The Progress of Prime Editing in The Treatment of Neurogenetic Diseases

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Abstract. Due to the non-renewable nature of central nervous system cells, genetic heterogeneity and the mechanism of dominant negative mutations, neurogenetic diseases have long lacked effective radical treatment methods. Traditional gene editing techniques such as CRISPR-Cas9 rely on DNA double-strand breaks (DSB), which are inefficient and pose safety risks in non-dividing neurons. Prime Editing (PE), as a disruptive "search - replace" type of precise gene editing technology, realizes all 12 base conversions, small fragment insertions and deletions without introducing DSB, bringing unprecedented opportunities for the treatment of neurogenetic diseases. This article will elaborate on the technical principles of PE and its unique advantages in the treatment of neurogenetic diseases by combining experimental research and literature reports in recent years. It will focus on summarizing the latest breakthrough progress of PE in the preclinical research of alternating hemiplegia (AHC) in children, especially its symptom reversal and functional recovery ability demonstrated in in vivo treatment. Meanwhile, this article delves deeply into the core challenges faced by the clinical transformation of PE, such as in vivo delivery efficiency and interference with mismatch repair (MMR) systems, and looks forward to the future directions of addressing these challenges through carrier engineering innovation. PE technology is expected to drive the transformation of neurogenetic diseases from symptomatic treatment to one-time radical cure in a new era.

Keywords: prime editing: Gene editing, neurogenetic diseases, AHC.

1. Introduction

Neurological diseases are the leading cause of disability and the second leading cause of death worldwide [1]. Over the past 30 years, the absolute number of deaths and disabilities due to neurological diseases has increased significantly, especially in low - and middle-income countries. Moreover, due to population growth and aging, it is expected to increase further globally [2]. Neurogenetic diseases are those with a distinct genetic predisposition or a high incidence rate within families. These diseases result from alterations in genetic material, leading to abnormal nervous system functions. They are diverse in type and mostly quite dangerous, posing a significant health threat and care burden in human society. Moreover, the treatment of such diseases is often difficult, and the existing treatment methods and approaches are also relatively limited. Therefore, it is necessary to explore new methods, new approaches and new measures to alleviate the burden and impact caused by neurogenetic diseases.

The existing treatment of traditional neurological diseases often faces many challenges. Take dominant negative effect mutations (such as ATP1A3, HTT) as an example. They show obvious resistance mechanisms to traditional therapies. The blood-brain barrier (BBB) and the delivery bottleneck brought about by the non-renewability of neurons are also problems and challenges that cannot be ignored in traditional treatment methods. With the development of gene sequencing technology, humans are now able to detect and determine the gene mutation sites of these neurogenetic diseases more quickly and accurately, providing new ideas for conquering treatment methods at the genetic level. The development of gene editing technology has fundamentally transformed the gene therapy paradigm for neurogenetic diseases. In recent years, CRISPR-derived technologies have been able to evolve from "break-dependent" to "precise repair". Among them, Prime Editing (PE), as a representative gene editing technology, does not rely on the double bond breaks (DSB) and donor DNA templates of traditional crisper technology. The realization of

insertions/deletions at target sites and all 12 types of point mutations has become a huge advantage in the development of gene editing treatment methods.

2. The advantages of PE in the treatment of neurogenetic diseases

2.1. The advantage of PE in terms of off-target rate during gene editing

PE is formed by the fusion of prime editing guideRNA (pegRNA), reverse transcriptase (RT), and nCas9 of the RT domain. When using PE for gene editing, there are generally three processes of gene recognition and pairing [3]: Firstly, the pegRNA in PE recognizes and guides to the target DNA, pairing with one strand of the target DNA. This pairing serves a positioning function. Throughout the catalytic editing process, under the guidance of pegRNA, PE is bound to a specific target DNA sequence, thereby promoting nCas9 to cut the single strand of the target DNA. The second pairing process occurs at the complementary sequence before the primer binding sequence (PBS) at the 3' end of pegRNA recognizes the cleavage site. Through hybridization with another strand of the target DNA, a new DNA single strand is synthesized catalyzed by RT. This extended DNA strand can encode the target gene according to the template specified on the pegRNA. The third pairing process occurs when a single strand of DNA encoded according to the template specified on pegRNA binds back to the original target strand of DNA. After DNA mismatch repair (MMR), if this new strand is introduced into the target site, gene editing of the target DNA can be achieved. These three pairing processes containing segRNA can significantly reduce the off-target efficiency of PE during gene editing and accurately target the mutation sites of neurogenetic diseases.

