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CRISPR-Cas9 Gene Editing for β-Hemoglobinopathies Therapy

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Abstract. β-hemoglobinopathies, comprising sickle cell disease (SCD) and transfusion -dependent β-thalassemia (TDT), represent monogenetic disorders caused by mutations in the β-globin gene, resulting in aberrant hemoglobin synthesis and severe clinical complications. CRISPR-Cas9, a revolutionary genome-editing tool, offers promising potential for correcting these mutations at the genetic level. This review employs a literature review methodology to explore the therapeutic application of CRISPR-Cas9 in treating β-hemoglobinopathies, focusing on its molecular mechanisms, experimental progress and clinical challenges. Key investigations confirm that ex vivo CRISPR-Cas9 editing of hematopoietic stem cells can effectively restores physiological hemoglobin production. However, challenges such as off-target effects, long-term, and cost safety remain barriers to clinical translation. Preclinical data highlight the need for optimized delivery systems—such as lipid nanoparticle-mediated transfection—to enhance editing efficiency while minimizing genotoxicity. Future research should prioritize optimizing delivery systems, enhancing specificity, and addressing long-term safety. This review concludes that CRISPR-Cas9 holds transformative potential for curing β-hemoglobinopathies but requires further refinement for widespread therapeutic use.

Keywords: CRISPR-Cas9, β-hemoglobinopathies, sickle cell disease, β-thalassemia, gene therapy

1. Introduction

β-hemoglobinopathies constitute a significant global health burden, with over 300,000 infants born annually with SCD or TDT [1]. Current therapeutic strategies, including chronic blood transfusions and allogeneic stem cell transplantation, are constrained by high costs, limited donor availability, and severe complications. Recent therapeutic advancements for β-hemoglobinopathies have centered on gene therapy, which involves ex vivo CRISPR editing of patient-derived hematopoietic stem cells (HSCs), followed by myeloablative conditioning and autologous transplantation to achieve durable genetic correction [2]. The CRISPR-Cas9 gene editing system has opened unprecedented opportunities to address the genetic root of these disorders. Unlike earlier gene therapy approaches reliant on viral vectors, CRISPR-Cas9 enables precise correction of HBB mutations or reactivation of fetal hemoglobin (HbF) through targeted epigenetic modulation. However, the clinical application of CRISPR-Cas9 technology continues to face significant

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challenges. Key obstacles include off-target effects, which refer to unintended genomic modifications. Immune responses to Cas9, as pre-existing antibodies or T-cell reactivity against the bacterial-derived protein could compromise efficacy or safety; inefficient delivery systems, particularly in achieving robust editing rates in quiescent HSCs without viral vector limitations and long-term safety concerns, such as potential genotoxicity or clonal dominance of edited cells over time. Through a comprehensive literature synthesis, this article summarizes cutting-edge approaches and mechanistic insights underlying CRISPR-Cas9-based therapies for β-hemoglobinopathies, while delineating persistent barriers to clinical implementation and proposing future research directions to advance this transformative technology.

2. CRISPR-Cas9 system overview

2.1. Structure and mechanism

The CRISPR-Cas9 editing platform relies on two essential components: the Cas9 endonuclease and a single-guide RNA (sgRNA). Cas9 is a bifunctional enzyme containing the HNH domain, which cleaves the complementary DNA strand, and the RuvC-like domain, responsible for cutting the non-complementary strand. Cas9 recognizes target DNA via sgRNA. The sgRNA is a complex formed by the hybridization of trans-activating crRNA (tracrRNA) and CRISPR RNA (crRNA). Here, the tracrRNA facilitates the processing of precursor crRNA (pre-crRNA) into mature crRNA and activates Cas9's crRNA-guided DNA cleavage activity. The guide sequence at the 5' end of the crRNA binds to the target DNA via complementary base pairing, while the 3' end of the complex adopts a double-stranded structure to stabilize its interaction with Cas9 [3-5]. Guided by sgRNA, Cas9 induces site-specific double-strand breaks (DSBs), triggering two DNA repair pathways:

·Homology-directed repair (HDR)achieves precise repair by using a sister chromatid or exogenous DNA template (e.g., via Rad51 nucleoprotein filaments and BRCA2), restricted to the S/G2 phases and requiring a template [6].

