

Application of CRISPR/Cas9 in the Disease Treatment

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Abstract. CRISPR/cas9 was widely used in medical study because it is fast, cheap, accurate, and efficient in knockout, knock-in, activation, interference and other editing functions. It has gained attention for precise and effect DNA pieces targeting and editing in different kind model systems, including rat, rabbit, and human cells. The paper will going to investigating how Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is used to solve human disease. Most of the DNA mutation linked to a hereditary illness can be edited using CRISPR and CRISPR-associated protein 9 technology. The following articles will not only talk about disease associated with gene mutations, but also solve disease by changing DNA sequences and removing allergy factor form food. To explain several of the above approaches, a few examples have been selected for this article, including Alzheimer's disease, Hemophilia, Tyrosine, Beta thalassemia, Parkinson's disease, Hepatocellular carcinoma, and Beta Lactoglobulin.

Keywords: CRISPR/cas9; blood disease, neurodegenerative disorder, cancer.

1. Introduction

The target-specific modification of an organism's genome using gene editing techniques is known as gene editing. Such modifications can alter their genetic information and expressive characteristics through precise and efficient gene insertions, deletions, or replacements. The changes can lead to some changes in physical and others, like hair color, and disease risk in different organisms. Researchers have discovered that mutations in a single gene can cause more than 6,500 human genetic diseases, and there are as many as 300 million people around the world who suffer from the disease throughout their lives or even die early. The last two decades have seen a growing trend of use CRISPR/cas9 in different aspect including biomedical, drugs and biomaterials. Gene editing technology is one of the weapon scientists are looking for to solve this pain. This technology can effectively modify genes without notice, correcting disease-causing mutated genes into the appropriate genes to reach the goal of eliminating human genetic diseases.

The main aim of this essay is to explore CRISPR/cas9 applications in different type of disease. The dissertation follows a few case-study designs, with in-depth analysis of how CRISPR/cas9 function in different aspect (Alzheimer's disease, Hemophilia, Tyrosine, Beta thalassemia, Parkinson's disease, Hepatocellular carcinoma, and Beta Lactoglobulin) and recent directions of progress. This project provided an important opportunity to advance the understanding of future applications of CRISPR/cas9. The first part of the paper begins by the basic principles of CRISPR/cas9 with horizontal analysis of applications value. It will then go on to CRISPR/cas9 applications in different aspects. The third part is challenges in cas9 applications and follow with outlook of CRISPR/cas9, which result in improving the efficiency of the reader, as well as quickly grasp the latest research progress.

2. CRISPR-Cas9

CRISPR means Clustered Regularly Interspaced Short Palindromic Repeats that was found in *Escherichia coli*. CRISPR-Cas9 has been using a lot as an genome editing technology in biomedical research for human and animal diseases. Genetic material to be altered, added, or removed at different locations in the genome by using this technology. The first clinical application occurred in 2016. At a hospital in China, a Chinese group used CRISPR/Cas9 to edit cells as a treatment for aggressive lung cancer. CRISPR-associated protein 9 (cas9) and single guide RNA (sgRNA). Small RNA

sequence known as "guide" RNA (gRNA) are involved in the process and have a "guide" sequence that can attach to specific DNA sequences. The function of a sgRNA is used to recognize DNA pieces that is complementary to its RNA and bind with it. If the bonding occurs, DNA will be cleaved at a precise location. The protospacer adjacent motif (PAM) which is a certain DNA sequence that is between two and five nucleotides long, must be located at 3' end of gRNA in order for it to cut. When repairing these brakes, cells can cause genes to undergo loss of function or mutation through different repair mechanisms. DNA has two ways to repair: homology directed repair (HDR), and non-homologous end joining (NHEJ).

Cas9 has a wide application because of its excellent gene editing efficiency. Compared with traditional gene editing technologies, Cas9 has higher editing precision and shorter editing time, which makes it widely applicable in a variety of fields such as gene therapy, agricultural breeding, and bioengineering. Furthermore, the editing process of Cas9 is can be highly controlled, this means that with the CRISPR/Cas9 system, it is possible to precisely alter any DNA sequence that matches the gRNA. This enables scientists to precisely edit the genome of an organism as needed to achieve specific therapeutic effects or breeding goals.

