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Editing Mutations of GABRB3 Using CRISPR-Cas9 as a Potential Treatment for Epilepsy and Autism Spectrum Disorders

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Abstract. The treatment of a variety of recurrent or resistant hematologic malignancies with chimeric antigen receptor T cell (CAR- T) cell therapy has seen significant success in recent years. The current CAR- T cell therapy approach is not without flaws, nevertheless, and there are still several issues with clinical treatment, including antigen escape, significant toxicity, and susceptibility to drug-resistant recurrence. This paper introduces the structural development and characteristics of CAR-T cells, reviews the limitations of CAR- T cell therapy, including antigen escape, toxicity, CAR-T cell depletion and drug-resistant relapse after treatment, and summarizes the related improvement and optimization strategies. The paper concludes that CAR-T cell immunotherapy has brought new hope to patients with hematologic malignancies, making a cure for refractory and recurrent hematologic malignancies possible. Although CAR-T cell therapy still has many challenges at present, such as immunogenicity, drug resistance, and toxicity.

Keywords: Chimeric antigen receptor, T cell, Structure, Toxicity, Drug resistance, T cell exhaustion

1. Introduction

In 2015, about 3.4 million people in the US have active epilepsy, who have reported being diagnosed with epilepsy, which is approximately 1.2% of the total population [1]. Temporary confusion, staring spells, rigid muscles, jerking movements of the arms and legs that cannot be controlled, loss of consciousness or awareness, and psychological symptoms such as fear, anxiety, or déjà vu are some of the signs and symptoms of epilepsy. Epilepsy can be classified as focal seizures and generalized seizures. As for focal seizures, abnormal activities can be found in only one area of the brain. Compared to focal seizures, generalized seizures are associated with all areas of the brain. Epilepsy has lots of risk factors, such as birth defects, low birth weight, seizures during the first month after birth, poor brain development, brain hemorrhage, severe brain damage, low brain oxygen levels, brain tumors, and infections in meningitis or encephalitis for example.

Autism spectrum disorders are composed of autism, Asperger's syndrome, pervasive developmental disorders, and other disorders. Three main symptoms of ASD include delayed or impaired speech, difficulties interacting with others, repetitive activities, and narrow interests. The Autism and Developmental Disabilities Monitoring (ADDM) Network of CDC estimated that 1 in 44 kids have an autism spectrum disorder diagnosis [2]. Siblings with ASD, specific genetic or chromosomal abnormalities,

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such as fragile X syndrome, problems after birth, elderly parents, and so on are risk factors for ASD. ASD has a high co-morbidity with epilepsy. And children and youth having epilepsy also have higher risks of developing ASD. For example, some people with ASD, who show reduced expression of GABRB3 and UBE3A, also have epilepsy. Another piece of evidence shows that Children with epilepsy are at a pooled risk of 6.3% for ASD; in samples with the greatest rates of comorbid intellectual impairments, the risk is nearly five times higher [3].

GABRB3 in chromosome 15 encodes the β3 subunit in the GABAA receptor, which play a primary role in the GABAA receptor assembly, trafficking, and brain development [4]. GABAA receptors, as ligand-gated chloride channels, play a part in the fast inhibition through rapid hyperpolarization in the postsynaptic dendrites [5]. GABAA receptors include five subunits, which are two α, two β, and a γ or δ subunit. After GABAA receptors on the postsynaptic dendrites are activated, chloride flows into the membrane, resulting in inhibition of action potential.

Several studies show the linkage between GABRB3 and epilepsy. GABRB3 mutation can weaken chloride currents and damage the channel involving the subunit hyperglycosylation. If the chloride is blocked, the action potential can be generated in the postsynaptic dendrite, which may lead to an imbalance between excitatory and inhibitory activities [6]. It is possible that this change in balance can lead to recurrent seizures, which correspond to the symptoms of epilepsy. Several recent studies found evidence supporting that GABRB3 can contribute to some of the phenotypes of epilepsy. A recent study found abnormal EEG during seizures in both homozygous and heterozygous mice with GABRB3 knocked out. Their EEG graphs have bursts of irregular high-amplitude slow and sharp waves, which coincided with the phenotype, such as stiffness of limbs and staring spells [7]. Some researchers speculate a new GABRB3 mutation mechanism, which is defective receptor localization to synapses, by conducting experiments showing that GABRB3+/− knockout mice have fewer γ2 subunits [8]. Since GABRB3 encodes the β3 subunit in the GABAA receptor, these studies suggest a potential pathway for epilepsy.

