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Evaluation of the application value of mRNA vaccine of monkeypox virus

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Abstract. With the advancement of technology in the global business environment, modern medicine has achieved significant milestones in vaccine development, particularly in combating various viral infections. In recent years, the global spread of the monkeypox virus has become a critical issue that the medical community urgently needs to address. This article assesses the potential value of mRNA vaccines in the application against monkeypox virus and provides a comprehensive comparison with traditional vaccines. Compared to traditional vaccines, mRNA vaccines exhibit high potential, safety, and efficacy; the paper thoroughly discusses the mechanism of action of mRNA vaccines, including their steps in the immune response, vaccine preparation, and the mechanism by which mRNA vaccines complete the immune response within infected individuals. Additionally, the article provides a detailed introduction to the synthesis steps and delivery systems of mRNA vaccines. Although the development of mRNA vaccines is still in the early stages and not yet commercially available, their application prospects in cell biology research and disease model construction are broad, and they are expected to become a powerful tool for future cell capture and analysis.

Keywords: mRNA Vaccines, monkeypox virus, biocompatibility, programmability, immune response.

1. Introduction

The evolving technological landscape has helped many industrial transitions in the global business environment. The research and development of modern medicine has made outstanding contributions to the technology of today's world. In the past few years, modern medicine has been lead technological revolution enabling it to develop vaccine that can fight different virus infection. Changing lifestyle factors, compromised immune systems and dietary habits have put humans at an exposed risk of contracting infections of different forms. For example, the global spread of monkeypox virus has made it one of the most important problems for the medical community to solve. Considering the complexity of the situation, through the research of medical researchers and continuous clinical trials, modern medicine has successfully developed an mRNA vaccine against monkeypox virus. The advent of the new mRNA vaccine has brought the difference between it and the traditional DNA vaccine to the attention of the general consumer. However, there are certain reasons attributed to this phenomenon.

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The monkeypox virus is spread by direct contact with the rash, blisters, or bodily fluids of a person with monkeypox. Prolonged close contact (more than 4 hours) with respiratory droplets from infected people, as well as sexual contact. The complications of monkeypox are similar to those of smallpox, with fever, severe headache, persistent fever, and the whole body progressing from rash to blisters, usually within 10 days. Monkeypox virus has morphologic features similar to other orthopoxviruses, with a size of 200–250nm, a brick-shaped virus that is enveloped and contains surface tubules along with a dumbell-shaped core component.). The good news is that in May 2022, Moderna, the world's leading mRNA vaccine developer, announced successful clinical trials of its monkeypox mRNA vaccine. mRNA vaccines have been demonstrated as a powerful alternative to traditional conventional vaccines because of their high potency, safety and efficacy, capacity for rapid clinical development, and potential for rapid, low-cost manufacturing. mRNA vaccine drug products undergo three typical steps in their manufacturing, which are upstream production, downstream purification, and finally formulation of the mRNA drug substance.

Unfortunately, although monkeypox virus mRNA vaccine has been successfully developed, the current development is still in the early stages, and this vaccine is not yet on the market. This paper will introduce the principle of mRNA, the steps of mRNA vaccines participating in immune response, and the preparation steps of vaccines as well as how monkeypox mRNA vaccine completes the immune response in infected people, the mechanism of virus clearance, and the commercial value of mRNA vaccine

2. The principle of mechanism of Monkeypox Virus mRNA vaccines

2.1. Major principal

The homology of VACV A27L is A29L. It joins the virus and attaches to the membrane of host's cell. The T cell Epitope of CD4+ and CD8+ are certain peptide segments of protein A27L. GAGs are able to regulate T cells and B cells. The binding region of GAGs (Glycosaminoglycans) is adjacent to the epitope of A29L protein, and they can prevent viruses from attaching to the surface of host cells by neutralizing virus particles.

The L1R is homologous proteins in both M1R and VACV. The targetting of neutralizing monoclonal antibodies are L1R. Homologous M1R is suspected to have the same function as L1R: the proper folding of the L1R protein is extremely important for inducing antibodies.

