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Applications of CRISPR in the Treatment of Autoimmune Diseases

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Abstract:

Autoimmune disorders are marked by the immune system's erroneous recognition and ongoing assault on the body's own tissues, impacting roughly 5-10% of individuals. Existing interventions, such as immunosuppressive drugs, can ease outward symptoms but cannot eliminate the root cause, while long-term use may bring about risks like heightened infection susceptibility or organ damage due to toxicity. CRISPR gene editing technology, which allows for accurate genomic modifications through Cas9 endonuclease and programmable guide RNA, has exhibited potential in treating monogenic diseases and certain tumors. However, its use in complex autoimmune conditions is still in the early investigative stage, with current approaches centered on editing genes that regulate immune cell activity and reestablishing immune tolerance. Notable research limitations include inefficient cell-type-specific delivery systems, unaddressed off-target modifications, and insufficient verification of long-term safety. This study conducts a comprehensive assessment of CRISPR-based therapeutic strategies for autoimmune diseases, encompassing the identification of disease-related genes (e.g., TLR7 in SLE and PTPN22 in RA), editing techniques (gene knockout, knock-in, and base editing), and the regulation of immune cell functions. Preclinical results show reduced production of autoantibodies, inhibited inflammatory responses, and mitigated tissue harm in models of SLE and RA. These findings offer a scientific foundation for the development of targeted, curative treatments, overcoming the drawbacks of traditional therapies. Future investigations should focus on improving editing accuracy, refining delivery systems, and performing extended safety evaluations to expedite the clinical application of personalized CRISPR-based treatments for autoimmune disorders.

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

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1. Introduction

Autoimmune diseases fall under the category of chronic pathology, and their core characteristics are manifested as the incorrect recognition and continuous attack by the body's immune system on its own tissues and organs. Currently, typical diseases such as rheumatoid arthritis, systemic lupus erythematosus (SLE), and multiple sclerosis affect approximately 5-10% of the population. The current clinical practice widely employs immunosuppressants and biologics as the main intervention methods. Although these drugs can alleviate the external manifestations of the disease, they are difficult to cure the root cause. It is worth noting that long-term use of such drugs may lead to various serious adverse reactions, such as increased susceptibility to infections and organ toxicity damage, highlighting the urgent need for more targeted and long-lasting new therapies for autoimmune diseases.

CRISPR is now widely applied in clinical research and other projects. It enables researchers to manipulate the genome with unprecedented precision. This system utilizes the Cas9 endonuclease and programmable single-guide RNA to achieve precise positioning and modification of specific DNA sequences. CRISPR has shown significant potential in the treatment of single-gene diseases and some malignant tumors, but its application in complex autoimmune diseases is still in the initial exploration stage. The current two major research goals and strategies are: (1) by editing the key genes that regulate the activation and differentiation of T/B cells, directly intervene in the pathogenic immune cell functions; (2) targeting immune checkpoint molecules or regulatory T cells, in order to restore the balance of immune tolerance. However, the realization of clinical translation still faces multiple key obstacles: an efficient delivery system for specific immune cell subsets needs to be optimized, off-target effects need to be further reduced, and the long-term efficacy and safety of the treatment also need to be fully verified.

This paper aims to systematically evaluate and optimize the treatment plans for autoimmune diseases based on CRISPR. By introducing advanced delivery technologies to enhance the targeting specificity, the therapeutic effects and potential risks of the treatment are comprehensively examined in relevant preclinical models. The expected outcome will provide crucial scientific basis for developing new-generation therapies that may achieve a cure.

2. CRISPR's Editing Program for Autoimmune-related Genes

2.1 Screening of pathogenic genes

The screening of pathogenic genes involves systematically examining and analyzing genes through techniques like sequencing to identify those linked to disease development, while autoimmune diseases often arise from disruptions in the regulation of multiple genes. The occurrence of autoimmune diseases is closely related to the abnormal regulation of multiple genes [1,2]. The application of CRISPR requires first precisely locating the key pathogenic genes [1,2]. Through methods such as genome-wide association analysis (GWAS), a large number of risk genes related to autoimmune diseases have been discovered. In SLE, the Toll-like-receptor-7-Gen (TLR7) and Interferon Regulatory Factor 5(IRF5)genes, and in RA, the Protein Tyrosine Phosphatase Non-Receptor Type 22 and Human Leukocyte Antigen-D Related Beta 1genes are among them. These genes participate in the occurrence and development of the diseases by influencing the activation of immune cells, the secretion of cytokines, or the process of antigen recognition [2].

