Exploring the Potential of CRISPR/Cas9 Technology in Autoimmune Disease Management

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Abstract. Autoimmune diseases are a group of diseases caused by the immune system mistakenly attacking its own tissues and cells. Autoimmune diseases are marked by the binding of autoantibodies to self-antigens, where the immune system mistakenly recognizes its own proteins, DNA or RNA as foreign substances and produces autoantibodies. There is a large inter-individual variation in the detection of autoimmune diseases, and in terms of treatment it is still only possible to alleviate the disease. But the development of gene editing technology, especially the CRISPR system, has made it possible to treat autoimmune diseases by gene editing using the CRISPR/Cas system. This research focuses on the application of CRISPR/Cas9 technology for the detection and treatment of autoimmune diseases. Relying on the high affinity of the dCas9 protein-sgRNA complex for specific sequences, abnormal nucleic acid sequences can be detected, and the use of the Cas12 system with exogenous ssDNA as a signal can make the results more obvious. In terms of therapy, according to previous experimental reports, the CRISPR/Cas9 system shows its potential in treating autoimmune diseases in three ways: directly knocking down the relevant disease-causing genes, modulating enhancers to activate or silence them by using CRISPR/dCas technology, and editing CAR T-cells to attack B-cells and plasma blasts by using CRISPR/dCas technology. This research will provide an in-depth discussion of these three aspects and their rationale, and make a discussion based on the current challenges of treating autoimmune diseases.

Keywords: CRISPR/Cas9, autoimmune disease, detection, treatment.

1. Introduction

Autoimmune diseases are a group of diseases caused by the immune system mistakenly attacking its own tissues and cells. Autoimmune diseases are marked by the binding of autoantibodies to self-antigens, where the immune system mistakenly recognizes its own proteins, DNA or RNA as foreign substances and produces autoantibodies. There are two types of autoimmune diseases. One is organ-specific autoimmune diseases and the other one is non-organ (systemic) autoimmune diseases, according to the extent and target of their effects. Organ-specific autoimmune diseases affect a specific organ or tissue in the body, such as Type I diabetes, which is caused by the immune system attacking pancreatic islet B cells. Systemic autoimmune diseases include rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), which affect multiple organs and tissues in the body. Autoimmune diseases are often multifactorial in nature, involving complex interactions between genes, the environment and the immune system. However, traditional diagnostic and therapeutic approaches are often limited in their targeting and understanding of the genetic factors underlying the disease.

Currently, the treatment of autoimmune diseases relies primarily on drugs, such as anti-inflammatory drugs, corticosteroids and immunosuppressants to suppress the inflammatory response. However, anti-inflammatory drugs, while prompt in their action, are taxing on the stomach and kidneys [1]. The use of immunosuppressive drugs is associated with many adverse effects and gonad toxicity. Emerging approaches include the use of biologics to target specific cells or immune factors to suppress the inflammatory response. This can provide effective treatment for critically ill patients, but is costly and are associated with risks of complications. There is also an innovative approach using mRNA vaccines that is still being investigated, the core of which includes, but is not limited to, decreasing B-cell activity, blocking T-cell activation, and potentially acting as an exogenous agent to induce immune tolerance [2].

The emergence of the CRISPR/Cas9 technology as a revolutionary gene editing tool has opened new avenues for biomedical research. It is mainly used as a molecular probe to bind specific sequence assays, as well as relying on its cleavage activity, to perform genomic perturbations to determine the effects of different genes on diseases [3]. Whereas autoimmune diseases have complex causative factors, and are even associated with the development of cancers, the knockout or silencing of potentially disease-causing genes or the editing of upstream elements can be achieved by using CRISPR/Cas9 technology, altering the amount and speed of gene expression, thus enabling the judgement of autoimmune disease causation at the genetic level. For the mRNA vaccines mentioned above, the editing of mRNA can also be achieved using CRISPR/Cas technology. In this research, the principles of using CRISPR/Cas9 technology will be discussed in depth, as well as the current situation and challenges in the field of autoimmune diseases, and most importantly, this review will highlight the potential of CRISPR/Cas9 technology in the detection and therapeutic approaches of these complex diseases.

