Adipocytes as a vehicle for *ex vivo* gene therapy: Novel replacement therapy for diabetes and other metabolic diseases

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ABSTRACT

Because of its availability and recent advances in cell biology, adipose tissue is now considered an ideal target site for the preparation of recipient cells and for the transplantation of gene-transduced cells for supplementation of therapeutic proteins. Inherited or acquired serum protein deficiencies are the ideal targets for gene therapy. However, to develop an effective *ex vivo* gene therapy-based protein replacement treatment, the requirements for the recipient cells are different from those for standard gene therapy that is intended to correct the function of the recipient cells themselves. To meet the requirements for such a therapeutic strategy, recent *in vitro* and animal model studies have developed new methods for the preparation, culture, expansion and manipulation of adipose cells using advanced gene transduction methods and transplantation scaffolds. In this short review, we introduce the progress made in novel adipose tissue-based therapeutic strategies for the treatment of protein deficiencies by our group and other investigators, and describe their future applications for diabetes and other metabolic diseases. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2011.00133.x, 2011)

KEY WORDS: Adipocyte, Gene therapy, Metabolic disease

INTRODUCTION

Since the first gene therapy trial against advanced melanoma using gene-transduced lymphocytes was published in 1990[1], numerous therapeutic clinical trials have been carried out, and inherited monogenic disorders represent approximately 8% of the diseases targeted by gene therapy applications (http://www.wiley.com/legacy/wileychi/genmed/clinical/). Recent studies on the biology of pluripotent stem or progenitor cells have suggested the sustained production of therapeutic proteins to be a potential treatment strategy for patients with a variety of genetic disorders[2–5]. The ability of cells to self-renew at a high proliferation rate has led to the expectations that these cells might be ideal targets for retroviral vector-mediated transgene delivery for permanent correction of the defect, not only for immunodeficiencies, but also for a variety of inherited or acquired metabolic diseases, including diabetes mellitus.

EX VIVO GENE THERAPY FOR IMMUNODEFICIENCIES

The most impressive outcomes of *ex vivo* gene therapy trials have been reported in subjects with immunodeficiencies as a result of monogenic disorders, including adenosine deaminase deficiency (ADA-SCID)[6,7], γc chain deficiency (X-SCID)[8,9] or X-linked chronic granulomatous disease (X-CGD)[10,11], where the treatments were combined with the infusion of *ex vivo* gene-corrected hematopoietic cells. Among these trials, the treatment for X-SCID caused the oncogenesis of gene-transduced cells through the clonal expansion of the cells with the activation of cellular oncogenes as a result of insertion of the MLV LTR sequence into the promoter region of the *LMO2* gene[12]. Clonal expansion was also reported in X-CGD gene therapy trials[11] and myelodysplasia with monosomy 7 was caused by the insertional activation of ecotropic viral integration site 1 (*EVI1*)[13].

To correct the immune disorder in these patients, it is necessary for the infused gene-corrected cells to grow, differentiate into multiple hematopoietic lineages and reconstruct the immune system. In the case of X-SCID, the introduced gene (γc) is essential for the maturation of T cells, hence, only the gene-transduced cells grow and mature into functional lymphocytes, causing *in vivo* selection of the gene-corrected cells[14], although the precise mechanisms underlying the development of leukemia in such patients are not completely understood[15].

EX VIVO GENE THERAPY FOR FAMILIAL HYPERCHOLESTEROLEMIA

The liver is one of the primary sites of metabolic activity, and is thus the target organ of the pathogenesis for many metabolic disorders. Hepatocytes are the major cell type in the liver and have the ability to proliferate after injury, making them seem

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Received 3 April 2011; accepted 6 April 2011

like an ideal target for *ex vivo* gene therapy purposes. Using essentially the same technique, in which a partial hepatectomy followed by MoMLV-mediated gene transduction and reinfusion of the cells was carried out, a total of five familial hypercholesterolemia patients were treated[16,17]. However, levels of serum cholesterol reduction in these patients were moderate, and metabolic responses after gene transfer varied substantially among the five recipients. Thus, the strategy has not been carried out again to date, as a result of the invasiveness of the procedure and ineffective cell engraftment in addition to difficulties in cell preparation steps[18], and the development of the treatment has been shifted to more efficient *in vivo* transduction methodologies[19]. The various gene therapy trials carried out for the treatment of various metabolic deficiencies are summarized in Table 1.

