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REVIEW

Nucleic Acid Therapy for β-Thalassemia

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Abstract: β-thalassemia is caused by mutations in the β-globin gene which diminishes or abolishes β-globin chain production. This reduction causes an imbalance of the α/β-globin chain ratio and contributes to the pathogenesis of the disease. Several approaches to reduce the imbalance of the α/β ratio using several nucleic acid-based technologies such as RNAi, lentiviral mediated gene therapy, splice switching oligonucleotides (SSOs) and gene editing technology have been investigated extensively. These approaches aim to reduce excess free α-globin, either by reducing the α-globin chain, restoring β-globin expression and reactivating γ-globin expression, leading a reduced disease severity, treatment necessity, treatment interval, and disease complications, thus, increasing the life quality of the patients and alleviating economic burden. Therefore, nucleic acid-based therapy might become a potential targeted therapy for β-thalassemia.

Keywords: RNAi, splice switching oligonucleotides, gene editing, targeted therapy, good health and well-being

Introduction

Genetic disease or DNA mutation is one of the major factors for developing diseases. β-thalassemia is one of the most common monogenic disorders found in the Mediterranean, China, Africa, East Asia and Southeast Asia, including Thailand and Indonesia. Approximately 5 to 10% of the world population comprise patients or carriers of thalassemia.[1] This disease is caused by mutations in the β-globin gene and causes impaired β-globin chain production.[2] Reduced or absent β-globin causes the excess free α-globin chain that can precipitate on the cell membrane, leading to the death of both erythroblast and erythrocytes resulting in anemia.[3] As a response to chronic anemic, the body produces erythropoietin (Epo) and induces intramedullary and extramedullary erythropoiesis.[4] Furthermore, blood transfusion and increasing iron absorption from the gastrointestinal tract contribute to iron deposition in many organs leading to organ dysfunction causing shorter life expectancy, lower quality of life, and health-economic burdens.[5,6]

In β-thalassemia, the underlying mechanism is a single point mutation or small deletion in the β-globin gene, so possibilities to target specific mutations might bring benefit for thalassemia therapy.[7] The major aim of the therapy is to achieve a balanced α/β ratio, thereby reducing the excess free α-chain. This review elaborates several nucleic acid based-strategies that might bring benefit as precision medicine for β-thalassemia, including reduced α-globin expression; restoration of β-globin expression by replacing the defective gene, modulating β-globin pre-mRNA splicing and correcting mutations using gene editing technology; and reactivating γ-globin expression (Figure 1).

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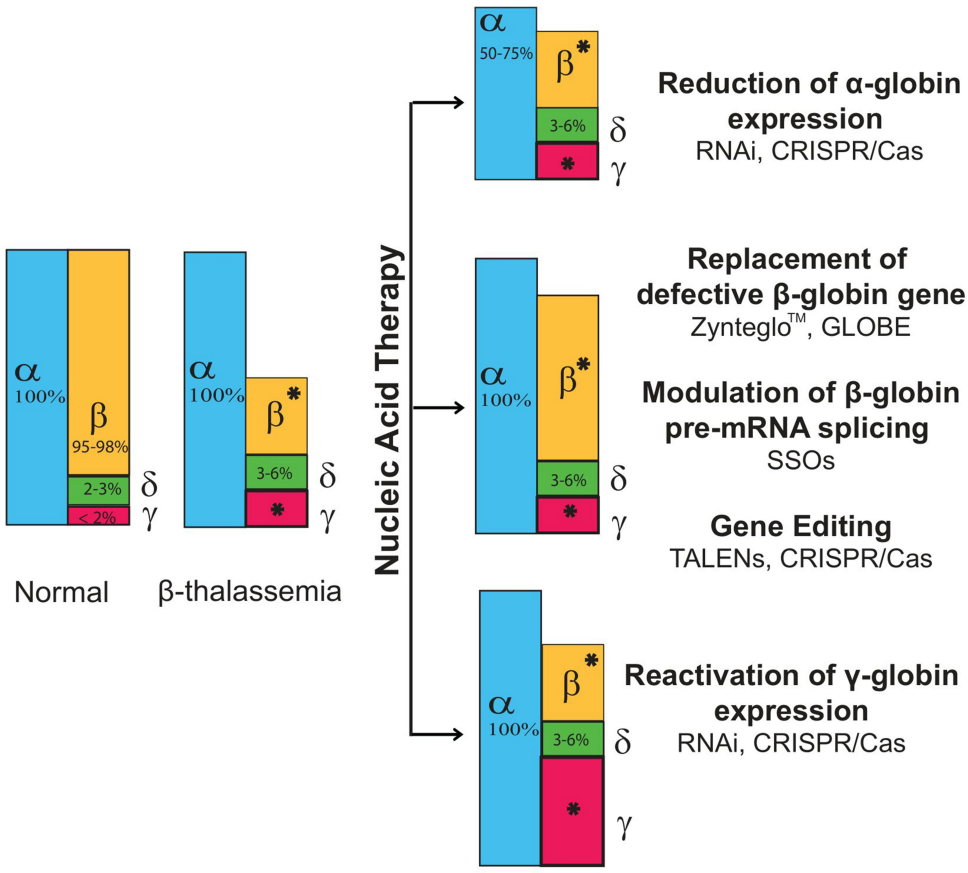

