

Application of CRISPR gene editing technology in cystic fibrosis treatment

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Abstract. CRISPR-Cas9 gene editing technology is to identify the target genome sequence through artificially designed sgRNA, guide Cas9 protease, so that it can effectively cut the DNA double strand, form a double strand break, repair after damage to lead to gene knockout or knock-in, and finally achieve the purpose of modifying genomic DNA which leading CRISPR a technology can effectively repair, correct or regulate genetic mutations associated with genetic diseases, providing patients with new treatment options. However, despite the remarkable progress, CRISPR technology still faces many challenges in its clinical application, including ethics and safety. This article reviews the principle and development of CRISPR technology, as well as its application in the treatment of cystic fibrosis, and summarizes the challenges of CRISPR in clinical applications. More importantly, this article focuses on some current improvements to CRISPR to make CRISPR gene editing technology more valuable and meaningful in the treatment of genetic diseases in the future.

Keywords: CRISPR, genetic disease, gene editing, cystic fibrosis.

1. Introduction

The alternation of genome could lead the changing of cellular process, which further may cause disease, which called genetic diseases. These diseases impose a significant health and economic burden on countless families around the world. This is despite the fact that traditional treatments have been able to alleviate some symptoms to some extent. But for most genetic diseases, curing them has been a huge challenge. CRISPR gene editing technology can directly repair or modify abnormal parts of a patient's genome, achieving a radical cure [1]. But problems such as its off-target effects and possible safety risks still need to be solved. This paper believes that machine learning can be integrated into the design of sgRNA, so as to optimize sgRNA to solve related problems.

2. Discovery and principle of CRISPR-Cas system

The CRISPR-Cas system was first discovered in *E. coli* in the 1990s. At the heart of this system, which has a defense against viral invasions, is a piece of repeating DNA sequence called CRISPR, and its associated Cas protein. The CRISPR region is interspersed with fragments from viruses, called spacers, that record the viral infections the bacteria once suffered. The process of the CRISPR-Cas system work can be broken down into three main steps: adaptation, expression and intervention [2].

2.1. Adaptation

When bacteria face a viral infection, they activate an adaptation mechanism that integrates portions of viral DNA into their CRISPR region. Multiple proteins and molecules are involved in this process. First, the Cas1 protein is involved in creating spacers (spacers) in the CRISPR region. Then, the Cas2 protein helps bind spacers to other DNA sequences to form unique CRISPR structures. These integrated Spacers will then become part of the bacteria's "immune system," which records the virus's genetic information for future confrontation.

2.2. Express

During the expression phase, spacer sequences integrated into the CRISPR region are transcribed into pre-crRNA (pre-crRNA). This step involves the bacteria's RNA polymerase, which is responsible for synthesizing pre-crRNA. However, pre-crRNA is too long to be used directly to intervene in viral infections. After this, another RNA enzyme, called Cas9, steps in to recognize and cut the pre-crRNA, splitting it into a series of shorter crRNA fragments (Fig.1).

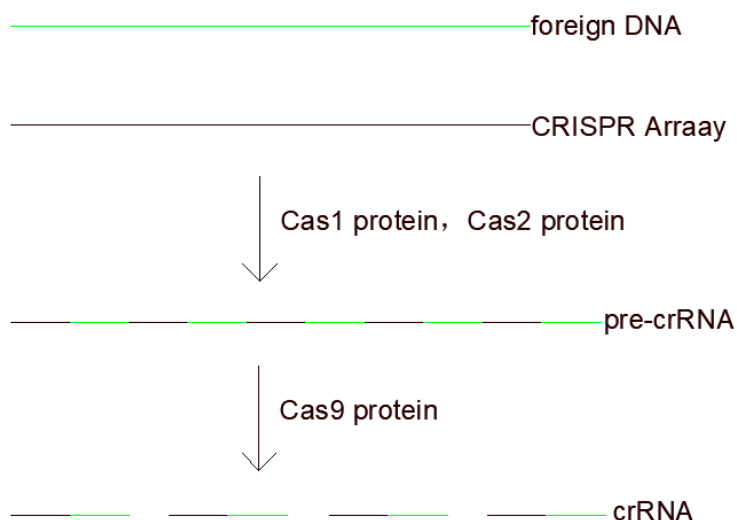


Figure 1. Adaptation and expressive flow charts

2.3. Intervene

When the bacteria again encounter a DNA sequence associated with a previous infection, the CRISPR-Cas complex is activated, with a specific type of Cas protein at its core guided by crRNA. This complex is known as the "guidance complex." The guide complex first recognizes the foreign DNA sequence and determines whether it matches crRNA. This match is determined by the spacer sequence in crRNA. Once the target is found, the Cas protein, through its nuclease activity, cuts the target DNA, creating a double-strand break. In this process, the Cas3 protein may also be involved, responsible for further degradation of the target DNA.

