

CRISPR/Cas9 in red blood cell-related diseases: breakthroughs in gene editing and therapy

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ABSTRACT

Red blood cell (RBC) disorders, including sickle cell anemia and β -thalassemia, are emerging as a significant global public health concern. Clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9)-mediated genome editing technology, commonly referred to as CRISPR/Cas9, is a precise and efficient genome-editing tool that has demonstrated considerable potential in advancing research on erythroid diseases. This review elucidates the molecular mechanisms underlying CRISPR/Cas9 technology and systematically illustrates its applications in generating animal models for RBC disorders, investigating erythrocyte differentiation pathways, and developing novel therapeutic strategies. Furthermore, the review critically evaluated the current challenges and future directions for CRISPR/Cas9-based approaches, providing insights to guide both fundamental research and clinical translation in the context of these hematologic conditions.

Keywords: CRISPR/Cas9, red blood cell, gene editing

INTRODUCTION

Erythrocytes play a vital role in key physiological processes, including oxygen transport, carbon dioxide removal, and acid-base homeostasis^[1]. Various types of red blood cell (RBC) disorders, such as sickle cell anemia^[2] and β -thalassemia^[3], are caused by genetic mutations that severely impair patients' quality of life and present life-threatening risks. Conventional treatment methods, including blood transfusion^[4], pharmacotherapy^[5], and hematopoietic stem cell transplantation (HSCT) are frequently limited by challenges such as transfusion dependence, elevated alloimmunization rates^[6], and donor scarcity^[7]. Given these constraints, there is an urgent need to develop more effective therapeutic strategies.

Since its discovery, the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system, or CRISPR/Cas9, has emerged as a transformative tool in life sciences due to its ease of operation^[8], high editing efficiency, and precise targeting capability. This revolutionary technology allows for accurate genome editing at designated loci, opening new avenues for genome-wide research and therapeutic advancements in RBC disorders^[9]. By facilitating precise modifications of erythrocyte-related genes, CRISPR/Cas9 demonstrates potential for correcting pathogenic mutations at their origin^[10], restoring normal erythrocyte function^[11], and ultimately offering more effective therapeutic options for patients^[12]. This review provides a comprehensive

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overview of recent advances in CRISPR/Cas9 applications within erythrocyte research ([Fig. 1](#)).

THE MECHANISM OF CRISPR/CAS9 TECHNOLOGY

The CRISPR/Cas9 system is derived from an adaptive immune mechanism found in bacteria and archaea^[13]. When infected by bacteriophages, these microorganisms integrate fragments of viral DNA into CRISPR loci as spacer sequences^[14]. These spacers are subsequently transcribed into CRISPR RNAs (crRNAs), which form duplex structures with trans-activating crRNAs (tracrRNAs) through complementary base pairing. This RNA complex guides the Cas9 endonuclease to identify and cleave foreign DNA sequences matching the spacer, thereby conferring protection against viral infections.

For gene editing applications^[15], researchers have combined the crRNA and tracrRNA into a single guide RNA (sgRNA). The 5' end of the sgRNA contains a 20-nucleotide targeting sequence that specifically binds to complementary DNA *via* base pairing. Guided by this sgRNA, the Cas9 endonuclease recognizes and binds to its target DNA sequence. The Cas9 protein subsequently induces double-strand breaks (DSBs) at precise genomic locations^[16]. Cells employ two primary repair pathways to address DSBs: non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ is an efficient yet error-prone process that directly ligates broken DNA ends^[17], frequently

generating insertions or deletions (indels) that disrupt gene function through frameshift mutations, thereby effectively achieving gene knockout. In contrast, HDR is a precise repair mechanism that necessitates a homologous donor DNA template. Using this template, cellular repair enzymes faithfully restore the DNA sequence, enabling precise gene modifications, including knock-in/ knock out or targeted mutation correction.

By leveraging these distinct repair pathways, researchers can perform diverse genomic modifications, including gene knockout, knock-in, and targeted replacements, providing powerful tools to advance RBC research and therapeutic development.

APPLICATIONS OF CRISPR/CAS9 TECHNOLOGY IN RBC RESEARCH

Construction of animal models for RBC diseases

The development of precise animal models that faithfully recapitulate human erythroid disorders is essential for investigating disease mechanisms and evaluating therapeutic interventions. CRISPR/Cas9 technology has revolutionized this process by enabling precise genome editing^[18]. Through targeted delivery of designed sgRNA and Cas9 protein into animal zygotes or embryonic stem cells^[19], researchers can efficiently introduce disease-relevant mutations in erythrocyte-related genes, creating genetically engineered models that closely mimic human pathophysiology.

