CRISPR-Cas9 System for of Type 1 Diabetes Treatment

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Abstract: Type 1 diabetes (T1D) is one of serious chronic metabolic illnesses, the reason behind is the hyposecretion of pancreatic β cells because of the autoimmune system, and traditional therapy mainly focuses on insulin replacement, which can not reverse the disease process. In recent years, the discovery and rapid development of gene editing technology like the CRISPR/Cas9 system has provided a new idea for the radical treatment of T1D. In this paper, the use of CRISPR/Cas9 technology for T1D treatment is systematically reviewed. First, based on the pathogenesis of T1D, the core links of immune disorder and β cell function loss are described. Secondly, we reviewed the development of CRISPR/Cas9 from a bacterial adaptive immune system to an efficient gene editing tool, and analyzed its targeted DNA cutting and repair mechanism guided by sgRNA. Further, two main application directions of the technique in T1D were discussed: editing immune cells to inhibit autoimmune attack, and inducing stem cells to differentiate into functional β cells or enhancing endogenous β cell regeneration. Current challenges, including off-target effects, low delivery efficiency, and immunogenicity, are analyzed, and some optimization strategies. Finally, we look forward to the future research direction, emphasizing the importance of interdisciplinary research in promoting clinical transformation and providing ideas for future research.

1 INTRODUCTION

Diabetes mellitus is one of serious metabolic illnesses, whose pathogenesis is the lack of insulin and hyperglycemia as an external manifestation. The diabetes mellitus is mainly divided into 2 types---type 1 diabetes and type 2 diabetes. Among them, the treatment of type 1 diabetes is the most difficult. T1D is an autoimmune disease caused by some relative susceptibility genes leading to the error recognition of T cells, they attack and damage the islet β cells then contribute to the abnormal secretion of insulin. Of all the diabetes patients around the world, T1D patients account for about 5%~10% and increase at the speed of 2%~3%. In the whole T1D patients, the major group is children, the T1D patients under 20 years old take up a percentage greater than 85% (Liang & Hu., 2013). They all require lifelong medication therapy.

We have some current developments on treating T1D. For example, insulin injection, which is the most common therapy method, but it requires long-term injection and there may be complications. Patients can also take medicine, and they need to take lifelong intake for treatment. The other methods are immunotherapy and pancreatic islet transplantation. However, immunotherapy has different effects on different patients with complications, and it always has a high price; pancreatic islet transplantation needs high cost as well, and the donor sources are limited. There are still no any complete cures at present.

Nevertheless, gene therapy as an emerging treatment in recent years has the hope to completely cure T1D. Gene editing technology can pinpoint a specific spot in the genome and use specific nucleases to achieve high profiling of the human genome's precise modification. CRISPR/Cas9 system has the most potential among all kinds of gene editing technology. Compared to most other gene editing technologies, the CRISPR/Cas9 system only needs to design 1 sgRNA to edit related genes, which has a simpler operation, lower cost and higher efficiency. CRISPR/Cas9 system can precisely modify stem cells to achieve treatment for T1D. This article will research the development of the CRISPR/Cas9 system in the treatment of type 1 diabetes.

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2 TYPE 1 DIABETES

2.1 Factors Cause Type 1 Diabetes

Type 1 diabetes is one type of diabetes mellitus and belongs to the autoimmune disease. The core mechanism is the attack of T cells (T lymphocytes) to pancreatic β cells. There are a few factors that lead to this mechanism: The first one is the hereditary factor, some susceptibility genes related to T1D may generate to the next generation, which is the necessary basis for T1D. It affects immune recognition, making an abnormal tendency of the recognized function of the immune system and cells, it may lead to the core mechanism above and increase the risk of T1D. The second factor is the environmental factor. Some environmental factors like virus infection and chemical substances may also increase the possibility of pancreatic β cells' dysfunction by mistakenly attacking T cells, finally raising the risk of T1D. The third factor is abnormal autoimmunity regulation. It is the immediate cause of T1D due to the interaction of hereditary genetic factors and environmental factors, which directly leads to T cells' attack on pancreatic β cells.

