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Application of gene editing technology in the research and treatment of type 2 diabetes

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Abstract. Type 2 diabetes (T2D) is a common chronic disease worldwide, and its incidence is increasing year by year. In general, decreased insulin sensitivity due to factors such as obesity and insufficient insulin secretion due to loss of pancreatic β-cell function are the main contributing factors to T2D. The emergence of CRISPR technology, a simple and efficient editing tool for generating mutations or corrections in genomic DNA, has provided an effective and powerful way to understand how identified mutations affect cell function, growth, and survival, making it possible to cure T2D at the gene editing level. This paper reviews the application and research progress of CRISPR technology in the treatment of T2D and assesses its potential for application in terms of repairing the function of the body's membrane protein receptor, enhancement of pancreatic β-cell activity and function, and regulation of genes related to insulin resistance. It provides a comprehensive reference for understanding the pathogenesis of T2D and the design and establishment of novel therapeutic regimens.

Keywords: CRISPR-Cas9, type 2 diabetes treatment, pancreatic β-cells.

1. Introduction

Diabetes mellitus is a metabolic disease caused by high blood sugar due to defects in insulin secretion, insulin action, or a combination of both. Significant symptoms of hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia,c and blurred vision. Generally, there are four types of diabetes mellitus, namely type 1 diabetes (T1D), Type 2 diabetes (T2D), gestational diabetes mellitus (GDM), and other specific types of diabetes mellitus. Of these, those suffering from T2D account for about 425 million people worldwide have T2D. And by 2045, this figure is expected to grow to 629 million. There is no doubt that T2D has become one of the global diseases that endanger human health. This makes it particularly important to design and establish new, safer, and more effective treatment options for T2D [1].

T2D, also known as non-insulin-dependent diabetes mellitus, is a form of diabetes mellitus that ranges from predominantly insulin resistance with relative insulin deficiency to defective secretion of predominantly insulin with insulin resistance. Two mechanisms cause T2D. One mechanism is due to the human body pancreatic islet β-cell function occurs sustained loss, insulin secretion is insufficient,

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and the hypoglycaemic effect is weakened; another mechanism, although insulin secretion is normal, is insensitive to insulin and cannot use insulin effectively, that is, insulin resistance and blood sugar rise [1]. Patients usually present initially with relative insulin deficiency and typically with a disturbance in glucose-dependent insulin secretion.

Dysfunction of pancreatic islets and pancreatic β-cell failure are important hallmarks of the pathogenesis of T2D. Typically, T2D involves insulin resistance, but ultimately it is the result of pancreatic β-cell failure [2]. Therefore, pancreatic β-cell activity is an important indicator of whether the body can restore homeostatic balance.

However, the current therapeutic options for T2D are mainly based on controlling the patient's blood glucose concentration, and no practical cure has been proposed. Therefore, in this paper, we will conduct in-depth research and analysis on the pathogenic mechanism of T2D at the genetic level, which will provide new ideas and new ways for the design and establishment of novel therapeutic solutions for T2D.

2. Gene editing normal function membrane receptor proteins

Functional genetics has now unraveled the insulin receptor targets in T2D, and most of them belong to receptor proteins on cell membranes. Studies have shown that a variety of membrane receptor proteins can influence insulin secretion and cellular sensitivity to insulin. For example, the LGR4 gene, a gene that directs the synthesis of leucine-rich G-protein-coupled receptors (LGR), is involved in the production of LGR receptors in pancreatic β-cell. LGR4 knockout mice fed a high-fiber diet were found to have improved glucose tolerance and insulin sensitivity, as well as reduced insulin levels, which provides strong theoretical support for treating T2D at the genetic level [3]. In the adipocytes of mice, the loss of the LGR receptor will cause the cell to partially lose its ability to recognize insulin, thus reducing the sensitivity of the cell to insulin, and at the same time, the secretion of insulin molecules in the whole organism will be interfered by this receptor, which is unable to properly regulate the normal maintenance of homeostasis, resulting in the emergence of the disease [4].

As an example, the OPRD1 gene directs the synthesis of the DOR receptor to regulate insulin secretion and cellular sensitivity to insulin under the action of opioids. The OPRD1 gene is expressed in human pancreatic islet β-cells and is directly related to the production of the opioid receptor DOR. The opioid receptor DOR can interact with enkephalins to produce neurostimulation, which leads to pleasure and gradual addiction.

