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Genome editing in mitochondria prevents inheritance of diseases

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Mitochondrial diseases are maternally inherited genetic disorders that cause a wide spectrum of debilitating conditions and which currently have no cure. In a study published April 23 in the journal *Cell*, Salk Institute researchers report the first successful attempt using gene-editing technology to prevent mutated mitochondrial DNA associated with multiple human mitochondrial diseases from being passed from mothers to offspring in mice.

"This technique is based on a single injection of mRNA into a mother's oocytes or early embryos and therefore could be easily implemented in IVF [in vitro fertilization] clinics throughout the world," said senior study author Juan Carlos Izpisua Belmonte of the Salk Institute for Biological Studies. "Since mutations in mitochondrial DNA have also been implicated in neurodegenerative disorders, cancer, and aging, our technology could potentially have broad clinical implications for preventing the transmission of disease-causing mutations to future generations."

Mitochondria are known as the powerhouse of the cell because they generate most of the cell's supply of energy. Each cell in the body contains anywhere from 1,000 to 100,000 copies of mitochondrial DNA, which is exclusively transmitted through maternal inheritance. In most patients with mitochondrial disease, mutated and normal mitochondrial DNA molecules are mixed together in cells. A high percentage of mutated mitochondrial DNA can lead to the degeneration and catastrophic failure of various organs, resulting in serious health

problems such as seizures, dementia, diabetes, heart failure, liver dysfunction, vision loss, and deafness.

Currently, therapies for preventing the transmission of mitochondrial diseases from mother to child are limited. While genetic screening of embryos only partially reduces the risk of transmitting mitochondrial diseases, an approach called mitochondrial replacement therapy, in which healthy mitochondria are provided by another donor, is being evaluated in the US and soon to be allowed in the UK but has raised ethical, safety, and medical concerns because it involves combining genetic material from three different individuals.

In the new study, Belmonte and his team demonstrated the therapeutic promise of an alternative approach that allows the direct correction of the mutated DNA in mitochondria by using DNA-cutting enzymes called restriction endonucleases and TALENs. This gene-editing approach might be safer, simpler, and more ethical than mitochondrial replacement therapy because it does not require donor eggs. The enzymes are designed to target a specific mutated DNA sequence and introduce a precise cut that destroys the mutated mitochondrial DNA while leaving the normal mitochondrial DNA intact, thereby shifting the balance toward a healthy genetic state in mitochondria.

To test this approach, the researchers used a mouse model that carries two different types of mitochondrial DNA and designed TALENs and restriction endonucleases to target and destroy only one type of mitochondrial DNA in the eggs of these mice. This approach decreased the levels of the targeted mitochondrial DNA, while sparing the untargeted mitochondrial DNA. The injected mouse embryos, which showed normal patterns of development, were then transferred to female mice, which gave birth to healthy pups that had low levels of the targeted mitochondrial DNA in various organs. In addition, the pups exhibited normal behavior, mitochondrial function, and genomic integrity.

Moreover, the offspring themselves gave birth to pups that showed barely detectable levels of the targeted mitochondrial DNA, demonstrating the potential of this approach for preventing the transgenerational transmission of mitochondrial diseases.

To confirm the clinical relevance of this strategy, the researchers next screened and tested TALENs designed to target human mitochondrial DNA mutations that cause two disorders, Leber's hereditary optic neuropathy and dystonia (LHOND) and neurogenic muscle weakness, ataxia, and retinitis pigmentosa (NARP). This approach resulted in a significant reduction in mutated mitochondrial DNA in mouse eggs that contained genetic material from patient cells. "We expect that this method will reduce the percentage of mutated mitochondrial DNA below the threshold for triggering mitochondrial diseases in humans," Belmonte says.

But before any clinical trials begin, it will be necessary to evaluate the safety and efficacy of the method in eggs from patients with mitochondrial diseases. Toward this goal, Belmonte's team is collaborating with several IVF clinics to test this technology in surplus human eggs that are donated by patients with mitochondrial diseases for research purposes.

"In our opinion, due to the hundreds of thousands of copies of mitochondrial DNA present in human eggs, and the fact that double-strand breaks in mitochondrial DNA generally lead to the elimination of these molecules, we believe that the selective elimination of mutated mitochondrial DNA in the germline could be safer than nuclear genome editing and therefore might represent a starting point for the study and use of these new technologies in human embryos," Belmonte says.

More information: *Cell*, Reddy et al.: "Selective Elimination of Mitochondrial Mutations in the Germline by Genome Editing"

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