Research in mRNA vaccines and delivery carriers

Wenxi Xie

Xi'an Technological University, Xi'an, Shaanxi, China

Abstract. Nowadays, mRNA vaccine in medicine, biology, chemistry and other fields play a huge role, but after decades of research, mRNA vaccine still has not been widely used, this is because mRNA vaccine stability is poor, easy to serumRNA hydrolase degradation, or by monocytic-macrophage system and unable to reach the target cells play a role. Moreover, the mRNA has a relatively large molecular mass and a negative charge, making it difficult to penetrate the phospholipid bilayer and enter the cell membrane, resulting in the decrease of cell permeability. Therefore, it is necessary to design appropriate delivery systems to ensure the smooth entry of mRNA into the target cells and the effective release into the cytoplasm for translation. This paper reviews the current status and progress of mRNA vaccines, and introduces several different delivery systems, their main roles and examples studied examples, and prospects the infinite potential of AI development on mRNA drugs.

Keywords: MRNA vaccine, target cell, Delivery system

1. Introduction

1.1. mRNA vaccine research progress

In 1989, Scientists study that mRNA can be expressed and transfected by eukaryotic cells under lipid wrapping, The concept that mRNA can be used as a drug (Malone et al., 1989); In 1990, The mRNA completes the in vitro transcription, And was successfully expressed in mice, Confirmed the scientific feasibility of mRNA drugs (Wolff.et al.,1990); In 1995, After an mRNA tumor vaccine developed in a mouse model, Prove that it has great prospects in the direction of tumor (Conry et al., 1995); In the early 2000s, The investigators use modified uridine synthesis mRNA to effectively resolve its fluctuations in vivo, Easy to decompose conditions, And addresses the problem of the immunogenicity of mRNA vaccines (Liu et al., 2023). In 2023, the emergence of COVID-19 vaccine promoted the rapid development of mRNA vaccines. The mRNA vaccines have a significant role in treating various diseases (Wang et al.,2021).

1.2. Introduction to the role of the mRNA vaccine

The mRNA vaccine is to vaccinate the mRNA containing the encoding antigen protein into the body, and form the corresponding antigen protein after translation, so as to induce the body to produce immune response and achieve the effect of preventing immunity. Currently, there are two main types of mRNA used for drug research: non-replicating mRNA from a viral source and self-amplified mRNA. MRNA therapy based on in vitro transcription has the following advantages: few mRNA infections, no risk of gene mutation insertion, and biodegradation; mRNA protein control accuracy, precise shape and stable time, and mRNA in vitro, with the potential for rapid, cheap and large-scale production (Chen et al.,2025).

The in vitro transcribed mRNA is structurally similar to the naturally occurring mature mRNA with a single-stranded structure. As shown in **Figure 1**, the mRNA vaccine consists of five major components, the 5 ′ cap, the 5 ′ translation region, the open reading frame (improved protein expression, codon optimization), the 3 ′ untranslated region, and the polyadenylate tail. For efficient optimization of the mRNA, the chemical modification of various mRNA structures was explored (Fan et al., 2024).

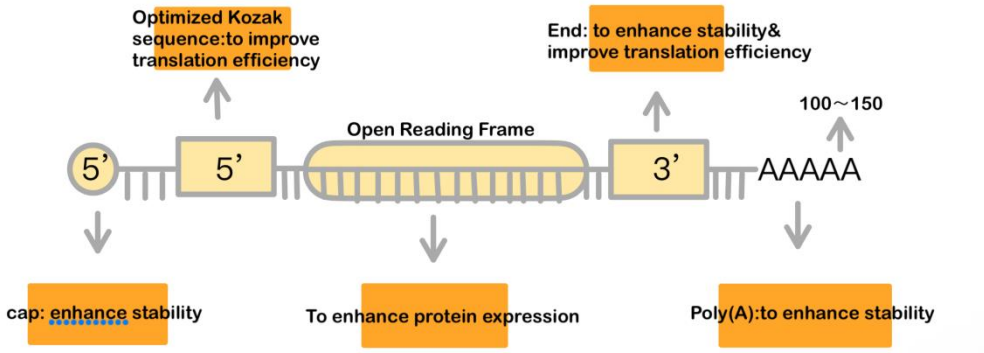

Figure 1. Basic structure and domain optimization strategy of mRNA

Alt Text for the figure: The five parts in the figure constitute mRNA vaccine: the head is used for enhancing stability, the untranslated region is mainly used for improving translation efficiency, the middle position is used for improving protein expression, and the polyadenylate tail at the end is mainly used in enhancing stability.

