Application of CRISPR Technology in the Treatment of Diabetes

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Abstract: The prevalence of diabetes is high worldwide (more than 800 million adults worldwide have diabetes). At present, CRISPR technology therapy is the best treatment effect and the most promising method. It can fundamentally solve the pathogenesis of diabetes by editing the WFS1 gene or knocking out the RNLS gene in pluripotent stem cells. This paper studies the causes of diabetes and proposes that CRISPR can realize the reversal of diabetes, which may have certain implications for the treatment of type 2 diabetes (T2D) and other diseases in the future. Noncoding genomic defects in diabetes are analyzed and CRISPER technology is used to reveal a diabetic regulatory circuit. Finally, the pathogenesis of T1D and T2D were analyzed, and new treatment strategies for type 1 and T2D were revealed, which provided references for future CRISPR application research in diabetes. However, there are still problems to be solved, such as technical research limitations, potential safety risks, and the balance between technical application and ethics. Future research can also focus on the combination of CRISPR and other emerging technologies (such as nanotechnology, gene therapy, etc).

1 INTRODUCTION

Diabetes is a chronic metabolic disease characterized by elevated blood sugar due to insufficient insulin secretion or insulin resistance. It can be divided into type 1 diabetes (T1D) and type 2 diabetes (T2D) according to different pathogenesis and clinical manifestations. Due to its serious harm to public health, it has attracted the attention of domestic and foreign researchers in recent years. According to relevant reports, the global prevalence of diabetes has increased significantly in the past 30 years. From 1990 to 2022, the number of adult diabetes patients over 18 years old in the world will surge from about 200 million in 1990 to 828 million (Zhou, et al., 2024).

With the development of medical technology, many new methods for treating diabetes have emerged, among which CRISPR is widely considered to be the most effective and promising method. CRISPR is a gene editing technology, which is regarded as an efficient and accurate treatment method for diabetes in recent years, and shows great application potential. It can fundamentally solve the pathogenesis of diabetes by editing WFS1 gene or knocking out RNLS gene in multi-functional stem cells. Traditional treatment methods generally rely on medication to control the patient's condition, with a focus on external regulation of blood sugar; CRISPR can repair the internal causes of diabetes from the genetic level (Maxwell, et al., 2020), and at the same time achieve personalized treatment to improve the treatment effect. It has significant advantages and broad prospects for development. If they can achieve coordinated development, they will be more and more widely used in the treatment of diabetes.

This study summarized the principle and application achievements of CRISPR in the treatment of diabetes at home and abroad in recent years, including editing WFS1 gene in multi-functional stem cells, up regulating mRNA of transcription factor HNF1A, and knocking out kidney enzyme protein gene and other therapeutic strategies to eradicate diabetes. The principles of these technologies were elaborated in detail, and their practical application level and potential negative effects were analyzed. Corresponding strategies have been proposed to address technical issues such as

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gene editing instability and off target effects, as well as ethical concerns. At the same time, we compared the traditional treatment of diabetes with CRISPR, and analyzed their advantages and disadvantages.

2 INTRODUCTION OF CRISPR

The CRISPR-Cas system provides adaptive immunity to bacteria and archaea. In the adaptation, Cas1-Cas2 inserts the original spacer from the foreign genetic element into the CRISPR array as a new spacer (represented as a rectangle of a different color), separated by CRISPR repeats (represented as a blue diamond). During crRNA biogenesis, CRISPR arrays are transcribed into pre-crRNAs, which are processed into mature crRNAs, each with an interval. crRNA (crRNA- transactive crRNA (tracrRNA)) is assembled with effector proteins or complexes to form a monitoring complex that recognizes and degrades foreign genetic elements that complement the crRNA interval during interference. The Type I CRISPR system is further subdivided into three seed types. They were based on the degree of homology between Cas9 proteins and the presence or absence of additional Cas proteins involved in adaptation. Type II-A and type II-B systems include Csn2 and Cas4, respectively, while most type II-C systems are characterized by the lack of Cas4 and Csn2II-C CRISPR arrays that embed internal promotors in each repeat sequence, generating pre-nested crRNA as a source of mature crRNA. Instead of processing a single pre-CRRNA transcript, another characteristic of all type II Cas9s is the need for PAM (progasm neighbor motif) sequences in the target DNA.

