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CRISPR-Cas9 and its Application in the Fields of Medics

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Abstract:

Gene editing technology has become a prominent objective within the fields of medical research, the clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR-Cas9) is one particular technology that has already been practiced in a set of clinical trials, which produced a satisfying result that have demonstrated its potential to be applied to a great range of treatments. CRISPR-Cas9 was derived from bacterial defense system, and has been applied in the field of medical research. For instance, the research practice of using CAR-T cells by CAR-T Therapy, and genetically modifying DNA strands to remove cancerous or mutated sequences. This research has great chance to promote treatments associated with genetic modification and provide new hope for patients in the future. Nevertheless, this technology is still immature, due to its potential drawbacks. Most researchers are still focusing on practices of CRISPR-Cas9 on animal models or isolated somatic cells. This article draws attention on different treatments of CRISPR-Cas9 and provides evaluation and suggestions as a reference for future practices of this technology. Overall, there are two discoveries of advancement to the CRISPR-Cas9 mechanism: modification to the guide RNA that improves the accuracy, and applying high-fidelity Cas9 protein variants to enhance its comprehensive accuracy.

Keywords: CRISPR-Cas9; mechanism; treatments; advancement.

1. Introduction

Gene editing technology is a commonly used biotechnology in medical research. CRISPR-Cas9 has been applied in a wide range of fields, such as agriculture, medicine, drug development, food development, microbial modification. The reason is because of its advantages of faster, more accurate and reduced expenditure, it also shows great application prospects in the future [1].

CRISPR-Cas9 is a powerful bacterial defense mechanism gene editing tool designed against phage infections and plasmid transmission spread in nature. It has now been modified into a capable RNA guided DNA targeting platform for genome editing [1]. The CRISPR-Cas9 system consists of two key molecules that introduce changes in the target gene sequence of DNA. An enzyme called Cas9 acts like a pair of scissors, cutting two DNA strands at specific locations in the genome, allowing for the addition or deletion of parts of DNA. A single guide RNA (sgRNA) is pre-designed RNA sequence to bind with the DNA strand, it is about 20 bases long. A sgRNA guides a Cas9 enzyme to the targeted genome. The sgRNA is able to ensure accurate binding, since this pre-designed RNA sequence is modified to the complementary of that exact DNA sequence, thus the single guide RNA will only bind to the targeted genome, not others. The Cas9 will follow the sgRNA to the location and cuts the two strands of the DNA.

Chemicals and Radiation are common ways to cause mutations in a gene, however, it is no way to control where the mutation occurs. Traditional gene targetingin involves scientists remove or add either a whole gene or a single base. Transcription activator-like effector nucleases (TALEN) [2], is a restricting endonuclease that can be engineered to cut specific sequences of DNA. These enzymes are found in prokaryotic organisms to provide a defense mechanism against invading viruses. This system can bind to practically any DNA sequence. Zinc-finger Proteins (ZNF), are the most common gene editing techniques [3]. Comparing to other systems, CRISPR-Cas9 protrudes as the most efficient and reliable for editing genes [4].

CRISPR-Cas9 has lots of potential to become a tool to treat a great range of medical conditions, for instance, cancer, hepatitis B or even cholesterol deposition. Most of the application of gene editing techniques involves modification of the genes of human cells. There are debates about the potential to edit germline cells, since this is illegal in most countries, although exceptions may exist in life-threatening conditions that CRISPR-Cas9 and other gene editing technologies are used [5]. This paper will analyze the application of CRISPR-Cas9 in order to provide

an efficient, site specific genome engineering technology for future research use.

