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Emerging Applications of CRISPR/Cas9 Technology in the Treatment of Diabetes Mellitus: A Narrative Review

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Abstract

Diabetes mellitus (DM), both Type 1 (T1DM) and Type 2 (T2DM), remains a global health threat. The causes of T1DM are autoimmune destruction of β cells, whereas T2DM is caused by insulin resistance and β-cell dysfunction. Current treatments manage the disease but do not address the genetic or cellular abnormalities. CRISPR/Cas9 is an extremely potent gene editing tool that can potentially cure diabetes by correcting genetic mutations, controlling gene expression, and regenerating β cells. In the CRISPR systems of T1DM, CRISPR technology has been employed to create immune-evasive β-like cells from iPSCs by editing HLA genes and immune checkpoints. In T2DM, specific disease-causing genes such as TCF7L2, GLUT4, and SLC30A8 have been edited to improve insulin regulation. Further, editing of PPARG and FOXO1 has been found to improve insulin sensitivity and suppress hepatic glucose production. GCK (glucokinase), one of the important regulators of glucose sensing in β-cells, has also been a target to increase insulin secretion and normalise glucose homeostasis. The delivery systems are adeno-associated viruses, lipid nanoparticles, and electroporation, among others. Novel tools like Cpf1 improve editing efficiency, particularly in non-dividing cells. Nanocarriers have also improved delivery

specificity and reduced degradation. Promising results have been seen with targeting metabolic enzymes like DPP-4 to preclude T2DM dysfunction. However, issues such as off-target effects, immune reactions, mosaicism, limitations in delivery, costs, and ethical viability still remain. Overcoming all these limitations, CRISPR-based approaches have high potential in overcoming treatment control for symptoms and offering long-term, possibly curative, diabetes therapies. Future research is required to refine delivery, enhance safety, and investigate long-term human effects.

Keywords: CRISPR-Cas Systems, Gene Editing, Diabetes Mellitus Type1, Diabetes Mellitus Type 2, Pancreatic Beta Cells, Insulin Resistance, Gene Delivery Systems

Introduction

Diabetes Mellitus (DM) is a metabolic disease in which hyperglycemia occurs due to either an inability to secrete insulin or an inability to use insulin. Insulin is a pancreatic beta-cell hormone responsible for mediating the entry of glucose into cells to derive energy. There are two major types of diabetes: Type 1 diabetes (T1DM) is an autoimmune disorder in which T-cells destroy pancreatic beta cells, leading to little or no insulin production. Type 2 diabetes (T2DM), the more common type, is characterised by insulin resistance and decreased insulin secretion, typically associated with obesity, physical inactivity, and genetics (1).

DM is a result of a combination of genetic and environmental factors that influence the disease. T1DM and T2DM are caused by multifactorial factors. Environmental factors that have been identified to cause DM include physical inactivity, obesity, exposure to certain drugs and toxins, and viral infections, while genetic factors have been identified to play an important part in the development of T2DM, with a concordance rate of nearly 100% in identical twins when both genetic and environmental factors are considered. It is important to note that even in the presence of genetic factors, DM is likely to develop only when the environmental factors affect gene expression. This highlights that genetic factors may predispose a person to the development of DM, but environmental factors may play an important part in triggering the development of diabetes mellitus (2).

Diabetes is a significant and increasing health issue in the world, which is closely associated with the increasing obesity and unhealthy lifestyles. According to a recent survey conducted by Saeedi *et al.* in 2019, 463 million individuals worldwide have diabetes, which corresponds to a prevalence rate of 9.3%. It was estimated that this prevalence rate would rise to 10.2% by 2030 and 10.9% by 2045. At over 116 million, China is leading in the number of diabetic patients among these nations (3). India is second on the list at 77 million, followed by the US at 31 million, indicating the US as one of the most vulnerable countries to diabetes in the coming decade (4).

Diabetes has potentially serious consequences, such as diabetic retinopathy, nephropathy, neuropathy, foot ulcers, and cardiovascular diseases, which need to be prevented or delayed through the early treatment of the disease (1).

