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CRISPR/Cas base-editing systems and their potential applications and prospects

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Abstract. CRISPR-Cas9 and its derivatives such as cytosine base editor, adenine base editor and prime editing are an important topic of research today. In recent years, with continuous development and updates by researchers, editing systems have been able to achieve various modifications of target genes. For example, base substitution, insertion and deletion of short fragment genes, etc. have wide applications in plants and animals, etc. However, these editing systems derived from the CRISPR/Cas9 system still face challenges in terms of editing efficiency and accuracy. This review describes the current status of research using editing systems in plant breeding, gene disease treatment, and knockdown of specific genes by establishing animal models, analyzes the advantages and disadvantages of editing technologies, and looks forward to their development.

Keywords: CRISPR/Cas system, DNA base editing, gene editing.

1. Introduction

The clustered regularly interspaced short palindromic repeats (CRISPR)/ CRISPR-associated protein system (Cas) is the latest gene editing technology developed with more flexibility and precision than zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALEN). The Cas protein in bacteria recognizes the proto-spacer motif (PAM) of exogenous DNA, and later transcribes the proto-spacer sequence of DNA into CRISPR RNA (crRNA) using CRISPR sequences. The new RNA forms an RNA complex with the trans-activated crRNA in the cell, which uses RNA-guided nucleases to produce double-strand breaks (DSB) [1]. The DNA base editing (BE) is usually a protein fusion structure that consists of a catalytically impaired dCas9, dCas12, or Cas9n, a nucleobase modifying enzyme that is specialized to single-stranded DNA, BEs are mainly formed by the fusion of Cas9 protein and base deaminases and are separate into two categories: cytosine base editors (CBEs) and adenine base editors (ABEs). Without producing DNA fragmentation and without the requirement for a DNA template, the single-base editing technique employs the single-base editing system to produce effective single-base replacement editing. The prime editing (PE) system is a newly developed target

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gene modification technology based on the CRISPR/Cas9 system, which can achieve arbitrary base swaps, short fragment insertions and deletions without the presence of donor DNA. PE system includes Cas9-nickase(nCas9), reverse transcriptase (RT), and prime editing guide RNA (pegRNA), including the spacer (sg RNA), primer binding site (PBS), and target sequence (which RT uses as a template to write new genetic information into the genome, called the RT template) that guide the nCas9 binding target site.

Both transcription activation-like effector nuclease technology (TALEN) and zinc finger nuclease technology (ZFN) are tools for genome editing and both consist of a chimeric nuclease encoding a sequence-specific DNA binding module and a non-specific DNA cleavage structural domain. By breaking the DNA double strand, TALEN and ZFN can perform a range of gene editing modifications, stimulating non-homologous end-joining with high error rates as well as site-specific homology-directed repair. Currently, TALEN is widely used for genomic modifications at the cellular level, such as editing in plant and animal cells, and in various research models, such as drosophila, zebrafish, and mice, while ZFN technology is heavily used to study target genes of great medical significance due to its ability to generate new species by modifying genetic background.

2. CRISPR/Cas base editing system

Homology dependent repair (HDR) is used by the CRISPR/Cas9 system to substitute single-base, however gene correction efficiency for single-base mutations is less efficient. Without producing DNA fragmentation and without the requirement for a DNA template, the single-base editing technique employs the single-base editing system to produce effective single-base replacement editing. Single-base editing technology uses a single-base editing method to achieve effective single-base replacement editing without generating DNA fragmentation and without the requirement for a DNA template. Single-base editing technology has proven to be highly precise since its introduction. Single-base editing technology has proven its great accuracy since its beginnings.

2.1. Base editing

DNA base editing is mainly used for point mutations and gene insertions or deletions and is more precise than its CRISPR/Cas-derived gene editing methods [2]. BEs are mainly formed by the fusion of Cas9 protein and base deaminases, and cytosine base editors (CBEs) and adenine base editors (ABEs) are the two basic categories of BEs.

2.1.1. Cytosine base editors. Cytidine deaminases, such as AID, APOBEC1, APOBEC3 APOBEC2, and APOBEC4, combine and deaminate single-strand DNA (ssDNA) and RNA. During the action of CBE, cytosine deaminase reaction converts cytosine (C) to uracil (U) in DNA and completes the gene editing that replace cytosine (C) by thymine (T) and change guanine (G) to adenine (A) as DNA is replicated.

2.1.2. Adenine base editors. Different from CBE, ABE system doesn't have a natural adenine deaminase. In turn, researchers modify RNA-specific deaminase to synthesize TadA, an enzyme that converts A to inosine. TadA could synthesize a protein, which fuse to nCas9(D10A) and a C-terminal NLS via the XTEN linker, by incorporating mutations A106V and D108N. With this reaction, ABE system can accomplish T-to-G editing though it has low efficiency [3, 4].

