PROJECT REPORT

A Review Article on

“Gene Therapy for Type 1 diabetes mellitus (T1DM)”

Submitted to:

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APPROVAL

This project, "**Gene therapy for type 1 diabetes mellitus (T1DM),**" was submitted to the Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University and has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy and has been approved as to its style and content.

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CERTIFICATE

This is to certify that the results of the investigation that are embodied in this project are original and have not been submitted before in substance for any degree at this university. The entire present work, submitted as a project for the partial fulfillment of the degree of Bachelor of Pharmacy, is based on the result of the author's (ID: 183-29-143) own investigation.

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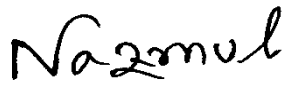
DECLARATION

I hereby declare that this project report was done by me under the supervision of **Sharifa Sultana**, associate professor and associate head, Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, in impartial fulfillment of the requirement for the degree of Bachelor of Pharmacy. I am declaring that this project is my original work. I am also declaring that neither this project nor any part thereof has been submitted elsewhere for the award of a bachelor's degree or any other degree.



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I would like to express my heartfelt gratitude to Almighty Allah for providing me with the ability to fulfill my project work and the chance to concentrate on this area.

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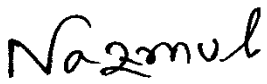
I would like to express my respect to all the teachers at the Department of Pharmacy, Daffodil International University and thank the other members for their excellent cooperation with us.



Author

DEDICATION

I dedicate this work to my parents, my teachers and my friends.

ABSTRACT

The autoimmune disease known as type 1 diabetes mellitus (T1DM) causes an insulin deficit and consequent hyperglycemia by targeting and destroying the body's own insulin-secreting islet cell. Without insulin, these people will eventually go into diabetic ketoacidosis (DKA), a potentially fatal condition. One of the emerging treatment options for treating T1DM is gene therapy. Insulin injections has been the sole therapeutic option for all type I diabetics for over eighty years. The usual treatment of type 1 diabetes is fraught with difficulties. Although whole-pancreas transplantation has been effective for some individuals, the procedure is technically challenging and has a high risk of complications. Research into diabetes has so focused heavily on finding ways to produce an endless supply of cells with glucose-responsive insulin secretion. This analysis focuses on the current state and prospective future of gene therapy in the management of T1DM. In this article, the potential of gene therapy for replacing beta cells is discussed. New findings in beta cell development and growth as well as gene delivery to beta cells, will be addressed. Implementing gene therapy for T1DM, selecting suitable vector, Potential therapeutic and preventative T1DM strategy for eliminate the need for daily insulin shots.

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CHAPTER 1

Introduction

Introduction

1.1 General information

Diseases may be treated with gene therapy, which is the therapeutic delivery or manipulation of genetic material within the cell. Disease onset and progression may be prevented or slowed by correcting the faulty genes responsible for it. Gene therapy generally employs one of three main forms of intervention: A new gene may be inserted into the body, damaged genes could be replaced with healthy ones, or the disease-causing genes could be inactivated. Somatic gene therapy directly targets somatic cells (in this example, the sick cells), unlike germline gene therapy, which targets reproductive cells (also known as gametes) to stop the spread of illness from one generation to the next [2]. Gene therapy is one of the most exciting developments in medicine right now because of its ability to treat many chronic conditions that have proven resistant to standard medical care, including autoimmune illnesses, diabetes, cancer, and heart disease [3, 4]. Insulin-producing islet cells in the pancreatic are destroyed by T cells in type 1 diabetes, an autoimmune illness [4]. T1DM has a complicated etiology that includes environmental and genetic components [5], much like those of other autoimmune disorders. Over the last several decades, scientists have identified many genes that play a role in the onset of type 1 diabetes [6]. Therefore, a more comprehensive disease management strategy or perhaps a cure for T1DM may be possible by the regulation of these genes using gene therapy. However, there may be risks involved with gene therapy despite its promising future. Using a viral vector to transport genes, for instance, has been linked to an exaggerated immune response and subsequent disease progression [7]. Additionally, gene therapy's safety characteristics have not yet been shown in people and most gene therapy experiments are being undertaken in animal models.

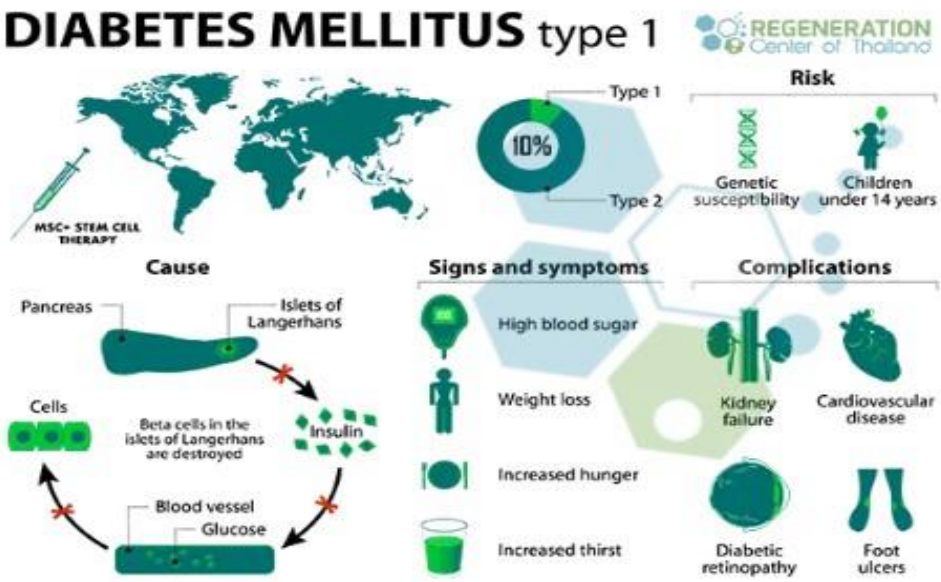


Figure-1

1.2. Immune intervention: Implementing gene therapy for T1DM

T-cell autoimmunity is responsible for the development of type 1 diabetes. The pancreatic beta cells, responsible for producing insulin, are the major focus [8]. Researchers believe that autoreactive CD4(+) and CD8(+) T lymphocytes are primarily responsible for pancreatic islet inflammation or insulitis [8,9]. Consequently, it seems that an imbalance between regulatory and auto-aggressive cell subsets may be at the root of type 1 diabetes [10]. According to data gathered during the Diabetes Control and Complications Trial, just 20% of people with a recent diagnosis of diabetes had any measurable insulin production (DCCT). It is possible that beta cell activity may be preserved with immunological intervention, and the patient's need for insulin therapy can be reduced [11]. Therefore, from an immunological perspective, there are a number of potential therapies that might be considered when creating an immunotherapy for T1DM. Potential treatments for this autoimmune disorder include gene therapy, immunoregulation and anti-inflammatory measures. Healing type 1 diabetes requires restoring insulin-producing -cell function and mending the loss in immunological tolerance [12].