Conventional types of medication for hyperglycemia, such as sulfonylureas, biguanides, peroxisome proliferator-activated receptor agonists (boost the action of insulin), and α-glucosidase inhibitors (interfere with absorption of glucose in the stomach), are either used exclusively or combined with other hypoglycemic medications. The major setbacks of the previously mentioned drugs include chronic hypoglycemia, obesity, decreased therapeutic effects due to unsuitable dosage, low potency, and side effects caused by drug metabolism and inadequate target specificity, solubility, and permeability issues. Even after the advancement of potential anti-hyperglycaemic medications, enhancing the current therapies to present optimal and steady glucose concentrations and decreasing the long-term adverse effects from diabetes are the major hurdles to successful diabetes therapy (1).

The effectiveness of traditional T2DM therapy in controlling blood sugar levels and averting long-term problems is limited. Gene therapy has come a long way in recent years, particularly with clustered regularly interspaced short palindromic repeats (CRISPR)–CRISPR-associated protein (Cas)9 to edit genes and create insulin (1,5). These tools, which allow us to quickly and effectively alter genomes, may hold the key to resolving the problems associated with treating T2DM.

To investigate developmental processes, gene expression regulation, and animal behaviour, CRISPR–Cas9 has been used for the recruitment of functional modules that either label specific genomic loci in living cells or animals or repress/activate gene expression. Certain genes linked to the pathophysiology of T2DM can be precisely modified using CRISPR-Cas9. For example, Maxwell et al. and Xue et al. presented the CRISPR/Cas9 system as a robust genome editing tool and applied it to diabetes research. Their application to edit genes relevant to diabetes came later. To investigate T2DM beta-cell dysregulation, CDKAL1, KCNJ11, and KCNQ1 genes were edited to study their functional roles in insulin secretion and β-cell function (5,6). Editing of the PTPN22 gene in mice accelerated the onset of T1DM. The editing of the INS gene in pigs and beta-cell cultures has helped in modeling insulin deficiency, while the knockout of the LEPR gene has helped in modeling obesity-diabetes in rats. While the technique is still in its infancy, the results indicate that the use of the CRISPR technique has potential applications in the modeling and analysis of diabetes (5,6).

In summary, diabetes mellitus is a global health problem whose prevalence is growing faster than imagined, and its aetiology is a multifactorial phenomenon, culminating in a situation where existing therapies fail to alleviate the complications of this condition. With the discovery of gene-editing tools like CRISPR–Cas9, we now have a unique opportunity to grasp the molecular basis of this disease and unlock its full potential for a more precise therapy.

Pathophysiology of Diabetes Mellitus

T2DM is a progressive, chronic metabolic disorder that is always accompanied by hyperglycemia. This condition is mainly due to the coexistence of β-cell dysfunction and insulin resistance (IR). This condition is gene-environment-molecule-lifestyle dependent (2).

In insulin resistance, muscle, adipose tissue, and liver become less sensitive to insulin, causing decreased glucose entry and increased hepatic glucose production. The pancreas's β-cells initially compensate for this by producing more insulin. Over time, the β-cells become dysfunctional, leading to β-cell exhaustion and the loss of the ability to regulate glucose homeostasis. At the cellular level, glucose enters the β-cells via GLUT2 transporters, leading to the secretion of insulin. But in T2DM, glucotoxicity, lipotoxicity, and persistent inflammation induce conditions of endoplasmic reticulum (ER) stress, oxidative injury, and mitochondrial impairment that impair insulin production and secretion (2).

Harmful dietary practices, especially those that are high in fat and sugar, exacerbate oxidative stress. This increases the levels of free fatty acid (FFA) and triglyceride-rich lipoproteins, which cause oxidative stress and activate harmful metabolic pathways (such as polyol, AGEs, PKC, and hexosamine). Such pathways induce inflammation, damage to the endothelium, and alterations in gene expression. FFAs in large amounts disrupt mitochondrial function. This results in insulin resistance and β-cell dysfunction (2).

Physical inactivity is a risk factor for T2DM, as it promotes obesity and low-grade inflammation. Exercise can stimulate inflammatory cytokines such as TNF-α and IL-6, which can disrupt insulin function. Exercise, on the other hand, increases insulin sensitivity by enabling anti-inflammatory cytokines (e.g., IL-1Ra), antioxidants (e.g., glutathione), and myokines like irisin that enable glucose metabolism (2).

Gut dysbiosis or gut microbiota imbalance has also been implicated in T2DM. Adverse lifestyle and diet lower beneficial short-chain fatty acids (SCFAs) while increasing harmful substances like lipopolysaccharide (LPS). Systemic inflammation, impaired gut barrier function, and dampened β-cell activity follow, and these contribute to insulin resistance and progression of T2DM (2).