2.2. The safety of PE in the treatment of neurogenetic diseases

Compared with the traditional crisper cas9 gene editing technology, the feature of gene editing in PE lies in that it does not produce DSBs, but only cuts a single strand of DNA. The principle of the former mainly relies on cutting the double strands of DNA to activate the DNA repair pathway of cells, while simultaneously connecting and repairing the two broken ends [4]. In contrast, PE performs gene editing in gene regions highly related to development and neural function without generating DBS, reducing unpredictable mutations caused by deletions, translocations, etc., which is obviously safer. In addition, traditional gene delivery relies on adenovirus vectors. However, common AAVs limit the size of the delivered genes to within 5Kb [5], while the gene size required by PE technology is basically above 6Kb [6]. If they are not split, normal delivery cannot be achieved, maintaining the integrity of the function during the delivery period. The consequence of splitting is that it may lead to a decline in editing efficiency and an increased risk of irrelevant genes being introduced into the vector. The engineered virus-like particles (eVLPs) developed by researchers have removed the limitation of gene size during delivery. The combination of PE and the novel delivery system has enabled the restoration of partial protein functions and vision in two mouse models with hereditary blindness [7]. By further optimizing PE-eVLP, the occurrence of off-target editing can be reduced and the possibility of carcinogenic transgenic integration during the editing process can be avoided [7], which undoubtedly represents a significant improvement in the potential safety of PE.

3. Breakthroughs and Applications of PE in Neurogenetic Diseases

3.1. Breakthrough of PE in the Treatment of AHC

AHC is a serious neurodevelopmental disorder that rarely occurs in infants and young children, and there is currently no cure for this disease [8]. The typical symptoms of this disease include hemiplegia (paralysis affecting one side of the body), dystonia (painful involuntary muscle contractions), abnormal eye movement and epileptic seizures [8]. It is worth noting that patients with AHC also present with non-paroxysmal hypotonia (low muscle tone), developmental delay and relatively severe intellectual disability [8]. The pathogenesis can be attributed to the pathogenic

variations of ATP1A3, among which the most common variant types are Atp1a3 D801N and E815K [9]. This variant encodes the catalytic α3 subunit protein complex of neuronal Na+/K+ ATPase proteins, which leads to the loss of sodium-potassium pump function. Research has found that by introducing corresponding mutant genes into mice and human cells to build models and directly injecting PE into the brain for gene editing treatment, the types of D801N and E815K in the ATP1A3 mutation of mice can be corrected and the neurological symptoms and lifespan of AHC can be significantly improved [10]. The research demonstrates the potential of PE as a one-time gene therapy for neurogenetic diseases in the future, and also symbolizes a major breakthrough in the in vivo treatment of PE.

3.2. The Application of PE in Alzheimer's Disease

Alzheimer's disease is one of the most common neurodegenerative disorders, mostly sporadic. Among them, the allele APOE4 of apolipoprotein E is the most powerful genetic risk factor for late-onset Alzheimer's disease [11]. The high content of GC bases at the APOE gene locus makes the traditional crisper-cas9 homologous directed repair (HDR) gene editing method inefficient and prone to unpredictable insertions and mutations. However, the emergence of PE technology has provided a new solution to this problem. Researchers successfully introduced the APOE4 gene mutation by optimizing pegRNA and constructed an in vitro AD model [12], providing an unparalleled isogenic control platform for in-depth study of the pathogenic mechanism of APOE4 in nerve cells. This is undoubtedly a major breakthrough in this field. However, it is worth noting that when PE is used to modify and edit APOE4, the editing efficiency is much lower than when PE is used to introduce APOE4 mutations [12]. During the process of editing high-risk APOE4 into medium and low-risk APOE3 using PE, the formation of the pegRNA stem-ring structure was hindered due to the change of bases, which affected the normal function of the entire PE component [12]. Although PE technology has demonstrated its great potential and value in the basic research of Alzheimer's disease and provided a powerful tool for the construction of AD disease models, its true transformation into a gene therapy method still faces the problems of low in vivo transformation efficiency and difficult delivery. In the future, how to conduct independent and targeted optimization for the PE strategy of therapeutic applications will be the key to its true success.

