Potential Applications and Research Exploration of CRISPRi in the Treatment of Autoimmune Diseases

Shaopu Huang*

School of Life Science, The Chinese University of Hong Kong, Hong Kong, China

*Corresponding author: 1155250772@link.cuhk.edu.hk

Abstract. Autoimmune diseases (AIDs) are chronic disorders driven by the breakdown of immune tolerance, where genetic susceptibility and environmental triggers converge to promote aberrant T and B cell activation, inflammatory cytokine release, and progressive tissue damage. Current therapeutic strategies, including corticosteroids, immunosuppressants, and biologics, can alleviate symptoms but remain limited by adverse effects, incomplete remission, and variable patient responses. CRISPR interference (CRISPRi), a novel gene regulation platform based on catalytically inactive Cas9 and guide RNAs, achieves precise, reversible suppression of target genes without inducing DNA double-strand breaks, thereby offering improved safety and controllability over conventional genome editing. Recent studies demonstrate that CRISPRi can effectively silence multiple inflammatory mediators and immune checkpoint molecules, enabling immune microenvironment remodeling and providing a feasible strategy for complex autoimmune conditions. Advances in multiplex CRISPRi, including the design of multi-sgRNA constructs and delivery via viral or non-viral systems, expand its capacity to simultaneously repress synergistic cytokine networks. Despite ongoing challenges in delivery efficiency, off-target risk, and long-term safety, CRISPRi holds considerable promise for network-level therapeutic intervention. This review aims to summarize the principles, technological progress, and potential applications of CRISPRi in autoimmune diseases.

Keywords: CRISPR interference; autoimmune diseases; multiplex gene silencing; immunotherapy.

1. Introduction

Autoimmune diseases (AIDs) are a group of conditions characterized by chronic inflammation and tissue damage resulting from the immune system losing tolerance to self-antigens. These include systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, and type 1 diabetes, among others [1]. Current treatment methods mainly include glucocorticoids, non-specific immunosuppressants, and single-target biological agents. Although they can alleviate symptoms to a certain extent, their efficacy is limited and their side effects are significant. Some patients respond poorly to existing drugs, highlighting an urgent need for more precise and controllable treatment strategies [2]. In recent years, CRISPR interference (CRISPRi) has attracted attention as an emerging gene regulation technology. This method achieves reversible and controllable gene silencing without introducing DNA double-strand breaks by fusing catalytically inactive Cas9 (dCas9) with a transcriptional repressor domain and targeting promoters or coding regions through specific single-guide RNAs (sgRNAs) [3].Compared with the traditional CRISPR-Cas9 gene knockout technology, CRISPRi offers higher safety and lower off-target risks, making it suitable for the precise regulation of immune-related gene expression.

Recent studies have shown that CRISPRi can precisely inhibit key inflammatory factors such as TNF-α and IL-6 in immune cells like T cells, and can also regulate the expression of immune checkpoint molecules such as PD-1 and CTLA-4. This further enables the remodeling of the immune microenvironment, providing a feasible strategy for multi-target intervention in autoimmune diseases [4]. For example, the dCas9-ZIM3(KRAB)-MeCP2 fusion protein developed by Kristof et al. significantly improved transcriptional repression efficiency and stability, laying a foundation for the combined regulation of multiple genes in complex diseases [5]. In addition, the CRISPRi system can achieve synchronous expression of multiple sgRNAs, enabling refined and synergistic regulation of

multi-gene networks to meet the intervention needs of complex inflammatory pathways in autoimmune diseases.

However, the translation of CRISPRi into the treatment of autoimmune diseases still faces challenges such as insufficient efficiency of delivery systems, long-term expression stability, off-target effects, and clinical safety. This review aims to review the technical principles of CRISPRi, the optimization of key tools, and its experimental exploration in autoimmune diseases, with a focus on discussing potential therapeutic targets and application prospects, so as to provide theoretical references and research basis for future precise gene intervention strategies.

