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Saturated Mutagenesis Surrounding Beta-globin Locus Identifies Novel Therapeutic Targets for Fetal Globin Induction and Treatment of Sickle Cell Anemia

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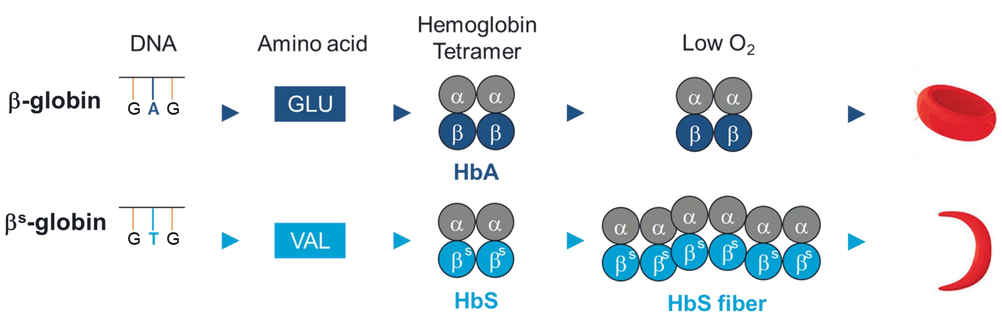
Background

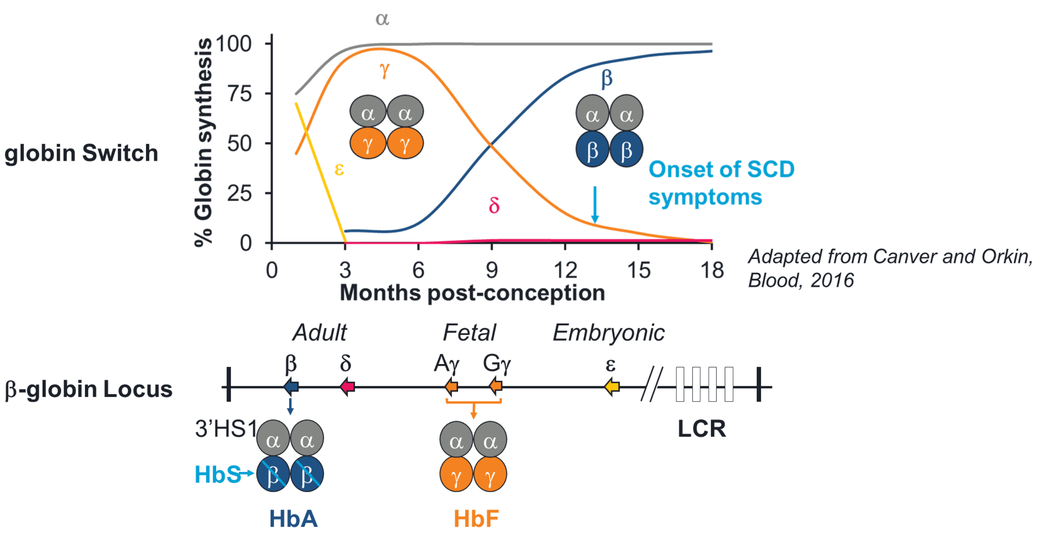
Etiology of SCD[1]

- Sickle cell disease (SCD) is caused by a single mutation (E6V) at position 6 of the beta-globin chain.
- This mutation creates a hydrophobic patch that leads to polymerization of hemoglobin molecules and formation of sickle hemoglobin (HbS) fibers when deoxygenated.
- Sickle red blood cells are rigid and prone to lysis, leading to anemia, acute chest syndrome, pain crises, and an array of other complications. Consequently, patients with SCD suffer significant morbidity and early mortality.



Fetal globin induction for the treatment of SCD

- Onset of SCD symptoms coincides with fetal to adult globin switch. Reactivating developmentally silenced fetal gamma globin inhibits polymerization of HbS and ameliorates SCD.[2]



Adapted from Canver and Orkin, Blood, 2016

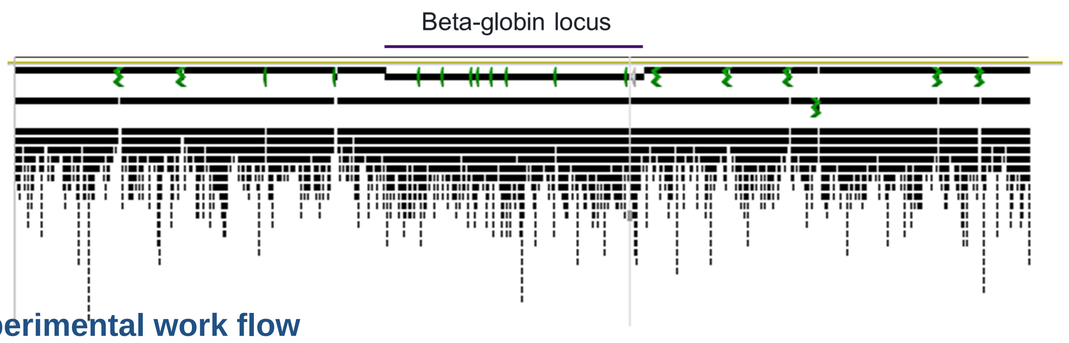
Objectives

- Identify cis-regulatory elements involved in repressing fetal globin expression.
- Develop a cell therapy product where a fetal globin repressor element is permanently disrupted using CRISPR technology that would lead to long lasting elevation of HbF for the treatment of SCD.