Figure 1 Nucleic acid-based targeted therapy for β-thalassemia Pathophysiology of β-thalassemia involves reducing or abolishing the β-globin chain, causing an excess of free α-globin. Therefore, three strategies can be employed to reduce the imbalance of α/β globin ratio: reducing α-globin expression, restoring β-globin expression and reactivating γ-globin. Several technologies can be used to achieve the aim, such as RNA interference (RNAi) to degrade the α-globin mRNA or to inhibit BCL11A to reactivate γ-globin production. Delivery of a normal β-globin gene to replace a defective gene is possible using the lentiviral vector. Moreover, modulating β-globin pre-mRNA splicing by splice switching oligonucleotides (SSOs) might reduce the possibilities of β-globin overexpression. Gene editing technologies such as Zinc Finger Nucleases (ZFNs), Transcription activator-like effector nucleases (TALENs) and Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein (CRISPR/Cas) has the ability to restore β-globin expression by correcting the mutation, to reduce α-globin expression by deleting specific α-globin enhancers, such as MCS-R2 and to induce HbF production by disrupting BCL11A, MYB or KLF1 genes. *Level of β-globin and γ-globin varies with the type of mutation.
Notes: Adapted from Fucharoen.[8]

Methods

An electronic search of medical literature regarding β-thalassemia, nucleic acid therapy, RNA interference, splice switching oligonucleotides, lentiviral mediated gene therapy, gene editing, ZNF, TALENs and CRISPR/Cas was made on PubMed and Embase. The studies were reviewed and summarized.

Nucleic Acid Therapy for β-Thalassemia

Reduction of α-Globin Chain Expression

The pathology of β-thalassemia is caused by an excess α-globin chain which precipitates in the membrane of erythrocytes and erythroid precursors. In the clinical setting, co-inheritance of α-thalassemia ameliorates the β-thalassemia phenotype by reducing the free α-globin chain.[9] Similar finding also found in the murine model of thalassemia.[10] To achieve a balanced α/β-globin ratio, 25 to 50% of α-globin mRNA expression should be reduced, without changes in β-globin mRNA expression.[11] Currently, two nucleic acid-based approaches have been explored to reduce the α-globin gene expression including post-transcriptional silencing through RNA interference (RNAi)[12–15] and genome editing to reduce the α-globin gene expression.[16]

The first technology is RNAi which regulates gene expression by mRNA degradation and inhibits its translation to protein.[17] The mechanism of this system has been reviewed extensively. Briefly, a small double-strand RNA (dsRNA) such