Effective delivery of mRNA in vivo is key to the vaccine effector, and exogenous mRNA must cross the lipid membrane barrier to reach the cytoplasm and convert into a functional protein. Currently, there are three main strategies for mRNA delivery: physical method, biological uptake mode based on viral vector, and non-viral vector based on lipid nanoparticles.

1.3. The advantages and challenges of mRNA vaccines

The mRNA vaccine has no infection and safe, There is no risk of infection or inserted genomic mutation; second, High levels of mRNA vaccine delivery, Wide coverage area, May deliver multiple antigens at a time, Very high efficiency, And for the tumor vaccines, Can override the relevant antigens such as TAA, And the characteristic antigen, TSA, To initiate an immune response in the host itself, To stimulate the generation of the antibodies, Enhanced anti-tumor efficacy; last, The mRNA vaccine can obtain high-yield mRNA by cell-free *in vitro* transcription, That is, once a mature mRNA vaccine is produced, You can stand on the platform to develop different mRNA vaccines (Liu et al.,2023).

Most of the mRNA vaccines today face production and safety challenges. In terms of production, the production and investment of plasmid are huge, with high requirements for production and fermentation process; the second is mRNA purification, large-scale production usually requires chromatography method, but different mRNA in the production process of different by-products, need to find the appropriate production process. In the process of mRNA production at home and abroad, in order to realize the internationalization of domestic vaccines, it is necessary further to strengthen the patent and development of domestic vaccines (Liu et al., 2023). In terms of safety, all kinds of new crown vaccine clinical trial safety research results show that the incidence of mRNA vaccine is significantly higher than inactivated vaccine, make vaccinated after vaccination, but in clinical trials did not appear serious or fatal more discomfort, so his safety remains to be studied, need longer safety test.

2. mRNA vaccine

2.1. As for the mRNA vaccine delivery vector

Due to the high infectious efficiency of the virus, its nucleic acid (DNA or RNA) can be delivered to the host cell and replicate. Early studies used the virus as a carrier for delivering nucleic acid. However, the adverse properties of some viral vectors, including potential carcinogenicity and plateau immunity, which can lead to serious clinical adverse events, have affected the research on the clinical application of viral vectors. The search for efficient, safe mRNA delivery systems is one of the hot topics for mRNA vaccines. Many types of cells accumulate in the melting media through the

spontaneous uptake of naked mRNA, but only a small part of mRNA can enter the cytoplasm and is prone to degradation by extracellular RNase, so appropriate strategies are needed to help transport mRNA to intracellular cells.

2.2. Lipid nanoparticles (LNP)

Lipid nanoparticles are the most well-studied and clinically advanced mRNA delivery carriers. The most widely used are cationic or ionizable lipid LNP, which usually contain cationic or ionizable lipids, cholesterol, helper lipids, and PEGylated lipids. mRNA LNP Vaccines trigger an immune response by transfection of antigen-presenting cells. After the mRNA vaccine is first endocytosed by antigen-presenting cells, escapes the connotation and enters the cytoplasm, the mRNA is translated into proteins by the ribosome, and the translated antigen protein can stimulate the immune system in various ways. The intracellular antigen is broken down into smaller fragments by the proteasome. These fragments are displayed on the cell surface by major histocompatibility composite class I molecules for presentation to cytotoxic T cells. Activated cytotoxic T cells kill infected cells by secreting perforin and granzymes, among others. Furthermore, antigen fragments can be presented to helper T cells via class MHCII molecules, and helper T cells promote the clearance of circulating pathogens by stimulating B cells to produce neutralizing antibodies. Most LNP formulations rely on cationic lipids, which is because the cationic lipids contain alkylated quaternary ammonium salt groups, which maintain their cationic properties even under the influence of pH changes, showing a stronger nucleic acid encapsulation capacity than neutral lipids. However, some studies have found that clinical trials of cationic lipids receive the limitation of cationic lipids with permanent positive charges because they can cause damage to cell membranes and adsorption of serum proteins (Gao et al,2018). However, until the emergence of ionization materials, the LNP delivery system has achieved a major breakthrough, retaining the effective transinfectivity based on the shortcomings of traditional cationic lipids in customer service. (Fenton et al., 2016). Its functions include: (1) helps to encapsulates negatively charged mRNA in the preparation of LNP; (2) positively charged lipids can interact with endosome membrane to release mRNA into cells; (3) reduce non-specific interaction with serum protein, reduce systemic toxicity and improve stability during physiological pH of 6-7. (McKinlay et al., 2018).