CURRENT PROGRESS IN OTHER DISEASES

Genetic and acquired disorders causing secreted serum enzyme deficiencies have also been postulated to be ideal targets for gene therapy applications. In these diseases, the deficient protein functions systemically, and its defect causes severe complications in target organs. Therapeutic genes expressed by a viral vector are directly infused into the target tissues (*in vivo* gene therapy), or therapeutic gene-transduced cells are transplanted (*ex vivo* gene therapy) and, subsequently, functional proteins are produced systemically to improve the symptoms through protein replacement therapy.

In the former strategy, the gene transduction efficiency might vary depending on the tissue and cell types, and unexpected ectopic gene transduction is not completely prevented. Acute toxicity has been observed after the clinical use of an adenoviral vector[20], leading to limited further use. The efficacy of the currently available AAV vectors was shown to be hampered by the pre-existing host immune system, resulting in limitations of their applications to a clinical trial for hemophilia B treatment[21].

In the latter strategy, these side-effects can be minimized by preparing the recipient cells *in vitro*, and gene transduction efficiency is controllable and checked before transplantation, although cell preparation steps are required. In addition, transplanted cells are required to reside and/or survive in the patient rather than replicate, in order to continue providing a therapeutic level of protein secretion. Hemophilia has been indicated to be one of most obvious candidates for protein replacement therapy. Although considerable efforts have been expended to apply *ex vivo* gene therapy to treat these patients, no obvious clinical benefits were observed[22–24]. However, transplantation of genetically-modified fibroblasts into the forebrain was shown to be effective in clinical gene therapy trials of Alzheimer's disease[25]. Another approach using encapsulated-cell biodelivery technology to provide nerve growth factor (NGF) release (the product name is NsG0202) is currently being studied in a clinical trial. In this strategy, cells are enclosed by an immunoprotective, semi-permeable, hollow fiber membrane, enabling the influx of nutrients and outflow of NGF, and preventing the direct contact of the cells with the host tissue and immune system. Preliminary results have shown good safety and tolerability with no serious adverse events, and an increase in the expression of cortical nicotinic receptors, and three patients have shown cognitive improvement[26]. However, these strategies were designed for local supplementation of NGF. There is thus an absolute necessity for a novel approach to systemic delivery of therapeutic proteins. Therefore, long-lasting protein replacement therapy using gene-transduced cells is needed to provide a sufficient therapeutic strategy for systemic metabolic diseases.

ADIPOSE TISSUE AS A TARGET TISSUE FOR *EX VIVO* GENE THERAPY

To develop life-long protein replacement therapy through transplantation of gene-transduced cells, adipose tissue has been explored as a suitable target for several reasons. First, aspirated fat is a common source of autologous tissue transplantation for the correction of tissue defects in plastic and reconstructive surgery[27–29]. Adipose tissue is well-vascularized, and now is recognized as an important endocrine and secretory organ[30–33], and thus could enable the systemic delivery of the therapeutic protein in cell-based gene therapy applications[34–37]. Fat cells have been shown to have a relatively long lifespan[38]. With regard to safety concerns, lipoaspiration or resection of adipose tissue and fat grafting are routinely carried out in the plastic and reconstructive surgery field with minimal risk. Adipocyte-based therapeutic strategy for enzyme replacement therapy is shown in Figure 1.

Recently, adipogenic potential has been shown to suppress the tumorigenic activity of *ink4a* knockout mesenchymal stem cells[39]. Furthermore, if the gene-transduced cells show an abnormal phenotype, the transplanted cells residing in the transplantation space could be easily excised. In fact, it has already been shown that the transplanted cells can be excised on occurrence of unexpected or abnormal effects[35]. These findings should encourage researchers to develop an adipose tissue-based life-long and risk-manageable treatment for patients with serum protein deficiencies.