Methods

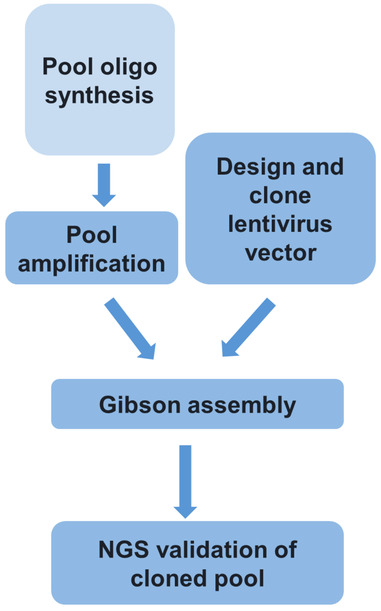
Lenti-mediated saturated mutagenesis of beta-globin locus and its surrounding regions

- > 26,000 guide RNAs (gRNAs) were designed to target a 320 kb genomic region centered around beta-globin locus for lenti-mediated saturated mutagenesis in HUDEP-2 cell line.
- Graph below shows the coverage of the library. Each black vertical line represents one gRNA.

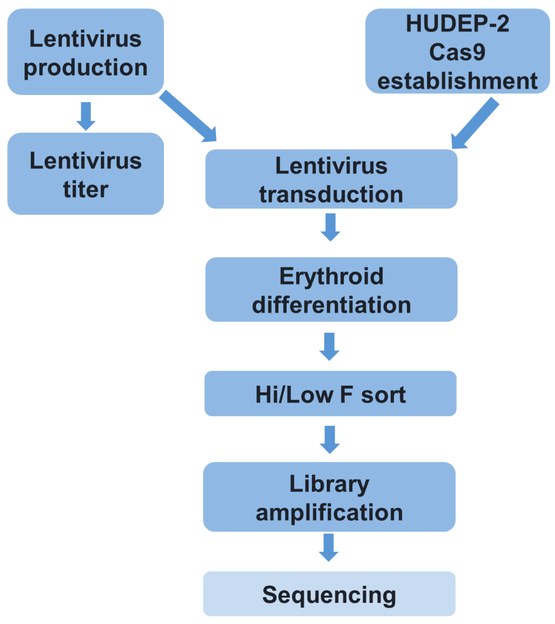


Experimental work flow

A. Lenti library cloning



B. Identification of HbF-inducing gRNAs



Results

Figure 1. Multiple gRNAs identified to induce HbF expression in HUDEP-2 cells

- >300 gRNAs were enriched in high HbF expressing HUDEP-2 cells.
- Most of the enriched gRNAs were mapped to beta-globin locus including HBG, HBD, and HBB genes.