3 DIABETES

3.1 Introduction to Diabetes

Diabetes mellitus is a group of disorders of carbohydrate, protein and fat metabolism caused by absolute or relative insufficiency of insulin secretion and/or insulin utilization disorders, with hyperglycemia as the main sign. The causes of the disease are diverse and can be roughly divided into the following: ethnic and genetic factors, long-term overfeeding, obesity factors, mental factors and autoimmune. Its clinical manifestations can also be divided into T1D and T2D. In general, T1D occurs more quickly, and the disease is often in adolescents and children. T2D has no obvious "three more and one less" symptoms, and is often a chronic disease, it is not easy to distinguish when and why the onset, and sometimes patients have been ill and failed to find treatment in time, only patients can be confirmed by blood sugar test.

3.2 Traditional Treatment of Diabetes

In the traditional treatment of diabetes, insulin replacement therapy is the main choice for patients with T1D, and changes in diet and lifestyle are considered to be the best choice for treatment and management of T2D (Bastaki, et al., 2005). Insulin is also important in the treatment of T2D when blood sugar levels cannot be controlled through diet, weight loss, exercise and oral medication.

3.2.1 Dietary Therapy and Exercise Therapy

The main treatment approach of dietary therapy is to accurately calculate the patient's daily calorie requirements and then control their diet to maintain blood sugar balance. Generally, the appropriate calorie intake value needs to be determined based on the patient's age, gender, weight, physical activity level, and other factors. For example, a 50-year-old male diabetes patient who is engaged in light physical labor, 170 cm tall and 75 kg in weight, is estimated to need about 1800 kcal of heat per day according to the formula. Among them, carbohydrate accounts for 50% -65%, protein accounts for 15% -20%, and fat accounts for 20% -30%. It focuses on unsaturated fatty acids and reduces animal fat intake (diabetes Branch of the Chinese Medical Association, 2021).

Aerobic exercises such as brisk walking, jogging, and swimming can all be the first choice for exercise therapy. During aerobic exercise, muscles continue to contract, thereby increasing the uptake and utilization of glucose in the blood; At the same time, exercise enhances insulin sensitivity, allowing insulin to function more efficiently and guiding glucose into cells for energy supply, resulting in a decrease in blood sugar levels. It is worth noting that patients with complications of diabetes need to exercise carefully to avoid additional damage to the body. For example, patients with retinopathy should avoid eyeground bleeding caused by intense exercise, and patients with neuropathy should prevent foot injury.

3.2.2 Drug Therapy

In the treatment of diabetes, the drugs used are generally divided into oral hypoglycemic drugs and insulin injection. The former is mainly used to treat T2D, and the latter is used to treat T1D. For T2D patients, insulin is also needed when oral drugs cannot control blood sugar. Oral hypoglycemic drugs include sulfonylureas, biguanides, alpha glucosidase inhibitors, etc.

The most common treatment drug is metformin, which is the first-line drug for T2D. Under normal circumstances, the liver can convert non sugar substances into glucose and release it into the bloodstream, while metformin can interfere with this pathway, reducing liver glucose output and subsequently lowering fasting blood glucose levels. On the other hand, metformin can enhance the sensitivity of peripheral tissues (such as muscles and adipose tissue) to insulin. Insulin is like a "key" to open the "door" for cells to absorb glucose. However, in patients with diabetes, especially in patients with T2D, the role of this "key" is often blocked. Cells are not sensitive to insulin, and it is difficult for glucose to enter cells for energy supply. Metformin can improve this insulin resistance state, allowing insulin to function better, promoting muscle cells to uptake glucose from the blood for energy metabolism, and also increasing the utilization of glucose by adipose tissue, thereby effectively reducing blood sugar. Both postprandial blood sugar and overall daily blood sugar levels can be well regulated.