SCAFFOLD DEVELOPMENT FOR CELL TRANSPLANTATION

For the successful treatment of such cell transplantation-based therapies, it is important to select suitable scaffolds for the transplanted preadipocytes, adapting the transplantation site to optimize their survival, differentiation and protein expression. These materials must fulfill several requirements, including mechanical support and the ability to guide tissue reconstruction, as well as biocompatibility, biodegradability and easy handling[40,41]. In this context, fibrin glue is capable of supporting the secretion of the exogenously transduced gene product from preadipocytes *in vivo*[42]. Considering the previous reports showing the importance of various cytokines for the regulation of cell function and the surrounding matrix conditions[43–50], these combinations with our fibrin gel condition could improve the outcomes of adipocyte-based gene therapies.

Table 1 | Clinical gene therapy trials for metabolic diseases

Diseases and transgene	Gene delivery	Vector	Administration route	Trial country	Phase	Number	References
Alpha-1 antitrypsin deficiency							
Alpha-1 antitrypsin	*In vivo*	Adeno-associated virus	Intramuscular	USA	Phase I	2	60–62
					Phase II	1	
		Naked DNA	Intranasal	USA	Phase I	1	
Cystic fibrosis							
Alpha-1 antitrypsin	*In vivo*	Naked DNA	Intranasal	USA	Phase I	1	63–66
Cystic fibrosis transmembrane conductance regulator	*In vivo*	Adeno-associated virus	Intrabronchial	USA	Phase I	2	
					Phase II	2	
			Intranasal	USA	Phase I/II	3	
		Adenovirus	Intrabronchial	France	Phase I/II	1	
				USA	Phase I	3	
			Intradermal	USA	Phase I	1	
			Intranasal	Switzerland	Phase I	1	
				USA	Phase I	4	
					Phase I/II	1	
			Intranasal + intrabronchial	USA	Phase I	1	
		Naked DNA	Intrabronchial	UK	Phase I	1	
			Intranasal	UK	Phase I/II	4	
				USA	Phase I	5	
			Intranasal + intrabronchial	UK	Phase I	1	
Familial hypercholesterolemia							
Low-density lipoprotein receptor	*Ex vivo* (Hepatocytes)	Retrovirus	Intrahepatic	USA	Phase I	1	16–18
Gaucher's disease							
Glucocerebrosidase	*Ex vivo* (CD34 + PBC)	Retrovirus	Bone marrow transplantation	USA	Phase I	1	67,68
				USA	Phase I/II	1	
			Intravenous	USA	Phase I	1	
Huntington's disease							
Ciliary neurotrophic factor (CNTF)	*Ex vivo* (BHK)	Naked DNA	Intracerebral	Switzerland	Phase I	1	69,70
				France	Phase I	1	
Lipoprotein lipase deficiency							
Lipoprotein lipase (LPL)	*In vivo*	Adeno-associated virus	Intramuscular	Netherlands	Phase I/II	1	19,71,72
				Canada	Phase I	1	
Mucopolysaccharidosis type I (Hurlers syndrome)							
Alpha-L-iduronidase	*Ex vivo* (BMC)	Retrovirus	Bone marrow transplantation	UK	Phase I/II	1	73,74
	Ex vivo (Fibroblasts)		Intraperitoneal	France	Phase I	1	
Mucopolysaccharidosis type II (Hunter disease)							
Iduronate-2-sulfatase	*Ex vivo* (PBC)	Retrovirus	Intravenous	USA	Phase I	1	75
Mucopolysaccharidosis type VII							
Beta-glucuronidase	*Ex vivo* (CD34+PBC)	Lentivirus	Intravenous	USA	Phase I	1	76–78
Ornithine transcarbamylase deficiency							
Ornithine transcarbamylase	*In vivo*	Adenovirus	Intrahepatic	USA	Phase I	1	20,79
Pompe disease							
Acid-alpha glycosidase	*In vivo*	Adeno-associated virus	Intramuscular	USA	Phase I/II	1	80–82
Familial lecithin-cholesterol acyltransferase deficiency							
Lecithin-cholesterol acyltransferase	*Ex vivo* (Adipocytes)	Retrovirus	Subcutaneous	Japan	Phase I	1	42,55,59

