

Innovative CRISPR-Cas9 Enhances Cell Rejuvenation, Addressing Diseases

Linfeng Zhang^{1,a,*}

¹*Zhejiang University, Zhejiang, China*

a. linfeng.22@intl.zju.edu.cn

**corresponding author*

Abstract: CRISPR-Cas9 technology, a groundbreaking gene editing tool, has shown promise in various fields such as genome modification, gene expression regulation, and functional screening. Despite its potential, the mechanisms by which CRISPR-Cas9 regulates cell fate and reprogramming remain incompletely understood, hampering its broad application in regenerative medicine and the treatment of age-related diseases. To address this, this study systematically reviews recent advancements in CRISPR-Cas9 and CRISPRa systems, focusing on their roles in cellular reprogramming, fate selection, and age-related disease therapy. We delve into the intricacies of sgRNA design, which significantly impacts the efficiency and specificity of gene editing, highlighting the importance of optimizing sgRNA sequences to enhance the precision and effectiveness of CRISPR-Cas9 in targeting specific genes. Furthermore, we examine the potential applications and challenges of CRISPR technology in cell cycle regulation and disease therapy. We underscore the transformative potential of CRISPR in precision medicine, enabling more targeted and effective treatments for various conditions. However, we also acknowledge the obstacles, including off-target effects and technical limitations, that need to be overcome for widespread clinical application. By synthesizing the existing literature, our study provides a comprehensive understanding of CRISPR technology's role in cell function regulation and the development of precision medicine. We propose directions for further optimization and improvement, such as enhancing sgRNA design and reducing off-target effects. Our findings serve as a theoretical foundation and practical guideline for advancing the translation of gene editing technology into clinical practice, ultimately paving the way for innovative treatments that can improve the quality of life for patients with various diseases.

Keywords: Rejuvenation, Age-related diseases, CRISPRa, iPSCs, Cancer Therapy.

1. Introduction

CRISPR-Cas9 technology, a groundbreaking gene editing tool, has shown promise in various fields such as genome modification, gene expression regulation, and functional screening. However, its specific mechanisms in cell fate regulation and reprogramming have not been fully revealed, a research gap that limits its application in regenerative medicine and disease treatment [1]. In recent years, cell reprogramming and rejuvenation research has become a hotspot in the fields of regenerative medicine and anti-aging, especially through the generation of induced pluripotent stem cells (iPSCs) or partial reprogramming techniques, which provide new possibilities for tissue repair

and aging delay [2]. Due to its precise editing ability of key gene loci, it is expected to significantly improve reprogramming efficiency. However, the CRISPR technology still needs to be deeply explored in existing studies in terms of cell cycle dynamics regulation, fate selection and its interaction with chromatin state. In addition, as the global population aging problem intensifies, the complexity of age-related diseases such as Alzheimer's disease, cancer and metabolic disorders puts higher demands on precision therapy [3]. Although CRISPR-Cas9 technology has provided novel ideas for the treatment of these diseases, its off-target effects, long-term safety, and delivery efficiency remain major obstacles [4]. In this paper, we systematically sorted out the application and optimization strategies of CRISPR-Cas9 technology in cellular reprogramming, explored the technological advances of the CRISPR system in the study of cell-cycle monitoring and fate selection, and its significance in unraveling cellular mechanisms. By focusing on the potential applications of CRISPR-Cas9 technology in the treatment of age-related diseases (e.g., neurodegenerative diseases and cancer) and its challenges and risks, this review will reveal the cutting-edge advances of CRISPR technology in the regulation of cellular functions and the development of disease therapeutic options, provide theoretical support for related fields and offer guidance for future directions.