In the 1970s, it was discovered that enkephalin not only acts as an addictive agent but is also a contributing factor to T2D [5]. Opioid addicts generally show a lower BMI (Body Mass Index) than normal, and mice injected with opioids for long periods also show significant food loss [6]. The opioid receptor DOR is often considered an important regulator of heroin and other opioid addictions [7], which is modulated by the recognition of enkephalins released by endopeptidergic neurons. DNA sequencing has shown that OPRD1 and its variants are naturally expressed in cells and direct the synthesis of the receptor.

Examination of the physiological form of each DOR receptor variant in the organism revealed the presence of both intact and defective expression variants of the OPRD1 gene. After balancing the age, gender, and BMI of the subjects, it was found that the majority of carriers of the defective variants had symptoms such as obesity, overweight, and diabetes; in contrast, carriers of the DOR receptor who were able to function normally tended to be free of symptoms such as diabetes [8]. Furthermore, the OPRD1 gene is the only gene that is normally expressed in human islet cells and synthesizes the opioid receptor, and its expression is weaker in the pancreas of patients with T2D than in the pancreas of normal subjects [9], as demonstrated by the introduction of diseased cells into the normal organism. Therefore, the DOR receptor generated by OPRD1 gene expression is not only related to the addictive properties of enkephalin but also to the suppression of the phenotype of T2D.

In treatment, gene editing techniques can be used to ensure normal expression of the OPRD1 gene and generate DOR receptors with normal regulatory functions, thus improving the tolerance of T2D in the general organism, but the intake of opioid agents needs to be controlled during treatment.

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3. Gene editing improves the activity and function of pancreatic β-cells

3.1. Gene editing of normal functioning pancreatic β-cells

Studies have shown that heritable variants that code for or mis-express genes highly associated with pancreatic islet β-cell function may lead to changes in insulin secretion, thereby increasing the risk of T2D in humans. It has been found that exogenous importation or shearing of a specific gene segment using techniques such as CRISPR/Cas9 can control the amount of insulin secreted, and it is possible to develop a therapeutic solution based on this.

An example of induced pluripotent stem cells directed to form gene-edited corrected pancreatic islet β-cells. The differentiation of pluripotent stem cells from patients often exhibits general abnormality, making them suitable for mass differentiation in vitro for the construction of in vitro disease models [10]. However, when patient stem cells were cultured in vitro, the number of pancreatic β-cells differentiated from them was much lower than the number of β-cells differentiated from normal stem cells [11]. It was found that mice carrying islet β-cells differentiated from patients' stem cells had a much slower drop in blood glucose after eating than normal mice, and had a shorter average lifespan, with most of them dying from complications caused by obesity.

It was found that abnormal mice carry a special gene, the WFS1 gene, which promotes endoplasmic reticulum stress, leading to massive stacking of unfolded proteins and slowly causing the death of pancreatic β-cells. After editing this gene using CRISPR/Cas9 technology, on the one hand, the content of β-cells produced by stem cell differentiation was detected in vitro after abnormal, normal, and correction, and on the other hand, the physiological characteristics and the average lifespan of mice carrying different genes in the same period were observed, and a clear discrepancy between before and after correction could be observed: the content of β-cells produced by stem cells after correction was significantly increased, and the amount of insulin secretion was also largely returned to normal levels. Since the prevalence of T2D in mice is greatly reduced, the survival rate of mice carrying the corrected gene is also significantly higher.

Therefore, inhibiting the production of abnormal or defective pancreatic β-cells by gene editing technology can enhance the body's tolerance to T2D thus achieving the therapeutic goal.

3.2. Correction of human induced pluripotent stem cell (ipsc) point mutations

Experiments have found that there are many variants associated with T2D, such as Modi gene variants, variants identified in protein-coding regions by genome-wide association analysis (GWAS), and genetic variants identified in non-coding genomic locations. Taking human iPSCs for gene editing as an example, by performing in vitro disease modeling of iPSCs from patients with T2D and using CRISPR technology to correct point mutations in patient-derived iPSCs, the purpose of targeting T2D-related gene defects is achieved.

To date, the most effective method used in iPSCs is CRISPR/Cas9-based homology-directed repair (HDR). This approach has been successfully applied to study signal transducer and activator of transcription 3 (STAT3) and GATA-binding protein 6 (GATA6) mutant iPSC lines generated by reprogramming patient cells expressing heterozygous mutations.

The use of CRISPR/Cas9 technology can correct gene mutations and completely reverse the phenotype of the disease [12], so that the body's insulin regulatory system can be restored, and the effect of treating T2D can be achieved.