For patients with diabetes, when their islet function is impaired, they cannot secrete enough insulin, or the body is resistant to insulin, and they cannot effectively use insulin, they need to inject exogenous insulin to make up for this deficiency. Insulin can bind to specific insulin receptors on the surface of muscle cells and adipocytes, activating a series of signaling pathways within the cells, allowing glucose from the blood to enter the cells smoothly, providing energy to the cells and reducing the glucose content in the blood. Insulin not only inhibits glycogen breakdown and gluconeogenesis in the liver, but also stimulates liver and muscle cells to synthesize excess glucose in the blood and store it as glycogen, ultimately achieving the goal of lowering blood sugar.

3.3 Relationship between CRISPR and Treatment of Diabetes

CRISPR, as an accurate gene editing tool, has made great contributions to the exploration of the pathogenesis of diabetes. Researchers can use it to knock out and modify specific genes in cells, so as to analyze the role of genes related to insulin resistance and abnormal pancreatic β cell function in the pathogenesis of diabetes.

For T1D, the patient's own islet beta cells are damaged due to the attack of the immune system. The researchers imagine to use CRISPR to edit the patient's immune cells so that they will not attack the islet beta cells by mistake, or to convert induced pluripotent stem cells (iPSCs) into functional islet beta cells through gene editing, and then transplant them back into the patient's body (Staedtke, et al., 2020) to restore normal insulin secretion. For T2D, this technology can be used to correct the genetic defects that lead to insulin resistance, enhance the sensitivity of the body to insulin (Venkatrama, et al., 2024), and improve the disease from the root.

According to existing research, the traditional treatment of diabetes focuses on regulating blood sugar from the outside, such as drugs to stimulate insulin secretion, supplement insulin or improve insulin sensitivity, while CRISPR is committed to repairing the internal root cause of diabetes from the genetic level (Bora, et al., 2023). If the two can develop together, they may provide more accurate and thorough treatment paths for diabetes patients in the future.

4 APPLICATION OF CRISPR IN THE TREATMENT OF DIABETES

4.1 Realization of Reversal of Diabetes

Diabetes may be caused by gene mutation caused by acquired factors. It is possible to reverse diabetes through CRISPR. A mutation in the WFS1 gene in patients with Wolfram syndrome 1 causes a rare inherited form of insulin-dependent diabetes. To develop a treatment for this form of diabetes, Dr. Jeffrey R. Millman, assistant professor of medicine and biomedical engineering at the University of Washington, and his team selected cells from a patient with Wolfram syndrome 1 (WFS1) and derived them to induce and differentiate into pluripotent stem cells

(IPscs). CRIPSR/Cas9 was then used to edit the WFS1 gene in patient-derived IPscs to produce autogene-corrected SC-β. The researchers then compared the gene-edited cells with the same batch of insulin-secreting beta cells that had not been CRISPR-edited. In mice with severe diabetes, the CRISPR-edited, newly grown beta cells secreted glucose more efficiently. The CRISPR-edited cells placed under the skin made diabetes disappear quickly in mice, and the animals' insulin secretion improved and their blood sugar stayed within normal ranges during a six-month monitoring period. CRISPR was first used to repair a genetic defect that caused diabetes in patients and successfully reversed diabetes (Maxwell, et al., 2020).

The research extends a strategy for tackling T1D and may have implications for the treatment of T2D and other diseases in the future.

Looking forward to the future, the researchers extract cells from human skin for differentiation, if the use of human metabolite cells for experiments, not only reduce the difficulty of raw material acquisition and reduce the cost of the experiment, in addition, the success of the experiment may also help solve other complications of Wolfram syndrome 1 (WFS1) patients.