3. Similarities and differences between the different types of CRISPR-Cas systems

The CRISPR-Cas system is divided into different subtypes based on the type and function of its Cas protein. These subtypes are divided into two main categories: Class 1 and Class 2, Class 1 and Class 2 can be further subdivided into different subtypes. Let's explore the various CRISPR-Cas systems in detail.

Class 1 CRISPR-Cas systems: Includes the Type I, III, and IV systems, whose complexes consist of multiple Cas proteins, resulting in complex structures. These systems use multiple Cas proteins during target recognition and intervention, often requiring more energy. Such systems have been less studied in most fields due to their complexity and relatively high difficulty.

Class 2 CRISPR-Cas systems: This category includes Type II, V, and VI systems, which typically involve only a single Cas protein to achieve target recognition and cutting. Class 2 systems have received a lot of attention for their simplicity and better operability, especially Type II systems. The best known of the Class 2 systems is the Type II system, which includes the Cas9 protein [3].

4. Different types and functions of Cas proteins

The Cas proteins within the CRISPR-Cas system play a key role in different systems, with different functions depending on the type and purpose of the system.

Type I Cas proteins: The Type I system consists of multiple Cas proteins that work together to form a complex complex. One of these Cas proteins is a complex called Csm/Cmr that recognizes foreign DNA or RNA and cuts it. The Csm/Cmr complex recognizes the target by its match to crRNA, which in turn activates the Cas3 protein, causing it to cut and degrade foreign nucleic acids [4].

Type II Cas protein: Typical of the Type II system is the Cas9 protein, which is currently the most widely used Cas protein for gene editing. The Cas9 protein precisely recognizes and cuts the paired DNA bases recognize by the guide RNA. Guide RNAs (including tracrRNA and crRNA) combine with Cas9 to form complexes, which together recognize the sequence of target DNA and guide Cas9 to make the cut, thus enabling editing of the genome [5,6].

Type V and VI Cas proteins: The Cas proteins of the Type V and VI systems play a role in target recognition and cutting. The Type V system typically includes the Cpf1 (also known as Cas12) protein, which, along with guide RNA, recognizes and cuts DNA. The C2c2 protein (also known as Cas13) in the Type VI system is specifically designed to target RNA, recognizing and cutting the target RNA by matching it to the sequence of the guide RNA [7].

The Cas proteins in different types of CRISPR-Cas systems have different functions and properties, giving scientists the tools to choose the right ones for gene-editing and other applications. A deeper understanding of these Cas proteins could help better develop and optimize applications of CRISPR-Cas technology.

5. Applications of CRISPR-Cas system

CRISPR-Cas technology shows fancy applications in several fields, most notably gene editing, gene regulation, and disease treatment. The following highlights two of the main applications: Knockout and Knock-in, and how they work.

5.1. Knockout

Knockout is the process of disabling a target gene by altering its DNA sequence. Researchers can precisely knock out specific genes through the CRISPR-Cas system to study the function of the gene in the organism. The process of knockout is the following steps:

Step 1. Acquisition of CRISPR sequence: After being infected by a virus, bacteria will embed a part of the genome fragment of the virus into their own genome to form CRISPR sequence. These sequences serve as a memory bank so that they can be quickly identified and defeated in future encounters with the same virus.

Step 2. Intervention of CRISPR-CAS: CRISPR sequences consist of two main components: a repeats and unique segments in between, called spacers. The CRISPR-Cas system identifies and destroys invading viral genomes by matching spacers to the virus's genetic information.

Step 3. sgRNA design: The researchers used the principles of the CRISPR-Cas system to design sgRNA. This is a molecule that recognizes a specific target gene sequence, similar to the spacers in CRISPR. sgRNA consists of two parts: one that complements the target gene sequence, and the other that binds to the Cas protein to enable gene editing.

Step 4. What the Cas protein does: The Cas protein in the CRISPR-Cas system acts as a "scissor," able to cut strands of DNA. By directing the sgRNA to a specific location in the target gene, the Cas protein triggers a DSB in the target DNA sequence.