H3L will stick onto the surface of the virus by its hydrophobic C-terminal structure; it will bind to the HS on the surface of the host cell as well, thereby, helping the virus enter. H3L is not only a target of cellular immune response, but also an important element that triggers host cells to produce T and B cells. H3L, just as L1R, is a target for neutralizing monoclonal antibodies.

A33R protein is shown on the surface of lipid membranes, but not on the surface of IMV. A33R is a necessary and indispensable protein for eliminating repeated infections. It is different from H3L, L1R is not the target for neutralizing monoclonal antibodies, but a target for neutralizing lipid membrane antibody reactions.

MPXV and VACV are highly similar (highly homologous), but their differences can lead to a cross protection of MPXV by smallpox vaccines. Therefore, scientists believe that developing a multi antigen binding vaccine against MPXV can greatly help suppress the monkeypox virus epidemic. For this virus study, scientists selected mRNA with vaccine target antigens of M1R, A35R, A29L, and H3L. There are three mRNA vaccine alternatives: M1R-A35R, A29L-H3L-A35R, and L1R-A33R. These all three sets contain EEV protein and IMV protein. The immune effect of vaccines can be enhanced by EEV and IMV proteins.

2.2. Weakness of mRNA vaccines compared to DNA vaccines

The shortcomings, and weakness, of mRNA vaccines are their storage methods and instability because mRNA vaccines are not stable enough to be stored at higher temperatures, therefore, they need to be

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stored in colder, more complex, cumbersome conditions. Many countries that do not meet these conditions will face certain restrictions.

2.3. The advantages of mRNA vaccines compared to DNA vaccines

Firstly, according to the literature, its risk is lower than that of DNA vaccines. DNA vaccines can cause many unexpected side effects. Scientists have conducted experiments on experimental mice. Moreover, DNA vaccines may lead to insertional mutations through activating oncogenes. Lastly, DNA vaccines may cause chromosomal instability through setting off rearrangements or chromosomal breaks. In addition, it has a less complicated reaction process.

3. Synthesis of mRNA vaccines

3.1.1. Gene synthesis

The antigen sequence of the vaccine is designed and optimized based on the genome sequence of the pathogen, and then inserted into the plasmid DNA. By querying the gene sequences of MPXV and VACV in the NCBI database, three sets of candidate vaccine genes M1R-A35R, A29L-H3L-A35R and L1R-A33R were designed and synthesized.

3.2. Construction of recombinant plasmid

Let M1R-A35R, A29L-H3L-A35R, A35R-Fc and L1R-A33R genes were connected into the pGEM-3Zf-n3 vector through restriction endonucleases Pac Ⅰ and Cla Ⅰ. The ligation reaction includes T4 ligase, target gene and vector, and is carried out at 16°C for 16 hours [1].

3.3. Linearization of recombinant plasmids

The recombinant plasmid was digested with restriction endonuclease Xho Ⅰ to obtain a linearized recombinant plasmid, and the linearized product was recovered through a gel recovery experiment. The single enzyme digestion system includes Xho Ⅰ, recombinant plasmid, 10×CutSmart Buffer and water, and each plasmid is repeated three times [1].

3.4. In vitro transcription, purification, and capping

3.4.1. Structure of mRNA. Non-replicating mRNA vaccines encode immunogenic antigens and contain 5’ and 3’ untranslated regions (UTR), open reading frames (ORF) and poly(A) tails. The self-amplifying mRNA also includes additional ORFs encoding the viral replication machinery, allowing for continuous RNA amplification within the cell and enhanced antigen expression. In vitro transcription (IVT) transcribes linearized DNA plasmids into mRNA sequences [2].

3.4.2. Capping. There is a 7-methylguanosine (m7G) at the 5' end of the mRNA, followed by the triphosphate part of the first nucleotide (m7G), which is called the 5' cap. The 5′ cap protects RNA from exonuclease cleavage, regulates pre-mRNA splicing, and initiates mRNA translation and nuclear export. By introducing a variety of post-transcriptional modifications, the effectiveness and stability of mRNA can be improved. The 3′ end of IVT mRNA has a poly(A) tail, which is essential for determining the longevity of the mRNA [3, 4].

