The Development, Application and Prospect of Gene Editing Technology

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Abstract. The recombinant DNA technology that emerged in the 1970s, the in vitro amplification technology of specific DNA sequences, and the gene cloning technology have become the routine techniques in today's laboratories, greatly promoting the rapid development of modern life sciences and bringing them into the era of modern molecular biology. With the continuous advancement of sequencing technology, driven by the Human Genome Project, the genomic maps of multiple species have been resolved, various omics have emerged, and various bioindustries such as gene chips, bio-cloud computing, and personalized molecular diagnosis have flourished. Furthermore, life science has entered the post-genomic era, and humans have gained a further understanding of various complex life phenomena. Gene editing technology is an engineering technique that can precisely "edit" the target genes of the organism. Recent years, it has developed very rapidly and has impacted all fields of life sciences. It is the most fiercely competitive next-generation core disruptive biotechnology worldwide. At present, gene editing mainly includes four types.This article reviews the development history of this technology and application of CRISPR/Cas9 technology in the disease treatment, the food industry and agriculture. Meanwhile, it looks forward to the existing problems and challenges.

Keywords: Gene editing; CRISPR/Cas9; Disease; Agriculture; Industry.

1. Introduction

Genetic engineering technology enables the bridging of insurmountable gaps between biological species and breaking the species barriers that conventional breeding struggles to overcome. It has opened up new frontiers for modifying the genetic traits of organisms in a short period. Zhu Jiankang once remarked "Genetic engineering employs gene editing, genetic engineering, recombinant DNA technology, molecular cloning, and gene cloning, and is regarded as the fastest-developing, most innovative, and widely applicable core technology in biotechnology."

Gene editing, particularly the CRISPR-Cas9 system, has advanced the field by leaps and bounds, enabling precise cutting and modification of DNA sequences and bringing revolutionary changes to the biosciences [1]. Currently, most discussions on gene editing focus on a single specific field, with relatively limited research on its applications across multiple disciplines. Therefore, this paper provides the evolution of this technology, elaborates on its applications in various fields, and offers insights into the future prospects of gene editing, aiming to serve as a reference for researchers and policymakers in related fields.

2. Evolution

2.1. Homing Endonucleases

In the late 1980s, researchers discovered that introducing double-strand breaks (DSBs) at target sites could significantly enhance the integration efficiency of the desired gene. Homing endonucleases were the first tools used to create specific DSBs in the genome. These enzymes recognize DNA fragments ranging from 14 to 40 base pairs (bp), and after cleavage, the breaks are predominantly repaired via Non-Homologous End Joining (NHEJ).

However, this system has notable limitations: First, it exhibits strict sequence specificity, with each enzyme recognizing only a unique, extended sequence, making it difficult to universally apply for

editing arbitrary target genes. Second, the NHEJ repair mechanism operates without the involvement of an exogenous DNA template, thereby preventing precise genomic modifications [2].

2.2. ZFN and TALEN Technologies

The ZFN (Zinc Finger Nuclease) technology utilizes a fusion nuclease composed of zinc finger proteins (ZFPs) and the FokI restriction endonuclease. ZFPs can recognize and bind to the specific DNA sequences, while dimerized FokI, possessing endonuclease activity, cleaves the recognized sequence, thereby inducing DSBs. Subsequently, the DSBs are repaired via either NHEJ or HR.

Similar to ZFN, the core components of the TALEN are DNA-binding proteins and the FokI restriction endonuclease. The key difference lies in the fact that TALEN employs TALEs, rather than ZFPs, fused with the FokI [3].

The sequence-specific DNA recognition in ZFN primarily relies on ZFPs. However, the design cost of ZFN is relatively high while the efficiency is low. In contrast, TALEN's sequence specificity depends on the repeat-variable diresidues (RVDs) within TALE proteins. Due to the modularity and ease of construction of TALE/TALEN systems, artificially engineered TALE proteins have broader applications in gene editing and transcriptional regulation compared to ZFNs. For instance, Yang et al. conducted the first systematic analysis of base-recognition preferences across all 400 possible RVD combinations, while Zhang's team further elucidated the mechanisms by which TALE proteins specifically recognize epigenetic modifications. Nevertheless, both the two technologies rely on the protein-mediated DNA sequence recognition, and complexity of the assembly remains a major obstacle limiting their widespread application in gene editing [4].

2.3. CRISPR/Cas9 technology

CRISPR/Cas9 based on RNA-guided endonucleases, which can modify the naturally occurring bacterial immune system. The clustered intergranular short palindromic reduplication sequence (CRISPR) was first discovered in the genome of the Escherichia coli in 1987. For the following nearly 20 years, the functions remained unclear. In 2007, Horvath et al. from Danisco Company in the United States first confirmed through viral infection experiments that it plays a role in resisting the viral infection in the bacteria [5].

