Perspective

Stem Cell INVESTIGATION

Genome editing and stem cell therapy pave the path for new treatment of sickle-cell disease

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Abstract: Sickle-cell disease (SCD), also known as sickle-cell anemia, is a hereditary blood disorder characterized by the presence of abnormal hemoglobin, the oxygen-carrying protein found in red blood cells. This devastating hematologic disease affects millions of children worldwide. Currently the only available cure is an allogenic hematopoietic stem cell transplant (HSCT) which is limited by the scarcity of fully-matched donors. SCD is caused by a single nucleotide mutation in the beta-globin gene. Correction of this genetic defect would provide a cure for the disease. Two recent murine studies have provided proof of principle for such a strategy by correcting the mutation in hematopoietic stem cells (HSC) using genome editing techniques. With transformative advances being made in the genome editing field, effective and precise manipulation of cellular genomes is becoming highly feasible. Genome editing techniques in combination with stem cell therapy should provide a safe and curative treatment of various genetic diseases such as SCD.

Keywords: Sickle-cell disease (SCD); genome editing; stem cell therapy

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Sickle-cell disease (SCD) was first reported in 1910 by Herrick who described "peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anemia". Substantial knowledge about SCD, including its molecular etiology, has been gained since Herrick's first report (1,2). Adult hemoglobin (HbA), the oxygen-carrying protein of erythrocytes, is comprised of four globin chains: two alpha and two beta chains ($\alpha_2\beta_2$). Globin chain production is controlled by genes located in the beta-globin gene cluster of chromosome 11 and the alpha-globin gene cluster of chromosome 16. SCD is caused by a single nucleotide substitution in the seventh codon of the beta-globin gene, producing hemoglobin S (HbS). Under low oxygen conditions, HbS polymerization deforms erythrocytes, obstructs small blood vessels, and impairs oxygen delivery to tissues. These vasoocclusive events are acute and painful crises which cause anemia, functional asplenia and irreversible organ damage. Approximately 275,000 children are born each year worldwide with SCD, their average lifespan ranges from 36 to 40 years of age in high-income countries, while most children die before age 5 in low-income countries. Currently, the two most widely available disease-modifying therapies are hydroxyurea and long-term transfusions (2). The only available cure for SCD is an allogenic hematopoietic stem cell transplant (HSCT) (3). Due to the scarcity of fully-matched donors, many patients receive mismatched transplants and are at risk for graft rejection or graft-versus-host-disease. Current advances in gene therapy are paving the path for overcoming these obstacles.

Curative treatment of SCD, rather than palliative treatment, must correct the inherited genetic defect. Previous therapeutic approaches for genetic disorders like SCD have included transplantation to replace hematopoietic stem cells (HSCs) or gene therapy using viral vectors to express normal copies of the affected gene in hematopoietic cells. However, early efforts were met with many obstacles due to unstable gene expression, transplant-related toxicities, and leukemia transformation induced

by the integration of viral vectors into the host genome. Although great progress has been made in addressing these issues, safety, durability, and cost-effectiveness remain a major concern (2,3). Various genome editing techniques have emerged in recent years, which can effectively and precisely manipulate cellular genomes and correct mutations without the use of viral vectors. These techniques take advantage of nucleases that can target, cleave, and repair specific genomic sequences at the sites of inherited disease-generating mutations. The nuclease systems include zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the Clustered Regularly Interspaced Short Palindrome Repeats and associated Cas proteins (CRISPR/Cas9).

Recently, Hoban *et al*. engineered a ZFN that specifically cleaves the sickle mutation at the beta-globin locus (4). By co-delivering a homologous donor template, they obtained high levels of gene modification in $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) from SCD patients. More importantly, the modified $CD34^+$ cells were able to produce wild-type hemoglobin tetramers both *in vitro* and *in vivo* when engrafted into immune-deficient NOD.Cg-PrkdSCIDIl2rgtm1Wjil/SzJ (NSG) mice. When expressed in cells, ZFNs cleave DNA in a sequence-specific manner and lead to double-strand breaks. The resulting double-strand break can then be repaired by one of two major cellular DNA repair mechanisms: non-homologous end joining (NHEJ) or homology-directed repair (HDR). When the ZFN is co-delivered alongside a homologous donor template containing the corrective base [either an integrase-defective lentiviral vector (IDLV) or a DNA oligonucleotide (oligo)], the cell may perform HDR to repair the break and permanently correct the cell's genome. Insertion and deletion errors are introduced when the break is repaired by NHEJ. Ideally, HSCs must be transduced to provide long-lasting therapeutic benefits to patients with SCD; committed progenitors and mature cells must be replenished by HSCs and lack the proliferative capacity to reconstitute and sustain the hematopoietic system (5). Unfortunately, slow cycling HSCs (G_0/G_1) are more likely to utilize the NHEJ pathway while committed progenitors and more mature cells (S/G_2) are more likely to utilize the HDR pathway (6). Factors imperative to achieving clinical relevance include ZFN specificity for cleaving the beta-globin gene with minimum off-target cleavage, maximization of HDR in HSCs, sufficient levels of gene correction *in vitro*, colony-forming ability, multipotent lineage, engraftment and long-term maintenance of gene correction *in vivo*.