2. Basic principles of CRISPR/Cas9

The CRISPR/Cas system consists mainly of crRNA, trans-activated crRNA (tracr RNA), and Cas proteins. crRNA and trans-activated crRNA combine to form an RNA complex known as the guide RNA. The guide RNA binds to the Cas9 protein to localize the Cas proteins to specific sites, relying on two endonuclease domains of the Cas9 protein to shear the PAM site. In fact, the fusion of the crRNA with a portion of the tracr RNA to form a single guide strand of approximately 80 bp (single guide RNA, sgRNA) gives it the properties of both crRNA and tracr RNA, and subsequent experiments revealed that the sgRNA can also guide Cas9 to cleave the target DNA. As shown in Fig. 1, double-strand breaks (DSBs) can be repaired mainly by non-homologous end-joining (NHEJ). And another approach of repairing DSB is homologous recombination repair (HDR), which is an important means of exogenous gene insertion [4], where base pairs are added by importing the corresponding exogenous DNA sequence as a template.

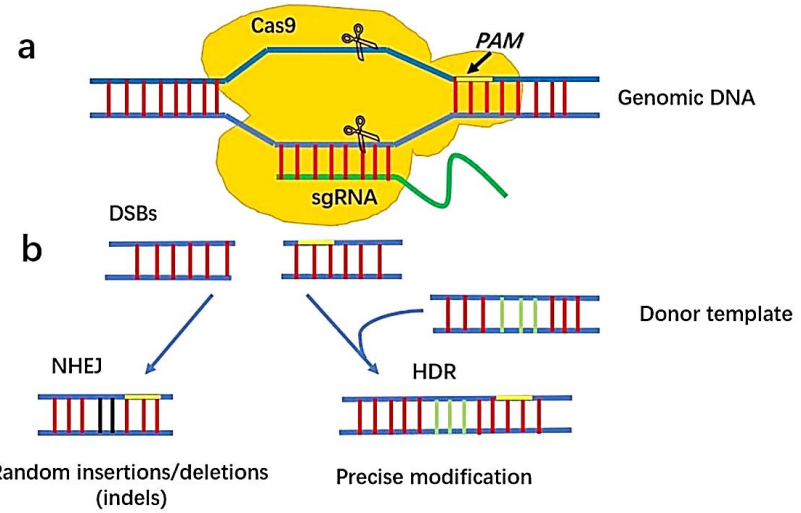


Figure 1. Mechanisms of CRISPR/Cas9 genome editing method [5]. (a) The Cas9 nuclease to a specific genome sequence with sgRNA and (b) Two DNA repair pathways for DSBs

In addition to performing gene editing, another application of CRISPR technology is genetic screening. CRISPR-Cas9 and its related variants, such as inactivated Cas9 (dCas9), have been applied to various genomic screening studies. By constructing CRISPR/Cas9 libraries, a large number of sgRNAs are designed to silence or shear different genes or regulatory sequences as a way of observing the characterization of individual cells [6]. CRISPR/Cas9 library screening enables the identification of key components of various biological processes, including the identification of disease-causing genes, the function of related genes, and the study of mechanisms of drug resistance. The emergence of CRISPR/Cas technology has not only dramatically changed the means of gene editing, but also

brought new hope for the detection and treatment of autoimmune diseases, tumors, cancers and other difficult diseases.

3. CRISPR in autoimmune disease detection

The Cas protein complex binds to the target DNA or RNA in a sequence-specific manner, allowing different target sequences to be targeted by using different sequences of the sgRNA, without the need for any protein editing or additional optimization of experimental conditions. Although the CRISPR/Cas9 system mainly works to cut the target sequence for sequence cleavage and gene deletion and insertion. However, some laboratory and clinical diagnostics based on the CRISPR system mainly focus on the sequence-specific binding features and do not rely on cleavage activity, thus requiring alteration of the structural domains of Cas9 that are cleavage-active to make them de-cleavage-active (dCas9), which shows a strong affinity for dsDNA. Similarly, to target RNA and avoid binding to DNA, it is necessary to provide PAM oligonucleotide sequences (PAMmers) that enable sgRNA to target Cas9 to the target RNA molecule [7]. PAMmers stimulate site-specific nucleic acid endonucleolytic cleavage of ssRNA targets, as PAM-mediated Cas9 catalyzes DNA cutting.