2.2 The Detailed Mechanism behind

Under the influence of the above factors, T cells attack β cells mainly through 2 ways respectively. The first and also the core pathway is the direct killing by the $CD8^+$T cells which is one type of T lymphocytes. $CD8^+$T cells recognize an antigen on the surface of pancreatic β cells, release perforin and granzymes to form membrane pores and let the granzymes enter the cell, activate the caspase cascade, and induce β cell apoptosis (Atkinson et al.,2011). The second pathway is the indirect killing by the $CD4^+$T cells. On one hand, $CD4^+$T cells secret chemical substances like IFN-γand TNF-α to activate macrophage and aggravate the damage to pancreatic β cells. On the other hand, $CD4^+$T cells can also assist B cells to produce the specific antibodies to damage pancreatic β cells again (Chow et al., 2014). These abnormal responses of T cells are the result of abnormal gene expression.

2.3 The Difficulties of Treating T1D

According to the nosogenesis of T1D, the difficulty of the treatment of T1D is that the constant attack of the immune system is difficult to suppress completely. There are a number of T cells' attack pathways working together to cause the damage of pancreatic β cells. A single-target intervention may not be able to stop the attack altogether. Also the Existing therapies are easily destroyed by the inflammatory microenvironment in the body and are difficult to maintain long-term tolerance. Additionally, Long-term immunosuppression carries a risk of side effects, it can increase the risk of infection, and tumor, and needs long-term medication. However, using gene editing technology like CRISPR/Cas9 system, can make a personalized plan for each patient and achieve precision targeted therapy. Furthermore, it can also promote β cell regeneration and protection, and achieve the effect of long-term treatment.

3 CRISPR/CAS9 SYSTEM COMPOSITION AND WORKING MECHANISM

CRISPR/Cas9 system is a simple and useful tool that efficiently modifies endogenous genes in various species and cell types (Hryhorowicz et al., 2017). It enables targeted gene knockouts, base editing, epigenetic modulation, and therapeutic applications across eukaryotes. In 1987, Japanese scientist Yoshizumi Ishino's team has for the first time identified clusters of regularly spaced short palindromic repeats (CRISPR) in the E. coli genome, but the function is unknown (Ishino et al., 1987). Until 2012, Jennifer Doudna, in collaboration with Emmanuelle Charpentier, demonstrated that Cas9 can cut specific DNA under sgRNA (single-guide RNA) guidance, enabling CRISPR programmability for the first time (Martin et al., 2012), and won the Nobel Prize in Chemistry in 2020. At present, CRISPR/Cas9 technology was applied in multiple fields, including but not limited to the treatment of diseases such as cancer, agriculture and ecological applications.

3.1 Composition of the CRISPR/Cas9

CRISPR/Cas9 system is derived from an adaptive immune mechanism to resist the invasion of foreign genetic materials like bacteriophages. It consists of Cas9 nuclease and single guide RNA (sgRNA). Cas9 nuclease comes from bacteria and archaea, it plays a key role in recognizing and cutting both strains of foreign DNA. sgRNA is an artificially engineered RNA sequence from crRNA (CRISPR RNA) with specific targeting at the 3 'end and tracrRNA (tans-

acting CRISPR RNA) produced by trans-activated ribonucleic acid gene expression at the 5' end.

As for the design and synthesis of sgRNA. To design the sgRNA, first, Identify the target gene locus you want to edit according to the protospacer adjacent motif (PAM) sequence. Secondly, select an appropriate length and scaffold sequence for a specific secondary structure to make sure it can accurately match with the target DNA sequence. Next comes to the synthesis of sgRNA, there are 2 different approaches. One is chemical synthesis: by chemical synthesis technology, accurately controls the sequence and modification of nucleotides, to produce sgRNA, but it is difficult and low-yield (Hoy et al., 2022). Another one is In vitro transcription synthesis: the DNA template encoding sgRNA is synthesized and then transcribed in vitro by RNA polymerase to produce sgRNA. The design and synthesis of sgRNA make sure the sgRNA will only bind to the target sequence but not other regions of the genome.