Summarized according to the Clinical Trials Database provided by the *Journal of Gene Medicine* (http://www.wiley.com/legacy/wileychi/genmed/clinical/). Protocol of clinical trial for lecithin-cholesterol acyltransferase deficiency by our group is now under review by Ministry of Health, Labour and Welfare. BHK, baby hamster kidney cells; BMC, bone marrow cells; PBC, peripheral blood cells.

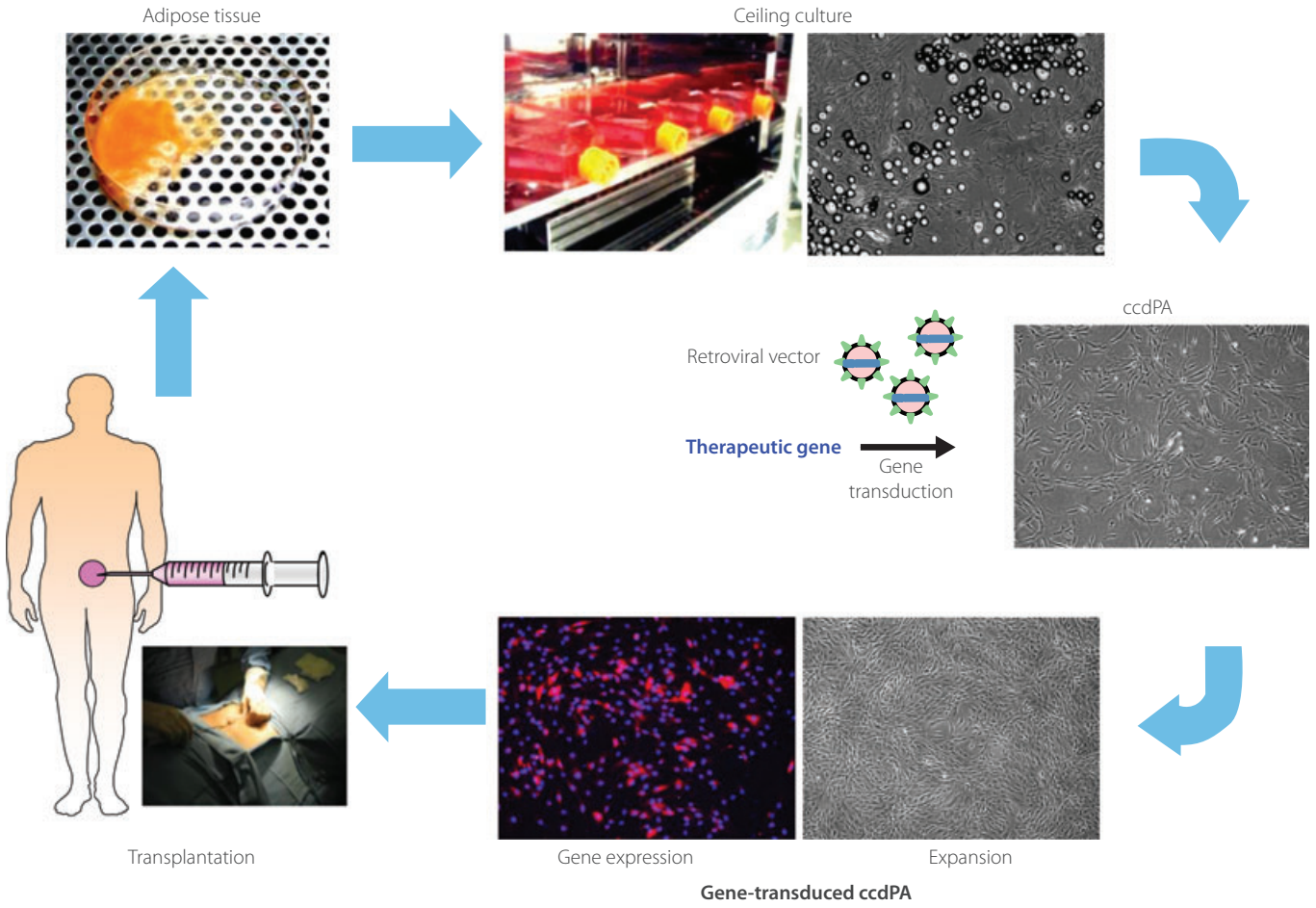

Figure 1 | Therapeutic strategy for adipocyte-based enzyme replacement therapy by *ex vivo* gene transfer. Adipose tissue is obtained by lipoaspiration from the patient. ceiling culture-derived proliferative adipocytes (ccdPA) are propagated by ceiling culture. The therapeutic gene is transduced by the retroviral vector. ccdPA stably secreting the therapeutic protein are expanded and harvested. Harvested cells are subcutaneously transplanted with the appropriate scaffold.

PREADIPOCYTES WITH HIGH ADIPOGENIC POTENTIAL

Recently, adipose tissue has been demonstrated to be a source of proliferative cells for cell-based therapies, such as regenerative medicine and gene transfer applications. Two types of preparation have been reported to be sources of adipose tissue-derived proliferative cells. One is stromal vascular fractions (SVF), which can be obtained as a sediment by the centrifugation of collagenase-digested fat tissue[9] and is the most commonly used technique. The adherent cells obtained from SVF are now recognized as adipose tissue-derived stem cells (ASC), which are pluripotent and can differentiate to yield various cell types, including cardiomyocytes, chondrocytes and osteoblasts, in addition to adipocytes, thus providing a relatively heterogeneous cell population appropriate for regenerative therapy[51–53]. However, these data show that SVF are heterogeneous, and therefore imply that SVF might not result in a stable therapeutic gene vehicle for gene therapy purposes.

