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Research Article

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Application of CRISPR-Cas9 Technology in the Treatment of Metabolic Diseases

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Abstract

Since its development, CRISPR-Cas9 technology has demonstrated significant potential for the research and treatment of metabolic diseases. This article first outlines the basic principles of CRISPR-Cas9 technology and its specific applications in the treatment of metabolic diseases. The application examples of this technology in the research of fat metabolism, obesity and diabetes in vivo, as well as its innovative progress in the transformation of zebrafish models with metabolic diseases, were discussed in detail. As a result of these applications, new insights into the pathogenesis of metabolic diseases have been gained, as well as a foundation for developing new treatment strategies has been laid. However, the application of CRISPR-Cas9 technology also faces some challenges, especially in terms of safety and ethics. Gene editing poses a number of safety, ethical, and legal issues, and norms and standards are being developed to ensure its responsible application. A final consideration in this article is to anticipate the direction in which CRISPR-Cas9 technology will develop in the future for the treatment of metabolic diseases, emphasize the need to continue exploring its potential applications, and anticipates that it will improve human quality of life and promote health.

Keywords: CRISPR-Cas9 Technology, Metabolic Diseases, Disease Treatment, Ethical Issues

1. Introduction

CRISPR-Cas9 is a groundbreaking gene editing technology that has attracted high attention in the biomedical field since its launch. This technology is based on the bacterial immune system, using specific RNA to guide Cas9 nuclease for precise DNA editing, thereby achieving detailed manipulation of the genome. CRISPR-Cas9 shows significant application prospect in metabolic disease research, especially in fat metabolism, obesity and diabetes. Through gene editing, researchers can gain a deeper understanding of the pathogenesis of these diseases and explore novel treatment options. However, with the widespread application of technology, safety and ethical issues are gradually emerging, prompting the scientific community to evaluate the use of gene editing more cautiously. This article will explore the application of CRISPR-Cas9 in the treatment of metabolic diseases and the challenges it faces, aiming to provide reference for future research and practice.

2. Overview of CRISPR/Cas9 Technology

CRISPR sequences are short palindromic sequences widely present in bacteria and archaea, forming regular intervals as a non-specific defense mechanism for bacteria to resist viruses. Its leading region is responsible for initiating transcription, generating pre cRNA, a non-coding RNA precursor, and also transcribing tracrRNA complementary to the repeat sequence. Subsequently, endogenous RNase III will cleave pre crRNA into mature crRNA containing repetitive and spacer sequences. Next, crRNA binds with tracrRNA to form sgRNA, which can tightly bind to the target DNA sequence through base complementarity. The CRISPR sequence co evolved with CRISPR related genes (Cas) to form a highly conserved CRISPR/Cas system. The protein encoded by the Cas gene usually has enzymatic activity, such as Cas endonuclease.

According to the different Cas proteins, the CRISPR system is divided into Type I, Type II, and Type III. Among them, the Type II system is relatively simple, which only requires sgRNA to guide Cas9 nuclease to locate 3 to 8 bases upstream of the PAM sequence and can cut foreign DNA and integrate it into the CRISPR sequence, forming immune memory. When the same exogenous DNA invades again, cells utilize their own repair mechanisms for targeted gene modification, including site directed mutagenesis and gene knock in. In 2013, scientists successfully used bacterial RNA to guide Cas9 protein for site directed mutagenesis of EMX1 and PVALB genes in 293T cells, validating the effectiveness of CRISPR/Cas9 in mammalian genome editing. Afterwards, this technology was used to modify genes for the treatment of genetic diseases such as sickle cell anemia and Duchenne muscular dystrophy, opening up new treatment opportunities [1].

Compared with the widely used LoxP Cre gene conditional knockout tool, the CRISPR/Cas9 system has significant advantages. In sgRNA synthesis, only 20 bp of DNA needs to be inserted into the vector, and the generated sgRNA is 20 nucleotides in size. Shorter sgRNA sequences can simplify operations and avoid complex issues that may arise during long fragment insertion. In addition, the system can modify multiple sites simultaneously, reducing experimental costs and shortening experimental time. Despite potential issues with off target effects, research has shown that using longer and stricter PAM sequences significantly reduces off target effects, providing a more reliable basis for gene therapy. In addition, the whole gene Digenome seq test of the newly discovered CRISPR/Cpf1 technology showed off target effects of less than 0.1% [2]. With the development of social economy, China has become a country with high incidence of metabolic diseases such as diabetes, hypertension, obesity and dyslipidemia. At present, there is no effective radical cure. The occurrence of these diseases is closely related to genes, and the emergence of CRISPR/Cas9 technology provides a new approach for studying their pathogenesis and treatment. This technology is currently mainly used to construct disease models and modify and treat pathogenic genes.

