SICKLE CELL DISEASE

Selection-free genome editing of the sickle mutation in human adult hematopoietic stem/progenitor cells

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Genetic diseases of blood cells are prime candidates for treatment through ex vivo gene editing of CD34$^+$ hematopoietic stem/progenitor cells (HSPCs), and a variety of technologies have been proposed to treat these disorders. Sickle cell disease (SCD) is a recessive genetic disorder caused by a single-nucleotide polymorphism in the β-globin gene (*HBB*). Sickle hemoglobin damages erythrocytes, causing vaso-occlusion, severe pain, progressive organ damage, and premature death. We optimize design and delivery parameters of a ribonucleoprotein (RNP) complex comprising Cas9 protein and unmodified single guide RNA, together with a single-stranded DNA oligonucleotide donor (ssODN), to enable efficient replacement of the SCD mutation in human HSPCs. Corrected HSPCs from SCD patients produced less sickle hemoglobin RNA and protein and correspondingly increased wild-type hemoglobin when differentiated into erythroblasts. When engrafted in immunocompromised mice, ex vivo treated human HSPCs maintain SCD gene edits throughout 16 weeks at a level likely to have clinical benefit. These results demonstrate that an accessible approach combining Cas9 RNP with an ssODN can mediate efficient HSPC genome editing, enables investigator-led exploration of gene editing reagents in primary hematopoietic stem cells, and suggests a path toward the development of new gene editing treatments for SCD and other hematopoietic diseases.

INTRODUCTION

Sickle cell disease (SCD) is a recessive genetic disorder that affects at least 90,000 predominantly African-American individuals in the United States and hundreds of thousands worldwide (*1*, *2*). The genetics and molecular basis of SCD have been understood for nearly 70 years, but curative treatments have lagged (*3*, *4*). SCD is caused by a single-nucleotide polymorphism (SNP) in the seventh codon of the gene for β-globin (*HBB*), one of two globins that make up the major adult form of hemoglobin. The resulting glutamate-to-valine substitution renders hemoglobin prone to polymerization under hypoxic conditions, producing characteristic "sickle"-shaped red blood cells (RBCs). Sickle RBCs have a markedly reduced life span in the bloodstream, damage the vasculature, and cause vaso-occlusion. Major clinical manifestations of SCD are chronic anemia, severe pain episodes, and progressive damage to vital organs such as the brain, lung, and kidney. In the United States, the disease causes a 30-year decrement in life span and a greatly diminished quality of life (*2*, *5*–*7*).

RBCs are produced from repopulating hematopoietic stem cells (HSCs) in the bone marrow (BM), and allogeneic hematopoietic cell transplantation (HCT) from an unaffected human lymphocyte antigen (HLA)–matched donor is currently the only lasting cure for SCD (*8*). However, HCT has been used sparingly because of the difficulty in identifying donors, the risks associated with the toxicity of the transplant regimen (requiring preparation with chemotherapy and immune suppression), and potentially fatal graft-versus-host disease (*9*, *10*). Recent transplant advances have reduced these risks in children (*11*) and have extended treatment to selected adults (*12*) and individuals for whom only a haploidentical HLA donor is available (*13*). Still, the vast majority of individuals with SCD do not pursue allogeneic HCT because of an unfavorable risk-reward profile, especially during early childhood. A curative treatment for SCD that can be safely applied to more people remains an urgent need.

Gene editing has recently emerged as a promising avenue to treat genetic diseases affecting hematopoietic cells (*14*–*16*). Ex vivo editing of autologous hematopoietic stem/progenitor cells (HSPCs) would be followed by reimplantation of edited cells, bypassing donor requirements and eliminating the risk of graft-versus-host disease and postgrafting immunosuppression. Because sickle RBCs have a markedly shorter life span in circulation compared to wild-type (WT) RBCs, even low levels of genotypic correction are predicted to generate a clinical benefit (*17*). Observations in patients after allogeneic HCT suggest that clinical improvement may occur when as few as 2 to 5% of long-term engrafted cells carry a normal *HBB* allele (*18*–*20*). An ideal gene editing treatment would exceed this modest target, but to date, even this level of gene editing has not been achieved (*14*, *21*).

During gene editing, a targeted nuclease creates a double-strand break (DSB) that can be repaired by one of two mechanisms: error-prone nonhomologous end joining (NHEJ) that results in genomic insertions and deletions (indels), or templated homology-directed repair (HDR) to precisely insert, delete, or replace a genomic sequence (*22*). The recent development of CRISPR-Cas9, a programmable RNA-targeted DNA endonuclease, has ignited an explosion of interest in gene editing to cure many genetic disorders, including SCD (*23*, *24*). Guided by a single guide RNA (sgRNA), the Cas9 nuclease can be programmed to cut a target locus within the genome, allowing rapid iteration and optimization not possible with other gene editing approaches (*23*, *25*).

Optimized methods for efficient ex vivo gene editing of human HSPCs are required to enable a CRISPR/Cas9-based treatment for blood disorders such as SCD. Recent work has demonstrated that Cas9 can be used for

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