During the screening process, researchers will combine multi-omics data (genomic, transcriptomic, proteomic) with clinical phenotypes. They will use models such as CRISPR library screening to verify the functions of genes. In the B cells of SLE patients, through screening, it was found that the abnormal expression of the TLR7 gene would enhance the activation of the type I interferon pathway. This is an important factor promoting the progression of the disease. In the synovial cells of RA, the functional mutation of the PTPN22 gene would lead to excessive activation of T cells. This would exacerbate joint inflammation.

2.2 The methods of gene knockout, knock-in or modification

The CRISPR mainly edits genes related to autoimmunity through three methods. The aim is to achieve disease intervention.

To perform gene knockout, one would use a guide RNA (gRNA) to direct the Cas9 enzyme to a specific pathogenic gene, where it cuts the gene's critical coding section—an action that results in breaks on both strands of the DNA molecule [3]. Cells repair these breaks through non-homologous end joining (NHEJ). The repair process leads to frameshift mutations. These frameshift mutations render the gene functionally silenced. In SLE model mice, knocking out the TLR7 gene can reduce the level of type I interferons in the serum [4]. It can also decrease the production of antinuclear antibodies and alleviate kidney damage.

To carry out gene knock-in, you would take advantage of the cell's homologous directed repair process. This involves introducing normal, functional genes or regulatory sequences into precise locations within the genome, where they become integrated as the cell repairs its DNA [3,5].

Inserting the IL-10 gene into the regulatory T cells (Tregs) of RA patients is an example of this [5]. This can enhance their ability to secrete anti-inflammatory factors. At the same time, it can inhibit the pro-inflammatory activity of effector T cells. To improve the knock-in efficiency, adeno-associated virus (AAV) is often used to deliver the donor DNA template. At the same time, proteins related to the NHEJ pathway (such as DNA ligase IV) are inhibited.

To perform base editing, you would use either a cytosine base editor (CBE) or an adenine base editor (ABE) to make direct corrections to single-letter mutations in disease-related genes. This method works without needing to create breaks in both strands of the DNA molecule. This reduces the risk of genomic instability. For functional gain mutations of the PTPN22 gene (such as R620W), ABE can be used to convert the mutated adenine (A) into guanine (G) [6]. This can restore its negative regulatory function on T-cell signaling. Consequently, it can inhibit autoimmune responses.

2.3 Molecular Mechanisms Regulating the Function of Immune Cells

Genetic editing of immune cells such as B cells and T cells

The abnormal activation of immune cells is the core feature of autoimmune diseases. The CRISPR can specifically edit the key genes of immune cells. It can reconfigure the functions of these cells.

When addressing B-cell editing, you would target overactive B cells—those that produce too many autoantibodies—by using CRISPR to modify their CD19 gene. This modification works to prevent the B cells from developing into antibody-secreting plasma cells, helping to reduce the harmful autoantibody production. Targeting the BLIMP1 gene (a key transcription factor regulating plasma cell maturation) can reduce the secretion of autoantibodies such as anti-dsDNA antibodies and rheumatoid factors [7]. In SLE models, the secretion of autoantibodies by edited B cells was significantly reduced. And this does not affect the normal humoral immune function.

When working with T-cell editing, you would focus on correcting the uneven distribution of T-cell subgroups—a condition that can trigger diseases like RA and SLE. This involves addressing issues such as an overgrowth of Th17 cells or impaired function of Treg cells, using editing techniques to restore a healthier balance among these cell populations [8]. Editing the Foxp3 gene of T cells can enhance the immunosuppressive function of Treg cells [8]. Knocking out the RORγt gene in Th17 cells can inhibit their differentiation. This will reduce the secretion of pro-inflammatory factors such as IL-17. Additionally, editing the PD-1 gene (an immune checkpoint molecule) of T cells can enhance their ability to eliminate autoreactive cells. However, excessive activation must be avoided to prevent immune damage.

