HIGH SCHOOL EDITION
Journal of Student Research
Volume 13 Issue 1 (2024)

Genome Editing Technologies in The Treatment of Diabetes

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ABSTRACT

This research paper will first introduce diabetes as a disease and briefly talk about all three types of diabetes as well. The symptoms, diagnosis, and consequences of having diabetes will also be discussed. Later, how the research question, "What are the various types of genome editing technologies that can be used to treat diabetes and what are their consequences?", relates to diabetes and why it is important would be explained, along with a possible hypothesis that it could answer. In addition to that, how the research question tries to resolve the problem of diabetes would be underlined and a few solutions would be skimmed upon, which are the genome editing technologies: ZFN, TALENS, and CRISPR.

Introduction

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces.[1] Insulin is a hormone that regulates blood glucose. This question is interesting because it explores novel and effective methods for treating diabetes, a prevalent chronic disease, mainly caused by heredity. Thus, it leads us to the method of dealing with genes, specifically via genome editing as in this study. Finding a cure for this disease is of utmost importance, because if left untreated, diabetes can also cause other predicaments such as heart disease, vision loss, and kidney disease. This study aims to investigate various genome editing technologies such as HR, ZNF, TALEN, and CRISPR to treat diabetes. This study hypothesizes that such genome editing technologies will successfully mitigate diabetes, but there will be side effects too.

Discussion

Types of Diabetes

A lower percentage of diabetics have type 1 diabetes (T1D), which is mostly caused by immune cells destroying pancreatic beta cells. Before a diagnosis is made, autoantibodies that target the beta cells that produce insulin may be present in the blood for years. However, having these autoantibodies does not ensure that a person would experience the illness. Individuals with type 1 diabetes need to regularly check their blood glucose levels and receive injections to replenish the insulin that their bodies are unable to produce. Early nutrition may also be important. There is evidence, according to a 2017 study, that breastfeeding reduces the risk of type 1 diabetes in children. In addition to having a higher chance of developing type 1 diabetes, people with this condition may also have Graves' disease, Hashimoto's thyroiditis, celiac disease, and pernicious anaemia. While having a family history of type 1 diabetes may make a person more likely to have it, the inheritance pattern is typically unclear and is still being studied. [2]

In most cases, type 2 diabetes is caused by insulin resistance, but pancreatic b cell loss is the ultimate cause. The pancreas initially produces more insulin to compensate for this, but eventually, is insufficient to maintain normal blood glucose levels. Type 2 diabetes affects 90 to 95 percent of the population overall, and it usually strikes those over 45. Obesity and being overweight are significant risk factors for type 2 diabetes. If a person in their ethnic group has a body mass index (BMI), a measurement of body weight based on weight in relation to height, of 25 or higher, they are at risk for type 2 diabetes. With type 2, the impact of family history on the likelihood of developing diabetes is more well-established than with type 1. Determining whether a shared environment and way of life or only genes are responsible for such influence can be challenging.[2]

Looking from a genetic perspective, diabetes can be further divided into two types – monogenic and polygenic. Monogenic refers to the condition being tested for a single gene. One of the most popular monogenic forms of diabetes is MODY (maturity-onset diabetes of the young). Certain types of MODY cause blood sugar levels to stay slightly elevated for the whole of a person's life, with no or very few symptoms and no problems. Insulin or a class of oral drugs known as sulfonylureas, which stimulate insulin release from beta cells, may be necessary for treating other types. Forms of MODY have been associated with at least ten genes. The GCK or HNF1A genes are the ones that have the highest mutations. Type 1 and type 2, the most prevalent types of diabetes, are polygenic, which means that alterations or defects in numerous genes are linked to the disease. Polygenic forms of diabetes are also influenced by environmental variables, such as obesity in the case of type 2 diabetes. Diabetes that is polygenic frequently runs in families. By monitoring blood glucose, sometimes called blood sugar, in people with diabetes risk factors or symptoms, doctors can identify polygenic variants of the disease.[2]

Methods of Genome Editing

Molecular "scissors" must be used to make targeted DNA sections accessible so DNA repair machinery can insert an interesting sequence. Zinc finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN) are two examples of such tools. The DNA binding domains of each engineered DNA restriction enzyme are combined with a DNA cleavage domain to create both. While TALEN binds to target DNA using the humanized bacterial transcription activator-like effector, ZFN employs the zinc finger protein repetitions. In both situations, a catalytically active FokI restriction endonuclease serves as the DNA cleavage domain, efficiently cutting both strands to cause a double-strand break (DSB) in the DNA. The cell then employs two DNA repair pathways to fix DSB: the non-homologous end-joining (NHEJ) pathway, which frequently results in mutations or deletions at the DNA cutting site, and homology-directed repair (HDR), a method for repair of DNA double-stranded breaks can be leveraged for the precise introduction of mutations supplied by synthetic DNA donors (Figure 1). [3]