Metabolic memory refers to ongoing cellular damage of initial hyperglycemia despite glucose normalisation. This is owing to ongoing oxidative stress, inflammation, AGEs, and epigenetic alterations, e.g., DNA methylation, histone acetylation/deacetylation, and miRNA modifications (e.g., miR-375, miR-7) that affect insulin secretion and β-cell health (2).

Finally, there is mitochondrial dysfunction, which results from aging and overnutrition, and which can reduce ATP and increase ROS, thus destroying the mitochondria. Dysfunctional mitophagy allows for the buildup of abnormal mitochondria, worsening insulin resistance. Certain mtDNA mutations (e.g., A3243G) are also linked with an increased risk of T2DM development (2).

Mechanism of the CRISPR–Cas9 technology

CRISPR-Cas9 is a gene-editing technology that enables its wide use in science and exhibits potential for gene therapies in the near future. The Cas9 endonuclease recognizes DNA next to a specific trinucleotide called a protospacer adjacent motif (PAM), most commonly NGG, using a single guide RNA (sgRNA). The Cas9 endonuclease creates a double-stranded DNA break, enabling the homologous

recombination pathway, gene disruption, or homology-dependent gene correction using non-homologous end-joining. CRISPR-Cas9 also has the potential for simultaneous or multiple gene-editing capacity, making it a powerful tool for the investigation of complex diseases such as diabetes; it has already been used in the investigation of β-cell function and glucose metabolism. Factors such as efficient delivery of CRISPR components, particularly into pancreatic β-cells, prove to be an obstacle in the process. This has been addressed using various techniques, including physical methods and virus-based systems, especially adeno-associated viruses (AAV), which are widely used in vivo due to efficient cellular uptake and relatively low immunogenicity, though immune responses can occur. However, off-target effects and immune responses have to be refined (1).

A second viral vector system using lentiviral vectors (LVs), besides AAV, is also employed in CRISPR gene editing. LVs can be used to deliver Cas9 and sgRNA cassettes in a single transfection and accommodate larger DNA cargos than AAVs. To improve the safety profile of LVs, non-integrating lentiviral vectors (NILVs) have also been developed. Although viral systems achieve efficient in vivo delivery of CRISPR/Cas9 systems, they still pose challenges, including immunogenicity, integration, and off-target effects. A new perspective on CRISPR/Cas9 delivery, using non-viral gene carriers, is based on nanoparticles, which include lipid nanoparticles, cationic polymers, liposomes, vesicles, and gold nanoparticles. The use of nanoparticles has shown promising outcomes in preclinical studies, offering more versatile delivery options for CRISPR/Cas9 applications (1).

Applications of CRISPR/Cas9 in Diabetes

Insulin, insulin analogs, and non-insulin oral hypoglycemic agents have been used as medications in patients with DM. However, due to internal pharmaceutical demerits and limitations on transportation methods (subcutaneous administration or oral distribution), resulting in challenges with oral administration because of subpar gastrointestinal absorption, proving their limitations (7). CRISPR gene therapy, along with nanocarriers provide a favourable result in treating DM, which transports CRISPR to the allocated locations using liposomes, polymer-based nanoparticles, and inorganic nanoparticles. Nano-carriers shield CRISPR from enzymatic degradation in the stomach, provide in vivo stability, and enhance bioavailability. The risk of hypoglycemia is reduced by the use of CRISPR-Cas complex and a nanocarrier, and increases patient acceptance while recreating the inherent insulin delivery through foreign stimulation. However, this treatment could be targeted to specific areas and released gradually over an extended period, thereby reducing complications and hence utilising the complete efficiency of the therapeutic results for the treatment of DM (8).

