Research and Application of CRISPR/Cas9 Technology in Oncology and Blood Diseases

Hengyi Zhang
Guiyang No.1 Middle School, Guiyang, 550000, China

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Abstract: The CRISPR/Cas system has been widely studied and applied to various fields as an efficient and simple gene editing tool. So far, CRISPR/Cas9 systems have evolved from the initial Cas9 to a variety of systems such as 12a and 13a; and also from the initial targeting of DNA and RNA to transcriptional regulation and DNA recycling. In addition, with the recognition of target sequences, certain CRISPR/Cas systems, such as Cas12, Cas13, and Cas14 proteins, exhibit non-specific cleavage activity on other single-stranded DNA or RNA molecules indirectly after the recognition of target sequences to initiate targeted cleavage activity under the guidance of guide RNA. This paper introduces the principle of CRISPR/Cas9 and discusses its application in oncology and hematological diseases, laying the foundation for the application of CRISPR/Cas9 technology in diseases, which also shows that CRISPR/Cas9 has a vast prospect of development.

1 INTRODUCTION

Some diseases are so specific that the virus is present in a baby's body at birth, and treatment becomes a major problem. Even if cured in the body, if it is present in the genes or not completely eradicated, it is bound to recur or get worse later, and even future generations may be affected. Thanks to the efforts of many scientists around the world, gene editing systems were introduced in the 1980s, an emerging genetic engineering technique or process capable of modifying specific target genes in an organism's genome, and are now widely used in the study of diseases such as tumors and blood disorders. 2020, French scientist Emmanuel Carpentier and American scientist Jennifer Doudna researched the "CRISPR gene editing technology" was even awarded the Nobel Prize in Chemistry. However, gene editing technology involves random homologous recombination of cells, and the efficiency of recombination is extremely low (one in a million), thus limiting the widespread use of gene technology.

The CRISPR/Cas system based on RNA-guided recognition of DNA has the advantages of simultaneous editing of multiple sites, simple operational design, and ease of operation. The main feature is the recognition and specific degradation of invaded exogenous DNA, and the variety of these DNAs. The CRISPR/Cas9 system, a key element of the type II CAS proteins, has enabled scientists to efficiently and precisely modify DNA or insert substitutions, allowing for the rapid and efficient construction of microbial models. The tremendous developments achieved by the CRISPR system provide important opportunities for treating genetic diseases and designing desirable genetic traits, as well as new methods for imaging living cells, etc., laying the foundation for the next applications of gene editing technologies and new discoveries.

Tumor development is accompanied by a combination of multiple genes in multiple ways and processes that are continuous and slow (Liu, 2015). Both different genes at the same stage of development and the same gene at different stages of development play different biological roles, so research on tumors requires interference with different gene expression in the same cell at different times or in different cells at the same time. To achieve this goal, rapid and effective gene editing technology is needed to artificially control gene expression, and the newly developed CRISPR gene editing technology can better fill this gap and more rapidly advance the research in many aspects.

The occurrence of blood disorders is mostly associated with genetic mutations. Gene editing technology, makes it possible to cure genetic mutation diseases. It is mainly a recombination

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pathway triggered by exogenous introduction of chromosomes with internal homologous recombination through nucleotides in the absence of nucleases, coupled with the use of viral genes as templates for editing. However, because of the low correction probability of conventional gene editing techniques, it is clinically limited, and the CRISPR/Cas9 system can effectively remedy this aspect. This paper will address the principles of CRISPR/Cas9 and its applications in cancer and hematological diseases, laying the foundation for the application of CRISPR/Cas9 technology in diseases, which is seen to have a vast future.

2 PRINCIPLE OF CRISPR/CAS9 SYSTEM

The CRISPR/Cas9 system mainly consists of Cas9 protein, crRNA and tracrRNA, while the first three were uniformly replaced by sgRNA as research progressed (sgRNA consists of base complementary pairing region, Cas9 handle and terminator) (Li, 2017). The adaptive immune system of bacteria and archaea for defense against foreign invading viruses and phages is the CRISPR/Cas9 Cas9 origin (Li, 2018). As an adaptive immune system, the RNA of CRISPR directs Cas proteins to recognize invading exogenous genomes in the form of base complementation and to shear exogenous DNA (Liu, 2015). The protein sequence structures can be classified into three types based on the difference in the sequence structure of Cas proteins and are noted as: type I, type II, and type III. Because type II contains only Cas9 protein and type I and III have multiple protein sequences, this section focuses on type II (Liu, 2015). Cas9 nucleic acid endonuclease has a large structural domain, in which the RuvC nucleic acid endonuclease-like domain at the N-terminus and the HNH nuclease-like activity in the middle play an important role in cutting DNA. When the Cas9 protein cleaves the DNA double strand resulting in a broken cut, HNH shears the complementary strand while RuvC cuts the non-complementary strand. And the binding of the CRISPR/Cas9 complex to exogenous spacer sequences is not affected when both endonuclease sites are mutated (Liu, 2015).