1.2.1 Cell-mediated gene therapy via immunological precursors

Gene replacement treatments, including organ and cell transplantation, were at risk for rejection due to the introduction of neoantigens, which might be countered by humoral or functional T-cell immune responses [13]. As a result, there are limited options for employing gene therapy to treat type 1 diabetes without causing harmful immune reactions [13]. The first strategy for reducing undesirable immune responses is gene transfer into immunologic progenitor cells to make the immune system recognize the therapeutic protein as'self [13]. This demonstrates that a persistent antigen-specific tolerogenic milieu may be created and Preventing CD8+ memory T-cell-mediated attack of islet-expressed antigen [14]. Thus, studies have shown that immunoprecursor cell-mediated gene therapy may be useful as an immunotherapy method for treating type 1 diabetes.

1.2.2 Immunotherapy with insulin

Insulin immunotherapy may also be practiced by delivering the hormone via the mucosal membrane. A resurgence of interest in this field, whose primary function is to increase immunological tolerance towards insulin antigens, has led to its application in clinical studies. An increase in regulatory T cells (Treg) is considered to be the mechanism behind this effect. Nonetheless, there are studies that show its effectiveness through the elimination of antigen-specific T cells. Insulin antigens are administered in combination with intestinal CD103+ dendritic cells (DCs) expressing retinoic acid and indoleamine 2, 3-dioxygenase. These cytokines are suppressive in character and work in tandem with insulin antigens. However, it has been shown to fail because antigen spreads before it reaches the intended spot [15].

1.2.3 T-cell receptor-specific gene therapy

T-cell receptors derived from human islet- and viral-specific CD4+ T cell clones may enable the targeting of a specific illness via gene therapy [16]. It has been shown that cells treated with islet-specific TCRs did not interact with the antigen as well as cells treated with viral-specific TCRs [17]. In a separate experiment with diabetic and non-obese mice, T-cells were used to target both the old and new islets. One possible explanation for this behavior is that the mAb targets the T-cell receptor (TCR). After receiving Ngn3-Btc and anti-TCR mAb therapy, the liver's periportal insulin-producing cells were kept in better condition [9]. Nearly 80% of insulin-producing cells were lost in untreated mice, according to the study [17]. Mice with diabetes were reportedly treated with gene transfer and an antibody [17]. This snippet of DNA activated insulin-specific regulatory T cells, which slowed down the immune system's assault on insulin-producing cells [17]. It was also found that in untreated mice, about 80% of insulin-producing cells are destroyed [9]. Because of this, NOD mice may be protected against or perhaps cured of type 1 diabetes by administering a mAb directed against TCR [9]. The use of lentiviruses for the transfer of genes for T cell receptors (TCRs) has been shown to be an efficient gene transfer approach [18]. The main objective of avoiding further cell death via tissue-targeted induction of antigen-specific intolerance [18] may be achieved by the lentiviral gene transfer of TCRs that detect autoantigens connected to type 1 diabetes. The T cell receptor and other chimeric MHC molecules are to blame for this [19]. We report on the electroporation of peptide/2 m/CD3- genes into a sensor T cell line in this paper [19]. Because endogenous MHC-I chains bound to peptide/2 m/CD3-products, MHC-I cross-linking resulted in strong activation signals [19]. NOD mice did not get diabetes when they were injected with InsB15-23/2 m/CD3-mRNA into their primary CD8 T cells [19]. So, T cell receptor-focused gene therapy is a promising way to treat type 1 diabetes.

1.3 Implemented vectors for gene therapy

1.3.1 Systems for delivering genes

The development of gene delivery technology raises the possibility of successfully administering a specific gene to cells while preventing or treating type 1 diabetes. The administration route, targeting strategy, and gene delivery technology are all crucial for efficient gene expression at specified locations. There are both viral and non-viral vector alternatives now available. The several vector possibilities that might be used are covered in this section of the review [20].

1.3.2 Gene therapy in diabetes using viral vectors

It is possible to think of a viral vector as being a very effective method for transporting genes to cellular components. It has been engineered in such a way that it is no longer infectious, that its ability to replicate has been severely reduced, but that its capacity to transmit genes has been preserved. While viruses are undoubtedly superior than plasmids or adeno-associated viruses as a means of delivering genetic material, there are still issues to consider when building a viral vector system, including the potential for cytotoxicity, inflammation, and immunogenicity. Without specialized targeting moieties, endocytosis is the primary route for the absorption of viral vectors. Invading the cell via the cell membrane, the viral vector complex deposits its payload deep within the cell after escaping the endosome. Following that, the plasmid may be released in one of two ways. Either the complex is internalized into the nucleus, where unpacking and release of the plasmid occur, or the complex dissolves in the cytoplasm, where the plasmid is liberated and gene expression occurs in the nucleus. In the last few years, many viral vectors have been made, and each has its own pros and

cons. The choice of viral vectors and the subsequent optimization should be guided by the intended therapeutic use [20].

1.3.3 Adenovirus

Adenovirus, often known as "Ad," is a member of the Adenoviridae family and ranges in size from 90 to 100 mm. It is a non-enveloped virus that ranges in size from 90 to 100 mm and contains double-stranded DNA. Humans Ads have been shown to have at least 57 different serotypes, which may be divided into seven distinct "species," ranging from A to G. Though there are several serotypes, species C (Ad1,2,5,6) is the most common [21]. Ad5 has undergone genetic alteration to produce the great majority of ad vectors, which may be categorized into two main groups: replication-defective (RD) and replication-competent (RC) [21]. Compared to other viral vectors, the adenoviral vector, sometimes referred to as the AVV, has a number of significant advantages. Since the majority of human cells include secondary integrin receptors and primary adenovirus receptors, an adeno-associated viral vector (AVV) is the most effective vector for transferring a gene in vivo. As a consequence, AVV is easily able to infect human cells, increasing the expression of transgenes [22]. Additionally, AVV has been extensively used in several therapeutic applications, and both the most secure delivery route and the right dose have been established. In fact, AVV is one of the vectors that is used most often in clinical trials. It is used in more than 20% of all gene therapy studies done around the world [22].
The use of AVV is a suitable vector for the delivery of the GK gene into liver cells. Genetic engineering was used to insert the human HGF gene (hHGF) into AVV. HGF has the power to promote the growth of pancreatic beta-cells since it has strong mitogenic features. The creation of insulin depends on this skill. According to the results, diabetes may be prevented, which also prevents the

degeneration of the pancreatic beta cells. This confirms the efficacy of adenoviral-mediated hHGF gene therapy for the treatment and prevention of type 1 diabetes [22,23]. The CTLA4-FasL gene may potentially be transferred via AVV, in addition to the possibility of transferring the hHGF gene. It has been shown that CTLA4Ig may increase T-cells' susceptibility to Fas-dependent apoptosis by blocking the CD28-B7 pathway, but CTAL4-FasL can successfully cause apoptosis in T-cells. Both of these skills have been proven. In order to conduct this study, adenoviruses containing the CTLA4-FasL gene (AdCTLA4-FasL) were created and injected into the tail veins of mice that had received several mild doses of STZ. The results showed that the number of people with autoimmune diabetes went down a lot, but the amount of insulin in the islets didn't change [24].