2. CRISPRa System in Cell Cycle and Fate Selection

2.1. Applications of CRISPR-induced Cellular Reprogramming

The CRISPRa system, which builds on the principles of CRISPR-Cas9, has emerged as a powerful tool for induced cellular reprogramming. By mutating the Cas9 enzyme at specific points (D10A and H840A) to create dead Cas9 (dCas9) and then fusing it with transactivating structural domains like VP64 and P300, researchers have developed a synergistic activation-mediated (SAM) strategy known as the coactivation mediator. This CRISPR-based activation (CRISPRa) approach enables direct and endogenous activation of individual gene expression in a single-gene-per-cell manner, making it a valuable tool for gain-of-function screening. CRISPRa system targets the transcription start sites (TSS) of different genes using sgRNAs in hybrid screens, which makes it more scalable and accessible than traditional gene expression methods that rely on donor DNA or open reading frames (ORFs). The system holds potential as a method for cellular reprogramming by initiating the expression of endogenous gene networks, allowing for the silencing and activation of endogenous genes to facilitate appropriate cell type conversion. This leads to the emergence of partially reprogrammed stable cell populations. Studies have demonstrated the successful application of this method in mediating the differentiation, trans-differentiation, and reprogramming of various mouse and human cell types [5]. Recent advancements have shown that primary adult skin fibroblasts can be reprogrammed and induced into pluripotent stem cells using CRISPRa. The efficiency of this approach is contingent on specific targeting of embryo genome activation (EGA) motifs by dCas9. The binding of dCas9 to the high-affinity guide site of the EGA-motif locally disrupts chromatin, enabling more efficient activation of neighboring genes. This ultimately results in the activation of a subset of endogenous genes that function as reprogramming factors, including NANOG and ZFP42 (REX1), leading to improved reprogramming efficiency [5]. Another study demonstrated the potential of the CRISPRa system to target cell type-enriched regulatory elements to control cell fate. By activating endogenous cardiac factors such as GATA4, HAND2, MEF2C, and TBX5 (G, H, M, and T; GHMT), researchers were able to reprogram human fibroblasts into induced cardiomyocyte-like progenitor cells (iCPCs). These cells showed the capacity to differentiate into cardiomyocytes, smooth muscle cells, and endothelial cells in vitro [6]. These findings illustrate the versatility of the CRISPRa system in developing a range of reprogramming methods for human cells. The ability to reprogram cells into progenitor cells, rather than terminally differentiated cells, offers exciting

possibilities for the regeneration of diseased or damaged tissues, representing a significant step forward in regenerative medicine.

2.2. CRISPRa screens and regulates reprogramming factors

CRISPRa is an ideal tool for screening the numerous chromosomal loci associated with pluripotency that undergo remodeling during the induction of pluripotent stem cells. Early studies utilizing CRISPR activation demonstrated its capacity to precisely target and remodel endogenous gene loci, such as Oct4 and Sox2. It was revealed that single-site targeting of Sox2 was sufficient to remodel and activate the gene, which in turn induced the expression of other pluripotent genes, leading to the establishment of a pluripotency network [7]. It was observed that Sox2 remodeling precedes the activation of other key pluripotency genes, and the efficiency of reprogramming is highly dependent on Sox2 levels. Simultaneously, remodeling the Oct4 promoter and enhancer also triggered cellular reprogramming, resulting in the generation of pluripotent cell lines in both cases. The additional activation of Oct4 can enhance the synergistic nature of reprogramming at pluripotency sites. The remodeling of enhancers is also essential for the further induction of Oct4 at later stages of pluripotency induction. Overall, these findings underscore the powerful potential of CRISPRa in targeting and remodeling endogenous gene loci associated with pluripotency, which can lead to the generation of pluripotent stem cells.

Our understanding of the molecular basis of cellular senescence is still incomplete, despite knowing some of the epigenetic basal loci involved. Mutations in the WRN gene cause Werner syndrome (WS), a disease characterized by accelerated senescence. In a study, human mesenchymal precursor cells (hMPCs) derived from human embryonic stem cells with a homozygous deletion of the Werner syndrome protein (WRN) gene were used to explore the mechanisms of WS through a genome-wide CRISPR activation (CRISPRa) screen [8]. The results revealed that overexpression of the transcription factor SOX5 induced significant epigenetic and transcriptional remodeling, leading to the activation of age-protective genes such as HMGB2 and alleviating cellular senescence. Additionally, SOX5 enhanced HMGB2 expression by increasing enhancer activity and specific histone modifications (H3K27ac and H3K4me1). These findings suggest a crucial role for SOX5 in cellular rejuvenation and the attenuation of senescence, including in aged brain endothelial cells.

