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Original Article

CRISPR and Gene Therapy Applications– A Comprehensive Review

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ABSTRACT

Gene therapy is emerging as a revolutionary approach for the treatment of genetic and acquired disorders. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology, combined with Cas Proteins, has transformed the field of genome editing due to its simplicity, precision, and efficiency. This review provides an overview of CRISPR mechanisms, recent advances in its application to gene therapy, current clinical trials, associated challenges, ethical considerations, and future prospects. With particular emphasis on monogenic disorders, cancer, infectious diseases, and regenerative medicine, this article highlights how CRISPR has accelerated progress towards personalized and curative therapies.

Keywords: CRISPR, Cas9, Gene therapy, Genome editing, monogenic diseases, Clinical trials

INTRODUCTION

Gene therapy refers to the modification of genetic material within a patient's cells to treat or prevent diseases. Traditional methods relied on viral vectors for gene addition, but these approaches were limited by lack of precision [1]. The discovery of CRISPR-Cas systems, particularly CRISPR-Cas9, revolutionized gene therapy by offering a programmable, efficient, and versatile genome-editing platform [2]. CRISPR was first identified as part of the adaptive immune system of bacteria, where it provided defense against invading viruses. In 2012, researchers Jennifer Doudna and Emmanuelle charpentier adapted CRISPR-Cas9 into a genome editing tool. Since then, this technology has rapidly advanced towards clinical applications. Gene therapy using CRISPR aims to correct defective genes, silence harmful mutations, or introduce protective modifications, providing a curative approach for many previously untreatable conditions [3-4].

2. Mechanism of CRISPR-Cas Systems

The CRISPR-Cas system works by utilizing a guide RNA (gRNA) to direct the Cas nuclease to a specific DNA sequence. Once bound, Cas introduces a double-stranded break (DSB) in the DNA. The cell's natural repair mechanisms – non-homologous end joining (NHEJ) or homology-directed repair (HDR) – then edit the sequence [1].

2.1 CRISPR-Cas9

Cas9 is the most widely used nuclease. It requires a protospacer Adjacent Motif (PAM) sequence to bind DNA. Cas9 cuts both DNA strands, enabling gene knockout, insertion, and correction [1, 5].

2.2 CRISPR-Cas12

Cas12 (Cpf1) introduces staggered cuts and recognizes different PAM sequences, expanding targeting Range [6].

2.3 CRISPR-Cas13

Unlike Cas9/12, Cas13 targets RNA, enabling transient gene regulation without altering the genome permanently [7].

2.4 Base Editing and Prime Editing

Base editors enable precise single nucleotide changes without causing double-stranded breaks. Prime

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editing, developed in 2019, allows versatile "search-and-replace" editing, broadening therapeutic Applications [4].

3. Applications of CRISPR in Gene Therapy

3.1 Monogenic Disorders

Many genetic diseases result from single-gene mutations, making them ideal candidates for CRISPR-based therapies. - Sickle Cell Disease and β-Thalassemia. CRISPR has been successfully used to edit hematopoietic stem cells to restore normal hemoglobin production [2]. In 2023, the first CRISPR-based therapy (Casgevy/Exa-cel) received regulatory approval for sickle cell disease [5].

Cystic Fibrosis: Efforts are ongoing to correct CFTR mutations using CRISPR.

Duchene Muscular Dystrophy (DMD): CRISPR can excise defective axons in the dystrophin gene to restore functional protein [8].

3.2 Cancer Therapy

CRISPR is applied to modify immune cells for targeted cancer therapy. - CAR-T Cells: CRISPR enhances CART cell therapy by knocking out inhibitory receptors and improving tumor recognition [7]

Oncogene Editing: Targeting oncogenes (e.g., KRAS, MYC) or tumor suppressors (e.g., TP53) is under Investigation [8].

3.3 Infectious Diseases

CRISPR is being explored to combat viral infections. - HIV: CRISPR can excise integrated HIV proviral DNA from host genomes [7].

Hepatitis B Virus (HBV): Cas9 disrupts viral cccDNA, reducing viral replication [7].

COVID-19: CRISPR-based diagnostics (SHERLOCK, DETECTR) and antiviral strategies have been developed [7].

3.4 Ophthalmology and Neurological Disorders

Leber Congenital Amaurosis (LCA): In vivo CRISPR therapy (EDIT-101) has entered clinical trials to restore Vision [6].

Neurological Disorders: CRISPR holds potential in Huntington's disease and amyotrophic lateral sclerosis (ALS) [8].

3.5 Regenerative Medicine

CRISPR is applied to engineer stem cells for tissue regeneration. Applications include diabetes (insulin secreting cells), cardiovascular repair, and organ transplantation with reduced rejection [7, 8].

4. Recent Clinical Trials and Studies (2021–2025)

Several CRISPR-based therapies are advancing in clinical development.

Table 1: Selected CRISPR Clinical Trials (2021–2025) [2-8]

Disease	Approach	Status/Outcome
Sickle Cell Disease (Exa-cel)	HSC editing to up regulate fetal Hb	FDA approved in 2023
β-Thalassemia	Similar to Exa-cel	Ongoing trials
Leber Congenital Amaurosis (EDIT-101)	In vivo retinal editing	Phase I/II underway
HIV	CRISPR excision of provirus	Preclinical, early trials
Cancer (CAR-T with CRISPR edits)	Immune cell modification	Multiple Phase I trials
Cystic Fibrosis	Correction of CFTR mutations	Preclinical

5. Challenges and Ethical Issues

Despite enormous potential, several challenges remain:

5.1 Delivery Methods

Efficient and safe delivery of CRISPR components to target tissues is critical. Viral vectors, lipid nanoparticles, and novel delivery systems are being optimized [3].

5.2 Off-target Effects

Unintended DNA cuts can cause mutations, increasing safety concerns. Improved gRNA design and engineered Cas variants aim to minimize these risks [8].

5.3 Immunogenicity

Immune responses against Cas proteins (derived from bacteria) can limit therapeutic applications [8].

5.4 Ethical Considerations

Editing germ line cells raises concerns about heritable modifications, designer babies, and societal impacts. Strict ethical guidelines govern clinical applications [9].

5.5 Regulatory Challenges

Different countries follow different regulatory frameworks, making global acceptance of CRISPR therapies Complicated [9].

FUTURE PROSPECTS

The future of CRISPR in gene therapy is promising: - Refined Editing Tools: Development of high-fidelity CAS enzymes, base editors, and prime editors will increase precision [4, 8].

AI Integration: Artificial intelligence can improve gRNA design and predict off-targets [8].

Personalized Medicine: Patient-specific genetic editing could provide tailored therapies [8].

Combination Therapies: CRISPR may be combined with conventional drugs, immunotherapy, or stem cell transplantation [8].

Global Access: Efforts are needed to make CRISPR therapies affordable and accessible worldwide [9].

Next-generation Diagnostics: CRISPR tools could also expand in real-time disease monitoring.

CONCLUSION

CRISPR technology has revolutionized the landscape of gene therapy. From treating monogenic disorders like sickle cell anemia to enhancing cancer immunotherapy and developing novel antiviral strategies, CRISPR demonstrates unparalleled potential. While challenges such as off-target effects, ethical concerns, and delivery barriers remain, ongoing research and technological innovations promise to overcome these hurdles. As the first CRISPR therapies gain regulatory approval, the next decade is expected to witness broader clinical translation, making personalized and curative gene therapies a reality [2-9].

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