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Research on the Application of CRISPR Technology in the Treatment of Type 1 Diabetes (T1D)

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Abstract: Diabetes is a chronic disease with a rapid increase in incidence, which is a general problem around the world. Two main types of diabetes are included. Polygenic diabetes includes type 1 diabetes (T1D) and type 2 diabetes (T2D), and their monogenic forms are juvenile mature diabetes (MODY) and neonatal diabetes (NDM). CRISPR technology is a gene therapy technology with low cost but very high feasibility. At present, researchers have envisioned the use of CRISPR technology in the treatment of T1D, but this technology has a high off-target rate, immune rejection and no abundant clinical trials in the human body to verify this technology, resulting in the fact that this technology is not really used in clinical treatment. In the middle. This article mainly analyzes and summarizes the problems and solutions encountered by CRISPR-cas9 technology from basic theory to idealized models. This article provides new solutions for the treatment of T1D with CRISPR technology and also provides references for this technology. In addition, problems such as immune rejection in the human body have not been solved. Future research can focus on the solution to the problem of the suppression of cellular immune rejection after treatment and the impact of re-mutation.

Keywords: CRISPR technology, Type 1 diabetes, cell therapy, gene editing.

1. Introduction

Type 1 Diabetes (T1D) is one of the diseases that affect people's health all over the world. It is a chronic disease, mainly affecting adolescents and people with family genetic history. It is a disease caused by an autoimmune reaction, mainly because the immune system mistakenly attacks the beta cells of the pancreas, resulting in the destruction of the beta cells of the pancreas, resulting in insufficient insulin secretion. Traditional treatments include insulin injections and pancreatic transplants, but these carry significant risks and do not cure the disease at all, nor do they address the loss of beta cells in the body. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a gene-editing technique that enables precise cutting and repair of DNA in and out of the body. Through guide RNA (gRNA), the target DNA sequence is specifically recognized and cut, and subsequently modified through the cell's repair mechanism. And rapid development in the field of biomedicine, mostly used to treat genetic and genetic diseases. So scientists envision using CRISPR technology to treat T1D to repair pancreatic beta cells, so that T1D can be genetically fundamental

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treatment, but also reduce the likelihood of genes passed on to future generations. Gene editing technology is currently mainly applied in four aspects: repair cells, immune system, direct replacement and stem cells. One of the major breakthroughs in the field involves a collaboration between CRISPR Therapeutics and ViaCyte aimed at creating insulin-producing cells that can be implanted in T1D patients. These stem cells are gene-edited using CRISPR to make them immune evasive. At present, the common problems are easy off-target and immune rejection in the process of gene editing [1]. The purpose of this review is to summarize and discuss the basic ideas, challenges and future potential of CRISPR technology in the treatment of T1D, so as to provide an effective reference for the treatment of T1D.

2. The technical principle of CRISPR

The CRISPR system is an immune mechanism derived from bacteria, through the synergistic interaction of Cas9 nuclease and guide RNA (gRNA). It can achieve precise cutting and editing at specific sites of target genes.

CRISPR sequences are composed of short repeat sequences and interval sequences. The spacer sequence is derived from the DNA of the virus, which allows the bacteria to remember previous infections. Cas proteins (such as Cas9) are enzymes that perform the cutting of DNA. The Cas9 protein is responsible for finding and cutting DNA that matches the CRISPR sequence [1].

Most current CRISPR-Ca9 systems are designed and built in a four-step process. Step one is to design a gRNA, which is a short RNA sequence that is usually complementary to the target DNA sequence. It is designed to ensure that the CRISPR system can pinpoint specific genes or genomic regions. In the second step, the CRISPR complex is formed, and the gRNA binds to the Cas9 protein to form the CRISPR-Cas9 complex. In step three, the CRISPR-Cas9 complex recognizes a DNA sequence in the cell that is complementary to the guide RNA sequence and binds to this target DNA. Finally, DNA cleavage is achieved, and the Cas9 protein cuts at a specific site in the target DNA sequence, causing a double-strand break (DSB).