The CRISPR/Cas9 system is simplified and consists of two parts: Cas9 protein and sgRNA. The principles are as follows: 1. The successfully expressed sgRNA forms a complex through its own Cas9 handle and Cas9 protein; 2. The sequence of the base complementary pairing region of the complex sgRNA pairs with the target sequence of the target gene through the principle of base complementary pairing; 3. Cas9 uses its own nucleic acid endonuclease activity to cleave the target DNA sequence, as shown in Figure 1:

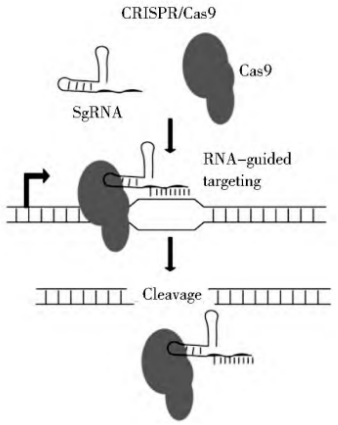

Figure 1: Cas9 cleaved DNA sequence (Li, 2017).

Cas9 proteins have multiple functions and large molecular weight, SpCas9 and SaCas9 are now the main widely used Cas9 proteins that have been applied to Streptococcus pyogenes and Staphylococcus aureus (Pan, 2020). In the type II system, crRNA becomes mature crRNA under the action of RNaseIII, followed by complementation with the homologous repeat sequence in tracerRNA hybridized into a double-stranded RNA dimer structure, which then binds to the Cas9 protein to form a cleavage complex, the targeting of which can be specialized for gene editing such as knockdown, insertion and targeted mutation of exogenous genes (Pan, 2020). The second part of CRIPS/Cas9 is a non-protein-coding RNA, tracrRNA, which is used to complete the maturation of crRNA and subsequent DNA shearing. In the case of Streptococcus pyogenes, for example, this RNA is transcribed from two initiation sites to produce a 171 nucleotide and 89 nucleotide sequence precursor tracrRNA of length, respectively, both of which are then further processed into a 75 nucleotide sequence mature tracrRNA, the precursor portion of which can complement the crRNA precursor, thereby facilitating its maturation (Li, 2018).

The cleavage of Cas9 produces DSBs (double-strand breaks) in eukaryotic cells by two repair pathways: non-homologous end joining and qualitative homologous repair (Li, 2017). In many bacteria without the NHEJ (nonhomologous ending joining) pathway, DSBs can also be repaired using a

cellular homologous recombination system with a chromosome or plasmid-borne template However, the process often results in arbitrary nucleotide insertions or deletions near the cleavage site. (Li, 2017) Thus, the NHEJ pathway typically alters the reading frame of the target gene cleaved by Cas9, prompting a shift in the target gene sequence and triggering the premature appearance of a stop codon, leading to the previously mentioned knockout (Li, 2017). The HDR pathway allows for precise Cas9 protein editing of the cleavage target, with specific nucleotide sequence editing, insertion, deletion and substitution of specific nucleotide sequences. However, since an efficient NHEJ pathway does not exist in many bacterial genomes, the DBS formed by Cas9 cleavage leads to cell death (Li, 2017).

3 CRISPR/CAS9 GENE EDITING TECHNOLOGY IN ONCOLOGY

Malignant tumor muscle, which can also be cancer, refers to a disease caused by abnormalities of cells These proliferating cells also invade other healthy parts of the body, resulting in a malfunction of the mechanisms that control cell division and proliferation.

Treatment of cancer is variable depending on many factors, including the type, location and amount of disease as well as the health status of the patient. Most treatments kill/remove cancer cells directly or cause their eventual death by depriving them of the signals needed for survival. Traditional treatments include: radiation therapy, surgery, and systemic therapy (chemotherapy). While radiation therapy is relatively safe (no anesthesia required) and can kill a large number of even invisible tumor cells in a specific area, it is prone to post-cure wound complications and poor healing; surgery has the ability to remove all cancer cells in a small area, but cannot kill microscopic lesions at the edge of the tumor; chemotherapy has the ability to kill cancer cells throughout the body, but cannot kill the tumor alone as well as systemic Toxicity makes this treatment option not the best choice either.

