Application and Prospect of Gene Editing Technology in Immunotherapy

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Abstract. Clinical medical practitioners are increasingly embracing CRISPR/Cas9 technology. While CRISPR/Cas9 technology provides powerful editing capabilities, it is also simple to operate, low in cost, and highly specific. Furthermore, this technology is capable of accurately and efficiently screening the entire genome in a short period of time, which is extremely valuable for gene diagnosis. This article focuses mainly on presenting the latest developments in CRISPR/Cas9 technology from four perspectives: homologous recombination, single base gene editing, off target effects, and epigenetic modifications. CRISPR/Cas9 technology is explored from three perspectives: cancer animal model construction, cancer mechanism research, and human embryo editing. In conclusion, CRISPR/Cas9's therapeutic significance in immune diseases is described from two perspectives: gene repair and knockout as well as breaking collective immune tolerance.

Keywords: Gene editing technology, immunotherapy, tumor treatment, immune diseases.

1. Introduction

Rapid development and widespread application of CRISPR/Cas systems are currently revolutionizing gene editing. In comparison with traditional gene editing techniques, the CRISPR/Cas system offers high efficiency, simplicity, and ease of optimization. It uses a natural immune mechanism produced by bacteria and archaea to edit genomes precisely. The system has shown great potential for applications in a variety of fields, including gene expression, gene regulation, cell imaging, nucleic acid labeling, and epigenetic modification.

In the field of immunotherapy, CRISPR/Cas9 technology could improve treatment of many complex diseases. Traditionally, cancer, genetic diseases, and infectious diseases require inefficient and side-effect-filled treatment methods.Using CRISPR/Cas9, we can treat these difficult diseases. Personalized treatments can be enabled by precisely modifying the genes of immune cells. This allows the immune system to detect cancer cells and attack them.

Although CRISPR/Cas9 technology is still being developed, it has potential applications in immunotherapy. Studies and clinical trials are showing that this technology is safe and effective in treating a variety of diseases. With maturation and optimization, CRISPR/Cas9 is expected to become a powerful tool in the medical community, transforming how disease is understood and treated. Describes the current application status, challenges, and future developments of CRISPR/Cas9 as it pertains to immunotherapy.

2. Overview of CRISPR/Cas9 Technology

CRISPR/Cas9 is a system that was discovered by humans in bacteria years ago and is used as a defense mechanism. There has been a recent application of CRISPR/Cas9 technology to editing the genomes of mammals. This was done in 2013. Based on the properties and functions of the CRISPR/Cas9 system, it can be said that the most important technology for gene editing is the type II CRISPR/Cas9 system, which consists of three components: the Cas9 protein, the CRISPR RNA, and the transactivation RNA.

3. The Latest Progress of CRISPR/Cas9 Technology

In 1987, humans first discovered the CRISPR gene cluster. In 2002, scientists discovered the function of CRISPR gene clusters within genes through research. The CRISPR system can be seen

as an adaptive immune mechanism that primarily functions in bacteria or archaea by resisting the entry of exogenous nucleic acids into cells. Until 2012, two women who later shared a Nobel Prize together, found and developed the Crispr-cas9 system in prokaryotic cells but had not been visited for its usage in eucalyptus [4-5] Later in 2013, Zhang Feng applied this into eucalyptic cells and then its mammals [6-7]. Subsequently, more and more scholars began to explore CRISPR/Cas9 technology, and numerous laboratories began to study this gene editing technique. Studies have shown that this technology can be used for gene transcription activation regulation [8-9], cell imaging [10], animal model construction [11-12], and so on.