2. Mechanism of CRISPR-Cas9

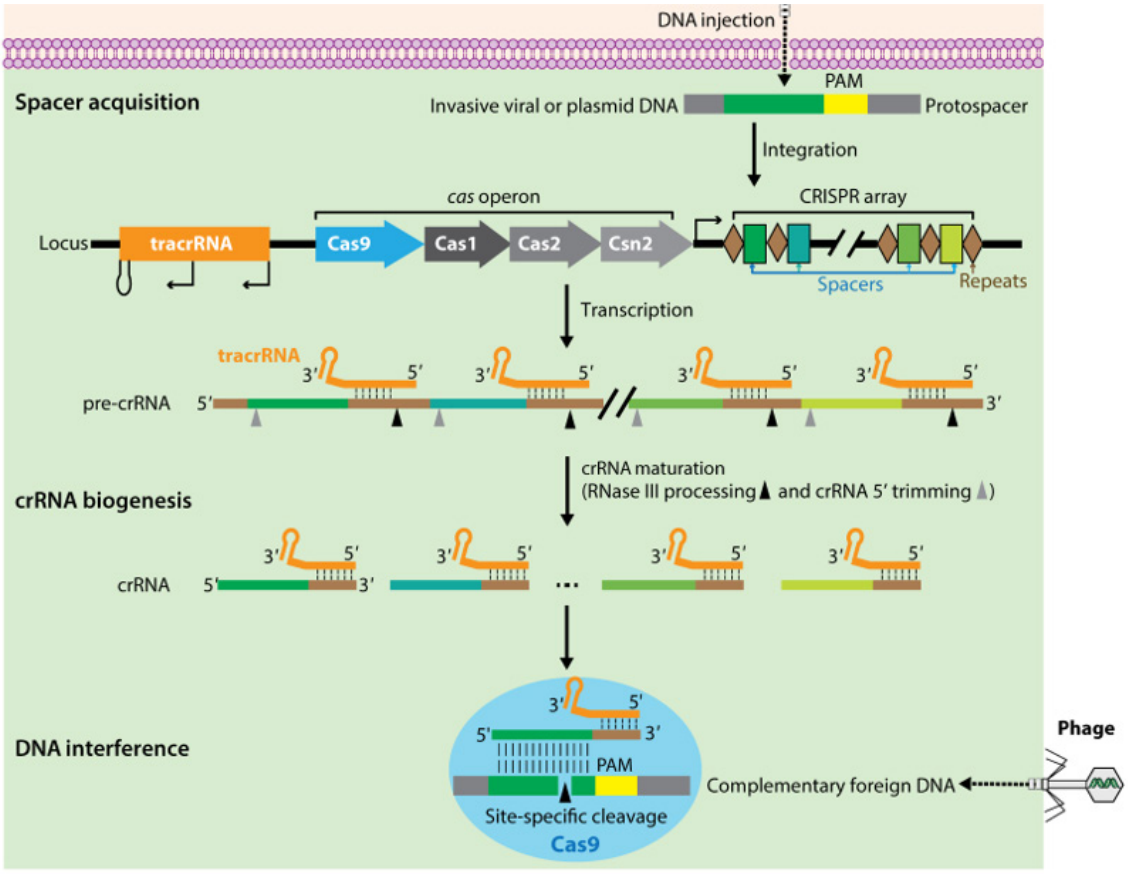

Figure .1 CRISPR–Cas9 structures and mechanisms [6].

The diagram demonstrates the mechanism of CRISPR-Cas9 used by bacteria to protect themselves, just like our immune system fights against viruses. The CRISPR locus inside the bacteria contains repetitive sequences (brown lines), which includes Cas genes, there are also some interval sequences between the Cas genes (colored spacers) that are marks left by viruses previously invaded the bacteria. Whenever a new virus attacks the bacteria, it will capture the virus's compartments and then place them in its locus, so that next time the virus comes back, the bacteria can recognize them. Next, the bacteria read out these compartments and turn them into crRNA, simultaneously, the bacteria also makes tracrRNA, which joins together with crRNA and then processed by RNase III. Now, the crRNA tracrRNA combination and the Cas9 will work together to identify and trim any virus compartments, resulting in the dysfunction of the virus invasion [6].

The researching CRISPR system model generates an sgRNA trough transcription and processing, this in which consists of two parts: a Cas enzyme for cutting the target DNA strand and a sgRNA, which binds to the target sequence of 20-base pair, the target sequence is necessarily complementary to that the sequence of the sgRNA. First, the promoter sequences illustrate the direction of transcription and point out which DNA strand will be transcribed. The sg RNA or crRNA guides a Cas9 protein to the specific DNA sequence, then the gRNA further directs

the Cas9 protein to propose a double strand break (DSB) in the marked DNA strands.