The common lipids that ionize cations include: 1,2- dimethoxy- N, N- dimethyl-3-aminopropane (DLin-DMA) (Heyes et al.,2005), 3- dioxylame-4-aminoethane (DLin-KC 2-DMA) (Semple.2010) and 4- (N, N-dimethylamino) butyric acid (dioleyl) methyl ester (DLin-MC3-DMA) (Dong.2019). Using DLin-MC3-DMA as an excipient (Semple et al.,2010), DLin-MC3-DMA. Ionizable lipid materials have been widely used for siRNA / LNP manufacturing. Kacuffman et al. (Kauffman et al.,2015) used the previously used siRNA / LNP formulation as a starting point to select the C12-200 LNP formulation capable of efficient mRNA delivery by changing the proportion and structure of lipids in the LNP. Based on the ionizable lipids of diketone piperazine, cKK-E12 has been shown to selectively deliver siRNA to liver parenchymal cells in nonhuman primates and is by far the most efficient siRNA non-viral delivery system for gene silencing in hepatocytes. Drawing on the experience of successful delivery of siRNA by cKK-E12, IVT mRNA / cKK-E12 LNP was injected in mice and found that the nanoparticles mainly accumulated in the liver 6h after injection, confirming the ability of cKK-E12 to deliver mRNA *in vivo*.

Currently, The LNP-based mRNA vaccine developed by the Moderna Company is also undergoing clinical trials. The mRNA-4157 (NCT03897881) is now clinically available as a tumor vaccine for the treatment of high-risk melanoma. The mRNA-5671 (NCT03948763) is a KRAS vaccine used for the treatment of pancreatic cancer, colorectal cancer, and Non-small cell lung cancer, which has been performed to clinical stage I (Du et al.,2024).

2.3. Pectin-modified lipid nanoparticles

Lipid nanoparticle delivery systems are one of the most promising drug carriers, with good biocompatibility, immunity, phytopicity and high drug loading efficiency. However, unmodified lipid

nanoparticles still have problems of poor stability, easy hydrolysis and easy rapid removal. To overcome the above shortcomings, the researchers modified lipid nanoparticles with polysaccharides, as a class of natural polymer, showing good biocompatibility after modifying lipid nanoparticles, but also have the advantages of targeting target organs and low toxicity. Polysaccharide-modified lipid nanoparticles have the potential to play an important role in clinical treatment (Ma et al., 2024). Hu Qingjuan et al. found that chitosan-modified liposomes can significantly increase the amount of liposome-enclosing drugs into cells, thus improving the bioavailability of oral drugs. Polysaccharide-modified liposomes have adjuvant activity, which can stimulate the body's immune response and enhance the immune response. Compared with conventional adjuvants, polysaccharide-modified LNP has better safety, can slowly release antigens or immune enhancers at the injection site, and only show mild inflammation (Hu et al., 2019). Gao et al. used the polysaccharide-modified LNP for the delivery of Newcastle disease vaccine and found it to have a good adjuvant effect (Gao et al., 2012). Bo et al. found that polysaccharide-modified LNP can upregulate related stimulatory molecules and activate immature mouse dendritic cells to induce their maturation (Bo.et al.,2019).

