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Principles and research progress of gene editing technology

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Abstract. Gene editing is a new genetic engineering technology. Given the quick development of molecular biology technology, genome editing tools have made great progress from the initial ZFNs and TALEN technology to CRISPR-Cas9 technology in recent years. Gene editing is the modification of specific targets in the genome of an organism to change its genetic information and phenotypic characteristics. This study analyzed the principle and research progress of gene editing technology through the data. For example, CRISPR-Cas9 can protect against secondary viral attacks, and ZFNs can be cleaved on any genomic sequence. Studies have found that there are still great challenges in the clinical field of genetic technology. Including the need for standardized safety protocols, regulatory transparency, and pricing frameworks, which increase the risk of further mutations. This article elaborates on the role and future development of gene editing in medicine, and provides a reference for the future field of gene editing technology.

Keywords: Gene editing technology, clinical, ZFN, CRISPR-Cas9.

1. Introduction

Gene editing is a new type of genetic engineering technology that has become the focus of attention in the biological world. Gene editing is the modification of specific targets in the genome of an organism to change its genetic information and phenotypic characteristics.

In the past decade, gene editing technology has made unprecedented progress, bringing a new era to the field of genetics. Gene editing has been around for over 30 years, but newer technologies like CRISPR have made a significant change. The innovative CRISPR-based gene editing treatment Casgevy, developed by Vertex Pharmaceuticals and CRISPR Therapeutics, was approved by the U.S. Food and Drug Administration (FDA) on December 8, 2023, for the treatment of sickle cell disease (SCD).More such treatments are expected in the future [1].There are also other recent developments in gene editing. One of them is gene modification therapies (GMTs).GMTs are making progress toward clinical use. Hemophilia, retinitis pigmentosa-related blindness, and severe combined immunodeficiency have all been treated with the use of clinical trials. As more and more rare diseases are identified as having genetic causes, GMTs are becoming increasingly important. Meanwhile, gene editing is already being used to improve crops. For example, one researcher edited 10 varieties of cherry and grape tomatoes to create dwarf versions that don't require stakes. The emergence of genetic technologies, such as CRISPR-Cas9, represents a great role in almost all biological fields, especially in genetics, evolution and clinical. In March 2017, a significant genetic research finding that used the CRISPR-Cas9 technique to save blind mice was published in the British journal "Nature Communications." The CRISPR-Cas9 technique has the potential to treat a variety of underlying genetic disorders that result in retinitis pigmentosa, as

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demonstrated by its ability to stop retinal deterioration in mice [2].Zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) are two significant gene editing methods among many other cutting edge technologies. One of the earliest methods for editing genes in a variety of organisms, including bacteria, fungus, plants, and animals, is zinc-finger nuclease (ZFN). They are made up of a Fok1 domain that cleaves the target sequence and a synthetic zinc-finger DNA-binding domain that is intended to accurately bind the target DNA sequence. Any genomic sequence can be sliced with ZFN [3].TALE nucleases (TALEN) sparked the revolution in genome editing by imitating Zinc-finger nucleases (ZFN). It was the first device to be relatively easy to design and manufacture, and to target gene editing sites with high precision, efficiency, and specificity. TALEN was quickly applied to crop, livestock, and a variety of other genome edits.

However, the therapeutic promise of genome editing has not been fully explored and many challenges remain, including the need for standardized security protocols, regulatory transparency and pricing frameworks, which Increases the risk of further mutations. The exploration of these questions can have great implications for clinical treatment and genetics. With a large number of gene editing studies, few of them have been applied to clinical practice, so it is necessary to review current progress in this area, especially the application of genome editing in vivo and clinical practice, in order to better study the future trend of gene editing field [4] .In order to comprehend the evolution of gene editing technology in recent years, this study will employ data analysis to examine the and research advancements of the technique.

2. Principles of gene editing

Gene editing, sometimes referred to as genome editing or genome engineering, is a kind of genetic engineering in which genetic material is added, removed, modified, or replaced to change a living organism's DNA. Today's gene editing tools allow scientists to target specific regions of the genome instead of randomly inserting genetic material, in contrast to older genetic engineering techniques. The process involves using an enzyme called a nuclease to target specific DNA sequences and cut them, so that scientists can delete, add or replace existing DNA. There are also many steps involved in gene editing. The steps go from design to deliver, to repair, and finally to analysis, as shown in Figure 1.

