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# *Research on Phage-based Influenza Vaccine Utilizing Hemagglutinin Protein*

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***Abstract:*** Influenza virus is a major threat to global public health, and its high variability and diversity bring great challenges to vaccine development. Hemagglutinin (HA) protein, as an important surface antigen of influenza virus, plays a key role in virus invasion and immune escape, and is the focus of vaccine development. Although the traditional influenza vaccine has certain effect, it is unable to cope with the rapid mutation of the virus. As a new technology, phage vaccine uses phage display platform to display foreign antigens and induce specific immune response. This article summarizes the structure, function and variability of HA protein of influenza virus, and the limitations of traditional influenza vaccine. Then the basic principle of phage display technology and its application potential in vaccine research and development are introduced. Subsequently, the research and development process, immunogenicity evaluation and advantages of influenza phage vaccine based on HA protein are expounded, and the challenges it faces are analyzed. Finally, the potential of phage vaccine in influenza prevention and control is summarized and the future research direction is prospected.

## 1. Introduction

Influenza virus is a kind of respiratory pathogen with high infectivity and variability, which has long posed a serious threat to global public health security [1]. There are many kinds of influenza viruses, among which the most well-known one is influenza A virus. Because of its wide host range, high-frequency gene recombination and antigenic variation, it has become the main culprit of influenza pandemic [2]. The structural characteristics of influenza virus include two glycoproteins, HA and neuraminidase, in its outer layer [3]. The transmission route of influenza virus mainly relies on air droplets and contact transmission, and its pathogenic mechanism involves a series of processes such as virus adsorption, invasion, replication and release of respiratory epithelial cells [4]. After influenza virus infection, patients may have fever, cough, myalgia, fatigue and other symptoms, and severe cases may be complicated with pneumonia, respiratory failure and even death [5]. It is particularly noteworthy that the high frequency variation of influenza virus enables it to escape the immune surveillance of the host.

Traditional influenza vaccines mainly include inactivated vaccine, attenuated live vaccine and subunit vaccine. Inactivated vaccine induces immune response by injecting inactivated influenza

virus particles, but its immunogenicity is relatively weak, and it needs multiple vaccinations to produce sufficient protective effect [6]. Attenuated live vaccine uses attenuated influenza virus strains to stimulate the body to produce immunity. Although it has strong immunogenicity, it has potential safety hazards, especially for people with low immunity, which may cause adverse reactions [7]. Subunit vaccine is prepared by extracting specific antigenic components of influenza virus (such as HA protein). Although its safety is high, its immunogenicity still needs to be further improved [8].

With the constant variation and evolution of influenza virus, the existing vaccines are often unable to cope with the emerging influenza virus strains. On the one hand, antigen variation of influenza virus may lead to antigen mismatch between vaccine and epidemic strain, thus reducing the protective effect of vaccine [9]. From another point of view, the existing vaccine production process and preparation process are relatively complex, and it is difficult to produce it on a large scale in a short time and deal with the sudden epidemic [10]. Based on this reason, developing new influenza vaccine technology to improve the immunogenicity, safety and production efficiency of the vaccine has become a research hotspot in the field of influenza prevention and control.

Phage is highly stable and safe, its genome is not easy to mutate, and it is not infectious to human cells. More importantly, phage can display foreign proteins on its surface by means of surface display technology, thus forming a new vaccine form-phage vaccine. Phage vaccine retains the immunogenicity of foreign proteins, and can also deliver antigens to the immune system through the natural infection route of phage, thus stimulating a stronger immune response. The research on phage vaccine of influenza vaccine based on HA protein has important scientific significance and application value.

## 2. Overview of HA protein of influenza virus

The diversity and variability of influenza virus surface antigens are the key factors for the virus to escape the host immune response and lead to repeated infection. As one of the most important surface antigens of influenza virus, HA protein plays a vital role in the process of virus invading host cells [11]. It is also the main target to induce the body to produce neutralizing antibodies and form immune protection.

HA protein is a glycoprotein on the envelope of influenza virus, and its molecular structure is complex and fine. Structurally, HA protein consists of head and stem [12]. The header area contains the receptor binding site(RBS). This is the key site where the virus binds to the surface receptor of the host cell. Influenza viruses bind to sialic acid receptors on host cells by means of the head of HA protein, thus starting the process of virus invasion into cells [13]. The stem region is responsible for anchoring HA protein on the virus envelope and maintaining its stability. The head and stem of HA protein are interdependent in function and structure, which together constitute an important machine for influenza virus to invade cells.

