

Application prospect of CRISPR-Cas9 gene editing in epilepsy

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Abstract. Epilepsy, a chronic noncommunicable brain disorder, is characterized by abnormal electrical activity in the brain, leading to seizures and disruptions in normal brain functions. Despite various known causes, a significant proportion of epilepsy cases remain unexplained. In recent years, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR-Cas9) technology has emerged as a powerful tool for genetic engineering. Compared with the first two generations of gene-editing technology, it has the advantages of low cost, easy design and easy operation. Utilizing CRISPR activation (CRISPRa), researchers have explored the potential of increasing the expression of genes involved in regulating synaptic interactions to control epileptic activity. Studies on transgenic mice have shown that upregulating the Kv1.1 gene (Kcna1), which encodes for a voltage-gated potassium channel responsible for regulating neuronal excitability, can reduce seizures and improve cognitive function. Additionally, CRISPR-Cas9 has been instrumental in creating animal models to study epilepsy, providing insights into gene functions, disease mechanisms, and potential therapeutic interventions. However, further research is needed to fully explore the potential of CRISPR-based therapies for targeted treatment of epilepsy. This review systematically introduces the pathogenesis of epilepsy, including the origins and causes of epilepsy and the mechanism of seizure formation and further discusses the application of the CRISPR/Cas9 system in epilepsy.

Key words: Epilepsy, CRISPR-Cas9 system, pathogenesis, gene therapy.

1. Introduction

Epilepsy (Seizure Disorder) is a chronic noncommunicable brain disorder that could be induced by stopping activity between synaptic and voltage-gated inhibitory conductance or by increasing activity between synaptic and voltage-gated excitatory conductance [1]. Both interactions would result in excessive neuron discharge and cause seizure symptoms and interrupt normal brain functions. Patients with seizures perform involuntary movement of the parts of the body, including muscle jerks, loss of control of bowel or bladder function, and loss of awareness and consciousness [2]. Seizures could be controlled by inhibition of the excitatory section and activation of inhibitory conductance [3]. Epilepsy is one of the most common neurological diseases, over 50 million population worldwide are diagnosed with this disease [2]. Among these patients, many have developed drug resistance, in Western Europe, shows that 22.5% of all patients with epilepsy have been reported to develop such resistance [4].

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR-Cas9) is a type of genetic engineering system where the sequence of the short guide RNA (sgRNA) is modified to guide the binding of nucleic acid and Cas 9. This system has been widely applied in gene editing by deletion, addition and replacement of nucleic acid in DNA sequence[5]. Reduction of epileptic action could be reduced by using CRISPR activation (CRISPRa); it regulates gene activity through promoter regulation; to increase gene expression that codes for the regulation of synaptic interaction.

2. Pathogenesis of epilepsy

2.1. origins and causes of epilepsy

Currently, 70% of the cases of epilepsy have no clear causes. The commonly known causes of epilepsy that have been analyzed are genetics, head injuries, brain infections, immune disorders, developmental disorders, metabolic disorders, brain conditions and brain vessel abnormalities (Fig. 1). Despite the numerous ways to trigger epilepsy, the seizures of epilepsy are only caused by abnormal electrical activity in the brain. Moreover, there are types of epilepsy, each can be caused by different reasons [6]. It is reported that juvenile myoclonic epilepsy and childhood absence epilepsy are mostly inherited, which means that the genes that would result in a rising likelihood of seizures are passed on to the next generation [7]. Meanwhile, head injuries which are more specifically Traumatic Brain Injury (TBI) are another cause of epilepsy. TBI can be a result of falls, or car accidents — there is a violent collision between an object and the head, which may then damage the brain tissue [8]. The seizures appeared could be early (within the first week of the brain injury) and late (more than a week after the brain injury). The recurrence probability of late seizures is relatively higher. In addition, brain infections, including meningitis can lead to epilepsy. There are many types of meningitis — bacterial meningitis, viral meningitis, fungal meningitis, parasitic meningitis, etc. They are all detrimental to the brain. For instance, bacteria would be carried by the blood stream and travel to the brain and spinal cord, which then invade the meninges, causing seizures [9]. Immune disorders can cause autoimmune epilepsy. In this case, people's immune systems may start to attack their own brain cells. This change is basically a result of dysfunction of the immune system [10]. Apart from the above reasons, epilepsy can also be caused by developmental disorders. These abnormalities include focal cortical dysplasia (FCD), polymicrogyria and tuberous sclerosis. In FCD, a particular area of the brain may have abnormal brain cells, which raises the possibility that seizure would occur and the dysfunction of the brain in that area. On top of these, metabolic disorders can be a kind of genetic cause, as it is sometimes inherited. Besides this, it can be developed from malnutrition and an unhealthy lifestyle. Finally, brain conditions and brain vessel abnormalities are some brain health issues that then cause epilepsy with brain tumours, strokes or dementia.

