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Methodological exploration of using gene editing technology to study gene function and disease mechanism

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Abstract. This paper discusses the application of gene editing technology, especially CRISPR-Cas9 system, in the study of gene function and disease mechanism. By modifying the genome sequence at a fixed point, gene editing technology uses specific nucleases to induce double strand breaks, trigger DNA repair mechanism, and realize accurate gene modification. This paper introduces the working principle of CRISPR-Cas9 and its application in establishing gene knock-out and knock-in models, and emphasizes the importance of this technology in revealing gene function and molecular mechanism of diseases. However, gene editing technology also faces challenges such as off-target effect and technical accuracy, and involves ethical issues. Therefore, the article puts forward that we should strengthen ethical review, improve laws and regulations, and enhance public awareness. In a word, gene editing technology provides a powerful tool for life science and medical research, and shows a broad application prospect.

Keywords: disease mechanism; gene editing technology; gene function; CRISPR-Cas9.

1. Introduction

Gene editing technology, especially the CRISPR-Cas9 system, has provided a powerful tool for researchers to reveal the mysteries of life science [1]. Genes are the basic units of genetic information of organisms. They encode all protein and RNA molecules that make up organisms, thus controlling various properties and functions of organisms. A deep understanding of gene function is the basis of biological research and the key to develop new therapies and drugs in the medical field. The study of disease mechanism also depends on an in-depth understanding of how specific genes and their mutations affect the health of organisms. Therefore, the technology that can accurately modify genes is very important to promote these studies.

Gene editing technology is just such a method that can modify the genome sequence at a fixed point. By designing a specific nuclease, it causes double strand breaks at the target gene position, thus triggering the DNA repair mechanism in the cell [2]. Scientists can use this process to introduce specific gene mutations, or provide repair templates to accurately modify gene sequences. The appearance of this technology has greatly promoted the study of gene function and disease mechanism.

This paper discusses the methodology of using gene editing technology, especially CRISPR-Cas9 system, to study gene function and disease mechanism. This paper analyzes how gene editing technology can help scientists reveal the specific functions of genes, and deeply discusses its application in analyzing the mechanism of disease occurrence and development.

2. Principles and methods of gene editing technology

Gene editing technology is a method that can precisely modify the genetic material of organisms. Its basic principle mainly relies on specific nuclease systems, such as ZFNs (Zinc-finger Nucleases), TALENs (Transcription Activator-Like Effector Nucleases), and the widely used CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats - CRISPR associated protein 9) system in recent years [3]. These technologies allow researchers to cut specific DNA sequences, thereby triggering the cell's DNA repair mechanism (Table 1).

Table 1 Classification and comparison of gene editing techniques

category	name	Description	advantage	disadvantage
first generation	ZFNs	Composed of a zinc finger DNA binding domain and a FokI nuclease domain	High specificity, avoiding immune response	Genetically engineered zinc refers to DNA binding proteins that are difficult and easy to miss the target
second generation	TALENs	Composed of a TAL effector DNA binding domain and a FokI nuclease domain	The design and construction are relatively simple, and the miss effect is low	Expensive and time-consuming, which may cause immune response
third generation	CRISPR-Cas9	Utilizes CRISPR sequences and Cas9 nuclease	Wide application range, high editing efficiency, simple operation and low cost	Low efficiency of homologous recombination, PAM sequence dependence, off-target effect

Among these technologies, the CRISPR-Cas9 system has received widespread attention due to its simplicity and efficiency. The CRISPR-Cas9 system consists of two main components [4-5]: one is a complex formed by the CRISPR RNA (crRNA) and the trans-activating crRNA (tracrRNA), which can specifically recognize the target DNA sequence; the other is the Cas9 protein, which has nuclease activity and can cleave the target DNA under the guidance of the crRNA-tracrRNA complex.

