EUROPEAN HEMATOLOGY ASSOCIATION

Update on hemoglobinophaties - Section 1

Genome editing in hemoglobinopathies

Mitchell J. Weiss

St. Jude Children's Research Hospital, Memphis, USA

Take-home messages

- Genome editing is initiated by programmed nucleases that introduce a double-stranded DNA break at a specified sequence.
- Cells repair double-stranded DNA breaks through error-prone non-homologous end joining (NHEJ) or through homology directed repair (HDR), which utilizes an exogenous DNA template to create precise nucleotide alterations. Strategies to treat β-hemoglobinopathies include NHEJ to induce fetal hemoglobin or HDR to repair the pathogenic mutation.
- In hematopoietic stem cells, NHEJ occurs at approximately 10-fold greater frequency than HDR.
- Challenges to clinical genome editing include optimizing programmed nuclease delivery into cells, defining and minimizing deleterious off target effects, and maximizing the efficiency of on target editing.

β-hemoglobinopathies, including sickle cell disease (SCD) and ß-thalassemia are common, devastating autosomal recessive disorders caused by mutations in the *HBB* gene, which encodes the β-globin subunit of adult hemoglobin (HbA, α_2 β_2).[1,2] Medical therapies extend the lives of patients and reduce suffering, but do not eliminate major clinical problems. Allogeneic bone marrow transplantation (BMT) is curative for up to 90% of selected patients, but serious complications can occur, particularly for BMT recipients without an HLA-matched sibling donor. Gene therapy via lentiviral vector replacement of *HBB* is promising, but has not yet proven to be fully effective.

We are poised to cure SCD and β–thalassemia by genome editing of hematopoietic stem cells (HSCs), either to repair the causal mutations or to create new mutations that suppress disease phenotypes by inducing fetal hemoglobin (HbF) production in adult red blood cells (RBCs). Genome editing is based on programmable sequence-specific DNA nucleases that create targeted double-stranded breaks (DSBs).[3,4] Classes of gene editing nucleases include clustered regularly interspaced short palindromic repeats (CRISPR)/Cas, transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs). Cells typically repair nuclease-induced DSBs according to two distinct and competing endogenous enzymatic pathways: 1) error-prone non-homologous end joining (NHEJ) can rejoin the broken DNA ends, but often introduces insertions or deletion mutations (indels) that can disrupt coding genes or noncoding regulatory sequences; 2) in the presence of a user-supplied homologous "donor" DNA template, DSBs can be repaired precisely by homology directed repair (HDR). By varying the HDR donor template, it is possible to correct deleterious mutations. Both HDR and NHEJ-mediated genome editing strategies are under investigation for treating β-hemoglobinopathies. The general strategy is to remove HSCs from the patient, edit these cells *ex vivo* to induce therapeutic genetic alterations, then return them to the patient after administration of a myelotoxic "conditioning" agent to enhance engraftment of the altered HSCs. This therapy modifies somatic cells so that the induced genetic changes are not passed through the germline.

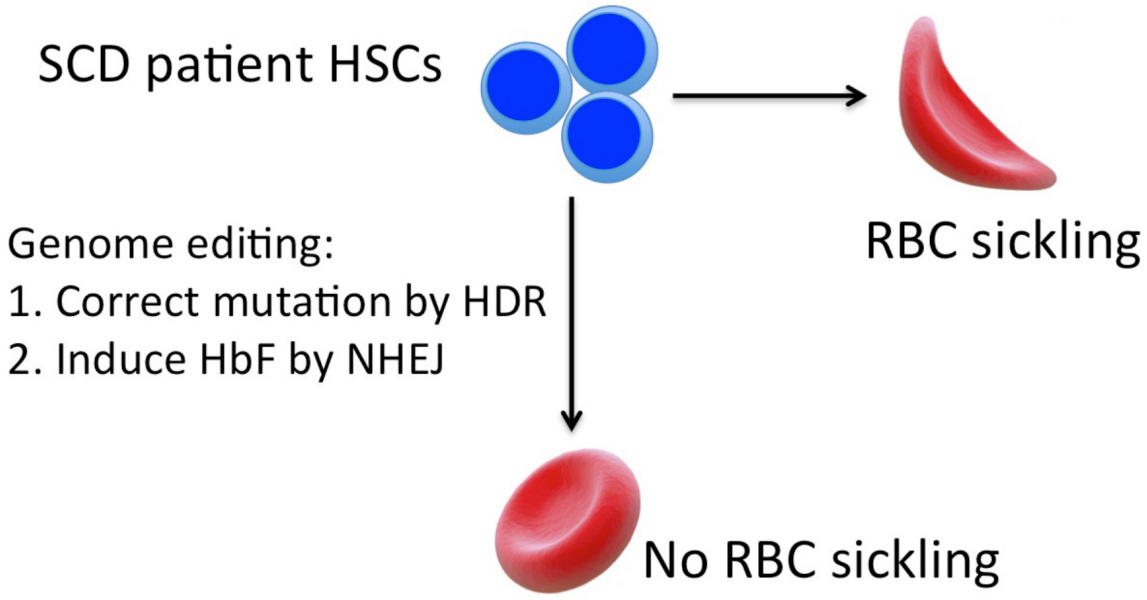
Correction of β-hemoglobinopathymutations by HDR