as small interfering RNA (siRNA) or short single-stranded RNA such as microRNA (miRNA) makes a complex with RNA-induced silencing complex (RISC) and further binds with the complementary mRNA. The mRNA will be cleaved with the help of RNAse to suppress the gene expression.[11,18] Use of this technique to reduce α-globin mRNA expression has been investigated comprehensively during the last decades. One in vitro study in murine erythroleukemic (MEL) cells and murine erythroid progenitor cells demonstrated that siRNA targeting α-globin mRNA reduced α-globin mRNA expression and protein production 50 to 65%. Moreover, the reduced α-globin chain led to declining reactive oxygen species (ROS) levels in thalassemic heterozygous β-KO erythroid progenitor cells.[12] A similar result was found in human erythroid progenitor cells, resulting in reduced α-globin mRNA ranging from 30 to 50% with no effect on β-globin chain mRNA expression. The siRNA also reduced the number of hemoglobinized cells in erythroid colony-forming cells (ECFCs).[13] Moreover, an in vivo experiment in Hbb^{th-4} mice, in which the murine adult β globin gene was replaced by the human $\beta^{IVS2-654}$-globin gene, showed that intravenous injection of siRNA targeting α-globin sequence and antisense oligonucleotides targeting β-globin sequence improved red cell pathology, such as decreasing the nucleated cells in the bone marrow, increasing reticulocytes number[15] and increasing the survival rate of homozygous Hbb^{th-4}/Hbb^{th-4} and heterozygous Hbb/Hbb^{th-4}.[14]

Another approach to reduce α-globin expression is using gene editing technologies that are able to insert, delete, disrupt or replace target DNA sequences.[19] A study used CRISPR/Cas9 to delete the MCS-R2 α-globin enhancer, which is one of the regulatory elements controlling the expression of α-globin RNA. With 70% deletion efficiency, the deletion of MCS-R2 demonstrated reduced α-globin mRNA expression and restored α/β-globin chain balance in patient-derived hematopoietic stem cells (HSCs) in vitro. Xenograft assay confirmed the editing of long term hematopoietic stem cells (LT-HSCs) because the deletion of MCS-R2 can be found in the primary and secondary transplantation.[16]

Thus, downregulating α-globin gene expression might bring benefits for β-thalassemia treatment. However, this approach has limitations such as the stability and the ability of siRNA to inhibit α-globin expression continuously. The editing efficiency and off-target of the CRISPR/Cas system are also crucial issues in the gene editing field. Moreover, the number of silenced α-globin expressions and no effect on β-globin expression are essential because the balance of α/β-globin ratio is the goal of this strategy. Even though this approach has not been implemented in the clinical setting, reduced α-globin chain expression using nucleic acid-based technology is a potential strategy as an alternative therapy for β-thalassemia.

Table 1 Lentiviral Vectors Used in Clinical Trials for β-Thalassemia

	HPV569	BB305	GLOBE
Promoter	U3	CMV	U3
LCR	HS2, HS3, HS4	HS2, HS3, HS4	HS2, HS3
β-globin gene	Whole β-globin gene, codon 87 mutation for anti-sickling properties	Whole β-globin gene, codon 87 mutation for anti-sickling properties	Mini β-globin gene, 257 bp of β-globin intron 2
β-globin promoter	+	+	+
β-globin enhancer	+	+	-
Insulator	Chicken HS4 insulator core	-	-
Ref	20	21	24

Abbreviations: HS, hypersensitive sites; LCR, locus control region; CMV, cytomegalovirus.

Restoration of β-Globin Gene Expression

Another approach to balancing the α/β-globin ratio is by restoring β-globin gene expression. Different from the last approach, reducing α-globin, this strategy works by increasing β-globin gene expression. Several methods are available to restore β-globin gene expression, such as replacing defective genes, modulating β-globin pre-mRNA splicing, and correcting mutations using gene editing technology which will be elaborated in this section.

Replacing the Defective β-Globin Gene

The gene therapy, using own patient hematopoietic stem cells carrying the correct β-globin gene, has been introduced to replace the defective gene. Starting with one patient with HbE/β-thalassemia, allogeneic stem cells were taken from the patient and transduced with the lentivirus carrying the correct β-globin gene and transplanted back to the patient. Several vectors have been used to deliver the correct β-globin gene (Table 1). The first was the $\beta^{A(T87Q)}$ vector, later known as HPV569 carrying U3 promoter/enhancer for