Liposomal particles are great candidate materials because of their high biocompatibility, simplicity in surface modification, and simple manufacturing method. Cho *et al.* recently reported a bioreducible lecithin-based liposomal nanocarrier combination for protein-based Cas9 genome editing. It was produced by protein-protein fusion, and electrostatic interactions between the negatively charged Cas9-RNP and cationic components enhanced loading efficiency (9). T2DM was chosen as the target

disease because it is a complex metabolic disease involving insulin resistance, chronic hyperglycaemia, impaired glucose-dependent insulin secretion, and altered glucose metabolism in the periphery, including adipocytes. Incretin hormone action, especially the glucagon-like peptide-1 (GLP-1) pathway, is involved in the control of insulin release during the postprandial state, and the pharmacological modulation of this pathway is an emerging therapeutic strategy in T2DM. Current knowledge on the biology of GLP-1 provides the foundation for the development of next-generation therapies for T2DM (10). Increasing glucagon-like peptide-1 (GLP-1), a key target hormone that stimulates insulin secretion, is one of the recent therapeutic approaches (10). However, this hormone has a short half-life due to its rapid breakdown by dipeptidyl peptidase-4 (DPP-4), which limits its therapeutic use (11). Several drugs that inhibit DPP-4, such as sitagliptin, vildagliptin, saxagliptin, and linagliptin, have been developed to prevent the breakdown of incretin hormones, including GLP-1 and GIP, increasing their levels and improving glycemic control in T2DM (12). Furthermore, due to their widespread use in clinical practise, DPP-4 inhibitors are being investigated as potential novel treatments to stop the development of hepatic fibrosis and steatosis (13). However, small-molecule antidiabetic drugs must be taken daily. They are also associated with adverse effects, such as damage to the liver. Type 2 diabetes may be treated with a low-risk approach, such as a CRISPR/Cas9-based strategy that efficiently downregulates the DPP-4 enzyme. Cas9-RNP, a ribonucleoprotein made up of recombinant Cas9 nuclease complexes and a sgRNA, is thought to modify the DPP-4 gene. A lecithin-based liposomal nanocarrier particle (NL) was created to carry the Cas9-RNP complex. To offset the negatively charged lipid structure of the NL and increase the efficiency of encapsulation, a cationic polymer was added to the Cas9-RNP complex. This occurs because electrostatic interactions between charged components significantly influence encapsulation efficiency (9). From a biodistribution perspective, NL is also ideal for treating liver diseases since lecithin is naturally metabolised by the liver (1).

The application of a positively charged polymer improved loading efficiency and Cas9-RNP complex encapsulation. When negatively charged lipids and charge-compensated complexes spontaneously interacted, NL spheres with a consistent size distribution self-assembled. In contrast to unprotected protein therapy approaches that have poor delivery efficacy due to enzymatic breakdown, the genome platform has outstanding biocompatibility, low cytotoxicity, and high solution stability, making it appropriate for treating hereditary and chronic human illnesses (1).

As indicated by the findings, the NL@Cas9-RNP system has a number of benefits, including successful treatment of liver disease, stable and effective in vivo administration, and highly efficient encapsulation of the Cas9-RNP complex. Further studies are needed to characterise and improve the pharmacokinetics, effectiveness, and safety of this DPP-4 gene editing method in animals (1).

Current development and emerging perspectives

CRISPR-based new tool Cpf1

In accordance with the CRISPR/Cas9 system type II and in order to simplify its application, a dual-RNA construct termed single guide RNA (sgRNA) has recently been developed by genetic engineering. It is made up of two components: trans-activating CRISPR RNA (tracrRNA) and CRISPR RNA (crRNA). When Cas9 binds to tracrRNA, CrRNA directs it to the target sequence. By using RNA instead of protein to assess target specificity, this approach greatly simplifies and increases the accessibility of the technology. Moreover, CRISPR/Cas9 is a superior nuclease-mediated genome editing technique, offering perks over meganucleases, ZFNs, and TALENs, such as ease of target design, efficiency, and the numerous mutations. A class of Cas9 orthologues known as the Cpf1 family proteins was identified. The HNH endonuclease domains of these proteins differ structurally from those of Cas9. By paving the way for more accurate genome editing, the discovery of Cpf1 technically offered a less complex and more constrained substitute for the CRISPR toolset. In terms of function, it belongs to the class 2 CRISPR systems. Cpf1 functions differently in the CRISPR system than Cas9. One RNA molecule would be sufficient to continue the process because Cpf1, in this case, leaves behind a sticky end after cleavage rather than a flat one, as shown in Figure 1. Thus, CRISPR/Cpf1 technology can be used to intentionally introduce desired genes into vectors. As a result, Cpf1 works better than Cas9. When it comes to genome editing in non-diverging cells, Cpf1 works over HDR's efficiency restriction. Gene editing is restricted at target sites with G-rich sequences because the CRISPR/Cas9 system can only target sites with PAMs containing NGG sequences. In the meantime, Cpf1 resolves this problem by locating the T-rich target areas. The inclusion of Cpf1 to the gene editing toolbox allows it to target a wider range of locations in the genome, which is another advantage over Cas9. To improve Cpf1's ability to induce INDEL mutations in target regions, researchers have recently produced Cpf1 proteins with uridine-rich 3′ ends and a complementary 20-bp target site (1).