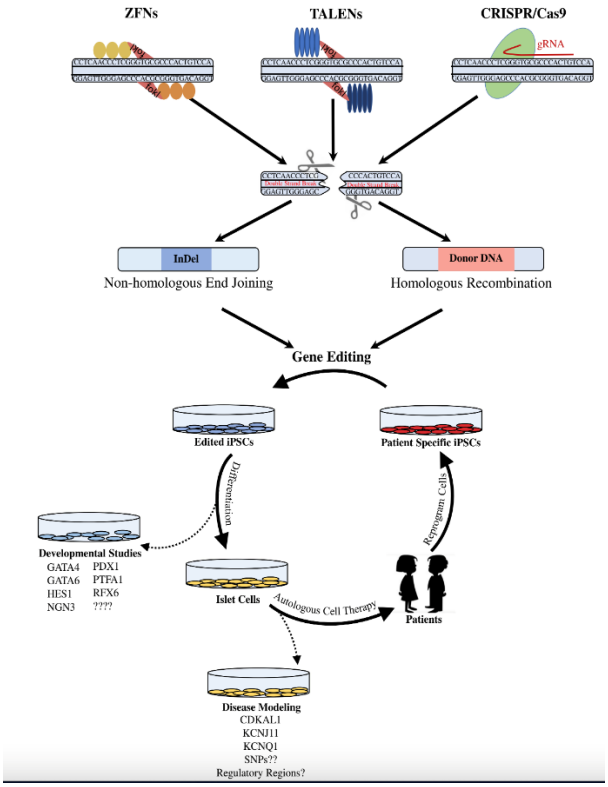

Figure 1. [3]

ZFN

ZFNs have been employed to alternate genes in various species and cell types. ZFNs allow researchers to introduce various genomic changes, such as point mutations, deletions, insertions, inversions, duplications, and translocations. This gives them access to previously unheard-of tools for manipulating the human genome. Additionally, ZFNs may have medicinal use, such as treating diabetes.

Insulin-stimulated glucose uptake is made easier in peripheral tissues, such as adipose, muscle, and the heart, by the glucose transporter GLUT4 (Figure 2). GLUT4 function is compromised in type 2 diabetes and obesity, causing hyperglycemia and raising the risk of cardiovascular disease, illness and nerve damage. Moreover, ZFP407 (zinc finger protein) controls adipocytes' glucose absorption in response to insulin stimulation. A reduction in GLUT4 mRNA and protein levels was thought to cause the decline in insulin-stimulated glucose absorption brought on by ZFP407 deficiency. Therefore, a novel treatment target for type 2 diabetes is ZFP407.

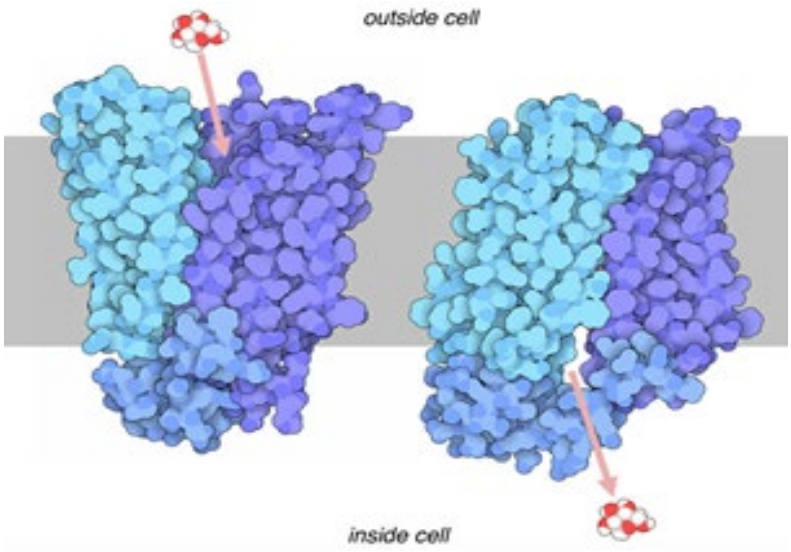

Figure 2.[10] Glucose transporters in cells

TALENs

Transcriptional activator-like effector nucleases (TALENs) are a new class of nucleases that target almost any sequence by fusing a nonspecific DNA-cleaving nuclease to a DNA-binding domain. The prospective application of TALENs to rapidly and effectively modify genes holds great promise for advancing biological research and producing novel treatment approaches for hereditary disorders.[5]

Targetable nucleases are employed to cause double-strand breaks (DSBs) at certain DNA regions. These DSBs are subsequently repaired by mechanisms that allow for the manipulation of sequences at the cleavage site.

