CRISPR/CAS9 gene editing
in diabetes mellitus: a future cure

Edición génica mediada por CRISPR/CAS9 en diabetes mellitus: una cura futura

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Abstract

Current advances in gene therapy have shown great potential in experimental studies for several disorders, including diabetes mellitus (DM). Site-specific gene editing has been enabled by clustered regularly interspaced short palindromic repeats (CRISPR) and its CRISPR-associated protein 9 (Cas9), a third-generation nuclease that causes double-strand DNA to break. This process allows the insertion, deletion or silencing of any desired gene. Studies in mice have demonstrated that diabetic status can be reversed by specific gene targeting. Other studies have targeted obesity and obesity-related genes as an alternative to improve insulin resistance and provide better metabolic homeostasis. There is ongoing investigation to identify the best possible strategy to treat DM with the CRISPR system. This review aims to analyze different experimental gene therapy studies mediated by CRISPR-Cas9 to determine their efficacy, safety profile and reliability as a possible future cure for DM. Likewise, basic concepts regarding CRISPR/cas9 will be reviewed.

***Keywords**: Diabetes mellitus, CRISPR-Cas9, gene therapy, gene editing, diabetes therapy.*

Resumen

Los avances actuales en la terapia génica han mostrado un gran potencial en estudios experimentales para varios trastornos, incluida la diabetes mellitus (DM). La edición de genes específicos del sitio ha sido posible gracias a las repeticiones palindrómicas cortas agrupadas y regularmente interespaciadas (CRISPR) y su proteína 9 asociada a CRISPR (Cas9), una nucleasa de tercera generación que hace que se rompa el ADN de doble cadena. Este proceso permite la inserción, eliminación o silenciamiento de cualquier gen deseado. Los estudios en ratones han demostrado que el estado diabético puede revertirse mediante la selección de genes específicos. Otros estudios se han centrado en la obesidad y los genes relacionados con la obesidad como una alternativa para mejorar la resistencia a la insulina y proporcionar una mejor homeostasis metabólica. Hay una investigación en curso para identificar la mejor estrategia posible para el tratamiento de la DM con el sistema CRISPR. Esta revisión tiene como objetivo analizar diferentes estudios de terapia génica experimental mediada por CRISPR-Cas9 para determinar su eficacia, perfil de seguridad y confiabilidad como una posible cura futura para la DM. Asimismo, se revisarán conceptos básicos sobre CRISPR/cas9.

Palabras clave: Diabetes mellitus, CRISPR-Cas9, terapia génica, edición de genes, terapia de la diabetes.

Introduction

Diabetes mellitus (DM) is a chronic non-communicable disease characterized by hyperglycemia resulting from impaired insulin secretion and insulin resistance, or complete lack of insulin due to pancreatic damage[1]. DM is among the top 10 causes of death globally, and remains one of the most prominent global public health issues[2]. The global economic burden of DM amounted to U.S $1.3 trillion in 2015, and it is expected to grow to over 2 trillion by 2030[3]. Individuals with DM have an increased risk of developing many comorbidities such as cardiovascular disease (CVD), chronic kidney disease (CKD), cancer, complicated infections and many others[4]. As a result, patients with DM have double to triple risk of all-cause mortality, higher admission rates to the intensive care unit (ICU) and higher in-hospital mortality[5]. Although progress has been made in promoting preventive measures for DM, it remains the second most significant condition to reduce life expectancy worldwide[6].

Current DM treatment recommendations focus on maintaining normoglycemia to prevent or delay adverse outcomes such as CVD and CKD. Treatment options vary according to the type of DM, going from different types of insulin in type 1 DM (DM1), to oral hypoglycemic drugs in type 2 DM (DM2)[7]. Although evidence shows that proper glycemic management decreases the overall risk of complications and mortality, to date, there is still no approved treatment capable of curing any form of DM[8]. In consequence, diabetic patients are forced to take medications permanently. Considering that most of these medications have considerable side effects, like hypoglycemia, and those administered parenterally on a daily basis may be cumbersome, it is not infrequent that patients show low adherence. Independent of the reason, low adherence is associated with suboptimal prevention of complications[9].