4.2 Reveal a Regulation Circuit of Diabetes

The onset of diabetes is often caused by the adverse effects of multiple factors, rather than the loss of a single isolated factor. The study found that HNF1A homeobox A (HNF1A) encodes the homeodomain transcription factor, whose haploid under-dose mutation induces diabetes. HNF1A's antisense lncRNA promoter, HASTER, can cis-regulate HNF1A transcription and maintain the cell-specific physiological concentration of HNF1A through positive and negative feedback loops. HASTER mutant mouse pancreatic beta cells exhibit HNF1A silencing or overexpression, which leads to hyperglycemia. After upregulation of Hnf1a mRNA by about 30-80% through the use of CRISPR-Cas9, Haster RNA increased by about 50-120%, revealing the mechanism of action of HASTER promoter as a regulator of HNF1A, with a negative feedback loop between them. That is, HNF1A positively regulates HASTER, while HASTER negatively regulates HNF1A, a finding that contributes to the development of novel diabetes treatments and facilitates our understanding of noncoding genomic defects in the disease (Beucher, et al., 2020).

In the future, there may be synthetic HASTER isofactor introduced into human body for treatment, which can not only be used as a regulatory factor of HNF1A, but also as a cofactor of other regulatory factors. It also provides clues to the genetic mechanism of diabetes.

4.3 Reveal New Treatment Strategies for T1D and T2D

T1D is mainly caused by immune system abnormalities that lead to the destruction of islet beta cells. CRISPR has been used to study genes associated with beta cell function. For example, CRISPR genome-wide screening revealed the effect of the RNLS (Renalase) gene on beta cell survival. Studies have found that knocking out RNLS can significantly reduce the sensitivity of beta cells to oxidative stress, thereby reducing the risk of T1D development (Cai, et al, 2020). At the same time, the research team further identified an already FDA-approved drug, Pargyline, whose mechanism of action is to bind to RNLS and inhibit it. The study also showed that Pargyline has good safety while protecting β cells and preventing diabetes in mice, and has considerable development significance in the future.

In addition, CRISPR has also been used to modify immune cells to suppress autoimmune responses against beta cells, providing new ideas for immunotherapy of T1D (Bevacqua, et al. 2021).

The team also established a genetic model in human islet cells and revealed the regulatory and genetic mechanisms by which a non-coding variation is associated with diabetes risk in humans.

The pathogenesis of T2D is complex, involving insulin resistance and beta cell failure. CRISPR is widely used to screen for genetic risk genes associated with T2D. For example, using CRISPR screening, it was found that the CALCOCO2 gene plays a key role in the regulation of beta cell function, and gene mutations are closely associated with T2D risk, and the experiment also completed a genome-wide CRISPR hybrid screening, providing a comprehensive perturbation dataset for future CRISPR research (Rottner, et al., 2023).

In addition, CRISPR has been used to edit fat cells to improve metabolic function and thus reduce the risk of T2D by promoting Browning of white fat (Tsagkaraki, et al., 2021), and also provides a future cellular therapy strategy for metabolic diseases, especially in larger animals.

5 CONCLUSION

CRISPR has demonstrated significant potential in the field of diabetes treatment, offering more options for patients. By precisely repairing genetic defects and inducing cell transformation, CRISPR holds promise for treating diabetes at its root cause and enabling personalized therapeutic approaches. Currently, CRISPR has achieved remarkable results in animal experiments, and some clinical trials are steadily progressing too. These make a credible foundation for its clinical application.

However, challenges and limitations remain in the application of CRISPR for diabetes treatment. Technically, issues such as off-target effects and instability in gene editing during the treatment process may impact the efficacy of diabetes therapy and even interfere with normal cellular physiological functions. In terms of safety, the Cas protein in the CRISPR system which derived from bacteria may be recognized as a foreign entity by the human immune system and trigger immune responses when introduced into human cells for gene editing. Ethically, gene editing technology continues to face significant controversy. Additionally, CRISPR-based diabetes treatments are still in the research and development phase, with high associated costs. This may result in only a small number of economically capable patients being able to afford such treatments in the future and this may exacerbate inequalities in the distribution of medical resources.

It can be seen that in the near future, collaboration among experts in biology, medicine, ethics, and law will be essential to advance CRISPR in diabetes treatment. It is believed that with continuous technological improvements and breakthroughs, CRISPR will bring revolutionary changes to diabetes therapy, offering genuine hope to hundreds of millions of diabetic patients worldwide and enable them to overcome the challenges of diabetes to regain a healthy life.

AUTHORS CONTRIBUTION

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

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