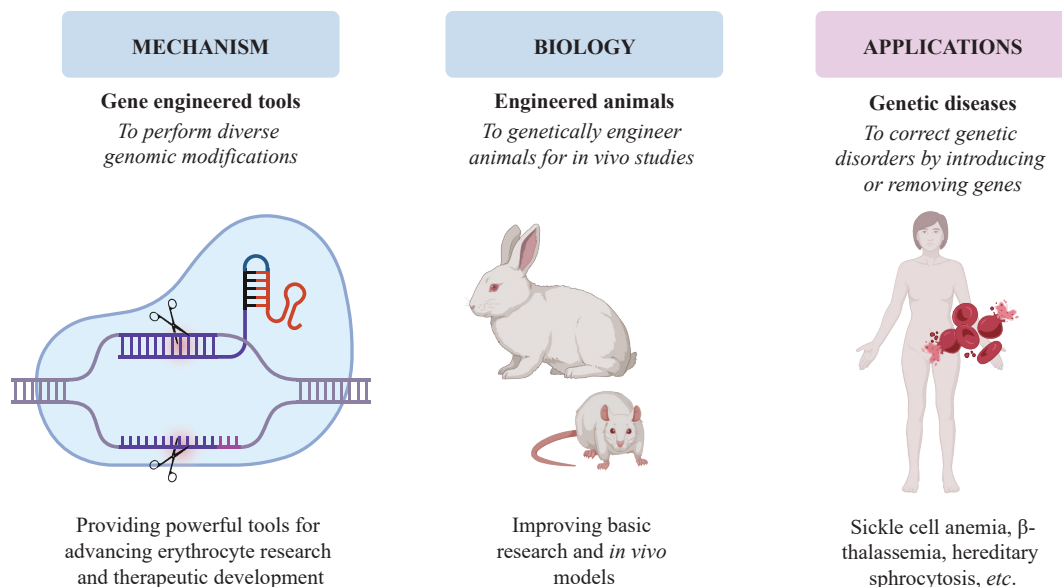


Fig. 1 Diagram of CRISPR/Cas9-mediated gene therapy approaches for inherited RBC diseases. CRISPR/Cas9: clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9); RBC: red blood cell. Created with BioRender.com.

In developing mouse models of sickle cell disease (SCD)^[20–21], researchers utilized CRISPR/Cas9 to introduce the human pathogenic point mutation into the murine β -globin (*HBB*) gene. The resulting models recapitulated key disease manifestations, including sickle-shaped erythrocytes, hemolytic anemia, and vaso-occlusive pathology. These genetically engineered models have become invaluable tools for investigating SCD pathogenesis and evaluating novel therapeutic approaches.

Similarly, CRISPR/Cas9 has facilitated precise modeling of β -thalassemia through various genetic manipulations in murine and other animal models^[22–24]. Researchers successfully generated animal models carrying various *HBB* gene mutations, including deletions and nonsense mutations, which reflect the genetic heterogeneity observed in human patients^[25–26]. This suite of models facilitates systematic investigation of genotype-phenotype correlations and provides robust platforms for therapeutic target discovery.

Compared to conventional gene-targeting approaches, CRISPR/Cas9 presents considerable advantages for the generation of animal models, including simplified experimental design, accelerated production timelines, and decreased costs. This technological advancement enables the rapid and efficient development of physiologically relevant animal models for erythroid disorders, substantially accelerating both fundamental research and therapeutic development in this field.

Exploration of the mechanism of RBC development

Erythropoiesis is a precisely orchestrated process governed by complex genetic networks and signaling pathways. CRISPR/Cas9 technology has emerged as a powerful tool for elucidating the molecular mechanisms underlying erythroid development^[27–29]. By performing targeted knockout or precise editing of erythropoiesis-related genes, researchers can systematically investigate how genetic perturbations affect specific stages of erythrocyte maturation^[30–32]. This approach allows for definitive characterization of gene function within the hierarchical framework of erythroid differentiation.

CRISPR/Cas9 technology holds promise for addressing global blood supply shortages. Among the 48 recognized human blood group systems, the ABO and Rh systems are of particular clinical significance in transfusion medicine due to their critical roles in determining donor-recipient compatibility. Type O RhD-negative erythrocytes constitute an especially

valuable resource as universal donor cells compatible with transfusion across most blood types.

