Genome Editing Used In Treatment Of Brain Disease

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Abstract- *Recent advancements in our understanding of the genetic basis of neurological disorders have spurred significant efforts to develop gene-based therapies. These therapies aim to correct the underlying genetic defects, either by introducing a functional copy of the mutated gene or by directly editing the genomic sequence. This review explores the main genetic strategies currently being investigated for the treatment of monogenic neurological disorders, highlighting the challenges and ethical considerations associated with these approaches.*

Keywords- neurogenetics; CRISPR/Cas9; genome editing; gene regulation; transcription; gene therapy

I. INTRODUCTION

The developing field of gene therapy holds promise for treating a wide range of brain diseases by targeting their underlying genetic causes. Emerging gene therapy research is already providing answers for some forms of amyotrophic lateral sclerosis (ALS) and has resulted in successful treatments for spinal muscular atrophy (SMA). Recent advances in DNA sequencing technology have led to an explosion of knowledge about the genetics of human disease, and the realisation that many more disorders are genetic in origin than previously thought. For example, Onasemnogene abeparvovec, sold under the brand name Zolgensma, is a gene therapy used to treat spinal muscular atrophy (SMA), a disease causing muscle function loss in children. It involves a one-time infusion of the medication into a vein. It works by providing a new copy of the SMN gene that produces the SMN protein. large-scale exome-sequencing projects, such as the Deciphering study of developmental disorders has identified Novel pathogenic de novo mutations in patients with undiagnosed neurodevelopmental conditions (Fitzgerald et al. [1]2015; McRae et al., 2017). The new knowledge has led to a surge of interest in the potential for therapies that address the genetic root cause of these disorders, rather than attempting to treat secondary consequences. These approaches include conventional gene therapy (also referred to as “gene transfer"), which aims to restore the function of the mutated gene.by introducing a functional copy into cells (Friedmann and Roblin 1972). In addition, advances in our ability to re-write DNA sequences via genome editing, particularly “clustered regularly interspaced short palindromic repeats” (CRISPR) technology, have sparked interest in their use for the treatment. of a variety of disorders. All of these approaches are particularly suited to monogenic conditions, which, in theory, can be cured by correction of the disease-causing mutation. In this review, we illustrate the growing therapeutic potential. of these developing technologies. We also consider the technical challenges still to be overcome, as well as some ethical issues posed by genetic interventions in the brain. Like many medical innovations, genetic therapies rely on basic knowledge acquired in model organisms. Importantly, There needs to be evidence that symptoms have the potential to to be alleviated or even cured. A disorder that highlights The value of pre-clinical research is Rett syndrome (RTT). a severe neurological disorder caused by mutations in the X-linked gene MECP2 (Amir et al., 1999). Mouse models recapitulate many features of the human condition (Chen et al. 2001; Guy et al. 2001), supporting the conclusion that The function of the MeCP2 protein is the same in mice as in humans. Importantly, the majority of symptoms can be reversed in adult Mecp2-null mice by restoring expression of the wild-type protein Guy et al. (2007); Robinson et al. 2012). This suggests that RTT is curable well after the onset. of symptoms. Phenotypic reversibility of a few other monogenic Neurological conditions have been tested using mice. models, with variable results. For example, restoration of Ube3a expression in young mice leads to the reversal of many adverse phenotypes in a model of Angelman syndrome, but Not all autism-related phenotypes are reversed when the gene is activated in older animals(Silva - Santosetal. 2015). These Findings point to an early window for therapeutic intervention. In all neurodevelopmental disorders of this type, basic understanding of the function of the mutated gene and its Time of action during life is an important pre-requisite for intervention.

How does genome editing work?

1. Genome editing uses a type of enzyme called an ‘engineered nuclease’ which cuts the genome in a specific place.
2. Engineered nucleases are made up of two parts:
   2.1. A nuclease part that cuts the DNA.
   2.2. A DNA-targeting part that is designed to guide the nuclease to a specific sequence of DNA.
3. After cutting the DNA in a specific place, the cell will naturally repair the cut.