Based on genome-wide association studies (GWAS), many autoimmune diseases have been found associated with mutations in genes leading to abnormalities in regulatory factors, which ultimately lead to aberrant T-cell activation to attack themselves [8]. Relying on the high affinity of dCas for DNA, it is possible to design sgRNAs that bind specifically to known mutant sequences. Kyeonghye Guk's team reported a conventional fluorescence in situ hybridization (FISH)-based detection of MRSA strains [9]. The dCas9 was used as a molecular probe, and after hybridization, dCas9/sgRNA complexes were isolated using Ni-NTA magnetic beads in a pull-down assay, and unbound genomic DNA was removed by a magnetically-assisted wash. Then dCas9 was used as a staining agent for isolated DNA with the DNA intercalating dye SYBR Green I, which fluoresces only in the presence of the target sequence. Samples fluoresce only when the target sequence exists. Similarly, using FISH and CRISPR/Cas9 technology together greatly improves the accuracy and efficiency of the test results by detecting dCas9 targeted mutant genes in patient cells or tissues.

The CRISPR/Cas12 system has also demonstrated superiority in the detection of disease. Compared to Cas9, Cas12 has the ability to cleave all surrounding ssDNA [10]. In the detection of autoimmune diseases, single-cell transcriptome sequencing can be used to capture the sum of all the mRNAs of a single cell at a given point in time to characterize the state or function of that cell [11]. Then, to make the results more accurate, it is an essential preparation step that DNA is amplificated using nucleic acid amplification techniques such as PCR and RPA before the CRISPR/Cas12 assay. PCR or RPA for DNA amplification is a necessary preparatory step before doing CRISPR/Cas12 detection. The gRNA then needs to be designed to bind to Cas12. Once Cas12 complexes bind to the target DNA, Cas12 shows an incidental capability of slicing and cuts off all the ssDNA present in the vicinity [10]. As shown in Fig. 2, in most diagnostic methods, this ssDNA is exogenous and serves as a short probe, to which a quencher and a fluorescent dye are attached at another end. Upon truncation of the ssDNA probe, the signal from the fluorescent dye can be detected [12], and the signal is amplified to determine the presence of the target sequence.

The current CRISPR/Cas-based assay can be used to detect RNA in autoimmune diseases caused by mutations in known sequences, or when gene expression is significantly different from that of normal immune cells, while nuclear antibody detection is preferred for autoimmune diseases whose pathogenesis is not yet fully understood. The advantage of CRISPR/Cas12 is its high specificity and sensitivity, and its ability to detect low levels of genetic variation or expression, which can be useful in the early diagnosis of disease.

Looking through the achievements of genetic studies, many common variants have been identified associated with autoimmune diseases. In performing large-scale assays, the CRISPR/Cas9 system is used to overcome incomplete target gene suppression and frequent off-target effects to a large extent

by synthesizing sgRNA libraries and deleting genes using Cas9 [13]. Transduction of sgRNA libraries into plasmids and enabling cells to express Cas9 proteins for gene deletion, and tagging of endogenous genes with reporter genes to select for gene Cells with altered expression are identified to identify gene regulatory elements, relying on massively parallel reporter gene assays (MRPA) to find promoters, enhancers and other elements associated with autoimmune diseases [3]. Based on this, gene editing can be used to enhance or inhibit the expression of cytokines and key genes affected by autoimmune diseases, which can lead to the alleviation of autoimmune diseases and the treatment of autoimmune diseases in the clinic.

