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Gene therapy for sickle cell disease: An update

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Abstract

Sickle cell disease (SCD) is one of the most common life-threatening monogenic diseases affecting millions of people worldwide. Allogenic hematopietic stem cell transplantation is the only known cure for the disease with high success rates, but the limited availability of matched sibling donors and the high risk of transplantation-related side effects force the scientific community to envision additional therapies. *Ex vivo* gene therapy through globin gene addition has been investigated extensively and is currently being tested in clinical trials that have begun reporting encouraging data. Recent improvements in our understanding of the molecular pathways controlling mammalian erythropoiesis and globin switching offer new and exciting therapeutic options. Rapid and substantial advances in genome engineering tools, particularly CRISPR/Cas9, have raised the possibility of genetic correction in induced pluripotent stem cells as well as patient-derived hematopoietic stem and progenitor cells. However, these techniques are still in their infancy, and safety/efficacy issues remain that must be addressed before translating these promising techniques into clinical practice.

Key Words: *BCL11A, fetal globin, gene addition, genome engineering, sickle cell anemia*

Introduction

Sickle cell disease (SCD) is a severe hereditary form of anemia that results from a single mutation at the sixth codon of the β-globin chain (from glutamic acid to valine) of the adult hemoglobin (Hb) tetramer ($\alpha_2\beta_2$) [1], which is prone to polymerization at low oxygen levels. It is one of the most prevalent and severe monogenetic disorders, and more than 100 000 individuals in the United States and several million around the world are affected by both acute and chronic manifestations of SCD, such as frequent pain crises, silent cerebral infarct, stroke, end organ damage and early death [2]. Polymerized sickle hemoglobin (HbS, $\alpha_2\beta^S{}_2$) interferes with red blood cell biconcave architecture and flexibility, resulting in crescent-shaped cells with enhanced adherence to the vascular endothelium, and hemolysis, which obstructs blood flow [3]. More detailed reviews on the pathophysiology [4] and genetics [5] of SCD are available in the literature for further reading.

Since its original description more than a century ago [6], treatments that only reduce the symptoms and complications of SCD such as blood transfusions, preventive therapies including penicillin prophylaxis and pneumococcal vaccination, and hydroxyurea therapy have been leveraged in the clinics. Blood transfusion, however, does not correct the phenotype and results in iron overload when not accompanied by aggressive chelation therapy. Hydroxyurea treatment provides clinical benefit through the induction of fetal globin (HbF, $\alpha_2\gamma_2$), which competes with sickle globin; thus, it reduces SCD symptoms, but response to hydroxyurea is not uniform among patients, and concerns for long-term use remain despite abundant evidence for safety [4]. On the other hand, substantial advances in cellular and molecular biology have led to some powerful tools that we have begun to employ. Of those, allogenic hematopoietic stem cell (HSC) transplantation is as of yet the only available curative option for patients with severe disease [7]. Despite the considerably high success rate of HSC transplantation, a significant proportion of the candidates (>80%) do not have a suitable matched sibling donor, and there remains a risk for graft rejection, graft-versus-host disease and transplant-related mortality [8–11]. Improvements have been made with reduced intensity condition; however, this approach also remains limited by donor availability [9].

For those lacking a suitable allogeneic HSC donor, genetic strategies targeting autologous HSCs remains an alternative (Figure 1). In theory, because genetically modified therapeutic cells are of patient origin, the risk for graft-versus-host disease and transplant rejection can be virtually eliminated, abrogating the need for immunosuppression as part of the

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conditioning regimen. Primary results obtained from clinical trials with genetically modified autologous HSCs expressing potential therapeutic genes for immunodeficiency disorders [12–21] have encouraged a focus in blood-related diseases. The globin disorders, although long held as a therapeutic target, have proven much more difficult because of the necessity of regulated, lineage-specific, high-level globin expression. In this review, general gene therapy approaches for SCD including gene addition and genome editing technologies for decreasing SCD symptoms by enhancing HbF or correcting the mutation in the β-globin sequence are outlined.