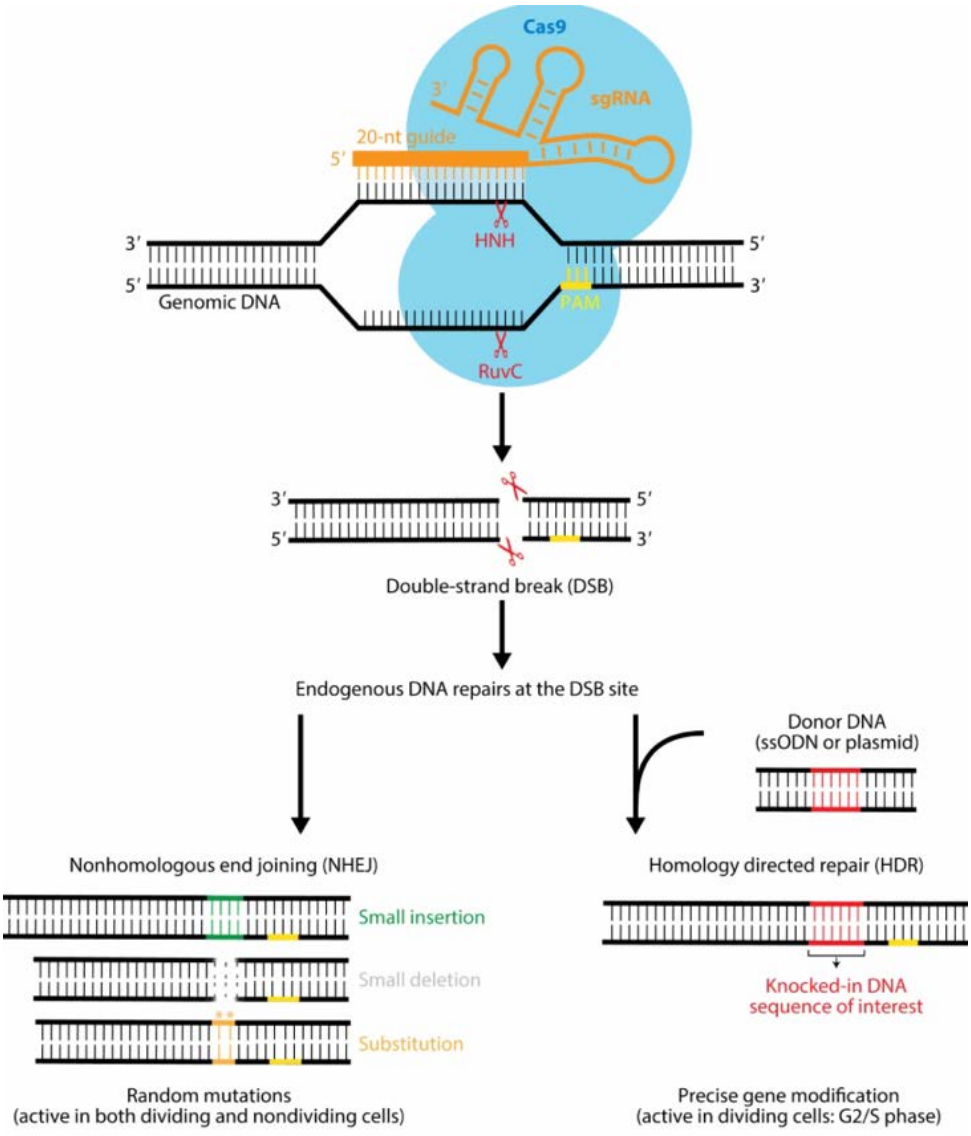

Figure. 2 CRISPR–Cas9 structures and mechanisms [6].

According to figure 2, two distinct Cas9 protein must be present at the appropriate strands to facilitate a DBS, for enhancing the accuracy of the DBS. Then the repairing template will be served to supply the empty slot, while in other situations, such as removing the mutation that has occurred in an DNA sequence. The DBS triggers the DNA repair mechanisms that include two types of gene modifications [1]: constitutive knockouts (KO), as the break ends directly separate; Knockins (KI), as two DNA sequences share an extensive nucleotide together.

3. Application in the Research of Medicines

This technology enables one to accurately measure practically any gene sequence stated by a guide RNA, which allows the expression of genes in a disease advancement, modification of mutations by diseases. It can also inactivate the oncogene, or activate the cancer suppressor genes [7].

3.1 Application

CRISPR-Cas9 can accurately repair tumor suppressor genes by mutations, thereby restoring their functions and inhibit the growth of tumor cells. In addition, CRISPR-Cas9 has the ability to genetically modify T-cells, and enhance the immune system to recognize and kill tumor cells.

CAR-T cells are genetically modified T-cells, with gene editing technologies such as CRISPR-Cas9, T-cells can gain ability to recognize specific cancerous cells and at-

tack them. CAR genes will be add into immunocytes, for instance, lymphocytes and leukocytes, these T-cells can specifically attack the target.

Within the therapy, doctors take T cells from the patient‘s blood and genetically altered in the laboratory by adding a receptor gene called chimeric antigen receptor (CAR) [8]. This helps T cells attach to specific cancer cell antigens. Then the modified T cells are reintroduced back to the patient’s immune system. The new T cells inserted with the matching receptor will attach to the antigens of cancer cells and destroy them.

CRISPR-Cas9 has the potential to treat genetic blood disorder such as β-thalassemia, β-thalassemia is an autosomal recessive genetic disorder. The causation of the β-thalassemia is due to mutations or removal of the β-globin gene, which results in incomplete synthesis of β-globin, and causing unstable accumulation of α-globin, which could produce cytotoxic oxygen species, and ultimately causing hemolytic anemia. CRISPR-Cas9 is able to locate the mutated sequence, then remove and replace the genome by DBS to cure β-thalassemia. Recently, researchers used CRISPR-Cas9 to knock out the beta globin gene, by constructing a similar disease model to human β-thalassemia using a mouse model. These models had validate the effectiveness of CRISPR-Cas9 in treating β-thalassemia, according to Researchers from Sarah Cannon Institute, Boston University School of Medicine, and other institutions [9].