The major form of SCD is caused by a homozygous A-to-T mutation resulting in a glutamic acid-to-valine substitution at β-globin amino acid 6. It is possible to correct this mutation through genome editing by creating a DSB in mutant *HBB* exon 1 and supplying a donor DNA template containing the normal sequence.[5-7] Preliminary results are promising, but several challenges exist for clinical application. First, NHEJ occurs more efficiently than HDR at DSBs,[8,9] particularly in quiescent HSCs, resulting in low rates of gene correction combined with NHEJ-mediated null (β^0) alleles. Second, HDR requires co-delivery of a donor template and may require selection of edited cells, both of which may complicate production of therapeutic cell products. In contrast to SCD, β-thalassemia is caused by many different mutations, making it more challenging to create a general correction strategy.

Induction of fetal hemoglobin via NHEJ

Fetal Hb (HbF, $\alpha_2\gamma_2$) is the main RBC oxygen carrier during late gestation. After birth, γ-γlobin synthesis declines and β-globin increases, thereby switching HbF to HbA. The γ-globin genes *HBG1* (Aγ) and *HBG2* (Gγ) are located upstream of *HBB* (β) and their relative expression is controlled by regional cis elements and key trans-acting transcription factors, both of which have been identified by human genetic studies.[10,11] Residual HbF in adult RBCs is expressed as a quantitative genetic trait and β-hemoglobinopathy patients with higher HbF levels experience fewer symptoms and reduced mortality.[10,12,13] Hydroxyurea, the only approved drug for SCD, acts partly by raising HbF levels though unknown mechanisms. Genome editing provides new opportunities to raise HbF levels therapeutically by manipulating known regulators. In a rare genetic condition termed hereditary persistence of fetal hemoglobin (HPFH), relative HbF levels exceed 20% in adult RBCs.[14] HPFH is a benign condition typically caused by deletions or point mutations in the extended β-globin locus. Individuals with SCD or β-thalassemia genotypes who co-inherit HPFH mutations experience few or no disease pathologies. Several groups, including ours, are using genome editing-mediated NHEJ to recapitulate HPFH mutations, with promising preliminary results.[15,16]

It is also possible to raise HbF by targeting transcription factors that repress γ-globin production. The leading candidate is BCL11A, which was identified through genome wide association studies to regulate HbF levels in adult RBCs.[17-19] Because HSCs and B cells require BCL11A,[20-23] therapeutic strategies to inhibit its expression must be specific to erythoid lineages. This may be achieved via NHEJ-mediated disruption of an erythroid-specific *BCL11A* enhancer, which raises HbF to potentially therapeutic levels in adult $CD34^+$ cell-derived erythroblasts.[19] Excision of the orthologous enhancer in mice increases embryonic/fetal globin expression in adult RBCs, with no deleterious effects on other cell lineages.[24] Thus, genome editing-mediated disruption of the*BCL11A* erythroid enhancer via NHEJ represents a potential therapeutic strategy.



Genome editing for sickle cell disease (SCD). Patient hematopoietic stem cells (HSCs) produce red blood cells (RBCs) that undergo pathological sickling due to adult hemoglobin (HbA, α2β2) polymerization at low oxygen tension. HSCs may be modified by genome editing to prevent sickling of their RBC progeny. Two approaches are under investigation: correction of the *HBB* gene SCD mutation by homology directed repair (HDR) and induction of fetal hemoglobin (HbF, α2γ2) by non-homologous end joining to de-repress γ-γlobin transcription from the *HBG1* and/or *HBG2* genes.

