Application and Development of Structural Vaccinology in Vaccine Molecular Design Steps

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Abstract: Structural vaccinology has a broad prospect, which takes genomic research as a basis, combines research directions from multiple disciplines, and advocates the use of surface proteins or three-dimensional structures of pathogens to guide vaccine design. However, there is still a lack of systematic knowledge about structural vaccinology's specific mechanism and application value in vaccine molecular design. This paper provides a brief introduction to structural vaccinology and the molecular design of vaccines, a review of the application of structural vaccinology in molecular design, an in-depth discussion of the role of structural vaccinology in the determination of antigenic epitopes, epitope synthesis and vector construction, and finally a brief overview of the current status and prospects of structural vaccinology. By collecting and analysing relevant data, this paper discusses in depth the application strategies, technological progress and future development direction of structural vaccinology in the key aspects of vaccine molecular design to provide new ideas and methodological references for vaccine research and development.

1 INTRODUCTION

A vaccine is a biological product that induces the host to develop immune resources against a particular antigen, interrupting the transmission of an infectious agent while helping the host to prevent infection. Vaccines also enhance the specific immunity of the inoculated population against an antigen, which is effective in preventing disease and treating it. The invention of vaccines is considered one of the triumphs of medical research. Immunisation stops the spread of infections in childhood and provides lifelong protection against certain diseases. Currently, the four types of vaccines available to humans are inactivated, live attenuated, subunit and nucleic acid vaccines. Live attenuated vaccines include the whole pathogen, which can replicate in the host and induce a strong immune response. Whole-pathogen inactivated vaccines are mostly safe and non-infectious and generally inactivated by physical heating and many chemical methods. Inactivated vaccines have now been tested and found to be deficient in inducing weak and short-term immunity and, therefore, require boosters to enhance their immune effect to achieve complete protection. Subunit vaccines contain purified or recombinant antigens consisting of only the antigenic portion of the pathogen, not the entire cell. They are, therefore, generally safe regarding toxicity and reactogenicity (Verma et al. 2023).

Structural vaccinology, as a discipline focusing on exploring the structure-function relationship of vaccines, plays a key role in understanding the working principle of vaccines and promoting the optimisation of vaccine design. With the help of X-ray crystal diffraction, cryo-electron microscopy and other cutting-edge structural biology techniques, researchers can precisely analyse the structure of vaccines and gain in-depth insights into the intrinsic mechanisms of vaccine-induced immune responses, thus building a solid theoretical foundation for the design and development of new vaccines. In addition, through computer-aided simulation and prediction, the interactions between vaccines and immune cells can also be studied, thus providing a more thorough understanding of the working mechanism of vaccines and helping to realise breakthroughs and developments in vaccinology.

After a long period of scientific research, analysing the three-dimensional structures of most protein antigens at the atomic level is now possible, thanks to the development of structural biology techniques. In addition, in-depth studies of structural

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biology have provided important structural information that can guide design modifications at the atomic level. In particular, the structures of antigen-protective monoclonal antibody complexes have revealed key epitopes for antigen recognition, which has led to a deeper understanding of the mechanisms of the host's protective immune response and thus guided the reverse design of vaccines. The structural biology-based vaccine design approach is a new research direction (Li et al. 2024). The development of synthetic vaccines can avoid the use of attenuated and inactivated disease-causing pathogens, further reducing the risks associated with their pathogenicity. These vaccines can be designed to trigger specific immune responses more precisely.

As an emerging field of vaccine research and development, the development of structural vaccinology has provided an important theoretical basis and technical support for enhancing vaccine effectiveness and promoting innovative breakthroughs in vaccine design technology. In this paper, we systematically integrate the research progress of structural biology in vaccine molecular design in recent years and discuss the basic principles, key technical paths and specific implementation steps of vaccine molecular design, aiming to enrich the research connotation of vaccine molecular design from the theoretical level and to provide scientific references and bases for the optimisation of vaccine molecular infrastructures and the improvement of vaccine efficacy.

2 DEFINITION AND MECHANISM OF ACTION OF VACCINES

Vaccines are automatic immunising agents for medical use. They are generally divided into two categories: prophylactic vaccines, which generally act on healthy individuals such as newborns, and therapeutic vaccines, which generally act on diseased individuals. According to tradition and custom, there are also types at the molecular level, such as live attenuated vaccines, inactivated vaccines, antitoxins, subunit vaccines (including peptide vaccines), vector vaccines, and nucleic acid vaccines.

The principle of action of vaccines is to treat pathogenic microorganisms and their metabolites using methods such as genetic engineering or artificial manipulation to reduce virulence so that they retain their properties of stimulating the immune system of the animal body. The immune system will produce certain protective substances, such as immune hormones, active physiological substances, unique antibodies, etc., when the animal body is exposed to such attenuated or inactivated pathogenic bacteria. When the protective cells in the animal's immune system recognise the antigen again, they will be stimulated to produce an immune response and make the same antibodies to prevent the pathogenic bacteria from harming the animal, which ultimately leads to immune protection of the body.

