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RNA-based vaccines – types, strategies of delivery and overview of RNA vaccines

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Abstract

The discovery of mRNA by Sydney Brenner dates back to 1961, but the in vivo expression of mRNA was successful only in 1990, which initiated the development of vaccines based on this molecule. During Sars-CoV-2 pandemy the interest in the use of nucleic acids in the production of drugs and vaccines has increased significantly. The success of mRNA vaccines against Sars-CoV-2 has particularly empowered the pharmaceutical industry to create newer and newer generation products based on RNA modification that could help not only in Covid-2019, but also in the prevention and treatment of other infectious diseases. RNA has a very high potential - it can be used in highly personalized therapies, furthermore the production of mRNA is cheaper, faster than the current therapeutics and the process of mRNA making is more flexible due to the great ease of producing mRNA in the process of transcription. Modifying of the structure of ribonucleic acid and the methods of its delivery leads to the creation of newer and newer vaccines. In this review, we present the potential of RNA molecule in producing vaccines, types of RNA vaccines, strategies of RNA delivery and review of existing RNA-based vaccines.

Key words: RNA; mRNA; vaccines; NRM; SAM

Introduction

Although the discovery of mRNA by the geneticist and molecular biologist Sydney Brenner dates back to 1961, the first evidence of the possibility of creating an mRNA vaccine was the successful in vivo mRNA expression in 1990, when an attempt was made to inject in vitro transcribed mRNA into the body of mice (visible expression in mouse skeletal muscle myocytes). Already then, research on the use of mRNA in vaccines began. Particular attention in the research was devoted to the structure of the messenger RNA - the instability of the molecule and high immunogenicity became a major limitation. The effectiveness of mRNA-based preparations was optimized thanks to modifications of the mRNA structure - it was noticed that the expression activity and half-life could be regulated as a result of modification of the mRNA sequence and delivery system. Although a long time has passed since 1991, the use of in vitro transcription in production is still a process that facilitates the production of mRNA vaccines. Once an antigen has been identified from a pathogen of interest, the corresponding gene is sequenced, synthesized and cloned to create a DNA messenger plasmid (pDNA) that is transcribed in vitro into mRNA and can be delivered to the patient [1]. The process itself is time-saving and economical, which makes it highly effective. Additionally, in vivo mRNA expression protects against contamination of the sample with protein and virus particles. The undeniable advantage of mRNA vaccines over DNA vaccines is the avoidance of the transcription process taking place in the cell nucleus, mRNA takes part only in the translation taking place in the cytoplasm of the cell, where one or more proteins are expressed depending on how many immunogens are encoded by the delivered transcript (antigen expression is directly proportional to the number of conventional mRNA transcripts successfully delivered during vaccination) [2,3]. In vitro, this matrix will be prescribed for an mRNA vaccine that can be delivered to an individual.

Moreover, the different structure of the mRNA and its absence in the structure of the CpG islets means that, compared to DNA-based vaccines, the risk of integrating the molecules with the host genome and immunological rejection are much less likely in the case of mRNA. Messenger RNA has self-adjuvanting properties, thanks to which a strong and long-lasting immune response is activated by tumor necrosis factor TNF-alpha, interferon-alpha and cytokines - this state was achieved thanks to the introduction of a modified nucleotide (pseudouracil) into the mRNA molecule, thanks to which there was a partial latency of mRNA molecules against the host's immune system (otherwise, toll-like receptors (TLRs) would be stimulated, which would trigger interferon-1 production and translation blockade). For comparison, for polypeptide and protein vaccines to be similarly effective, it would be necessary to use additional adjuvants (hence the great advantage of mRNA vaccines). Moreover, mRNA expression allows for the generation of complex proteins, which would be very difficult or even impossible to obtain using other techniques[4,5]. There are two types of mRNA constructs on the basis of which mRNA vaccines can be divided - NRM (non-replicating mRNA, conventional) and SAM (self-replicating; saRNA; which encodes replicase components capable of controlling intracellular mRNA amplification) - both have the same cap structure, regions 5 'and 3' untranslated (UTR), open reading frame (ORF) and 3 'poli(A) tail (the sequence of these regions determines the stability of the molecules), but SAM is further distinguished by the presence of replicase [5,6,7]. SAM is a molecule much larger than the mRNA itself, almost 5 times larger, so the delivery of saRNA is more difficult. The size of SAM is due to the encoding, in addition to the protein of interest (which replaces the viral structural protein, making the vaccine incapable of producing infectious viral particles) and the subgenomic promoter, of four additional nonstructural proteins encoding the replicase. After delivery to the cytoplasm of the cell, non-structural SAM molecules form RNA-dependent RNA polymerase (RDRP), which replicates genomic and subgenomic RNA - antigen expression is higher than in non-replicating mRNA. Therefore, a great advantage of SAM is the reduction of the required RNA dose [6,7]. The characteristics of NRM are simplicity and a small particle size, which greatly facilitates the transmission of molecules, while the disadvantages are low stability and activity, which in turn can be increased by optimizing the structural elements of RNA [7].