3.1 Homologous Recombination

Scientists are constantly striving to explore and practice CRISPR/Cas9 technology. At present, Homology Directed Repair (HDR) can be used to treat genetic diseases, but its efficiency is relatively low. In 2016, scholars Suzuki et al. from the Salk Institute proposed that by knocking in target genes that do not rely on homologous recombination, gene fragment knock in can be mediated using the Non Homologous End Joining (NHEJ) repair mechanism in non dividing cells [13]; In addition, the use of this technology can effectively treat rat models with retinal inflammation. Homology directed repair (HDR) is a mechanism within cells that repairs double stranded DNA damage. Non homologous end joining (NHEJ) is also one of the DNA repair systems, but unlike HDR, NHEJ is active in all cell cycles and does not require cloning homologous arms. Due to NHEJ being considered an error prone DNA repair system, scientists had not given much attention to its role in precise transgenic insertion prior to this. Scholars have also found that by regulating the cell cycle, multiple small molecules can affect the mechanism of DNA break repair [14-15]. The factor RS-1 in the HDR repair process can significantly enhance the efficiency of TALEN and the HDR mediated by this technology [16].

In China, many laboratories are working to improve the efficiency of HDR (homologous recombination repair) technology for more precise gene editing. HDR technology is a method of editing genes by guiding cells to self-repair. In the past few years, researchers have made significant progress, making this technology more efficient and reliable. In 2017, researcher Yao and his team conducted in-depth research on an improved HDR method. This method is called "long homologous end mediated repair", and in experiments on mice and monkeys, they found that this method can significantly improve the success rate of gene editing. Meanwhile, this method also reduces unnecessary mutations that may arise from another repair method - "short homologous end mediated repair" [17]. That is to say, their research has helped us to perform gene editing more accurately and reduce uncertainty. In the same year, another group of researchers, Zhang et al., also validated Yao's team's findings through experiments, further supporting the effectiveness of this improved method [18]. In 2018, the Yao team conducted further research on how to use linearized donor fragments to improve the efficiency of in vivo targeted gene editing. They found that this linearized donor fragment can significantly improve the success rate of gene editing, with an effect up to 12 times higher than traditional methods [19]. This progress means that we can perform gene editing more efficiently, which is of great significance for the development research and disease treatment of human embryos. In summary, these research findings mark a significant breakthrough in gene editing technology, providing us with new possibilities in medical research and treatment. These advances not only drive the development of science, but also provide valuable tools for possible future treatment methods.

3.2 Single Base Gene Editing

The experimental results demonstrate that the human genome contains 32000 single nucleotide polymorphisms (SNPs) [20]. In 2016, Komor et al. studied dead Cas9 protein coupled deaminase and found that it can achieve targeted single base gene editing (CBE) without cutting DNA. Specifically, it can edit C to T or G to A. In addition, it was pointed out that nickese Cas9 can replace dCas9, and it was found that coupling uracil glycosidase inhibitor (UGI) at the carbon end of the protein can effectively inhibit the DNA base cleavage mechanism and significantly improve the efficiency of

single base editing [21]. Afterwards, Komor et al. gradually optimized and updated the single base gene editing tool, not only adding Gam protein to the N-terminus of the fusion protein to reduce the probability of base insertion and deletion, but also copying UGI at the C-terminus of the fusion protein to improve the specificity of the edited product [22]. In addition, scholars such as Nishida from Japan [23] and Wang from China [24] have analyzed and explored single base editing tools with the aim of improving gene editing efficiency. In addition, Chinese scholars Zhou et al. also found that single base editing tools can treat thalassemia and have a good repairing effect on single base mutations in thalassemia patients [25].

3.3 Off-target Effects

In the clinical application of CRISPR/Cas9, the most important factor to consider is off target effects, which not only affect the quality of genes mediated by CRISPR/Cas9 technology, but also directly affect the safety of cell therapy mediated by CRISPR/Cas9 technology. In 2018, Hu et al. used in vitro evolution to screen xCas9, which is more efficient and has stronger specificity [26]. However, after investigation, it was found that there were errors in the experimental design of the article, so it was withdrawn from the journal Nature Methods. In addition, Genentech conducted detailed analysis and research on 1423 predicted off target sites in 81 gene editing projects targeting mice and rats, and used second-generation sequencing to determine 32 of these sites; We also analyzed off target mouse embryos edited with single targeted RNA (sgRNA) and identified 43 off target sites; In addition, the experiment also demonstrated that high fidelity Cas9 protein can significantly reduce off target effects [27].

