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Review

Revisiting Gene Therapy Approaches for Beta-Thalassemia

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Beta-thalassemia (β-thalassemia) is known as a blood disease that occurs as a result of a defect in the synthesis of the beta-globin (β-globin) chains of the Hb molecule. This condition prevents red blood cells (RBCs) from forming normally due to the lack or low levels of β-globin chains. The term thalassemia is derived from a combination of the Greek words “thalassa” (sea) and “haima” (blood).[1,2]

Beta-thalassemia is a genetic disease and follows an autosomal recessive inheritance. It is one of the rare diseases known as a blood disorder, with the Hb molecule being a mutation in the synthesis of β-globin chains. This condition prevents RBCs from forming normally due to the lack or low levels of β-globin chains. Under normal circumstances, the Hb beta (HBB) gene and the Hb alpha (HBA) gene are required for the synthesis of β-globin chains. Beta-thalassemia is caused by mutations in the HBB gene. Depending on the type and number of mutations, patients with β-thalassemia show different disease symptoms. One β-globin gene is inherited from each of the parents, and a person carrying two mutations may suffer from β-thalassemia. The weight of this disorder depends on the type and number of mutations.[3,4]

ABSTRACT

Beta-thalassemia (β-thalassemia) is known as a blood disease that occurs as a result of a defect in the synthesis of the beta-globin (β-globin) chains of the hemoglobin (Hb) molecule. The term thalassemia is derived from a combination of the Greek words "thalassa" (sea) and "haima" (blood). It is a genetic disease and follows autosomal recessive inheritance. One of the rare diseases known as a blood disorder, the Hb molecule is a mutation in the synthesis of β-globin chains. There are three types of β-thalassemia; β-thalassemia major, β-thalassemia minor, and β-thalassemia intermedia. There are hundreds of defined mutations in the Hb beta (HBB) gene. The next generation of treatment on the change of these mutations has led to studies in gene editing. Clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) technology was first introduced in 2012, causing a paradigm shift in the field of genome editing. Engineered and programmable bacterial nucleases enabled genome sequences to be edited. It can target genes in a given deoxyribonucleic acid region and make desired changes. Recent advances in genome sequencing methods and studies in the HBB gene have provided significant clinical benefits in its treatment, supporting new achievements in understanding molecular mechanisms and advances in gene editing technology. This review addresses the various aspects of β-thalassemia, including its genetic basis, classification, and the significance of mutations within the HBB gene. Furthermore, it highlights the transformative impact of CRISPR/Cas9 technology in advancing our understanding of molecular mechanisms and ushering in a new era of gene editing possibilities.

Keywords: Anemia, CRISPR/Cas9, gene therapy, iPSCs, mutations, thalassemia

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There is a balance between alpha (α) and β-globin chain synthesis in healthy individuals. In individuals with β-thalassemia, mutations lead to globin chain synthesis, which increases unstably, and an excessive amount of α chain. Non-binding globin chains cause cellular damage, with RBC predecessors accumulating and collapsing. This progressive painful process leads to defective erythroid maturation, ineffective erythropoiesis, and shortened RBC survival.

Hemoglobin beta mutations, which cause the absence of β-globin production, lead to β 0-thalassemia. Other mutations that disrupt β-globin synthesis to varying degrees are classified as β+ or β++ (silent) thalassemia.[5,6]

The probability of seeing β-thalassemia in the world's population is estimated at one in 100,000 live births. The origin of β-thalassemia is found in the Mediterranean, while major species are found mostly in the Middle East, Southeast Asia, India, and China. It has also been reported that the prevalence of β-thalassemia has increased in countries where malaria is endemic. The distribution of his disease has been made possible by migration. Human migration has contributed to the further spread and settlement of β-thalassemia around the world.[7,8]

TYPES OF BETA-THALASSEMIA

There are three types of β-thalassemia. According to their incidence; β-thalassemia major (TM), β-thalassemia minor (Tm), and β-thalassemia intermedia (TI). Beta-thalassemia major is the heaviest form in which β-globin chains are completely lacking. This condition can cause life-threatening anemia if left untreated. The disease is therefore known as "Mediterranean anemia". Beta-thalassemia is a milder form if small, and symptoms are often mild or non-existent.[9]

a. Thalassemia Major

Commencing in early childhood and known as 'Cooley's anemia,' the condition carries significant and severe repercussions. This form is the heaviest of all. It occurs in children born as a result of the mutated gene from surrogate parents.[10]