We can manipulate this repair process to make changes (or 'edits') to the DNA in that location in the genome[1]

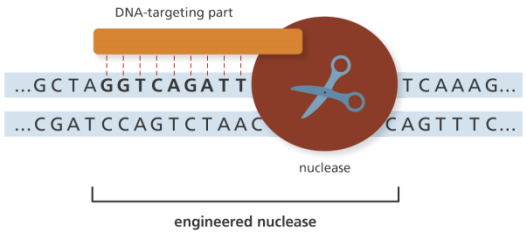

Fig.Illustration showing the basic structure and function of engineered nucleases used for genome editing.Image credit: Genome Research Limited[1]

Genome editing is a process where an organism's genetic code is changed. There are several available tools for gene editing, all of which use enzymes that act on DNA called nucleases. The different editing tools act on a similar basic principle: the enzyme is directed to a specific target site in the genome by either a guide sequence or by specific DNA binding domains within the nuclease itself. Once it recognizes and binds to the target DNA, the nuclease can be used for editing via several pathways. If the nuclease creates double-stranded breaks (DSBs), the cell will attempt to repair the break via non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ is error-prone and often causes insertion and deletion mutations (indels), which can result in the gene becoming non-functional (gene knockout). If a new DNA sequence with regions of homology to the target is provided, HDR will use this as a template for repair of the DSB. This allows for new gene sequences to be integrated into the genome (gene knock-in). Newer forms of gene-editing technology do not induce DSBs, and instead use engineered or inactivated nucleases to either cut only a single strand of DNA, or simply to identify and bind to the target sequence and direct a different enzyme for the modification of DNA. Each gene-editing system has a unique set of advantages and disadvantages.

Gene Editing Techniques: Tools to Change The Genome

Gene editing might sound simple on paper, but it is far from easy. The history of genome engineering goes back almost 70 years, to the initial discovery of the DNA double helix. Since then, scientists have spent decades trying to uncover ways to edit the genome that balance specificity with time and cost. Here are the 7 successful strategies that scientists have used to modify DNA so far.

1. Restriction enzymes: the original genome editors

The ability to edit genes became a reality with the discovery of restriction enzymes in the 1970s. Restriction enzymes recognize specific patterns of nucleotide sequences and cut at that site, presenting an opportunity to insert new DNA material at that location. Restriction enzymes are not commonly utilized for gene editing these days, since they are limited by the nucleotide patterns they recognize, but they remain widely used today for molecular cloning. Additionally, certain classes of restriction enzymes play key roles in DNA mapping, epigenome mapping, and constructing DNA libraries.[2]

2. Zinc finger nucleases: increased recognition potential

As time went on, the need for precision in genome editing became more evident. Scientists needed a gene-editing technique that recognized the site they wanted to edit, as off-target effects could be deleterious. The discovery of zinc finger nucleases (ZFN) in the 1980s addressed this issue.
ZFNs are composed of two parts: an engineered nuclease (Fokl) fused to zinc finger DNA-binding domains. The zinc-finger DNA-binding domain recognizes a 3-base pair site on DNA and can be combined to recognize longer sequences. Additionally, the ZFNs function as dimers, increasing the length of the DNA recognition site and consequently increasing specificity. However, while specificity increased with ZFNs, it was not perfect.One main hurdle with using ZFNs was that the 3-base pair requirement made the design more challenging. Guanine-rich target sites appeared to yield more efficient editing than non-guanine-rich sites. Additionally, since the ZFN interaction with DNA is modular (i.e., each ZF interacts with DNA independently), the editing efficiency was also compromised. Therefore, scientists needed to address these issues if they wanted to have more efficient genome editing. ZFNs showed real promise in the field of medicine. Notably, scientists used ZFN to disable CCR5 on human T-cells, a major receptor for HIV. Following ZFN-mediated editing, scientists found autologous CD4+ T-cells were safe to use and were an exciting potential for HIV therapy. Additionally, ZFNs have been used to edit tumor-infiltrating lymphocytes as a treatment strategy for metastatic melanoma. [2]