Some research shows the correlation between GABRB3 and ASD. For example, a study found a potential pathophysiological mechanism for ASD. Specifically, one of the factors contributing to the mechanism is the problem in phosphorylation of GABAA receptors composing β3 subunits. The researcher uses the mice whose serine residues 408 and 409 (S408/9) in the GABAA receptor β3 subunits are mutated to alanines (S408/9A) because the phosphorylation of S408/9 can make the receptor function in a more stable way, which can be simulated by mutating to S408/9A [9]. A study finds that the mutated mice's dendritic spine structures become abnormal; their behaviors show repetitive patterns; their social interactions are reduced; they exhibit epileptic phenotypes [9]. This study suggests a possible mechanism for ASD in humans.

There are limitations in current treatment for epilepsy and ASD, so a new method that can fix some issues is needed. One of the major problems people have epilepsy or ASD or both are facing is that there is no cure for either disease. Also, there are lots of side effects of some surgeries and medications. And if patients take medications, they may adapt to them. Additionally, even though some research shows the effectiveness of some treatments in a short term, there are few studies conducting research for long periods to demonstrate the effectiveness. As for other behavioral treatments, since some need to be individually customized, the doctors cannot promise whether the treatments the patients received are effective or not. Thus, these patients need a solution to solve some of these problems.

Researchers can edit specific regions of the genome using the novel technique called CRISPR-Cas9 by deleting, inserting, or changing certain genome sequences. It can potentially treat many diseases because it is currently the most straightforward, adaptable, and precise approach to gene editing. Additionally, the capability of genome editing to focus on particular cell types provides some extent of precision not previously existed with other therapies. Researchers may now target polygenic disorders like cancer and diabetes in addition to monogenic diseases because of recent developments in genome editing technology. With these capabilities, researchers can create potentially curative treatments that conventional techniques would not be able to produce.

DOI: 10.54254/2753-8818/3/20220273

2. Treatment for epilepsy and ASD

2.1 Epilepsy

2.1.1 Anti-epileptic drugs (AEDs). AED is the most widely used method of treating epilepsy. AEDs can aid about seven out of ten individuals who have seizures. AEDs function by altering the chemical composition of the brain. They can prevent seizures from occurring but cannot treat epilepsy. Sodium valproate, carbamazepine, lamotrigine, levetiracetam, and topiramate are examples of common AEDs. When patients began to use AEDs, side effects can be frequent. Some people's side effects might not show up for a few weeks, while some may arise shortly after therapy begins and disappear in a few days or weeks. Depending on the medication that the patients are taking, there may be different side effects. drowsiness, fatigue, agitation, headaches, tremors that are difficult to control, hair loss or unusual hair growth, swollen gums, and rashes are typical adverse effects of AEDs.

2.1.2 Surgically remove a small portion of the brain that is related to the onset of seizures. If AEDs are not managing seizures or if tests reveal that seizures are brought on by a condition in a tiny region of the brain that can be removed safely, surgically removing a part of the brain may be a possibility. In such circumstances, there is a strong possibility that the seizures will end entirely following surgery. The damaged portion of the brain is removed by the surgeon after making a tiny cut in the scalp and opening up the skull. After surgery, it may take patients a few weeks or months to feel normal again. Patients may need to continue taking AEDs for one to two years if the seizures don't end right immediately. Surgery carries a risk of consequences, including issues with memory, mood, or vision. These issues could get better with time or they might remain in this state forever

2.1.3 A procedure to put a small electrical device inside the body. Two kinds of stimulations, which are deep brain stimulation (DBS) and vagus nerve stimulation (VNS), are alternative techniques that may be helpful if AEDs are not managing seizures and brain surgery is not an option for people having epilepsy. Under the skin of the chest, a tiny electrical device resembling a pacemaker is positioned during VNS. The instrument is fastened to a wire that enters the skin and links to the vagus nerve, a nerve in the neck. Electricity is transmitted in short bursts along the wire to the nerve. It is believed that altering the electrical impulses in the brain can aid with seizure suppression. Although VNS often does not entirely prevent seizures, it can help make them less severe and less frequent. Most likely, patients still need to use AEDs. When the VNS is turned on, side effects might include a hoarse voice, a painful throat, and a cough. This typically lasts 30 seconds and occurs every 5 minutes. The VNS device's battery may normally be used for up to 10 years before needing to be replaced.

Deep brain stimulation (DBS) has similarities with VNS. However, the device implanted in the chest is wired to the brain directly. By altering the electrical impulses in the brain, electrical bursts transmitted through these lines can aid in the prevention of seizures. It is unclear how successful DBS is for treating epilepsy because it is a relatively new treatment and is not used frequently. Additionally, it has some severe side effects, including the possibility of brain bleeding, depression, and memory issues.

