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Progress and challenges of mRNA vaccines

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Abstract

Messenger RNA (mRNA) vaccines are third-generation nucleic acid vaccines developed after first-generation inactivated and live-attenuated vaccines and second-generation subunit and viral vector vaccines, characterized by a rapid response to pathogen mutation, simple production process, and high production capacity. The basic mechanism through which mRNA vaccines provide immune protection is the introduction into the body of mRNA expressing a target antigen through a specific delivery system and expression of the corresponding protein in vivo, which stimulates a specific immunological response. Multiple mRNA vaccine platforms against infectious diseases and cancers have shown encouraging results in both animal models and human subjects; in particular, mRNA vaccines against COVID-19 have been widely adopted around the world. However, the development of mRNA vaccines has not been straightforward. The rapid progress of mRNA vaccines would not have been possible without major recent advances in innate immune sensing and in vivo delivery strategies. Creative research in mRNA design, lipid/polymer/novel nanocarrier development, and coupling to wearable/implantable electrostimulation medical devices may drive the evolution of mRNA vaccines.

KEYWORDS
cancer vaccines, delivery vector, mRNA, sequence optimization

Messenger RNA (mRNA)-based vaccines are a recently emerging breakthrough technology.[1] The basic principle of mRNA vaccines involves the transfer of mRNA into the body via specific delivery systems, such as lipid nanoparticles (LNPs), which express antigenic proteins in the body and stimulate a specific immune response in the organism.[2,3] Multinational pharmaceutical companies have conducted several clinical trials of mRNA-based vaccines for the prevention of infectious diseases and oncology treatments.[4–6] Especially, mRNA vaccines against novel coronavirus infections were widely adopted during the COVID-19 pandemic. Clinical data showed that

COVID-19 mRNA vaccines offer protection rates of up to 95% (Pfizer/BioNTech & Moderna)[7,8] and also have high production capacity and a rapid development cycle. Overall, mRNA vaccine technology has significant benefits in terms of controlling outbreaks of infectious diseases.[9–11]

In addition to SARS-CoV-2, mRNA vaccines also have great potential for the prevention and treatment of other diseases.[12] For example, mRNA cancer vaccines have shown promising results in multiple clinical trials against a variety of aggressive solid tumors. One widely accepted mechanism by which mRNA vaccines are thought to disrupt tumor development is by boosting the patient's immune response by encoding tumor-associated antigens (TAAs) (Figure 1A). Following vaccination, mRNA vaccines efficiently express tumor antigens in antigen-presenting cells (APCs), thereby promoting APC activation and innate/adaptive immune stimulation, and subsequently inducing strong $CD8^+$ and $CD4^+$ T cell responses (Figure 1B).[13] In addition to the traditional universal vaccination approach, mRNA vaccines can also be used in cell therapy as personalized cancer treatment; that is, transfection of extracted and purified patient-derived cells with mRNA vaccines in vitro and transfusion of the treated cells back into the patient. The production of personalized vaccines represents a therapeutic application of mRNA vaccines against tumors. In addition to being used directly to vaccinate patients, mRNA can also be used in cell therapy, transfecting patient-derived cells in vitro and infusing the treated cells back into the patient. Using the above strategy, patient-derived dendritic cells (DCs) can be transfected with mRNA encoding TAA, which allows the presentation of TAA-derived peptides on DCs and activation of antigen-specific T cells in vivo. This technique