3 MOLECULAR DESIGN OF VACCINES

The molecular design of vaccines refers to the in-depth analysis of the structure of vaccines and their immune effects through modern molecular biology and immunology techniques, then design and optimise them to make vaccines safer and more efficient. The design concept is to identify the key components in the molecular structure of the pathogen that triggers the immune response and modify them according to the characteristics of its biology (Li et al. 2024). The development of synthetic vaccines can avoid attenuated disease-causing pathogens and reduce the risks they pose. At the same time, these vaccines can be designed to trigger specific immune responses more precisely.

The molecular design of vaccines encompasses a variety of techniques. The first is using computer-based simulation programs to predict pathogen proteins' spatial conformation and three-dimensional structure, providing strong evidence for the design of vaccine molecules that match them. The second is screening effective vaccine molecules through multiple comparative testing experiments. The third is to utilise bioinformatics tools and algorithms to design efficient vaccine molecules. The fourth is to design chimeric vaccine molecules by combining the principle of high structural similarity of different types of surface antigens. Fifthly, chemical synthesis and bioengineering techniques are used to assemble and modify antigens and other molecules so that they have vaccine functions. In conclusion, the molecular technology of vaccines requires an in-depth study of pathogens' structure and biological characteristics. It is a highly critical and complex process in vaccine development.

4 STRUCTURAL VACCINOLOGY AND ITS APPLICATION TO VACCINE MOLECULAR DESIGN

4.1 The Concept of Structural Vaccinology and Its Development

The concept of structural vaccinology (SV) was introduced in 2008, advocating the study of utilising surface proteins or three-dimensional structures of pathogens to guide vaccine design, and this research direction is considered to have a broad prospect.SV takes genome study as a foundation and combines the research directions of multidisciplinary fields such as molecular biology and structural biology. By utilising techniques such as X-ray crystallography, nuclear magnetic resonance spectroscopy and cryo-electron microscopy, researchers can dissect the molecular structure of pathogen antigens and further understand the mechanisms by which they interact with the host immune system (Li et al., 2024).

SV research suggests that machine learning algorithms rarely augment SV and are used as an aid. A potential extension of SV design is the use of evolutionary algorithms that evaluate assessments and replacements based on multiple rounds of data evaluation, provide variables by modifying inputs, and optimise and adjust for the function's results to improve its fitness after several iterations have been performed. However, there is still a lack of good scoring functions to ensure that the evolutionary algorithm will produce optimised vaccine candidates rather than maximising some arbitrary mechanism (Huffman et al. 2022).

4.2 Application of Structural Vaccinology to Specific Steps of Vaccine Molecular Design

4.2.1 Determination of Antigenic Epitopes

Antigenic epitope determination identifies key antigenic epitopes in viral proteins by molecular biology methods. African swine fever (ASF) is highly infectious and lethal, and its causative agent is the African swine fever virus (ASFV). Only in-depth studies of ASFV antigens can facilitate the development of effective vaccines and control measures. p22 protein, one of the major structural proteins of ASFV, can be detected at the early stages of ASFV infection, making it a potential candidate protein for detecting ASFV(A et al. 2021).To carry out the preliminary identification of antigenic epitopes recognised by monoclonal antibodies, firstly, it is necessary to use PSIPRED software to predict the secondary structure of the p22 protein and get that the protein is composed of four α-helices and seven extended chains; secondly, eight peptides were designed and synthesised according to the amino acid sequence and secondary structure of p22 protein, and Dotblotting preliminarily identified the antigenic epitopes of p22 protein. Then, TMHMM-2.0 software was used to predict the transmembrane structural domains of the protein and the tertiary structure of the p22 protein; finally, antigenic epitope analysis was carried out, and the antigenic epitopes obtained by identification were compared with the amino acid sequences of the p22 protein of 20 different ASFVs in GenBank. The results showed that the sequences of the two antigenic epitopes recognised by the monoclonal antibody were highly conserved, and there was no difference in amino acid sequences (Wang et al. 2025).

4.2.2 Epitope Synthesis

Epitope synthesis uses synthetic peptide technology to synthesise these antigenic epitopes artificially, and its methods include two major categories: chemical synthesis and biosynthesis. Chemical synthesis is divided into solid-phase synthesis and liquid-phase synthesis. Solid-phase synthesis is the step-by-step construction of peptide chains by connecting amino acids to solid-phase carriers one by one, usually used for the synthesis of short peptides, which is the most commonly used method of peptide synthesis; liquid-phase synthesis is carried out in solution, usually used for the synthesis of longer peptide chains with complex spatial structures. The biosynthesis method also consists of two methods: one is edited using genetic engineering technology, inserting gene fragments encoding antigenic epitopes into expression vectors, and finally extracting the target products in host cells so that epitopes with natural conformations can be synthesised; the other is to clone the gene fragments encoding polypeptides and introduce them into phages so that the final translated polypeptides are presented on the surface of the phages In the other case, the gene fragment encoding the peptide is cloned and introduced into the phage so that the final translated peptide is presented on the surface of the phage.

