず利用されるわけではありません．一つの薬剤ならマーケッ

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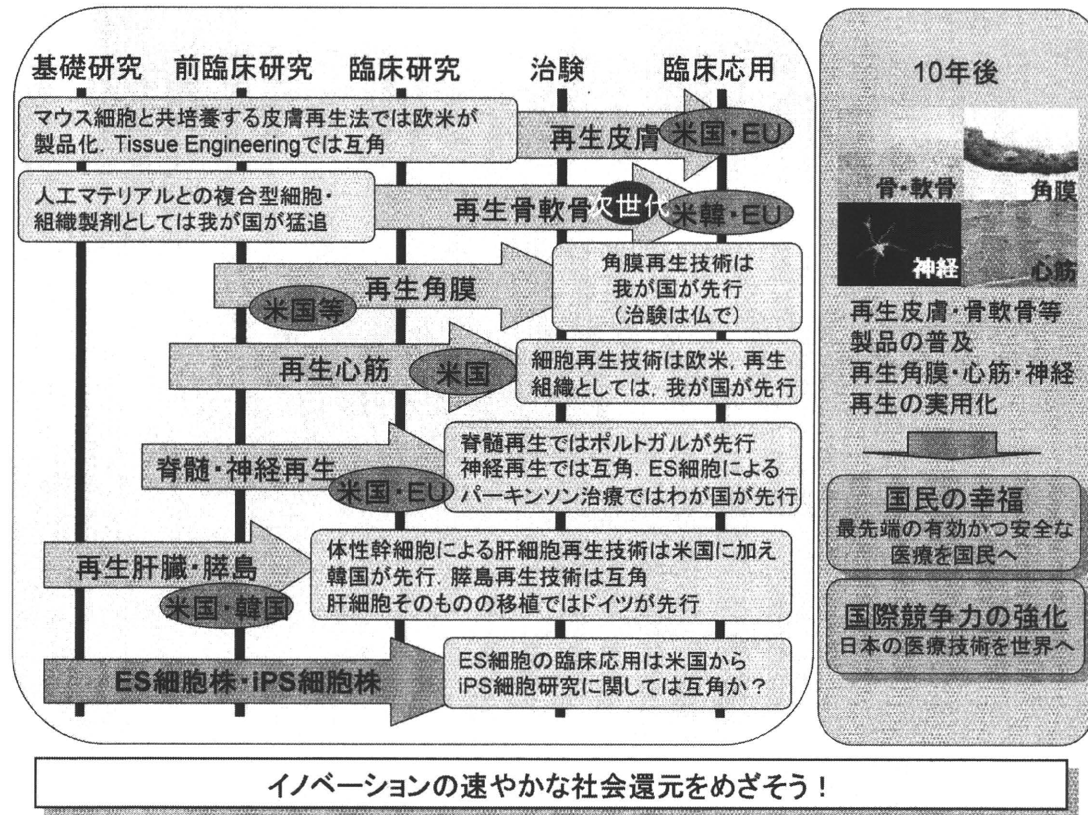

図2　再生医療俯瞰図

（松山晃文：再生医療に期待する―安全かつ有効な再生医療の実現をめざした産・官・学への期待. 再生医療　7：31-35, 2008より改変）

トに乗って，あたかも保険からの支出が増えるようにみえますが，そうではなくて，患者さんを治すことによってどのぐらい社会資産が上がったのか．すなわち，本来なら治療に1,000万円かかる人が再生医療と免疫抑制薬という新しい治療法を使うことによって500万円ですむのであれば，社会的にメリットがあるといえます．

現在，第3期科学技術基本計画が進行中で，これに乗るかたちで“新健康フロンティア”や“イノベーション25”ならびに前出の“革新的医薬品・医療機器創出のための5カ年戦略”があります．大学病院などは治験によって潤っているところがありますが，今後新しい薬剤はなかなか出てこないと思われ，そうするとお金が入らなくなる．ではどうするかということになります．

おそらく医療機器の治験が，今後は大学病院の主要な収入となる可能性があります．大学病院でなければ治験ができない医療機器は当然あって，医療機器に関しては医師の“腕”によるという問題がある．たとえば，特殊な機器を使うとなったら，やはり腕のいい医師がやらないとだめだということがあります．医療機器の治験が大学病院の収入の中心になっていくだろうと推測されます．

“革新的創薬等のための官民対話”という会議が設置されていますが，この“等”には，薬剤だけではなくて医療機器も入るということです．ですから，臓器移植のときに使うデバイスも，従来は医療雑貨であったのが，医療機器として承認される可能性がある．医療機器で承認されるときに，もし日本発のものであれば，JIS規格（日本工業規格），TS（technical standard）あるいはTR（technical report）というかたちで規制をつくっていくことによって，世界に打って出ていくことができるのです．

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表1　細胞組織利用医薬品医療機器の臨床開発

企業の所属国	Phase Ⅰ	Phase Ⅱ	Phase Ⅲ	合　計
日　本	1	1	0	2
米　国	22(19)	33(11)	9(3)	64(33)
欧　州	3	7	5	15
韓　国	3	0	0	3
その他	1	5	4	10
合　計	29	46	18	93

2008年10月末時点．米国のカッコ内の数字は2004年10月におけるデータ
(第4回PMDA国際バイオロジクスシンポジウム〈http://www.pmda.go.jp/event/file/200900908event_6.pdf〉より改変)

再生医療の社会還元に向けた行政施策

厚生労働省はこれまで，研究費の配分が基礎的研究に若干シフトしていたのではないかという反省がありました．そういう基礎的な部分ではなく，開発支援にウエイトを置きたいと．この開発とはなにかというと，1日でも早くいいものを患者さんにお届けすることです．

再生医療の社会還元に向けて，どうすればいいのでしょうか．まずなにか研究をするときには，自分がどの立ち位置にいるか認識しないといけません．わが国と諸外国を比較してみましょう(**図2**)．わが国でも皮膚領域において，J-TEC社のJACEという培養皮膚が承認されましたが，アメリカやEUにくらべると遅れています．骨や軟骨に関しても遅れていて，競争しているとすれば心臓と神経に関してです．

ES細胞(胚性幹細胞：embryonic stem cells)とiPS細胞の臨床応用が実現するかは疑問です．いまiPS細胞に注目が集まっていますが，先日ハーバード大学のある教授と「iPS細胞の臨床応用は可能か」について話をしたときに，「(iPS細胞の臨床応用の実現なんて)君たちはクレイジーだ」と言われました．

iPS細胞は，創薬スクリーニングには使えると思います．日本は世界でも数少ない薬剤を自己開発できる国です．隣の韓国ではできません．スイスやドイツ，フランス，アメリカ，イギリスなど，そうそうたる国の一つに入ります．ですから，iPS細胞が今後どのように生きるべきか考えると，創薬スクリーニングしかない．もしiPS細胞を創薬スクリーニングに使用できるとしたら，わが国発のガイドラインをつくっていくべきです．それならば日本が世界において勝つ余地があると思われます．

世界的に再生医療は，実質的には販売されています．ただ正規に承認されたものはほとんどありません．アメリカでも承認されているものは一つしかないし，ヨーロッパでも一つしかありません．日本でも一つしかありません．これをもって日本が遅れているのではなくて，アメリカでは有償治験ができるのです．それを考えて，実質的に販売されていると言わせていただきました．

再生医療の治験について日本は非常に寂しい状況で，ほとんど治験を行っていません．アメリカをみてみると，2004年のデータでは確実に治験プロトコール数が増えています(**表1**)．このPhase Ⅲの製品がアメリカやヨーロッパで承認されたあと日本に入ってくると思われますが，ではどうやって日本の科学技術産業に活かしていくかは，喫緊の課題です．

わが国で承認されたものは，J-TEC社の自己由来培養皮膚JACEしかありませんが，これはアメリカから技術導入しています．治験の段階に入っているのがMSC(間葉系幹細胞：mesenchymal stem cell)で，これはGVHD(移植片対宿主病：graft-versus-host disease)治療用です．これらはほとんど海外から持ってきたものですが，このよう

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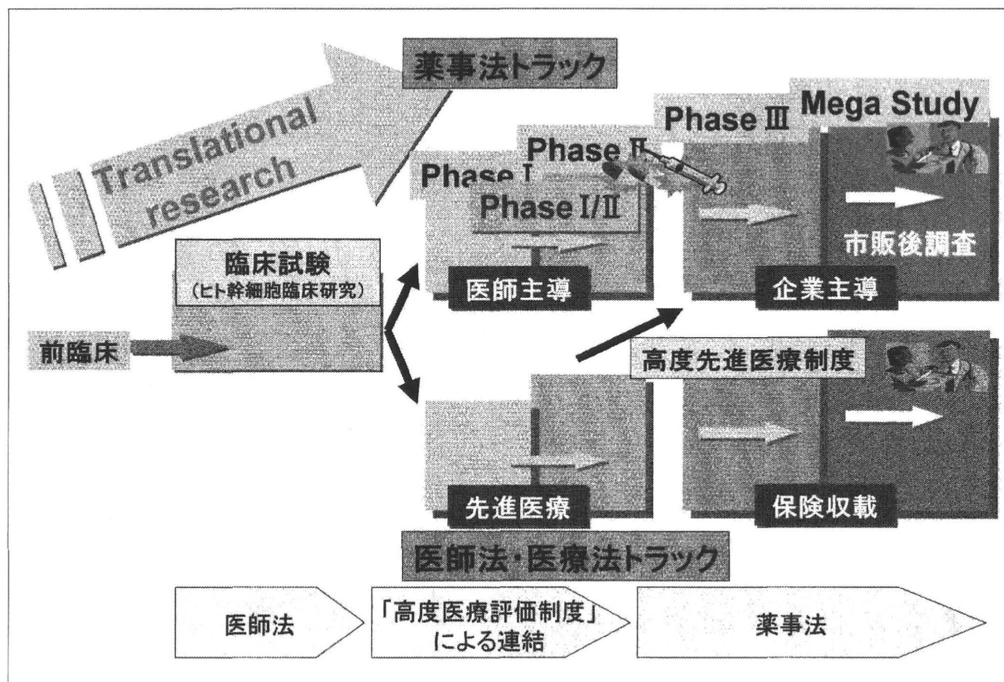

図3 わが国における再生医療の二つの出口
(松山晃文:再生医療の実現化─制度論的検討. 再生医療 8:85-89, 2009より改変)

な現状はなんとかしなければならず,行政的には喫緊の課題です.ですから,いかに臨床研究を行っているみなさんを導くかが課題ですし,シームレスな,臨床研究を行ったあとに企業に引き取っていただくためのシステムが必要です.