The search for a cure for DM has been a never-ending quest in the medical field, and significant progress has been made in the last decade. Current advances in gene therapy have shown great potential in experimental studies. Site-specific gene editing has been enabled by clustered regularly interspaced short palindromic repeats (CRISPR) and its CRISPR-associated protein 9 (Cas9), a third-generation nuclease that causes double-strand DNA to break. This process allows the insertion, deletion or silencing of any desired gene[10]. This review aims to analyze different experimental gene therapy studies mediated by CRISPR-Cas9 to determine their efficacy, safety profile and reliability as a possible future cure for DM. Likewise, basic concepts regarding CRISPR/cas9 will be reviewed.

CRISPR-CAS9 BASICS: STRUCTURE AND MECHANISM

The CRISPR/Cas9 system is a bacterial defense mechanism against bacteriophages and plasmids that mimics an adaptive immune system. The immunization process following the invasion of foreign genetic material from phages or plasmids consists of integrating short fragments of foreign DNA into the CRISPR sequence array within the host chromosome as new spacers[11]. The incorporation of genetic material serves as a means of recording prior infections, enabling the host to prevent future invasions[12]. Subsequent transcription of the CRISPR array will yield short mature CRISPR RNAs (crRNAs). At the 5′ end, the crRNA contains a spacer that complements a sequence from a foreign genetic element, and the 3′ end contains the CRISPR repeat sequence. Interaction between the crRNA with its complementary foreign target sequence will trigger the destruction of the invading DNA or RNA by Cas nucleases upon the second infection[13].

CRISPR systems have been divided into six groups (I-VI) according to the current classification of CRISPR-Cas loci. Every group has a unique set of Cas proteins and different means of aging[14]. The type 2 CRISPR system employs a single DNA endonuclease, Cas9, to recognize DNA sequences and cleave each strand with a distinct nuclease domain, namely HNH and RuvC domains[15]. Proper maturation of type 2 systems requires another noncoding RNA, called the trans-activating crRNA (tracrRNA), to pair with the repeat sequence in the crRNA to form a hybrid RNA structure[16]. This dual-RNA directs Cas9 to cleave any DNA containing a complementary 20-nucleotide (nt) sequence along with the corresponding adjacent protospacer adjacent motif (PAM)[15]. To simplify the previous components, it is possible to combine the crRNA and the tracrRNA into a single RNA transcript known as chimeric single guide RNA (sgRNA) while retaining complete functionality of the CRISPR-Cas9 system[17].

Furthermore, changing the spacer within the crRNA allows the two-component CRISPR-Cas9 system to target virtually any DNA sequence of interest to generate a site-specific blunt-ended double-strand break (DSB)[18]. The DSB created by the system is then repaired by error-prone nonhomologous end joining, resulting in small insertions or deletions (indels) at the breaking point[19]. Precision in this process depends on PAM recognition and later DNA interrogation, which needs to be entirely complementary to the sgRNA; otherwise, Cas9 will not separate the DNA strands and will continue to analyze DNA until it finds a perfect match[20]. Since DNA recognition is mediated by a 20-nt guide RNA sequence rather than a protein, this alternative offers a much simpler production process than other DNA editing techniques like zinc finger nucleases[21]. Given its simplicity, high efficiency and broad applicability, the scientific community has put significant effort into adopting the CRISPR-Cas9 system to treat or even cure several diseases, like DM.

CRISPR-CAS9 AND DIABETES MELLITUS: WHERE ARE WE GOING?

It is well known that genetics play a vital role, to a variable extent, in the development of all types of DM[22]. Many approaches have been proposed regarding CRISPR/Cas9 implementation in DM; however, since this method is rela-

tively new, evidence regarding its efficiency is still lacking in human models. For example, CRISPR systems have been applied to drive differentiation, transdifferentiation, and reprogramming of various mouse and human cell types. Notably, the variety of genes tested with the CRISPR system to target DM range from those implicated in obesity to DM itself, giving plenty of room for experimentation in animal models.

Wang et al.[23] designed four different sgRNA targeting around 50 to 150 base pairs (bp) upstream of the human *UCP1* gene; along with a synergistic activation mediator (SAM) to boost CRISPR activity. The uncoupling protein 1 (UCP1) is involved in non-shivering adaptative thermogenesis, and its expression is correlated with increased energy expenditure, making it a tentative option for anti-obesity treatment. The latter CRISPR-SAM model was applied to human white preadipocytes from two subjects. Results indicated that *UCP1* messenger RNA (mRNA) increased 6000-fold with a 20-fold increase in UCP1 protein expression. Obese mice that developed human brown-like adipocytes showed a sustained improvement in glucose tolerance and insulin sensitivity. Despite the study's encouraging results, the authors stated that there were some limitations and that further investigation is needed to achieve human applicability.