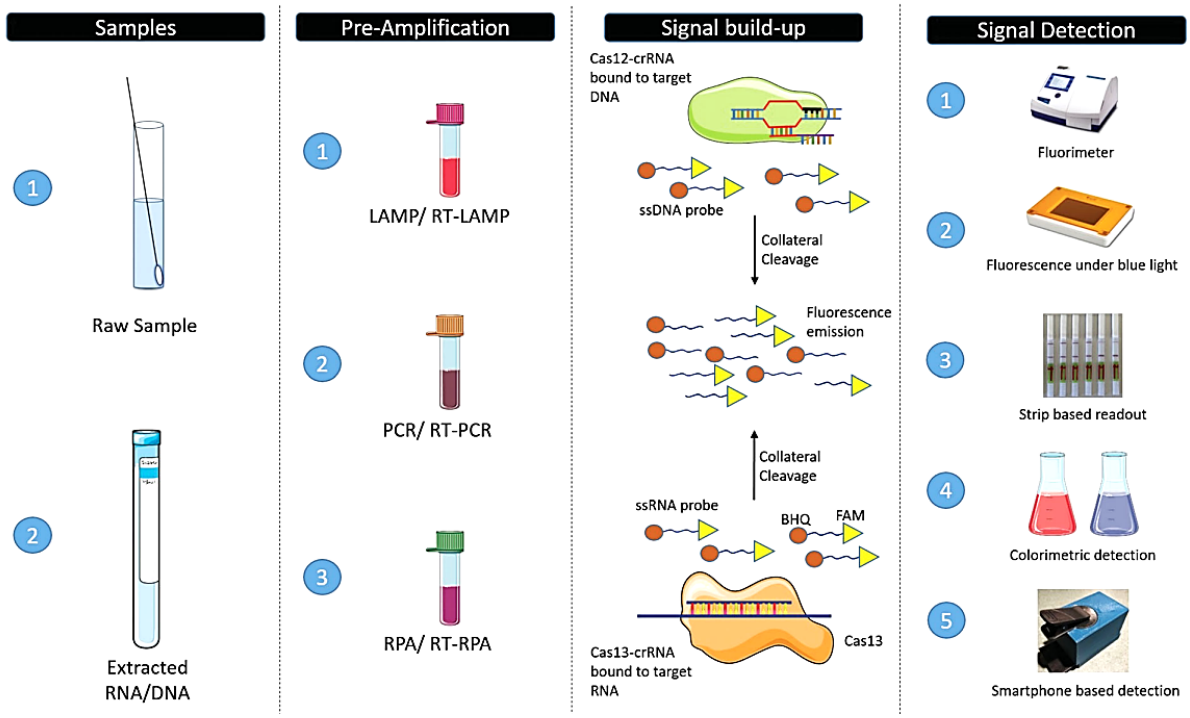


Figure 2. Schematic diagram of molecular detection workflow based on CRISPR-Cas12/Cas13 [12]

To construct a CRISPR/Cas9 system that makes corrections at the gene level. The sgRNA and Cas9 must penetrate the nucleus to function locus. Viral vectors and lipid nanoparticles are two main CRISPR-Cas9 delivery systems [14]. By correcting variants in disease-causing genes, it may be possible to reverse their associated autoimmune phenotypes. For example, by directly knocking out the relevant disease-causing genes. As an example, Zhu's team studied type 1 diabetes [15]. The lymphocyte-specific protein tyrosine kinase (LCK) encodes a kinase that is developmentally selective for and matures T cells as well as maturation, and influences their function. Autoreactive T cells contribute to the development of T1D by attacking pancreatic islet cells, leading to an absolute decrease in insulin levels in the body. LCK in peripheral blood mononuclear cells (PBMC) can be knocked down by CRISPR-Cas9. Experimental comparisons revealed that the G allele of the LCK SNP rs10914542 impairs TCR/CD3-mediated T cell activation, which means high risk of getting T1D. Therefore, knockdown of the G allele of SNP rs10914542 of LCK is useful for the treatment of T1D.

