The Principle and Derivation of Mrna Vaccine

Chenxi Wang *

Nanjing Zhonghua High School, Nanjing, Jiangsu, China

* Corresponding Author Email: peimingxi1346@gmail.com

Abstract. Research has focused on mRNA vaccine in recent years. The outbreak and spread of covid-19 have also spurred research on mRNA vaccines. The 2023 Nobel Prize in Physiology or Medicine went to Drew Weisman and Kariko Katalin in honor of their holding of US patents relating to the application of immunogenic-free nucleoside-modified RNA. When compared to conventional vaccinations, mRNA vaccines offer additional benefits, it is not only effective against infectious diseases, but also against some non-infectious diseases such as cancer. mRNA vaccines also have drawbacks, such as side effects from vaccination. This review will start with the characteristics of various traditional vaccines, and then introduce mRNA vaccines. 'Next, both the manufacture process and reactions after the administration of mRNA vaccine are discussed – Externally produced RNA is injected into the body, endocytosed by cells, and converted into immunostimulating proteins by ribosomes, then the body will identify and destroy the corresponding pathogen or cancer cells. By taking two diseases and corresponding mRNA vaccines as examples, the advantages and disadvantages of mRNA vacciniation are presented, like quick effect and side effects such as thrombosis, thrombocytopenia and so on. Finally, I will talk about the preparation methods of mRNA vaccines that are different from traditional vaccines.

Keywords: vaccines, mRNA vaccines, mRNA applications, BNT162b2, cancer vaccine.

1. Introduction

A vaccination is a biological product that provides active acquired immunity to defend against a particular infectious or malignant disease, which can help the host prevent or cure the occurrence of sickness by achieving long-term immune response.

There are numerous kinds of vaccinations: Living viruses that have been killed or attenuated, which means they have been changed or weakened so they can't cause illness; Inactivated toxins (for bacterial infections when the sickness is caused by the toxins produced by the bacteria rather than the bacteria themselves); or vaccinations containing only fragments of the pathogen (subunit and conjugate vaccines fall under this category) [2].

Different development strategies are needed for each type of vaccination. Here is the brief introduction to several traditional vaccines.

1.1.Live, Attenuated Vaccines

Attenuated vaccines are vaccines that reduce the virulence of pathogens but still maintain their activity. The resulting vaccine virus will not reproduce to the point where it may infect a human, but it will nonetheless elicit an immune response that can guard against infection in the future [2].

1.2.Killed or Inactivated Vaccines

The vaccine made by inactivating the pathogenic microorganism and retaining the whole microbe [2].

1.3.Toxoids

an inactivated toxin whose other characteristics, usually immunogenicity, are preserved but whose toxicity has been reduced by heat treatment or chemical means (formalin) [2].

1.4.Toxoids

Subunit and conjugate vaccines only include fragments of the pathogens they are intended to prevent [2].

1.5. mRNA Vaccines

Messenger RNA (mRNA) is a big, single-stranded molecule that mimics the genetic sequence of DNA in the nucleus of cells. It acts as a template for cells to synthesize proteins. Fig.1 shows central dogma, which shows the different structures of DNA and RNA.

mRNA vaccine is based on mRNA preparation, belongs to nucleic acid vaccine, is the third generation of new vaccines.

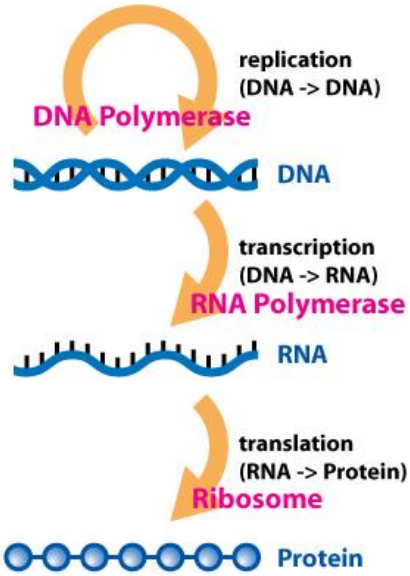

Fig. 1 Structure diagram of central dogma [1].

