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The Role and Impact of Gene Editing on Vaccine Development

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Abstract: The application of gene editing technology in vaccine development has brought new hope for dealing with emerging and reemerging infectious diseases. Currently, gene editing tools, represented by CRISPR/Cas9, have significantly accelerated the vaccine development process and improved the safety and efficacy of vaccines by virtue of their highly efficient and precise operational characteristics. However, gene editing technology still suffers from off-target effects and high costs, and its wide application in vaccine development has yet to be further explored. This paper analyzed the application of gene editing technology in accelerating the construction of vaccine candidates, improving vaccine safety, and facilitating the design of novel vaccines (e.g., nucleic acid vaccines, viral vector vaccines, and subunit vaccines), and found that gene editing technology reduces the pathogenicity of pathogens by targeting the disease-causing genes, which makes vaccine research and development more efficient and safer. Gene editing provides a more precise and adaptable tool for future vaccine design, which can help to respond to infectious disease outbreaks more rapidly. Despite the limitations of the current technology, future research can further improve the accuracy and cost of gene editing, as well as further explore multivalent vaccine construction and high-throughput screening techniques, which will lead to further breakthroughs in the field of vaccines.

Keywords: Gene editing, vaccine development, CRISPR/Cas9, viral vaccines, immunogenicity.

1. Introduction

Vaccines play a crucial role in the prevention of infectious diseases by stimulating the immune system to produce antibodies that enable individuals to quickly recognize and resist infection when exposed to pathogens. When a large portion of the population is vaccinated, the chances of transmission of infectious diseases are significantly reduced, protecting those who are unable to be vaccinated (e.g., those with compromised immune systems or allergies), and vaccination reduces hospitalization, complications, and mortality rates due to infectious diseases. Genome editing technologies, including CRISPR/Cas, zinc finger nuclease (ZFN), and transcription activator-like effector nuclease (TALEN), utilize nuclease enzymes to make precise modifications to specific nucleotides in the genome, thereby enhancing homologous recombination (HR) efficiency. These technologies can lead to gene disruption events that occur through non-homologous end joining (NHEJ) or homology-driven repair (HDR) mechanisms. CRISPR-Cas9 technology, in particular, has revolutionized the vaccine development process due to its ability to make precise modifications to the pathogen genome, significantly enhancing the efficacy and safety of vaccines. The technology not only facilitates customized optimization of antigens and improved vector design, but also deepens the understanding of the role of host genes in the vaccine response, which ultimately enhances the vaccine development and production process. Inactivated vaccines are made by killing the pathogen and their development challenges include ensuring that the vaccine remains immunogenic during the inactivation process without causing side effects. Live attenuated vaccines are made by weakening the pathogen so that it loses its pathogenicity, and the development challenge is to find the right attenuation method that is effective in stimulating an immune response without turning the vaccine back to pathogenicity. Subunit vaccines contain only specific parts of the pathogen (e.g., proteins) The challenge is to extract and purify an effective antigen while ensuring that the immune response is durable. These vaccines often require the addition of adjuvants to enhance the immune effect. mRNA vaccines stimulate the immune response by synthesizing the mRNA of the pathogen. Maintaining the stability of the mRNA and the efficacy of the delivery system is key to development, as well as the need to ensure long term safety and efficacy. Gene editing can effectively create and screen vaccine targets, remove pathogenic genes from pathogens, create safe attenuated vaccines, enhance the immunogenicity of vaccines, and rapidly design and produce vaccines against new pathogens. CRISPR has made the process of vaccine development much more efficient, allowing for the rapid construction of a wide range of vaccine candidates and enabling researchers to make precise modifications at specific gene loci. CRISPR/Cas9, has greatly advanced the process of vaccine development.