Toxicities

While gene editing is quite specific, nuclease-induced off-target DSBs can cause unintended mutations.[25] Additionally, genomic deletions or rearrangements can occur between two DSBs that occur simultaneously in the same cell. While some off-target DSBs are predicted by sequence homology to the on-target site, current *in silico* prediction algorithms fail to capture the majority of *bona fide* off-target effects.[25] Numerous cell-based unbiased genomic approaches can detect off target DSBs and genomic rearrangements in cells to a sensitivity of about 0.1%. Most low frequency off-target DSBs are likely to be inconsequential. However, a major concern is that rare off-target mutations in HSCs could confer selective growth advantages and thereby induce hematopoietic malignancies. In these cases, very low-frequency mutations occurring below current limits of detection could be deleterious. Ideal preclinical assays to assess such mutations are lacking. Currently, patients receiving any form of HSC gene therapy, including genome editing, must undergo myelotoxic conditioning prior to infusion of modified autologous HSCs in order to facilitate their engraftment. Conditioning regimens can cause acute organ damage, sterility, hair loss and leukemia.

How much correction is enough?

The fraction of correctly edited HSCs required to cure β-hemoglobinopathies is unknown. Genetic correction will enhance the survival of erythroblasts and RBCs over those with β-thalassemia or SCD mutations. Therefore, disease-free RBC populations can arise from relatively low levels of corrected HSCs. Assessment of mixed donor chimerism after allogeneic BMT for β-hemoglobinopathies[26-32] and mathematical modeling[33] estimate that as low as 20% corrected HSCs could be curative. However, these models assume that every gene-edited cell will undergo complete correction, which may not be the case. For example, HDR to correct the *HBB* SCD mutation will likely be accompanied by NHEJ-induced null alleles and gene editing to inhibit γ-to-β globin switching may not induce HbF to fully therapeutic levels in all RBCs. Thus, while preclinical benchmarks for % HSC editing are important to consider, clinical trials are necessary to define more precisely the therapeutic requirements for HSC modification.

References

1. Chakravorty S, Williams TN. Sickle cell disease: a neglected chronic disease of increasing global health importance. Arch Dis Child 2015;100:48-53.
2. Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis 2010;5:11.
3. Hoban MD, Orkin SH, Bauer DE. Genetic treatment of a molecular disorder: gene therapy approaches to sickle cell disease. Blood 2016;127:839-48.

*4. Hoban MD, Bauer DE. A genome editing primer for the hematologist. Blood 2016;127:2525-35.

Informative review of genome editing with emphasis on hematology applications.

*5. Dever DP, Bak RO, Reinisch A, et al. CRISPR/Cas9 beta-globin gene targeting in human haematopoietic stem cells. Nature 2016;539:384-9.

Correction of the sickle cell disease mutation by homology directed repair.

*6. DeWitt MA, Magis W, Bray NL, et al. Selection-free genome editing of the sickle mutation in human adult hematopoietic stem/progenitor cells. Sci Transl Med 2016;8:360ra134.

Correction of the sickle cell muation by homology directed repair.

7. Hoban MD, Lumaquin D, Kuo CY, et al. CRISPR/Cas9-Mediated Correction of the Sickle Mutation in Human CD34+ cells. Mol Ther 2016;24:1561-9.
8. Cong L, Ran FA, Cox D, et al. Multiplex genome engineering using CRISPR/Cas systems. Science 2013;339:819-23.
9. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Genome engineering using the CRISPR-Cas9 system. Nat Protoc 2013;8:2281-308.
10. Lettre G, Bauer DE. Fetal haemoglobin in sickle-cell disease: from genetic epidemiology to new therapeutic strategies. Lancet 2016;387:2554-64.
11. Smith EC, Orkin SH. Hemoglobin genetics: recent contributions of GWAS and gene editing. Hum Mol Genet 2016.
12. Platt OS, Brambilla DJ, Rosse WF, et al. Mortality in sickle cell disease. Life expectancy and risk factors for early death. N Engl J Med 1994;330:1639-44.
13. Musallam KM, Sankaran VG, Cappellini MD, Duca L, Nathan DG, Taher AT. Fetal hemoglobin levels and morbidity in untransfused patients with beta-thalassemia intermedia. Blood 2012;119:364-7.
14. Forget BG. Molecular basis of hereditary persistence of fetal hemoglobin. Ann N Y Acad Sci 1998;850:38-44.