lentivirus expression, with the whole β-globin gene with a mutation at codon 87 for its anti-sickling properties. This vector also carried β-globin promoter; HS2, HS3, and HS4 of LCR; and two copies of the chicken HS4 insulator core.[20] A patient with transfusion-dependent β-thalassemia with HbE/β^0-thalassemia genotype became transfusion independent one year after gene therapy and total Hb maintained between 9 to 10 g/dL, with 3.7 g/dL of the therapeutic HbA^{T87Q}. A partial clonal dominance upon vector integration within the high mobility group AT hook 2 (HMGA2) gene was observed and declined at 12 years after gene therapy.[20] However, due to the specific clonal expansion during the first study, some improvements were made to this vector to increase safety in clinical application. Its U3 promoter/enhancer was changed to CMV promoter/enhancer, and chicken HS4 insulator core was removed, creating the new vector, BB305 or Zynteglo™.[21] Moreover, the first phase of the clinical trials among 22 patients was performed to investigate the safety and the efficiency of this vector. Twelve of 13 patients with non β^0/β^0-thalassemia became transfusion-independent, while only three patients with β^0/β^0-thalassemia became transfusion-independent and another six still received blood transfusion with decreasing annual transfusion volumes. Importantly, no clonal dominance was observed among all patients.[22] The latest vector has been approved by the European Medicines Agency (EMA) in 2020.[23]

The second vector which has been used in a clinical trial was the GLOBE vector. This vector carried minimal LCR, namely, 2.7 kb fragment of HS2 and HS3 with no effect on β-globin expression, and the mini β-globin gene, including the β-globin promoter and absence of β-globin enhancer. Primary and secondary transplantation of GLOBE vector transduced cells resulted in increased hemoglobin level and improved clinical features in thalassemic mice.[24] Furthermore, clinical trials among three adults and six pediatric patients with homozygous β-thalassemia showed improved hematological parameters and disease pathology. The reduced transfusion interval and transfusion-dependence was achieved after treatment. Most pediatric patients with β^0/β^0-thalassemia have achieved transfusion-independence, while adult patients have reduced transfusion requirements.[25,26]

This breakthrough therapy gives hope to patients with thalassemia for a higher quality of life, even though the cost-benefit study for this approach still needs to be investigated. A clinical trial with a broad range of patients is needed before being clinically implemented and finding better alternatives and exploring safer approaches are always options. The limitation of this strategy is the overexpression of the β-globin gene which might lead to excess free β-globin chain.

Modulating β-Globin Pre-mRNA Splicing

One of the important molecular mechanisms of the defect in β-globin gene expression is mutation-induced aberrant splicing, which activates aberrant splice sites and alters the splicing pathways, even though the correct splice sites might function normally, for example, IVS1-5, IVS1-6, and IVS1-110 mutations in intron 1, IVS2-654, IVS2-705 and IVS2-745 mutations in the intron 2 of the β-globin gene.[9] In addition, abnormal hemoglobin such as HbE also has resulted from aberrant splicing.[27] Due to the activation of aberrant splice sites, the RNA is incorrectly spliced and some intron fragment is retained leading to nonfunctional β-globin chain production. Thus, this mutation reduces the production of correctly spliced β-globin protein and causes β-thalassemia.

Modulating β-globin pre-mRNA splicing is one potential approach to increase β-globin expression. Short synthetic oligonucleotides (15 to 25 nucleotides) to a specific pre-mRNA sequence might alter the recognition of splice sites by the spliceosome, leading to an alteration of splicing of the targeted transcript and interfering with the ratio of normal protein: RNA or RNA: RNA; therefore, called splice switching oligonucleotides (SSOs) (Figure 2).[28,29] However, a chemical modification of SSOs is necessary to prevent degradation of the pre-mRNA-SSOs complex by RNase H.[30] Modification of the phosphate backbone with phosphorothioate (PS) backbone modifications or the ribose component of the oligonucleotide might improve the stability of the SSOs in vivo and increase the binding affinity of SSOs to the specific sequence and proteins. This interaction further affects the pharmacokinetic and pharmacodynamic factors of SSOs, such as plasma and tissue distribution and cellular uptake, which further increases efficiency and reduces toxicity and immunostimulatory activity.[31] Chemical modification and its effects on SSOs have been reviewed comprehensively.[29,32]