2. Overview of gene editing technology

2.1. Basic principle

Gene editing technology is a tool for precisely modifying biological genomes, and its basic principle mainly relies on DNA double-strand breaks and the subsequent repair mechanism. The main repair pathways include HDR and NHEJ. HDR utilizes homologous DNA as a template in the repair process and is able to accurately insert or replace specific DNA sequences, whereas NHEJ triggers changes in gene function by directly connecting broken DNA ends, which may lead to insertions or deletions. The design of specific primers and selection of targets are crucial in the gene editing process. The design of primers needs to take into account the specificity and conservatism of the target sequence to ensure editing precision and effectiveness. At the same time, target selection should be based on the function, expression pattern and potential pathogenicity of the gene in order to realize efficient gene editing.

2.2. Characteristics and application of major gene editing technologies

Gene editing technology is in a stage of rapid development, and technologies such as CRISPR-Cas9 are powerful tools for precise modification of the genome Currently, the main gene editing technologies include CRISPR/Cas9, TALEN and ZFNCRISPR/Cas9 utilizes RNA-guided Cas9 nuclease to achieve highly efficient and easy gene editing. The CRISPR system has the advantages of easy operation, low cost, and the ability to target multiple genes. The CRISPR system has the advantages of easy operation, low cost, and the ability to target multiple genes, and is widely used in the fields of vaccine development and gene therapy. Using the Cas13 system to target the RNA of the new coronavirus, specific crRNAs can be designed to degrade the viral genome and thus inhibit viral replication [1]. TALEN is a gene editing technology that uses specific proteins to recognize and bind to specific DNA sequences, thereby directing FokI nuclease to cleave DNA. In the development of HIV and influenza vaccines, TALEN can precisely edit host cell genes to improve the immunogenicity and safety of the vaccine [2]. ZFN specifically binds DNA and activates FokI

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nuclease through zinc finger proteins. Although ZFNs are more complex to develop and apply, they still show potential in some specific applications. The use of ZFNs to modify t-cell receptors, which can improve immune responses in HIV vaccine strategies, and the use of ZFNs to edit the genomes of viral vectors to improve the safety and efficacy of vector-based vaccines by eliminating unneeded genetic material, thereby improving the accuracy of immune responses. These gene editing technologies not only provide powerful tools for scientific research, but also revolutionize areas such as vaccine development and gene therapy, offering unlimited possibilities for improving human health and quality of life.

3. Gene editing in vaccine development

3.1. Accelerate the process of vaccine development

The application of gene editing technology has significantly increased the speed of viral genome construction, making the efficiency of vaccine development dramatically improved. While the traditional vaccine development process usually requires a long period of virus culture and screening, gene editing technologies, such as CRISPR/Cas9, enable researchers to rapidly construct the genome of a target virus

The CRISPR-Cas9 system has been shown to exhibit great efficiency in modifying adenovirus genomes. The technology enables the rapid construction of recombinant viruses by introducing precise mutations or exogenous genes at specific sites in the adenovirus genome through the RNA-guided Cas9 nuclease. Compared with traditional viral gene editing methods, CRISPR-Cas9 has the advantage of being simple and efficient, which can significantly shorten the generation time of recombinant viruses and reduce the complexity of experimental steps. The application of this technology has greatly facilitated the study of gene function, the development of viral vaccines, and the advancement of viral genome modification, especially in the construction of viral strains with specific mutations or exogenous gene expression, which is much more efficient than the previous gene editing methods. For example, in the study of adenoviruses, the CRISPR-Cas9 system enables the editing and recombination of viral genomes by introducing targeted double-stranded cleavage, which triggers the non-homologous end-joining repair mechanism of the host cell. It was shown that virus strains constructed using CRISPR-Cas9 technology were able to maintain high genetic stability and genetic transmission of mutations during the transmission process. This finding provides a powerful tool for virology and vaccinology to rapidly generate recombinant viruses for downstream functional studies or vaccine design and development [3]. CRISPR screens have been successfully used to explore the interactions of viral genomes with host cells, allowing researchers to identify and manipulate genes critical to the viral life cycle. This process facilitates the rapid customization of viral strains for vaccine purposes, especially in the development of attenuated viruses, which are essential for the manufacture of safe and effective vaccines. Base editing based on CRISPR technology facilitates the generation of precise mutations in the viral genome, enabling the rapid production of vaccine strains that can be used for immunogenicity and safety testing. This approach provides a more efficient method compared to traditional vaccine development techniques. Gene editing technology makes it possible to construct multivalent vaccines. By inserting multiple antigenic sequences into the same viral vector, vaccines can be designed to provide protection against multiple pathogens. This strategy greatly improves the adaptability and efficacy of vaccines. The application of CRISPR/Cas9 technology focuses on the editing of viral genomes and the study of virus-host interactions. By precisely manipulating the viral genome, CRISPR technology not only helps to reveal the replication mechanism of viruses, but also screens out host factors that are critical to the viral life cycle. These research results have further promoted the development of multivalent vaccines against specific viruses. CRISPR functional studies have provided new ideas for vaccine

