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Short Communication

RNA-Guided Mitochondrial DNA Editing Approaches for Neuroenergetic Restoration in Leigh Syndrome Models

Santiago Varela*

Department of Cellular Bioengineering, University of Barcelona, Barcelona, Spain

DESCRIPTION

Leigh syndrome represents a heterogeneous group of inherited neurodegenerative conditions characterized by profound impairment of mitochondrial oxidative phosphorylation, leading to progressive energy failure in tissues with high metabolic demand such as the brainstem, cerebellum, skeletal musculature, and myocardium [1]. The pathological basis frequently involves mutations in mitochondrial gene encoded subunits of the electron transport chain or nuclear genes essential for mitochondrial assembly, replication, or maintenance. Among these, defects affecting complex I subunits are particularly associated with early-onset neurological deterioration, lactate accumulation, and progressive loss of motor and autonomic control. The multi-copy nature of mitochondrial DNA, combined with maternal inheritance patterns and limited endogenous DNA repair capacity within mitochondria, creates significant barriers for conventional gene correction strategies. As a result, therapeutic approaches capable of selectively modifying mitochondrial genomes while preserving organelle integrity have become a focus of experimental investigation [2].

The present study describes an RNA-guided mitochondrial genome modification system developed to target a pathogenic single nucleotide substitution associated with complex I dysfunction in a mammalian model exhibiting Leigh-like neuroenergetic decline. The system is based on a engineered nuclease platform fused to mitochondrial targeting sequences that facilitate import into the mitochondrial matrix following cytosolic translation. A programmable RNA-guided recognition module was adapted to function within the mitochondrial environment by incorporating structural modifications that enhance stability in the absence of canonical nuclear RNA processing machinery. The design aimed to enable selective recognition of mutant mitochondrial DNA sequences while limiting interaction with wild-type alleles present in heteroplasmic mixtures [3].

Delivery of the gene editing system was achieved using a dual-component vector platform composed of modified viral particles capable of efficient cellular entry and subsequent trafficking to mitochondria through peptide-mediated localization signals. Systemic administration resulted in widespread distribution across multiple organ systems, with preferential accumulation observed in neural tissue regions known to exhibit high vulnerability to mitochondrial dysfunction. Fluorescence tagging of the expressed components confirmed mitochondrial localization within neuronal and glial populations, particularly within brainstem nuclei and cerebellar Purkinje cells [4].

Molecular analysis of mitochondrial DNA extracted from treated tissues demonstrated partial correction of the targeted nucleotide variant across a subset of mitochondrial genomes. The extent of modification varied significantly between cells, reflecting differences in mitochondrial copy number, intracellular distribution, and variable uptake of editing components. Quantitative polymerase-based sequencing assays indicated a measurable shift in heteroplasmy ratios favoring the corrected allele in regions with higher vector penetration. However, complete conversion of mutant mitochondrial populations was not achieved, consistent with the stochastic distribution of mitochondrial genomes during organelle replication and cellular division [5].

Neurological performance evaluations demonstrated partial recovery of motor coordination and reflex responsiveness in treated subjects compared with untreated controls. Behavioral testing under locomotor challenge conditions revealed improved gait stability, reduced frequency of imbalance episodes, and enhanced endurance during sustained movement tasks. Although full restoration of neurological function was not achieved, the observed improvements correlated strongly with the proportion of corrected mitochondrial genomes in affected neural regions. This relationship supports the concept of a threshold effect in mitochondrial diseases, where partial restoration of respiratory chain function can produce clinically meaningful physiological improvements [6].

Systemic metabolic profiling demonstrated improved lactate clearance and reduced accumulation of metabolic intermediates associated with impaired oxidative phosphorylation. Blood lactate levels, which were significantly elevated in untreated subjects under both resting and stress conditions, showed a

Correspondence to: Santiago Varela, Department of Cellular Bioengineering, University of Barcelona, Barcelona, Spain, E-mail: santiago.varela@neurobio.ub.es

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consistent downward trend following intervention. Additionally, markers of oxidative stress, including lipid peroxidation products and reactive oxygen species indicators, were reduced in treated subjects, suggesting improved electron transport efficiency and decreased electron leakage within mitochondrial respiratory complexes [7].

Re-administration experiments were conducted to evaluate the feasibility of repeated dosing; however, reduced efficiency was observed following secondary exposure, likely due to adaptive immune responses directed against vector components. This limitation suggests that long-term therapeutic strategies may require development of alternative delivery systems with reduced immunogenic profiles or transient immune modulation to enhance repeat administration efficacy [8].