3.2 Working Mechanism of the CRISPR/Cas9 System

After having a specific sequence of sgRNA which is complementary to the target mutant gene sequence affecting pancreatic β cell in the genome, we extract the multipotential stem cells from a patient and introduce the CRISPR-Cas9 system into the extracted stem cells (through carrier). The sgRNA binds to the specific part of DNA, followed by Cas9 nuclease. The Cas9 binds to the sgRNA and corresponding DNA, and makes a cut across both strands of the DNA. The cell recognizes that the DNA is damaged and tries to repair it. It activates its own DNA repair mechanisms, then we start to intervene.

As the figure 1 shown, the process to repair the severed DNA also have 2 different methods. The first one is non-homologous end joining (NHEJ), it means when the double strains break, it quickly joins the two ends of the broken DNA together, and in this process, there is always some insertion or deletion of genes at the junction part. Thus genes that enhance the function of islet beta cells can be introduced in and abnormal genes that impair the function of pancreatic β cells can be knocked out. The second method is homologous directed repair (HDR), it needs to offer a foreign DNA template homologous to the sequence at both ends of the broken DNA. When the cell starts to repair, it will accurately integrate the genetic information on the template into the break site to achieve accurate gene editing-like replacement. It repairs the mutated gene sequence to a normal one, returning the pancreatic β cells' function to normal. After that, the edited stem cells are induced to differentiate into pancreatic β cells and transplanted back into the patient's body to return to the normal function of the genes, thus promoting the normal work of pancreatic β cells and synthesis of insulin. (Lotfi et al., 2023)

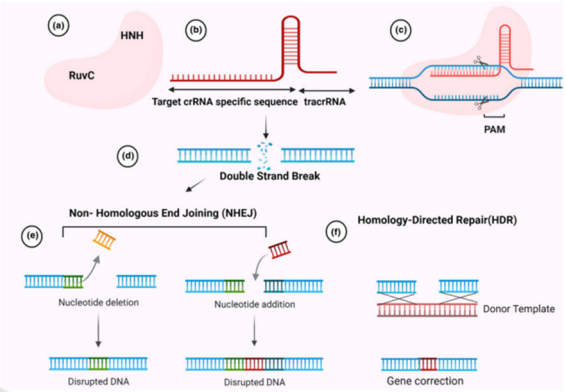

Figure 1: Two Process of the CRISPR/Cas9 System's Mechanism (Lotfi et al., 2023).

4 CURRENTLY USED IN THE TREATMENT OF T1D

The application of the CRISPR/Cas9 system in the treatment of T1D is currently in the early stage of research, focusing on gene-edited immune cells, islet cell regeneration, and immune tolerance induction.

4.1 Suppress Autoimmune Responses

Luo's team used the CRISPR/Cas9 system to knock down the expression of co-stimulatory molecules on the DCs surface. Cationic lipid-assisted PEG-b-PLGA nanoparticles (CLAN) were used as the delivery system and vector, then injected into the type 1 diabetes model mice, finally found that blood sugar levels were significantly reduced in the treatment group and damage to islet beta cells was reduced by 50 percent. It inhibits the ability to activate effector T cells and suppress the autoimmune responses (Luo et al., 2020). More innovatively, Martina S Hunt's team used CRISPR/Cas9 system through dual-locus, dual-HDR editing to achieve double HDR synergism, using the RNP complex formed by purified Cas9 protein and sgRNA. It enables Treg to obtain antigen-specific recognition ability while enhancing the expression stability of FOXP3, which prevents Treg

from transforming into effector T cells in an inflammatory environment. This directly inhibits the activation of autoreactive T cells, thereby restoring the immune system's tolerance to autoantigens. (Hunt et al., 2023)

