

# Development and Application of CRISPR Technology in the Treatment of Autoimmune Diseases

Xiang Zhang

Shanghai Institute of Technology, School of Perfume and Aroma Technology, Shanghai, 201418, China  
Corresponding author: 20151015641@mail.sdufe.edu.cn

## Abstract:

Autoimmune diseases (ADs) result from the immune system's misidentification of self-antigens, leading to harmful responses and pathological states. The CRISPR/Cas9 system, a groundbreaking gene-editing technology, offers promising avenues for addressing ADs. Drawing inspiration from prokaryotic immune mechanisms, CRISPR/Cas9 precisely targets and edits foreign DNA, providing unprecedented genome-editing capabilities. Manipulating immune cell genetics or regulating relevant protein expression with CRISPR/Cas9 shows significant therapeutic potential in managing ADs. Recent research and clinical studies emphasize the valuable role of CRISPR/Cas9 in understanding the underlying pathogenic mechanisms of ADs, especially concerning non-viral triggers. Through strategic and targeted approaches, substantial progress is anticipated in comprehending and managing ADs. CRISPR/Cas9 emerges as a safe and efficient tool for tackling a wide range of ADs with diverse etiologies, offering transformative advancements in treatment strategies. This technology holds immense promise in reshaping the landscape of autoimmune disease management, providing hope for improved outcomes and enhanced quality of life for affected individuals.

**Keywords:** CRISPR/Cas9; autoimmune diseases; systemic lupus erythematosus; rheumatoid arthritis.

## 1. Introduction

Autoimmune diseases (ADs) are a kind of diseases caused by the disorder of immune tolerance to self-antigens. The body's immune response to self-antigens leads the body to enter the disease state. The pathogenesis is often caused by multiple antigenic epitopes that cause polyclonal activation of the immune system, and the changes of T lymphocytes and B lymphocytes in the immune system are caused by the formation of corresponding antibodies. Therefore, congenital immune diseases are mostly polygenic diseases, causing complex autoimmunity and inflammation to interact.

ADs include organ-specific ADs and systemic ADs. Organ-specific adenovirus refers to the disease of patients that is generally limited to a specific organ, caused by the autoimmune response to specific organs, mainly including Hashimoto's thyroiditis, Graves' disease, myasthenia gravis and so on. Systemic lupus erythematosus (SLE) refers to the pathological damage of multiple organs and tissues caused by immune response, mainly including rheumatoid arthritis (RA), SLE, Sjogren's syndrome (SS), etc. AD is a complex disease that is difficult to cure. Once diagnosed, most patients need to take medication for a long time or even for life, which is very detrimental to their health.

This article will focus on the application of CRISPR/Cas9 gene editing technology in ADs, and the understanding pathogenic mechanisms and developing targeted therapeutic approaches. By leveraging CRISPR/Cas9's precise genome editing capabilities and its potential to modulate immune cell genetics, aims to elucidate the molecular basis of ADs and advance treatment strategies.

## 2. The CRISPR-Cas9 Technology

### 2.1 Mechanism

The CRISPR-CAS system is a natural immune system in prokaryotes, which recognizes and cuts off the invading virus the second time it invades by storing a small gene of the invading virus in its own DNA in a storage space called CRISPR. The sg RNA (guide RNA) was designed to guide CAS protease to effectively cleave the target DNA. Then, the mediated genome editing by CRISPR/Cas9 relies on the generation of Double-Strand Breaks(DSBs) and subsequent DNA repair processes. DSBs induced by CRISPR/Cas9 trigger cellular repair processes in DNA, such as Non-Homologous End Joining (NHEJ), which is error-prone and can lead to insertions and deletions (indels) involving small mutations at the target site, disrupting or eliminating the function of genes

or genomic elements (e.g., regulatory regions). Another repair process that can be triggered is Homology-Directed Repair (HDR), which can potentially correct congenital DNA errors without introducing mutations [1].

Genetic engineering of immune cells or the expression of related proteins to treat ADs has been a treatment method with considerable application experience. Recent research and clinical studies have indicated that employing this technology for the investigation of non-viral infections in ADs enables a better understanding of the pathogenic mechanisms underlying ADs. Furthermore, by utilizing the CRISPR/Cas9 system to design more targeted approaches, significant advancements can be made in both the research and treatment of ADs [2].

