of adult mice, showing significant promise for treating central nervous system (CNS)-related diseases with non-viral delivery vector.

269 The First Coacervate-based Delivery System for Advanced Gene Therapy

Xiaofei Gao[1]*, Renxia Zhang[2], Peipei Zhu[1], Qing Zhang[2], Xiaoqian Nie[1]
[1]Westlake University, Hangzhou, Zhejiang, China, [2]Nanoportal Biotech, Hangzhou, China
**Corresponding Author*

Gene therapy marks a significant breakthrough in the pharmaceutical field, necessitating the development of advanced delivery technologies to facilitate its clinical application. Here, we report the identification of a mammalian endogenous protein capable of forming coacervates with various nucleic acids. These coacervates exhibit an mRNA encapsulation capacity 1000-fold greater than lipid nanoparticles (LNPs), demonstrating efficient cellular entry, robust cytoplasmic release of nucleic acids, and broad compatibility with diverse cell types, including primary human and mouse immune cells. Based on these properties, we developed the first coacervate-based system for gene delivery, named EASY, utilizing human and mouse endogenous proteins (PCT/CN2024/124967). EASY supports a wide range of genetic cargoes for gene overexpression, knockdown, and knockout.

With EASY, we achieved over 90% transfection efficiency for mRNA encoding green fluorescent protein (GFP) in primary human T cells, while maintaining 90% cell viability. GFP expression persisted in 64% of cells up to 12 days post-transfection, highlighting its high payload capacity. EASY also achieved high transfection efficiency across various types of primary cells, including NK cells, B cells, and hematopoietic stem cells (HSCs). Notably, EASY facilitated efficient gene knockout by co-delivering Cas9 mRNA and sgRNA. For example, EASY achieved >80% efficiency in targeting the TRAC, B2M, and PDCD1 genes in human primary T cells, improving the safety and efficacy of T cell therapies. Indeed, EASY demonstrated a robust capacity for delivering large genetic cargoes, enabling simultaneous editing of up to ten genes by co-delivering Cas9 mRNA and the corresponding sgRNAs in human primary T cells, with consistent editing efficiency across all targets. To further explore its therapeutic potential, we targeted BCL11A in HSCs, a transcription factor that represses γ-globin and fetal hemoglobin expression in erythroid cells. The knockout of BCL11A increases fetal hemoglobin expression, offering a potential therapeutic strategy for hemoglobinopathies. Our results showed that approximately 80% of alleles at this locus were successfully modified, with no evidence of off-target editing in HSCs using EASY, highlighting its potential for advancing cell therapy.

In addition to gene knockout, EASY supports targeted genomic integration through homology-directed repair (HDR). By inducing double-strand breaks and delivering single-stranded DNA (ssDNA) templates, EASY achieved >30% efficiency in inserting an anti-CD19 chimeric antigen receptor (CAR) sequence into the PDCD1 gene locus. This approach generated CAR-T cells with sustained CAR expression and disrupted PD1 expression. These enhanced CAR-T cells exhibited potent anti-tumor activity in NSG mice xenografted with $CD19^+$ Raji cells.

In conclusion, EASY is a versatile, non-viral, LNP- and electroporation-free gene delivery platform that combines high efficiency, low toxicity, and user-friendliness. By eliminating risks such as insertional mutagenesis and dependence on specialized equipment, EASY represents a transformative tool for gene editing and cell therapy manufacturing, significantly expanding the therapeutic potential of CAR-T, HSC other cellular therapies.

270 Engineered Protein Delivery Vehicles Enable Single Intrastromal CRISPR-Cas9 Therapy for TGFBI Corneal Dystrophies with High Efficacy and Safety: A Comprehensive Preclinical Assessment in Non-Human Primates

Changchang Xin[1], Qian Luo[2], **Zihua Jiang[3]**, Xichen Wan[4], Hua Lu[5], Mingrui Chen[4], Xujiao Zhou[4], Martin Chan[6], Xingtao Zhou[4], Zongli Zheng[7], Jiaxu Hong[8]
[1]Fudan University, Fundan, China, [2]Karolinska Institutet, Solna, Sweden, [3]Karolinska, Solna, Stockholm, Sweden, [4]Fudan University, Shanghai, China, [5]Geneditbio Limited, Beijing, China, [6]Geneditbio Limited, Hong Kong, Sha