CRISPR/Cas9 can achieve therapeutics not only by knocking out genes, but also by modulation of enhancers. Taking systemic lupus erythematosus (SLE) as an example, systemic lupus erythematosus is a kind of complex autoimmune disease characterized by the appearance of autoantibody and aberrant activation of the type I interferon (IFN) pathway. The damage of IFN pathway is responsible for the pathogenesis of SLE, which tends to be elevated in SLE patients. MicroRNA miR-146a performs as a negative regulator in the IFN pathway, and Altered type I IFN pathway in SLE patients is caused by low expression of miR-146a, which influences the targeting key signaling proteins [16]. SLE risk variants located in the promoter and distal enhancers of miR-146a can lead to alterations in the miR-146a pathway in lupus patients by affecting binding affinity to transcription factors. binding

affinity leading to aberrant expression of miR-146a. By designing and synthesizing single guide RNAs targeting candidate enhancers and combining them with dCas9, it is possible to target upstream candidate enhancers, which can then be activated or inhibited, and the expression effect can be observed to find the true enhancers. In this way, in the face of SLE, it is possible to use the CRISPR/Cas9 system to target a certain region first, and through the enhancement or inhibition of this sequence or the direct knockdown of this sequence, it is possible to observe the expression effect of this sequence. direct knockdown, and observe the regulatory function of this sequence on the downstream target genes, e.g., if the deletion of the 32.5 kb enhancer significantly reduces the expression of miR-146a [17], then the SLE can be alleviated by targeting the 32.5 kb enhancer to activate the expression of miR-146a.

In patients with SLE, it may also be improved by administering CD19 CAR T cells. Conceptually, deep depletion of CD19 B cells and plasmoblasts in tissues may induce an immune reset in SLE, which could stop immunosuppressive therapy. Gene editing of T cells using CRISPR/Cas9 technology allows them to express CAR+14 that can bind to the specific antigen CD19 on target cells, which makes B cells and their malignant progeny targeted via the highly specific and ubiquitous surface antigen CD19. Clinical applications based on this technology have shown that three months after CAR T cell administration [18], all five patients showed sustained improvement in signs and symptoms of SLE, all immunomodulatory and immunosuppressive medications including glucocorticoids and hydroxychloroquine could be discontinued, and all five patients achieved drug-free remission.

4. Challenges in treating autoimmune diseases

The detection of autoimmune diseases mainly relies on indirect immunofluorescence with antinuclear antibodies, but due to manual operation, low standardization and the lack of a reference system, the test results are highly variable. As autoimmune diseases are often accompanied by tumors and other diseases, cytokine expression varies and is complex in different diseases, and there may be overlapping expression. Although the use of CRISPR/Cas9 technology is important for early diagnosis of diseases, most autoimmune diseases have complex pathogenic mechanisms, and screening at the genetic level is only an aid. The traditional treatment of autoimmune diseases includes the use of immunosuppressive drugs and biologics to suppress the immune response. These drugs are effective, but have adverse effects such as gastrointestinal symptoms, immunosuppression, myelosuppression, and neoplastic infections. Although CRISPR can theoretically cure autoimmune diseases at the genetic level, gene editing involves not only technical difficulties but also ethical issues, which makes it difficult for large-scale clinical application.

5. Conclusion

CRISPR/Cas technology for the detection of autoimmune diseases relies on the high specificity of dCas9 to locate the site of mutation and the use of fluorescence to amplify the signal. Cas12, although the mechanism is different, also requires the binding of sgRNA to the specific site. The most obvious aspect of autoimmune diseases is the aberrant expression of cytokines. By designing sgRNA and dCas9, targeting mRNA in cells, performing fluorescence quantitative analysis, and comparing with normal immune cells, we can initially determine whether the immune system is in trouble, and then go on to determine whether there is an immune disease.

The treatment of autoimmune diseases by CRISPR/Cas technology mainly includes the direct knockdown of relevant disease-causing genes; the modulation of enhancers by CRISPR/dCas technology to activate or silence them, and the editing of CAR T cells to attack B cells and plasm oblasts by CRISPR/dCas technology.

The clinical application of CRISPR technology to treat autoimmune diseases still has some limitations. However, CRISPR/Cas technology has brought new breakthroughs and perspectives for

autoimmune diseases. As the technology continues to be refined, more gene loci can be targeted, the efficiency and accuracy of gene editing can be improved, and patients can be exposed to more personalized treatments.

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