2.3. CRISPR Systems for Real-Time Monitoring of Cell Cycle Dynamics

The process of aging is intricately connected to the biological clock, as the circadian transcriptome is reprogrammed in a tissue-specific way with the passage of time. CLOCK, a crucial element of the molecular clock, partners with BMAL1 to control gene transcription [9]. Research involving the use of CRISPR/Cas9 technology to deactivate CLOCK in human mesenchymal stem cells (hMSCs) revealed a hastening of the aging process, highlighting CLOCK as a promising target for combating age-related illnesses. This study also demonstrated the potential of CRISPR to modify CLOCK in human embryonic stem cells, illustrating its ability to rectify age-related changes.

CRISPR-based barcoding systems in cancer research have revolutionized the monitoring of cancer cell subpopulations and treatment responses [10]. Through precise homology-directed repair, these systems can introduce or silence mutations, such as correcting ALK-F1174L mutations in neuroblastoma cells. This groundbreaking approach enables the repair or truncation of proteins, ensuring safety by preventing the development of new mutations. These innovative tools offer a versatile solution for genetic modification and monitoring cancer therapy effectiveness.

3. CRISPR-Cas9 gene editing in the treatment of age-related diseases

3.1. Major Approaches to Reprogramming to Treat age-related Diseases

Reprogramming technology is a scientific approach that involves redirecting cells to adopt specific functions for therapeutic purposes, such as replacing or repairing damaged tissues. This cutting-edge technology relies on gene editing and cell fate conversion to manipulate gene expression within cells, thereby altering their biological properties and activating or inhibiting key molecular pathways. By harnessing the power of genetic reprogramming, researchers have unlocked unprecedented treatment solutions for a wide array of complex diseases. One of the most promising applications of reprogramming technology is cell therapy using induced pluripotent stem cells (iPSCs). In animal models, iPSCs have shown remarkable potential for restoring tissue function in various disease conditions. For example, a recent study demonstrated that transplanting iPSCs into the brains of rats that had experienced a stroke resulted in a significant improvement in glucose metabolism in the affected region, consequently restoring neurological function [11]. The transplanted iPSCs successfully differentiated into neuronal and angiogenic cells, promoting the restoration of oxidative stress and mitochondrial function in the region. Furthermore, the integration of CRISPR-Cas9 technology with iPSCs has expanded the possibilities of reprogramming applications. By introducing specific genetic modifications into iPSCs, researchers can mimic the mechanisms of complex diseases, facilitating drug screening and gene therapy development. These modified iPSCs can be differentiated into patient-specific functional cells for transplantation therapy and tissue repair, offering personalized treatment options for a wide range of medical conditions.

3.2. Advances in the CRISPR System for Cancer Therapy

Recent advancements in relay T-cell therapies, particularly chimeric antigen receptor (CAR)-T-cell therapy, have shown promising results in treating relapsed and refractory cancers. By targeting specific genes such as PRDM1, PDCD1, TGFB2, and DNMT3A, the therapeutic efficacy of CAR-T cells can be significantly improved [12]. The use of the CRISPR/Cas9 system has revolutionized knockout technology for CAR-T cell therapy, allowing for precise gene editing. It has made it possible to prepare infused T cell products in vitro with knockout mutations through the transient transfection of Cas9 guide RNA (gRNA) ribonucleoprotein complexes. The selection of the appropriate gRNAs is crucial for the success of this process. Researchers have utilized CRISPR/Cas9 in human T cells to demonstrate the efficacy of the transient transfection of Cas9 gRNA ribonucleoprotein complexes in achieving knockout mutations. By pooling data from CRISPR/Cas9-mediated knockouts in human T cells, researchers have been able to identify the optimal gRNA for targeted gene editing. Furthermore, the combination of epigenetic information with sequence-based prediction tools has significantly improved gene editing efficiency. By utilizing tools that leverage dCas with CRISPR, researchers have been able to activate tumor suppressor genes in cancers such as colon, breast, and gastric cancer [13]. This approach has shown promise in enhancing the immune response in patients with immunogenic tumors. CRISPR-dCas9-mediated epigenetic modulation has shown potential in transforming non-immunogenic tumors into a more responsive, "hot" phenotype [14]. By targeting pro-immunogenic genes and repressing those involved in tumor immune evasion and immunosuppression, the immunogenicity of tumors can be enhanced. This approach may hold the key to improving outcomes in patients with non-immunogenic tumors, ultimately leading to better response rates and potential curative effects in cancer treatment.