Nonspecific DNA-splitting nuclease and an easily designed DNA binding domain are recent developments in TALEN technology that efficiently target diverse highly replicated gene sequences that cause diabetes such as TCF7L2, KCNQ1 and KCNJ11.

Comparable to ZFNs, TALENs consist of a customisable DNA-binding domain linked to a nonspecific FokI nuclease domain. The highly conserved repetitions that make up this DNA-binding domain come from proteins called transcription activator-like effectors (TALEs), which are produced by certain Xanthomonas species of bacteria. Following assembly, the TALEN constructs are introduced onto plasmids, which are subsequently transfected into the target cells. This allows the gene products to be produced and to reach the nucleus to access the genome. As an alternative, TALEN constructs can be introduced into the cells as mRNAs, eliminating the chance of the TALEN-expressing protein integrating into the genome. The success of introgression during gene editing and the degree of homology-directed repair (HDR) can be significantly increased by using an mRNA vector. DNA from either side of a double-strand break with little to no sequence overlap for annealing can be directly ligated by non-homologous end joining (NHEJ). Through chromosomal rearrangement or indels (insertions or deletions), this repair mechanism introduces defects into the genome that could make the gene products coding for that region non-functional.[4]

Thus, non-specialist researchers can use TALENs due to their high rates of cleavage activity, ease of construction, and nearly infinite targeting range.[4]

CRISPR

The adaptive resistance of prokaryotes to bacteriophages, invasive plasmids, or viruses is the source of CRISPR/cas. After processing, the RNA containing the leader sequence, palindromic sequence, and protospacer

is known as a CRISPR RNA (crRNA). The complementary protospacer region of these crRNAs directs them toward foreign DNA, and Cas proteins are responsible for their recognition. Nucleic acids complementary to the crRNA can be cleaved by mature Cas complexes. A second transactivating crRNA (tracrRNA) pair with the repetitive sequence in the crRNA triggers the Cas9 endonuclease activity in the instance of Cas9, a type II Cas protein. Research indicates that a single-guide RNA (sgRNA) containing both tracrRNA and crRNA could be specifically designed to target and activate Cas9 endonuclease activity across the mammalian genome at the same time. The capacity to coordinate the expression of the Cas9 protein is a versatile modular system that may effectively and affordably target genomic regions for editing by utilizing any number of custom-designed sgRNA. [6]

There are uses for CRISPR/Cas9 technology besides genome editing. The catalytically inactive cas9 (dCas9) can be attached to other proteins to alter gene expression if both endonuclease domains are deactivated. Through the dCas9-VP16 fusion, gene expression can be turned on, and RNA polymerase can be blocked by the dCas9-KRAB fusion, which represses gene expression. According to a recent publication, fibroblasts from patients with type 1 diabetes had their insulin gene expression activated using the dCas9 technology. [6]

dCas9 activators and multiple sgRNAs can target the endogenous activation of MSC chemokine receptors and pancreatic transcription factors in MSCs. This could lead to the differentiation of MSCs into surrogate IPCs that can be transplanted and expanded ex vivo while retaining their immunomodulatory characteristics. To do this, one study has shown that it is possible to successfully activate endogenous human insulin transcription in HEK293T, Hela, and human fibroblasts by using the dCas9-VP160 fusion and numerous insulin promoters targeting sgRNAs. The findings of this work lend credence to the unique application of endogenous genes, such as Pdx-1, Neurod1, MafA, and others, involved in pancreatic development being activated transcriptionally. The possibility of creating effective MSC-derived treatments for Type 1 diabetes increases when paired with the targeted activation and maintenance of genes involved in MSC immunomodulation, such as chemokine receptors and soluble factor synthesis. [6]