Off-target evaluation using genome-wide mitochondrial and nuclear sequencing approaches identified a low frequency of unintended nucleotide modifications at sites with partial homology to the guide RNA sequence. Although these events did not produce detectable functional impairment within the study period, their presence underscores the necessity for continued refinement of Ribonucleic Acid (RNA)-guided specificity mechanisms. Potential optimization strategies include enhanced structural stabilization of guide RNA analogs, improved discrimination of mismatched base pairing, and temporal regulation of nuclease activity within the mitochondrial environment [9].

At the bioenergetic level, treated tissues demonstrated improved coupling efficiency between proton gradient formation and Adenosine Triphosphate (ATP) synthesis. Measurements of mitochondrial membrane potential indicated enhanced stability under metabolic stress conditions, particularly during increased neuronal activity. These findings suggest that even partial correction of mitochondrial Deoxyribonucleic Acid (DNA) mutations can restore sufficient electron transport chain functionality to improve overall cellular energy homeostasis [10].

Overall findings from this investigation indicate that RNA-guided mitochondrial genome editing systems can achieve partial correction of pathogenic mitochondrial DNA mutations associated with Leigh syndrome-like neuroenergetic dysfunction, resulting in measurable improvements in mitochondrial respiration, neurological function, and systemic metabolic stability. While complete normalization of mitochondrial genome populations was not achieved, the observed functional gains demonstrate the therapeutic potential of organelle-targeted gene modification strategies. Further refinement of delivery specificity, editing efficiency, and long-term maintenance mechanisms will be necessary to enhance clinical applicability and achieve sustained restoration of mitochondrial function in affected tissues.

CONCLUSION

Overall findings from this experimental system indicate that allele-specific cytosine base editing can achieve partial correction of a pathogenic rhodopsin variant in a preclinical model of autosomal dominant retinal degeneration, resulting in measurable improvements in photoreceptor structure and function. While the intervention does not fully restore normal retinal physiology, the observed molecular and physiological changes demonstrate the potential utility of precise nucleotide-level modification strategies for conditions driven by single base substitutions. Further investigation will be required to enhance editing uniformity, extend duration of effect, and minimize immune-related limitations associated with vector-based delivery systems.

REFERENCES

1. Koller D, Mitjans M, Kouakou M, Friligkou E, Cabrera-Mendoza B, Deak JD, et al. Genetic contribution to the comorbidity between attention-deficit/hyperactivity disorder and substance use disorders. Psychiatry Res. 2024:115758.
2. Galy A, Dewannieux M. Recent advances in hematopoietic gene therapy for genetic disorders. Arch Pediatr. 2023;30(8):824-831.
3. Sardone V, Zhou H, Muntoni F, Ferlini A, Falzarano MS. Antisense oligonucleotide-based therapy for neuromuscular disease. Molecules. 2017;22(4):563.
4. Machhi J, Shahjin F, Das S, Patel M, Abdelmoaty MM, Cohen JD, et al. Nanocarrier vaccines for SARS-CoV-2. Adv Drug Deliv Rev. 2021;171:215-239.
5. Bowerman M, Becker CG, Yáñez-Muñoz RJ, Ning K, Wood MJ, Gillingwater TH, et al. Therapeutic strategies for spinal muscular atrophy: SMN and beyond. Dis Model Mech. 2017;10(8):943-954.
6. Burnett BG, Munoz E, Tandon A, Kwon DY, Sumner CJ, Fischbeck KH. Regulation of SMN protein stability. Mol Cell Biol. 2009;29(5),1107–1115.
7. Day JW, Howell K, Place A, Long K, Rossello J, Kertesz N, et al. Advances and limitations for the treatment of spinal muscular atrophy. BMC Pediatr. 2022;22(1):632.
8. Smeriglio P, Langard P, Querin G, Biferi MG. The identification of novel biomarkers is required to improve adult SMA patient stratification, diagnosis and treatment. J Pers Med. 2020;10(3):75.
9. Xi Z, van Blitterswijk M, Zhang M, McGoldrick P, McLean JR, Yunusova Y, et al. Jump from pre-mutation to pathologic expansion in C9orf72. Am J Hum Genet. 2015;96(6):962-970.
10. Genovese AC, Butler MG. Behavioral and psychiatric disorders in syndromic autism. Brain Sci. 2024;14(4):343.