The CRISPR-Cas9 genome editing has transformed the study of diabetes by allowing the accurate manipulation of genetic and regulatory factors in β-cell models. Maxwell et al. demonstrated that the correction of a WFS1 mutation in patient-derived iPSCs restored β-cell function and also improved glycemic control after transplantation in diabetic mice, and the single-cell analysis showed a decrease in ER stress and an increase in insulin production (6). In the same way, the direct association between regulatory variants and β-cell dysfunction was demonstrated by targeting the non-coding risk loci, including the ABCC8-KCNJ11 locus, which validates the use of CRISPR in functional dissection of GWAS-identified regions (14).

Based on these gene-specific correction and regulatory dissection methods, Guo et al. and Xue et al. modeled congenital hyperinsulinism through ABCC8 knockout in hESCs and were able to recapitulate disease phenotypes such as high basal insulin secretion and respond to pharmacological manipulation, which supports the applicability of CRISPR-edited hESCs in disease modelling and drug testing (5,15). Building on this, Atla et al. produced a panel of isogenic hESC-derived β-like cells with knockouts in T2D-related genes, revealing the essential functions of genes such as ABCC8 and TCF7L2 in β-cell

maturation, insulin content, and chromatin structure (Atla et al. 2022). Meanwhile, Bevacqua et al. applied multiplexed CRISPR editing to coding and non-coding elements in primary human islets, demonstrating the regulatory importance of loci such as PCSK1 in insulin processing (16).

Overall, these studies demonstrate the versatile capabilities of CRISPR not only in the correction of pathogenic variants but also in the study of gene activity and regulation, which can provide revolutionary insights and therapeutic opportunities in diabetes and β-cell diseases.

Limitations and Challenges

Despite CRISPR/Cas9 being identified as a promising strategy for the treatment of complicated diseases like diabetes, various limitations, technical, biological, and ethical challenges continue to persist in its clinical translation.

Off-Target Effects and Genomic Integrity

Off-target effects are still among the most significant issues. These off-target genetic alterations can cause harmful mutations in non-target genomic regions, possibly disrupting essential genes or inducing oncogenic changes. The spontaneity and frequency of off-target mutations raise concerns about safety and conflict with the accuracy of genome editing, particularly in therapeutic contexts.

Delivery Constraints

Delivery issues remain a hindrance. Delivery of the CRISPR components, specifically the Cas9 protein and guide RNA, efficiently to the target cells (such as pancreatic β-cells) is technically challenging. Viral vectors such as adeno-associated viruses (AAVs) provide high transfection efficiency but are limited by factors such as low packaging capacity, high cost of production, and potential for eliciting immune responses. Conversely, non-viral delivery systems, such as lipid nanoparticles and electroporation, are less immunogenic but usually experience poor tissue penetration and diminished editing capacity.

Immunogenicity

The Cas9 enzyme itself may induce an autoimmune response, particularly in subjects with previous exposure to Streptococcus pyogenes (the original organism that the SpCas9 is derived from). Such immune recognition may cause inflammation or elimination of the edited cells, diminish therapeutic efficacy, and pose potential risks of immune-mediated tissue injury.

Mosaicism and Editing Efficiency

Mosaicism, where only a portion of cells bear the genetic alteration, is possible when CRISPR editing is less than complete or less than efficient, especially in vivo. This causes patchy therapeutic results since only a portion of the pancreatic β-cells would be effectively edited, perhaps not sufficient to restore normal insulin secretion or glucose homeostasis. Mosaicism also makes clinical interpretation and effectiveness challenging, particularly in preclinical animal models, and can demand repeated or

combination treatment, both elevating risk and cost. Moreover, partially edited cells can act in unexpected manners or even induce immune reactions as a result of differential protein expression. Identifying mosaicism involves sophisticated methods such as single-cell sequencing, which are costly and not commonly available in clinical practise, further making quality assurance and monitoring challenging.