3.4 Epigenetic modification

During the normal development of living organisms and the occurrence of diseases, the changes that occur inside cells are not only DNA base mutations, but also epigenetic modifications of the genome [28-29]. Therefore, the technology of epigenetic modification and editing of specific genomic loci is of great significance in both scientific research and practical applications. In the past, scientists have studied targeted DNA methyltransferases for different diseases, analyzed proteins related to histone modifications, and developed a series of small molecule drugs. Clinical evidence has shown that the epigenetic modifications caused by these small molecule drugs are mostly not site-specific and are likely to have varying degrees of side effects during treatment [30-31].

After understanding the principle of how new lipoproteins can recognize DNA, scientists began to produce artificial new lipoproteins that bind to protein coupled DNA methyltransferases, truly achieving targeted epigenetic regulation. Eventually, they successfully used targeted methylation to reduce highly expressed genes in cancer cells, thereby inhibiting their growth [32]. However, currently, there are certain optimization obstacles in the design of artificial zinc finger binding proteins, which undoubtedly limits the practical application of this tool. Therefore, the use of transcription activator like effector (TALE) technology in the later design can be better optimized. Moreover, for specific genomic loci, each requires a specific TALE protein [33]. The characteristic of the CRISPR/Cas9 system is its simplicity and ease of application, which is undoubtedly an effective means of targeted expression regulation. In 2016, Liu et al. proposed that dCas9 protein coupled DNA demethylase can act on targeted epigenetic modifications, and DNA methyltransferase can also achieve targeted epigenetic modifications; Meanwhile, experiments have shown that targeting the distal promoter of the demethylated *MyoD* gene can facilitate the transformation of fibroblasts and the production of myotubes [34]. Liu and other research seniors studied CRISPR/Cas9 technology, developed the in vivo surface remodeling mediated by this technology, and used this technology to treat mice with diabetes, muscular dystrophy, acute kidney disease and other diseases. Research shows that it can successfully cure or alleviate the disease of diseased mice [35].

Domestic researchers have also conducted in-depth analysis and research on CRISPR/Cas9 technology in epigenetic modification. Zhou et al. pointed out that in mouse experiments, CRISPR/Cas9 technology can activate multiple genes in the mouse brain at the same time, and can

also induce small glial cells to differentiate into functional neurons [36]. Cheng et al. developed the Casilio technique through analysis and research, which can independently regulate multiple genes with multiple functions at the same time. It can also act on the OCT4 gene enhancer to affect its histone modification, with the aim of promoting the expression of endogenous genes [37].

4. The Application Value of CRISPR/Cas9 Technology in Tumor Immune Diseases

4.1 Construction of Cancer Animal Models

In 2014, Niu et al. successfully obtained the Ppar - γ and *Rag*1 dual gene knockout of crab eating macaques mediated by CRISPR/Cas9 technology for the first time [38]. In 2018, Yan et al. analyzed and explored CRISPR/Cas9 technology, constructed a Huntington's disease pig gene knock in model, and efficiently simulated the phenotype of human neurodegenerative disease patients [39].

In 2016, a research team from Stanford University proposed that CRISPR/Cas9 technology could repair *HBB* mutation sites in hematopoietic stem cells of patients with sickle cell anemia, providing theoretical support for the cure of sickle cell anemia [40]. In addition, CRISPR/Cas9 has been found to have a great potential for treating genetic hearing loss. Gao and other researchers used CRISPR/Cas9 technology to treat a hearing loss mouse model with *YMC1* mutation. This experiment suggests that CRISPR/Cas9 technology is likely to be used for genetic hearing loss.

4.2 Research on the Mechanism of Cancer Occurrence

In the research process of tumor immune diseases, ex vivo cell culture is a key means, and CRISPR/Cas9 technology can enable researchers to analyze and understand tumor cell lines more deeply [41]. Previous researchers have used Cas9 to cleave target DNA. When DNA double strand breaks, it is mainly repaired through non homologous end connections and homologous end connections [42]. If CRISPR/Cas9 technology can be used to target the highly expressed Pyk2 knockout in glioma cells, thereby controlling the migration and proliferation of glioma cells, it will provide help for senior researchers to understand the process of glioma cell occurrence and deterioration, and also lay a theoretical foundation for later research and treatment of glioma patients [43].