In this review, The principle of mRNA vaccines will be introduced, besides, an analyze on the advantages of mRNA vaccines will also be conducted.

2. Mechanism of mRNA Vaccine

The mRNA sequence of the target protein is synthesized in vitro. Antigen-presenting cells endocytose the injected mRNA vaccine in a few different ways. Then, mRNA is translated by ribosomes into proteins that stimulate the immune system (figure 2). Firstly, the protein is secreted and captured by receptor located on the outside of B cell, activating the production of specific antibody, which could recruit phagocytic macrophage to target the pathogens and perform clearance. Except for the activation of immune response by the intact protein, the translated intracellular antigens can also be broken down by proteasome complexes into smaller fragments, displaying by MHC-I molecule to CD8+T cells (cytotoxic T cells) or CD4+ T cells (helper T cells). Activated CD8+T cells kill infected cells by secreting cytolytic molecules. CD4+T cells can stimulate B cells like complete mRNA translated protein [3].

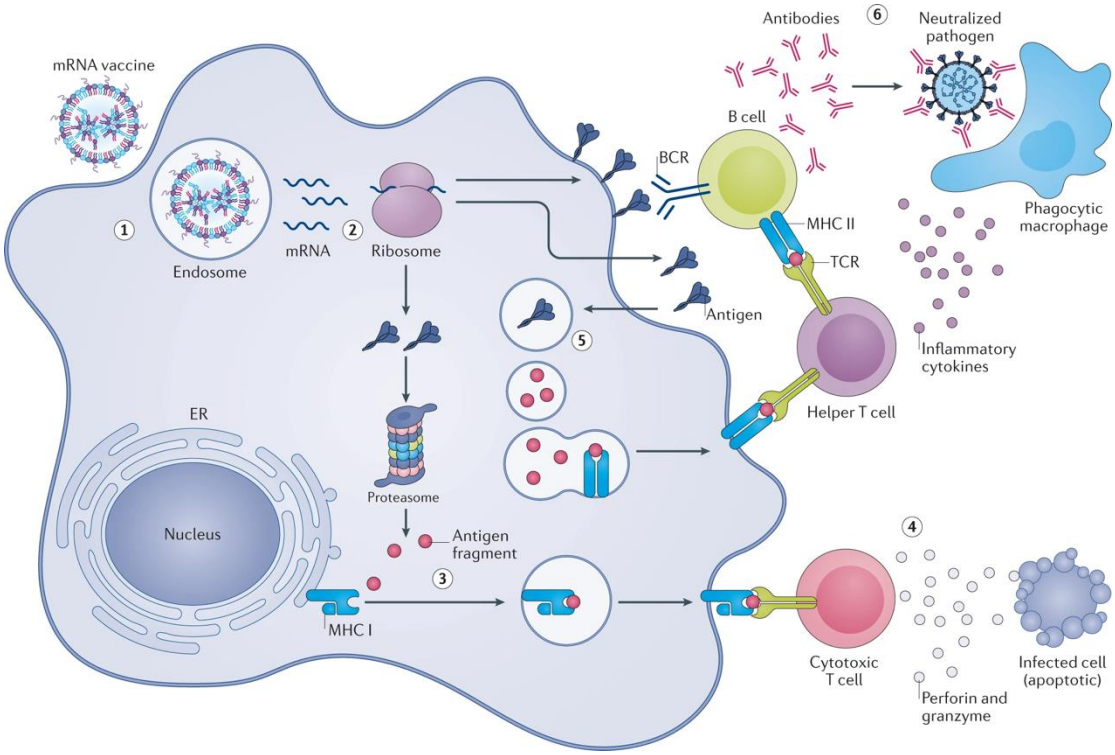

Fig. 2 Messenger RNA vaccines elicit immunity through transfection of antigen-presenting cells [3].

3. Application and Characteristic

mRNA vaccines currently in research or in clinical use can be divided into non-replicating mRNA vaccines, self-expanding mRNA vaccines and circular RNA vaccines according to their genetic characteristics. Here are some examples of mRNA vaccines.

3.1. COVID‐19 mRNA vaccine

3.1.1 The profile of COVID‐19 and SARS-CoV-2

The infectious disease known as coronavirus disease 2019 (COVID-19) is caused by the coronavirus SARS-CoV-2. Public health emergency status was given to COVID-19 by the World Health Organization (WHO).

