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Technologies and Challenges for MRNA: Current Situation Analysis and Outlook

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Abstract:

The development of mRNA technology in the field of medicine is receiving increasing attention, with its potential applications far exceeding traditional vaccines and protein expression systems. However, to achieve widespread application of mRNA technology, there are still many challenges to overcome. Firstly, the instability and immunogenicity of mRNA limit its stable delivery and effective expression in vivo. Secondly, large-scale production and purification techniques still need further optimization to meet market demands. Additionally, the long-term safety and efficacy of mRNA therapy also require more clinical validation and research. To overcome these challenges, researchers are continuously exploring new technologies and methods. For example, by modifying mRNA sequences and constructing carriers, the stability and immunotolerance of mRNA can be improved. At the same time, developing efficient delivery systems and production processes is also a key focus of current research. In conclusion, despite facing many challenges, the enormous potential and prospects of mRNA technology are highly anticipated. Through ongoing research and innovation, it is believed that mRNA technology will bring more groundbreaking advancements to the field of medicine.

Keywords: Overview of mRNA drugs, the definition of mRNA, applications of mRNA, the conclusion of mRNA

1. Introduction

1.1 Classification of mRNA

what mRNA molecules can be classified into
non-replicating mRNA
self-replicating RNA
cyclic RNA according to their characteristics.

Non-replicating mRNA is a complete mRNA encoding antigenic proteins transcribed in vitro, and its structure contains a 5′-cap structure, 5′and 3′untranslated regions (UTR), 3′-polyA and ORF regions. 5′UTR is mainly involved in the translation of ORF sequences downstream of the mRNA, and the 5′ end contains a 7-methyl guanosine cap structure (5′-cap, m7G), which prevents the mRNA from being degraded by exonucleases. mRNA from being degraded by exonuclease; while the function of 3′UTR is to maintain the stability of mRNA; the 3′ end contains poly (A) tail structure, which can protect mRNA from being degraded, enhance the stability of mRNA, and improve the translation efficiency. Non-replicating mRNA has a simple structure, short RNA sequence, and does not encode other proteins. Most of the mRNA vaccines that have been marketed or are undergoing clinical trials are non-replicating. However, non-replicating mRNA vaccines also have some drawbacks, such as short half-life in vivo and unsuitable for application scenarios such as protein replacement.

In addition to the structure of non-replicating mRNAs, self-replicating RNAs encode four additional viral non-structural proteins (nsP1-4) and a subgenomic promoter (which is derived from the genome of alphaviruses). nsP1-4 encodes a replicase, which is responsible for self-replication of RNAs in the cell. Upon entering the cytoplasm, the self-replicating RNA binds to host cell ribosomes and produces RNA replicase ((RDRP). RDRP formation is a complex, multi-stage process, with each of the nsPs having multiple functions, e.g., nsP2 serves as an RNA deconjugating enzyme and a protease for multiprotein processing; nsP3 mediates a variety of viral interactions with host proteins; and nsP4 is an RNA dependent RNA polymerase. RDRP replicase first synthesis complementary antisense strand RNA using the positive-strand RNA as a template, and then synthesis and replicates

multiple positive-strand RNAs using the antisense strand RNA as a template, a process that results in higher and sustained levels of antigenic expression of self-replicating RNAs relative to non-replicating mRNAs, and one of the reasons that self-replicating RNA vaccines only require a lower dose of RNA. . It has been shown that self-replicating RNA can extend the antigen expression cycle to about half a month [36]. Self-replicating RNAs have many advantages and also face some difficulties to overcome; excessive inflammatory response, non-structural proteins produced by alphavirus replicons that may interfere with normal signal transduction in host cells, and longer sequences and complex structures that raise the difficulty of process development. Cyclic RNAs are a class of single-stranded, closed-loop RNA molecules that can be prepared by cyclisation of mRNA precursors by alternative splicing; and by enzymatic reactions. Since cyclic RNA does not contain 5′-cap and 3′-poly(A) and lacks free ends, it is less susceptible to degradation by nucleic acid exonucleases, is more stable than linear RNA [37], and is favourable for storage and transport. Cyclic RNA lacks the basic elements for cap-dependent translation, and initiates translation by adding IRES elements to the 5′UTR region, so the translation efficiency is low; in addition, in industrial applications, one of the major preparation challenges for cyclic RNA is the purification step, and high-purity, large-scale preparation is particularly difficult.