The process of epithelial-mesenchymal transition (EMT) is a significant factor in driving cancer progression, allowing cancer cells to become more aggressive. One key player in this transition is the transcription factor ZEB1. Recent studies have focused on using CRISPR/dCas9 technology to target ZEB1 in triple-negative breast cancer (TNBC) models. The results have shown that by utilizing the dCas9 approach, ZEB1 can be effectively silenced *in vivo*, leading to a significant reduction in tumor growth and progression [15]. By combining dCas9 with a structural domain known as KRAB, researchers were able to identify specific changes in gene expression and methylation patterns related to ZEB1. This resulted in the reactivation of key cellular adhesion sites and enhanced accessibility of chromatin, ultimately promoting a more epithelial-like state. Overall, this research highlights the potential of CRISPR technology in epigenetic engineering, specifically in reversing the effects of EMT and potentially improving outcomes for TNBC patients.

3.3. Treatment options and evaluating risks for patients.

CRISPR-based technologies have revolutionized the way we can reprogram the immune response to combat tumors more effectively. The precision of CRISPR-Cas9 gene editing has shown promising results in enhancing T-cell killing by modifying key cell surface molecules like PD-1 and TCR. However, concerns have been raised about the potential off-target effects that could lead to genomic instability or disruption of normal genes. That's where the newly developed dCas9 systems come into play. These systems work transiently, are reversible, and eliminate the risk of long-term consequences, making them safer for *in vivo* applications and more clinically viable. The advantages of dCas9 and other technologies extend beyond just gene editing. They also allow for the direct up-regulation or down-regulation of transcriptional and epigenetic markers within the same platform, significantly broadening their application in cancer therapy. However, challenges remain in finding safe and precise delivery mechanisms, as well as addressing potential adverse immune responses in clinical CRISPR applications [12]. Recent studies have shed light on the complexity of depleted T-cell and NK-cell development, with chronically-stimulated cells showing significant epigenetic changes that make them unresponsive to anti-PD-1 therapy. By using targeted epigenetic CRISPR editing of key genes, researchers have been able to revitalize these depleted and dysfunctional cell subpopulations, such as TCF1, which plays a crucial role in T cell persistence and NK cell survival [16]. While the full extent of CRISPR-based technologies' impact on altering the tumor landscape is still unknown, the hope is that through a variety of approaches, durable immune responses can be achieved in the fight against cancer.

The potential of CRISPR/dCas9 in cancer treatment through epigenetic editing is promising. However, designing sgRNAs poses a challenge in utilizing CRISPR-based tools effectively [17]. Although CRISPR/Cas demonstrates precise gene editing abilities, both the Cas9 system and dCas9 are susceptible to off-target effects. To mitigate this issue, modifications such as altering PAM specificity of Cas9 proteins, rationally designing Cas9 proteins for improved charge interactions with non-targeted DNA sites or using truncated sgRNAs can enhance Cas9 specificity and reduce off-target risks. Numerous CRISPR/Cas9-based therapies are already undergoing clinical trials, primarily focusing on cancer treatment, while others are being researched for hematologic diseases. This indicates that CRISPR/Cas gene editing technology is advancing towards clinical applications, especially in cancer therapy. Similarly, emerging technologies like CRISPR/dCas for epigenetic editing may soon follow suit.

4. Discussion

The article explores how the system's crucial applications revolutionize disease treatment. It discusses cell fate selection, disease modeling, drug screening, and gene editing technologies, particularly in cancer therapy, showcasing their significance in advancing medical breakthroughs.