Helicobacter pylori (Hp) is a Gram-negative bacterium typically found in the gastric epithelium of

humans (Xu et al. 2021).In 2022, a study was conducted to synthesise the core undecanoate, outer core pentasaccharide, outer core pentasaccharide, inner core trisaccharide, phosphorylated inner core trisaccharide, and α-1,6-glucan in the structure of the Hp lipopolysaccharides using a chemical method, and the antibody affinity of the synthesised oligosaccharides was evaluated using a glycan chip technique. The experimental results showed that α-1,6-glucan could bind well to serum IgG antibodies of most Hp-infected patients, and this study demonstrated that α-1,6-glucan may be an important antigenic epitope of Hp lipopolysaccharide (Zou et al. 2022). A series of oligosaccharide molecules formed from monosaccharide units linked by glycosidic bonding were first generated by a catalytic reaction of tens of steps using tribenzyl oxidised boron benzoate (Zhao et al. 2020) as a starting material from monosaccharides to pentasaccharides, with the yields of each molecule being 90%, 88%, 90%, 92%, and 89%, respectively. Each sugar unit is a pyranose ring structure and carries a hydroxyl group, and the pentasaccharide molecule carries an amino group at the end (Zhao et al. 2024).

4.2.3 Carrier Construction

To enhance immunogenicity, vector construction is the insertion of synthetic antigenic epitopes into appropriate vectors, such as DNA vaccines or virus-like particles. In order to prevent and reduce losses, a study utilised Red homologous recombination technology, using bacterial artificial chromosome (BAC) as a gene editing platform, to integrate the F gene of NDV into the genome of MDV double deletion strain Md5BACΔmeqΔLorf9, thereby knocking out the F gene of NDV and inducing I-SceI enzyme expression. The F gene of NDV was integrated into the genome of MDV double deletion strain Md5BACΔmeqΔLorf9, which induced the expression of the I-SceI enzyme, thereby knocking out the kanamycin resistance gene, resulting in the successful construction of the recombinant live-vector vaccine candidate strain Md5BACΔmeqΔLorf9-F (Gong et al. 2024). In order to construct a recombinant adenovirus with the replication-defective human adenovirus type 5 (Ad5), the capsid protein of duck tambuusu virus (DTMUV), another study inserted the DTMUV Capsid gene into the pShuttle-CMV-Hsp70 plasmid containing the heat shock protein 70 (mHsp70) of Mycobacterium tuberculosis as the adjuvant by a one-step cloning technique and constructed a recombinant adenovirus able to The DTMUV Capsid gene was inserted into the pShuttle-CMV-Hsp70 plasmid containing Mycobacterium tuberculosis heat shock protein 70 (mHsp70) as an adjuvant, to construct the recombinant shuttle plasmid pShuttle-DTMUV Capsid, which can express DTMUV Capsid and mHsp70 proteins (Wu et al. 2024).

5 CONCLUSION AND OUTLOOK

This paper summarises the definition of a vaccine and its principle of action, as well as describes the concept, application, and prospects of structural vaccinology. Structural vaccinology plays a pivotal role in many steps of vaccine molecular design, which can not only determine antigenic epitopes by predicting and comparing the secondary and tertiary structures of proteins but also artificially synthesise antigenic epitopes by using synthetic peptide technology, which helps to construct carriers for antigenic epitopes to enhance immunogenicity.

However, although structural vaccinology has made certain breakthroughs and development, it still faces serious challenges: first, the difficulty of structural analysis, some antigens are difficult to crystallise or complex structure, which limits its application; second, the pathogen will escape the immune response through mutation, resulting in immune escape phenomenon, which makes the vaccine effect decline; third, the high cost of structural vaccinology technology, which restricts its large-scale applications Thirdly, the high cost of structural vaccinology technology limits its large-scale application, and how to reduce the cost is also an urgent problem to be solved.

Vaccine design methods based on structural biology have become a promising research direction. In vaccine production and supply, innovations in vaccine molecular design will not only reduce costs and increase efficiency but also promote the improvement of vaccine production technology, which on the one hand, will enable the development of broad-spectrum vaccines capable of responding to a wide range of pathogen variants, and on the other hand will enable the design of personalised vaccines based on the differences in the individual's immune system, so that the efficiency of the vaccine supply can be increased rapidly to meet the needs of global public health. In response to new outbreaks, the increasingly mature vaccine molecular design technology can rapidly analyse the pathogens and design targeted vaccines at the early stage of an

outbreak, and combined with the development of new adjuvants and delivery systems, it can improve the immunogenicity and stability of vaccines and achieve rapid prevention and control of infectious diseases. In addition, with the continuous development of structural biology technology, the precision and efficiency of 3D structure analysis of antigenic proteins will continue to improve. Based on the high-resolution structural information, the immunogenicity and specificity of antigens can be improved through strategies such as antigen epitope modification and protein multimerisation design, laying a solid foundation for developing new-generation vaccines.

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