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Protection of β Cells through Renalase (RNLS) and CRISPR/Cas9 as a Potential Therapeutic Approach in Diabetes

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Abstract. Type 1 diabetes mellitus (T1DM) is a metabolic disease caused by the destruction of pancreatic βcells, with various complications. RNLS (Renalase) is a protein expressed in the kidneys and is strongly associated with complications of cardiovascular disease and other diseases. Multiple studies have proved the association between RNLS and T1DM, showing that RNLS plays an important role in the protection of pancreas islet β cells. RNKS can reduce damage caused by immune attacks and oxidative stress and regulate energy metabolism. Current studies used gene-editing techniques, such as CRISPR/Cas9, as a potential therapeutic approach for T1DM through targeting multiple genes. Researchers also used *in vivo* CRISPR screen and identified the role of RNLS in β cells. However, future research is required to provide evidence of whether and how targeting RNLS with gene editing tools can restore β cell function, aiming to provide novel therapeutic approaches and personalized clinical applications for heterogeneous T1DM patients.

Keywords: Type 1 diabetes mellitus (T1DM), RNLS, CRISPR/Cas9.

1. Introduction

Diabetes occurs when a person's blood sugar is too high, affecting millions of people. Type 1 diabetes mellitus (T1DM) is a metabolic disorder syndrome characterized by high blood sugar due to an absolute lack of insulin. This disease is mainly caused by the destruction of islet β cells through immune-mediated. In some patients, there may be no evidence of autoimmune destruction of islet β cells, which is called idiopathic type 1 diabetes. Thirst, polydipsia, polyuria, polyfood, and weight loss are common symptoms. Diabetes has many complications, such as retinopathy, kidney disease, and neuropathy. Some patients who have had diabetes for a long time, including insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM), are most likely to develop complications [1]. Therefore, the economic burden posed by diabetes is also substantial. The total cost of diagnosing diabetes in the United States is approximately $4129 billion, of which direct medical expenses are approximately $3066 billion, with indirect costs of $1063 billion. Medical costs are significantly higher for people with diabetes, costing an average of $19,736 per year, of which about $12,022 is directly linked to diabetes [2]. Thus, it can be seen from this data that the economic burden caused by diabetes is still huge, and the impact on society is very significant.

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RNLS, or Rnalase, is one of the proteins expressed in kidney, also related to diabetes. Studies found that there are many single nucleotide polymorphisms (SNPs) in this gene, especially in functional regions. These SNPs have been linked to a variety of diseases, including coronary heart disease, stroke, high blood pressure and end-stage kidney disease [3]. Autoimmune destruction in pancreatic β cells is a major cause of T1DM. Therefore, RNLS plays an important role in β cell vulnerability, avoiding β cell loss in T1DM, and RNLS is a potential therapeutic target in T1DM [4]. Thus, gene editing targeting RNLS has been approved the therapeutic role on diabetes. However, the role of RNLS and How RNLS can be used to treat T1DM remains unclear.

This review summarizes the role of RNLS in T1DM and current applications of gene editing tools in T1DM, aiming to provide respective for the treatment of diabetes.

2. Function of RNLS

2.1. RNLS gene and protein expression

Renalase is the gene code located on chromosome 10 (10q23.33) and contains 10 exons. The total length is about 311,000 base pairs. Renalase is a 38 kDa protein containing the flavin adenine dinucleotide (FAD) domain, which functions as both a cytokine and an isomerase. It is now recognized as a regulator that can improve cardiometabolic disease. renalase is secreted mainly in the kidneys and has the ability to reduce and regulate blood pressure. The RNLS has a cardiovascular effect that affects lipid metabolism. Some experimental evidence shows that disruption of lipid metabolism homeostasis can alter RNLS expression in nephropathy. Lipid metabolism disturbance may lead to vascular injury and promote the onset and development of atherosclerosis [5].

2.2. RNLS in cardiovascular diseases.

Renalase is closely related to cardiovascular, Chronic Kidney Disease (CKD)is an important risk factor in cardiovascular diseases such as left ventricular hypertrophy, heart failure and coronary heart disease. The cardiovascular risks associated with renalase include endocrine dysfunction, inflammation, interrupted calcium and phosphate metabolism, arterial calcification, and RAAS activation. Therefore, the effect of renalase treatment on the heart is great, renalase treatment can improve the heart function to some extent, and reverse the problem of left ventricular hypertrophy associated with CKD [5].

Renalase also has a significant role in cardiovascular protection. Some studies have shown that Renalase can not only protect cardiomyocytes through antioxidant mechanisms, but also play a cardioprotective role by directly regulating vascular tension and myocardial metabolism. Renalase greatly reduces the risk of cardiovascular events in hypertensive patients by enhancing the function of endothelial cells and promoting vasodilation [6].

