Proceedings of ICEGEE 2025 Symposium: Sensor Technology and Multimodal Data Analysis
DOI: 10.54254/2753-8818/110/2025.AU23156

Analysis of Disease Treatment Efficacy Based on CRISPR

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Abstract: CRISPR-Cas9 gene editing technology has become a revolutionary tool in the field of life sciences. Its mechanism is to guide RNA (gRNA) to accurately target DNA sequences and Cas9 nuclease is used for cutting, so as to achieve efficient genome editing. Compared with traditional technologies (such as ZFN and TALEN), CRISPR has the advantages of simple design, low cost and wide applicability, and is widely used in genetic function research, disease treatment and agricultural improvement. This research will discuss the application of CRISPR in the treatment of diseases. In the field of biomedicine, CRISPR has been successfully applied to the treatment of genetic diseases, such as repairing dystrophin gene mutations to repair Dull's muscular dystrophy (DMD) and activating fetal hemoglobin in patients with β-hemoglobin disease. In addition, the emergence of base editing technology has further improved safety, such as the realization of single base repair in hereditary tyrosinemia (HT1). CRISPR also shows potential in antiviral treatment (such as HSV keratitis) and tumor immunotherapy (such as CAR-T cells knocked out by PD-1). Despite the broad prospects, CRISPR still faces challenges such as off-target effect, delivery efficiency and ethical controversy. In the future, with the development of new Cas protein, the optimization of the delivery system and the improvement of the ethical framework, CRISPR technology is expected to make greater breakthroughs in the fields of precision medicine, agriculture and synthetic biology, and provide innovative solutions for human health and sustainable development.

Keywords: CRISPR, Disease treatment, Efficacy, Safety

1. Introduction

Recent years, clustered regularly interspaced short palindromic repeats (CRISPR) have produced revolutionary effect in the field of life science rapidly. The CRISPR is first found in the adaptive immune system of bacteria and archaea, and used for defense the attack of phage. In this system, bacteria protect integrity of self-DNA by recognize and cut the exogenous DNA. In 2012, the team of Jennifer Doudan and Emmanuelle Charpentier first put CRISPR-Cas9 transfer to a gene editing tool, which can precisely edit gene in eukaryotic organisms. This breakthrough discovery not only lead the two scientists becoming the winners of Nobel Prize in Chemistry, but also provide a new direction of the development of gene editing.

The CRISPR shows a high-efficient operation mechanism. The Cas9 nuclease can recognize and cut target DNA sequence precisely by design specific guid RNA (gRNA). Compared to conventional technology for gene editing (like TALEN and ZFN), the CRISPR is easier to design and implement at lower cost and suitable for variety of biological systems [1]. Due to these advances, the CRISPR

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implement in many fields rapidly, including the research of gene function, disease treatments, agriculture improvement and environmental protection. In biomedicine, the CRISPR is widely used to treat inheritance diseases, cancer and infectious diseases. Now, scientists can treat the inheritance disease, which produce by the mutate of single gene, by use CRISPR to *in vivo* or *in vitro* gene editing, such as the sickle cell anaemia and duchenne muscular dystrophy (DMD). And the CRISPR also can be used to develop new cancer treatments method and increase the tumor defensive activation of T cell. In the field of agriculture, the CRISPR promote the development of gene improvements food plant, like increase the disease resistance, environmental adaptation and yield of food plant [2].

Although the future of CRISPR is promising, there are some potential challenges that it cannot avoid. Off-target effect is the dominant limitation of the CRISPR, which may cause non-target gene mutate and lead to unpredictable biological consequences. And the CRISPR face a lot of ethical challenges, especially in the monitoring problem of human embryos gene editing and reproductive gene editing. How to balance the technology potential and possible ethical challenges becomes the common focus of academic field and society [3].

Atopic dermatitis (AD) is a chronic, relapsing and inflammatory skin disease. The symptoms are the damage of skin barrier, immune dysfunction, and the anomalous change of microbiota. The AD produces significant effects to the life quality of the patient, and increase the social medical burden. The pathogenesis of the AD is really sophisticated, promoted by inheritance issues, environmental issues and abnormal of immune system. Filaggrin (FLG) gene mutate cause the function of skin barrier decreases, and the over expression of cytokines like IL-4 and IL-13 promote the Th2 immune dysfunction. This mechanism plays an important role in the development and take place of the AD. Although current treatment plan (like topical hormones, immunomodulator and biological agents) can relieve the symptoms, it limits treatment course and may have sub effects, so it urgently needs to find a treatment plan that more efficient and precisely [4]. The CRISPR bring a new hope on AD treatment. The CRISPR can repair mutation of gene precisely and control the expression of gene. In recent years, the potential application of CRISPR in repairing skin barrier-related genes, regulating the expression of immune factors and optimizing skin microbiota has received wide attention. However, the off-target effect, delivery efficiency and ethical issues of CRISPR in clinical application are still the main obstacles that need to be overcome in the process of its promotion. Here, this research will discuss the application performance of CRISPR in treating different diseases.