3.4.3. Purification. The IVT reaction mixture contains residual NTPs, enzymes, misformed mRNA, and DNA plasmid templates. Laboratory purification methods include DNase enzyme digestion to remove DNA and lithium chloride (LiCl) precipitation [5]. Inefficient purification techniques can reduce the translation efficiency of mRNA vaccines and increase unnecessary immune stimulation. For example, purification of modified mRNA by reversed-phase HPLC can significantly increase mRNA transfection and protein yield [6]. Chromatography is a commonly used purification process in the biopharmaceutical industry. SEC offers selectivity, scalability, versatility, and high purity, but it cannot remove impurities

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of the same size. Ion-pair reversed-phase chromatography (IEC) is an excellent mRNA vaccine purification technology that relies on the charge difference between target mRNA and impurities, with higher binding capacity and cost-effectiveness [7].

4. Delivery system

4.1. Development status of mRNA vaccine delivery systems

Because mRNA is different from DNA, it has no double helical structure or hydrogen bonds between base pairs. It is a single-stranded structure with many species, very active metabolism and a short half-life, and is decomposed a few minutes to a few hours after synthesis. Meanwhile, it is a hydrophilic macromolecular nucleic acid rich in anionic groups that is difficult to pass through the negatively charged lipid bilayer cell membrane. Therefore, a safe and efficient delivery system is the core and key technology for developing mRNA vaccines. Currently, non-viral vectors are mainly used for mRNA delivery. Non-viral vectors include protamine, liposomes, dendritic cells, inorganic nanoparticles, cationic cell-penetrating peptides, etc., but liposomes and their derivatives are most widely used, including liposomes complexes, cationic nanoemulsion (CNE), liposomal nanoparticles (LNP), etc.

Lipid delivery carriers were first used because the positively charged LP and mRNA (anionic groups, negatively charged) form multilayer vesicle complexes through simple electrostatic interactions. Compared with naked messenger RNA, messenger RNA wrapped in the delivery vector is not susceptible to degradation by nucleases, which allows messenger RNA to be transported through the cell membrane for the next step of translation. The preparation is simple, quick, reproducible and biodegradable; because cationic lipids are also positively charged in the human environment, with certain toxicity and relatively short half-life, they may combine with other non-specific negatively charged molecules in the body, reducing delivery efficiency.

CNE is composed of nanoemulsions combined with cationic lipids. Utilizing hydrophobic and hydrophilic surfactants that stabilize the oil core in the aqueous phase, nanoemulsions can generate particles through vigorous stirring, sonication, and microfluidics. The delivery effect is good and it is in the early research stage. This section will focus on the application value of LNP in monkeypox vaccine

4.2. LNP

Currently, the most popular and clinically used mRNA vaccine delivery system is LNP composed of four components: cationic or ionizable lipids, polyethylene glycol lipids (PEG), auxiliary phospholipids and cholesterol. This system has made rapid clinical progress due to its advantages such as easy preparation, self-assembly, good biocompatibility, high bioavailability and large loading capacity. The monkeypox virus mRNA vaccine uses this delivery system.

In LNPs, the head group of cationic lipids carries a permanent positive charge, which may lead to toxic side effects and low delivery efficiency. In contrast, the head group of ionizable lipids remains neutral or has a small positive charge under physiological conditions (pH 7.4), thereby reducing non-specific interactions and improving safety; while under acidic conditions (pH 7.4) $\leqslant$5.0), the head group is positively charged, and binds to the cell membrane phospholipid bilayer, destroying its stability, thereby achieving mRNA delivery.

Each of the four components in LNP has its own role:

1) Cationic lipids or ionizable lipids affect the speed and efficiency of mRNA entry into cells.

2) PEG lipids embedded in the outer surface of LNP structure prevent the aggregation or fusion of LNPs by steric hindrance of macromolecules, reduce the interaction of LNP and plasma proteins, and delay the clearance of LNP, and thus prolong their circulating half-life.