2. Overview of AIDs

Autoimmune diseases (AIDs) are generally driven by the interplay of genetic susceptibility and environmental triggers. In genetically predisposed individuals, genome-wide association studies (GWAS) have identified a large number of susceptibility loci, most of which lie within non-coding regulatory regions associated with immune cell activation. These loci further influence the thresholds of key signaling pathways and cellular states, thereby lowering the balance threshold between "tolerance and response" and laying the groundwork for subsequent environmental stimuli [6]. Against this genetic background, antigenic stimuli such as infections can break peripheral tolerance through mechanisms like "molecular mimicry," "bystander activation," and "epitope spreading." Lifestyle factors, such as smoking, can promote antigen modification (e.g., citrullination) and enhance autoantibody responses. Additionally, dysbiosis of the mucosal microbiota (e.g., in the gut) drives the upregulation of the Th17/Th1 axis and B cell activation through impaired barrier function and crosstalk between innate and adaptive immunity, accelerating the onset and progression of autoimmunity [7].

At the effector level, abnormal activation of T cells and B cells triggers a cascade of proinflammatory responses involving cytokines such as TNF-α, IL-6, and IL-17. These cytokines form self-sustaining inflammatory circuits with dendritic cells, fibroblast-like synoviocytes, and myeloid cells: TNF-α, as an upstream hub, promotes the expression of adhesion molecules and chemokines; IL-6 drives plasma cell differentiation and acute-phase reactions via STAT3; IL-17, in synergy with TNF-α, induces tissue stromal cells to produce more chemokines and metalloproteinases, leading to persistent tissue damage and organ-specific pathological changes [2, 8]. In terms of treatment, glucocorticoids and conventional immunosuppressants (e.g., methotrexate, azathioprine, mycophenolic acid) can rapidly suppress inflammation and induce symptom relief. However, they are limited by dose- or duration-related side effects, such as metabolic disorders, skeletal complications, cardiovascular issues, and infections, as well as their limited ability to re-establish long-term immune tolerance. Biological agents (e.g., TNF inhibitors, IL-6R inhibitors, IL-17 inhibitors, B cell-targeted drugs, or co-stimulation pathway-targeted drugs) have significantly improved prognosis but still face practical limitations, including drug-resistant antibodies due to immunogenicity, increased risk of secondary infections, diminished efficacy over time, and high-cost burdens. Moreover, complete remission and drug-free maintenance remain unstable in heterogeneous patient populations [9].

Thus, future advancements are expected to improve the rate of durable remission and reduce the burden of systemic immunosuppression through more refined stratification based on genetic/epigenetic profiles, microbiota-mucosal immune interventions, and the combination of antigen-specific immune tolerance induction with multi-pathway synergistic therapies.

3. Principles and Advantages of CRISPRi Technology

CRISPRi achieves site-specific binding to sequences near the promoter or upstream/downstream of the transcription start site (TSS) of target genes by pairing endonuclease-inactivated Cas9 (dCas9) with sgRNA, thereby blocking the recruitment or elongation of RNA polymerase through spatial

hindrance. When dCas9 is further fused with a transcriptional repressor domain (e.g., KRAB), the complex can silence enhancers/promoters at the local chromatin level, significantly enhancing repression intensity and achieving controllable suppression at the epigenetic level [10, 11].

Unlike CRISPR-Cas9 knockout, which induces double-strand breaks (DSBs), CRISPRi does not introduce DNA breaks. Thus, it generally avoids DSB-associated genotoxicity and irreversible mutations caused by repair processes. It also exhibits "switch-like" reversibility: when dCas9/sgRNA is removed or its expression is downregulated, target gene transcription can partially or fully recover [11]. In terms of specificity and controllability, the targeting of CRISPRi is determined by ~20 nt sgRNAs. Through optimization of TSS-proximal sites and multi-sgRNA design, stable, potent transcriptional repression effects with good reproducibility across multiple cell lines can be achieved. Large-scale library studies have shown that well-designed CRISPRi libraries have a high signal-to-noise ratio when detecting "essential genes" and do not exhibit the non-specific toxicity reported in some nuclease-based CRISPR experiments [12].