2.4. Exosomes

Exosomes are extracellular discal vesicles measuring from 40 to 100 nm in diameter. With a nanoscale size and a membrane structure similar to the cell membrane, mRNA delivery as a natural carrier is considered one of the most promising delivery tools (Li.et al., 2024). Lipid nanoparticles (Lipid nanoparticles, LNPs) have been approved by the FDA for mRNA delivery and preparation of anti-SARS-CoV-2 mRNA vaccine. LNPs were found to be dose-dependent and caused cytotoxicity, while exosomes did not cause any adverse effects. Moreover, as exogenous substances, LNPs may have side effects on the body, instead, exosomes are nanoscale vesicles secreted by cells and do not have side effects on the body. Thus, exosomes are more suitable for mRNA delivery in vivo compared to LNPs. Exosomes are highly biocompatible, less cytotoxic and immunogenic, and small exosomes can cross barriers difficult to cross by conventional delivery carriers. For example, there are four main types of anti-SARS-CoV-2 mRNA vaccines, depending on the source of the exosomes. On the basis of RBD protein vaccine, Professor Cheng team loaded mRNA into human LSCs-Exo against SARS-CoV-2 mRNA vaccine (Popowski et al., 2022); plant-derived exosomes have gastrointestinal immunity and low immunogenicity, often used to deliver small molecule drugs; Pomatto prepared the mRNA vaccine against SARS-CoV-2 by delivering mRNA encoding S protein, S1 protein and N protein (Pomatto et al., 2022). Milk-derived exosomes can tolerate the strong acidic environment of the gastrointestinal tract and can be used for oral delivery of drugs. Zhang et al. prepared an oral vaccine against SARS-CoV-2 mRNA by loading mRNA encoding RBD into milk exosomes (Zhang et al.,2023).

Vaccination is undoubtedly the best way to prevent the SARS-CoV-2 virus, which has a very high transmission rate. As of April 26,2023,382 vaccines are under development worldwide, including inactivated vaccines, protein vaccines and mRNA vaccines. As of April 16,2023, the global 13321463740 doses of vaccine, but vaccine production and vaccination rate has not met the global demand, the existing vaccine delivery adjuvant also has some defects, need further research to solve exosomes belongs to cell secretion of nanoscale vesicles, can be used to deliver nucleic acid, protein, with high biocompatibility, low immunogenicity and stability. As early as 2007, Kuate used the exotract system to prepare a new vaccine against SARS-CoV infection, and found that the vaccine could induce high levels of neutralizing antibodies to enhance immunity. Therefore, researchers often develop vaccines based on exosome delivery of SARS-CoV-2-related antigens, which are divided into RBD protein vaccine and mRNA vaccine (Pesce et al.,2021).

2.5. Lipid / Polymer hybridized Nanoparticles (LPHNs)

LPHNs are nanostructures composed of a lipid shell and a polymer inner core, with an infectious efficiency of up to 80% to mRNA. LPHNs' "ride" mRNA vaccines can cross the cellular barrier and achieve efficient in vivo release and protein translation, including being stable, durable, and

biodegradable. Moreover, LPHNs have the advantages of both polymer nanoparticles and liposomes, and they are widely used to contain drugs of different properties (hydrophilicity, hydrophobic, amphiphilic) for the diagnosis and treatment of diseases. LPHNPs As a drug delivery carrier, it can be carried separately, as a dual vehicle, or as a modified active targeted delivery.

In single drugs, LPHNPs is widely used to deliver tumor chemical drug molecules to cancer cells. The results of Chu and Zhang showed that the uptake of LPHNP in both cervical and prostate cancer cells was higher than that of unhybrid nanoparticles (Chu et al.,2009). Liu prepared LP HNPS containing paclitaxel (PTX) with PLGA and diuryl phosphatidylcholine (DLPC). In vitro release study showed that drug 7d can release the cumulative release of unhybrid nanoparticles at the same time was only 30%, and the in vitro cytotoxicity experiment showed that the cytotoxicity of loaded LPHNPS to MCF-7 human breast cancer cells was 6~7 times that of drug solution, thus effectively reducing half inhibitory concentration (IC5o) (Liu et al., 2010).