This technology offers new hope for patients, especially in treating tumor. Traditional tumor therapy is long term, expensive and psychologically stresses the patients, while CAR-T therapy usually only requires a single injection, and has a shorter treatment cycle, in which greatly reduces the patient’s burden.

3.2 Existing Problems of CRISPR-Cas9

Most of the research has been engaging in its application within animal models or isolated human cells, the ultimate goal of applying CRISPR-Cas9 is to treat various kinds of syndromes . There is still much work to eliminate “off-target” effects, improve its precision, and reduce the chances that the mechanism could make mistake, as of taking other genes as the one that was intended to be edited. The problem with this is that, for instance, in most cases, a guide RNA consists of a specific sequence of 20 bases , while 19 of the 20 complementary bases could exist somewhere completely different in the genome, since the Cas9 must locate that specific 20 base pairs of target sequences within millions to billions of the genome. This means that the guide RNA could bind to other sites, and end up with a false cut, thus causing a mutation at the wrong location of the strand. The consequence is either small or large, as mutation could happen at a crucial part of a genome. In addition, after a DBS, if there is an absence of a repair template, random insertion and deletions will occur, even substitution at the site of where the DBS occurred [5]. This regularly disturbs the typical functions of the gene’s function. The efficiency of the guide RNA is also concerning, due to its relatively low stability, which potentially reduce the efficiency of the complex formed with Cas9 protein, it is necessary to establish other accurate and efficient Cas9-based tools.

3.3 Improvements on “off-target”

Scientists have found two approaches to ensure the accurate binding and cuts of CRISPR-Cas9, including the use of high-fidelity Cas9 variants, modified gRNA. One type is chemically modified gRNA. Studies have shown that chemically modified synthesized gRNA can improve efficiency of the genome editing of T cells, CD34+hematopoietic stem cells, and progenitor cells. Generally, chemical modifications are introduced in the gRNA structure during synthesis or post synthetically. These modification can include backbone modifications (phosphorothioate linkages), 2’-modifications (2’-O-methyl, 2’-fluoro), or nucleotide analogues such as LNAs. Afterwards, the modified gRNA is delivered into cells, binds with the Cas9 endonuclease. Together this CRISPR complex formed are found to enhance stability and allows the gRNA to persist longer to reach its target sites, therefore improving the efficiency of the CRISP-Cas9 mechanism [10].

Another discovery is a few high-fidelity Cas9 protein variants that could reduce the “off-targets” effect, by modifying the genetic sequences and their interaction with DNA and RNA, these variants focuses on the stability of the Cas9-sgRNA complex to bound and select. Some of the alternatives have been ranked as: Sniper-Cas9, eSpCas9, SpCas9-HF1 and other variants [11]. Nevertheless, selecting any of these advancements of Cas9 variants remains a challenge.

4. Conclusion

This article provides an overview of CRISPR-Cas9 and its applications in medical researches. CRISPR-Cas9 is a powerful tool derived from effective bacterial defense mechanisms in nature, as it stands out in the field of gene editing due to its advantages of greater efficiency, accuracy, and lower cost. This paper delves into its working mechanism, including how the Cas9-sgRNA complex work together to precisely cleave DNA double strands, and trigger DBS to the target sites. Then introduces its potential applications in the treatment of cancer, hereditary diseases such as β - thalassemia, and more similar genetic modification-required treatments. Especially when apply-

ing it in the CAR-T therapy, the enormous potential of CRISPR-Cas9 in enhancing the immune system‘s ability to remove cancerous cells has been demonstrated.

The significance of this paper lies in emphasizing the new hope that CRISPR-Cas9 could bring to the treatment of genetic diseases and tumors, as it provides a comprehensive reference framework for researchers. At the same time, it also points out the challenges faced by current technology, such as off target effects and high-efficiency improvements despite a number of advancements, therefore providing a direction for future research. This paper has unique insights and concluded various valuable references for the further development and clinical application of gene editing technology.

However, this paper also acknowledge that current research is mostly focused on animal models and isolated human cells, and there is still insufficient exploration of more practical and specific human applications and cases. Future research needs to focus on addressing off target effects, improving gene editing efficiency, and exploring its potential in the treatment of a wide range of other human diseases. Looking ahead, with the continuous advancement and improvement of gene editing technology, CRISPR-Cas9 is expected to play a more important role in the medical field, and bring good news to more patients.

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