SSOs modulate RNA splicing by redirecting alternative splicing that can be categorized in four mechanisms: exon skipping, exon retention, restoration of correct splicing and displacement of splicing factors from triplets repeats which have been reviewed extensively.[28] The SSOs have been used to correct aberrant splicing in β-thalassemia alleles including the mutations IVS1-110,[34,35] IVS2-

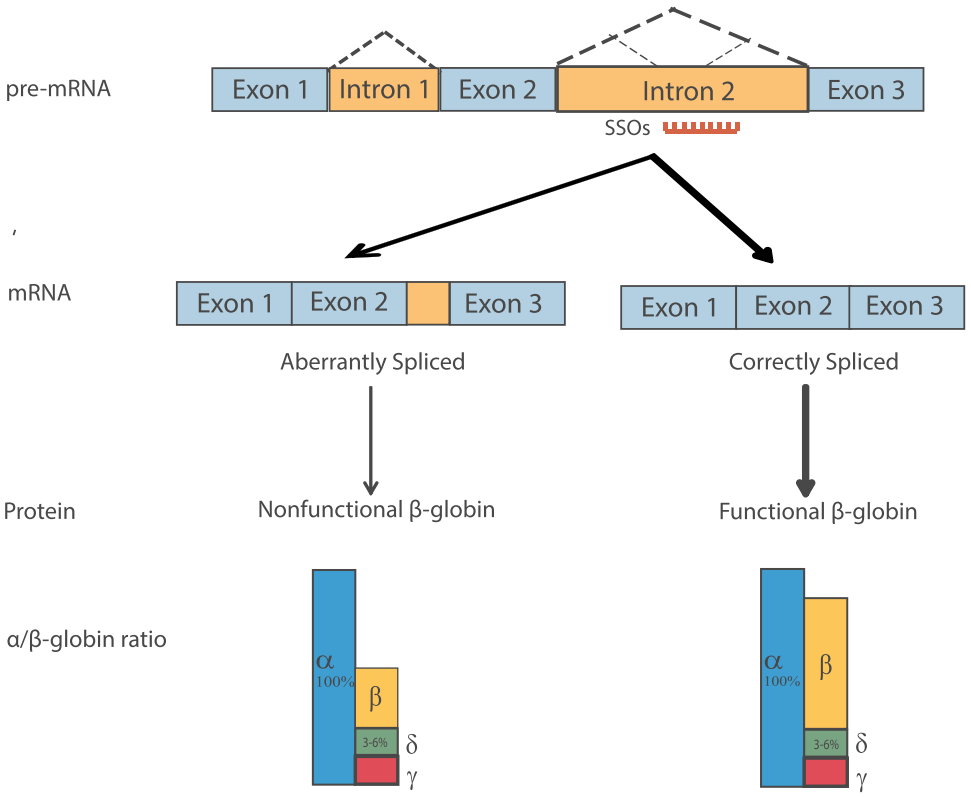

Figure 2 Mechanism of splice switching oligonucleotides (SSOs) Mutation in the intron region may activate aberrant splice sites leading to retaining the intron fragment in the mature mRNA and translated to a nonfunctional β-globin chain. SSOs targeted to the aberrant splicing elements block spliceosome to recognize the pseudo-exon and restore correct splicing which is translated to fully functional β-globin protein. Even though 100% of correct splicing cannot be achieved, the correctly spliced β-globin balances the α/β globin ratio, reduces the excess α-globin and increases the HbA level, thus alleviating the disease pathology.
Notes: Adapted from Svasti et al.[33] Copyright (2009) National Academy of Sciences.

654,[36–38] IVS2-705,[39] IVS2-745,[40] and βE.[36] SSOs successfully induced correct splicing and increased normal β-globin production in cell-free extracts, stable cell lines with a mutated β-globin gene, erythroid mononuclear cells from peripheral blood of patients, thalassemic mouse erythroid progenitors, human iPSCs and β-thalassemic mouse model.[36–38,40,41] Among patients with $\beta^{IVS2-654}$ thalassemia, erythroid cells, free uptake and syringe load of SSOs into cells increased correct β-globin mRNA up to 77% and HbA production up to 54%.[36] Moreover, in the $\beta^{IVS2-654}$-thalassemia mouse model, intravenous injection of SSOs restored 12% of correct β-globin mRNA in mice peripheral blood or increased 6-fold compared with untreated controls. Surprisingly, chimeric mouse and human β-globin were found in 1 to 5% of peripheral blood samples.[33]