Current research has pursued two primary strategies to increase the supply of universally compatible blood: enzymatic modification of blood group antigens^[33–34] and genetic engineering of erythroid precursors. The latter approach utilizes CRISPR/Cas9 to generate RHD-knockout erythroid progenitor cells, which can then be expanded and differentiated into mature erythrocytes^[35]. These combined efforts in enzymatic conversion and genetic editing aim to both expand the available blood supply and enhance transfusion safety through the production of universal donor blood products.

CRISPR/Cas9 has emerged as a powerful tool for investigating the regulatory roles of erythrocyte membrane proteins in cellular morphology and function^[36]. By precisely editing genes encoding key cytoskeletal components, including spectrin and ankyrin^[37], researchers have demonstrated that these proteins are essential for maintaining normal erythrocyte morphology. Genetic perturbations induce characteristic abnormalities such as ovalocytes and stomatocytes, while concurrently altering fundamental physiological properties including cellular deformability and osmotic fragility^[38–39]. These findings provide mechanistic insights into how membrane-cytoskeleton interactions preserve erythrocyte integrity and establish a molecular framework for understanding related hemolytic disorders. Such CRISPR/Cas9-enabled studies not only advance fundamental scientific understanding but also identify potential therapeutic targets for hereditary spherocytosis and other erythrocyte membrane pathologies.

Research on the treatment of RBC-related diseases

SCD

SCD is an inherited hemoglobinopathy resulting from a point mutation in the *HBB* gene, which leads to the production of abnormal hemoglobin S (HbS)^[40]. Under hypoxic conditions, HbS undergoes polymerization, inducing erythrocyte sickling^[41]. These rigid, sickle-shaped cells cause microvascular occlusion and chronic hemolysis, thereby contributing to the progressive multiorgan damage characteristic of SCD pathophysiology.

CRISPR/Cas9-based therapeutic strategies for SCD primarily concentrate on two approaches: direct correction of *HBB* gene mutations or reactivation of fetal hemoglobin (HbF) expression^[42]. The first strategy employs CRISPR/Cas9-mediated HDR to precisely replace pathogenic mutations with wild-type

sequences, thereby restoring normal adult hemoglobin (HbA) production^[43]. However, clinical translation of this approach faces significant challenges, including the inherently low efficiency of HDR *in vivo* and the necessity for co-delivery of donor DNA templates to target tissues.

Another notably promising therapeutic approach involves CRISPR/Cas9-mediated disruption of the *BCL11A* gene^[44], a key transcriptional repressor of γ -globin (*HBG*) expression. By targeting *BCL11A* in hematopoietic stem and progenitor cells (HSPCs), this approach reactivates HbF production. The elevated HbF exhibits high oxygen affinity and inhibits polymerization, thereby compensating for the defective HbA in SCD.

Clinical trials have demonstrated that reinfusion of CRISPR/Cas9-edited HSPCs into SCD patients following *ex vivo* modification leads to a durable elevation of HbF^[45]. This results in a marked reduction of erythrocyte sickling and substantial clinical improvement.

Furthermore, alternative CRISPR/Cas9 strategies targeting the *HBG1/HBG2* gene promoters have showed comparable success in HbF induction, providing multiple therapeutic avenues for SCD treatment^[45]. These genome editing approaches collectively represent a paradigm shift from symptom management toward potential disease modification.

β -thalassemia

β -thalassemia is an inherited hemolytic anemia resulting from mutations in the *HBB* gene that impair β -globin chain synthesis. The clinical severity varies substantially based on the specific mutation type, ranging from transfusion-dependent thalassemia major to milder thalassemia intermedia phenotypes^[46].

CRISPR/Cas9-based therapeutic strategies for β -thalassemia primarily target two approaches^[47]: direct correction of *HBB* gene mutations to restore normal β -globin production and reactivation of *HBG* expression to compensate for β -globin deficiency.

While HDR-mediated gene correction has encountered challenges such as low editing efficiency and limitations in donor template delivery^[48], current research efforts are predominantly focused on *HBG* induction strategies^[49]. This approach parallels successful SCD therapies and leverages HbF's capacity to functionally replace deficient HbA.

