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Prime editing and its application in the treatment of diseases

Ruiyan Yang

School of Chemistry and Life Sciences, Beijing University of Technology, Beijing, 100083, China

hycz0326@emails.bjut.edu.cn

Abstract. With the rapid development of biotechnology, gene editing technologies have become an important research direction in the field of biomedicine, and their potential application in disease treatment has become increasingly prominent. As a new type of gene editing technology, prime editors have received widespread attention. In recent years, gene editing technologies have made remarkable progress. For example, CRISPR-Cas9 system has been widely used, which provides new ideas and methods for the treatment of hereditary diseases, cancers, etc. The application scope of PE is even wider, which enables researchers to successfully achieve the intervention and treatment of many diseases by precisely editing the target genes. Although the application of PE in disease treatment shows great potential, there are still technical challenges in its editing efficiency as well as delivery efficiency, which need to be further studied and explored. The text in this article analyses the composition, principles, and optimization strategies of PE, followed by an in-depth discussion of its application in the treatment of Hereditary tyrosinaemia type (HT1) and α1-antitrypsin deficiency(A1ATD). By reviewing and analysing the use of PE in different diseases, this paper reveals the potential and challenges of PE and looks into its future. The study in this paper provides a reference for the use of PE in editing efficiency enhancement as well as in disease treatment. However, there are still many issues that need to be addressed, such as improving the accuracy and efficiency of PE and reducing off-target effects. Future research can focus on these directions to further promote the application and development of gene editing technologies in the medical field.

Keywords: PE, HT1, A1ATD.

1. Introduction

PE has the ability to convert any base to another base and insert DNA fragments precisely into the genome, thus replacing genes that cause disease.PE can make generation of single point mutation, simultaneous introduction of multiple point mutations, precise insertions, precise deletions, simultaneous introduction of insertions and deletions, directed gene evolution, generation of gene knockout, modification of cis elements [1], which can well correct the genes that cause diseases. The use of gene editing tools to directly repair disease-causing mutations can cure human genetic diseases.PE has the characteristics of high precision and high specificity, which makes it a great potential for gene therapy, and it can correct most diseases due to human gene mutations, so PE is a new gene editing technology that attracts a lot of attention nowadays, and it has a high potential for development.

As a new type of gene editing tool, PE has attracted the attention of many researchers and has been thoroughly researched and developed, with higher precision, specificity and versatility than other gene

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editing technologies. Due to its all-purpose gene editing ability, PE is now used in various research fields such as zoology, botany and genetics. In botanical research, PE can knock out genes, modify homeopathic originals, and introduce or replace amino acids to improve yield, disease resistance, stress resistance, and the content of a certain chemical substance in plants [2]. In research in the animal field, PE is a tool to establish animal models of human genetic diseases, and today there are several PE-based mouse disease models. In addition, PE can be used for gene editing breeding to modify key functional genes affecting economic traits to obtain animals with good quality [3]. In human gene therapy, PE has been used in cellular and individual animal level studies, and has successfully corrected a variety of disease-causing genes.PE also has the potential to be used in screening for gene mutations. However, the editing efficiency of PE is limited, and the large size of PE also affects its delivery efficiency. Numerous studies have been conducted to evaluate the safety of PE and to improve its editing and delivery efficiency.

In this paper, we summarise the optimisation strategies for improving the editing efficiency of PE, and demonstrate its different delivery routes by introducing its application in disease treatment, so as to provide a reference for future research and suggestions for further optimisation of PE.

2. Overview of PE

2.1. Components of PE

PE consists of a fusion protein and prime editing guide RNA (pegRNA). The fusion protein consists of nSpCas9 (H840A) and the reveese transcriptase of Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV-RT). Where H840 of SpCas9 is mutated from G to A, thus inactivating its HNH structural domain, only the RuvC structural domain of nSpCas9 (H840A) is shear active, singularly shearing the non-target strand (NTS) in the target dsDNA and does not shear the target strand (TS). As shown in Figure 1, pegRNAs include a reverse transcription primer binding site (PBS) that directs reverse transcriptase to extend target DNA, a reverse transcription template (RTT), a sequence that binds to Cas9, and a spacer sequence, which is a guideRNA that is associated with directing the pegRNA to bind to target DNA.

