A review of progress in the CRISPR/Cas9

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Abstract: CRISPR/Cas9 is the third generation of genome site-specific editing technology after Zinc-finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN). With the features of simple design and easy operation, CRISPR/Cas9 plays an important role in modern genome engineering. This mini review mainly introduces the principles and development of genome editing technology based on CRISPR/Cas9 system, discusses the related ethical issues of genome editing technology, and puts forward the prospect of its application in various fields..

1. Introduction

Genome editing technology is a kind of genetic operation technology that can modify DNA sequence precisely and edit DNA sequence directionally at the gene level. As the main and widely used genome editing technology, artificial endonuclease technology has developed through o Zinc-finger nuclease (ZFN), the first generation and transcription activator-like effector nuclease (TALEN),the second generation. Nowadays, CRISPR/Cas9, the third generation of artificial endonuclease technology, which is easy to operate and can be targeted precisely with low methylation sensitivity, has become the most widely used one. Clustered regularly spaced short palindrome repeats (CRISPR) is a natural immune system conducted by bacteria and archaea to fight against invasive viruses and exogenous RNA. Compared with ZFN and Talen, CRISPR/cas9 enjoys the following significant advantages: ZFNs and Talens are based on protein-guided DNA cutting, which requires complex and time-consuming protein engineering, selection and verification. On the contrary, CRISPR/cas9 only needs a short programmable gRNA for DNA location, which is relatively cheap and easy to design and produce[1]. CRISPR system mainly includes three types: I, II and III [2]. In recent years, two new types of CAS system, type IV and V, have been found. Among them, type I and III need a variety of complex CRISPR related proteins (CAS proteins) to work together, while type II system only needs a CAS protein and sgRNA to edit the target[3], which provides an important condition for its wide application. CRISPR has been found for 20 years. With more profound and comprehensive research, cas9 system has become one of the most mature ones, which has been widely used in fields of life science such as medicine, agriculture and so on in recent years.

2.1 Discovery of CRISPR/Cas9 system

Since the late 20th century, people began to explore the genome editing technology. In 1987, a Japanese research group found the tandem spacer repeat near the alkaline phosphatase gene of K12 E.coli [4], and then found that it widely existed in the genome of bacteria and archaea. In 2002, the sequence was officially named of clustered regularly interspaced short palindromic repeats (CRISPR) [5]. In 2005, people first found the source of foreign spacer sequence and speculated that bacteria may resist the invasion of foreign genetic material through CRISPR system, and in 2007, people first confirmed its role in acquired immunity through experiments[6]. In 2008, the function of CRISPR system to resist the transfer of foreign plasmids was first verified[7]. In 2012, Cas9 was first found to be able to target DNA cleavage in vitro [8], which indicated that CRISPR gene editing technology has been applied in eukaryotic gene modification. Until 2013, Zhang Feng published an article in science magazine, using CRISPR/Cas9 technology in human cells, and realized the use of type II CRISPR system to complete gene editing in mammalian cells for the first time, which greatly promoted the

development of genome editing technology. Since then, a large number of CRISPR/Cas9 related research results have emerged. It has found the whole genome screening function of Cas9[9,10], as well as the crystal structure of apo-cas9, gRNA and target DNA multiplex. It has been identified that several small molecular compounds can improve the gene editing efficiency of CRISPR/Cas9 system.

2.2 Structure of CRISPR/Cas9 system

The basic structure of CRISPR/Cas9 system consists of four parts, including the CRISPR clusters, which are made up of discontinuous repeats(R) and spacers with similar length, leader and a series of related protein genes Cas. The 5 'end is a reverse activated CRISPR RNA gene, with a series of Cas protein coding genes (Cas9, Cas1, Cas2 and Csn2) in the middle; the 3' end is a CRISPR locus composed of a sequence of spacers and repeats, in which the length of the repeats is usually 21-48bp, and the spacers used to identify the CRISPR system and the target gene is usually 26-72bp; the Cas (CRISPR Associated) is a double stranded DNA nuclease located near CRISPR site. Cas9 protein contains two domains, which are involved in the maturation of crRNA and can modify specific DNA sequences under the guidance of guide RNA.

