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ACTIVATION OF HOMOLOGY-DIRECTED DNA REPAIR PLAYS KEY ROLE IN CRISPR-MEDIATED GENOME CORRECTION IN FETAL AND NEONATAL CELLS

Clara T. Nicolas, MD, PhD[1], Gourish Mondal[2], Caitlin VanLith[3], Whitney Thompson[3], Robert Kaiser[3], Daniel O'Brien[3], Lori Hillin[3], William Cao[3], Joseph Lillegard[4]

[1]University of Alabama at Birmingham, Birmingham, AL, USA, [2]UCSF, San Francisco, CA, USA, [3]Mayo Clinic, Rochester, MN, USA, [4]Mayo Clinic / Children's Minnesota, Rochester, MN, USA

Purpose: Gene editing for treatment of inborn errors of metabolism (IEMs) has been limited by poor efficiency of adult hepatocyte targeting. Our study was designed to test these hypotheses: 1) in utero gene editing cures hereditary tyrosinemia type 1 (HT1) in a small animal model, 2) increased gene editing efficiency in developing hepatocytes relates to abundance of DNA repair gene expression.

Methods: Direct fetal intrahepatic injections of adeno-associated virus (AAV)-Cas9 and AAV-homology template (HT) were performed in Fah-/- mice at gestational day 15±1. We isolated hepatocytes from fetal, neonatal, and adult Fah-/- mice, transfected them with Cas9, a guide RNA, and a HT to introduce guided DNA breaks and repair at the Fah locus, and performed Sanger sequence analysis of this locus. After confirming increased efficiency of gene correction through homology-directed repair (HDR) in fetal/neonatal hepatocytes compared to adult hepatocytes, we performed a gene expression analysis to explore the characteristics of fetal/neonatal hepatocytes that make them more susceptible to efficient gene editing and investigated whether these conditions can be replicated through cell synchronization and liver regenerative stimulus.

Results: In utero CRISPR/Cas9-mediated gene editing in an HT1 mouse model results in complete liver repopulation with corrected hepatocytes, providing stable cure. We showed that fetal/neonatal livers are comprised of proliferative hepatocytes with abundant expression of HDR genes, key for efficient CRISPR/Cas9 gene editing. The same is true of hepatocytes after undergoing partial hepatectomy, where post-hepatectomy cells show a higher efficiency of HDR than pre-hepatectomy cells. Specifically, HDR-related genome correction is most effective in the replicative phase, or S-phase, of an actively proliferating cell.

Conclusion: We show that taking advantage of or triggering cell proliferation, specifically DNA replication in S-phase, may serve as a tool to improve liver CRISPR/Cas9-mediated genome editing efficiency and provide curative therapy for IEMs in both children and adults.