The other cell preparation is obtained from the floating mature fat cell fraction obtained after the centrifugation, followed by a ceiling culture[54]. Because the cells are propagated using the buoyant properties of mature adipocytes in this preparation, the progeny cells are more homogeneous than ASC. Proliferative adipocytes were propagated by the ceiling culture technique from the mature adipocyte fraction, and the cells were designated as ceiling culture-derived proliferative adipocytes (ccdPA)[55]. The ccdPA are nearly homogeneous and show only a trace of mature adipocytes by analysis of surface antigen profiles. On stimulation to induce differentiation, the ccdPA showed increased lipid droplet accumulation accompanied with higher adipogenic marker gene expression compared with the ASC, even after *in vitro* passaging, suggesting the commitment of ccdPA to the mature adipocyte lineage[56].

GENE-TRANSDUCED ADIPOCYTES AS VEHICLE CELLS

MoMLV-mediated gene transduction in human ccdPA resulted in a high gene transduction efficiency[55]. In search of optimal transplantation conditions, the 3-D long-term culture system using fibrin gel, a tissue sealant utilized in the clinic, was established. The gene-transduced ccdPA spontaneously accumulate lipid droplets without any artificial stimulation in 3-D culture using the fibrin glue (Aoyagi Y, Kuroda M, Asada S, Tanaka S, Konno S, Tanio M, Aso M, Okamoto Y, Nakayama T, Saito Y, Bujo H, unpublished observations, 2010). Interestingly, the fibrinogen concentration was shown to affect the lipid accumulation in the cells. The expression of the transduced gene was correlated with cell differentiation (Aoyagi Y, Kuroda M, Asada S, Tanaka S, Konno S, Tanio M, Aso M, Okamoto Y, Nakayama T, Saito Y, Bujo H, unpublished observations, 2011).

In one study, the insulin gene-transduced cells were propagated, and the efficacy of these cells was evaluated in a diabetic mouse model[35]. The transplantation of the cells improved hyperglycemia and blood HbA_{1c} concentrations in a manner that was dependent on the cell number, without causing hypoglycemia. The plasma insulin concentration was dependent on the implanted cell number, and the systemic effect of the circulating insulin was confirmed by a marked improvement in bodyweight reduction and liver glycogen content. Thus, the autotransplantation of gene-transduced ccdPA could serve as a novel clinical application for a variety of systemic metabolic disorders.