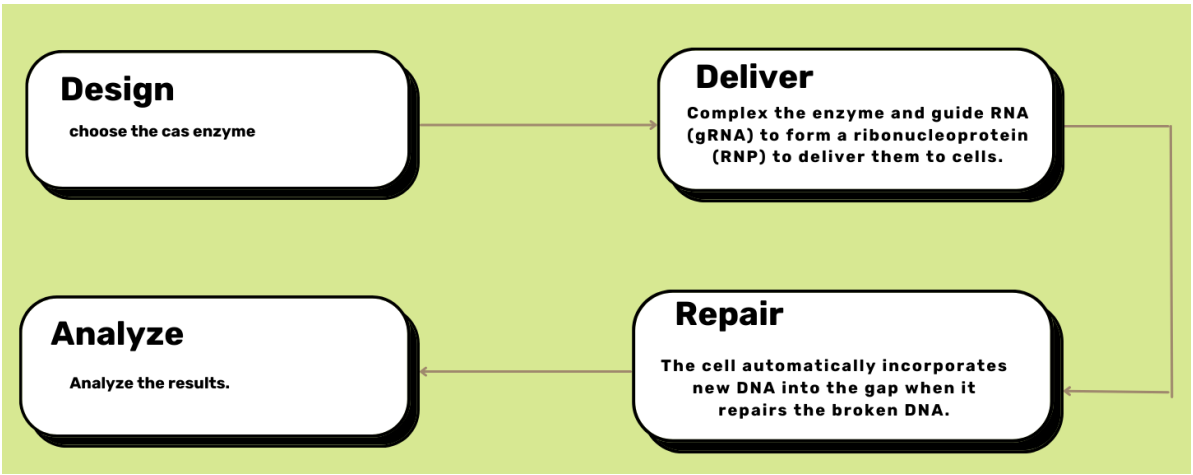

Figure 1. Steps involved in gene editing (Picture credit: Original)

Site-specific double strand breaks (DSBS) are produced at certain genomic regions during the gene editing process. Simultaneous gene editing and DSBS repair are accomplished by homologous recombination (HR) or non-homologous end joining (NHEJ). One method for repairing different types of DNA damage, such as interstrand crosslinks, single-stranded DNA gaps, and double-strand breaks (DSBs), is homologous recombination (HR). Among these, single unrepaired double-strand breaks can result in aneuploidy, genetic abnormalities, or even cell death. Double-strand breaks are particularly

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hazardous. The strand exchange protein, also referred to as Rad51 in eukaryotic cells, plays a crucial part in HR. Pre-, synaptic, and post-synaptic stages of HR are where Rad51 operates. Single-stranded DNA (ssDNA) is loaded with Rad51 during the presynaptic stage. The resulting right-handed RAD51-ssdna filament, also known as the presynaptic filament, is made up of six Rad51 molecules, each of which has 18 nucleotides turned in a single helix. Rad51 facilitates the creation of heteroduplex DNA (D-loop) by fostering the physical fusion of invasive DNA substrates with a homologous double-stranded DNA template during synapses. Here, the donor and invading ssDNA strands are accommodated within the filaments to produce Rad51-dsDNA filaments. Ultimately, Rad51 separates from the double-stranded DNA during post-synaptic DNA synthesis, which uses the 3' end of the invasion as a primer. This exposes the 3' -OH needed for DNA synthesis [5]. When used in its traditional or classical form, NHEJ operates in around 30 minutes, which is a very quick operation.

It does not have the inherent ability to restore the original sequence close to the DSBS; instead, it functions by merely connecting DNA ends. The three subunits of the DNA-pk complex (Ku70, Ku80, and DNA-PKcs) and the LIG4/XRCC4/XLF complex (8–12) are crucial elements of NHEJ. [6]

3. Current gene-editing technologies

Nowadays, there are many kinds of gene editing technologies in development, such as CRISPR-Cas9, ZFN and TALENs. These gene editing techniques can be used in gene modify and immune virus attack.

3.1. CRISPR-Cas9

CRISPR-associated protein 9 and clustered regularly interspaced short palindromic repeats are referred to as CRISPR-Cas9. By creating site-specific double-strand breaks in the target DNA, the CRISPR-associated (Cas) system thwarts conjugative plasmids and invasive phages by the use of the double-RNA-guided DNA endonuclease Cas9 [7]. TracerRNA and crRNA joined to generate functional sgRNA. The sgRNA directs the Cas9 nuclease to particular nucleotide sequences, while longer RNA scaffolds bind to DNA targets mostly through the crRNA portion of the sgRNA. An sgRNA can be created to attach to particular genomic regions by modifying its crRNA sequence to be complementary to the target DNA sequence.

The naturally existing genome-editing mechanism that bacteria employ as an immune system is the model for CRISPR-Cas9. Bacteria that are virus-infected seize tiny bits of viral DNA and splice it into their own DNA in predetermined ways to create what are known as CRISPR arrays. Bacteria can "remember" viruses (or closely related viruses) thanks to CRISPR arrays. The bacteria create RNA fragments from the CRISPR array that may identify and adhere to particular viral DNA areas in the event that the virus strikes again. The virus is then rendered inoperable by the bacteria's employment of Cas9 or a related enzyme to cleave the DNA.