The immune process mediated by CRISPR/Cas9 is divided into three steps: in the adaptation phase, foreign sequences are integrated into the proximal end of the CRISPR array to achieve adaptability. In the expression and interference phases, elements within the repeat spacer are transcribed into precursor CRISPR RNA (pre-crRNA) molecules, which are then cleaved enzymatically to produce short crRNAs. These crRNAs can pair with complementary protospacer sequences of invading viruses or plasmids. crRNA binds with trans-activating CRISPR RNA (tracrRNA) to form a double-stranded RNA structure, which is processed to form mature crRNA-tracrRNA complexes. The spacer region of crRNA serves as memory, containing segments derived from virus or plasmid DNA. The crRNA-tracrRNA complex binds with Cas protein to form ribonucleoprotein (RNP) complexes, guiding Cas protein to silence foreign sequences and thus achieve an immune response [2,3]. Studies have found that the SpCas9 nuclease can bind and cleave DNA sequences without the formation of an RNA complex. Therefore, by programming the single guide RNA (sgRNA) to direct the Cas protein to the desired genome, double-strand breaks (DSBs) can be created. These DSBs are typically repaired through error-prone non-homologous end joining (NHEJ) or homology-directed repair (HDR) mechanisms. Despite the repair of DSBs, small insertion/deletion mutations at the breakpoint disrupt the open reading frame of the gene. It is possible to achieve gene knockout by creating indels within the coding exons. If the target encoded protein domain is an essential functional region, indels induced by CRISPR/Cas9 will result in a high frequency of mutations [4]. CRISPR/Cas9 gene editing technology has great potential and clinical validation space. For a series of ADs with independent pathogenesis, its strong specificity can be more efficient and safe to achieve the purpose of treatment.

## 2.2 CRISPR/Cas9 Therapeutic Applications

The gene editing technology has faced challenges due to its unpredictable results, but it has gained increasing attention in terms of feasibility with more and more research. Past gene editing tools such as zinc finger nuclear (ZFNs) and transcription activator-like effector nucleases (TA) were based on protein structural domains and nucleotide pairing, targeted by Cys2-His zinc finger proteins or transcription activator-like effectors (TALE). After being designed and assembled for co-use, the DNA sequence domains that could be recognized were difficult to achieve the expected results, making it inconvenient for researchers to design and study. However, CRISPR/Cas9 has more flexible and stronger targeting capabilities, such as using the CRISPR interference system to block transcription, which has increased the efficiency of regulating the targeted gene group by 1,000 times [5]. In the application of therapy, the virus delivery vector is widely used. Adeno-associated virus (AAV) has been proven to have a low probability of introducing exogenous genes, and has been shown to be safe. After selecting the targeting site and suitable Cas9 protein, for example, using double-stranded self-complementary AAV to load sgRNA that mediates the Dmd exome to deliver it to mice, effective improvement of muscle integrity and function has been achieved [6].

## 2.3 ADs

Research on Targets of ADs Studies have shown that the IL-36 cytokine family is an important part of the mechanism of autoimmune diseases such as RA, SLE, and SS. In this study, it was found that (miR-155) is a regulatory factor that leads to autoimmune responses and the long-term production of pro-inflammatory factors. By designing sgRNA against miR-155, successful reduction of pro-inflammatory cytokine production in cloned cells was achieved. This suggests that miR-155 may play an important role in autoimmune diseases, and targeting miR-155 may become a potential strategy for treating such diseases. This method can be achieved through CRISPR/Cas9 technology, providing useful information for developing new treatment approach. CRISPR/Cas9 was used to inactivate the known myeloid differentiation primary response gene 88 (MyD88) adapter protein, simultaneously lowering the activity of differentially expressed genes induced by MyD88 adapter protein, including IL-3B and IL-36G. Using the same CRISPR/Cas9 tool to study known genes related to autoimmune diseases, a deletion was generated at upstream of the TNFAIP3 gene at a distance of 140kb, resulting in multiple gene changes, including the coding of the IL-20RA, providing evidence of IL-20RA as a risk factor for autoimmune diseases [7]. By suppressing

the expression of known pathogenic genes with strong correlation and studying the genomic regions known to be associated with the action of pathogenic genes to find evidence of undiscovered pathogenic genes. Similarly, the reverse study of CRISPR/Cas9 technology has become a key factor in the treatment of autoimmune diseases or genetic diseases [8].