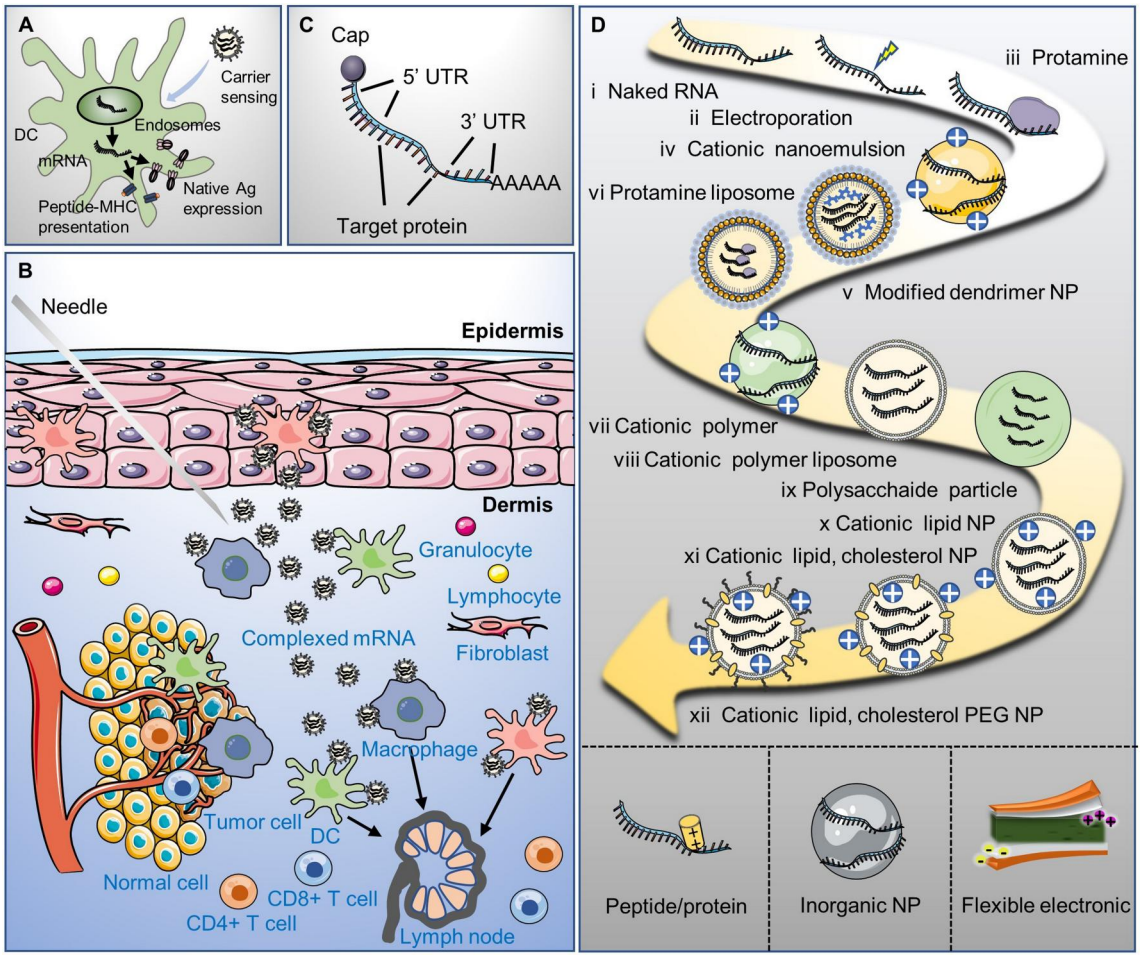

FIGURE 1 Mechanism and types of mRNA vaccines. (A) Innate immune sensing of mRNA cancer vaccines; (B) Mechanism of direct injection of mRNA vaccine effectiveness; After direct injection of mRNA vaccine, antigen expression is expressed on antigen-presenting cells (APC), dendritic cells (DC) are stimulated to mature and migrate, and the transmitted information stimulates lymph nodes to produce $CD8^+$ T cells and $CD4^+$ T cells, thereby killing tumor cells. (C) Universal sequence information for mRNA vaccines; (D) Major delivery methods for mRNA vaccines.

also allows mRNA encoding a chimeric antigen receptor to be transfected with patient-derived T cells, enabling engineered T cells to directly recognize specific antigens expressed on tumors.[14–16]

Sequence optimization and delivery vector construction are two key elements of the mRNA vaccine technology platform. Due to its high degree of programmability, in principle an mRNA vaccine can only contain the nucleic acid sequence information encoding the protein. However, after the mRNA enters the body, it is easily degraded by a variety of nucleases, triggering a strong innate immune response. Therefore, it is necessary to modify the bases of mRNA transcribed in vitro, to reduce its immunogenicity and improve its stability in vivo. Based on bioinformatics methods, optimizing the sequence and secondary structure of mRNA can improve its translation efficiency and protein expression, thereby enhancing the effects of vaccines.[17] The 5 and 3′ UTR elements flanking the coding sequence profoundly influence the structure and function of mRNA.[18] The 5′ cap structure is required for efficient protein production by mRNA, and the poly(A) tail plays an important regulatory role in mRNA translation and stability (Figure 1C). Replacing rare codons with common synonymous codons of homologous tRNAs, which are abundant in the cytoplasm, may increase protein production from mRNAs. Enrichment of G:C content has also been shown to increase steady-state mRNA levels in vitro and protein expression in vivo.[5,19] In addition, the concatenation of sequences is also the development trend of mRNA vaccines. Gao et al. developed methods to design chimeric receptor structure domain (RBD) dimers protein vaccines adaptable to SARS-CoV-2 variants. As two different RBD sources in series form, chimeric RBD dimers are more likely to stimulate broad-spectrum antibody response in animals compared to homologous RBD dimers, thus providing better protection. We have added this section to the third paragraph.[20] The above improvements to mRNA vaccines could increase protein translation, modulate innate and adaptive immunogenicity, and improve delivery efficiency.