2.1.4 Ketogenic diet. This is a kind of diet that require patients to take a high amount of fat and a low extent of carbohydrates and proteins. The diet is supposed to decrease the chance of having seizures in children by altering the brain's chemical composition. Before AEDs were widely used, the ketogenic diet was one of the primary methods for treating epilepsy. However, due to the association between a high-fat diet and major medical disorders including diabetes and cardiovascular disease, it is no longer often utilized in adults. When AEDs are unable to manage a child's seizures, a ketogenic diet can be considered as a treatment because there is evidence revealing that it helps some children have fewer seizures.

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2.2 ASD

2.2.1 Behavioral . There is no cure for ASD and no treatment that works for everyone. And researchers have not found the causes of ASD. The purpose of treatment is to reduce symptoms, enhance children's functioning, and foster growth and learning. The social, linguistic, and behavioral challenges linked to ASD are addressed in a few ways. There are programs concentrating on reducing problematic behaviors and delivering new knowledge. Other initiatives concentrate on instructing children on how to behave in society or enhance their ability to interact with others. Applied Behavior Analysis (ABA) is a popular behavioral therapy for people with ASD. In order to enhance a number of abilities, ABA promotes desired behaviors while discouraging undesirable behaviors. Children's progress will be monitored and quantified. Discrete Trial Training (DTT) and Pivotal Response Training are two ABA teaching approaches (PRT). DTT teaches a desired behavior or reaction through detailed instructions. Lessons are simplified, and desired responses and actions are rewarded. Unwanted responses and actions are disregarded. Instead of taking place in a clinic, PRT occurs outdoors. PRT aims to enhance a few "crucial abilities" that will aid in the children's acquisition of numerous other skills. The ability to start a conversation with someone is an illustration of a crucial ability.

2.2.2 Developmental. Developmental methods concentrate on enhancing a narrow range of interrelated developmental abilities, such as language or motor capabilities, or a variety of developmental skills. Behavioral and developmental techniques are frequently integrated. For people who have ASD, speech and language treatment is chosen the most often among all developmental therapies. speech and language are both improved through speech and language therapy. Some people having ASD use verbal communication. Others may use signs, gestures, images, or an electronic device to communicate.

The goal of occupational therapy is to help the patient live as independently as possible. Dressing, eating, and engaging with other people are examples of skills, which are intended to improve through this therapy. Physical therapy and sensory integration treatment are both included in occupational therapy. The use of sensory integration treatment can enhance how the body reacts to overwhelming or constricting sensory input [10]. Physical abilities, such as little finger motions or bigger movements of the trunk and torso, can be improved through physical therapy.

2.2.3 Educational. Educational programs with a strict framework frequently work effectively for kids with ASD. These programs are often composed of a group of experts and various activities to enhance social, communicative, and behavioral skills. Children in preschool can make good progress if they can get focused, customized behavioral treatments.

Treatments for education are often provided in a classroom environment. The Treatment and Education of Autistic and Related Communication-Handicapped Children (TEACCH) approach is one of the educational strategies. The basic notion of TEACCH is that persons with ASD can benefit from consistency and visual learning [11]. It gives teachers options to change the arrangement of a classroom so that they can enhance children's outcomes in academic and other aspects. For instance, daily schedules can be written down and displayed in an obvious position.

2.2.4 Pharmacological. Specific drugs can help regulate symptoms but cannot alter the fundamental symptoms of ASD. For instance, children can take some medication if they are hyperactive. And anxiety may be treated with antidepressants. Co-occurring symptoms are treated by several drugs, which can improve ASD patients' mental functioning. For instance, these medications may be used to regulate excessive energy, difficulty concentrating, or self-harming tendencies. Children who suffer from ASD may also experience health difficulties including seizures, sleep abnormalities, restricted dietary preference, or digestive concerns [12].

2.2.5 Psychological. For teens and adults, ASD is frequently accompanied by other mental health conditions including anxiety and sadness. One psychological strategy that emphasizes understanding

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the relationships between ideas, feelings, and behaviors is called cognitive-behavior therapy (CBT). In CBT, the patient and therapist jointly decide on objectives before the patient changes how they think about a situation in order to alter how they react to it.