アメリカでは,なぜこんな中途半端なことが起こっていないのかというと,FDA(食品医薬品局)という,日本でいえばPMDA(医薬品医療機器総合機構)に相当する組織が一括してfirst-in-manの部分を管理しているためです.ただ,アメリカでも「それは厳しすぎる」という意見があって,Phase Ⅰ GMP(good manufacturing practice)ガイダンスというガイドラインが出てきました.これはわが国のヒト幹細胞臨床研究のレベルとほぼ一緒です.世界的にみて,アメリカは厳しすぎたのが下がってきて,日本は甘すぎたのが上がってきたということで,ほぼ同じ水準に終息してきていることは興味深いといえます.

日本では再生医療のトラックは二つあって,臨床研究を行ったあとに治験に入っていくトラックと,医師法・医療法のなかで医療技術として保険医療化を行うトラックがあります(**図3**).薬事法トラックから企業主体でいけば償還価格,薬価がつくという流れになるし,医師法・医療法トラックでいけば手技料として保険収載がされます.ただ,いいものは企業主導に持っていきたいということがあって,ここを結びつける制度として,高度医療評価制度,いわゆる第3項先進医療が2008年4月にスタートしました.みなさんが頑張って得たデータを拾い上げて,なんとか企業に使ってもらえるように持っていくことが行政施策の一環です.

なかなかそれが通らないので,PMDAは厳しすぎるのではないかといわれています.ですが,「そうではない」といいたい.たとえばiPS細胞ではなにをクリアしなければいけないかリストアップしてみると,さまざまなことがあります.iPS細胞は奇形腫形成が非常に多く,iPS細胞を未分化のままで維持することが非常に難しいのです.

現在,細胞組織加工製品の評価基準の見直しを

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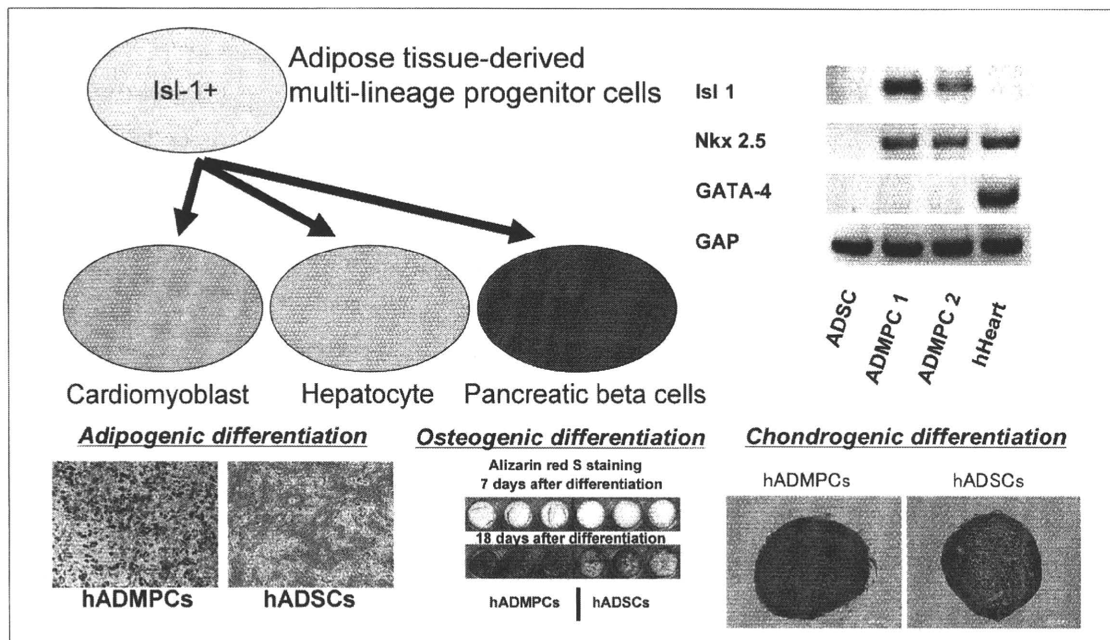

図4 新規脂肪組織由来幹細胞

行っています．ディスカウントするのではなくて，「確かに，昔のガイドラインていうのはわかりにくかったね」という反省で，自家細胞と同種細胞にわけて，わかりやすく書き下しをしたものが2008年に発出されています．現在，幹細胞由来製品の安全性評価ガイドラインとして，PMDA顧問で，近畿大学総合薬学研究所所長の早川堯夫先生たちと一緒に，ES細胞ならびにiPS細胞を使うときのガイドラインをつくっています．こういうものは公開性を持たないと，本当にOKかどうかわからないので，作成されたガイドラインをオフィシャルな形で公開し，みなさんの目でチェックしていただくことになっています．

それから現在進行形ですが，甘利明先生が規制改革担当大臣であったときに日本再生医療学会が中心になって行っていた，臨床研究における保険外併用療法と臨床研究費の問題があります．これは喫緊の課題で，現在のようにあまりにも規制をきつくすると，東京大学ならPhase Ⅲまでできる，京都大学ならPhase Ⅱまでできるけれど，そのほかの大学ではPhase Ⅰができるかできないかというレベルになってしまう．混合診療ができなかったら臨床研究なんかできませんし，臨床研究に気合いを入れて，たとえば再生医療や臓器移植でやろうと思ったら，1億円，2億円の研究費ではどう考えても無理なわけです．これがいまホットな話題で，日々熱い議論がなされています．なかなか難しいのですが，アイデアの一つとして，DPC（診断群分類：diagnosis procedure combination）をやっているところは，包括診療のなかで臨床研究費をみたらいいのではないかという議論があります．

脂肪組織由来幹細胞の新展開

こんな話ばかりしていると，「おまえ，なにも研究していないだろう」というおしかりを受けそうですので，私が神戸で行っている研究について発表させていただきます．神戸発世界になるのか，神戸発神戸になるのか正直いってわかりませんが，いま頑張ってやっていることをお話しします．

この写真は，実は私のおなかです．これを出すと，私の研究室の女の子からは，「松山先生がいつも発表で，これを話のつかみにするのは恥ずかしくてしょうがない」と言われるのですが，この研

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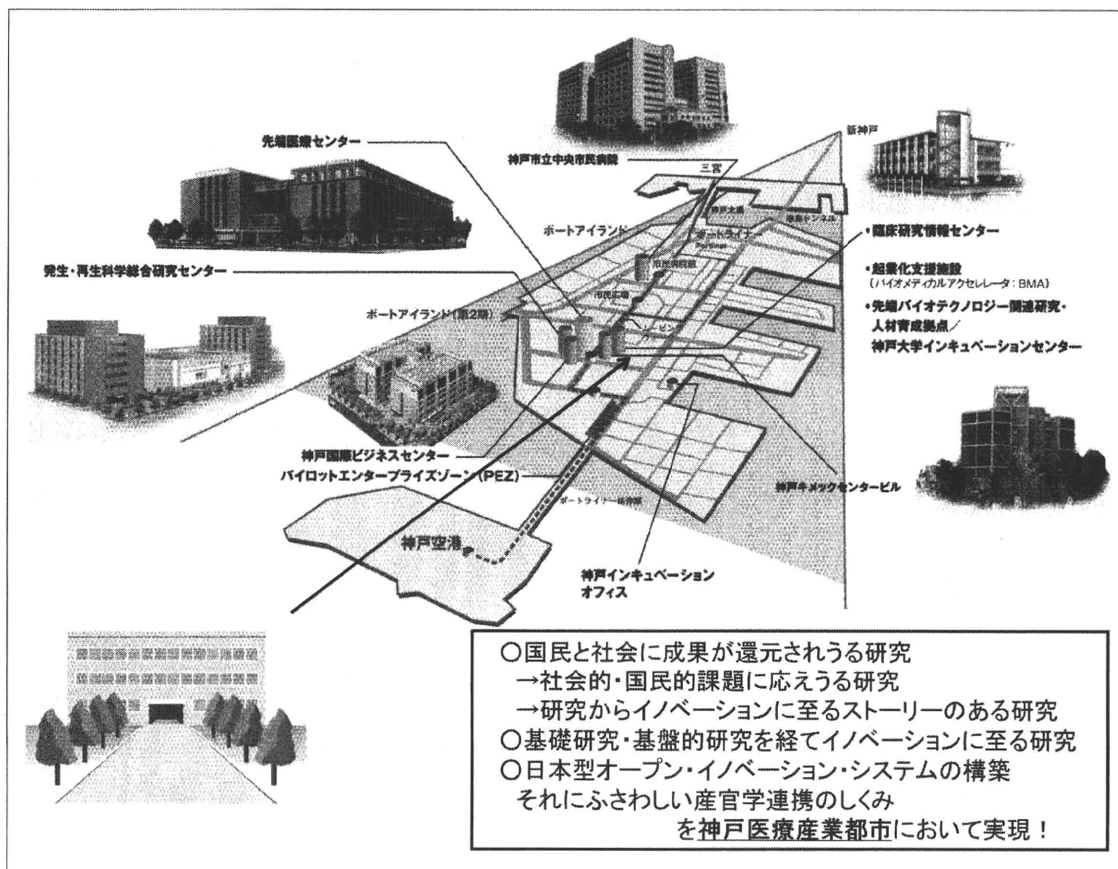