The antigenicity of HA protein is the basis of its use as vaccine antigen. Antigenicity refers to the ability of antigen to stimulate the immune system to produce specific antibodies or sensitized lymphocytes. The antigenicity of HA protein is mainly determined by the amino acid sequence and spatial conformation of its head region. Because of the high genetic variability of influenza virus, the amino acid sequence of HA protein often varies, which leads to the change of its antigenicity.

Antigenic drift refers to the gene mutation during the replication of influenza virus, which makes the HA protein sequence slightly change, escapes the host immunity, and causes local outbreak or epidemic of influenza [14]. Antigenic drift is a common form of influenza virus variation, and it is also the main cause of seasonal influenza epidemic. Due to the frequent occurrence of antigenic drift, the influenza vaccine needs to be updated every year according to the data of the global

influenza surveillance network to ensure the matching between the vaccine and the epidemic virus strains.

Antigenic transformation refers to the large variation of HA protein of influenza virus, which leads to the emergence of new subtypes or strains of influenza virus. Antigenic transformation usually occurs in the process of cross-species transmission of influenza virus, such as the transmission of avian influenza virus to humans [15]. Due to the lack of immunity of human beings to new influenza virus subtypes or strains, antigenic transformation often leads to a global influenza pandemic.

## 3. Foundation and application of phage vaccine technology

The basis of phage vaccine technology lies in the biological characteristics and display technology of phage. Phage is highly specific and infectious, and can accurately identify and infect its host bacteria. This characteristic provides the possibility for the application of phage in vaccine research and development. Phage display technology is a technology of fusing foreign protein or polypeptide to phage surface protein, so as to display it on phage surface. This technology enables phage to be used as a carrier to display various antigen proteins, which provides a new idea for vaccine development. Phage itself has a certain immune stimulating effect, which can activate the immune system of the body and produce an immune response to the antigen displayed on the surface of phage. When phage displays the antigen protein of a pathogen, the body will produce specific antibodies against the pathogen, thus forming immune protection.

In the preparation of phage vaccine, it is necessary to select the appropriate phage vector and antigen protein first. Phage carriers usually choose those phages that are safe to human body, non-pathogenic and have high display efficiency. The antigen protein is selected according to the target pathogen of the vaccine, such as HA protein of influenza virus. Then, with the help of genetic engineering technology, the gene sequence of antigen protein is inserted into the genome of phage, so that antigen protein can be fused with the surface protein of phage and displayed on the surface of phage. Finally, phage vaccine products were obtained by means of culture and purification. The selection of some key factors in the preparation of phage vaccine is shown in Table 1.

Table 1: Key Elements in the Preparation Process of Phage Vaccines

| Element | Selection Principle | Example |
|---|---|---|
| Phage Vector | Safe for humans, non-pathogenic, high display efficiency | λ Phage, M13 Phage, etc. |
| Antigen Protein | Selected based on target pathogen, immunogenic | Influenza Hemagglutinin, Bacterial Surface Antigen, etc. |
| Genetic Engineering | Insert antigen gene into phage genome, fuse with surface protein | Gene cloning, site-directed mutagenesis, etc. |
| Manufacturing Process | Culture phages, purify vaccine product | Cell culture, chromatography purification, etc. |

In the research and development of influenza virus vaccine, phage vaccine technology has also shown great potential. Traditional influenza vaccine is mainly based on inactivated virus or subunit vaccine, and its preparation process is complicated, and it is difficult to completely match the constantly changing influenza virus [16]. Phage vaccine technology can quickly screen vaccine candidates with high immunogenicity by displaying antigens such as HA protein of influenza virus with phage. This vaccine can not only induce the body to produce specific antibodies against influenza virus, but also enhance the immune response of the body with the help of the immune stimulation of phage itself.

However, the development of phage vaccine also faces some challenges. For example, the

immunogenicity and protective efficacy of phage vaccine may be affected by many factors, such as the type of phage, the selection of display antigen and the display efficiency. For this reason, these factors need to be fully considered and optimized in the development of phage vaccine. The safety of phage vaccine also needs strict evaluation and monitoring to ensure that it is safe and harmless to human body.

## 4. Influenza phage vaccine based on HA protein

The unique biological characteristics and safety of phage make it have a wide application prospect in vaccine research and development. Phage display technology is a technology to insert foreign genes into phage genome and display the encoded proteins on the surface of phage [17]. By inserting the HA protein gene of influenza virus into the phage genome, a candidate phage vaccine strain displaying HA protein can be constructed.

The development process of influenza phage vaccine based on HA protein first involves the construction of phage display library. Scientists need to select a suitable phage vector and insert the gene sequence of influenza virus HA protein into a specific position in the phage genome to ensure that HA protein can be correctly expressed on the phage surface and maintain its natural conformation. This process requires fine molecular biology operation and strict control of experimental conditions.