3. Application of CRISPR/Cas9 Technology in Metabolic Diseases

3.1. Application of CRISPR/Cas9 Technology in Lipid Metabolism Research in Vivo

The PCSK9 gene is the third gene associated with autosomal dominant familial hypercholesterolemia, in addition to LDLR and APOB. Functional acquired mutations can lead to a decrease in LDL receptor levels, resulting in an increase in LDL-C levels. In the ARIC observational study, it was found that PCSK9 function deletion mutation can reduce the LDL-C level of African Americans and whites, and is related to the reduction of the incidence of cardiovascular events. Therefore, PCSK9 has become a new target for the treatment of hyperlipidemia. Although PCSK9 is widely expressed in multiple organs, only PCSK9 in the liver can enter the bloodstream.

Researchers have successfully introduced the CRISPR/Cas9 system into mouse liver using nonpathogenic adeno-associated virus (AAV) to target and cleave the DNA strand of PCSK9 gene. After 3 to 4 days, the mutation rate of PCSK9 gene exceeded 50%, the PCSK9 level in plasma significantly decreased, the LDL receptor in the liver increased, the LDL-C level decreased, and no off target effects were observed. Another study also used AAV to introduce Cas9 and gRNA targeting PCSK9, and the results were consistent with the former. This marks the first successful modification of the PCSK9 gene, laying the foundation for the treatment of familial hyperlipidemia [3].

In addition, the TRIB1 gene is associated with PCSK9 and plays an important role in liver cell lipid metabolism. When TRIB1 is overexpressed, fatty acid oxidation increases, cholesterol and VLDL synthesis decrease, and HDL levels increase; Knocking out TRIB1 can lead to high blood sugar. To investigate the role of TRIB1 in lipid metabolism, Nagiec et al. used CRISPR/Cas9 to disrupt the TRIB1 gene. The results showed an increase in PCSK9 transcription and protein secretion, a decrease in hepatic LDL receptors, and an increase in LDL-C levels, confirming the regulatory role of TRIB1 in lipid metabolism.

CREB3L3 is a membrane-bound transcription factor primarily expressed in the liver and small intestine. It co activates the expression of fibroblast growth factor 21 (FGF21) in the liver with PPAR α, promoting fat breakdown and heat production, thereby reducing insulin resistance and blood triglyceride levels. Nakagawa et al. used CRISPR/Cas9 to obtain CREB3L3 conditional knockout mice for the first time. The experiment showed that CREB3L3 knockout mice in the liver had elevated cholesterol levels, but the small intestine had no such effect, indicating that CREB3L3 mainly regulates lipid metabolism in the liver. This further demonstrates the effectiveness of CRISPR/Cas9 technology in modifying target genes and establishing animal models to explore the pathogenesis, providing a new approach for the treatment of cardiovascular diseases.

3.2. Application of CRISPR/Cas9 Technology in Obesity Research

Obesity is regarded as an independent risk factor for many diseases (such as type 2 diabetes and cardiovascular disease), and it is also an important component of metabolic syndrome. Gene therapy provides a new approach for the treatment of obesity. Research has found that the FTO gene is closely related to obesity, as it inhibits mitochondrial thermogenesis in adipocyte precursor cells and its mutations hinder the conversion of white fat to brown fat. The thermogenic pathways regulated by FTO genes include factors such as ARID5B, rs1421085, IRX3, and IRX5. By using CRISPR/Cas9 technology to repair rs1421085, ARID5B can be inhibited, thereby reducing the expression of IRX3 and IRX5 and promoting weight loss. This method has opened up new avenues for the treatment of severe obesity.

Lep is a hormone secreted by white adipose cells that acts on the hypothalamus through the central leptin receptor (LepR), suppressing appetite and regulating energy balance. Obese patients often exhibit leptin resistance, characterized by elevated leptin levels and a feedback decrease in LepR. Elevated leptin also promotes free radical generation. Animal models targeting Lep and LepR, such as ob/ob mice and db/db mice, are widely used to study their importance in glucose and lipid metabolism. Bao et al. successfully constructed LepR knockout mice using CRISPR/Cas9 technology, showing obesity, hyperglycemia, insulin resistance and other characteristics. Long term deletion may also lead to complications of diabetes. This model improves the deficiency of existing mouse models and provides convenience for the study of the pathogenesis of obesity and diabetes [4].

In China, researchers have successfully knocked out the Lep and LepR genes using CRISPR technology, establishing a Lep gene knockout rat model and filling a gap in this field. In addition, they also constructed cytochrome P450 (CYP) 2E1 gene knockout mice

to study the role of this gene in metabolism and related diseases (such as diabetes and alcoholic cirrhosis), and no off-target effect was observed.