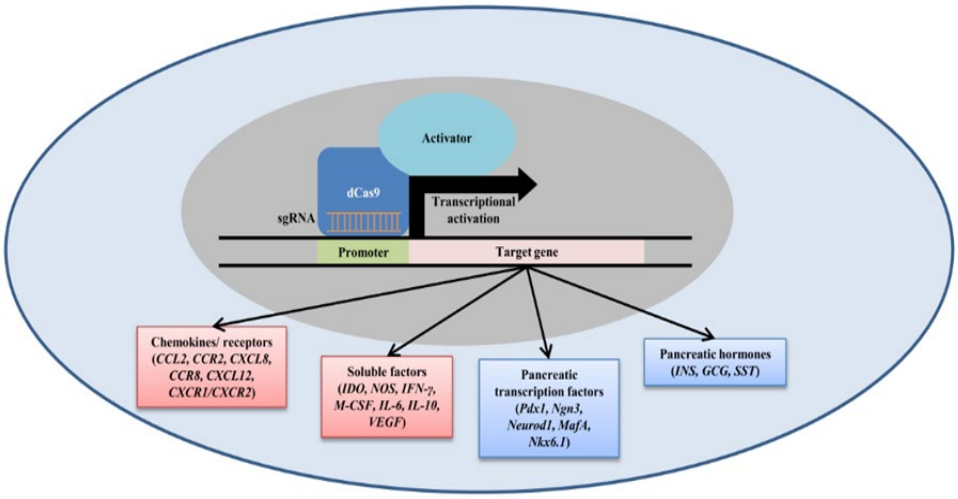

Figure 3. [7]

If the cells were not protected in a macroencapsulation vessel, current allogenic SC-β replacement therapies would require lifelong immunosuppression of the patient. While encapsulation devices offer a potentially excellent long-term solution to the autoimmunity issue in type 1 diabetic patients, CRISPR/Cas9 gene editing may be used to create beta cells that are immune system resistant.[7]

When combined, the modularity of the CRISPR/Cas9 system creates an adaptable, multipurpose platform that can be used to research disease, development, and drug discovery by varying gene editing, activation, and repression.

Evaluating The Three Types of Gene Editing Tools

TALEN is more commonly used than ZFN because of its more sophisticated DNA binding design, which allows it to be tailored to bind to almost any desired DNA target. TALEN has been utilized to inactivate many transcription factors related to diabetes in human iPSC cells.

The complexity of both ZFN and TALEN-based techniques' designs is a drawback. To cleave DNA, the FokI endonuclease needs two ZFNs or TALENs to target non-palindromic DNA locations since it needs to dimerize at the DNA binding domain. Designing the DNA binding domain is extremely challenging, particularly when using ZFN. As a result, creating the necessary DNAs calls for a high level of proficiency in molecular cloning and computational design.[8]

CRISPR/Cas9 has better targeting efficiency and less cytotoxicity than ZFN and TALEN gene editing, particularly when several sgRNAs target the same gene. Despite being extremely targeted, CRISPR technology also exhibits off-target binding, which can result in off-target insertions, deletions, or translocations. This phenomenon may be caused by mismatch tolerance between genomic DNA and gRNAs. However, off-target binding can be reduced by using Cas9 binding specificity in the bioinformatic design of sgRNAs. Cas9 mutant variations have more accurate targeting.

Conclusion

It has now been determined that approximately ten sites in the human genome appear to be vulnerable to Type 1 diabetes especially. These include two genes: the glucokinase (GCK) gene on chromosome 7, which is important for glucose metabolism and helps regulate insulin secretion, and the gene at the locus IDDM2 on chromosome 11. Using candidate gene and linkage-based investigations, only a few Type 2 diabetes risk genes were found; however, with the development of genome-wide association studies, several genes have been found, including many that were not previously thought to be involved in Type 2 diabetes: KCNQ1, KCNJ11, and TCF7L2.[9] Here, all the genome editing techniques mentioned above can be used to attempt treating diabetes.

CRISPR is by far one of the best genome editing techniques compared to the already existing ones. Target site recognition is mediated solely by the gRNA, making CRISPR-Cas9 the most versatile and intuitive genome editing method. This eliminates the need to design additional proteins to recognise every possible target site. Even though immunogenicity, off-targeting, polymorphism, delivery method, and ethics are several major concerns with the CRISPR/Cas9 system, there are also possible strategies that could be used to tackle these problems such as implementing the CRISPR/Cas system for gene editing early in a lifetime, targeting immune-privileged organs, bioinformatics tools to design more accurate gRNA and predict off-targeting, use of Cas9 nickases (Cas9 nickase has a different breaking mechanism than the normal Cas9 protein), and adding anti-CRISPR proteins.[9] Therefore, its strengths outweigh its weaknesses, making it the ideal genome editing tool for treating diabetes.

Acknowledgments

I would like to thank Ms. Danya Chowdhury for being my mentor and piquing my curiosity in the field of medicine, thus inspiring me to write this paper.

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