Polygenic Disease Complexity

Both T1DM and T2DM involve complex polygenic and multifactorial etiologies influenced by environmental factors. Unlike monogenic disorders, targeting a single susceptibility gene is unlikely to achieve sustained clinical improvement. Multiplex gene-editing strategies may be required, but simultaneous editing increases technical complexity and potential off-target risks. Translating CRISPR-based interventions from proof-of-concept models to polygenic metabolic disease, therefore, remains scientifically demanding.

Economic and Accessibility Considerations

CRISPR-based therapies are currently associated with substantial financial costs, including personalized guide RNA design, vector manufacturing, quality control, and regulatory evaluation. Contemporary gene therapies frequently exceed six-figure per-patient costs, raising concerns regarding accessibility and global health equity. Given that diabetes disproportionately affects low- and middle-income countries, economic feasibility remains a critical consideration. Demonstration of long-term durability and clear superiority over established treatments will be necessary to justify widespread clinical adoption.

In spite of these limitations, CRISPR/Cas9 is a formidable and multidisciplinary genome editing tool. Future research will need to strive to make it more precise and safer through better delivery systems, the development of high-fidelity Cas9 variants, the reduction of mosaicism, and the integration of effective off-target screening mechanisms. These developments will be key to unlocking its complete therapeutic value in treating diabetes and other diseases of major complexity.

Ethical issues and safety concerns of CRISPR/Cas9 technology

Until recently, human genetic modification was restricted to gene therapy for somatic cells. The advent of CRISPR as an accurate and efficient genome-editing tool reignited fresh debates on human germline modification. It led to extensive genome-editing research across all species, though in humans it was at first confined to non-viable, tripronuclear zygotes. In a recent analysis of research on CRISPR-Cas9 editing in human embryos, Wiley *et al.* expressed that concerns still persist about low editing efficiency, mosaicism, and off-target mutations posing ethical concerns on potential future clinical use in viable embryos (17).

Major concerns

Off-target mutations

A major issue with CRISPR/Cas9 genome editing is off-target mutations, where similar DNA sequences in large genomes may lead to unintended cuts, causing cell death or abnormal changes.

Earlier gene-editing tools like TALENS and ZFN required custom-designed proteins that took years to develop. In contrast, CRISPR only needs a complementary RNA to target DNA, making it much faster and more efficient. However, this ease of use also increases the risk of unintended and potentially harmful outcomes.

The possibility that the technology could be exploited to produce a bioweapon, such as an infectious disease that harms people or crops, is the most terrifying scenario. It is important to stress the legal criteria for using CRISPR. Safety and security procedures should be developed to control any creature that has the potential to harm the environment (18).

Genetic enhancement

CRISPR/Cas9 has been a simple, precise, and rapid genome-editing tool with deep potential in applications ranging from agriculture to medical therapeutics. But it presents serious ethical, moral, and safety concerns, most notably the risks of unintended destructive effects, concerns over informed consent, and the potential for eugenic exploitation. Therefore, the technology requires stringent regulations, international dialogue, and widespread societal engagement (18).

Patient safety

Patient safety is critical when considering germline editing to prevent inherited diseases (19). Though it may relieve parents' concerns, extreme caution is required to avoid unintended effects. While pairing CRISPR with improved enzymes could reduce risks, therapeutic use in humans remains distant. Somatic gene therapies degrade over time and can be dose-controlled, but germline changes are irreversible, requiring stricter clinical trial standards. CRISPR's risks vary by application; some may soon be acceptable, while others remain too dangerous. Still, it holds great potential for improving health and the environment (18).

Generation of chimeric animals for organ transplantation

CRISPR can create chimeric animals to grow human-compatible organs, reducing wait times for transplants (20). However, bioethical concerns arise as chimeras may contain human neurons or germ cells. Critics fear this could blur the line between human and animal identity, while others argue such organisms lack human consciousness and won't impact dignity or the environment (21).

Ultimately, once genome editing reaches safe clinical standards, extensive social, legal, and ethical discussions must guide its use (22,23). Regulatory frameworks need reevaluation to prevent misuse (24–26). Despite ongoing research, CRISPR-Cas9's medical potential is expected to grow rapidly, making it crucial to balance innovation with public conscience and proper laws (21).

Future Recommendations

While CRISPR/Cas9 gene editing has shown promising results in the treatment of Type 1 and Type 2 diabetes mellitus, certain hurdles have to be overcome to render this treatment effective in a clinical setting. In the future, greater emphasis should be placed on the refinement of CRISPR/Cas9 gene editing in pancreatic β-cells and other insulin-responsive cells, particularly through the use of RNPs and Cas9 with high specificity to prevent off-target effects. Advances in the gene editing of primary human islet cells and CRISPR/Cas9-mediated approaches have been made, providing a platform to improve gene correction mechanisms (14).