In order to complete a more efficient and accurate base editing, the research team combined the target AID (a CBE) and ABE to develop the adenine and cytosine base editor (ACBE), which allows C to T and A to G gene editing acting on the same target simultaneously. The primary editing window of the original spacer sequence of the target AID is at 1-5 and its cytidine deaminase binds at the C-terminus of Cas9-nikase. In contrast, the primary editing window of ABE is located at proto-spacer sequences 4-8, and the binding site of adenine deaminase to Cas9-nikase is N, which is structurally complementary to the target AID. Therefore, these two base editors fuse to form ACBE [5]. This can be achieved by SPACE system or ref.

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2.2. The prime editing system

The prime editing (PE) system is a newly developed CRISPR/Cas9 system's derivative technology, which can achieve arbitrary base swaps, short fragment insertions and deletions without the presence of donor DNA. The PE system includes Cas9-nickase (nCas9), reverse transcriptase (RT), and guide editing guide RNA (pegRNA) includes the spacer (sg RNA), primer binding site (PBS), and target sequence (which RT uses as a template to write new genetic information into the genome, called the RT template) that guide the nCas9 binding target site. The pegRNA guides the nCas9-RT complex to bind to the gene target site. nCas9 creates a cut 3 nt upstream of the PAM sequence, and the 3' end of the cut strand is complementarily bound to the PBS, after which RT uses the RT template for extension to synthesize new base information. Containing the 3' end of the flap, the edited product is stably balanced with the product that has the non-edited flap's 5' end on it, where the 5' end of the non-edited flap is excised by the nuclease to obtain the product of one edited strand and one year of the non-edited strand, resulting in stable edited DNA after cellular repair. The PE system does not require donor DNA and does not require double strand breaks (DSBs) to achieve free base substitution and precise insertion and deletion of bases at the target site (up to 44 bp for insertion and up to 80 bp for deletion). Compared with single-base editing systems, the PE system is less efficient for CG or AT, which are located in the editing window of single-based editings (4-8 t) and attracts more insertion-deletion mutations at most sites. However, in terms of precision, the PE system can accurately edit the target base, while the single-base editing with multiple editable CGs or ATs in the edit window will produce multiple editing products, of which only the products of the target base will account for a relatively small percentage. For precise editing of small fragment insertions and deletions, the PE system does not require donor DNA and produces fewer DBS-induced insertion-deletion mutations than the CRISPR/Cas9-mediated HDR, while the editing efficiency is similar to that of HDR [6].

3. Application of CRISPR/Cas base editing system

3.1. Cultivation disease-resistant crops

Traditional mutation breeding is done by identifying rare spontaneous mutations and then inducing mutations in the plants or crossing them with wild species to screen out superior varieties, but this method consumes a lot of resources and takes a long time, which is no longer suitable for the development of modern society [7]. Thus, efficient and rapid breeding methods have emerged.

Firstly, the gene to be edited is selected and targets for its protein-coding region and gene expression regulatory region are designed, and the constructs are prepared according to the targets and become a generation of plants as transgenic crops. The application of base editing technology is also expanding in order to enable gene mutations to occur in plants with relative ease and reduce resource consumption. Based on the analysis of a large amount of data from wild and cultivated species, single nucleotide differences are a major cause of phenotypic differences in crops. With base editing technology, arbitrary substitution of bases as well as insertion and deletion of bases can be achieved, which in turn allows amino acid substitution or translation termination, and great potential to change crop phenotypes and obtain new varieties.

CRISPR/Cas-based base editing technology has great significance in gene editing of crops. Base editing technology was first applied to the sugar content of strawberries, a fruit crop. The open reading frame (uORF) upstream of the transcription factor gene FvebZIPs1.1 was targeted using the CBE base editing system (A3A-PBE) and APOBEC3A deaminase. A total of 66 T0 transgenic plants were obtained, of which 60 were pure alleles and bialleles, accounting for 90.9% of the observed genetic changes. The gene-edited strawberries had a higher sugar content than the control, and more importantly, the gene-edited strawberries were GMO-free. This paper reports the application of base editing system in uORF region for the first time. In this study, the base editing system was applied to the target gene uORF region of strawberry for the first time, which provides a powerful tool for gene activation without the introduction of transgenic elements and provides a broad prospect for quantitative character improvement and germplasm innovation of strawberry and other fruit crops [8].

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At the same time, because base editing allows precise and efficient nucleotide substitution, a study attempted to use stable or transient expression of CRISPRCas9 in two tetraploid potato varieties (Solanum tuberosum) to knock out the amyloid-producing stGBSSI gene, resulting in a mutant, the loss of function of StGBSSI protein was confirmed by the production of quaternary gene mutants with impaired amylose biosynthesis. The experiment also validated the concept of CRISPR-Cas9 base editing tetraploid potato by targeting two coding sites of the StGBSSI enzyme catalytic motif, opening a new approach for potato genome engineering [9]. Thus, CRISPR/Cas-based base editing has a significant and pioneering role in breeding crops to achieve improved quality of agricultural products, breeding superior variants, and developing gene editing pathways.

