
# Mitochondria-targeted catalytic RNAs for selective silencing of mutant transcripts in mitochondrial diseases

Paweł Głodowicz1 , Julia Latowska-Łysiak1 , Adriana Grabowska1 , Julia Misiorek1 , Konrad Kuczyński1 , Marek Figlerowicz2 , Katarzyna Rolle1 1Department of Molecular Neurooncology, Institute of Bioorganic Chemistry Polish Academy of Sciences, Poznan, Poland, 2Department of Molecular and Systems Biology, Institute of Bioorganic Chemistry Polish Academy of Sciences, Poznan, Poland

Background: Mitochondrial diseases are rare disorders frequently caused by pathogenic mitochondrial DNA (mtDNA) mutations that impair mitochondrial gene expression and oxidative phosphorylation. The heteroplasmic nature of many mtDNA mutations provides an opportunity for selectively targeting mutant transcripts while preserving their wild-type counterparts. However, RNA-targeting therapies remain challenging due to limited nucleic acid delivery into mitochondria. Method: We developed a mitochondria-targeted system based on catalytic ribozymes designed to selectively reduce mutant transcripts. A genetic construct enabling intracellular ribozyme production and mitochondrial delivery was evaluated in control and patient-derived cells carrying pathogenic mtDNA mutations. Ribozyme activity, target RNA and protein levels, cytotoxicity, and mitochondrial function were assessed. Results: The developed system enabled efficient mitochondrial delivery and functional activity of ribozymes, resulting in a significant reduction of targeted mitochondrial transcripts. Importantly, ribozymes preferentially reduced mutant RNA while preserving the corresponding wild-type transcript, demonstrating mutation-selective activity. This selectivity was confirmed at both RNA and protein levels. No detectable cytotoxicity was observed in control or patient-derived cells. Moreover, selective reduction of mutant mitochondrial gene expression resulted in functional improvement, with treated patient-derived cells showing an improved mitochondrial respiratory profile. Conclusion: Mitochondria-targeted ribozymes can selectively suppress pathogenic mitochondrial transcripts while preserving wild-type RNA and improving mitochondrial function without compromising cell viability. These findings provide proof of concept for a genetically encoded, mutation-selective RNA-targeting platform and support its further development as a precision therapeutic strategy for mitochondrial diseases caused by pathogenic mtDNA mutations. Funding: 2021/ABM/05/00004, Medical Research Agency, Poland