図5 神戸医療産業都市構想：神戸であればできること，神戸でなければできないこと

究を始めたとき，私には研究費がなかったので，10万円で研究をはじめました．研究費が10万円だとラットも試薬も買えません．以前勤務しておりました大阪大学医学部附属病院未来医療センターに，たまたま残っていた使い古しの薬剤と，自分のおなかの脂肪を使ってスタートした研究がここまできて，なんとか花開きつつあります．

この脂肪から新しい脂肪組織由来幹細胞を採取しましたが(**図4**)，従来の方法よりも効率よく脂肪にも分化し，骨化もして，islet-1という転写因子が発現しています．非常に未分化なマーカーを発現している細胞だけをとることに成功しました．

このislet-1があると心臓や肝臓，膵臓になったりするので，「ではその心臓をつくろう」という話になりました．私のもとで仕事をしてくれていたのは大倉華雪さんという，もと薬剤師だった人ですが，「再生医療をやりたい」と大阪大学大学院心臓血管外科の門をたたいてくれました．しかしながら，「脂肪細胞から心臓をつくる」というテーマを大学院の何年間でやれと言われて，そのとき彼女は泣きそうでしたが，「先生，そんなの無理です」と言いながらもなんとか研究をやり遂げました．

Cardiomyoblast-like cellsといいますが，心臓マーカー蛋白質であるalpha-cardiac actin，myosin light chainが発現してきます．0.1% DMSOをかけただけで心筋芽細胞に分化します．その細胞を細胞シートにして，澤芳樹先生のテクニックを使ってラットに移植をしました．移植すると生命予後が非常によくて，28例に移植して全例が生存しています．一方，DMSOで処理しない群では，ある程度死んでいます．16週間後に比較してみ

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ると，心臓は動きつづけていました．

つぎに膵臓ができるかどうか検討しました．非常に苦労して，6個のステップでなんとかインスリンを分泌する細胞をつくることができました．これは大変試行錯誤しましたが，大学院生たちには，「私の青春を返してくれ」と言われたくらい研究はしんどかったです．膵島，膵臓の場合，周りにグルカゴンがきて，真ん中にインスリン分泌細胞，β細胞がくるのですが，きちんとそういうクラスターをつくることができて，インスリンが分泌しています．糖尿病マウスに移植後には血糖がきれいに下がりました．

ここで，成功だとシャンパンを開けたのですが，2週間ちょっとしか血糖値が下がらないことと，あとになってわかったのですが，1匹のマウスを治すのに200万円近くかかっていて，人に換算すると2週間治療するだけで2億円かかるのです．「これはどう考えても無理だよ」と．この話を行政官にすると真っ青な顔になって，「申しわけないけれど，その研究はやめてくれ」と言われました．現在はもう少し安く，なんとか700万円ぐらいで治療できるというプロトコールを開発しつつあります．

肝細胞への分化では，同じようにクラスターをつくって，oncostatinとか，FGF(fibroblast growth factor)，HGF(hepatocyte growth factor)を入れます．これも全部大倉さんがやってくれました．というのは，私の下についてくれたのが彼女しかいなかったんです．毎晩11時ぐらいにディスカッションしたり，彼女が家に帰ったあともノートの内容がわるいと夜中の1時でも電話したので，「人権無視だ」とよく言われました．それでも6本の論文が出来上がったから，「許してくれ」と言えるかなと．ちゃんとアルブミンも出ています．

それからLDLの取り込みを蛍光脂質でみていますが，きれいに細胞が赤色に染まり，索状で肝臓の構造ができているのがある程度わかります．これのいいところは，尿素もちゃんとつくっていることです．

この再生した肝細胞をマウスに移植しました．3カ月にわたって毎週2回，四塩化炭素を腹腔内に打つという実験系です．これで慢性肝炎のモデルをつくりました．実際に移植すると，アルブミン，ビリルビンの値がともに改善しました．ただ残念なことに，実際にこれを人に投与すると1億円かかるんですね．それを考えると，いまのところはスクリーニングしかない．脂肪組織由来の幹細胞で，肝臓，膵臓，心臓ができるのですから，現実にはiPS細胞はいらないともいえます．

大阪大学では特任准教授をさせていただいていたのですが，大学では循環器なら循環器，肝臓なら肝臓と，特定の疾患しかやらせてもらえないということがありました．それならと，脂肪を源にしていろいろな再生医療研究をしたところ，たまたま神戸市の医療産業都市(**図5**)から，「ぜひともこれを臨床応用してほしい」という話があり，アカデミアから出ることになりました．アカデミアで私が一番やりたかったのは教育で，アカデミアにはいつか帰りたいと思っていますが，神戸ではどうやって大学発シーズを臨床に持っていくか，社会還元するかということを，OJT(on the job training)という形でやっていきたいと考えています．

ハッピーなことに，当研究所の周りには神戸市立中央市民病院や理化学研究所など多くの施設があります．みなさんにサポートしていただき，神戸の医療産業都市構想を実現する，私の持っているシーズというものをアウトプットできるように，より努力したいと思っています．

本研究においては，特に肝臓，膵臓，心臓に関して，さまざまな先生方のサポートをいただきました．なかでも，話に出ました大倉華雪さんは日本学術振興会の研究員ですが，大変おもしろい経歴の持ち主です．6年間引きこもりで家にいましたが，ご家族が病気をされたことから一念発起して薬剤師になり，その後再生医療で頑張りたいということで大阪大学大学院心臓血管外科に来ました．修士2年間と大学院3年間，「私に人権はないのか」と言いながらもよく頑張ってくれました．本研究にサポートしていただきましたみなさまに感謝申し上げます．

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TISSUE ENGINEERING: Part C
Volume 17, Number 2, 2011
© Mary Ann Liebert, Inc.
DOI: 10.1089/ten.tec.2010.0139

Transplantation of Human Adipose Tissue-Derived Multilineage Progenitor Cells Reduces Serum Cholesterol in Hyperlipidemic Watanabe Rabbits

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Familial hypercholesterolemia (FH) is an autosomal codominant disease characterized by high concentrations of proatherogenic lipoproteins and premature atherosclerosis secondary to low-density lipoprotein (LDL) receptor deficiency. We examined a novel cell therapy strategy for the treatment of FH in the Watanabe heritable hyperlipidemic (WHHL) rabbit, an animal model for homozygous FH. We delivered human adipose tissue-derived multilineage progenitor cells (hADMPCs) via portal vein and followed by immunosuppressive regimen to avoid xenogenic rejection. Transplantation of hADMPCs resulted in significant reductions in total cholesterol, and the reductions were observed within 4 weeks and maintained for 12 weeks. ^{125}I-LDL turnover study showed that the rate of LDL clearance was significantly higher in the WHHL rabbits with transplanted hADMPCs than those without transplanted. After transplantation hADMPCs were localized in the portal triad, subsequently integrated into the hepatic parenchyma. The integrated cells expressed human albumin, human alpha-1-antitrypsin, human Factor IX, human LDL receptors, and human bile salt export pump, indicating that the transplanted hADMPCs resided, survived, and showed hepatocytic differentiation *in vivo* and lowered serum cholesterol in the WHHL rabbits. These results suggested that hADMPC transplantation could correct the metabolic defects and be a novel therapy for inherited liver diseases.

Introduction

Familial hypercholesterolemia (FH) is characterized by premature and accelerated development of atherosclerotic lesions caused by elevated levels of cholesterol-rich lipoproteins in plasma. The disease is caused by mutations in the low-density lipoprotein (LDL) receptor gene that result in a significant decrease in receptor-mediated uptake of lipoproteins from the circulation.[1–3] Patients homozygous for defects in LDL receptors have serum cholesterol levels 5–10 times those of normal and suffer as early as the first two decades of life from complications such as coronary artery disease.[4,5] In homozygous FH patients, conventional drug therapy cannot treat the condition, and therapeutic recourses are limited to chronic plasmapheresis or orthotopic liver transplantation.[1] Although liver transplants lower LDL levels, the procedure is life threatening; in addition, donor livers are in short supply. Cellular transplantation has been proposed to provide functional LDL receptors for the treatment of hypercholesterolemia. Transplantation of allogenic and xenogenic hepatocytes has been shown to be effective in lowering serum cholesterol in the Watanabe heritable hyperlipidemic (WHHL) rabbit,[6–9] which is an animal model for homozygous FH. Further, a number of gene therapy approaches have shown some promises in animal models and human,[10–13] and the therapies will cure a number of patients with FH in near future. As an alternative to whole-organ transplantation and/or gene therapy, we have investigated the ability of human adipose tissue-derived multilineage progenitor cells (hADMPCs) to differentiate into hepatocytes *in vitro* and to replace critical liver functions[14] as well as previous reports,[15,16] because the *in vitro* differentiation of hADMPCs into various kinds of cell types in now well reported and hADMPCs can be easily and safely obtained in large

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quantities without serious ethics issues.[17,18] In this study, we are investigating whether hADMPCs could differentiate into hepatocytes *in vivo* and replace critical liver functions as considerable therapeutic potential for cellular replacement.

