Genome Editing with ZFN, TALEN and CRISPR/Cas Systems: The Applications and Future Prospects

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Introduction

With the rapid development of zinc finger nucleases (ZFN), transcription activator-like effector nuclease (TALEN) and Cluster regularly interspaced short palindromic repeat (CRISPR)/Cas systems, manipulating genome becomes relatively easy and efficient. There are two major purposes for genome manipulation: one is for understanding novel genes function and their regulation roles in cells. Recent genomic and genetic approaches for disease genes have identified many novel loci underlie the disease phenotypes. More than 2,000 robust associations have been found in more than 300 complex diseases and traits in recent years by genome-wide association studies (GWAS) [1]. Understanding the function roles of genetic loci, particular novel genes will bring new insight of the diseases mechanisms. The second major application for manipulating genome is providing a new therapeutic means for many genetic disorders. A disease causing mutation could be replaced with normal allele in several Mendelian monogenic diseases.

Classic gene targeting relies on the homologous recombination when DNA fragment is introduced in the genome. Although recombination with LoxP/Cre and FRT/Fpe systems is widely used for gene targeting, it requires many steps of cloning and selections [2]. The lab work is still time consuming and complicated and targeting efficiency sometimes is low. In the past decade, the development of ZFN, TALEN systems dramatically increase the efficiency of genome editing. These approaches uses engineered nucleases that are composed of sequence-specific DNA-binding domains and are fused to a nonspecific DNA cleave module FokI. These chimeric nucleases enable efficient and precise genetic modifications by inducing targeting DNA double strand breaks (DSBs). All eukaryote cells repair DSBs through the efficient and precise genetic modifications by inducing targeting DNA double strand breaks (DSBs). All eukaryote cells repair DSBs through

The basic structure for ZFN and TALEN consists of sequence-specific DNA binding domain and a nonspecific DNA cleavage module [4]. The Zincfinger is an ideal platform for the design of novel DNA binding domain. The ZFP region provides a ZFN with the ability to bind a specific base sequence. This region contains a tandem array of Cys-His2 fingers, each recognizing approximately 3 base pairs of DNA. The DNA binding domain of a typical TALE comprises a tandem arrays of 15.5-19.5 single repeat, each one consist of about 34 highly conserved residues. The DNA binding specificity of each repeat is essentially driven by a polymorphism at position 12 and 13 within the module called repeat variable di-residual (RVDs). Eg, HD for C, NI for A, NG for T and NK for G [8]. For FokI is only active as a dimer. The artificial nucleases ZFN and TALEN are composed of active pairs in which two monomers bind adjacent target sites separated by a DNA spacer which allows for the formation of an active dimer to cleave the target locus [9].

Recent further another nuclease system CRISPR/Cas provides an alternative gene editing platform. It has potential for multiplexed genome editing. In Bacteria, Cas protein, CRISPR RNAs (crRNA) and trans-activating crRNA form ribonucleoprotein complexes, which target and degrade foreign DNA. The process is guided by crRNAs [10]. For construction of the targeting nucleases, short segments of foreign DNA as spacer are integrated with the CRISPR genomic loci and transcribed and processed into short CHRISPR RNA, these crRNAs anneal to trans-activating crRNAs and direct sequence-specific cleavage and silencing of pathogenic DNA by Cas protein. The system can be retargeted to cleave virtually any DNA sequence by redesigning the crRNA [11].

The Applications of ZFN, TALEN and CRISPR/Cas in Biologic Research

The rapidly development of endonucleases systems of ZFN, TALEN and CRISPR/Cas has greatly impacted on the biomedical research. Gene disruption is the useful means to understand the gene’s function in model organisms’ when taking advantage of errors introduced during DNA repair to disrupt or abolish the function of a gene or genomic region. Gene addition is normally used inducing the addition allele in the genome, for example adding three endogenous loci (AAVS1, OCT4 and PITX3) in mammalian ES and iPS cells [12] and the cells retained characteristics of pluripotency. Gene correction or replacement refers to replacing disease-causing mutation allele with

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no mutation allele in the genome. ZFN, TALEN have been widely used in the gene disruption, gene addition and gene correction. From human, mouse, zebrafish, pig, rat to hamster, Arabidopsis and rice.

CRISPR/GAS can be directly portable to human cells by co-delivery of plasmid expressing the Cas9 endonuclease and the necessary crRNA components. One step generation of mice carrying mutations in multiple genes that has greatly accelerate the in vivo study of functionally redundant genes and epistatic gene interaction [13].

Correction of an allele of a disease gene could be curative in several Mendelian genetic disorders. ZFN-induced HDR has been used to directly replace the disease mutations of X-linked severe combined immune deficiency (SCID) [14], sickle cell disease [15] and alpha-1-antitrypsin deficiency [16]. ZFN based approaches has also been taken place to the clinical for treatment of leukaemia [17]. It was also used for HIV treatment by delivery of CCRA32 gene that showed to confer resistance to the virus [18].

The Limitations and Future Directions

Off target effects refer the cleavage of nucleases at undesired location. This could happen as the DNA binding domain to recognize target sites; the dimerization of the natural binding domain and be fused to enzymatic domains. The obligate heterodimer Fok I domains can be easily combined with the TALE arrays to prevent homodimerization of TALE monomers. It also showed high DNA cleavage activity only at spacers of 10-15 base pairs [19]. Another attempt was exchanging the non-specific Fok I cleavage domain with the PvuII restriction nuclease, not like Fok I this enzyme will only cleave targets that contain a site in the spacer sequence [20].

The viral and nonviral gene delivery of the site-specific nucleases is the other hurdle for the applications of the genome editing. Electroporation, lipid-based regents can be toxin for the cells. Viral vectors also have limitation as they are complex, difficulty to produce, potentially immunogenic. AAV is a promising vector for the efficiency of ZFN-mediated HDR, and can drive ZFN-mediated gene correction. AAV allows packing expression cassette less than 4.2 kb, it is enough to accommodate both ZFN monomers and an engineering donor construct. But only a single TALEN monomer with a minimal promoter sequence can be inserted into this vector [21].

ZFN proteins are capable of crossing cell membranes and inducing endogenous gene disruption. This provides a great opportunity to deliver the nucleases without virus transfection. For CRIPSR Cas system, the requirement for a NCG PAM sequence of S progenies Cas9 limits the targets space in the mouse genome [13]. Exploiting different Cas9 protein may enable to target most of the mouse genome. It is also interesting to know if Cas9 endonuclease can be integrated as a DNA-binding domain and be fused to enzymatic domains.

In summary the rapid development of genome editing with the endonuclease systems has dramatically changed the biomedical research. These systems are not only great platforms for investigating the genes’ functions, but also provide a valuable means to treat many diseases from Mendelian disorders to cancers. The researches on nucleases for genome manipulation will revolutionize medical cares for many complex genetic diseases in the future.

References