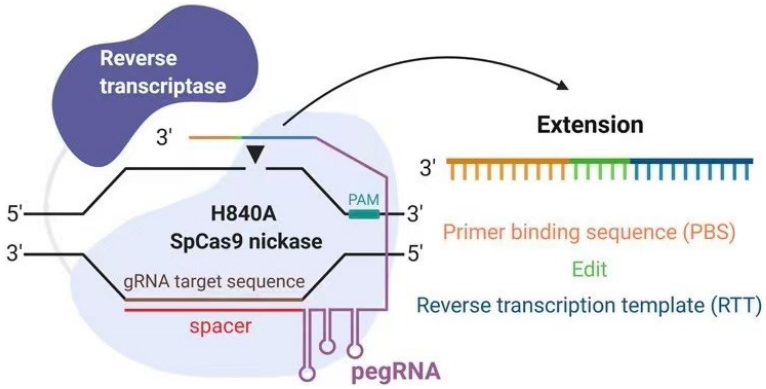

Figure 1. Components of pegRNA [4].

2.2. PE mechanism

The pegRNA-directed fusion protein binds to the TS of the target DNA while unravelling the DNA double helix, exposing the NTS. nSpCas9 (H840) binds to the NTS and specifically cuts at the target site, exposing the 3' end (Fig. 2A), whose cleavage site is located within the target sequence, 3 bases away from the PAM region. the PBS sequence binds to the NTS , and then RT was reverse transcribed according to RTT thereby forming the edited sequence, producing an edited 3‘flap as well as an unedited 5’flat (Fig. 2B). the 3‘flap and 5’flap competed for binding to the TS. The specific endonuclease recognises and excises the 5‘flap and retains the 3’flap, yielding a DNA double strand with one single

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strand with the target mutation and one single strand in its original state (Fig. 2C). Since their sequences do not match each other, the mismatch repair mechanism of DNA can be utilised thus obtaining the target sequence and achieving precise gene editing (Fig. 2D) [4].

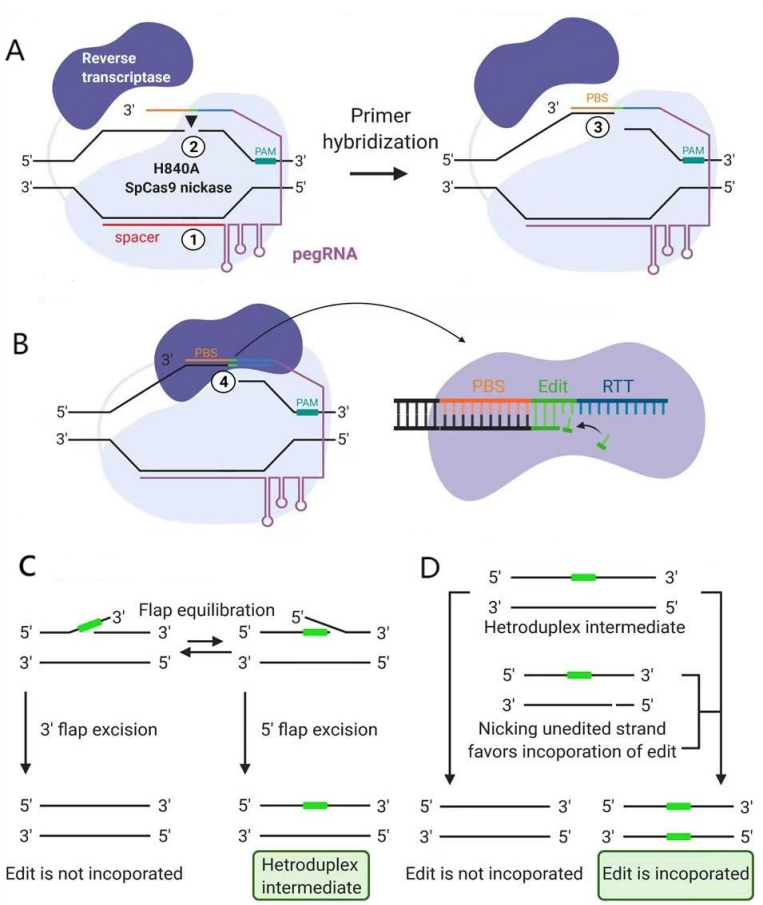

Figure 2. PE mechanism [4].

PE uses a special genetically engineered enzyme to singly cleave a single strand of DNA and combines it with pegRNA to reverse transcribe the DNA strand, utilising the DNA mismatch repair mechanism to perform precise gene editing.