Considering the polygenic nature of T2DM, future studies should aim to improve combinatorial and regulatory genome editing rather than using single-gene knockouts. Functional validation of diabetes risk genes in stem cell-derived β-like cells has shown the intricate relationships between genes in the regulation of insulin secretion and β-cell maturation (5). This may be further expanded to include epigenome editing, such as CRISPRa and CRISPRi, to modify gene expression without permanent alterations to the genome.

Moreover, further optimization of viral and non-viral delivery systems is critical to enhance tissue-specific targeting of the liver and β cells of the pancreas. Even though the pre-clinical outcomes of genome editing are highly promising, existing limitations include immunogenicity, packaging capacity, and the efficiency of genome editing. Long-term studies on genome stability, immune responses to Cas9, mosaicism, and the longevity of metabolic correction are critical to assess the safety and efficacy of genome editing (1).

Overall, these approaches to combine high-precision editing tools with advanced β-cell models and delivery systems are likely to be instrumental in bringing CRISPR-based diabetes therapies from mouse models to humans.

Conclusion

Diabetes Mellitus is a major health issue in the world today, with current treatments only able to alleviate the symptoms of the disease rather than addressing the genetic or cellular defects in the disease process. Genome editing using the powerful tool CRISPR/Cas9 has shown promise in addressing genetic defects in diabetes through precision medicine approaches that correct genetic defects, modulate metabolic pathways, and regenerate functional beta cells in the pancreas. With continued improvements in delivery systems of the next-generation genome editing tools, precision medicine approaches using genome editing are expected to be powerful in addressing Type 1 and Type 2 diabetes

in the future. However, several hurdles in genome editing, such as off-target effects, immunogenicity, delivery issues, ethics, and costs, must be overcome to translate genome editing into the clinics in the future. With continued improvements in genome editing, it is expected to become a powerful tool for addressing diabetes in the future, either preventing the disease or modifying it in a long-term fashion.

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Conflict of Interest

The authors declare no competing financial interests or personal relationships that could have influenced the work reported in this paper. This narrative review was conducted independently and did not receive funding from any commercial entity or organisation.

Tables/Figures

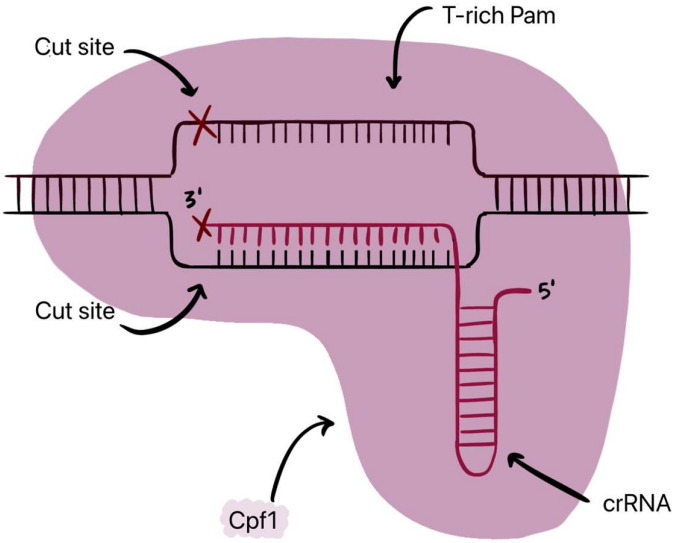

Figure 1. The CRISPR/Cpf1 technology's molecular processes. Although it differs from CRISPR/Cas9 technically, CRISPR/Cpf1 technology expands the toolkit thanks to its enzyme. Component. A class 2 nuclease, the Cpf1 enzyme exclusively recognises the target site using one strand of RNA. Based on the location of a T-rich PAM with a TTTN sequence at the 5′ end of crRNA, this enzyme can identify the target sequence. As can be observed, one strand is exactly split at 19 base pairs (bp) after the PAM sequence, and the opposing strand is cleaved at 23 base pairs (bp). Compared to CRISPR/Cas9, CRISPR/Cpf1 sticky ends enhance specificity and functional efficiency.