In practical applications, to simplify operations, crRNAIn practical applications, to simplify operations, crRNA often fused into a single crRNA and tracrRNA are often fused into a single guide RNA (sgRNA), allowing for the design of just one sgRNA molecule to direct the Cas9 protein to cleave specific DNA sequences. After cleavage, the cell attempts to repair the broken DNA, at which point researchers can provide a repair template that introduces specific genetic variations during the repair process [6].

The operational method of gene editing technology typically involves designing an sgRNA that can specifically recognize and bind to the target gene sequence based on its sequence. This step is crucial for gene editing technology as it determines the precision and efficiency of the edits. Through transfection or other methods, the expression vectors for sgRNA and Cas9 protein are introduced into the target cells. It is important to balance transfection efficiency with cell toxicity during this step. Once the sgRNA-Cas9 complex forms within the cell and identifies the target DNA sequence, the Cas9 protein will cleave the DNA [7]. Subsequently, the cell initiates its intrinsic DNA repair mechanisms. In the absence of an exogenous repair template, the cell usually resorts to non-homologous end joining (NHEJ) for repair, which may result in random insertions or deletions (indels), leading to a loss of gene function. If a repair template is provided, the cell may employ homologous recombination (HR) for precise repair, thereby introducing specific genetic variations.

3. Application of gene editing technology in studying gene function

3.1. Establish gene knock-out and knock-in models

Gene knockout refers to the complete or partial removal of a specific gene in an organism using gene editing technology to study the impact of the loss of function of that gene on the organism. The CRISPR-Cas9 system introduces double-strand breaks (DSBs) by recognizing a specific DNA sequence of the target gene through guide RNA (gRNA) and cleaving it with Cas9 nuclease.

Subsequently, the cell repairs the break using the NHEJ repair mechanism, resulting in insertions or deletion mutations and achieving gene knockout. For example, knocking out the tumor suppressor gene PTEN in mice can lead to a significant increase in the frequency of tumor formation within the mice, demonstrating the crucial role of the PTEN gene in inhibiting tumor formation [8]. Similarly, knocking out the WEE1 gene in rice can enhance its salt tolerance and drought resistance, providing new insights for crop improvement.

Gene knock-in refers to inserting a foreign gene into a specific position of an organism through gene editing technology to study the influence of over-expression or ectopic expression of the gene on the organism. Also using CRISPR-Cas9 system, by designing specific gRNA and donor DNA templates, the precise insertion of foreign genes can be achieved. Knocking in human eye development-related gene PAX6 in Drosophila melanogaster, it can be observed that the compound eye of Drosophila melanogaster turns into bifocal eye, which provides an important clue for studying the mechanism of gene regulation of eye development [9]. In addition, knocking in the green fluorescent protein gene in zebrafish can directly observe the gene expression pattern and cell behavior, which greatly facilitates biological research [10].

The efficiency and accuracy of CRISPR-Cas9 system make gene editing easy. Compared with the traditional gene knockout technology, CRISPR-Cas9 can obtain a high proportion of mutants in a short time, and the off-target effect is low. Gene editing technology is not only suitable for gene knock-out and knock-in, but also can realize many types of genome modification such as gene replacement and point mutation. At the same time, the technology has strong universality among different species, and can be effectively applied from yeast to mammals.

3.2. Discovery of new gene functions by gene editing technology

Using gene editing technology, such as CRISPR-Cas9, scientists can specifically "knock out" the target gene, that is, delete or inactivate the gene, and observe the performance of cells or organisms after losing the function of the gene [11]. This knock-out strategy helps scientists identify the key role of genes in development, metabolism and disease occurrence, and provides direct evidence for understanding complex physiological mechanisms and disease mechanisms. Through gene editing technology, foreign genes or specific mutations can be accurately inserted into the cell genome to study their effects on cell physiology. This method not only verifies the functional hypothesis of genes, but also explores how specific gene variation causes diseases, providing theoretical basis for the treatment and research of genetic diseases.