In CRISPR/Cas9, pre-crRNA is processed into mature crRNA by the Cas9 and the RNaseⅢ, and forms complexes with tracrRNA and Cas9. This complex is precisely located through complementary pairing of crRNA and target DNA and PAM recognition, inducing DNA unwinding to form an R-loop. Subsequently, the HNH domain of Cas9 cuts the complementary chain, and the RuvC domain cuts the non-complementary chain, resulting in the breakage of the double chain. If PAM exists, cells can be repaired through the HR or NHEJ mechanism to achieve gene editing [3,6].

After the emergence of CRISPR technology, it has demonstrated great application value and potential in disease treatment, agricultural and food industry. Scientists around the world have also been actively conducting research on the technological upgrade and application.

3. Application of CRISPR/Cas9

3.1. Disease Treatment

As a high-throughput genetic screening tool, it has been successfully applied to analyze the functions and biological pathways of cancer-related genes, such as screening potential disease treatment targets. By leveraging the large-scale genomic screening function of CRISPR/Cas9, genes or genomic regions related to diseases can be identified, and the roles in the occurrence and development of diseases can be deeply explored. This will enable a more comprehensive understanding of the molecular mechanisms of disease occurrence and development of new targeted therapeutic sites [7].

Human papillomavirus 16 (HPV16) is recognized as the primary cause of cervical cancer. Utilizing this system to disrupt the E7 gene of HPV16 can promote apoptosis in cervical cancer cell lines, inhibit cell proliferation, and thereby treat malignant cervical carcinoma caused by HPV infection [8]. Furthermore, similar CRISPR/Cas9-edited T-cell therapies have been trialed in patient populations with multiple myeloma, synovial sarcoma, and myxoid liposarcoma, indicating broad prospects for treatment of the various solid tumors.

In addition to the cancer,it can specifically target and cut the conserving sites of hepatitis B virus (HBV) genome, strongly inhibiting its expression and replication. The key reason why hepatitis B is difficult to cure lies in the long-term existence of a covalentially closed circular DNA in HBV-infected cells, which is the original template for RNA replication of the HBV genome and pregenome. Directly targeting cccDNA with Cas9 endonuctidase to cause DSB can achieve the effect of inactivating cccDNA to resist HBV infection, and there is no off-target effect [9,10].

This technology also plays a significant role in the field of new drug research and development. It can address the issue of individual nucleotide variations causing diseases and can also be applied to source treatment, triggering a "revolution" in new drug development. With the continuous progress and application of technology, gene editing technology is expected to play a more important role in the future research and development of new drugs.

As safety concerns are systematically addressed, the application of CRISPR therapeutics in treating infectious diseases in humans will become increasingly viable.

3.2. Agricultural Field

At present, CRISPR/Cas9 gene editing has become an important tool for improving crop traits. Taking acetyllactate synthase as an example, researchers successfully replaced two specific amino acid residues through this technology and cultivated several new herbicide-resistant rice varieties. This technology can not only utilize the NHEJ repair mechanism to create mutant populations of rice and wheat, providing materials for functional genomic research and genetic improvement, but also achieve precise modification of specific protein sites through base editing. For example, through targeted mutagenesis of the amino acid transporter genes, the quality of rice has been effectively improved. These advancements have significantly expanded the editing range of genes related to important traits in rice and wheat, providing innovative technical strategies for crop breeding [11].

This technology holds great potential in enhancing plant resistance. With the continuous increase in global climate change and ecological pressure, the stress faced by plants is also becoming increasingly severe. These stresses include biological stresses (such as diseases, etc.) and abiotic stresses (such as drought, salinization, high temperature, etc.). Targeted editing of genes related to disease resistance, insect resistance, drought resistance and salt tolerance in plants can cultivate crop varieties that are more adaptable to complex environmental conditions. For example, editing the GmTAP1 gene in soybeans can make the plants show enhanced resistance to three soybean Phytophthora (P. sojae) strains P231, P233 and P234. Editing ZmGA20ox3 to disable its function can enhance the drought tolerance of corn seedlings and increase the plant height of corn [12].

In addition, if temperate fruit trees break the dormancy, they need to rely on the accumulation of low temperatures, and the warm winters caused by climate change may disrupt this process. Voogd et al. identified a MADSbox gene AcFLC-like homologous to Arabidopsis thaliana FLC. Its expression was induced by low temperature and the germination time was regulated through epigenetic modifications (such as demethylation of histone H3K27me3). By encoding the activation sub-region of this gene through CRISPR/Cas9, its low-temperature dependence can be broken, enabling kiwifruit to germinate normally even in non-optimal climates, providing an adaptive solution for addressing climate change [13].

