Proceedings of the 3rd International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/2024.KA19745

# *Application of CRISPR/Cas9 System in Type 2 Diabetes Mellitus*

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***Abstract:*** With the development of the social economy, the quality of people's living standards has improved. The risk of Type 2 diabetes mellitus (T2DM) is greatly increased. CRISPR/Cas9 system can provide more accurate and effective treatment for T2DM. Nowadays, the CRISPR/Cas9 system is still in the scientific research stage in the field of treatment of T2DM, and still has many uncertainties. For example, the off-target problem, the lack of clinical trials and ethical issues. This paper mainly analyzes and summarizes the research of CRISPR/Cas9 system on the treatment of T2DM, so as to provide a more systematic reference for the treatment method for future research. However, this treatment method also has the problem of Off-target. Although CRISPR/Cas9 system has high accuracy, it still has the problem of off-target, which may lead to the accidental injury of normal genes, thus causing unpredictable consequences. Technical problems: Although CRISPR/Cas9 system is relatively simple, it still needs superb technology and complex conditions in actual operation, which increases the treatment cost and time, and the sequelae of later treatment is still unclear. Both need further research by researchers.

***Keywords:*** CRISPR/Cas9 system, Type 2 diabetes mellitus, gene editing.

## 1. Introduction

Diabetes mellitus(DM) is mainly divided into Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM) . T1DM is an autoimmune disease caused by T cell-mediated damage to pancreatic beta cells by the autoimmune system [1]. T2DM is associated with genetic factors, unhealthy lifestyle habits, infections, and imbalanced gut microbiota, leading to insulin resistance and insufficient insulin secretion. T2DM is the most common type of diabetes, accounting for about 90%. The patients are mainly adults, which is a serious public health challenge worldwide [2]. T2DM patients are prone to various chronic complications under long-term hyperglycemia, such as cardiovascular disease, neuropathy, kidney disease, etc. [3-4]. Not only does it harm the patient's health, but it also increases economic expenses.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system, where interval sequences in the CRISPR system are transcribed into CrRNA that guide the system to bind to specific target virus sequences, and cut the target DNA or RNA through an endonuclease encoded by the CRISPR system. The CRISPR/Cas9 system (clustered regularly interspaced short palindromic repeats Cas9) is the first CRISPR gene editing technology to be used. The CRISPR/Cas9 system uses sgRNA to guide Cas9 nuclease to the PAM region of the target DNA sequence to cause double-strand

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breaks, which then triggers the DNA repair mechanism of the cell to achieve precise gene editing. This technology has been widely used in the biomedical field for its high efficiency, accuracy and programmability [5]. Compared to traditional treatments, CRISPR therapy has the potential to provide a radical solution for patients with greater accuracy and efficiency.

## 2. Pathogenesis of T2DM

### 2.1. Genetic factors

Genetic factors play an important role in the pathogenesis of T2DM. The pathogenesis of T2DM is complex and involves the variation of multiple genes, which mainly affect β cell dysfunction, insulin resistance, insulin secretion deficiency and impaired sugar resistance [6]. The genes associated with β cell dysfunction include human insulin (INS) gene, pancreatic insular amyloid polypeptide (Amylin) gene, tumor necrosis factor receptor (TNFR5) gene, liver nuclear factor 4α (HNF4α) gene, GATA binding protein 6 (GATA6) gene, and zinc finger 1 (JAZF1) group Thyroid adenomata-associated (THADA) genes cause β cell defects and T2DM development. Insulin resistance-related gene variants may affect susceptibility to T2DM, and this effect is mainly mediated by paternal alleles [7]. Deficiency of insulin secretion is one of the important links in the development of T2DM. Impairment of islet β cell function leads to the dysfunction of insulin synthesis and secretion, and the increase of blood glucose level, which results in T2DM and the deficiency of insulin secretion is also one of the important links in the occurrence of T2DM. Impaired islet beta cell function leads to impaired insulin synthesis and secretion, elevated blood glucose level, and promotes T2DM formation.

### 2.2. Environmental factors

Environmental factors are also related to the onset of T2DM. The worsening of air and chemical pollution in cities can increase the possibility of changes in endothelial function, inflammation, and insulin resistance. Traffic noise can inhibit insulin secretion and reduce peripheral insulin sensitivity. Nowadays, living areas are highly urbanized, and the heat island effect caused by it can lead to poor glucose metabolism, decreased brown adipose tissue, and increased nighttime light, which may also lead to elevated blood sugar levels. In addition, the rapid development of the economy will lead to people being sedentary for a long time and having less exercise time, which will lead to the metabolic imbalance of the body and further increase the risk of diabetes [8].