3. Current status of CRISPR applications in T1D

3.1. Principles of CRISPR technology in T1D treatment

3.1.1. Repairing pancreatic β-cell function

The cause of T1D is the immune system attacking and destroying pancreatic β-cells. Therefore, restoring or replacing the function of these cells is key to treatment. Researchers can utilize CRISPR to transform the patient's own somatic cells (such as skin cells) into induced pluripotent stem cells (iPSCs), and then modify them through gene editing to create β-cells with immune evasion properties. These modified β-cells can be transplanted back into the patient's body to replace the destroyed cells, enabling physiological insulin secretion [2].

3.1.2. Regulating immune system response

The fundamental cause of T1D lies in the autoimmune system's erroneous attacks. Thus, CRISPR technology can also be used to regulate the patient's immune system. For instance, knocking out genes associated with autoimmune responses, such as PD-1 or CTLA-4, in T cells can reduce T cell attacks on β-cells, thereby protecting the function of remaining pancreatic cells. Additionally, editing the patient's hematopoietic stem cells to differentiate into immune-regulatory cells can help restore the immune system's self-tolerance.

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3.1.3. Gene replacement therapy

Through CRISPR technology, researchers can insert therapeutic gene sequences, such as the insulin gene or regulatory genes related to insulin secretion, at specific gene loci. This method not only enables the restoration of insulin secretion functions in patient cells but also allows for precise regulation of insulin levels, thereby avoiding adverse reactions like hypoglycemia. The discovery of CRISPR-Cas9 has opened new prospects for gene therapy, providing a powerful tool for precise gene editing. The core of this technology lies in matching single guide RNA (sgRNA) with the target gene sequence, using RNA-guided DNA nucleases (like Cas9) to induce DSBs at specific locations. This process leads to the inactivation of the target gene or, in the presence of appropriate template DNA, allows the insertion of the desired gene sequence through homology-directed repair (HDR). Compared to traditional gene editing technologies such as zinc-finger nucleases (ZFN) and transcription activator-like effector nucleases (TALENs), CRISPR-Cas9 has quickly become the preferred tool for mammalian genome editing due to its simplicity and high efficiency. While ZFN and TALENs are effective, their design and application are relatively complex, especially in recognizing specific DNA targets. The flexibility of CRISPR technology enables researchers to more easily make precise modifications to genes. In diabetes research, CRISPR technology has been widely applied to explore the functions and gene regulation of β-cells. These studies typically focus on genes related to insulin synthesis and secretion, allowing researchers to gain deeper insights into the pathogenesis of diabetes by editing these genes. For example, research has shown that pancreatic and duodenal homeobox 1 (PDX1) is a key regulator of the INS gene; modifying PDX1 expression using CRISPR-Cas9 can significantly impact glucose-induced calcium influx and insulin production. Additionally, CRISPR has been used to screen multiple genes associated with insulin regulation, advancing our understanding of insulin metabolism control. These studies not only provide new insights for basic science but also lay the groundwork for developing potential diabetes treatment strategies. With the continuous development of CRISPR technology, it is expected to play an even greater role in gene therapy, disease model establishment, and biomedical research across multiple fields [3,4].

3.1.4. Stem cell technology

Generating Insulin-Secreting β Cells: By combining CRISPR technology with stem cell techniques, insulin-secreting cells can be generated from iPSCs as an alternative treatment.

Cell Reprogramming: Research is being conducted on how to use CRISPR to reprogram other types of cells into β cells to replace damaged ones.

In recent years, controlled clinical trials on stem cell therapy for T1D have shown promising results and safety. Studies indicate that mesenchymal stem cells (MSCs) can improve diabetic symptoms in animal models of T1DM. In 2014, Carlsson et al. confirmed that MSC therapy could preserve β cell function in newly diagnosed T1DM patients. In one study, 20 newly diagnosed adult T1DM patients were randomly divided into an MSC treatment group and a control group. After one year of follow-up, the results showed that the treatment group had significantly reduced C-peptide peak levels with no observed side effects.