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design, making it possible to develop effective vaccines against multiple viral strains, thereby improving the effectiveness of public health protection.

3.2. Improving vaccine safety

Gene editing technology also plays an important role in improving vaccine safety. tools such as CRISPR/Cas9 and CRISPR/Cas13 can effectively introduce specific mutations (e.g., insertions or deletions) by precisely cutting the RNA or DNA of viruses, thereby causing viruses to lose their disease-causing ability. Recent studies have shown that CRISPR technology has broad application prospects in the suppression of viral infections and vaccine development. By utilizing CRISPR technology, the pathogenic mechanism of viruses can be better understood, laying the foundation for the development of novel antiviral strategies. Such technological advances provide new ideas for addressing global public health issues, especially for vaccine development against emerging and reemerging viruses.

Leishmaniasis is a parasitic disease caused by Leishmania protozoa against which conventional vaccines provide limited protection and there are challenges in developing safe and effective vaccines. To address this challenge, researchers used CRISPR-Cas9 gene editing to successfully reduce the toxicity of Leishmania protozoa, allowing it to act as a live vaccine to induce an immune response without causing disease. With this technique, scientists were able to precisely delete or suppress specific genes associated with pathogenicity, allowing the modified parasite to both maintain antigenicity, and thus be able to stimulate the host immune system, and lose its ability to infect or cause disease. The high specificity of the CRISPR-Cas9 system is crucial in this process. Typically, live vaccines may carry some risk of infection, especially in individuals with weakened immunity [4]. However, precise genetic modification using CRISPR can effectively remove key pathogenicity genes of Leishmania protozoa, minimizing the potential threat of vaccines to humans. This approach not only reduces the risk of infection, but also ensures that only the antigenic components required to trigger an immune response are retained, resulting in a vaccine that is well balanced in terms of safety and immune efficacy [5]. This breakthrough opens up new directions for the development of safe live vaccines using gene editing techniques, providing a powerful tool in the fight against parasitic and other infectious diseases.

With the CRISPR-Cas9 system, scientists were able to genetically reprogram B cells to consistently produce antibodies against specific viruses. Rather than simply stimulating the immune system, this process fundamentally modifies the genes of B cells so that they can actively produce antibodies after exposure to a virus. This technique not only extends the duration of immune protection but also reduces the frequency of vaccinations, making vaccine effects longer lasting. Traditional vaccine methods usually produce antibodies in the body in the short term through inactivation or attenuation [6]. However, for complex and persistent viruses such as HIV, the effectiveness of traditional methods is often limited, in part due to HIV's ability to mutate rapidly and evade recognition by the immune system. By directly targeting and modifying viral genes through CRISPR, such vaccines can precisely elicit a targeted immune response from the body, effectively reducing the risk of off-target reactions and side effects [7]. This not only improves the safety of vaccines but also provides a novel solution for long-term immune protection. This technology is particularly promising against recalcitrant viruses such as HIV, and holds the promise of overcoming viral infections that are difficult to combat with conventional vaccines.