When working with other immune cells, one approach involves modifying the CD14 gene in macrophages. This editing process is used in situations where macrophages, after taking in the body's own antigens, might trigger excessive inflammatory reactions—by altering this gene, such inflammatory responses can be suppressed. Editing the CD80/CD86 genes in dendritic cells can reduce their ability to present their own antigens to T cells. This will decrease the activation of T cells.

The influence on the differentiation, activation and proliferation of immune cells CRISPR editing directly affects the differentiation, activation and proliferation of immune cells by regulating the genes that determine cell fate.

When regulating cell differentiation, one method involves targeting the T-cell development phase. Here, you would disable the RORγt gene, a step that works to lower the percentage of Th17 cells in the overall T-cell population [7]. At the same time, it can promote the differentiation of Treg cells. Editing the PAX5 gene of B cells can inhibit their differentiation into plasma cells. This will reduce the source of autoantibody production.

When regulating T cell activation, you would focus on the processes that trigger T cell activity. This involves managing the signaling pathways from the TCR-CD3 complex and the interactions with co-stimulatory molecules—key steps that determine whether and how T cells become activated [9]. Editing the CD28 gene can block the co-stimulatory signal. This will inhibit excessive activation of T cells [9]. B cell activation requires the BAFF/BAFF-R signal. Knocking out the BAFF-R gene can reduce its sensitivity to BAFF. This will decrease activation.

The immune system normally restrains excessive growth of cells that react to the body's own tissues, but when this control fails, their unchecked proliferation worsens tissue damage. Editing genes that regulate the cell cycle (such as p21) can inhibit their proliferation rate. In the synovial T cells of RA patients, after editing the p21 gene, the number of inflammatory cells infiltrating the joint was significantly reduced. This is an example.

2.4 Application of CRISPR in the Treatment of Various Autoimmune Diseases

2.4.1 SLE

SLE is characterized by the production of autoantibodies and the deposition of immune complexes. The application of CRISPR technology focuses on inhibiting the over-activated immune signals and the generation of autoantibodies.

Targeting the type I interferon pathway is using CRISPR to knockout the TLR7 or IRF5 genes in B cells or plas-

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macytoid dendritic cells (pDC) of SLE patients, the secretion of type I interferons can be reduced [10]. Animal experiments have shown that the survival period of SLE model mice with TLR7 knockout is prolonged. Their proteinuria levels decrease and the pathological damage to the kidneys is alleviated. The study included 50 SLE patients. Their B cells were split into two groups. One group was the control group; no treatment was given. The other group had the TLR7 gene in B cells removed using CRISPR [11,12]. Both groups of cells were grown under the same conditions. Then, the amount of type I interferons they released was checked. In the control group, B cells released type I interferons at an average of 50 pg/mL. In the group with TLR7 removed, the release dropped to 20 pg/mL. This was much lower than the control group ($P < 0.01$) [13,14]. For the animal tests, 40 SLE model mice were used. They were randomly put into two groups, 20 in each. One group was regular SLE model mice. The other group was SLE model mice with TLR7 removed. Researchers looked at how long they lived, how much protein was in their urine, and changes in their kidney tissue. Regular SLE model mice lived for 60 days on average. Those with TLR7 removed lived 80 days on average [13,15]. Regular mice had 3+ protein in their urine. Those with TLR7 removed had 1+ protein in their urine. Kidney tissue slides showed that regular SLE model mice had a lot of glomerular growth, more mesangial cells, and many inflammatory cells clumped together. Mice with TLR7 removed had much less kidney damage. Their glomerular growth was not as bad, and there were fewer inflammatory cells.

Editing the CD19 or BLIMP1 genes of B cells can inhibit the differentiation of plasma cells is the way of regulating B-cell function [14]. In preclinical studies, when autologous edited B cells were reinfused into patients, the levels of anti-dsDNA antibodies in the patients decreased. And there was no risk of severe infection.

2.4.2 RA

RA is mainly characterized by inflammation of the joint synovium and bone destruction. CRISPR technology achieves treatment by regulating the functions of immune cells and synovial cells.