Materials and Methods

Cells

hADMPCs were prepared as described previously[19] with some modifications.[14,17,18] Adipose tissues from human subjects were resected during plastic surgery in five subjects (four males and one female, age, 20–60 years) as excess discards. Ten to 50 g of subcutaneous adipose tissue was collected from each subject. All subjects provided informed consent. The protocol was approved by the Review Board for Human Research of Kobe University Graduate School of Medicine, Osaka University Graduate School of Medicine, and Foundation for Biomedical Research and Innovation. After five to six passages, the hADMPCs were used for transplantation. Human cryopreserved hepatocytes were purchased from Invitrogen (Lot number: HuP81) and cultured as indicated by the manufacturer's protocol. Human adipose tissue-derived fibroblastic cells were obtained according to previous report.[20]

Flow cytometric analysis

hADMPCs isolated from adipose tissue were characterized by flow cytometry. Cells were detached from culture dishes by 0.25% trypsin/ethylenediaminetetraacetic acid (EDTA) and suspended in Dulbecco's phosphate-buffered saline (DPBS; Nacalai Tesque) containing 0.1% fetal bovine serum. Aliquots (5×10^5 cells) were incubated for 30 min at 4°C with fluorescein isothiocyanate-conjugated mouse monoclonal antibodies to human CD31 (BD PharMingen), CD105 (Ancell Corporation), CD133 (R&D Systems), phycoerythrin-conjugated mouse monoclonal antibodies to human CD29, CD34, CD45, CD73 (BD PharMingen), CD44, or CD166 (Ancell). Isotype-identical antibodies served as controls. Further, the cells were incubated with mouse monoclonal antibodies against human stage-specific embryonic antigen-4 (from Chemicon International, Inc.), ABCG-2, or CD117 (BD PharMingen) with nonspecific mouse antibody used as a negative control. After washing with DPBS, cells were incubated with phycoerythrin-labeled goat anti-mouse Ig antibody (BD PharMingen) for 30 min at 4°C. After three washes, cells were resuspended in DPBS and analyzed by flow cytometry using a FACSCalibur flow cytometer and CellQuest Pro software (BD Biosciences).

Adipogenic, osteogenic, and chondrogenic differentiation procedure

For adipogenic differentiation, cells were cultured in the differentiation medium (Zen-Bio, Inc.). After 3 days, half of the medium was changed with adipocyte medium (Zen-Bio) every 2 days. Five days after differentiation, adipocytes were characterized by microscopic observation of intracellular lipid droplets by Oil Red O staining. Osteogenic differentiation was induced by culturing the cells in Dulbecco's modified Eagle's medium containing 10 nM dexamethasone, 50 mg/dL ascorbic acid 2-phosphate, 10 mM β-glycerophosphate (Sigma), and 10% fetal bovine serum. Differentiation was examined by Alizarin red staining. For Alizarin red staining, the cells were washed three times and fixed with dehydrated ethanol. After fixation, the cells were stained with 1% Alizarin red S in 0.1% NH_4OH (pH 6.5) for 5 min and then washed with H_2O. For chondrogenic differentiation, hADMPCs were first trypsinized and 2×10^5 cells were centrifuged at 400 *g* for 10 min. The resulting pellets were cultured in the chondrogenic medium (alpha-minimum essential medium (alpha-MEM) supplemented with 10 ng/mL transforming growth factor-β, 10 nM dexamethasone, 100 μM ascorbate, and 10 μL/mL 100×ITS Solution) for 14 days. For Alcian Blue staining, nuclear counterstaining with Weigert's hematoxylin was followed by 0.5% Alcian Blue 8GX for proteoglycan-rich cartilage matrix.

hADMPC transplantation and immunosuppression regimen

WHHL rabbits (8 weeks old; purchased from Kitayama-labes, Inc.) were anesthetized with pentobarbital (50 mg/kg). An incision distal and parallel to the lower end of the ribcage was made. The peritoneum was incised, and hADMPCs ($n = 5$) or human adipose tissue-derived fibroblastic cells ($n = 3$) (3×10^7 cells) suspended in 3 mL of Hanks' balanced salt solutions (HBSS) (20°C) or 3 mL of control saline ($n = 6$) were infused in 5 min into the portal vein via a 18-gauge Angiocath™ (BD). The immunosuppression regimen (Fig. 1A) consisted of the following: (1) intramuscular injection of cyclosporin A (6 mg/kg/day) daily from the day before surgery to sacrifice; (2) intramuscular injection of rapamycin (0.05 mg/kg/day) daily from the day before surgery to sacrifice; (3) methylprednisolone at 3 mg/kg/day (days 1–7), followed by tapering to 2 mg/kg/day (days 8–14), 1 mg/kg/day (days 15–21) and 0.5 mg/kg/day (day 22 to the time at sacrifice); (4) intravenous injection of cyclophosphamide (20 mg/kg/day) at days 0, 2, 5, and 7; (5) ganciclovir (2.5 mg/kg/day intramuscular injection (i.m.)) was also administrated to avoid viral infection in the immunocompromised host.

DNA extraction and quantification of human-derived cells

Total DNA of WHHL rabbit liver, which was obtained at the time just after hADMPC transplantation, and 2, 4, 8, and 12 weeks after transplantation, were isolated using a NucleoSpin Tissue kit (Macherey-Nagel) according to the manufacturer's instructions. hADMPCs and rabbit hepatocytes were mixed at the ratios of 100:0 (100%), 10:90 (10%), 1:99 (1%), 0.1:99.9 (0.1%), 0.01:99.99 (0.01%), and 0.001:99.999 (0.001%), and DNA was isolated. Seven hundred nanograms of each samples of extracted DNA was quantified by real-time polymerase chain reaction (PCR) using the ABI Prism 7900 Sequence Detection System (Applied Biosystems), primers for the 82 bp *Alu* amplicon (forward, 5′-GTCAGGAGATCGA GACCATCCC; reverse, 5′-CCACTACGCCCGGCTAATTT), and SYBR Green (TOYOBO) dye using a previously published protocol.[21,22] Reactions were performed in quadruplicate and the *Alu* levels were calculated by the standard curve.

Assay for lipid profiling

Serum samples were obtained from nonfasting rabbits before and after transplantation. Serum total cholesterol was measured in each sample using assay kits from Wako Pure Chemical Industries. Serum lipoproteins were analyzed by an on-line dual enzymatic method for simultaneous quantification of cholesterol and triglycerides by high-performance