Although the majority of patients have mild illness, some may suffer shock, kidney failure, liver failure, arrhythmias, respiratory failure, or cardiovascular damage. These symptoms are especially common in patients with additional underlying disorders[4].

3.1.2 The profile of the vaccine

Approved by the US government as the first COVID-19 vaccine, BNT162b2 is produced by Pfizer-Bi-Ontech. In order to control the COVID-19 pandemic, using it to battle SARS-CoV-2 infection has proven to be essential[5].

The vaccine known as BNT162b2 consists of a lipid nanopreview (LNP) encoding the full-length spike protein with two proline modifications (in positions 986 and 987) in the S2 subunit to maintain the protein in the prefusion shape. The mRNA-1273 vaccine is similar in that it consists of a lipid nanopreview capsule consisting of four lipids. The whole spike glycoprotein of SARS-CoV-2, including proline alterations in the S2 subunit and an undamaged furin cleavage site, is encoded by an mRNA found inside the capsule. These vaccinations have been approved for up to three doses, and in susceptible individuals, up to four doses. [5]

ALC-0159 and DSPC make up the lipid nanopreview (LNP) of BNT162b2, and they are crucial to the development of a stable lipid-bilayer nanopreview. Cholesterol provides structural support for

the LNP. Lastly, the key element in the delivery of mRNA into the cell is ALC-0315. Moreover, sugar is added to the vaccine to prevent freezing and salt buffers to maintain pH balance. Similarly, polyethylene glycol (PEG) 2000 DMG and DSPC stabilize mRNA-1273 LNP by forming a lipid bilayer that is physically supported by cholesterol. Unlike BNT162b2 LNP, it carries lipid SM-102 to aid in the release of mRNA into the cell. This immunization is supplemented with sucrose, which serves as a cryoprotectant, and salt buffers to preserve pH balance[5].

3.1.3 Advantages of the vaccine

The advantages of the BNT162b2 vaccine are obvious. Its effect is rapid, and this is due to different reasons. For instance, the stimulation of T-cell responses, the generation of high titers of neutralizing antibodies, and a track record of effectiveness across a variety of demographic categories, including the elderly and other susceptible populations[5].

3.1.4 Adverse reactions to the vaccination

Although the BNT162b2 vaccine was authorized and no significant side effects were reported in clinical testing, but after millions of doses of the vaccine have been given, side effects are also happening[5].

According to the survey, With the mRNA vaccines under study, Cardiovascular problems, including myocarditis, thrombocytopenia, and thrombosis, commonly occur[6].

3.2.Tumor/cancer mRNA vaccine

3.2.1 The profile of cancer

One of the greatest threats to human health and cancer is the leading cause of death worldwide. People have been looking for effective ways to treat cancer, which has a high incidence and high mortality [7].

3.2.2 The profile of the cancer vaccine

Researchers have been working to create messenger RNA (mRNA) vaccines for therapeutic purposes, particularly those that can be used for anti-tumor therapy, ever since mRNA vaccines proved to be helpful in preventing COVID-19.

By administering mRNA encoding tumor-associated or tumor-specific antigens, tumor mRNA vaccines either initiate or strengthen an efficient anti-tumor immune response. When compared to conventional treatments, mRNA cancer vaccines have reduced side effects, increased efficacy, and enhanced specificity, making them a promising new strategy in cancer immunotherapy[7].

3.2.3 Advantages of the cancer vaccine

The mRNA cancer vaccine's distinct mode of action has made it possible to provide benefits over traditional cancer treatments including radiation, chemotherapy, and surgery.

powerful immunogenicity of the mRNA cancer vaccine is one of its main advantages. It fosters a robust humoral immune response as well as a cell-mediated immune response, both of which have a powerful anti-tumor effect. Because the mRNA cancer vaccine has the ability to trigger a systemic immune response, it has shown promise in treating metastatic tumors that are resistant to surgical resection. In addition to the previously mentioned benefits, mRNA cancer vaccines have the ability to create and preserve long-term immune memory, which may help prevent tumor recurrence. In preclinical cancer models, a number of mRNA cancer vaccines have demonstrated strong therapeutic efficacy for both primary tumors and metastases[7].