While CRISPR technology holds immense potential in revolutionizing the field of medicine, concerns regarding its safety and efficacy persist. One of the major obstacles in the widespread clinical application of CRISPR/Cas9 technology is the issue of off-target effects. These unintended alterations in the genome can result in unexpected mutations or even the development of cancer. Efforts to mitigate off-target effects include optimizing the design of the single guide RNA (sgRNA), modifying the prototypical adjacent motif (PAM) specificity, and utilizing high-fidelity Cas9 variants such as SpCas9-HF1 and eSpCas9 [18]. Despite these strategies, eliminating off-target effects, particularly in complex in vivo settings, remains a formidable challenge. It also has been observed that the chromatin state also plays a crucial role in influencing the off-target effects of CRISPR/Cas9. Variations in the chromatin structure can significantly impact the efficiency of target editing, highlighting the complexity of gene editing attempts.

Efficiency in cellular reprogramming using the CRISPRa system relies heavily on the accurate recognition and binding of sgRNA to the target region. However, several factors can impact this process, such as the complexity of chromatin structure, the expression level of the target gene, and the accessibility of transcription factors. Dense chromatin surrounding the transcription start site (TSS) of certain genes can hinder dCas9 from binding effectively, ultimately diminishing the activation efficiency of the CRISPRa system [19]. Additionally, when attempting multigene reprogramming, it becomes challenging to activate several key genes simultaneously to produce synergistic effects. Overcoming these obstacles remains a technical challenge in the field of cellular reprogramming. Researchers continue to investigate and develop strategies to improve the precision and efficiency of the CRISPRa system for more effective cellular reprogramming processes.

Despite the promise shown by CRISPR-enabled CAR-T cell strategies in cancer therapy, long-term efficacy is limited by challenges such as immune escape, T cell depletion, and tumor heterogeneity. The suppressive immune cells, molecular signals, and hypoxic conditions present in the tumor microenvironment (TME) complicate CRISPR-based therapies by hindering delivery and targeting efficiency [14]. These obstacles must be overcome to maximize the potential of this innovative treatment approach.

The translation of CRISPR technology into clinical use requires resolving key challenges such as off-target effects, chromatin efficiency, and adapting to tumor complexity. Potential solutions could involve optimizing sgRNA with the help of artificial intelligence, improving delivery systems, and integrating multi-omics data to enhance CRISPR approaches for precision medicine.

5. Conclusion

The research highlighted in this paper underscores the potential of CRISPR technology in cell fate manipulation and disease management. However, there are hurdles that must be cleared before its widespread clinical application. One key area that necessitates improvement is the targeting accuracy and off-target effects control of CRISPR systems by enhancing techniques like employing dCas9 fusion proteins for precise epigenetic modulation and creating more intelligent sgRNA design tools. Additionally, to handle the diverse nature of various diseases, it is imperative for future studies to concentrate on crafting personalized treatment plans to maximize the adaptability and versatility of the CRISPR system. Ultimately, these advancements will be crucial in realizing the full therapeutic potential of CRISPR technology.

The CRISPR/dCas9 system is revolutionizing tumor immunotherapy and shows promise in treating chronic diseases like Alzheimer's and age-related disorders. As we continue to advance this technology, it is crucial to thoroughly examine the potential risks and refine delivery methods to ensure maximum effectiveness. Through epigenetic editing, we can unlock new breakthroughs in medical treatment. Moving forward, research must focus on understanding the full scope of CRISPR's capabilities and developing precise techniques for delivering this innovative therapy.

The CRISPR system presents a revolutionary opportunity for the advancement of precision medicine and cell therapy. Its potential applications in regulating cell cycles, selecting cell fates, and treating diseases offer immense promise for improving human health. However, transitioning CRISPR from the realm of laboratory research to clinical practice requires ongoing commitment to enhancing its technical capabilities, ensuring its safety, and establishing ethical guidelines. Only through these sustained efforts can CRISPR fulfill its potential as a transformative tool in healthcare.

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