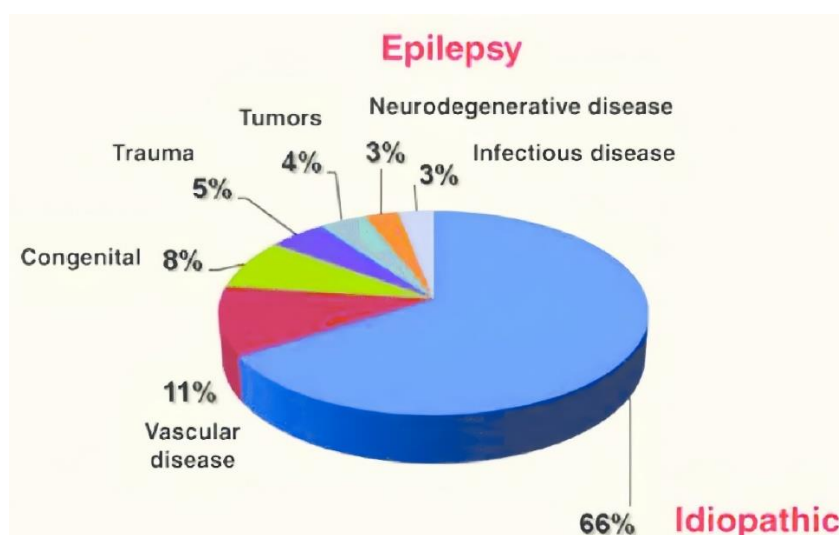


Fig. 1 Percentage of different causes of epilepsy [5].

2.2. Mechanism of seizure formation

In general, there are 3-6 layers of neurons in the human cerebral cortex, and the neurons can be divided into two categories which are projection neurons and interneurons. Project neurons are those cells that are responsible for transmitting information to the remote neurons in the brain. Other than just sending, this neuron and its networks process and store the information. Interneurons are one kind of nerve cell. They can be found in integrative areas of the central nervous system (CNS), and

they could only reach a limited distance of the brain area. Interneurons contain local axons which govern ensemble activity [11]. The excitatory synapses on post-synaptic neurons are composed of projection neurons; whilst inhibitory synapses on principal cells or other inhibitory neurons are composed of interneurons. One single change in one cell can bring effects on both adjacent or distant neurons. This is because of that neuronal excitability is controlled by the ways that the neurons are connected and organized [12]. Local interneurons can be directly activated by the input and also by accepting the information transported from the projection neurons. This explains why hypersynchronous discharges, a manifestation that can be seen on an electroencephalogram (EEG), can spread and thus affect a population of neurons. Apart from hyper synchronization, seizures can also be initiated by high-frequency bursts of the neuronal population. After seizure initiation is the seizure propagation. Propagation is the process of spreading a partial seizure within the brain. It would occur once the activation is enough to recruit the neurons. This results in a loss of peripheral inhibition, with seizure activity spreading to adjacent areas via local cortical connections and to more distant areas via long association pathways such as the corpus callosum [11].