CRISPR-Cas9 technology has obvious merits by comparison of other gene editing technologies. For example, compared with traditional gene editing technologies like: ZFNs and TALENs. It has higher efficiency and a wider range of applications. At the same time, CRISPR-Cas9 technology requires lower costs and is easier to popularize [1].

3. Application of CRISPR/cas9

3.1. Blood Disease

3.1.1 Hemophilia

An inherited bleeding disorder of coagulation dysfunction called Hemophilia, can cause the body's inability to clot and form blood clots to stop bleeding. It is characterized by impaired production of blood enzymes and a long clotting time, causing patients to bleed for a longer period after injury. Common manifestations are redness and pain, limited joint mobility, and muscle atrophy. A mutation or alteration in one of the genes that codes for the manufacture of the clotting factor proteins—which are necessary for the formation of blood clots—is typically the cause of hemophilia. The clotting proteins may stop functioning altogether or function improperly if this instruction is altered. Hemophilia has two different types; one is hemophilia A and the other one is hemophilia B. The cause of Hemophilia A is a deficiency or decrease in coagulation factor VIII, and the cause of hemophilia B is by a deficiency or decrease in coagulation factor IX.

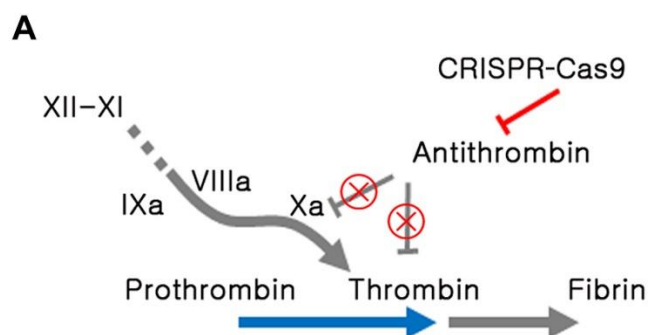


Fig. 1 How Cas9 increases thrombin production (unoriginal, cite from [2]).

As shown in the figure 1 the Antithrombin (AT) can be a sustainable treatment of hemophilia A and B patients by genome editing because it is thrombin generation's endogenous negative regulator [4]. To explain that Cas9 mRNA and a sgRNA were delivered to the mouse hepatic AT by lipid nanoparticles (LNPs). Delivery of CRISPR-Cas9 by LNP resulting in increased thrombin production by inhibiting AT which been proved as an effective and safe method for treating hemophilia [2].

3.1.2 Beta(β)-thalassemia

Each year approximately 60,000 symptomatic individuals are born to the global population, of which about 1.5% are beta thalassemia carriers. The probability of developing the disease differs from region to region. It is more prevalent in the Mediterranean countries. In the contract, the overall annual incidence of symptomatic individuals is 1 in 10,000 in the European Union. And the probability is 1 in 100,000 worldwide. The Maldives has the highest prevalence of thalassemia in the world at 18%. It is noteworthy that both males and females are affected by β -thalassemia [3]. Beta thalassemia is an inherited hemolytic anemia that is the result of a mutation or absence of a gene for the hemoglobin peptide chain, which results in reduced synthesis of the hemoglobin peptide chain or a complete absence of the hemoglobin peptide chain, which reduces the production of hemoglobin. For patients with beta thalassemia, low hemoglobin levels result in insufficient oxygen transport by red blood cells. Patients are associated with weakness, pallor, weakness, and more serious complications.

Beta thalassemia results from variations in the HBB gene, commonly referred to as mutations. The HBB gene codes to produce the beta-globin protein. Certain HBB gene variations inhibit the synthesis of any beta-globin at all. One therapeutic approach to alleviate β -hemoglobinopathies caused by mutations in the HBB gene is the use of gene editing techniques to damage the GATA1 binding site on the +58 BCL11A erythrocyte enhancer, thereby inducing γ -globin expression [4].