A new type of RNA vaccine are vaccines using trans-amplifying RNA (taRNA) based on a double vector system - one encoding a vaccine antigen derived from the saRNA from which the replicase has been removed (as a result of deletion of the sequence coding for replication, a transreplicon is generated), while the other one provides replicase activity (standard saRNA or non-replicating RNA no.). This type of vaccine is still influenced by scientific research, and the safety, versatility and manufacturing method seem to be particularly promising in terms of the method of developing taRNA vaccines [8].

Delivery strategies of RNA vaccines

There are several delivery strategies for mRNA vaccines - naked mRNA, viral vectors, polymer-based vectors, lipid-based vectors, peptide-based vectors, and hybrid lipid-polymer nanoparticles. The naked RNA delivery system is the simplest - transmission takes place by intramuscular injection, but the plasma half-life of such a molecule is short, and the unprotected mRNA, in turn, is susceptible to enzymatic degradation by ribonuclease or RNAse. Viral vectors use genetically modified viruses and their replication ability and ease of expression in the cytoplasm of cells, however, the disadvantage of such a delivery system is the difficulty of integrating the genetic material into the host's genetic material, possible rejection, cytotoxicity and immunogenicity. Polymer-based vectors employing diethylaminoethyl dextran (DEAE) are sometimes used - now being superseded by more efficient lipid vectors. Biopolymer nanoparticles (e.g. PLGA) have tolerable toxicity and efficient transfection, but the disadvantage is the higher molecular weight that hinders delivery, as is the case with block copolymers (e.g. polyethylene glycol methacrylate, dimethylaminoethyl methacrylate (DEAEMA), DEAEMA-co-n-butyl methacrylate) [9,10].

Among the vectors, the most effective and the most clinically advanced are lipid nanoparticles (LPNs), which may contain molecules that further facilitate and guide the uptake by APC cells.[10] LPN consists of ionized lipid, phospholipid, cholesterol and PEGylated lipid. The vaccines against Sars-CoV2 - Pfizer/BioNTech and Moderna, which were popular during the pandemic, have such a synthetic vector [6].

RNA-vaccines overview

Covid-19 vaccines are aimed at introducing the viral S protein into the body (Spike surface protein, a type I transmembrane glycoprotein involved in receptor binding and membrane fusion through interaction with ACE2 receptors on the host cell surface) - in the case of mRNA vaccines this is due to uptake of mRNA by translational cells, resulting in the spike antigen protein of the coronavirus Spike - which triggers the host's immune response and, in effect, the production of antibodies and memory cells that will recognize and respond to the pathogen in the event of an actual Sars-CoV-2 infection. Messenger RNA is synthetically produced, which does not require a time-consuming process of cell culture to produce the virus or desired protein, as is the case with other types of vaccines. Both the mRNA and the LPN vector are synthetically produced, therefore the very process of creating mRNA vaccines is fast and effective [5,6].