At the same time, viral infection is also a key factor. Studies have shown that viral infection can promote insulin resistance in skeletal muscles and even the whole body [9]. Viruses are easily stimulated, and individuals with genetic susceptibility can lead to increased secretion of adrenal cortex hormones and catecholamine hormones. The virus can directly invade pancreatic beta cells or indirectly attack beta cells, leading to a decrease in their quantity or function, thereby causing T2DM.

### 2.3. Dietary factor

Long-term intake of foods high in oil and sugar will lead to the accumulation of fat, increase the body's blood lipids, accelerate the formation of fat and cause the occurrence of T2DM. Excessive consumption of carbonated beverages, uncontrolled eating habits, and fast eating speed, as well as an unreasonable dietary structure, can all lead to excessive intake of carbohydrates, which can then be converted into sugar, causing elevated blood sugar levels and accelerating the formation of T2DM. With the rapid development of the socio economy, insufficient intake of whole grains and excessive consumption of refined carbohydrates and processed foods can lead to T2DM.

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## 3. The working principle of CRISPR/Cas9 system formatting the text

The core principle of the CRISPR-Cas9 system lies in the RNA mediated DNA recognition mechanism. Firstly, the CRISPR system undergoes transcription and subsequent processing steps to generate a key molecule - single directed RNA (sgRNA). SgRNA is formed by fusing a portion of CRISPR RNA (crRNA) with a transcription promoter sequence. Among them, the crRNA segment originates from the matching process between CRISPR sequences and exogenous DNA fragments, while the transcription initiation sequence provides a guiding signal that can be recognized by RNA polymerase. After binding to Cas9 protein, sgRNA forms a complex that can be precisely located at the target DNA sequence. Through the principle of complementary base pairing, sgRNA guides Cas9 protein to specific DNA sites. The cutting function of Cas9 protein is the key to its function. Once the Cas9 protein binds to the target DNA sequence, its intrinsic cleavage activity is activated. Specifically, the nuclease region of Cas9 protein possesses double stranded cleavage enzyme activity, which can cleave the target DNA strand complementary to the sgRNA guiding sequence. This cleavage activity depends on two nuclease active sites: the HNH domain and the RuvC like domain. When the Cas9 protein binds to the target DNA, its nuclease region cleaves the target DNA strand, causing double strand breaks (DSBs). DSB serves as a signal of cellular DNA damage, triggering the initiation of DNA repair mechanisms in cells. Cells mainly perform DNA repair through two methods: non homologous end joining (NHEJ) and homologous directed repair (HDR). Using the CRISPR-Cas9 system, scientists are able to precisely delete specific genes in animal models. This process typically involves designing and synthesizing sgRNAs that match the target gene, followed by the introduction of Cas9 protein. After binding to sgRNA, Cas9 protein is guided to the location of the target gene. Once the Cas9 protein binds to the target gene, it will cause DSBs, thereby activating the cell's DNA repair mechanism. If the NHEJ repair mechanism is used, cells may undergo base insertion or deletion at the target gene, thereby achieving the knockout of the target gene [10].

## 4. Applications

1) β cell regeneration and functional recovery:
The main characteristics and causes of diabetes are the loss or reduction of the function of islet β cells caused by attack, resulting in insufficient insulin secretion. CRISPR/Cas9 system can achieve β cell regeneration or improve insulin secretion in vivo by accurately editing related genes and combining them [11].

The University of Washington research team used CRISPR/Cas9 system combined with stem cell therapy to treat the mutant genes of diabetes and the attacked beta cells. In this study, induced pluripotent stem cells (IPSC) technology was used to transform human stem cells into islet β cells using skin cells from patients with Wolfram syndrome, and CRISPR/cas9 system was used to correct the mutated genes leading to Wolfram syndrome. The experimental results showed that the diabetes of mice receiving cell transplantation disappeared rapidly after implantation of CRISPR edited stem cells, and their blood glucose levels remained in the normal range on average for six months, without obvious side effects or rejection [12]. Gene therapy technology has long-term effectiveness and can maintain long-term stability [11]. However, because this technology is in the experimental stage, and the experiment has more uncertainties and risks, it is necessary to further optimize and improve the technology.