### 3. RA

In RA patients, CD4+ cells usually express higher autophagy. The Yang team stimulated activated CD4+ cells for 24 hours, collected and measured the ATAC-seq, Hi-C, captured Hi-C, and nuclear RNA data of activated CD4+ cells, and found that the MYC and FOXO1 genes may be the causative factors of RA. MYC is a central regulator of autophagy enhancement pathway for T cell subpopulations, which may aid inflammatory T cells in arthritic joints [9]. The Fan team identified five immune signature genes, including CXCL13, SDC1, LGLC1, PLXNC1, and SLC29A3, using two machine learning algorithms, and independent data sets validated that they were highly expressed in RA samples [10]. After validating the deletion or inhibition of the signature genes using CRISPR/Cas9 technology, it can be used to establish the feasibility of developing therapeutics for ADs such as RA. It was also discovered that by combining CRISPR/Cas9 technology with past therapies, such as Treg therapy, it is possible to break through the long-standing bottlenecks and difficulties in RA treatment.

### 4. Psoriasis

Psoriasis is a chronic inflammatory skin disease that has long posed challenges in the medical field. Recent research has shown that utilizing lipoic acid (LA) as an efficient carrier to synthesize polymer-delivered RNPs provides enhanced stability. By combining LA with macromolecular NBC, the GBLA polymer was obtained. It was found that delivering GBLA-22/Cas9 RNP to the NLRP3 gene significantly alleviated symptoms in psoriasis patients, including redness, scaling, and hard nodules. Following treatment, the expression of related cytokines such as IL-1 $\beta$ , IL-18, and TNF- $\alpha$  in psoriasis mice was suppressed, with TNF- $\alpha$  confirmed as the primary pathogenic mechanism target for treatment. Additionally, the pathogenesis of psoriasis is associated with changes in multiple genes, including the C-REL gene linked to the activation pathway of the innate immune system's NF- $\kappa$ B, the TRAF3IP2 gene associated with keratinocyte proliferation, and the overexpression of the IFHI and DDX58 genes involved in the interferon pathway and antiviral response, as well as the DEFB4 gene regulating skin barrier

function. These genes are all located at the PSORS4 locus, suggesting their involvement as risk factors for psoriasis. Overall, CRISPR/Cas9-based therapeutic approaches offer promising avenues for treating psoriasis, providing hope for addressing this persistent ailment.

### 5. Systemic Lupus Erythematosus

In the study investigating the association between the IRF5 risk locus and pathogenic genetic variants in SLE, the intervention effect of regulating IRF5 gene expression using a composite approach was evaluated in monocytes from SLE patients through CRISPRi [11]. The results indicated a close correlation between the rs4728142 allele and increased IRF5 expression, demonstrating the rs4728142 locus as an enhancer regulating IRF5 expression. Additionally, CRISPRi interference on this enhancer resulted in a reduction in the production of disease-related cytokines. This study assessed the intervention efficacy of modulating IRF5 gene expression in monocytes from SLE patients through CRISPRi, investigating the association between the IRF5 risk locus and pathogenic genetic variants. The findings revealed a significant correlation between the rs4728142 allele and heightened IRF5 expression, highlighting the rs4728142 locus as an enhancer regulating IRF5 expression. Furthermore, CRISPRi interference on this enhancer led to a decrease in the production of disease-related cytokines [12]. Therefore, utilizing CRISPR solely to intervene in the expression of associated genes can serve as a means to regulate the production of pathogenic cytokines in patients and be incorporated into therapeutic research purposes.

### 6. Conclusion

ADs arise from the immune system's failure to tolerate self-antigens, encompassing organ-specific and systemic conditions. While organ-specific ADs primarily affect individual organs like the thyroid or muscles, SLE targets multiple organs. The advent of CRISPR/Cas9 gene editing technology presents promising prospects for understanding and treating ADs. CRISPR/Cas9, a prokaryotic immune system, offers precise genome editing capabilities by recognizing and cleaving foreign DNA. Its application in modifying immune cells and regulating protein expression has shown significant therapeutic progress. Recent research indicates CRISPR/Cas9's potential to deepen our understanding of ADs' pathogenic mechanisms and refine treatment strategies. Therapeutic advancements, including viral delivery vectors like AAV, demonstrate promise in animal models. Additionally, CRISPR/Cas9 research opens avenues for discovering disease-causing genes and innovative therapies. In summary, CRISPR/Cas9 technol-

ogy offers novel approaches to addressing ADs' complexity and improving treatment outcomes.

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