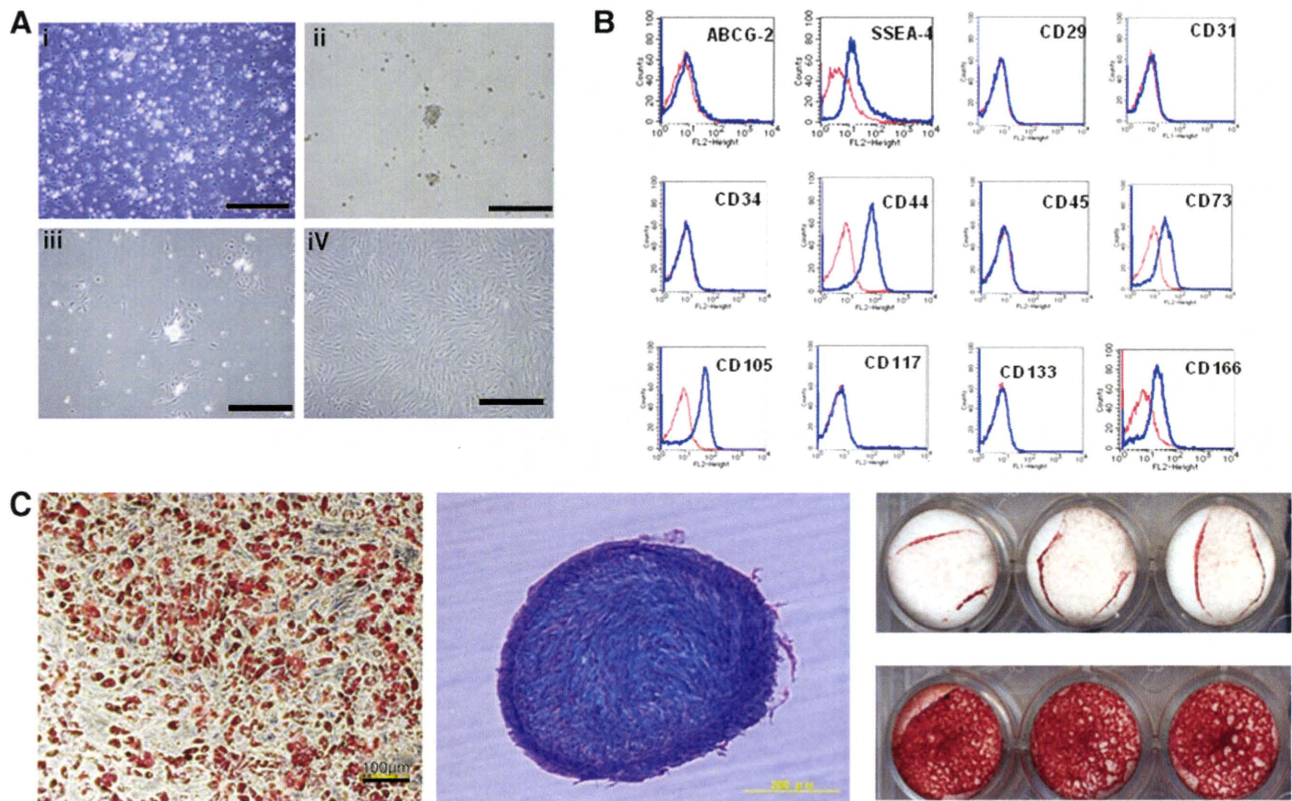


FIG. 1. **(A)** Morphological characters of human adipose tissue-derived multilineage progenitor cells (hADMPCs). The cells obtained from adipose tissue were seeded and incubated for 24 h **(i)**. After incubation, the adherent cells were treated with ethylenediaminetetraacetic acid solution, and the resulting suspended cells were replated at a density of 10,000 cells/cm^2 on human fibronectin-coated dishes (BD BioCoat) **(ii, iii)**. Within two to three passages after the initial plating of the primary culture, hADMPCs appeared as a monolayer of large flat cells (25–30 μm in diameter). As the cells approached confluence, they assumed a more spindle-shaped, fibroblastic morphology **(iv)**. i) Bar = 499 μm, ii) bar = 201 μm, iii) bar = 502 μm and iv) bar = 202 μm. **(B)** Cell surface markers expressed on hADMPCs. The cells were negative for markers of the hematopoietic lineage (CD45) and of hematopoietic stem cells, ABCG-2, CD34, and CD133. They were also negative for CD31, an endothelial cell-associated marker, and the surface antigen c-Kit (CD117). However, they stained positively for a number of surface markers characteristic of mesenchymal and/or neural stem cells, but not embryonic stem (ES) cells, including CD29, CD44 (hyaluronan receptor), CD73, CD105 (endoglin), and CD166. hADMPCs also were positive for stage-specific embryonic antigen (SSEA)-4. **(C)** Adipocytic, chondrocytic, and osteocytic differentiation potentials of hADMPCs. Adipocytic differentiation potential of hADMPCs was confirmed by Oil Red O staining (the left panel) (bar = 100 μm). Chondrocytic differentiation potential of hADMPCs was estimated by extracellular matrices with Alcian Blue staining (the middle panel). Osteogenic differentiation potential of hADMPCs was confirmed by Alizarin red S staining for mineralized nodules (the right panel).

liquid chromatography at Skylight Biotech, according to the procedure as described.[23]

Immunohistochemical staining of WHHL rabbit liver sections

The WHHL livers were harvested and fixed immediately with 10% formalin. They were placed into optimal cutting temperature compound (Sakura Finetechnical Co.), frozen immediately, and then sectioned at 7 μm thickness. The sections were then incubated with blocking solution (Blocking one; Nacalai Tesque) for 1 h. The samples were incubated with rabbit anti-human-specific albumin antibody (MBL), rabbit anti-human-specific alpha 1 anti-trypsin antibody, and rabbit anti-LDL receptor antibody, followed by Alexa Fluor 488-labeled goat anti-rabbit IgG (Molecular Probes). To show the colocalization of human CD90 and albumin, the samples were incubated with the rabbit anti-human CD90 monoclonal antibody (Epitomics, Inc.) and then with Alexa Fluor 488-labeled goat anti-rabbit IgG (Molecular Probes), and washed extensively. Then, the specimens were incubated with rabbit anti-human-specific albumin antibody (MBL), followed by Alexa Fluor 546-labeled goat anti-rabbit IgG (Molecular Probes). The treated sample was examined with a BioZero laser scanning microscope (Keyence).

PCR analysis of WHHL rabbit liver for human liver-specific genes

Total RNAs of WHHL rabbit liver, hADMPCs, and human hepatocytes were isolated using an RNAeasy kit (Qiagen). After treatment with DNase, the cDNA was synthesized using Superscript III RNase H-minus Reverse Transcriptase (Invitrogen). Real-time PCR was performed using the ABI Prism 7900 Sequence Detection System (Applied Biosystems). About 20×Assays-on-Demand™ Gene Expression Assay Mix for human alpha-1-antitrypsin (Hs01097800_m1), human albumin (Hs00609411_m1), human factor 9, human GATA-binding

protein 4 (GATA4) (Hs00171403_m1), human hepatocyte nuclear factor 3 beta (Hs00232764_m1), human LDL receptor (Hs00181192_m1), and human glyceraldehyde-3-phosphate dehydrogenase (Hs99999905_m1) were obtained from Applied Biosystems. It was confirmed that human detectors and rabbit detectors do not cross-react with the other species. TaqMan® Universal PCR Master Mix, No AmpErase® UNG (2×), was also purchased from Applied Biosystems. Reactions were performed in quadruplicate and the mRNA levels were normalized relative to human glyceraldehyde-3-phosphate dehy-