The S protein is composed of two subunits, S1 and S2. The S1 subunit contains an N-terminal domain (NTD) and a C-terminal domain (CTD) - the C-terminal domain (CTD) has the receptor binding domain (RBD) involved in binding to the ACE2 receptor. The S2 subunit, on the other hand, consists of the intimal fusion peptide (FP), two 7-peptide repeats (HR), the outer area of the proximal membrane (MPER) and the transmembrane domain (TM) - this subunit is responsible for the fusion of the viral membranes with the host cell membrane. Full-length S protein (containing the exact conformation of the protein), RBD domain, S1 subunit, NTD and FP are used in vaccine design. The RBD domain is widely used in the development of Sars-CoV2 vaccines due to the presence of multiple conformational neutralizing epitopes capable of inducing the production of high titer antibodies that would neutralize the coronavirus.

On the other hand, the RBD domain is the domain with the most variability in the genome. There is a furin cleavage site at the interface of the S1 and S2 subunits, which allows cleavage of the S protein subunits and allows the RBD domain to specifically bind to the ACE2 receptor, facilitating fusion and subsequent viral entry into the host's respiratory cells [11].

BNT162 is an mRNA vaccine developed by the German concern Pfizer and BioNTech. Among the four candidates, the company presented nucleoside-modified mRNAs (modRNA - BNT162b1 (using RBD) and BNT162b2 (using the S protein), based on mRNA containing uridine (uRNA) and based on self-amplifying mRNA (saRNA) - each connected with a lipid nanoparticle. In November 2020, the first study published by Pfizer showing 90% efficacy of BNT162b2 vaccine in Phase 3 clinical trials involving more than 38,000 people [11]. Few days later Pfizer announced 94% efficiency of vaccination in high-risk groups without serious side effects. The only downside is distribution - vaccines must be stored at 80 ° C. The vaccine gained FDA (Food and Drug Administration) approval in December 2020 and was the first vaccine against Sars-CoV-2. In April 2021, the company announced a 91.3% success rate in for symptomatic infection and 100% success rate for severe infections as defined by the CDC and 95.3% as defined by the FDA. In August 2021, the FDA granted its full approval to the use of this vaccine in people over the age of 16 and the elderly (also as a first vaccine). Currently, after two vaccines 21 days apart (full immunity after 14 days from the second dose), a booster dose is used after six months, similar to the vaccine from Moderna. After vaccination with vaccine made by Pfizer/ BioNTech side effects often include chills, headaches, weakness, fever, redness and swelling at the injection site, but usually disappear after 1-2 days. Vaccines are much less likely to cause anaphylaxis - hence the CDC's recommendation to stay and monitor patients at the vaccination site for 15 minutes, or 30 minutes for patients with a history of shock. The FDA labeled the Pfizer / BioNTech preparation with a warning information about the possibility of myocarditis in young adults, however, such incidents, although significant, occur very rarely and the condition often disappears without medical intervention [12,13].

The American concern Moderna has patented the mRNA-1273 vaccine, which causes the synthesis of a pre-linked form of the coronavirus S protein, recognized by the host's immune system, and allows for the generation of a specific immune response. On November 16, Moderna presented the results of the study, informing about 94.5% effectiveness of the vaccine in the 3rd phase of clinical trials involving 30,000 people. The advantage, unlike the vaccine from the Pfizer / BioNTech concern, is the stability of the vaccine in conventional refrigerators for one month and in the freezer for up to six months. The vaccine by Moderna was the second vaccine (after the Pfizer / BioNTech vaccine) approved by the FDA for emergency use in December 2020. According to the company's data, the Moderna vaccine six months after vaccination showed an effectiveness of 90% against infection and over 95% effectiveness in the development of a severe infection. The side effects of the vaccine are similar to those of the competitor's vaccine, the FDA also placed a warning on the labels of the Moderna preparation about the possibility of myocarditis in young adults, but it is a highly unlikely side effect, often reversing without the need for hospitalization [14].