*15. Traxler EA, Yao Y, Wang YD, et al. A genome-editing strategy to treat beta-hemoglobinopathies that recapitulates a mutation associated with a benign genetic condition. Nat Med 2016;22:987-90.

Induction of fetal hemoglobin by genome editing of the HBG genes in hematopoietic progenitors.

16. Ye L, Wang J, Tan Y, et al. Genome editing using CRISPR-Cas9 to create the HPFH genotype in HSPCs: An approach for treating sickle cell disease and beta-thalassemia. Proc Natl Acad Sci U S A 2016.
17. Sankaran VG, Menne TF, Xu J, et al. Human fetal hemoglobin expression is regulated by the developmental stage-specific repressor BCL11A. Science 2008;322:1839-42.
18. Xu J, Peng C, Sankaran VG, et al. Correction of sickle cell disease in adult mice by interference with fetal hemoglobin silencing. Science 2011;334:993-6.

*19. Canver MC, Smith EC, Sher F, et al. BCL11A enhancer dissection by Cas9-mediated in situ saturating mutagenesis. Nature 2015;527:192-7.

Induction of fetal hemoglobin by genome editing of the BCL11A gene in hematopoietic progenitors.

Update on hemoglobinophaties - Section 1

20. Luc S, Huang J, McEldoon JL, et al. Bcl11a Deficiency Leads to Hematopoietic Stem Cell Defects with an Aging-like Phenotype. Cell Rep 2016;16:3181-94.
21. Tsang JC, Yu Y, Burke S, et al. Single-cell transcriptomic reconstruction reveals cell cycle and multi-lineage differentiation defects in Bcl11a-deficient hematopoietic stem cells. Genome Biol 2015;16:178.
22. Liu P, Keller JR, Ortiz M, et al. Bcl11a is essential for normal lymphoid development. Nat Immunol 2003;4:525-32.
23. Brendel C, Guda S, Renella R, et al. Lineage-specific BCL11A knockdown circumvents toxicities and reverses sickle phenotype. J Clin Invest 2016.
24. Smith EC, Luc S, Croney DM, et al. Strict in vivo specificity of the Bcl11a erythroid enhancer. Blood 2016.
25. Tsai SQ, Joung JK. Defining and improving the genome-wide specificities of CRISPR-Cas9 nucleases. Nat Rev Genet 2016;17:300-12.
26. Marziali M, Isgro A, Sodani P, et al. Peripheral red blood cell split chimerism as a consequence of intramedullary selective apoptosis of recipient red blood cells in a case of sickle cell disease. Mediterr J Hematol Infect Dis 2014;6:e2014066.
27. Wu CJ, Krishnamurti L, Kutok JL, et al. Evidence for ineffective erythropoiesis in severe sickle cell disease. Blood 2005;106:3639-45.
28. Iannone R, Casella JF, Fuchs EJ, et al. Results of minimally toxic non-myeloablative transplantation in patients with sickle cell anemia and beta-thalassemia. Biol Blood Marrow Transplant 2003;9:519-28.
29. Andreani M, Testi M, Battarra M, Lucarelli G. Split chimerism between nucleated and red blood cells after bone marrow transplantation for haemoglobinopathies. Chimerism 2011;2:21-2.
30. Hsieh MM, Wu CJ, Tisdale JF. In mixed hematopoietic chimerism, the donor red cells win. Haematologica 2011;96:13-5.
31. Walters MC, Patience M, Leisenring W, et al. Stable mixed hematopoietic chimerism after bone marrow transplantation for sickle cell anemia. Biol Blood Marrow Transplant 2001;7:665-73.
32. Wu CJ, Gladwin M, Tisdale J, et al. Mixed haematopoietic chimerism for sickle cell disease prevents intravascular haemolysis. Br J Haematol 2007;139:504-7.
33. Altrock PM, Brendel C, Renella R, Orkin SH, Williams DA, Michor F. Mathematical modeling of erythrocyte chimerism informs genetic intervention strategies for sickle cell disease. Am J Hematol 2016;91:931-7.