With the development of biomolecules and immune experimental technology, biological therapy, such as immunotherapy and gene therapy, has gradually developed into the fourth type of cancer treatment after the three methods of radiotherapy, chemotherapy, and surgery, which is often used as an adjuvant therapy combined with the three conventional methods. Chemotherapy is a systemic treatment method. Traditional chemotherapy drugs have problems such as multidrug resistance (MDR), low selectivity and low accumulation concentration in tumor area, and toxicity and side effects on normal tissue cells. Therefore, continuous innovative treatments need to be developed to improve the problem. It has attracted much attention due to its unique core and shell structure and its properties of programmed release drugs. This system can wrap one drug in the polymer core by different preparation methods, and the phospholipid layer packs the nanoparticle nucleus in the lipid shell, controlling the release rate of two independent drugs while enhancing the efficacy (Mu et al., 2018).

2.6. Others

The combination of inorganic materials and polymers enables the efficient compression and delivery of mRNA. Gold nanoparticles (gold nanoparticles, AuNP) are one of the most commonly used inorganic nanoparticles due to their easy synthesis and modification and low cytotoxicity, showing their potential for clinical application as a drug delivery platform. Functionalization of AuNP; can further expand its applicability, such as designing dendrimers PAMAM grafted AuNPs, the nanocomplexes shows good nucleic acid protection and tolerance. Au-[PDL-g-PEG]NP was functionalized using PEG (poly-D-lysine-graft-polyethylene glycolPDL-S-PEG); with the ability to preferentially localize preferentially to the tumor mitochondria

The polymer-lipid hybrid nanoplatform has the advantages of both liposomes and polymer carriers. It has been shown that serum stability may limit the effectiveness of systemic administration of PAES, and Kaczmarek developed PAES-PEG-lipid nanoparticles with improved serum stability, capable of delivering mRNA to mouse lung cells (Jing et al.,2022).

3. Conclusion

After the emergence of the novel Coronavirus vaccine, mRNA vaccines and lipid nanoparticle delivery systems have made significant progress, and mRNA vaccines have played a crucial role in the prevention and treatment of various diseases. LNP delivery system can protect mRNA from degradation by nucleic acids, enhance its half-life, play a more stable and longer time in the host, stimulate the immune system to protect the body, greatly improve the effectiveness of mRNA drugs, and solve the challenges of mRNA clinical experiments to a certain extent. But there are still many problems that we need to solve. For example, marketed mRNA vaccines have also exposed the current limitations of mRNA drugs, that is, since the cold chain storage and transportation, how to transport mRNA vaccines around the world or with the help of transportation to understand the progress of mRNA vaccine technology in other countries is challenging. If in the future, lyophilized powder

preservation technology can be used to store and transport mRNA vaccines to improve the accessibility of the vaccine. At the same time, mRNA vaccines and their vectors also have potential toxicity and immune response, and further chemical modification of mRNA or the development of better delivery platforms to improve their stability will remain the focus of future research. In addition, intestinal and digestive cancer, which is the most common disease in Chinese people, can also be combined with mRNA vaccines in the future. Nowadays, artificial intelligence is developing rapidly and can be combined and applied manually. The intelligent algorithm optimizes the mRNA sequence to produce more stable, safe, and effective mRNA vaccines.

References

[1] Malone, R.W et al.,1989. Cationic liposome-mediated RNA. Proc Natl Acad Sci USA86, (16):6077-6081.

[2] Wolff, J.A et al., 1990. Direct gene transfer into mouse muscle. Science247(4949):1465-1468.

[3] Conry, R.M et al., 1995. A carcinoembryonic antigen polynucleotide vaccine for human clinical use. Cancer Gene Ther, 2(1):33-38.

[4] Liu, C.W et al.,2023. mRNA Overview of vaccine technology and industrial development. China Food and Drug Administration, (12): 74-84.