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3.3. Development of novel vaccines

3.3.1. Nucleic acid vaccines

The core of nucleic acid vaccines is to cause the body to generate its own immune response by delivering DNA or mRNA fragments encoding antigenic proteins. Gene editing technology plays a key role in the optimization of nucleic acid sequences. With precise gene editing tools, such as the CRISPR/Cas system, scientists are able to modify nucleic acid sequences to improve the efficiency of vaccine expression and immune effects. RNA vaccines are usually unstable in the body and are susceptible to enzymatic degradation. CRISPR-Cas9 is able to accurately edit the genome of viruses or pathogens, helping to design and optimize vaccines. By editing specific genes in viruses, researchers can enhance immunogenicity or adjust the targeting of vaccines to develop more effective vaccines. For example, CRISPR-Cas9 can be used to modify the surface antigenic genes of a virus to induce a stronger immune response. CRISPR-Cas9 is not just used for gene editing, but can also regulate gene expression. In the development of nucleic acid vaccines, CRISPR-Cas9 technology can be used to modulate the expression of key genes in the immune system, thereby enhancing the immune effect of the vaccine or reducing side effects. Many nucleic acid vaccines use viral vectors to deliver vaccine components. With CRISPR-Cas9, these viral vectors can be edited to make them safer, more stable and more efficient to deliver. Gene editing technology can also help reduce the adverse reactions caused by these vectors in vivo and improve vaccine availability. CRISPR-Cas9 can also be used to test and optimize the safety of vaccines. By precisely editing vaccine-associated genes, researchers can simulate different mutation scenarios and assess the effectiveness of vaccines against various pathogen variants while reducing potential risk factors [8].

3.3.2. Viral vector vaccines

The development of viral vector vaccines relies on the construction of suitable vector viruses to introduce antigenic genes for expression in host cells. Gene editing technologies, such as CRISPR/Cas9, can modify the viral genome by precise knockout or insertion of exogenous genes, thereby improving the efficiency and safety of vector delivery. Adeno-Associated Viruses (AAVs) are a widely studied type of viral vectors favored for their low immunogenicity and cytotoxicity. They are capable of safely delivering CRISPR components and enabling tissue-specific delivery. This makes AAVs an ideal tool for targeting different cell types such as lung, heart, and neural cells. The application of the CRISPR/Cas9 system in vaccine development is mainly based on its ability to target and modify the viral genome, thereby reducing viral pathogenicity. The advantage of this technology is its high efficiency and accuracy against a wide range of viruses for which effective treatments are currently lacking: In some preclinical studies, delivery of CRISPR/Cas9 by recombinant AAV vectors successfully ameliorated atherosclerosis in low-density lipoprotein receptor (LDLR) mutant mice [9]. Despite the series of successes of AAV vectors in clinical trials, their limited cloning capacity remains a major challenge. This allows vectors to carry only small amounts of genetic material, limiting the use of CRISPR components

3.3.3. Recombinant subunit vaccines

Recombinant subunit vaccines rely on the design and expression of specific viral or bacterial protein fragments to induce an immune response. CRISPR-Cas9 technology is being applied to accelerate the development of subunit recombinant protein vaccines by improving antigen selection and vaccine optimization through precise gene editing. This technology could simplify vaccine production by enabling scientists to target specific pathogen proteins that trigger immune responses without having to use the entire pathogen, making these vaccines safer and easier to manufacture [9]. CRISPR could

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also improve the efficiency of parallel antigen development and shorten the time from discovery to clinical application.

4. Impact of gene editing on the cost and efficiency of vaccine development

The use of CRISPR technology in vaccine development is transforming the traditional R&D process, especially in the enhancement of DNA vaccine platforms. In the development of the COVID-19 vaccine, researchers succeeded in shortening the time required to create an effective vaccine through the use of CRISPR-based enhancers. Compared to traditional vaccine development methods, this technology not only enables precise antigen design, but also rapid adaptation to emerging pathogens, thus providing a scalable and efficient solution. Such advances allow for rapid deployment during viral outbreaks by reducing the time for vaccine development from months to weeks

Additionally, CRISPR plays an important role in creating genetically engineered animal models, which is critical for vaccine testing. Researchers have developed a one-step CRISPR method to generate mice with human-like immune responses [10]. The rapid generation of such models greatly accelerates the testing phase of potential vaccines and improves the accuracy of preclinical trials. This integration not only simplifies the process of vaccine development, but also enhances the reliability of vaccine studies, allowing scientists to more effectively evaluate and optimize the safety and efficacy of vaccines.