In another study, 42 T1D patients were assigned to a stem cell transplant group (combining umbilical cord MSCs with autologous bone marrow mononuclear cells) or a standard insulin treatment group. One-year follow-up results indicated that the treatment group experienced increases in C-peptide levels and insulin requirements, while the control group saw decreases. Additionally, the treatment group had lower HbA1c and fasting blood glucose levels, with a significant reduction in severe hypoglycemic events. Despite these studies demonstrating the potential of stem cell therapy, limitations such as small sample sizes and short follow-up periods remain. Current challenges in

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treating T1D include: how to generate mature β-like cells from human pluripotent stem cells (hPSCs) in vitro, improve differentiation efficiency, protect implanted cells from autoimmune attacks, produce sufficient cell quantities, and achieve complete insulin independence. Despite these technological obstacles, stem cell-based therapies are still considered a forefront approach for curing T1D [5].

3.2. The application of CRISPR technology in treating T1D

3.2.1. Mouse model research

The non-obese diabetic (NOD) mouse is widely recognized as one of the leading spontaneous models for type 1 diabetes (T1D), displaying characteristics that closely mimic the onset of the disease in humans. This model originates from the genetically predisposed inbred strain, Jcl: ICR, which is noted for its susceptibility to cataracts, and it possesses the critical H-2g7 haplotype essential for T1D research. The MHC class II molecule I-Ag7 is pivotal in the manifestation of type 1 diabetes due to its specialized peptide-binding capabilities, which may promote the development of various autoreactive T cells. Furthermore, over 20 non-MHC loci have been implicated in the onset of diabetes in NOD mice, including genes like IL2 and CTLA-4, underscoring the intricate genetic networks involved in the autoimmune diabetes process in these subjects. To enhance the study of T1D, scientists are endeavoring to "humanize" NOD mice by incorporating genes linked to T1D in humans, thereby establishing a flexible platform for evaluating therapies customized for diverse high-risk T1D groups. As of now, researchers have employed CRISPR-Cas9 technology to create 13 distinct transgenic mouse models, altering specific genes related to immune modulation or β-cell performance to replicate various facets of T1D. For example:

In the year 2016, scientists developed a groundbreaking rodent prototype with an inherited alteration in its genetic code—the Ptpn22 (R619W) transformation—which was the initial instance of a type one diabetic animal model created through the innovative technique known as CRISPR-Cas9 engineering. This novel creation involved a singular change at the molecular level within the natural structure of the PTPN22 gene, which accelerated the spontaneous development of the disease process associated with insulin production impairment. Notably, this modification resulted in heightened levels of autoantibodies against insulin in both fully affected and partially altered female subjects, providing additional evidence supporting the correlation between the genetic abnormality and the advancement of diabetes mellitus.

Researchers used CRISPR-Cas9 technology to develop a NOD mouse model with AID gene knockout to investigate the role of the AICDA gene. The research indicated an elevated expression of AID in the B cells of wild-type NOD mice. The suppression of AID through knockout methods significantly postponed the development of type 1 diabetes without compromising the overall diabetogenic function. Additionally, the conjunction of an inducible AICDA knockout with RAD51 inhibitor therapy potentiated the stimulation of CD73+ regulatory B cells, efficiently repressing the pathogenic T cell response. Consequently, the AID/RAD51 pathway emerges as a promising therapeutic target, with the potential to avert the onset of type 1 diabetes by transforming B cells into a regulatory phase marked by the CD73+ attribute.

While these models are crucial for preclinical research, they have limitations and differ from human diseases, necessitating comprehensive studies that integrate animal model findings with human clinical observations to advance understanding and treatment of T1D [6].

3.2.2. T1D cell therapy

The scientific community has invested significant resources into investigating cellular treatments for type 1 diabetes mellitus (T1DM). Lantidra—a pioneering cell-based intervention developed by Donislecel and manufactured by CellTrans Incorporated in Chicago—is the initial therapeutic

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regimen to attain regulatory approval from the Food and Drug Administration (FDA) for the management of T1DM. This groundbreaking approach involves transplanting portions of pancreas tissue derived from recently departed individuals onto affected persons. To initiate the procedure, medical professionals will typically implant anywhere from one to three sets of allogeneic pancreatic beta cells sourced from these generous benefactors. According to data collected during a comprehensive examination involving thirty volunteers, an impressive twenty-one subjects managed to sustain their autonomy regarding insulin administration over a period of twelve months or longer; eleven were free of insulin dependence for intervals ranging from one to five years; while ten continued to enjoy freedom from reliance upon exogenous insulin injections beyond the threshold of half a decade. Despite these encouraging results, it was observed that five test subjects failed to realize complete liberation from insulin supplementation. A critical constraint associated with such regenerative medicine protocols revolves around finding precise matches between donated tissues and individual hosts, thereby limiting the widespread utility of this innovative technique within contemporary clinical settings.