3. CRISPR-Cas9 System

The CRISPR-Cas9 system is a revolutionary gene-editing technology that makes precise modifications to DNA sequences in living cells. The Cas9 protein and the guide RNA (gRNA) are the two key elements of the mechanism. Cas9 is directed by gRNA to a specific region of the genome where it breaks DNA. This can result in either gene knockout by adding a new sequence or gene knockout that results in the target gene's destruction. Cas9 is a nuclease that is guided by short RNA by pairing with Watson-Crick bases of target DNA). The system is significantly easier to develop, highly specific, effective, and well-suited for high-throughput and multichannel gene editing across a variety of cell types and organisms. This promising tool enables the ability to create biological systems and organisms, and it has great potential for applications in basic science, medicine, and biotechnology [11].

3.1. Editing Mechanisms

CRISPR-Cas9 is a clear two-part system for effective targeted gene editing. The single-effect Cas9 protein, which has the endonuclease domains RuvC and HNH, is the first part. HNH cuts complementary DNA strands while RuvC only cuts DNA strands that are not complementary to spacer sequences. When these domains work together, the target DNA experiences double-strand breaks (DSBs). Simply put, the Cas9 protein then cuts two DNA strands at the target site to produce the DSB. A single guide RNA (sgRNA) that carries a scaffold sequence that allows it to adhere to Cas9 and a 20-base-pair spacer sequence that complements the target gene and is next to the protospacer adjacent motif (PAM) region is the second element of efficient targeted gene editing. The CRISPR-Cas9 complex is directed to the correct chromosomal region by this sgRNA. The editing mechanism then utilizes either the homologous directed repair (HDR) or non-homologous end joining (NHEJ) endogenous DNA repair pathways (Fig.2) [12].

Most cell types experience the pathway of NHEJ more frequently, which involves random base pair insertions and deletions or insertion deletions at cut sites. It depends on the Ku protein to cling to each damaged DNA strand's end. In order for nucleases to trim or fill the ends with polymerase and optimally connect via the DNA ligase IV complex, Ku recruits the enzymes and complexes required. Frameshift mutations, which frequently result in premature stop codons and/or non-functional peptides, are frequently caused by this error-prone mechanism.

HDR is a more precise DSB repair method since it uses homologous sequences as a repair template. The method is typically error-free if the DNA template used for repair matches the DNA sequence that was present at the DSB, or if these alterations are present in the template DNA, particular mutations can be introduced into the damaged DNA. The primary stage in each template repair approach is depicted below. To create the 3' protruding end of ssDNA, nuclease excises the broken

5' DNA end. This will serve as a starting point for DNA repair synthesis as well as a substrate for the required protein's chain invasion. The homologous DNA donor's one strand is subsequently replaced by the ssDNA strand, which can also template-repair another strand. This results in the construction of DNA structures known as replacement rings (D-rings). As a result, the DNA repair procedure can be finished by disassembling the recombinant intermediate [13].

