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Mini review

Utilization of TALEN and CRISPR/Cas9 technologies for gene targeting and modification

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Abstract

The capability to modify the genome precisely and efficiently offers an extremely useful tool for biomedical research. Recent developments in genome editing technologies such as transcription activator-like effector nuclease and the clustered regularly interspaced short palindromic repeats system have made genome modification available for a number of organisms with relative ease. Here, we introduce these genome editing techniques, compare and contrast each technical approach and discuss their potential to study the underlying mechanisms of human disease using patient-derived induced pluripotent stem cells.

Keywords: TALENs, CRISPR/Cas9, gene editing, disease modeling, gene therapy

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Introduction

The development of recombinant DNA technologies has provided scientists with the fundamental tools to modify DNA sequences. These tools paved the way for the introduction of conditional alleles at specific genomic loci. To edit the genome, the introduction of a targeting construct coupled with homologous recombination (HR) was traditionally employed. Unfortunately, this approach could prove inefficient and labor intensive.[1–3] Moreover, genomic targeting in eukaryotes was restricted to model organisms due to the ease of embryonic stem cell manipulation.[2,4,5] Recently, the development and application of the sequence-specific endonucleases transcription activator-like effector nucleases (TALENs)[6–10] and the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system[11–15] have made a revolutionary contribution to the genome editing toolbox. The advent of these technologies now enables researchers to readily manipulate any gene of interest in numerous model organisms.

TALEN and CRISPR technologies provide precise and efficient genetic modification by inducing a double-strand break (DSB) at a specific target site – an essential step for performing targeted genomic editing. The presence of a DSB activates innate cellular DNA damage repair mechanisms,[16] including the dominant error-prone non-homologous end joining (NHEJ) pathway[17,18] and the less frequent homology recombination-directed repair (HDR) pathway.[19] DNA repair by NHEJ has the potential to lead to gene disruption by introducing deletions or mutations, while the HDR-repair pathway can be used to introduce precise changes in the presence of a donor DNA template.

In this review, we introduce TALEN and CRISPR technologies and review the extensive scientific progress made using these novel approaches. We also summarize the application of these tools in modeling human disease and discuss the future prospect of utilizing these techniques coupled with induced pluripotent stem cells (iPSCs) for future gene therapies.

TALEN-mediated genome editing

TAL (transcription activator-like) effectors or TALEs are site-specific DNA-binding proteins derived from the plant pathogen *Xanthomonas* sp., which uses TALE proteins to weaken host defenses by activating genes favorable to bacterial infection.[6–8] Each DNA-binding module of a TALE protein typically consists of 34 amino acids arranged in tandem. These repeats are nearly identical in sequence except for two highly variable amino acids in the 12th and 13th amino acid positions (termed the repeat variable diresidue or RVD). The RVD position establishes base-recognition specificity, and thus distinct RVDs allow TALEs to recognize a specific target DNA base (NI = A, HD = C,

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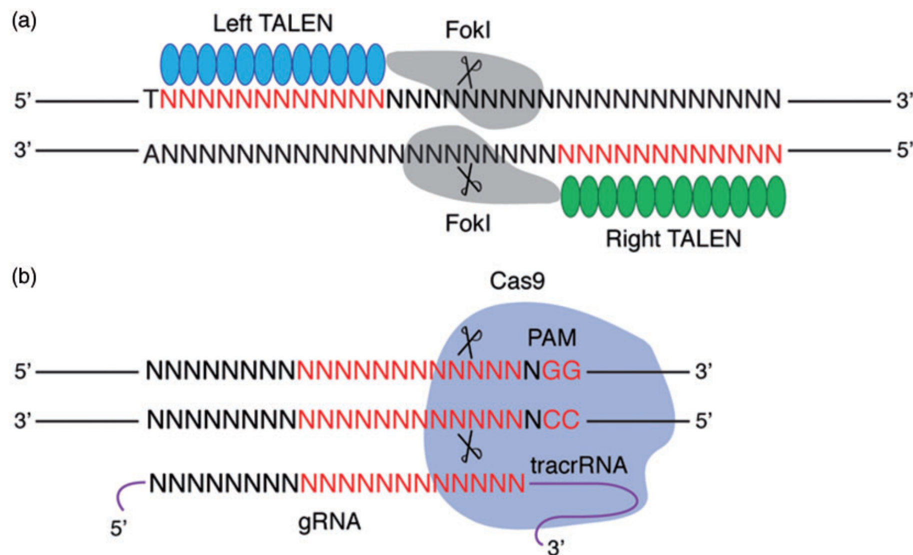