1.2 Mechanism of action

mRNA drug in vivo role can be roughly divided into six steps: the first step, the injected mRNA drug is endocytosed by the antigen-presenting cells; the second step, the mRNA enters the cytoplasm, translated by the ribosome into proteins, translated antigenic proteins through a variety of pathways to activate the immune system; the third step, the intracellular antigens are broken down by the proteasome complex into smaller fragments, the fragments through the major histocompatibility complex (MHC) class I molecules to cytotoxic T cells; in the fourth step, activated cytotoxic T cells kill infected cells by secreting cytokines such as perforin, granzyme, etc.; in addition, secreted antigens can be ingested by the cells, degraded in the nucleus endosomes, and delivered to helper T cells through MHC class II molecules; finally, helper T cells stimulate B cells to produce antibodies and activate phagocytes to clear pathogens through inflammatory cytokines. activate phagocytosis to clear pathogens.

2. What is mRNA

RNA is an important ribonucleic acid molecule, which plays an important role in transmitting genetic information in living organisms. mRNA is the full name of “messenger RNA”, which is mainly responsible for transcribing the genetic information in DNA into the information required for protein synthesis. mRNA molecules translate this information into proteins through ribosomes in the cell. Inside the cell, mRNA molecules translate this information into proteins via ribosomes, thus enabling the expression of genetic information. mRNA has a relatively simple structure, which is mainly composed of nucleotides, including adenine (A), cytosine (C), guanine (G) and uracil (U). These nucleotides are arranged in chains to form the molecular structure of mRNA. At each end of the mRNA, there are 5’ and 3’ ends, which indicate the start and termination ends of the mRNA molecule, respectively. In addition, mRNA contains a number of non-coding and coding regions. The non-coding regions mainly include promoters and terminators, while the coding regions contain specific information required for protein synthesis.
Overall, mRNA, as an important ribonucleic acid molecule, its definition and structure are important for understanding the transmission and expression of genetic information in living organisms. Through the study of mRNA, one can gain a deeper understanding of the genetic mechanisms in organisms and provide an important theoretical basis for the development and progress of the life science field.

3. RNA drug delivery strategies in vivo

3.1 Chemical Modification

RNA is a macromolecule composed of phosphate backbone, ribose and bases (Fig. 3a), chemical modification of its parts can increase its enzyme stability, strengthen the binding of nucleic acids and proteins, and weaken the recognition of the immune system. The phosphate backbone of RNA (Fig. 3a) can be degraded rapidly by nuclease, and this is the main limiting factor for the development of its medicinal use. The first generation of RNA modification strategies focused on the modification of the phosphate backbone, i.e., replacing the non-bridging oxygen in the phosphate backbone with a partial group.

3.2 Coupling ligands

RNA drugs can be covalently coupled to lipids, galactose, proteins/peptides or aptamers to prolong the circulation time in vivo and to increase the accumulation and uptake in specific tissues and cells. The use of specific coupling ligands not only modulates the binding of RNA drugs to plasma proteins and improves the tissue distribution of the drugs, but also enables delivery to specific tissues or cells by targeting the ligands to cell surface receptors.

Meanwhile, by designing the linkage between the ligand and the RNA drug, such as adopting acid-sensitive amide bonds, disulfide bonds or Dicer-sensitive bonds that can be easily broken by reduction in the cytoplasm, etc.[32] , the RNA can be separated from the coupler after entering into a specific physiological environment, and effectively adapted to a specific therapeutic mechanism.