There are many promising mRNA-based vaccines against other infectious diseases undergoing clinical trials. In the USA, a vaccine against the Zika virus containing modified mRNA encoding the ZIKV variant and a wild-type variant, the vector of which is lipid nanoparticles (LNP) was developed - this vaccine was tested for immunogenicity and safety in mice that were observed. The experiment showed that the administration of two doses of the prM-E coding preparation allowed to obtain high titers of antibodies conferring protection and immunity against the Zika virus, which gives promising prospects for the use of vaccines in humans [14,15].

In the third phase of clinical trials there is a vaccine against CMV - Moderna mRNA-1647 designed to prevent congenital infections and to prevent CMV in transplant patients. The vaccine contain six mRNA fragments encoding two cytomegalovirus surface proteins. Five of them encode a pentamer attached to the viral cell membrane, the sixth encodes glycoprotein B (gB) - the dominant antigenic determinant of CMV - both elements are necessary for the virus to enter the host cell and, as a result, infection. Phase III clinical trials, which will end in early 2023, are expected to evaluate the safety and efficacy of the vaccine against primary CMV infection in women aged 16 to 40 years, which will involve approximately 8,000 people, including 6,900 women of childbearing age. This phase will be performed with a dose of 100 mg of mRNA-1647 [16,17].

Positive results are presented by the HIV vaccine of the IAVI companies, Scripps Research, using eOD-GT8 60-mer which can serve as improved primary immunogens to induce highly neutralizing VRC01 class bnAb antibodies in humans. They target the conserved CD4 binding site (CD4bs) of HIV. Studies in mice have proven the effectiveness of the nanoparticle for activation, multiplication and mutation to produce antibodies similar to VRC01. In the first phase of the research, the possibility of stimulating the human immune system to the generation of bnAbs (VRC01) class by eOD-GT8 60m was to be investigated. In February 2021, the concern announced the success in stimulating the production of B cells necessary for the generation of bnAb against HIV - the answer was detected in 97% of the participants who received the vaccine. The study allowed for the continuation of work on further clinical trials improving the efficacy and safety of the vaccine and offering promising prospects for the development of vaccines against other pathogens. In recent months, Moderna announced cooperation with IAVI, Scripps Research on research on the mRNA-1644 and mRNA-1644v2-Core vaccine in humans, which would be the first mRNA vaccine against HIV - the advantage over other types is the ease of mRNA modification, which is necessary for the presence of multiple variants of HIV. In the upcoming study, which will run until May 2023, 56 people between the ages of 18 and 50 will be divided into four groups and receive either the 1644 mRNA vaccine, the 1644v2 mRNA core antigen, or both. If the study shows positive results, it will move to more advanced clinical trials and become a great hope in the fight against HIV [18,19,20,21].

In the initial phase of clinical trials there is also a rabies virus vaccine (CV7201) composed of mRNA encoding rabies virus glycoprotein (RABV-G) along with with protamine (as an adjuvant and stabilizer), which in vivo testing showed safety and good tolerance, while it turned out that the effectiveness of a given vaccine largely depends on the route of administration - here, much higher effectiveness was obtained by administration without a needle using a special Pharmajet device Tropis ID ™ - intradermally or intramuscularly than conventionally using a needle and syringe. A study report by Aldrich et al. on the vaccine using dose of 1 μg or 2 μg showed an immune response up to four weeks after the second dose, however study participants will be monitored for 2 years to assess the long-term safety and persistence of the immune response. Using a dose of 5 μg had unacceptable reactogenicity for a prophylactic vaccine [9,22,23].

Conclusion

RNA is a unique macromolecule with great potential in preventing infectious diseases. Continuous work on modifying ribonucleic acid and the methods of its delivery leads to the creation of newer and newer vaccines and thus brings hope for the prevention of some dangerous diseases and the same epidemics, which was also noticeable during the coronavirus pandemic.

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