2. Application performance of CRISPR

2.1. Duchenne muscular dystrophy (DMD)

DMD is a fatal X-linked genetic disease, and the dystrophin genes mutate and then cause muscle degeneration. The conventional therapy method includes steroid and gene substitution, which shows no significant effect. The gene editing method-CRISPR-Cas9 provides a new way for precise repair of pathogenic mutations. Using adeno-associated virus (AAV) to deliver CRISPR-Cas9 system, the mutant exon 23 in the dystrophin gene was targeted [5], and some functional protein expression was restored. The subjects are mdx mice (DMD model mice), divided into treatment group and control group. The evaluation indicators are dystrophin protein expression level, muscle tissue pathology, exercise ability (treadmill test). This method shows a gene repair effect. The expression of dystrophin protein in the muscle tissue of mice treated with CRISPR, was restored to 3%-15% of the normal level. The function improvement is muscle fiber necrosis is reduced and muscle strength is enhanced in mice in the treatment group (running endurance is improved by 20%-30%). It has a long-term effect. After a single treatment, dystrophin expression can last for at least 6 months, and no obvious immune rejection or off-target effect has been observed. This study proves for the first time that

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CRISPR-Cas9 can repair DMD pathogenic mutations in living animals, laying the foundation for clinical transformation.

2.2. B-hemoglobin disease

B-hemoglobin disease (such as β-thalassemia and sickle cell disease) is caused by mutation of the β-bed protein gene. Fetal hemoglobin (HbF) can replace defective β-bead protein, but HbF expression in adulthood is inhibited by BCL11A. This study reactivates HbF through CRISPR-targeted BCL11A enhancers [6]. It can design sgRNA-targeted BCL11A red blood cell-specific enhancers, and electrotransfer CRISPR-Cas9 can be used for patient hematopoietic stem cells (HSCs), as shown in Figure 1. In vitro differentiation evaluates HbF expression, and it is transplanted to immunodeficient mice to verify long-term hematopoietic reconstruction ability. It can be used to increase editing efficiency, where more than 80% of HSCs achieved targeted editing, and the expression of BCL11A decreased by 70%.

It can be also used to increase HbF level. The proportion of HbF in red blood cells after editing increased from <10% to 30%-40%. Animal model maintained high HbF levels for 6 months after transplantation, and did not affect the function of hematopoietic stem cells. The study provides preclinical evidence for the “one-time treatment and lifelong cure” strategy of β-hemoglobin disease, and the relevant therapies have entered clinical trials such as CTX001.

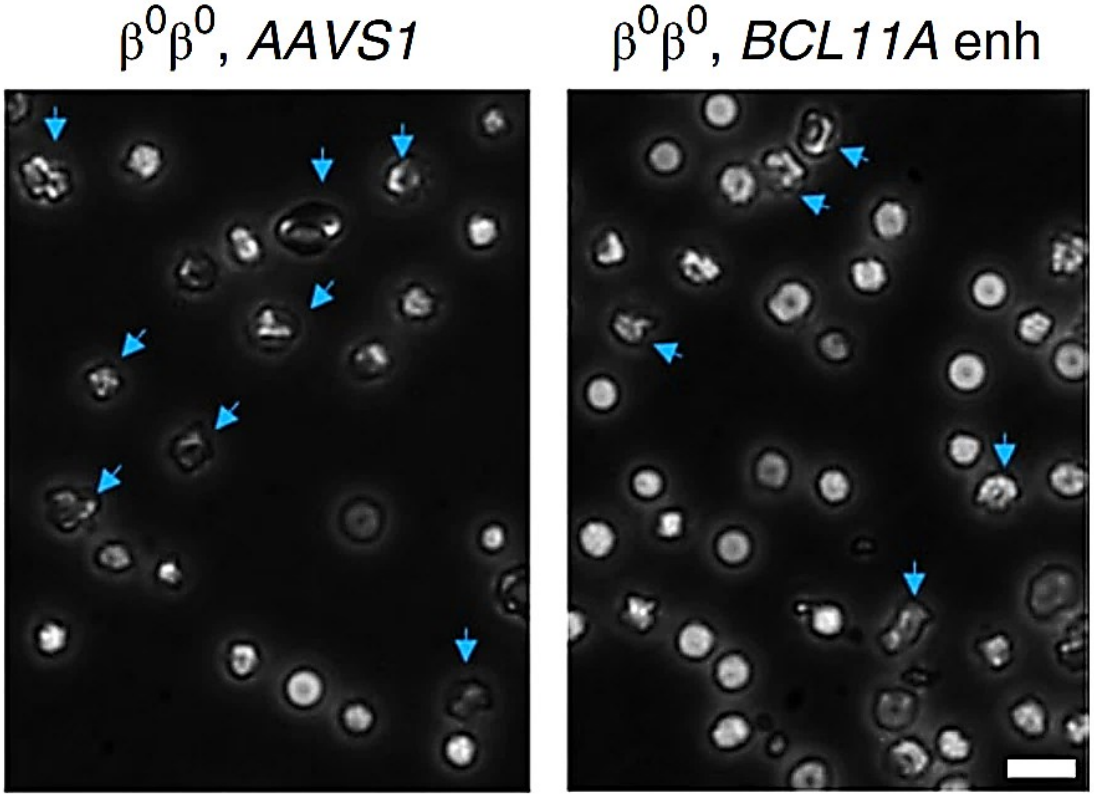

Figure 1: The appearance of enucleated red blood cells after BCL11A enhancer editing [6].