However, to maintain the correct splicing, chemical SSOs require life-long and periodic administrations. For that reason, researchers have endeavored to explore vectors that can constantly express the oligonucleotides. Intracellular expression of the antisense sequence is preferable for chronic diseases which can be achieved by the embedding antisense sequence in vectors, such as U7, U1 or U2 small nuclear RNAs (snRNAs).[42] In 1998, a study by Gorman proposed using U7 small nuclear RNA (snRNA) which serves as a mediator of histone pre-mRNA processing as a vector to carry antisense sequence for splicing modulation. Because of its location inside the nucleus, this vector demonstrated stability, expressed at a relatively high level and was quite effective to deliver SSOs.[43] Using this approach, correctly restoring spliced β-globin pre-mRNA in HeLa cells expressing several mutations in the intron 2 β-globin gene could be observed for at least six months.[43–45] However, the correction level depended on the target sequence.[45] Double-target engineered U7 snRNAs produced higher correction in HeLa IVS2-654 cells compared with a single targeted sequence (40 and 3%, respectively). Moreover, correctly spliced β-globin mRNA could be translated to hemoglobin A synthesis, improving the thalassemic erythroid cell pathology of HbE/$\beta^{IVS2-654}$-thalassemia erythroid progenitor cells.[37] The latest construct could restore approximately

80% of correctly spliced $\beta^{IVS2-654}$- thalassemic human iPSCs.[38] Not only in β-thalassemia, but the modified U7 snRNAs has also been extensively explored in several RNA mis-splicing diseases including DMD and SMA.[46–52]

Moreover, tagging SSOs in snRNAs improved the nucleus delivery and prevented SSOs from degradation because snRNAs are naturally located and work in the nucleus. Hence, this modification enhances the efficacy of the modulation of the splicing process. Thus, modulating β-globin pre-mRNA splicing might be beneficial in β-thalassemia caused by mutations that create aberrant splicing. Unfortunately, several studies showed a modification of U7 snRNA resulting in two isoforms, full length and truncated, of U7 snRNA that might interfere with the antisenses function to block the splicing machinery in certain animal models.[46,53] A similar finding also found in the erythroid progenitor cells of thalassemic mice (unpublished data). Therefore finding the best model to test the SSOs would prove very important before its further application.

Mutation Correction Using Gene Editing Technologies

Gene editing is technology to modify DNA, including deletion, insertion or correction, of living cells, including human, animal and plant.[54] This technology combines DNA-binding domains and DNA cleavage nucleases, resulting in creating a double-strand break (DSB). Three well-known techniques in this field are ZFNs, TALENs and CRISPR/CRISPR/Cas9).[55]

ZFNs are artificial restriction enzymes consisting of a zinc finger DNA binding domain and FokI DNA cleavage domain. The DNA binding domain or so-called finger recognizes a specific DNA triplet (3 bp), and each ZFN usually contains 3 fingers, and is thus able to recognize 9 base pairs.[56] Since FokI needs dimerization to functionally cleave the DNA, two individual ZFNs are constructed to recognize DNA on the left and right arms of the target sequence with 5–6 bp spacer between two FokI endonucleases. After dimerization of FokI endonuclease, DSB and DNA repair mechanisms occur.[57] Using ZFN technology, reactivation of γ-globin is achieved by inducing indel mutation of the SOX6 binding domain, and γ-globin expression increased up to 6 fold.[58]

Similar to ZFNs, transcription activator-like effector nucleases (TALENs) also constitute an artificial restriction enzyme composed of a TAL effector DNA-binding domain combined with a FokI endonuclease DNA cleavage domain. The TAL effectors contain 33–35 amino acids and at 12–13 amino acids, known as Repeat Variable Diresidue (RVD), can be modified to recognize specific target sequences. Each TALEN usually contains 13–20 TALE repeats, and similar to ZFNs, to work functionally, FokI endonuclease need dimerization. Therefore, two individual TALENs must bind on opposite sides of the target sequence with a spacer of 14–20 nucleotides. With this characterization, TALENs are very specific to recognize as a specific target sequence. Unfortunately, the size of TALENs might limit delivery to the cells.[55–57] In thalassemia, TALENs have been shown to correct $\beta^{IVS2-654}$-thalassemia in vitro in human iPSCs and in vivo in double heterozygous $TALENs^{+}/Hbb^{th-4}$ mice. In these studies, the correction efficiency ranged from 32 to 50%, and the expression of β-globin was comparable to normal controls. Interestingly, no off-target mutation was detected in both studies.[59,60]