3.2. ZFN

Zinc finger DNA-binding domains (ZFNs) can be designed to cleave on any sequence of DNA. The monomer creates a dimer and cleaves the target DNA spot when it is joined to the zinc finger array by the Fok1 nuclease domain. The zinc finger's site-specific binding to the target DNA sequence is mostly caused by the amino acid at the beginning of its alpha helix, which may be changed to attach to any specific target sequence. Two ZFNs are needed to bind opposite DNA cleavage sequences and cleave the target DNA site by dimerizing the fok1 nuclease domain when the ZF-binding site is palindromic. In the spacer area between the two opposing strands, Fok1 dimerizes and creates a double-strand break. Natural repair mechanisms including homologous recombination (HR) and non-homologous end joining (NHEJ) are then used to fix DSBS.

In one study, xeno-donor DNA molecules were introduced into maize cells and ZFNs were successfully incorporated into the targeting gene IPK1 at a specific position in the maize genome to increase the frequency of gene targeting. Furthermore, heritable gene targeting events that were carried over to the following generation were seen in 20% of the chosen lines.

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Furthermore, because ES cell culture technology was lacking, gene targeting in animals like rats and cattle had previously been challenging to accomplish using conventional methods. However, ZFN technology has made it possible for researchers to carry out effective gene knockout or knockin in these animals [3].

3.3. TALENs

Because TALEN technology has better sequence accuracy than other DNA-binding proteins like ZFNs, it has revolutionized applied biology. TALENs, a second-generation gene editing tool, have configurable DNA-binding repeats and the fok1 nuclease domain found in Transcription Activator-Like Effector (TALE) proteins. Targeted base editing of the mitochondrial genome is now possible thanks to the recent successful replacement of C:G pairs with T:A in the Arabidopsis thaliana mitochondrial genome using mitochondrial targeting TALEN cytidine deaminase, which did not alter the genome's structure and was stably passed down to the following generation.

The localization of TALEs and TALEN has been made simpler by the production of TALEN-terminal domain variants that identify all bases by directed evolution techniques. Repeat specificity can be freely selected to match any target sequence that is required, however in TALE or TALEN targets, it is advised to contain at least two or three Cs or Gs and incorporate rvd HD and NN, respectively. TALEN pairs can be precisely cut to the appropriate nucleotides in the target DNA sequence because the amount of TALEN repeats can be varied somewhat widely [8].

4. Future trends in gene editing technology

First off, modifying the human genome through genome editing with tools like CRISPR-Cas9 raises ethical concerns. The majority of the alterations brought about by genome editing are restricted to somatic cells, or cells other than sperm and eggs. These alterations are unique to certain tissues and are not inherited by subsequent generations. Nonetheless, alterations to the genes of sperm, egg, or embryo cells may be inherited by subsequent generations. Many ethical questions are raised by genome editing in germline cells and embryos, such as whether or not it is appropriate to permit the use of this technology to improve traits that are often associated with humans, like height or intelligence. Due of ethical and security concerns, embryonic genome editing and germline cell editing are now prohibited in the United States and many other nations. Therefore, we need to conduct additional study on CRISPR-Cas9 and apply it in appropriate contexts in order to aid in its development in the future. The second major issue with zinc fingers (ZFN) is that their specificity might overlap and be dependent on the environment of the surrounding DNA. There are also only a few suitable targeting locations, and every modification must create a zinc-finger array. Complex, pricey, and technically demanding modules with a certain level of complexity are among ZFNs' drawbacks [3].

As a result, the nation and government must allocate enough money for a thorough examination of this technology. Thirdly, the issue with TALENs technology is that the thymidine nucleotide at the 5'end of the target DNA influences the half-repeated sequence's attachment capacity, which restricts the ability to select the appropriate target sequence. The selection of mutant TALEN N-terminal domain variants that bind to A, G, or C, however, may be able to overcome this restriction.

5. Conclusion

Through research, the paper found that gene editing has been widely studied, but has not yet been fully developed in some aspects. For example, in clinical practice, gene editing technology has problems in both safety and technology. It seems that there is still a lot of work to be done, so more efforts need to be spent on researching gene editing in the clinical field. Scientists should spend more energy on gene editing research in the clinical field. At the same time, people also need to consider ethical issues about gene editing, such as whether to design babies. This study lets scientists know that more energy needs to be spent on researching clinical gene editing technologies in the future. Future clinical trials rely on these gene edits Technology's advancement and rapid growth have also aided in the growth of this subject and will continue to present chances for the advancement of genetics, molecular biology, and

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biotechnology. Future gains in the field of genome editing are highly likely given the potent genome editing tools' ongoing diversification, extension, and competition. The current gene editing technology cannot be widely used in clinical practice. This study can be further refined in the future to facilitate in-depth research on this topic and make more discoveries in clinical gene editing technology.

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