3. Transcription activator-like effector nucleases: single-nucleotide resolution

In 2011, a new gene-editing technique emerged, which was an improvement over ZFNs. Transcription activator-like effector nucleases (TALENs) are structurally similar to ZFNs. Both methods use the Fokl nuclease to cut DNA and require dimerization to function, however, the DNA binding domains differ. TALENs use transcription activator-

like effectors (TALEs), tandem arrays of 33-35 amino acid repeats. The amino acid repeats possess single-nucleotide recognition, thereby increasing targeting capabilities and specificity compared to ZFNs. Even with the single-nucleotide resolution, using TALENs as a gene-editing tool was still time and cost-intensive and possessed certain design restrictions. The structure of TALENs meant the target site required a 5' thymine and 3' adenine, limiting target customizability. Further, TALENs displayed decreased editing efficiency in heavily methylated regions. Delivery into cells was also challenging, since TALENs are much larger than ZFNs (~6kb vs. ~2kb), increasing the amount of time and money required to create a successful edit. While TALENs showed improvement in genome editing technology, the high labor and monetary cost still hindered its widespread adoption. Like its predecessor ZFNs, TALENs have been used in the field of medicine, as well as agriculture. Scientists used TALENs to correct COLA7A1 dysfunction in epidermolysis bullosa, a disease characterized by loss of skin integrity leading to potentially fatal skin blisters and increased risk for skin cancer. In agriculture, scientists found a way to create pathogen-resistant rice using TALENs[2].

4. CRISPR-Cas9 gene editing: genome editing revolutionized

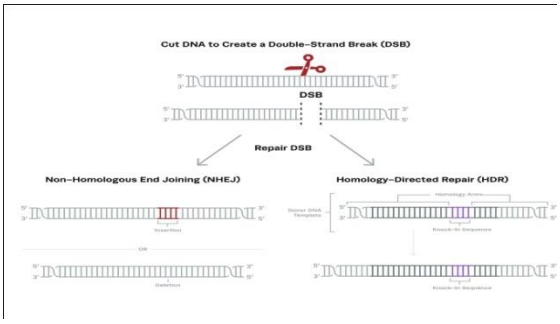

Fig.How genome editing is accomplished using CRISPR-Cas9[2]

Scientists were still searching for an easier and faster way to edit genes. Fast forward to 2012, when everything changed. Scientists discovered a new method of genome editing derived from CRISPR-Cas9, a system that has long existed in bacteria to help them fight off invading viruses. The teams led by Jennifer Doudna and Emmanuelle Charpentier rocked the science world with the first biochemical description of CRISPR. In 2013, Feng Zhang described how CRISPR could be used to edit eukaryotic DNA. Since these discoveries, CRISPR as a gene-editing tool has seen unprecedented popularity. CRISPR is an elegant two-component system consisting of a guide RNA and a Cas9 nuclease. The Cas9 nuclease cuts the DNA within the ~20 nucleotide region defined by the guide RNA. With CRISPR, scientists can customize their guide RNAs, and algorithms have been developed to assess the chances of off-target effects (i.e., does this sequence exist in other places of the genome). However, CRISPR is much more customizable and cost-effective, making it more accessible to scientists that may have budget and time constraints. The advancements in genome editing techniques have opened up new doors for what genome editing can do to address issues in medicine, agriculture, and beyond. CRISPR has completely revolutionized what genome editing can mean for our future by increasing the speed and breadth of science. We are already feeling the impact of CRISPR in its role in drug discovery, diagnostics, and gene drives, just to name a few. At this rate, don't be surprised if you see more talk about genome editing in the near future. [2]

5. Base editing: single nucleotide substitutions

Base editing is a relatively new method of genome editing derived from CRISPR-Cas9. Unlike traditional CRISPR systems, base editors (BEs) do not induce double-stranded breaks in the genome. Base editing systems, developed by David Liu's lab at the Broad Institute, use a 'catalytically dead' Cas9 (dCas9), which cannot cleave DNA, fused to bacterial enzymes called DNA deaminases. Cytidine deaminases, which induce C to T substitutions, are naturally occurring in bacteria, while adenine deaminases, which induce A to G substitutions, were engineered from bacterial enzymes specifically for base editing purposes. Fusing dCas9 to either a cytidine deaminase (CBEs) or an adenine deaminase (ABEs) and providing a sgRNA to direct it to the target sequence, allows researchers to introduce substitutions in DNA. The ability to induce single nucleotide substitutions was a major step forward for the field, both because a majority of human diseases are caused by single nucleotide polymorphisms and because editing without creating double-stranded breaks avoids many of the potential risks of other forms of editing such as CRISPR-Cas9. However, the current CBE and ABE systems only cover four of 12 possible transition mutations, a fact that led to the development of prime editing systems. [2]

