Deletion of the RNLS Gene using CRISPR/Cas9 as Pancreatic Cell β Protection against Autoimmune and ER Stress for Type 1 Diabetes Mellitus

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Abstract

BACKGROUND: Type 1 diabetes mellitus (T1DM) is a chronic disease in children which is usually caused by autoimmunity that damages pancreatic a and b cells which have functions as blood glucose regulators. Some studies stated that Renalase (RNLS) gene deletion will protect these b cells from autoimmune reactions and Endoplasmic Reticulum (ER) stress. RNLS deletion by genome editing Clustered Regular interspersed Short Palindromic Repeats-CRISPR-related (CRISPR/Cas9) is believed to have the potential to be a therapy for T1DM Patients. AIM: This research was conducted to know the potential of RNLS deletion using the CRISPR/Cas9 as an effective therapy and whether it has a permanent effect on T1DM patients.

METHODS: The method applied in this research summarized articles by analyzing the titles and abstracts of various predetermined keywords. In this case, the author chose a full-text article published within the past 10 years by prioritizing searches in the last 5 years through PubMed, Google Scholar, Science Direct, Cochrane, American Diabetes Association, and official guidelines from IDAI.

RESULTS: RNLS deletion using CRISPR/Cas9 in mice weakened the response of polyclonal -cell-reactive CD8+ T cells and disrupted the immune recognition to cells so that autoimmune killing did occur. In addition, such deletion prevents RNLS ER stress by increasing the threshold, triggering the unfolded protein response so that ER stress is difficult to occur. RNLS mutations in b cells also increase b cell survivability to oxidative stress.

CONCLUSION: b cells RNLS deletion by genome editing CRISPR/Cas9 is effective in protecting b cells from autoimmune reactions and RE stress. However, further research is needed to determine the side effects and safety of its use.

Introduction

Type-1 diabetes mellitus (T1DM) is a chronic disease characterized by impaired insulin production due to damage to pancreatic islet cells, either by autoimmunity or idiopathic processes, resulting in T1DM sufferers being unable to produce insulin, so it is known as insulin-dependent diabetes mellitus. Several risk factors cause T1DM, including genetic, environmental, obesity, birth weight, and some ethnic differences [1], [2], [3], [4], [5].

World epidemiological data in 2017 recorded 425 million people having diabetes, which is predicted to grow to over 600 million by 2045 [6]. Based on data from the Indonesian Pediatrician Association (IDAI), there were 1220 T1DM patients in Indonesia until 2018. In 2015 and 2016, there were 63% of children with T1 diabetes were first diagnosed with Diabetic Ketoacidosis, where this percentage increased to 71% in 2017 [7].

T1DM is divided into 2 types, namely, type 1A diabetes mellitus caused by damage to pancreatic cells due to an autoimmune reaction (more than 95% of cases), while type 1B diabetes mellitus is a b cell damage that is not caused by autoimmune (<5% of cases), but due to idiopathic causes. In general, the various types of T1DM can lead to complications such as acute and chronic disorders of macrovascular and microvascular. If there is a long-term disturbance effect on the disease, it can lead to a more progressive diabetes disease followed by complications such as retinopathy, nephropathy, neuropathy at the risk of developing diabetic foot ulcers, and other disorders. Many factors can trigger the occurrence of T1DM. In terms of genetic factors, it is currently known that gene damage occurs at more than 40 gene loci; while environmental factors are related to viral infection and diet [2], [7], [8].

Until now, exogenous insulin therapy is still the main therapy for patients suffering from T1DM. This insulin therapy is usually given by several injections every day [9], [10]. An appropriate daily insulin regimen is also required to achieve optimal glycemic control [11]. In addition to therapy, regulation of adequate physical
activity and exercise is also important for people suffering from T1DM [7]. However, the lack of patient compliance in exercising and undergoing insulin therapy often becomes an obstacle in the treatment of T1DM [10], [12].

With the development of technology in recent years, many studies have focused on how to influence immune attacks on cells in T1DM. Starting from rituximab, teplizumab, abatacept, to combination therapy of Antithymocyte Globulin (ATG) Plus G-CSF. Unfortunately, this therapy only delays the initial occurrence of T1DM [9], [11], [13], [14].

Treatment trials using stem cells such as Autologous Non-myeloablative Hematopoietic Stem Cell Transplantation using cyclophosphamide, granulocyte colony-stimulating factor, and rabbit ATG; autologous ex vivo expanded polyclonal Tregs; and Autologous Hematopoietic Stem Cell is also less effective because only temporary [9], [15].