5. Conclusion

This paper has systematically analyzed the application of gene editing technologies in vaccine development, covering how tools such as CRISPR/Cas9, ZFN, and TALEN can accurately modify viral or host genes to accelerate vaccine research and development, and enhance vaccine safety and efficacy. Gene editing technologies, especially CRISPR/Cas9, can rapidly construct vaccine candidates and reduce viral pathogenicity by targeting disease-causing genes, making vaccines safer and more reliable. CRISPR technology has also significantly shortened vaccine development time and played an important role in the design of nucleic acid vaccines, viral vector vaccines, and subunit vaccines.

These advances demonstrate the far-reaching impact of gene editing technology on vaccine development, providing strong support for global public health through the rapid construction of novel vaccines to address unexpected viral infections, such as COVID-19. Gene editing enables more targeted and adaptive vaccine design, helping to enhance vaccine immunity and reduce side effects.

There are still limitations to the use of gene editing technology in vaccine development. Existing technologies have not yet been able to completely avoid the off-target effects of gene editing, which may have potential impact on vaccine safety. Due to the complex operation and high cost of CRISPR, it remains a challenge to generalize it for practical clinical applications.

Future research could focus on improving the accuracy and lowering the cost of gene editing to promote its widespread use in vaccine development. Increased research on gene editing technology in multivalent vaccine construction and high-throughput screening will bring further breakthroughs in the field of vaccines, laying a more solid foundation for dealing with emerging and re-emerging infectious diseases.

References

[1] *Shenyang, K., Zhiquan, Z., & Cai, S. (2015). CRISPR/Cas9 system in disease modeling and gene therapy.* Chinese Journal of Biochemistry and Molecular Biology, 31*(8), 9.*

[2] *Bhardwaj, A., & Nain, V. (2019). TALENs - an indispensable tool in the era of CRISPR: A mini review.* Journal of Genetic Engineering & Biotechnology, 19*(1), 125.*

DOI: 10.54254/2753-8818/82/2025.KA21015

[3] *Bi, Y., et al. (2014). High-efficiency targeted editing of large viral genomes by RNA-guided nucleases.* PLoS Pathogens, 10*(5), e1004090.*

[4] *Hlavova, M., Turoczy, Z., & Bišová, K. (2015). Improving microalgae for biotechnology — From genetics to synthetic biology.* Biotechnology Advances, 33*(6), 1194-1203.*

[5] *Media, F. (n.d.). Frontiers in Cellular and Infection Microbiology.* Cellular & Infection Microbiology.

[6] *Shyr, D. C., et al. (2023). One year follow-up on the first patient treated with Nula-Cel: An autologous CRISPR/Cas9 gene corrected CD34+ cell product to treat sickle cell disease.* Blood, 142*, 5000-5000.*

[7] *Fiumara, M., et al. (2024). Author correction: Genotoxic effects of base and prime editing in human hematopoietic stem cells.* Nature Biotechnology*,* 42*(6), 986-986.*

[8] *Bock, C., et al. (2022). High-content CRISPR screening.* Nature Reviews Methods Primers, 2.

[9] *Vilela, J., Rohaim, M. A., & Munir, M. (2020). Application of CRISPR/Cas9 in understanding avian viruses and developing poultry vaccines.* Frontiers in Cellular and Infection Microbiology, 10*, 581504.*

[10] *Massachusetts General Hospital. (2020, December 15). One-step method to generate mice for vaccine research.* ScienceDaily. *Retrieved from www.sciencedaily.com/releases/2020/12/201214123538.htm.*