Similarly, Chung et al.[24] developed a sgRNA to target the *Fabp4* gene in white adipocytes. This gene was targeted based on the evidence that silencing the fatty acid binding protein 4 (FABP4) was proven to be an effective strategy to induce body weight loss and metabolic recovery in high-fat diet-induced mice[25]. Targeted delivery of the CRISPR system against FABP4 in white adipocytes reduced the expression of this protein. The latter resulted in body weight reduction and reduced inflammation with the restoration of hepatic steatosis in obese mice. Moreover, insulin sensitivity was significantly improved in the treated mice. The authors stated that this method provided a simple and safe approach to treat obesity and obesity-induced complications while simultaneously improving several metabolic variables such as insulin sensitivity[24].

On the other hand, implementing induced pluripotent stem cells (iPSCs) offers an exciting alternative for the treatment of DM. Previously, Nandal et al.[26] designed a step-by-step protocol for deriving iPSCs from human pancreatic cells using the CRISPR-Cas9 system. Different studies have demonstrated that the CRISPR system can be used to reduce the time and cost of chimeric organ generation significantly. However, immune tolerance must improve before the technique can be used in humans[27]. Moreover, The Rezania group demonstrated that their human embryonic S7 cells significantly outperformed the iPSCs from pancreatic progenitors in several aspects. Firstly, the S7 cells displayed proper glucose responsiveness while reversing DM four times faster than iPSCs. Furthermore, iPSCs did not produce as many insulin-producing cells as their human embryonic stem cells counterpart. More investigation is needed to optimize the viability of the CRISPR system in cell therapy[28].

Approaching from another perspective, Bevacqua et al.[29] designed a CRISPR/Cas9 model to target the *PDX1* gene and applied it through lentiviral transduction in a culture of islet cells. Authors chose *PDX1* because it encodes a transcription factor crucial for β-cell function and which is mutated in human diabetes[30]. Indels were detected in approximately 66% of sequences by PCR. This method reported a significant reduction of the expression of PDX1 protein in the group of cells treated with the CRISPR system; thus, specific targeting of *PDX1* was possible. Data reported that *PDX1* loss led to impaired physiological function of β-cell. Similar results were found when targeting the *KCNJ11* gene, which resulted in β-cell loss of function. Overall, the author concluded that CRISPR/Cas9 was an effective method to target any gene of interest in human islet cells and that it was equally possible to improve β-cell function as it was to impair it[29].

The major challenge that opposes CRISPR/Cas9-based therapeutics is the delivery to the target site. Viral delivery is the primary choice for gene therapy, but there is a significant possibility of severe immunogenicity[31]. For that reason, nanoparticles (NP) have been proposed to fulfill the challenge of targeted delivery in genome editing. Polymer-based NP has been used to transport CRISPR systems and other silencing techniques, targeting genes like *BMP9* and *PEPCK*, effectively reducing glucose levels and improved glucose tolerance while reducing inflammation and insulin resistance[10]. Although there has been extensive ongoing research to explore an effective method for the possibility of curing DM, more effort is needed to overcome the limitations inherent to this method and to achieve the target of curing this disease.

Conclusion

CRISPR/Cas9 has opened many possibilities as a genome editing tool. These possibilities have been explored to target important diseases like DM. Current evidence supports that CRISPR/Cas9 can effectively target any gene in virtually any cell type. This has led researchers to provide different approaches to treat or even cure DM. Studies in mice have demonstrated that diabetic status can be reversed by specific gene targeting. Other studies have targeted obesity and obesity-related genes as an alternative to improve insulin resistance and provide better metabolic homeostasis. There is ongoing investigation to identify the best possible strategy to treat DM with the CRISPR system. However, evidence in humans is still lacking and will be for a considerable amount of time until the challenge of tissue-specific delivery of CRISPR system in the absence of viral mediators is addressed. Although experimental models have shown encouraging results, more research is needed before CRISPR becomes a reality.

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