Preventive therapy performed using TRIGR, BABY DIET, and FINDIA also showed no difference [16].

In this case, one solution that can be offered to treat patients suffering from T1DM is gene therapy. Nucleases are the primary tool in gene therapy. This modern therapy was done by injecting genetic material into the patient's cells to replace the damaged gene or to insert therapeutic transgenes. The idea in implementing gene therapy is to add normal genes to the genome that is mutated or damaged so that these genes can function properly. This therapy provides the theoretical advantage that a single treatment will achieve lasting clinical benefits. Lately, clinical gene therapy test has proven therapeutic benefits and exceptional security. Furthermore, gene therapy has been widely used to treat cancer, cardiovascular disease, infections, decreased metabolic function, lymphatic diseases, radiation-induced injuries, and post-surgical therapy. Gene therapy can also be a solution to treat diabetes mellitus, although the efficacy and safety of this therapy is still a problem [17], [18], [19].

Therefore, there has been no effective therapy to treat DMT. Therefore, through this literature review, the researchers aimed to explore the potential of gene therapy as a therapy that has a permanent effect on T1DM patients.

Methods

In this study, the articles were searched through PubMed, Google Scholar, Science Direct, Cochrane, and the American Diabetes Association to filter by filtering the past 5 years’ study published to obtain actual results as well as by the official guidelines from IDAI. If no or fewer references were found from the past 5 years, researchers would broaden the search by filtering up to the past 10 years. Article searches were performed using keywords T1DM, ER stress, Clustered Regular interspersed Short Palindromic Repeats-CRISPR-related (CRISPR-Cas9), genome editing, Renalase (RNLS), and RNLS deletion.

Meanwhile, article screening was done by adjusting the title and abstract to the research topic. When the title and abstract matched, it was then preceded by checking the availability of the article in full-text. Eventually, the researcher then read the entire article to determine whether the article is useful and appropriate to the topic.

Results and Discussion

T1DM

T1DM is divided into several types, those are type 1A caused by autoimmune and type 1B (idiopathic) caused by non-autoimmune, which is also caused by secondary diseases such as pancreatitis. The occurrence of T1DM is characterized by a loss of β cell mass of about 80–90%, causing clinical symptoms such as hyperglycemia. It is known that 10–30% of patients suffering from T1DM have relatives who also have the same disease. This is probably caused by a genetic predisposition related to the major histocompatibility complex (MHC). In this case, MHC involves HLA Class II alleles known as HLA-DQ and HLA-DR. The increase in risk factors for T1DM is also caused by mutations involving genes inside or outside the MHC [2], [5].

The occurrence of T1DM starts from the pancreatic cells, those are cell α and cell β. Insulin produced by cell β has the function of influencing the body's cells so that they can use glucose as energy. The hormones secreted by the pancreatic cells are formed to balance blood glucose levels, in which when the blood glucose level is low, then the pancreas cells will produce glucagon and vice versa. Insulin and glucagon have opposite work in regulating and maintaining the balance of blood glucose levels. T1DM patients experience other autoimmune disorders, such as celiac disease and thyroid disorders.

T1DM is divided into three stages, 2 of them are subclinical and 1 is a late-stage with symptoms. Stage 1 in T1DM is known as the subclinical stage where 2 or more antibodies are present (IAA, IA-2A, or ZnT8A). At this stage, the blood glucose level is normal (normoglycemia). Then, at Stage 2, blood glucose levels become abnormal (dysglycemia) due to the death of cells β. In Stage 3 which is the clinical stage, the occurrence of cell β death results in insulin deficiency, abnormal blood glucose levels, and the occurrence of hyperglycemia symptoms [2], [5].
**Endoplasmic reticulum (ER) in T1DM**

Many studies found that the ER can be targeted to prevent the death of cell β on T1DM. Like other secretory cells, pancreatic cell β island contains more ER than non-secretory cells, so they are more susceptible to ER stress due to their physiological function, namely insulin biosynthesis. As a cell organelle, ER has several functions, including; protein folding of newly synthesized secretory proteins, calcium storage, and pro-apoptotic and anti-apoptotic signal transducers [20], [21], [22].