RNLS also have an effect on the heart. Left ventricular hypertrophy (LVH) is a common condition in people of high blood pressure, it is associated with a risk of heart failure and sudden death. Patients with end-stage renal failure have a high probability of contracting this disease, and RNLS plays an important role in the development of left ventricular disease. In a study of neonatal rats, it was found that neonatal rats with kidney injury had a higher heart weight to body weight ratio, increased plasma norepinephrine levels, and decreased cardiac RNLS expression. Therefore, it can be seen that RNLS deficiency may disrupt myocardial homeostasis, RNLS can reverse structural and functional changes in the heart [5].

2.3. RNLS in diabetic kidney diseases.

Renalase may slow the progression of diabetic kidney diasease (DKD) by inhibiting glomerular hypertrophy and reducing interstitial fibrosis. In diabetic patients and db/db mice, reduced renalase expression is associated with increased kidney damage, while overexpression of renalase improves renal function and reduces the expression of fibrosis markers. In addition, Renalase can be used as a biomarker for chronic kidney disease (CKD) and acute kidney injury (AKI), and can be used to help treat kidney disease. Due to the conflicting results of different studies, its exact physiological and pathological effects

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still need to be further clarified. After overcoming these challenges, Renalase is expected to be used in clinical treatment [5].

3. RNLS in diabetes

3.1. RNLS mutations in diabetes

Renalase is expressed in pancreatic β cells and may influence glucose metabolism and diabetes. Gene polymorphisms (such as SNP rs10509540) are associated with type 1 diabetes (T1D). Deleting the renal enzyme gene via CRISPR has a protective effect on pancreatic β cells, which may be beneficial for T1D therapy[5].

In type 2 diabetes (T2D), certain renal enzyme gene variants increase the risk of high blood pressure, and higher serum renal enzyme levels are associated with insulin resistance. Elevated renal enzyme levels in T2D patients may also reflect a compensatory response to increased dopamine.Renalase may link diabetes and hypertension through the renin-angiotensin-aldosterone system (RAAS), which is expected to be a new target for diagnosis and treatment.[5]

Renalase can also be a biomarker to help identify people with type 2 diabetes who have a higher risk of developing diabetic retinopathy. The Glu37ASP polymorphism in the kidney enzyme gene was found to be associated with susceptibility to this complication, suggesting that diabetes-induced retinal problems are more likely to appear in people with specific genetic variants. By detecting these genetic polymorphisms, doctors can identify patients at risk of infection earlier and provide them with a more personalized treatment plan, thereby reducing the chance of retinopathy and improving the overall health of patients [3].

3.2. Oxidative stress

Research indicates that Renalase appears to have multiple functions in cardiovascular protection, metabolic regulation, immune response modulation, and oxidative stress resistance. Recent studies have further identified specific functions and potential utilization value of these functions. First, Renalase plays an important role in regulating oxidative stress. Oxidative stress is a major feature of many chronic diseases, including diabetes and cardiovascular disease. Renalase, with its oxidation-reduction activity, can reduce the level of Reactive Oxygen Species (ROS) in cells. This can reduce cellular oxidative damage. Renalase may reduce ROS generation by regulating the activity of NADPH oxidase, resulting in significant reduction of beta cell damage in an experimental diabetes model [7]. This antioxidant function is of great importance for protecting the survival and function of pancreatic beta cells.

3.3. Metabolic regulation

RNLS plays an important role in metabolic regulation. Specific functions include regulating energy metabolism, fatty acid oxidation, and glucose homeostasis.Some studies have shown that Renalase can activate AMPK (Adenosine 5'-Monophosphate-activated Protein Kinase), thereby promoting the extraction and metabolism of glucose in liver and muscle cells and improving human insulin sensitivity[8]. Furthermore, there is an important role of Renalase in promoting lipid metabolism in fat cells through regulating the expression of PPARy (Peroxisome Proliferator-Activated Receptors) [9]. These experimental results both can prove that Renalase has a great role in the treatment of metabolic diseases, and also will be helpful in the future treatment of chronic diseases.

In addition, the function of Renalase in immune regulation has received great attention. Studies have shown that renalase can reduce tissue damage and inflammation by inhibiting the release of inflammatory factors. Mutations in Renalase alter the immune cell response and T cell activity , reduce the production of pro-inflammatory cytokines, thereby alleviating the autoimmune response in type 1 diabetes [10].