Step 5. DNA repair mechanisms: When a DNA break occurs, cells activate their own DNA repair mechanisms, and there are two main repair pathways: Nonhomologous end junction (NHEJ): The cell attempts to connect the broken end directly, but this process can lead to the insertion or deletion of some DNA bases, triggering genetic mutations. Homologous recombination (HDR): Using foreign DNA templates for repair, precise gene editing can be achieved, but it is more complex [8].

By directing the CRISPR-Cas system to the location of a specific gene, using the NHEJ repair mechanism, the cell may delete a segment of the target gene sequence during the repair process, resulting in the loss of function of the gene to achieve the purpose of gene knockout.

5.2. Gene insertion (Knock-in)

Knock-in is the process of inserting a foreign DNA sequence precisely into a target gene. This process can be used to introduce a new gene or repair a gene mutation. The process of knocking in is following steps:

Step 1. sgRNA design: Similar to gene knockout, the researchers first design single guide RNA (sgRNA), which is able to pair with each other at specific locations in the target gene sequence. One part of the sgRNA complements the target sequence, and another part binds to the Cas protein to guide editing.

Step 2. The role of Cas proteins: The Cas protein in the CRISPR-Cas system also plays a key role in gene insertion. The Cas protein has a "scissor" function that can cut strands of DNA. In gene insertion, Cas proteins typically cut two parts of the target genome, on either side of the insertion point.

Step 3. Foreign DNA template: Unlike gene knockout, gene insertion requires a foreign DNA template. This DNA template is a fragment of DNA containing the desired gene to be inserted, which will be used during the editing process to replace the target gene sequence cut by the Cas protein.

Step 4. Repair process: Once the Cas protein has cut both sides of the target gene, the cell attempts to repair the pair of strand breaks. There are two main repair pathways at this point: Non-homologous end joining (NHEJ): Similar to gene knockout, cells attempt to directly join the broken end, but this process may result in the insertion or deletion of some DNA bases, triggering mutations. Homologous recombination (HDR): In gene insertion, researchers want the cell to use a foreign DNA template for repair. The cell will match the foreign DNA template to the cut site of the target gene to insert the new gene sequence into the genome [9].

By directing Cas proteins and Sgrnas to specific locations in the target gene while providing the foreign DNA template, the cell may use the HDR repair pathway to insert foreign DNA into the target genome. This will result in the insertion of new sequences in the target genome, thus achieving the purpose of gene insertion.

6. A brief introduction of Cystic fibrosis

Cystic fibrosis is a common genetic disorder caused by mutations in the CFTR gene. The protein encoded by this gene function as an ion channel that regulate the flow of chloride ions and water across the cell membrane, thereby maintaining normal concentrations of fluid on the cell surface. However, in people with cystic fibrosis, mutations in the CFTR gene prevent chloride ions and water from flowing properly, causing thick mucus to build up in the airways and digestive tract, causing serious lung infections and other symptoms [10]. CRISPR gene-editing technology offers a potential therapeutic avenue for fixing CFTR gene mutations.

7. The main advances CRISPR has made in the treatment of cystic fibrosis

Use the CRISPR-Cas9 system to precisely edit the CFTR gene and fix its mutations. Restore the normal function of the CFTR gene by directing the Cas9 protein and the appropriate sgRNA to accurately locate the mutation site, and then inserting the correct base into the genome via the NHEJ or HDR repair mechanism. In addition to directly repairing gene mutations, researchers also try to modify the cutting pattern of the CFTR gene by modifying modification shear to produce normal CFTR proteins. This approach reduces the risk of off-target effects and avoids inaccurate genome editing. The CRISPR-Cas9 system can induce clipping in non-target areas, leading to undesirable genetic variation. To reduce off-target effects, researchers have optimized Sgrnas to select more

specific Sgrnas, while developing novel Cas9 protein variants, such as the highly specific Cas9 (eSpCas9). The researchers took the patient's cells, such as lung epithelial cells, made gene-editing repairs, and then implanted these repaired cells into the patient. This approach opens up new possibilities for treating cystic fibrosis.

8. The advantages and current shortages of CRISPR in cystic fibrosis

8.1. Advantages of CRISPR

CRISPR gene editing technology holds great promise in treating genetic diseases, but it also faces some limitations and challenges.