4. Gene editing of insulin resistance-related genes

Studies on insulin resistance have found that the innate immune transcription factor interferon regulatory factor (IRF3) adversely affects glucose homeostasis by inducing the endogenous hydroxy fatty acid ester (FAHFA) hydrolase androgen-induced gene 1 (AIG1) in adipocytes. Adipocyte-specific knockout of IRF3 protects male mice from high-fat diet-induced insulin resistance, while overexpression of IRF3 or AIG1 in adipocytes promotes high-fat diet-induced insulin resistance.

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In addition, in the case of adipocyte IRF3 overexpression, drug inhibition of AIG1 can reverse obesity-induced insulin resistance and restore glucose homeostasis. The experiment has determined that the adipocyte IRF3/AIG1 axis is a key link between obesity-induced inflammation and insulin resistance and proposed a method to limit metabolic dysfunction associated with obesity [13].

Other studies have shown that zinc finger FYVE type 28 (ZFYVE28) mediates insulin resistance by increasing the production of late endosomes and promoting the degradation of phosphorylated insulin receptors [14]. At the same time, some studies have discovered insulin resistance gene loci in the whole genome by using the ratio of serum triglycerides to high-density lipoprotein cholesterol as a representative and conducted comprehensive genomic identification. They found that the PLA2G12A gene and the PLA2G6 gene can release arachidonic acid and improve insulin sensitivity; the transcriptional cofactor degeneration-like protein 3 (VGLL3) can increase insulin resistance by increasing fat; and the anti-apoptotic gene TNFAIP8. This is a sexually dimorphic insulin resistance factor that can exert its effect by increasing visceral fat content [15]. Experiments have shown that these genes and cell regulatory factors can improve the body's insulin resistance and are likely to become candidate targets for gene editing.

CRISPR technology can be used to edit and regulate the expression of insulin resistance-related genes and influencing factors, thereby increasing the body's insulin sensitivity and improving the body's T2D symptoms.

5. Challenges and prospects of gene editing

Although CRISPR-Cas9 technology has shown great potential in the treatment of T2D, especially in repairing pancreatic β cell function and regulating related gene expression, it still has shortcomings such as off-target effects and delivery efficiency.

Off-target effect refers to the fact that the CRISPR-Cas9 may produce unexpected DNA cuts at non-target locations during gene editing, leading to genomic instability and potential safety risks. In addition, the exogenous nucleic acids used in the CRISPR-Cas9 may be recognized by the host immune system and trigger immune responses, which may affect the safety and effectiveness of the treatment. In terms of delivery efficiency, it is still a technical challenge for this technology to effectively deliver to specific cell types such as pancreatic β cells, which may affect the feasibility of treatment. Although CRISPR-Cas9 has shown therapeutic potential in the short term, its long-term safety and potential long-term effects are not yet fully understood. Ethical issues are also an important factor that cannot be ignored in considering the adaptability of this technology. The use of gene editing technology involves ethical disputes, especially when it involves human embryos and germ cells, which require strict ethical review and supervision. The optimization and application of the CRISPR-Cas9 requires sophisticated design and adjustment, including the design of guide RNA and the selection of Cas9 variants to improve the specificity and efficiency of editing.

Therefore, future research may focus more on how to ensure the accuracy of gene editing, develop effective delivery systems, and evaluate long-term safety.

6. Conclusion

According to the two pathogenic mechanisms of T2D, how to improve the body's sensitivity to insulin and restore the body's pancreatic islet β-cell function are the fundamental problems that need to be solved to cure T2D. It has been found that gene editing technology can be used to improve the symptoms of T2D in three ways, namely, gene editing of membrane protein receptors to enable the body to obtain normal function, gene editing to improve the activity and function of pancreatic β-cells, and gene editing of insulin resistance-related genes.

Although the mechanism regulating the delivery efficiency of insulin in the organism is still unknown, it is still possible to cure T2D at the genetic level using gene editing techniques such as CRISPR. On the one hand, gene editing technology can improve insulin sensitivity and pancreatic β-cell function by correcting and knocking out abnormal genes, and on the other hand, it can help to find safer and more

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efficient gene editing candidate targets, which will lay a good foundation for a deeper understanding of the insulin regulation mechanism.

Currently, existing studies have already made basic inquiries into the treatment of T2D at the genetic level, and with the application of advanced gene editing technology, it is inevitable to further deepen the understanding of the pathogenic mechanism and treatment of T2D. This will not only help to understand the body's insulin regulation mechanism but also be of great significance to the design and establishment of safer and more effective therapeutic programs for T2D.

Authors Contribution

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

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