1.3.4 Adeno-associated virus

For the purpose of overexpressing a fake PDL1-CTLA4Ig polyprotein or IL10, vectors based on the Adeno-associated virus serotype 8 (AAV8) were used [25]. These vectors target particularly. It was discovered that the islet cells were protected from rejections for at least one hundred twenty days [25]. intramuscular injections of rAAV vectors that were encoded with murine IL-10 were performed. The findings provide credence to the use of immunoregulatory cytokines gene therapy that is selectively given by rAAV for the purpose of avoiding the return of autoimmune illness after islet transplantation in the treatment of type 1 diabetes [26]. Leptin and human alpha-1 antitrypsin were among the additional genes that were encoded utilizing rAAV vectors to treat type 1 diabetes [22,27].

Adenovirus and Adeno-associated virus viral vectors for gene therapy applications have the following characteristics:

Viral vector	Packaging capacity	Length of expression	Relative viral titer	Transduction efficiency	Infect both dividing and non-dividing cells	Immunogenicity
Adenovirus	7.5 kb	Transient	+++	+++	Yes	High
Adeno-associated virus	4.5 kb	Transient and Stable	++	++	Yes	Low

1.3.5 Retrovirus

A retrovirus is yet another kind of vector that is often used in gene delivery systems (RV for short). A single strand of RNA that makes up the genome of RV is present in two copies. The DNA sequence for both the structural and catalytic viral proteins is present in this genome. Proviral sequences are found in retroviral vectors, which make it possible for them to successfully splice the desired gene into the target cells. This is made feasible by the presence of these sequences in retroviral vectors. In retroviral vectors, viral and cellular promoter regions further enhance the production of the gene of interest in target cells [28]. When RV is used as a vector, it provides various advantages, including steady integration over a long period of time and a high capacity for cloning. However, there are also drawbacks associated with the use of RV vectors. For instance, RV can only transduce cells that are actively dividing, and there is a possibility of insertional mutagenesis when employing RV. On the other hand, an RV that activates itself has been designed so that the possibility of mutagenesis may be eliminated [28].

1.3.6 Electroporation

Gene electro-transfer, often known as GET, is a technique that involves the use of electric fields with the purpose of inducing gene transfer [29,30]. The

permeability of the cellular membrane is thought to be increased as a result of this. This method has been used in a number of investigations, and the results have shown that plasmid DNA can be effectively transported in vivo to skeletal muscles and cardiac tissues, as well as into cells of pig heart in vivo [30–32]. Several of these research have been described.

1.3.7 Plasmid

This vector has the ability to separately express two genes that are included on a single plasmid [33]. It has been observed that the administration of pReg/PI improved the symptoms of streptozotocin-induced type 1 diabetes by enhancing the regeneration of beta cells and reaching immunological self-tolerance [34]. There are several plasmid vector types that may be employed, including the pVAX plasmid [35]. In liver parenchymal and non-parenchymal cells, this type of gene transfer may cause the transient expression of genes of interest without the introduction of viruses [35]. In this study, it was shown that the pVAX plasmid expresses IGF-1, and normoglycemic mice no longer required any further treatment after 10 injections [35].

1.4 Development of the pancreas

Beta cell progenitors are excellent candidates for use as a source of tissue for transplantation since mature beta cells have a limited capacity for self-replication. When adopting methods that induce differentiation in vitro, using beta cell progenitors requires a detailed comprehension of the beta cell growth and development process. By a dorsal and lateral expansion of the epithelium immediately posterior to the growing stomach, the pancreas emerges from the upper duodenal region of the developing intestine. The embryonic gut's

endodermal and mesodermal cells need intercellular contact in order to develop into the pancreas and small intestine, respectively [36–39]. The growth of pancreatic endocrine cells relies on a complex interplay between extracellular soluble factors, cell-matrix interactions, and cell-cell communication, which will eventually be regulated by transcription factors. This is similar to how the pancreas as a whole develops. Numerous new findings have been made concerning this process, such as the discovery of transcriptional factors that increase and sustain beta cell activity, but many issues remain. Gene therapy may be used to target the genes that code for the elements necessary for beta cell formation in order to enhance beta cell proliferation from progenitors both in vivo and up vitro [40]. Therefore, one of the main objectives of diabetes research is to close these gaps. To address these gaps is one of the top goals for diabetes research.

1.4.1 Produced by secreted growth and differentiation factors

To produce new sources of transplantable insulin-producing cells, a great deal of attention has been paid to the extracellular signals that promote the proliferation of endocrine cells [41]. Using intercellular signaling molecules like integrin beta B and fibroblast growth factor 2, research on chicks has shown that the notochord has the capacity to inhibit the synthesis of sonic hedgehog (shh), allowing for pancreatic differentiation [41]. Other lines of evidence also point to an activin role in the pancreas' development. The pancreatic islets of mice models expressing mutant activin receptors exhibit hypoplasia and aberrant development [42]. Additionally, the protein follistatin, which binds to activin, may mimic the detrimental effects of the mesenchyme on the growth of rat pancreatic endocrine cells [43]. It has been shown that activins sometimes work in conjunction with other stimuli to promote the growth of endocrine cells. A mouse pancreatic beta