CRISPR/Cas9 technology enables precise genome editing of regulatory elements controlling *HBG* expression, including transcriptional promoters, enhancers, and repressive binding sites. Targeted modification of these genomic regions has been shown to effectively reactivate HbF production in

both animal models and human erythroid cell cultures^[50–51]. The clinical potential of this approach is exemplified by exagamglogene autotemcel (exa-cel), an investigational CRISPR/Cas9-edited cell therapy. By disrupting the *BCL11A* erythroid enhancer in patient-derived HSPCs, exa-cel treatment induces sustained elevation of HbF levels ($\geq 20\%$ of total hemoglobin), significantly improves total hemoglobin concentrations, and markedly reduces transfusion requirements^[52]. Ongoing clinical trials have further demonstrated that CRISPR/Cas9-edited HSPCs can successfully engraft and produce HbF-expressing erythrocytes, leading to increased hemoglobin level in transfusion-dependent patients, reduced transfusion frequency in responders, and improved iron homeostasis parameters^[53–54]. These findings establish CRISPR-based induction of *HBG* expression as a promising therapeutic strategy for β -thalassemia, with the potential to transform disease management from chronic supportive care to definitive treatment.

Other RBC-related diseases

The therapeutic potential of CRISPR/Cas9 extends beyond SCD and β -thalassemia to encompass other erythroid disorders. A notable example is glucose-6-phosphate dehydrogenase (G6PD) deficiency, an X-linked enzymopathy affecting approximately 400 million people worldwide^[55]. This condition stems from pathogenic variants in the *G6PD* gene that diminish enzyme activity, compromising erythrocyte antioxidant defenses and increasing susceptibility to oxidative stress-induced hemolysis. Emerging CRISPR/Cas9-based strategies seek to address the underlying genetic defect through precision gene editing to correct disease-causing mutations and transcriptional modulation to enhance residual *G6PD* expression. These approaches can effectively restore G6PD activity in erythroid precursors, offering the potential for sustained clinical improvement. By directly targeting the genetic basis of G6PD deficiency, CRISPR/Cas9 technology may overcome the limitations of current supportive therapies and provide a definitive therapeutic intervention.

CRISPR/Cas9 technology has demonstrated therapeutic potential for hereditary erythrocyte membrane disorders including hereditary spherocytosis and hereditary elliptocytosis. These conditions arise from mutations in genes encoding critical membrane structural proteins, such as ankyrin-1, band 3, and spectrins, leading to defective erythrocyte morphology and function^[56]. Current research indicates that CRISPR/Cas9-mediated correction of these genetic defects can restore normal membrane architecture and cellular integrity in preclinical models. Compared to

conventional treatments such as splenectomy, which only alleviate symptoms, this gene-editing approach targets the underlying genetic pathology, offering the possibility of definitive treatment.

THE CHALLENGES FACED BY CRISPR/CAS9 TECHNOLOGY IN RBC RESEARCH

Off-target effects

While CRISPR/Cas9 technology demonstrates remarkable targeting precision, off-target effects remain a significant challenge for its therapeutic application^[57]. These effects arise when the Cas9 nuclease induces unintended double-strand breaks at genomic loci other than the intended target, potentially causing deleterious mutations^[58]. Such off-target activity may disrupt normal cellular function through various mechanisms, including the inadvertent activation of oncogenes or suppression of tumor suppressor genes, thereby raising substantial safety concerns for clinical translation^[59]. Current research efforts focus on enhancing the specificity of CRISPR/Cas9 systems to minimize these risks while preserving therapeutic efficacy.

The occurrence of off-target effects is primarily attributable to the interplay between sgRNA specificity, Cas9 protein activity, and cellular repair processes. Partial sequence complementarity between the sgRNA and non-target DNA sites allows for base mismatch tolerance, potentially enabling Cas9-mediated cleavage at unintended loci when sequence similarity is sufficiently high. Furthermore, sustained or excessive Cas9 nuclease activity within cells has been shown to elevate off-target mutation frequencies^[60].

To address these limitations, several optimization strategies have been developed. Computational sgRNA design algorithms now enable the selection of highly specific guide sequences with minimal predicted off-target activity. Protein engineering has produced enhanced Cas9 variants (*e.g.*, eSpCas9, SpCas9-HF1) that maintain on-target efficiency while significantly reducing off-target cleavage^[61]. An alternative approach employs paired Cas9 cleavage enzymes to create offset single-strand breaks that only generate double-strand breaks when properly aligned, thereby improving overall targeting precision^[62].

Limitations of delivery systems

The clinical translation of CRISPR/Cas9-based therapies critically depends on the development of

efficient and safe delivery systems for target cells. Current approaches primarily utilize viral vectors and non-viral methods, each presenting distinct advantages and limitations that require careful consideration for specific applications^[63].