To address the issue of donor cell matching, researchers have turned to stem cells in hopes of providing treatment for more diabetes patients. Currently, several other cell therapies for type 1 diabetes (T1D) are also under development. For example, the VX-880 clinical trial is in phase I/II, and preliminary results show that two patients achieved insulin independence after at least one year of treatment, with HbA1c levels of 5.3% and 6.0%, respectively. VX-880 is an allogeneic therapy, where the pancreatic cells are derived from fully differentiated stem cells capable of producing insulin. The treatment involves infusing the cells into the portal vein, while an immunosuppressive regimen is used to prevent cell rejection.

A notable breakthrough in the realm of Type 1 diabetes cell therapy is the introduction of a gene-edited allogeneic stem cell method. VCTX211, a product of the partnership between CRISPR Therapeutics AG and Viacyte, is presently in a phase I/II clinical trial (ClinicalTrial NCT05565248). This trial is structured in two segments: the application of CRISPR/Cas9-edited allogeneic pancreatic endoderm cells, alongside a detachable perforated device aimed at both administering and safeguarding these cells.

VCTX211 is characterized by the knockout of two genes, B2M and TXNIP, alongside the insertion of four others: PD-L1, HLA-E, TNFAIP3, and MANF, all intended to enhance cellular performance. In contrast to its predecessor, VCTX210A—whose clinical trials have already wrapped up (ClinicalTrial NCT05210530)—the newly developed VCTX211 has undergone genetic modifications to mitigate immune rejection by T-cells and NK cells, as well as to shield the endoplasmic reticulum from oxidative damage. Furthermore, the introduction of the A20 (TNFAIP3) gene facilitates engraftment and safeguards against cell apoptosis triggered by cytokines, while the addition of the MANF gene promotes the proliferation of β-cells and bolsters resistance to inflammatory stress [7].

4. Problems and development prospects

4.1. Problems

Although CRISPR technology has shown great potential in the treatment of T1DM, it still faces many challenges in clinical application due to its certain uncontrollability

4.1.1. Off-target effect

The CRISPR/Cas9 system has an off-target effect during the gene editing process, which can cause unintended effects and side effects by applying the method to cells outside the target, causing unwanted genetic mutations and increasing the risk of cancer. Therefore, how to improve the

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specificity and accuracy of editing, so that the cas9 system can quickly and accurately bind to the receptor cells is still the focus of current research. Secondly, gene-edited cells or non-target cells caused by gene therapy products or off-target effects do not belong to things that already exist in the body, and it is necessary to explore whether long-term survival in the body will bring risks to the body

4.1.2. Rejection Reaction

In the process of clinical transformation, the problem of immune rejection is also an obstacle that cannot be ignored. Immune rejection is one of the body's own protective mechanisms that destroys foreign cells through specific responses. There are also chronic rejection and acute rejection. The reaction time of acute rejection is relatively short, usually within a week, and can be alleviated by immunosuppressive treatment, but chronic rejection may not respond until 3 months later, and it is difficult to alleviate through immunosuppressive treatment, or even pose a threat to life. So preventing immune rejection is also very important. Although editing cells in the body using CRISPR technology can reduce the rejection of the immune system to a certain extent, immune escape cannot be fully achieved, and achieving it is challenging. To this end, researchers are trying to combine CRISPR technology with other immunomodulatory tools, such as genetically engineered "invisible" cells or nanoparticle delivery systems, to improve the survival rate of CRISPR-treated cells and the risk of rejection.