After the construction of phage display library, the next step is to screen candidate phage vaccine strains with immunogenicity. This process is usually realized by immunological methods, such as enzyme-linked immunosorbent assay (ELISA) and western blot. Scientists will use the specific antibody or serum of influenza virus to react with phage display library to screen phage clones that can specifically bind to antibody or serum. The HA proteins displayed by these clones have good immunogenicity and can induce the body to produce specific antibodies against influenza virus.

The screened candidate phage vaccine strains need further evaluation and optimization. Scientists will evaluate the immunogenicity of these candidate strains and observe their ability to induce antibodies and immune duration with the help of animal experiments. Futhermore, the safety of vaccine candidates will be evaluated to ensure that they will not have adverse reactions or potential risks to the human body. In this process, scientists may genetically engineer phage vaccine candidate strains. The development process of influenza phage vaccine based on HA protein involves many complicated steps (as shown in Table 2).

Table 2 Development Stages of Phage-Based Influenza Vaccine

| Development Stage | Main Content | Key Technologies/Methods | Evaluation Indicators |
|---|---|---|---|
| Phage Display Library Construction | Select phage vector, insert influenza hemagglutinin gene | Molecular cloning, genetic engineering | Diversity of library, expression level & conformational correctness of hemagglutinin |
| Immunogenicity Screening | React specific antibodies or sera with phage display library | ELISA, immunoblotting | Number & binding strength of phage clones binding to antibodies/sera |
| Vaccine Candidate Evaluation & Optimization | Evaluate immunogenicity & safety of selected phage vaccine candidates | Animal experiments, immunological assays | Ability to induce antibodies, immune duration, safety indicators |
| Genetic Engineering | Genetically modify | Site-directed | Improvement in |

| | | | |
|---|---|---|---|
| Modification | vaccine candidates to enhance immunogenicity, stability & safety | mutagenesis, protein engineering | immunogenicity, stability & safety of modified phage vaccine candidates |
| Preclinical Studies | Conduct pharmacodynamics, pharmacokinetics & toxicology studies | Animal model experiments, in vitro experiments | Vaccine efficacy, safety, & pharmacokinetic parameters |
| Clinical Trials | Conduct Phase I, II, & III clinical trials to verify safety & efficacy | Randomized controlled trials, double-blind trials | Safety, immunogenicity, protective efficacy & durability of vaccine |
| Production & Quality Control | Establish large-scale production process, conduct QC & stability studies | Biopharmaceutical processes, quality control techniques | Vaccine yield, purity, stability & quality control indicators |
| Registration & Marketing | Submit registration application, obtain regulatory approval for marketing | Pharmaceutical registration regulations, approval process | Registration approval status, market performance & user feedback after launch |

The research and development of influenza phage vaccine based on HA protein also faces some challenges. The variability and diversity of influenza viruses make vaccines need to be constantly updated to adapt to new virus strains [18]. This requires scientists to closely monitor the variation of influenza virus and adjust vaccine components in time. The immune mechanism and effect of phage vaccine in human body need further study. Although phage vaccine has shown good immunogenicity and safety in animal experiments, its effect in human body needs to be verified by clinical trials.

In order to solve these problems and challenges, scientists are actively carrying out related research. On the one hand, with the help of the global influenza surveillance network, they closely track the variation of influenza virus, obtain new virus strain information in time, and provide scientific basis for vaccine update. From another point of view, they are constantly exploring the immune mechanism and effect of phage vaccine in human body, and verifying its safety and effectiveness with the help of clinical trials.

## 5. Conclusions

Influenza virus is a highly mutated respiratory pathogen, and its surface antigen HA protein plays a key role in the process of virus invading host cells. The variability and diversity of influenza viruses make the development and application of traditional influenza vaccines face many challenges.

Phage, as a virus that can infect bacteria specifically, has a wide application prospect in vaccine research and development because of its unique biological characteristics and safety. With the help of phage display technology, the HA protein gene of influenza virus can be inserted into the phage genome, and a candidate phage vaccine strain displaying HA protein can be constructed. This technology provides a new idea for the research and development of influenza vaccine, which enables the vaccine to simulate the natural infection process more accurately.

Compared with traditional influenza vaccine, influenza phage vaccine based on HA protein has many advantages. It can not only display the natural HA protein of influenza virus, but also induce the body to produce specific antibodies against the virus more effectively. It also has the advantages of simple preparation, low cost, easy mass production and popularization. However, the

development of phage vaccine also faces some challenges, such as the variability and diversity of influenza virus, the immune mechanism and effect of vaccine in human body and so on.

In order to solve these problems, scientists are actively carrying out related research. With the help of the global influenza surveillance network, they closely track the variation of influenza virus, obtain new virus strain information in time, and provide scientific basis for vaccine update.

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