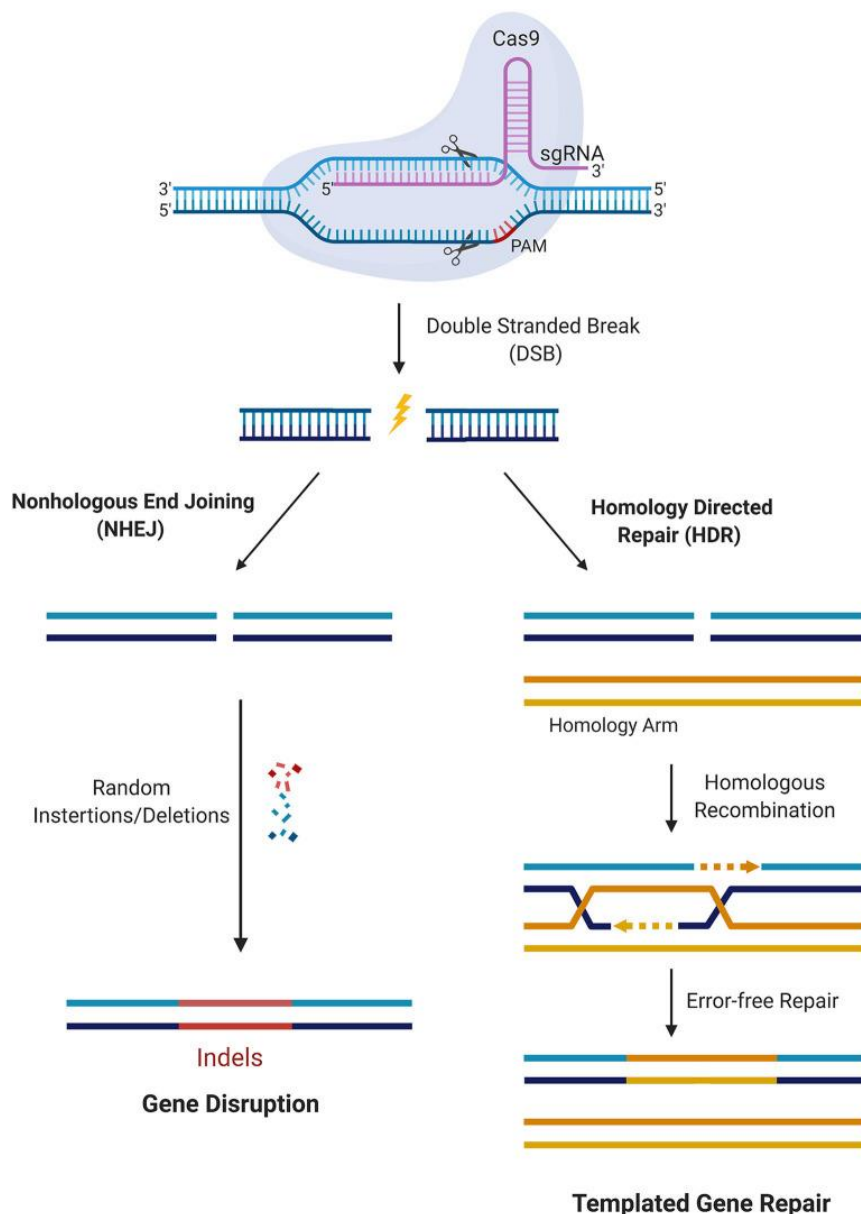


Fig. 2 Two comparing processes of NHEJ and HDR [13].

3.2. Advantages

There are two major competitors of CRISPR-Cas9 in the field of genome editing, which are Zinc finger nucleases (ZFNs) and Transcription activator-like effector nucleases (TALENs) respectively. Nevertheless, the CRISPR-Cas9 system has several advantages over them, including its ease of use, versatility, efficiency, specificity, and low off-target rate, which have made it a popular tool in the scientific community.

ZFNs, one of the first enzymes used in targeted genome engineering, are produced when zinc finger nucleases are fused to a restriction endonuclease (usually FokI3) [14]. Three base-pair DNA sequences are recognized by zinc finger domains; hence, a chain of connected zinc finger domains can detect longer DNA segments, resulting in the necessary targeting specificity. Double-stranded DNA is only cut at the two locations where the ZFN attaches to the opposing DNA strand when the

FokI endonuclease functions as a dimer (Fig. 2). This requires simultaneous identification and binding of both ZFNs, which limits off-target effects. These two ZFNs are made to detect separate, closely spaced nucleotide sequences inside the target (Figure.3A).

Target specificity is derived from protein-DNA interactions, just as ZFN. A single TALE motif in the context of TALEN recognizes a single nucleotide, whereas many TALE motifs can be connected to a longer sequence (Figure 3B). TALEN is simpler to construct than ZFN because each TALE domain's activity is restricted to a single nucleotide and has no impact on the binding specificity of nearby tales. The TALE motif, like ZFN, is connected to the FokI endonuclease and necessitates dimerization for cleavage to take place. The complexity of the application is increased because two different skills must be united on opposing strands that are just next to the target DNA [15]. (Fig.3B)