With the rapid development of high-throughput measurement technology and biological information technology, researchers have obtained a large amount of genetic information in tumor cells. In the process of tumor development, different genes play different roles at the same stage or the same gene at different stages (Liu, 2015). Therefore, studies related to tumor gene function need to effectively interfere with different gene expression at different stages of cell differentiation. Therefore, the study of tumor gene function requires effective interference with the expression of different genes at different stages of cell differentiation. On this basis, the effect of the gene on tumor development should be investigated so as to artificially and effectively control the level of gene expression within the cell. CRISPR/Cas9 is currently being investigated for three applications: 1. targeted editing of target genes using this gene editing technology, which has been widely used in genetic engineering of eukaryotes and prokaryotes; 2. genome-scale editing based on this technology, coupled with high-quality sequential technology screening in combination with phenotypic gene-related technologies; 3. Use of Cas9 (dCas9) after inactivation of nuclease activity to transform it into a device that uses RNA guidance to develop a wider range of uses by fusing effectors with dCas9 (Liu, 2015).

In 2014, Torres et al. first initiated the study of the CRIPR/Cas9 technique to construct a muscle model of malignancy. cas9, guided by specific sgRNA, cleaves outside the site-specific DNA, causing inversions and ectopics in the chromosome where the cleaved DNA is located, thus accurately mimicking the formation of some tumors such as Ewing's sarcoma (Qu, Li, Jiang, etc. 2015). In the same year, Xue et al. used CRIPS/Cas9 technology to successfully suppress double mutations in two oncogenes (p53 and pten), and animal liver cancer models were constructed. (Qu, Li, Jiang, etc. 2015) Platt et al. published a mouse tumor model in which a DNA plasmid expressing Cas9 nucleic acid endonuclease and sgRNA was injected into the liver of mice using a hydraulic tail vein injection technique; the Pten and P53 oncogenes were also edited in mice (Liu, 2015), and the targeting AVV subtype vector was designated as a CRISPR/Cas9 delivery system, allowing Cas9 to be specifically expressed in the liver and lung. The mouse model of lung adenocarcinoma was successfully constructed (Qu, Li, Jiang, etc. 2015). In addition, a study on the relationship between rectal cancer and the PIK3R1 gene reports the application of functional studies of solid tumor-related genes. The researchers used CRISPR/Cas9 technology to knock down the PIK3R1 gene at the level of rectal cancer cell lines, and later examined the changes in interepithelial stromalization, proliferation, and stem cell properties of tumor cells in the knocked-down cells and wild-type cell lines, respectively, thereby demonstrating that the PIK3R1 gene has the function of regulating

invasion, metastasis, and proliferation of rectal cancer cells (Liu, 2015).

4 CRISPR/CAS9 GENE EDITING TECHNOLOGY IN HEMATOLOGICAL DISEASES

Most of the hematological diseases are associated with genetic mutations and have limited and ineffective therapeutic techniques, while the application of gene editing technologies has made another possibility for the treatment of hematological diseases. Zinc finger ribonucleases and transcription activator-like effector nucleases were found to be less easy to design and less specific, as well as cytotoxic, making these two techniques not widely available. CRISPR/Cas9 is widely used in the field of disease research because of its simplicity, targeting specificity and affordability. In this section, we present the progress of CRISPR/Cas9 technology in two hematological diseases, thalassemia and hemophilia (Li, 2018).

Thalassemia results in impaired hemoglobin synthesis with symptoms similar to those of anemia 1. Fatigue and pallor due to low red blood cell hematocrit, as well as skeletal disorders, splenomegaly, and yellow fever. β - Thalassemia is caused by mutations or small fragment deletions of HBB (human bead protein) on chromosome 11, which affects the transcription, shearing, and translation of MRNA, resulting in β-hemoglobin deficiency 1. This results in a deficiency of β-hemoglobin.1 An abnormal excess of cells results in an excess of α-protein chains and in damage to the cell membranes of red blood cells, which may form toxic aggregates if the damage is too great. The only treatment currently available is hematopoietic stem cell transplantation, but this is expensive and difficult to match.1 In a 2012 clinical study in the United States, researchers transfected β-thalassemia patients with autologous CD34+ hematopoietic cells using a wild-type β-globin transgenic TNS9.3.55 vector (chronic viral vector), but no genetic markers were detected in any of the four subjects.1 In recent years, Liu et al. In recent years, Liu et al. used the CRISPR/Cas9 system to screen for optimal gRNA as well as a small molecule compound (L755507), in combination with ssODN, to transfect iPSCs from β - thalassemia patients, thereby repairing the deletion mutation in β 41-42 (TCTT).1 Unlike the former, Mettananda et al. used the α and β two-bead imbalance protein chains resulting in ineffective erythropoiesis and hemolysis in β -thalassemia principle, greatly reduced the excess free α -bead protein, thus greatly alleviating the clinical manifestations of the patient.1 In addition, they used the CRISPR/Cas9 system to delete mutations in the α -bead protein MCS-R2 enhancer on the patient's human hematopoietic stem cells to form α - thalassemia.1 After editing, CD34+ cells differentiated into mature erythrocytes, and the reduction of α-hemoglobin corrected the physiological imbalance between the two, thus effectively improving the patient's symptoms (Li, 2018).