cell tumor line was used to produce betacellulin for the first time. Rat insulinoma cell line INS-1 growth has been shown to be accelerated by it [44]. Initially, a mouse pancreatic beta cell tumor line was used to isolate betacellulin. It is a member of the epidermal growth factor (EGF) family and may be identified in human pancreas [45]. Both betacellulin and the EGF receptor (EGFR) have been proven to be expressed in the human pancreas, and it has also been shown that mice lacking the EGFR exhibit aberrant pancreatic islet formation [45,46]. Additionally, it has been shown that betacellulin is required for the insulin gene to be produced in clonal alpha cells that have had the PDX-1 gene transfected [47]. Exocrine AR42J cells are converted into insulin-producing cells by the synergistic action of betacellulin and activin A [48]. This was also shown to be the case when activin A and hepatocyte growth factor were administered to exocrine cells [49]. However, studies have shown that betacellulin and activin A both have distinct actions on human developing pancreatic epithelial cells. Betacellulin was discovered to promote cell proliferation whereas Activin A was identified to induce endocrine differentiation, which was followed by an increase in insulin expression [50]. The protein known as HGF/SF, or hepatocyte growth factor/scatter factor, is derived from mesenchyme and acts on epithelial cells through the c-met receptor, a membrane-spanning protein tyrosine receptor. During the early stages of pancreatic development, the expression of the HGF/SF and c-met genes is highly expressed; however, it rapidly declines after puberty and continues to decline throughout adulthood [51–53]. The c-Met receptor protein is found in the same cells in the islet that convey insulin, according to studies utilizing immunofluorescence. The epithelial cells seen in the human fetal pancreas have also been demonstrated to be mitogenic by HGF/SF [52]. When given alone, HGF/SF may also transform pancreatic acinar AR42J cells into cells that produce insulin [49]. HGF/SF overexpression in the islets of transgenic mice may also result in an increase in beta cell proliferation and islet mass as well as a mild state of hypoglycemia [53]. Additionally, it has been shown that exposure

to HGF/SF increases the synthesis of Reg, a protein associated with pancreatic regeneration, in human fetal islets [54]. Other compounds, including glucagon-like peptide 1 (GLP-1) and its more stable counterpart exendin-4, as well as prolactin, which stimulates the growth of islet cells significantly in vitro [55], may also be crucial for the proliferation and differentiation of endocrine cells. In diabetic rats, exendin-4 boosted beta cell proliferation and neogenesis [57], while GLP-1 induced the differentiation of AR42J cells into insulin, pancreatic polypeptide, and glucagon-positive cells [56]. Furthermore, it was shown that stimulation of the GLP-1 receptor activated the insulin gene in a human beta cell line in concert with the transcription factor PDX-1 and cell-cell contact [58].

1.4.2 Increasing the Number of Primary Pancreatic Beta Cells and Precursors

Although adult beta cells and beta cell precursors may be induced to multiply by hepatocyte growth factor/scattered factor (HGF/SF) in combination with complex extracellular matrices, beta cells are known to have a limited capacity for reproduction. However, growth stimulation causes a loss of differentiation, which is followed by a rapid decline in insulin expression [60,61]. Additionally, primordial beta cells can only divide through 10 to 20 population doublings before experiencing a growth stop due to cellular senescence [60,62]. Expanded primary cells have been shown to express more of the cyclin dependent kinase inhibitor p16 INK4a and to have shorter telomeres, higher levels of senescence-related beta-galactosidase, and higher levels of beta-galactosidase [63]. This has been shown via a variety of trials. Beta cell precursors have been suggested as a potential source of tissue for transplantation since they may have a better propensity for proliferation [64]. But as of yet, this hasn't been officially established. Additionally, the bulk of the physiologically relevant determinants are still unknown, despite the extensive research that has been done to identify

the processes that control the development and maturation of human beta cell precursors [59-67]. After undergoing a number of laboratory procedures, such as a 90% pancreatectomy, pancreatic wrapping in rodents [68,69], and transplantation with fetal mesenchyme into naked mice [70], the neogenesis of endocrine islets from ductal epithelium has been shown in vivo. These experimental circumstances include, among others: As a method of boosting the amount of endocrine tissue obtained from adult cadaveric pancreases for transplantation, the neogenesis of endocrine islets from ducts with the use of matrix and growth factors has been suggested [71]. Growth factors and a matrix would be used throughout this in vitro procedure.

CHAPTER 2

Literature Review

2.1 Chellappan, D. K., Sivam, N. S., Teoh, K. X., Leong, W. P., Fui, T. Z., Chooi, K., ... & Dua, K. (2018). Gene therapy and type 1 diabetes mellitus. *Biomedicine & Pharmacotherapy*, *108*, 1188-1200.

The burden of treating type 1 diabetes, which is already challenging, might become much more challenging with conventional medications. Gene therapy is one of the newly available therapeutic options for T1DM. The main subjects of this research include gene therapy's possible future and current use in treating T1DM. The vast majority of published trials on gene therapy for the treatment of T1DM are conducted on animals and in preclinical settings. Additionally, there has been no study done on the security of these medications in real patients. A few of the gene level interventions under investigation right now include genetic vaccination, immunological precursor cell-mediated gene therapy, transplantation of cells expressing the genes against T1DM, stem-cell-mediated gene therapy, overexpression of genes and proteins necessary against T1DM, and vectors.

2.2 Irwin, D. M. (2021). Evolution of the Insulin Gene: Changes in Gene Number, Sequence, and Processing. *Frontiers in Endocrinology*, *12*, 649255.

Insulin has played an essential role in the evolution of fundamental molecular biology, in addition to its many other contributions to clinical medicine. Insulin has different roles in different vertebrate species, although being essential to their existence. Genome sequencing has shown a surprising variety of insulin coding sequences among vertebrate species, as well as changes in gene content and order that may have practical consequences for biological function. Genomes with several copies of the insulin gene may be better equipped to specialize one of them. Modifications in proteolytic processing have shown that inulin's typical two-chain hormone structure is not required for all of its biological actions.

2.3 Yechoor, V., & Chan, L. (2005). Gene therapy progress and prospects: gene therapy for diabetes mellitus. *Gene therapy*, *12*(2), 101-107.

Although it has long been researched, gene therapy has not yet shown promise in the treatment of diabetes mellitus. Significant barriers have been shown to include the toxicity of the vectors themselves as well as the lack of physiological regulation of the generated insulin. Recent advances in the knowledge of the b-cell developmental biology and the transcriptional cascade that regulates it have led to the effective use of in vivo and ex vivo gene therapy together with cell therapy in animal models of diabetes. In light of related advancements in stem cell biology and immunology, new opportunities for employing gene therapy to treat autoimmune diabetes have arisen.

2.4 Xu, R., Li, H., Lai-yin, T., Hsiang-fu, K., Lu, H., & Lam, K. S. (2003). Diabetes gene therapy: potential and challenges. *Current gene therapy*, *3*(1), 65-82.