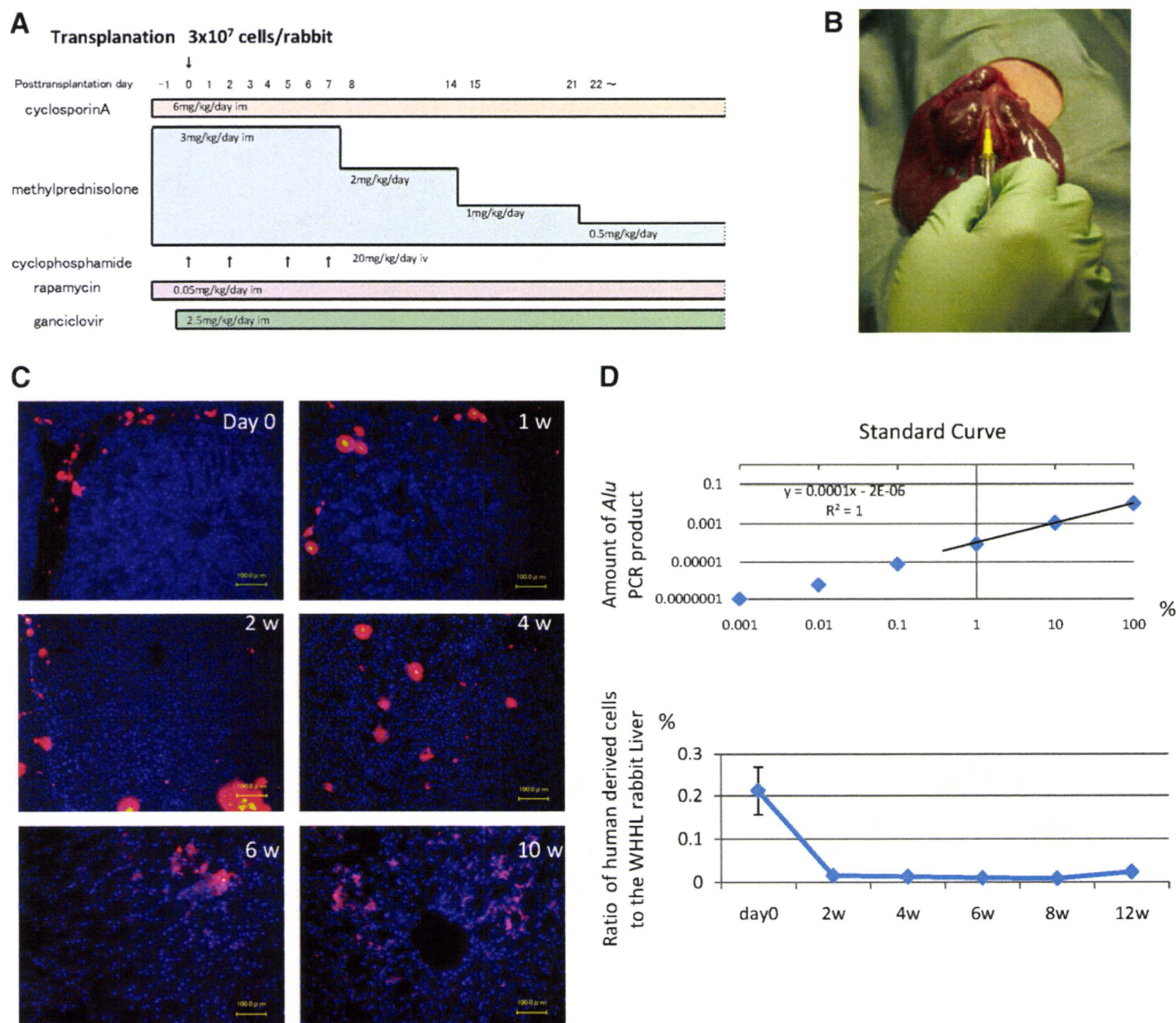


FIG. 2. **(A)** Immunosuppression regimen. Cyclosporin A (6 mg/kg/day) and rapamycin (0.05 mg/kg/day) were administered intramuscularly daily from the day before surgery to sacrifice. Methylprednisolone was administered at 3 mg/kg/day (days 1–7), 2 mg/kg/day (days 8–14), 1 mg/kg/day (days 15–21), and 0.5 mg/kg/day (day 22 to sacrifice). Cyclophosphamide (20 mg/kg/day) was injected intravenously at days 0, 2, 5, and 7. Ganciclovir (2.5 mg/kg/day) was also injected intramuscularly to avoid viral infection in the immunocompromised host. **(B)** Surgical procedure. Watanabe heritable hyperlipidemic (WHHL) rabbits were anesthetized with pentobarbital. An incision was made distal and parallel to the lower end of the ribcage. The peritoneum was incised and hADMPCs, and human adipose tissue-derived fibroblastic cells (hADFCs) (3×10^7 cells/rabbit) or controls were infused into the portal vein using an 18-gauge Angiocath. **(C)** Localization of transplanted hADMPCs in the WHHL liver. At the day of and 1, 2, 4, 6, and 10 weeks after transplantation of DiI-labeled hADMPCs via the portal vein, the WHHL rabbit liver was examined histologically. DiI-fluorescent labeled-hADMPCs resided and distributed in the portal area at the day of transplantation. One to 2 weeks after transplantation, the DiI-stained hADMPCs-derived cells were localized near the portal areas. Four weeks after transplantation some of the DiI-stained cells resembled innate hepatocytes morphologically. Six and 10 weeks after transplantation, DiI-positive transplanted cells were dispersed in a centrilobular direction, resembling the mature innate hepatocytes. Bars = 100 μm. **(D)** Quantification of repopulation of the transplanted cells in the liver. The ratios of human-derived cell repopulation were examined by analyzing an *Alu* repetitive DNA sequence at the day of and 2, 4, 8, and 12 weeks after transplantation. In upper panel the standard curve was indicated, and in lower panel the ratio of repopulation of human cells was shown in time course after transplantation of hADMPCs.

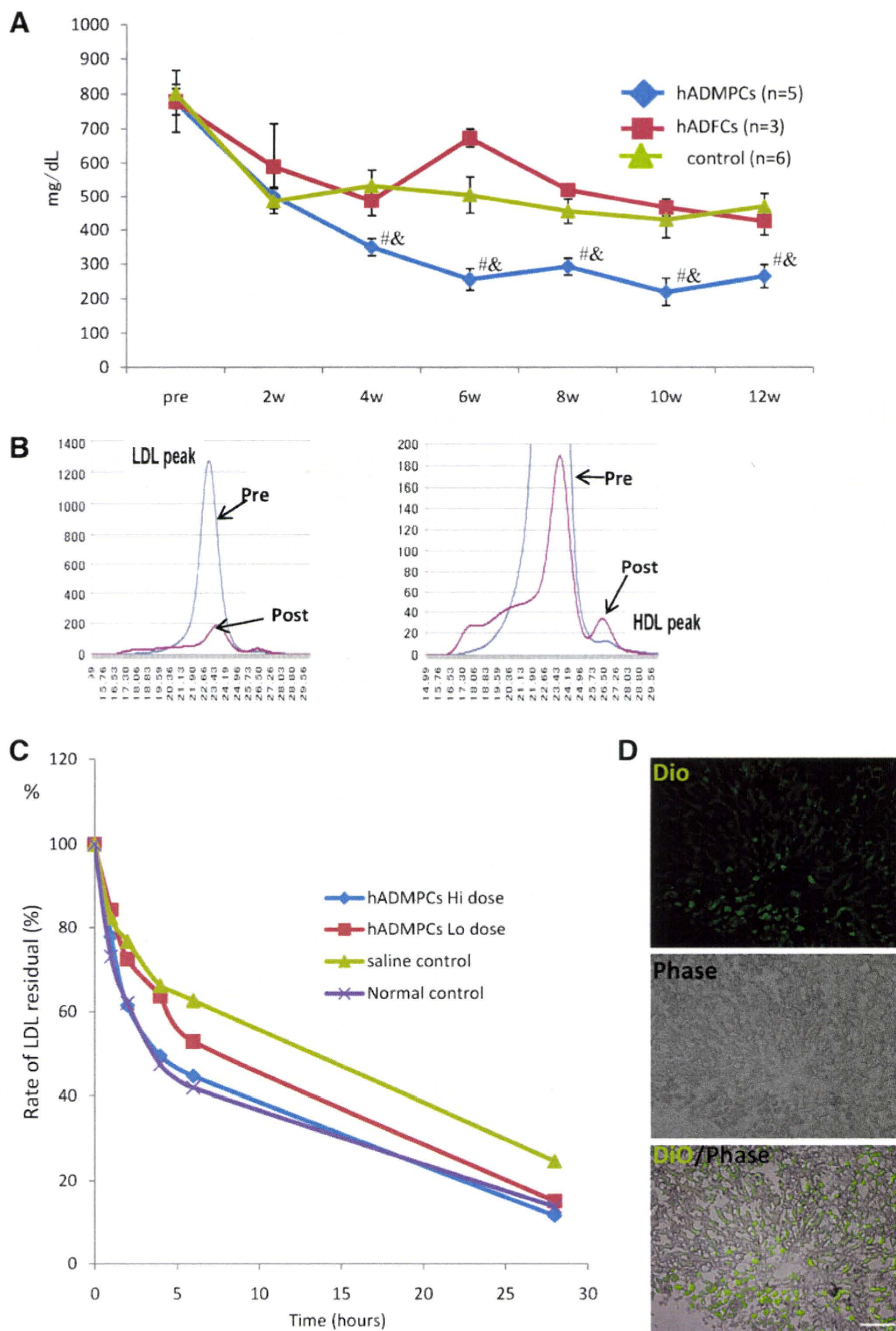
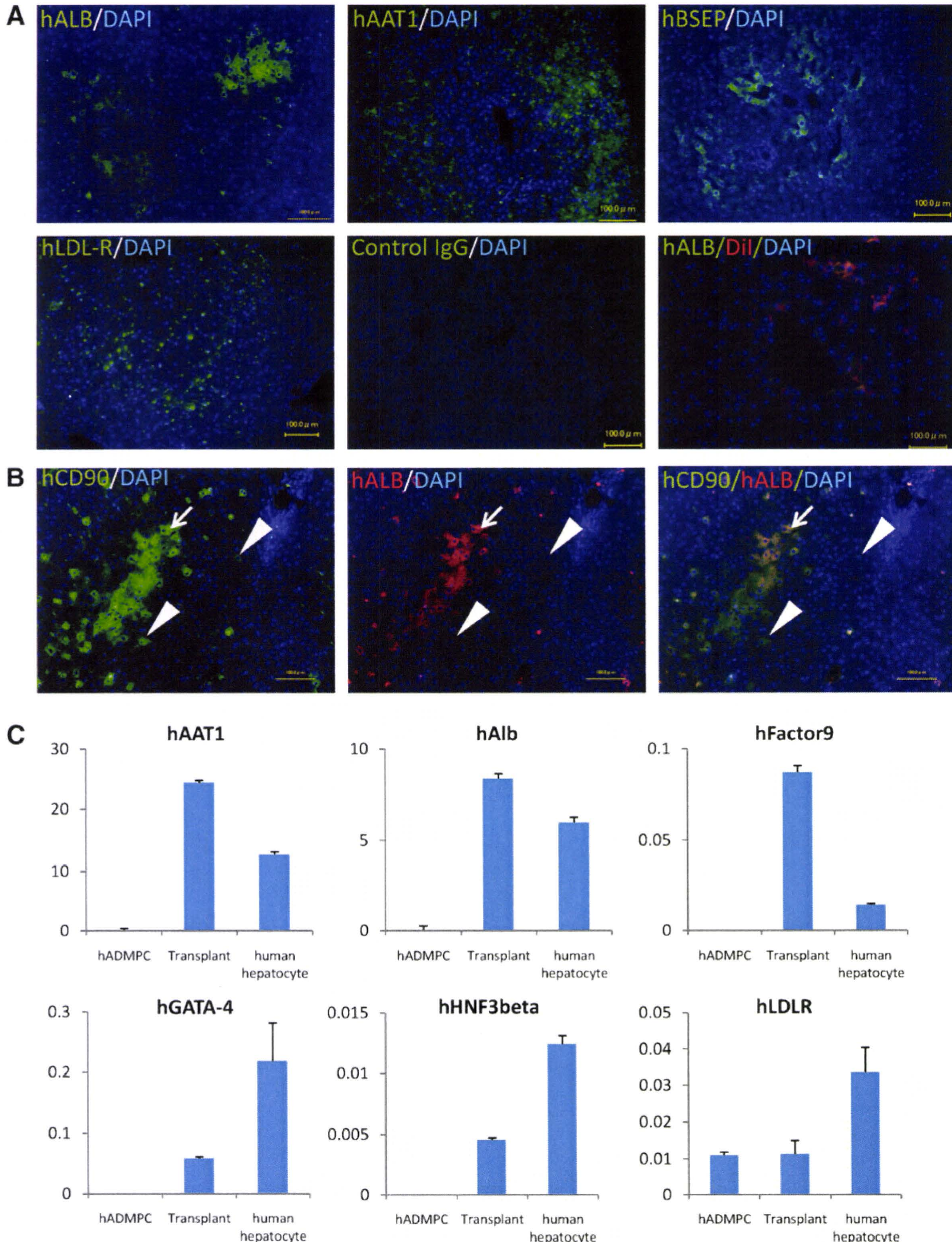