3.1.3 Tyrosinemia

Tyrosine is a component of most proteins, and tyrosinemia is an uncommon autosomal recessive hereditary metabolic condition caused by defective tyrosine breakdown. Tyrosine and its byproducts build up in tissues and organs as a result, endangering several organs including the kidneys, liver, brain, and bones.

There are three kinds of tyrosinemia which can be distinguished based on the malfunctioning enzyme. When mutations in the FAH gene cause deficiencies in fenugreek acetoacetate hydrolase, the final enzyme in the tyrosine metabolism pathway, and buildup of tyrosine and its metabolites, including succinyl acetone, 4-hydroxyphenyllactic acid, and 4-hydroxyphenylpyruvic acid. These events cause tyrosinemia type I [5]. About 1 in 100,000 people have type I tyrosinemia. Tyrosine catabolism disorder brought on by tyrosine aminotransferase insufficiency is the etiology of tyrosinemia type II. Less than 1 in 250,000 people have Tyrosinemia II. Tyrosinemia type III is caused by 4-hydroxyphenylpyruvate dioxygenase deficiency. Only a few cases appear in the world. Mutation in FAH, TAT, and HPD genes are related with this disease.

In HT1 rabbits, the precise FAH gene repair mediated by CRISPR-Cas9 can recover the lethal phenotype and restore liver function and kidney function [6]. Research show HT1 rabbits treated with gene therapy had normal levels of all six parameters (creatinine, aminotransferase, total bilirubin, blood urea nitrogen, aspartate aminotransferase and direct bilirubin) as same age wild-type rabbits, whereas HT1 rabbits not treated of AAV had shown a trend of higher levels of renal and liver damage indices.

3.2. Neurodegenerative Disorder

3.2.1 Alzheimer's Disease

Alzheimer's disease (AD) is a progressive, evolving neurodegenerative disorder with an insidious onset. The most common type of dementia is AD in old age, and patients suffer from impaired thinking, memory and independence as a result, affecting quality of life as well as death. This disease is a heterogeneous group of diseases, while its formation may be due to a composition of factors. According to the research, there is no precise cause, regardless of factors like age, head trauma, thyroid illness, high or low mother reproductive age, or viral infections. Early-onset and late-onset is the represent two types of AD. Less than 10% of cases are early-onset Alzheimer's disease (EOAD), which is highly less common than the late-onset Alzheimer's disease (LOAD) variant. Alzheimer's disease patients typically only can live for approximately 8 to 10 years after symptoms first appear, while the illness can last from 1 to 25 years. While 95% of LOAD patients are approximately equal

or higher than seventy years of age, the typical age at which EOAD symptoms first appear is approximately ~46. The average death age in LOAD is usually 82 or older, while the average death age in EOAD is about 52.

Given the fact that it is still unknown what processes underlie the pathophysiology of AD. However, it is generally acknowledged that the pathophysiology of and treatment may benefit from A β [7].

There is an extremely rare type of Alzheimer's disease, which are brought on by dominantly inherited pathogenic mutations in the Presenilin 1 (PSEN1), Presenilin 2 (PSEN2) genes, and amyloid precursor protein (APP).

The reason of that is thioredoxin-interacting protein (Txnip) effectively attenuates A β -induced oxidative modification of protein cysteines, down-regulating Txnip levels in HT22 cell via the CRISPR/Cas9 system allowed Txnip have potential to be identified as a therapeutic target that can used in AD treatment [1]. Meanwhile, When the PSEN1 genes are knocked out in mouse neuronal N2a cells using the CRISPR/Cas9 method, the endogenous γ -secretase 's background is eliminated. It is also discovered that the generation of A β 42 and A β 40 is decreased when recombinant protein produced from PSEN1 mutations is added exogenously [1].