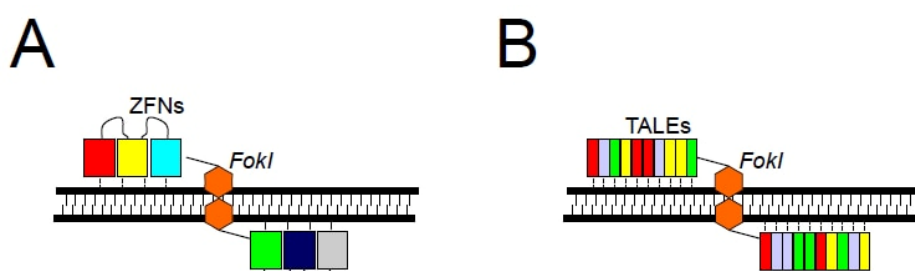


Fig. 3 The nuclease editions [15].

With the emergence of gene-editing technologies, they have greatly expanded the ability to treat diseases. For each target sequence, ZFNs and TALENs demand the creation of a new protein, which can be time-consuming and expensive. Specific protein-DNA interaction properties place limits on the targeting's adaptability and specificity. Furthermore, the delivery of ZFNs and TALENs into cells is difficult and necessitates unique techniques like electroporation or viral vectors. These techniques, nevertheless, are pricey, time-consuming, and may harm cells. Contrarily, the CRISPR-Cas9 system merely requires the creation of a new gRNA target, which can be given to cells via a variety of techniques, including plasmid transfection and viral vectors, and may have its specificity increased by changing the gRNA sequence [16].

4. Application of CRISPR in epilepsy

CRISPR-Cas9 is widely used in the construction of various biological models, including cell lines, animal models, and disease models, to study gene functions, disease mechanisms, and potential therapeutic targets.

The mouse model has been a large factor included in the made up of CRISPR-Cas9 system-based experiments, the CRISPR-Cas9 system found in bacteria and archaea has recently been harnessed for genome editing in various model organisms. By directly delivering CRISPR-Cas9 reagents into the mouse zygote, researchers can generate mutant animals with specific genetic alterations [17]. This system consists of key components, including the guide RNA, responsible for target specificity, the Cas9 nuclease, which induces DNA double-strand breaks, and the donor oligonucleotide or plasmid carrying the desired mutation flanked by sequences that match the target site. In this case, both vitro and vivo models are intimated with the application of CRISPR in epilepsy [18].

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Vitro and in vivo are two common research models used in biomedical research, including the study of epilepsy. It could be conducted that vitro Models are mostly about cells and Organoids, examples of in vitro studies include the isolation, growth and identification of cells derived from multicellular organisms (in the cell or tissue culture); subcellular components (e.g. mitochondria or ribosomes); cellular or subcellular extracts (e.g. wheat germ or reticulocyte extracts); purified molecules it provides an insight into cells' and microorganisms' behaviour [19,20]. While Vivo

models are experiment conducted 'within the living' and occurs on a living organism, such as living animals.

4.1. In vitro

CRISPR-Cas9 also plays a significant role in constructing organoids, three-dimensional mini-organs derived from stem cells. Genetic alterations using CRISPR-Cas9 create disease-specific organoids, providing valuable tools to study disease mechanisms and drug responses in a more human-relevant context [21]. Additionally, CRISPR-Cas9 enables the creation of animal models to study viral diseases, such as introducing human-specific receptors into animals to investigate viral infections primarily affecting humans.

Many experiments focus on the methodological details related to vitro preparations of study within seizures and epilepsy, focusing on establishment and utilization. In vitro, preparations offer valuable and effective methods for investigating epilepsy and epileptic seizures. These preparations can be derived from various species, with mammalian brain slices being the most commonly utilized for studying epileptiform activity [22]. These thin brain slices can be used acutely, immediately after preparation, or cultured over days or weeks in an incubator (organotypic brain slice preparation). Additionally, intact brain models, such as whole hippocampus or whole brain preparations, are also highly valuable. In vitro, epileptiform activity can be induced through ionic or pharmacological manipulations on preparations from healthy animals, animals with a history of epileptogenic insults, or through genetic models that exhibit epilepsy-like characteristics in vitro.