3.2. Treatment of genetic diseases

The CRISPR/Cas system has super extensive applications and prospects in the treatment of diseases due to its ability to make precise edits to genes. BE, as an emerging gene editing technology, has many advantages in CRISPR-derived systems such as avoiding DSB double-strand breaks, and has great potential in the treatment of genetic diseases. Single nucleotide variants are currently one of the major factors causing genotypic diseases. The prerequisite for the treatment of such diseases obviously is the editing of the single base.

Although not yet used in clinical studies, based on many animal experiments, researchers have found that the function of DNA base editing to correct point mutations in genes would be helpful in the treatment of monogenic diseases. For example, mutations or deletions in the Rpe65 gene cause the Rpe65 enzyme to work abnormally, causing diseased animals to exhibit impaired eye function. In contrast, the ABEmax system restored Rpe65 expression in 32% of retinal cells. In addition, other in vivo experiments have found that base editing technology can help treat hearing loss-related disorders, neuromuscular diseases, blood disorders, neurodegenerative diseases, metabolic diseases, Hutchinson-Gilford premature aging syndrome (HGPS), and other diseases caused by single-base mutations. So far, the therapeutic role of base editing in several diseases has been validated in in vitro experiments, such as cystic fibrosis, facioscapulohumeral muscular dystrophy, Tay-Sachs and Marfan syndromes, sickle cell disease, Alzheimer's disease, and prion diseases [10].

Based on the two most basic BE tools, ACE and BCE, researchers can derive many different functional gene editings or related products to achieve the treatment of more complex genetic diseases. For example, ACBE is a combination of two tools to achieve the effect of editing two clips simultaneously. In addition, there is an online gene editing tool, ACE of BASEs, developed by a team to detect the role and effect of base editing at different positions. Researchers can design therapeutic regimens applying base editing through online assays [11].

3.3. Construction of novel animal models

The CRISPR/Cas system occupies a key role in the construction of animal models because of its high efficiency, fast operation and high flexibility of use. For example, Yixin Xu et al. deepened the application of CRISPR/Cas9 technology in the study of knocking out the exon 4 region of the Golgi GP73 gene to obtain animals with low GP73 expression. As a reference model for physiological functions and metabolic mechanisms, this model has a high knockdown rate and good experimental reproducibility. In terms of application, GP73 occupies an important position in the pathogenesis of liver diseases and affects immune regulation and metabolism in humans. Therefore, the construction of the corresponding mouse model is of guiding significance for future studies of water-soluble GP73 in vivo experiments [12].

And Tan Zhixia et al. used CRISPR/Cas9 technology to construct targeting sites using ZIFIT, adding protection bases and corresponding promoters, and synthesizing forward and reverse primers according to the pUC57-gRNA backbone sequence to perform Hand2 knockdown. Although the survival rate of the F2 generation propagation was not high, the mutant pure-sibling model was successfully preserved, which is important for studying the effect of this gene on right ventricular progenitor cells and morphology [13].

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Also, with the continuous upgrading of novel tools single-base editing systems have taken an important place in livestock breeding and genetic research. It facilitates gene modification methods, improves precision and efficiency, and makes base substitutions more targeted. At the same time, its presence accelerates the emergence of superior traits, and the Kazakh sheep model with low expression of the MSTN gene was obtained using the AncBE4max system, which was highly efficient in lambs by Yao Xudong et al [14].

However, although the newer iterations and validation of various editing technologies and the combination with animal creation methods have promoted the construction of various animal models for research and practical application, their development is relatively rapid. However, it still does not meet the real needs and needs to be improved in terms of accuracy and efficiency, and the restricted editing window will become an important research issue [15].

4. Conclusions

Nucleotides targeting ability on either the plus or complementary minus DNA strand greatly opens the therapeutic applications of Base Editing. From crop breeding, genetic disease treatment and other aspects have very broad prospects. However, there are still many pressing problems with base editing technology. On the issue of editing efficiency, single gene editing systems are more efficient compared to the bootstrap editing techniques, but most of them have an efficiency of 20%-40%, which cannot meet the need of screening a large number of new varieties [16]. Meanwhile, how to reduce the generation of non-target products has also become an important issue. With the upgrade of the prime editing system generation by generation, the novel PE3 caused an increase in editing efficiency by creating another sgRNA, but it also played a reverse role in reducing non-target products. More often, it causes less efficiency in preventing off-target because it is not possible to predict the off-target effect caused by sgRNA guiding cas9 protein binding to the site by designing PAM to calculate its mismatch probability occurring at the 5' end and at the 3' end.

Although the new generation of bootstrap editing technology reduces the dependence on PAM and expands the variety of PAMs, it is still some distance away from the practical needs. In addition to this, the scope of application of the PRIME EDITING system is more oriented towards plants (due to its low editing efficiency in animal cells in general) [6,17], and its editing efficiency can be improved by expanding its dominant position in plants, such as optimizing the upgraded system with specificity in combination with the characteristics of specific plants. Along with modern technological iterations, the directions of development have diversified and become widespread. For example, further unlocking the evolutionary potential through PLSM, combined with single-base editing libraries for optimization will enable targeted evolutionary studies based on in situ saturating mutations in important crop genes to be provided more efficiently, reliably, and conveniently [18].

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