In their study, Hoban *et al*. employed electroporation to introduce *in vitro* transcribed mRNA encoding the ZFN and either an IDLV or oligo donor (4). $CD34^+$ HSPCs obtained from SCD patients were treated with ZFN and IDLV containing the correction for the sickle mutation. The modified $CD34^+$ cells were able to differentiate into erythroid, myeloid, and lymphoid cell types *in vivo* when engrafted into immune-deficient NSG mice. Genomic analysis showed gene correction of the SCD mutation in 18% of the reads. Analysis of the globin tetramers by high pressure liquid chromatography revealed an average protein correction level of 5.3%, demonstrating the ability to correct $CD34^+$ cells from the bone marrow of SCD patients. Although gene modification levels are somewhat low, it may be clinically significant (7,8). Patients with beta-thalassemia are transfusion independent with chimerism levels as low as 20-30% (7). Based on data from SCD patients who received allogenic HSCTs, chimerism levels as low as 10% are effective for treating SCD. A recent study of patients with mixed chimerism following HSCT found significantly high proportions of donor-derived erythrocytes despite low levels of donor-derived HSPCs (8). The enrichment of donor erythrocytes is believed to be an *in vivo* selective advantage that is not completely understood. Furthermore, patients heterozygous for the SCD mutation generally lack clinical manifestation of the disease, suggesting that correction of one allele per HSC would provide significant relief. Finally, while the ZFN was specifically designed to target the beta-globin gene, off-target cleavage was revealed at the highly homologous delta-globin gene. Although the delta-globin gene is considered dispensable, the impact of this off-target cleavage needs further investigation.

Although the study by Hoban *et al*. provided the groundwork for a potential therapy to treat SCD by using site-specific gene correction with ZFNs in HSPCs, efficiency and specificity of the technique need further improvement. Among various genome editing techniques, the CRISPR/Cas9 system has significantly advanced in recent years and is attracting much attention (9). The system offers highly flexible genome editing with striking efficiency. Cas9 requires extensive homology for cleavage to occur and could potentially minimize off-target cleavage.

By using the CRISPR technique, Canver *et al*. approached a curative treatment of SCD disease from a different angle than Hoban *et al.* (10). Canver *et al.* focused on a stretch of enhancer DNA that controls the molecular switch BCL11A. BCL11A determines whether a red blood

cell produces the adult form of hemoglobin HbA ($\alpha_2\beta_2$) or the fetal form HbF ($\alpha_2\gamma_2$). HbF is the main oxygen transport protein found in the developing human fetus and persists in the newborn for roughly 6 months. Normally, production of HbF is switched off in the newborn and HbA is switched on. In children with SCD, HbA is replaced by HbS due to a mutation of the betta globin chain. Symptoms associated with SCD are relieved if HbF remains the predominant form of hemoglobin well after birth. In fact, hydroxyurea is effective in treating SCD at least partly by promoting the production of HbF (11). To bring back the expression of HbF, Canver and colleagues used the CRISPR-based genome editing technique to systematically cut out small sections of DNA along the entire length of the enhancer in human HSCs. The cells were then allowed to mature into red blood cells and the amount of HbF produced was determined. This led to the identification of a specific location in the enhancer that when cut results in production of high levels of HbF in adult cells. These data provide proof of principle that targeted editing of BCL11A enhancer in HSCs could be an attractive approach for curing SCD and related conditions. However, fixing the sickle mutation in the beta chain may be a more straightforward approach to curing SCD, but this was found to be more challenging technically.

Advances in genome editing techniques continue to provide researchers with an increasing ability to manipulate gene expression, making hematologic disorders ideal targets for therapeutic development. Foremost, HSPCs can be readily obtained from patients, easily manipulated *in vitro*, and then safely transfused back to patients. Furthermore, existing treatment approaches have shown that only partial restoration of normal gene function is required to significantly ameliorate the clinical manifestation of many hematologic diseases. Genome editing techniques in combination with stem cell therapy are paving the path for a safe and curative treatment of various monogenic diseases. Patients afflicted with hemoglobinopathies including SCD, beta-thalassemia, and coagulation disorders such as hemophilia may be the first to benefit from such development.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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