Compared with RNAi, CRISPRi acts at the transcriptional level, avoiding seed-dependent off-target effects and expression profile noise caused by non-specific activation of the interferon pathway—common issues in RNAi. In large-scale comparisons, CRISPRi generally exhibits lower systemic off-target effects and higher consistency. Under rigorously controlled conditions, both techniques can yield similar functional readouts at the pathway level, but RNAi requires additional off-target correction, whereas CRISPRi imposes a lighter correction burden [13]. Meanwhile, in contrast to the permanent allele loss caused by CRISPR-Cas9 knockout, the "reversible, tunable, and DSB-free" properties of CRISPRi make it particularly suitable for studies on essential genes, dose-sensitive genes, non-coding regulatory elements, and recoverable functions. It also facilitates graded and combinatorial multi-target silencing (e.g., dual-sgRNA or multi-sgRNA architectures), providing a safer and more controllable tool framework for refined functional analysis and therapeutic target validation [10, 12]. In summary, CRISPRi, centered on the programmable targeting of dCas9–sgRNA and (optional) epigenetic regulation via repressor domains, combines reversibility, high specificity, and low off-target risk, demonstrating unique advantages in functional genomics, non-coding regulation analysis, and potential therapeutic development.

4. Design and Delivery of Multiplex CRISPRi Systems

The development of multiplex CRISPRi systems aims to address the challenge of synergistic interactions within multi-gene networks in complex diseases. The core idea is to construct tandem vectors capable of expressing multiple sgRNAs simultaneously, enabling parallel transcriptional repression of multiple genes within a single cell. Common strategies include utilizing tRNA-sgRNA polycistronic architectures or Csy4 endonuclease recognition sites, which allow precise release of sgRNA arrays through host endonuclease activity or post-transcriptional cleavage. This ensures stable expression and functional independence of each sgRNA, thereby achieving efficient multi-target intervention without increasing vector quantity [14].

In terms of delivery strategies, viral vectors remain the most commonly used platform. Lentiviruses, with their large cargo capacity and ability to integrate into the host genome, are widely applied in establishing stable in vitro cell lines and analyzing multi-gene networks. For example, Gilbert et al. utilized lentiviral delivery of dCas9-KRAB and multiple sgRNAs to effectively suppress the synergistic effects of several transcription factors, achieving stable phenotypic readouts at the population level [10]. However, the inherent risk of genomic integration limits their application prospects in certain clinical scenarios. Additionally, non-viral nanoparticle vectors such as lipid nanoparticles (LNPs) have demonstrated advantages in delivering multiplex CRISPRi, including rapid action, controllability, and low integration risk. LNPs can co-encapsulate dCas9-KRAB mRNA and multiple sgRNAs, enabling transient and reversible gene silencing while avoiding the risk of genomic integration. As reviewed by Lino et al., the application of LNPs in CRISPR systems has gradually matured, with their programmable lipid compositions conferring tissue tropism, making

them particularly suitable for localized CRISPRi interventions in the liver, immune system, and mucosal barrier tissues [15].

The effectiveness of multiplex CRISPRi is typically evaluated at three levels: molecular, cellular, and organismal. At the molecular level, RT-qPCR, RNA-seq, or ChIP-qPCR are used to detect changes in target gene transcription levels and chromatin modifications. At the cellular level, flow cytometry analysis of phenotypic changes or functional assays (such as cytokine secretion and metabolic state alterations) verify the effects of synergistic silencing. In animal models, comprehensive evaluation of multi-gene silencing effects on disease phenotypes requires combining histopathology, immune phenotypic changes, and long-term safety testing. Furthermore, delivery efficiency, immunogenicity, off-target binding, and long-term expression stability are key parameters determining translational potential, which must be systematically quantified and optimized in early-stage research.