3.3. Application of CRISPR/Cas9 Technology in Diabetes Research

The pathogenesis of type 2 diabetes is relatively complex, mainly involving the reduction of islet beta cells and dysfunction. Therefore, it is very important for the diagnosis and treatment of diabetes to deeply explore the role of pancreatic islet β cells in diabetes. Researchers have developed a novel Cre tool mouse using CRISPR/Cas9 technology for gene editing of pancreatic beta cells. They used the termination codon sequence of the Ins1 promoter of the insulin gene as a target for Cre insertion and injected plasmids encoding gRNA and Cas9, as well as donor DNA. By mating with flox mice, F1 mice labeled as Cre loxP recombinant were obtained. This recombination phenomenon was only observed in the pancreatic islets of insulin positive cells, and was not expressed in other tissues, indicating successful β - cell specific recombination. Meanwhile, there was no significant difference in glucose tolerance between these mice and wild-type mice. Therefore, the use of CRISPR/Cas9 technology to create dual cis trans knock in Cre tool mice provides important support for studying pancreatic beta cell function and glucose metabolism [5].

Human induced pluripotent stem cells (hiPSC) have also been widely used in disease model construction. With CRISPR/Cas9 technology, we can modify hiPSC for diabetes related genes, establish a model more similar to human disease, and explore the molecular mechanism of diabetes. For example, hiPSC can be isolated from monogenic diabetes patients with MODY, and use CRISPR technology to edit genes such as HNF4A, GCK, PDX-1 and INS, and then induce differentiation into pancreatic progenitor cells and transplant them back into patients. For neonatal diabetes, KCNJ11 gene can be edited; Type 1 and type 2 diabetes are involved in the editing of HLA DR-DQ, HLA-B, HLA-A, INS, TCF7L2, IGF2BP2, IRS1 and KCNQ1 genes. These artificial transcription factors can selectively regulate the differentiation of pluripotent stem cells into pancreatic cells by introducing specific gRNAs into transcriptional regulatory regions, thereby overcoming the maturation barrier of pancreatic islet like cells derived from stem cells in vivo and providing abundant donor resources for pancreatic transplantation.

With the continuous development of CRISPR/Cas technology, CRISPR interference technology (CRISPRi) and CRISPR activation technology (CRISPRa) have emerged. CRISPRi is an improved gene regulation tool based on CRISPR/Cas technology, which silences genes by introducing the fusion transcription factor inhibitor dCas9 into the promoter region near specific genes without cutting DNA. CRISPRa upregulates gene expression by introducing the activator dCas9 near specific genes. The emergence of these two technologies provides the possibility for precise regulation of gene expression. Considering the polygenic characteristics of diabetes, CRISPRi and CRISPRa technologies can be used to establish gene libraries in the future to screen out genes related to promoting or inhibiting the growth and apoptosis of pancreatic beta cells.

3.4. Application of CRISPR/Cas9 Technology in Metabolic Disease Zebrafish Model Modification

Zebrafish are considered an ideal animal model for studying metabolic diseases because they possess major metabolic organs similar to humans, such as the hypothalamus and pancreas. Although brown fat has not yet been found as a temperature changing animal, zebrafish has been widely used in the study of obesity, diabetes and fatty liver. In 2013, Hwang et al. first injected Cas9 mRNA and specific gRNA into the fertilized eggs of zebrafish, successfully achieving site directed mutagenesis of drd3 and gsk3b genes. Subsequently, Cheng Cheng and others in China used CRISPR/Cas9 technology to design the gRNA targeting RFX6 gene and construct a knockout model. They found that the mutant grew slowly, was small and had diabetes related characteristics, indicating the importance of RFX6 in pancreatic development. In another study, researchers successfully inserted the Gal4 sequence into the GFP gene by co injecting Cas9 mRNA, sgRNA, and Gal4 plasmid. These results demonstrate the effectiveness of CRISPR/Cas9 in zebrafish genome editing, providing a powerful tool for the study of endocrine system diseases [6].

CRISPR/Cas technology has brought convenience to genome research, and its high efficiency and multi-site editing capabilities provide new possibilities for precision medicine and personalized treatment. At present, nearly 60 susceptibility genes related to diabetes have been identified, such as TcF7L2 mutation, UCP2 overexpression and SLC16A11 mutation. These genes can be used to construct lentivirus mediated CRISPR-Cas9 gene knockout library and combined with high-throughput sequencing to screen more type 2 diabetes pathogenic genes. In addition, Cas9 can also interact with modifying enzymes to change chromatin structure, so it is also a new trend to apply Cas9 to the treatment of diabetes in epigenetics. Overall, CRISPR/Cas9 technology has brought new opportunities and development prospects for metabolic disease research.