Severe anemia, retardation of development, jaundice, Feeding problems, diarrhea, irritability, recurrent bouts of fever, and progressive abdominal growth caused by spleen and liver growth can occur. among the symptoms. If treatment is not put in place effectively in the first place, it can result in heart failure. For treatment, blood transfusions and iron chelation therapy are required regularly. These treatments include regularly giving blood transfusions to replace normal blood cells in the body with those that are missing, and administering iron chelation therapy to prevent iron buildup. A bone marrow transplant could be a treatment option for TM patients. Survival of individuals treated for transfusion and proper chelation (removal of heavy metals) with regular and appropriate intervals extends beyond the age of 40.[10,11]

b. Talasemi Intermedia

Individuals with thalassemia intermedia are diagnosed later than TM. They show milder symptoms than TM. It doesn't require transfusion, or only rarely does. At the severe end of the clinical spectrum, patients are located between ages two and six, and although they can survive without regular blood transfusions, signs of growth and development retardation are dominant. The observed clinical characteristics encompass symptoms such as yellowing of the skin (jaundice), the formation of gallstones (cholelithiasis), enlargement of the liver and spleen, the presence of leg ulcers, the formation of masses in the bone marrow that produce red blood cells, the development of weakened bones with lower mineral density (osteopenia) and increased susceptibility to fractures (osteoporosis), various bone-related disorders, significant and noticeable changes in the skeletal system, and the occurrence of blood clotting issues due to the deposition of excess iron, resulting in a state of heightened blood clotting. Bone marrow transplant remains the only definitive treatment currently available. Research is still underway. Individuals with thalassemia intermedia can undergo splenectomy (spleen removal), folic acid supplementation, extra modules, treatment of erythropoietic masses and leg ulcers, and the treatment of thromboembolic (clot formation) events. [12,13]

c. Thalassemia Minor

Patients are found in carrier condition. Their clinical properties are often asymptomatic. When both parents are carriers, they have a 25% risk of having children with homozygous thalassemia for each pregnancy.[14,15]

Patients with β-thalassemia should be monitored by a hematologist for care and treatment. These doctors consult on managing symptoms of the disease, blood transfusions and other treatment options. β-thalassemia is an inherited disease among families. Therefore, genetic counseling is recommended to families at risk of β-thalassemia. Genetic counseling can help identify disease risk, manage symptoms, and prevent the transmission of the disease to future generations.

GENETIC STRUCTURE OF BETA-THALASSEMIA

The HBB gene spans a length of 1606 base pairs and is composed of three exons. It is located on the

short arm of chromosome 11 (11p15.4). Within the HBB gene, there are regulatory elements such as TATA box, CAAT box, CACCC boxes, and a locus control zone situated at the 5' end. Additionally, this gene is influenced by a neighboring promoter region that contains regulatory elements, contributing to the control of its expression. A number of transcription factors, most notably the erythroid Kruppel-like factor that connects the proximal CACCC box, regulate the function of HBB. It settles towards the 3' end of a 70 kb zone. Beta-like globin is typically encoded by a group of epsilon (ε) globin genes, including γ [gamma (HBE)], Gγ (HBG2), Aγ (HBG1), δ [delta (HBD)], and β (HBB). These genes are found on chromosome 11 (11p 15.15) to make various Hb tetramers such as embryonic Hbs (Hb Gower-1 (ζ2ε2), Hb Gower-2 (α2ε2), and Hb Portland (ζ2β2)), fetal Hb (α2γ2), and adult Hbs (HbA, α2β2 and HbA2, α2δ2). The HBB gene encodes β-globin, a protein that is a fundamental component of normal adult Hb. During childbirth, fetal Aγ and Gγ globin expression are silenced. Once the person becomes an adult the β-globin gene is predominantly expressed; this process is called γβ-Hb transition. The BCL11A responsible for this transition interacts with the γ, SOX6, GATA1, and NuRD complex to suppress the expression of FOG1-globin genes in adult erythroblasts. BCL11A binding regions are mapped in γ-globin promoters and a default 3.5kb HbF silencer. More than 1400 Hb variants, including more than 900 mutation variants in the HBB gene, have been demonstrated by recent studies. The most common β-globin structural variants are hemoglobin E (HbE [β26 Glu>Lys]), sickle hemoglobin (HbS [β6 Glu>Val]), and hemoglobin C (HbC [β6 Glu>Lys]). It is one of the most common β-thalassemia mutations that produce HbE (βE). Beta is an abnormal Hb variant that affects globin pre-mRNA (pre-messenger ribonucleic acid) binding and occurs during the gene's çevrilmesi to the protein as a result of the extraction of introns in pre-mRNA through the consolidation process. βE (CD26) is common among Southeast Asians. HbE/β-thalassemia shows a highly variable phenotype. While many patients may remain transfusion-free throughout their lives, some may start transfusions at an early age.[16-18]