3. Potential treatment --- crispr-cas9

3.1. Side effects or disadvantages of conventional treatments

As mentioned before, conventional treatments for epilepsy can have side effects. Specifically, many treatments can control epilepsy to some extent, but cannot stop it permanently. For example, the anti-convulsant medication used as a preventative measure after a head injury cannot stop long-term post-traumatic epilepsy even though it can lower the likelihood of early posttraumatic seizures within the first few weeks following the injury [13]. Moreover, current AEDs have a major drawback known as seizure exacerbation. For instance, patients with idiopathic generalized epilepsies (IGEs) are especially vulnerable to seizure aggravation. Another wildly existing issue is that patients can get adapted to the AEDs they are taking. It has been demonstrated experimentally that all AEDs that lose efficacy over time due to extended administration cause pharmacodynamic tolerance in individuals with epilepsy. This "adaptation" of AED targets, for example via loss of receptor sensitivity, is the cause of this phenomenon. Functional tolerance can result in cross-tolerance to different AEDs or even a complete lack of AED activity. According to credible experimental data, almost all first- to third-generation AEDs can become less effective over time, but to varying degrees [13].

As for ASD, many of the studies taken into consideration had rather brief treatment durations [14]. Given that ASD is a chronic disorder and longer periods of therapy are to be expected, a prolonged follow-up is essential, especially for examining safety outcomes. In several research, follow-up was conducted throughout the open-label treatment's "maintenance" periods [15, 16]. These methods increase clinical relevance but lack the controlled environments needed for a more thorough assessment of effectiveness [17]. Furthermore, although it has been demonstrated that adapting CBT to use with children who have ASD can be successful, there are some restrictions on these modifications. To be specific, the generalization of the modifications across research can be impacted by the child's level of functioning, the varying application of each adjustment, and the use of various CBT programs [18]. Thus, it can be difficult to find a generalized treatment for ASD. And the modifications for each person are hard to be precisely determined.

3.2. Current development stage of CRISPR-Cas9

Genome editing can make modifications to an organism's DNA. At specific sites in the genome, these technologies enable the addition, removal, or modification of genetic material. There are several methods for genome editing that have been developed. CRISPR-Cas9, which stands for clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9, is a well-known example. Because CRISPR-Cas9 is faster, less expensive, more accurate, and more effective than other existing genome editing techniques, the technology has received a substantial amount of attention among scientists.

Two crucial components, which are RNA guide RNA and the enzyme Cas9 (gRNA), make up the CRISPR-Cas9 system. In order to add or remove DNA fragments, Cas9 functions as a pair of "molecular scissors" that can cut the two DNA strands at a precise position in the genome. A brief segment of a pre-designed RNA sequence (approximately 20 bases long) enclosed in a larger RNA scaffold makes up gRNA. The pre-designed sequence "guides" Cas9 to the appropriate region of the genome while the scaffold component binds to DNA. This guarantees that the Cas9 enzyme produces a cut at the proper location in the DNA.

The guide RNA is intended to locate and attach to a particular DNA sequence. The complementary RNA bases in the guide RNA match those in the genome's target DNA sequence. As a result, the guide RNA should, in principle, only attach to the target sequence and not to any other parts of the genome. The Cas9 cut both strands of DNA following the guide RNA to the same spot in the DNA sequence. At

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this point, the cell knows that the DNA has to be repaired and makes an attempt. It is possible to change one or more genes of interest using the DNA repair technique (see Figure 1) [19].

Nowadays, CRISPR-Cas9 is applied in research labs to study disorders in cells and animal models. Researchers now are figuring out whether this method is secure and efficient for usage in people. Various kinds of disorders, including monogenetic diseases like cystic fibrosis, hemophilia, and sickle cell disease, are being investigated in research and clinical studies. Additionally, it is promising for treating and preventing more complicated illnesses, such as cancer, heart diseases, mental disorders, and HIV.