FIG. 3. **(A)** Total serum cholesterol levels. hADMPC transplantation in WHHL rabbits was followed for 12 weeks. Total serum cholesterol was measured in five rabbits that each received 3×10^7 hADMPCs, three rabbits that each received 3×10^7 hADFCs, and in six rabbits that received saline (control). Bars indicated mean $\pm$ standard error of the mean (SEM) ($^{\#}p < 0.05$; control vs. the hADMPC-transplanted WHHL rabbit; $^{\&}p < 0.05$; the hADFC-transplanted WHHL rabbit vs. the hADMPC-transplanted WHHL rabbit). **(B)** Lipoprotein profiles in a representative WHHL rabbit with hADMPC 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 high-density lipoprotein (HDL) peak. **(C)** Rate of clearance of LDL from the serum of rabbits with and without transplantation of hADMPCs. Animals were injected with ^{125}I-labeled human LDL, and the time course of clearance was monitored following trichroloacetic acid precipitation of serum at time 5 min, 1 h, 2 h, 4 h, 6 h, and 28 h. Residual ^{125}I-LDL was expressed as percentages of that at 5 min. $^{\#}p < 0.05$ (control vs. the hADMPC-transplanted WHHL rabbit [low dose]) and $^{*}p < 0.05$ (control vs. the hADMPC-transplanted WHHL rabbit [high dose]). **(D)** DiO-LDL uptake into hADMPC-derived hepatocytes in the WHHL rabbi liver. Thin-sliced recipient liver was incubated with DiO-labeled LDL in the serum-free medium for 24 h. After washing and fixation, the incubated slices were applied for fluorescent microscopy. DiO-LDL uptake cells (green) and no uptake parenchymal cells were observed in the section. Bar = 100 μm.

drogenase expression. To confirm that hADMPCs differentiated into hepatocytes *in vivo*, the cells before transplantation and human primary hepatocytes (Invitrogen, Lot number; HuP81) were applied for quantitative PCR as control.

Clearance of ^{125}I-LDL from rabbit serum

WHHL rabbits (8 weeks old) were anesthetized with pentobarbital (50 mg/kg). The peritoneum was incised and hADMPCs (high-dose; 3×10^7 cells/rabbit, $n=2$, low-dose; 5×10^6 cells/rabbit, $n=2$) suspended in 3 mL of HBSS (20°C) ($n=5$) or 3 mL of control saline ($n=2$) were infused into the portal vein via a 18-gauge Angiocath (BD). The rabbits were immunosuppressed using the protocol illustrated in Figure 1A. Eight weeks later, the animals were tested by the LDL turnover assay. ^{125}I human LDL (BT-913R, Lot No. 9130709; Biomedical Technologies Inc.) was delivered via the marginal ear vein of the WHHL rabbits and normal control



rabbits in physiological saline containing 2 mg/mL bovine serum albumin. Blood was collected from the opposite ear after injection at 5 min, 1 h, 2 h, 4 h, 6 h, and 28 h. ^{125}I-labeled apolipoprotein B-containing LDL was precipitated with 20% of trichloroacetic acid (Wako Pure Chemical Industries) (serum; 320 μL, 100% w/v trichloroacetic acid (TCA) 80 μL), and then the precipitants were applied for counting.

Uptake of DiO-labeled LDL by transplants ex vivo

Human LDL (1.019–1.063 g/mL) was isolated by sequential ultracentrifugation from normolipidemic donors as previously described,[24] dialyzed against saline-EDTA, and then sterilized by filtration through a 0.2 μm filter. Lipoproteins were labeled with 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO; Sigma) by incubating the LDL in 0.5% bovine serum albumin/PBS with 100 mL DiO in dimethyl sulfoxide (3 mg/mL) for 8 h at 37°C. The lipoproteins were obtained by sequential ultra centrifugation (1.019–1.063 g/mL) as described,[14] and then dialyzed against PBS and filtered before use. To evaluate the uptake of DiO-LDL by transplants *ex vivo*, thin-sliced WHHL rabbit liver tissue were incubated with serum-free Dulbecco's modified Eagle's medium containing 10 μg/mL DiO-LDL for 24 h at 37°C. Finally, the incubated slices were rinsed, fixed with 10% formalin, sectioned into 5 μm thickness, and mounted with Perma-Flour (Japan Tanner Corporation). The slides were examined using a BioZero laser scanning microscope (Kyence).

Statistical analysis

Values were expressed as mean ± standard error of the mean. Differences between mean values of treated and untreated groups were evaluated using the Student's *t*-test. A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using the SPSS Statistics 17.0 package (SPSS Inc.).

Results

Characteristics of hADMPCs

The cells obtained from adipose tissue were seeded and incubated for 24 h (Fig. 1Ai). After incubation, the adherent cells were treated with EDTA solution, and the resulting suspended cells were replated at a density of 10,000 cells/cm^2 on human fibronectin-coated dishes (BD BioCoat) (Fig. 1Aii and 1Aiii). Within two to three passages after the initial plating of the primary culture, hADMPCs appeared as a monolayer of large flat cells (25–30 μm in diameter). As the cells approached confluence, they assumed a more spindle-shaped, fibroblastic morphology (Fig. 1Aiv). After passaging five to six times, the hADMPCs were applied for transplantation. We used flow cytometry to assess markers expressed by hADMPCs (Fig. 1B). The cells were negative for markers of the hematopoietic lineage (CD45) and of hematopoietic stem cells, ABCG-2, CD34, and CD133. They were also negative for CD31, an endothelial cell-associated marker and the surface antigen c-Kit (CD117). However, they stained positively for a number of surface markers characteristic of mesenchymal and/or neural stem cells, but not embryonic stem cells, including CD29, CD44 (hyaluronan receptor), CD73, CD105 (endoglin), and CD166. hADMPCs also were positive for stage-specific embryonic antigen-4. Next, adipogenic, osteogenic, and chondrogenic differentiation potential of hADMPCs were examined (Fig. 1C). Adipogenic differentiation was induced by culture with differentiation medium containing 1-methyl-3-isobutylxanthine (a peroxisome proliferator-activated receptor γ agonist), dexamethasone, and insulin. Induction was confirmed by the accumulation of intracellular lipid droplets that were stained with Oil Red O. After 7-day induction for osteogenesis, hADMPCs were stained with Alizarin red S for mineralized nodules. hADMPCs showed intense Alcian Blue staining, indicating chondrogenic induction capability of hADMPCs.

Serum cholesterol in WHHL rabbit with transplants

hADMPCs were separated from human subcutaneous adipose tissues, cultured for five to seven passages, and applied for transplantation into WHHL rabbits. WHHL rabbits received immunosuppressants and an antiviral agent as illustrated in Figure 2A, and then were transplanted 3×10^7 hADMPCs by portal vein infusion (Fig. 2B). At the day of and 1, 2, 4, 6, and 10 weeks after transplantation of hADMPCs via the portal vein, we examined whether the cells reside or not in the liver after transplantation. Typical

FIG. 4. **(A)** Immunohistochemical identification of human hepatocytic marker cells in liver sections of WHHL rabbits after hADMPC transplantation. Twelve weeks after hADMPC transplantation, human albumin-, human alpha-1-antitrypsin-, human bile salt export pump (BSEP)-, and LDL-receptor-positive cells were dispersed within the perivenous regions of the liver parenchyma, where they made contact with and integrated among the host cells with cell–cell interactions between hADMPC-derived cells and diseased hepatocytes pair. Ten weeks after transplantation of DiI-stained hADMPCs, copresence of human albumin (green) and pretreated DiI-fluorescence (red) on the same cells was observed. Bar = 100 μm. **(B)** Differentiation of transplanted hADMPCs into hepatocyte-like cells. Twelve weeks after transplantation, almost but not all human CD90-positive cells expressed human albumin, indicating that major population of transplanted hADMPCs could differentiate into hepatocyte-like cells (left panel: human CD90; middle panel: human albumin; right panel: merge). Arrows indicate human CD90 and human albumin double-positive cells; arrowheads indicate human CD90-positive but human albumin-negative cells. **(C)** Human hepatic gene expression in WHHL rabbit liver after hADMPC transplantation. RNA was prepared from the WHHL rabbit liver 12 weeks after hADMPC transplantation. We used the following hepatic markers: human alpha-1-antitrypsin, human albumin, human factor IX, human GATA-binding protein 4 (GATA-4), human hepatocyte nuclear factor 3 (HNF-3) beta, and human LDL-receptor. Their expression levels were examined by quantitative real time-polymerase chain reaction (RT-PCR) using Assays-on-Demand Gene Expression Assay Mix. The livers of WHHL rabbits that received saline ($n = 3$) were negative for human hepatic genes. The mRNA levels were normalized based on human glyceraldehyde-3-phosphate dehydrogenase expression as housekeeping gene and data are mean ± SEM of triplicate experiments. The livers of WHHL rabbits that received hADMPC transplantation ($n = 3$) were positive for human hepatic genes, and their expression levels were similar to those of human primary hepatocytes but not hADMPCs *per se*. Data are mean ± SEM.

distribution patterns of transplanted hADMPCs were followed in Figure 2C. DiI-fluorescent labeled-hADMPCs resided and distributed in the portal area at the day of transplantation. Six and 10 weeks after transplantation, DiI-positive transplanted cells migrated into centrilobular direction. Next, to demonstrate certain percentage of repopulation of the transplanted cells in the liver, the ratios of human-derived cell repopulation were examined by analyzing a repetitive DNA sequence at the day of and 2, 4, 6, and 12 weeks after transplantation (Fig. 2D). To indicate standard curve, we mixed the indicated percentage of hADMPCs with rabbit hepatocytes and plotted the obtained the amount of *Alu* PCR products, and estimated the amount of repopulation of the transplanted cells in the liver. At the day of transplantation, the ratio of hADMPCs to whole WHHL rabbit liver cells was $0.21\% \pm 0.056\%$ (mean $\pm$ standard error of the mean) and the ratio decreased to $0.016\% \pm 0.002\%$, $0.011\% \pm 0.001\%$, and $0.009\% \pm 0.0001\%$ after 2, 4, and 8 weeks of transplantation, respectively. After 12 weeks of transplantation, the ratio was increased to $0.024\% \pm 0.00005\%$ as indicated (Fig. 2D).