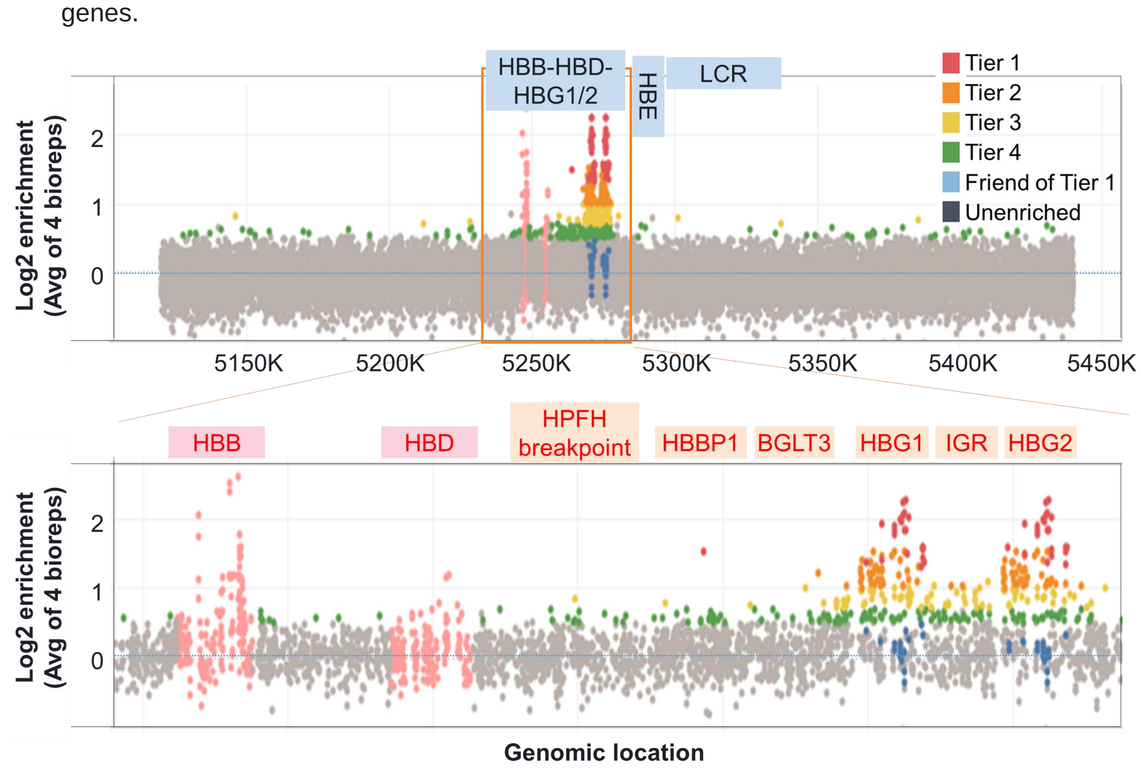


Figure 2. Indel analysis revealed multiple clusters of HbF-inducing elements

- Indel analysis of high HbF-expressing and low HbF-expressing HUDEP-2 cells identified multiple novel cis-elements involved in the suppression of HbF expression in the adult cells.

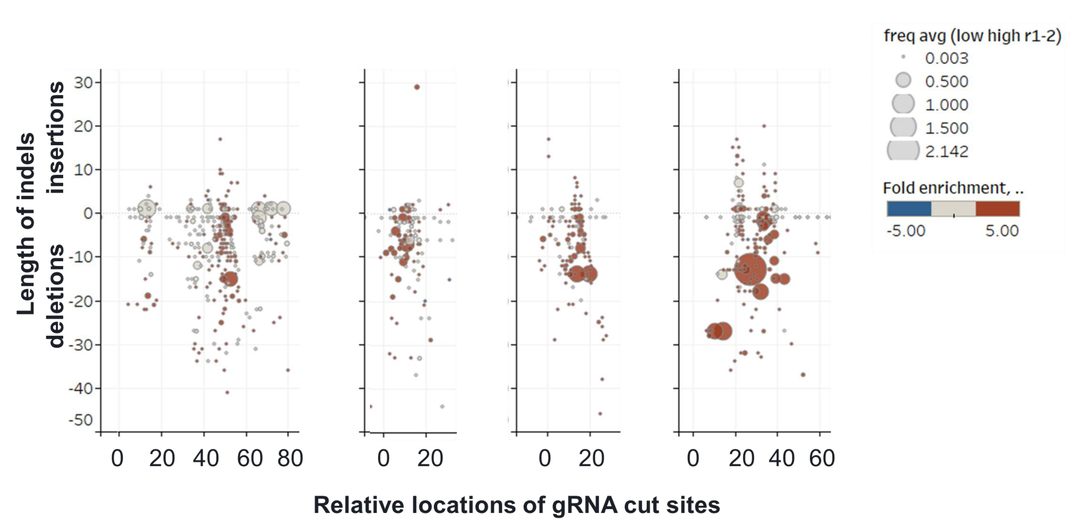
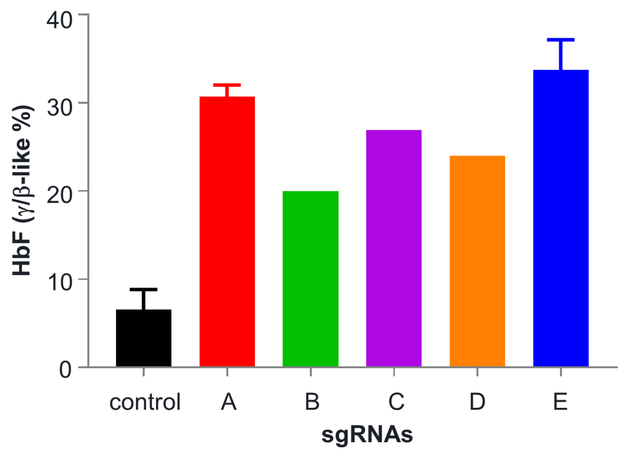


Figure 3. Validation of HbF-inducing sgRNAs in primary CD34+ cells



- A subset of sgRNAs targeting the cis-elements identified in the HUDEP-2 screen were transfected into mobilized peripheral blood CD34+ hematopoietic stem and progenitor cells as ribonucleoprotein (RNP) complex.
- Elevated gamma globin expression as measured by reverse phase UPLC was confirmed following erythroid differentiation.

Summary

- Lenti-mediated saturated mutagenesis led to discovery of novel HbF-inducing sites at the beta-globin locus.
- Indel analysis of HbF-inducing gRNAs further demonstrated discrete clusters that were associated with fetal globin induction.
- High levels of HbF achieved when transfecting mobilized peripheral blood CD34+ cells from healthy volunteers with RNPs of SpCas9 and individual gRNA targeting these cis-elements.

References

1. Bender MA. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A, editors. Gene Reviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2018.
2. Fetal haemoglobin induction in sickle cell disease. Paikari A, Sheehan VA. Br J Haematol. 2018 Jan;180(2):189-200.

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