As a result of the Human Genome Project and the quick advancements in molecular biology, gene therapy is one of the most intriguing new medical advancements of the twenty-first century. The major objective of gene therapy for diabetes mellitus is to maintain constant blood sugar levels in spite of significant dietary changes (DM). For diabetes mellitus, gene therapy provides a better risk-benefit ratio than islet transplantation, which is hampered by a lack of donors, rejection, and the need for ongoing insulin administration. In addition to other recent breakthroughs in gene therapy for insulin-dependent diabetes, current attempts to get beyond the drawbacks of adenovirus, adeno-associated virus, and retrovirus vectors and to target gene delivery for maximum efficiency of gene expression are covered in this review. New advancements in the use of stem cells for potential use in diabetic gene therapy are also emphasized.

CHAPTER 3

Purpose of the study

3. Purpose of the study

The aim of this study is to eliminate the need for ongoing treatments, relieving patients of the constant burden of dealing with their conditions.

- Find out the possibility of eliminating the need for daily insulin shots.
- Fixing faulty genes, which are at the root of type 1 diabetes, replacing them, and increasing the immune system's sensitivity to unhealthy cells.
- Find out an effective gene therapy for Type 1 diabetes mellitus.
- Prompts the injected DNA to produce insulin and Type1 Diabetes permanently reversed.
- Determine the role of gene therapy in beta cell replacement techniques.
- Find out of new vector technologies would make it possible to eliminate toxicity and simplify the process of cell/tissue-specific homing.

CHAPTER 4

Methodology

4. Methodology:

I looked for appropriate research and papers by searching online databases that are freely available to the public. The database of the organization was used to search for published literature and research and among the databases used were Science Direct, ProQuest, PubMed, Scopus, Chemical abstracts, Clinical trial databases and Wiley Online Library. Research was done from October 3, 2022, until November 18, 2022. T1DM and gene therapy are utilized as keywords. The scope of the search was limited to peer-reviewed scientific publications that published papers reviewing preclinical trials, randomized clinical studies and other review articles. Further honing of the findings was accomplished by restricting the search to works published in English and to the years 2010 through 2022. When I collect information, I divide the research into numerous categories. In order to carry out this separation, I searched for shared terms or content. Excluded studies didn't include either gene therapy or type 1 diabetes. Forty-six papers met the criteria and were included in the analysis. For this review article, I used Microsoft Word, Quillbot, and the Grammarly application.

CHAPTER 5

Result & Discussion

5.1 A combination strategy that integrates gene therapy and genetic engineering

Combining gene therapy and genetic engineering may lead to the development of novel treatments for type 1 diabetes [74]. In one such study, the approach was to induce the manufacture of human insulin in a K-cell line derived from malignancies [73,74]. This was accomplished by joining the mouse glucose-dependent insulinotropic polypeptide (GIP) gene to the human insulin gene at the 5′-regulatory region [74]. In its intestinal K cells, this mouse produced human insulin [74]. It is conceivable to transduce the human insulin gene into those cells to enable insulin production without the patient needing to regularly administer insulin injections since humans have a considerable number of K cells along the lining of the gut epithelium [74]. Human adipose mesenchymal stem cells (hAMSCs), in addition to K-cells, are also genetically modified with the pancreatic duodenal homeobox1 (PDX-1) gene in the hopes that they would differentiate into cells that can secrete insulin [72]. The pancreas' growth and the function of beta cells may both be influenced by a transcription factor called PDX-1 [72]. Additionally, it has been shown that it may influence the expression of the glucokinase gene, insulin, and Glut-2 [72]. There is no indication that any pancreatic tissue forms in animals missing this transcription factor [72]. The preferred method for delivering the PDX-1 gene to hAMSCs was by lentivirus [72]. These adipose-mesenchymal stem cells underwent morphological changes after being treated with PDX-1. Cells started to aggregate and form clusters, both of which are critical milestones in the path toward fully formed pancreatic tissue [72]. These stem cells may be developed into insulin-producing cells via further study and development since they can express the PDX-1 gene and are easily accessible [72]. Additionally, by transducing a specific line of cells to express our gene of interest, followed by genetic engineering and gene therapy, it is conceivable to reprogramme healthy cells inside an organism. Through the

activation of three genes and the use of a PPAR agonist, liver cells may be reprogrammed to become insulin-secreting ducts [75]. Their genes, more especially Pdx1, Ngn3, and MafA, were used in this study together with the PPAR agonist WY14643 [75]. Ad-PNM, a single-dose adenovirus that only contained the three genes, promoted the production of insulin and reduced blood glucose levels in the hepatocytes of CD1 mice [75]. Sadly, this was only temporary; after a few weeks, these cells were eliminated, which led to a return of the hyperglycemia [75]. However, alternative insulin-secreting duct structures formed in the liver and the diabetes went into remission when these three genes were co-administered with a PPAR agonist [75]. This is because the genes Pdx1, Ngn3, and MafA are in charge of the pancreas' growth, which therefore made it possible for the development of endocrine progenitor cells and the maturation of beta cells as a result [75]. An additional substance known as the PPAR agonist is required for the development of liver cells creating ducts that generate insulin [75]. Although WY14643's toxicity to humans is a negative, it has prompted researchers to advise utilizing fibrate drugs instead since they have similar pharmacological effects [75].

5.2 Potential therapeutic and preventative T1DM strategy: Genetic vaccination

Over the last several years, genetic vaccination has received a lot of interest as a possible cure as well as a preventative for type 1 diabetes. Genetic vaccination, also known as DNA vaccination, is the process of inducing or suppressing an immune response to an antigen in a host by administering to that host antigen-encoding plasmid DNA (pDNA) [76,77]. This kind of immunization is also known as DNA vaccination. It enables direct adjustment of the tolerogenicity of targeted site and provides a great deal of flexibility in terms of regulating the character of T cell responses [78]. pDNA, a viral vector-based vaccine, and a