6. Prime editing: editing without double-stranded breaks

Prime editing systems allow for all possible transition mutations, as well as small insertions of up to 50 nucleotides and deletions of up to 80 nucleotides. The system, also developed by the Liu lab, works using a Cas9 nickase, which induces single-stranded breaks in DNA, fused to a reverse transcriptase enzyme. Rather than using an sgRNA and a donor template for repair, prime editing uses a single engineered construct known as a prime editing guide RNA (pegRNA), which is

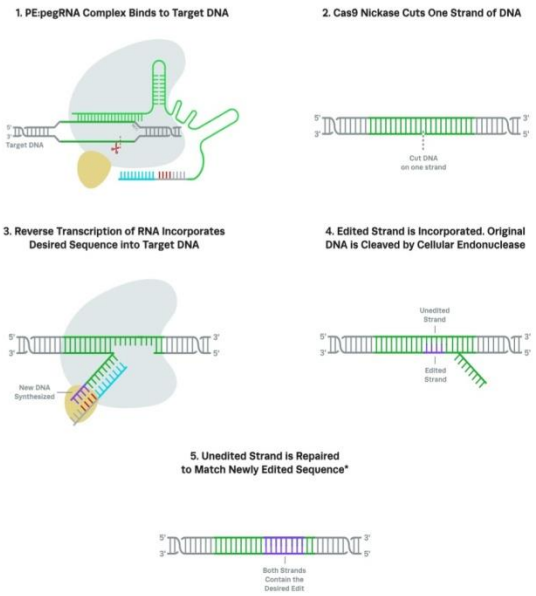

Fig.Steps involve in prime editing:editing without double-standed breaks[2]

made up of the primer binding site (PBS) sequence and a sequence containing the desired edit. After it finds the target, the Cas9 nickase creates a cut in one strand of the DNA and the reverse transcriptase uses the pegRNA as a template for reverse transcription, attaching the corresponding nucleotides to the nicked DNA end. The original sequence is naturally excised by endonucleases within the cell, however, this leaves a mismatch between the two strands of DNA that must be resolved for the editing to be complete. This is achieved by using a different guide RNA to direct the prime editor to nick the opposing unedited strand. When this occurs, the cell repairs the nick using the new DNA from the first edit as a template for repair, resulting in a complete edit.

The fact that prime editing can generate all possible transition mutations, insertions, and deletions without inducing DSBs makes it much safer to use and therefore highly desirable for therapeutic purposes. Base editors and prime editors are currently being investigated for the treatment of blood disorders such as sickle cell disease, and they have many other potential therapeutic applications. [2]

7. PASTE: 'Drag-and-Drop' Editing for Large Insertions

Programmable Addition via Site-specific Targeting Elements (PASTE) is the new kid on the block when it comes to genome editing. Developed by Jonathan Gootenberg and Omar Abudayyeh, this system allows for the targeted insertion of large DNA sequences, also without creating double-stranded breaks.

PASTE harnesses the power of serine integrase proteins from bacteriophages to incorporate new genetic information - up to 36 kb - into the genome. For this to work, however, the integrase's attachment site (AttP) must first locate and bind to the correct landing site (AttB), and these are not common in genomes.

To overcome this obstacle, PASTE incorporates prime editing to copy these AttB sites into the genome near the target sequence via reverse transcription. Conveniently, prime editing systems can insert up to 50 bp of DNA, and the AttB landing sites recognized by integrases are around 46 bp. Once the AttB site has been incorporated near the target sequence via prime editing, it acts as a beacon for the integrase, which will be recruited to the site to perform the insertion of the attached desired DNA sequence.

The development of the PASTE tool is certainly exciting in the field of gene editing because it allows for large-scale gene knock-in without creating DSBs. This indicates its potential in therapeutic applications, such as treating diseases that are caused by multiple pathogenic mutations in large genes. Like base and prime editing systems, PASTE's ability to edit DNA without creating DSBs increases the safety profile of any potential therapeutic application of this technology. [2]