3. Optimization of the PE

3.1. PE2 PE3

The PE2 system genetically engineered MMLV-RT to introduce six-point mutations, namely H9Y, D200N, T306K, T330P, and L603W. The aim was to improve its reverse transcription activity at high temperatures as well as to increase the affinity and stability of the pegRNA to DNA.

Since the DNA mismatch repair mechanism may act on both edited and unedited sequences. Therefore PE3 designed a second guideRNA which recognises the NTS and directs nSpCas9 (H840) to bind the TS and then make a second cut at a different position. Since the cuts are made on the TS and the TS is stimulated, the editing efficiency will increase, but at the same time there will be a risk of indel mutation.PE3b can solve this problem well by designing the guideRNA that recognises the NTS that has been introduced with the target mutation. This artificially creates a time gap between the two cuts and thus reduces the indel mutation. [5]

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3.2. Chromatin and cis-chromatin environment of edited loci

PE efficiency can be effectively manipulated by cis-chromatin editing; PE efficiency is positively correlated with H3K79me2 and can be effectively predicted by epigenetic traits. cis-chromatin environment can interact with trans-acting elements. Moreover, the cis-chromatin environment around the editing site can be manipulated to change the editing efficiency. The transcription of the edited gene can be reduced by CRISPR off, thus reducing the PE efficiency. And pre-activation of the editing gene promoter can substantially increase the PE efficiency [6].

3.3. Other Optimization of the PE

The pegRNA is the guide sequence and RTT of PE system, which plays an important role in the editing efficiency of PE. PE can be effectively optimised by changing the length of PBS and RTT to reduce accidental loop formation or optimising the promoter of pegRNA or adding special structures to protect the 3' end of pegRNA from degradation [7].

Alternatively, PEs can be optimised by inhibiting DNA mismatch repair. For example, PE4 and PE5 are PEs that allow cells to express MLH1 dominant loss-of-function protein (MLH1dn), which lacks three amino acids at the C-terminal end of its C-terminal end, thus missing the nucleic acid endonuclease, and therefore has a reduced DNA mismatch repair activity as a way of protecting the DNA that introduces the target mutation.PE4 is the result of an addition of MLH1dn to PE2, and PE5 is the result of an addition of MLH1dn to PE3 [8].

PE, as a more all-purpose gene editing technology, is of great research significance, but its initial editing efficiency is low, which limits the scope of its application. Currently, PE has been optimised from various aspects, such as MMLV-RT, pegRNA, and epigenetics, to greatly improve its editing efficiency. These optimisation strategies can break through the limitations of research and improve the application potential of PE systems.

4. Line thickness PE utilization in HT1

HT1 is an inherited metabolic disorder caused by a mutation in the gene for fenugreek acetoacetate hydrolase (Fah), which results in an inability to degrade tyrosine, leading to the accumulation of toxic metabolites, resulting in weight loss and liver, kidney, and peripheral nerve disease in patients. In severe cases, patients may develop cirrhosis and liver cancer.

Several studies have shown that PE can be effective in treating HT1 in mouse models. Mutation of the last nucleotide in exon 8 of the Fah gene, from the G-point to A, results in loss of Fah function. In response to this, the PE system can treat the disease in vitro by editing transplantable cell populations. In response to this, PE can be used to treat the disease in vitro by editing transplantable cell populations. In one study, HT1 mouse hepatocytes were utilised to reprogramme into mCdHs. A PE3b system was designed to modify the mutation location of the Fah gene. In order to improve the editing efficiency of the PE system, two kinds of guide RNA were designed for different targets, and then different lengths of PBS were designed, and the editing efficiency and indel frequency of each length of PBS were determined, and then two kinds of sgRNA were designed for each pegRNA, corresponding to the PE3 and PE3b methods, respectively. After transfection to mCdHs, the scheme with the highest editing efficiency and smaller indel frequency was chosen, with PBS lengths of 11 nucleotides for pegRNA1 and nicking sgRNA1b, and the editing efficiency of this method was at 2.3% with no bystander effect. The PE3b system was transfected into mCdHs by electroporation, and the modified mCdHs were transplanted into the livers of HT1 mice, which showed good survival after transplantation [9].

The plasmid carrying PE3 could enter HT1 mice by hydrodynamic injection and repaired the Fah gene, which altered the disease phenotype of the mice and did not appear to accidentally injure non-target genes [10]. PE3 was also carried by two adenovirus-associated viral vectors (AAVs) in the form of split-PE, one AAV carrying nSpCas9, and the other AAV carrying pegRNA, RT. The AAV vectors were introduced into HT1 mice by hydrodynamic injection to repair the point mutation and treat the mice [11].