·Non-homologous end joining (NHEJ) directly ligates broken ends via the Ku70/80 complex, often introducing insertions/deletions (indels) without a template. This pathway is error-prone but active in G1 phase, making it suitable for gene knockout [7].

CRISPR-Cas9's dominance in genome engineering stems from its design simplicity, high efficiency, and broad applicability.

2.2. Applications in genome editing

CRISPR/Cas9 technology harnesses two primary DSB repair pathways NHEJ and HDR—to enable diverse genome-editing strategies. Gene knockout exploits the error-prone NHEJ pathway, where Cas9-induced DSBs trigger insertions or deletions that disrupt gene function through frameshift mutations or premature stop codons, effectively silencing disease-causing genes such as oncogenes. Conversely, gene knock-in or replacement relies on HDR, using exogenous DNA templates to precisely integrate or replace sequences at target loci, though this method is restricted to dividing cells. To overcome the dependency on DSBs, base editing combines a Cas9 nickase with deaminases to directly convert single bases (C→T or A→G), enabling correction of point mutations like the Glu6Val substitution in sickle cell anemia. Prime editing further expands precision by integrating nCas9 with reverse transcriptase and a prime editing guide RNA, which encodes both target specificity and desired edits, allowing versatile modifications without donor DNA. This approach minimizes off-target effects and addresses complex mutations, such as excising latent HIV

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proviruses. Collectively, these strategies—ranging from disruptive gene knockout to nucleotide-level precision editing—highlight CRISPR/Cas9's versatility in treating genetic disorders, cancer, and infectious diseases. By tailoring DNA repair mechanisms, this technology offers transformative potential for previously untreatable conditions [5].

3. Pathophysiological mechanisms of β-hemoglobinopathies

Hemoglobin, a tetrameric protein ($\alpha_2\beta_2$), transports oxygen in red blood cells (RBCs). The α- and β-globin chains are synthesized during erythropoiesis under tight genetic regulation. Mutations in the β-globin gene disrupt this process, leading to either structurally abnormal hemoglobin (as in SCD) or reduced β-globin production (as in TDT).

3.1. Pathophysiological mechanisms of sickle cell disease

Sickle cell disease (SCD) arises from a point mutation (Glu6Val) in the β-globin gene (HBB), leading to the production of abnormal hemoglobin S (HbS). Under hypoxic conditions, HbS polymerizes into insoluble fibers through hydrophobic interactions, causing RBCs to adopt an irreversible sickle shape, lose deformability, causing microvascular obstruction. Vaso-occlusion triggers ischemia-reperfusion injury, activating endothelial adhesion molecules and promoting the formation of adhesive clusters between sickled RBCs, leukocytes, and platelets, which exacerbate inflammation and oxidative stress. Chronic hemolysis releases cell-free hemoglobin and heme, which deplete nitric oxide and generate reactive oxygen species, further damaging vascular function and contributing to complications such as pulmonary hypertension, thrombosis, and multi-organ injury. Concurrently, damage-associated molecular patterns (e.g., HMGB1) activate TLR4 and NLRP3 inflammasomes, driving the release of pro-inflammatory cytokines like IL-1β and perpetuating a vicious inflammatory cycle. Current therapies aim to disrupt this pathological cascade: hydroxyurea or gene editing induces fetal hemoglobin (HbF) to inhibit HbS polymerization, anti-adhesion agents improve microvascular flow, antioxidants and anti-inflammatory therapies mitigate oxidative damage and inflammation. CRISPR-Cas9-based gene-editing technologies aim to directly correct the mutation or reactivate γ-globin expression, offering a transformative path toward curing SCD [5].