4.2 Regeneration of Pancreatic β Cells

In 2006, for the first time, Samson S L and Chan L's team systematically demonstrated the feasibility of gene therapy PDX-1, NeuroD/BETA2 and Neurogenin 3 to promote the regeneration and functional reconstruction of β cells by transdifferentiation of non-endocrine cells. (Samson S. L. & Chan L. 2006). Although limited by the technology of the time, it laid the theoretical foundation for the breakthrough technology of CRISPR/Cas9. After that, Saleh's team reviews the potential of pancreatic α cell to β cell transdifferentiation in the treatment of diabetes mellitus, focusing on the mechanism of reprogramming α cells into insulin-secreting cells induced by gene editing (such as overexpression of PDX-1) or small molecule compounds. The effect of improving blood glucose homeostasis in animal models was verified. (Saleh et al., 2021)

5 CHALLENGS AND PROSPECTS

5.1 Limitation of CRISPR/Cas9 System

The first limitation is the delivery challenge. Until now, there are many ways to introduce the CRISPR/Cas9 system into the cells. For example, viral vectors like lentivirus and non-viral vectors like plasmid vectors. However, all of them have their own positive and negative aspect. We still need to find a safer and more efficient method to deliver the CRISPR/Cas9 system. Next the immunoreaction challenge is also important. The injection of Cas9 nuclease as a foreign substance, it will activate the immune reaction inside the human body, attacking and destroying the Cas9 enzyme, also prevents the gene editing and affects CRISPR/Cas9 system effectiveness. The third one is an off-targeting challenge. CRISPR/Cas9 has a high rate of off-targeting. The sgRNA may bind with incorrect DNA sequence and lead to gene editing errors with a high rate of gene mutation. The fourth restriction is an ethical challenge. There are still many ethical and social concerns about using CRISPR/Cas9 technology to change the gene sequences in embryo cells. The final one is a technical challenge. The technology and machinery need to be very advanced. In addition, facing different patients, we need to consider changing situations and produce different gRNA, which will contribute to a high cost of time and money. We still need to find a cheap and more accurate way to use the CRISPR/Cas9 system and make sure everyone's type 1 diabetes can be treated. (Cheng et al., 2023)

5.2 Improvement

To deal with these limitations, we can search for new delivery carriers to optimize the delivery system, or attempt to deliver Cas9 mRNA instead of protein, reducing immunogenicity and improving expression efficiency and delivery efficiency. Try a double sgRNA design. using two sgRNA to simultaneously target both ends of the target region may improve specificity. Try to scale and automate production, and simplify the production process, reduce the production cost. Let everyone afford this treatment.

6 CONCLUSION

To sum up, CRISPR/Cas9 technology for Type 1 Diabetes (T1D) treatment represents a groundbreaking frontier in both gene editing and diabetes research. This review has systematically explored the pathogenesis of T1D, the historical development and mechanistic principles of CRISPR/Cas9, and its transformative potential in addressing the challenges of T1D. Key findings highlight the versatility of CRISPR/Cas9 in targeting immune dysregulation, promoting β-cell regeneration, and inducing immune tolerance, offering hope for a curative approach to this chronic autoimmune disease. Apart from its promise, CRISPR/Cas9 still faces challenges such as off-target effects, immunogenicity, and delivery efficiency. The design of high-fidelity Cas9 variants, new delivery systems, and immune escape strategies is gradually addressing these issues. The research on the CRISPR/Cas9 system for treating type 1 diabetes treatment can show the potential of this technology in treating type 1 diabetes and promoting the clinical transformation. While significant progress has been made, we still need to explore the way to improve specificity and safety, optimize delivery strategy, and more multi-disciplinary collaboration to try to reduce

the cost of treatment, so that everyone can achieve the treatment of disease.

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