Mechanism that allows bacteria to infect plants may inspire cure for eye disease

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Investigators from the University of Miami Miller School of Medicine used mitoTALENS to eliminate mutated DNA inside mitochondria. From left to right are Milena Pinto, Carlos T. Moraes, Sandra Bacman, Sion Williams, and Susana Peralta. Their technique could lead to treatments for mitochondrial diseases like the blinding eye disease Leber hereditary optic neuropathy. Their research is co-funded by the National Eye Institute, a part of the National Institutes of Health.

Credit: Byron Maldonado

By borrowing a tool from bacteria that infect plants, scientists have developed a new approach to eliminate mutated DNA inside mitochondria—the energy factories within cells. Doctors might someday use the approach to treat a variety of mitochondrial diseases, including the degenerative eye disease Leber hereditary optic neuropathy (LHON). The research, published online today in *Nature Medicine*, was funded by the National Eye Institute (NEI), a part of the National Institutes of Health (NIH).

Mitochondria convert fuel from food into a form of energy that cells can use. They also make enzymes for a variety of cell functions, and in humans they are the only cell component other than the nucleus that houses genes. Mitochondrial gene mutations can lead to a variety of health problems including muscle weakness, heart disease, and blindness in the case of LHON. Most cells contain thousands of mitochondrial DNA (mtDNA) copies. People with mitochondrial disease often have both mutant and normal mtDNA within their cells. No cures exist for mitochondrial diseases and few effective treatments are available.

Searching for strategies to repair mitochondrial gene defects, a group of investigators at the University of Miami Miller School of Medicine explored proteins called transcription activator-like (TAL) effectors. In nature, TAL effectors are found only in certain types of plant-infecting bacteria. They enable the bacteria to use plant DNA to multiply and spread infection.

Scientists recently began using TAL effectors to modify DNA in a variety of organisms. In the lab, TAL effectors can be fused with DNA-breaking proteins called nucleases. These TAL effector nucleases

(TALENs) can be used to add or remove specific genes or correct gene mutations—techniques that fall under the broad category of genome editing. During the past few years, scientists have begun adapting TALENs and other genome-editing tools for gene therapy. Until now, scientists had only used TALENs to edit genes in the cell nucleus. Today's report marks the first time TALENs have been used to edit mitochondrial genes.

"Mitochondrial-targeted TALENS (mitoTALENs) represent the most promising hope for an effective treatment of diseases caused by mutations in mtDNA," said Carlos T. Moraes, Ph.D., a professor of neurology and cell biology at the University of Miami Miller School of Medicine and principal investigator of the study. "Our research demonstrates that mitoTALENs can substantially decrease or eliminate mutant mtDNA without harming normal mtDNA."

Using cells in the lab, the investigators designed mitoTALENs to bind and cut mitochondrial DNA that had a specific mutation in the gene Complex I, which causes LHON. The scientists then tested whether the mitoTALENs eliminated the mutant mtDNA.

Analysis revealed a temporary drop in cells' total mtDNA, which was due to a reduction in mutant mtDNA. "Once the mitoTALENs bound and cut the DNA at the specified target, the mutant mtDNA was degraded," said Moraes. "The drop in total mtDNA stimulated the cells to increase their mtDNA by replicating the unaffected molecules. Two weeks later, mtDNA levels had returned to normal. But since the mutant mtDNA was destroyed, the cells had mostly normal mtDNA."

Reducing but not necessarily eliminating all mutant mtDNA from a person's cells would be sufficient to treat many mitochondrial diseases, Moraes said. Mutant mtDNA typically does not cause signs of disease until it makes up 80 percent or more of the total mtDNA in a cell, which

helps explain why age of onset, the constellation of symptoms, and disease severity varies among individuals with the same mutation. "A modest reduction in mutant mtDNA is likely sufficient to effectively treat disease," he said.

"The science in this project advances an imaginative and very clever approach that may one day lead to a therapeutic strategy for mitochondrial diseases," said Houmam Araj, Ph.D., director of the lens/cataract and oculomotor/neuro-ophthalmology programs at the NEI.

Symptoms of LHON include an abrupt loss of central vision at about age 20 in one eye followed shortly thereafter by the other eye. Although a variety of gene mutations are linked to the disease, most cases involve Complex I mutations, which cause degeneration of the nerves in the back of the eye that transmit visual information to the brain. Neurological problems such as tremors may also occur.

Getting mitoTALENs into cells in tissues presents a formidable challenge, however. The scientists plan next to test the approach in animals.

More information: Bacman SR, et al. Specific elimination of mutant mitochondrial genomes in patient-derived cells by mitoTALENs. *Nature Medicine*. Published online Aug. 4, 2013. dx.doi.org/10.1038/nm.3261

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