[5] Wang Y et al., 2021. mRNA vaccine: A potential therapeutic strategy. Mol Cancer20(1):33.

[6] Chen, H.M et al., 2025.mRNAVaccine-moleculamechanisms of molecular design, presentation, and immune activation. Chinese Journal of Zoonosis, 1-7.

[7] Fan, Y.C et al.,2024 Progress on mRNA vaccines and lipid nanoparticle delivery carriers. Scientific Bulletin, (33):4813-4823.

[8] Du, X.L et al.,2024. Development and application of lipid nanoparticles in cancer therapy. Pharmaceutical Research, (11): 1116-1124 + 1140.

[9] Ma, Y.Q et al.,.2024. Advances in the drug delivery system for polysaccharide-modified lipid nanoparticles. Journal of Bioengineering, (12): 4339-4350.

[10] Hu, Q.J et al., 2019.Inhibitory effects and primary mechanism of polysaccharide from Portulaca oleracea L. on growth of HepG2 cells. Food Research and Development. 40(3): 38-44 (in Chinese).

[11] Gao, H et al., 2012.Optimization on preparation condition of epimedium polysaccharide liposome and evaluation of its adjuvant activity. International Journal of Biological Macromolecules, 50(1): 207-213.

[12] Bo, R.N et al., 2019. Mechanism of lycium barbarum polysaccharides liposomes on activating murine dendritic cells. Carbohydrate Polymers, 205: 540-549.

[13] Li, Z. W et al., 2024.mRNAProgress in the vaccine delivery system.Animal Husbandry and Veterinary Medicine of China, (11): 4932-4942.

[14] Popowski, K.D et al., 2022. Inhalable dry powder mRNA vaccines based on extracellular vesicles. 5(9):2960-2974.

[15] Pomatto, M. A. C et al., 2023. Plant - derived extracellular vesicles as a delivery platform for RNA - based vaccine. feasibility study of an oral and intranasal SARS-CoV-2 vaccine. Pharmaceutics, 15 (3).

[16] Zhang, Q et al., 2023. Intraduodenal delivery of exosome-loaded SARS-CoV-2 RBD mRNA induces a neutralizing antibody response in mice. Vaccines (Basel) 11 (3).

[17] Pesce, E et al., 2021. Exosomes recovered from the plasma of COVID-19 patients expose SARS-CoV-2 spike-derived fragments and contribute to the adaptive immune response. Front Immunol 12:785941.

[18] Chu, C.H et al., 2011. UltrafinePEG-coatedply. Nanotechnology, 22(18):185601.

[19] Liu, Y et al., 2010.Nanoparticles of lipid monolayer shell and biodegradable polymer core for controled release of pacli. Int J pharmaceyt, 395(1):243-250.

[20] Gao, X.P et al., 2018. DNA vaccines: mechanisms of action. Chinese Journal of Tissue Engineering Research, 22(8):1281-1286.

[21] McKinlay, C.J et al., 2018. Enhanced mRNA delivery into lymphocyts enabled by lipid-varied libraries of charge-altering releasable transporters. Proceedings of the Natiional Academy of Science of United States of American.115(26): E5859-E5866.

[22] Heyes, J et al., 2005.Cationic lipid saturation influences intracellular delivery of encapsulated nucleic acids. Journal of Controlled Release, 107(2):276-287.

[23] Semple, S.C et al., 2010.Rational design of cationic lipids for siRNA delivery. Nature Biotechnology, 28(2):172-176.

[24] Dong, Y. Z et al., 2019.chemistry in siRNA delivery systems. Advanced Drug Delivery Reviews, 144:133-147.

[25] Fenton, O. S et al., 2016.Bopinspire dalkenyl amino alcohol ionizable lipidterials for highly potene in vivo mRNA delivery. Advanced Materials, 28(15):2939-2943.

[26] Mu, J et al.,2018. Progress of lipid polymer hybrid nanoparticles. Chinese Journal of Hospital Pharmacy, (08): 891-896.

[27] Jing, G.T et al., 2022. Nonviral vector delivery system for mRNA vaccines. Chinese Journal of Bioengineering, (09), 58-66.