Development of Gene Editing Technology

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Abstract

Gene editing technology is a kind of technology for specific modification of genomic sequences, Double-strand breaks are caused by cleavage of a specific site sequence by an artificial endonuclease, Gene knockout, site-directed mutagenesis or gene repair of the target sequence is achieved by deletion, insertion or substitution of the DNA fragment, This paper reviews the basic principles of gene editing technology, zinc-finger nuclease(ZFN), Transcription activator-like effector nuclease(TALEN), and CRISPR/Cas9 technology. Finally, the paper discusses the application history and limitations of gene editing technology, as well as the future development trend of gene editing technology.

Keywords

Gene editing, ZFN, TALEN, CRISPR/Cas9.

1. Introduction

Gene editing technology is a kind of technology for specific modification of DNA sequences at the level of biological genome, including gene knockout, gene addition, and gene replacement. With the continuous improvement and development of gene editing technology[1-2], three generations of gene editing technology, namely artificial zinc finger nucleases technology, transcription activator-like effector nucleases technology, and CRISPR/Cas9 technology, have been developed successively. Compared with traditional gene editing technology, the new gene editing technology has higher accuracy, faster efficiency and lower cost. No matter which kind of gene editing technology, the principle is similar, will cause the DNA double strand breaks, thus activating DNA repair mechanism. DNA repair mechanism include Homology-directed repair(HDR) and non-homologous end-ligation repair(NHEJ), homology-directed repair(HDR) has high fidelity, in the case of a homologous arm, the foreign gene will be completely integrated into the target site by homologous recombination, thereby realizing the replacement of the original gene, and not cause random base insertion or base loss. Non-homologous end-ligation repair(NHEJ) has low fidelity, random insertion or loss of bases is likely to occur during the DNA repair process, and resulting in a frameshift mutation to inactivate the gene and achieve knockout of the target gene. Non-homologous end-ligation repair will also connect the exogenous gene sequence into the repair site, enabling targeted insertion of the gene, if an exogenous gene sequence exists.

2. Zinc finger nucleases(ZFN)

Zinc finger nuclease was discovered in 1995[3], it was the first generation of synthetic nucleases. Zinc finger nucleases are composed of a zinc finger protein (ZFP) that specifically recognizes a DNA sequence and a cleavage domain of a FokI endonuclease[4]. Zinc finger structure (ZF) is the basic structural unit of zinc finger protein, which is present in most proteins, but also has structural differences between different species. Zinc finger protein (ZFP), which composes of zinc finger structure, can specifically recognize DNA sequences for cleavage and lead to double-strand breaks. Each ZFP domain recognizes a 3 bp DNA sequence, we can construct different sequence sizes of ZFP structures to achieve the purpose of identifying different DNA sequences. FokI is a restriction endonuclease expressed by F. faecalis, which can specifically recognize the GGATG nucleotide sequence, thereby to cause the organism repair itself. Zinc finger nuclease technology is widely used

in a variety of animal genetic modifications, including mice, rats, zebrafish, pigs, and so on[5-7], however, zinc finger nuclease technology still has certain problems, such as high cost, cytotoxicity and off-target phenomenon.

3. Transcription activator-like efector nuclease(TALEN)

This technology was found in the phytopathogenic bacteria Xanthomonas[8], similar to zinc finger nuclease, TALEN are composed of tale protein and FokI endonucleases that specifically recognize and bind DNA. The tale protein contains three components, a C-terminal contains a nuclear localization signal and a transcriptional activation domain, a N-terminal contains a transport domain and a intermediate domain contains a repeating amino acid sequence that specifically binds to DNA (Figure 1). The intermediate domain consists of 1.5-33.5 tale units, each of which contains 33-35 amino acid residues. The difference between 12 and 13 residues determines the specific recognition of tale protein and DNA sequence, it is called a repeat variable diresidue[9], and the other residues are basically the same. As an emerging gene editing technology, TALEN technology has higher recognition rate and lower off-target rate than zinc finger nuclease technology, but TALEN also have some problems, such as the toxicity of cells is not very clear, large amount of work in sequence design, this affects the wide-ranging extension of the technology.

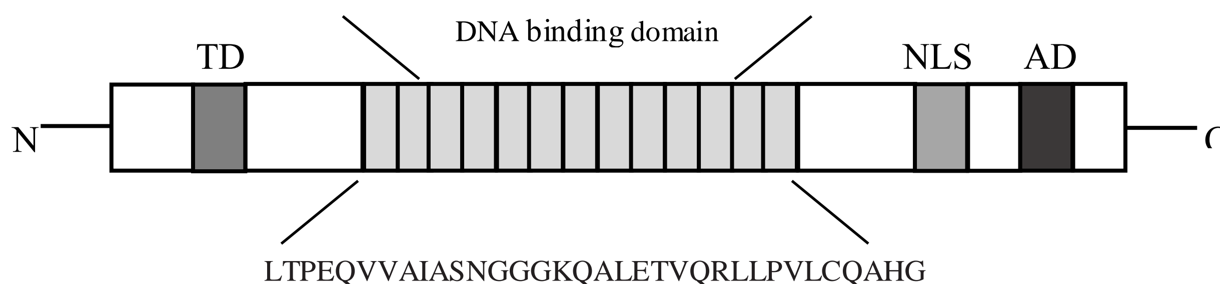

Fig. 1 Structure of tale protein

4. Clustered regularly interspaced short palindromic repeats/Cas endonuclease(CRISPR/Cas)

Shortly after the advent of TALEN technology, scientists developed a CRISPR/Cas-based genome editing technology[10-11]. The CRISPR/Cas9 system originates from the naturally occurring acquired immune system during the long-term evolution of archaea and bacteria to protect its own genes from the interference and destruction of viruses, bacteriophages and other exogenous nucleic acids[12]. The researchers finds a highly conserved gene that encoding a CRISPR-associated protein(Cas) in the vicinity of the active CRISPR genome, the RNA transcribed from the CRISPR binds to the Cas protein to form a nucleic acid protein complex, which recognizes and cleaves the exogenous DNA, and play immune function of the CRISPR/Cas system. At present, the CRISPR/Cas9 system comprises the following three parts: a transactivation sequence region, a Cas protein coding gene sequence region, and a CRISPR sequence region. The leader sequence in the upstream of the CRISPR sequence acts as a promoter to initiate transcription of the immature CRISPR RNA(crRNA), binds to trans-activating CRISPR RNA(tracrRNA) and matures, and forms a complex with Cas protein, and then guides the Cas protein to recognize and cleave the target sequence[13]. CRISPR/Cas9 technology is widely used in tumor immunotherapy and biological model establishment[14-16].

5. Conclusion

With the continuous development and progress of gene editing technology, there is more space for applications in various fields. As the most popular gene editing tool, CRISPR/Cas9 technology has made significant progress in design difficulty and cost of use compared to ZFN and TALEN, it is becoming the tool of choice for many laboratories. The CRISPR/Cas9 technology has made

tremendous breakthroughs and progress in the biomedical field in the past five years. Although there are still problems such as off-target effects, with the continuous development and improvement of researchers, these new technologies will bring real value and benefits.

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