CRISPR/Cas9 technology could model AD-associated mutations in human neural cells. Mutations that cause AD: APP and PSEN1, were introduced into human induced pluripotent stem cells (iPSCs). consequently, higher levels of A β could be detected in neurons transformed with mutant human iPSCs (pure, heterozygous) compared to homozygous controls [1].

3.2.2 Parkinson's Disease

It (also know as "tremor palsy") is a neurological disorder that primarily affects the elderly, typically beyond the age of 65. It is defined by motor symptoms such as bradykinesia, myotonia, resting tremor, and postural balance abnormalities. The primary disease-causing change in Parkinson's disease is due to denaturing and death of dopamine (DA) neurons which located in substantia nigra of the midbrain, leading to a marked reduction in the amount of DA in the striatum and ultimately the disease. However, age, oxidative stress, genetics, and the environment are other variables that can contribute to PD [8].

A model of PD diseased cells generated by CRISPR Cas9 technology revealed that PD diseased cells exhibited higher levels of p62 in DA neurons than wild-type homozygous control cells, demonstrating that adapter protein p62's S-nitrosylation promotes α -synuclein concentration and disease spread [8].

Using the model, it was also found that midnolin (MIDN), a regulator of PARKIN expression, has association of PD pathology, and when MIDN is knocked down, a loss of Parkin expression is observe [8]. Since 10.5% of patients with sporadic PD lack the MIDN gene, drug molecules targeting an increase in the MIDN gene may be considered to reduce Parkinson's disease symptoms.

3.3. Cancer (hepatocellular carcinoma(HCC))

Around 85-90% of primary liver cancer is hepatocellular carcinoma (HCC), with a five-year survival rate of 19.6% on average for patients in the US. There are about 6 new cases of hepatocellular carcinoma per 100,000 people per year (U.S). If diagnosed at an early stage, it can be treated with localized therapy. However, in advanced stages, only tyrosine kinase inhibitors (TKIs) can be used, which have a 30 percent success rate and develop resistance within six months.

By building paired-guide RNA (pgRNA) CRISPR libraries, high-throughput screening of long noncoding RNAs in human hepatocellular carcinoma cells was accomplished. Which created a library with a target of 671 lncRNAs that had 12,472 pgRNA pairs. Through screening, 51 long non-coding RNAs that can either positively or negatively influence the proliferation of cancer cells were found, and nine of these had their biological roles confirmed [9].

Construction of a knockout model of AT-rich interaction domain 2 (ARID2) in SK-Hep1 hepatocellular carcinoma cell line by CRISPR/Cas9 system, show ARID2 is involved in

hepatocellular carcinoma as an oncogene because hepatocellular carcinoma cells proliferated and moved more freely after ARID2 was knocked down [10].

3.4. Others(protein β -lactoglobulin (BLG))

Beta Lactoglobulin (BLG) in milk can cause an allergic reaction, milk intolerance, so people with allergies should not consume dairy products. But the primary whey protein which found in the most mammals' milk is BLG; however, camel, rabbit, rodent, and human milk do not contain BLG. In bovine milk, BLG represents ~50% of the whey proteins, ~12% of the total proteins. By using CRISPR/Cas9, zygote microinjection, ZFNs gene editing, and SCNT, the BLG protein can be eliminated from milk. Cas9 mRNA and small guide RNA (sgRNA) were co-injected into goat single-cell stage embryos to produce BLG knockout (BLG KO) goats. Furthermore, it is no longer present in BLG KO goat milk. The relative expression of BLG in the mammary gland of BLG knockout goats was statistically significant reduced (p value < 0.01)(fig.2 and fig.3) [11]. The three target sites (sg1, sg2, and sg3) had single sgRNA targeting efficiencies of 8.00%, 8.33%, and 9.09% [11].