To reveal the effects of hADMPC transplantation onto the lipid profiles of the WHHL rabbit, serum cholesterol levels were monitored over 12 weeks (Fig. 3A). Significant reductions in total serum cholesterol were observed within 4 weeks of the transplantation, and the reductions were maintained for the entire period. The reduction in serum cholesterol in the animals that received hADMPC transplantation was significantly greater than that of the control group. To determine the effects of hADMPC transplantation on the fractions of high-density lipoprotein and LDL in recipient animals, fractionation by fast protein liquid chromatography was performed (Fig. 3B). Transplantation of hADMPCs 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 hADMPCs contributed 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 hADMPCs than WHHL rabbits without transplanted hADMPCs (Fig. 3C). Rabbits with hADMPC transplants showed $\sim$2.4-fold (high-dose; 3×10^7 cells/rabbit) and 1.4-fold (low-dose; 5×10^6 cells/rabbit) increase in the rate of LDL cholesterol clearance.

To evaluate the uptake of DiO-LDL by transplants *ex vivo*, thin-sliced WHHL rabbit liver was incubated with DiO-labeled LDL for 24 h and the uptake was examined as clearance experiment (Fig. 3D). DiO-LDL was uptaken by some but not all of the cells in the WHHL rabbit liver transplanted with hADMPCs. The DiO-LDL-uptaking cells were seen dispersed, contacted, and integrated among the nonuptaking parenchymal cells, suggesting that hADMPCs differentiated into hepatocytes *in vivo*, lowered of serum cholesterol via LDL uptake.

hADMPCs reside, survive, and differentiate into hepatocytes in vivo

After establishment of the graft as indicated by long-term lowering of serum cholesterol, human-specific hepatocytic proteins, such as albumin, alpha-1-antitrypsin, bile salt export pump, and LDL-receptor, positive cells were identified dispersed within perivenous regions of the liver parenchyma, where they have contacted and integrated among the host cells (Fig. 4A), with cell–cell interactions conserved between hADMPC-derived hepatocytes and diseased hepatocytes pair. Ten weeks after transplantation of DiI-prestained hADMPCs, copresence of human albumin (green) and pretreated DiI-fluorescence (red) on the same cells was observed (Fig. 4A), indicating the transplanted hADMPCs might differentiate into hepatocyte-like cells. To confirm transplanted hADMPCs might differentiate into hepatocyte-like cells and to reveal the efficacy of differentiation, the colocalization of human CD90 and human albumin was examined. As shown in Figure 4B, almost but not all human CD90-positive cells expressed human albumin, indicating that about 80% or more of transplanted hADMPCs could differentiated into human albumin-positive hepatocyte-like cells 12 weeks after transplantation. Next, to confirm the differentiation of hADMPCs into hepatocytes *in vivo*, expression of hepatocyte markers was analyzed by quantitative RT-PCR. The WHHL rabbit liver that was transplanted with hADMPCs expressed higher levels of human-specific alpha-1-antitrypsin, albumin, and coagulation factor IX than hADMPCs (Fig. 4C). The expression levels of human GATA-4, human hepatocyte nuclear factor 3 beta, and LDL-receptor were also higher in the WHHL rabbit liver than hADMPCs (Fig. 4C). These results indicate that hADMPCs differentiate into mature hepatocytes *in vivo*.

Discussion

We have used the WHHL rabbit to study the ability of hADMPC-derived hepatocytes to lower serum cholesterol in an animal model of FH. Our results have shown that hADMPCs transplanted into the rabbit liver differentiate into hepatocytes *in vivo* and effectively clear LDL from the circulation.

The reductions in cholesterol brought about by the engrafted hADMPC-derived hepatocytes suggest that human LDL receptors can act as replacement for the mutant LDL receptors in the WHHL rabbit. This capacity of hADMPC-derive hepatocytes is not unexpected, as the liver is the most important site of LDL uptake, accounting for >50% of total removal from the circulation, and the liver is only organ capable of converting cholesterol to bile for excretion. The substantial decrease in serum cholesterol achieved suggests that the hADMPC-derived hepatocytes both internalize LDL and metabolize the cholesterol to bile for excretion. The correlation between cholesterol and coronary heart disease has been well documented, and decreases in serum cholesterol of the magnitude that we have demonstrated would be expected to decrease morbidity and mortality in the patients with severe FH.[25]

The appearance of the hADMPC-derived hepatocytes as revealed by immunohistochemistry and RT-PCR indicated that the hADMPCs differentiated into hepatocytes and integrated into the liver parenchyma. The perivenous migration of the differentiated hepatocytes derived from hADMPCs along the portal-venous axis and suggests that hADMPCs recognize conserved signals on host cells and matrix. There are some reports describing the hepatogenic differentiation potential of hADMPCs.[15,16] These studies

described that hepatocytes differentiated from hADMPCs *ex vivo* engrafted in the liver and functioned, and that the hADMPCs could be resided and changed their characters into hepatocyte-like cells only in the chemically damaged liver. These reports, revealing that hADMPCs have capabilities to differentiate into hepatocytes, hinted us that hADMPCs might differentiate into hepatocytes in liver. Hepatogenic signals from the microenvironment such as cell-to-cell connections or intermediates are probably important factors that dictate the type of functional hepatocytes in hepatic differentiation.[26] We are currently investigating the mechanism for the differentiation hADMPCs into hepatocytes.

The choice of cell source is critical for realizing success in cellular therapy. Liposuction surgeries yield a massive amount of lipoaspirate adipose tissue from 100 mL to >3 L as cell sources.[27] A major advantage of hADMPCs is their availability in safe and easy with few ethical issues, as compared with the shortage of human livers for orthotopic transplantation, which has been shown to be effective for the treatment of FH.[25] Our serum cholesterol reduction studies and *in vitro* studies demonstrated that human LDL binds to the hADMPC-derived hepatocytes receptor, indicating that this therapy will be useful in humans. Previous attempts to study the efficacy of hepatocyte transplantation in the WHHL rabbit model have employed allogenic hepatocytes, xenogenic hepatocytes, or hepatocytes transduced *ex vivo* with a recombinant retrovirus containing the LDL receptor cDNA.[6–13] The lowering effects of hepatocyte transplantation on serum cholesterol have been reported, but there was some problems. First, hepatocytes could not be expanded *ex vivo* with functional potentials; second, the cell viability reduced after cryopreservation; third, the many injected hepatocytes are supposed to be cleared by the reticuloendothelial system or lose viability during early phase. The rate of LDL clearance was returned to normal in LDL receptor knockout mice by introduction of an adenoviral construct containing an LDL receptor cDNA, and similar approaches have lowered serum cholesterol levels in the WHHL rabbit.[10,12,13] However, sustained expression of the LDL receptor from viral vectors can be difficult to achieve.[11,13] Moreover, hepatocytes derived from hADMPCs have the advantage that the LDL receptor is expressed from an endogenous gene with intact regulatory sequences. Such control of LDL receptor levels would not be expected after treatment of hypercholesterolemia with LDL receptor cDNA construct that lack the regulatory regions of the gene.[28]

Our experiments have shown that the hADMPCs expressed hepatocyte markers after transplantation *in vivo* and the integrated cells into parenchyma provide functional LDL receptors, indicating that they differentiated into hepatocytes and might lower serum cholesterol in the WHHL rabbit. These results suggested that hADMPC transplantation via portal vein could correct the metabolic defects of FH patients and that hADMPC-derived hepatocytes could function as supplier with plasma proteins derived from liver, giving us an idea that hADMPC-transplantation might be a novel cell therapy for hemophilia, alpha-1 antitrypsin deficiency, mucolipidosis, and other diseases caused by genetic defects for liver function. In near future, the therapy will be a novel therapy for kinds of inherited liver diseases.

Acknowledgments

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), RIKEN Program for Drug Discovery and Medical Technology Platforms, and Kobe Translational Research Cluster, the Knowledge Cluster Initiative, Ministry of Education, Culture, Sports, Science and Technology (MEXT).

Disclosure Statement

All of the authors stated no conflict of interest.

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Received: March 7, 2010
Accepted: August 9, 2010
Online Publication Date: September 21, 2010