3.3 Nanodelivery carriers

In addition to the above strategies, researchers have also designed and developed various delivery systems to carry nucleic acids into target tissues and cells. The delivery systems can be classified into viral and non-viral vectors. Viral vectors have high efficiency of nucleic acid delivery but obvious limitations [56], such as: 1 some viral loaded nucleic acids can only be integrated into dividing cells; 2 limited nucleic acid loading capacity (<10 kb); 3 high immunogenicity and toxicity; and 4 wild-type or helper viruses may be generated with the risk of teratogenicity and mutagenesis. As viral vectors have been extensively reviewed, this article will focus on lipid nanoparticles (LNP), polymeric nanoparticles, exosomes and other non-viral nanocarriers [introducing the chemical composition and physical properties of the relevant nanodelivery vectors, as well as their in vivo RNA delivery mechanisms and characteristics].

3.4 Other delivery strategies

In addition to the already introduced delivery strategies, there are many other options for RNA delivery, such as spherical nucleic acid (SNA) particles that can penetrate various biological barriers, DNA nanostructures with flexible and precise structural design, porous nanomaterials such as metal- organic frameworks (MOFs), and electrostatic nanomaterials. The structural morphology of these nanostructures (Fig. 5d~g) and the RNA loading modes of these nanoparticles are different, and each of them has its own distinct advantages, which have attracted a lot of attention.

4. Use in vaccines

4.1 Vaccines for infectious diseases

The use of mRNA vaccines to cope with infectious diseases generally by encoding pathogen-specific structural proteins, which are used to prevent pathogenic infections. For example, type II severe acute respiratory coronavirus SARS-CoV2, respiratory syncytial virus (RSV), herpes zoster virus (VZV), cytomegalovirus (CMV), influenza virus (Flu), HIV, Zika virus, dengue virus, and monkeypox virus, etc [64]. The expression of defective or modified oncoproteins by mRNAs to modulate the tumour immune microenvironment for cancer immunotherapy has also attracted much attention. Currently, more attention is focused on the use of mRNAs as therapeutic vaccines to stimulate the training of the immune system to kill cancer cells by encoding tumour-associated antigens (TAA) and tumour-specific antigens (TSA) and to facilitate the recognition and attack of tumour cells by the immune system, thus providing new opportunities for mRNA products. new opportunities for mRNA products. Unlike protein vaccines, mRNAs can encode entire antigenic proteins and are able to deliver multiple epitopes without being restricted to specific HLA epitopes in peptide vaccines [66]. In addition, multiple antigens can be expressed simultaneously using multiple mRNA strands or in the same mRNA strand, inducing a broader immune response against tumour types capable of producing multiple neoantigens.

4.2 Worldwide marketed mRNA products

BNT162b2 (Comirnaty®), co-developed by Pfizer and BioNTech, received EUA in the US on 11 December 2020 and a Biologics licence Application (BLA) in the US on 31 August 2021, and is the first globally marketed mRNA neocollagenesis vaccine. moderna's mRNA-1273 (Spikevax®), which received EUA in the U.S. on 18 December 2020 and BLA in the U.S. on 1 February 2022, is the second mRNA neocollagenesis vaccine to be marketed globally. Shiyao's SYS6006 New Crown mRNA vaccine, which received EUA in China on 22 March 2023, is the first of its kind in China. The mRNA new crown vaccine AWcorna, co-developed by Suzhou Aibo and Watson Biologicals, was granted an EUA by the Indonesian State Food and Drug Administration on 29 September 2022, the first overseas EUA granted by China. mRNA new crown vaccine of Sweeper was granted an EUA by Laos on 8 December 2022, the second overseas EUA granted by China.

4.3 Differences in the regulation of the production of mRNA vaccines for the prevention of infectious diseases in China and abroad

Generally, for a drug to be marketed and distributed, the manufacturing site must pass the GMP compliance inspection [10]. At the same time, the investment in GMP production plants for mRNA drugs is huge, and the operation and maintenance costs are very high. However, there are differences in the management of preventive vaccine production among countries: China implements a strict entry system for vaccine production and strictly controls the establishment of new vaccine production enterprises. In addition to meeting the conditions for the establishment of vaccine production enterprises, a preventive biological product manufacturer applying for a drug production

licence should also comply with the relevant policies of the national vaccine industry authorities. The holder should have its own vaccine production capacity, and commissioned production is generally not allowed [11,12]. Therefore, in mainland China, if research institutes want to become holders of preventive mRNA vaccines (e.g., Xin Guan mRNA vaccine), they must build their own GMP production lines, and have the ability to produce and inspect the vaccine for release; however, as long as the vaccine is not a preventive mRNA vaccine, it is not subject to this restriction, and it can be entrusted to be produced. However, as long as the vaccine is not a preventive mRNA vaccine, it is not subject to this restriction and can be commissioned for production. Against this background, Chinese and foreign mRNA pharmaceutical companies have adopted completely different strategies in the layout of GMP production bases for preventive mRNA vaccines.