The HBB gene has another mutation β IVS2–654, β IVS2–705 and β IVS2–745 globin in intron 2. Human β-globin (β IVS2-654) 654, a cytosine-thymine mutation in nucleotide has been passed into literature as one of the most common mutations in China and Southeast Asians that causes β-globin pre-mRNA binding β-thalassemia. β (C>G), another abnormal insertion mutation contained in intron 2 of IVS2-745 thalassemia, causes the termination code by blocking β-globin production. Other HBB abnormal splicing mutations are CD19 and CD27 mutations in exon 1, IVS1-5, IVS1-6, and IVS1-110 mutations in intron 1.[19-21]

The mutation causes this disease; includes spot mutations, deletions, and larger gene rearrangements.

Point mutations fall into three different categories:

1. Promoter and 5 'UTR regions contained in mutations leading to beta gene transcription,

2. Mutations affecting mRNA processing strain splice-junction and consensus mutations, polyadenylation, and other 3'UTR mutations,

3. Includes nonsense, frameshift, and initiation codon mutations that result in faulty mRNA translation.[1]

GENE EDITING AND BETA-THALASSEMIA TREATMENT

Engineered and programmable bacterial nucleases have enabled the editing of genome sequences. Several gene editing tools are available. Firstly, the zinc finger nucleases (ZFNs); are used to intercept the specific 3-4 bp deoxyribonucleic acid (DNA) array region of the genome by targeting it. In this method, SekI is a group of engineered and chimeric nucleases developed to combine a bacterial endonuclease (a double-stranded DNA nickname) with DNA-binding zinc finger fields.[22]

Transcription activator-like effector nucleases (TALENs), non-specific nucleases FokI linked to the TALE region for the DNA recognition zone, are artificial proteins that contain a division area. TALEN activity is associated with two DNA binding sites surrounding an unallocated 12-20 bp spacing sequence.[23]

TALENs are commonly used protein-based genome engineering tools. In terms of design and cost, it is a very powerful tool with ZFN. Repeated studies should be carried out to test the cutting efficiency of TALENs. Moreover, DNA methylation and histone acetylation may have an impact on their effectiveness. Clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9), on the other hand, is not limited to these restrictions, it is practical and easy to manufacture. The main goals of gene therapy are to transfer a healthy copy of HBB or recreate γ-globin expression and therefore HbF.[24,25]

The CRISPR/Cas9 technology was first introduced in 2012 and has resulted in a paradigm shift in the field of genome editing. CRISPR/Cas9 is a defense mechanism used by bacteria and archaea. This defense mechanism prevents viruses from attacking bacterial or archaeal cells. CRISPR refers to the repeated DNA sequences in the genome, which activate the cell's defense against viral attacks. The repeated DNA sequences in the CRISPR region contain fragments from the viruses' DNA, known as spacers. CRISPR spacers are used to recognize and eliminate the virus DNA. CRISPR spacers bind to the Cas9 protein after the DNA of the virus enters the cell. This causes Cas9 to cut sharply into the virus DNA. In this way, the replication of the virus is blocked and the defense of the cell is activated.[26,27]

How Does CRISPR/Cas9 Work?

CRISPR/Cas9 is a technology used to make genetic changes. It can target genes in a given DNA region and make desired changes. The working mechanism of CRISPR/Cas9 is based on the defense mechanism used to prevent viruses from attacking the cell. CRISPR/Cas9 includes the Cas9 protein and an RNA guide, along with the targeted DNA sequence. The RNA guide allows Cas9 to bind to its targeted DNA region. Cas9 cuts double-strand DNA in the targeted DNA region and makes changes to genetic material.[28]

Gene editing studies in β-Thalassemia

Beta-thalassemia induced pluripotent stem cells (iPSCs) with CD17 a homozygous dot mutation in HBB (A>T) have been fixed with CRISPR/Cas9, which produces gene iPSCs that have the normal karyotype and maintain pluripotency without any off-target effects.[29]

Xiong et al.[30] studied through a study IVS2-654 the combination effect of CRISPR/Cas9 and single-chain oligodeoxynucleotides in iPSCs derived from β-thalassemia patients. CRISPR/Cas9 targets IVS2-654 mutation sites and double-strand breaks in HBB. Redesigned iPSCs have the advantage of maintaining genome stability. Furthermore, the expression of the HBB gene can be restored *in vitro* after hematopoietic differentiation. Our findings therefore suggest that gene correction strategies will facilitate the development of cell therapy for genetic disease in iPSCs.