Stable gene addition with lentiviral vectors

HSCs are limited in the human body, and prolonged cultivation of HCSs in *in vitro* conditions changes the potential of stem cells. Therefore, determining efficient gene transfer systems providing stable expression of a target gene at therapeutic levels without leading to any safety concerns after modification, such as an immunogenic response or oncogenesis due to random insertional mutagenesis, is one of the main hurdles to clinical application. After the first gene therapy trial using a γ-retroviral vector to transduce mobilized $CD34^+$ cells for the treatment of severe combined immunodeficiency (SCID) was reported [22], the potential of these viral vectors has been widely investigated for various hematological disorders. However, the use of the γ-retroviral vectors in clinical trials was marred because they are not capable of transducing non-dividing cells, including HCSs in quiescent state; cannot carry large gene sets such as β-globin and its regulatory elements required for high-level expression; and present relatively instable RNA to be reverse transcribed and delivered to the nucleus [23,24]. Most important, treatment with γ-retroviral vector-transduced HSCs in various disorders led to leukemia or myelodysplasia, which was attributed to vector insertion near proto-oncogenes, activated due to enhancer sequences in the retroviral long terminal repeats [25]. The development of human immunodeficiency virus type 1 (HIV-1)-derived vectors, belonging to the lentivirus family, circumvent these safety and efficacy issues. Apart from transducing non-dividing cells and transferring large sequences, they have displayed a safe profile without any sign of insertional oncogenesis or mutagenesis in SCD and *β*-thalassemia patients for 4 to 30 months [26,27]. The self-inactivating design in the U3 region of the 3′ long terminal repeat region, removing accessory genes, and separating packaging components, have made lentiviral vectors a relatively safer option, reducing the possibility of insertional oncogenesis, genotoxicity, and the development of replication competent counterparts [28]. Although lentiviral vectors have the confidence of the scientific community, recent work suggests that foamy viral vectors might also provide a safe option [29,30].

β-globin gene addition

The initial idea of inhibiting HbS polymerization in gene therapy applications emerged with the introduction of a functional β-globin transgene into HSCs. However, efficient and erythroid-specific expression of transgene in reconstituting HSCs remained insufficient in *in vivo* studies [31,32]. Incorporation of the human β-globin locus control region (LCR)

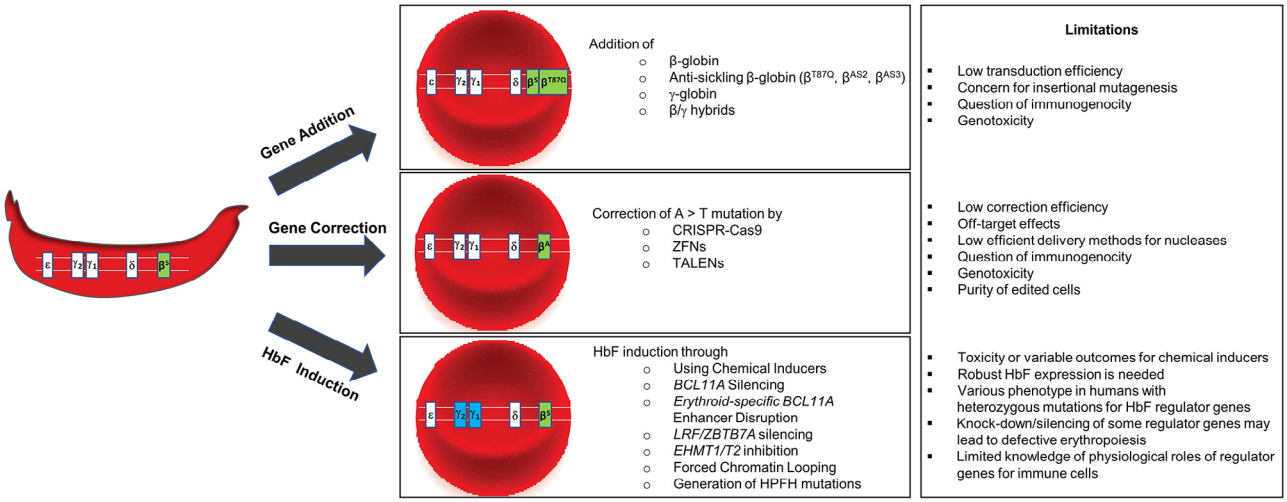

Figure 1. Genetic strategies for sickle cell disease. Anti-sickling protein coding gene addition, fetal globin induction via knocking-down/silencing of repressors of γ-globin gene, and sickle mutation correction with genome engineering tools, particularly CRISPR/Cas9, are the main genetic approaches for sickle cell disease. However, low efficient gene transfer methods, editing rates and safety issues are critical issues that needs to be addressed before starting clinical trials.