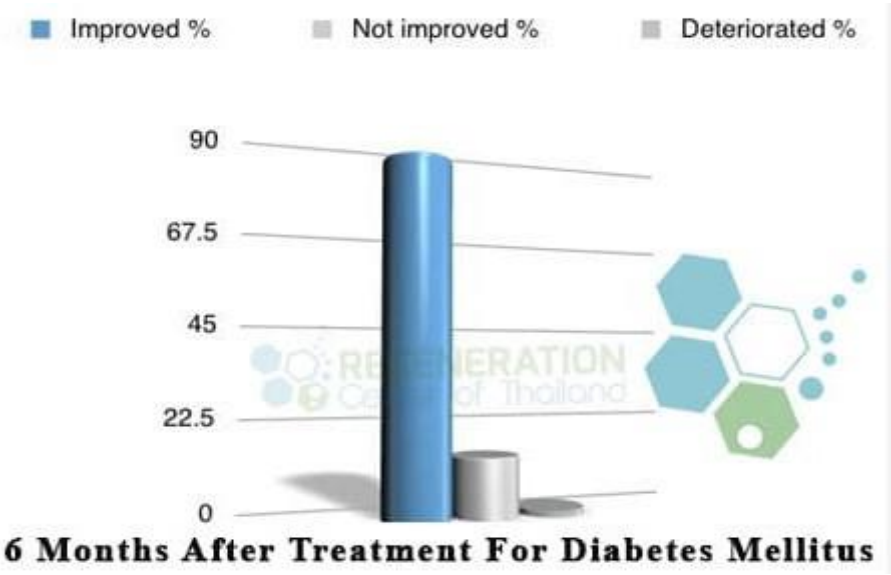
more contemporary strategy including antisense oligonucleotides (AS-ODN) are all examples of strategies that are used in DNA vaccination [78]. pDNA, which is the approach that has been explored the most, has shown that it may be effective in preventing and treating type 1 diabetes in NOD mice. For instance, it has been shown that giving NOD mice pDNA expressing CXCL10 or CCL4 may help them from developing type 1 diabetes. This is done by promoting the development of specific neutralizing antibodies that are directed against each of the mice's unique chemokines [79,80]. Additionally, it has been shown that pDNA carrying a cell-specific regulatory T cell (Treg) autoantigen, an anti-inflammatory cytokine, or both may promote Treg formation [78]. Through intramuscular injection or the use of a gene cannon, pDNA may be delivered into the muscle [78,81]. The practice of blasting the skin's epidermis with particles covered with pDNA is referred to as "gene gun vaccination" [78,81]. It was shown that gene gun-mediated pGAD65 selectively induced IL-4 secreting CD4+ T cells and significantly delayed the development of diabetes. The gene gun approach is superior to intramuscular injection in preventing diabetes in NOD mice, as opposed to intramuscular delivery of pGAD65, which elicited a predominant type-1 like T cells response and did not stop disease progression [81]. This shows that the gene gun strategy is more effective at avoiding diabetes in NO than intramuscular injection. To increase the efficacy of combination medicines even further, researchers are looking into the potential use of genetic vaccination as a component. Combine the GAD65 DNA vaccine with an anti-IL-1 antibody while treating diabetic RIP-GP mice. RIP-GP mice are creatures that express both the rat insulin promoter (RIP) and the lymphocytic choriomeningitis virus glycoprotein (LCMV-GP) [82]. According to these findings, anti-IL-1 monotherapy does not have the same protective effects against diabetes as the anti-IL-1/GAD65 DNA vaccine combination [82]. The down regulation of diabetogenic T cells and the activation of CD4+CD25+Foxp3+ Treg by GAD65 are likely events occurring below the surface [82]. However, some remaining beta

cells are required for this protective action to take place [82]. When compared to pIL-10, pGAD65, SGAD65/IL-10, SGAD65/750/IL-10, or INS/IL-10, pDNA combining GAD65 fragment gene and IL-10 gene (SGAD65190-315/IL-10) is the most efficient strategy for avoiding diabetes in NOD mice [82]. This is due to the fact that pDNA carries both the IL-10 and GAD65 fragment genes. Generic immunization has generally shown outstanding outcomes in the prevention and treatment of type 1 diabetes in mouse models, suggesting that it may have potential as a future therapeutic and/or preventive option for individuals with the condition.

5.3 Stem cell-mediated gene therapy

Exogenous insulin therapy and islet transplantation into the portal vein are two treatment options that have been available to individuals with type 1 diabetes (T1DM) for many decades now [83]. However, as was mentioned earlier, there are other treatments available, such as stem cell therapy, which helps in the restoration of lesions in pancreatic tissue, manages blood glucose levels, and secretes insulin by inducing stem cells to distinguish into insulin-producing molecules [83]. Stem cell therapy is just one example of an alternative treatment. These types of therapy eliminate the problems of a reduced ratio of donors, the need for harmful immunosuppressive medicines to be taken for the rest of the patient's life and transplant rejection [84]. The results of a research project that was carried out in 2014 by shown that innovative cellular replacement treatments that are based on stem cells, such as human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC), may be utilized to treat type 1 diabetes [83]. This is due to the fact that T1DM only affects a single cell type. The findings of this investigation also demonstrated that both of these stem cell treatments had a number of drawbacks [83]. The application of these methods is restricted to in vitro tests or pre-clinical models because of the drawbacks

associated with them, which include ethical concerns, transgenic tactics, and epigenetic failure [83]. However, a different study that was carried out by Wu demonstrated that one of the benefits of stem cell therapy is the potential to solve the problem of hypoglycemia, which is characterized by the inconvenient requirement of multiple daily injections or life-long subcutaneous injections of insulin [84]. In this study, the hypoglycemia problem was examined. These two distinct kinds of stem cells need to go through a process of multiple differentiation in order to develop into -cells that are capable of functioning [85]. During this process, the cells must go through the stages of endoderm, pancreatic endoderm, and endocrine development. Because of these drawbacks, one of the new procedures that was proposed was called trans-differentiation. This entails the transformation of one somatic cell type into some other type without going through any pluripotential phases [83].



After six months treatment: Insulin Producing functional beta steam cell therapy For T1DM

5.3.1 Gene therapy administered by hematopoietic stem cells

It has also demonstrated that islet transplantation might be harmful owing to an assault mediated by memory T-cells [86]. Stem cells are employed to create

replacement beta cells in order to get around this restriction and to stop memory autoimmune attacks on transplant islets or replacement -cells [86]. Stem cells are also used to avoid memory autoimmune attacks. According to the findings of the study [86], one of the stem cell therapies, specifically the hematopoietic stem cell-mediated gene therapy, has the potential to be of great benefit and could help reduce the destruction of antigen-expressing islets. This is accomplished by inhibiting antigen-specific memory T-cell responses.

