Research Progress and Advantages of RNA Bridge Gene Editing Technology

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Abstract: The purpose of this paper is to deeply discuss the principle, application, and comparative advantages of gene editing technology, especially RNA bridge gene editing technology. Firstly, the background and significance of the development of gene editing technology were introduced, and then the principle and application fields of RNA bridge gene editing technology were elaborated, including the achievements in disease treatment and agriculture. Then, the principles, challenges, and applications of traditional gene editing technologies (ZFN, TALEN, CRISPR-Cas9) are summarized. Through comparison, the advantages of RNA bridge gene editing technology compared with traditional technology, such as high specificity, high editing efficiency and strong flexibility, were analyzed. Finally, this paper summarizes and prospects the RNA bridge gene editing technology, and points out that although it has potential, it still needs to be further studied on its feasibility, safety, efficiency, and specificity in different species and cell types.

***Keywords*:** gene editing technology; RNA bridges; traditional techniques; Comparison of advantages

1. Introduction

The development of gene editing technology is of critical importance for an in-depth understanding of gene function and advances in basic research, medicine, and agriculture. To meet the demand for precise gene manipulation, traditional gene editing technology has emerged and continues to develop. With the deepening of research, it has been found that gene editing has great potential in the fields of medicine, pharmacy and agriculture, so it is of great significance to explore and study more advanced gene editing technologies [1].

2. Basic overview of RNA bridging technology

2.1 Principle

RNA bridging is a new type of gene editing method. It contains a region that specifies the donor DNA sequence and another region that specifies the genomic insertion site. First, it guides the recombinase to a specific location, and the recombinase cleaves the upper strand of the target DNA and the donor DNA under its guidance to form a special structure, then the sheared DNA strand is exchanged, and finally the recombinant enzyme cleaves the lower strand of the DNA to complete the recombination, to achieve precise insertion, inversion, or deletion of the donor DNA into the target genomic locus [1][2].

2.2 Research Progress and Application

On June 26, 2024, Patrick Hsu et al. from the Arc Institute, in two concurrent research papers in Nature, detailed a bridge RNA-guided recombinase. This recombinase can insert, invert, or delete long DNA sequences at specific genomic loci, which directly enables novel genome editing capabilities. These capabilities are of great significance in the field of disease treatment and medicine. By precisely manipulating the genome, it has the potential to correct the genetic defects underlying genetic diseases and cancer, thus providing new ideas and methods for their treatment. This, in turn, paves the way for the development of new biological drugs. In agriculture and animal husbandry, the precise gene editing ability allows for the acceleration of the improvement of agricultural and livestock breeds.This study demonstrates the feasibility of manipulating RNA for applications.

3. Basic overview of gene editing technology

3.1 ZFN gene editing technology

3.1.1 Principle

ZFN editing technology recognizes the binding target DNA sequence by designing and synthesizing a specific ZFP and

then uses Fokl to account for the cleavage activity of the endonuclease to achieve double-strand breaks and gene editing of the DNA.

3.1.2 Challenges and Application Limitations

The use of ZFN technology in clinical treatment presents many challenges. Although simple and easy, the success rate is low due to the interaction between zinc fingers [5]. The introduction of ZFN protein may trigger an attack on the immune system, and the current technology seems to be only able to manipulate cells extracted from patients in vitro, and direct injection of genes in vivo is inefficient. The precision of ZFN manipulation needs to be carefully evaluated, as even the smallest errors can cause cells to become cancerous. However, the ZFN cleavage mechanism leads to less accuracy than expected, and its cleavage requires FokI cleavage region dimerization and unit - bound DNA, and heterodimers, homodimers, and single ZFN unit - bound DNA can cause cleavage, resulting in different recognition sequences, which may cleave genomic pseudopalindromic sequences and bring ZFN toxicity.

3.2 TALEN gene editing technology

3.2.1 Principle

TALEN editing technology recognizes and binds the DNA sequence of interest by designing and synthesizing specific TALE, and then utilizes the cleavage activity of FokI endonuclease to achieve double-strand breaks and gene editing of the DNA.

3.2.2 Application in the study of mutant mitochondrial DNA

In the study of mutant mitochondrial DNA, TALEN technology mainly plays the role of targeted recognition and cleavage of mutant mitochondrial DNA. The researchers used an AAV9 to deliver mito-TALENs. The two parts of TALEN are targeted to recognize sequences on either side of mutant mitochondrial DNA, and each TALEN is fused with a FokI endonuclease domain. When administered to a mouse model harboring heterogeneous mtDNA mutations, FokI was targeted by TALE to form a dimer and then cleave the target, effectively reducing the load of mutant mitochondrial DNA in muscle and heart that was stable over time [7].