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5. PE utilization in A1ATD

A1ATD is an inherited disorder. PiZZ of E342K in the Serpin peptidase inhibitor family A member 1 (SERPINA1) gene mutated from the G point to A leads to misfolding of alpha-1 antitrypsin in hepatocytes, which causes endoplasmic reticulum stress and alpha-1 antitrypsin deficiency, ultimately leading to the disease, which severely affects liver and lung function, and even life of the patient. PiZZ mice are the mouse model of A1ATD, and the improved PE2* can well correct the wild-type SERPINA1 allele, thus achieving pathogenic gene correction in mice [12].

In another study, PE in human pluripotent stem cells (hPSCs) was thoroughly tested and the ability of PE to correct the position of mutations in induced pluripotent stem cells (iPSCs) from patients with A1ATD was determined. hPSCs, which include embryonic stem cells (ESCs) and iPSCs, can be differentiated into cells from all three germ layers of the embryo, and are important cells for genetic engineering. The results showed that PE could induce all types of nucleotide substitutions as well as small insertions and deletions in hPSCs, and PE corrected mutations in i PSCs from patients with A1ATD, but it was less efficient in editing, possibly due to low transfection efficiency or low pegRNA editing activity [13].

6. Conclusion

This article provides an in-depth discussion of the components, principles, and optimisation strategies of PE, as well as its application in disease treatment. The article first outlines the components of PE as well as the working steps. Subsequently, the article analyses in detail the existing optimization strategies of PE, mainly in terms of pegRNA as well as epigenetic aspects. In addition, the article discusses the application of PE in the treatment of A1ATD and HT1, demonstrating its great potential and practical application value in the medical field. The results of this article provide help in understanding the principle of PE technology, provide a reference for medical research and clinical practice of A1ATD and HT1, provide suggestions for the future development of PE technology, and help to promote the innovation and development of gene editing technology, as well as provide a direction for future research and application in related fields. As gene editing technology such as PE is a rapidly developing field with new technologies and methods emerging constantly, this paper cannot cover all the latest research results, and due to space limitations, this paper cannot provide in-depth case studies of diseases, but only a general introduction of its application.PE, as a revolutionary biotechnology, brings new hope for disease treatment, and through continuous research and application, it is believed that it will play a more important role in the future.

References

[1] Anzalone A V et al 2019 Search-and-replace genome editing without double-strand breaks or donor DNA Nature 576 7785 149-157
[2] Hassan M M et al 2020 Prime editing technology and its prospects for future applications in plant biology research Biodes Res 2020 9350905
[3] Liu Y et al 2022 Prime editing: A new technology breaking through the type of base editing Hereditas 44 11 993-1008
[4] da Costa B L Levi S R Eulau E Tsai Y T & Quinn P M J 2021 Prime editing for inherited retinal diseases Front Genome Ed 3 775330
[5] Kantor A et al 2020 CRISPR-Cas9 DNA base-editing and prime-editing Int J Mol Sci 21 17 6240
[6] Li X et al 2023 Chromatin context-dependent regulation and epigenetic manipulation of prime editing bioRxiv 20230412536587
[7] Huang Z & Liu G 2023 Current advancement in the application of prime editing Front Bioeng Biotechnol 11 1039315
[8] Chen P J et al 2021 Enhanced prime editing systems by manipulating cellular determinants of editing outcomes Cell 184 22 5635-5652e29
[9] Kim Y et al 2021 Adenine base editing and prime editing of chemically derived hepatic progenitors rescue genetic liver disease Cell Stem Cell 28 9 1614-1624e5

DOI: 10.54254/2753-8818/58/20241359

[10] Jang H et al 2022 Application of prime editing to the correction of mutations and phenotypes in adult mice with liver and eye diseases Nat Biomed Eng 6 2 181-194
[11] Jiang T et al 2022 Deletion and replacement of long genomic sequences using prime editing Nat Biotechnol 40 2 227-234
[12] Liu P et al 2021 Improved prime editors enable pathogenic allele correction and cancer modelling in adult mice Nat Commun 12 1 2121
[13] Habib O et al 2022 Comprehensive analysis of prime editing outcomes in human embryonic stem cells Nucleic Acids Res 50 2 1187-1197