2. How CRISPR/Cas9 system works?

The working principle of CRISPR/Cas9 system can be summarized as follows: (1) the invasion of exogenous DNA to form new spacer sequence; (2) the synthesis of crrna to form RNase protein RNA CAS ternary complex; (3) the exertion of crispr-cas9 system activity: using crrna to recognize exogenous DNA, and cutting the exogenous DNA through Cas enzyme. When the external DNA invades for the first time, this sequence is stored in the CRISPR gene locus of bacteria, and a new spacer is formed to integrate into two repeats at the 5 'end. This process is called adaptation stage. The spacer formed in the adaptive stage is highly conserved, and the foreign DNA sequence corresponding to the spacer sequence is protospacer. There are several extended base sequences at the 5 'or 3' end of the spacer sequence and they are very conserved, which is called PAM (protospacer adjacent motif). When phage or virus infect bacteria, the host can recognize the invading nucleic acid and scan the potential PAM of external DNA, and take the adjacent PAM sequence as the candidate prospacer; then, the repeat sequence is synthesized at the 5 'end of CRISPR locus, and the new interval sequence is integrated into the two repeat sequences. Under the regulation of its precursor region, the spacer sequence is transcribed into crrna precursor (pre crrna). With the participation of trans activated tracrrna and Cas protein, it is processed into short mature crRNA including repeats and spacers. Under the action of endonuclease, crRNA-tracrRNA-cas9 complex with active and cutting power is formed. When exogenous DNA invades again, crispr-cas9 system can play its interference role on exogenous genetic material [3、6]. In 2012, Gasiunas and others found that it can mediate cas9 targeted DNA fragments effectively as well as natural crRNA and tracrRNA, and gradually optimize the CRISPR/Cas system to a Cas9/sgRNA system that is easier to operate by using single guide RNA (sgRNA) .

3. Applications of CRISPR/Cas9 system in the fields of life science

CRISPR/Cas9 technology has developed into the hottest new generation of genome editing technology with its advantages of convenient operation, simple design, high efficiency and multi-point editing, and has been widely used in all aspects of life science in recent years.

4.1 Medicine

4.1.1 Research on model biology

CRISPR/Cas9 can induce genome modification at multiple independent sites at the same time with the use of Cas9 and several different target gRNAs, which plays an increasingly important role in the construction of the model. Since 2012, CRISPR/Cas9 technology has been successfully applied to

gene modification of drosophila[12], mouse[13], arabidopsis[14] and other model organisms. For example, using CRISPR/Cas9 system to generate and detect gene mutation of drosophila has become a simple and efficient method. Bassett et al. [12] effectively induced up to 88% of drosophilas to produce yellow and white gene targeted mutations by directly injecting Cas9 system mRNA and sgRNA into the embryo, finding that this mutation could be passed on to the next generation, and the efficiency of CRISPR/Cas9 system was at least 10 times higher than previously reported.

4.1.2 Clinical medicine

In tumor therapy, CRISPR/Cas9 technology can knockout of oncogenes targetly, interfere with the expression of related proteins, affect the activity of oncoproteins, and thereby inhibit the growth of tumor. Chimeric antigen receptor-T cell (CAR-T cell) immunotherapy is to introduce the fusion gene CAR into the host T lymphocyte genome in the form of nucleic acid, so that CAR-T cells can be unrestricted as a major histocompatibility complex The form specifically recognizes and kills cancer cells expressing specific antigens[15]. On the other hand, CRISPR/Cas9 technology can also be used to targetly repair the tumor suppressor genes to restore their function and activity, so as to achieve the purpose of suppressing tumors.

As a genetic scissors, the CRISPR / Cas9 system can excise any genome, and by targeting HIV-1 long terminal repeats or highly conserved regions of basic viral genes, it provides a huge opportunity to eradicate the HIV-1 genome and eliminate latent viruses[16,17]. For example, a 2018 study[17] found that LTRs targeting HIV-1 infected T cells using the CRISPR/Cas9 system to target can effectively reduce the level of provirus in the cell. This gene editing method can also prevent subsequent HIV infection. In addition, the modified CRISPR/Cas9 can directly bind and activate the HIV proviral gene latent in the cell, and at the same time, combine the antiviral drugs to kill the activated HIV virus. CRISPR/Cas9 can directly act on the cell surface co-receptor CCR5 to protect cells from HIV invasion, which may also indicate that the technology can contribute to the prevention of HIV infection.

4.2 Agriculture

In 2019, Chinese Academy of Agricultural Sciences [18] used CRISPR/Cas9 gene editing technology to knock out PAI1, REC8, OSD1 and MTL these four rice endogenous reproduction-related genes of hybrid rice Chunyou 84 (CY84), resulting in hybrid rice hybridization reproductive traits, and thus cloned seeds of hybrid rice. The specific operation is: use CRISPR/Cas9 to edit REC8, PAIR1 and OSD1 meiosis control genes to produce diploid gametes and tetraploid seeds, and knock out the MTL gene of diploid gametes (MiMe) (during double insemination) Haploid seeds. In the end, hybrid heterosis and haploid induction were fixed, and 4 genes were edited at the same time to produce hybrid seeds through selfing, which achieved the fixation of heterozygous genotypes. With the continuous deepening of research on plant metabolic pathways, the use of molecular technology to improve crop quality has also achieved significant results. It not only solved the shortcomings of traditional breeding techniques and molecular marker breeding, but also broadened the genetic background of breeding varieties and avoided safety issues brought about by genetic breeding technology[18,19].

