Editorial

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 traitsin recent years by genome wide association studies (GWASs) [1]. Understudying 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 re-combineering with *LoxP*/Cre and *FRT*/Flpe systems is widely used for gene targeting,it requires many steps of cloning and selections [2]. The lab work is till 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 andare fused to a nonspecific DNA cleave module *Fok* l. These chimeric nucleases enable efficient and precise genetic modifications by inducing targeting DNA double strand breaks (DSBs). All eukaryote cells repair DSBsthrough DNA repair mechanisms, including error-prone nonhomologous end joining (NHEJ) andhomology-directed repair [3].There are three kinds of nucleases systems are widely used for gene editing:ZFN, TALEN and CRISPER-associated based RNA-guided DNA endonucleases. The systems are combined with simplicity and flexibility for the system and have been widely used for the genome editing in recent years.

The Basic Structures of ZFN, TALEN and CRISPER/Cas Systems

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, previous studies individual ZFNs used three fingers to construct a 9-bp target, which enabled ZFN dimmersto specify 18bp of DNA per cleavage site [5]. More recent studies added up to six fingers per ZFN for increasing the speciality [6]. The Fok l DNA-cleavage domain is fused to DNA binding domain. It can cleave both DNA strands when two nuclease domains unite to form a functional endonuclease. ZFNs can be assembled as modules that are custom designed to recognize specifically customer-designed DNA, typically 18-24 base pairs DNA for3-4 zinc finger each sides with a 4-7 base pair spacer between two half sizes [7].

The customer-designed domain engineering is time consuming and may associate with high rate of failure in the lab for ZFN engineering. Since the effectiveness of the endonuclease depends almost solely on their DNA binding domain, the TAL effector proteins from number of bacterial pathogens for plant represent a potential source of modularly constructed. 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 unit 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]. As *Fork* l 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 emerge another nuclease system CRISPR/Casprovides 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 CHRISP RNA, these crRNAs anneal to trans-activating cRNAs 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 CRIPSR/Cas has greatly impacted on the biomedical research. Gene disruption is the useful means to understand the gene's function in model organism's 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

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 alpha1-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 CCRΔ32 gene that showed to confer resistance to the virus [18].

The Limitations and Future Directions

Off targeteffects refer the cleavage of nucleases at undesired location. This could happen as the DNA binding domain to recognize target sites; the dimerization potential of the natural *Fok* l cleavage, the promiscuity of the short linker. Many optimized protocols to produce zinc finger arrays and TALE with higher specificities. The obligate heterodimer *Fok* l 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* l cleavage domain with the *Pvull* restriction nuclease, not like *Fok* l this enzyme will only cleave targets that contain a *Pvull* 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-mediate 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 CRIPSPR Cas system, the requirement for a NGG 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

1. Manolio TA (2013) Bringing genome-wide association findings into clinical use. Nat Rev Genet 14: 549-558.

2. Liu P, Jenkins NA, Copeland NG (2003) A highly efficient recombineering-based method for generating conditional knockout mutations. Genome Res 13: 476-484.

3. Ward RL, Packham D, Smythe AM, Murray J, Anderson-Stewart P, et al. (2000) Phase I clinical trial of the chimeric monoclonal antibody (c30.6) in patients with metastatic colorectal cancer. Clin Cancer Res 6: 4674-4683.

4. Patel SD, Moskalenko M, Tian T, Smith D, Mc Guinness R, et al. (2000) T-cell killing of heterogenoustumor or viral targets with bispecific chimeric immune receptors. Cancer Gene Ther 7: 1127-1134.

5. Goldstein G, Manson K, Tribbick G, Smith R (2000) Minimization of chronic plasma viremia in rhesus macaques immunized with synthetic HIV-1 Tat peptides and infected with a chimeric simian/human immunodeficiency virus (SHIV33). Vaccine 18: 2789-2795.

6. Onori A, Pisani C, Strimpakos G, Monaco L, Mattei E, et al. (2013)UtroUp is a novel six zinc finger artificial transcription factor that recognises 18 base pairs of the utrophin promoter and efficiently drives utrophinupregulation. BMC Mol Biol 14: 3.

7. Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD (2010) Genome editing with engineered zinc finger nucleases. Nat Rev Genet 11: 636-646.

8. Sun H, Biggs JC, Smith GM (2000)Antitumor activity of a chimeric antibody against the leucocyte antigen CD48. Cancer Immunol Immunother 48: 595-602.

9. Smith J, Bibikova M, Whitby FG, Reddy AR, Chandrasegaran S, et al. (2000) Requirements for double-strand cleavage by chimeric restriction enzymes with zinc finger DNA-recognition domains. Nucleic Acids Res 28: 3361-3369.

10. Gasiunas G, Barrangou R, Horvath P, Siksnys V (2012) Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc Natl Acad Sci U S A 109: E2579-E2586.

11. Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, et al. (2013) Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell 152: 1173-1183.

12. Hockemeyer D, Soldner F, Beard C, Gao Q, Mitalipova M, et al. (2009) Efficient targeting of expressed and silent genes in human ESCs and iPSCs using zinc-finger nucleases. Nat Biotechnol 27: 851-857.

13. Wang H, Yang H, Shivalila CS, Dawlaty MM, Cheng AW, et al. (2013) One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell 153: 910-918.

14. Urnov FD, Miller JC, Lee YL, Beausejour CM, Rock JM, et al. (2005) Highly efficient endogenous human gene correction using designed zinc-finger nucleases. Nature 435: 646-651.

15. Sebastiano V, Maeder ML, Angstman JF, Haddad B, Khayter C, et al. (2011) In situ genetic correction of the sickle cell anemia mutation in human induced pluripotent stem cells using engineered zinc finger nucleases. Stem cells 29: 1717-1726.

16. Yusa K, Rashid ST, Strick-Marchand H, Varela I, Liu PQ, et al. (2011) Targeted gene correction of alpha1-antitrypsin deficiency in induced pluripotent stem cells. Nature 478: 391-394.

17. Provasi E, Genovese P, Lombardo A, Magnani Z, Liu PQ, et al. (2012) Editing T cell specificity towards leukemia by zinc finger nucleases and lentiviral gene transfer. Nat Med 18: 807-815.

18. Holt N, Wang J, Kim K, Friedman G, Wang X, et al. (2010) Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 in vivo. Nat Biotechnol 28: 839-847.

19. Mussolino C, Morbitzer R, Lutge F, Dannemann N, Lahaye T, et al. (2011) A novel TALE nuclease scaffold enables high genome editing activity in combination with low toxicity. Nucleic Acids Res 39: 9283-9293.

20. Schierling B, Dannemann N, Gabsalilow L, Wende W, Cathomen T, et al. (2012) A novel zinc-finger nuclease platform with a sequence-specific cleavage module. Nucleic Acids Res 40: 2623-2638.

21. Gaj T, Gersbach CA, Barbas CF, 3rd (2013) ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol 31: 397-405.