3.2. Pathophysiological mechanisms of β-thalassemia

Transfusion-dependent β-thalassemia (TDT) pathophysiology stems from mutations in the HBB gene, including point mutations or deletions, which reduce or abolish β-globin synthesis, leading to α/β-globin chain balance. Excess α-globin chains form insoluble aggregates that damage erythroid precursors in the bone marrow, causing ineffective erythropoiesis and peripheral hemolysis, which manifest as severe anemia. β-Thalassemia exacerbates iron metabolism dysregulation through a dual mechanism. First, the reduction or absence of β-globin chains triggers ineffective erythropoiesis, suppressing hepcidin synthesis. As a key regulator of iron homeostasis, decreased hepcidin levels promote excessive intestinal iron absorption. Second, many patients undergo long-term blood transfusions to manage anemia. During this process, iron enters the body with transfused red blood cells and is released upon erythrocyte degradation, contributing to systemic iron overload. Excess iron saturates the iron-binding capacity of transferrin, and surplus iron persists in the serum as non-transferrin-bound iron (NTBI). NTBI catalyzes the generation of free radicals, resulting in lipid peroxidation, DNA damage, and iron deposition in critical organs. This ultimately drives heart

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failure, liver fibrosis, and endocrine dysfunction.. This cascade forms a self-reinforcing cycle—anemia promotes iron overload, while iron toxicity further impairs erythropoiesis and accelerates organ damage. Current therapeutic strategies focus on genetic correction to restore α/β chain balance, combined with iron chelators to remove toxic free iron, aiming to disrupt the pathological cycle and improve clinical outcomes [6,7].

4. CRISPR-Cas9 in β-hemoglobinopathies therapy

4.1. CRISPR-Cas9 treats β-hemoglobinopathies by targeting the BCL11A

Studies have demonstrated that elevated HbF levels can reduce morbidity in patients with TDT and SCD, positioning it as a promising therapeutic target for β-hemoglobinopathies. The zinc-finger transcription factor encoded by the BCL11A gene suppresses γ-globin expression in erythroid cells, thereby inhibiting HbF synthesis [8-10]. Preclinical research has shown that CRISPR-Cas9-mediated editing of the erythroid-specific enhancer region of BCL11A holds substantial promise. In a landmark study, electroporation-based delivery of CRISPR-Cas9 into $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) from healthy donors achieved modification of approximately 80% of alleles at the target locus, with no detectable off-target effects. In immunodeficient mouse models, edited cells demonstrated engraftment efficiency equivalent to unedited cells, with stable editing patterns maintained for 16 weeks. In clinical trials, a patient with transfusion-dependent TDT and another with SCD received CTX001 (autologous CRISPR-Cas9-edited $CD34^+$ HSPCs). Both patients showed high levels of edited alleles in bone marrow and blood, pancellular distribution of elevated HbF, and sustained clinical benefits: the TDT patient achieved transfusion independence, while the SCD patient experienced elimination of transfusion requirements and cessation of vaso-occlusive episodes. These findings indicate that CRISPR-Cas9-mediated editing of the BCL11A enhancer effectively enhances HbF production, offering a promising therapeutic strategy for β-hemoglobinopathies [11]. In a major milestone for the field, Vertex and CRISPR Therapeutics announced that exa-cel (brand name: Casgevy), their CRISPR-Cas9 gene-editing therapy, received conditional approval from the UK's Medicines and Healthcare products Regulatory Agency (MHRA). This approval marks exa-cel as the world's first CRISPR-based gene-editing drug to reach the market, signifying a transformative advancement in the treatment of β-hemoglobinopathies.