2.3. Hereditary tyrosinemia type I (HT1)

The HT1 leads to the accumulation of toxic metabolites in the liver due to FAH gene mutation. Traditional treatment requires lifelong medication and is prone to recurrence. This study tries to permanently repair mutations through base editing which without DNA double-strand breakage [7]. Using AAV to deliver adenine base editor (ABE), it can target and correct the pathogenic point

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mutation (A→G) of the FAH gene. And it can evaluate liver function, metabolite levels and survival rate in adult HT1 model mice. It can increase editing efficiency, where about 30% of alleles in hepatocytes are corrected and FAH protein expression is restored. It shows a good therapeutic effect, where blood toxic metabolites (succinylacetone) decreased by 90% and the survival rate increased from 0% to 80%. In addition, this method shows a great safety. No off-target editing or elevated liver damage markers were detected. For the first time, it has been proven that base editing can treat metabolic liver disease, providing a safer gene therapy strategy for single-base mutation diseases.

2.4. Herpes simplex virus (HSV) keratitis

HSV keratitis can cause blindness, and existing antiviral drugs cannot remove latent infections. This study explores the possibility of CRISPR directly cutting the HSV genome to block the recurrence of the virus [8]. It can use Cas9-sgRNA to target the UL8 gene of HSV and deliver it to the mouse cornea through nanoparticles, post-infection treatment, detecting viral load, corneal lesions and immune response. It shows a good antiviral effect. The viral DNA of the CRISPR group was reduced by 95%, and the corneal inflammation score was reduced by 70%. The problem of latent infection is relatively small. The HSV reactivation rate of the treatment group is significantly lower than that of the control group (20% vs 80%). High safety does not cause corneal scars or autoimmune reactions. It provides concept verification for CRISPR antiviral treatment, which may be applied to other DNA virus infections (such as hepatitis B and HPV) in the future.

2.5. PD-1

PD-1 is a T cell immune checkpoint molecule, and the tumor microenvironment inhibits T cell function through the PD-1/L1 pathway. This study combines CRISPR and CAR-T technology to enhance anti-tumor immune response [9]. T cells were isolated from NSCLC patients, PD-1 was knocked out with CRISPR, and in vitro amplification was reinfused to the mouse model. It can monitor tumor volume, T cell infiltration and cytokine secretion. Good tumor control reduced the tumor of the PD-1 knockout group by 60% and extended the survival by 2 times. The secretion of IFN-γ in T cells after editing is increased, and the regulatory T cells (Treg) are reduced in the tumor microenvironment. There is a good safety. No cytokine storm or autoimmune toxicity has been observed. It has created a new paradigm of CRISPR combined with cell immunotherapy, and a number of CAR-T clinical trials edited by PD-1 are currently underway.

3. Conclusion

CRISPR gene editing technology has made breakthrough progress in the treatment of many diseases in recent years. In terms of genetic diseases, this technology has not only successfully repaired the dystrophin gene mutation of the mouse model of DMD and significantly improved muscle function, but also accurately edited the BCL11A enhancer of hematopoietic stem cells. It brings hope of cure to patients with β-thalassemia and sickle cell disease. What's more exciting is that the new generation of base editing technology can efficiently correct pathogenic mutations in hereditary tyrosinemia without causing DNA double-strand breakage, showing higher safety. In the field of antiviral treatment, CRISPR significantly reduced the recurrence rate of viral keratitis by targeting the HSV genome. In terms of tumor immunotherapy, combining CRISPR and CAR-T technology, the killing effect of T cells on non-small cell lung cancer has been significantly enhanced by knocking out the PD-1 gene. CRISPR technology is expected to make greater breakthroughs in the following aspects. With the optimization of the delivery system (such as new AAV carriers and lipid nanoparticles), the editing efficiency will be further improved and the side effects will be significantly reduced. The maturity of new technologies such as single-base editing and pilot editing will greatly expand the

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therapeutic and the scope of genetic diseases. In addition, the application potential of CRISPR in infectious disease prevention and control, agricultural breeding, synthetic biology and other fields has yet to be fully exploited. However, the technology still faces challenges such as off-target effect, immunogenicity and ethical norms. With the gradual resolution of these problems, CRISPR is expected to become a conventional treatment in the next 10-20 years, bringing revolutionary changes to human health.

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