Viral delivery systems, particularly lentiviral and adeno-associated viral (AAV) vectors, demonstrate high transduction efficiency across diverse cell types, making them attractive vehicles for CRISPR/Cas9 delivery. However, their clinical application encounters considerable safety challenges. The inherent immunogenicity of viral vectors may provoke host immune responses ranging from localized inflammation to systemic organ damage. Furthermore, the integration of vectors carries additional risks due to random genomic insertion, which may disrupt tumor suppressor genes or activate proto-oncogenes, thereby increasing tumorigenic potential^[64]. These safety concerns necessitate careful risk-benefit evaluation when employing viral delivery systems in therapeutic genome editing.

In contrast to viral vectors, non-viral delivery systems including liposomal formulations and polymeric nanoparticles, offer improved safety profiles with reduced immunogenicity^[65–67]. However, these platforms face significant translational challenges, particularly their relatively low transfection efficiency compared to viral counterparts. The *in vivo* delivery of CRISPR/Cas9 components *via* non-viral vectors is further complicated by multiple physiological barriers, including rapid clearance by the reticuloendothelial system and enzymatic degradation in circulation, which collectively limited the therapeutic payload reaching target tissues^[65]. These fundamental limitations currently restrict the clinical efficacy of non-viral CRISPR/Cas9 delivery approaches.

The development of safe and efficient delivery systems remains a critical challenge for the clinical translation of CRISPR/Cas9-based therapies in hematological disorders. Recent advancements have focused on innovative delivery platforms, including exosome-encapsulated formulations and cell-penetrating peptide conjugates, which show promise for enhancing both the targeting specificity and biosafety of CRISPR/Cas9 delivery in erythroid cells^[68]. These emerging technologies aim to overcome the current limitations of viral and non-viral vectors while maintaining high editing efficiency.

Ethical and social issues

The expanding clinical applications of CRISPR/Cas9 technology for erythroid disorders have

brought concomitant ethical and societal concerns to the forefront^[69]. While current therapeutic approaches focus on somatic cell editing, the potential extension to germline modifications has raised substantial ethical debates. Germline editing poses unique challenges as it induces heritable genetic alterations that may persist across generations, with uncertain consequences for the human gene pool^[70]. These irreversible modifications demand careful consideration of both immediate clinical benefits and long-term population-level implications.

The clinical implementation of CRISPR/Cas9-based therapies faces significant socioeconomic challenges, as high treatment costs may exacerbate healthcare disparities by restricting access to affluent patients. Furthermore, this technology raises profound ethical concerns regarding the potential misuse for non-therapeutic genetic enhancement ("designer babies") and the risk of genetic discrimination. These issues underscore the urgent need for comprehensive ethical frameworks and robust regulatory policies to guide the responsible development and equitable deployment of CRISPR/Cas9 technology in hematologic applications^[71].

SUMMARY AND PROSPECT

CRISPR/Cas9 has emerged as a transformative gene-editing platform that has significantly advanced both the understanding and treatment of erythroid disorders. This technology has enabled precise disease modeling, provided novel insights into erythropoiesis mechanisms, and offered promising treatment strategies for various hemoglobinopathies and erythrocyte membrane defects. However, several critical challenges remain to be addressed before clinical translation, including off-target effects, delivery system limitations, and unresolved ethical considerations surrounding genetic modification.

Future advances in CRISPR/Cas9 technology are expected to focus on the following three key areas. Firstly, improved understanding of its molecular mechanisms should enable enhanced editing precision and reduced off-target effects. Secondly, the development of novel delivery systems may overcome current limitations in targeting efficiency and biosafety. Finally, establishing international consensus guidelines through the collaboration of researchers, policymakers, and ethicists will be crucial for clinical translation. Collectively, these advancements are expected to accelerate the therapeutic application of gene editing while addressing both technical and ethical challenges.

Upon addressing current limitations, CRISPR/Cas9 technology is poised to revolutionize the treatment of erythroid disorders and significantly advance fundamental research in hematology. This genome-editing platform offers unprecedented opportunities to develop curative therapies for previously intractable hemoglobinopathies and erythrocyte membrane disorders. Furthermore, its continued application in basic science will deepen our understanding of erythropoiesis and hemoglobin regulation, significantly advancing both translational medicine and fundamental biological knowledge.

Author contributions

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Ethics statement

Not applicable.

Informed consent statement

Not applicable.

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Declaration of competing interest

The authors declared no conflict of interests.

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