Furthermore, the latest gene editing technology is CRISPR/Cas9 which originally found in bacteria as a defensive system from phage.[61,62] Nowadays, with advanced knowledge about CRISPR/Cas9, genome editing becomes a rising star in the field. Editing the diseases caused mutation and introducing the correct donor template have become much more simple and affordable. There are several components for genome editing utilizes CRISPR/Cas9, which are gRNA followed by PAM sequence, Cas9 enzymes, and with or without donor template to promote DNA repair.[63] CRISPR/Cas9 recognizes a specific sequence and creates a double-strand break (DSB) on the DNA becoming effective tools for gene editing.

In general, the two types of donor templates are donor plasmid (dsDNA) and single-stranded oligonucleotides (ssODNs).[64] The donor template carries the correct sequence with downstream and upstream homologous arms. Donor plasmid can carry several base pairs of homologous arms, including selection markers such as drug resistance genes or the EGFP gene. Moreover, it can carry site-specific genetic manipulation tools such as the Cre-lox system and piggyBac transposase which can be removed after homologous recombination occurs.[65–67] In the Cre-lox system, Cre recombinase recognizes 34 bp of loxP resulting in removal sequence between loxP including selection markers. Unfortunately, using this system leaves 34 bp of loxP recognition site on the gene of interest. This remaining sequence is an advantage for the silencing gene of interest but raises a concern for mutation correction.[68] This limitation is overcome by piggyBac,

a donor plasmid providing flexibility to remove any sequence between ITR of piggyBac, including the selection gene, using transposase and providing a perfect corrected gene. For example, the piggyBac donor, carrying the puromycin resistant gene and ΔTK gene for selection has been used to correct a mutation in CD41/42 β-thalassemia iPSCs.[69] With this system, the selection gene can be removed completely and creates seamless correction. Unfortunately, due to its content, the donor plasmid is large and might interfere in its delivery into the cells.

Using ssODNs, which is a single strand donor carry 40–90 bp of upstream and downstream of the mutation site, the delivery rate into the cells might be more effective. However, ssODNs are unable to carry the selection marker; therefore, the selection of the corrected clone might present a challenge. For example, ssODN has been used to correct the mutation in βE and IVS1-110.[70,71] In β-thalassemia, CRISPR/Cas9 has been investigated in several mutations such as 4 bp deletion or CD41/42, −28, IVS2-654, IVS1-110 and βE[59,65,69-73] using patient-derived iPSCs or CD34+ cells. In CD41/42 and −28 mutation, using a piggyBac system to carry homologous arms, the correction efficiency was 23%.[69] In HbE/β-thalassemic patient-derived iPSCs transfected with double expressed plasmid carrying gRNA and Cas9 nuclease and 180 bp of ssODN, HDR efficiency was 2.9%.[71] Moreover, in the IVS1-110 mutation, using CD34+ hematopoietic stem cells, 8% of correction can be achieved.[70] Surprisingly, in the absence of a donor template, CRISPR/Cas9 successfully induced indel mutation in human IVS1-110 mutation. DNA repair occurred via the nonhomologous end joining (NHEJ) pathway resulting in indel mutation (66.2%) and HbA production increase up to 75.6%. A similar result is achieved when using Cas12 and its suitable gRNA in $\beta^{IVS2-654}$-thalassemic erythroid progenitor cells. Without the presence of a donor template, DNA repair occurred via the NHEJ pathway resulting in indel mutation (76.6%) which was sufficient to restore correctly spliced β-globin mRNA (70.1%) and HbA production (9.9–59.1%).[73]

