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Perspective

Engineered Extracellular Vesicles for Therapeutic mRNA Delivery to Hepatocytes: Treating Metabolic Liver Diseases

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DESCRIPTION

Messenger RNA (mRNA) therapeutics offer tremendous potential for addressing genetic disorders; however, efficient delivery to specific cell types remains challenging. We have developed engineered Extracellular Vesicles (EVs) specifically designed for hepatocyte-targeted delivery of therapeutic mRNA to treat metabolic liver diseases. These biomimetic nanocarriers, approximately 100 nm in diameter, were derived from modified human cells and engineered to express targeting ligands and stabilizing proteins that enhance mRNA delivery efficiency while minimizing immunogenicity. This approach was evaluated using Ornithine Transcarbamylase (OTC) deficiency as a model disease, a urea cycle disorder characterized by toxic ammonia accumulation due to enzyme deficiency.

The production platform utilized HEK293T cells genetically modified to overexpress specific EV biogenesis factors, including certain tetraspanins and Endosomal Sorting Complexes Required for Transport (ESCRT) proteins, increasing vesicle yield approximately 8-fold compared to unmodified cells. Surface engineering was achieved through expression of a fusion protein combining the exosomal membrane protein *CD63* with Asialoglycoprotein Receptor (ASGPR) targeting peptides, creating EVs with high specificity for hepatocytes. The EV loading process employed a transient cell permeabilization technique during EV biogenesis, achieving approximately 45% encapsulation efficiency for modified OTC mRNA featuring optimized untranslated regions and nucleoside modifications to enhance stability and translation efficiency while minimizing innate immune activation.

Comprehensive characterization confirmed consistent size distribution, with over 90% of vesicles falling within the 80nm-120nm range as measured by nanoparticle tracking analysis. Cryo-electron microscopy revealed intact vesicular structures with characteristic bilayer membranes and internal heterogeneity consistent with RNA-protein complexes. Proteomic analysis confirmed enrichment of desired targeting ligands and depletion of immunogenic components through CRISPR-mediated knockout of specific genes in the producer cells. RNA sequencing demonstrated predominant presence of the therapeutic mRNA with minimal contamination by host cell RNAs, confirming the specificity of the loading process.

In vitro studies using primary human hepatocytes demonstrated efficient cellular uptake, with approximately 65% of cells showing significant mRNA delivery within 6 hours as assessed by flow cytometry. Functional translation was confirmed through enzyme activity assays, with OTC enzyme levels reaching approximately 38% of normal hepatocyte activity well above the 10% threshold typically associated with phenotypic correction in urea cycle disorders. Importantly, immune activation studies using peripheral blood mononuclear cells revealed minimal induction of inflammatory cytokines compared to lipid nanoparticle controls, suggesting reduced potential for immune-mediated toxicity.

In vivo evaluation utilized the spfash mouse model of OTC deficiency, characterized by minimal residual enzyme activity and susceptibility to hyperammonemic episodes. Intravenous administration of engineered EVs demonstrated predominant hepatic distribution, with approximately 28% of the injected dose accumulating in the liver within 4 hours as assessed by near-infrared fluorescence imaging. Liver cell-type analysis confirmed preferential accumulation in hepatocytes rather than non-parenchymal cells, validating the targeting strategy. Therapeutic efficacy was demonstrated through significant reduction in plasma ammonia levels following protein challenge, with treated animals maintaining ammonia concentrations below neurotoxic thresholds. Enzyme activity assays on liver tissue confirmed restoration of approximately 25% of normal OTC activity, persisting for approximately 5 days following a single treatment.

CONCLUSION

Safety assessment revealed transient, mild elevation of liver enzymes that resolved within 48 hours without intervention. Complete cytokine profiling demonstrated minimal inflammatory

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activation compared to lipid nanoparticle controls, with no evidence of complement activation or significant changes in hematological parameters. Repeated administration at 7-day intervals maintained therapeutic enzyme levels without evidence of diminished efficacy or increased adverse effects, suggesting absence of neutralizing immune responses. These engineered extracellular vesicles represent a promising platform for hepatocyte-targeted mRNA delivery, potentially enabling treatment of various metabolic liver disorders while addressing the immunogenicity and targeting challenges associated with current synthetic delivery systems.