Efficiency: The efficiency of the CRISPR-Cas9 system stems from its ability to precisely identify and bind to a specific sequence of the target gene, which is then triggered by the cutting activity of the Cas9 enzyme to trigger a DNA double-strand break. The cell then attempts to repair these breaks, usually through NHEJ or HR. By harnessing the cellular self-repair processing, CRISPR-Cas9 enables accurate gene editing that can correct inherited mutations.

Accuracy: CRISPR-Cas9's accuracy lies in designing appropriate guide Rnas that are highly complementary to specific sequences of target genes. This complementarity allows the Cas9 enzyme to cut at specific DNA locations, reducing the risk of affecting other genes.

Diversity: The diversity of the CRISPR-Cas9 system manifests itself in the design of the guide RNA. By properly designing the guide RNA, CRISPR-Cas9 can enable different kinds of editing, such as correcting genetic mutations, inserting new genes, or regulating gene expression levels. This flexibility gives CRISPR a wide range of potential for a variety of gene therapy applications.

Customizability: The customizability of the CRISPR-Cas9 system stems from the design of the guide RNA. Depending on the specific genetic mutation, the guide RNA can be designed to direct the Cas9 enzyme to cut or repair at the exact location. This customization allows gene therapy programmes to be personalized for different genetic diseases.

Dual use of research and treatment: CRISPR's mechanism allows it to be used not only for gene therapy, but also for scientific research. Researchers use CRISPR gene editing technology to reveal the role of genes in disease development, understand disease mechanisms, and formulate corresponding treatment strategies.

Potential reversibility: The reversibility of CRISPR editing is determined by the cell's own DNA repair mechanisms. In some cases, cells can restore the edited DNA to return it to its original state. This mechanism provides flexibility for gene editing, with the ability to reverse it if needed.

8.2. The current shortages of CRISPR

Although CRISPR has made some important advances in the treatment of cystic fibrosis, there are still some challenges to its clinical application. On the one hand, processing efficiency and off-target effects need to be further optimized. On the other hand, long-term safety and efficacy have yet to be validated in larger clinical trials. However, these advances offer new hope for the treatment of cystic fibrosis and show the great potential of CRISPR as a potential gene therapy tool.

Non-specific effects: The CRISPR-Cas9 system may bind to similar non-target sequences when looking for a target DNA sequence, triggering non-specific gene editing. This can lead to undesirable side effects and even trigger unexpected mutations.

Unknown side effects: The DNA breaking and repair process during CRISPR-Cas9 editing may trigger problems such as DNA damage, instability and genome rearrangement in cells, which may affect the normal function of cells.

Limitations: The efficiency and accuracy of the CRISPR-Cas9 system may vary across genes, cell types, and tissues. Certain cells are difficult to edit, or less efficient at editing, which may limit the broad applicability of the treatment.

Extracellular pathways: Introducing the CRISPR-Cas9 system into the body and getting it into a specific cell or tissue is a challenge. Efficient delivery of the editing tool into the target cells, as well as ensuring its stability and persistence in the body, require technical issues to be addressed.

Immune response: The body may develop an immune response to the CRISPR-Cas9 system because the bacteria-derived Cas9 protein is considered foreign in the body. This can lead to failure of treatment or side effects.

Off-target effects: Although CRISPR-guided RNA is designed to specifically identify the target gene sequence, there are still possible off-target effects, where editing occurs on non-target genes. This can have unexpected effects on the normal function of the cell.

8.3. The improvement of CRISPR

Improve editing efficiency: By improving the design of sgRNA and variants of Cas9 protein, find more efficient editing methods, such as using highly specific Cas9 variants such as eSpCas9, to improve editing efficiency.

Control off-target effects: Develop more accurate sgRNA design algorithms to reduce off-target effects. Using methods such as machine learning, improve the specificity of sgRNA while developing new Cas9 protein variants with higher specificity and lower off-target rates.

9. Summary

This paper mainly describes the principle and mechanism of crispr gene editing technology and its application in cystic fibrosis and its advantages and disadvantages. Through the understanding of crispr, it can be concluded that although CRISPR technology has made important progress and potential application value in the treatment of genetic diseases, it still needs further research and verification to apply it in clinical treatment. By means of machine learning, sgRNA is designed to reduce off-target effects. Addressing these challenges will require the concerted efforts of scientists, policymakers and the public around the world to ensure the safe and effective application of CRISPR technology to bring real benefits to patients with genetic diseases.

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