4. The challenges faced by PE in the treatment of neurogenetic diseases

4.1. The difficulties of PE in delivery

The unique anatomical structure of the nervous system constitutes the primary obstacle. The BBB selectively prevents macromolecular substances from entering the central nervous system, greatly limiting the intracranial delivery efficiency of PE components. Although viral vectors (such as AAV) are widely adopted, they have significant limitations. There are many existing optimization methods for PE, including PE2, PE3, PE4, PE5, etc. [13-14]. However, due to the poor compatibility of PE itself with AAV vectors, the capacity of AAV vectors is limited, while PE systems usually exceed the capacity of a single AAV vector. It is necessary to split it into dual AAV delivery. Splitting the dual AAV vector delivery undoubtedly affects the editing efficiency and the safety during the editing process, which puts forward requirements for the development of new delivery systems such as viral itch particle vectors and nanoparticle vectors. Or in the future, further modifications can be made to the PE system, reducing it to a size that can be delivered by a single AAV carrier while retaining its editing effect.

4.2. The challenges brought by the MMR

MMR is a key link in repairing base mismatches or deletions during DNA replication and recombination [15]. However, during the process of gene editing in PE, the newly introduced mutations inevitably mismatch the bases on the target DNA strand, thereby triggering MMR. This leads to two possibilities: MMR uses the strand with the introduced mutation as a template for repair

and retains the gene editing result, or MMR uses the strand without the introduced mutation as a template for repair, resulting in the failure of gene editing. The existence of this mechanism greatly affects the gene editing efficiency of PE. Therefore, achieving the inhibition of MMR or reducing the impact of MMR on gene editing has become the key to improving the success rate of PE.

Researchers have made certain improvements to PE by introducing new gRNA and crisper systems [16] and interfering with MMR to ensure that the mutated strand introduced during the editing process of PE can have an advantage in competing with the non-mutated strand for binding to the DNA target strand, thereby increasing the probability of successful editing to a certain extent. Therefore, how to control the response efficiency of MMR has become the key point for balancing and improving the editing efficiency of PE. It should also be particularly noted that the introduction of new grnas may to some extent cause new indels, which poses a significant risk and hidden danger during the critical period of nervous system development, especially during the period when neural precursor cells rapidly separate in the embryonic or infant stage, when MMR is more active [17]. Unexpected new mutations that occur during the gene editing process can cause potentially huge harm.

4.3. Heterogeneity of PE cells and their dependence on developmental stages

The diversity of the pathogenesis of neurogenetic diseases and the cellular diversity of the nervous system have raised the demand for precise regulation of the application of PE. Different neuronal subtypes have significant differences in response to PE, and different neurons are highly sensitive to different delivery systems. Even the capsid that has been engineered There are also differences of orders of magnitude in the transduction efficiency of excitatory neurons, inhibitory neurons, astrocytes, oligodendrocytes, etc. [18] This means that the biological distribution of PE components through systemic or ventricular injection within the brain is uneven. Some cell populations may efficiently receive editing tools while others are almost inaccessible, which may directly lead to potential inhomogeneity in subsequent editing efficiency. In addition, the sensitivity of the developmental time window cannot be ignored: during the embryonic period, neural precursor cells undergo high-frequency division, and the risk of indel mediated by MMR is high, while the risk of mature neurons is relatively low [17]. Existing studies have only demonstrated therapeutic effects on experimental animals at specific times. However, most AHC patients are diagnosed or show symptoms of neurogenetic diseases significantly later than the period when PE intervention for gene editing treatment occurs [10]. Whether this will lead to the ineffectiveness and weakening of subsequent gene editing treatment remains unknown. Similarly, due to the lack of long-term data tracking of PE in the treatment of neurogenetic diseases, we cannot guarantee whether this one-time gene editing treatment technology will cause irreversible impacts and harm to the lives of animals and humans in the long run.

5. Conclusion

An efficient, safe and non-invasive delivery system is a prerequisite for the successful treatment of neurogenetic diseases with PE. Current AAV-based delivery strategies are limited by packaging capacity, immunogenicity and potential genomic integration risks. Future research directions can focus on developing novel non-viral delivery platforms. By optimizing the lipid components and targeted peptides of engineered virus-like particles, they can efficiently penetrate the blood-brain barrier and specifically target neuron and glial cell subsets, while achieving instantaneous editing to eliminate long-term expression risks, or making miniaturized modifications to the PE system. While retaining the editing capabilities, adapt to the existing delivery system. In addition, an intelligent regulation system is designed to control the spatiotemporal specific activation of PE components by using small molecule drugs or optogenetic tools, achieving precise manipulation of editing time and space and minimizing the risk of off-target.

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