Gene editing technology, especially the method combined with Qualcomm screening technology such as CRISPR screening, enables scientists to analyze the functions of thousands of genes at the same time, so as to quickly identify gene sets that play a key role in specific cell physiological processes, which not only accelerates the understanding of complex physiological networks and disease mechanisms, but also provides valuable gene target information for developing new therapeutic strategies [12]. In addition, by accurately replicating gene mutations related to human diseases in cell or animal models, gene editing technology also promotes in-depth discussion of disease mechanisms, and provides an efficient model tool for the development of new drugs, making it possible to construct highly accurate disease models.

4. Application of gene editing technology in the study of disease mechanism

Gene editing technology has shown a wide application prospect in the study of disease mechanism, especially in the establishment of disease model. As a key tool of modern biomedical research, it significantly promotes the understanding of the molecular mechanism of diseases and provides a new method for drug screening and development. The application of gene editing technology in the study of disease mechanism is shown in Table 2.

Table 2 Application of gene editing technology in the study of disease mechanism

application area	application example
Neurodegenerative diseases	Animal models of Alzheimer's disease and Parkinson's disease were prepared by CRISPR/Cas9 technology to study the pathogenesis of these diseases [13].
Hypertrophic cardiomyopathy	A mouse model of hypertrophic cardiomyopathy was established by gene editing technology to study the molecular mechanism of heart disease [14].
cancer	Using CRISPR technology, animal models of various cancers (such as lung cancer and liver cancer) were established to study the molecular mechanism of tumor occurrence and development [15].
Immunodeficiency diseases	Using gene editing technology to prepare immune deficiency mouse model, study the function of immune system and the treatment strategy of related diseases [16].
metabolic disease	Animal models of diabetes and obesity were constructed by gene editing technology to study the molecular mechanism of metabolic disorder [17].
infectious disease	CRISPR genome editing technology is used to construct cell and animal models infected by viruses (such as HIV and influenza virus), and to study its pathogenesis and prevention methods [18].
genetic disease	Gene editing technology is used in gene therapy research of hereditary diseases such as blood system, liver, muscle and nervous system, showing its potential in disease treatment [19].
Ophthalmic diseases	CRISPR gene editing therapy is used to treat Leber congenital amaurosis type 10 (LCA10) and other ophthalmic diseases, and clinical trials have been carried out [20].

4.1. Establish a disease model

Cystic fibrosis (CF) is a hereditary exocrine gland disease, which mainly affects the respiratory tract and digestive system. The fundamental reason is the mutation of CFTR gene (the transmembrane conductance regulator of cystic fibrosis) [21]. Using gene editing technology, scientists can accurately simulate various mutations of CFTR gene, so as to reproduce the pathological characteristics of CF in cell or animal models. This model not only helps to understand the pathogenesis of CF, but also provides an experimental platform for new drug development and efficacy evaluation.

Sickle cell anemia is a hereditary hemoglobinopathy. The mutation of β -globin gene leads to the abnormality of hemoglobin S(HbS), which leads to the formation of sickle cells under hypoxia, and then causes a series of symptoms [22]. Through gene editing technology, researchers can accurately introduce this mutation and create a disease model of sickle cell anemia. These models are very important for studying the formation mechanism of sickle cell, the process of vascular occlusion and organ injury, and also provide a powerful tool for developing new treatment methods.

When establishing these disease models, gene editing technology allows accurate simulation of human genetic diseases, so as to understand the pathogenesis of diseases more deeply. This not only helps to understand the disease itself, but also provides a more realistic experimental environment for drug research and clinical trials. With the continuous development of gene editing technology, its application in the study of disease mechanism will be more extensive and in-depth.

