A magic bullet to specifically eliminate mutated mitochondrial genomes from patients’ cells

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When mitochondrial diseases result from mutations found in the mitochondrial DNA, engineered mitochondrial-targeted nucleases such as mitochondrial-targeted zinc finger nucleases are shown to specifically eliminate the mutated molecules, leaving the wild-type mitochondrial DNA intact to replicate and restore normal copy number. In this issue, Gammage and colleagues successfully apply this improved technology on patients’ cells with two types of genetic alterations responsible for neuropathy ataxia and retinitis pigmentosa (NARP) syndrome and Kearns Sayre syndrome and progressive external ophthalmoplegia (PEO).

See also: PA Gammage et al (April 2014)

Mitochondrial diseases are very heterogeneous, both clinically and genetically. Central nervous system and muscle are commonly affected due to their high-energy demands (Schon et al, 2012). Many of these disorders are caused by mutations in nuclear genes and follow a Mendelian pattern of inheritance and expression. Others are caused by mutations in the mitochondrial DNA (mtDNA), which behave differently in many aspects. Besides being exclusively maternally inherited or sporadic, deleterious mutations are usually present in a heteroplasmic condition. This happens because a cell contains approximately 1000 copies of mtDNA molecules, and mutated mtDNAs can co-exist with wild-type ones. The balance between mutated and wild-type mtDNA has a major impact on the development of a disease, as more than 80% mutated mtDNA is usually required for a biochemical and clinical phenotype (Schon et al, 2012).

Because of this unique aspect of mitochondrial diseases, the ability to reduce the levels of mutated mtDNA has been the goal of various laboratories. In this issue of EMBO Molecular Medicine, Gammage and colleagues describe the use of mitochondrial-targeted zinc finger nucleases (mtZFN) to alter the mitochondrial DNA (mtDNA) heteroplasmy balance in cells derived from patients with mitochondrial diseases (Gammage et al, 2014). ZFNs are modular proteins that can be engineered to recognize new DNA sequences. They also contain a FokI moiety at the C-terminus that cleaves the DNA, adjacent to the zinc finger-binding site. Because the FokI nuclease moiety is functional as a dimer, for each region to be cleaved, two juxtaposed DNA recognition/nuclease monomers are required to promote site-specific double-strand breaks (Palpant & Dudzinski, 2013).

Gammage and colleagues developed two ZFN that can bind specific regions of the mtDNA. The authors had to modify the ZFN so that the protein was localized to the mitochondrial matrix (mtZFN), where they can physically interact with the mtDNA. To increase specificity of DNA cleavage, they used an obligatory heterodimeric form of FokI. They tested the approach with two different mutations, a point mutation associated with a neurological disease known as neuropathy ataxia and retinitis pigmentosa (NARP) syndrome and a large deletion breakpoint associated with disorders known as Kearns Sayre syndrome and progressive external ophthalmoplegia. In both cases, expression of the specific mtZFN molecular pairs led to a decrease in the relative levels of mutated mtDNA. These results are similar to those recently obtained for a different class of designer nucleases, known as mitoTALEN (Bacman et al, 2013).

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The ability to reduce the levels of mutated mtDNA by designer mitochondrial nucleases can have a major effect on the biochemical phenotype of affected cells. A small decrease in the levels of mutated mtDNA would induce the residual mtDNA to proliferate and repopulate the mitochondria with a higher ratio of wild-type to mutated mtDNA (Fig 1). mtDNA levels are controlled by a poorly understood mechanism, but likely by factors responsible for mtDNA replication, such as transcription factors (required for replication priming), helicase twinkle, DNA polymerase gamma, and others (Carling et al, 2011). Because the wild-type mtDNA is very protective in heteroplasmic cells, a decrease in the percentage of mutant from 80% to 60% can completely eliminate the biochemical defect (Sciacco et al, 1994). Indeed, Gammage and colleagues showed an improvement in...
Elimination of mutated mtDNA

Partial elimination of mutated mtDNA

Repopulation with remaining mtDNA

Conflict of interest
The author declares that he has no conflict of interest.

References

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