A balance between protein load and ER folding capacity is required to maintain the quality of insulin synthesized. To maintain this balance, the RE has a quality control called the unfolded protein response (UPR). The imbalance between the protein that must be folded and the folding capacity of the ER results in a large number of misfolded proteins. The misfolded protein will accumulate in the lumen of the ER which then causes ER stress and leads to the activation of the UPR. In physiological conditions, the main function of the UPR is to restore homeostasis RE by decreasing the RE stress, improving insulin production, and preventing the death of cell β. However, in certain pathological conditions and continuous occurrence of UPR activation will cause a malfunction in RE function to the death of cell β. UPR deficiency can also lead to the death of cell β and T1DM [20], [21], [23].

As ER protector, UPR has three protein agents that act as stress sensors when quality control is not up to standard. These three agents comprise protein kinase R - like ER kinase (PERK), inositol requiring enzyme 1a, and activating transcription factor 6 (ATF6). These three sensors are usually kept in an inactive state by binding to the chaperone binding immunoglobulin protein (BiP). Active BiP increases protein folding, inserts polypeptides, and removes misfolded proteins leading to endoplasmic-reticulum-associated protein degradation. When misfolded proteins accumulate in the lumen of the ER, BiP separates from PERK, IRE1, and ATF6 which is then activated. IRE1 and PERK can switch from monomeric inactive conformations to oligomers allowing autophosphorylation and activation. Meanwhile, ATF6 is cleaved into an active form and enters the nucleus to increase the transcription of genes that respond to ER stress [23], [24].

Environment and genetics are also assumed as factors that can induce ER stress in cell β. This is indicated by the increase in free fatty acids, cytokines, viral infections, and the occurrence of hyperglycemia which can cause ER stress and eventually lead to diabetes. The ability of cell β in treating the ER stress may be the cause of some individuals being prone to ER stress [20].

ER stress can also be caused by ER calcium depletion which is caused by hyperglycemia, free fatty acids, cytokines, and thapsigargin (TG). ER calcium depletion occurs when cytosolic calcium increases in the ER. The leak of ER calcium into the cytosol causes an increase in post-translational modification (PTM) in cells β. PTM protein may cause loss of immune tolerance because it is seen as a neoantigen in the immune system. Once an antibody is against cell PTM β, this will lead to the deployment of an antigen that led to the various positivity of patient antibodies and eventually leads to the death of cell β and the T1DM [20].

In several studies conducted on mice, genetic knockout/deletion of the PERK, IRE1, Elf2α, and ATF6 genes showed the role of physiological and pathological responses. Although PERK KO mice were able to survive, they had growth defects such as pancreatic dysfunction, skeletal dysplasia, and hyperglycemia because they could not maintain the integrity of the ER [23], [24].

In studies conducted in humans, mutation on PERK gene causes Wolcott-Rallison syndrome which is indicated by permanent neonatal diabetes and epiphyseal dysplasia. Wolfram Syndrome gene mutations whose function is to regulate transmembrane proteins to reduce ER stress are also related to T1DM [23].

PERK has a dichotomous role, in which it can distinguish the severity of ER stress and promote growth stop to allow repair if possible or apoptosis when ER stress is severe. Elf2 is the main target of PERK. Once activated, Elf2 stops the protein synthesis, allowing cells to overcome the excessive misfolded, and restoring the ER homeostasis. However, in prolonged ER stress response, PERK/EIlf induces cell death through the factor of C/EBP Homologous Protein CHOP transcription [24].

The effects of a lack of IRE1/XBP1, PERK, and ATF6 give different responses in different environments. Under the conditions of glucose deficiency, PERK will encourage the formation of respiratory super complexes in the mitochondria as alternative energy. In contrast, on low-glucose exposure to CD4 T cells in the tumor environment under ER stress, mitochondrial respiration is reduced. Therefore, it can be found that RE stress regulates the onset, progression, and severity of a wide range of pathological diseases such as diabetes, cancer, and obesity [24].

**Genes associated with type-1 diabetes**

Genetic factors are one of the causes of T1DM. It has been identified by Genome-wide association studies (GWAS) that dozens of genes influence T1DM. GWAS also reported the relationship between T1DM and the viral RNA receptor gene region, thus finding IFIH-1 as the first gene for suspected T1DM. In addition, GWAS identified multiple gene loci using meta-analytical studies combined with population studies. There were 50 gene loci associated with T1DM including PTPN22, RGS1, CD55, AFF3, IFIH1,
RNLS is a newly discovered flavoprotein that is strongly expressed in the kidney and heart with the functions of metabolizing the catecholamines in the kidney [28], [29]. In addition, RNLS functions regulate blood pressure and cardiovascular function [30].