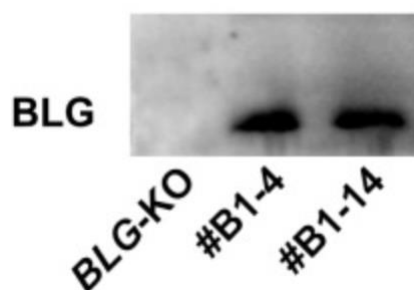


Fig. 2 Compare the expression of BLG in the mammary glands of wild-type and BLG-KO goats.
(unoriginal, cite from [11])

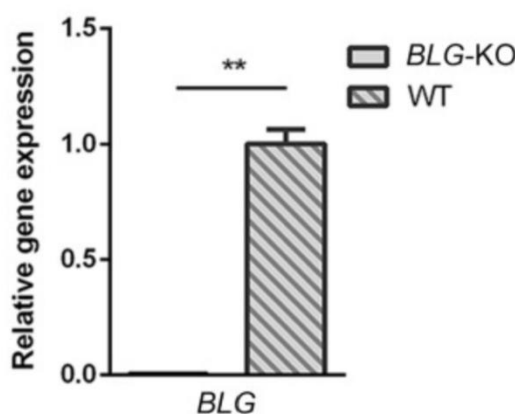


Fig. 3 Compare the BLG protein's presence in whey using a western blot
(unoriginal, cite from [11]).

4. Challenges in Cas9 Applications

Although CRISPR-Cas9 has a wide range of application prospects in gene editing, there are some technical difficulties. For example, there may be problems such as off-target phenomenon and difficult penetration of the technology in actual operation, which requires scientists to further study and improve the technical details. Secondly, many cas9 experiments use mice or models, and there are no real clinical trials to test whether this method is effective or feasible.

In response to these technical difficulties, some strategies can be adopted to deal with them. For example, the occurrence of off-target phenomenon can be reduced by optimizing the experimental conditions and improving the precision of operation; at the same time, tools such as new carriers and

transduction systems can be developed to improve the penetration efficiency and safety of the technology.

5. Cas9 Application Outlook

5.1. Disease Treatment

It has been shown CRISPR-Cas9 gene-editing technology able to fix harmful base mutations or tamper with genes that cause disease, opening new therapeutic options. There are still a lot of potential barriers to targeted delivery of CRISPR-Cas9 technology for the treatment of disease, such as the inability to do efficiently vivo transfer the CRISPR system to diseased cells. At the same time, it is crucial to select proper gene editing techniques and transfer vectors for various diseases. Future study may concentrate on non-viral vectors that possess the ability to recognize targets. The CRISPR system could be used to create precise cellular models, reveal cellular mechanisms, and identify new drug targets, leading to the development of more efficient and safer drugs.

5.2. Livestock

CRISPR-Cas9 has the ability to edit the genes of microorganisms, thereby altering their genetic characteristics. This could help in the development of new drugs and biomaterials, as well as improving the productivity of crops and livestock.

6. Conclusion

The main target of the current study was to relatively systematically and comprehensively understand the past situation, achievements reached, correctly treat advantages and disadvantages of CRISPR-Cas9, think about how to improve and progress CRISPR-Cas9 technology; at the same time, the project can help related workers and those who are interested in CRISPR-Cas9 to have a general framework and understanding of the actual application of cas9, and have a clear goal and direction after reading this article. This research also shows that CRISPR-Cas9 has demonstrated great promise as an up-and-coming gene editing technology in several fields, like

The major limitation of this study is that it only talks around a few diseases in some type, the study may not be comprehensively and systematically. Moreover, it is unfortunate that the study did not include all recent related experiments and the selected experiment or article may not be representative. Future research should consider more about the representative of selected studies and comprehensively of selected topic. Not only does this article has a lot of limitations, but the technique itself has certain flaws and limitations.

In contrast to its great capabilities, this technology still has many uncontrollable factors, including off-targeting. One of the most controversial issues is the ethics of gene editing. Many experiments cannot be tested in clinical trials due to ethical issues, and the effectiveness of the treatments is questionable. However, CRISPR-Cas9 still gives the possibility of treating rare diseases as well as improving human health. In the future, these may be improved or even solved.

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