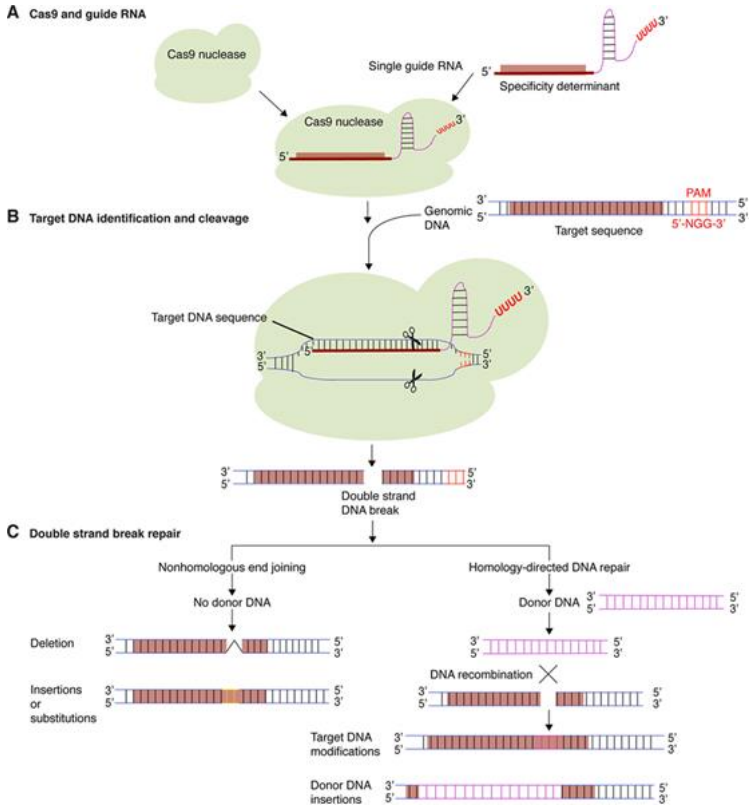

FIGURE 1. The mechanism of CRISPR-Cas9 editing targeted sequence. From Principles, Applications, and Biosafety of Plant Genome Editing Using CRISPR-Cas9, by K. El-Mounadi, M. L. Morales-Floriano, & H. Garcia-Ruiz, 2020, Front. Plant Sci. Copyright 2020 by Kaoutar El-Mounadi, Maria Luisa Morales-Floriano, & Hernan Garcia-Ruiz

CRISPR-Cas9 has several benefits when treating diseases. CRISPR-Cas9 offers a low-cost, highly effective, and user-friendly gene-editing system compared to alternative gene-editing methods and some other treatments. Also, CRISPR-Cas9 does not need complicated protein design and engineering. Another advantage of the CRISPR-Cas9 technology to simultaneously disrupt many genes, through the use of several gRNAs targeting various genes [20], opens up new opportunities in the investigation of complicated polygenic diseases.

The xCas9 form, compared to SpCas9, has a higher target efficiency, higher specificity, lower off-target rates, and more PAM compatibility. SpCas9 can be less effective than recent CRISPR-Cas variants because it requires a 5-NGG-3 PAM right next to a target 20-nucleotide long DNA sequence where it only recognizes NGG, which is the PAM site [21]. Additionally, scientists are making progress in improving some disadvantages of CRISPR-Cas9. Specifically, even though CRISPR-Cas9 may change the genome by generating various mutations of random off-target [22], by recognizing various PAMs, novel CRISPR-Cas variants have enhanced the editing effectiveness of targeted bases in the genome of interest [23]. Additionally, CRISPR-Cas9 can induce mutations at non-specific loci which have similarities to homology to the target sites [21].

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However, CRISPR-Cas9 has some disadvantages which need to be improved. Specifically, the off-target effects need to be detected and prevented. Creating a CRISPR system that is well-engineered can greatly decrease the off-target impacts [24]. For example, by strengthening the cleavage specificity of the nucleases or decreasing the duration of the functional activity, off-target effects can be controlled. Scientists are developing new Cas proteins to enhance the on-target rates. For example, the Cas proteins eSpCas9, HF-Cas9, HypaCas9, and Sniper Cas9 have been modified and displayed improvements in on-target specificity [25, 26, 27]. Another method involves the use of Cas9 nickases. This functions to inactivate an endonuclease domain so that the off-target effects can be evaluated [28, 29]. Furthermore, by reducing the time that Cas9 is active, off-target effects brought on by CRISPR can be lessened. For instance, compared to other vector systems like lentiviral or plasmid cargo delivery ways, the Cas9 system supplied through electroporation has a lower half-life.

4. Conclusion

ASD and epilepsy are often co-occurring. As a result, these two disorders are frequently studied together. However, there are certain drawbacks to the ASD and epilepsy therapies that are currently on the market. And neither condition has a treatment. There are lots of side effects from mile to sever extent after taking medications. Also, patients can have adaptations to the medications. while some studies have demonstrated the usefulness of some treatments for a short period of time, there are few studies show whether they can be effective for a long duration. Numerous studies show a connection between GABRB3 and both epilepsy and ASD, pointing to potential paths for the pathophysiological mechanisms underlying the two disorders. This study reviews the benefits and drawbacks of CRISPR-Cas9 to examine its possibility as a treatment for both epilepsy and ASD. In the future, CRISPR-Cas9 may be used as a therapy to address some issues in traditional treatments.

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