Adaptation of CRISPR/Cas9 system for directed elimination of mitochondrial DNA copies with mutations

P-01.1-40

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Most pathogenic mitochondrial DNA (mtDNA) mutations are commonly heteroplasmic, whereby wild-type and mutant genomes co-exist in the same organelle. An increase in the number of mutant molecules can lead to the reaching of a certain threshold of heteroplasmy and the manifestation of mitochondrial disease. We propose a strategy for modifying the components of the CRISPR/Cas9 system to manipulate the mtDNA heteroplasmy level in a cell. Using the cybrid cell line of heteroplasmic mitochondrial disease, bearing the point mutation m.8993T>G in mitochondrial *ATP6* gene we initially confirmed intramitochondrial localization of modified Cas9 nuclease, expressed from the cell nucleus by immunocytochemistry, western blot and electron microscopy. Stable and uniform expression of Cas9 nuclease in the cells was obtained by integrating the *Cas9* gene, which contains the mitochondrial localization signal, into the genome of cybrid cells by Sleeping Beauty transposon system. To deliver the second component of the system, we made several modifications of the guide RNA (gRNA) using described determinants of nucleic acids import into mitochondria. In vitro cleavage analysis showed that such modifications do not affect the assembly and functional activity of the CRISPR/Cas9 system. During subsequent immunocytochemical analysis of the localization of fluorescent-labeled variants of modified gRNA we did not detect their import into the mitochondria. Now we aim to solve this problem by adapting self-complementary adeno-associated viruses type 2 (scAAV2) to deliver the gene encoding the gRNA as part of the viral genome into the mitochondria. The results of our work will contribute to the further development of the technology for treatment of mitochondrial diseases.