3.2.3 Operational Limitations

TALEN editing technology is cumbersome and slow to splic, making it impossible to build in large quantities [6].

3.3 CRISPR - Cas9 Gene editing technology

3.3.1 Principle

CRISPR-Cas9 editing technology utilizes sgRNAs in the CRISPR-Cas9 system to recognize and bind the DNA sequence of interest, which then leads the Cas9 endonuclease to cleavage and edit the DNA.

3.3.2 Application and Potential Problems

Application in producing healthy animals with beneficial economic traits: structures required for CRISPR/Cas9 function can be microinjected directly into fertilized eggs to obtain farm animal offspring with desirable traits. The technology, which inactivates genes and generates site-specific mutations, has been used to improve livestock quality, create large numbers of genome-edited animals, and significantly improve meat yield, disease resistance, and more. Application in viral genome modification: CRISPR/Cas9 has been successfully used to generate new viral mutants, purify cell cultures and sensitive organisms, and obtain attenuated virus variants as vaccine prototypes in the absence of viral replication in cells.

4. Advantages of RNA bridging compared to traditional gene editing technologies

RNA bridge gene editing technology has many advantages over previous gene editing technologies.

(1) Higher precision: More precise edits can be made in the genome, reducing off-target effects and unwanted gene mutations.

(2) Higher editing efficiency: Multiple gene loci can be edited at the same time to improve editing efficiency.

(3) More flexibility: Different editing effects can be achieved by changing the RNA sequence, with higher flexibility and programmability.

(4) Wider application: It can be applied to a wider variety of organisms and cell types, including cells that are difficult to handle with traditional gene editing techniques.

(5) Easier to operate: This technique uses RNA as a guide and is easier to modify than existing gene editing techniques that use conventional recombinases, which utilize more complex protein-DNA binding sites.

5. Summary and outlook

RNA bridge gene editing technology is a new biological editing mechanism, which can generally modify the genetic material through sequence-specific embedding, excision, inversion, etc., and realize the single-step rearrangement of basic DNA, which is a simpler genome editing method. This technique has currently demonstrated genome editing in bacteria, but further evaluation of its feasibility and safety in different species and cell types, including mammalian cells, is still needed. Compared with traditional editing technology,it is more accurate and effective,and has great potential for development in the fields of medicine, pharmacy, biology, etc., and the "RNA bridge" is expected to perform more accurate and effective large-scale genome editing than existing technologies, and can mediate recombination rather than cause fractures that need to be repaired. However, this technique is currently only validated in bacteria, and further research is needed on its efficacy and safety in other organisms.

References

[1] Zhao Xixi. "RNA Bridge" New gene editing technology is introduced — Journal of Medical Science:009.

[2] Li Qi, An Hailong. ZFN/TALEN Technology and Crop Genetic Improvement. Chinese Journal of Plant Physiology. 2013;49(07):626-636.

[3] Durrant MG, Perry NT, Pai JJ, et al. Bridge RNAs direct programmable recombination of target and donor DNA. Nature. 2024;630(8018):984-993.

[4] Yu Q, Liu S, Yu L, et al. RNA demethylation increases the yield and biomass of rice and potato plants in field trials. Nat Biotechnol. 2021;39(12):1581-1588.

[5] Khalil AM. The genome editing revolution: review. J Genet Eng Biotechnol. 2020;18(1):68.

[6] Zhang HX, Zhang Y, Yin H. Genome Editing with mRNA Encoding ZFN, TALEN, and Cas9. Mol Ther. 2019;27(4):735-746.

[7] Bacman SR, Kauppila JHK, Pereira CV, et al. MitoTALEN reduces mutant mtDNA load and restores tRNA(Ala) levels in a mouse model of heteroplasmic mtDNA mutation. Nat Med. 2018;24(11):1696-1700.

[8] NI Peiling, LIU Chang, CHEN Lingyi. DNA scissors — TALEN□CRISPR/Cas. Chinese Journal of Cell Biology. 2014;36(01):5-11.

[9] Janik E, Niemcewicz M, Ceremuga M, et al. Various Aspects of a Gene Editing System-CRISPR-Cas9. Int J Mol Sci. 2020;21(24).