4.2. CRISPR-Cas9 treats β-hemoglobinopathies by targeting the HBB gene

CRISPR-Cas9 gene editing technology offers a revolutionary strategy for treating β-hemoglobinopathies. Researchers have achieved precise correction of diverse HBB gene mutations in patient-derived induced pluripotent stem cells (iPSCs) using a dual-guide RNA system combined with CRISPR-Cas9, thereby restoring normal β-globin expression. For instance, co-delivering Cas9, guide RNAs, and a donor DNA template carrying the wild-type HBB sequence into iPSCs from TDT patients significantly restored HBB protein expression in erythroid cells. In SCD research, high-fidelity Cas9 variants and chemically modified guide RNAs, combined with recombinant adeno-associated virus 6 (rAAV6)-mediated delivery of donor templates, enabled up to 60% allelic correction in autologous $CD34^+$ hematopoietic stem cells. Transplantation of these edited cells into immunodeficient mice demonstrated stable multilineage chimerism, where even low-level chimerism (20%) substantially increased normal hemoglobin (HbA) and reduced hemoglobin S (HbS). Mechanistically, the sgRNA directs Cas9 to the mutation site of the HBB gene. Cas9 cleaves the HBB gene, inducing DSB. This break thereby allows HDR to utilize a homologous donor

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template to correct the original mutation in the target gene. Ultimately, this process restores normal beta-globin production, resolving the α/β-globin chain imbalance in beta-thalassemia or preventing HbS polymerization in sickle cell disease. Preclinical studies demonstrate that gene-edited hematopoietic stem cells in murine models exhibit no genotoxicity or tumorigenicity, confirming safety and reproducibility. These advancements establish a foundation for universal therapies addressing diverse mutation types but also provide critical support for upcoming Phase I/II clinical trials, heralding an era of long-term cure for β-hemoglobinopathies through autologous transplantation [12-14].

5. Conclusion: current challenges and future perspectives of CRISPR-Cas9 gene editing for β-hemoglobinopathies therapy

CRISPR-Cas9 gene editing represents a groundbreaking therapeutic strategy for β-hemoglobinopathies, such as TDT and SCD, by directly correcting pathogenic mutations in the HBB gene or reactivating HbF through targeted disruption of the BCL11A erythroid-specific enhancer. Despite remarkable preclinical and early clinical successes, several critical challenges must be addressed to enable its broad clinical application. A primary technical hurdle involves mitigating off-target effects. Although high-fidelity Cas9 variants and optimized guide RNA designs have advanced, even low-frequency unintended edits in long-lived HSCs pose potential oncogenic risks. Delivery inefficiency further complicates translation: while viral vectors like AAV6 are widely utilized, their immunogenicity and limited cargo capacity hinder scalability, and non-viral alternatives require refinement to achieve robust editing rates in HSCs. Additionally, long-term stability of edited cells remains uncertain, as clinical trial data reveal a gradual decline in edited allele frequencies in patient bone marrow over time. Ethical and accessibility challenges persist, including germline editing concerns, insufficient long-term safety data, and high costs that limit access in resource-constrained regions with high β-hemoglobinopathy prevalence.

However, technological innovations and strategic optimizations hold promise for overcoming these barriers. Next-generation editing tools, such as base and prime editors, may circumvent double-strand break risks by enabling precise single-nucleotide corrections—prime editing has already demonstrated efficacy in rectifying the SCD-causing Glu6Val mutation. Innovations in delivery systems, including nanoparticle-based platforms or ex vivo HSC expansion protocols, could enhance editing efficiency and scalability. Combination therapies integrating CRISPR editing with pharmacological HbF inducers or anti-inflammatory agents may synergize to address both genetic defects and secondary pathologies. Personalized approaches tailored to specific mutation types further optimize clinical outcomes. Concurrently, global collaboration to standardize protocols, reduce costs, and address ethical dilemmas is essential. Pilot programs in high-prevalence regions, coupled with community engagement, may accelerate real-world implementation.

In conclusion, while CRISPR-Cas9 has redefined the therapeutic landscape for β-hemoglobinopathies, its clinical translation demands resolution of technical, safety, and ethical complexities. Advances in precision editing, delivery technologies, and multimodal therapies, supported by robust regulatory frameworks, are poised to transform experimental breakthroughs into mainstream treatments. Ongoing clinical trials underscore the feasibility of durable cures, offering hope for millions worldwide. Through interdisciplinary collaboration and global equity initiatives, CRISPR-based therapies may soon transition from bench to bedside, heralding a new era of genetic medicine for these devastating disorders.

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