One-Shot, One Cure With Genome Editing for Dyslipidemia

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Study Hypothesis

One of the most inspiring examples of rapid bench-to-bedside translation has been the development of proprotein convertase subtilisin/kexin 9 (PCSK9) inhibitory antibodies for the reduction of serum low-density lipoprotein and prevention of coronary artery disease. Astute genetic analysis of gain-of-function mutations in patients with elevated low-density lipoprotein (LDL) levels built the case for the role of PCSK9 in LDL receptor binding and lysosomal destruction. Now the prospect of lipid-lowering therapy delivered monthly via injection has been on the horizon with successful clinical trials for the 2 leading monoclonal antibodies. The logical next expectation would be for a permanent disruption of the PCSK9 gene, rendering continued therapy unnecessary because the naturally occurring and protective loss-of-function mutation could be given as a 1-time treatment. Ding et al. present evidence that the technology for this now exists with genome editing and the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) 9 nuclease. CRISPR/Cas9 is the latest nuclease with demonstrated ability to generate double-strand breaks in mammalian cells at a specified target site, and thereby create frame-shift mutations when error-prone repair processes insert or delete nucleotides at the break site. The authors demonstrate the ease with which a PCSK9-specific nuclease system can be designed, validated for editing efficiency, and delivered to hepatocytes with an adeno viral vector.

How Was the Hypothesis Tested?

Ding et al. designed synthetic guide RNAs (gRNA) that hybridize a 20-nucleotide DNA sequence in exons 1 and 2 of the PCSK9 gene and assessed the ability to generate mutations using a Surveyor assay in 293 cells. This assay uses a mismatch-specific DNA endonuclease to cleave DNA at sites of base-substitution and other distortions. It makes for a rapid and simple tool to evaluate the editing efficiency of a designed gRNA. The authors then injected adenoviruses expressing Cas9, the CRISPR-associated nuclease derived from Streptococcus pyogenes, and an active gRNA into mice via the tail vein. The efficiency of in vivo mutagenesis of Pcsk9 was assessed in genomic DNA harvested from mouse livers 3 to 4 days after injection with the Surveyor assay. Finally, a test for clinical efficacy was conducted with 5 mice receiving the CRISPR-Pcsk9 virus, and 5 mice each receiving either no virus or GFP virus as negative controls. Pcsk9, triglyceride, cholesterol, and alanine transaminase levels were measured in all mice to evaluate the response to treatment.

Principal Findings

The efficacy of CRISPR/Cas9-mediated generation of mutations in Pcsk9 was ~50% both in vitro using 3T3-L1 cells and in vivo with harvested mouse hepatocytes 3 to 4 days after tail vein injection. Although not complete loss of function, the authors surmised that this would be sufficient to see a clinical effect and proceeded to test therapeutic efficacy without further optimizing the gene targeting or delivery methods. There was indeed a substantial reduction in plasma PCSK9 levels, with a >5-fold reduction in the CRISPR-Pcsk9 group. As a result, there was a 35% to 40% reduction in total cholesterol, which is consistent with the 36% to 52% reduction in cholesterol reported in Pcsk9-knockout mice. There was no statistically significant reduction in triglyceride level or alanine transaminase, confirming that the cholesterol reduction was not the result of a toxic or inflammatory effects on the liver.

Implications

The efficiency by which a single injection of CRISPR/Cas9 targeting the mouse Pcsk9 gene reduced serum cholesterol levels is a remarkable proof-of-concept for the potential for genome editing in the prevention of cardiovascular disease. The ability to target multiple genes with a single injection of Cas9 nuclease makes it an attractive platform by which to deliver gene therapy. The prospect of using this approach to immunize a high-risk patient for life-long protection from atherosclerosis is mentioned as a feasible goal by the authors.
and is certainly a paradigm shifting technology for the field. Technical hurdles certainly exist before such dreams can be realized. Most significantly, the efficient delivery of the CRISPR-Cas9 system remains a challenge given the immune response induced by adenoviral delivery used in this article. As with all genome editing technology, the off target effects of the nuclease remain a mystery. Although this study did not observe significant off target mutagenesis at 10 predicted off target sites, the possibility of low-frequency events in vivo remains. Even with these sizeable concerns, the rapid rate of innovation in the field and the dramatic effect seen in this study make for an exciting future for the therapeutic potential of genetic editing.

Disclosures

Dr Gupta is a member of the Early Career Committee of the American Heart Association Functional Genomics and Translational Biology Council.

Key Words: gene, suppressor • gene targeting • genetic therapy • lipids • PCSK9 protein, human
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