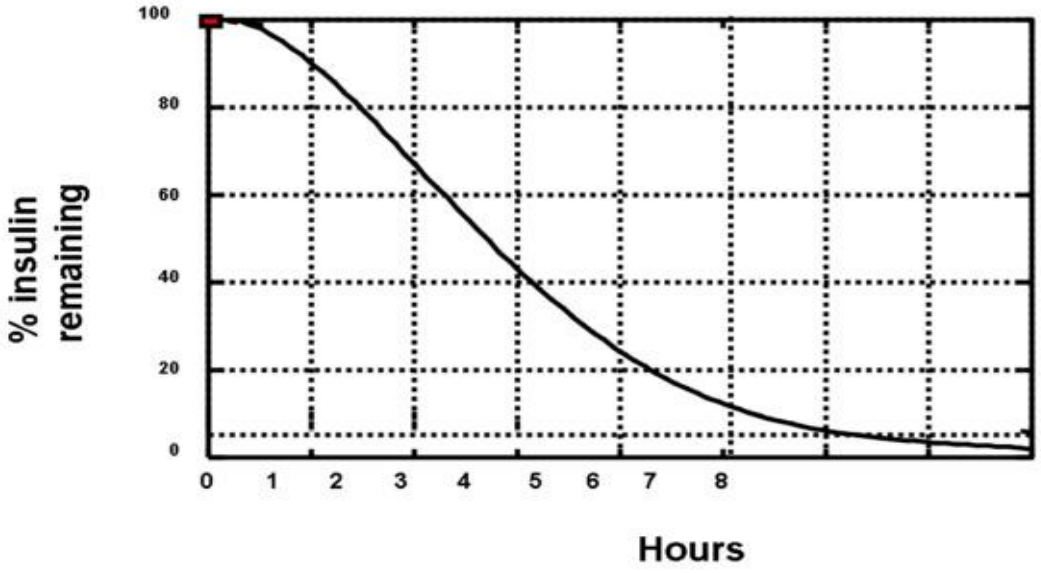
5.3.2 Adipose-derived stem cells (ADSCs)

Adipose-derived stem cells (ADSCs) are a different kind of mesenchymal stem cell that may one day treat type 1 diabetes [87]. Adipose tissues may include ADSCs, which may develop into a variety of cell types with little immune rejection [87]. Additionally, ADSCs possess a substantial capacity for proliferation. ADSCs are also a useful source of autologous stem cells since their surface markers, CD73 and CD90, encourage prolonged incubation durations and high proliferative capacity [87]. Treatment with ADSCs may reduce inflammation and cell infiltration while also boosting insulin and Pdx1 synthesis in the pancreas [87]. This is in addition to the advantages already discussed.

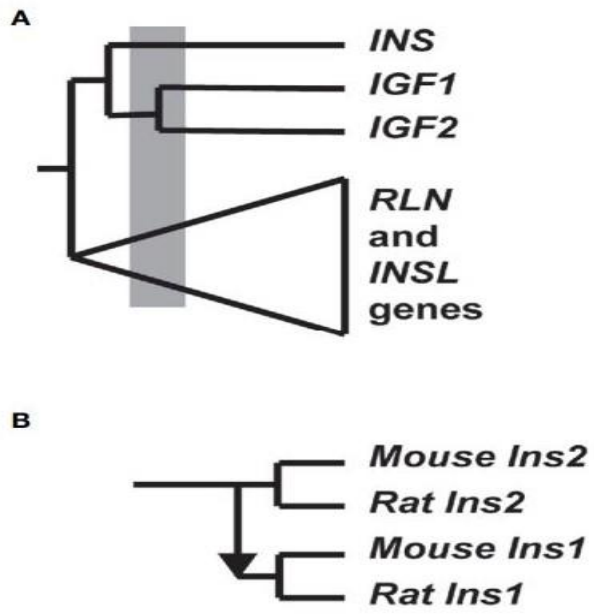
5.4 Evolution of the Insulin Gene: Modifications to the Number, Sequence and Function of Genes

Insulin's role in controlling blood sugar levels in vertebrate blood has been extensively studied [88]. One hundred years ago, the discovering of insulin allowed for a real therapy for diabetes, which sparked a medical revolution [89]. Insulin, insulin analogues, and other peptides have been used to treat diabetes since its discovery, and this therapy has developed and is developing further [90]. The disease remains incurable. Insulin has been instrumental in the advancement

of numerous ground-breaking technologies that are now standard in molecular biology, such as protein sequencing [91] and the derivation of the three-dimensional proteins structures [92]. Proteolytic processing's function in controlling the biological activity of insulin is a significant finding with broad implications for other bioactive peptides [93,94]. Numerous insulin protein sequences have been established since the sequencing of human insulin more than 60 years ago [91]. This is likely owing to insulin's medical significance, its modest size, and its relative ease of isolation [95,96]. Since the beginning of the genomic era 20 years ago, several insulin sequences have been predicted from whole genome sequences. Improved knowledge of human genetics and illness [97,98] is only one example of how genomic sequences have impacted the biological sciences as a whole [99]. New insulin sequences found from genome sequences have shown that the number of insulin genes within a species is more than previously thought, and that variations in the proteolysis process of the proinsulin precursors likely contribute to the wide range of insulin's biological effects.



This graph was made using information from a euglycemic clamp (0.2 U/kg intra-abdominally) and displays the onset and duration of insulin aspart's activity. Only effective insulin gene therapy can lengthen the time that insulin remains in the body.



5.4.1 Insulin-like gene duplication in vertebrates

(A) Phylogeny of insulin supergene members of the family based on sequences and genomic location [100-103]. The triangle represents the divergence of numerous human relaxins (RLN1, RLN2, and RLN3) and insulin-like peptides (INSL3, INSL4, INSL5, and INSL6). The grey area represents two genome duplications (2R) that happened towards the beginning of vertebrate evolution. (B) The origins of the duplicated insulin genes in rodents. The Ins1 gene was created by a retroposition event in the common ancestor of the mouse (Mus musculus) and rat (Rattus norvegicus), as shown by the arrow, while Ins2 is found at the locus-of-origin [104-108].

5.4.2 Evolution of insulin sequences

Along with changes to insulin gene abundance and structure, insulin gene sequencing has also changed. Genes normally change at a nearly constant rate, but sometimes they show bursts of more rapid change that are considered to signal a change in gene function. Mammalian insulin research has provided evidence in

favor of this hypothesis. It is widely known that the guinea pig's (Cavia porcellus) and its cousins' (rodents of the suborder Hystricomorpha) insulin sequences are very variable [109,110]. The biological effects of these insulins differ as well, acting more like a growth factor than a metabolic hormone [111,112]. The rate of evolution of the insulin protein sequence in the guinea pig and its relatives has been sped up by these changes in sequence and function [110,113,114]. Other New World monkey species [114,115], which have animals with lesser potency insulin hormones [116], have experienced similar, though less dramatic, occurrences of fast evolution of insulin sequences.