Xu et al.[31] found that the abnormal merger site targeting the mutation IVS1-110G > A using the Cas9 ribonucleoprotein (RNP) and IVS2-654 C>T mutation is Cas12a/Cpf1 by RNP from β to primary CD34+ changes in hematopoietic stem and progenitor cells (HSPCs). Designed for the high effectiveness and penetration of Cas9 and Cas12a. Patient HSPCs showed a reversal of abnormal addition and new effectiveness of β-globin expression. Specifically, up to 73% InDel was observed, and sequentially adding 1-bp to the IVS1-110 region was enough to restore the normal β-globin merger. The application of this gene-editing technology offers a bright future for the treatment of transfusion-dependent β-thalassemia genotypes.

It was applied in CD34+ cells from Egyptian IVS1-110-thalassemia patients with the β mutation through CRISPR/Cas9 gene editing technology. Cas9 along with guide RNA (gRNA) was introduced into CD34+ cells, resulting in the introduction of double-strand breaks at the specific target site in the DNA. With this fracture, the IVS1-110 mutation was disabled. This study supported current studies into the CRISPR/Cas9 application to treat β-thalassemia.[32]

A strategy has been developed to fix the -28 in codons 41 and 42 found in exon 2 with 4-bp (TCTT) deletion in the (A>G) region. It was developed by reprogramming patient-derived iPSCs with a combination of CRISPR/Cas9 technology and piggyBac transposon cut and paste.[33]

Two different research groups, Liu et al.[34] and Niu et al.[35] conducted 4-bp deletion (-TCTT) and (-CTTT) in β mutation on CD41/42 -globin, respectively, to CRISPR/Cas9-mediated hematopoietic stem cells derived from β-thalassemia patients carrying this disease. Homology-directed repair-based treatment was selected. It demonstrates the most effective and secure method for genetically correcting CD41/42 4-bp deletion in iPSCs.

Hereditary persistence of fetal hemoglobin (HPFH) is a condition with high HbF production that occurs in adulthood depending on mutations in β or a globin gene cluster or γ promoter gene region. The target site-specific β has been selected to remove part of the SaCas9 globin locus. By mimicking the HPFH genotype in bone marrow-induced adult CD34+ HSPCs by repairing it with non-homologous end-joining, it was successfully regulated following the differentiation of erythroid cells with 13 kb of HPFH-5 deletion.[36]

These transcription factors from BCL11A, SOX6, LRF/ZBTB7A, and KLF1 γ-globin gene expression links have been recently documented in genome-wide association studies. Another option for treating β-thalassemia could be gene therapy.

This treatment aims to replace the abnormal β-globin gene with a healthy gene. However, this treatment is not yet in clinical practice and is in the research phase.[37-40]

In conclusion, thalassemia syndromes, which are in the population, are among the leading diseases in sickle cell anemia and genetic counseling and prenatal diagnosis. Data from clinical trials investigated in the past and now underway lead to the design of further clinical and preclinical trials based on gene editing. Fundamental studies on the possibility of globin change and new technology advances are leading to the study of new gene therapy approaches. Erythropoietic stem cells also preferred in the past, have been the cellular therapy of choice to date, taken from β-thalassemia patients. With recent studies, it offers the ability to increasingly benefit from gene therapy and induced pluripotent stem cells. It has been nearly three decades since gene therapy was recommended as a method to treat diseases deemed difficult or incurable. It has now entered between treatment methods where gene editing is an effective tool. It uses various strategies for disease treatment based on the basis of the disease caused in this method. This method is; to disable an imperfect allele, delete it, add it, or make an accurate copy of an allele among completely modified treatment varieties. Recent advances in genome sequencing methods and studies in the HBB gene have underpinned new achievements in understanding the molecular mechanism and advancements in gene editing technology, delivering significant clinical benefits in its treatment.

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