Figure 1: Application of CRISPR/Cas9 technology in metabolic diseases

4. Challenges and Ethical Issues of CRISPR/Cas9 Technology

4.1. Safety Issues of Gene Editing Technology

Although CRISPR/Cas9 technology has shown great potential in gene editing, its safety remains a key issue. Research has shown that this technology may lead to unexpected off target effects, i.e. unnecessary editing on non-target genes, which could result in potential biosafety risks. This risk is particularly important when treating human diseases, as inaccurate gene editing may trigger new diseases or worsen existing ones. In addition, the long-term effects of CRISPR/Cas9 have not been fully evaluated, so in-depth research and rigorous testing are needed before clinical application to ensure its safety in various applications [7].

4.2. Ethical and Legal Issues of Gene Editing Therapy

The rapid development of gene editing technology has raised many ethical and legal issues. Firstly, there is widespread controversy over whether gene editing should be applied in germ cells, as this could have irreversible impacts on future generations [8]. The legality and ethics of such operations must be fully discussed and reviewed. Secondly, the accessibility and fairness issues of gene editing have also raised social concerns, especially whether economically disadvantaged groups can access corresponding treatments. In addition, the existing legal framework may not be able to effectively address the new challenges brought by gene editing, so it is necessary to update relevant laws to meet the needs of this emerging technology.

4.3. Future Development Direction and Standardization of CRISPR/Cas9 Technology

In the future, the development direction of CRISPR/Cas9 technology may include research on improving editing accuracy and reducing off target effects. Meanwhile, with the continuous advancement of technology, standardization and regulation will become particularly important. Scientists, ethicists, and legal experts should collaborate to establish a comprehensive ethical and legal framework to guide the research and application of gene editing. In addition, the public's awareness and acceptance of gene editing technology will also affect its future development. By enhancing public participation and education, the relationship between scientific innovation and ethical responsibility can be better balanced.

Figure 2: Challenges and ethical issues of CRISPR/Cas9 technology.

5. Conclusion and Prospect

In summary, CRISPR-Cas9 technology has brought revolutionary changes to the research and treatment of metabolic diseases, and its potential applications in gene editing, disease model construction, and treatment strategy development are encouraging. Through targeted editing of genes related to lipid metabolism, obesity and diabetes, researchers not only deepened their understanding of the pathogenesis of metabolic diseases, but also paved the way for new treatments. However, despite the significant advantages of CRISPR-Cas9 technology, its safety and ethical issues urgently need to be addressed to ensure its feasibility and sustainability in clinical applications. In the future, further in-depth research on the potential and potential risks of this technology, as well as the development of relevant norms and standards, will be the key to promoting its healthy development. I hope that in the near future, CRISPR-Cas9 technology can bring good news to more patients with metabolic diseases and improve human health.

References

1. Li, T., Yang, Y., Qi, H., Cui, W., Zhang, L., Fu, X., ... & Yu, T. (2023). CRISPR/Cas9 therapeutics: progress and prospects. *Signal transduction and targeted therapy, 8*(1), 36.
2. Guo, C., Ma, X., Gao, F., & Guo, Y. (2023). Off-target effects in CRISPR/Cas9 gene editing. *Frontiers in bioengineering*

and biotechnology, 11, 1143157.
3. Wang, S. W., Gao, C., Zheng, Y. M., Yi, L., Lu, J. C., Huang, X. Y., ... & Ke, A. W. (2022). Current applications and future perspective of CRISPR/Cas9 gene editing in cancer. *Molecular cancer, 21*(1), 57.
4. Xu, X., Liu, C., Wang, Y., Koivisto, O., Zhou, J., Shu, Y., & Zhang, H. (2021). Nanotechnology-based delivery of CRISPR/Cas9 for cancer treatment. *Advanced drug delivery reviews, 176,* 113891.
5. Zhang, H., Qin, C., An, C., Zheng, X., Wen, S., Chen, W., ... & Wu, Y. (2021). Application of the CRISPR/Cas9-based gene editing technique in basic research, diagnosis, and therapy of cancer. *Molecular cancer, 20,* 1-22.
6. Hillary, V. E., & Ceasar, S. A. (2023). A review on the mechanism and applications of CRISPR/Cas9/Cas12/Cas13/Cas14 proteins utilized for genome engineering. *Molecular biotechnology, 65*(3), 311-325.
7. Zhang, S., Shen, J., Li, D., & Cheng, Y. (2021). Strategies in the delivery of Cas9 ribonucleoprotein for CRISPR/Cas9 genome editing. *Theranostics, 11*(2), 614.
8. Gupte, P., Dhingra, K., & Saloni, V. (2024). Precision Gene Editing Strategies with CRISPR-Cas9 for Advancing Cancer Immunotherapy and Alzheimer's Disease. *Journal of Knowledge Learning and Science Technology ISSN: 2959-6386 (online), 3*(4), 11-21.