3.2.4 Adverse reactions to the vaccination

Among the frequent side effects are headaches, myalgia, pruritus, fever, nausea, diarrhea, rashes, and dyspnea.

Although they are less frequent, serious adverse events can include pulmonary embolism, immune system problems, and psychological issues.

Although uncommon, different degrees of toxicity have occasionally been noted. Additional side effects from vaccines and their adjuvants include elevated liver enzymes, hyponatremia, anemia, colitis, and elevated creatinine levels[8].

4. Production of mRNA vaccines

The fact that mRNA is comparatively easy to produce is one of its main advantages over conventional vaccinations. To generate mRNA products with desired quality qualities, a set of manufacturing procedures have to be followed[9].

The production process can be divided into two parts: the unit operations needed to purify the mRNA product and the enzymatic mRNA creation that makes up the upstream processing[9].

Three enzymatic stages comprise the upstream process: mRNA transcription, DNA template digestion, and plasmid linearization. Important steps in developing the upstream process were choosing easily obtainable raw materials and figuring out how to adjust the process parameters to produce the desired yield and quality profile of the medicinal ingredient. To get the finest in vitro transcription yields, you must start with good quality template DNA. A minimum of 70% supercoiled ratio, a single band of linearized DNA, and a correctly lengthened poly(A) tail confirmed by sequencing are among the essential quality criteria that must be present before usage[10].

The downstream platform process's de novo design included buffer selection for purification, resin selection, and precipitation mitigation[10].

Following the previously described downstream and upstream platform technique, the mRNA bulk drug material can be supplied for early phase clinical research and encapsulated into LNP[10].

At this stage, the sample comprises protein, brief abortive transcripts from abortive cycling during initiation, oligodeoxynucleotides, and the necessary mRNA transcript inside a complex combination of various nucleotides. These contaminants can be removed from the sample by combining extraction and precipitation techniques[5].

5. Conclusion

Research and use of vaccines have been going on for hundreds of years, but mRNA vaccines have only emerged in the last few years. Although it is a new vaccine, it is very promising because it has many advantages that traditional vaccines do not have - safer, easier to mass produce, and can target different types of diseases such as cancer, which are advantages that traditional vaccines do not have. Clinical trials of mRNA vaccines during the COVID-19 pandemic in recent years have also demonstrated that, because to their distinct advantages, mRNA vaccines may eventually supplant conventional vaccinations. [11]

Although mRNA vaccines have many advantages, they also have shortcomings that need to be improved, for example, excessive inoculation dose may lead to excessive immunity, and mRNA is easily degraded by enzymes.

In view of these drawbacks, corresponding solutions are needed:

To solve the problem of excessive immunity, Self-amplifying RNAs(saRNA) can be used. Self-amplifying RNAs have been shown to express antigens more strongly at lower concentrations than conventional mRNA, indicating that this technique may enhance immunity[12].

Additionally, we can optimize the structural features of mRNA, specifically the 5'cap, 5' and 3'UTRs, the coding region, and the poly(A) tail, to increase the stability of mRNA's control of immune responses. This will result in higher translational efficiency, which will ensure an adequate immune response [13].

Lipid Nanopreviews (LNPs) are intelligent nano-sized lipid-based carriers that can effectively transport mRNA into the cytosol. During systemic circulation, LNPs can also shield the mRNA from RNAase[14].

mRNA vaccines offer excellent scientific value and considerable potential for the prevention or treatment of additional diseases due to their ability to elicit a wide spectrum of host immune responses. In order to break through limitations such as excessive immunity, mRNA vaccines will improve on these limitations in future development.

References

[1] https://en.wikipedia.org/wiki/Central_dogma_of_molecular_biology。

[2] https://historyofvaccines.org/vaccines-101/what-do-vaccines-do/different-types-vaccines。

[3] Chaudhary, N., Weissman, D. & Whitehead, K.A. mRNA vaccines for infectious diseases: principles, delivery and clinical translation. *Nat Rev Drug Discov* **20**, 817–838 (2021).