4.2. Reveal the molecular mechanism of disease occurrence

Gene editing technology is widely used to explore the mechanism of activation of oncogenes and inactivation of tumor suppressor genes. Through systems such as CRISPR-Cas9, researchers can accurately knock out or modify genes related to cancer, and observe how these changes affect key biological processes such as cell proliferation, differentiation and apoptosis. This research method not only helps to identify new cancer-related genes, but also provides scientific basis for developing targeted therapy for specific gene mutations.

Gene editing technology also plays an important role in the research of neurodegenerative diseases. For example, by knocking out the genes related to Alzheimer's disease or Parkinson's disease, scientists can simulate the pathological process of these diseases in cell or animal models, thus gaining a deeper understanding of the molecular mechanisms of these diseases. This research method is helpful to find potential disease markers and provide new ideas for early diagnosis and treatment.

In addition, gene editing technology has also been applied to study the pathogenesis of hereditary diseases. Many genetic diseases are caused by mutations in specific genes. Simulating these mutations through gene editing technology can help scientists reveal how these mutations lead to diseases. This research method not only helps to deeply understand the pathophysiological process of hereditary diseases, but also provides strong support for developing therapeutic methods for specific gene mutations.

4.3. Drug screening and development

Using precise gene editing techniques such as CRISPR-Cas9, researchers can introduce or correct specific gene variations in cell or animal models, so as to simulate the state of human diseases and understand the biological basis of diseases more accurately. By creating a model with known gene mutations, scientists can not only observe how these mutations affect cell function and tissue structure, but also evaluate the effects of different therapeutic interventions, which is very important for revealing complex pathological processes.

Gene editing technology also plays an important role in drug screening. For example, through the CRISPR screening experiment of Qualcomm quantity, the contribution degree of thousands of genes in specific diseases can be evaluated at one time, and then the key genes that may be drug targets can be locked [23]. This method greatly improves the efficiency of finding new drug targets, helps to find potential therapeutic targets, and speeds up the process from laboratory to clinical trials. In addition, gene editing can also be used to create cell lines that are sensitive or resistant to specific drugs, which provides a basis for the design of personalized medical programs and promotes the development of precision medicine.

5. Challenges and ethical issues faced by gene editing technology

Gene editing technology, such as CRISPR-Cas9, although powerful, faces a series of technical challenges and ethical issues in practical application. Technically, the "off-target" effect will lead to editing in non-target positions, causing unknown genetic variation, reducing editing accuracy and bringing potential health risks; At the same time, it is still a difficult problem to realize gene editing with high accuracy and efficiency in complex genome environment. Ethically, the use of gene editing to create "design babies" has triggered a debate about the naturalness and fairness of human beings, and the popularization of gene editing technology may also lead to social discrimination based on

genetic information, especially in the fields of employment and insurance, further aggravating social inequality.

In order to meet the challenges brought by gene editing technology, it is necessary to establish a strict ethical review mechanism to ensure that research and application follow ethical principles and protect personal dignity and rights; Formulate and improve relevant laws and regulations, define the boundaries of technology application, prevent abuse and protect public interests; Improve the public's awareness of gene editing technology and its ethical issues, promote the participation and discussion of all walks of life, and strive to form a broad social consensus; At the same time, because the influence of gene editing technology transcends national boundaries, it is necessary to strengthen international cooperation, jointly formulate unified standards and guidelines, and eliminate the "gray zone" in ethics and supervision.

6. Conclusion

Advanced gene editing tools such as CRISPR-Cas9 provide unprecedented opportunities for biological and medical research. Through precise genome modification, scientists can establish gene knock-out and knock-in models to reveal the role of specific genes in organism development, metabolism and disease occurrence. In addition, the application of gene editing technology is not limited to basic research, but also extends to the establishment of disease models, drug screening and development, and the exploration of genetic disease treatment. However, although gene editing technology has great potential, its application still faces technical challenges such as "off-target" effect and ethical issues, such as the debate on human naturalness and fairness. Therefore, future research needs to continue to optimize gene editing technology, while strengthening ethical review mechanism and public participation to ensure the rational and safe use of technology.

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