The selection of a suitable vector for effective delivery of mRNA vaccines in vivo is also critical for highly efficient therapy. Exogenous mRNA must penetrate the lipid membrane to reach the cytoplasm before it can be successfully translated into protein. Although naked mRNA has been used successfully for in vivo immunization, previous studies have shown that intratumoral injection of mRNA mixtures encoding four cytokines (IL-12sc, GM-CSF, IFN-α, and IL-15 sushi) can generate a potent antitumor immune response leading to tumor regression;[21] there are still many limitations to the application of naked mRNA. For example, it is only suitable for intratumoral injection, which has partial effects. Multiple approaches have been used to deliver mRNA vaccines. The most widely accepted strategy is the use of self-assembled nanoparticles based on amphiphilic molecules, such as lipids and polymers, as delivery vehicles. A number of vectors for delivering RNA have been developed, including protamine, positively charged oil-in-water cationic nanoemulsions, chemically modified dendrimers compounded with polyethylene glycol (PEG)-lipid, protamine-complex in PEG-LNPs, cationic polymers, cationic polymers complexed with lipid components, polysaccharide granules or gels, cationic LNPs, cationic lipids complexed with cholesterol, and cationic lipids complexed with cholesterol and PEG-lipid (Figure 1D).[5] The COVID-19 mRNA vaccines currently administered in most parts of Europe and North America use liposomal vectors based on cationic lipids complexed with cholesterol and PEG-lipid, and have achieved significant prophylactic results. As fundamental components of biological systems, peptides and proteins are natural carriers for the delivery of nucleic acids. Peptide/protein carriers form nanoparticles with mRNA through the electrostatic interaction of enriched positively charged lysine and arginine residues with negatively charged nucleic acids.[22] Moreover, virus-like particles have also been used to deliver nucleic acid vaccines with remarkable results.[23] In addition to organic-based carriers, inorganic nanocarriers have been attracting increasing interest in recent years. Inorganic nanoparticles have highly stable structures, uniform and tunable particle scales, and readily modifiable surface chemistry. For example, Au nanoparticles can be used for genetic immunity based on in vitro DC transfection through electrostatic adsorption of nucleic acids.[24] Nanoparticles of another inorganic material, mesoporous silica, have biodegradability and unique large porosity, which allows the modification of more active sites to carry nucleic acids effectively.[25] Although inorganic materials have shown great potential in basic research on mRNA vaccines, more clinical data are required to eliminate concerns about nanotoxicity. In addition to chemical carriers, physical stimulation is another strategy to increase mRNA uptake efficiency in vivo. Electroporation has been used to increase the uptake of therapeutic RNA, but conventional electrical stimulation devices have limitations. For example, overloaded currents can lead to massive cell death, and it is also difficult to apply electrical stimulation precisely to the target cells or tissues. With the rapid development of portable powered devices and flexible electronics, a range of

wearable/implantable devices based on wireless transmission, fuel cells, and nanogenerators have recently been shown to output biologically safe electrical currents and successfully modulate cell and tissue functions, and thus have promise for the expansion of mRNA vaccine delivery systems.

There has been a rapid explosion in both basic and clinical research on mRNA vaccines. Over the past 3 years alone, dozens of preclinical and clinical reports have demonstrated the efficacy of mRNA therapeutic platforms. Although preclinical studies are encouraging regarding the prospects and advantages of mRNA-based vaccines, many challenges remain that cannot be ignored. Studies have shown that people vaccinated with AstraZeneca, Johnson & Johnson, Pfizer, or Moderna mRNA COVID-19 vaccines are at risk of venous thrombosis that even though the side effects are not common, the risk is significantly higher than the general population.[26] This side effect may be attributed to the different responses of the immune system to vaccine-induced spike proteins inside the blood vessels. Exposure of spike proteins to platelet factor 4 (PF4) induces the human immune system to synthesize PF4 antibodies, resulting in a rare autoimmune thrombocytopenia mediated by platelet-activating antibodies.[27,28] It follows that mRNA cancer vaccines may also encounter unpredictable problems and risks. For example, while much of the early work on mRNA vaccines has focused on cancer therapy, difficulties in antigen prediction and poor immunogenicity have limited clinical application. Further, major obstacles to the development of effective anticancer vaccines are the identification and efficient delivery of highly immunogenic tumor-specific antigens. Tumor antigens vary greatly between individuals, with some antigens being less immunogenic and even evading recognition by the host immune system. Even if the antigen is immunogenic, the inhibitory immune microenvironment can prevent efficient T cell infiltration and lead to T cell exhaustion. Finally, therapeutic vaccines for chronic diseases, such as cancer, require multiple higher doses than for preventive vaccines, resulting in more stringent safety standards for mRNA and its vector.[15] The above problems suggest that we should be more cautious about clinical trials of mRNA cancer vaccines. Fortunately, transnational pharmaceutical companies have recently announced that they will launch mRNA tumor vaccines as planned; the staged R&D progress in the industry has undoubtedly encouraged academic research on mRNA vaccines. In conclusion, mRNA is a powerful and versatile vaccine platform that promises to play an active role in defeating chronic and infectious diseases despite many challenges to overcome.

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CONFLICT OF INTEREST

The authors declare no competing interests.

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