4. Promising Role Of CRISPR/Cas9 In Brain Diseases

Application of CRISPR/Cas9 in Parkinson's disease:-

Parkinson's disease (PD) is the second most prevalent neurological disorder in humans, following Alzheimer's disease. It is a heterogeneous neurodegenerative condition identified by impaired bodily movements (Troncoso-Escudero et al., 2020). PD is characterized by the progressive loss of dopaminergic neurons (DN) in the substantia nigra pars compacta (SNPC), which leads to a significant decrease in dopamine levels reaching the striatum and subsequent functional impairment of the motor circuit, resulting in motor symptoms like rest tremors, bradykinesia, and rigidity that constitute the core of its clinical features (Blesa et al., 2012; Magrinelli et al., 2016). Additionally, the presence of intracytoplasmic Lewy bodies (LB), primarily consisting of α-synuclein and ubiquitin, is also a defining characteristic. While α-synuclein gene mutations have only been linked to infrequent familial instances of PD, it is worth noting that α-synuclein is present in all Lewy bodies (Blesa et al., 2012). Approximately 90 % of PD patients have no known cause (idiopathic), while the remaining 10 % have familial PD

caused by mutations in specific genes like SNCA, PRKN/PARK2, PINK1, LRRK2, PARK7, DJ-1, GBA, UCH-L1, and MAPT/STH. It is possible that these mutations could also be associated with sporadic PD (Cota-Coronado et al., 2020; Nalls et al., 2019). The expression of α-synuclein is closely linked to SNCA gene, which is one of the most important predictive locations for sporadic PD (Ferreira and Massano, 2017). The missense mutation called Ala53Thr (A53T) in SNCA is recognized as one of the most prominent risk factors for early-onset PD. SNCA has several mutations, but A53T is particularly noteworthy in its association with Parkinson's disease (Spira et al., 2001). In 2022, Yoon et al. conducted a study which showed that using the CRISPR-Cas9 system to delete the A53T-SNCA gene significantly improved conditions related to Parkinson's disease, such as the overproduction of α-synuclein, reactive microgliosis, dopaminergic neurodegeneration, and motor symptoms associated with Parkinson's (Yoon et al., 2022).

In another study Kantor and colleges have developed a novel all-in-one lentiviral vector that employs CRISPR-Cas9 technology to specifically downregulation of SNCA mRNA and protein expression led to the reversal of disease-related phenotypic perturbations (Kantor et al., 2018). Additionally, Chen and colleagues examined the mechanism by which SNCA operates in the nucleus using neurons derived from human-induced pluripotent stem cells from Parkinson's disease patients with A53T and SNCA-triplication autosomal dominant mutations, as well as their CRISPR-edited corrected counterparts. The study demonstrated that the absence of SNCA leads to resistance against Lewy pathology, indicating the possibility of utilizing CRISPR/Cas9n-mediated gene editing as a potential treatment for PD (Chen et al., 2020). In another study Zhou et al. examined the PARK2 and PINK1 genes by utilizing CRISPR-Cas9 and somatic cell nuclear transfer techniques in a domestic pig model. The scientists revealed that they were able to acquire approximately 38 % successful outcomes in obtaining homozygous cell colonies that had a double-knock-out for PARK2 and PINK1 genes (Zhou et al., 2015). Furthermore, a fascinating research conducted on nigral dopaminergic neurons (DN) involved the use of CRISPR/Cas system to delete PARKIN (PRKN), DJ-1 (PARK7), and ATP13A2 (PARK9) genes. By analyzing transcriptome and proteome data, it was found that oxidative stress is a shared dysregulation pathway among all isogenic cell lines (Ahfeldt et al., 2020). Loss-of-function mutations in DNAJC6, the gene responsible for producing HSP40 auxilin, have recently been found in individuals with early-onset PD. Human embryonic stem cells (hESCs) were used with CRISPR-Cas9-mediated gene editing to uncover these mutations. Through transcript analysis and experimental validation, it was discovered that defects in DNAJC6-mediated endocytosis can hinder the WNT-LMX1A signal during mDA neuron development. This, in turn, can lead to the generation of vulnerable mDA neurons with pathological symptoms as a result of reduced expression of LMX1A during development (Wulansari et al., 2021).

II. CONCLUSION

The genome editing/sequencing is best for the treatment of neurogenrative brain disease like Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Amyotrophic Lateral Sclerosis (ALS), Angelman Syndrome, MECP2 Duplication Syndrome, Fragile X Syndrome. The CRISPR/Cas9 technique is best treatment of all type of neurogenarative brain disease specially in alzheimer's disease and parkinson' disease

III. RESULT

At present time the many treatment is available for treatment of brain disease but the overall the genome editing is best, because of its effect and low side effect by comparison of other treatment and very effective in treating the brain disease

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