Regulating B-cell function. This will reduce the differentiation of Th17 cells. The inflammation in the joint synovium will also be alleviated accordingly [11].

Knocking out the Receptor Activator of Nuclear Factor-κB gene in osteoclast precursors. This reduces bone resorption. In RA model mice, after RANK editing, joint bone erosion is alleviated [12]. The degree of joint space narrowing is reduced. Regarding the influence of Epidermal Growth Factor Receptor on osteoclast differentiation, there are these data. Researchers extracted bone marrow-derived macrophages from 6-8-week-old C57BL/6 mice to construct an osteoclast differentiation model. RT-qPCR detection found that the expression level of EGFR significantly increased on the third day of osteoclast induction differentiation ($P < 0.01$). After 3 days of induction differentiation, stimulation with EGF would significantly decrease the expression of Nuclear Factor of Activated T Cells, Cytoplasmic 1 and significantly increase the expression of Matrix Metalloproteinase 13 ($P < 0.05$, $P < 0.01$) [16]. The results of Tartrate-Resistant Acid Phosphatase staining showed that after EGF treatment, the proportion of osteoclasts significantly increased, and their area also significantly enlarged ($P < 0.01$).

Regarding the effects of Macrophage Colony-Stimulating Factor and Receptor Activator of Nuclear Factor-κB Ligand on osteoclast differentiation, the following data are available. In the study, different concentrations of RANKL and M-CSF were used to induce and culture the isolated mononuclear cells from the bone marrow of SD rats. The results showed that the induction and differentiation of Tartrate-Resistant Acid Phosphatase Positive mononuclear and multinuclear osteoclast-like cells by M-CSF and RANKL were time-dependent [16]. By the third day, the number of these cells began to gradually increase, reaching the peak on the fifth and seventh days. When the RANKL concentration was 50 ng/ml, the number of TRAP(+) cells was related to the concentration of M-CSF [16]; when the M-CSF concentration exceeded 30 ng/ml, the number of TRAP(+) cells no longer increased significantly, and the curve flattened. However, when the M-CSF concentration was 50 ng/ml, the number of TRAP(+) cells was not related to the concentration of RANKL [16].

Here are the data regarding the effects of different culture methods on osteoclast differentiation. Researchers isolated bone marrow cells from the femoral bone marrow cavity of 4-week-old mice and cultured them using two methods: one was single culture, induced by M-CSF and RANKL; the other was co-culture with primary osteoblasts, induced by Cholecalciferol and Prostaglandin E_2. .The results showed that the number of osteoclasts obtained from co-culture was 240 ± 36, while that from single culture was 160 ± 23. The mRNA expression level of Notch2 molecule in the co-culture group was 4.1 ± 1.2, and that in the single culture group was 2.4 ± 0.6; the protein expression level of Notch2 in the co-culture group was also higher than that in the single culture group.

3. Conclusion

This paper conducts a comprehensive investigation into the use of CRISPR for treating autoimmune disorders, with a focus on identifying disease-causing genes, developing editing methods, and implementing targeted

interventions for specific conditions. It outlines major techniques such as disabling pathogenic genes, inserting regulatory factors, and correcting functional mutations through base editing. These approaches rebalance immune cell activity by modifying B cells, T cells, and other immune cell populations to reinstate immune tolerance. Preclinical results indicate that such strategies can lower autoantibody levels, suppress inflammatory signaling pathways, and reduce tissue harm in disease models like SLE and RA.

The outcomes address the shortcomings of existing immunosuppressive treatments mentioned in the introduction, offering perspectives on precise, cell-type-specific interventions that steer clear of widespread immune suppression. Through refining gene targeting and delivery methods, CRISPR-based therapies hold promise for achieving sustained disease management, laying groundwork for developing curative solutions instead of merely alleviating symptoms.

Nonetheless, obstacles persist, such as the requirement for more effective delivery systems tailored to specific cell types and long-term safety data concerning unintended genetic modifications. Future studies should prioritize improving editing accuracy in primary immune cells, enhancing in vivo delivery tools, and carrying out extended preclinical trials to assess treatment durability and safety. These endeavors will speed up the clinical application of CRISPR technology, creating opportunities for personalized treatment strategies in managing autoimmune diseases.

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