In methodological practice, existing studies have provided replicable protocols for achieving efficient multi-gene CRISPRi in stem cells and complex systems. For instance, Satoshi Matsui et al. established a doxycycline-inducible CRISPRi system capable of simultaneously silencing multiple genes in human induced pluripotent stem cells (hPSCs). Host cells were constructed by knocking a dCas9-KRAB expression cassette into the AAVS1 safe harbor locus, while four sgRNAs targeting FOXA1, FOXA2, and FOXA3 were integrated into a single lentiviral vector using Golden-Gate cloning, enabling efficient co-silencing of these three functionally redundant transcription factor genes. Experimental results showed that this system could suppress FOXA family gene expression by over 95% within 3 days and successfully generate multiple monoclonal CRISPRi hPSC lines. This protocol provides a reliable tool for rapidly, reversibly, and efficiently studying multi-gene functions in hPSCs [16]. In summary, multiplex CRISPRi systems, combined with refined sgRNA design and appropriate delivery platforms, offer powerful means for multi-gene synergistic regulation and complex disease mechanism research.

5. CRISPRi-Based Multi-Factor Anti-Inflammatory Strategies in AIDs

In autoimmune diseases, multiple pro-inflammatory factors (such as TNF-α, IL-6, and IL-17) exhibit a synergistic amplification effect. Inhibition of a single target often has limited efficacy due to pathway compensation. Therefore, the concept of CRISPRi-based synergistic silencing of multiple pro-inflammatory factors is proposed, which utilizes dCas9-KRAB and multi-sgRNA arrays to synchronously suppress multiple genes at the transcriptional level, thereby weakening the inflammatory network from the source [4]. This strategy emphasizes starting from the network rather than isolated targets, aiming for systemic inhibition of the inflammatory cascade.

Three key points are highlighted in the design of this strategy: first, target combination, which involves selecting combinations of upstream key factors such as TNF-α and downstream effector factors such as IL-6 and IL-17 based on disease pathway maps. Second, redundancy and optimization, which entails designing multiple sgRNAs for each target and achieving efficient release through tRNA/Csy4 architectures. Third, controllability, which involves using inducible or transient expression systems to avoid infection risks caused by long-term immunosuppression [17].

In terms of delivery, lentiviruses are suitable for in vitro mechanism verification but are limited in clinical applications due to integration risks. Adeno-associated viruses (AAVs) have shown good safety in animal models but are constrained by cargo capacity, which can be partially addressed by split inteins and small dCas9 variants. Lipid nanoparticles (LNPs) encapsulating dCas9 mRNA and sgRNAs offer advantages of transience, reversibility, and non-integration, making them particularly suitable for short-term intervention in acute inflammation. For functional evaluation, multi-level indicators should be integrated: molecular level (RT-qPCR, RNA-seq), epigenetic level (ChIP-seq to track repressive marks such as H3K9me3), cellular functional level (Th17/Treg ratios and cytokine profiles), as well as histopathology and disease scoring in animal models, to verify the therapeutic potential of synergistic silencing [4, 17]. Future clinical pathways may include short-term

intervention in the acute phase, local targeted drug delivery, and combination with cell therapies. However, immunogenicity and off-target effects must be strictly controlled, and clinical exploration can only proceed after validation through three levels of evidence: molecular, cellular, and animal studies [18, 19].

6. Conclusion

CRISPRi has emerged as a versatile platform for precise gene regulation with significant promise in autoimmune diseases. In contrast to conventional genome editing or broad immunosuppressive therapies, CRISPRi enables reversible, tunable, and non-destructive silencing of pathogenic genes, thereby minimizing risks associated with DNA double-strand breaks and long-term genomic instability. Multiplex CRISPRi strategies, which simultaneously repress key pro-inflammatory mediators such as TNF-α, IL-6, and IL-17, offer a network-level approach to overcome pathway redundancy and compensatory signaling that often undermine single-target interventions. Recent advances in sgRNA optimization, engineered repressor domains, and improved delivery technologies, including lipid nanoparticles and viral vectors, further expand the translational potential of CRISPRi and support its development as a next-generation modality for precision immunotherapy.