Unfortunately, low correction efficiency becomes a concern in CRISPR/Cas technology; however, using the appropriate form of Cas nucleases such as DNA plasmid, lentiCRISPR, Cas RNA or Cas protein, and selecting the most suitable delivery methods might overcome this problem.[65,74-76] Moreover, small molecules such as β-3 adrenergic receptor agonist or NHEJ inhibitor (RAD51, SCR7) were also found to increase the HDR event.[77] In CD41/42 β-thalassemic patient-specific iPSCs, 54% correction efficiency was achieved when combining CRISPR/Cas, ssODN, and β-3 adrenergic receptor agonist.[72] Taken together, current advances in the gene editing technology for β-thalassemia are remarkable and show a possible future application to correct mutation causing β-thalassemia, hence restoring the normal β-globin chain.

Reactivating γ-Globin Expression

The clinical manifestation of β-thalassemia usually starts in the first year of life, when fetal hemoglobin (HbF) is replaced by adult hemoglobin (HbA) and declining HbF levels occur. HbF is a tetramer consisting of 2 γ-globin chains incorporating 2 α-globin chains.[78] It has a higher oxygen affinity providing advantages for the fetus during pregnancy.[79] In clinical settings, a high level of HbF ameliorates the clinical severity of β-thalassemia such as co-inheritance with the hereditary persistence of fetal hemoglobin (HPFH) or Xmn1-HBG2 polymorphism.[9] The increasing γ-globin chain binds with the excess α-globin chains, improving the balance of globin chains, and lessening the disease pathology.

Recent findings showed three transcription factors; BCL11A, KLF1, and MYB, regulate the γ-globin gene expression by repressing its expression after birth. Several studies have investigated the possibility to downregulate their expression as a treatment for β-thalassemia.[9,80,81] Two powerful tools, RNAi and gene editing technology, are employed to disrupt the BCL11A gene. In vitro study in CD34+ derived erythroid cells demonstrated ability of siRNA to inhibit BCL11A production and successfully increased the HbF levels.[82] Studies using a lineage-specific and miRNA-embedded expression of BCL11A-targeting shRNAs have reported that the shRNAs successfully reduced BCL11A mRNA expression up to 90%, induced 60 to 70% γ-globin mRNA expression, and further increased HbF production in cell cultures[83] and animal models.[84] Unfortunately, one study using RNAi targeting KLF1 gene did not show a reduction of KLF1 mRNA expression.[85] Interestingly, study in murine erythroleukemic (MEL) cell line containing an intact 183-kb human β-globin locus showed that MYB-targeting shRNAs successfully reduced 40–70% of BCL11A and KLF1 mRNA expression, however, the MYB knockdown failed to increase γ-globin mRNA expression.[86]

In addition to correcting mutation causing diseases, gene editing technology, including CRISPR/Cas, ZFNs, and TALENs also can be used to knockdown BCL11A,[87–89] KLF1,[90] and HBG1/2 genomic regions or

promoter[91,95] to derepress γ-globin expression, thereby increase HbF production. With the increasing of HbF, the ratio of α-globin and β-like globin can be restored, thus reducing the excess of free α-globin. In 2019, the first clinical trial of CRISPR/Cas9 to disrupt the BCL11A gene was started to treat β-thalassemia and sickle cell disease.[96] Thus, modulating the expression of γ-globin using nucleic acid-based therapy might offer an alternative therapy for β-thalassemia in the future.

Conclusion and Future Perspectives

As technology continues to advance, disease-caused mutations might become a target for disease treatment, as specific treatments might provide different benefits for different mutations. In β-thalassemia, the aim of the therapy is to balance the α/β-globin ratio which can be achieved by modulating α-, β-, and γ-globin expression. Several approaches have been investigated to achieve this goal using nucleic acid-based therapy, such as RNA interference, virus-mediated gene therapy, and gene editing. However, limitations such as the stability, delivery methods, suitable vectors, and optimum doses of nucleic acid should be carefully considered. By understanding this nucleic acid-based therapy, personalized medicine or targeted therapy for β-thalassemia can be developed and applied in the clinical setting. Nevertheless, the risk of health inequities and its potential misuse raise concerns among health practitioners. Thus its development and application should be tightly regulated and monitored.

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Disclosure

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