Blue ear disease is one of the most serious infectious diseases in pig industry in the world, but the use ofCRISPR/Cas9 technology can effectively produce safe genetically modified pigs with good resistance to blue ear disease. Whitworth et al. [20] experimented with somatic cell transplantation to knock out pig differentiation cluster 163 (CD163), a receptor gene that is considered to be a porcine blue ear disease virus. Studies had shown that this gene editing pig underwent blue ear disease. The transgenic pigs showed no clinical symptoms after being infected with blue ear disease strains, but pigs of the control group did. Besides, in 2018, the CRISPR/Cas9 system has begun to be used for the genome modification of African swine fever virus, and the knockout efficiency has been improved significantly [21]. It has also solved some problems of the traditional ASFV vaccine, such as not providing complete immune protection [22] or having toxic side effects. In addition, due to the proximity of evolutionary distance, size of certain organs, morphological structure, physiological

metabolism and immune system, etc., domestic animals, especially pigs, also provide a better model for the study of human diseases and the development of targeted drugs, which has an important application value in the field of biomedicine. [23-25]

4. Ethical concerns of CRISPR/Cas9 system

Burgeoning science and technology has always been a double-edged sword. We should not ignore its negative effects while enjoying its benefits. In the clinical application of this technology, the ethical issues of gene therapy can not be ignored. Ethical problems are mainly the safety problems faced by life individuals: Currently, there are still many potential safety risks in technology to eliminate diseases through gene editing [26]. Modification of disease genes that may be inherited in germ cells [27] requires complex techniques to ensure the existence and accuracy of gene modification in gametes, but current gene editing techniques are likely to inadvertently increase the possibility of interfering with or changing other genes, leading to gene mutation. Moreover, the germ cells of gene editing will be affected by the interaction between genetic variation and environment in the later development. This complexity will make it more difficult to predict the harmful effects of gene changes on human beings. These unpredictable risks may produce bad by-products and do unpredictable harm to the next generation.

Because of the unpredictability of the consequences, gene editing of embryos is also controversial in current technological and social background. A few of them hold the opinion that the modification or editing of embryonic DNA can deepen people's understanding of human early development. Meanwhile, researchers hope that in a long time, experimental research on embryos can be used in clinical to repair gene mutations that cause disease, so as to avoid the transmission of genetic diseases to the next generation. However, most ethicists and many researchers are still worried that these technologies will be used for non-medical purposes. Due to the complexity and particularity of the potential risks of human embryo gene editing technology, if improperly used, it is likely to cause serious consequences out of control, and even put human beings in a dangerous situation that is difficult to save. At present, about 30 countries in the world have directly or indirectly banned all clinical use of germline gene editing legislation, and many scientists have reached a general consensus in the field of gene ethics on human germ cell gene editing: gene therapy for the purpose of eliminating diseases is morally acceptable, but it must follow the basic ethical principles and norms of treatment Because ethical acceptability is the basis of the feasibility of the progress of gene editing technology, we should strengthen and standardize the ethical review of gene editing in medical clinical research[25,30], while giving full play to its potential, we should minimize the potential risks.

5. Prospects

Survival and development is the eternal theme of mankind. Since the 21st century, people of the world are facing a series of enormous tests. Current novel coronavirus pneumonia in 2020 is another world-class acute crisis since World War II. How to survive this crisis is closely related to biotechnology. In 2019, the world's population has exceeded 7.7 billion, which brings unprecedented challenges to global food security. It is an urgent need for mankind to improve crops faster. For this reason, the investment in biotechnology of all countries in the world is increasing day by day. The technology update brought by CRISPR/Cas9 is a buffer and an opportunity for these human challenges.

It can't be ignored that off-target effects is still the most essential safety problem of CRISPR/Cas9 in human disease treatment[26,27]. Although selecting segments with low homology with human gene to design the target of gene editing virus can reduce the Miss mutation rate to a certain extent, off-target effects has seriously restricted the wide application of CRISPR/Cas9 technology, especially in the clinical application. The main direction of further improvement is to reduce the off-target effects and solve the problem that multiple different genes can be precisely edited at the same time. With the deepening of research on CRISPR/Cas9 system, some limitations of

CRISPR/Cas9 technology will be overcome. In the near future, CRISPR/Cas9 system will be widely used in model biology research, clinical medicine and agriculture.

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