5. Advantages and disadvantages

5.1 Disadvantages:

Although mRNA drugs or vaccines have made significant progress in the prevention and treatment of tumours, autoimmune diseases and respiratory disorders, research is still at an early stage, and a number of difficulties hamper the development of mRNA therapies. For example, LNPs are susceptible to oxidation and degradation, making it difficult to achieve large-scale industrial production, and the lipid component of the LNP delivery system can cause inflammation and allergic reactions in mice [16]. Virus-like particles, biomimetic nanoparticles, polymer nanoparticles, and inorganic nanoparticles are also not approved by the FDA for clinical use for various reasons. The ideal delivery system should have high transfection efficiency, adequate safety, protection of mRNA from rapid degradation and targeted delivery, which are far from being achieved.

5.2 Advantages

First, mRNA drugs can be customised and adapted to suit different diseases and individual characteristics. This personalised approach provides patients with more effective treatment options. Second, mRNA drugs have a shorter production cycle and can respond faster to new diseases and viruses. In addition, mRNA drugs can stimulate the body's own immune system and improve treatment efficacy. Overall, mRNA drugs have great potential in therapy and will revolutionise the medical field.

6. Other fields of application

mRNA drugs are a new type of biotechnological drugs with a wide range of application prospects. In the field of cosmetics, mRNA drugs can be used to improve skin texture, promote cell regeneration and repair damaged tissues. By applying mRNA drugs in cosmetics, the efficacy and absorption rate of the products can be improved, thus bringing better beauty effects to users.

The use of mRNA drugs in cosmetics requires rigorous research and testing to ensure their safety and efficacy. At the same time, relevant regulations and regulatory policies need to be continuously improved to protect consumers' rights and health. In the future, with the advancement of technology and in-depth research, the application of mRNA drugs in cosmetics will be further promoted and developed.

Overall, the use of mRNA drugs in cosmetics brings new opportunities and challenges to the beauty industry. Through continuous exploration and innovation, we can make better use of this technology to provide consumers with safer and more effective beauty products.

7. Outlook and Summary

As a next-generation disruptive technology in the field of biomedicine, mRNA vaccines have been ranked at the top of the "Top 10 Breakthrough Technologies in the World" list by MIT Technology Review in 2021, and their innovations have been described as the "Third Wave of Pharmaceuticals" after small-molecule drugs and antibody drugs, with the potential to prevent and treat many diseases. As a next-generation disruptive technology in the field of biopharmaceuticals, its innovative drugs have been called the "third wave of pharmaceuticals" after small molecule drugs and antibody drugs, and have the potential to prevent and treat a variety of diseases. China's "14th Five-Year Plan" for the development of the pharmaceutical industry clearly states that, in the R&D and industrialisation of new vaccines, the establishment of technical platforms such as mRNA vaccines, new adjuvants for vaccines, and new delivery systems should be supported, and the development and industrialisation of related products should be promoted. mRNA technology, as a breakthrough technological platform, is expected to partially replace traditional drugs and vaccines, and open up new opportunities for the development and industrialisation of new drugs and vaccines. As a breakthrough technology platform, mRNA technology is expected to partially replace traditional drugs and vaccines, open up new therapeutic fields and bring about new therapeutic changes. At present, preventive mRNA vaccines have been validated, and good efficacy has been achieved in the field of tumour immunotherapy, but the joint efforts of scientists in various fields are still needed. It is believed that, with more and more

mRNA drugs undergoing in-depth research, mRNA drugs will show strong vitality in human health.

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