These results indicated that the newly developed MV vector had the significant character as the new gene transfer vector compared with conventional viral gene transfer vectors including Sendai virus vector, which belongs to the same RNA virus vector.

We could successfully generated induced pluripotent stem cells (iPS cells) from human fibroblasts or peripheral blood T cells using MV-dF-OSKL-EGFP. These iPS cells expressed the pluripotent markers of NANOG and Tra-1-60 and were demethylated. These iPS cells also differentiated into three germ line tissues in vitro and in vivo. Importantly, we also could establish the ground state like pluripotent cell (GSL-iPS cells) from hematopoietic cells by using MV-dF-OSKL-EGFP. In the presence of human leukemia inhibitory factor (LIF), GSK-3 inhibitor (CHIR99021), and MEK inhibitor (PD0325901), GSL-iPS cells were able to be cultured from a dissociated single cell with rapid cell growth. GSL-iPS cells also expressed pluripotent markers of NANOG and Tra-1-60, and were able to differentiate into three germ line cells. In conclusion, our newly developed MV vector may induce revolutional advance in the field of gene and cell therapy using iPS cells.

6. Targeted Genome Editing of Cell Lines for Improved and Scalable Production of Lentiviral Vectors for Human Gene Therapy

Alessio Cantore,[1] Michela Milani,[1] Johannes Lengler,[2] Sara Bartolaccini,[1] Tiziano Di Tomaso,[1] Philip D. Gregory,[3] Friedrich Scheiflinger,[2] Angelo Lombardo,[1,4] Luigi Naldini.[1,4]
[1]San Raffaele Telethon Institute for Gene Therapy, Milan, Italy; [2]Baxter Innovation GmbH, Vienna, Austria; [3]Sangamo BioSciences, Inc, Richmond; [4]Vita Salute San Raffaele University, Milan, Italy.

Lentiviral vectors (LVs) represent efficient and versatile vehicles for gene therapy. The manufacturing of clinical-grade LVs relies on transient transfection of vector components. This method is labor and cost intensive and becomes challenging when facing the need of scale-up and standardization. The development of stable LV producer cell lines will greatly facilitate overcoming these hurdles. We have generated an inducible LV packaging cell line, carrying the genes encoding for third-generation vector components stably integrated in the genome under the control of tetracycline-regulated promoters. In order to minimize the immunogenicity of LVs for in vivo administration, we set out to remove the highly polymorphic and antigenic class-I major histocompatibility complex (MHC-I) expressed on LV packaging cells and subsequently incorporated on the LV envelope. We performed genetic disruption of the β-2 microglobulin (B2M) gene, a required component for the assembly and trafficking of the MHC-I to the plasma membrane in LV producer cells, exploiting the RNA-guided Cas9 nuclease. We generated B2M-negative cells devoid of surface-exposed MHC-I, which retain the ability to produce LVs. In order to insert the LV genome of interest in the packaging cell line, we performed site-specific integration in predetermined loci of the genome of these cells, chosen for robust expression, exploiting artificial nucleases and homology-directed repair. In several independent iterations of this process, we generated producer cell lines both for LV expressing marker genes and a therapeutic gene, i.e. coagulation factor IX (FIX), the gene mutated in hemophilia B. We show that these LV producer cells are stable in culture and can produce several liters of LV-containing conditioned medium. These LVs have comparable or only slightly lower infectious titer and specific infectivity than LVs produced by state-of-the-art transient transfection process and can transduce therapeutically relevant target cells, such as hematopoietic stem/progenitor cells and T lymphocytes to high efficiency. Moreover, we intravenously administered FIX-expressing LVs produced by the cell line to hemophilia B mice and established therapeutic levels of circulating FIX. These data indicate that site-specific integration is an efficient, rapid and reproducible method to generate LV producer cells, starting from a universal stable inducible LV packaging cell line. Overall, we provide evidence that rationally designed targeted genome engineering can be used to improve the quality, safety and sustainability of LV production for clinical use.

Translational Studies in Cardiovascular and Pulmonary Therapy

7. Lentiviral Vector-Mediated CFTR Gene Transfer to CF Pig Airways Corrects the Anion Transport Defect In Vivo

Ashley L. Cooney,[1] Mahmoud H. Abou Alaiwa,[2] David A. Stoltz,[2] Michael J. Welsh,[2,3] Patrick L. Sinn,[4] Paul B. McCray.[1,4,5]
[1]Microbiology, University of Iowa, Iowa City, IA; [2]Internal Medicine, University of Iowa, Iowa City, IA; [3]Howard Hughes Medical Institue, University of Iowa, Iowa City, IA; [4]Pediatrics, University of Iowa, Iowa City, IA; [5]Genetics, University of Iowa, Iowa City, IA.

Cystic fibrosis (CF) is the most common autosomal recessive disorder in Caucasian populations and is caused by mutations in CFTR. Complementation of CFTR rescues the anion transport defect in vitro, making this monogenetic disease a candidate for CF gene therapy. Lentiviral vector-mediated CFTR gene transfer has long been discussed as a promising strategy for gene therapy. Lentiviral vectors can efficiently transduce non-dividing cells and persistently express a therapeutic transgene. We previously demonstrated that the non-primate lentiviral vector derived from feline immunodeficiency virus (FIV) and pseudotyped with a GP64 envelope confers gene transfer to porcine airway epithelia both in well-differentiated primary cultures in vitro and in wild-type pig lungs in vivo. The CF pig model recapitulates many features of human disease and provides a new platform for preclinical gene therapy studies. Here, we show that lentiviral-mediated delivery of CFTR to nasal and lung epithelia corrects the anion channel defect in the CF pig model. We delivered GP64-FIV-pigCFTR to the nose and lung of 2-week old gut-corrected CF pigs using a MADgic atomizer. Two weeks post-transduction, we harvested sinus, trachea, and bronchus tissue to assay for gene correction. In freshly excised trachea and bronchus tissue explants, we observed a short-circuit current response to forskolin and IBMX (F&I) and inhibition by GlyH-101, consistent with CFTR function. Compared to non-transduced animals, cultured ethmoid sinus epithelia also showed evidence of correction by short-circuit current measurements. Loss of CFTR results in reduced bicarbonate transport and a lower ASL pH. A reduction in ASL pH impairs bacterial killing. Following gene transfer, we observed an increase in nasal and tracheal pH and rescue of bacterial killing. Together, these exciting results demonstrate the first evidence of functional in vivo correction of CFTR by a lentivirus in the CF pig model. These findings support the utility of a lentivirus for CF gene therapy.