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4. Gene editing technology in diabetes

β cells are destroyed in T1D by the immune system.β cells in T2D become functionally impaired due to stress caused by amyloid deposition and insulin resistance. β cells In both T1D and T2D, β cells are affected by hyperglycemia, ER stress, and oxidative stress. Studies have shown that β cells can be protected, and inhibiting the Rnls gene reduces the stress response and protects β cells from damage [7]. Table 1 summarizes the different studies on CRISPR/Cas9 and the effects of diabetes-related genes on beta cell function. The CRISPR (Clustered Regularly spaced Short palindromic repeats) /Cas9 gene editing technology has made great strides in biomedical research in recent years. CRISPR/Cas9 system, originally derived from the immune defense mechanism of bacteria, has been transformed into a highly specific genome editing tool. The technology uses Cas9 nuclease to cut DNA and guide it to identify specific gene sequences through Single-guide (SgRNA), enabling precise gene knockout, insertion, and replacement. The CRISPR/Cas9 technology is efficient, precise and simple. It has quickly become a powerful tool for studying multiple genetic diseases and developing gene therapy strategies.

In the study of diabetes, the application of CRISPR/Cas9 technology has demonstrated its great potential. CRISPR/Cas9 has been used to create more accurate models of diabetes, so that to study the pathogenesis of diabetes and to select new therapeutic targets. For example, some studies showed that use CRISPR/Cas9 to knock out specific genes--GLUT2 genes and INS1 genes in mouse models to study the molecular mechanisms of beta cell function and insulin secretion, showing how specific genes lead to β cell dysfunction and diabetes [11]. This approach improves the specificity and reproducibility in this study, providing a basis for future understanding of the causes of diabetes and the development of new treatments.

In addition, CRISPR/Cas9 may also be used to directly repair genetic defects caused by diabetes, providing a new direction for gene therapy of diabetes. Herman et al. used CRISPR/Cas9 technology to knock out genes associated with immune responses in beta cells, reducing the expression of antigens on the surface of these cells. This genetic modification makes beta cells less likely to be recognized and attacked by the immune system. The researchers observed increased cell survival in the gene-edited β cells and the insulin secretion function was not loss. Moreover, the study also found that genetically modified beta cells have stronger antioxidant capacity and are better able to resist cell damage caused by inflammatory responses and oxidative stress. This result also supports the idea which genetic modification of beta cells using CRISPR/Cas9 technology can improve the tolerance in immune attack and environmental stress of T1D.

Overall, CRISPR/Cas9 gene editing technology to modify β cells not only protected these cells from being destructed by the immune system but also enhanced cell survival percentage, demonstrating the value of this gene-editing approach in T1D therapy [12].

This discovery shows the potential of CRISPR/Cas9 in reshaping and enhancing β cell function, opening up new possibilities for treating diabetes through gene editing in the future. In general, CRISPR/Cas9 can help express the molecular mechanism of diabetes and can also be used as a new tool for diabetes treatment, especially in the field of personalized therapy, which can be developed in the future.

Table 1. Applications of gene editing technology on diabetes

Targets	Disorders	Methods	Results	Ref
GLUT2 gene	β cell dysfunction	Knockout with CRISPR/Cas9	GLUT2 gene leads to beta cell dysfunction and impaired insulin secretion	[11]
INS1 gene	β cell dysfunction	Knockout with CRISPR/Cas9	Demonstrated the role of INS1 gene in beta cell function and diabetes	[11]
Immune response genes	T1DM	Knockout with CRISPR/Cas9	Reduced expression of antigens on beta cells, increased survival, and preserved insulin secretion	[12]

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Table 1. (continued).

The genes about oxidative stress	T1DM	Modification using CRISPR/Cas9	Genetically modified β cells exhibited stronger antioxidant capacity and resistance to inflammation	[12]

5. Conclusion

T1DM remains a big problem for human health and has a significant impact on the global economy. RNLS plays a very important role in diabetes, especially the function of RNLS in the protection and metabolic regulation of pancreatic islet β cells, which can reduce the oxidative stress and immune destruction of pancreatic islet β cells. RNLS is expected to become an important therapeutic direction for T1DM. Based on the application of CRISPR/Cas9 gene editing technology, some studies have found that deleting or modifying RNLS genes may enhance the survival of βcells in the pancreas and also improve tolerance to immune attack and environmental stress. This not only provides a new method for the treatment of T1DM, but also provides a new method for the treatment of other metabolic diseases. However, although RNLS has shown good therapeutic effects in animal experiments, clinical trials have not yet been experienced. Thus, the specific mechanism of action and clinical application in humans still need to be further studied. In the future, by exploring the mechanism of action of RNLS and the application of gene editing technology, it is expected to develop more personalized, more accurate, and better diabetes treatment pipelines.

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