Hemophilia is an inherited bleeding disorder associated with the X chromosome, which manifests itself by patients bleeding longer and bruising easily after surgery, along with an increased chance of bruising and brain bleeding. Due to the difference in genetic mutations, it can be divided into hemophilia A and hemophilia B. Both have the same symptoms with joint, muscle, and deep tissue bleeding. Replacement therapy is currently the mainstay, but the high cost of treatment and the production of antibodies to clotting factors by frequent intravenous infusions of clotting factor concentrates prevent replacement therapy from being a long-term applied and effective treatment.1 Hemophilia A is caused by mutations in the F8 gene that encodes clotting factor VIII. Researchers have induced pluripotent stem cells (iPSC) from patients containing inversion mutations in which the inversion gene was repaired to a normal genotype using the CRISPR/Cas9 system and no off-target mutations occurred throughout.1 Hemophilia B is caused by mutations in the clotting factor IX (F9) gene. The only current treatment is infusion of clotting factor concentrate, which has a short half-life and requires prolonged infusion. in 2011, researchers at a children's research hospital and the University of London, used a diphasic adeno-associated virus (scAAV) carrying the optimized codon FIX gene for treatment. After one year, stable expression of activated IX factors was detected in all six subjects, and the next 7-10 weeks, all showed specific T-lymphocyte immune responses induced by the viral capsid, accompanied by elevated transaminases. Factors such as hepatotoxicity, reduction or loss of introduced genes, and unmet demand for vector production limited clinical application.1 Ohmori et al. first abandoned conventional gene therapy by injecting adenoviral vectors expressing Streptococcus pyogenes Cas9 mRNA (Streptococcus pyogenes Cas9) and sgRNA targeting exon 8 of the F9 gene in mice8 (AVV8) was injected into the liver of wild adult mice. A double-stranded DNA break occurred

at the target site of the F9 gene, and the introduction of homologous recombination repair (HDR) at this site sufficiently enhanced FIX activity. In addition, insertion of F9cDNA in introns and repair with NHEJ or HDR was effective in restoring coagulation function (Li, 2018).

Currently, gene editing technology is not mature enough to be fully applied in the clinical setting. However, studies have shown that CRISPR/Cas9 gene editing technology will become an effective solution for gene therapy for the treatment of hematological diseases.

5 CONCLUSION

CRISPR/Cas9 systems are widely used in life sciences, agriculture, medicine, and industry because of their simple design and ease of operation. Scientists have used gene editing to construct animal disease models to study a variety of difficult clinical conditions. In addition, in agriculture, CRISPR/Cas9 has also accelerated genetic breeding in plants and animals, etc (Wang, 2017).

With the continuous improvement of CRISPR/Cas9 technology system, its application will be expanded in the fields of energy, environmental protection, and health. The technology can also be combined with other types of technologies, such as gene sequencing, gene expression analysis, disease modeling, and drug delivery, thus making the application of various technologies more extensive. In addition to the construction of animal models, it has been shown that CRISPR can also be involved in the regulation of bacterial metabolism. In addition, CRISPR technology is also widely used in other fields, for example, editing the mosquito genome to obtain specific antibodies to control the transmission of Plasmodium. The application of improving the growth rate and temperature resistance of fish, the research and application of pet size and coat color formulation, etc. also enable CRISPR technology to be widely used in daily life (Wang, 2107).

Despite the rapid development of CRISPR technology, there are still some problems. Scientists are refining this technology by modifying the editing proteins, using direct homologous enzymes, using material assistance, and other measures to make CRISPR/Cas9 technology applicable to more fields.

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