4.2. In vivo

In animal models, the CRISPR-Cas9 gene-editing system is extensively used to generate genetically modified mice and rats. By introducing specific mutations or deleting target genes, researchers can gain valuable insights into gene functions and observe disease phenotypes in living organisms. This powerful tool enables scientists to study the effects of genetic alterations and provides a means to explore potential therapeutic interventions for various conditions.

One important application of CRISPR-Cas9 in animal models is the generation of conditional gene knockouts using the Cre-lox system. This approach allows for precise spatial and temporal control of gene inactivation in specific tissues and at different developmental stages. Such conditional knockouts provide researchers with a valuable platform to study the function of genes in specific tissues or during particular stages of development.

Additionally, CRISPR-Cas9 has proven instrumental in creating animal models for studying various diseases. By introducing disease-associated mutations into the genome, scientists can develop animal models that accurately mimic human genetic disorders. These models are valuable for understanding the underlying mechanisms of diseases and testing potential therapeutic approaches. Referring with figure 2. CRISPR/Cas9 technology allows for gene editing that can introduce mutations in specific organs or tissues in postnatal mice [23]. To create knockout (KO) mouse models, the Non-Homologous End Joining (NHEJ) approach is used with optimized sgRNAs co-injected with Cas9 mRNA. This results in mice with frame-shifting insertion/deletion (indel) mutations, leading to gene disruption.

The Indel-based knockout model is a common strategy employed in CRISPR-Cas9 gene editing. It involves introducing small insertions or deletions (Indels) at specific genomic loci. This type of genetic alteration often leads to the disruption of gene function, effectively "knocking out" the gene. On the other hand, the HDR-based knock-in model involves using a donor oligonucleotide or plasmid carrying the desired mutation flanked by sequences homologous to the target site. This approach allows for precise genome modifications, such as incorporating point mutations, inserting tags or transgenes, or replacing the mouse sequence with its human ortholog.

In summary, CRISPR-Cas9 technology has revolutionized the field of genetic research in animal models, enabling scientists to create precise genetic modifications and explore the functions of specific genes. These advances have significantly contributed to our understanding of gene function

and disease mechanisms and hold promise for the development of novel therapies for various genetic and neurological disorders.

5. Conclusion

In summary, this review delved into the intricate world of epilepsy, uncovering the disruptions in neural activity and seizures that define the condition. Through the lens of CRISPR-Cas9, a transformative gene-editing tool, we navigated the molecular intricacies of epilepsy's origins and mechanisms. The precision of CRISPR-Cas9's DNA editing, orchestrated by Cas9 endonuclease and guide RNA, allowed us to decipher the genetic underpinnings, shedding light on the balance between excitatory and inhibitory conductance. yet, CRISPR-Cas9's significance extends beyond epilepsy, propelling us into a genomic era with far-reaching implications. Beyond the realm of this disorder, CRISPR-Cas9 serves as a beacon illuminating the path to precision medicine, promising targeted interventions for an array of genetic maladies. Our microcosmic exploration of epilepsy's genetic tapestry is a steppingstone to a macrocosm of genetic revolution. at the crossroads of scientific advancement and ethical contemplation, CRISPR-Cas9 beckons us to a wider dialogue. The implications of harnessing this technology span beyond the laboratory, prompting us to navigate the complex terrain of ethical considerations and societal ramifications. Balancing innovation with responsibility becomes paramount, ensuring CRISPR-Cas9's enduring contribution to human welfare.

The intersection of CRISPR-Cas9 and epilepsy research propels us toward personalized therapeutic possibilities, reshaping the contours of neurological care. As the canvas expands beyond epilepsy, CRISPR-Cas9 paints a larger picture of potential, heralding a future where the language of genetics is translated into precision healing.

Author Contributions

All the authors contributed equally and their names were listed in alphabetical order.

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