Gene Editing Strategies to Create Human Derived Erythroid Progenitor Cells with SC Mutation

Lubna Hamdan, MD, Mengna Chi, PhD, Punam Malik, MD

Cincinnati Children's
changing the outcome together

INTRODUCTION

- Sickle cell disease (SCD) is caused by homozygous Beta-sickle mutation on both alleles or compound heterozygous with Hemoglobin (Hb)S on one allele and HbC or a Beta-thalassemia mutation on the second allele.
- Hemoglobin sickle-Hemoglobin C disease (HbSC) accounts for 30% of SCD genotypes. HbS results from A→T mutation in the sixth codon of the HbB gene while HbC results from G→A mutation in the same codon of the HbB gene.
- HbSC patients also have substantial morbidity and mortality. Hence, creating HbSC cell models is crucial to understand disease pathophysiology and developing novel therapies.

OBJECTIVE

- The aim of the project is to optimize a CRISPR/Cas9 gene editing strategy in Human Umbilical Cord Blood-Derived Erythroid Progenitor (HUDEP)-S cell line from a sickle patient to create HUDEP-C and HUDEP-SC cell line.

METHODS

- Two different single stranded oligonucleotide templates (ssODN donor A and B) were trialed with at least 2 different concentrations.
- Delivery of ribonucleoprotein (RNP) complex of CRISPR guide RNA (gRNA) and Cas9 with ssODN templates was done through electroporation.
- Transfected cells were cultured and incubated for recovery before harvested for examination.
- Two methods were used for confirming gene editing:
  1) PCR with a digestion reaction using a restriction enzyme (HhaI): If editing is successful and homology directed repair (HDR) occurs, two bands (250 and 207 Bp) should be detected during gel electrophoresis.
  2) TIDE (Tracking of indels by Decomposition) assay coupled with sanger sequencing to examine if non-homologous end joining (NHEJ) occurs

RESULTS

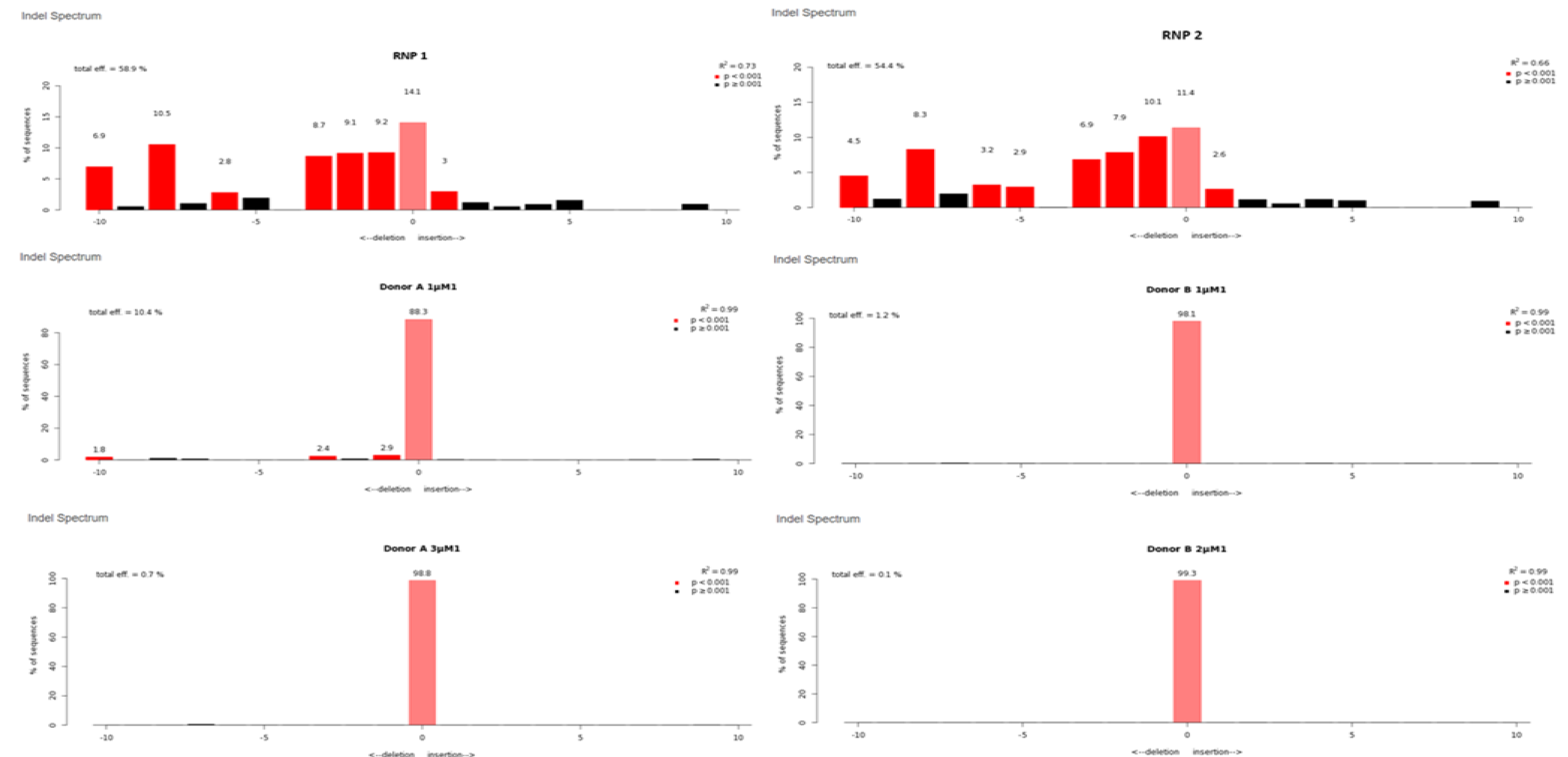
ssDON Templates- Edits needed to result in HBC highlighted in red

Donor A
TCTGACACAACTGTGTTCACTAGCAACCTCAAACAGACACCA
TGGTGCATCTGACTCCTaagGAGAAGTCTGCGGTTACTGCGC
TGTGGGGCAAGGTGAACGTGGATGAAGTTGGTGGTGA

Donor B
TCAGGGCAGAGCCATCTATTGCTTACATTTGCTTCTGACACA
ACTGTGTTCACTAGCAACCTCAAACAGACACCATGGTGCAtCT
GACTCCTaagGAGAAGTCTGCGGTTACTGCGCTGTGGGGCAA
GGTGAACGTGGATGAAGTTGGTGGTGAGGCCCTGGCAGGT

Gel electrophoresis after digestion reaction with HhaI- 100 bp ladder used

TIDE Assay Results: RNP1 and RNP2 samples with indels. Donor A at 1µM with indels, at 3 µM no indels. Donor B at 1 µM and 2 µM with no indels



CONCLUSION

- Our experiment did not result in HDR in HUDEP-S cells while resulted in indels in RNP control groups. This could be attributable to ssODN toxicity, instability or electroporation conditions. Further experiments are needed to optimize gene editing in HUDEP-S cells.