Figure 1 Genome targeting by TALEN and CRISPR/Cas9 systems. (a) TAL (transcription activator-like) effector (TALE) proteins (shown as blue and green spheres) bind to target sequences (shown in red) to generate a site-specific double-stranded break (DSB) upon dimerization of fused FokI endonucleases (shown in grey). TALE targeting sites are typically preceded by a thymine (T) at the 5′ end. N is any base. (b) The CRISPR/Cas9 system relies on an engineered guide RNA (gRNA) to target DNA. The gRNA is constructed from the fusion of a crRNA and a tracrRNA (transactivating CRISPR RNA) (see text). The gRNA complexes with Cas9 (shown in purple) to induce cleavage of target DNA sites complementary to 20 nucleotides of the gRNA and located adjacent (5′) to the PAM sequence. Studies have demonstrated that the 12 base pairs closest to the PAM sequence are the most important for specificity (shown in red). N is any base

NG = T, NH = G or NN = G/A). An array of four different repeat units has been shown to be sufficient to generate TALEs with novel DNA recognition sites.[9,10,20,21]

The ability to recognize and subsequently cut DNA at specific sites is accomplished by fusion of the catalytic domain of the FokI endonuclease to TALE repeats,[22] which subsequently generate a TALEN protein (Figure 1(a)). Since dimerization of the catalytic domain of FokI is mandatory for nuclease activity, a pair of TALENs must be designed to recognize DNA sequences to the left and right of the intended cut site.[6–10] Consequently, TALENs can be employed to generate site-specific DSBs to facilitate genome editing through NHEJ or HDR.

To date, TALENs have been used to target genomic loci in a number of human cell lines, including embryonic stem cells (hESCs) and iPSCs.[7,23–27] Furthermore, TALEN-mediated genome targeting has been shown to also function in plants,[6] fruit flies[28,29] worms,[30] frogs,[31] and zebrafish.[8,32–34] Most recently, TALEN proteins have been used for rapid gene modification in mouse, rat, and rabbit by embryo microinjection.[35–37]

CRISPR/Cas9-mediated genome editing

CRISPR are segments of repetitive DNA sequence found in the bacterial genome.[38] These segments serve to protect the organism from invading foreign nucleic acids, such as viruses or plasmids.[39–41] CRISPR systems have been shown to integrate invading foreign DNA between repeats. This integration provides a novel DNA template for the transcription of hybrid RNA molecules (crRNAs) that contain sequences from both the adjacent CRISPR arrays and the invading DNA (termed the protospacer sequence). Following transcription, each crRNA hybridizes with a second RNA (known as the transactivating CRISPR RNA or tracrRNA), and together, these molecules form a complex with the Cas9 (CRISPR associated protein 9) nuclease.[42,43] DNA cleavage by the Cas9 nuclease targets DNA by relying on the protospacer-encoded region of the crRNA to direct the complex to a region called the protospacer adjacent motif or protospacer adjacent motif (PAM).

The type II CRISPR/Cas9 system that has been adapted from *Streptococcus pyogenes* is capable of inducing sequence-specific DSBs that allow targeted genome editing (Figure 1(b)). This modified CRISPR/Cas9 system requires the interaction between the Cas9 nuclease and a newly engineered guide RNA (gRNA).[11,12,43] The gRNA is a single RNA chimera that is constructed from the fusion of a crRNA and a tracrRNA, and modification of 20 nucleotides at the 5′ end of the gRNA (corresponding to the protospacer region of the crRNA) serves to guide Cas9 to putative cleave sites to generate DSBs. CRISPR/Cas9 has been demonstrated to induce targeted cleavage at predicted sites in a number of different mammalian cell lines, including stem cells, and in a number of eukaryotes.[13–15,44–56] Moreover, Cas9 has also been engineered into a nicking enzyme to increase specificity and facilitate homology-directed repair with minimal off-target rates.[57–60] Additional studies have demonstrated that the CRISPR/Cas9 system is additionally capable of multiplex genome engineering by simultaneous introduction of multiple gRNAs, easing programmability and exhibiting broad applicability of this system.[14,15,44]

Efficiency and specificity of TALENs and CRISPR/Cas9 systems

The efficiency of genome targeting has always depended on a number of technical factors, including the cell type being targeted and the location of the targeting site.

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Importantly, the targeting efficiencies of TALEN and CRISPR/Cas9 technologies are much higher in a number of different cell types compared to traditional genome editing methods.[7,8,12,14] However, when targeting hESCs and iPSCs, studies have shown that use of the CRISPR/Cas9 system is substantially better at promoting NHEJ and HDR to generate mutant clones than TALENs.[61,62]

One significant concern related to the use of TALENs and/or CRISPR/Cas9 is the risk of off-target mutagenic effects that can be introduced during the genome targeting processes. In principle, TALENs only function when dimers between the FokI nuclease domains come together, thus, the specificity is determined by the combination of two TALEN DNA-binding domains. The specificity of the CRISPR/Cas9 system is instead determined by the presence of the PAM sequence and the 20 nucleotides upstream of the PAM site in the target genome (included in the gRNA). Recent studies on CRISPR/Cas9 have demonstrated that the 12 base pairs closest to the PAM sequence are the most important for specificity.[11,63]