4.3 Application of Human Embryo Editing

The characteristics of CRISPR/Cas9 technology are simple design, easy operation, and efficient editing, so it is widely used in various fields, especially in gene therapy. Scientists have explored and studied CRISPR/Cas9 technology for a long time, and conducted multiple experiments in human three prokaryotic embryos, including gene knockout and gene repair. In 2015, Liang et al. first pointed out that CRISPR/Cas9 technology could be used to edit HBB genes in human three prokaryotic embryos, and found through experiments that the technology could also knock out HBB genes quickly and accurately. However, they also found that HDR mediated repair efficiency was low, and significant off target effects were found through deep sequencing [44]. In 2016, Fan Yong's research group discovered that CRISPR/Cas9 technology can efficiently edit the *CCR5* gene in human three prokaryotic embryos. If a template for homologous repair is introduced, the *CCR5* Δ 32 genotype can be obtained, allowing individuals with mutations in this genotype to effectively prevent HIV infection [45]. In 2017, researchers from Oregon Health Sciences University in the United States pointed out that CRISPR/Cas9 technology can help repair the mutated gene *MYBPC3* related to early embryonic hypertrophy in the human body, and explored the safety of CRISPR technology [46]. British scholars have also conducted gene editing experiments on normal human embryos, exploring the role of OCT4 gene in pre implantation embryo development in humans [47].

5. The Therapeutic Significance of CRISPR/Cas9 in Tumor Immune Diseases

5.1 Gene Repair and Knockout

The occurrence of tumors is associated with the activation of oncogenes and/or the inactivation of tumor suppressor genes. The prevention and treatment of tumors can start from the activation of oncogenes or inhibition of tumor suppressor genes, that is, the activation and increase of tumor suppressor gene expression [48]. The pathogenesis of chronic myeloid leukemia is closely related to mutations in the tumor suppressor gene ASXL1 in vivo. Gomez et al. used CRISPR/Cas9 technology to repair ineffective ASXL1 mutations in KBM5 cells, thereby inhibiting the proliferation rate of KBM5 cells, improving cell differentiation ability, and prolonging the survival time of tumor bearing mice in the experiment [49].

5.2 Breaking Down Immune Tolerance in the Body

Once tumor cells are present in the body, they will continuously adjust and modify due to changes in the body's state and its own tissue compatibility complex, and can also avoid the inhibitory and killing effects of the immune system. Therefore, Yu et al. believe that breaking immune tolerance can achieve the effect of treating tumor diseases [50].

At present, there are two main methods of tumor cell immunotherapy: one is to use T cells modified with specific T cell receptors for treatment, and the other is T cell therapy modified with chimeric antigen receptors [51]. The principle of these two treatment methods is to recognize the expression of specific receptors on the surface of T cells, and then recognize specificity to reduce the immune response of target cells. Through experiments, it has been proven that T cells modified with chimeric antigen receptors can significantly treat hematological malignancies and non Hodgkin lymphoma diseases [52]. However, the problem with this treatment method is that it may make it difficult to control the modified cell genes after reaching the recipient cell genome, so using this treatment method may result in various adverse reactions in the later stage [53]. In 2017, Ma Yan et al. used CRISPR/Cas9 technology to construct updated chimeric antigen receptor modified basic T cells [54], effectively improving the therapeutic effect and experience of tumor cell diseases.

6. Conclusion and Prospect

Since its emergence and application, CRISPR/Cas9 technology has had a significant impact on gene editing, promoting its development and practice and simplifying, convenient, and efficient construction of animal disease models. The field of immunotherapy is currently undergoing a significant amount of research on gene editing. Additionally, CRISPR/Cas9 gene editing human trials have been initiated [55] and [56].

In addition to its potential impact on clinical immunotherapy, CRISPR/Cas9 technology will open up new fields, such as cell imaging, gene expression regulation, and epigenetic modification, due to its simplicity. It is currently necessary to study and optimize CRISPR/Cas9 technology's off target effects. The advancement of life sciences can once again be achieved with additional human analysis and research, collaboration, and exploration by scientific teams around the world.

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