[4] Alimohamadi Y, Sepandi M, Taghdir M, Hosamirudsari H. Determine the most common clinical symptoms in COVID-19 patients: a systematic review and meta-analysis. J Prev Med Hyg. 2020 Oct 6;61(3): E304-E312. doi: 10.15167/2421-4248/jpmh2020.61.3.1530. PMID: 33150219; PMCID: PMC7595075.

[5] Echaide M, Chocarro de Erauso L, Bocanegra A, Blanco E, Kochan G, Escors D. mRNA Vaccines against SARS-CoV-2: Advantages and Caveats. Int J Mol Sci. 2023 Mar 21;24(6):5944. doi: 10.3390/ijms24065944. PMID: 36983017; PMCID: PMC10051235.

[6] Yasmin F, Najeeb H, Naeem U, et al. Adverse events following COVID‐19 mRNA vaccines: a systematic review of cardiovascular complication, thrombosis, and thrombocytopenia. *Immun Inflamm Dis*. 2023;11: e807. 10.1002/iid3.807

[7] Wang B, Pei J, Xu S, Liu J, Yu J. Recent advances in mRNA cancer vaccines: meeting challenges and embracing opportunities. Front Immunol. 2023 Sep 6; 14:1246682. doi: 10.3389/fimmu.2023.1246682. PMID: 37744371; PMCID: PMC10511650.

[8] Kaczmarek M, Poznańska J, Fechner F, Michalska N, Paszkowska S, Napierała A, Mackiewicz A. Cancer Vaccine Therapeutics: Limitations and Effectiveness-A Literature Review. Cells. 2023 Aug 28;12(17):2159. doi: 10.3390/cells12172159. PMID: 37681891; PMCID: PMC10486481.

[9] Sara Sousa Rosa, Duarte M.F. Prazeres, Ana M. Azevedo, Marco P.C. Marques, mRNA vaccines manufacturing: Challenges and bottlenecks, Vaccine, Volume 39, Issue 16, 2021

[10] Whitley J, Zwolinski C, Denis C, Maughan M, Hayles L, Clarke D, Snare M, Liao H, Chiou S, Marmura T, Zoeller H, Hudson B, Peart J, Johnson M, Karlsson A, Wang Y, Nagle C, Harris C, Tonkin D, Fraser S, Capiz L, Zeno CL, Meli Y, Martik D, Ozaki DA, Caparoni A, Dickens JE, Weissman D, Saunders KO, Haynes BF, Sempowski GD, Denny TN, Johnson MR. Development of mRNA manufacturing for vaccines and therapeutics: mRNA platform requirements and development of a scalable production process to support early phase clinical trials. Transl Res. 2022 Apr; 242:38-55. doi: 10.1016/j.trsl.2021.11.009. Epub 2021 Dec 4. PMID: 34871810; PMCID: PMC8641981.

[11] Gote V, Bolla PK, Kommineni N, Butreddy A, Nukala PK, Palakurthi SS, Khan W. A Comprehensive Review of mRNA Vaccines. Int J Mol Sci. 2023 Jan 31;24(3):2700. doi: 10.3390/ijms24032700. PMID: 36769023; PMCID: PMC9917162.

[12] Bloom, K., van den Berg, F. & Arbuthnot, P. Self-amplifying RNA vaccines for infectious diseases. *Gene Ther* **28**, 117–129 (2021). https://doi.org/10.1038/s41434-020-00204-y

[13] Kim SC, Sekhon SS, Shin WR, Ahn G, Cho BK, Ahn JY, Kim YH. Modifications of mRNA vaccine structural elements for improving mRNA stability and translation efficiency. Mol Cell Toxicol. 2022;18(1):1-8. doi: 10.1007/s13273-021-00171-4. Epub 2021 Sep 20. PMID: 34567201; PMCID: PMC8450916.

[14] Ramachandran S, Satapathy SR, Dutta T. Delivery Strategies for mRNA Vaccines. Pharmaceut Med. 2022 Feb;36(1):11-20. doi: 10.1007/s40290-021-00417-5. Epub 2022 Jan 30. PMID: 35094366; PMCID: PMC8801198.