Transformed human cell lines, such as embryonic kidney 293T cells and erythromyeloblastoid leukemia K562 cells, have been used to study the off-target effects.[64–66] Higher levels of off-target mutagenesis suggested that the CRISPR/Cas9 system is susceptible to cleavage at off-target loci due to mismatches in the gRNA. However, controlling the concentration of the Cas9 mRNA appears to diminish these off-target effects. The inducing single-stranded DNA breaks (nicks) generated by the CRISPR/Cas9-D10A nickase nuclease have been shown to reduce the off-target rate by more than 1500 fold.[57,64] Notably, whole-genome sequencing of TALEN and CRISPR/Cas9 gene targeted cells has also shown that off-target mutations are extremely rare in hESCs and iPSCs.[67–69]

Generation of disease models using TALENs and CRISPR/Ca9 targeting

On-going genome targeting in human cells using TALENs and/or CRISPR/Cas9 will help establish disease models that more accurately reflect the pathogenesis observed in patients and provide proof-of-principle approaches for future gene therapies. To that end, iPSCs derived from the somatic cells of patients can provide a useful tool for studying the underlying mechanism of human disease and may serve as a promising source for cell replacement therapies.[70,71]

Due to the unpredictable genetic variations of patient-derived iPSCs, researchers have been challenged to distinguish minor, disease-related phenotypic changes from otherwise normal variations in different genetic backgrounds. Therefore, one of the best ways to study the function of disease-related gene mutations is to utilize TALENs or CRISPR/Cas9 technologies to introduce mutations in a more controlled environment. Recently, mutations in 15 genes related to metabolic diseases were introduced using TALENs to provide isogenic control cell lines and to demonstrate cell-autonomous phenotypes.[25] The generation of additional isogenic iPSCs that differ exclusively at disease causing genomic loci will provide invaluable tools towards finding a solution to this problem.

Conversely, other laboratories have utilized TALENs and CRISPR/Cas9 technologies to repair mutations in patient-derived iPS cells. TALENs have been used to correct a mutation found in patients suffering from alpha-1 antitrypsin (AAT) deficiency, a genetic liver disorder that predisposes patients to liver cirrhosis and hepatocellular carcinoma, cystic fibrosis, and Gaucher's disease.[72,73] Moreover, CRISPR/Cas9 has been used to study cystic fibrosis by correcting a mutation found in the transmembrane conductor receptor (CFTR) gene in primary adult stem cells.[74] TALENs and the CRISPR/Cas9 system have also been used to target mutations found in iPS cells derived from Duchenne muscular dystrophy (DMD) patients. This correction leads to the generation of skeletal muscle cells that express a wild type version of dystrophin, a protein that is fundamental in supporting muscle fiber strength. Together, these reports demonstrate that genome-modifying systems provide an invaluable tool for generating and investigating models of human disease.

Generation of mouse models using TALENs and CRISPR/Cas9

TALENs and CRISPR/Cas9 are being employed to quickly and directly generate mouse models of human disease. The direct microinjection of TALENs and CRISPR/Cas9 into zygotes enables instant germline modifications and accelerates the generation of mouse models.[75–77] In addition to germline manipulation, a Cre recombinase-(Cre) dependent Cas9 knock-in mouse model was recently shown to enable the introduction of genetic alterations via the expression of gRNAs in specific tissues (brain, bone marrow, and lung) through adeno-associated virus (AAV), lentivirus or particle-mediated delivery.[78] This technology allows genetic modifications to be introduced in the genome of somatic cells (*in vivo* or *ex vivo*) for immediate analysis of phenotypes associated with disease-causing mutations. Moreover, another study has shown viral-mediated delivery of the CRISPR/Cas9 system to alter the somatic cells of mice to introduce the fusion of the EML4-ALK oncogene. This fusion is detected in a subset of human non-small cell lung cancers (NSCLC). The fusion of EML4 (echinoderm microtubule-associated protein like 4) and ALK (anaplastic lymphoma kinase) results from an inversion of the short arm of chromosome 2, and modeling of this particular fusion event in the mouse (via traditional methods) had been previously problematic.

Conclusions and future perspectives

TALENs and CRISPR/Cas9 genome editing technologies have dramatically boosted the ability to manipulate a diverse set of genomes. These novel approaches are aggressively being applied to study a wider set of biological questions, including several human disorders. Naturally, limitations exist for each system, including the requirement of generating TALEN pairs to target one site and slightly higher off-target rates for CRISPR/Cas9.

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Targeting efficiencies between TALENs and/or CRISPR/Cas9 in certain cell types is an additional problem that will need to be resolved by further study. Moreover, the application of these technologies in the clinical setting has yet to be established, and it will be imperative to understand potential complications after genetic correction. In summary, TALENs and CRISPR/Cas9 systems are invaluable genetic manipulating tools that will continue to be improved and applied to countless future studies. These systems hold the key to revolutionize biological research and facilitate the promise of personalized medicine in the future.

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