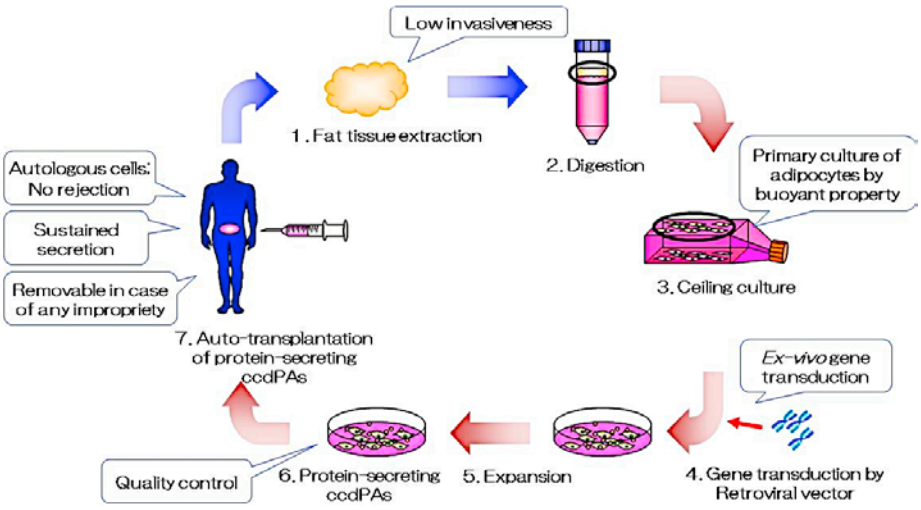
Adipocyte-based gene therapy for serum protein deficiencies

1. Overview

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, our research group has developed new methods for the preparation, culture, expansion and manipulation of adipose cells using advanced gene transduction methods and transplantation scaffolds. The novel adipose tissue-based therapeutic strategies developed by our group could be applicable for the treatment of various protein deficiencies.

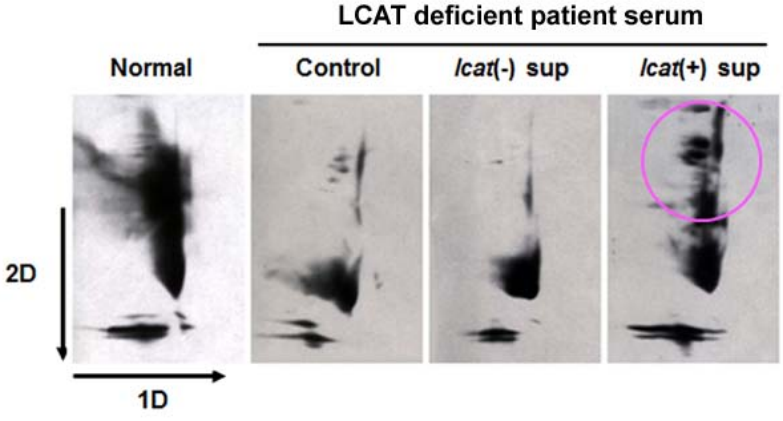
2. Proliferative Adipocytes as Therapeutic Gene Vehicle

Adipose tissue is a great source of proliferative cells that can be used in cell-based therapeutics. It is abundant in the human body and can be easily excised with minimal risks. Most commonly used are the stromal vascular fractions (SVF), which are obtained from the sediment after collagenase digestion of the adipose tissue. There are numerous reports showing that these cells are heterogeneous and have potential to differentiate into multi-lineage progenitors, thus suitable for regenerative medicine applications. But for the purpose of gene therapy, more homogeneous cell preparations are required for stable gene transduction and therapeutic gene expressions. Therefore, we focused on the floating mature adipocytes after collagenase digestion and centrifugation to develop therapeutic protein secretion machinery. Following the centrifugation, we harvested a fraction of lipid-containing floating cells for the therapeutic gene vehicle. Using a ceiling culture technique, we have developed a preparation procedure of highly proliferative adipocytes from this fraction. We call them “ceiling culture-derived proliferative adipocytes (ccdPAs).” The transduction efficiency of ccdPAs for MoMLV-based amphotropic retroviral vectors was as high as 40-50%, with less than two integrated copies of viral genomes per cell on average. The cells retained a high level of adipogenic potential in comparison to SVF even after viral transduction or consecutive *in vitro* passages. These features of ccdPAs make them ideal for use as a therapeutic gene vehicle.



3. Clinical application for LCAT deficiency

Lecithin:cholesterol acyltransferase (LCAT) deficiency is an autosomal recessive inherited metabolic disorder caused by a mutation in the *lcat* gene. Patients show impaired lipoprotein metabolisms, which eventually cause renal failure, the most important complication of the disease in the 4^{th}-5^{th} decades. Currently, symptomatic therapies using reno-protection treatment with fat-restricted diets or renal transplantation have been reported in some cases. The most reliable and principal therapy to prevent serious renal damage is the continuous supplementation of the deficient LCAT enzyme. Infusion of recombinant LCAT or blood transfusion has shown sufficient, but transient, recovery of abnormal lipoprotein profiles. Thus, the development of a sustained protein replacement therapy has been long awaited as a curative therapy. Our gene/cell therapy technology using ccdPAs seems suitable for this purpose. We have developed a GMP-based procedure to produce ccdPAs. We transduced *lcat* genes into ccdPAs. They secreted functional LCAT protein, correlating with the integrated copy number of the vector genome. The secreted LCAT protein clearly ameliorated the disturbed high-density lipoprotein subpopulation profile caused by impaired LCAT function in the patient's serum by the *in vitro* incubation assay, strongly suggesting the feasibility of our strategy. It was possible to produce nearly 10^{12} gene-transduced cells from 1 g of fat tissue within one month of fat tissue preparation. The presence of therapeutic LCAT protein in serum was immunologically identified upon transplantation of the *lcat* gene-transduced ccdPAs in mice models using a clinically applicable fibrin scaffold. Thus, ccdPAs would provide an excellent platform for developing a novel adipocyte-based protein replacement therapy for a variety of patients with serum protein deficiencies who require long-term therapeutic protein supplements as well as LCAT deficiencies. With these results, we are preparing to launch clinical research as soon as we receive approval from the Japanese Ministry of Health, Labour and Welfare.



4. Perspectives for Proliferative Adipocytes Therapy

With our method, we might be able to cure patients with hemophilia, lysosomal diseases and neuro-degenerative diseases. Our novel technique using ccdPAs can also be used for diseases such as diabetes. Together with strong partners such as CellGenTech, Inc. and Chiba University Graduate School of Medicine, we are on our way to accomplish our mission. We are seeking collaborators in projects for treatment of patients with other genetic disorders. We have also been collaborating with other laboratories on basic research in adipocyte biology using the sophisticated "ceiling culture" technique.