3.3. Industrial Field

Escherichia coli (E. coli) is widely used in industrial fermentation due to its simple cultivation methods, short proliferation cycle, and strong environmental tolerance. Yang et al. utilized CRISPR-

mediated homologous recombination technology to simultaneously knockout up to 3 gene loci in E. coli [14].

The industrial strain of Saccharomyces cerevisiae maintains stable production performance in various production environments due to its excellent fermentation conditions and is applied to a wide range of biological products. The technology has greatly shortened the construction time of the Saccharomyces cerevisiae cell factory. Meanwhile, some studies have systematically explored the two major bottleneck problems existing in the application of this system in Saccharomyces cerevisiae - the scarcity of sgRNA high-efficiency expression promoters and the insufficient transformation efficiency of exogenous DNA. A CRISPR/ Cas9 system driven by the PoI Ⅱ promoter for tRNA-sgRNA expression was constructed. By optimizing the thermal shock time parameters of yeast chemical transformation, the results showed that the efficient editing of single gene loci was achieved in the diploid Saccharomyces cerevisiae. Among them, the gene knockout efficiency was the highest (82.23%), which was similar to the editing efficiency obtained by using PoI Ⅲ class promoters and showed more excellent expression stability [15].

4. Challenges Currently faced by Gene Editing Technology

4.1. Off-target Effects and Toxic Reactions

Correspondingly, the editing of non-target genes, known as off-target effects, may lead to serious consequences such as genomic toxicity, inactivation of gene functions, genetic alterations and even carcinogenesis. Therefore, when using gene editing technology, the risks of off-target effects of genes should be fully understood and evaluated. To reduce off-target efficiency, researchers have currently obtained high-fidelity mutants through structural optimization or random mutations. However, the targeting efficiency of these mutants is usually low. Therefore, improving the specificity of Cas9 while maintaining the target editing activity is crucial for exploring high-fidelity and efficient Cas9 tools. Sun et al. recently obtained a novel SuperFi-Cas9 by mutating seven residues based on Cas9. The rate at which this mutant protein lyses target DNA is comparable to that of the wild type, but it has higher off-target recognition performance. The lysation activity of SuperFi-Cas9 was only tested in vitro. Clinical application still requires further research. Continuing to optimize and develop truly high-fidelity mutants will be the research focus in the future [16].

In addition, how to enhance the safety of gene editing technology has always been a difficult and necessary problem to be faced. Toxic reactions caused by gene editing are also very common. Among them, evaluating the sources of genotoxic impurities involved in the production of gene editing tools, analyzing the manufacturing processes, and considering starting substances, intermediate substances, solvents, etc., to avoid the generation and doping of toxic substances, can inspire clinical scholars on how to reduce genotoxic effects

4.2. Ethical Issues

In 2015, the CRISPR/Cas9 technology was first applied to human embryo editing, triggering intense discussions on the ethics and regulation of this technology. The "gene-edited baby incident" involving He Jiankui in 2018 once again brought the ethical issues of gene editing and the related discussions on the construction of governance systems to a climax. After the incident, the global scientific research community responded strongly to it. The organizing committee expressed unexpectation and unease over the incident and emphasized that He Jiankui's behavior was irresponsible and inconsistent with international norms.

In response to the ethical risks arising from human gene editing technology, if people aim to properly address the issue, should not only approach it from a technical perspective but also incorporate a "human" perspective. People should not only focus on the present of individuals but also on the future of the entire human race, while taking into account the interests of both humanity and the entire Earth's ecology. When it comes to the governance of gene editing technology, it is

necessary to change the previous post-event governance approach and incorporate the concepts of prevention, responsibility and mutual benefit into the governance process of the technology.

5. Conclusion

Gene editing technology has broad application prospects and has been applied in multiple aspects such as gene function research, developmental biology research, molecular breeding, and cancer treatment at present. Worldwide, it has also received attention. For instance, the National Institutes of Health (NIH) in the U.S. announced in the early 2018 that it would launch a $190 million research program for somatic cell gene editing. However, when it comes to applying gene editing in clinical practice, there are still some urgent problems to be solved. However, with the continuous efforts of scientific workers, the in-depth research on the regulatory mechanism of gene editing and the more profound discovery of the principles of human diseases, new gene editing tools and methods will keep emerging. Eventually, scientists will obtain ideal gene editing tools that are highly efficient, highly specific, non-off-target, easy to use, and have low sequence requirements.

It is necessary to fully and objectively evaluate gene editing technology and solve the negative problems it brings. While constantly improving and perfecting the technology, legislative supervision and management should also be strengthened. Clinical researchers should cross the river by feeling for the ethical bottom line, and conduct endless exploration of life steadily and comprehensively within the framework of bioethics. Only when it is beneficial to both individuals and society can gene editing technology shine brightly.

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