Nevertheless, significant challenges remain before CRISPRi can be applied clinically, including optimization of delivery efficiency, mitigation of immunogenicity, control of long-term expression, and comprehensive evaluation of off-target effects. Bridging these gaps will require integrated efforts in molecular engineering, biomaterial development, and rigorous preclinical validation in relevant autoimmune models. Looking ahead, the convergence of CRISPRi with personalized medicine and systems-level analysis of immune networks may enable more precise, patient-tailored therapies. In this way, CRISPRi not only represents a technological advance but also highlights a paradigm shift toward rational, multi-target, and safer interventions for complex autoimmune disorders.

References

[1] Nautiyal G, Sharma I, Pandey P, et al. Autoimmune diseases: recent insights on epidemiology, pathogenesis, and prevalence rate. Artificial Intelligence and Autoimmune Diseases, 2024, 1133: 33-58.

[2] Song Y, Li J, Wu Y. Evolving understanding of autoimmune mechanisms and new therapeutic strategies of autoimmune disorders. Signal Transduction and Targeted Therapy, 2024, 9(1): 263.

[3] Israr J, Kumar A. Current progress in CRISPR-Cas systems for autoimmune diseases. Progress in Molecular Biology and Translational Science, 2024, 208: 231-259.

[4] Eladl O. CRISPR interference-mediated silencing of pro-inflammatory genes in autoimmune models: functional and biophysical evaluation with sustained effects in primary human PBMCs. International Immunopharmacology, 2025, 163: 115257.

[5] Kristof A, Karunakaran K, Allen C, et al. Engineering novel CRISPRi repressors for highly efficient mammalian gene regulation. Genome Biology, 2025, 26(1): 164.

[6] Gutierrez-Arcelus M, Rich S, Raychaudhuri S. Autoimmune diseases connecting risk alleles with molecular traits of the immune system. Nature Reviews Genetics, 2016, 17(3): 160-174.

[7] Pisetsky D S. Pathogenesis of autoimmune disease. Nature Reviews Nephrology, 2023, 19(8): 509-524.

[8] Christovich A, Luo X M. Gut microbiota, leaky gut, and autoimmune diseases. Frontiers in Immunology, 2022, 13: 946248.

[9] Davis J S, Ferreira D, Paige E, et al. Infectious complications of biological and small molecule targeted immunomodulatory therapies. Clinical Microbiology Reviews, 2020, 33(3): e00035-19.

[10] Gilbert L A, Horlbeck M A, Adamson B, et al. Genome-scale CRISPR-mediated control of gene repression and activation. Cell, 2014, 159(3): 647-661.

[11] Qi L S, Larson M H, Gilbert L A, et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell, 2013, 152(5): 1173-1183.

[12] Horlbeck M A, Gilbert L A, Villalta J E, et al. Compact and highly active next-generation libraries for CRISPR-mediated gene repression and activation. Elife, 2016, 5: e19760.

[13] Smith I, Greenside P G, Natoli T, et al. Evaluation of RNAi and CRISPR technologies by large-scale gene expression profiling in the connectivity map. PLOS Biology, 2017, 15(11): e2003213.

[14] McCarty N S, Graham A E, Studená L, et al. Multiplexed CRISPR technologies for gene editing and transcriptional regulation. Nature Communications, 2020, 11(1): 1281.

[15] Kazemian P, Yu S Y, Thomson S B, et al. Lipid-nanoparticle-based delivery of CRISPR/Cas9 genome-editing components. Molecular Pharmaceutics, 2022, 19(6): 1669-1686.

[16] Matsui S, Shiley J R, Granitto M, et al. Protocol for establishing inducible CRISPR interference system for multiple-gene silencing in human pluripotent stem cells. STAR Protocols, 2024, 5(3): 103221.

[17] Schmidt R, Steinhart Z, Layeghi M, et al. CRISPR activation and interference screens decode stimulation responses in primary human T cells. Science, 2022, 375(6580): eabj4008.

[18] Lee M H, Shin J I, Yang J W, et al. Genome editing using CRISPR-Cas9 and autoimmune diseases: a comprehensive review. International Journal of Molecular Sciences, 2022, 23(3).

[19] Ewaisha R, Anderson K S. Immunogenicity of CRISPR therapeutics critical considerations for clinical translation. Frontiers in Bioengineering and Biotechnology, 2023, 11: 1138596.