Novel gene therapy approach creates new route to tackle rare, inherited diseases

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Nonsense mutations are single-letter errors in the genetic code that prematurely halt the production of critical proteins. These unfinished proteins are unable to function normally, and nonsense mutations cause 10-15 percent of all inherited genetic diseases, including Duchenne muscular dystrophy, spinal muscular atrophy, cystic fibrosis and polycystic kidney disease. There is currently no cure or broadly effective treatment for these often devastating conditions that are individually rare

but estimated to collectively affect up to 30 million people worldwide.

A new study, led by Christopher Ahern, Ph.D., at the University of Iowa Carver College of Medicine, reveals a novel approach and robust technology platform for suppressing nonsense mutations using engineered transfer RNA (tRNA) molecules. The research by Ahern, his UI colleagues, and collaborators at The Wistar Institute in Philadelphia, the Cystic Fibrosis Foundation Therapeutics Lab in Lexington, Mass., and Integrated DNA Technologies Inc. in Coralville, Iowa, shows that modified tRNAs can efficiently and accurately repair nonsense mutations with any amino acid. The findings were published Feb. 18 in *Nature Communications*.

"Because nonsense mutations cause a wide range of severe, life threatening diseases, there is a significant unmet medical need to efficiently repair these stop codons in people having these inherited genetic alterations," says Ahern, UI professor of molecular physiology and biophysics and a member of the Iowa Neuroscience Institute. "Our unique gene therapy approach takes advantage of the built-in fidelity of the translation process but reengineers tRNAs to turn disease-causing stop signals back into the correct amino acid. Basically, our anticodon engineered tRNA technology turns 'stops' into 'gos' and hopefully one day may be used to correct defective genetic sequences in people."

The process of turning genetic code into protein is called translation. Transfer RNAs (tRNAs) match up with the blueprint code of the messenger RNA and deliver the correct amino acid in the correct order to build the protein. The code sequences of the messenger RNA, which dictate the order of amino acids, are called codons. The matching sequence on the tRNAs are called anticodons.

At the end of every protein coding sequence there is a genetic stop signal—a stop codon—that tells the protein production machinery to

halt. Nonsense mutations occur when a mistake in the genetic sequence turns an amino acid codon in the middle of the protein into a stop codon.

Ahern and his UI team, including lead study author John Lueck, Ph.D., who is now at the University of Rochester, systematically tested the engineered tRNA molecules for their ability to repair premature stop codons with each of the 20 natural amino acids. The high-throughput screen efficiently identified multiple potent engineered tRNAs for each amino acid and stop codon type.

To demonstrate that the approach could work in more complex and physiologically relevant systems, Ahern lab members, together with collaborators at the Cystic Fibrosis Foundation Therapeutics (CFFT) lab and the laboratory of David Weiner, Ph.D., at The Wistar Institute showed that the engineered tRNAs when encoded and formulated for efficient delivery are expressed at high levels and are effective at correcting nonsense mutations in living mouse muscle tissue. Interestingly, the tRNA activity persisted for weeks in the delivered forms, suggesting this sustainable gene therapy approach may have potential for being used in the clinic one day.

Importantly, the team at the CFFT lab under William Skach, MD, showed that the tRNAs were selective in their activity and did not affect normal stop codons that signal the true end of the protein sequence.

And at the UI, Ahern with postdoctoral fellows Lueck and Danny Infield, Ph.D., and UI professor of pediatrics and cystic fibrosis expert Paul McCray, MD, showed that the approach could correct a CF-causing nonsense mutation and accurately produce a functional CFTR protein.

"What I like about this study is that a number of different labs with different expertise all verified our engineered tRNA technology in a variety of contexts," Ahern says. "That suggests the approach is robust."

Although he is excited about the potential for anticodon engineered tRNAs to tackle diseases caused by nonsense mutations, Ahern notes there are many scientific questions to answer and technical hurdles to overcome to find out if this approach can be translated into human therapies.

"For many diseases caused by nonsense mutations, even correcting a small percent of the mutated protein could be enough to be therapeutic to the patient," Ahern says. "If this were to work as a human therapy, we would have a way to target every known stop codon disease."

More information: John D. Lueck et al, Engineered transfer RNAs for suppression of premature termination codons, *Nature Communications* (2019). DOI: 10.1038/s41467-019-08329-4

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