AN *EX VIVO* GENE THERAPY TRIAL USING EXOGENOUS GENE-TRANSDUCED ADIPOCYTES

Lecithin-cholesterol acyltransferase (LCAT) deficiency has been identified as a genetic metabolic disorder. Cholesteryl ester levels are markedly reduced in lipoproteins, and abnormal cholesterol deposition is observed in the tissues of these patients, who often develop severe complications including corneal opacity, anemia, proteinuria and renal failure[57]. LCAT deficiency is caused by mutations in the *lcat* gene, and more than 40 different mutations have been identified to date[58]. Protein replacement treatment was suggested to be effective; however, no approach for the permanent correction of the symptoms has been reported.

However, in a previous study, the human *lcat* gene was transduced into human ccdPA by a retroviral vector. The transduced cells secreted functional LCAT protein *in vitro*, correlating with the integrated copy number of vector genomes[55]. The secreted LCAT protein clearly ameliorated the disturbed high-density lipoprotein subpopulation profile caused by impaired LCAT function in patients' serum by the *in vitro* incubation assay, strongly suggesting the feasibility of our strategy[59]. An application of this *in vitro* assay system to evaluate the responsiveness of patients is now under investigation. The LCAT delivery achieved in the mouse model with the clinically available fibrin scaffold was enough to suggest the efficacy of the *ex vivo* gene therapy strategy to prevent a poor prognosis in those patients[41].

The potential safety issues related to the ccdPA have been carefully addressed[55]. Gene transduction did not affect the cell growth, adipogenic differentiation or surface antigen profiles of the cells. The averaged integrated copy number was stable during the *in vitro* expansion process, and clonal expansion was not observed, indicating no predominant growth of gene-transduced cells. The transplantation experiments showed no signs for side-effects.

CONCLUSION

There are high hopes that a successful gene therapy approach can be developed in the future to treat rare genetic defects. Numerous studies have been carried out to develop such treatment strategies, both on the basic level and in the clinic. Although hematopoietic cells are proven target cells for *ex vivo* gene therapies, especially for immune-related diseases in which those cell functions are primarily affected by the gene defects, they might not be suitable targets for the many metabolic diseases that result in impairment of multiple organs. The physiological functions and applicability of adipose tissue would enable researchers to develop a novel therapeutic strategy to deliver therapeutic proteins systemically.

Mature adipocytes have been explored as a source of target cells for *ex vivo* gene therapy. Propagated ccdPA would provide an excellent platform for a novel adipocyte-based protein replacement therapy for patients with serum protein deficiencies who require long-term therapeutic protein supplements. A good manufacturing practice production procedure has been established, and the gene-transduced cells can be expanded up to nearly 10^{12} cells from 1 g of fat tissue within 1 month after fat tissue preparation[55]. To further expand the adipocyte-based therapeutic strategy for the supplementation of other proteins, it will be necessary to evaluate the characteristics of ccdPA from various kinds of fat diseases, such as those from subjects with metabolic syndrome, which might affect the secretion function of adipose tissues, and to develop an allogeneic transplantation method for patients with lethal conditions in childhood, as well as to establish the necessary transplantation procedure. After the careful consideration of the safety in combination of efficacy, the novel transplantation therapy developed using adipocytes might be applicable not only for genetic deficiencies, but also for lifestyle-related diseases, including diabetes mellitus.

ACKNOWLEDGMENTS

We thank Dr Itsuko Ishii, Dr Fumiaki Matsumoto, Dr Masaharu Ichinose and Dr Kaneshige Satoh for helpful and valuable suggestions for our research. We also thank Sakiyo Asada, Yasuyuki Aoyagi, Yoshitaka Fukaya, Shunichi Konno and Shigeaki Tanaka for their assistance in preparing this manuscript. This study was supported in part by Health and Labour Sciences Research Grants for Translational Research, Japan (HB).

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