2) Insulin gene editing:

CRISPR/Cas9 system can replace or repair the mutated genes in islet β cells [12], which can improve the function of insulin receptor, improve the sensitivity of the body to insulin, and then reduce blood sugar.

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The research team of Lund University removed the activity of that gene by using CRISPR/Cas9 system in rat insulin producing cells, successfully reduced the activity of TXNIP gene, reduced cell death, and increased insulin production. Because when the human body is at high blood sugar levels, that will lead to the death of beta cells, thereby reducing the production of insulin. This study not only clarified the core role of hat in the regulation of TXNIP, but also further pointed out that the intervention strategy for this mechanism is expected to optimize insulin secretion and prevent cell apoptosis. This has far-reaching significance for the prevention and treatment of diabetes. The reason is that by fine regulating the expression pattern of related genes, it may be able to promote the function of islet β cells to return to normal, and then enhance the insulin secretion potential of patients. However, the technology is still in the experimental stage, and the CRISPR/Cas9 system has high activity and specificity, so there are still many problems to be solved, such as potential problems such as Off-target effect [13].

3) Insulin receptor gene repair:

With the development of CRISPR/Cas9 system, scientists try to directly use the cell repair mechanism to achieve precise gene repair and restore its normal function. CRISPR/Cas9 system has high precision, high efficiency and versatility. It can achieve precise editing of specific genes through accurate positioning and efficient cutting mechanisms. However, the CRISPR/Cas9 system still faces many problems, such as ethical issues, off target effects and technical challenges. Ethical issues are mainly based on the direct editing of human genetic material involved in gene editing technology, which has certain sensitivity and will cause a certain social impact. The off-target effect is mainly caused by the diversity of CRISPR/Cas9 system and the precision of human genetic material. Although researchers have used a variety of methods to reduce the occurrence of off-target effects, eliminating the mop effect remains a difficult challenge. The technical challenges reflect that CRISPR/Cas9 system still needs to be refined and improved. Ensure the security of the technology.

4) Stem cell therapy:

By combining CRISPR/Cas9 system with stem cell therapy, stem cells are transformed into β cells and transplanted into patients to replace the damaged islet β cells.

The University of Washington research team used CRISPR/Cas9 gene editing system combined with stem cell therapy to treat mutant genes of diabetes and attacked β cells. In this study, IPSC technology was used to convert human stem cells into islet β cells using skin cells from Wolfram syndrome patients, and CRISPR/Cas9 system was used to correct the mutated genes causing Wolfram syndrome. The experimental results showed that the diabetes of mice receiving cell transplantation disappeared rapidly after implantation of CRISPR/Cas9 edited stem cells, and their blood glucose levels remained in the normal range on average during the six months of monitoring, without obvious side effects or rejection [12]. Although the experiment is still in the experimental stage, it also provides an effective demonstration that CRISPR/Cas9 system can provide a more accurate and effective treatment for T2DM. In the future, with the development and improvement of science and technology, this technology is expected to become one of the main treatment methods for T2DM.

## 5. Conclusion

CRISPR/Cas9 system has the characteristics of high efficiency, accuracy and flexibility, which shows great potential in the treatment of T2DM. Through CRISPR/Cas9 system, researchers can repair or replace the defective genes in the human body, so as to restore the function of pancreatic β cells and improve the secretion ability of insulin, providing a new possibility for the radical cure of T2DM. In the treatment of T2DM, CRISPR/Cas9 system can not only directly edit the defective genes to achieve the direct treatment of T2DM, but also provide a more accurate and effective treatment method by combining with stem cell therapy. This paper summarizes the current situation of CRISPR/Cas9 system in the treatment of T2DM and provides two specific examples of researchers using

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CRISPR/Cas9 system to treat T2DM, as well as the existing problems of CRISPR/Cas9 system, so that people can systematically understand the application of CRIPR/Cas9 system in the treatment of T2DM. At the same time, this paper has certain limitations, and the conclusions obtained by analyzing the previous data may conflict with the new findings of researchers. To sum up, CRISPR/Cas9 system shows great potential for T2DM treatment in the scientific research stage, but further evaluation and experiment on its safety and efficacy are still needed. In the future, we need more in-depth detection and evaluation of the effectiveness and potential risks of this technology in clinical trials.

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