3) Auxiliary lipids provide support for the lipid bilayer structure, maintain the stability of the LNP structure, and the mRNA crosses the cell membrane into the cells.

4) Cholesterol is distributed in the gap of lipid particles, enhancing the mobility and structural stability of LNP.

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At present, there are relatively few targeted studies on LNP. Although prevention of messenger RNA vaccines, only effective delivery of cells and expression of sufficient amounts of antigen is sufficient for the application of therapeutic messenger RNA vaccines, LNP and other delivery technologies need to consider corresponding targeted solutions such as target tropical modification or promoting lesion drug accumulation, etc. In addition, the possible problem of enrichment in an organ caused by LNP also raises higher requirements, hoping to find better solutions in the future.chemical modification of mRNA, intracellular delivery of mRNA remains a major obstacle. Clinical translation of mRNA-based therapies requires delivery technologies that ensure stable mRNA delivery under physiological conditions [8].

Although liposomes are the most developed mRNA delivery carrier, and the success of the COVID-19 vaccine has increased enthusiasm for its research, poor storage stability, high immunogenicity, limited tracking and detection capabilities, and difficulty in detaching nucleic acid molecules also limit its use worldwide. large-scale applications within. In short, conventional vectors can no longer meet the growing demand for mRNA delivery [9].

4.3. A novel vector for mRNA delivery

Research has revealed that numerous biological molecules inherently exhibit characteristics conducive to mRNA delivery vehicles, thereby making them a popular choice among scientists for constructing vectors. The pursuit of drug safety necessitates that mRNA carriers exhibit excellent biocompatibility and minimal toxicity – attributes that biomacromolecules innately possess.

Among these biomolecules, cell-penetrating peptides and protamine, along with other proteins or peptides, have emerged as efficacious conveyors of mRNA. A key advantage of protein/peptide carriers lies in their capacity to meticulously regulate the constitution of each functional unit, i.e., amino acids, enabling the synthesis of peptides with diverse functionalities with ease and versatility. Furthermore, the monodispersity of protein peptide particles allows for precise regulation not only of their size but also the quantity of mRNA they encapsulate, contributing to a more controlled and efficient delivery system [10].

Aside from protein-based vectors, natural polysaccharides emerge as alternative carriers due to their inherent biological vitality, compatibility, and degradability as polymer materials. These compounds, characterized by their exceptional hydrophilic nature, reactivity, and amenability to chemical alterations, have piqued substantial interest among biomedical researchers for the development of nanoscale drug delivery systems. The presence of numerous functional groups (like hydroxyl, amine, and other chemically manipulable sites) and the complex branched architecture along the polysaccharide chains facilitate their bonding with smaller molecules or nucleic acids. This versatility has led to various polysaccharides, including starch, alginates, and chitosan, being explored for nucleic acid transportation, positioning them as promising candidates for messenger RNA (mRNA) delivery platforms [11].

5. Conclusion

At present, Chinese researchers have made great contributions to the research and development of mRNA monkeypox virus vaccine, and have also made a lot of results. China's mRNA vaccine research and development field has entered the forefront of the world. This scientific research on the development of monkeypox mRNA vaccine in China is only an exploration and experimentation on new exploration and developed field of orthopoxvirus-like mRNA vaccine. Under the support from national science and technology department for the development of emerging vaccine technology, the Chinese mRNA vaccine team has enhanced the level of technological research and development a lot, from the level of a follower to the level of a leader. The relevant research will not only do a good job in product and technical reserves to prevent the resurgence of smallpox and biological anti-terrorism, but also significantly improve China's independent innovation ability and international influence in the field of vaccine research and development. Meanwhile, mRNA vaccines also face many challenges: mRNA design with optimization, stability, delivery, protein translation, and so on. Despite researchers are facing these challenges, they continue to work hard and to address these issues; researchers believe that

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with the continuous advancement of technology, mRNA vaccines will better bring benefits to human-beings.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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