5.4.3 Changes in proteolytic processing

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                          Signal peptide      ↓          B-chain              ⇓
Human                   -MALWMRLLPLLALLALWGPDPAAA FVNQHLCGSHLVEALYLVCGERGFFYTPKT RR EAE
Japanese medaka         MATLWIHTASLLILLVMSFP-TTQA TTLQHLCGSHLVEALYIVCGDNGFFYNPQS AA GSP
Clownfish               MAALWLHTAALLVLLVTSCP-GSRA ISTQHLCGAHLVDALYLVCWENGFTYNPGS NN GRA
Little brown bat        -MALWTRLLPLLALLALWAPAPAQA FNHEHLCGEDLVDIMTIICGDQGFK-NPKA AR ELP
Aardvark                -MALWVRLLPLLALLAIGAPPPARA FVSQHLCGSHLVEALYLVCGERGFFYTPKT RR ETE
SGIV VILP               ------------------------- THQLQVCGGELIDALTEHCGDRGVYTPPRR GR RTR
                                                        C          C

                                  C-peptide                    ⇓      A-chain
Human                   ----DLQVGQVELGGGPGAGSLQPL-ALEGSLQ KR GIVEQCCTSICSLYQLENYCN
Japanese_medaka         VQSLLPNTGRALSAGGETEGAP--FKEQMKAIA KR NILERCCYMPCTIYDLASFCS
Clownfish               LRFLPPKTGRATSSGGENEAPEFAFNDAMEMLV KP NIVEQCCARPCSIYDLSAYCN
Little brown bat        ----DPQEGEVDMG----AGGPKAL-TVEELLQ NT DIVEVCCTNICSFYDMETYCN
Aardvark                ----DLQAGMVGA------GGPQPF-PAEVARQ QR GIVEQCCTSVCSLYQLENYCN
SGIV VILP               -------------------------------- SV GLADACCKNECDENELDRYCN
                                                              CC   C        C

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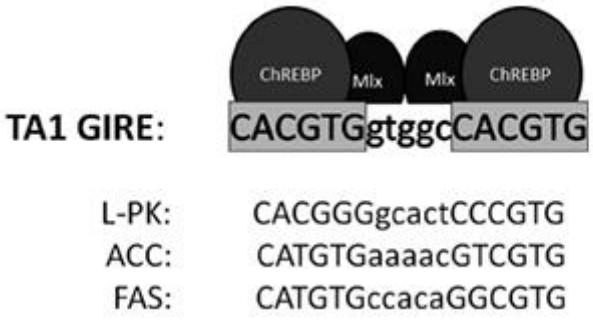
Vertebrate organisms show variation in the way they metabolize proinsulin-like sequences. The proinsulin protein sequence from Homo sapiens is compared to that of a few other species whose proteins may undergo somewhat different proteolytic processing. Two fish [117], the Japanese medaka (Oryzias latipes) and the clownfish (Amphiprion ocellaris), as well as two mammals [114], the little brown bat (Myotis lucifugus) and the aardvark (Orycteropus afer), all encode insulin proteins, and there is also a viral insulin-like peptide (SGIV-VILP) from the Singapore grouper iridovirus [118]. Vertebrate cells infected with Singapore

grouper iridovirus would generate SGIV-VILP. The signal peptidase cleavage site for human insulin is denoted by a and the prohormone convertase processing sites are denoted by an in the single-letter system used to represent protein sequences. Above the alignment, domains of the human proinsulin sequence are shown. Substitutions of anticipated protease inhibitory amino acids in the sequences are highlighted in boldface when compared to their human counterparts. Below the alignment, we can see the conserved cysteine residues that participate in disulphide bridging.



Expression of transcriptional activators and glucose-inducible response elements (GIREs): By incorporating GIREs, target genes may be transcriptionally regulated in response to blood glucose levels. The consensus sequence for GIREs is CACGTG, which divides into two 5-bp spaces and two 6-bp motifs. A tetramer of the transcription factor ChREBP-Mlx binds to each GIRE and promotes transcription in response to high blood glucose levels. Acetyl-CoA carboxylase (ACC), fatty acid synthase, and L-pyruvate kinase (L-PK) are just a few of the liver-specific genes that have been revealed to include GIREs (FAS).

5.5 Limitation of Gene Therapy:

Genetic therapies hold promise to treat type 1 diabetes mellitus, yet, these treatments are still novel and not without their potential drawbacks.

- An abnormal response from the immune system. The body's immune system may mount an assault on the foreign viruses if it perceives them as invaders. Organ failure or inflammation may result from this.
- Main drawbacks of using virus vectors are its immunogenicity and cytotoxicity. (Adenovirus)
- Aiming for the incorrect cells to destroy. These modified viruses may infect other cell types since viruses may have systemic effects on the body. In such a scenario, normal cellular processes may be disrupted, potentially leading to the emergence of new illnesses.
- Patients often need numerous courses of treatment after receiving a gene therapy due to its limited duration of effect. A lasting treatment requires therapeutic DNA to be effective over the long term and for the cells carrying it to be durable and long lived.
- Uncontrollable population growth and Higher risk of increasing allergies.
- Expensive. Not approved by FDA.

CHAPTER 6

Conclusion

5. Conclusion

Type 1 diabetes affects a large and growing population across the globe. Gene therapy is an approach used to maintain a near normal BG level in a manner that is effective, safe, and specific; this is the basic objective of any treatment for type 1 diabetes. In addition, gene therapy for immune treatments is another promising therapeutic strategy for type 1 diabetes. Patients' need on insulin might be lessened with immunological therapies aimed at protecting beta cells from autoimmune damage.

The use of gene therapy is warranted for this objective since surgical or procedural methods are unable to alter gene expression. Integrating genes into cells and creating new forms of gene therapy are both areas where genetic engineering plays a significant role. In addition, genetic vaccination shows promise for the management of type 1 diabetes since it allows for extensive manipulation of the T-cell response.

This review also covers many systems of viral and non-viral vectors, each with its own set of benefits and drawbacks. For gene therapy to be effective, vectors are essential because they allow for the controlled and precise delivery of genes to their intended locations. Consider the therapeutic goal while selecting vectors to utilize. There has to be more research done on non-viral vectors since they are safer to employ in people and do not cause antigenicity. For these treatments to be successful, the right vector must be chosen, and viral vectors must be further optimized to reduce the inevitable side effects, such insertional mutagenesis and host immunogenicity. Non-viral vector construction should be further studied in depth to boost the transfection effectiveness and practicality of non-viral systems.

Understanding the biology of cytokines implicated in T1DM is also critical for developing safe and effective immunotherapy, and more in-depth research are needed to prove the efficacy of combination immunological therapies due to a lack of data. Finally, it is important to search for candidate genes that might reduce unwanted side effects, opening the door to the creation of a unique and secure therapy for type 1 diabetes.

In this overview, we looked at the benefits and drawbacks of overexpressing critical genes and proteins to cure type 1 diabetes via gene therapy.

CHAPTER 7

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