4.1.3. Ethical challenges

A biotechnology must be evaluated from the perspective of risk-benefit and ethics. Because of the limitations of CRISPR technology and complex biological relationships, changing genes may cause ethical conflicts. The CRISPR genome-editing technique presents ethical dilemmas for several key reasons. Firstly, there are significant limitations associated with CRISPR, such as ineffective on-target edits, the possibility of mosaicism, and the risk of unintended off-target modifications. Secondly, there are questions about the long-term viability of species that have been altered: it's crucial to consider whether these changes will have lasting impacts and whether the edited traits could be passed down to future generations, potentially leading to unforeseen consequences [3]. Therefore, if it is not possible to make accurate predictions about species that have carried out CRISPR technology, this technology may also cause many new problems. It proves that this technology is still challenging [8]

4.2. Prospects

4.2.1. Personalized medicine

Autologous cell therapy: Autologous cell therapy combines gene-editing technology with one's own cells and modifies them in vitro to improve their structure and function. Cell therapy offers the opportunity to prevent or reverse TID. Adoptive transfer of autologous cells with enhanced immunomodulatory properties can suppress autoimmunity and preserve islet B cells. This therapy has been made possible through a combination of CRISPR technology in genome editing technology and tolerant cell transplantation. Modified in vitro and perfected on its own. Somatic hematopoietic stem cells and tolerant dendritic cells can protect endogenous and newly generated islet B cells from the patient's autoimmune response without hindering immune monitoring of infectious agents and malignant cell transformation [9].

Control of Epigenetics: Epigenetics is the field that explores histone and DNA variants that alter gene expression without altering the DNA sequence. Researchers have developed ways to express

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and regulate genes without changing their sequence, such as the CRISPR off technique. Heritable changes in gene activity are reversible and can stem from modifications like DNA methylation and histone alterations. These changes predominantly target the gene's promoter region, leading to fluctuations in gene expression levels. It's crucial to recognize that environmental factors can trigger these modifications, which in turn may result in variations in protein production. While controlled epigenetic modifications are vital for cellular growth and division, unchecked alterations can contribute to various health issues. Furthermore, because these epigenetic markers can be passed down to subsequent generations, there is a heightened risk of disease susceptibility. Recent research suggests that epigenetic factors, including microRNA, histone modifications, and DNA methylation, may play a significant role in regulating genes associated with complications from Type 1 Diabetes Mellitus (TIDM) [10]. This technique is highly specific and highly controllable, making it of great potential in treating T1DM. Potential.

4.2.2. Multi-centre clinical research

Find a large number of patients with T1D around the world and classify them according to gender, age and severity of disease. Let them use the same experimental scheme to conduct clinical trials in different locations and units at the same time and end the experiment at the same time, then collect and sort out data from various places and analyse them, and finally summarise the advantages and disadvantages of treating T1D with CRISPR technology, such as off-target rate, rejection rate, etc.

Adjusting the CRISPR-cas9 system according to the advantages and disadvantages is a more accurate and perfect system, which can also make the risk of CRISPR technology lower. Retain these analysis data and establish a large database for future reference.

As CRISPR technology develops, there will be an increasing focus on ethical issues to ensure patient rights and safety. Despite these challenges, CRISPR technology holds great promise for the treatment of TIDM. With the continuous improvement of gene editing tools and the exploration of new therapeutic strategies, it is expected to achieve radical therapy of TIDM in the future. At present, some preclinical studies based on CRISPR technology have achieved remarkable results, such as the successful restoration of normal insulin secretion and blood sugar regulation function in a mouse model in which insulin gene mutation was repaired by gene editing.

This article mainly describes the principle, current situation, experimental model, problems and solutions of T1D. This literature provides us with the idea of completely curing T1D by changing genes. This literature not only provides new ideas for the treatment of T1D, but also provides a basis for the application of personalized medicine in the future. However, before applying CRISPR technology to clinical practice, we still need to solve a series of challenges, including the evaluation of off-target effects, research on long-term safety, immune rejection and discussions on ethical issues. Future research will focus on these problems to ensure the application of CRISPR in the treatment of T1D under the premise of ensuring safety and effectiveness. CRISPR technology has opened up a new direction for the treatment of T1D, but it still needs to be further explored and verified. It is expected that in the near future, this technology can be transformed into clinical application, which will truly benefit the majority of patients and bring higher quality of life and hope for treatment. This also makes it possible to treat T1D fundamentally.

5. Conclusion

This article mainly describes the principle, current situation, experimental model, problems and solutions of T1D. This literature provides us with the idea of completely curing T1D by changing genes. This literature not only provides new ideas for the treatment of T1D, but also provides a basis for the application of personalized medicine in the future. However, before applying CRISPR

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Authors Contribution

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

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