Based on the results of the study, the deletion of RNLS in mice may exacerbate cerulein-induced pancreatitis. This was proven by the provision of recombinant RNLS that can reduce the severity of pancreatitis. RNLS also has anti-inflammatory effects, so it can protect against tissue damage from pancreatitis. Therefore, RNLS deficiency may increase the risk of pancreatitis [31].

In the liver, RNLS can reduce the risk of fatty liver disease (FLD). The provision of exogenous RNLS is also useful for protecting the liver from ischemia/reperfusion (IR) injury by reducing oxidative stress, improving mitochondrial function, reducing apoptosis in liver tissue, and increasing the hepatocyte defense when experiencing oxidative stress. Therefore RNLS deficiency can cause susceptibility to FLD and IR injury [32].

A study also shows that RNLS deficiency is associated with hypertension. The study proved that the deletion of RNLS can increase the pulse rate and blood pressure in mice. On the other hand, another research that was conducted in Swedish urban communities claimed that there was no evidence of an association between the RNLS gene and hypertension or other cardiovascular diseases [33].

Other studies further stated that the deletion of RNLS only occurs on cell β which will be transplanted to protect these cells from autoimmune reactions. This study was carried out by injecting splenocytes from diabetic NOD mice (Figure 1). As a result, the cell β with RNLS mutations may survive for only 2 months compared to cells b plain that only last 1–2 weeks. Cell-β may survive due to RNLS mutations, in which the immune recognition of the mutation of cells b is disturbed. Such mutations cause the weak response of T polyclonal β-cell-reactive CD8+ in diabetic NOD mice. However, the RNLS mutation did not affect the response of all-rejection, so that the mutation of cell β is still destroyed by the response of allogenic. Thus, it can be concluded that RNLS mutations in cells are not immune to immune detection, but only resistant to autoreactive stimulation of T CD8+ cells. The deletion of RNLS also does not interfere with the differentiation of stem cells into cell β and does not affect insulin secretion [27].
Potential of pargyline as an RNLS inhibitor

Pargyline is a drug that has been approved by the Food and Drug Administration of the United States which has the same effect as the removal of RNLS on β cells. These drugs may protect endogenous cells β or autoimmune-transplanted cell β from ER stress [27].

Genome editing technology as a method for deleting RNLS in cell b

In addition to using drugs, the deletion of RNLS can also be done by genome editing methods [27]. In 2010, genome editing methods have been applied universally, such as Zinger-Finger Nucleases, meganucleases, and transcription activator-like effector nucleases. In 2013, a study found that clustered regularly interspaced short palindromic repeats-CRISPR-related (CRISPR/Cas9) derived from the bacterial species Streptococcus pyogenes has been shown to cleave target gene sequences and allow new avenues for gene modification in mammals [34].

CRISPR-Cas is an adaptive immune system found in bacteria and archaea. This technology is a third-generation artificial nuclease technology based on the CRISPR-Cas system and is believed to be a powerful gene-editing tool [35].

Genome editing using CRISPR/Cas9 has several advantages, in which the process is simpler, faster, and cheaper than another gene editing. Nuclease engineering is efficient in a wide variety of organisms, including mammals. Therefore, there will be a significant possibility of using genome editing tools for developing cell-based human therapies that can provide life-saving therapy for various diseases such as human immunodeficiency virus infection, sickle cell anemia, and cancer. In addition, CRISPR/Cas9 technology can also correct mutations and prevent transmission of genetic diseases from parents to offspring. Studies using human trinuclear zygotes concluded that CRISPR/Cas9 can effectively correct a mutated human-globin gene, a gene that encodes a subunit of adult hemoglobin [18], [34], [36], [37].

Although this technology has many advantages in disease prevention strategies, CRISPR/Cas9 is also still being debated, one of which is in terms of the code of ethics. In addition, the concern of off-target mutagenesis and mosaicism in the resulting animals could be a drawback of this technology [34], [36].

Conclusion

T1DM is a chronic disease in children that has a fairly high incidence and often becomes a global problem. This disease is characterized by impaired insulin production due to damage to pancreatic cells a and b. Insulin injection is currently a lifelong therapy for people with T1DM.

The deletion of RNLS using the CRISPR/Cas9 genome editing method can be a promising solution as a current therapy for T1DM. Apart from being faster, cheaper, and highly effective, this therapy is also
believed to have great potential to provide therapy with a permanent effect.

It is expected that further research will be carried out to determine the effectiveness of RNLS removal therapy with the CRISPR/Cas9 method which was clinically tested to determine the side effects that can be caused by its use. So that later this therapy can be used as the main therapy for people with T1DM (Figure 2).

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